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Organizers/Conveners: n/a

PLENARY LECTURES' ABSTRACTS

Plenary Talks

Monday Plenary Talks
Oct 10, 8:50 - 10:20

PL-01

Applying in vivo electroporation to large scale vaccination and immunotherapies: Is this a moon shot?

Matti Sallberg
Karolinska Institutet, Sweden

A major obstacle in the wider use DNA-based vaccines is the poor uptake into human cells. The so far best technology to promote immunogenicity of DNA vaccines is in vivo electroporation (EP). However, this technology currently has limitations for a wider use in vaccinology due to the dependence on advanced devices, stable electricity, and that the treatment often is associated with a transient pain. These are issues that needs to be overcome before in vivo EP is a widely used and accepted technology for DNA vaccine delivery. During the COVID-19 pandemic, several new technologies have been put to a wider use in vaccine development including in vivo EP. The mRNA has proven to be safe and effective in fighting COVID-19, although the longevity of the immune response are still not clear. Adenoviral vectors were rapidly developed but have been found to have limitations as a platform for multiple doses. Thus, as a complementary technology to mRNA, plasmid DNA is highly attractive. The two platforms have different limitations and different advantages, with the ease of delivery with mRNA being and obvious significant advantage. We are currently developing DNA-based vaccines for homologous and heterologous prime-boost strategies. For COVID-19, we have developed a booster vaccine with a plasmid expressing three receptor bonding domain loops corresponding to three SARS-CoV-2 variants (WH1, Alpha and Beta), combined with the highly conserved membrane and nucleoproteins (M/N). This will be delivered using a new delivery device that allows for single-step intra-muscular DNA injection and EP treatment. However, will this be applicable to mass vaccination? In parallel we are developing DNA vaccines to prevent infections with Crimean-Congo Hemorrhagic Fever Virus, and to treat chronic hepatitis B and D virus infections. For these indications the use of in vivo EP is mot likely and easier concept to introduce and to use. Thus, what is needed from in vivo EP to be a widely accepted technology for DNA vaccine delivery. This will be discussed in the current presentation.

PL-02

Pulsed electric fields for winemaking: from the test tube to glass of wine

Javier Raso
University of Zaragoza, Food Technology, Spain

The ability of pulsed electric fields (PEF) to electroporate the membranes of grape skin and microbial cells can be used by wineries to improve winemaking. The release of polyphenol from the grape skins in the maceration-fermentation stage represents the stage with the highest requirements in energy and manpower during red winemaking. The electroporation of the grape skins by PEF increases the extraction rate of phenolic compounds that are responsible for the sensory properties (colour, flavour, astringency, and bitterness), aging performance, and beneficial effects on health attributed to the moderate consumption of wine. The improvement in polyphenolic release permits a reduction in the duration of the maceration-fermentation by 2 to 5 days resulting in an increment of the production capacity of a winery and energy-saving. In the case of white wine, wineries can also take advantage of PEF to improve the extraction of varietal aroma precursors that are located in the skin of some white grape berries. This attractive effect may prevent the use of macerating enzymes and/or save energy by shortening the duration of cold maceration in the production of white wine. The capability of PEF to inactivate spoilage microorganisms while preserving physicochemical and sensorial properties of must and wines may help enhance wine quality by guaranteeing reproducible fermentations and reducing or replacing the use of SO2 for wine stabilization. It has been also demonstrated that PEF triggers yeast autolysis thereby accelerating the release of mannoproteins from cell walls and decreasing the duration of aging on lees.
The current availability of commercial PEF units capable of responding to the processing capacity demanded by the wineries and the authorization by the European regulation of the PEF technology as a new oenological practice for white and red winemaking constitute a definitive impulse for the implementation of the PEF technology in the wineries.

PL-03

Gene Electrotransfer: Better understanding for better utilization

Marie-Pierre Rols
CNRS, IPBS, France

Cell membranes can be transiently permeabilized by application of electric pulses. This process, called electropermeabilization or electroporation, allows hydrophilic molecules, such as anticancer drugs and nucleic acids, to enter into targeted cells and tissues. Electroporation has been successfully developed in human and veterinary clinics to treat cutaneous and subcutaneous cancers. It is also promising for gene therapy and vaccination. The knowledge of the processes involved in membrane permeabilization and in gene transfer is mandatory for the method to be efficiently and safely used in clinics. As will be presented, our strategy to address these processes is to use different biological models with increasing complexities and implement different imaging tools. The description of the full mechanisms takes benefit from studies performed on different biological models (lipid vesicles, cells in 2D and 3D culture, mice) and from different microscopy tools that allow to visualize the processes. Single cell imaging experiments revealed that the uptake of molecules (antitumor drugs, nucleic acids) takes place in well-defined membrane regions and depends on their chemical and physical properties (size, charge). Small molecules can

freely cross the electropermeabilised membrane and have a free access to the cytoplasm. Heavier molecules, such as plasmid DNA, face physical barriers (plasma membrane, cytoplasm crowding, nuclear envelope) which engender a complex mechanism of transfer and is a multi-step process steps including the initial interaction with the electropermeabilised membrane, the crossing of the membrane, the transport within the cell towards the nuclei and finally gene expression. In a tissue, other barriers are present such as cell-cell contact, junction, extracellular matrix…To better understand the limits and improve the transfer of molecules, we have developed 3D spheroids models that more accurately mimic the in vivo complexity of tumors and reconstructed human skin containing a differentiated dermis and epidermis. Since microscopes have a limited penetration in tissue, we implemented a clearing technique to accurately and completely analyze and quantify the transfection rate in the whole spheroids. All these models allow to optimize the pulse parameters to obtain a high gene expression rate with minimum side effect and therefore improve the development and use of electroporation in clinics.

Plenary Talks

Tuesday Plenary Talks
Oct 11, 8:30 - 10:00

PL-09

Achieving non-invasive therapy and drug/vaccine delivery: Challenges and prospects

Hamid Hosano

Kumamoto University, Japan

The use of non-invasive or less-invasive medical procedures has numerous advantages, including cost, side effects, and post-procedure care, over conventional methods, particularly for the aging population. Recent advances in the control of electromagnetic/sonic waves propagation/interaction in deep tissue, have opened a new horizon for moving from non-invasive diagnosis to therapy, or even combining the two. Since the 1980s, extracorporeal shock wave lithotripsy (ESWL) with focusing shock waves has been a long-standing clinical practice. Currently, this technique is utilized in orthopaedics for bone formation/pain management and offers promising opportunities in cardiovascular medicine and cancer therapy as well. Image guided high-intensity-focused-ultrasound (HIFU) therapy has been successfully used to ablate benign and malignant tumors. Focusing of electromagnetic waves has also attracted considerable attention in this respect. In this talk, we will summarize our group's experiences with electromagnetic and ultrasound waves focusing (shock waves and HIFU) used for non-invasive therapies and needle-free pain-free transcutaneous vaccine/drug delivery. The current challenges and future opportunities will be discussed.

PL-10

Preclinical Insights into Electroporation and the Myocardium: What have we achieved and what do we hope to achieve? Preclinical insights

Jacob Koruth

Mt Sinai Medical Center, United States

The presentation will cover a state of the art review of electroporation and its application on myocardium as it relates to cardiac ablation. We will discuss the effects on atria and ventricles and review what has been demonstrated, what has been translated to clinical practice and what we hope to do in the near future (with a focus on ventricular myocardium). We will also briefly touch upon the role of reversible electroporation.

PL-04

Irreversible electroporation for the treatment of brain cancer: History and Future Directions

Rafael V. Davalos

Virginia Tech, United States

Irreversible Electroporation (IRE) is a minimally invasive surgical therapy we invented to treat unresectable tumors using low-energy microsecond pulses. IRE is unique among tissue ablation techniques in affecting only the cell membrane while tissue molecules, everything encompassing collagen structures to proteins; remain intact, thereby making treatment near critical structures such as major blood vessels and nerves possible. We are developing an advanced form of the technology, high frequency irreversible electroporation (HFIRE) for the treatment of glioblastoma (GBM). This new therapy preferentially targets cancer cells over healthy cells, transiently disrupts the blood-brain barrier for delivery of therapeutics, and induces a positive immune response. Our preclinical work focuses on helping canine patients with naturally occurring GBM, which are excellent translational models of human GBM. Results of our ongoing trials have been extremely positive, supporting that HFIRE is effective for the treatment of GBM, including tumors refractory to surgery, radio- and chemotherapies. Additionally, I will discuss our use of bioelectrics to develop technologies with applications in rare cell isolation, medical device design, 3D printing, and focal cancer therapy.

Plenary Talks

Wednesday Plenary Talks
Oct 12, 8:30 - 10:00

PL-05

Electrochemotherapy and IL-12 gene electrotransfer in veterinary medicine

Maja Čemažar

Institute of Oncology Ljubljana, Slovenia

The first electrochemotherapy (ECT) in veterinary medicine was performed in cats in the late 1990s, and since then ECT has become gradually used and recognised as a standard treatment modality for different cutaneous and

subcutaneous tumors in various animal species, predominantly in dogs. Some canine tumors, such as mast cell tumors and perianal tumors respond excellent to ECT, while others, such as oral tumors responds poorly and in these cases the main focus of ECT is on improvement of the quality of life. In addition to dogs, various tumor types in cats and horses can be treated by ECT. Specifically, squamous cell carcinoma which is a common malignancy in cats and requires invasive treatments, has a pronounced response to ECT with bleomycin. In horses, ECT with cisplatin has proven to be an effective treatment for sarcoids, the most common neoplasm in horses. In the last decade ECT has also been described in treating various tumors in small exotic animals, such as sea turtles, green turtles, ferrets, rats, hedgehog and cockatiel, where surgical removal of tumor would be difficult due to the size of the animals or location of the tumor. Therefore, ECT is an effective local treatment of tumors in various animal species, however it lacks the systemic component of treatment, which is essential in combating the metastatic disease. The cancer progression and metastasis are strongly connected to the insufficiency of antitumor immune response. For this reason, the use of interleukin 12 (IL-12) – a promising candidate for eliciting immune response – in combination with electroporation, called IL-12 gene electrotransfer (IL-12 GET) has been introduced to preclinical and clinical research in veterinary medicine more than 10 years ago. Several studies have been published using IL-12 GET alone or in combination with ECT for the treatment of various tumors, predominantly in dogs. IL-12 GET has proven to be mostly effective in round cell tumor, specifically mast cell tumor. In other tumor types such as squamous cell carcinoma, oral melanoma, osteosarcoma, adenocarcinoma, fibrosarcoma, the success of IL-12 GET alone or in combination with ECT is limited. The underlying mechanisms of the combined therapy are, besides direct cytotoxicity, antiangiogenic action and antitumor immune response. Moreover, IL-12 GET in combination with ECT showed to be a safe treatment with no, or minimal side effects and with minimal plasmid DNA shedding. The future of ECT alone and in combination with IL-12 GET in veterinary medicine is focused towards the finding the appropriate biomarkers for selection of tumors/patients that will respond to these types of treatment and to optimization of the treatment modality, specifically the frequency of IL-12 GET and/or ECT to obtain the optimal tumor response.

PL-11

Local intratumoral electroporation delivery of interleukin 12 immunotherapy leads to systemic anti-cancer response

Adil Daud

University of California, Melanoma and Cutaneous Oncology Department, United States

Systemic administration of potent anti-tumor immunotherapies is often associated with profound tumor responses but also with serious and sometimes irreversible treatment-related adverse events. Optimizing efficacy of treatment approaches while reducing severity of adverse events has become the primary focus in development of novel anti-cancer therapies and combination treatment approaches. Intratumoral delivery of plasmids through electroporation (EP) represents a promising local treatment approach that generates systemic immune responses while minimizing treatment related side effects. OncoSec Medical System (OMS) pairs a generator and an applicator to induce localized expression of recombinant proteins by EP in palpable tumor lesions. Intratumoral delivery of interleukin 12 (IL-12) tavokinogene telseplasmid (tavo) has demonstrated clinical utility to safely treat cancer patients across solid tumor disease states. IL-12 is a pleiotropic cytokine that has been extensively studied as a potential cancer immunotherapy candidate due to its ability to engage multiple immune effectors and reverse tumor-induced immunosuppression. However, systemic administration of IL-12 has proven to be exceedingly toxic in clinical trials, ultimately limiting its clinical utility. Intratumoral delivery of tavo via OMS EP resulted in local and systemic anti-tumor effects while inducing only minimal treatment-related adverse events in multiple clinical trials. Here, we summarize findings from two of our trials designed to evaluate a novel anti-tumor treatment combination of pembrolizumab (immune checkpoint inhibitor) and intratumoral tavo-EP.

KEYNOTE-695 study is a single-arm, phase 2, open-label, multicenter study of tavo-EP plus pembrolizumab in patients with unresectable or metastatic melanoma progressing on standard of care immune checkpoint inhibitor(s). Patients had a 27.8% objective response rate (ORR, n=54), with 47% of responding patients having durable response lasting over 1 year. Median overall survival (OS) was 23.5 months (n=56). Grade 3 treatment-related adverse events (TRAEs) were reported for 6.7% patients. No grade 4 or 5 TRAEs were reported. KEYNOTE-890 study cohort 1 is a phase 2, open-label, multicenter study assessed the safety and efficacy of tavo-EP in combination with pembrolizumab as 2L+ treatment for advanced triple negative breast cancer (TNBC). Patients had a 17.4% ORR (n=26), with a median duration of response of 16.6 months. Median OS was 11 months (n=26). Grade 3 TRAEs were reported for 23.1% patients. No grade 4 or 5 TRAEs were reported. In comparision to nanoparticle and mRNA delivery, it is associated with reduced side effects.

In summary, EP delivery of tavo allows for safe administration of IL-12 treatment in patients with solid tumors. Findings from OncoSec clinical trials support continued development of an electroporation-based delivery of potent medicines as a promising approach to local delivery of treatment with systemic effects while minimizing the severe toxicities invariably associated with systemic immunotherapy.

PL-06

Back to basics: Electroporation of artificial cells and what we can learn from them

Rumiana Dimova

Max Planck Institute of Colloids and Interfaces, Germany

Giant vesicles are a fascinating model membrane sys-

tem, which has been initially established and used as a workbench for studying basic properties of simple lipid bilayers (The giant vesicle book, Eds. Dimova & Marques, CRC Press, 2019). Nowadays, they are increasingly employed by biophysicists to unravel the mechanisms driving various biological processes occurring at the level of the cell membrane. Furthermore, giant vesicles provide exceptional biomembrane models for systematic studies on the effect of electric fields because the membrane response, in terms of deformation, poration and permeation can be directly visualized under the microscope (Dimova et al., Soft Matter 3:817, 2007; Dimova et al., Soft Matter 5:3201, 2009). Methodologies for assessing the membrane material properties and effects of membrane remodeling factors as deduced from measurements on giant vesicles become increasingly important (Dimova, Annu. Rev. Biophys. 48:93, 2019). In this talk, we will introduce such methods and showcase approaches for measuring properties such as membrane capacitance (Salipante et al., Soft Matter 8:3810, 2012; Vitkova et al. Colloid Surf. A-Physicochem. Eng. 557:51, 2018; Faizi 2021), bending rigidity (Gracia et al., Soft Matter 6:1472, 2010; Faizi et al. Electrophoresis, 42:2027, 2021), pore edge tension (Portet and Dimova, Biophys. J. 99:3264, 2010; Leomil et al., Bioinformatics Advances 1:vbab037, 2021), permeation and membrane viscosity (Faizi et al., Biophys. J. 121:910, 2022); all these approaches are solely based on observing the response of giant vesicles to electric fields. We will show that the presence of charged lipids and gangliosides in the membrane, lowers the edge tension, thus increasing pore lifetimes and rendering membranes less stable against electroporation (Lira et al., Adv. Sci., 8:2004068, 2021; Aleksanyan et al., Biophys. J. in press, 2022). Presence of calcium ions reverses the propensity of membrane disruption.

Plenary Talks

Thursday Plenary Talks
Oct 13, 9:00 - 10:00

PL-07

A snapshot of clinical research on electrochemotherapy: progress and future directions

Luca G. Campana

Manchester University NHS Foundation Trust, United Kingdom

In medicine, the use of electrochemotherapy (ECT) has been enabled by standardisation of the technique and continuous collaborative efforts across specialities. In clinical practice, this is having an impact at four levels: (1) for clinicians, via expansion of the available locoregional therapies; (2) for multidisciplinary teams, by increasing the chance of novel combined approaches; (3) for patients, through the availability of a low-demanding, quality-of-life-friendly treatment; finally, (4) for health systems, improving workflow by application of a straightforward and safe procedure.
Next, the current limitations (including bias, level of supporting evidence, and lack of shared indications) along with the future directions of investigation will be discussed. As reported by the National Institute for Health and Care Excellence (NICE) and recent meta-analyses, the current level of evidence of ECT remains low in quantity and quality and its place in guidelines marginal. Hence, the need for standardisation and continuous rigorous evaluation through comprehensive data collection and monitoring of indications, outcomes, and costs. To this aim, large databases will be essential. The International Network for Sharing Practices of ECT (InspECT) registry (https://insp-ect.eu) was established in 2008 to assess the outcome of patients treated with ECT. Since then, it has provided relevant contributions to the advancement of clinical ECT and, recently, has been structured into seven dedicated working groups (melanoma; basal cell and squamous cell carcinoma; rare histotypes; quality of life; elderly patients; breast cancer; calcium electroporation). As the current InspECT chair, I will present the main findings from the most recent publications, with particular emphasis on basal cell carcinoma, immunotherapy-ECT combination in melanoma and care of elderly patients.
Over the last few years, the improvements in ECT equipment and treatment delivery, along with the introduction of novel systemic therapies, have opened new exciting avenues for translational and clinical research. However, whereas the beneficial effects of ECT for superficial tumours and, more recently, deep-seated malignancies are widely accepted, the variability in responses across histotypes needs to be addressed. Currently, patient selection relies on clinical factors (tumour size, histotype, and exposure to previous oncological treatments); however, predictive biomarkers are lacking. To this aim, researchers will need to re-explore the biological factors underpinning tumour response to ECT, including cancer cells themselves and the tumour microenvironment, in the frame of a robust research roadmap. Notably, identifying biomarkers of response may improve the ECT technique by customising treatment parameters, improve patient outcomes by manipulating the tumour and its microenvironment, and allow the exploration of novel rational therapeutic combinations. However, whether these opportunities will be exploited to produce clinically meaningful impact and practice-changing results remains to be seen.

PL-08

Tumor Treating Fields: A Novel Treatment Modality for Solid Tumors

David D. Tran

University of Florida College of Medicine, United States

Tumor Treating Fields (TTFields) is a novel approved therapy for glioblastoma (GBM) and malignant mesothelioma and presently under phase 3 studies in several other solid cancers. TTFields employ non-invasive, external application of low-intensity, intermediate-frequency, alternating electric fields to disrupt the mitotic spindle, leading to chromosome mis-segregation and apoptosis. TTFields also target several DNA damage repair mechanisms and trigger ER stress-dependent autophagy. More recently, TTFields has been shown to induce blood brain barrier permeability, plasma membrane perforation, and immun-

ogenic cell death thought to result in peritumor inflammation that is routinely observed in TTFields-treated patients. A potential mechanism of this property centers on TTFields-induced focal disruption and perforation of the nuclear envelope, leading to cytosolic release of large naked micronuclei clusters that recruit and intensely activate major DNA sensors and type 1 interferon (T1IFN)-dependent anti-tumor innate and adaptive immune stimulation. In a study involving patients with newly diagnosed GBM (ndGBM) treated with TTFields, robust T cell activation was detected specifically via the T1IFN trajectory, which highly correlated with T cell receptor clonal expansion, a hallmark of antigen-specific adaptive immune reactions. The combination of TTFields and an immune checkpoint inhibitor created a potential therapeutic synergy, demonstrating highly promising efficacy with acceptable toxicity in ndGBM. However, resistance to TTFields eventually occurs in many patients. The mechanism of resistance follows TTFields' effect on cellular membranes leading to mis-localization and mis-association of key master regulators controlling cancer stem cells and pro-tumor inflammation. Thus, current TTFields-based cancer immunotherapeutic strategy in solid tumors should be focused on maximizing its T1IFN-stimulated adaptive immunity while also disrupting its stemness and inflammatory dysregulation.

ORAL PRESENTATIONS' ABSTRACTS

Educational Session

Sunday Educational Session Track
Oct 09, 13:00 - 17:00

OR-03

Pulsed electric fields

Antoni Ivorra

Universitat Pompeu Fabra, Department of Information and Communication Technologies, Spain

If living organisms are exposed to long electric fields, most of the observable physiological effects appear to be of thermal origin. On the other hand, if the electric field exposure is brief (i.e., pulsed), it is possible to apply high electric fields without causing significant heating and two remarkable biophysical phenomena can be observed: electrical stimulation and electroporation.

Electrical stimulation consists in nonphysiologically inducing action potentials by delivering electric fields. (Action potentials are sudden transitions in transmembrane resting voltage that propagate along the cell membrane and are the basis of nerve impulses.) Electrical stimulation only occurs in excitable cells such as neurons or muscle cells. On the other hand, electroporation is a universal phenomenon that occurs in all living cells. Electroporation consists in nonphysiologically increasing the plasma membrane permeability to ions and molecules by exposing the cells to high electric fields. Such increase in permeability is, presumably, related to the initial formation of nanometric pores in the cell membrane; from which the term electro-"poration" stems. Remarkably, both phenomena occur when the transmembrane voltage is artificially increased above a threshold due to the presence of the electric field. (However, as we will see in the talk, the concept of transmembrane voltage threshold to initiate electroporation is debatable.)

The degree of permeabilization caused by electroporation depends on the characteristics of the exposure (e.g., field magnitude and duration) and on the sort of cells or tissues and their environment. It can result in viable cells (reversible electroporation) or it can result in cell death (irreversible electroporation). Many biomedical and biotechnological treatments are based on electroporation. In vitro, reversible electroporation is now commonly used for gene transfection of cells in culture whereas irreversible electroporation is used for cold pasteurization of liquid media or for facilitating the extraction of cellular contents. In vivo, reversible electroporation is used in living tissues for gene therapy and to enhance the penetration of anticancer drugs or calcium into undesirable cells. Also in vivo, irreversible electroporation (IRE) is used in minimally invasive procedures to ablate undesirable tissues for cancer treatments or for managing cardiac arrhythmias.

After an overview of fundamental concepts on electricity and bioelectricity, the talk will be focused on biophysical aspects of electroporation. However, electrical stimulation and other phenomena that may accompany electroporation will also be presented. In particular, Joule heating and electrochemical reactions will be introduced as these phenomena are sometimes neglected by the newcomer. On the other hand, the applications of electroporation will be overlooked as these will be presented in other talks in the educational session.

OR-192

Applications of pulsed electric field in the food industry - Educational session

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The modern food industry is facing crucial challenges amid an increased global population, and the demand of high quality, safe, healthy, and nutritious products. Food production should also be sustainable and with low environmental impact. These challenges can only be achieved through research and innovation on food systems that include novel and nutrition-driven technologies.

Pulsed electric field (PEF) is a technology that is already being implemented in the food industry with clear advantages on low-energy consumption for processing, improved retention of nutrients, flavour or texture in comparison with traditional processing technologies. PEF applications in the food industry include "cold pasteurization" of juices, extraction of cellular compounds, meat tenderization and optimization of fermentation processes as well as pre-treatment of vegetables prior to unit operations such as slicing, frying, and drying.

OR-01

Pulsed Electric Field (PEF) Technology for Environmental Applications

Christian Gusbeth

Karlsruhe Institute of Technology (KIT), Institute for Pulsed Power and Microwave Technology (IHM), Germany

The environment is the external conditions, including all the biotic and abiotic factors that interact and affect the survival and development of an organism or population. The aim of environmental applications is the reduction of hazardous waste, water contamination, air and atmospheric pollution and soil degradation. The dramatic increase in the world's population, combined with industrialisation, urbanisation, intensification of agriculture and water-intensive lifestyles, is increasingly leading to shortages in water supply. Currently, about 20% of the world's population has no access to safe drinking water. While water is a renewable resource, it is also a finite one. Global freshwater consumption has increased sixfold in the last century. The discharge of industrial effluents, sewage and sludge into water bodies is responsible for infection risks and health effects from contaminated drinking water. Therefore, improved technologies need to be developed to control chemical and microbial contamination and the high consumption of this valuable resource. This lecture will discuss the environmental impact of bacterial contamination of industrial water circuits and hospital wastewater and how the application of PEF technology can help solve these environmental problems. In the last decays

PEF techniques gained increasing importance in cellular biology, in gene technology, in medicine, in food production and in biotechnology. The significant part of the lecture will focus on the PEF treatment for bacterial decontamination of hospital wastewater effluents. The stringent necessity of decontamination of such wastewater effluents relies on the fact that these effluents are loaded with pathogenic and increasingly with antibiotic-resistant bacteria. One important issue addressed during the lecture is the safety of the PEF technology, related to mutagenicity and induced electro tolerance in reference bacteria. In the second part of the presentation, a promising application of PEF treatment in electrocoating systems of paint shops will be presented. The aim of this application is to prevent the bacterial contamination in the pretreatment and coating process to eliminate the use of biocides and drive down both freshwater consumption and wastewater generation. In addition, by efficiently monitoring the bacterial load in process liquid, a high finish quality can be maintained, reworking is avoided, and less paint consumption make more efficient use of resources and lower operating costs. We have found that PEF treatment with short bipolar pulses and even with a high specific treatment energy, which is required in extreme cases to achieve maximum bacterial inactivation, does not affect the quality of the coating. PEF treatment is therefore a suitable method for automation and effective in bacterial inactivation, does not affect the quality of the coating. PEF treatment is therefore a suitable method for automation and effective in bacterial decontamination of paints.

OR-02

Biomedical applications of electroporation

Gregor Serša

Institute of Oncology Ljubljana, Department of Experimental Oncology, Slovenia

Exposure of cells or tissues to an electric field can induce structural changes in the cell membrane that allow inflow of molecules into the cells. At the same time, the outflow of molecules is induced. Under specific conditions, the changes in the membrane are reversible; therefore, we call that reversible electroporation. This condition is useful for the introduction of cytotoxic drugs with hampered transport into the cells, such as bleomycin and cisplatin. This is called electrochemotherapy. Additionally, DNA or RNA can be transported into the cells, so it is useful as a delivery system for gene therapy. This approach is called gene electrotransfer. When multiple pulses are applied to the cells or tissues, irreversible changes are induced in the cells, which lead to cell death. This is called irreversible electroporation.

Electrochemotherapy is used as tissue ablation therapy for either superficial tumors or deep-seated tumors. Based on the specific mechanism of action, electrochemotherapy is effective in tumors with different histologies. In addition, electrochemotherapy is effective also for treatment of vascular malformations. Currently, it is used in many EU cancer centers and also in veterinary oncology around the world. Due to the induction of immunogenic cell death, this locally induced immune response contributes to the eradication of tumors and can be considered an in-situ vaccination.

To further improve the therapeutic effect, electrochemotherapy can be used in combination with an immunotherapeutic approach, it can be used either in combination with immune checkpoint inhibitors or immunostimulants. One of the approaches that is being explored is electrochemotherapy in combination with gene electrotransfer with a plasmid coding for interleukin 12 (IL-12). Electrochemotherapy in combination with gene electrotransfer is being used in veterinary oncology for the treatment of dogs. These combinations are also being explored with irreversible electroporation.

Due to their effectiveness, these approaches are constantly being explored and applied in many different ways. In addition to application in oncology, gene electrotransfer can also be used for the delivery of vaccines into tissues. The technology with new skin applicators and plasmids or RNA vaccines coding for proteins that trigger an immune response are being tested. One of the applications is also the SARS-CoV-2 vaccine. To make all these biomedical applications even more effective or to refine their use, we need to learn more about the biological factors that govern the treatment effects. These factors will open new possibilities for selection of patients suitable for treatment and help to refine specific treatment approaches or even personalize electroporation-based treatments.

OR-126

Electrochemotherapy- from Bench to Bedside and Beyond

A. James P. Clover

Cancer Research @UCC., University College Cork, Ireland

Electrochemotherapy is the combination of electroporation and low dose cytotoxic drug to achieve localised tumour control. This treatment had risen to become a well-established treatment for cutaneous malignancies both of skin and non-skin origins. It is now delivered in many Cancer Centres across Europe and has become part of national treatment guidelines. This educational session talk will explore the journey of this development.

Early investigators on both sides of the Atlantic realised the potential for electrochemotherapy to add an additional option in the tool box for Cancer treatments by combining electroporation and low dose cytotoxic treatments. Early treatment successes lead to the development of standard operating procedures and a consortium of clinicians across Europe that aimed to produce the experience and evidence base to validate this treatment. Now the efficacy and durability of treatment effect is increasingly established. However, both bench side and clinical researchers continue to strive to develop and expand treatment options available to patients. Current developments include many exciting treatment variations. These include treating solid organs as well as skin, altering the cytotoxic agent to include novel agents such as calcium and investigating pulse parameters.

This educational talk will give a comprehensive introduction to Electrochemotherapy by covering the principles of treatment, the evidence base for effectiveness and explor-

ing where future developments are likely.

OR-223

Pulsed electric fields and the cardiovascular system: cardiac ablation and beyond

Elad Maor
Sheba Medical Center, Israel

Pulsed field ablation (PFA) with irreversible electroporation has emerged as a promising technique for catheter ablation of cardiac arrhythmias. While commercial devices are being used clinically for the treatment of atrial fibrillation, there are significant knowledge gaps and contemporary industry-independent basic data is needed. In addition, there is an increasing interest in other possible application of PFA in the cardiovascular field. These applications include treatment of ventricular fibrillation, septal ablation, targeting other vascular structures and cardiovascular neuromodulation. PFA has appealing characteristics for cardiologists, including its ability to be tissue specific and its nonthermal nature. The waveform details of commercially used PFA systems are not been disclosed by the industry due to intellectual property concerns. These waveform properties (e.g., pulse intensity, waveform shape, number of pulses, electrode configuration and geometry) are critical for treatment planning, and can affect cardioselectivity, safety and efficiency of the treatment. In this talk we will provide information on the fundamentals of electroporation relating to the cardiovascular system, summarize key studies and applications to date, and provide insight into future applications. Specifically, we will discuss (1) electroporation cardioselectivity including its effect on blood vessels and concerns regarding coronary vasospasm, (2) importance of high-frequency-based protocols, (3) how human induced pluripotent stem cells models are used to study PFA effect on cardiomyocytes, (4) review contemporary clinical data.

P1 - General Applications for Food Processing

Monday morning Track A
Oct 10, 10:50 - 12:10

OR-84

Dielectric analysis of Gram-positive and -negative bacteria subjected to pulsed electric fields

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Kumamoto University, Japan

Pulsed electric field (PEF) is a promising method for sterilizing conductive liquids at low temperatures. Bacterial resistance to PEF depends on the type of bacteria, including the shape, size and membrane structure classified by the Gram staining. Gram-positive bacteria have a thick cell wall (tens of nm) with a membrane, while Gram-negative bacteria have a thin cell wall sandwiched between the inner and outer membranes. Several experimental studies for example H. Htilsheger and B. Mazurek, have shown that Gram-positive bacteria are more resistant to PEF than Gram-negative bacteria. However, there is little discussion about the physical effect of PEF and resistance to PEF at the cellular level based on their structures and physical properties. In this study, we measured the dielectric properties of bacteria subjected to PEF using an impedance spectroscopy, and evaluated the degree of the PEF-induced bacterial damage. Listeria innocua as Gram-positive and Klebsiella aerogenes as Gram-negative were compared for the resistance to PEF by considering the change in their dielectric properties measured using an impedance spectroscopy, together with the survivability in our sterilization experiment. Besides, we discussed the recovery of both bacteria damaged by PEF by the time course of their dielectric properties. Our sterilization experiment showed that Gram-positive bacteria were more resistant to PEF, as was previously reported. The impedance spectroscopy indicated that Gram-positive bacteria, compared to Gram-negative one, had a smaller change in dielectric properties and a larger electric field threshold at which dielectric property changed. A numerical study of electric field distribution on Gram-positive or Gram-negative bacteria subjected to PEF implies the microscopic effect of PEF on the bacterial membrane.

OR-85

Assessment of microbial inactivation and lipid oxidation using non-thermal plasma on mussels

Cinzia Mannozzi[1], Roberta Foligni[1], Lama Ismaiel[1], Silvia Tappi[2], Romolo Laurita[2], Luca Belleggia[1], Cristiana Cesaro[1], Andrea Osimani[1], Massimo Mozzon[1]
[1]Università Politecnica delle Marche, Italy
[2]University of Bologna, Italy

Mediterranean mussels (Mytilus galloprovincialis) farming has a great impact on the economy of many European countries. However, this kind of shellfish is a filter-feeding organism that can accumulate bacteria in very high quantities, thus plasma-activated water (PAW), as a non-thermal technology, can be a potential tool able to guarantee food decontamination.

However, considering that the food decontamination occurs through the activity of the highly reactive species (e.g. hydrogen peroxide, nitrates, nitrites), this one can be induced undesired chemical changes as well, thus leading to a deterioration of quality and nutritional features, such as peroxidation of food lipids.

The aim of this work was thus to assess the microbial inactivation and investigate the oxidation degree of mussels processed with PAW obtained by exposing 500 ml of distilled water for 4 min to a pulsed corona discharge driven by a high voltage power generator (AlmaPulse, AlmaPlasma s.r.l.) using peak voltage of 18 kV and a pulse repetition frequency of 5 kHz. PAW dipping times of 5, 10, and 15 minutes were explored vs controls dipped, for the same times, in demineralized water.

Total lipids were extracted from PAW-treated samples and controls by the Bligh and Dyer (1959) method and analysed for non-volatile (peroxides, oxysterols) and volatile lipid oxidation products. Twelve oxygenated derivatives from the cleavage of the fatty acid hydroperoxide isomers were detected in the headspace of mollusc lipids

(C5-C9 aldehydes, alcohols, and ketones) and six cholesterol oxidation products (COPs) were identified in the unsaponifiable matter. Peroxide value, total fatty acid composition, volatile levels, cholesterol and COPs content were not significantly affected by PAW soaking. A total amount of 110-140 μg of COPs/g of total lipid were observed in the analysed samples, corresponding to 10-20 μg of COPs/110 g of fresh matter. However, the efficacy of the microbial decontamination (total mesophilic aerobes, Enterobacteriaceae, Pseudomonadaceae, and Escherichia coli) was very limited as well.

The present work is part of the project "PRIN 2017 -PLASMAFOOD - Study and optimization of cold atmospheric plasma treatment for food safety and quality improvement" founded by MIUR - Ministero dell'Istruzione dell'Università e della Ricerca.

OR-87

Application of moderate electric fields to improve mass transfer steps in fruit processing

Anne Kathrin Baier, *Justus Knappert*, Cornelia Rauh

Sustainable food processing demands for holistic usage of raw materials and energy-efficient processing concepts. Moderate electric fields (MEF) provide an innovative and sustainable way of food processing due to the direct and very efficient usage of electrical energy. The combination of electrical effects on cells and tissue as well as increased processing temperatures due to ohmic heating have a high potential for the improvement of mass transport steps. The research project MEFPROC aimed at generating knowledge on the application of MEF in mass transfer operations to facilitate MEF uptake in industrial food processing.

The chain of fruit processing provides several options for MEF integration and was taken as an example of possible application. Juice recovery via mechanical pressing can be improved by previous MEF application due to cell rupture and facilitated flow of liquid from the tissue. An increase in cell disintegration of fruit mash by MEF could be determined dependent on the previous degree of mechanical milling. For coarse mashes, an increase by factor 2 was measured while for fine mashes with already high degree of disintegration no further cell opening was possible. Depending on the mash fineness an increase in juice yield by up to 20 % was found. Disintegration of apple mash could as well be demonstrated in pilot scale. Besides the MEF treatment itself, the design of the subsequent juicing showed to be of high importance.

For processing of red currants, marked increases in the polyphenol content and the antioxidant capacity of the obtained juices after MEF treatment were observed. This was traced back to the temperature dependent extractability of phenols from the plant matrix. The MEF treatment thus could potentially replace time consuming mash incubation before pressing and /or improve nutritional quality of the juices.

MEF led to significant increases in extraction yield for polyphenols from red currant press cakes using aqueous solvents. The highest differences between treated and untreated samples were found directly after treatment for an extraction time of 0 min. MEF also increased yields during further extraction at temperatures of 42 °C or 85 °C by 50 and 30 %, respectively. This indicates that improved extractability is a result of altered cell and tissue structure, not only of an increase in temperature. These effects could be used to decrease extraction time or to partly replace extraction steps with organic solvents. The polyphenol contents of the extracts were in good correlation with the antioxidant capacity.

MEF application is possible as an additional processing step before pressing or extraction as well as by installing electrodes into existing concepts for extraction units. Thorough optimization of MEF parameters as well as adaption of the previous and subsequent processing steps is required to fully benefit from MEF effects on mass transfer.

OR-88

Enhancement and Reversion of Irreversible Electroporation via Osmotic Shock Treatment

Greta Gančytė, Povilas Šimonis, Arūnas Stirkė
Center for Physical Sciences and Technology, Department of Functional Materials and Electronics, Lithuania

Saccharomyces cerevisiae yeast employs the HOG pathway to recover after dangerous shape modifications and intracellular water disbalance caused by environmental osmotic pressure changes. Pulsed electric field (PEF) treatment is known to cause plasma membrane permeabilization, an effect known as electroporation. Yet, there is no information on whether the HOG biochemical pathway has a role in yeast response to PEF treatment.
It was investigated if post-PEF osmotic pressure change of the media affects yeast cells' viability, size and if the HOG pathway is involved in recovery. Experiments were performed with wild type (WT) Y00000 yeast and a mutant strain derived from WT, Y02724, with no active HOG1 gene. Yeast suspension was exposed to a single electric field pulse with a duration of 150µs and field strength of up to 10 kV/cm. Electroporation buffer was used as a reference point to represent isoosmotic conditions. Swift osmotic pressure change is defined as osmotic shock. After PEF treatment, cells were transferred to hyperosmotic, isoosmotic or hypoosmotic solution. Viability was assesed by counting colony forming units. Cell size was evaluated by measuring the turbidity of the solution. Protein and DNA efflux was observed by measuring light absorbtion of the media at 260 and 280nm wavelengths.
We showed that post-pulsed electric field treatment by a sudden change of osmolarity of the media has a significant impact on the yeasts' viability, cell size and leakage of intracellular components into the media when compared to isoosmotic conditions. We show that electroporation efficiency depends not only on electric field strength, duration and the number of impulses applied, but also on whether post-PEF treatment was applied. While it is known that electric fields of 8-10 kV/cm cause irreversible damage to the membrane, after incubation in hyperosmotic conditions after PEF, viability increased by ˜30% and the radius of the cell was reduced by ˜2 µm. After weaker field strengths of 2 and 4 kV/cm PEF and hyperosmotic shock treatment, the viability of WT yeasts was restored to 100

%, for hog this result was achieved only after 2 kV/cm treatment. Hipoosmotic shock caused the opposite effect: after exposure to pulse of 4 kV/cm viability decreased by 12 % (WT) and 39 % (hog); after exposure to 6 kV/cm pulse, by 16 % and 25 %. The size of the cells almost doubled, after 10 kV/cm treatment. Protein and DNA leakage evaluation revealed that amount of intracellular compounds in the media decreased after hyperosmotic shock and increased after hipoosmotic shock, supporting the hypothesis that mechanical cell shape alteration influences cells' reaction to PEF. Furthermore, hog strain was more sensitive to treatments suggesting that HOG pathway is of relevance to recovery.
To summarise, yeast membrane recovery after exposure to PEF can be altered by subsequent change in osmolarity of media. HOG pathway involvement was linked to recovery after electroporation.

P12 - Electroporation and Cellular Pathways

Monday morning Track B
Oct 10, 10:50 - 12:10

OR-167
Identification of the ion channels affecting membrane permeabilization by nanosecond electric pulses

Giedre Silkuniene, Uma Mangalanathan, Alessandra Rossi, Andrei G. Pakhomov, *Olga N. Pakhomova*
Old Dominion University, United States

Several studies reported that electric fields could suppress the function of ion channels, possibly by electroconformational changes in the channel proteins. We hypothesized that electrically induced alterations in ion channel structure contribute to cell membrane permeabilization. This work aimed at identifying ion channels affecting electroporation with nanosecond electric pulses (nsEP).
We used a LentiArray CRISPR library to knockout (KO) 328 ion channel genes in U937 human monocytes stably expressing Cas9 nuclease. In the library, gene-specific guiding RNAs (gRNAs) are packed into lentiviruses and arrayed in a 96-well format. Each well contains four gRNAs designed to recognize different regions of the same gene and facilitate a permanent gene KO. A total of 328 U937 cell derivatives were generated, each with a KO for a single ion channel gene. Each KO was treated in electroporation cuvettes with trains of 20 or 40 pulses (300 ns, 7 kV/cm, 20Hz) in the presence of a membrane-impermeable fluorescent dye YoPro-1 (YP). Then the cells were placed in a 96-well plate and, in 50 minutes, imaged to access membrane permeabilization by YP entry. Imaging was performed using an Olympus IX83 microscope configured for high-throughput screening with automated stage repositioning and auto-focusing. Nine fields of view in each well were scanned and stitched in one image, yielding 300-600 cells per sample. YP fluorescence in individual cells was quantified with the Advanced CellScoring package of MetaMorph, then averaged and normalized to the control transduced with scrambled gRNA.
In the first screening, all 328 KOs were exposed to 20 pulses. Based on the average of four independent experiments, we selected KOs with lower- and higher-YP uptake than in the control. The selected KO cell lines were generated de novo, and the new screening was performed two weeks after transduction. Forty and twenty pulses were applied for lower-YP and higher-YP groups, respectively. In the lower-YP group, thirteen KOs responded to nsEP with at least 8% YP uptake reduction, thus reproducing the first screening results. For three gene KOs (CLCA1, KCND3, and SHROOM1), YP reductions were statistically significant ($p<0.05$), and the overall effect was 15-27% lower than in the control. Unusually, ATP1A1 gene KO initially showed a strong reduction of the YP effect but weakened with time after the transduction. Increased YP uptake (>8%) was detected for at least fifteen gene KOs. For five genes (ATP2C2, CACNA1G, CHRNA10, KCTD17, and SCNN1B), the effect was significant ($p<0.05$) and 15-26% higher than in the control.
The genes whose KO resulted in lower YP uptake are likely producing proteins predisposed to injury by nsEP, either structural or through interactions with lipids and other proteins. The KOs leading to higher YP uptake point to genes involved in plasma membrane stabilization or electric injury repair.

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OR-168
Denaturation of proteins subjected to 1 ns-long MV/cm electrical pulses

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The electric field on the plasma membrane of a cell exposed to a PEF of several kV/cm exceeds 1 MV/cm, which permeabilizes the membrane and induces trans-membrane ion flow. Also, membrane proteins as dielectric materials with diverse structures are subjected to the huge fields. Molecular dynamics simulations have predicted that MV/cm-class PEF stimulates membrane proteins and activates their functions, and there is growing interest in the effects of PEF on proteins.[1] However, due to the significant biochemical response following the PEF induced Ca2+ influx, it is difficult to explore the effect of PEF on proteins. Previously, we experimentally demonstrated that intense electrical pulses on the order of 200 kV/cm destroy urease protein in its aqueous solution using the specially adjusted electrophoresis.[2] Moreover, we reported the MV/cm class electrical pulses disintegrate transthyretin aggregate.[3] Here, we investigated the primary, secondary and tertiary structures in three kinds of proteins, lysozyme, albumin, and urease, characterized by structures and molecular weights, exposed to 10 pulses of 1.3 MV/cm. Electrophoresis indicates that the primary structure in all three kinds of proteins were altered. Circular dichroism

(CD) analysis, applied to albumin protein, indicated the number of α-helices was significantly decreased by the exposure to the pulse. Through the experiment, we carefully monitored the change in pH, temperature, and hydrogen peroxide concentration, which could affect the protein conformation, none of these factors affected any of proteins.

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OR-170

Pulsed electric fields as tool for human skin remodeling

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Impairment of extracellular matrix (ECM) remodeling is observed in tumour microenvironment or fibrosis and results in excessive collagen production and/or its decreased degradation by metalloproteinases (MMPs). Due to its pivotal role in tissue architectures and functions, ECM components and the proteins that regulate ECM remodeling are thus promising therapeutic targets for human healthcare. Physical stimuli appear as attractive tools to remodel ECM owing to their local and space-time control effects1.
For that purpose, we assessed the potential of pulsed electric field technology, classically applied to drug and plasmid delivery, to modulate cutaneous cells behavior as well as remodel collagen at tissue scale. Two distinct calibrated electric protocols classically used for gene electrotransfer (GET) (10 square-wave pulses of 5 ms, 1 Hz) and electrochemotherapy (ECT) (8 square-wave pulses of 100 μs, 1 Hz) were applied in our studies without addition of any drugs. It appeared that dermal fibroblasts proliferation and migration properties were not affected when grown in monolayer, whatever the electric field intensity applied2. We assessed the ECM remodeling after electroporation was applied onto a tissue-engineered human dermal substitute model, by examining genes modulation by transcriptomic and proteins synthesis, as well as MMPs activity. Fourier-Transform Infrared-Attenuated Total Reflectance and Differential Scanning Calorimetry were used as complementary tools to analysed collagen structure. We demonstrated3 that pulsed electric fields induced 1) a rapid modulation (4h after electrostimulation) of mRNA's genes composing the matrisome, particularly a down-regulation of pro-collagens and ECM maturation's enzymes; 2) a transient decrease in pro-collagens production and hydroxyproline tissue content within a week after electrostimulation; 3) a long-lasting ROS-dependent over-activation of MMPs and especially collagenase's family for at least 48h and 4) a down-regulation of TGF-β1, a key player in pathological fibrosis. First results also indicate that pro-angiogenic processes occur after pulsed electric field application.
Taken together, our results open up realistic and relevant prospects for pulsed electric field technology as a local and effective treatment of skin abnormal ECM.

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OR-171

Electropermeabilization and the packing of bilayer lipids: a problem addressed by real-time fluorescence measurements

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Although there are studies that link electropermeabilization (EP) to phenomena like: increased production of reactive oxygen species (ROS), enhanced endocytosis, lipid peroxidation, etc., the mechanisms are yet to be elucidated. The goal of our work was to obtain pertinent data regarding the molecular changes in the biophysical parameters of the cell membrane due to the application of EP pulses, mainly related to the lipid bilayer packing and peroxidation [1].
We used generalized polarization (GP) which is a parameter sensitive to changes in membrane lipids arrangement. Membrane GP is measured using liposoluble fluorophores (like: laurdan) having a high spectral sensitivity to the presence of water molecules in their proximity. The emission spectra of laurdan shift their maxima towards longer wavelengths if the lipid environment becomes less packed, allowing water molecules to penetrate deeper in the hydrophobic core of the membrane. In a less packed membrane, GP decreases.
We used a specially designed system of electrodes which allowed performing electropermeabilization of cells in

suspension simultaneously with time-dependent measurements of fluorescence and of temperature.
NIH 3T3 cells were electroporated with 1 to 50 bipolar pairs of rectangular pulses. GP, ROS production and temperature were monitored in real time before and after pulse delivery. The GP behavior was analyzed in conjunction with the temperature variation of the cell suspension. In order to distinguish between thermal and non-thermal effects of EP pulses, various buffer conductivities (0.01, 0.04, 0.14 S/m) have been used for various number of pulses. Concerning the correlation of peroxidation with the GP parameter evolution, ROS production was inhibited by using N-Acetyl L-Cysteine as a ROS scavenger. Endocytosis inhibitors (Chlorpromazine and Filipin III) were used to check whether the variation of GP was caused by a specific clathrin/caveolin endocytosis process triggered by EP pulses.
Two categories of effects were observed: i/ a thermal effect, consisting in an increased bilayer disorder (a deeper penetration of water into the hydrophobic core), and ii/ a nonthermal effect, leading to a higher degree of lipids packing, the latter being attributed to a peroxidation process (if EP was conducted in the presence of the ROS scavenger, a reduction of the GP deflection was observed). A correlation between the kinetics of ROS production and that of the unpacking of membrane lipids triggered by EP was demonstrated, and this correlation showed not to be linear. The membrane lipids packing, as observed by GP, was not affected by the inhibition of endocytosis processes. An analysis of the permeabilization conditions in which the changes in the packing of the bilayer lipids and their peroxidation occur, was performed.

[1] Tivig I et al., Bioelectrochemistry 138 (2021) 107689

OR-172
Microsecond pulsed electric fields: effects on U87-cell line in different culture conditions

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Glioblastoma multiforme (GBM) is the most common brain cancer in adults. GBM starts from a small fraction of poorly differentiated and aggressive cancer stem cells (CSCs) responsible for aberrant proliferation and invasion. Notably, the canonical CSC paradigm has been recently revised, and it is now widely accepted that the CSC phenotype is characterized by dynamic cellular state rather than a fixed population. Due to this extreme tumor heterogeneity, actual therapies provide poor positive outcomes, and cancers usually recur.
Therefore, alternative approaches, possibly targeting CSCs, are necessary against GBM. Among emerging therapies, high intensity ultra-short duration pulsed electric fields (PEFs) are considered extremely promising and a previous investigation by our group demonstrated the ability of a specific electric pulse protocol (named PEF-5: 0.3 MV/m, 40 us, 5 pulses) to selectively affect medulloblastoma CSCs preserving normal cells.
Here, we tested the same exposure protocol (PEF-5) to investigate the response of U87 GBM cells.
According to the dynamic nature of CSCs, we evaluated the effects of PEF-5 exposure on U87-MG cells maintained in standard culture conditions (U87 ML) or as neurospheres (U87 NS).
Our data, obtained by analyzing different endpoints (i.e., cell viability, cell permeabilization, reactive oxygen species (ROS), full transcriptomic analysis and mRNA expression of different genes, as well as clonogenic and invasion potential), showed that PEF-5 exposure substantially influenced the fate of GBM CSCs. The exposure seems responsible of a differential regulation of many genes involved in hypoxia, inflammation and P53/cell cycle checkpoints, with consequent reduction of the cell's ability to form new neurospheres and to transmigrate in vitro. Due to the well-recognized radio-resistance reported for GBM, in this work, we assessed the PEF-5 capability to affect GBM cells also in combination with ionizing radiations (IRs). Combined PEF-5 exposure and IRs showed an additive effect of the two physical stimuli with a dose-dependent effect of IR on the cell clonogenic potential. Further, cell invasiveness was suppressed in both U87 ML and NS exposed cells. Of note, PEF-5 treatment alone was significantly more effective in decreasing cell migration and invasion than radiation exposure in both U87 ML and NS cells independently of the delivered dose.
Globally, our results confirm ultra-short pulsed electric fields as a promising treatment to destabilize GBM, opening up the possibility of developing effective PEF-mediated therapies.

P34 - Development of pulse generators and electrodes

Monday morning Track C
Oct 10, 10:50 - 12:10

OR-47
Piezoelectric Transformer based High-Voltage Pulse Generator using Wide-bandgap Semiconductors for Electroporation

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The demand for nano-second to micro-second range HV pulse generators for electroporation is growing as evidenced in the recent literature, where the application of electroporation is of significantly increasing interest in the field of medicine and biotechnology. The HV pulse amplitude, pulse-width and repetition frequency are decided depending on the specific application.
In this work, a new application of Piezoelectric Transformer (PT) based power converters to generate high-voltage (HV) bipolar pulses for electroporation in can-

cer treatment applications is proposed. In the first stage of power conversion, the PT power converter with GaN FETs and SiC diodes accepts a relatively low-voltage input of 24 Vdc and converts it to a HV output, charging a capacitor to 2 kV. In the second stage, the charged HV capacitor is used as a source for a SiC MOSFET based inverter to generate bipolar pulses with pulse widths of 600 ns. The detailed PT based converter design and selection of wide bandgap semiconductor (WBG) switches such as GaN FETs, high-voltage SiC diodes and SiC MOSFETs, as well as simulation results to demonstrate proof-of-concept using LTSpice are presented. Work is ongoing to develop hardware prototype to determine the performance, size, and weight of the proposed HV pulse generator experimentally, and to compare it against commercially available electroporation power supplies.

PTs are a special type of transformer that do not make use of electromagnetic energy transfer mechanisms. The principle is to use vibration as a coupling medium to transfer input electrical energy to mechanical energy and then again back to electrical energy at the output with a different voltage amplitude.

Compared to traditional electromagnetic transformers for high step-up and high-voltage applications, PTs have advantages such as high power-density, low electromagnetic interference, reduced weight, high galvanic-isolation, and comparable efficiency. Almost all power converters proposed for HV electroporation pulse generation in the literature make use of either an inductor and/or a transformer to boost the input voltage. For a high-gain step-up conversion from a relatively low-voltage input, PTs have been demonstrated in the past as a better alternative to magnetics-based power conversion. Furthermore, they may enable the removal of magnetic components from the system which is advantageous for medical applications.

The objective of this work is to explore and extend the application of PTs to generate HV pulses for electroporation at a better performance, size, weight, and cost compared to existing magnetics-based power converters. The potential for extending the pulse voltage and repetition rates will also be considered. The performance of such a PT based HV pulse generator using WBG devices specifically for electroporation cannot be found in the published literature.

OR-98

Four channel 12 kV, 50 A, 100 ns - 100 µs generator for biomedical and biotechnological applications

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Pulsed electric fields in the sub-microsecond range are being increasingly used in biomedical and biotechnology applications, where the demand for high-voltage and high-frequency pulse generators with enhanced performance and pulse flexibility is pushing the limits of pulse power sol-id-state technology. In the scope of this article, a new pulsed generator, which includes four inde-pendent MOSFET based Marx modulators, operating individually or combined, controlled from a computer user interface is described. The generator is capable of applying different pulse shapes, from unipolar to bipolar pulses into biological loads, in symmetric and asymmetric modes, with voltages up to 12 kV and currents up to 50 A, in pulse widths from 100 ns to 100 µs, including short-circuit protection, current and voltage monitoring. This new scientific tool can open new re-search possibility due to the flexibility it provides in pulse generation, particularly in adjusting pulse width, polarity, and amplitude from pulse-to-pulse. It also permits operating in burst mode up to 5 MHz in four independent channels, for example in the application of synchronized asymmetric bipolar pulses, which will be shown together with other characteristics of the generator.

OR-48

Super boosting for sub-nanosecond switching of high voltage SiC MOSFET

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Fast HV generators were traditionally based on gas switches with all their associated limitations. Recent progress in HV Si and furthermore SiC MOS transistors opened the door to replacing the gas switches; this progress lowered their turn-on times in the range of 10's of ns and with up to 6.5 kV per component (i.e. SiC MOS). Further switching performance improvement can be achieved by so called gate super-boosting technique with commutation time approaching nanosecond level.

This technique is based on applying of very short overvoltage pulse of up to 300V on the MOS gate to counterbalance the effects of the leads inductance and internal parasitic capacitance. In this way super boosting a 1.7 kV rated SiC MOS allows to reduce the MOS rise time (tr) by a factor of > 26 (datasheet tr = 20 ns vs. measured tr < 800 ps), resulting in an output voltage slew rate > 1 kV/ns and an amplitude > 1 kV into a 50 Ohm load.

Similarly, our test with super-boosting of a 3.3 kV rated SiC MOS resulted in an accelerated rise time by a factor of 20 (datasheet tr = 24 ns vs. measured tr < 1.2 ns), with an

output voltage slew rate > 1.77 ns and an amplitude $>$ 2.5 kV into a 50 Ohm load.

To achieve this, the SiC MOS gate driver needs to be extremely compact, using state of the art components in low inductance packages and with good decoupling of its power

supply.

Further increasing of the output voltage is possible either by multiple series connected MOS transistors or by adding a Marx generator with sufficient number of stages to meet the desired output voltage.

We propose a fast generator based on the boosted com-

mutation of cheap commercially available HV SiC MOS and a Marx topology to trigger thyristors to increase the output
voltage. The whole prototype uses commercial off-the-shelf (COTS) components, and the printed circuit board (PCB) is planned in a very compact design.

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OR-97

Histopathology study of nsPEF from a novel electroporator

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A novel nanosecond electroporation system was developed for in-vivo investigation of nsPEF, in the nanosecond pulse duration regime with amplitudes in the region of 1 kV and rise times less than 2ns. This system was used for the primary investigation of the effect of nsPEF on live porcine liver tissue.
The fast nsPEF electroporation system developed for this work utilised relatively slow charging and rapid discharging of an open circuit coaxial transmission line through a stack of avalanche transistors operating as a fast-switching element. The nsPEF duration was directly dependent on the charged line length whilst the maximum nsPEF amplitude and transition time were dependent upon the number of avalanche transistors that were stacked in series. Timing control was managed by an external trigger signal from a pulse generator with repetition frequency limited by the associated charging time of the charged line.
A range of nsPEF was applied to a porcine liver at a range of settings. High voltage nsPEF were applied from the novel electroporator through a parallel plate system where both plates were positioned on parenchymal surfaces approximately 10mm apart with liver tissue occupying the entire space between the plates. Samples from the experimental sites were received as stained histology sections of liver mounted one tissue per slide. One section of each tissue was stained with haematoxylin and eosin and an additional section was stained with picro-sirius red combined with Millers elastin stain. All sections were scanned in their entirety and comments on any features of interest noted and documented with a representative photomicrograph.
In conclusion, the electroporation device as configured in this study was able to effect irreversible (and possibly reversible) electroporation associated cellular changes within liver tissue. The electroporated cellular features were well demarcated from normal tissue, within close tolerance borders and related directly to the instrument application sites. Morphologically, the effect of treatment is limited to cells. Collagen within the treated areas is unchanged from normal.
The design of the novel electroporator used in this pre-clinical investigation is detailed in another abstract titled 'Design of a versatile nanosecond pulsed electric field (nsPEF) system.

OR-114

Microfluidic chip with multi-detection modules for treatment of viable cells by pulsed electric field

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The possibility to artificially adjust and fine-tune gene expression is one of the key milestones in bioengineering, synthetic biology, and advanced medicine. The effects of proteins or other transgene products depend on the dosage, therefore controlled gene expression is required both for laboratory use and therapeutic applications, where even slight fluctuations of the transgene product impact its function and cell viability. In this context, physical techniques demonstrate optimistic perspectives and pulsed electric field technology is a potential candidate for a non-invasive, biophysical regulator. It is challenging to integrate the biological technology with mechanical and electric engineering. The main task of engineers is to apply their scientific and engineering knowledge to the solution of technical problems. Fusion of biology, mechatronics and physics should further address biocompatibility guidelines to ensure complete functionality and reliability. However shorter nanosecond pulse duration pulsed electric field (nsPEF) has only been studied for the past two decades, but has unique properties not observed with the longer pulses already described. Millisecond PEFs are commonly used in life sciences, especially for DNA transfection to generate pores on the cell membrane. On the other hand, nsPEFs can directly reach intracellular components without cell membrane destruction, and thus nsPEFs have emerged as a unique therapeutic tool for intracellular manipulation without any chemical intervention. Although nsPEFs are now recognized as a drug-free and purely electrical cancer therapy, the molecular mechanism of nsPEF action remains largely unclear. In the past few decades advances in the field of microfluidics and microfabrication have contributed to the development of dynamic cell culture systems and in vitro models recapitulating the human organs substitutes. Aside from improving the cellular and biological contents of the microfluidic systems, monitoring the tissues with integrated sensors and improved microscopy techniques will help generate more data to gain a better understanding of the cellular behaviour in the device. We exposed mammalian cell lines, transfected with NF-κB pathway-controlled transcription system, to a range of microsecond to nanosecond pulsed electric fields parameters. Some possible designs of potential microfluidic chip will be presented as well.

P8 - Mechanisms of Cell Death in Electroporation-based Therapies

Monday morning Track D
Oct 10, 10:50 - 12:10

OR-57

Acute ATP Loss During Irreversible Electroporation Supports Caspase Independent Cell Death

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Introduction: Mode of cell death following tumor ablation influences the type and strength of the immune response, wherein IRE has been reported to variably cause apoptosis, necrosis, oncosis and pyroptosis. Intracellular ATP is a key substrate for apoptosis and is rapidly depleted during Irreversible electroporation (IRE). The objective of this study was to understand whether intracellular level ATP determines the mode of cell death following IRE.

Material and methods: Mouse bladder cancer cell line (MB49) was treated in vitro with an increasing number of pulses (0, 10, 30, 50 or 90) with a duration of 100 µs and a frequency of 1 Hz at a fixed field strength (1500 V/cm) in 4mm gap cuvette. Cell proliferation and viability were assessed with Cell Counting Kit-8 (CCK-8) and trypan blue staining respectively at 4 and 24-hours following treatment. ATP levels in culture were measured using Cell-Titer-Glo® Assay at serial timepoints (30 min, 2 hours, and 24 hours). The mode of cell death was evaluated by combined Annexin-V/Propidium Iodide/ staining. Caspase 3/7 activity was measured at 4 hours following pulse application. Change in the expression of caspase-3 and caspase-8 were investigated by Western blotting. For blocking condition, apoptosis was inhibited by a pan-caspase inhibitor (z-VAD-fmk, 50 µM). IRE treated cells were supplemented with exogenous ATP (1.5 µM) to determine whether it can alter or reverse the mode of cell death.

Results: Cell proliferation and viability decreased at both assessed timepoints proportional to the number of pulses applied and were not rescued by treatment with the z-VAD-fmk. Caspase 3/7 activation and apoptosis were predominant when treating cells with 10 pulses, where treatment with z-VAD-fmk rescued cells 24 hours after treatment. Such findings were muted in the groups receiving higher number of pulses. Annexin/PI assay demonstrated most cells stained positive for both stains after IRE treatment, indicative of both apoptotic/necrosis process which was not rescued by the treatment with z-VAD-fmk. Decrease in intracellular ATP was observed in all treatment conditions and all timepoints. ATP loss was greater during IRE compared to RE and the addition of z-VAD-fmk did not alter intracellular ATP levels. Exogeneous ATP supplementation did not impact proliferation or viability. Western blots revealed reduced pro-caspase 3 cleavage in the presence of exogenous ATP. The addition of z-VAD-fmk increased caspase 8 activity, but did not impact levels of cell death, suggesting initiation but not the completion of apoptotic cascade during IRE.

Conclusion: Rapid and acute ATP loss during IRE impedes completion of the apoptosis cascade, resulting in necrosis predominant cell death. This effect could not be rescued by ATP supplementation and was insensitive to pan-caspase inhibitors. Cell necrosis from IRE is expected to be immunostimulatory and effective in cancer cells that carry mutated or defective apoptosis genes.

OR-58

Characterization of the immunogenic cell death for electroporation treatments

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Introduction: When used as a focal ablation treatment, irreversible electroporation (IRE) and high frequency irreversible electroporation (HFIRE) induce cell death by membrane destabilization and loss of homeostasis. In IRE, monopolar pulses are delivered into the tissue of interest while HFIRE utilizes shorter bipolar pulses. Recent in vivo studies suggest that both IRE and HFIRE treatments can stimulate an immune response in addition to tissue ablation[1,2]. The extent of immunogenicity is often a determining factor in patients' post-treatment outcomes with potentially desirable consequences such as abscopal effects. Yet, the level of electroporation-induced inflammatory response is not well-understood in correlation with the treatment protocol.

Here, we aim to study whether the extent of inflammation resulted from IRE and HFIRE treatments can vary based on the applied treatment protocol.

Materials and Methods: As the first step for characterizing the electroporation-induced inflammatory response, the level of released ATP was measured from human brain tumor cells after treatment with IRE and HFIRE. ATP is one of the damage associate molecular pattern (DAMPs) proteins that plays a role in activation of innate and adaptive immune system. Cells were cultured in well plates and treated with the IRE and HFIRE pulses. To keep the levels of cell death constant among treatment groups, the applied voltage was normalized to the electric field thresholds for each waveform. ATP concentration was measured in the supernatant of the treated cells using a luminescence-based assay (Abcam).

Results and Discussion: Our preliminary results indicate that applying IRE and HFIRE pulses lead to the release of ATP from the treated cells. The ATP release increases when higher electric fields are applied. Furthermore, our results suggest a direct correlation between the applied pulse width with the levels of released ATP. The release of DAMPs is the first line of triggering an inflammatory response. Hence, our results can provide a guideline for

choosing the most optimum pulse parameters based on the desired inflammatory outcomes. Our results can also provide perspective on the temporal dynamics of the inflammatory response after IRE and HFIRE treatments. An important determining factor in the level of immunogenicity after local tumor treatments is the cell death mechanism. Often a combination of accidental and programed cell death can be observed in a single IRE or HFIRE treatment [3]. The future perspective of this study is to more precisely quantify the cell death mechanism in response to IRE and HFIRE both in vitro and in vivo.

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OR-59

The Dynamics of Extraction and Impact of Intracellular DAMPs Released from Irreversibly Electropermeabilized Cells on the Viability of Electroporated Cells

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Cells undergoing exposure to electric fields can experience no electroporation, reversible electroporation (EP), or irreversible electroporation (IRE) resulting in immediate cell death. The interplay of cell death and EP is modulated by pulse parameters such as pulse strength, duration, pulse number, and pulse repetition frequency. IRE uses brief pulsed electric fields to damage cell plasma membrane irreversibly thus leading to instant cell death and inflammation due to DAMPs released and is applied for tissue ablation.
Whereas intracellular DAMPs are known to be released from cells following IRE, the influence of IRE extracted intracellular compounds (ICs) on cancer cell viability has never been studied. We created an in vitro model to investigate an impact of IRE extracted ICs on viability of cancerous cells. We discovered that ICs generated from IRE cells significantly increased the viability of reversibly electropermeabilized cancer cells. Furthermore, results revealed that our EP parameters and conditions caused no cell lysis. We determined that no more than 60 % of intracellular proteins could be extracted due to IRE. The main amounts of RNA extracted were 26S and 18S ribosomal RNA subunits and some messenger RNA. Surprisingly, we did not detect any differences in the qualitative composition of proteins extracted in the EP supernatants probably due to the low sensitivity and specificity of the methods used. Controversially, we were able to obtain only slight DNA amounts in EP supernatants.
In conclusion, the levels of extracted ICs as proteins and RNA corresponded to intensifying electrical pulses despite the release of DNA. Secondly, the process of electric field assisted extraction of ICs we attributed to the electroporation induced pores and electrophoretic forces occurring during the pulses that facilitated extraction of ICs.
We hypothesize, that our results contribute to the explanation of a bigger picture of processes that may occur in the tumor microenvironment after IRE.

OR-60

Nano- and microsecond electric field thresholds for killing epithelial and smooth muscle cells

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Pulsed electric field (PEF) treatments are advantageous in their ability to non-thermally target cellular components while sparing structural and vascular components. We compared the electric field thresholds for cell death in three human esophageal cell lines: normal epithelial (ATCC CRL-2692), pre-malignant epithelial (ATCC CP-52731), and smooth muscle (H-6089, Cell Biologics). Thresholds were measured by placing two cylindrical electrodes orthogonal to a dense cell monolayer and delivering trains of 100 uni- or bipolar pulses, 200 ns to 10 µs duration, at 10 Hz. Propidium iodide was added two hours post-exposure to label dead cells, allowing time for transiently electroporated cells to recover their membrane integrity. The electric field strength which killed 50% of cells (LD50) was considered the cell death threshold. Unipolar pulses were significantly more effective at killing pre-malignant compared to normal epithelial cells, with an average 20% lower LD50 for all tested pulse durations. Conversely, bipolar pulses with a single phase of less than 1 µs were less efficient in killing pre-malignant compared to normal epithelium. Smooth muscle cells were the most sensitive to both unipolar and bipolar PEF, particularly in the nanosecond range, where the LD50 was over 1.5-fold lower in the smooth muscle than in the normal epithelium . Unexpectedly, the response of smooth muscle cells to PEF was not determined by the electric field intensity alone, a phenomenon that has not been previously reported or expected. It suggested that other factors play a role in smooth muscle sensitivity to PEF, such as cell orientation relative to the electric field, cell-to-cell communication, and/or calcium signaling between cells. To explore this, we delivered ten 300 ns pulses to individual cells on a stage of a confocal microscope in either perpendicular or parallel orientation to the electric field and measured the YO-PRO-1 dye uptake by a time-lapse imaging for the next 5 min. Smooth muscle cells aligned perpendicular to the electric field were 5-times more permeable than those aligned parallel to it. Additionally, we found that just a single pulse caused a calcium response lasting up to 200 seconds, which could be spread between cells and cause a propagation of effects outside of the electric field threshold. Overall, the higher killing efficiency of unipolar pulses in pre-malignant compared to normal epithelium suggest PEF is a promising treatment modality for ablation of pre-malignant cells to prevent the progression to cancer. Further studies will focus on the anomalous sensitivity and mitigation of damages to smooth muscle

cells.

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OR-61

Spatial distribution of permeabilization, pH fronts and temperature in a 2D tissue model induced by electrolytic electroporation: a numerical study

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Electrolytic electroporation is a new tissue ablation technique that is giving excellent results in the treatment of tumors. This technique consists of coupling electroporation with electrolytic ablation. The goal of this study is to simulate the spatial distribution of permeabilization, pH fronts and temperature generated by different electrode arrays and to analyze how these parameters impact tissue damage when electrolytic electroporation is applied. A two-dimensional bicarbonate buffer model is used to simulate the biological tissue. The numerical model solves the two-dimensional Nernst-Planck equations for ion transport in a nine-ion electrolyte. In addition, the bioheat transfer equation of Pennes was used to calculate the temperature distribution in tissue. Numerical simulations show that extreme pH fronts reach values of pH $<$ 3.8 (around the anodes) and pH $>$ 11.6 (near the cathodes). These pH values affect cell permeabilization levels, resulting in the entry of high concentrations of molecules into the treated tissue and, consequently, its destruction. Temperature gradients produced in the medium are not significant to induce severe tissue damage (maximum values were $<$ 41 °C). Spatial pattern of pH fronts, permeabilization and temperature depend on the exposure time, applied charge, shape electrodes array, polarity and separation distance between electrodes. Results obtained in this study were contrasted with those obtained in in vitro and in vivo experiments of electrolytic electroporation reported in the international literature. We conclude that acid-base pH fronts and action they exert on tissue permeabilization play an important role in tissue damage when electrolytic electroporation is applied, being remarkable for arrays of four and five electrodes arrays with 2 cm separation between them.

P13 - Biological Processes Induced by Electrotransfer of Molecular Cargo

Monday afternoon Track A
Oct 10, 13:30 - 14:45

OR-49

Palmitoylation of STING following DNA electroporation in mouse skeletal muscle

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Skeletal muscle is ideally suited as a target for therapeutic gene delivery due to its abundance, high vascularization, and high levels of protein expression. Intramuscular plasmid DNA (pDNA) injection is an easy but not particularly efficient delivery method. In electroporation or electrotransfer, defined electric pulses increase the permeability of the cell membrane, facilitating the uptake of pDNA into cells. Although clinical trials using pDNA electrotransfer are increasing, the molecular mechanisms that underlie this delivery technology remain unclear. One of the consequences of pDNA electrotransfer is the activation of DNA-specific pattern recognition receptors or DNA sensors. These sensors may signal via the adaptor protein stimulator of interferon genes (STING), which relies on the post-translational modification palmitoylation for activation. We investigated STING palmitoylation in mouse skeletal muscle and C2C12 myocytes after pDNA electroporation. Muscle gene expression by RNA sequencing and protein expression by bead array was assessed four hours after delivery. DNA sensing was revealed by the upregulation of specific pro-inflammatory cytokines and chemokines after pDNA injection alone, electroporation alone, or the combination. The mRNAs of 14 putative DNA sensors were significantly upregulated in the muscle. STING was not significantly regulated, confirming that other mechanisms are involved in its activation. We performed bioinformatical predictions and identified high confidence palmitoylation sites on STING as well as the putative DNA sensors cGAS (cyclic GMP–AMP synthase), Ddx58 (DExD/H-box helicase 58), Dhx36 (DEAH-box helicase 36), Dhx9 (DExH-box helicase 9), Ifi204 (interferon-activable protein 204) and Zbp1 (Z-DNA binding protein 1). STING conserved cysteine residues, highlighted by a cross-species alignment, suggest the evolutionary importance of protein palmitoylation. STING palmitoylation stoichiometry was evaluated using the novel acyl-PEGyl exchange gel-shift assay. Two previously characterized palmitoylation sites represented by a mobility shift in the presence of PEG-5k and hydroxylamine-treated samples in the immunoblot were detected in all experimental groups. Uniquely, one additional shift band was detected in all pulsed groups, indicating increased STING palmitoylation following electroporation. Palmitoylation plays a role in STING signaling, which suggests that palmitoylation sites are potential therapeutic targets that may have differential effects on immune signaling and regulatory functions. Understanding the molecular mechanisms of skeletal muscle gene therapy and creating safety biomarkers will be made possible by identifying unique post-translational modifications in the muscle.

OR-51

Electroporation as an aid in obtaining extracellular vesicles from cancer cells. Increasing the efficiency of EVs isolation using differential centrifugation

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Electroporation in biomedical sciences is especially useful as a method that facilitates the introduction of various substances into the cells. By overcoming the cell membrane barrier, numerous possibilities of using this method in diagnostics and therapy have arisen. Yet most of the scientific reports focus on the combination of electroporation with gene therapy or chemotherapy, a less explored topic seems to be the possibility of using this method to obtain various of its components from the cell, including extracellular vesicles (EVs), important participants in intercellular communication. There are many methods of isolating EVs from cells, but can they be improved? Ongoing studies investigate the utility of using reversible electroporation as an additional step in the extracellular vesicles isolation protocol with the differential centrifugation method. Using, inter alia, the IncuCyte imaging system, we determined that the application of electrical impulses enhances the outflow of cell contents. More detailed research, including flow cytometry and dynamic light scattering (DLS), confirmed that electroporation facilitated the isolation of EVs from cells. We conducted the research on two melanoma cell lines (A375 and Me45) and immortal keratinocytes (HaCaT) with the use of the BTX Square Wave Electroporator.

OR-52

Improving Electrotransfer with Nonreducing Sugars

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Electric pulses have been widely used in basic research and preclinical studies for transferring molecular cargo (plasmid DNA, mRNA, and ribonucleoprotein) into cells. To improve the efficiency of electrotransfer, we investigated effects of non-reducing sugars (NRS), such as sucrose, trehalose, and raffinose, on cargo transport in cells. In experiments, the cells were pretreated with an NRS at different concentrations for various periods. Then, the cargo was electrotransferred into the treated cells. At 24 hours post electrotransfer, we measure the transgene expression and cell viability. Our data showed that the treatment could induce the formation of amphisome-like bodies (ALBs) and enlarge lysosomes. As a result, cargo degradation was reduced and the efficiency of electrotransfer was increased, compared to the untreated controls. These data suggest that the NRS can be used to improve electrotransfer of molecular cargo in various applications.

OR-53

Gene electrotransfer: comparison of parameters used in electrogenotherapy and high frequency electroporation on gene expression

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Studied for more than 30 years, transfer of molecules in cells in vivo or in in vitro models mediated by the application of electric fields has been the subject of many advances in the medical field. By electrical stimulation of cells or tissues, it is possible to introduce therapeutics molecules and thus use electroporation for the treatment of cancers or diseases such as muscle dysfunction, a process called electrogenotherapy (EGT). These treatments are possible using a precise protocol of long pulses and high-voltage: 10 pulses of 5 ms at 0.6 kV/cm. One of the objectives of our work is to compare this protocol of long pulses with high frequency protocols presenting short pulses of less than 5 µs, that may decrease side effects such as muscle contraction and pain. To proceed, a new design of electrodes coupled to a high frequency generator has been developed. Another pulse sequence, High Voltage – Low Voltage (HV-LV) protocols, has been also tested for comparison. We first used HCT-116 2D colorectal cancer cells in suspension and submitted them to electric pulses in the presence of propidium iodide or of a Tomato-labeled plasmid to address the effects of these different electric pulses protocols on electrotransfer efficiency. Ongoing experiments show that the pulse duration plays a dramatic role on gene expression. Short-duration pulses can permeabilize the cells but do not lead to more than 1.5% of transfection rate. HV-LV protocols lead to 6 to 15% transfection, while the EGT protocol lead to 40 to 50% of transfection. We are now performing the same experiments on 3D spheroids models that more accurately mimic the in vivo complexity of tumors. In order to accurately and completely analyze and quantify the transfection rate in the whole spheroids, we are implementing clarification, allowing to observe all the cells in the spheroid whatever their localization, from the surface to the core. Altogether, these experiments should be of interest to optimize the pulse parameters of gene electrotransfer to obtain a high gene expression rate with minimum side effect.

OR-54

Electrotransfer of molecules in the reconstructed skin model

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Pulsed electric fields are used in medicine for electrochemotherapy and DNA electrotransfer. Their use is mainly localized in the skin, which is an organ of choice, due to its accessibility, large surface area, vascularization and immune system. To better understand the limits and improve the transfer of molecules, we have developed a 3D system of reconstructed human skin containing a differentiated dermis and epidermis. This model has been

validated by different methods (biological markers, electrical parameters), enabling us to have access to a complex model mimicking the in-vivo conditions.
In this project, we have used this model to study the transfer of molecules of different sizes and charges by electroporation. Molecules by modulating EGT electrical parameters and employing different types of electrodes. The first results show that the corneal layer becomes permeable to small molecules (less than 500 Da) when applying electric field pulses, which is not the case for large molecules such as plasmid DNA.
This work will help answer questions such as:
- Is there a change in the "barrier" function of the skin?
- Is there a disorganization of the intercellular junctions at the level of the epidermis?

P2 - Electric Fields in Fermentation and Wine Production

Monday afternoon Track B
Oct 10, 13:30 - 14:45

OR-50

Inactivation of resistant Escherichia coli by combining antibiotics with electroporation

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Several bacterial species has developed resistance to antibiotics, which is increasingly becoming serious concern and a global health threat (1). Development of novel approaches for efficient fight against infectious bacterial diseases is therefore crucial (2). One of promising approaches is electroporation, where exposure of bacteria to short electric pulses of sufficient strength renders their membranes permeable, thus significantly facilitating transport of otherwise impermeable molecules through the membrane (3).

The aim of our study was to investigate electroporation as a potentiator of antimicrobial efficacy against bacteria E. coli ER2420, resistant to tetracycline and chloramphenicol. In the study we compared bacterial inactivation potentiated by electroporation for antibiotics with different modes of action: tetracycline (inhibits protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor site), chloramphenicol (inhibits protein synthesis by preventing transfer of amino acids to the growing peptide chains) and ampicillin (inhibits cell wall synthesis by attachment to penicillin-binding proteins). Antibiotics were added before pulse application at concentration achieving three different inactivation rates, where 80-90%, 50-60% or 10-15% of bacterial cells survived. Bacterial cells with antibiotics were transferred to plate electrodes (d = 1 mm) and exposed to a train of eight pulses, 100 µs of duration of different electric field strength (5, 10, 15 or 20 kV/cm). After the treatment cells were incubated again with antibiotic for 3 h with shaking at 37 °C in order for antibiotic to enter permeabilized bacterial membrane. Bacterial viability was determined after 24 h hours with plate count method on Luria broth agar plates without antibiotic.

We showed that electroporation can potentiate the efficiency of all antibiotics. The most profound effect was observed at higher electric field strength (10 kV/cm and higher) and antibiotic concentration (where at least 50-60% of bacteria died when treated with antibiotics only). Our results also suggested that for tetracycline, this potentiation can be higher than for other antibiotics. Nevertheless, further studies are needed in order to understand the dependence of the antibiotic efficacy potentiation by electroporation. Our findings provide the basis for development of new antimicrobial treatments in which electroporation could be combined with antimicrobials, for inactivation of bacteria, particularly in contaminated fluids (e.g. hospital wastewaters).

(1) Klein EY, et al., 2019; doi: 10.1136/bmjgh-2018-001315

(2) Juma A, et al., 2020; doi: 10.3389/fmicb.2020.01477

(3) Kotnik T, et al., 2015; doi: 10.1016/j.tibtech.2015.06.002

OR-118

Microbial stabilization of wine by Pulsed electric fields (PEF)

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Winemaking is a complex process in which the presence of spoilage microorganisms may represent an enormous risk to the final quality of wine with dramatic economical losses. Current strategies implemented in wineries for microbial control are the use of sulphur dioxide (SO2) and sterilizing filtration prior to bottling. However, the extensive use of SO2 is being questioned due to their potential toxic effect and sterilizing filtration involves high operational costs and it is detrimental to the wine colour. The ability of Pulsed Electric Fields (PEF) to inactivate microorganisms at lower temperatures than those used in thermal processing could represent a suitable alternative for microbial control in wineries. Although the potential of PEF for wine microbial decontamination has been reported, most of these studies have been conducted under laboratory conditions that cannot be transferred to the wineries. In this investigation, the potential of PEF for microbial stabilization of wine in different winemaking steps has been investigated under processing conditions simulating an industrial process.
The PEF-resistance (15-25 kV/cm; 25- 400 µs; 40-170 kJ/kg) of three target microorganisms (Saccharomyces bayanus, Brettanomyces bruxellensis and Oenococus oeni) suspended in wine without SO2 and with added SO2 (5 - 40 ppm) was characterized. A 4-month shelf-life study was performed comparing decontamination by PEF (15 kV/cm; 40 and 115 kJ/kg) with SO2 (5-40 ppm) addition. In this study, it was evaluated the microbial stability and the oenological parameters of white wine, rosé wine and red wine after the alcoholic fermentation and red wine after the malolactic fermentation. Finally, physicochem-

ical and sensory analysis was conducted comparing sterilizing filtration with PEF decontamination (15 kV/cm; 85, 127 and 168 kJ/kg) using a red wine ready for bottling. Results showed that up to 4.0 log cycles of inactivation can be obtained by PEF for all the microorganisms studied S. bayanus, B. bruxellensis and O. oeni being the latter the most resistant. Furthermore, a synergetic effect in the lethality was observed by combining moderate PEF treatments with small doses of SO2. The shelf-life studies performed in wines revealed the capacity of PEF to eliminate or reduce SO2 doses used for microbial control in wines with no impact on the quality parameters of wines. Finally, a triangle sensory test revealed that an expert panel was not able to discriminate the wines treated by PEF at different intensities from the wine that was treated by sterilizing filtration. Therefore, these results reveal the great potential of PEF as a physical process to be used in the wineries as for replacing or reducing SO2 doses or as an alternative for sterilizing filtration.

OR-117

Improved Pulsed Electric Field processing units for decontamination of thermosensitive protein-rich foods

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Pulsed Electric Fields is an emerging technology for the pasteurization of liquid foods. The technology's working principle requires small electrode gaps to create electric fields with magnitudes being high enough to inactivate foodborne microorganisms. In typical treatment chambers being applied in industry, inhomogeneous electric fields and local temperature peaks are found. When treating protein-rich foods, this leads to local denaturation and agglomeration of proteins and finally to the formation of a fouling layer, which affects the product flow, electric field and temperature distribution and can lead to the total clogging of the treatment zone as well as an ineffective inactivation. Consequently, Pulsed Electric Fields for pasteurizing protein-rich liquid foods at industrial scale is currently limited.
The present contribution presents the results of a systematic study on how to avoid protein fouling while ensuring a sufficient pasteurization at the same time. Methodologically, numerical simulations and experiments were performed using the example of milk pasteurization. A model was developed to simulate the treatment conditions in a PEF treatment chamber, which can be used to identify the points where fouling occurs. To demonstrate the microbial efficiency the model solution was inoculated with Lactobacillus plantarum. Different parallel configurated treatment chambers, suitable for a continuously operating pilot scale PEF system, were simulated and stepwise optimized to avoid protein denaturation and the occurrence of protein fouling. The efficiency of each treatment chamber was experimentally studied focusing on protein denaturation and microbial reduction. The results showed less fouling and protein denaturation using the newly designed parallel treatment chamber compared to the current standard for industrial PEF systems at the same inactivation rate. Moreover, real food products, such as milk, were tested to demonstrate the usage for PEF technology for thermosensitive food products.

OR-05

Effectiveness of Pulsed Electric Field Treatment on Spices on Quality Properties, Aflatoxin Decomposition and Its Mutagenity: Red Pepper Case

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As one of the most popular and most consumed spices all over the World, red pepper (Capsicum annuum L.) is commonly used as spice and flavoring agent providing unique flavor and attractive red color to food products. One of the major issues associated with red pepper is aflatoxin (AF) contamination in almost ¼ of the vegetables that causes 0.5-1.5 billion dollars lost in trade each year. Aflatoxin degradation because of their high heat stability and resistance to most of the food processing technologies is still a big challenge. Thus, efficacy of pulsed electric fields by energies in the range of 0.97 and 17.28 J was applied to red pepper flakes for the determination of changes in quality properties of red pepper flakes, inactivation of aflatoxin producing Aspergillus parasiticus as well as aflatoxin decomposition, and reduction in mutagenity. While 64.37% inactivation with 17.28 J was obtained in mean initial A. parasiticus number; 99.88, 99.47, 97.75, and 99.58% reductions were obtained on the mean initial AfG1, AfG2, AfB1, and AfB2 concentrations. Depending on the applied PEF energy and aflatoxin concentration, mutagenic effect of aflatoxin is completely eliminated.

P34 - Development of pulse generators and electrodes

Monday afternoon Track C
Oct 10, 13:30 - 14:45

OR-99

A novel electrode for Transurethral Electroporation Based Treatments for Bladder Tumors

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The limitation of electroporation-based technologies for the treatment of tumors is that specific electrodes are needed in order to be able to deliver the electric pulses to the particular organ. Our group has previously investigated the potential use of Electrochemotherapy (ECT) and Calcium Electroporation (CaEP) for the treatment of bladder tumors with promising results in vivo. Therefore, we have designed a novel electrode that can be used endoscopically (through the urethra) in the urinary bladder.

Our electrode assembly comprises a bipolar array of 6 (3 x 2) needle shaped electrode elements, in which

both central electrodes are off-set from an imaginary line between the two external electrodes. The novelty of this design is that it produces an optimal electric field distribution and allows direct visualization of the areas to be treated. Linear electrode arrays produce butterfly shaped electric fields with some cold spots. In this case, in order to ensure exhaustive treatment of all interested areas substantial overlapping of the treated areas may be required, leading to potential tissue damage. Our electrode provides a homogeneous square shaped electric field. Thus, large overlapping during treatment is avoided. This solution leads to faster and more efficient application of the electroporation treatments, with potentially minimal tissue damage.

Bladder tumors are common and highly prevalent. Treatment requires transurethral surgery (TURBT) (often multiple) and multiple adjuvant intravesical treatments with instrumentation of the urinary ways, all these associated with considerable side effects and high costs.

TURBT is normally performed with a resectoscope. These devices use different ex-changeable instruments such as a loop electrode for cutting or resection of the tumor, also a roller ball for coagulation. The design of our electrode fits standard resectoscopes. This way, we can take biopsies, inject drugs directly to tumors, and apply the electric pulses under direct visualization, anywhere in the bladder. This procedure is similar TURBT, making it easy for urologists to learn, facilitating the introduction of electroporation as a potential treatment of bladder tumors. We expect that ECT or CaEP as a single treatment will be an effective ablative treatment for bladder tumors, thus leading to significant reduction in costs and side effects.

In conclusion, our electrode shows a novel design that provides an optimal electric field distribution, with the possibility of direct visualization of the bladder during endoscopic treatments, using standard urological resectoscopes, opening the possibility of electroporation based treatments in the bladder. However, the novelty of the off-set electrode element, which provides a homogeneous square shaped electric field, can also be implemented in other types of electrodes for different uses. The electrode is under initial development and clinical trials are currently under planning.

OR-111

Effect of electrically insulating the tip and/or a part of the circumference of the active needle length on the electric field line pattern and temperature gradient during irreversible electroporation

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Purpose: If temperature effects occur during irreversible electroporation (IRE) treatment, they are expected near the electrodes, especially near the tip. Electrical insulation of the electrode sides where the highest electric field line density occurs might contribute to a better control of the ablation volume and might suppress temperature effects at the 'backside' and tip of the electrode. Therefore, the effect of additional electrical insulation applied to the original IRE electrodes, tip and/or active needle length (ANL), on the electric field line pattern and temperature gradient during IRE pulse delivery was investigated.

Methods: IRE was performed in a polyacrylamide gel and in a semolina in castor oil model by two monopolar 19G IRE electrodes (AngioDynamics). Five designs of electrical insulation were investigated, the original electrode and insulation of: the electrode tip, a part of the circumference of the ANL or both the tip and a part of the circumference of the ANL. The ANL was insulated for half or three quarters of the electrode circumference. All types of shielding were compared to the original unshielded IRE electrode. The color Schlieren method, an optical method to visualize a temperature gradient, was used to image the temperature development. The (pulse) protocol used consisted of 20 pulses with 90 µsec pulse length, 10 pulses/s, 1.5 cm inter-electrode distance, 1.5 cm ANL and a potential difference of 1500 V (BTX Gemini X2) between the electrodes. The resistance was measured by the BTX system, before and after pulsing. A static electric field of 5.4 kV (MPL 500-10.000 V, FuG Elektronik GmbH) in combination with cameras placed in three dimensions were used to visualize the electric field line pattern, appearing by alignment of semolina.

Results: Around the tip, the electric field lines showed the highest density and most strongly fanned out. The semolina and color Schlieren results were in line, the highest change in temperature gradient was present around the tip, at the transition between the insulation and ANL for tip insulation and along the negative electrode. The temperature gradient showed locations where the highest temperatures were reached. These locations could be at risk for thermal damage. The more insulation was applied, the higher the measured resistance. This resulted in a more intense and enlarged area that showed a change in temperature gradient. An enhanced parallel electric field line pattern was realized when both the electrode tip and part of the ANL were insulated in comparison with the original electrode.

Conclusion: Electrically insulating the electrode tip in combination with a part of the circumference of the ANL gives rise to a better parallel electric field and predictable ablation zone in between the electrode pairs. Since the tip had the largest share in the electric field disturbance and showed the highest electric flux density in comparison to the ANL, tip insulation is preferred over both tip and ANL insulation.

OR-112

An intradermal, hand-held electroporation device for in vivo applications

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The development of DNA-based vaccines has been the focus of extensive research and development efforts. DNA vaccines and DNA based therapeutics have significant advantages over other platforms including avoidance of cold-chain requirements and a superior safety profile.
A major challenge for DNA vaccination platforms is the need for a secondary delivery mechanism to promote cellular transfection. Several approaches have been developed for such purpose, and electroporation (EP) is the leading platform and state-of-the-art. To this end we have developed a hand-held, battery powered, simple-to-use electroporation device with an intradermal microneedle-electrode (MNE) array attachment for in vivo electroporation. The initial implementation is an 8x8 configuration where the MNEs are spaced equidistant at 750 µm. This configuration allows for the creation of many zones for EP with each zone consisting of a 2x2 mini-array to focus transfection on immediate peri-electrode volumetric region. The prototype device can produce up to 50 kHz programmable electrical pulses with a maximum output voltage of 35 V at pulse intervals of 10 µs. The use of lower voltage and current that avoids tissue damage.
Electrical testing for the device has been performed on rat carcass skin and compared to a function generator-amplifier system, to ensure proper pulse generation in air and in skin. Further studies have been performed in vivo on a rat model, delivering a green fluorescent protein (GFP) encoding plasmid. FITC images, 24 hrs post pulsation, show distinct marks of the electroporation chip with GFP expression localized around the needle locations signifying the application of the electric field. The pulsing sequence is also tracked during the experiment and associated with the corresponding GFP intensity. Numerical simulation is pursued in synergy with experimental tests, where we simulate the realistic MNE array and skin tissue geometry for the prediction of intradermal field and current distribution. These results are compared with impedance spectroscopy measurements and are used to optimize device and protocol design.

OR-113

An in vivo Electrotransfer Instrument that Incorporates Tissue Heating and Impedance Feedback-Based Electropulsation

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Pulsed electric fields have broad potential for molecular delivery applications relative to gene therapy, protein therapies, vaccination, and chemotherapy. When electric fields are used for gene delivery (gene electrotransfer or GET), pulsed fields are generally used to induce the uptake of exogenous plasmid DNA by cells comprising a tissue. Wide ranges of electrical parameters have been used and are typically specific to the electrode(s) being used and the target tissue. These electrical parameters are pulse width, voltage, number of pulses, and period. Historically, these pulse parameters have been empirically determined to arrive at optimal electrical conditions for delivery. This technology has not been improved in several decades. Recently, however, localized moderate heating (to ˜43°C) during GET has been shown to increase expression of a delivered foreign gene. Tissue impedance measured during electrical treatment has been used in a feedback manner to adjust electrical treatment in real-time to optimize the electroporation process. This resulted in increased expression too. This study focused combining the use of heat and impedance to improve GET.
A device that could be used to locally heat the target tissue, apply electric pulses, and use impedance feedback to guide pulsation was designed, constructed, and tested. This system included an electrode array that contained up to 16 individually addressable electrodes, a heat source, and a thermal camera (FLIR Lepton 3.1) to monitor tissue temperature. The camera and heat source were integrated into a handle that contained the electrodes. Infrared, microwaves, and warm air were investigated as mechanisms to transfer heat that could be integrated into the device. Warm air proved to be as efficient as the other two methods with respect to heating times in phantom tissue and porcine skin. It proved to be much simpler to implement and integrate into a handle that contained electrodes. Images from the camera were used in a control algorithm to heat tissue safely and to maintain temperature during treatment. The final system was able to heat tissue 5 mm below the skin surface to 43°C in under a minute. Electric pulses were created by electronically switching the output of a commercial power supply (Magna-Power, Flemington, NJ, USA). Impedance measurements were made using the same electrodes as were used for applying electroporation pulses. Relays were used to disconnect the high voltage supply immediately after a pulse was applied to and allow impedance measurements using an impedance analysis chip (AD5933, Analog Devices). Electroporation pulses could be applied at a maximum rate of about 3 Hz while making impedance measurements between each pulse. The system could reproducibly detect impedance reductions in porcine skin that result from electropulsation. The combined heating and impedance-based pulsing are currently being tested for the delivery of DNA to Guinea pig skin.

OR-201

Design of a versatile nanosecond pulsed electric field (nsPEF) system

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The nsPEF system developed in this work is based on the relatively slow charging and rapid discharging of an open circuit coaxial transmission line through a stack of avalanche transistors operating as a fast-switching element. This methodology allows for the generation of high voltage nsPEF with a wide variety of nsPEF parameters. The duration of the nsPEF produced is determined by the parameters of the charged or transmission line's associated time delay. The duration of the nsPEF is twice the associated time delay of the transmission line

affiliated with the system. The amplitude of the nsPEF is restricted by the number of avalanche transistors that are stacked in series and their collective collector-emitter breakdown voltage. The extent of the amplitude and the reflection coefficient of the system is determined by the impedance ratio between the load impedance and the characteristic impedance of the transmission lines. For a system with zero reflection coefficient, the load and characteristic impedance should match and results in a nsPEF amplitude that is half the voltage level the charged line is charged to. The transition times of the nsPEF are determined by the avalanche transistors that operate as the fast-switching elements. Published data suggests that they can produce transition times of 300 ps. A trigger, or a control signal, with an amplitude less than the base-emitter breakdown voltage, can be applied to the base terminal of the lowest stacked transistors, Q1, to dictate when a nsPEF is produced, the number of nsPEF that is produced and the repetition frequency between the nsPEF that is produced. Through manipulating the current loop within the circuit and placing a load, or loads, across various potential differences in respect to the ground plane of the system, a positive, negative, or bipolar (generation of positive and negative pulses simultaneously) nsPEF can be generated. Changing the location of the load or loads in relation to the transmission line allows the selection of the polarity of the nsPEF generated. The developed nsPEF electroporation system can generate well-defined and specific numbers of symmetrical high voltage nsPEF, of various polarities, with rise and fall times less than a nanosecond, with a wide variety of durations and repetition frequencies. The resulting histopathology study of nsPEF generated from this electroporator is highlighted in another abstract titled 'Histopathology study of nsPEF from a novel electroporator'.

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P9 - Electroporation-based vaccines and immunogenic effects of electroporation

Monday afternoon Track D
Oct 10, 13:30 - 14:45

OR-203

Impact of electrochemotherapy on immune cells in the context of cancer – In vitro study

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Introduction: Electrochemotherapy (ECT) is an established cancer therapy that delivers cytotoxic drugs with the help of electroporation (EP) into the cells. It is widely used for the treatment of melanoma and is under investigation for treatment of internal malignancies. ECT is locally very effective, leading to sustained reduction of the treated tumour with little side effects to the patients. In contrast, systemic effects of this treatment have been rarely observed.
A systemic and long-lasting immune response is necessary to generate immunological memory, thus targeting metastatic disease and disease reoccurrence. The tumour microenvironment consists of a variety of cells including cancer and immune cells, all of which get impacted by ECT treatment. Currently the direct effects of ECT on immune cells regarding their viability and functionality is still unknown.
The aim of this study is to investigate the impact of EP and ECT on different immune cell in vitro permeability, survival, immune profile and functionality in the context of lung cancer in order to identify a means of understanding and improving the response to ECT.
Method: Several immune cell subtypes were treated with various EP and ECT conditions and the effect of these treatments on cell permeability and survival as well as metabolic activity were established. Further, immune cell subtypes were treated with cisplatin-ECT (based on established lung cancer cells in vitro results) and changes to their viability, metabolic activity, immune profiles and functionality were observed.
Results: T cell viability and metabolic activity was unaffected by all tested EP and ECT conditions. However, the viability of both macrophages and dendritic cells (DCs) was negatively impacted with increasing electric field strength for both EP and ECT treatments. Additionally, the metabolic activity of DCs and macrophages decreased with increasing electric field strength. Data from currently ongoing experiments for immune profiles and functionality after treatment will also be presented.
Taken together, these findings suggest that failure to generate an effective systemic immune response following ECT administration is due, at least in part, to the detrimental effect of the voltage currently used to treat patients on the antigen presenting cells present in the tumour microenvironment.

OR-202

Immunological effects of electrochemotherapy in b16f10, 4t1 and ct26 murine cell lines in vitro

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Electrochemotherapy (ECT) elicits an immune response in the treated tumors, however the effects of different cytostatics and time frame of changes in different tumors models are still unknown. Usually described cell deaths after ECT are apoptosis, necrosis and immunogenic cell death (ICD). ICD effectively induces adaptive immune response against neo-antigenes, which are released by dying or dead cells. The inflammatory response is promoted by DAMPs (damage-associated molecular patterns) released from the cells and serve as danger signals. Some of the DAMPs that can activate ICD are exposure of calreticulin (CRT) on the cell surface, the release

of ATP and high mobility group box 1 (HMGB1) from the cells. Immunologically important changes in tumor cells like loss of antigens, defects in the process of antigen presentation (MHC I, MHC II) or changes in immunologically important cell markers such as PD-L1 and CD40 also affect the immune response. The aim of the study was to determine the changes in expression of DAMPs (CRT, HMBG1) and specific cell markers (MHC I, MHC II, PD-L1 and CD40) after ECT in vitro. We used three murine cell lines that form immunologically distinct tumor models: B16F10 (melanoma), 4T1 (mammary carcinoma) and CT26 (colorectal carcinoma). They were treated with ECT with three different cytostatic at IC50 concentrations (concentration that induces death of 50 % of cells) – cisplatin (CDDP), oxaliplatin (OXA) and bleomycin (BLM). HMGB1 release was determined with an ELISA kit, and for the expression of cell markers we used flow cytometry. All values were measured 4, 24 and 48 h after the treatment. We demonstrated that ECT with CDDP caused the most potent changes of measured immunological markers in all tested cell lines. An increase in HMGB1, CRT, MHC I, PD-L1 and CD40 in all three cell lines within 48 h after the treatment was detected. ECT with OXA induced similar but less pronounced changes as ECT with CDDP. ECT with BLM induced only a few of the tested markers and the response was the most potent in B16F10 melanoma cell line, however more immunologically important changes were detected compared to 4T1 and CT26 cell line. In conclusion, ECT induced changes in the expression of immunologically important cell markers. In general, CDDP and OXA induced more changes than BLM. For exact evaluation of immunological effects of ECT, further in vitro and in vivo studies are needed.

OR-204

Skin dendritic cell mobilization upon electrochemotherapy: a dynamic analysis by fluorescence microscopy and flow cytometry

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Electroporation (EP) of tissues in humans is feasible, efficient and tolerable. Its most advanced routine clinical use is electrochemotherapy (ECT). EP increases drug delivery into tumor cells that results in up to 80% of complete responses in different tumor types.
T cell contributes to transitory or complete clearance of tumors post-treatment (1). ECT with bleomycin induces an immunological cell death of tumor cells (2), which is key for efficient tumor Antigen presentation by dendritic cells (DC) to T cells. Poor systemic protective responses may be due to an insufficient anti-tumoral polarization of T cell responses by DC at the tumor site. Investigation of the impact of ECT on DC mobilization and activation in the tumor should provide keys to improve current treatments.
We assessed the impact of ECT with bleomycin on the growth of subcutaneous melanoma and on mobilization of DC in C57Bl/6. We found that ECT treated mice display an oedema at the tumor site as early as 6 hours, which resorbs progressively until day 3-5. We found a substantial decrease in the frequency of tumor infiltrating DC (TIDC) as early as 6h and during the first 72h in ECT-treated tumors. This decrease correlates with an increase of activated DC expressing CCR7 frequency. A slight decrease in TIDC frequency could be detected during the first 24h upon EP only, while bleomycin only had no effect. These results suggest that ECT induces an efficient activation/maturation of TIDC with a departure of DC infiltrating the tumors through the upregulation of CCR7.
We then hypothesized that in ECT-treated tumors, new infiltrating DC could be recruited mainly from the normal blood vessels adjacent to the tumor. To address this question, we had set up different strategies using CD11c-DTR-GFP or CD11C-YFP mice bearing melanoma.
First, we analyzed the DC mobilization in the tumor of CD11c-YFP mice in dorsal skin window chambers using a DSD-macroscope. This technology allows us to quantify DC over time in each tumor region and to follow their behavior during the first days after treatment. We observed a decrease in DC density in the tumor and in the periphery with ECT. Upon ECT, we also observed an increase in DC speed in the tumor but also in the periphery when compared to control mice. These findings are consistent with an activation of DC induced by the treatment.
In parallel, the analysis by fluorescence microscopy on tumor sections of CD11c-DTR-GFP mice injected with a fluorescent anti-CD31 antibody that stains the endothelial cells of blood vessels, confirmed the decrease of DC in tumor after ECT. Seven days later, the re-infiltration of DCs in the tumor occurred through the blood vessels and took place mainly in the periphery of tumor.
Our data provide new insights in the induction of immune responses against solid tumors upon ECT. Defective pathways could be overcome upon delivery of therapeutic/immunostimulatory molecules at proper timings to induce a systemic protective response.

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OR-205

Release of damage-associated molecular pattern molecules in vitro

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Activation of the immune response appears to affect the outcome of electroporation-based therapy. Activation of the immune system can be triggered by immunogenic cell death, in which damage / danger-associated molecular pattern molecules (DAMPs) are actively or passively released from cells. In the extracellular space, DAMPs act as an endogenous damage signal that is recognized by cells of the immune system, promoting the release of pro-inflammatory mediators and recruiting other immune

cells. Along with inflammation, the adaptive immune response is activated, resulting in long-term protection against previously introduced molecules or cancer cells.
Since in both electrochemotherapy and irreversible electroporation the immune system response plays an important role in the outcome of cancer therapy, we investigated if and when specific DAMPs are released in response to electroporation in vitro. Eight 100 µs long pulses with a repetition frequency of 1 Hz, as commonly used in tumor treatment and soft tissue ablation, were used in the experiments. We examined the release of ATP, high mobility group box 1 protein (HMGB1), and externalization of calreticulin after electroporation because they are the gold standard for predicting immunogenic cell death in cancer cells. However, since other DAMPs are also known, we extended our study to the detection of released nucleic acids and uric acid. In this study, different cell lines were used (CHO - hamster epithelial cell line, B16F1 - murine melanoma and H9C2 - cardiomyocytes) to investigate whether the release of DAMPs in different cell types is different.
Although detection of certain DAMPs remains uncertain (i.e. uric acid), successfully detected DAMPs show a strong correlation with cell survival/irreversible electroporation and a much weaker correlation with membrane permeabilization/reversible electroporation. The trends of release of DAMPs appears to be similar in different cell lines. However, the exact amount of released DAMPs varies, which may be attributed to different physiological and metabolic needs of the cell type.

OR-10

The Influence of New High-Frequency Nanosecond Electrochemotherapy on the Elimination of LLC1 Tumours, Prolonging C57BL/6J Mice Survival and Positively Affecting Changes in Immune Cell Subpopulations

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This work focuses on anti-tumour immune response after nanosecond electrochemotherapy (ECT) to provide new data and further support to employ nanosecond ECT in clinics. Carcinoma tumours were induced by subcutaneously injecting C57BL/6J mice with LLC1 cells. For tumour ablation, bleomycin electrochemotherapy (ECT) was used with 4 different conditions of high-frequency nanosecond ECT (nsECT1-4) in comparison with μsECT procedure (1.3 kV/cm x 8 pulses, 100 μs, 1 Hz). nsECT parameters (3.5 kV/cm × 200 pulses) – nsECT1 (200 ns, 1 kHz), nsECT2 (200 ns, 1 MHz), nsECT3 (700 ns, 1 kHz) and nsECT4 (700 ns, 1 MHz). Afterwards, mice survival and tumour growth dynamics were evaluated. For the determination of anti-tumour antibody titers, mice blood sera were collected 10 days after the treatment. Spleens, lymph nodes and tumours were isolated from euthanized mice, and cell suspensions were prepared. Immune cells were identified in organ suspensions and antibody titers were assessed by multi-colour flow cytometry.
High-frequency nanosecond electrochemotherapy with nsECT3 and nsECT4 parameters has shown to significantly reduce carcinoma tumour progression and prolong the lifespan of C57BL/6J mice. After nsECT, the level of anti-tumour antibodies doubled in treated mice compared to untreated tumour-bearing mice. The expression of suppressor and activation receptors on immune cells was investigated. Changes in immune cell populations were observed after nsECT3 and nsECT4. Changes in myeloid cells: increased expression of CD31 on splenic monocytes, relatively more M1 macrophages and a higher percentage of CD11b dendritic cells in tumours. Changes in lymphocytes: plasma, memory B and CD4 CD8 T cells were increased in the spleens of nsECT-treated mice. Newly identified CD3 CD4 CD8 B220 Gr-1 CD11b CD11c DX5 CD1d MHCII CD86 CD80 "negative lymphocytes" were increased in spleens and tumours after nsECT3 and nsECT4. These "negative lymphocytes" had higher expression levels of antigen presentation markers (MHCII, CD80, CD86) and decreased expression of suppressor PD-L1. As opposed to nsECT3 and nsECT4 treatment, μsECT treatment led to decrease in cytotoxic CD8 T cells and no increase in plasma cells.
It was shown that nanosecond electric pulses result in successful nano-electrochemotherapy and an immunogenic response. Ultrashort pulses could be used to develop effective anticancer treatment strategies, gradually replacing standard microsecond procedures.

P6 - Microalgae Biorefinery

Monday late afternoon Track A
Oct 10, 16:00 - 17:40

OR-41

Integration of Pulsed Electric Field (PEF) Technology into the Microalgae Biorefinery

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In recent years, many studies have shown that the use of pulsed electric field (PEF) technology to extract valuable compounds from microalgae is suitable for energetic and nutritional purposes and meets economic requirements. This was only possible through the implementation of the microalgae biorefinery concept, which involves the use of the residual biomass after the extraction of high and medium value products (e.g., pigments, polysaccharides, and proteins) for biofuel production. The International Energy Agency, IEA, defines biorefinery as "the sustainable processing of biomass into a spectrum of marketable products and energy". A major advantage of PEF treatment is the bypassing of drying and the immediate processing of the wet concentrated biomass. Moreover, PEF treatment can be used as a mild and effective method of cell disruption, facilitating recovery of unaltered constituents at low energy costs and allows cascade processing for multiple component recovery, since it does not destroy cell shape and maintains gravimetrical biomass separab-

ility after single extraction step. However, cell components are not immediately available since electroporation does not conduct to cell disintegration. It was assumed that irreversible electroporation of the cell membrane and the subsequent increased permeabilization are the main effects which allows proteins and other ingredients to pass through the membrane. We found in our studies that efficient protein extraction after PEF treatment requires an incubation step, and that the progress and kinetics of this release depend on the biomass concentration and the incubation temperature. In addition to diffusion in a chemical gradient, a second biological, enzyme-driven process within the PEF-treated biomass was confirmed to facilitate the release of more than 40 % of total proteins from the cells. This is mainly the water-soluble protein fraction, but proteins from the cell organelles such as nucleus, mitochondria and chloroplast are also released. It is not exactly known which biological processes are triggered by PEF treatment in the cell, which lead to the release of valuable components - apart from the enzymatic autolysis. It seems that the type of cell death (programmed cell death, necrosis, or apoptosis) induced by PEF treatment influences the efficiency of protein extraction. Therefore, a better understanding of cell death in response to PEF treatment could lead to possible improvements in protein extraction efficiency. This presentation will give an overview about the various factors, such as the post PEF treatment incubation parameters, which have an impact on protein release, as it could be possible to optimize the incubation to obtain higher yields. In addition, the role of an important discovery is discussed, namely a water-soluble factor that is released by PEF-treated cells and triggers cell death in untreated cells. Based on the cell death inducing factor released after treatment, a working model for the recovery of compounds from microalgae is proposed.

OR-40

Microfluidic devices for the optimization of pretreatments in the context of microalgae-based productions

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In the present context where sustainable and renewable carbon-neutral biofuels are needed as an alternative to fossil fuels to meet the rising energy demands and while facing environmental issues, microalgae have gained increasing attention both in the scientific and industrial communities. These unicellular photosynthetic organisms owe this particular attention to their capacity to use sunlight, CO2 fixation, nutrients, and water to generate biomass at a high rate with rich energy content. However, the production chain when using algae as feedstock is energy-intensive and provides a poor product quality since component fractions are mixed or emulsified.
As a first part, this lecture will demonstrate the potential of pretreatments, such as Pulsed Electric Fields and mechanical stress to increase the extraction efficiency of lipids produced by microalgae cells.
We will show that microfluidic technology is a suitable approach as it allows the analysis of the impact of electrical and mechanical treatments at the single-cell level, with high throughput. Specific architectures of microfluidic devices devoted to electrical or mechanical solicitation on cells will be presented.
As a single-cell level study, we will investigate the impact of PEFs on the rigidity of the cell wall. Indeed, previous results have revealed the major role of the cell wall as a barrier to efficient lipid extraction.
Gaining knowledge at the single-cell level will further lead to the development of optimized pretreatment extraction and culturing techniques, therefore economically viable large-scale production.

OR-42

No downstream without upstream: Challenges for PEF treatment of Arthrospira platensis and studies on the mechanism of phycocyanin release

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The cyanobacterium Arthrospira platensis is a promising sustainable source for edible proteins and other high valuable substances such as the blue pigment-protein complex phycocyanin. The technology of Pulsed electric field (PEF) was recently studied to permeabilize the cell membrane, enhancing the mass transfer of water-soluble cell metabolites. A major drawback for PEF is the high electrical conductivity of the cultivation medium, which precludes a direct treatment. An exchange of the medium on the other hand makes the process unnecessary complex. Further, the question of the release mechanism for water- soluble substances is not sufficiently clarified in the published literature. The two stated problems represent a hurdle for the implementation of PEF for cell disruption of Arthrospira platensis on a commercial level. In the first part of this study, the extent to which the cultivation of Arthrospira platensis in salt-reduced medium is possible was investigated. In the second part, the relation between cell permeabilization and phycocyanin release was analyzed.
It was shown that a comparable growth rate and pigment composition can be achieved in modified salt-reduced medium by controlling the pH with CO2 gassing. The electrical conductivity of 4.6 mS cm-1 of the modified medium significantly reduces the dilution factor before PEF, in comparison to full medium (20 mS cm-1). To investigate the mechanism of phycocyanin release, the degree of cell permeabilization (cell disintegration index) was directly measured by means of a new method using the fluorescent dye propidium iodide (PI). The novel method allows conclusions to be drawn about the effects of treatment time, electric field strength and treatment temperature. Using a self-developed algorithm for image segmentation, the disintegration of trichomes was observed over a period of 3 hours. This revealed a direct correlation between the cell disintegration index and the decay of the trichomes. This decay, in turn, could be brought into a direct temporal relationship with the release of phycocyanin.
This work shows that it is necessary to consider the en-

tire process to implement PEF for cell disruption of Arthrospira platensis. The relation between permeabilization and the kinetics of particle decay and phycocyanin extraction is demonstrated for the first time. Thus, the results presented contribute to a deeper understanding regarding the release of cell metabolites in response to PEF. This facilitates the design of new processes to produce sustainable products from Arthrospira platensis.

OR-43

Nanosecond Pulsed Electric Fields selectively foster cell proliferation in heterotrophic microalgae

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Nanosecond pulsed electric fields (nsPEF) are a promising tool to increase upstream efficiency in cellular agriculture. Several biological effects including gene expression and growth stimulation have been reported for photoautotrophic microalgae species. The process window tailoring such effects is defined by several electrical and biological parameters. Optimization protocols have been limited to photoautotrophic organisms; however, heterotrophic species currently have a higher relevance owing to improved consumer perception and industrial-relevant productivity.

This research highlights nsPEF-related advances in the process optimization of heterotrophic microalgae (Auxenochlorella protothecoides and Chlorella vulgaris), leveraging biomass productivity. Advanced statistical modeling using Plackett Burman's design was established to identify optimal process parameter combinations (pulse width, -repetition frequency, -number, electric field strength). The screening study (P=0.0002, R2 =0.8) showed a significant effect of microalgae species (P= 0.00002) and electric field strength (P= 0.00167) on the percentage of biomass difference compared with the pumped control. The pulse width, -number, and time between treatments did not have a significant effect on the final biomass yield. The best productivity resulted in 12.28±0.028 g/L of C. vulgaris biomass harvested 96 h after inoculation and 72 h after nsPEF treatment (15 kV/cm, 5 Hz, 40 ns, 2 treatments after 5 minutes). It was 26.22±1.25% higher than the control 9.73 ±0.55 (P=0.009). The model maximization determined an increase of 22.77% with a 95% confidence interval [16.02%-29.52%] for C. vulgaris by applying two treatments after 5 minutes each with 15 kV/cm, 5 Hz, 100 ns pulses. Auxenochlorella protothecoides did not show a significant increase in biomass 95%-CI [-3.7%, 2.22%]. The treatment effect on lipids and protein content was analyzed while pore formation, calcium flux, and transcriptomics were used to study underlying mechanisms.

This work lays the foundation for improved microalgae productivity and, therefore, affordability of this potentially more sustainable resource. It develops further knowledge on using experimental design for screening several combined factors in nsPEF-research and opens the door to scrutinize underlying mechanisms and transferability to multiple organisms.

OR-44

Scaling microalgal biomass extraction for industrial application

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Downstreaming of microalgae and their industrial application has become a growing field of interest. Microalgae can serve, for instance, as supplier for biofuel, fine chemicals, pharmaceuticals, cosmetics, or food applications. However, the extraction of these compounds is challenging due to their sturdy cell wall. Standard extraction methods, e.g. bead-milling, sonication, chemical, or enzyme extraction suffer from several drawbacks. They are often ineffective against the cell wall, inacceptable heat development occurs, long treatment times are necessary, or environmental harmful solvents are used. Moreover, scaling of these methods are often difficult or uneconomic [1].

Based on our previous studies [2] [3], we developed a spark discharge treatment in industrial scale for the extraction of Cyanidium caldarium, which is an extremophile microalga. The alga is used for the production of cosmetics due to its high amounts of polyphenols and amino acids, which are postulated to have anti-aging properties [4]. The developed system was created modular to meet requirements from the industry partner concerning varying treatment volumes. Extraction processes were conducted with a pulse amplitude of 35-40 kV at 11 Hz, and a pulse length of 100 ns. The algae suspension was moved through the system by a peristaltic pump, which ensured uniformly treatment of the bulk liquid and made external cooling unnecessary. Treatment times depended on the volume of biomass (dry weight 100g/l), usually between 30 and 300 minutes. During the extraction process, the bulk temperature did not exceed 25 °C, which is preferable for thermolabile extractives, such as proteins or pigments.

Additionally, under the set treatment parameters, it was possible to extract the blue pigment phycocyanin without the addition of chemicals or freeze thawing cycles. The extraction success was compared to several conventional techniques. For comparing studies, two sonication treatment schemes and pulsed electric fields treatment with equal energy input in each case as for spark discharges were conducted. Moreover, a protocol of freeze-thaw-cycles were performed as most common extraction method for phycocyanin. For PEF treatment and freeze-thawing, no noteworthy extraction yields of phycocyanin were achieved in comparison to spark discharge treatment. For one sonication scheme, an elevated extraction yield (13mg/g dw) was achieved compared to 3mg/g dw of purified extract after spark discharge treatment t. However, proteomic analysis revealed that

especially freeze-thaw cycles and sonication cause the most modifications on the protein level of phycocyanin, whereat spark discharges showed only minor changes, indicating that this method is much gentler to sensitive extractives, such as pigments.

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OR-45

Application of pulsed electric fields and mechanical cell disintegration techniques in biorefinery cascade of microalgae

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Owing to the wide variability in structural features characterizing the plethora of existing microalgal strains, this work investigated the applicability of pulsed electric field (PEF) technology to promote the release of intracellular compounds from two model species (e.g., A. platensis, and C. vulgaris) in combination with different mechanical cell disruption techniques, namely high-shear homogenization (HSH), and high-pressure homogenization (HPH).
For this purpose, two different routes were followed, based on delivering PEF treatments of constant intensity (20 kV/cm, 100 kJ/kgSUSP.) to i) A. platensis after a gentle HSH step (20.000 rpm, 96 kJ/kgSUSP.), and ii) C.vulgaris prior to an ultimate HPH process (5 passes at 150 MPa, 750 kJ/kgSUSP.). Cell disruption efficiencies associated with either single or combined techniques were properly assessed through morphological (optical/scanning electron microscopy, and particle size distribution), quali-quantitative (content of water-soluble carbohydrates, and proteins in the achieved supernatant after aqueous extraction step), and economical (calculation of the specific energy consumption per unit mass of dry weight target compound) analyses.
As a general trend, single PEF treatments induced no measurable effect on the cell shape/structure, but only a surface shrinkage could be detected, which was likely attributed to the occurrence of intracellular compounds leakage during water diffusion step. Interestingly, the application of PEF in a sequential mode with mechanical treatments boosted the yield and selectivity of extraction from A. platensis and C. vulgaris cells, with an outstanding additive effect detected towards low molecular weight compounds (e.g., carbohydrates).
The cascaded combination of PEF and HSH/HPH techniques led to comparable and, in some cases, lower specific energy requirements for proteins and carbohydrates recovery than those granted by single HPH processing, with the latter causing cell debris formation and an undifferentiated release of intracellular compounds, which complicated the subsequent separation/purification stages.
This work demonstrated the feasibility of coupling electrical and mechanical technologies in the frame of microalgal biorefinery, with the aim to develop a tunable technological platform capable of efficiently valorizing disparate microalgal strains, thus increasing the availability of high-added value molecules to feed multiple industrial sectors.

P10 - Gene Electrotransfer for Immunotherapy

Monday late afternoon Track B
Oct 10, 16:00 - 17:30

OR-67

Transfection by electroporation using pulses from nano- to millisecond duration in vitro

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Gene electrotransfer (GET) or gene transfection by electroporation is a widely used method in which electric pulses cause temporary increase in cell membrane permeability and thus enable the entry of nucleic acids into cells. The purpose of this work was to explore, evaluate and demonstrate potential use of different pulses for introducing plasmid DNA (pDNA) into cells in vitro and compare efficiency and dynamics of gene expression after GET.
We performed experiments on cell suspensions of immortalized human skin fibroblasts 1306 cell line and on C2C12 murine myoblasts with four ranges of pulse durations (nanosecond, high frequency bipolar pulses (HF-BP), micro and millisecond). Cells were exposed to electric pulses in 2 mm cuvettes and transfected with pDNA encoding green fluorescent protein (GFP). Six pDNA concentrations, namely 40, 60, 80, 100, 250 and 500 µg/ml, were tested. After 24 hours cell survival was detected with MTS assay and percentage of GFP positive cells and their median fluorescence intensity (MFI) with flow cytometry. To determine time dynamics of GFP expression after GET with different duration of pulses percentage of GFP positive cells and their MFI were measured every 8 hours for 6 days.
Overall GET, which represents the percentage of transfected cells relative to the initial population and considers both the efficiency of transfection and cell survival, increased with increasing pDNA concentration for all pulse durations and both cell lines. Using the highest pDNA concentration, i.e. 500 µg/ml, we achieved between 35% of overall GET with millisecond pulses in C2C12 myoblast. Overall GET with millisecond pulses was however significantly lower in 1306 fibroblast, 15%. Interestingly, overall GET with microsecond pulses was comparable in both cell lines, at approximately 30%, as well as for HF-BP pulse protocol, at 20%. On the contrary, with nanosecond pulses overall GET was higher in 1306 fibroblast

compared to C2C12 myoblast. Also, MFI of GFP positive cells was significantly higher in 1306 fibroblast with all pulse durations except for millisecond pulses showing that GET efficiency depends on cell line. Our measurements also showed that time dynamics of the onset of GFP (both percentage of GFP positive cells and their MFI) are comparable for nanosecond, micro- and millisecond pulses in 1306 fibroblasts.

OR-68

Skin dendritic cell mobilization upon Gene electrotransfer (GET)

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Electropermeabilization/electroporation (EP) is one of the non-viral methods based on the native transmembrane electric potential modulation of the cell by applying electric pulses. This physical method thus enables the efficient and local delivery of chemotherapeutic drugs into tumor cells in Electrochemotherapy (ECT) (1) or nucleic acids for interleukin-12 gene-electro transfer in Immuno-Gene Electro-Therapy (IGET) (2). For Gene Electro-Therapy (GET), we set up electric field parameters to obtain a high expression of both fluorescent reporter and therapeutic genes while showing full safety in living animals (3). The combination of pIL-12 GET and partial irreversible electroporation (IRE) not only enhanced survival but also bring a curative effect in wild type mice (2). This two-step treatment, named Immuno-Gene Electro-Therapy (IGET), led to the activation of the adaptive immune system and the development of an anti-tumor immune memory (2). We are now using GFP transgenic mice to evaluate the effect of EP parameters on the skin dendritic cells by intravital microscopy (unpublished data).

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OR-69

Electrophoretic Infusion of DNA into Gels and Tissues: Experimental and Theoretical Analysis

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Gene electrotransfer is a process in which pulsed electric field is used to increase cell membrane permeability and introduce foreign genetic material into cells for gene therapeutic and gene editing applications. While most research in gene electrotransfer is dedicated to electrophoresis of DNA at the microscopic length scale close to cell membrane and DNA translocation across the cell membrane, the issue of DNA mobility over macroscopic length scales and DNA distribution in tissues has not been explicitly explored. The current method of pressure-based injection of DNA solution using a syringe does not allow precise control over the macroscopic distribution of DNA in tissues [1,2], leads to inhomogeneous distribution [2], results in tissue damage due to excess pressure [3] and presents a challenge in scaling up from small animals (mice) to large animals and humans [1]. In this work, we use electrophoretic infusion through a micro-pipette to transport DNA over macroscopic distances in-vitro in tissues models (3D gels). We show that long-duration low-intensity electric currents allow control over the macroscopic spread of DNA through electrophoresis, and theoretical models can be used to predict the macroscopic distribution. Further, permeabilizing electric field can be subsequently applied and its distribution can be modelled to provide a maximum overlap with the macroscopic distribution of DNA, leading to efficient gene electro-transfer without excess or insufficient coverage of electric field.

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OR-70

Immunomodulatory effects of radiotherapy and gene electrotransfer of plasmid DNA encoding chemokines CCL5 and CCL17 in murine tumors

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Chemokines are small signaling proteins belonging to the superfamily of cytokines, which regulate immune cell migration. The degree and the type of infiltrated immune cells into the tumor microenvironment affects the disease progression and correlates with the efficacy and outcome of immunotherapies. Similarly, beneficial immunomodulatory effects were also observed after irradiation. Therefore, we sought to investigate gene electrotransfer (GET) of proinflammatory chemokines CCL5 or CCL17 in combination with irradiation, as a potential therapeutic strategy for cancer therapy. Specifically, the chemotactic properties of investigated chemokines were first examined on cell cultures in vitro in murine CT26 colon and 4T1 breast cancer models. Next, extravasation

inducing ability of chemokines was determined using intravital microscopy of fluorescently labeled splenocytes in CT26 and 4T1 dorsal window chamber models in vivo. Murine tumor models were chosen based on their immunophenotype – CT26 corresponding to inflamed and 4T1 to immunosuppressive tumor model – allowing for a comparison of different tumor predisposition to immune response. The antitumor effectiveness of combined therapy utilizing GET of chemokines and two irradiation regimes (single dose of 10 Gy and fractionated dose of 3x 5 Gy) was determined in vivo. Lastly, qRT-PCR was used to evaluate gene expression of several cytokines in tumors after therapies, while changes in the abundance of CD4+, CD8+ cells and vasculature (CD31+ cells) were determined with immunofluorescent staining. Both chemokines CCL5 and CCL17 were able to induce the migration of mouse macrophages RAW264.7 in vitro. Similarly, both CT26 and 4T1 dorsal window chamber models showed increased retention of splenocytes after GET of chemokines compared to control. CT26 tumor growth delay after combined therapy of GET of chemokines and both irradiation regimes was significantly longer compared to control and even led to tumor cures. In the case of 4T1 tumors, only GET of chemokines combined with fractionated irradiation led to a pronounced tumor growth delay but without tumor cures. Gene expression analysis showed increased expression of both chemokines after corresponding therapies. Moreover, increased expression of CXCL9 and CXCL10, two potent chemoattractants of cytotoxic CD8+ T lymphocytes, was determined in most combined therapies. Comparison of the degree of infiltrated immune cells into the tumors showed increased numbers of helper CD4+ and cytotoxic CD8+ T lymphocytes after GET of chemokines, however their numbers decreased whenever irradiation was used. Reduced numbers of immune cells were recapitulated in tumors after combined therapy along with the decreased area of tumor vessels. Our results show the potential of chemokines in cancer immunotherapy, however additional optimization of combined therapy is needed before translation into clinics.

OR-71

OncoSec's 2nd Generation Therapy: Amplification of the CXCR3/CXCL9 axis by intratumoral electroporation of plasmid CXCL9 synergizes with plasmid IL-12 therapy to elicit robust anti-tumor immunity

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Previous studies have shown that intratumoral electroporation of plasmid IL-12 (tavokinogene telseplasmid, or IT-TAVO-EP) leads to upregulation of IFN-γ, a critical driver of tumor infiltrating lymphocytes (TIL) through the action of its target genes, CXCL9, CXCL10, and CXCL11. Signaling of these chemokines through the CXCR3 receptor is hypothesized to improve the clinical response to anti-PD-1 therapies, including pembrolizumab (ref 1). Biopsies collected during a Phase 2 clinical trial evaluating IT-TAVO-EP plus pembrolizumab in melanoma patients predicted to be refractive to anti-PD-1 immune checkpoint inhibitors (NCT03132675) showed that clinical outcome was closely tied to intratumoral CXCR3 mRNA levels, emphasizing the mechanistic importance of tumor-infiltrating CXCR3+ immune cells. Therefore, we asked if maximizing intratumoral chemokine levels by electroporation of pCXCL9 could augment the anti-tumor immune responses driven by intratumoral IL-12.

In this study (ref 2), we show that the IT-pIL12-EP leads to an increase in CXCR3+ lymphocytes in the local lymph node and that blocking the CXCR3 signaling axis abrogates IL-12 anti-tumor efficacy. Intratumoral electroporation of CXCL9 with IL-12 augments the anti-tumor responses seen with intratumoral IL-12 alone. Consistent with these findings, transcriptomic analyses of treated lesions demonstrated upregulation of signatures for interleukins, interferons, GPCR activation, antigen presentation machinery, and TCR signaling, suggesting that IL-12/CXCL9 reshapes the TME to promote dendritic cell licensing and CD8+ T cell activation. Finally, we show that the combination of IL-12 and CXCL9 drives anti-tumor responses in distant untreated lesions and significantly improves anti-PD-1 response. These findings show that the CXCL9/CXCR3 axis synergizes with IT-pIL12-EP therapy. Maximizing CXCL9 expression through intratumoral electroporation may enhance the efficacy of intratumoral IL-12 therapies in the clinic.

References:

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OR-139

Differences in Nano-Electrotransfection Efficiency Between Cancer and Primary Murine Immune Cells

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In this work we compared nano and microsecond range electroporation protocols for electrotransfection of cancer cell lines (4T1) and primary murine dendritic cells (DC). The efficiency of electrotransfection was evaluated using two different molecular sizes and different detectable protein-encoding plasmids: green fluorescent protein (GFP) encoding plasmid (4.7 kbp; p-EGFP-N1) and a plasmid expressing firefly luciferase and red fluorescent protein (tdTomato) (8.5 kbp; pcDNA3.1(+)/Luc2=tdT)).

First of all, the permeabilization of the cells was studied to ensure that both μsPEF and nsPEF protocols trigger saturated permeabilization. The susceptibility to PEF of

all the cell lines involved in the study was similar. Two different protocols were selected: nanosecond pulsed electric fields (nsPEF) (7 kV/cm x 300 ns x 100, 1 MHz) and microsecond (μsPEFs) (1.2 kV/cm x 100 μs x 8 pulses), which trigger high permeabilization. After that we used CHO-K1 cell line, as a model cell line, to determine the optimal concentration of used plasmids for further usage in 4T1 cell line and primary murine DCs electrotransfection protocols. The efficiency of electrotransfection of CHO-K1 cells was similar for both plasmids even though the size of the plasmid is different. Also, nsPEF and μsPEF returned comparable results. It was not the case for 4T1 and DCs. The transfection rate with bigger plasmid was reduced several-fold and at the same time the nsPEF protocol ensured significantly better transfection efficiency than μsPEFs. Afterward, we evaluated primary DCs transfection efficiency using immature and mature DCs, where for maturation LPS and TNF-α were used. However, there were no significant differences in transfection efficiency between immature and mature DCs.
It was shown that the used nsPEFs protocol ensured equivalent or better transfection efficiency than μsPEFs. However, the plasmid size, concentration and the cell type affect the transfection efficiency even though the sensitivity to electroporation itself is similar in all used cells. In this study, we demonstrate the applicability of nsPEFs range protocols for electrotransfection of primary murine DCs. The results of this study are appliable in gene therapy and DNA vaccination studies, where ultrashort pulses methodologies can be used. The nsPEF can be an alternative to available gene electrotransfection methods and protocols.
Acknowledgement: The project was funded by the Research Council of Lithuania (No. S-MIP-19-22).

P15 - Nanosecond Pulse Stimulation and Electropermeabilization (MURI AFOSR)

Monday late afternoon Track C
Oct 10, 16:00 - 17:30

OR-04

Focusing nanosecond pulse effects away from electrodes

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There is a tremendous promise in focusing bioeffects of electric stimuli away from electrodes, with applications such as non-invasive deep brain stimulation and uniform tumor ablation. The challenge is avoiding bioeffects near electrodes, where the electric field is the strongest, while achieving them remotely with a (much) weaker electric field.

Effects of nanosecond electric pulses (nsEP) are reduced ("canceled") when the pulses are made bipolar. This phenomenon of bipolar cancellation enables interference targeting of nsEP effects, when two bipolar waveforms combine into a unipolar nsEP remotely from stimulating electrodes. The change in pulse shape, from bipolar (inefficient) near the electrodes to unipolar (efficient) at a distance, cancels the bipolar cancellation (CANCAN effect). We showed CANCAN efficiency for preferential permeabilization and ablation of cell monolayers in the center of a quadrupole electrode array. CANCAN focusing was demonstrated in CHO, HEK, and BPAE cell lines, using different pulse parameters and permeabilization markers.

A world's first single-body nsEP generator designed specifically for CANCAN targeting, EPULSUS-FPM4-7, was built by EnergyPulse Systems in Lisbon, Portugal. This generator utilizes a second-generation CANCAN concept, with four channels of unipolar nsEP replacing two channels of bipolar nsEP. Four electrodes, forming a quadrupole, are alternately energized by unipolar nsEP in a series which creates multiphasic, bipolar electric field oscillations near and between the electrodes. The electric field in the center of the quadrupole stays unipolar and is more efficient at producing bioeffects. The second-generation CANCAN is more versatile and confines nsEP effects to smaller remote targets.

We coupled the EPULSUS generator with a one-of-a-kind strobe laser workstation for imaging cell membrane potential with nanosecond time resolution. It is utilized to explore complex effects of CANCAN stimulation and the dependence of bipolar cancellation on the angle change of the electric field vector. Fast strobe imaging may also provide answers to vital questions that have been debated for decades, such as the kinetics of electropore formation and resealing. We also plan to use strobe imaging to establish how CANCAN activates Na+ and Ca2+ voltage-gated ion channels, to support future CANCAN applications such as non-invasive deep brain stimulation.

Support: AFOSR MURI grant FA9550-15-1-0517 to AGP.

OR-06

Nanosecond electric pulses target the alpha-1 subunit of membrane Na,K-ATPase

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Experimental evidence suggests that electric pulses (EP) can alter ion channel conformation. The structural alterations in channel proteins likely impact membrane permeability, providing pathways for transporting ions and molecules across the plasma membrane. We performed a high-throughput screening to find ion channels affecting membrane permeabilization by nanosecond EP (nsEP).
Using lentiviral CRISPR/Cas9 gene editing we generated gene knock-outs (KOs) in cell human monocytes U937 and obtained a total of 328 derivatives with KO for a single ion channel gene. Each KO was exposed in cuvettes to 20 or 40 pulses (300ns, 7 kV/cm, at 20

Hz). In 50 min, cells were imaged for Yo-Pro-1 (YP) uptake on an Olympus IX83 microscope configured for high-throughput screening. We found that KO of the ATP1A1 gene coding for the alpha-1 subunit (ATP1A1) of Na,K-ATPase (NKA) reduced YP uptake by up to 30 % in two independent series of experiments. The subsequent work aimed to validate and further explore this findings by other methods.
First, YP uptake within 5 min after nsEP was measured in ATP1A1 KO cells by time lapse imaging on a scanning confocal microscope. Ten 300ns pulses (7 kV/cm, 5 Hz) were delivered to cells in the field of vision with a pair of tungsten electrodes. YP fluorescence in ATP1A1 KO cells was ˜20% lower than in control cells, confirming the data obtained with the high-throughput screening.
Next, ATP1A1 was suppressed by transduaction of U937 cells with shRNA. The ATP1A1 knock-down (KD) was validated with qPCR, and KD cells were accessed for YP uptake. We measured a reduction of YP uptake in ATP1A1 KD cells by 20% and 25% in high-throughput and confocal setups, respectively.
Next, we tested if NKA inhibition with ouabain affects the YP uptake. U937 cells were incubated with 1 µM ouabain for 15 min, then exposed to 10 pulses (300 ns, 7 kV/cm, 5 Hz) and assayed for YP uptake using the confocal microscope setup. We found that the presence of ouabain did not reduce YP uptake but increased it by 21%. This result confirms the role of ATP1A1 in YP uptake after nEP and indicates that this role is not related to NKA physiological function as an ion pump.
During the high-throughput screening, we noticed that the initially strong inhibition of YP uptake diminishes over time. We hypothesized that the function of the ATP1A1 gene was compensated by paralog genes typically present in U937 at low expression levels. Using qPCR we checked the expression level of other NKA's alpha subunits and found that ATP1A4 expression was upregulated in ATP1A1 KD cells. Therefore, upregulation of NKA paralog genes can compensate for inhibited ATP1A1.
Overall, our data point to ATP1A1 as a potential protein target (or one of protein targets) for cell membrane permeabilization by nsEP.

Support: AFOSR MURI grant FA9550-15-1-0517 to AGP.

OR-07

Ultrafast Imaging enabling the direct observation of the charging dynamics caused by pulsed electric field effects

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Introduction: Ultrafast Imaging tools have the potential to enable direct visualization of cellular response to pulsed electric fields. Previously, we demonstrated the ability to image changes in membrane potential during cellular exposure to unipolar and bipolar electric pulses using streak camera microscopy (SCM). More recently, we constructed a strobe illumination microscopy system for use in conjunction with FlouVolt™ dye for imaging variations in cell membrane potential during the application of electric pulses. The system allows flexible variation of trigger timing in order to image any time point of a cell-electric pulse interaction. The system has minimum time resolution of 10 ns. While comparable to SCM, strobe photography requires the delivery of multiple electric and illumination pulses to visualize the full time course of an exposure event, but affords the ability to acquire full 2 dimensional image sequences. These tools are complimentary in nature, and provide the ability to directly measure responses never before possible. Potential applications for the system include resolving the charging and discharging times of a cell membrane and measuring the time a cell takes to exhibit a physical response to an electric pulse.
Method: We imaged CHO-K1 cells, loaded with FluoVolt™, exposed to pulsed electric fields. Imaging was conducted with both strobe microscopy and SCM. Electric pulse energies were chosen such that no apparent cell damage was observed, enabling acquisition of multiple images from the same cell in order to resolve the onset, duration, and passing of each pulse. Using these systems, we captured data observing the membrane charging response to both square wave and AC waveforms.
Discussion: We demonstrated the ability to resolve a whole cell interaction with an electric pulse with temporal resolution of sub-microsecond. Our systems enables the capture of the full 2D spatial scene with strobe and a single spatial dimension with SCM. Hypothetically, the strobe system should be able to resolve cellular response to frequency up to 30 MHz, given sufficient signal to noise for the fluorescent dye. Previous work with streak camera microscopy demonstrated a similar limit of 5 MHz as is reported here, based on limitations of the fluorescent dye. By obtaining a full 2D image, we are able to resolve the charging kinetics of individual cells across a wide field of view and directly correlate that response to electric field modeling results. This enables the validation of modeling results for cell size, orientation, cell position relative to the electric field and when combined with other techniques, cell cycle, pharmaceutical treatment and other physical stressors (mechanical fields, temperature, environmental buffer).
Conclusion: Ultrafast imaging tools such as SCM and strobe photography are capable of resolving membrane charging dynamics in a variety of cell types with sub-microsecond temporal resolution. Combined, these tools can enable visualization of charging rate dynamics, estimation of frequency dependent dielectric properties, among others.

OR-08

Excess Efficacy of Nanosecond Stimulation in Cardiomyocytes

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It is generally assumed that when an electric field is applied to a cell or tissue, the induced drift of ions and

accumulation of charges at the membrane is the main mechanism by which the field changes the transmembrane voltage. Since theory predicts a drift velocity proportional to the applied field, the charging current, too, should be proportional to the applied field. If a certain charge accumulation is required, e.g. to excite a cell, the required field strength should be proportional to the duration of the field application. In other words, the basic theory of the drift of charges in an electric field predicts that the strength-duration curve for the stimulation of an excitable cell has slope -1 in a log-log plot.
In experiments, this theoretical prediction has generally been confirmed for nerve stimulation, but not for cardiomyocytes, for which the log-log slope of the strength-duration curve has been reported to be between -0.75 and -0.5. This implies that short, strong pulses are more effective at charging the membrane than predicted by basic theory.
To understand what mechanisms can cause this excess efficacy, we developed a model of membrane charging that considers all know mechanisms by which the field affects the transmembrane membrane: 1) Propagation of the applied field itself; 2) dielectric displacement; 3) drift/electrodiffusion; 4) active membrane response (opening of ion channels); 5) ballistic ions, i.e. ions that are sufficiently close to the membrane to be able to reach it without collisions with water or other molecules. We also considered different criteria for whether a pulse achieved excitation: a) reaching a certain transmembrane voltage, e.g. -45 mV or -55 mV; b) maintaining a certain depolarization for a minimum time (11 µs) to allow for channel opening; c) when considering active membrane responses, whether the sodium activation variable m reaches the value 0.5.
We found that the combined mechanisms of field propagation, dielectric displacement, and drift lead to strength-duration curves with a plateau for short pulse durations that transitions into a log-log slope -1 for longer durations, consistent with experimental data for nerve stimulation. The inclusion of an active response caused a variety of interesting effects, including a profound reduction of the stimulation threshold for microsecond duration pulses (by more than 50%). The inclusion of an active response did not, however, substantially change the slope of the strength-duration curve. Our analysis of ballistic ions suggests that they may help explain the excess efficacy of short, strong pulses.

OR-09

Cellular Excitability and nsPEFs: Involvement of lipid oxidation in action potential activation

Aurianne Rainot[1], Lea Rems[2], *Mounir Tarek*[1]
[1]Université de Lorraine, France
[2]University of Ljubljana, Faculty of Electrical Engineering, Slovenia

Several experimental studies have shown that nanosecond pulsed electric fields (nsPEFs) can stimulate neuronal cells. Excitable cells stimulation results in general in a depolarization - repolarization of the membrane of these excitable cells, called action potential. The latter involves activation of voltage-dependent (voltage-gated) ion channels (VGIC), ubiquitous transmembrane proteins, that engage in the propagation of the electrical signal. Under normal conditions, this activation is induced by a change in transmembrane potential in the order of few tens of mV and takes place on time scales of the order of ten to a hundred microseconds. Hence, the mechanism of excitable cells' activation by nsPEFs, i.e. pulses of few nanoseconds duration, is still puzzling, because of the significant difference in the time scales involved. There is no clear consensus to date on the underlying mechanism leading to electrostimulation when cells are subject to nsPEFs.
In this study we focus on a mechanism that might potentially be implicated in the activation of VGICs. nsPEFs were shown experimentally to generate both intracellular and extracellular Radical Oxygen Species (ROS). Moreover, it has been suggested over two decades ago, that model as well as cell membranes can be oxidized when subject to PEFs. There is experimental evidence that pulsed electric fields can increase the extent at which unsaturated lipid acyl chain peroxidation occurs.
Here, we first present results from computational chemistry methods, in particular molecular dynamics simulations of model lipid membranes that show that lipid oxidation induces changes in the properties of the membranes that can be drastic and long-lived enough to trigger activation of an action potential. Our results show indeed that under certain conditions, lipid oxidation of a patch model membrane might lead to a spontaneous membrane depolarization which could be large enough to activate voltage-gated sodium channels and generate an action potential.
We then resorted to modelling of the action potential at the cell level with Hodgkin-Huxley models. We present here results from equivalent circuit models' implementation in Comsol Multiphysics to gain better understanding of the macroscopic response to the physical changes induced by lipid oxidation. Interestingly enough, these models confirmed as well, the predictions from the microscopic (MD simulations) investigation.

P3 - Pulsed Electric Fields (PEF)
Strategies in Enhancing Health-promoting Properties of Plant-based Foods

Monday late afternoon Track D
Oct 10, 16:00 - 17:30

OR-220

Pulsed electric fields (PEF) for the preservation and bioproduction of health-related compounds and properties in plant-based foods

Robert Soliva-Fortuny, Olga Martin-Belloso, Pedro Elez-Martinez

University of Lleida, Food Technology, Spain

The concentration and biological activity of phytochemicals, as well as of several compounds closely related to food quality, is dramatically affected when high temperatures are applied during food processing. Electropermeabilization, cell wall disruption and other structural changes caused by pulsed electric field (PEF) treatments may lead to significant microbial inactivation and increased shelf-life extension while preserving quality characteristics. In addition, PEF can also be applied to enhance the release of phytochemical compounds from the food matrix and, consequently, can be considered as a strategy to tune the characteristics of processed food products.

Applied as a non-thermal preservation method, PEF allows a significant reduction of the impact of processing on the fresh-like characteristics of fruit juices. At the same time, recent applications of low intensity PEF stand out as a way either of stimulating the production of secondary metabolites in fresh commodities, hence increasing their antioxidant potential, or as a strategy to improve the bioaccessibility of desirable compounds.

This presentation will review several of the latest advances regarding the application of PEF for the development of safe, nutritious and high-quality food products. Key examples of the PEF application to liquid plant-based foods with the aim of improving stability while preserving quality and health-related value will be discussed/shared. In addition, application of PEF treatments to solid matrices in order to obtain plant foods with enhanced and more bioaccessible phytochemical contents will be presented and discussed.

OR-221

PEF for the extraction and recovery of health-related compounds from vegetable by-products and wastes

Giovanna Ferrari[1], Serena Carpentieri[2], Farid Soltanipour[2], Gianpiero Pataro[2]

[1]University of Salerno and ProdAl S.c.a.r.l., Italy

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Agricultural residues as well as food by-products and side steams represent a cheap and rich source of valuable bioactive compounds, that, if properly recovered, can be utilized in different industrial sectors, such as the food, pharma and cosmetic, to respond to the increasing consumers demand for natural ingredients with no potential toxicity. The number of product with novel formulations incorporating natural compounds, which are replacing synthetic additives, available in the market is increasing making the plant extracts market constantly growing.

The valorization of agro-food residues through the recovery of high value added bioactive compounds is therefore strategic not only from the economic standpoint but also to increase the sustainability of the agriculture and food processing industry allowing mitigating the environmental burdens of these sectors.

The recovery of bioactive compounds from agro-food biomasses with sufficiently high yields is typically carried out via conventional solid-liquid extraction using organic solvents, which are mostly toxic, hazardous and harmful.

However, being these compounds embedded in the plant cells envelop, to reduce the resistance to mass transfer of intracellular compounds the implementation of efficient cell membrane permeabilization steps complementing conventional solvent extraction has been proposed. Among the technologies successfully tested, namely microwave, ultrasound or ohmic heating, pulsed electric field (PEF) gained great attention being a gentle and scalable cell disruption method of plant biomasses.

The ability of PEF to intensify the selective recovery of target intracellular compounds from various vegetable matrices with reduced extraction costs, thanks to the reduction of energy and solvent consumption, and processing time has been demonstrated and valuable results are reported in many papers available in the literature.

In this lecture the recent findings on the utilization of PEF for solid liquid extraction process acceleration will be presented and the effect of different combinations of the PEF processing variables on extraction efficiency will be discussed, with particular focus on the results obtained in the frame of the European Project Accelwater. The economic viability of the PEF assisted extraction process with respect to conventional methods will be also demonstrated via process simulation.

OR-125

PEF-assisted processes for improving the health-related properties of plant foods

Ignacio Alvarez-Lanzarote, Javier Raso

University of Zaragoza, Spain

Pulsed electric fields (PEF) is a non-thermal process characterized by the electroporation of cell membranes which is used for several applications in the food industry including improving the extraction of intracellular compounds of interest from plant-based foods. There are several processes in which the application of PEF has improved not only the yield but also the content of compounds associated with health-related properties such as polyphenols, pigments, vitamins, etc. when producing or obtaining fruit juices, olive oil, wine, etc.

In this work, examples of processes (juice extraction, drying, freeze-drying, etc.) in which PEF technology has been implemented will be presented centering when PEF was used as a pre-treatment of the product (olives, grapes, apples, tomatoes) and its consequence in the obtained final product (oil, juice, wine, etc.) and the possible enhancing increment of health-related compounds. In some cases, new opportunities or products can be produced, such as grape or apple juices with enhanced concentrations of polyphenols when PEF-treating the fruits previously to process them. In other cases, the application of PEF for other purposes, such as peeling of tomatoes, can result in by-products rich in lycopene which can be the

base of recovery processes of health-related compounds. But not always and in all circumstances, PEF increase the extraction of compounds or the effects are clear. The introduction of PEF technology in a standardized process implies the adaptation of the connected processing steps which need to be adjusted, or also changes in properties of the raw material treated by PEF could affect those steps. Also in this work, some limitations of the PEF technology will be commented, i.e. when obtaining extra virgin olive oil (EVOO) or juice extraction.

OR-124

Evaluation of the beneficial effects of PEF-treated artichoke residues extract on THP-1 human cell lines

Serena Carpentieri[1], Giuseppina Augimeri[2], Jessica Ceramella[2], Adele Vivacqua[2], Stefania Sinicropi[2], Gianpiero Pataro[1], Daniela Bonofiglio[2], Giovanna Ferrari[1]

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Artichoke (Cynara scolymus L.), a typical Mediterranean edible plant, and its processing by-products, including external bracts, leaves, and stems, have been extensively studied for their nutritional values and therapeutic properties. Recovery and valorization of agri-food residues, still rich in valuable intracellular compounds, have emerged as a strategy to obtain high value-added compounds at low cost for the formulation of functional foods.

However, the recovery of bioactives from plant cells with sufficiently high yields, requires the adoption of an efficient cell membrane permeabilization step before conventional solvent extraction, in order to reduce the mass transfer resistance of intracellular compounds from the inner part of the cell.

To this purpose, pulsed electric fields (PEF) is gaining great interest as a gentle and scalable cell disruption technique of plant biomass, showing great potential to intensify the selective recovery of target intracellular compounds from various plant matrices, while reducing the energy, the solvent consumption, and the treatment time. However, to date, no studies have been carried out on the application of PEF-assisted extraction of phenolics from artichoke stems, nor on the subsequent evaluation of the biological effects of the extracts using human cell lines. Therefore, in this study, PEF-assisted aqueous extraction was utilized to recover effectively and sustainably valuable intracellular compounds from artichoke stems. The extracts were then subjected to membrane separation and subsequent stabilization by freeze-drying. The biological effects of the obtained artichoke extracts (AE) on human Lipopolysaccharide-stimulated THP-1 macrophages, with specific emphasis on their antioxidant and anti-inflammatory activities, were investigated.

Interestingly, AE from PEF pre-treated (E=1 kV/cm; WT=5 kJ/kg) artichoke stems resulted to possess significantly higher content of total polyphenols (+122% on average) and antioxidant power (+155% on average), as compared with the untreated samples.

Moreover, from HPLC-PDA analysis the chlorogenic acid was found to be the most abundant phenolic compound in AE, representing 53% of the total polyphenol content, followed by naringin, catechin, epicatechin, sinapic acid, phlorizin, cynarine, and gallic acid. The potential biological effects of AE were also investigated using THP-1 macrophages stimulated by Lipopolysaccharide (LPS), as an in vitro model system of oxidative stress. A reduced reactive oxygen species production upon treatment with AE was found. Moreover, AE was able to reduce the secretion of the potent pro-inflammatory mediators Interleukin-6 and Monocyte Chemoattractant Protein-1 in LPS-stimulated macrophages.

These results highlight the anti-inflammatory and antioxidant properties of the extracts from PEF-treated artichoke by-products, corroborating their potential application as a source of functional ingredients obtained through a feasible and sustainable process.

OR-123

Effect of Pulsed Electric Fields (PEF) on the extraction of phenolic compounds in orange by-products

María del Carmen Razola-Díaz[1], Robert Sevenich[2], Eduardo Jesús EJGH Guerra-Hernández[1], Vito Verardo[1], Oliver Schlüter[2]

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Orange peel is the main by-product of the orange juice industry. This by-product is a well-known source of bioactive compounds widely studied for its antioxidant, anti-inflammatory, anticancer, antirheumatic, antidiabetic, and cardioprotective activities. Therefore, the revaluation of this by-product is of great importance. In this context, this study focuses on establishing the optimal conditions for pulsed electric field (PEF) technology as a pretreatment for the orange peel extraction process to obtain extracts rich in phenolic compounds, an objective framed in the European Project SHEALTHY (https://www.shealthy.eu/project/). For this, a Box-Behnken design of 15 experiments with 3 independent factors has been used: pulse width (µs), number of pulses and field energy (kV/cm). In each experiment the phenolic content was extracted by ultrasound technology and analyzed by HPLC-MS. The response variables analyzed were the content of total phenolic compounds, total flavonoids, total phenolic acids, and the two major compounds, Hesperidin and Narirutin. The validity of the experimental design was confirmed by ANOVA and the optimal conditions were established by response surface methodology. With the optimal conditions of a PEF of 1.4 kV/cm and 30 pulses of 110 µs, a recovery of 41.80 mg/g dry weight of total phenolic compounds was obtained from the orange by-product, a content 20% higher than that obtained with ultrasonic extraction without CEP as pretreatment.

P11 - Immunogenic Effects of Electroporation-based Treatments

Tuesday morning Track A
Oct 11, 10:30 - 12:10

OR-206

Adjuvant immunogenicity of nanosecond pulsed electric field anti-cancer treatments

Claudia Muratori, Flavia Mazzarda, Alexandra E. Chittams-Miles, Julia Pittaluga, Andrew Ojeda, P. Thomas Vernier

Old Dominion University, Frank Reidy Research Center for Bioelectrics, United States

Using different tumor models, we found that treatment with nanosecond pulsed electric fields (nsPEF) enhance the host antitumor immune response. The strongest effect was measured in mice bearing CT-26 colon carcinoma tumors where the local treatment with nsPEF protected 78% of the animals from a second tumor cell challenge and delayed the growth of established distant lesions. Protection in nsPEF-treated mice correlated with an increased antitumor effector activity in the spleen. Notably mice cured by nsPEF remained protected even one year post treatment. We hypothesize that nsPEF-treated tumors act as an in-situ cancer vaccine by initiating innate immunity while molding and sustaining adaptive immunity. Indeed, we provide evidence that the damage created by these short electrical stimuli is sensed in tumors by the innate immune platform known as inflammasome. Upon assembly, the inflammasome induces membrane pore formation and proinflammatory cytokine processing, leading to a form of inflammatory cell death known as pyroptosis. We found that nsPEF triggered pyroptosis in macrophages accompanied by caspase-1 activation and release of the highly proinflammatory cytokine IL-1β. Concurrently, to sustain adaptive immunity, nsPEF in tumor cells activate persistent ER-stress accompanied by phosphorylation of translation initiation factor 2α (eIF2α): a biomarker of immunogenic cell death. Tumor antigen-release from nsPEF-treated tumor cells comes with the release of ATP, a chemotactic factor for dendritic cells (DCs), HMGB1, the DC maturation signal, and externalization of the "eat me" signal calreticulin. Understanding and manipulating the cross talk between innate and adaptive immunity after nsPEF is expected to improve the immunotherapeutic response triggered by these treatments.

OR-200

Induction of bystander and abscopal effects after electroporation-based treatments

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Local delivery of electric field pulses can lead to reversible (EP) or irreversible electroporation (IRE) of tumor cells. Both types of electroporation have been thoroughly investigated and applied for in vivo and clinical studies as novel modalities of antitumor treatment. To achieve antitumor responses using EP, anticancer drugs, such as bleomycin or cisplatin, or calcium locally or systemically are administered prior to delivery of electric pulses. These antitumor therapies correspondingly are known as antitumor electrochemotherapy and calcium electroporation. On the other hand, IRE causes dramatic changes in membrane and subsequently cellular homeostasis that leads to cell death without the administration of any exogenous substances.

We have demonstrated that following IRE cells release various substances, like ATP, proteins, and RNA, but not DNA. It seems that released molecules promote cell survival in vitro and might have an immunoregulating effect in vivo. On the other hand, a dramatic negative effect on nonelectroporated cell viability was observed when the medium was collected from cells treated with bleomycin or calcium electrotransfer and applied to directly unaffected cells. These in vitro studies were translated into in vivo studies where we aimed to test antitumor responses of combined treatments. Experiments were performed on BALB/c mice bearing two 4T1 tumors on both back flanks. To find out whether the combined treatments can induce an abscopal effect one of the tumors was treated and another was left untreated. Tumor treatment was performed by i.v. injection of bleomycin (200 µl, 470 µM) or/and i.t. injection of calcium chloride (half tumor volume, 168 mM)) followed by tumor electroporation using eight 100 µs pulses at 1200 or 1500 V/cm pulse strength. Some mice were additionally treated with IL2-coding plasmid electrotransfer into tibialis cranialis muscles for possible immune response and abscopal effect enhancement.

We have shown the feasibility to obtain various biological effects on directly unaffected cells after calcium electroporation and/or bleomycin electrotransfer both in vitro and in vivo. Possible interplays of these effects are discussed.

OR-207

Tumor Treating Fields-based Vaccination in Solid Tumors

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Tumor Treating Fields (TTFields), a novel approved therapy for glioblastoma (GBM) and malignant mesothelioma, employ non-invasive application of low-intensity, intermediate-frequency, alternating electric fields to disrupt the mitotic spindle, leading to chromosome missegregation and apoptosis. Emerging evidence suggest that TTFields may also induce plasma membrane perforation, blood brain barrier disruption and inflammation. However, the mechanism of these properties and whether they can be harnessed therapeutically are unclear. Recently, we reported that TTFields induce focal disruption of the nuclear envelope, leading to cytosolic release of large naked micronuclei clusters that recruit and intensely activate the 2 major DNA sensors – cGAS and AIM2, and their cognate cGAS/STING and AIM2/Caspase-1 inflammasomes, thereby releasing large quantities of proinflammatory cytokines and type-1 interferons (T1IFNs) that promote development and maturation of DCs and

cytotoxic T cells. In murine models of GBM, TTFields-treated GBM cells provide both tumor immunogens and danger signals to induce anti-tumor memory immunity both intratumorally and systemically, producing a cure rate approaching 66% and partial immunity in an additional 25% in a STING- and AIM2-dependent manner. In patients with newly diagnosed GBM patients, we detected robust post-TTFields activation of adaptive immunity specifically via the T1IFN trajectory anchored by plasmacytoid DC activity, which was strongly correlated with T cell receptor (TCR) clonal expansion. Importantly, we also defined a T cell-based gene signature predictive of TTFields effects on T cell activation and TCR clonal expansion. Collectively, these studies defined a therapeutic strategy using TTFields as cancer immunotherapy in GBM and potentially other solid tumors.

OR-208

Nanosecond Electric Pulses Overturn Immunosuppression in the Tumor Microenvironment

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Immunosuppression in the tumor microenvironment (TME) not only promotes tumor growth but also plays a critical role in cancer resistance to immunotherapy. We and other groups demonstrated that an electrical engineering technology, nanosecond electric pulses (nsEPs) could effectively ablate local tumor meanwhile result in a long-term immune protection in several animal models. Further characterization of local and systemic immune profiles in two cancer models treated with nsEPs with one leading to a strong vaccine effect but the other resulting in no immune protection suggests changes in immunosuppressive cells in the TME but not in blood are associated with antitumor immunity.

This study is to explore mechanisms how nsEPs mount a potent immune response in a predominately immunosuppressive breast tumor. An orthotopic 4T1-luc mouse mammary tumor model was treated with nsEPs (100ns, 50kV/cm, 3Hz and 1000 pulses). Blood, spleen, draining lymph node (dLN) and tumor were harvested at 4-hour, 8-hour, 1-, 3- and 7-day posttreatment for the analysis of frequencies, death and activation markers of various immune cells and suppressor function of regulatory T cells (Tregs).

We found nsEP treatment greatly reduced immunosuppressive cells including Tregs, tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) locally and systemically. Contrarily, effector T cells were spared. The suppressor function of Tregs was greatly impaired and this was in line with a significant reduction of activation markers 4-1BB and TGFβ and the selective elimination of active Tregs by nsEPs. Although all three types of immunosuppressive cells were diminished, the cell death in tumor occurred only to TAMs whereas Treg death was observed in the dLN. Nevertheless, no death of MDSCs took place in both tumor and dLN. Furthermore, a switch of TAMs from MHC-II- M2-like to MHC-II+ M1-like was observed. Noticeably, a continuing rise of tissue resident CD8 T cells indicated the initiation of anti-tumor specific cytotoxic T cells.

Our discoveries support nsEPs are a potent TME modifier reversing the immunosuppressive barrier in the TME, thus, are a promising in situ vaccination approach for immunosuppressive cells dominated cancers.

OR-209

Employing the Electrochemical Side Effects of Pulsed Electric Fields Within the Tissue Microenvironment

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Introduction: Irreversible Electroporation (IRE) is a promising focal ablation procedure used clinically for the treatment of unresectable tumors. [1] IRE involves the application of short, high-voltage pulses across the target area via locally advanced electrodes. While the non-thermal cell death mechanism of IRE can mitigate the thermal side effects seen in other focal ablation modalities, the pulsed electric fields can instead cause electrochemical side effects. This is primarily a result of electrolytic reactions occurring around the electrode-medium interface. These reactions form basic and acidic by-products at the cathode and anode respectively, which can generate substantial pH change. Some groups have studied these effects to minimize the role of electrochemical fronts in their desired treatment,[2] while others use the electrolytic effects to increase ablation area. Understanding how to control these effects could prove beneficial in improving electroporation treatments. [3]

Methods: Agar (1% w/v) tissue phantoms containing bromothymol blue (10% v/v) were used to investigate the effects of various pulse parameters (pulse duration, pulse number, and voltage) as well as electrical conductivity on local pH changes during IRE. This specific dye was chosen as it conveniently shows areas of an appreciable pH change ($\pm$1.0). Thereafter, we translate our pulse parameters into an ex vivo porcine liver model to provide a physiologically relevant environment. As we cannot accessibly visualize pH change within a liver, here we utilize a pH electrode to provide point measurements on the surface of the liver after treatment.

Discussion: Our results show that pH change, an indicator of electrochemical effects, can be manipulated to desired magnitudes in contained areas using extended, low-voltage pulses. While previously known to be a side effect of pulsed electric fields, these effects, if controllable, could be utilized to intentionally manipulate pH and induce electrochemical reactions. This could affect the body's acid-base relationship, which is critical to homeostasis as it governs a multitude of micro and macroscopic functions.[4] With proper parameter selection, the ability to control electrochemical reactions within the body could be clinically applicable across electroporation treatments.

[1] Davalos, Mir, and Rubinsky, "Tissue Ablation with Irreversible Electroporation."
[2] Maglietti et al., "The Role of Ph Fronts in Tissue Electroporation Based Treatments."

[3] Klein et al., "Single Exponential Decay Waveform; a Synergistic Combination of Electroporation and Electrolysis (E2) for Tissue Ablation."
[4] Hopkins, Sanvictores, and Sharma, "Physiology, Acid Base Balance."

OR-210

Immunogenicity of nucleic acid delivery to skin

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Skin is the largest organ in the body. With its heterogeneous population of cells, it provides a passive physical barrier against infection and also contains elements of the innate and adaptive immune systems. Hence, it is an active immune organ and attractive target for DNA vaccination. Various cells can be found in the skin, including keratinocytes, fibroblasts, endothelial cells and several types of immune cells. This diversity of cell types in skin could be important for DNA vaccination or other gene therapies because DNA transfection could elicit different immune responses in different cell types present in the skin. The experience with different cell types and tumor models demonstrated that electrotransfer of plasmid DNA with or without the therapeutic gene (pDNA) activated several cytosolic DNA sensing pathways and resulted in induction of different cytokines. Based on this research and the innate immunogenicity of skin, here we correlated the effects of electrotransfer of gWizBlank pDNA in mouse skin to fibroblasts and keratinocytes in vitro using reverse transcription real-time PCR (RT-qPCR) and several types of protein quantification. After electrotransfer of pDNA, the mRNAs of the putative DNA sensors DEAD (AspGlu-Ala-Asp) box polypeptide 60 (Ddx60), Absent in melanoma 2 (Aim2), DNA-dependent activator of interferon regulatory factor/ Z-DNA binding protein 1 (Dai/Zbp1), Interferon activated gene 202 (Ifi202); Interferon-inducible protein 204 (Ifi204) were upregulated in keratinocytes, while Ddx60, Zbp1 and Ifi204 were upregulated in fibroblasts. Increased levels of the mRNAs and proteins of several cytokines were detected and varied based on cell type. Mouse skin experiments in vivo confirmed our in vitro results with increased expression of the mRNAs of putative DNA sensors along with several cytokines and chemokines. Finally, with immunofluorescent staining, we demonstrated that skin keratinocytes, fibroblasts and macrophages contribute to the immune response observed after electrotransfer of pDNA. In conclusion, our results confirm the essential immune functions of both immune and non-immune cells in the skin which are essential for vaccination and gene therapy using pDNA electrotransfer.

P14 - Cytoskeletal Changes and their Implications in the Different Types ofPulsed Electric Fields (PEF) based Technologies and Treatments

Tuesday morning Track B
Oct 11, 10:30 - 12:10

OR-191

Suspended Nanonets Enable Precise Quantitation of Forces and Cytoskeletal Changes Post Electroporation

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Intracellular cargo delivery by applying electrical pulses was first demonstrated in 1982. Since then, electroporation (EP) has seen a meteoric rise with the successful delivery of a variety of cargos inside cells of multiple lineages, both in vivo and in vitro. With EP events, the loss of cytoskeleton integrity and contractility is well established. However, after decades of advancement in EP, a description linking loss and recovery of contractility with cytoskeleton is still incomplete. In this talk, I will describe how single cells attached to nanofiber nanonets experience a loss and recovery of forces post EP event, correlated with loss and recovery of the actin cytoskeleton structure. I will describe our Nanonet Force Microscopy (NFM) method for measuring single cell forces. Small diameter fibers (˜ 200 nm) capable of deflecting and acting as force sensors are deposited orthogonally on larger diameter non-deformable fibers (˜ 2 µm) in a suspended two-layer fiber system and fused at the intersections to achieve fixed-fixed boundary conditions. Cell forces are estimated inversely by applying force vectors that originate at focal adhesion clusters on fibers and are directed along the tension-bearing actin stress fibers inside the cells. We integrate nanonets within a microfluidic device and deliver ten 100 μs pulses at three voltage magnitudes (500, 1000, and 1500 V) and two directions (parallel and perpendicular to cell orientation), exposing cells to electric fields between 441 V cm−1 and 1366 V cm−1. Post electroporation, cellular contractility is lost and recovered in three distinct stages that coincide with actin-cytoskeleton remodeling. In stage 1, cells round up, followed by an unusual and unexpected biphasic stage (stage 2) characterized by increased contractility tens of minutes post-electroporation, followed by force relaxation. The biphasic force behavior is concurrent with the remodeling of actin stress fibers and membrane blebbing. Finally, in stage 3, cells elongate and regain their pre-electroporation morphology and contractility in 1−3 hr. We observe a significant drop in cell viability at high voltages applied perpendicularly to the cell long-axis. Our experiments with multiple healthy and cancerous cell lines demonstrate that contractile force is a more dynamic and sensitive metric than conventionally accepted cell shape to electroporation. A mechanobiological understanding of cellular cytoskeleton remodeling linked with contractility post-electroporation provides a

new twist to exploring the mechanisms that drive membrane recovery with potential implications for molecular medicine, genetic engineering, and cellular biophysics.

OR-31

Molecular dynamics simulation studies of intense electric field on cytoskeleton components

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Cytoskeleton is an ensemble of self-assembled protein fibers which are essential for the life of a cell. We focus on microtubules, one type of cytoskeleton fibers, which enable cell division and intracellular transport. Here we demonstrate that molecular dynamics simulations enable predictions of the potential effects of nanosecond scale intense electric field effect on tubulin (a building block of microtubules), microtubules, or kinesin.

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OR-33

Chip platform for PEF delivery to microtubules on total internal reflection fluorescence microscope

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Pulsed electric field (PEF) technology is promising for the manipulation of biomolecular components and has potential applications in biomedicine and bionanotechnology. Microtubules, nanoscopic tubular structures self-assembled from protein tubulin, serve as important components in basic cellular processes as well as in engineered biomolecular nanosystems. Recent studies in cell-based models have demonstrated that PEF affects the cytoskeleton, including microtubules. However, the direct effects of PEF on microtubules are not clear. In this work, we developed a lab-on-a-chip platform integrated with a total internal reflection fluorescence microscope system to elucidate the PEF effects on a microtubules network mimicking the cell-like density of microtubules. The designed platform enables the delivery of short (microsecond-scale), high-field-strength ($<$ 25 kV/cm) electric pulses far from the electrode/electrolyte interface. We showed that microsecond PEF is capable of overcoming the non-covalent microtubule bonding force to the substrate and translocating the microtubules. This microsecond PEF effect combined with macromolecular crowding led to the aggregation of microtubules. Our results expand the toolbox of bioelectronics technologies and electromagnetic tools for the manipulation of biomolecular nanoscopic systems and contribute to the understanding of microsecond PEF effects on a microtubule cytoskeleton.

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OR-30

Unraveling the interplay between DNA transport and cytoskeletal elements during/after electroporation

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Translocation and transport of genetic cargoes into eukaryotic cells is an essential step for the manipulation and genetic engineering of cellular activities medicine and fundamental biology. The cytoskeletal elements (e.g. actin cortex, microtubules) play critical roles in the several steps associated with gene-electro transfer processes (from translocation across the cell membrane to the transport inside the cytosol), but the underlying mechanisms remain unclear and vague. This talk will describe how genetic cargoes with different sizes form aggregates during and after electric pulses. Next, this talk will also explain how to understand the chaotic and active transport of genetic cargoes (with different sizes) through the cell cytoplasm. Different modes of cargo transport within the cytoskeletal meshwork have been demonstrated for various mammalian cells with different activities. We demonstrate that the electrotransferred DNA cargo undergoes anomalous diffusion for different DNA sizes and cell types (non-cancerous and cancerous cell lines). All of these new insights and information will allow us to provide and develop a more predictable theory for gene-electrotransfer by implementing the contribution of cytoskeletal elements during and after electro-permeabilization.

OR-82

Transient disruption of blood-brain barrier on rat brains at low-voltage high-pulse using non-invasive electrode approach

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Introduction: High voltage pulses used for reversible (RE) and irreversible electroporation (IRE) are known to induce Blood Brain Barrier (BBB) disruption when delivered using invasive needle electrodes. We investigated the feasibility of recapitulating pulsed electric field (PEF) induced BBB disruption using non-invasive, scalp mounted electrodes conventionally used for brain stimulation in animal models and human patients.
Methods: Non-invasive application of PEF between a skull mounted electrode and distal grounding pad was tested in Sprague Dawley rats. Combinations of pulses were applied by varying the voltage (1000 or 1500 V), pulse numbers (10 or 100), pulse width (20 or 100 µs) and frequency (1

or 10 Hz). BBB disruption was quantified by extravasation of intravenously administered Evan's Blue (EB) dye. Histology was performed to ascertain injury to the brain and compared to invasive pulse application with a needle electrode. Correlative COMSOL simulation studies were performed on a rat head model to estimate electric fields in the brain using our electrode configuration. 2D monolayer of b.End3 cells in a 24 well plate were treated at electric field strength levels estimated in the brain by simulation models at different pulse numbers, and compared with conventional RE pulse parameters (1000 V/cm, 10 pulses; positive control). Changes in cell viability (CCK-8) was measured at 2- and 24-hours post-treatment, while disruption of barrier function was determined by quantifying alteration in the actin cytoskeleton and zona occludin (ZO-1) expression at cell-cell junctions. Changes in transcapillary transport of IgG isotype control FITC antibody and 70 kDa Rhodamine dextran dye following RE and the test pulse parameters were studied with a microfluidic model of the BBB.
Results: The percentage area of the brain with EB dye was sensitive to increasing voltage, pulse number, with the greatest coverage (24.55 %) occurring when maximum energy was delivered (1500 V/cm, 100 pulses, 100 µs). Correlative simulations suggested that the electric field strength demarcating the region of BBB disruption ranged between 43 – 62 V/cm, where the area of disruption correlated with the total electrical charge deposited into the brain. Disruption of actin cytoskeleton with nuclear translocation of ZO-1at RE pulse parameters was recapitulated at lower electric field strengths (250 V/cm) with 160 pulses, without overt loss of cell viability or evidence of electroporation. These pulse parameters reproduced alterations in permeability in the microfluidic setup as well to both antibody and small molecule dyes.
Conclusion: PEF delivery with non-invasive electrodes can produce sizeable BBB disruption in rat models at electric field strengths that are substantially lower than the required threshold for RE. Simulation models suggest that similar outcomes can be achieved in human brain.

OR-32

Cell shape, orientation, and pulsation buffer affect electroporation lethality for elongated cells aligned on suspended nanofibers

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Cell membrane permeabilization by pulsed electric fields, known as electroporation, has applications in a variety of fields, yet the impact of cell shape has not yet been fully understood. For many electroporation applications, cell survival and recovery post-treatment is desirable, as in gene transfection, electrofusion, and electrochemotherapy. In other applications viability is undesirable, as in tumor and cardiac ablations. A complete understanding of how cell shape and cytoskeletal morphology affect cell viability post-electroporation may lead to improved electroporation methods and treatment selection. In this study, we use suspended nanofiber networks within a microfluidic device to reproducibly generate elongated cells (5-25x length over width) with precisely controlled orientations with respect to an applied electric field. We show that post-electroporation cell viability is highly dependent on cell orientation with respect to the electric field. Cells oriented parallel to the electric field had significantly higher viabilities than seen in suspension or on a 2D surface. Subsequently, cells perpendicular to the electric field had significantly lower viabilities. Additionally, the conductivity of the electroporation buffer significantly impacts viability outcomes in elongated cells. When the pulsation buffer conductivity (0.1 mS/cm) is an order of magnitude lower than the internal conductivity of the cell (2.5 mS/cm) we saw a trend reversal, with perpendicular cells having a significantly higher viability. By individually analyzing cells attached to 1- or 2- fibers, we show that there are minimal differences in post-electroporation viability despite differences in their cell adhesion and cytoskeletal structure. Further, we show that cell survival for elongated cells is still supported by the predictions of the standard pore model of electroporation, applied with accepted model properties. More pores formed at the tips of the cells elongated with the electric field and more pores formed at the flank of cells oriented perpendicular to the electric field. An improved understanding of cell shape and pulsation buffer conductivity may lead to improved methods for enhancing cell viability post-electroporation by engineering the cell morphology, cytoskeleton, and electroporation buffer conditions.

P21 - Electroporation for Cardiac Ablation: Mechanisms and Scientific Advances

Tuesday morning Track C
Oct 11, 10:30 - 12:10

OR-173

Pulsed field ablation cardio-selectivity: Differential effect of High-Frequency Electroporation on myocardium and other tissues

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Background: Pulsed field ablation (PFA) is an emerging non-thermal ablation method based on the biophysical phenomenon of electroporation. While PFA is associated with less collateral damage compared to thermal ablation, data on its cardiac-selectivity nature and on its tissue-specific thresholds is lacking. Therefore, this study aimed to compare the in-vivo differential effect of high frequency electroporation protocols (HF-IRE) on various tissues. Methods: 23 Sprague-Dawley rodents were allocated to 3 different HF-IRE protocols with amplitudes of 300-, 600- and 900-volts. All protocols used twenty 100-µs bursts at a frequency of 150 kHz. The in-vivo experiment included HF-IRE ablation of cardiac muscle, skeletal striated muscle, liver, carotid artery and sciatic

nerve. Animals were euthanized after 14 days. Lesions were evaluated quantitatively by histologic analysis using staining for fibrosis and by morphometric evaluation of tissue damage extent. Results: There were 8, 7 and 8 animals in the 300-, 600- and 900-volts protocols, respectively. HF-IRE protocols demonstrated a graded effect on myocardial tissue with larger lesions in the 900V protocol compared with the other two protocols. Lesion width was 428±315µM, 694±493 µM and 1348±892 µM for 300-, 600- and 900-volts respectively ($p<.05$), with similar trend in lesion length measurements ($p = .01$). No damage to the carotid artery was observed in all protocols. Partial damage to the sciatic nerve was observed in only 2 samples (25%) in the 600-volts group and in one sample (14.3%) in the 900-volts group. Liver and skeletal muscle tissues demonstrated ablation-induced fibrosis in all protocol groups with no graded effect between group. Conclusions: Electroporation effect is tissue-specific such that myocardium is more prone to electroporation damage compared to neural and vascular tissues. Our results suggest no neural or vascular damage with using a low amplitude HF-IRE protocol. Further investigation is warranted to better identify other tissues specific thresholds.

OR-174

A Novel Single-Shot Pulsed Field Ablation System Is Associated with Large and Durable Ventricular Lesions In Vivo: A Preclinical Assessment of Safety and Efficacy

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Pulsed field ablation (PFA) is a non-thermal ablative strategy that achieves cell death in cardiac tissue by irreversible electroporation.

The objective of this study is to recognize that a novel single-shot PFA catheter system (CRC EP Inc., Tustin, CA) is capable of creating large and durable ablation lesions, without catheter repositioning, in both the atria and the ventricles without any adverse effects on adjacent structure or thromboembolic events based on swine rete mirabile examinations. In this context, the safety and efficacy of PFA was investigated using a novel 8-Fr, 16-electrode, bidirectional, 25-, 30- or 35-mm spiral with the larger catheters containing 2 distal mapping electrode pairs.

In total, 14 pulsed field applications were delivered in 5 swine (55–92 kg) under general anesthesia, without paralytic agents, including: 7 lesions in the right (RV) and 7 in the left ventricle (LV). The PFA catheters were inserted through commercially-available 8.5-Fr steerable introducers. Bipolar PFA (2.5–4.0 kV) was performed using 56±18 sec, single-shot, QRS-gated applications under intracardiac echocardiographic guidance. The intensity of skeletal muscle activation was quantified by measuring the absolute acceleration of muscular contractions using a calibrated Galaxy S6 accelerometer sensors and an app (phyphox, Aachen, Germany). The phone was taped to animal's chest. Lesions were assessed by pre- versus post-EGM analysis, pacing threshold, 3D voltage mapping (EnSite, Abbott, Chicago, IL), necropsy and histology. The swine rete mirabile model was used to investigate for embolic events related to PFA.

All applications were single-shot without repositioning the catheter. No to minor microbubbling, but no skeletal muscle stimulation were observed. Acceleration was measured at < 0.5 m/s² (noise level). No tachycardiac rhythms were induced by PFA applications. There was marked reduction in post- versus pre-PFA EGMs (0.7 ± 0.4 mV vs. 1.8 ± 1.5 mV; $P<0.0001$ – or 0.5 ± 0.2 mV vs. 2.0 ± 0.9 mV when catheter was sized to ventricular dimensions) and pacing threshold (7.5 ± 2.9 mA vs. 20 mA; $P<0.0001$). All lesions were large and durable up to 7 – 28 days of follow-up. The lesions measured: 32.1 ± 4.7 mm in length, 26.6 ± 7.8 mm in width, 8.4 ± 3.1 mm in depth, 62.9 ± 2.1 mm in circumference (when catheter and ventricular axes aligned) and 13.9 ± 4.7 cm³ in volume. Despite the higher waveform voltage and prolonged applications used, no or minor superficial thermal effects were detected at necropsy or histology. Moreover, gross and microscopic examinations of the rete mirabile and kidneys revealed no evidence of thromboembolism in any of the animals.

In conclusion, the novel, single-shot PFA catheter system is capable of creating large and durable ventricular lesions using 56-sec applications in vivo. Despite the presence of minor microbubbles, examination of the rete mirabile and kidneys revealed no thromboembolic events in any of the animals.

OR-175

Direct and alternating current pulse filed ablation lesion comparison

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Background: Pulse field ablations (PFA) are introduced into clinical practice. A number of generators and catheters are emerging. while there is little known about lesion formation, maturation, and long-term effects on the myocardium. Gross pathology inspection and examination with 9.4 T magnetic resonance imaging (MRI) are standards for morphological evaluation. We aim to compare lesions from alternating current (AC) PFA, direct current (DC) PFA, and radiofrequency (RF) lesions in swine heart tissue.

Methods: Animals underwent AC, DC, and RFA ablation in the left ventricle. After animal euthanasia, hearts were explanted, fixed, and scanned by 9.4 T MRI, then sliced. Slices were photographed and measured. The pathological evaluation included staining alcian blue and yellow Masson trichrome. Evaluated were depth, width, and lesion estuary, the volumes of the lesions were calculated using a

formula and directly measured from MRI with the "point-by-point" method.
Measurements obtained from gross pathology inspection and MRI did not differ, while distinct changes o for each ablation energy could be traced and attributed to different current characteristics.

OR-181

Multiscale Modeling of Pulsed Field Ablation in Anisotropic Myocardium

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Pulsed Field Ablation (PFA) is an electroporation-based treatment modality to perform cardiac tissue ablations. In this technique, a catheter is typically placed in contact with the endocardium where the electric pulses are delivered to ablate a target area of the myocardium. The cardiac parenchyma is principally constituted by elongated myocytes organized in fibers oriented, mainly, perpendicular to the endocardial surface. This anisotropic morphology results in a higher electric conductivity at the fibers direction, becoming a preferential pathway for the electric current to flow along. According to that, wide and shallow lesion morphology could be expected when monopolar PFA applications are delivered focally. Contrary to that, some recently published pre-clinical data present a deep elongated lesions morphology not aligned to the expected distribution of the electric field. This study presents a multiscale simulation approach able to identify factors needed to be considered when electroporation treatments are applied in a high anisotropic tissue such as the myocardium. First, a model was implemented where multiple cylindric myocytes were arranged mimicking the microscopic conformation of the cardiac tissue. Using that geometry, longitudinal and transversal electric fields at different frequencies and magnitudes were applied to assess the interactions between the electric currents, the ionic solutions, and the cell membranes. The results of the simulations were employed to describe the expected anisotropic behavior at tissue level in terms of electric conductivity and expected membrane disruption for each of the fiber orientations. Second, a macroscopic model of the heart cavity was implemented. In this model, a focal ablation catheter in contact with the myocardial tissue was defined to simulate the delivery monopolar PFA treatments. The microscopic simulations results confirmed the anisotropic properties of the myocardium and reveal a complex behavior of this high structured tissue. Specifically, when low frequencies and low electric field magnitudes are applied, the induced membrane disruptions are predominantly appearing when field is applied parallelly to the cardiac fibers. However, intense high frequency pulse waveforms are commonly required to mitigate skeletal contractions during PFA treatments. In that situation, the results of the microscopic simulations reveal a demarcated higher sensitivity to the electric fields in perpendicular orientation. The results provided by the macroscopic model and the microscopic behavior, can explain the elongated lesion morphology reported in some recent PFA studies. The multiscale model and approach presented in this study have been successfully employed to identify relevant factors able to improve the current understanding and predictive tools and the overall PFA clinical outcomes.

OR-182

Preventing cardiac cell damage after electric injuries

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The only effective therapy for ventricular fibrillation, electric shock, is also known to cause electroporation of cell membranes, which may be injurious to the myocardium. A higher defibrillation dose increases the severity of post-resuscitation myocardial dysfunction, contributing to the high mortality of post-cardiac arrest syndrome. Understanding the membrane integrity and restoration process is crucial for defibrillation without adverse electric injuries. This motivates the ongoing research to investigate mechanisms of electropore resealing and the search for its possible refinement.
Myocyte cell membrane repair can take minutes. During cardiopulmonary resuscitation (CPR), this delay can be fatal due to the reduced cardiac output resulting in cell hypoxia. Block copolymers can represent a straightforward solution to maintain membrane integrity and preserve cardiac myocyte viability, thus yielding a new opportunity for a treatment of high clinical impact.
Regarding cardiac injury during CPR, the intervention with Poloxamer 188 (P188) has been evaluated only as part of a bundle therapy with sevoflurane and standard advanced life support. Therefore, its role in preventing cardiac electric damage has not been established. Here, for the first time, we demonstrate that P188 can prevent cardiac electric injury by reducing the death of human cardiomyocytes caused by electroporation.
We used nanofiber multiwell plates to create monolayers of AC16 human cardiomyocytes. A 3D printer customized with an electrode holder was employed to precisely position stimulation electrodes orthogonal to cell monolayers. This technique also ensured the maintenance of time intervals between exposure and agent administration. Contact electrodes produced the electric field gradually decaying with distance from them, allowing the comparison of cell killing by a range of electric field strengths in a single sample.
We used both wide-field and scanning confocal fluorescence microscopy for measuring sarcolemma damage (by YO-PRO1 dye uptake) and cell viability (by propidium staining at 2- to 24 h after exposure) in monolayers treated by micro- and nanosecond electric pulses (µs and ns EP). Further, we evaluated the impact of P188 on membrane resealing and, consequently, cell survival.
Our study revealed different time dynamics of cardiac cell death evoked by ns and µs EP. After a train of 200, 10

kV/cm, 300-ns pulses, the cell death rose from 74 after 2 h to 90 percent after 8 h. Exposure to µs EP resulted in cell death delayed up to 24 h.
Application of 1% P188 solution seconds after ns pulses reduced cell death about 1.5 times. We plan to use confocal microscopy with a mounted chamber perfusion system to establish how the incorporation of P188 in the cell membrane enhances its resealing.
These novel findings broaden the possibilities for future prevention of electric injuries caused by defibrillation. Subsequent in vivo studies can warrant a further clinical trial.

Support: This research has been made possible by the Kosciuszko Foundation.

P4 - Electric Fields for Ohmic Heating in Food Processing

Tuesday morning Track D
Oct 11, 10:30 - 12:10

OR-115
Differentiation of Thermal and Electric Field Effects During Ohmic Heating
Felix Schottroff
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Electrotechnologies use the direct application of electric fields to an electrically conductive product, which is placed in-between at least two electrodes. Due to the occurrence of an electrical current flow and the dissipation of electrical energy into thermal energy, an associated temperature increase (Ohmic heating) will occur during these treatments. Although the primary objective of ohmic heating is a fast and uniform temperature increase, additional effects of the electric field on enzymes and biological cells and tissues are discussed as well. Especially for the design of decontamination processes, i.e. pasteurization and sterilization, by electrotechnologies, knowledge of the individual contribution of heat and electric field toward the inactivation of microorganisms is crucial. Both are directly interconnected and the ultimate aim of these processes is to find a balance between product quality loss and microbiological safety, thus ideally leading to a minimization of the thermal load. Also, design and optimization of processes distinctly depend on knowledge of these mechanisms, enabling the utilization of the full potential of these technologies. However, in order to gather this knowledge, pronounced efforts in terms of experimental design and simulation have to be undertaken. Thus, this presentation will focus on these issues, taking into account experimental designs and considerations in terms of simulation, enabling the differentiation of thermal and non-thermal effects of electrotechnologies.

OR-166
Electric Fields and their Effects on Vegetative Microorganisms, Spores and Enzymes
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Interest in the use of electrotechnologies for food processing was revived in the 1980s due to industry interest in delivering improved food quality while assuring safety. A notable development has been the discovery that under the right temperature conditions, low-frequency (< kHz) electric fields result in inactivation of bacterial spores at a greater rate than purely thermal methods. The efficacy of combined electrical and thermal (electrothermal) approaches suggests that the electric field interacts with specific components within the spore to cause inactivation. Studies on the effects of electric fields a number of enzymes, including pectin methylesterase, alpha amylase, peroxidase and cellulase, have shown activation of the enzyme at sub-optimal activity temperatures, and accelerated inactivation at above-optimal activity temperatures. Further, studies on the effect of frequency have shown enhanced activity below specific frequencies.

OR-178
Industrial Applications of Ohmic Heating
Henry Jaeger
University of Natural Resources and Life Sciences (BOKU), Austria

Compared to conventional heating methods, ohmic heating can achieve shorter heating times while avoiding hot surfaces and can reduce temperature gradients. Benefits may result for the processing of high viscous and particulate foods as well as for solid foods. Although the technology had its first industrial application almost a century ago, the current use cases are still scarce.
Challenges are resulting from the electrical, thermophysical and rheological properties of the products that have to be mastered in order to achieve uniform heating and the full benefit resulting from the technology. In addition to the product parameters, process parameters such as the current frequency used, the electrode material and the geometry of the treatment chamber are also relevant and need to be considered for specific applications. Furthermore, equipment availability still depends on a small number of providers.
Nevertheless, successful applications have been achieved, e.g. for cooking and baking as well as for the pasteurisation and sterilisation of sensitive products. Above mentioned limitations have been addressed in these cases and have resulted in optimized concepts regarding product formulation as well as process setup.
The presentation will highlight existing and future application scenarios. Product and process variables will be addressed in order to emphasize the need for a re-engineering of existing processes when being replaced or complemented by ohmic heating. Furthermore, aspects related to process validation will be discussed in order to exemplify

particularities resulting from the different heating principle.

OR-55

Ohmic heating of patatin enriched potato protein: Influence of moderate electric fields on thermal induced gel properties

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Heating of proteinacious foods is an elementary step during food processing as it commonly determines structural properties of the product. Ohmic Heating (i.e. heating by the use of an electric field) is an emerging heating technology, which offers several advantages over conventional heating processes, e.g. a higher primary energy conversion or faster and more uniform heating. However, the influence of Ohmic heating during thermal gelation of globular plant proteins and their functionality is not fully understood yet. This study aims to fill this lack of knowledge and to investigate possibilities of Ohmic heating to affect protein functionality and thereby properties of thermal induced gels.

Therefore, 9 wt % patatin enriched potato protein solutions at neutral pH and an ionic strength of 25 mM NaCl were heated from 25-65°C via Ohmic heating at several electric field strengths and conventional heating, resulting in come-up times of 240 and 1200 s as well as holding times of 30 and 600 s. Gels were then physically characterised (texture, moisture, water holding capacity) and the level of protein denaturation was determined. To reveal deviances between both heating methods on micro and molecular scale, an SDS-PAGE, gel permeation chromatography, gel solubility tests, FTIR-spectroscopy and scanning electron microscopy were performed.

Results indicated less proteins denatured and participated in gel network structures when heated via Ohmic heating. Conversely, more proteins remained in a partially native state and less hydrophobic interactions were measured within ohmically treated gel network structures. Scanning electron microscopy revealed the protein network to be more gap-like and frayed when an electric field was present. Gel properties showed ohmically heated gels to have tendencies of lower gel rigidity, moisture content and water holding capacity, but higher gel elasticity.

The present study demonstrated that Ohmic heating can be used as a tool to target and control protein denaturation and thereby functionality. Thermal induced gels by Ohmic heating possess different gel properties (compared to conventional heating) which can be of interest for novel food products or biomedical applications. Further, Ohmic heating is applicable in case less denaturation during thermal treatment of food products containing globular plant proteins is desired.

OR-56

Pulsed Electric Fields as a new ohmic heating system for vegetable blanching

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University of Zaragoza, Spain

Blanching is a thermal process widely used in the food industry, which is applied to vegetables and some fruits prior to freezing, heat sterilization or dehydration. Traditional food blanching technologies include the use of boiling water or steam, which are very time-consuming and energy-intensive treatments, potentially affecting the sensory quality of the products. Moreover, in the case of large vegetables pieces, the fact that heat transfer occurs by conduction from the hot medium to its core results in a slow heat penetration rate leading to a problem of thermal uniformity.

Therefore, volumetric heating systems are being investigated. Pulsed Electric Fields (PEF) is one of the most recently proposed alternatives for applying ohmic heating, especially in processes where mass transfer occurs in addition to heating. Therefore, the aim of this work was to optimize the PEF-assisted blanching of carrots immersed in calcium chloride solutions.

PEF heating of electric field strengths and frequencies up to 2 kV/cm and 150 Hz where applied to carrot samples immersed in calcium chloride solution at electrical conductivities and temperatures up to 3.5 mS/cm and 80 °C. Heating curves of both matrices were used to characterize the influence of PEF parameters in the process and to compare with the conventional heating. A commercial kit was used to study the inactivation of the peroxidase enzyme and the final texture of the freeze-thawed carrots was measured.

The results showed that increasing the frequency and electric field strength did not improve the heating uniformity resulting in higher heating rates in carrots. However, increasing the initial electrical conductivity of the saline medium improved heating uniformity, achieving 80 °C in both matrices after 60s when starting at an electrical conductivity of 6 mS/cm (equal to the conductivity of the electroporated carrot). Nevertheless, in order to find a more feasible industrial application, the influence of the initial temperature of the saline medium (20-80 °C) was evaluated, determining a better heating uniformity applying PEF at high temperatures. The best results were obtained when applying PEF-heating of 1.33 kV/cm, 100 Hz, an initial medium temperature of 80 °C and 3.5 mS/cm (at 80 °C) reaching 85 °C in 90 s in the carrot and in the saline medium. A holding heating phase (85 °C for 50 s) was required for complete peroxidase inactivation after the PEF heating-up phase. Blanching assisted by PEF shortened the heating time by 60 % compared to conventional blanching by immersion in hot water. In addition, PEF-blanched samples in a medium containing calcium chloride showed higher (18.5 %) hardness values (determined after being freeze-thawed) than those traditionally blanched (also with CaCl2 added).

Based on the obtained results, it can be concluded that PEF allowed reducing the blanching time processing of

carrots improving their texture after a freezing-thawing process.

P20 - Electroporation-based Treatments in Veterinary Medicine II

Tuesday afternoon Track A
Oct 11, 13:30 - 14:45

OR-75

Linear DNA amplicons delivered by electro-gene-transfer as veterinary Covid-19 vaccine candidate

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Introduction. As reported by the American Veterinary Medical Association, besides human-to-human transmission, human-to-animal transmission of SARS-CoV-2 has been observed in some wild animals and pets. With animal models as an invaluable tool in the study of infectious diseases combined with the fact that the intermediate animal source of SARS-CoV-2 is still unknown, researchers have demonstrated that cats and ferrets are permissive to COVID-19 and are susceptible to airborne infections. To address this issue, we have developed a genetic vaccination platform based on a linear DNA amplicon (Linear DNA COVID-19 vaccine) encoding RBD region of SARS-CoV-2 Spike protein and delivered by electro-gene-transfer (EGT). In clinical trials conducted in cats and ferrets in USA, we have investigated safety, immunogenicity and efficacy of Linear DNA COVID-19 vaccine.
Methods. In feline trial, 11 client-owned Covid-19 negative adult cats received 1 mg prime-boost vaccination (at days 0-28), intramuscularly via EGT. To assess vaccine immunogenicity, blood samples were collected before and one month after boost. In ferret trial, 25 Covid-19 negative adult ferrets received two different vaccine doses (0.25 and 1 mg), either with prime-boost or only prime, and were challenged with $5x10^5$ PFU of live SARS-CoV-2 intranasally two weeks after boost. Immune response and vaccine protective efficacy were assessed before and after challenge. In both trials, we exploited a clinically validated device for veterinary electroporation called VetePorator, based on Cliniporator technology currently approved for ECT applications in humans, adapted to EGT and already used in many animal studies.
Results. No severe adverse effects related to EGT procedure were revealed in cats and ferrets administered with two doses vaccination regimen. Linear DNA COVID-19 vaccine proved to be safe in both trials. Measurement of binding/neutralizing antibodies against many SARS-CoV-2 variants and T cell immune response specific for RBD region of Spike protein showed the induction of B and T cell response in vaccinated cats and ferrets. Moreover, in ferret clinical study, the two doses vaccination regimen showed not only to elicit B and T cell immunity, especially in 0.25 mg prime/boost vaccinated group, but also to protect from viral challenge, with no detectable infectious virus in nasal swabs three days post challenge.
Conclusions. Here we demonstrate that Linear DNA COVID-19 vaccine delivered by EGT is safe and immunogenic in cats and induces protective immune response from viral challenge in ferrets. These results warrant further investigations and hold promise for the development of veterinary vaccines to fight SARS-CoV-2 in animals and potential transmission to humans.

OR-76

Interleukin 12 gene electrotransfer to skin: experience from studies on pigs

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Gene electrotransfer (GET) of plasmid encoding interleukin 12 (IL-12) has already been used in clinical trials in combination with electrochemotherapy for the treatment of various spontaneous tumors in dogs. Although combination therapy proved to be effective, there is a need for the optimization of gene therapy in order to improve the treatment outcome. Specifically, the optimisation concerning the different concentration of plasmid DNA, the use of invasive or non-invasive electrodes and the use of plasmid DNA lacking the antibiotic resistance gene. In recent years, we have developed a plasmid encoding human interleukin-12 (phIL12) that is currently in a Phase I clinical study enrolling patients with basal cell carcinoma of the head and neck (Clinicaltrials.gov: NCT05077033). Plasmid phIL12 is free of the antibiotic resistance gene and has therefore been developed in accordance with the EMA guidelines for advanced therapy medicinal products. The plasmid was already evaluated in a preclinical study in the mouse tumor model CT26, where a plasmid with a transcript for the mouse ortholog IL-12 was used, which demonstrated its biological activity, safety, efficacy, pharmacokinetic and pharmacodynamic properties. The aim of this study was to investigate the use of different IL12 GET modalities (different plasmid DNA concentrations and use of invasive or non-invasive electrodes) on IL-12 expression in the skin as well as confirm the pharmacokinetic characteristics of plasmid phIL12 in another animal species. The porcine model was selected due to the fact that human IL-12 is biologically active in pigs and the skin characteristics are similar between the pigs, dogs and humans. The study was approved by national Animal Ethics committee (U34401-2/2021/5). Gene transfer of phIL12 in the skin was performed on 9 pigs. Different concentration of plasmid phIL12 (0 mg/ml, 1 mg/mL and 2 mg/mL) and two different types of electrodes: plate (non-invasive) and needle (invasive) were used. Animals were euthanized 7, 14, and 28 days after GET. The expression of IL-12

in the skin was performed on mRNA level by RT-qPCR and on protein level by ELISA test. Next, the distribution of plasmid DNA was evaluated in several different organs as well as in skin by RT-qPCR. The results of our study showed that the phIL12 GET with invasive electrodes induced higher expression of IL-12 on protein level as well as on mRNA level 7 days after GET compared to non-invasive ones. The distribution of the plasmid DNA showed the presence of plasmid in all of the samples tested with the highest plasmid copy number in the treated skin 7 days after GET. However, plasmid copy number in all of samples decreased over time and was at the minimum 28 days after GET. The results of this study demonstrated the expression of phIL12 in porcine skin and similar pharmacokinetics properties as with the mouse ortholog.

OR-77

A combination of electrochemotherapy and gene electrotransfer in canine stage III melanoma: Initial experience from peru

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Introduction: Electrochemotherapy (ECT) and Gene electrotransfer (GET) are valuable tools in the treatment of cancer. The aim of this study is to combine ECT and GET in three dogs with metastatic melanoma.
Patients and methods: All patients presented a confirmed stage III melanoma. They were treated using ECT combined with GET. For the ECT intravenous bleomycin was used. The electric pulses (8 100µs long of 1,000 V/cm at 5,000Hz for ECT and at 1 Hz for GET) were delivered using an EPV-100 electroporator (Biotex, Argentina). Disposable needles electrodes were used. For GET, a plasmid coding canine IL-2, and a plasmid coding canine IL-12 were used, and the procedure was repeated 28 days later.
Results: Case 1: A 10 years old, cross breed, spayed female, presented with a mass on the gingiva of the premolar area on the right mandibula. The patient had cytoreductive surgery, where an amelanotic melanoma was diagnosed. Within the first month a relapse was observed. The tumor measured 3 cm, was ulcerated and hemorrhagic. The owners rejected surgery, and only accepted ECT. A complete response was achieved presenting a reduction of the mandibular grith and bone remodeling, confirming bone involvement. Three months later, a follow-up ultrasound revealed a heterogeneous lymph node. Surgical resection of it combined with ECT on the tumoral bed and on the scar of the original tumor was performed. GET was performed using IL-2 near the original tumor and IL-12 in a close muscle.
No side effects were observed. The patient remains free of disease, reaching 649 days.
Case 2: An 11 years-old, cross breed, spayed female, was presented with a mass on the soft palate extending to the nasopharynx, presenting difficulty to breath and eat. A 3 cm pedunculated mass infiltrating the tonsil was observed. The patient had lymph node involvement and a suspicious nodule in Chest radiographs.
ECT combined with GET was performed. The patient developed a mild inflammation in the treated area during the first week. After the second GET an oronasal fistula developed with no long-term symptoms. Two months later, in follow-up radiographs, the suspicious nodule was not present. The patient remains disease free for 469 days.
Case 3: A 10 years old, schnauzer, spayed female, presented for lameness of the right anterior leg. A 1cm pigmented tumor on the nailbed was found. The cytology suggested the diagnose of melanoma. Digit amputation with lymphadenectomy was performed. The lymph node involvement was confirmed. GET treatment was recommended to improve survival and disease-free times. After the treatment the patient did not have any side effects and recovered uneventfully. After 261 days the patient remains free of disease.
All the patients remain disease free up to the writing of this work.
In conclusion, ECT combined with GET is a promising tool for the treatment of metastatic melanoma in dogs.

OR-218

From Cancer to COVID-19: gene electrotransfer as a versatile tool to design innovative veterinary vaccines

Luigi Aurisicchio

Takis S.r.l., Italy

We have recently developed Tel-eVax, a vaccine targeting Telomerase which was shown efficacious for treatment of canine B-cell lymphoma and other tumor types. Telomerase is overexpressed in >90% tumor types and is a potential universal antigen.
The current pandemic demonstrated the urgent need to develop versatile vaccination platforms that could be quickly implemented for infectious diseases. It is of utmost importance to provide a vaccine in a time-critical manner for such diseases, as the frequency of such epidemics and pandemics as we see them today, will be heavily increasing due to rise in global travel, global warming, increase in population density, penetration into previously uninhabited areas and animal trade. Nucleic acid vaccines, such as those based on mRNA are endowed of these features.
To address the urgent need to find solutions to the SARS-CoV-2 Pandemic, Takis has developed COVID-eVax, a vaccine approach based on genetic engineering and DNA electroporation as part of the X-eVax platform, previously developed. The project started in 2020 and consisted of the molecular design of the vaccine, the development of the reagents and tests necessary to test its effectiveness and the experiments in animal models. Subsequently, GMP-grade material (Good Manufacturing Practices) was produced, all regulatory studies were conducted (toxicology, biodistribution, immune response) and finally a phase 1 study in humans, which ended in December 2021, achieving all the objectives set and providing the basis for eval-

uations in Phase 2 and 3 studies.
DNA vaccines advantages are: (1) simple and quick production of DNA encoding the antigens by PCR or synthetic methods (potential game-changers for new variants especially vaccine resistant strains), (2) easy large-scale production, (3) safety compared to inactivated virus vaccines, and (4) higher thermostability (minimal cold-chain requirements), which is an issue with some vaccines. The DNA-based platforms offer great flexibility in manipulating the encoded vaccine antigen and have a great potential for accelerated development. Recently, the first DNA vaccine against SARS-CoV-2 (ZyCov-D) has been registered in India for human use; moreover, DNA vaccines have been extensively tested in multiple clinical trials in the oncology field and are commonly used in veterinary medicine. These vaccines (as opposed to mRNA-based vaccines) are stable, do not require cold-chain supply, and can easily be produced in large amounts in bacteria. All these advantages make this platform technology an attractive tool, as it overcomes several shortcomings of alternative approaches (e.g., complex production processes, stability issues, purchase price).
In this presentation, opportunities and challenges of DNA-based vaccines will be discussed and their potential use in Veterinary medicine.

P28 - Electroporation for Cardiac Ablation: Clinical Use and Development

Tuesday afternoon Track B
Oct 11, 13:30 - 14:45

OR-46
Epicardial high-density electrogram mapping dynamics during Pulsed Field Ablation (PFA)

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The use of irreversible electroporation (IRE) as a method for cardiac ablation in the treatment of cardiac arrhythmias, known as Pulsed Field Ablation (PFA), has rapidly moved from preclinical studies to clinic. One of the major advantages of this novel ablation technique over radiofrequency or cryoablation is safety. Although efficacy and long-term lesion durability have been demonstrated in limited groups of patients, studies in larger groups of individuals are still under development.
The reference indicator of acute lesion formation during cardiac ablation with thermal techniques consists in recording the local electrical activity changes with electroanatomical mapping systems. In the case of PFA, the modifications produced in the reversible and irreversible damaged areas are still unknown. Of particular interest from a clinical perspective is to determine how and when the electrical activity of the reversible areas recovers after PFA. Studying the dynamics of this process can help to understand the optimal use of electroanatomical mapping systems in the PFA framework.
In this study we acquired high-density local electrograms before and during 60 min after PFA application. The measurements were performed in different points of the right and left ventricular epicardial surface of swine using an open chest approach following a protocol approved by the Sant Pau Hospital Animal Experimentation Ethical Committee. Under general anesthesia, a median sternotomy was made to create a surgical window. Unipolar electrograms were recorded using a 128-channel electrode matrix (BIOSEMI) attached to the epicardial surface (interlectrode distance=0.75 mm, total dimensions 9.5 $\times$ 9 mm). For PFA ablation, a monopolar catheter (Biosense Webster Thermocool STSF) was positioned in the region corresponding to the center of the electrode array and a return electrode patch was attached to the back of the animals. After the procedure, and at least 3 hours from the last application, tissue was processed and lesion areas were stained with Triphenyltetrazolium Chloride (TTC) to assess acute lesion morphology.
Our results show an immediate change from basal narrow biphasic electrograms to wide monophasic electrograms with marked ST-segment elevation. Narrow QRS-complexes gradually reappear while ST-elevation recovers back to basal conditions from the periphery towards the center of the mapped area The extent and dynamics of the observed changes depend on the voltage and the position, i. e., depend on the electric field intensity. After tissue staining, we observe that the area where the electrograms are modified is beyond the area of acute damage. This confirms that the electrical activity of the reversible electroporation area is also acutely modified. The preliminary analysis of the results suggest that the different recovery dynamics observed could help in differentiating between reversible and irreversible electroporation areas. This study could also help in establishing the optimal acute time for remapping after treatment.

OR-176
Characterization of the effects of cryoablation, RF ablation or pulsed field ablation on compound action potentials of porcine phrenic nerves

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Introduction: Atrial fibrillation is conventionally treated by electrical isolation of the pulmonary veins. Phrenic nerve injury (PNI) has been reported as a complication associated with ablations performed with cryo and radiofrequency (RF) ablation. Electroporation has been increasingly explored as a potential substitute to these modalities, and while early research has demonstrated promising safety results, temporary phrenic nerve stunning has been observed. The purpose of this study was to investigate the dose response of phrenic nerve damage

from the direct application of cryoablation, RF ablation, and electroporation ex vivo.
Methods: Phrenic nerves (PN) were dissected by removing extraneous connective tissues, fat and associated vasculature from healthy anesthetized swine (n = 44). Subsequently, PN were placed in oxygenated buffer and maintained at 37°C. Each PN was then stimulated proximally with a 1 V, 0.1 ms square wave pulse. Biphasic compound action potentials (CAPs) were recorded both proximally and distally along the PN: i.e., on either side of the site of applied ablations. After stable baseline recordings were obtained, the following ablative therapies were administered: focal cryo at -80 C for 30 and 60 seconds (n=4); focal RF at 55, 60, and 65°C for 1 minute (n=4), and electroporation energies of 500, 600, 800, 1200, 1800, and 2000 V applied with 90, 100µs, monophasic pulses with probes placed 10 mm apart (5 mm on either side of the nerve, n=4). CAPs were then recorded at 15-minute intervals for 3 hours post-therapy. Waveforms were recorded before and after ablation, then analyzed to determine changes in latencies, amplitudes, and slopes of elicited CAPs.
Results: All nerves subjected to cryo ablation or radiofrequency ablations at a temperature of 65° C elicited complete loss of function. Amplitudes of CAPs were reduced over 90% from pre-ablation levels. RF ablations at 55° C and 60° C caused moderate damage in nerve conduction after 30 minutes, with an average CAPs amplitude reduction of 47%. The two highest voltage levels of electroporation (1800 V and 2000 V) showed a significant reduction of CAP amplitude of 35% and 30% respectively compared to baseline ($p < 0.05$). The other doses of electroporation showed no statistically significant changes from baseline ($p > 0.05$). Reductions in conduction velocities were not significant with p values > 0.05 for all treatments including control group.
Conclusion: This study demonstrates that depending on ablative therapy applied, the impacts on nerve activities can vary widely in an ex vivo experiment. In this isolated swine phrenic nerve preclinical research model, direct cryoablation induced the greatest losses of function, RF was dose dependent still showing a potential complete loss of function. Electroporation showed decreased phrenic nerve function only at high doses and was associated with preserved conduction at all voltage levels tested.

OR-177

Utilizing Human Induced Pluripotent Stem Cells to Study Cardiac Pulsed-Field Ablation

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Background: Cardiac electroporation is a novel promising non-thermal ablation method. Nevertheless, significant knowledge gaps exist regarding its electrophysiological consequences in human cardiomyocytes, including; protocol and delivery optimization, irreversibility threshold, cell recovery time-constants, and the mechanistic nature of its cytolytic and anti-arrhythmic properties.
Methods and Results: Healthy-control hiPSC-derived cardiomyocytes were enzymatically dissociated and seeded as circular cell sheets (hiPSC-CCSs). Electroporation pulse-field ablation (PFA) experiments were performed by delivering high-voltage electrical pulses via two needle-shaped electrodes. Detailed voltage-mapping studies were subsequently conducted. PFA application generated electrically isolated lesions within the hiPSC-CCSs. Further characterization reveled that; (1) lesions persisted over prolonged periods of time, demonstrating cell death and indicating irreversible electroporation, (2) cell death occurred within 24hrs following PFA, and was localized in areas exposed to the highest field-intensities, (3) a reversible electroporation component was also documented with a temporal decrease in lesion-dimensions in areas exposed to lower field-intensities, (4) most tissue recovery had occurred within the first ˜30 minutes following PFA, (5) per single pulse, high-frequency PFA was less efficacious than standard monophasic PFA, (6) increasing pulse-number had augmented lesion area, due to cumulative field intensity amplification, and (7) electroporation sensitization was achieved by increasing extracellular Ca2+, indicating its role in PFA-mediated cytolysis.
Finally, evaluating for potential anti-arrhythmic properties, we targeted arrhythmic rotors created in the hiPSC-CCSs. PFA abolished arrhythmic rotors directly and allowed the generation of sustained line-blocks isolating the remaining arrhythmogenic substrates within the hiPSC-CCSs.
Conclusion: This new model for the study of cardiac PFA provides novel insights into its temporal and electrophysiological characteristics, and facilitates electroporation protocol optimization, screening for potential PFA-sensitizers, and studying the mechanistic nature of its anti-arrhythmic properties.

OR-179

Open-Chest Pulsed Electric Field Ablation of Cardiac Ganglionated Plexi in Acute Canine Models

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Objectives: This study aimed to evaluate the safety and acute effect on markers of cardiac autonomic tone following pulsed electric fields (PEF) delivered to epicardial ganglionated plexi (GP) during a cardiac surgical procedure.
Background: Ablation of GP as a treatment for atrial fibrillation (AF) has shown promise but thermal ablation energy sources are limited by risk of inadvertent collateral tissue injury.
Methods: In acute canine experiments, median sternotomy was performed to allow identification of 5 epicardial GP regions using an anatomy-guided approach. Each site was targeted with saline-irrigated PEF (1000 V, 100 µs, 10 ECG-synchronized pulse sequences). Atrial effective

refractory period (AERP) and local electrogram (EGM) amplitude were measured before and after each treatment. Histology was performed post-treatment from samples of treatment adjacent structures.
Results: In 5 animals, 30 (n=2) and 60 (n=3) pulses were successfully delivered to all 5 target sites). There was no difference in local atrial EGM amplitude before and after PEF at each site (1.83 ± 0.41 mV vs. 1.92 ± 0.53 mV; p = 0.72). Mean AERP increased from 97 ± 15 ms at baseline to 115 ± 7 ms following treatment at all sites (18.6% increase, 95% CI [1.9-35.2%]; p = 0.037). There were no sustained ventricular arrhythmias or acute evidence of ischemia following delivery. Histology showed complete preservation of adjacent atrial myocardium, phrenic nerves, pericardium, and esophagus.
Conclusions: The use of PEF to target regions rich in cardiac GP in open-chest canine experiments was feasible and effective at acutely altering markers of cardiac autonomic tone.

OR-28
Early clinical experience with cardiac pulsed field ablation

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Catheter ablation is used increasingly to prevent recurrent atrial fibrillation (AF). The primary ablation strategi is pulmonary vein isolation (PVI) but succesrates with this strategy has been limited in part by insufficient durability of ablative lesions formed by conventional thermal ablation methods. These methods also carries a risk of collateral damage to extracardiac tissue such as the esophagus and the phrenic nerve. Pulsed field ablation (PFA) is emerging as an efficient and safe method to produce durable PVI. The presentation will focus on the implementation in clinical practice of a commercially available PFA system for PVI, and review our clinical experience as well as early published data on safety and efficacy for AF ablation.

P37 - Models for in vitro electroporation

Tuesday afternoon Track C
Oct 11, 13:30 - 14:45

OR-127
Chitosan-based breast cancer cell cultures: a promising tool for in vitro evaluation of anticancer treatments

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In drug development, two-dimensional (2D) cell cultures are widely used to choose the most effective drug candidates that are then tested in animal models. Nevertheless, these cultures, almost completely lacking extracellular matrix (ECM), may lead to unreliable results because they do not reflect the tissue architecture. Thus, several three-dimensional (3D) in vitro models, such as spheroids and hydrogel-based cultures, have been developed to resemble the tumour microenvironment and reduce the use of animals in testing according to the 3R rule (Reduction, Replacement, Refinement).
Herein, a 3D in vitro model of breast cancer has been obtained by seeding HCC1954 cells on lyophilized scaffolds composed of chitosan that derives from partial deacetylation of chitin and presents a structural similarity to the glycosaminoglycans (GAG) of ECM. Cultures were characterized at various time points (1, 3, and 7 days from seedings): cell morphology, cell growth, stemness, and matrix deposition were evaluated by means of phase contrast microscopy, cell viability, histochemical staining, and mRNA expression analysis. Then after, the responses of breast cancer cells to anticancer drugs and electroporation (EP) were evaluated. All results were compared to that obtained on 2D cell cultures. Finally, the electrical properties of the hydrated with medium and non-hydrated scaffolds were determined in order to compare them with typical tissue electrical characteristics.
Our data have shown that chitosan hydrogels allowed the formation of 3D cultures where breast cancer cells organized themselves to form not only monolayer but also spheroids and produce ECM molecules, such as collagen1A1 and laminin B1. In 3D cultures cancer stem cell population was enhanced, as demonstrated by increases in the expression of stemness markers, such as Nanog, SOX2, OCT4, and in sphere-forming ability. Furthermore, the N-cadherin/E-cadherin ratio was significantly higher than that detected in 2D cultures. Finally, chitosan-based cultures were less sensitive to doxorubicin and electroporation than adherent cell cultures.
Collectively, results suggest that chitosan-based breast cancer cel cultures may represent a promising model for in vitro evaluation of anticancer treatments.

OR-128
Skin electroporation for non-invasive drug delivery:Electrical properties of skin models and fluorescent molecule delivery

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The skin presents an accessible and convenient administration route for non-invasive drug delivery. Transdermal delivery platforms, such as nicotine patches, can effectively administer drugs through the epidermis in a controlled manner. Advantages include increased bioavailability, systemic delivery and painless administration, which in turn improve patient compliance and quality of life.

However passive diffusion of drugs through the skin is only achieved for low MW (<400-500 Da), relatively lipophilic molecules (logP around 2 to 3). Reversible skin electroporation can temporarily increase the permeability of the epidermis allowing bigger or more hydrophilic molecules to overcome the skin barrier.
We have developed a drug delivery platform, consisting of a nanocomposite, electrically-conductive hydrogel, acting as a drug reservoir and an electrode for the application of electrical pulses. It is based on an agarose hydrogel, reinforced with carbon nanotubes to increase its electrical conductivity. The hydrogel can be dried and then reswollen in a liquid solution with a molecule of interest (e.g. a therapeutic macromolecule or a fluorescent dye).
Two skin models are used: freshly-isolated extracts of hairless mice skin and lab-grown, reconstructed human skin layers. The conductive hydrogels are placed on the skin models and a pulsed electric field is applied through a generator. A multi-sided approach is employed to evaluate electroporation effectiveness and drug delivery. We measure the real-time electrical response of the system through a digital oscilloscope. The dynamic electrical resistance is calculated from the tension and current measurement. In parallel, we evaluate the macromolecule delivery through fluorescence microscopy of the skin surface and histological observations of fluorescent molecule penetration.
Our results show that the dynamic resistance of the skin rapidly decreases during the application of the pulsed electric field, due to the electroporation of the lipid bilayers and the subsequent increase in ionic charge transport. Depending on the electrical parameters applied (intensity and duration of electric field), this decrease in resistance may be temporary or permanent, indicating us the reversibility of electroporation. The fluorescent microscopy confirms that application of a pulsed electric field increases the delivery rate of hydrophilic molecules (MW of 450 Da up to 4 kDa). The histological observations show that the delivery of smaller molecules is greatly enhanced but, for the time being, bigger molecules (4 kDa) tend to accumulate in the outermost skin layers.
We are going to present our latest results regarding the effect of pulsed electric fields on the electrical properties of our skin models, coupled with observations on fluorescent molecule delivery.

OR-129

High-throughput cell transfection in a microfluidic electroporation chip

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A technology of microfluidics is an emerging tool in the field of biology, medicine and chemistry. Microfluidic device is also known as 'lab-on-a-chip' technology [1]. In moving from macro- to microscale, there is unprecedented control over spatial and temporal gradients and patterns that cannot be captured in conventional Petri dishes and well plates [2]. However, there is not a single standard microfluidic chip designated for all purposes – every different field of studies needs a specific microchip with certain geometries, inlet/outlet, channel depth and other parameters to precisely regulate the required function.
Since our group is studying an effect of pulsed electric field to the cells, we have manufactured a microfluidic chip designated for high-throughput electroporation of cells. In our microchip, a cell culture chamber is divided into two parallel channels by a membrane, meanwhile electrodes for electroporation are attached to the wall of the channels. Both microchannels have their own inlet and outlet, enabling injection of transfection material separately. Our perspective is to perform electroporation of mammalian cells in two different ways: (1) plasmid and cells are injected in the same microchannel and (2) injected into separate microchannels.

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OR-130

Inorganic nanoparticles as physical aids for local thermal ablation and electroporation enhancement: efficacy assessment in 2D and 3D cellular models

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Cancer cells and the tumor microenvironment play a key role in cancer development, progression, and resistance to treatment. In order to tackle both components, often referred to as "the seed" and "the soil", we herein propose the use of different inorganic nanoparticles as physical effectors to improve cancer treatment.
Conducting (gold) and semiconducting (iron oxide) nanoparticles have both: an excellent heating yield upon exposure to light [1], and a non-negligible potential to locally enhance the electric field [2].
Herein we compare different classes of commercially available spherical and rod-shaped gold nanoparticles, as well as custom-made spherical and chain-like magnetic anisotropic iron oxide nanoclusters [3].
In our study, we evaluated three types of pulsed electric field protocols: the ones used for electrochemotherapy, for irreversible electroporation, and for gene electrotransfer. The effects of pulsed electric fields, combined with spherical or elongated nanoparticles, were compared: 1) in different aqueous media, where we evaluated the heating of the suspensions, 2) in a type IV collagen gel, where we evaluated nanoparticles suspensions potential for collagen denaturation, and 3) in cells grown in 2D and multicellular spheroids (3D) made with murine hepatocellular carcinoma cells (HEPA 1-6).
Compared to electric pulses alone, the combination of pulsed electric fields with nanoparticles can disrupt the collagen gel and has a more pronounced detrimental effect on cells. The comparison of iron oxide-based

nanomaterials with gold nanoparticles indicates that magnetic nanoparticles can, upon specific conditions, dramatically outperform gold nanoparticles in different electroporation protocols.
By simultaneously acting as physical (magnetic, photothermal and electric) effectors, the nanochain-based platforms offer original multimodal possibilities for prospective cancer treatment, affecting both, the cells and the extracellular matrix.

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OR-131

Scanning electrochemical microscope as a tool for the electroporation

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The influence of pulsed electric fields (PEF) on living cells is associated with the phenomenon of electroporation when pores open in the plasma membrane of cells. Recently it was shown that scanning electrochemical microscopy (SECM) and electrochemical impedance spectroscopy (EIS) can be used for the determination of quantitative electrochemical characteristics before and after the electroporation. The results show that it is possible to use SECM for targeted electroporation at the selected area of tissue. The advantages of SECM over other standard methods used in electroporation studies are that the SECM methodology allows not only the study of cellular responses to PEF at the population level but also single cells and enzymatic reactions. Moreover, due to the small diameter of the ultramicroelectrodes (less than 25 µm), the electrochemical signal settles quickly, within a few nanoseconds, so SECM allows recording signal changes at an extremely high frequency. Using SECM micro and nanoelectrodes it will be possible to measure the signal of intracellular molecules coming out of electroporated cells at an extremely high frequency, and this will allow seeing the processes taking place in the plasma membrane of cells immediately after electroporation. It is expected that after applying SECM, it will be possible to observe the beginning of cell permeation or the dynamics of the formation and closing of primary pores. Next, microelectrodes and SECM will be used to evaluate the dynamics of cell death after electroporation over a period of hours and even days by evaluating the electrochemical properties of the growth medium.

This project has received funding from the European Regional Development Fund (project No 01.2.2-LMT-K-718-03-0063) under a grant agreement with the Research Council of Lithuania (LMTLT).

P39 - Electroporation for clinical use

Tuesday afternoon Track D
Oct 11, 13:30 - 14:45

OR-119

Electrochemotherapy of portal vein tumor thrombus as dowstaging to liver transplantation

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Liver transplantation (OLT) is contraindicate in Portal Vein tumor Thrombosis (PVTT) from Hepatocellular Carcinoma at hepatic hilum(pH-HCC) Surgery,Thermal ablation and chemotherapy show poorer outcomes Electrochemotherapy (ECT) has been successfully used in patients with pH-HCC with PVTT. We report the results of ECT as downstaging aimed to definitive cure by OLT.
F.P. 53 years HBV related Cirrhosis Child-Pugh B7 class; EGDS F2 aesophageal Varices. Diabetes. April 2016 : Enhanced Computed Tomography (CT) detected HCC(n.3 nodules in VII-VIII-VI;diameter range=2–5 cm) and PVTT of right portal vein. The patient was considered ineligible for OLT. May 2016: first ablation session with percutaneous Radiofrequency-ablation(RFA) of 3 HCC-nodules . August 2016: second ablation session with ECT of PVTT. CT october 2016: disappearance of PVTT and patent right portal vein. No intraparenchymal recurrence. CT march 2017: No recurrence in portal vein and in the left lobe. local recurrence in the VII-VIII segments. May 2017 : transarterial chemoembolization (TACE) of right lobe recurrences. CT October 2017: patent right portal vein. No recurrence. The patient was reconsidered for OLT. He underwent OLT in April 2018. At 36-months follow-up , no recurrence of HCC occurred. March 2021: enhanced CT and PET/CT detected a single small nodule (1.5 cm) uptaking tracer in the left upper pulmonary lobe, no hepatic recurrence . CT-guided FNB showed metastasis from HCC . June 2021: left lung upper lobectomy . At the current time the patient is alive and recurrence-free at 44 monts follow-up.
ECT Could be aneffective technique as pre-OLT dowstaging in HCC with PVTT.

OR-121

Response to Calcium Electroporation in Cancers Affecting the Skin – a Phase II Clinical Study

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many
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Background: Up to one in five cancer patients present cutaneous malignancy. When cancer is present in the skin, symptoms and distress can effect patients' quality of life. Surgical and medical treatment can be challenging and local treatments are warranted. Calcium electroporation (EP) uses intratumoral calcium injection and electrical pulses applied directly to target tissue. EP creates transient membrane pores that facilitate diffusion of otherwise non-permeant calcium, into target cells. The targeted cancer cells may die due to toxic calcium levels whilst normal cells restore homeostasis. The method is safe and effective with low cost. Furthermore, calcium EP has low treatment toxicity without mutagens and spares healthy tissues.

Materials and methods: This ongoing study aims to investigate response to calcium EP in a non-randomized phase II trial in 30 patients with cutaneous or subcutaneous malignancy of any histology. Patients must have been without response in cutaneous metastases over a two-month period, and are allowed systemic treatment. Patients are treated once and followed at month 1, 2, 3, 4, 5, 6, and 12. The primary endpoint is response two months after treatment. The overall response rate will be defined as number of responding lesions (partial or complete response) relative to treated lesions evaluated by changes in size (mm) by clinical examination with caliper measurement, further documented by clinical photography. The trial is a collaboration between three cancer centres in Næstved, Vejle (Denmark) and Lübeck (Germany). In one patient subset, magnetic resonance imaging is used to verify treatment area. In another subset, qualitative interviews have been performed to describe patient experience.

Results: To date 16 patients with a total of 45 metastases of different cancer types have been in enrolled in the study; breast cancer (n=10), lung cancer (n=3), pancreatic- (n=1), gastric- (n=1) and endometrial cancer (n=1). Median follow-up is 2.5 months (1-12). Few side effects have been observed and healthy tissues have been spared without sign of hyperpigmentation. Nine patients have been interviewed and the qualitative data of this subgroup is being analysed. MRI has been used to verify treatment areas in three patients. Preliminary results from the first eight patients show an ORR of 42% (CI 23-63 %,) across all treated tumours after two months (n = 24). Whereas efficacy of calcium EP for breast cancer metastases has previously reported, this is the first account on treatment for lung, pancreatic, gastric and endometrial cancer.

Conclusion: This study seeks to define response rates for different types of malignant skin tumours treated with calcium EP in order to uncover the potential of calcium EP as a standard treatment option. Future studies regarding mechanisms of action will further aid in predicting responses. The study is part of the Changing Cancer Care consortium supported by the European Fund for Regional Development.

OR-83

Electrochemotherapy of Posterior Resection Surface for Lowering Disease Recurrence Rate in Pancreatic Cancer (PanECT Study)

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Pancreatic cancer remains one of the deadliest cancers despite numerous research in the past decades. The only curative option is radical surgical resection. To improve survival an earlier diagnosis or a more efficient treatment method is needed.
Study was designed as a prospective pilot study to assess feasibility, safety, and efficacy of electrochemotherapy (ECT) of the posterior resection surface after a surgical resection of pancreatic head carcinoma. ECT will be performed within 8-28 min after intravenous in bolus administration of bleomycin (15 mg/m2). Plate electrodes will be used for ECT treatment, the electrodes will be placed between choledochal cut-end, truncus celiacus, remaining of the pancreas and aortal lymph nodes. A phase I clinical study is designed to include 20 patients that meet the inclusion criteria. Every patient will be closely followed-up after operation, findings will be noted and reported in line with Clavien-Dindo classification of surgical complications. Treatment effectiveness will be evaluated by US or CT imaging, to detect early local recurrence of the disease.
At the present time 4 patients were already included in the study. Preliminary results will be presented at the Congress.
Further research is needed to improve early detection and radical treatment of pancreatic cancer. After enrolment of 20 patients the analysis of feasibility, safety and efficacy will be performed. We expect the local recurrence rate to be lower after hybrid surgical and electrochemotherapy treatment in comparison to surgical resection alone.

OR-22

Burst Sine Wave Electroporation for Large Blood-Brain Barrier Disruption for Efficient Drug Delivery—A Feasibility Study

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Introduction: Glioblastoma multiforme (GBM) is a difficult tumor to treat due to its highly invasive nature and its intratumoral heterogeneity. Additionally, the presence of the blood-brain barrier (BBB), responsible for restricting the passage of systemic neurotoxins, macromolecules, and infectious particles from the brain parenchyma, poses a unique challenge. This selectivity often leads to essential drug molecules designed to target malignant cells in the brain to be screened out by the epithelial-like tight junction proteins coupled with molecular efflux transporters.
Despite major progress in the development of

electroporation-based ablation therapies, several challenges and gaps in knowledge exist. Up until this point, a large focus of high-frequency irreversible electroporation (H-FIRE) has been dedicated to inducing tumor cell death through the use of rectangular pulses, but a protocol for maximizing BBB disruption has not yet been developed. This study investigates the use of burst sine wave electroporation (BSWE), to induce larger volumes of BBB disruption while maintaining controlled volumes of tissue ablation.
Methods: Four male Fischer rats were treated with either a standard rectangular wave H-FIRE protocol (n=2, 5-5-5 µs, 480V), or an equivalent characteristic frequency sine wave BSWE protocol (n=2, 50 kHz, 680 Vpeak = 480VRMS) with 200 bursts of 100 µs of energized time. Rodents were administered Evans Blue Dye (EBD) as well as Gadolinium (Gd) contrast agent immediately prior to treatment. The solutions were allowed to circulate for one hour at which the rodents were then sacrificed. Rodents underwent T1 weighted contrast-enhanced imaging to quantify volumes of BBB disruption as demonstrated by hyperechoic areas. Additionally, a craniotomy was performed, and the rodent brain was sectioned to measure areas of EBD uptake.
Results: Our results demonstrated larger volumes of BBB disruption produced by 50 kHz BSWE (MRI: 152.8 mm^3 ± 11.17; EBD: 25.27 mm^2) compared to equivalent 5-5-5µs H-FIRE protocols (103.2 mm^3 ± 7.99; EBD: 10.36 mm^2) as measured through both MRI and EBD quantification respectively.
Conclusions: We have demonstrated a potential to capitalize on the frequency-dependent nature of cell permeabilization by utilizing narrowband sinusoidal electrical waveforms to induce larger volumes of BBB disruption. While standard H-FIRE utilizes traditional rectangular wave electrical pulses to induce cellular permeabilization, our preliminary results suggest that BSWE for nonthermal ablation of malignant gliomas and large BBB disruption may allow for the passing of large therapeutic molecules into the brain parenchyma for longer treatment windows and promote more diffuse regions of reversible electroporation to induce more efficient drug uptake into malignant cells.

OR-24

Pain sensation and muscle contractions during delivery of high-frequency electroporation pulses

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To minimize neuromuscular electrical stimulation during pulse delivery in electroporation-based treatments, it was proposed to replace long monophasic pulses of 50-100 μs with bursts of biphasic high-frequency pulses in the range of microseconds to reduce muscle contraction and pain sensation. These pulses, often referred to as high-frequency (HF) electroporation pulses, proved to be potentially useful for some clinical applications of electroporation such as tissue ablation, electrochemotherapy, and gene electrotransfer. In cardiac tissue ablation, where irreversible electroporation is used, the treatment has become known as Pulsed Field Ablation. While the reduction of muscle contractions has been confirmed in several in vivo studies, the reduction of pain sensation has not yet been confirmed in humans, nor has the relationship between muscle contraction and pain sensation been investigated. The data obtained by cell/animal experiments, modeling, and theoretical considerations, although of great value, do not allow evaluation of pain reduction during HF electroporation therapy. Therefore, in our study, we investigated the pain sensation elicited by short biphasic HF pulses in healthy subjects using the short-form McGill Pain Questionnaire. Twenty-five healthy subjects were subjected to electrical stimulation of the tibialis anterior muscle with biphasic HF pulses in the range of a few microseconds and both symmetric and asymmetric interphase (between positive and negative phase) and interpulse delays (between pulses). We also examined the relationship between muscle contraction and pain sensation while varying the pulse parameters (pulse width, interphase, and interpulse delays). In addition, we analyzed which pain fibers were more likely to be excited based on the pain descriptors selected by the subjects from the pain questionnaires (A-delta or C fibers). Our results confirm that biphasic high-frequency pulses (1 and 2 µs) reduce muscle contraction and pain sensation in comparison to the longer monophasic pulses currently used. In addition, we established that interphase and interpulse delays play an important role in reducing muscle contraction and/or pain sensation. This study has shown that the range of optimal pulse parameters can be increased depending on the prerequisites of the therapy.

P19 - Electroporation-based Treatments in Veterinary Medicine I

Tuesday late afternoon Track A
Oct 11, 16:00 - 17:30

OR-217

The use of electrochemotherapy in combination with other oncological therapies

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Cancer therapy seeks to improve the quality of life of veterinary patients and prolong their survival. In general, this is achieved by an efficient local control of the disease. Surgical oncology plays an important role for this aim, but when used alone it can't provide the best results. The combination of different treatment modalities,

with different mechanisms of action, is what allows to prolong survival times, maintain a good quality of life, and provide very good long-term control of the disease. Electrochemotherapy, now widely available across the world, plays a fundamental role in veterinary oncology. It cannot replace surgery in most of the cases, but it can complement it effectively. Allowing to achieve better results when combined than when used separately. The same goes for chemotherapy, cryosurgery, immunotherapy and radiotherapy, which are also found among the existing cancer treatments. There is very few scientific evidence regarding the combination of electrochemotherapy with other therapies in in veterinary medicine, however, from the theoretical point of view there is no impediment for it. In this work we present results of treatments that include a combination of therapies including electrochemotherapy, in order share some light in this matter.

OR-74

From Bench-to Kennel-to Bedside: Deploying Novel Preclinical Animal Models of Cancer in the Development of Irreversible Electroporation for Human Patient Applications

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Over the last decade, a multitude of focal tumor ablation methods have emerged as potential treatment options for cancer. Unfortunately, almost all of these modalities have high complication risks and many patients are not candidates for these strategies due to tumor progression, tumor size, and location near critical structures. The overwhelming majority of clinically available ablation modalities are either ionizing or thermal-based and have critical limitations, such as limited control in precision, inability to treat large or multiple tumor nodules, and incomplete ablation near major vessels. The risk of injury to critical structures near the ablation zone is also a major concern, particularly damage to blood vessels, nerves, or ducts. To address these limitations, our research team has deployed irreversible electroporation (IRE) and High Frequency Irreversible Electroporation (H-FIRE) for cancer therapy. IRE and H-FIRE deliver a series of electric pulses through electrodes inserted directly into the tumor. The induced electric field distribution is nonthermal and produces structural defects in the target cell membrane that causes cell death. Unfortunately, the lack of clinically and physiologically relevant pre-clinical cancer models is often a significant limitation with the development of novel biomedical devices and implementation of focal tumor ablation strategies. To date, the majority of studies testing electroporation-based modalities for cancer treatment have focused on rodent models, which have been critical in moving this field forward and will continue to be essential for providing mechanistic insight. However, while these small animal models have notable translational value, there are significant limitations in terms of scale and anatomical relevance. To address these limitations, our research team and collaborators have developed a diverse range of large animal models and spontaneous tumor studies in veterinary patients to complement existing rodent models. These preclinical models and veterinary patients are excellent at providing realistic avenues for testing electroporation-based tumor ablation modalities, perfecting surgical and treatment techniques, and developing treatment strategies for future use in human patients. Here, we provide an overview of results generated using IRE and H-FIRE across a broad spectrum of preclinical animal models and spontaneous tumors in veterinary patients. Our findings demonstrate the effectiveness of electroporation-based therapeutics on local tumor ablation under clinically relevant conditions. We also define mechanisms associated with systemic anti-tumor effects and immune system activation following focal tumor ablation. Finally, we discuss findings related to combination therapeutic approaches that successfully enhance local tumor ablation and augment systemic anti-tumor immunity.

OR-73

Electrochemotherapy in a pancreatic neuroendocrine tumor in a dog: A case report

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Introduction: The aim of this study is to use ECT in a case of an insulinoma, to improve the hypoglycemic condition of the patient.

Case: A 10 years-old, Shih tzu, spayed female presented with a history of a sudden seizure, with plasma glucose of 30g/dl. An ultrasound revealed a 2.66 cm mass in the right pancreatic branch. An insulinoma was suspected and derived to our clinic.

The patient was received in a depressed mental state, and three more episodes of seizures occurred. Blood samples were obtained, and a bolus of 33?xtrose was administered. Isotonic fluid therapy plus dextrose was started. The owners reported that the patient was weak and listless during the past few months. The patient did not have a seizure again; however, her blood glucose did not rise above 50g/dl.

Lab results were unremarkable. The differential diagnosis was an insulinoma or a IGF – II producing tumor.

Chest radiographs were normal. A CT-scan was performed for staging and treatment planning, revealing no metastasis.

Prior to surgery, the patient showed clinical decompensation. She presented a blood glucose level of 18g/dl, which was treated with dextrose supplementation upon stabilization. The patient was admitted to surgery for a partial pancreatectomy. However, during surgery the tumor was firm, vascularized, with a semicircular shape located next to the duodenum, rendering surgery very risky. . Then, ECT was performed as a safer option.

For the ECT, intravenous Bleomycin was used. The electric pulses (8 100µs long of 1,000 V/cm at 5,000Hz) were delivered using an EPV-100 electroporator (Biotex, Argentina) using gold plated plates electrode. .
Results: After the intervention the patient was evaluated using Glasgow Composite Measure Pain Scale, showing mild to moderate discomfort. Specific canine pancreatic lipase at 24 and 48 hours; the former was suspicious for pancreatitis (225.34 ug/L) but normalizing at 48 hours (150.3 ug/L). The blood glucose values stabilized in the first 48 hours, allowing the cessation of dextrose supplementation. Blood glucose was stable between 75g/dl to 85g/dl. Upon discharge blood glucose presented values of 140g/dl. In subsequent evaluations, fasting blood glucose was 270g/dl, then NPH insulin was started at a dose of 0.25U/kg every 12 hours.
The histopathological examination with immunohistochemistry confirmed an insulinoma.
During follow-up, ultrasonography revealed a reduction of more than 50% of the tumor one month after the procedure (measurement of 1.1cm), confirming a partial response. The quality of life was improved due to the cessation of the hypoglycemia.
At time of writing, the patient remains free of clinical signs or progression of the disease. This is the first case of an insulinoma treated with ECT in veterinary medicine.
In conclusion, ECT was an effective approach for treating a patient with an insulinoma.

OR-138

Longer duty cycle effects of irreversible electroporation on pig's pancreatic tissues: a pilot study

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A conventional irreversible electroporation (IRE) uses a pulse width of the duty cycle ranging 0.01–0.04% to cause irreversible permeabilization on the membrane of a cancer cell. However, such a duty cycle of pulsing may leave residual liver cells within the ablated tissue. The relationship between the duty cycle of pulse width with the feasibility and safety of IRE remains unclear. This study demonstrated the effect of varying the duty cycle of 50–83% on the feasibility and safety of applying IRE in the pig's pancreas. Triphenyltetrazolium chloride staining demonstrated that during the 50% duty cycle of IRE, the ablation area was significantly dependent on time after IRE. Furthermore, no ablation was observed 14 days after either duty cycle 50%-IRE or 83%-IRE. This indicated that the ablated tissues had recovered. For histopatholocial evaluation, most parameters of interstitial edema, hemorrhage, necrosis, and infiltration decreased 14 days after IRE of 50% duty cycle, except for fibrosis. For hematological evaluation, levels of aspartate transaminase, alanine transaminase, total bilirubin, amylase, and lipase were the same as those in the control group after 14 days of duty cycle 50%-IRE. The pathological values were reportedly lower than that of the 50% duty cycle 14 days after IRE for the 83% duty cycle of IRE. Moreover, the electrocardiogram did neither depict arrhythmia after the 50% duty cycle nor 83% of IRE in all the animals. Together, these data suggest that a longer duty cycle of pulsing for IRE ensures the feasibility and safety for its application in clinical trials.

OR-213

Veterinary Guidelines for Electrochemotherapy of superficial tumors

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Electrochemotherapy (ECT) consists in the application of electric pulses to increase chemotherapeutic drug intake (bleomycin, cisplatin, or calcium) into the tumor cells. It has become a very valuable treatment option in veterinary oncology. It is an effective and safe treatment modality, which is not only beneficial as a palliative treatment, but also for a curative approach. Performing the treatment adequately will ensure the best results possible, in the minimum number of sessions, and reduce complications. Usually, only one session is enough to achieve excellent results, but the treatment can be repeated. Several sessions can be necessary in the case of incompletely treated or very extended lesions, as well as in the occurrence of new lesions. ECT is effective for superficial or oral tumors of any histology that are accessible to the electrodes. Intravenous bleomycin is the preferred drug and route of administration, leaving other ways of administration and drugs for selected cases. The guidelines presented here are destined to veterinarians who want to develop their understanding of the basis of ECT and wish to perform it adequately and effectively. In this paper, we also discuss common problems and how to solve them, and we include practical tips to improve the treatment results based on common questions and mistakes of beginner users.

P22 - Irreversible Electroporation (IRE) and Immunotherapy

Tuesday late afternoon Track B
Oct 11, 16:00 - 17:30

OR-186

A phase II-study of electroporation potentiated immunotherapy in liver metastatic pancreatic cancer (EPIC-1)

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Introduction: Pancreatic cancer (PC) is the fourth leading cause of cancer death. This is largely due to late diagnosis, aggressive tumor biology and resistance to chemotherapy. Immune checkpoint inhibitors (ICI) have shown impressive results in several tumor types, but not in PC. Animal trials have, however, shown that ablation of a tumor lesion using irreversible electroporation (IRE) can alleviate resistance to ICI in PC and other cancers. The aim of this trial was to examine the efficacy and safety of combined pembrolizumab and IRE ablation in liver metastatic PC.
Materials & Methods: Patients (PS 0-1) with progression on or intolerance to first or subsequent lines of chemotherapy was included in a prospective single-arm trial. Patients were administered pembrolizumab (400mg every 6 weeks) and treated with IRE of a single liver metastasis after 10 days. CT scans were performed at baseline, 14 days after IRE and every two months during follow-up. Blood and tissue samples were collected at baseline, on the day before IRE and on the first postoperative day.
Results: Inclusion was stopped after eight patients due to progression in all eight patients during interim analysis. No serious adverse reactions to pembrolizumab or surgical complications were recorded. Flowcytometric classification of circulating immune cells and RNA-sequencing of tumor samples are currently ongoing.
Conclusions: Combined IRE and pembrolizumab does not have a clinically relevant effect size in previously treated metastatic PC. The cancer immunological impact of IRE is currently being investigated.

OR-187

Irreversible Electroporation and Immune Checkpoint Inhibitor Immunotherapy provides improved primary and systemic cancer control

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Accumulating evidence suggests that focal therapies combined with immunotherapies can provide systemic anti-cancer immunity. Here, we assessed the ability of combined irreversible electroporation (IRE) and Immune Checkpoint Inhibitor (ICI) to improve primary and systemic tumor control in various murine cancer models.
Hind limb tumors (TRAMP-C2, MC-38 and CT26) were treated with IRE (1500 V/cm, 50 μs pulse width, 50 pulses at 1Hz) by a set of parallel electrodes, followed by ICI immunotherapy with anti-CTLA-4 (200 μg on day 1, 100 μg on day 4, 7 and 10) or anti-PD-1 (100 μg on day 1, 3 and 5).
In the TRAMP-C2 prostate cancer model, IRE followed by anti-CTLA-4 demonstrated superior outcomes than either monotherapy. Anti-CTLA-4 failed to control tumor growth and did not alter SPAS-1 T cell distribution while IRE-treated tumors showed a brief delay of growth but were unable to successfully control the tumor. In contrast, the combinatorial regimen of IRE followed by anti-CTLA-4 therapy led to complete tumor regression in 46% of mice. In addition, combination IRE and anti-CTLA-4 therapy increased intratumoral SPAS-1+ T cells and increased SPAS-1+ tissue-resident CD8+ memory T cells (TRM) in non-lymphoid tissues including skin. Mice that had previously achieved complete remission following dual IRE + anti-CTLA-4 therapy were 100% protected from secondary tumor challenge, suggesting that enhanced tumor antigen-specific TRM in the skin are associated with tumor protection. IRE immediately followed by anti-PD-1 did not show an effect on tumor growth or SPAS-1+ T cell levels in blood. However, the anti-PD-1 group showed sustainable tumor regression (compared to IgG control, $p<0.05$) when given 10 days later, after both IRE and anti-CTLA-4 treatment.
In the MC-38 colon cancer model, primary tumor control was significantly improved by combining IRE with anti-PD-1 treatment (median survival days: control: 8, anti-PD-1: 9, IRE: 16, IRE+anti-PD-1: 26). This was correlated with a higher frequency of CD8+ tumor infiltrating lymphocytes. IRE + anti-PD-1 also achieved a rate of secondary tumor rejection rate of 95%. In addition, CD8 depletion after IRE + anti-PD-1 treatment led to 0% rejection upon rechallenge (n=10). In another colon cancer model, CT26, IRE + anti-CTLA-4 led to complete tumor regression in 25% of the mice (n=8), compared to continued tumor growth with IRE or ICI monotherapy.
In summary, our results show that IRE and ICI can lead to a synergistic and durable antitumor response, prolong overall survival, and induce long-term immune protection. This success appears to rely heavily on CD8 T cells (i.e., population, location, and phenotype). Insufficient priming of CD8 T cells can be addressed by focal therapy and early intervention of anti-CTLA-4 such as shown in the TRAMP and CT-26 models. On the other hand, T cell exhaustion can be mitigated by engineering of focal therapy and anti-PD-1 immunotherapy as shown in the MC-38 model and delayed administration of anti-PD-1 in TRAMP.

OR-188

Irreversible electroporation selectively lyses cancer cells while preserving function and promoting tumor infiltration of chimeric antigen receptor (CAR) engineered T cells

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Purpose: We investigated optimal IRE pulse parameters for the selective depletion of cancer cells while sparing Chimeric Antigen Receptor (CAR) T cells in the tumor microenvironment. We also studied the impact of IRE on CAR T cell function and its recruitment into tumors.
Materials and Methods: Following IRE (at varying voltage, pulse width and numbers) on mouse (AB12) and human (MSTO-GM) mesothelioma cells, T cells (CAR and control) were co-cultured. Cell survival and proliferation were quantified at 2-, and 24-hours post-treatment. Pulse parameters with greatest differential cytotoxicity between cancer and T cells was termed selective IRE (sIRE) and further tested in a 3D tumor mimic with Propidium Iodide/Calcein staining. Numerical models were constructed in COMSOL to estimate induced transmem-

brane voltage in different cells, the electric field gradient, and statistical probability of cell death in a 3D in vitro tumor mimic. Impact of repeated sIRE on CAR T cells was evaluated with a 51Cr release and bioluminescent cytotoxicity assay at various effector-to-target (E:T) ratio. Chemokine release from cancer cells and its effect on CAR T cell migration were measured with a multiplex Luminex bead immunoassay and transwell co-culture, respectively. Outcomes of sIRE on intratumor T cell population and cancer ablation was studied in BALB/cJ mice bearing AB12 subcutaneous tumors using flow cytometry and immunohistochemistry. Trafficking of intravenously administered CAR T cells from chemokine release by sIRE was assessed in NOD scid gamma mice bearing MSTO-G/M tumors.
Result: Simulations suggested that cancer cells develop 1.5 – 2 fold higher transmembrane voltage when treated with the same pulse parameters, increasing their susceptibility to IRE. Cuvette model studies confirmed this, where sIRE (1000 V/cm, 10 μs pulse width, 125 pulses) provided greatest differential cytotoxicity (MSTO-GM: 43.2% vs. CAR T cell: 84.6%, $p < 0.01$), with similar outcomes in a 3D gel model. sIRE effectiveness in 3D gel models containing mixed cell populations could also be predicted by simulation models. sIRE parameters slightly affected the proliferation, but not function of CAR T cells as measured on bioluminescent cytotoxic assays (p not significant). CAR T cells remained potent following repeated treatment (3x doses at 2:1 E:T ratio cytotoxicity for sIRE: 45% vs. IRE: 29% & Sham: 75%). sIRE of murine mouse tumors resulted in greater preservation of T cells (CD3+: 0.7%) at 4 hours post-treatment when compared to IRE (CD3+: 0.46%), with greater recruitment at 24 hours post-treatment (CD3+: 6.7%). Robust release of chemokines by sIRE induced greater migration in a transwell assay, findings that were validated in mouse bearing MSTO-G/M tumors.
Conclusion: sIRE selectively lyses cancer cells while preserving function of CAR T cells; sIRE-induced chemokine gradient promotes CAR T-cell tumor infiltration, thereby augmenting the E:T ratio and anti-tumor efficacy.

OR-214

Experimental study on enhancing the bioelectric effect of tumor cells using the combination of nanosecond and microsecond pulsed electric field

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Based on the different targets on cells and bioelectrochemical mechanisms of the nanosecond pulsed electric field (nsPEF) and microsecond pulsed electric field (μsPEF), this paper studied the enhanced bioelectric effect of tumor cells using the combination of nsPEF and μsPEF. With human malignant melanoma cells (A375) as experimental subjects, single-cell fluorescence assay, flow cytometry double staining assay and monolayer cell ablation experiment were carried out to analyze the targeting characteristic and enhancement effect of nano-microsecond pulse electric field (nμPEF). The results showed that nano-microsecond pulse electric field could simultaneously target the nuclear membrane and the cytomembrane on cells. Compared with nsPEF or μsPEF alone, the nμPEF could significantly promote the fluorescence dissipation degree of single cell, produce increasing apoptotic and necrosis of cells, and achieve the wider-range and low-residue ablation area. Besides, when the applying sequence of nanosecond pulses and microsecond pulses was reversed, the enhanced bioelectric effect was weaker than that of nμPEF. Therefore, the multi-target characteristic and enhancement effect of nμPEF could provide the theoretical support for greater killing effect of tumor cells and complete ablation of tumor tissues, which is expected to become a novel therapy of pulsed electric field for tumors.

OR-215

Decellularized intestinal tissue as a potential graft for bladder reconstruction therapies

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Introduction: Irreversible electroporation (IRE) has previously been reported to kill cells without disrupting the extracellular matrix (ECM), with potential applications in tissue engineering. Here, we developed a workflow and investigated the application of IRE for ex vivo decellularization of the small intestine, a widely used scaffold material for reconstructive surgery.
Methods: Wistar rats (16-20 weeks old) were sacrificed to extract the small intestine (SI, 1 inch length). IRE and detergent wash (DT - positive control) were used for tissue decellularization, and results compared to sham treatment. IRE of SI in cold PBS was performed in a 4mm gap cuvette with following pulse parameters: 1500 V/cm, 100 µs, 50 pulses at 1 Hz. IRE treated SI was then transferred to Iscove's Modified Dulbecco's Medium (IMDM) with supplements and incubated for 4h, followed by 4x PBS wash (12 h), 1x PBS wash (1 h) and DNase wash (24 h). DT was performed by washing the SI in triton X-100 solution (52 h), sodium deoxycholate (26 h), DNase (1 h), 1x PBS (1 h), saline solution (1 h) and DI water (1 h). H&E and Masson's Trichrome stains were used for assessing morphological status and collagen in SI samples. Colorimetric quantitative assays were used to measure residual DNA (Quantifluor dsDNA system), glycosaminoglycan (Blyscan) and collagen (Sircol) levels in the tissue. Ultimate tensile test was performed on control and treatment groups to determine changes in mechanical properties from treatment. IRE treated IS was lyophilized and made into a slurry to form scaffolds and seeded with bladder smooth muscle cells. Cytotoxicity assay was performed on the scaffolds and compared to Matrigel cell encapsulation (positive control).
Results: Outcomes of IS decellularization with IRE (41 hrs) was comparable to DT (82 hrs) with shorter processing time. H&E and Masson's trichrome stained IRE samples demonstrated complete removal of cellular components with preservation of collagen (ECM), equivalent to DT. Total residual DNA in IRE samples (183 ng/mg)

group was considerably lower than sham group (2990 ng/mg of tissue) but higher than DT (10 ng/mg). IRE samples had significant preservation of ECM content (collagen – 3.5 µg/mg, GAG – 0.27 µg/mg), comparable to native IS (sham: collagen – 3 µg/mg, GAG – 0.77 µg/mg) and higher than DT (collagen – 1.3 µg/mg, GAG – 0.05 µg/mg). Mechanical stiffness of IRE (13.2 MPa) and DT treated IS (10.38 MPa) group was higher than native tissue (5.3 MPa) without statistically significant difference. Cytotoxicity assay showed that the cells grown in IRE scaffolds were healthy and viable, equivalent to Matrigel culture.
Conclusion: IRE is a novel and effective approach for ex vivo decellularization of bulk tissue with outcomes comparable to conventional detergent wash at considerably shorter time requirement. IRE provides added advantage of not using harsh chemicals and better preservation of ECM.

P32 - Pulsed electric field effects on neural tissues and the brain

Tuesday late afternoon Track C
Oct 11, 16:00 - 17:30

OR-17
Characterization of Ca2+ fluxes modulation by nanosecond pulsed electric fields in neuroblastoma and mesenchymal stem cells

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To assess if any correlation can be established between the frequency content of different pulsed electric stimulations and the permeabilization to calcium ions, the response of two different cell cultures to a set of different nanosecond electric pulses was analyzed. These observations seem particularly interesting, as calcium is a fundamental second messenger in cells. In particular, both SH-SY5Y neuroblastoma cell line and mesenchymal stem cells (MSCs) were analyzed. The use of two cellular models allows us to observe if the results obtained are cell type-dependent and so to deepen which specific biological activity can be modulated with pulsed electric stimulations to better address different therapeutic applications. We also investigated if spreading the pulses in multiple groups enabled the increase of SH-SY5Y and MSCs membrane permeabilization. In fact, some authors observed, looking at the electric field-mediated uptake of specific fluorescent dyes, cells "sensitization" if the delivered electric pulses dose was split in a few fractions, but the mechanism of this phenomenon is still debated and unclear.
Our experiments were conducted using fluorescence microscopy and acquiring fluorescence variations in real-time using FURA-2 ratiometric dye for calcium detection. Cells were pulsed using coplanar electrodes of a planar waveguide integrated in an inverted microscope stage. Electric pulses lasting 10 ns in different numbers and different repetition frequencies were used. Fluorescence images were acquired every 10 seconds starting from 1 minute before the exposure up to 10 minutes after the end of the exposure to monitor complete calcium fluxes dynamics after the exposure. Sham exposures were performed during each set of experiments.
The experiments were performed in the presence and in absence of external Ca2+ ions to exclude the implication of internal cell organelles in regulating the cytosolic Ca2+ levels. Moreover, in order to demonstrate that the Ca2+ levels modulation is mainly due to the pores formation induced by the electrical stimulations, two different Ca2+ channels inhibitors were also used and the results were compared with experiments without inhibitors.
Our results demonstrate that Ca2+ modulation seems independent on the frequency content of the different pulsed electric fields patterns applied. Similarly, no additive effects on cytosolic Ca2+ increase were observed when the pulse number was spread in multiple fractions, if compared to the fluorescence reported when the same number of pulses was applied consecutively. The SH-SY5Y cells seemed more sensitive to pulses stimulation, may be due to their neuronal and tumor nature because Ca2+ is a strong regulator of the functionality of such cells. Our results are interesting to better understand permeabilization-mediated Ca2+ dynamics to possibly control associated cell functions for new therapeutic applications in cancer and non-cancer diseases.

OR-18
RISEUP: Regeneration of Injured Spinal cord by Electro pUlsed bio-hybrid imPlant

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Spinal Cord Injury (SCI), a major cause of paralysis, currently has no effective therapies. Every year almost 500.000 people are diagnosed with SCI worldwide. In Europe, the average investment is up to 2 M€ per patient in health are [1]. The difficulty on the neuronal restoration after SCI is based on the complex cascade of events that inexorably cause a degenerative chronic stage mainly favored by the non-permissive environment and limited capacity for axonal regrowth. Multifaceted

strategies are considered the unique solution for functional restoration by including cell substitution, neuroprotection and axonal growth promotion. RISEUP project proposes to attain neuronal functional regeneration after SCI by an unprecedented and unique bio-hybrid-compatible electro-activated and wireless rechargeable implantable technology. RISEUP introduces high voltage microsecond electric pulses (micropulses) stimulations and low amplitude direct currents on a combination of stem cells (induced neural stem cells and multipotent stromal cells), whose transplantation is facilitated by an innovative scaffold biomaterial. The RISEUP concept is that micropulses, being able to impose and control cytosolic Calcium oscillations [2, 3], will facilitate cell maturation, survival and neurotrophic factors secretion. Because Calcium signaling is essential for neuronal activity, endogenous neuronal reconnections will also be favored. RISEUP goal, even if ambitious, is concrete due to the multidisciplinary partners' competences, initiating from TRL1 a radically new line of technology (electro-activated, remotely controlled, biocompatible, biodegradable cell-containing implants for the repair of neuronal lesions) establishing its proof-of-principle (TRL3). The long-term vision of RISEUP is the radical change in SCI treatment modality to assure the cure delivery without any machinery connection, dramatically improving patients' quality of life.

OR-19

Effects of electrical stimulation in neural stem cells and mesenchymal stem cells cell fate

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CNRS, Gustave Roussy, Metabolic and Systemic aspects of the oncogenesis (METSY), France

Spinal cord injuries remain a significant therapeutic challenge due to the inability of the central nervous system to regenerate lost neurons and restore functional connections. Because of the enormous potential in the treatment of central nervous system disorders and injuries, Neural Stem Cells (NSCs) transplantation is an attractive therapy that demonstrates its ability to replace lost cells or repair host neural circuits, as well as its ability to differentiate into any required neural cell type [1]. Mesenchymal Stem Cells (MSCs) are considered a competent cell source for tissue engineering applications. Transplantation of MSCs into spinal cord injury has an immunoregulatory effect on the tissue that contributes to structuring regenerative microenvironments in areas of injury and has the potential to generate multiple differentiated progenies [2].

Studies with NSCs and MSCs, either separately or in combination, show improved functional recovery after transplantation, providing therapeutic benefit in terms of decreasing the inflammatory environment. However, the low survival rate and uncontrolled graft differentiation requires the use of combination therapies. In this context, cell-based therapies still need improvements such as the enhancement of the cell survival and the neuronal differentiation and maturation, by complementary treatments, for example electrical stimulation. For this purpose, exogenous electrical stimulation consists the most often in triggering action potentials with low electric fields. This allows us to non-chemically alter the niche signals of NSCs or MSCs to promote proliferation and differentiation towards specific lineages.

Activation of calcium signalling is essential in neuronal development, including neuronal induction [3] and regulates physiological functions in the cell such as proliferation and differentiation [4]. Therefore, in this work, intracellular calcium oscillations pattern has been studied under conditions of proliferation and neuron-induced differentiation in NSCs and MSCs. Using electroporation to control calcium oscillations [4], we studied the effects of the oscillations on proliferation and induced neural differentiation of NSCs and MSCs. Calcium oscillations were recorded in time lapse microscopy with the calcium dye Fluo-4 AM. Morphological changes during the differentiation process were studied by immunofluorescence techniques and brightfield microscopy.

Manipulation of calcium patterns through electroporation might be a potential therapy to promote cell proliferation as well as neuron-induced differentiation in a simple and easily controllable way. Electrical stimulation is a feasible and flexible methodology in vitro and it is easily transferable to in vivo in order to rescue central nervous systems injuries.

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OR-16

High-rate nsPEF bursts stimulate neurons at paradoxically low electric field thresholds and without electroporation

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Nanosecond Pulsed Electric Field (nsPEF) is a new modality to achieve bioeffects, including cell stimulation. However, use of high electric field is required for neuron stimulation using nsPEF, thus increasing a risk of plasma membrane disruption. Here we demonstrate that the high pulsed electric field requirement can be bypassed by exploiting temporal summation when multiple subthreshold stimulatory nsPEF are compressed to high-rate bursts. Threshold electric field and neuron's ability to respond to multiple stimulation attempts was evaluated in a wide range of nsPEF burst parameters.

nsPEF burst stimulation was tested for excitation of dissociated rat hippocampal neurons. A stimuli-induced action potential (AP) was detected using optical membrane potential registration at standard FITC settings and transmembrane potential-sensitive fluorescent probe FluoVolt™. nsPEF bursts enabled neuron stimulation at 0.1±0.01kV/cm (400ns/pulses at 2MHz for 500µs), which was a substantial reduction compared to a single 400ns pulse that required 3±0.2kV/cm. Furthermore,

for constant burst duration the efficacy of nsPEF bursts (reduction of threshold electric field) increased with the duty cycle, which could be achieved by an increment in pulse repetition rate or pulse duration. Also, all tested nsPEF bursts stimulated neurons at a lower time-average electric field than a single matching pulse (both stimuli had identical time-average electric field and duration), indicating an unknown specific nsPEF burst effect.
nsPEF bursts exhibited the lowest time-average threshold values (indicating the most effective temporal summation) when the duty cycle was <10%. At the same time, these nsPEF bursts required the highest electrical field/pulse (≈1-3kV/cm) to induce AP. These circumstances suggest that AP generation could be mediated due to membrane disruption and increased permeability-mediated ions fluxes (depolarization). Furthermore, the ability of neurons to generate responding AP to every stimulation attempt (up to 100) was investigated. Neurons stimulated using single µs-range pulses (conventional stimulation) responded by producing AP to all applied stimuli, while to a single 400ns pulse responded only once. This revealed, that single pulse nsPEF was unsuitable for multiple stimulations. Although, in general, nsPEF burst could be used for successful multiple stimulations (e.g., 250 x 400ns/pulse at 500kHz or 150 x 100ns/pulse at 250kHz) responded to all stimuli.

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OR-20

Microsecond electric pulses effects on induced neuronal stem cells for regeneration of spinal cord injuries

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Spinal cord injury (SCI) is a neurological and pathological state characterized by motor, sensory and autonomic dysfunctions, which affects around 500,000 people every year. So far, several therapeutic strategies based also on stem cell transplantation have been studied, but the results are poor and an effective protocol for spinal cord regeneration is still missing.
RISEUP project (fully described in another abstract submitted to WC2022), funded by the European community in the H2020 FET-OPEN program, provides for the development of an innovative system for the regeneration of SCI based on the transplantation and the microsecond (µsec) electric pulses stimulation, through a biocompatible, biodegradable, and electrified support, of mesenchymal (MSCs) and induced neuronal (iNSCs) stem cells. The hypothesis is that, by modulating the calcium fluxes of iNSCs through µsec stimulation, these cells will differentiate in mature neurons acquiring the ability to regenerate the lesioned area. Moreover, MSCs stimulation could induce their differentiation in neuronal-like cells, able to release neurotrophic factors necessary for neurons survival.
Here we present the first results obtained by applying different exposure protocols in terms of repetion frequency, pulses number, amplitude, duration, and polarity.
The purpose of these preliminary results is to evaluate the survival, homeostasis and differentiation of cells in response to the different protocols of stimulation.

OR-21

Low pulsed electrical fields for inducing transient BBB disruption in a mouse model

Shirley Sharabi, Yael Mardor, David Last, Dianne Daniels, Sigal Liraz-Zaltsman, Itzik Cooper
Sheba Medical Center, Israel

Brain diseases are extremely hard to treat, mainly due to poor penetration of therapeutics across the blood-brain barrier (BBB). It was previously demonstrated by us and others that BBB disruption (BBBd) can be achieved by applying electroporation (EP). Unfortunately, applying high voltage pulses can induce significant adverse effect and require invasive surgical procedures. In the past years, we have discovered that low pulsed electric fields (L-PEFs), an order of magnitude below the threshold of electroporation, can induce transient BBBd non-invasively. Here, we treated mice with 25-400 pulses (100-300V, electrode gap 1.2-1.3 cm, 50µs pulses at 4Hz) using 2 plate electrodes pressed against the skull. BBBd was studied by MRI-based BBB maps developed uniquely for L-PEFs, and by quantifying the accumulation of different molecular weight therapeutics to the brain.
MRI studies: Contrast agent (Gd-Dota) was administered to the tail vein 5 min to 4 hours post L-PEFs. BBBd volumes and intensities were calculated using BBB maps, based on delayed-contrast MRI. A pixel by pixel analysis of the contrast intensity was used to evaluate BBBd and the volume and intensity of pixels above a certain threshold were calculated based on a 3 exponential model. Correlation between the results and a finite elements simulation of the electric fields distribution in the brain was studied.
BBBd was depicted in the BBB maps Following 100V/100 pulses. The simulations showed that the electric field in the disrupted volume was 28-65 V/cm, significantly below known EP threshold. A significant correlation was found between BBBd volume and the number and amplitude of the pulses (r2=0.96/0.91 respectively). BBB fully recovered at 1h for 100V and 4hrs at 400V.
In order to evaluate drug accumulation in brain tissues, Doxorubicin (580Da), a biologic drug (ICTK, 50 kDa), and IgG (150 KDa) were administered to the tail vein of sham and L-PEFs-treated (200V/100 pulses) mice in 3 separate experiments, and were allowed to circulate for 4hrs. The mice were then perfused and the brains were removed and processed for drug concentration. Doxorubicin was detected by a fluorimeter, ICTK was detected

by ELISA and IgG was detected by immunofluorescence staining of brain slices.
Drug accumulation studies showed increased concentrations of all tested drugs in the brain. Doxorubicin concentration, 4 hours post L-PEFs, was 690±259 nM, which is X431 its IC50 in GL261 glioma cells, while undetected in the sham group. ICTK showed x13 increase ($p<0.0001$) compared to sham. IgG staining revealed significant extravasation into brain tissue while undetected in sham mice brains.
Our results demonstrate the feasibility of applying L-PEFs, non-invasively, for inducing transient and safe BBBd in a mouse model. We further showed that this rapid treatment provides efficient delivery of small/large therapeutics into the brain, thus leading the way to efficient treatment of various CNS disorders.

P5 - Mechanisms and Applications of PEF in the Food Industry

Tuesday late afternoon Track D
Oct 11, 16:00 - 17:30

OR-89
The role of post-electroporation recovery on the survival of Thai basil leaves during drying

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Horticultural crops have a low tolerance to dehydration. In this paper, we show that the reversible electroporation of Thai basil leaves followed by 24 h resting before hot air drying at 40 °C enhanced the survivability of the tissues at certain levels of dehydration (moisture ratio = 0.2 and 0.1). However, this increased survival was rather limited. Through measurements of metabolic heat production during resting, rehydration kinetics, respiration and photosynthesis of the rehydrated leaves, we show that resting after the application of a reversible pulse-electric field (PEF) may allow a phase of hardening that has a protective effect on the cells, thus decreasing damage during the subsequent drying phase. Increased preservation of cell vitality would be associated with a more turgid and fresh-like rehydrated product, as cells would have the capacity to retain the rehydration water.

OR-90
Evaluation of the Extraction Yield of Phenolic Compounds in Olive Leaf Treated with Pulsed Electric Fields

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The olive leaf is one of the main by-products from the olive oil industry. This by-product is a rich source of phenolic compounds that have been shown to have beneficial health activities, which are due in part to their antioxidant activities. Therefore, the revaluation of this by-product would be of great importance for the food industry. For this reason, this study focuses on establishing the optimal conditions for a technology based on the use of pulsed electric fields (PEF) as a pretreatment in the olive leaf in order to obtain an extract enriched in phenolic compounds, an objective framed in the Project European SHEALTHY (https://www.shealthy.eu/project/). For this, a Box-Behnken design of 15 experiments with 3 independent factors has been carried out: frequency (Hz), treatment time (s) and field energy (kv/cm). In each experiment, the phenolic compounds were extracted using ultrasonic technology. The response variables were the content of total phenolic compounds, and the two major compounds, hydroxytyrosol and oleuropein, measured by HPLC-MS. The validity of the experimental design was confirmed by ANOVA and the optimal conditions were established by using the response surface methodology. The optimal conditions by CEP were 0.6 kv/cm at 110 Hz for 11 seconds to obtain the maximum content of total phenolic compounds, which was 24 mg/g of dry leaf. The final data confirmed that the treatment with CEP under these optimal conditions has proven to be an effective pretreatment in improving the extraction of phenolic compounds in olive leaves, probably due to the increase in the permeability of the cell membrane.

OR-212
Suitability of different Electrode Materials for Pulsed Electric Field (PEF) Application

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Pulsed Electric Fields (PEF) is industrially used technology for preservation of liquid products. Due to the direct contact of electrode surface and products, safety and quality concers related to metal particle migration have been raised in case of stainless steel. This resulted in common use of titanium electrodes, recently also regarded as critical by EFSA. In this study suitability of six different electrode materials for PEF application was assessed. The selected materials were: titanium, tantalum, super duplex stainless steel, silicon doped with phosphorus, graphite, and glassy carbon. The main criteria for the selection of the materials were: electrical conductivity, electrical resistance, corrosion resistance, and possible toxicity.
The continuous parallel plate PEF chamber for liquid application with electrode distance of 10 mm was built, designed to be operated in a pilot-scale machine (PEFPilot™ Dual, Elea GmbH, Germany). All electrode pairs were tested for a period of 40 hours (5 cycles of 8 hours), at electric field strength of 15 kV/cm, specific energy input of 120 kJ/kg, and 2 bar backpressure. The pulse form was rectangular with width of bipolar pulses set to 7 µs. The electrode behaviour was tested in a model solution (pH=4, 20L) recirculated continuously at a flow rate of 80 L/h. The model solution was changed between cycles, meaning that each cycle had 32 PEF runs. After each

cycle concentration of eroded material in the solution was determined by inductively coupled plasma – optic emission spectroscopy (ICP-OES), while electrode surface changes were examined by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) after 8 and 40 hours of operation.
The utilization of titanium electrodes resulted in the lowest migration rate of metal into the medium. During the first cycle, around 7.14 μg/L of titanium was released per PEF run. The titanium concentration seemed to increase with increase in electrode operation time, being 17.05 μg/L per PEF run in the fourth PEF cycle. A similar tendency was observed in stainless steel, with release of Fe, Cr and Mo. On contrary, the concentration of silicon was 67.86 μg/L after the first and 55.80 μg/L after the fifth cycle per PEF run. According to the obtained results graphite and tantalum seem unsuitable for PEF application in the tested set-up.
The changes on electrode surface observed under microscope were visible for all tested electrode materials, with exception of glassy carbon after the first cycle. The changes of cathode surface compared to anode surface were more pronounced in case of titanium and stainless steel, where already after 8 hours of operation the surface of electrode was completely altered. The surfaces of both silicon electrodes were altered after 8 hours, while more pronounced changes on the surface of glassy carbon anode were observed when compared to cathode.
The obtained results indicate that electrodes made from silicon doped with phosphorus or glassy carbon could be a possible alternative for titanium electrodes currently used for PEF application in food and other similar areas.

OR-95

Physical and chemical characterization of freeze-dried strawberries and red bell peppers pretreated by pulsed electric fields (PEF)

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Freeze-dried is considered a gentle drying process and it is largely used for the production of foods such as dried soups, dried snacks, breakfast cereals and cereal bars. The freeze-drying process better maintains the physical characteristics and the nutritional and sensory properties of the raw materials compared to hot drying. However, during the freeze-drying, phenomena as collapse and shrinkage are likely to happen in sugar-rich plant materials.
In this study, the effects on chemical and physical properties of pulsed electric fields (PEF) pre-treated freeze-dried strawberries and red bell peppers was investigated. PEF treatments at fixed electric field strength and frequency (E= 1.0 kV/cm and f = 1 Hz) were performed. Variable number of pulses (20, 50, 100 and 200) and thereby energy inputs between 0.3 and 6.0 kJ/kg on fresh products have been applied. The effects on the process efficiency and the quality of freeze-dried fruits and vegetables were evaluated by cell disintegration index, freezing kinetics, freeze-drying kinetics, shrinkage measurement, rehydration capacity, mechanical properties, color determination and chemical analyses (ascorbic acid, polyphenols, antioxidant compounds and anthocyanin content).
Results showed that the PEF treatment positively affected the physical characteristics of the samples better preserving the original shape of the fresh fruits and vegetables. Compared to the untreated samples, a significant reduction of the shrinkage phenomenon for both, bell peppers and strawberries, was detected by a reduction of the volume loss of 30% and 50%, respectively. Pre-treated samples showed a lower firmness and increased rehydration capacity. Moreover, PEF treatment does not appear to have effects on the analyzed nutritional compounds compared to untreated samples.
The improved shrinkage behavior of the PEF treated vegetables could represent an important feature for marketing purposes as, due to the less volume losses, they resulted in more similar to the corresponding fresh products and likely more desirable for customers. Overall, the results of this study suggest that PEF could be an effective pretreatment to improve the freeze-drying process and the final quality of freeze-dried fruits and vegetables.

OR-91

Electrically conductive biocomposite film for in-pack pulsed electric field food sterilization

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Pulsed electric field (PEF) is an emerging non thermal food sterilization technology. The preservation of nutritional and organoleptic food properties turns this technology very attractive to extend the shelf life of minimally processed food. The microbial inactivation is achieved through electroporation, due to the application of high voltage pulses to food during microseconds. Currently, food is sterilized inside a treatment chamber before packaging. However, this process has a risk of recontamination, that is preventing the industrial PEF implementation.[1] The in-pack food sterilization would overcome this obstacle. Nonetheless, suitable electrically conductive food packaging materials are lacking. In this context, electrically conductive biocomposite materials might be a customizable and sustainable solution.[2] Herein, biocomposite films were prepared with alginate/zein biopolymers and carbon-clay electrically conductive fillers. A caramel-sepiolite nanocomposite was graphitized at 550/800 ºC under N2 flow during 1 h.[3] Multiwalled carbon nanotubes were used to improve the electrical conductivity. The fillers were characterized by X-ray Diffraction (XRD), Raman spectroscopy, Solid State 13C Nuclear Magnetic Resonance (NMR), and Scanning Electron Microscopy (SEM). The XRD revealed the maintenance of sepiolite structure during pyrolysis at 550 ºC and Raman and 13C NMR confirmed the conversion of caramel into a graphitic material. The SEM showed a homogeneous dispersion of sepiolite clay and caramel. The filler with the highest electrical conductivity was dispersed into the alginate/zein matrix and the films

obtained by solvent casting. The film containing 70 wt% filler reached a maximum electrical conductivity of 329 and 6 µS/cm in-plane and through-plane directions, respectively. However, the mechanical resistance of films decreased in comparison with the control due to the high loads of carbon-clay filler. The electrically conductive films will be used as food packaging electrodes to validate the in-pack PEF sterilization concept.

Acknowledgements:
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OR-120

Optimization through Response Surface Methodology of Pulsed Electric Fields-Assisted Extraction of bioactive compounds from red grape pomace

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Grape pomace is the major by-product generated during the winemaking process, with a production of 20 kg for each hectoliter of wine. The recovery of high-added value intracellular compounds from grape pomace might represent a strategy to improve the sustainability of wine production and promote economic opportunities. However, in order to weaken the cell membrane, which constitutes a physical barrier that hinders the recovery of these compounds from plant residues via conventional solvent extraction (SLE), an efficient permeabilization of the cell envelopes should be necessary.
Pulsed electric fields (PEF) is gaining great interest as a mild and easily scalable cell disruption technique, enhancing the extractability of intracellular compounds from various plant matrices, with reduced solvent, time, and energy consumption.
However, to maximize the benefits of PEF-assisted extraction over the SLE, an optimization step of the whole PEF-assisted extraction process should be carried out. To date, few studies addressed the optimization of all the factors involved in the extraction process, made of a PEF pre-treatment followed by a subsequent SLE, applied on grape pomace.
Therefore this study was focused on the optimization of the PEF-assisted extraction process using a central composite design for response surface methodology from response surface methodology (RSM) with the aim to sustainably intensify the extractability of phenolic compounds from red grape pomace. The cell disintegration index (Zp) was used as a response variable to identify the optimal PEF pre-treatment conditions of grape pomace in terms of field strength (E = 0.5–5 kV/cm) and energy input (WT = 1–20 kJ/kg), to be applied prior to the subsequent solid-liquid extraction (SLE) process. For both untreated and PEF-treated samples, SLE was optimized to determine the most effective combination of extraction temperature (20–50 °C), extraction time (30–300 min), and solvent concentration (0–100% ethanol in water).
Total phenolic content, flavonoid content, antioxidant activity, anthocyanin content, and tannin content of the red grape pomace extract, were determined. The extracted compounds from untreated and PEF-treated samples at the optimal conditions were analyzed via HPLC-PDA analysis.
Results revealed that under the application of the optimized PEF-assisted extraction conditions the extracts obtained from PEF-treated red grape pomace showed higher TPC (6%), FC (25%), FRAP (12%), DPPH (14%), TAC (54%), and TC (15%), as compared to the control extraction. HPLC analyses revealed that epicatechin was one of the main phenolic compounds extracted, and no degradation phenomena occurred due to PEF application.
These results strengthen the feasibility of the innovative approach investigated in supporting the valorization and potential reutilization of grape processing by-products in the food industry.

P16 - New Technologies for Cells and Tissues Electropermeabilization

Wednesday morning Track A
Oct 12, 10:30 - 12:15

OR-132

(Elongated) gold nanoparticles: injectable antennas locally amplifying the electroporation or just a thorn in our flesh?

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Gold nanoparticles have been described as promising materials, which could locally enhance the electric field via the “lighting rod effect”. While theory predicts that individualized elongated conducting nanoparticles with an aspect ratio (length/width) of about 15.7 could locally enhance the electric field up to 100 times [1], experimental results obtained by our colleagues and us indicate this goal still remains elusive.
In order to master gold nanoparticles behavior around

and within living cells, we have to understand their behavior in space and time. Upon administration to cells, gold nanoparticles quickly undergo aggregation and cellular confinement, hampering nanoparticles potential for electroporation enhancement.
As soon as gold nanoparticles meet proteins, either in cells or within (bodily) fluids, their surface structure and properties rapidly change. Conversely, their gold-based core is rather inert and can persist in vivo over years and decades.
In this work, we present gold nanoparticles potential for electroporation enhancement, focusing on theory and practice, summarizing the current state of the art. In the prospect of exploiting the potential of gold nanoparticles for electropermeabilization enhancement, we herein present gold nanoparticles behavior in vivo over a period of one year [2], and we provide an overview of gold's fate in humans, treated with ionic gold salts [3]. These salts, used in the eighties to treat rheumatoid arthritis, have been shown to locally generate gold nanoparticles and elongated gold nanoparticles microstructures.
While we do not provide the miraculous recipe for gold nanoparticles application in electroporation, we provide a general review of the (lack of) knowledge in the use of gold-based particular and molecular compounds for future studies and potential field development.

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OR-137

Application of a new electroporation microsystem to the study of the impact of tumor microenvironment on electrochemotherapy efficiency

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Pancreatic cancer has a very poor prognosis, with a percentage of survival inferior to 7% at 5 years [1]. Its tumor microenvironment (TME) presents a high density of fibrotic tissues, inducing a low penetration of drugs in cells. Electrochemotherapy (ECT) appears as a promising treatment to enhance anticancer drug efficiency, and more investigations are needed to improve ECT protocols and drug selection. However, there are only a few studies comparing the effect of ECT on relevant 3D cell models such as spheroids produced with several types of cells, including fibroblasts. We believe that the microsystem we recently developed [2], enabling culture, monitoring and electroporation (EPN) of a large number of spheroids sharing similar characteristics will be a key asset to analyze the effect of a dense fibrotic TME on ECT efficiency, using pancreatic cancer as a pathological model.
A 2% agarose hydrogel is molded to form microwells [3] and bonded to an ITO coated glass slide, corresponding to an electrode for EPN. Spheroids are obtained by seeding PANC-1 pancreatic cancer cells with MRC-5 fibroblasts (ratio 2:1) transduced using NucLightTM green lentivirus, supplemented with 0.1 mg/mL of type I collagen. To perform EPN, another electrode is placed 1 mm above the one with the hydrogel (Figure 1c) with tubing for the injection of low conductive EPN buffer (300 µS/cm). This buffer will be supplemented with a standard drug for pancreatic cancer treatment, gemcitabine, for ECT tests. EPN is performed by applying 2 sine wave bursts (10 kHz, 600 V/cm, 5 ms) whose relevance has recently been pointed out [4].
The developed microsystem allows obtaining cohesive co-cultured spheroids after 3 days. As fibroblasts appear in green, it will enable to differentiate them from cancer cells for further immunostaining analysis. First tests have been performed to determine the EPN threshold using propidium iodide (PI, 15 µM) to label electroporated cells and then fluorescein di-acetate to label living cells. They demonstrated an efficient and reversible EPN for 600 V/cm.
We plan to perform ECT test and analyze its effect on the produced co-cultured spheroids thanks global growth follow-up and in-situ proliferation analysis, revealed by confocal microscopy. We will compare these results to those obtained with spheroids made of cancer cells only. It will enable to evaluate the effect of TME on ECT efficiency, which presence will be studied by labeling collagen in the co-cultured spheroids. We also intend to optimize the microsystem design to enable the monitoring of spheroid growth with bio-impedance measurement.

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OR-133

Spatially resolved, high efficiency electrotransfection on a CMOS microelectrode array

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Electroporation faces the same hurdles typically encountered by any intracellular delivery technology: higher transfection efficiencies are often achieved by submitting cells to more aggressive treatments, which in

turn results in lower cell viability. In this work, we take advantage of a CMOS microelectrode array chip (MEA) [1] to perform spatially resolved electroporation of the cells grown on its surface. Due to the limited cellular area affected, electroporation with microelectrodes inherently has low toxicity and a large range of pulse parameters can be modulated without compromising cell viability. Thanks to the ability to test different electroporation conditions on different areas of the MEA, large screening experiments designed to yield optimal electroporation parameters can be performed in parallel. We used this method to define parameters that maximize the delivery efficiency of an mCherry-encoding mRNA in primary fibroblasts while keeping cell toxicity to a minimum. After electroporation with those optimized conditions, expression of the fluorescent protein could be detected after one hour and, six hours after electroporation, successful transfection was observed on almost all the electrodes used to electroporate the cells.
By sequentially introducing different fluorescent molecules or nucleic acids in the extracellular medium, and electroporating cells at different locations on the MEA, different patterns of cell fluorescence could be created. Our results showcase the promising applications that electroporation on MEAs can serve, such as screening of large libraries of molecules (e.g., mRNA delivery in dendritic cells) or creation of engineered tissues on chip for drug screening applications.

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OR-136

The fabrication and operation of a continuous-flow microfluidic device for single-cell electroporation

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The ability to transform cells via the introduction of genetic regulatory elements is now central to nearly every aspect of biomedical research and development. Current therapeutic innovations, such as CAR T-cell therapy, have traditionally relied on viral-mediated gene delivery. Although efficient, viral transformation techniques limit the payload size within the viral coat, may have mutagenesis risks, and have high manufacturing costs. These drawbacks have renewed interest in alternative cell transduction methods such as electroporation. Electroporation is an efficient electro-physical, non-viral approach for the intracellular delivery of exogenous materials. Microscale electroporation is being investigated because it allows lower pulse voltages, better control over the uniformity of electric fields, along with the ability to monitor cell membrane permeabilization in real-time, leading to higher cell viability and electro-Transfection Efficiency (eTE).

Here, we report the fabrication and operation of a flow-through electroporation system, consisting of a microfluidic channel along with coincident pulsing electrodes placed along the channel. As a cell passes between an electrode set, the applied field is controlled by the electrodes bias and spacing, while the pulse length is determined by the cell velocity and electrode width. Two sets of electrodes are used to perform dual-pulse High voltage/Low voltage (HV/LV) electroporation. The HV electrodes are narrow and biased to allow a short permeabilizing pulse while the LV electrodes are wider to enable longer term molecular transport into the permeabilized cells. Voltage is applied as a pulse train at high frequencies in order to avoid solution electrolysis seen with continuous DC pulses.

This system is able to electroporate single cells in a serial fashion at a throughput of >200 cells/sec. HEK293 cells, hydrodynamically focused in the middle of the channel, were successfully transfected by delivering a GFP encoding plasmid DNA via electroporation. The number of pulses experienced by a cell were increased from 22 to 113 pulses by reducing the cell velocity (increasing residence time), showing a linear increase in eTE from 30% to 44% when applying a 40 V (E=216 kV/m) pulse train of 10 us pulses at 10 kHz, 10% duty cycle. Applying a dual pulse scheme of 52 pulses at 40 V (E=216 kV/m) of 10 us at 10 kHz, 10% duty cycle followed by 340 pulses at 12.3 V (E= 66.5 kV/m) of 200 us at 1 kHz, 20% duty cycle, increased the eTE from 36% to 50%. The highest eTE achieved to date was 68 ± 9% with cell viabilities approaching 100% up to 120 hours post-electroporation with voltages less than 40 V. Future work includes the utilization of impedance cytometry electrodes to electrically monitor the degree of cell membrane permeabilization following the application of the electroporation pulses, as well as the use of closely spaced interdigitated electrodes using lower voltages to avoid solution electrolysis.

OR-134

Localized single-cell electroporation using U shaped microstructures in a microfluidic channel

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Localized single-cell electroporation allows non-toxic, controlled and efficient delivery of biomolecules to living cells. To obtain localized electroporation, it is required to apply the electric pulses through miniaturized structures. Since the concentrated electric fields are localized only to the vicinity of the miniaturized structures, the target cell must be attached to the miniaturized structures. This means single cells should be micro-manipulated to these structures by an external force (by optical or magnetic tweezers) or adhered to these structures (limiting to adherent cell lines) for successful localized electroporation. A major barrier to the routine operation of localized electroporation in biological laboratories is the cumbersome manipulation and loading of single cells for drug and gene delivery.

To overcome this limitation, we fabricated a localized single-cell electroporation chip consisting of a scalable hydrodynamic U-shaped micro-post array integrated into a microfluidic channel. In our microfluidics device, the cells are trapped in a microtrap array by a gentle flow, after which target molecules are supplied to the device and electrotransferred to the cells under electric pulses with perfect cell viability. The system provides the ability to monitor the electrotransfer of exogenous biomolecules in real-time. We reveal through numerical simulations that localized electroporation is the mechanism of permeabilization in the microtrap array electroporation device. We demonstrate the simplicity and accuracy of this microtrap technology for electroporation by delivery of both small molecule electrotransfer using propidium iodide and large molecule electrotransfer using plasmid DNA for gene expression, illustrating the potential of this minimally invasive method to be widely used for precise intracellular delivery purposes (from bioprocess engineering to therapeutic applications).

OR-135

Smart, solid-state, nanosecond pulsed power techniques for medical, agro and environmental applications

Guus Pemen

Eindhoven University of Technology, Netherlands

Rapid developments in solid-state pulsed power technology offer opportunities to precisely adjust HV pulse shapes, even on a pulse-by-pulse basis. In this paper we provide an overview of our research on solid-state, repetitive nanosecond pulsed power techniques. We are able to "program", set and adjust HV waveforms even during a pulse cycle. We will review several realized topologies, like our implementation of a solid-state impedance matched Marx generator, and show the capabilities of these realizations to generated flexible and adjustable high-voltage waveshapes.

OR-66

Optimization of Ablation Region and Electrode Positioning in H-FIRE via Machine Learning

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Authors recently demonstrated that the use of Machine Learning (ML), and more specifically Artificial Neural Networks (ANN), is an appropriate way to improve the effectiveness of irreversible electroporation applied to cancer ablation. Indeed, modern ML techniques propose themselves as a smart strategy to identify optimized sets of parameters to be used in medical applications based on irreversible electroporation. Investigations based on empirical trials of the different involved parameters are the rule so far, but they cannot represent a sustainable approach in terms of costs and therapy effectiveness towards extensive clinical applications.

In this abstract, we focus on the so-called high-frequency irreversible electroporation (H-FIRE). The adoption of H-FIRE in oncologic care is more and more frequent due to the reduction of heavy side effects as muscle and nerve contraction.

H-FIRE effectiveness is the result of a complex and long process, involving numerical modeling defining the so-called treatment planning protocol. However, a large margin of uncertainty remains between prediction by treatment planning and the experiments, and the practice and experience of the surgeon is the best guarantee for the safe and effective outcome of the treatment.

As experimental results are already largely available in the current literature, we selected a number of data for the creation of a knowledge base and their preparation to accurately train an ANN with a well-controlled and solid methodology. Specifically, the focus is on two groups of target (output) parameters: 1) expected ablation area, and 2) the expected electrode placement. Then after feature (input) definition and data assumption, two different artificial neuronal network typologies were defined. The first one was tasked to estimate the ablation area given a specific experiment setup. The second one is aimed at determining the needed electrode configuration given a specific experiment setup and it involves the edge-to-edge electrode distance, the electrode application depth, and the electrode diameter as target parameters.

Both the two ANNs were fed with a dataset including 152 experiments ("Ablation ANN") and 192 experiments ("Electrode ANN"), respectively. After algorithm iterations, the residuals of the Ablation ANN are close to zero, thus indicating that the estimated ablation areas are very reliable. Similarly, the residual for the Electrode ANN, which reports the estimation errors in [mm] for the diameter, depth, and edge-to-edge distance of the electrodes, shows that the majority of the residuals concentrates around zero providing a successful and reliable estimation of these parameters.

To conclude, the building up of a complete, high quality and robust knowledge base, combined with an adequate design strategy of ANN, led to the identification of the optimum choice of important H-FIRE parameters, interesting for a rapid optimization of future H-FIRE protocols.

P24 - Calcium Electroporation

Wednesday morning Track B
Oct 12, 10:30 - 12:10

OR-110

Phase II Investigation of the Histopathologic Effect of Calcium Electroporation on Cancer in the Skin – CaEP-B

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Zealand University Hospital, Denmark

Background: Many patients with cancer may present with cutaneous or subcutaneous tumours that can be difficult to manage with surgery, systemic treatments or radi-

ation. Calcium electroporation (EP) is a novel local treatment using intratumoral calcium and pulsed electric fields, with the first clinical trials displaying safety and efficacy as well as limited side effects. Reports of long-term local disease control and systemic responses following treatment in the initial small-cohort trials imply a positive effect on cancer immunity caused by calcium EP-induced cell death. This study investigates the histopathological effect of calcium EP and immune responses of the tumour microenvironment , ClinicalTrials.gov Identifier: NCT04259658.
Materials and methods: This non-randomized phase II study will include 24 patients with cutaneous or subcutaneous malignancy. Tumours are treated once and re-treated after one month with sequential biopsies taken at baseline and day 2, 7, 28, 30, 60 and 90 after initial treatment, depending on the number of included tumours. The primary endpoint is the change in proportion of tumour infiltrating lymphocytes two days after treatment compared to before treatment. The samples will be analysed for immune markers of the innate and adaptive immune system as well as tumour necrosis, changes in vasculature and inflammation. Circulating tumour DNA from blood samples (baseline and day 28, 60, 90) will be analysed in a subgroup of patients . Secondary endpoints include clinical response rate, PD-L1 expression of different tumour histological subtypes and importance of previous irradiation. Patients are followed up to 12 months.
Results: This study is still ongoing. Fourteen patients with metastases of different tumour histology have been treated including breast cancer (n=11), lung cancer (n=1), malignant melanoma (n=1) and bladder cancer (n=1) - the first bladder and lung cancer cases treated with calcium EP. Both formalin-fixated, paraffin-embedded samples (n=83) and frozen biopsies (n=21) have been obtained. Preliminary results of HE-stained samples show examples of tumour necrosis, residual tumour, inflammatory reaction and change in tumour infiltrating lymphocytes, mitosis rate, nuclear size and elastoid degeneration from sequential biopsies. The patient with bladder cancer metastases had progressive disease on immunotherapy, but clinically showed remission when re-treated with immunotherapy after calcium EP treatment.
Conclusion: This is the most comprehensive clinical study to date investigating the effect of calcium electroporation on malignant tumours and their microenvironment. The results of this trial may illuminate mechanisms underlying this promising new treatment for cancer in the skin across different tumour histological subtypes. Understanding changes in the tumour microenvironment could uncover synergistic effects with the immune system and determine whether calcium EP has the potential to turn immunogenic “cold” tumours into “hot” tumours to benefit targeted therapies.

OR-160

Endoscopic calcium electroporation in patients with Barrett’s esophagus high-grade dysplasia: A first-in-man phase 1 study

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Background and aims: Barrett’s esophagus high-grade dysplasia (BE HGD) can transform into esophageal adenocarcinoma (EAC) in 19-28% of the cases. Once resected, a shift in diagnosis from HGD to EAC happens in 21-38%. Calcium electroporation combines a local injection of calcium with electrical pulses to increase the individual cells’ permeability and flux of ions leading to necrosis. This study presents the first results with calcium electroporation in the esophagus.
Methods: Six patients with BE HGD scheduled for an endoscopic submucosal dissection (ESD) were treated with Ca-EP six weeks before the planned ESD. All side effects and adverse events (AEs) were registered, and the patients were later evaluated with gastroscopy.
Results: The observed adverse events included retrosternal pain, throat irritation, coughing, and headache. No serious adverse events were observed. A hyperemic area was observed in four patients after Ca-EP corresponding to treated areas. A fibrinous coating (three patients) and ulcers (four patients) were observed up to a week after treatment. One patient had to undergo two CTA scans after treatment due to pain and a visually large fibrinous clot, which showed no perforation.
Conclusion: Ca-EP is safe and feasible in patients with BE HGD. This study paves the way for more extensive studies to investigate the effect on dysplasia cells and the safety and feasibility of treating esophageal adenocarcinoma.

OR-161

Effectiveness of calcium electroporation on human sensitive and resistant breast cancer cells

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Breast cancer ranks among the top three most common malignant neoplasms in Poland. The use of calcium ion-assisted electroporation is an alternative approach to the classic treatment of this disease. The studies conducted in recent years confirm the effectiveness of electroporation with calcium ions. They are signal transducers in many intracellular pathways, therefore, disturbance of intracellular calcium ion homeostasis can lead the cell to death. Electroporation is a method that uses short electrical pulses that create transitional pores in the cell membrane. They allow the penetration of certain drugs that are not normally able to penetrate the cell membrane.
The aim of this study was to investigate the antitumor effect of electroporation alone and calcium ion-assisted electroporation on human mammary adenocarcinoma cells, sensitive (MCF-7/WT) and resistant to doxorubicin (MCF-7/DOX). Cell viability was assessed using independent tests: MTT and SRB. The consumption of the intracellular ATP pool was tested with the ATP-lite assay. The type of cell death after the applied therapy was determined by TUNEL and flow cytometry (FACS) methods. The expression of α-1G and α-1H proteins was assessed by immunocytochemistry, and changes in the morphology of CaEP-treated cells were visualized using a holo-

tomographic microscope.
The obtained results confirmed the effectiveness of the investigated therapeutic method. The results of the work constitute a good basis for planning research at the in vivo level, and in the future, perhaps, to develop a more effective, safer, and more acceptable method of breast cancer treatment for patients.

OR-162

Endoscopic Calcium Electroporation for Colorectal Cancer: a phase I study

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Background: Colorectal cancer is one of the most common malignancies worldwide, with approximately 20% of the patients having an advanced disease. Local symptoms from the tumor, such as pain, bleeding and stenosis, remains a common issue and affects quality of life. Electroporation is a method to permeabilize cell membranes with high voltage pulses, allowing increased passage of otherwise poorly or non-permeating substances such as calcium (calcium electroporation). Recent studies have shown that calcium electroporation efficiently eliminates cancer cells. Additionally, studies show that cancer cells are vulnerable to the treatment, whereas normal cells are more resistant to calcium electroporation. The aim of this study is to determine safety of calcium electroporation for advanced colorectal cancer.
Method: This exploratory phase 1 study investigated safety of endoscopic calcium electroporation for colorectal cancer. The study included six patients with inoperable rectal and sigmoid colon cancer, all presenting with local symptoms from the primary tumor. Patients were offered endoscopic calcium electroporation and were followed with follow-up endoscopy and CT/MR scans. Additional treatments were performed when appropriate. Secondarily, biopsies and blood samples were collected before and after treatment. Biopsies were examined for histological changes and CD3/CD8 and PD-L1. Additionally, blood samples were examined for circulating cell free DNA.
Results: A total of ten procedures were performed, and no serious adverse events occurred. All treatments were performed as an out-patient procedure, and patients were discharged within one hour after treatment. Prior to inclusion, patients reported local symptoms, such as bleeding, pain, and stenosis. After treatment, five of six patients reported symptom relief within a few days after treatment.
In one patient, calcium electroporation was offered followed by systemic chemotherapy. Complete visual response of primary tumor was seen. Follow-up biopsies after 12 weeks showed granulation tissue and ulcerous tissue and no adenocarcinoma was described. In addition, complete response of lung- and partial response of liver metastasis.
Biopsies were analyzed for changes in CD3/CD8 as well as PD-L1 and no clear trend of the density appeared. Blood sample analysis found no trend towards changes in circulating cell free DNA
Conclusion: This first study on calcium electroporation for colorectal tumors shows that calcium electroporation is a safe and feasible treatment modality in colorectal cancer. The treatment can be performed as an out-patient treatment and may potentially be of great value for the fragile patient with limited treatment options.

OR-163

Impact of variety type of calcium electroporation protocols on selected cellular attributes in human colon cancer

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Electroporation is now commonly accepted as method using wide-range electric field pulses to rapidly increase of transmembrane voltage and thereby rise of cell membrane conductance, which supported molecular transport through cell membranes. According to type of pulses (duration, amplitude, number of pulses) reversible electroporation is divided into: 1) millisecond-, 2) microsecond-, 3) nanosecond-electroporation. The promising approach gives combination of calcium ions with electroporation (CaEP). The aim of our studies was evaluation the effects of different type of PEF pulses combined with Ca2+ on cell structure and homeostasis. We tested different protocols of EP (µs- and nsPEF) +/- Ca2+ and observed its impact on cellular stress response, tumour biomarker expression and viability for colon cancer cell lines.
The LoVo (sensitive human colorectal adenocarcinoma cell line) and LoVoDX (doxorubicin-resistant human colorectal adenocarcinoma cell line) were tested. As control the Hs738st/int (normal human intestine fibroblast) were used. The following CaEP protocols were used: (1) µs: 1.2 kV/cm; (2) ns: 37.5-50 kV/cm; +/- 2nm of Ca2+. The YO-PRO-1 uptake was assessed by flow cytometry. The viability was measured by MTS assay and ROS level analyzed by bioluminescent assay. The expression of aspartate-β-hydroxylase (ASPH) protein and heat shock proteins (HSPs) were visualized using confocal laser scanning microscope (CLSM). The expression of CD133 marker was evaluated by immunocytochemistry.
The YO-PRO-1 uptake depended on protocols parameters. MTS assay shown a viability decrease of malignant cell caused by µs- and nsCaEP. Normal cells were sensitive only to µsCaEP parameters. We obtained the most promising survival ratio between normal and malignant cells for nsCaEP (2 mM of Ca2+ with 50 kV/cm). Moreover, in malignant cells the significant release of ROS after nsCaEP was detected and this was linked with increase of HSP 27 and HSP 70 expression. ASPH signal was clearly observed in untreated LoVoDX and LoVo cells what is correlated with the aggressive tumorigenesis. The ASPH signal decreased after each of CaEP parameters. The opposite effect occurred in Hs378st.int cells which

presented a low level of ASPH in untreated cells and it was higher after the nsCaEP application. Expression of CD133 is different between normal and malignant cells as well as is modulated by CaEP. The obtained results confirmed that nsCaEP originates significant reduction of colorectal cells viability, increases ROS/HSPs and reduces ASPH signal that possibly is associated with cell overloaded by calcium influx and cell death.

Financing: This work was supported by the Polish National Science Centre project SONATA BIS 6 (2016/22/E/NZ5/00671, PI: J. Kulbacka).

OR-164

Antitumor effects of nanosecond PEFs with calcium ions in colon cancer in vitro and in vivo

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Background. Colon cancers are still poorly diagnosed; thus, their therapy does not have satisfactory outcomes. The main problem is the primary and acquired resistance, characteristic of these types of cancers [1]. As chemotherapy is not as effective alone, pulsed electric fields (PEFs) seem to support conventional anticancer methods. Despite enhanced drug delivery, varied parameters of PEF such as duration and number of pulses and the intensity of electric field can be responsible for regulating specific cellular responses [2]. NsPEF may induce oxidative stress in cells by stimulating ROS production and disrupting the balance of oxygenases, and antioxidant enzymes, which in turn cause cell damage with increased oxidative markers in membrane lipid peroxidation [3].
Material and methods. In this study, we have used cancer cell lines (LoVo, LoVoDX, MC38/D1) and murine models. For the electroporation process, 200 pulses of 10ns and high voltage (12.5 – 50 kV/cm) were used. In the case of in vivo experiments, 400 and 1200 pulses were applied. The efficacy of nsPEFs protocols with and w/o calcium ions was determined by clonogenic and MTT assay. The cell response was validated by analyzing morphology by SEM, AFM, and confocal microscopy. Proteasomal activity, GSH/GSSG assay, and ROS production were evaluated as markers of oxidative stress and protein damage. Finally, an animal model (C57BL/6 mice) was used in the study. NsPEF with CaCl2 or bleomycin as an ECT standard was performed. The observations were carried up for four weeks.
Results. Our results revealed that PEFs protocols significantly reduced cell viability, particularly with calcium ions. We have observed an increase in oxidative stress markers in colon cancer cells. NsPEF application in colon cancer in vivo, caused tumor size to decrease and stimulated an increase in heat shock proteins (HSP27, HSP40, and HSP70) expression. Moreover, ns pulses stimulated the growth of CD45+ cells in tumor tissue. Conclusions. The application of nsPEF with calcium ions seems to be an auspicious method for efficacious cancer elimination and significantly facilitates the action of conventional cytotoxic pharmacological agents.
Financing: This work was supported by the Polish National Science Centre project SONATA BIS 6 (2016/22/E/NZ5/00671).

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P30 - Cancer Immunotherapy and Pulsed Electric Fields (PEF)

Wednesday morning Track C
Oct 12, 10:30 - 12:10

OR-144

Local intratumoral electroporation delivery of potent anti-cancer interleukin 12 immunotherapy leads to systemic anti-cancer response

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Systemic administration of potent anti-tumor immunotherapies is invariably associated with undesirable rates of treatment-related adverse events. Optimizing efficacy of treatment approaches while reducing severity of adverse events has become the primary focus in development of novel anti-cancer therapies and combination treatment approaches. Intratumoral delivery of expression plasmids by electroporation (EP) represents a promising local treatment approach to reduce toxicities observed with systemic treatments. OncoSec Medical System (OMS) pairs a generator and an applicator to induce localized expression of recombinant proteins by EP in palpable tumor lesions. Specifically, intratumoral delivery of interleukin 12 (IL-12) tavokinogene telseplasmid (tavo) has demonstrated clinical utility to safely treat cancer patients across solid tumor disease states. IL-12 is a pleiotropic cytokine that has been extensively studied as a potential cancer immunotherapy candidate due to its ability to engage multiple immune effectors and reverse tumor-induced immunosuppression. However, systemic administration of IL-12 has proven to be exceedingly toxic in clinical trials, ultimately limiting its clinical utility. Intratumoral delivery of tavo via OMS EP resulted in local and systemic anti-tumor effects while inducing only minimal treatment-related adverse events in multiple clinical trials. Here, we summar-

ize findings from two of our trials designed to evaluate a novel anti-tumor treatment combination of pembrolizumab (immune checkpoint inhibitor) and intratumoral tavo-EP.KEYNOTE-695 study is a single-arm, phase 2, open-label, multicenter study of tavo-EP plus pembrolizumab in patients with unresectable or metastatic melanoma progressing on standard of care immune checkpoint inhibitor(s). Patients had a 27.8% objective response rate (ORR, n=54), with 47% of responding patients having durable response lasting over 1 year. Median overall survival (OS) was 23.5 months (n=56). Grade 3 treatment-related adverse events (TRAEs) were reported for 6.7% patients. No grade 4 or 5 TRAEs were reported.
KEYNOTE-890 study cohort 1 is a phase 2, open-label, multicenter study assessed the safety and efficacy of tavo-EP in combination with pembrolizumab as 2L+ treatment for advanced triple negative breast cancer (TNBC). Patients had a 17.4% ORR (n=26), with a median duration of response of 16.6 months. Median OS was 11 months (n=26). Grade 3 TRAEs were reported for 23.1% patients. No grade 4 or 5 TRAEs were reported.
In summary, EP delivery of tavo allows for safe administration of IL-12 treatment in patients with solid tumors. Findings from OncoSec clinical trials support continued development of an electroporation-based delivery of potent medicines as a promising approach to local delivery of treatment with systemic effects while minimizing the severe toxicities invariably associated with systemic immunotherapy.

OR-145

Nano-Pulse Stimulation™ (NPS™) in combination with the TLR7/8 immune adjuvant resiquimod eliminates murine Pan02 pancreatic tumors and inhibits the growth of rechallenge tumors

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Pancreatic cancer is associated with an extremely poor prognosis and immunotherapy alone has not demonstrated sufficient efficacy in the treatment of nonresectable tumors. However, when a treatment modality capable of inducing tumor cell death is given in combination with an immunotherapy, the two treatments may synergize, significantly increasing the response rate. Nano-Pulse Stimulation™ is a bioelectric modality that initiates a cascade of events within cells that leads to regulated cell death (RCD), exposing tumor antigen to the immune system. We conducted studies to determine if NPS™, with or without the addition of the immune adjuvant resiquimod, could eliminate a murine Pan02 pancreatic tumor, while also preventing the growth of a Pan02 rechallenge by inducing a CD8+ T memory cell response. After tumor antigen is released and taken up by dendritic cells (DCs), resiquimod further promotes adaptive immune recognition by binding to TLR7 and TLR8 on DCs, stimulating the production of type 1 IFNs and helping to prime and expand the population of tumor-specific CD8+ T cells. We found that using a medium dose of NPS™ (180 mJ/mm3) in combination with resiquimod (50ug; i.t.) was the optimal treatment regimen for both eliminating a primary Pan02 tumor (93%) as well as inhibiting growth of a Pan02 cell rechallenge tumor (60% TGI). In addition, when we depleted the CD8+ T cells with an anti-CD8 antibody delivered in vivo before rechallenge, it reduced inhibition of rechallenge tumor growth by 35% (NPS™ (180 mJ/mm3) + resiquimod + aCD8ab = 25% TGI). After assessing efficacy, we analyzed rechallenge tumors for the presence of tumor infiltrating CD8+ T cells using immunohistochemistry. We found that rechallenge tumors in mice that received the NPS™ (180 mJ/mm3) + resiquimod treatment had, on average, 6X (avg.=219) the number of CD8+ T cells than in tumors rechallenged after surgical resection of the primary tumor (avg.=27) and 3X more than in tumors whose efficacy was depleted using an anti-CD8 antibody (avg.=67). This number was also inversely correlated with tumor size, suggesting that the infiltrating CD8+ T cells were responsible for inhibiting tumor growth. Overall, NPS™ (180 mJ/mm3) + resiquimod was the treatment condition most effective at eliminating a primary tumor and inhibiting a rechallenge tumor. This observed effect was likely due to CD8+ T cell priming events that resulted from the synergy between NPS-induced regulated cell death and the immune adjuvant properties of resiquimod. We plan to continue to optimize our treatment approach by exploring the use of different adjuvants in combination with NPS™ treatment energies to maximize efficacy. Our hope is that the NPS™ may one day become a novel candidate for the treatment of pancreatic and other hard to treat cancers.

OR-143

Delivery of Immune Modulators Using Gene Electrotranser to Induce a Robust Immune Response Against Solid Tumors

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Immunotherapy has the potential to be an effective therapeutic approach for cancer. The utilization of immune checkpoint inhibitors (ICIs) has enhanced the anti-tumor effectiveness of immunotherapy approaches. While encouraging, the therapy is not without shortcomings. The tumors of many patients are initially refractory to checkpoint inhibitor therapy or acquire resistance. Effectiveness of ICIs may be related to the amount of CD3 and CD8 cells within the tumor or tumor periphery. Tumors can be categorized as cold (low checkpoint expression and minimal immune cell infiltrate), altered (excluded (lymphocytes at the margin not infiltrating) or immunosuppressed (low level of infiltrate but a suppressive environment) and hot (significant infiltrate and not a suppressive environment. A cold or altered tumor would be resistant to ICI therapy. One approach to overcoming resistance is to modify the tumor microenvironment (TME) from cold or altered state to hot in order to enhance the efficacy of ICI therapy. We have been evaluating a gene-based approach to modify the TME to increase T-cell infiltration and change from a suppressor environment to a

pro-inflammatory environment. To accomplish this, we have used gene electrotransfer (GET) to deliver proinflammatory cytokines directly at the tumor site. Both interleukin-15 and interleukin-12 have been effective in modifying the TME and inducing a robust immune response. Utilizing two tumor models B16.F10 melanoma model in C57Bl/6 mice and 4T1 breast cancer model in BalbC mice, we observed a significant reduction in T regulatory cells and myeloid derived suppressor cells while T effector cells were significantly increased within the TME following intratumor delivery. This approach also resulted in prolonged disease-free survival as well as long term immune memory in a single tumor model. We further tested this approach in combination with anti-PD-1 using a two-tumor model consisting of a subcutaneous B16F10 tumor and B16F10 cells expressing luciferase injected via the intraperitoneal route in a C57Bl/6 mouse. Intratumor delivery of pIL-12 GET as a monotherapy resulted in reduction or elimination of the subcutaneous tumor. However, the monotherapy approach was only successful in reducing the peritoneal spread in about 50% of the mice. When pIL-12 GET was combined with anti-PD1 administered via an intraperitoneal injection not only was there an elimination of the subcutaneous tumor, but it resulted in the elimination of intraperitoneal metastatic growth. In both tumor models, upregulation of MHC class 1 and PDL1 expression was observed. The results have this study suggest that the combination of delivering plasmids encoding cytokines combined with ICIs could be utilized as an effective combination therapy for solid tumors.

OR-141

Electroporation Efficacy in breast cancer cell line co-cultured with T lymphocytes

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3D scaffolds composed of hyaluronic acid and ionic-complementary self-assembling peptides can allow heterogeneous cell-cell contact and the appropriate support of spatially organized extracellular matrix, thus evoking more clinically relevant experimental conditions for growing cancer cells, as compared to conventional cultures. These 3D cultures may therefore represent a valuable system to study the anticancer effect of Electroporation (EP) protocols, with translational potential.

Our working hypothesis is that EP of cancer cells growing in 3D scaffolds may elicit increased antigen exposure, in turn improving T lymphocyte migration and targeting of cancer cells for their elimination.

To this aim, we cultured breast carcinoma cells (HCC1954) with T cells (Jurkat) in 3D scaffolds, to partially recreate a tumor microenvironment. Two different EP conditions (600 V/CM and 1000 V/cm), commonly employed in electro-chemotherapy protocols, were applied, followed by the addition of PHA-M- or mock-stimulated T cells, to test whether the potential adjuvant effect of EP could be modulated by T lymphocyte activation.

Our results demonstrated that EP alone and EP of co-cultures of HCC1954 cells with resting T cells affected significantly number and size of cancer cell-containing 3D structures. This reduction in 3D cancer cell spheroids paralleled the T-lymphocyte infiltration and the induction of cell death as evidenced by PI staining. This effect was overall, largely improved when PHA-M activated T cells were added. These results prove that EP may exert anticancer effects by increasing the cell killing by activated T-lymphocytes. We suggest this may be facilitated by EP-mediated antigen exposure on cancer cells.

Further experiments to evaluate Cancer Stem Cells markers in such experimental setting are on-going and the results will be presented and discussed.

OR-142

Protein sampling with electroporation facilitates profiling of spatial differential protein expression in breast tumors in vivo

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Excision tissue biopsy, while central to cancer treatment and precision medicine, presents risks to the patient and does not provide a sufficiently broad and faithful representation of the heterogeneity of solid tumors. Here we introduce e-biopsy – a novel concept for molecular profiling of solid tumors using molecular sampling with electroporation. As e-biopsy provides access to the molecular composition of a solid tumor by permeabilization of cell membrane, it facilitates tumor diagnostics without tissue resection. Furthermore, thanks to its non tissue destructive characteristics, e-biopsy enables probing the solid tumor multiple times in several distinct locations in the same procedure, thereby enabling the spatial profiling of tumor molecular heterogeneity.We demonstrate e-biopsy in vivo, using the 4T1 breast cancer model in mice to assess its performance, as well as the inferred spatial differential protein expression. In particular, we show that proteomic profiles obtained via e-biopsy in vivo distinguish the tumors from healthy breast tissue and reflect spatial tumor differential protein expression. E-biopsy provides a completely new molecular sampling modality for solid tumors molecular cartography, providing information that potentially enables more rapid and sensitive detection, at lesser risk, as well as more precise personalized medicine.

OR-140

Experimental and theoretical Brownian Dynamics analysis of Ion transport during cellular electroporation of E. coli bacteria

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The motion of ions through pores formed in the inner and outer plasma membranes of Escherichia coli cells during electroporation is simulated in 3-D space using a Brownian dynamics model, which is mostly deterministic following Newtonian mechanics, but has some stochastic properties to account for elastic ionic scattering in water. The pore's conductance, diffusion coefficient, mobility and translation time of Ca2+, Mg2+, Na+, K+ and Cl- ions are estimated from the numerical model and validated with experiments conducted at the Gustave Roussy. The results from this work provide a better understanding of the electroporation process, aiding in the design of electrical pulses and waveforms for maximizing the throughput of DNA, drugs and gene materials into cells, primarily for application in cancer treatment.

P7 - Pulsed Electric Fields (PEF) for Recovery of Components from Microorganisms

Wednesday morning Track D
Oct 12, 10:30 - 12:10

OR-63

Combination of plasma-activated water with non-lethal pulsed electric field on bacteria inactivation

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Pulsed electric fields (PEF) and Plasma-activated water (PAW) are known for their antibacterial effects. PEF effects are usually present in the plasma discharges applied in biomedicine. Our mild PEF treatment of 200 ns, 12.5 kV/cm, at 100 Hz applied for 100s on E. coli in planktonic form did not result in bacteria inactivation. Two different types of Plasma-activated water made by a transient spark in atmospheric air were prepared. The first type of PAW prepared in a closed-air reactor is rich in NO2- and poor in H2O2, while the second one prepared in an open-air environment is richer in H2O2. NO2- and H2O2 in PAW react together to form peroxynitrites (ONOO-/ONOOH), which further degrade into OH and NO2 radicals.

We tested the antibacterial effects of PAW, PEF, and their coupled effect applied to the decontamination of E. coli in water, PAW, and PAW + additional H2O2. The reinforcement in H2O2 is to simulate the production of radicals through peroxynitrite chemistry. The radicals are known to damage the cell membrane and induce phospholipid peroxidation fragilizing the membranes to the PEF treatment. We observed an antibacterial synergy of PAW with the PEF treatment only in presence of both H2O2 and NO2- in the PAW.

The TBARS method which measures the membrane lipid peroxidation showed a significantly stronger peroxidation of the lipids in PAW rich in H2O2 and NO2-.

Furthermore, we analyzed Giant unilamellar vesicles (GUV) which are a simple spheroid membrane model composed of lipid bilayer only. Observed GUV degradation consecutive to PAW, PEF, and PAW+PEF treatments indicated potential bacterial membrane damage by lipid degradation. We observed a strong degradation of the GUV for PAW+H2O2+PEF by optical microscopy. The lipid peroxidation induced by PAW chemistry seems to be an important parameter of bacteria inactivation by plasma and plasma-activated water combined with a pulsed electric field.

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OR-64

Continuous Extraction of Proteins from Microbial Cells by Pulsed Electric Fields

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In food and bio technology, proteins are increasingly produced in microbiological production systems, by means of fermentation. In conventional bioprocesses, downstream processing is usually initiated by cell disintegration, using high-pressure homogenization. This step is followed by extensive purification steps due to the resulting high loads of host cell impurities.

In order to reduce impurities of the resulting protein solutions, pulsed electric field (PEF) treatment and the resulting electroporation was investigated for permeabilization of cell membranes and the selective release of target proteins from E. coli. For this purpose, continuous electroporation was employed to selectively extract recombinant Protein A from the periplasm of E. coli. For this purpose, a specifically designed flow-through PEF treatment chamber was deployed, operated at 1.5 kg/h, using rectangular pulses of 3 ms at specific energy input levels between 10.3 and 241.9 kJ/kg. Energy input was controlled by variation of the electric field strength (28.4–44.8 kV/cm) and pulse repetition frequency (50–1000 Hz). The effects of the process parameters on cell viability, product release, and host cell protein (HCP), DNA, as well as endotoxin (ET) loads were investigated.

It was found that a maximum product release of 89 % was achieved with increasing energy input levels. Cell death also gradually increased, with a maximum inactivation of -0.9 log at 241.9 kJ/kg. The conditions resulting in high release efficiencies while keeping impurities low were electric field strengths 30 kV/cm and frequencies 825 Hz. In comparison with high-pressure homogenization, PEF treatment resulted in 40 % less HCP load, 96 % less DNA load, and 43 % less ET load.

Therefore, PEF treatment can be an efficient alternative to the cell disintegration processes commonly used in downstream processing. Ultimately, PEF can contribute

to design continuous or circular, more efficient bioprocesses for future applications in food and bio technology.

OR-65

Sequential extraction of different compounds of interest from yeast biomass assisted by Pulsed Electric Fields

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Yeast biomass generated during alcoholic fermentation represents the second most important by-product of oenological and brewing industries. Although yeast biomass is a valuable product, it has traditionally been used for applications that do not add economical value or are discarded, which represents an environmental problem due to the high biological and chemical oxygen demand. Biomolecules with different applications in the food, pharmaceutical and cosmetic industries can be obtained from the yeast cytoplasm (protein, nucleic acids, glutathione, polyphenols) and from their envelopes (mannoproteins, β-glucans).

The objective of this study was to evaluate a strategy based on the application of Pulsed Electric Fields (PEF) for the development of a cascade process, which allows obtaining a spectrum of valuable products from yeast biomass.

Biomass of Saccharomyces cerevisiae 3D Viniferm was treated by PEF at 15 kV/cm for 50 µs (26.0 kJ/kg), 150 µs (96.8 kJ/kg) and 200 µs (129.5 kJ/kg) to electroporate 20, 85 and 99% of the population. After 24 hours of incubation at 25 ºC, the concentration of extracted intracellular compounds (proteins, amino acids, polyphenols and glutathione) and the antioxidant capacity of the supernatant were determined. The cells were incubated again for obtaining mannoproteins and β-glucans due to the cell wall autolysis triggered by the PEF treatment.

After 24 h incubation, hardly any extraction of cytoplasmic compounds was detected in the untreated cells (5-19%). However, the electroporation caused by the PEF treatment facilitated the release of intracellular compounds. Between 33 to 100% of the total content of the analysed compounds were extracted. Significant differences in the amount of extracted compounds were not detected between the two most intense PEF treatments applied. PEF-induced autolysis resulted in a mannoprotein concentration in the supernatant of the three PEF-treated suspensions approximately 8 times higher than in untreated cells. After the separation of the mannoprotein-rich extract, an insoluble fraction with a concentration in β-glucans ranging from 269 to 300 mg/g of dry extract was obtained from the PEF-treated yeast cells.

The results obtained in this research have shown that the electroporation of yeast cells caused by the application of PEF technology could be a useful tool in the development of an efficient, economical and sustainable method for the valorisation of yeast used in the winemaking and brewing process.

OR-148

The effect of nanosecond pulsed electric field on the production of metabolites from lactic acid bacteria

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Watermelon juice is sensitive to heat, oxygen, and light which makes it difficult to preserve using processing operations that may degrade its functional ingredients and generate unpleasant flavors. However, through an optimized fermentation process, watermelon juice can be preserved at the same time as the nutritional value is improved. In this study, nanosecond pulsed electric field (nsPEF) was applied to the probiotic strain Lactobacillus plantarum DSM 9843 used to ferment watermelon juice. The object of this research is improving efficiency of the fermentation process by nsPEF-induced stress.

Fermentation of watermelon juice with L. plantarum DSM 9843 provoked a pH reduction from pH 5.6 to pH 3.8 and the production of 2.34 g/L D-lactic acid, 1.88 g/L L-lactic acid and 0.075 g/L acetic acid after 20 h incubation without nsPEF treatment. nsPEF treatment was performed during the logarithmic growth phase and the nsPEF conditions (repetition frequency, voltages and total number of pulses) were changed. After the frequency increased from 1 Hz to 50 Hz, an increase of 9.4% D-lactic acid and 7.5?etic acid were observed. It is noteworthy that nsPEF treatment (5.0 kV, 700 pulses) resulted in 19% increase ($p< 0.01$) in L-lactic acid compared to the control. Significant increases in D-lactic acid (6.8%, $p< 0.05$) and acetic acid (15%, $p< 0.01$) were also observed after nsPEF treatment (D-lactic acid: 4.5 kV, 700 pulses and acetic acid:4.5 kV, 1000 pulses) compared to the control. The nsPEF treatment did not affect the viability of the cells and sufficient numbers remained in the product after fermentation.

These results suggest that the metabolism of lactic acid bacteria was affected by the nsPEF treatment. This research demonstrates the potential of this novel electrotechnology for the increased efficiency of fermentation processes.

OR-159

PEF-Processing of Microbial Biomass at KIT-IHM

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This contribution will give a brief overview of current R&D activities at KIT-IHM:

In the past, PEF-processing was shown to allow recovery of multiple cell components from microalgae by cascade processing. In collaboration with European partners, cascade processing of Scenedesmus was implemented for the production of biostimulants and biofertilizer for agricultural applications and lipids as an aquafeed additive.

At lab- and pilot-scale, lipid extraction from microalgae is under focus. For lipid extraction from microalgae it is advantageous to include an incubation step after PEF treat-

ment. This allows to reduce the treatment energy from 1.5 MJ/kgDW to 0.25 MJ/kgDW. Despite this reduction in treatment energy, economic efficiency is dominated by the solvent extraction step. A techno-economic analysis of PEF-assisted lipid recovery pathways revealed, that the major part of energy expenses is caused by solvent recycling and not by biomass processing. First results indicate that the application of biphasic solvent systems, which use a minimum content of azeotropic mixtures of water and Ethanol, are advantageous, even at slightly reduced lipid yields.
Compared to lab-scale batch cultivation, continuous large-scale open pond cultivation of microalgae results in elevated conductivity values of the microalgae suspension after harvest. Energy efficiency of PEF-treatment depends on suspension conductivity. To overcome this general hurdle in PEF-assisted microalgae processing, a new approach for microalgae pretreatment by pulsed microwave treatment (PM) was tested. A cavity-based applicator was manufactured and tested. Lipid extraction efficiency was well comparable to PEF-processing.
Heterotrophic cultivation of microbial biomass offers advantages over autotrophic cultivation of microalgae, i.e. higher biomass density and local independence from sunlight. In particular, oleaginous yeasts can utilize residues from organic industries, such as glycerol or molasses, as a carbon source, producing considerable lipid contents. For component recovery, yeasts also require a cell disruption step prior to lipid extraction. First results indicate that PEF-assisted lipid extraction allows complete lipid recovery from oleaginous yeasts.

P18 - Electroporation and cellular processes

Wednesday afternoon Track A
Oct 12, 14:00 - 15:00

OR-149

Urine protects urothelial cells against nanosecond pulsed electric fields damage

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Recent advances in understanding bioeffects of nanosecond pulsed electric fields (nsPEF) suggest that they could be safer than longer pulses and more efficient in causing a cytotoxic effect in tumor cells. For urothelial cancer treatment, nsPEF might minimize the neuro-muscular excitation while preserving the benefit of complete tumor ablation.
Permeabilization of cells results in the loss of intracellular K+ and ATP and the influx of extracellular Na+ and Ca2+. Since ablation efficiency depends on the tumor environment, and the bladder is constantly exposed to urine, we evaluated the effect of urine presence at the ablation site.
We prepared artificial urine (AU) with compounds commonly present in the healthy human urine at physiological concentrations. Parameters known to influence the therapy outcome, such as the concentration of Ca2+, conductivity, and pH, were the same in all tested solutions.
Electric field thresholds for complete ablation were measured in monolayers of cancer and healthy urothelial cells. The monolayers were treated by electric pulses using two needle electrodes placed orthogonal to the monolayer and generating the electric field, which gradually decreased with distance from the electrodes. The region of cell death was measured by staining with propidium iodide added to the cell monolayer at 2 hours after the nsPEF treatment. Cell death thresholds were determined by matching the stained areas to the simulated electric field intensity. Our study compared the cell death thresholds for nsPEF exposures in a standard physiological solution and the AU. We also conducted a separate confocal microscopy study to explore the impact of AU on membrane permeabilization.
AU had a significant protective effect, increasing the cell death threshold 1.4 times for healthy urothelial cells and 1.8 times for cancer cells. We proposed and investigated several explanations for this effect, including the high content of urea resulting in higher osmolarity and the higher concentrations of Mg2+ and K+. Omitting urea from AU, thus decreasing its osmolarity, resulted in a decline in cell killing threshold for healthy urothelial cells almost to the level achieved in physiological solution, but for cancer cells the threshold remained 1.6 fold higher. We also found that the higher concentration of Mg2+ in AU was not responsible for increased cancer cell survival. The impact of other AU components is under investigation.
Novel results of our study may support future clinical applications of nsPEF for bladder cancer ablation.

Support: This research has been made possible by the Kosciuszko Foundation.

OR-13

Synergistic Gene Electrotransfer and 3D Bioprinted Implants for Improving Biomanufactured Implant Biological Integration for Enhancing Musculoskeletal Tissue Regeneration

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In vivo gene therapy approaches and biomanufacturing, or 3D bioprinting, have independently shown promise in regenerative medicine, yet their synergistic potentials have not been well explored. Musculoskeletal (MSK) tissue injuries—from tendon tears, volumetric muscle loss, and myotendinous junction injuries—are commonplace and often lead to permanent disability and deformation. While there are current clinical regenerative treatments

for many MSK injuries, they have relatively long recovery times and often do not restore native function. To prospectively progress MSK tissue regeneration, we explore combining gene electrotransfer (GET) with novel fibrous 3D bioprinted collagen microfibers to promote advanced healing and tissue integration. First, GET parameters for delivery to the skin were optimized with monophasic and biphasic pulses with reporter and effector genes towards optimizing underlying MSK tissue healing. Tissue twitching and damage, as well as gene expression and distribution, were evaluated, quantified, and optimized. Bioprinted collagen microfiber constructs, mimicking healthy tendon structures, were then implanted subcutaneously for biocompatibility and angiogenesis analyses as implants alone or combined with GET for human fibroblast growth factor-2 (FGF2). GET of FGF2 significantly increased angiogenesis and histological biocompatibility of the bioprinted implants when compared to bioprinted construct implant-only sites. The combination of biomanufactured collagen-microfiber-based implants and angiogenic GET therapy may together lead to better graft biocompatibility and prospectively tissue regeneration in MSK repair.

OR-151

Combinatorial treatment with nsPEF and antibiotics increases Methicillin-Resistant Staphylococcus aureus inactivation

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Staphylococcus aureus (S. aureus) is a virulent bacterium that is one of the primary causes of hospital and community-acquired skin and soft tissue infections (SSTIs). S. aureus has become antibiotic-resistant over time, leading to the evolution of Methicillin-Resistant S. aureus (MRSA). The increasing prevalence of MRSA has put further strain on medical centers, and patients infected with this type of bacteria have worse outcomes.
In this study, we are investigating the potential synergistic effects of dual treatment with nanosecond pulsed electric fields (nsPEF) and antibiotics. While nsPEF has been extensively used to treat eukaryotic cells, the effects of these shorter pulses on bacteria have been less investigated. MRSA planktonic cells in exponential growth phase were exposed to increasing number of either 300 or 600 ns pulses (3 MV/m, 1 Hz) in Luria Broth in electroporation cuvette at room temperature. The highest pulse dose tested, namely 120 pulses of 600 ns duration, caused 0.5 log10 inactivation. While these nsPEF doses were not enough to completely inactivate MRSA planktonic cells, they undoubtedly caused a reasonable level of damage. We anticipated that the perturbation created by nsPEF would increase antibiotics efficacy. To test this hypothesis, we used two antibiotics approved to treat SSTIs: doxycycline, an inhibitor of bacterial protein synthesis, and daptomycin, a lipophilic peptide that intercalates into the cell membrane causing membrane destabilization, ion flux, and membrane depolarization. In our experiments, we compared the cytotoxic effects of these antibiotics administered either before or after nsPEF. While doxycycline caused 2 log inactivation only when cells were pretreated with nsPEF, combining nsPEF with daptomycin greatly potentiated the effects of each monotherapy regardless of the treatment order. Altogether our results show that nsPEF impacts MRSA viability, potentiates the effect of specific classes of antibiotics and that the order of the combined treatment (antibiotic/nsPEF or nsPEF/antibiotics) can have major impact on the cotreatment efficacy. Results from ongoing experiments looking at the effects of other antibiotics (vancomycin), investigating the efficacy of the cotreatment on MRSA biofilms, and in vivo treatments of SSTIs will also be discussed.

OR-165

Calcium Oscillations And Mesenchymal Stem Cells Fate: Characterization and Control Through Electroporation

Leslie Vallet, Franck M. André, Marina M. Sanchez Petidier, Romain Fernandes, Lluis M. Mir

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Mesenchymal Stem Cells (MSCs) are adult stem cells whose multipotency was initially described to cover various cell types constituting connective tissues, such as osteoblasts, adipocytes, or chondrocytes [1]. Nevertheless, under specific conditions, more recent studies shed light on extended differentiation abilities to other types of specialized cells such as muscle [2] or neuron-like cells [3]. Not only due to this promising multipotency, but also to interesting secretory activities [4] as well as rescuing functions exerted towards damaged cells within the body [5], these cells have attracted more and more interest in the context of regenerative therapies these last decades. In another respect, it was observed that MSCs naturally display spontaneous calcium (Ca2+) oscillations whose frequency can vary over the course of differentiation or proliferation processes. Ca2+ is known to be an important cellular second messenger, often signaling in an oscillatory fashion. Frequency and/or amplitude of these Ca2+ oscillations can embed important information subsequently decoded by some proteins in the cell whose activity is Ca2+-sensitive [6]. This signaling mechanism allow for specific stimuli to modulate precise cellular response [6]. More precisely, in this work, we focused first on the changes in Ca2+ oscillations triggered by stimuli involved in cellular proliferation or differentiation events. In a second part, we aimed to assess whether controlling Ca2+ oscillations frequency and/or amplitude might mimic or potentiate the effects of these stimuli. To this end, our laboratory has developed a technique to homogeneously control Ca2+ oscillations within a cell population by physical means. This technique implies the use of microsecond pulsed electric fields (µsPEFs) capable of generating slight permeabilization of the cell membrane to the Ca2+ present in the surrounding medium. This subsequently triggers Ca2+ oscillations similar to the

natural ones (in shape, amplitude and duration) due to the Ca2+ induced - Ca2+ release response [7]. This work carries both fundamental and applicated aspects: on the one side, to assess the role and the importance of the Ca2+ oscillations in various cellular processes in MSCs, and on the other side, to develop a suitable device to control them on the long-term, in the perspective of therapeutic applications.

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P23 - Irreversible Electroporation (IRE)

Wednesday afternoon Track B
Oct 12, 14:00 - 15:00

OR-189

Does imaging response after irreversible electroporation correlate with survival in localized pancreatic cancer?

Rasmus Virenfeldt Flak, Rune Vincents Fisker, Niels Henrik Bruun, Mogens Tornby Stender, Louise Stenholt, Ole Thorlacius-Ussing, Lars Jelstrup Petersen
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Introduction: Irreversible electroporation (IRE) and other local ablative therapies are being increasingly studied for the treatment of pancreatic cancer (PC), when surgical resection is not possible. Most published studies use imaging outcomes as the primary endpoint for evaluating efficacy. However, there are no consensus on how to evaluate the response and the commonly applied techniques have not been scientifically validated for this use. The aims of the current study were to examine the use of response evaluation in this setting, to identify the knowledge-gaps in the published literature and to test the validity of the most used methods in a cohort of PC patients.
Methods: First, a systematic literature search was performed in PubMed. Studies reporting comparative imaging outcomes in ablation-treated locally advanced PC were included. Studies were excluded if the outcomes could not be differentiated between different disease stages, histology or surgical approaches. Secondly, PET/CT-scans acquired from a prospective cohort of localized PC patients treated with IRE, were reexamined by two nuclear medicine and radiology experts. Semiautomated registration of tumor size, 18-FDG-standard uptake values (SUV), metabolic tumor volume (MTV), total lesion glycolysis (TLG) and remote metastases were performed. Comparative imaging outcomes on the lesion- and patient-level were tested for correlated with mortality rate (MR) by Poisson regressions with the Huber/White sandwich estimator.
Results: Of the 34 studies included in the systematic review, 14 used standardized response criteria, 14 used self-determined criteria or absolute size comparison and six did not report the response evaluation method. One study statistically tested CT imaging outcomes with survival using a self-determined categorization and found a significant correlation. Data from the clinical trial revealed a significant correlation between patient-level progressive disease (PD) based on RECIST 1.1. and MR in all time intervals. Patient-level PD based on EORTC PET response criteria was correlated with MR in the three to six-month post-IRE period but failed to correlate in the other time intervals. Lesion-level PD based on changes in tumor size, SUVmax, MTV and TLG failed to correlate with MR. However, non-dichotomized lesion-level changes of the same variables revealed a significant correlation between MR and changes in tumor size, MTV and TLG.
Conclusions: There was a notable variation in the use of imaging response evaluation methods across the published literature. Only sparse evidence exists showing that comparative imaging findings correlates with survival. Patient-level PD based on RECIST 1.1. is correlated with poorer survival in PC patients in the current study but results should be validated in an independent cohort. Lesion-level analysis is inappropriate for evaluation of efficacy, at this point in time, but several promising parameters should be examined in future trials.

OR-190

Toward large ablation volumes with single insertion high-frequency irreversible electroporation

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Introduction: Hepatocellular carcinoma (HCC) is the fastest growing type of cancer diagnosed in the United States and the third leading cause of cancer-related mortality worldwide. Additionally, the liver is a common site of metastasis from primary tumors in other organs. However, most patients are not candidates for surgical resection or liver transplantation, the only two definitive therapies available for liver cancer [1].
High-frequency irreversible electroporation (H-FIRE) is an emerging technology that utilizes pulsed electric fields to generate nanoscale defects in the membranes of targeted cells, disrupting homeostatic equilibrium and generating cell death over several hours following

pulse delivery. H-FIRE offers numerous advantages over thermal ablation and its predecessor, conventional IRE, such as reduced muscle contractions and electrochemical effects, potentially more predictable ablation volumes, and more consistent electric field distributions. However, H-FIRE generated ablation volumes have struggled to reach clinical relevance (˜3 cm). In this study, we examine the ability of internally cooled applicators and newly introduced waveform manipulations to generate large ablations in vivo.
Methods: Six female Yorkshire pigs (50–55 kg) were used in experiments (Palmetto Research Swine, Reevesville, South Carolina), all of which were approved by the Institutional Animal Care and Use Committee. In all cases, a custom generator (Voltmed, Inc., Blacksburg, VA) was used to deliver H-FIRE waveforms via an 18-gauge single-insertion monopolar or bipolar applicator inserted into the liver. In experiments using a monopolar applicator, a grounding pad was placed distally ˜30 cm from the insertion site. An accelerometer (Analog Devices, Inc., Norwood, MA) was affixed to the skin approximately 15 cm from the insertion site. In all cases, 300 bursts were delivered at a rate of 1 Hz, and animals were recovered for 24 hr prior to sacrifice. Variables under study included applied voltage, probe irrigation, H-FIRE waveform, and probe type.
Results: In all cases, treatments were well tolerated with no side effects; all bursts were delivered in the absence of cardiac synchronization, and no cardiac abnormalities were observed. Muscle contraction severity was significantly stronger for the monopolar electrode configuration and was dependent upon grounding pad placement location. By manipulating the interpulse delay (5-2-5-100), applied voltages were increased by ˜500 V over the standard waveform (5-2-5-2), and with internal probe irrigation, large (˜ 5 cm3), completely non-thermal ablations were achieved with a maximum treatment voltage up to 3,300 V.
Conclusions: In this study, we optimized several parameters toward maximizing ablation volumes with H-FIRE while retaining its advantages over conventional IRE and thermal ablation. To our knowledge, the ablation volumes reported in this work are the largest reported with H-FIRE and were achieved with a single insertion device.

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OR-211

Optimization of Irreversible Electroporation Needle Electrode Placement Using Ultrasound-dependent Respiratory Motion Tracking Filters

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Background: Irreversible electroporation is a novel ablation technique that uses needle electrodes to improve percutaneous interventions. The optimal probe placement increases the treatment efficacy and reduces the exposure to radiation.
Method: We developed a needle insertion navigation system that optimize the electrode configurations in terms of location, insertion depth, distance between electrodes and number of electrodes. The navigation system designed based on 3D slicer open-source medical images processing and analysis software. The system utilizes Computer Tomography (CT) medical images for needle location identification, while the needle insertion and placement are fully controlled by respiratory organs motion tracking using real-time ultrasound images. The needle location tracked by the Channel and Spatial Reliability tracking algorithm (CSRT) for respiratory organ motion tracking, while the insertion was controlled by real-time distance and orientation estimation.
Results: The navigation system was tested and validated on CT and US images data of 10 patients with liver and renal cell carcinoma (RCC) diseases with an average cancer diameter of 6 and 5 cm respectively. The results indicated that the average insertion and tracking accuracy was 88.2% and 85.7% for liver and renal cancers, with an average location error of 1.6 ±0.65 mm and 1.83±0.4 mm respectively.
Conclusion: The control of patient respiratory motion during the electroporation needle electrode placement decreases the placement errors and increases the probe placement accuracy which in turn will improve the treatment quality. Our finding can enhance the Irreversible electroporation clinical treatment planning by providing real-time needle placement tracking and monitoring system.

P29 - Electroporation-based Therapies - Head and Neck Cancer

Wednesday afternoon Track C
Oct 12, 14:00 - 15:00

OR-102

Electrochemotherapy for the treatment of cutaneous squamous cell carcinoma: the INSPECT experience (2008-2019)

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Introduction: Cutaneous squamous cell carcinoma (cSCC) is a frequent skin cancer with a high risk of recurrence characterized by tumor infiltration and poor prognosis. ECT (electrochemotherapy) is an alternative treatment option for locally advanced or recurrent cSCC that is not suitable for surgical resection. The aim of this study was to evaluate the data in InspECT (International Network for Sharing Practice on ECT) registry of the referral centers and to clarify the indications for use of ECT as treatment modality for cSCC.
Materials and methods: Patients with primary, recurrent or locally advanced cSCC from 18 European centers were included. They underwent at least one ECT session with bleomycin between February 2008 and November 2020, which was performed following the European Standard

Operating Procedures.
Results: The analysis included 162 patients (mean age 80 years; median, 1 lesion/patient). Side effects were mainly local and mild (hyperpigmentation, 11%; ulceration, 11%; suppuration, 4%). The response to treatment per patient was 62% complete and 21% partial. In the multivariate model, intravenous drug administration and small tumor size showed a significant association with a positive outcome (objective response). One-year local progression-free survival was significantly better (p<0.001) in patients with primary tumors (80% (95% C.I. 70%-90%) than in patients with locally advanced disease (49% (95%C.I. 30%-68%).
Conclusion: In the present study, ECT showed antitumor activity and a favourable safety profile in patients with complex cSCC for whom there is no widely accepted standard of care. Better results seem to be obtainable in primary and small tumors (<3 cm) and using intravenous administration of bleomycin.

OR-100

Treatment of Basal Cell Carcinoma with Electrochemotherapy: Insights from the InspECT Registry (2008-2019)

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Background: This report describes electrochemotherapy (ECT) modalities in basal cell carcinoma (BCC) patients and evaluates the efficacy, safety, and predictive factors.

Methods: This is a cohort study. The International Network for Sharing Practices of Electrochemotherapy (InspECT) multicenter prospective database was queried for BCC cases treated with bleomycin-ECT between 2008 and 2019.

Results: The analysis included 330 patients. There were 623 BCCs (median number: 1/patient, range: 1-7; size: 13 mm, range: 5-350), 85% primary, and 80% located in the head and neck region. The procedure was carried out under local anaesthesia in 68% of cases, with the addition of mild sedation in the remaining 32%. Out of 300 evaluable patients, 242 (81%) achieved complete response (CR). No previous therapies (OR 0.35, 95% C.I. 0.19-0.67, p = 0.001) and deep tumour margin coverage with electric pulses (OR 5.55, 95% C.I. 1.37-21.69, p = 0.016) predicted CR achievement. Toxicity included skin ulceration (16%; G3, 1%) and hyperpigmentation (8.1%; G3, 2.5%). After 17 months, 28 (9.3%) patients developed local recurrence/progression.

Conclusion: Despite no convincing evidence that ECT confers improved outcomes compared with surgical excision, still, it might be considered in patients who are not fit for invasive interventions. BCC treatment naivety and ability to cover deep cancer margin with electrodes predict tumour clearance and may help clinicians select patients.

OR-101

Calcium electroporation for low risk basal cell carcinoma – a proof of concept study

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Introduction: Basal cell carcinoma (BCC) is the most common type of cancer with increasing incidence rates. In electrochemotherapy (ECT) permeabilization of the cell membrane by electric pulses (electroporation) allows chemotherapeutics such as bleomycin to enter the cell increasing the anti-tumor effect. ECT appears to be an effective treatment of BCC. In calcium electroporation (CaEP) chemotherapy is replaced by calcium chloride injection resulting in severe ATP depletion leading to necrosis. The effect of CaEP has been shown to be comparable to ECT in the treatment of cutaneous breast cancer and malignant melanoma metastases. The aim of the study was to evaluate the effect of CaEP in the treatment of BCC using either high or low frequency electroporation.
Materials and Method: Patients with low risk primary BCCs were treated in local anesthesia with injection of calcium chloride (225 mM) directly into the tumor including a safety margin of 5 mm, followed by electroporation (ePORE, Mirai, Ireland) with pulse frequencies of either 250 kHz (high) or 5 kHz (low). The higher frequency was designed to limit muscle contraction and discomfort during treatment. Histology proven non-complete responders were retreated after 3 months. Tumor demarcation, tumor depth and efficacy were evaluated using dermoscopy and optical coherence tomography OCT.
Results: 25 patients with superficial (7) or nodular (18) BCCs with a diameter of 5-30 mm were included. Seven patients (28%) were in complete response at 3-month follow-up. Of the 13 patients treated with high frequency 3 had complete response after one treatment with no recurrence within 12-month. Ten patients were retreated resulting in 4 partial responses and 6 with no response. Nine out of 12 patients treated with low frequency received two CaEP treatments, 2 patients declined re-treatment due to pain during the first treatment and 1 patient dropped out due to internal cancer. 4 patients had complete response at 3-month follow-up. At 12-month follow-up 2 patients had microscopic recurrences, one was lost to follow-up and one have not yet reached 12-month follow-up. Of the remaining 5 patients treated with low frequency 4 patients had partial response and 1 patient no response. High frequency CaEP was significantly less painful than low frequency with a median pain score of 2 compared to 5 (p=0.03). One patient had severe necrosis resulting in scar formation. The cosmetic result in patients with complete response was good in 2 patients, fair in 2 patients and poor in 3 patients.
Conclusion: Using CaEP with a new type of electroporator the previously described effect of ECT on BCCs could not be reproduced. Low frequency CaEP were more painful

than high frequency. Further studies should be initiated to explore the mechanism of low efficacy of CaEP on BCCs and to optimize treatment procedures to obtain efficacy rates as reported for ECT of BCC using bleomycin.

OR-109

Effects of Pulse Repetition Frequency on Nanosecond Electrochemotherapy

Veronika Malyško-Ptašinskė[1], Eivina Radzevičiūtė[2], Irutė Girkontaitė[2], Jurij Novickij[1], Julita Kulbacka[3], Nina Rembiałkowska[3], Vitalij Novickij[1]

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This work focuses on nano-electrochemotherapy with bleomycin and doxorubicin and the electroporation dependence on pulse amplitude (6–10 kV/cm), duration (100–500 ns), and pulse repetition frequency (10 kHz, 100 kHz, and 1 MHz) with bursts of 10 pulses on murine Lewis lung carcinoma (LLC1) cell line. For electroporation, 0–3 kV, 100 ns–1 ms square wave high voltage pulse generator [1], and a commercially available 1 mm gap electroporation cuvette were applied. For detection of cell permeabilization, Yo-Pro-1 and flow cytometry were employed. Cell viability was evaluated 24-, 48-, and 72-hours post-electroporation. As a reference, ESOPE (1.3 kV/cm x 100 µs x 8) protocol was used, and optimal concentrations of bleomycin and doxorubicin were determined.

It was shown that the permeabilization rate of LLC1 cells using nsECT could be manipulated by changing pulsing protocols. As expected, the cell permeabilization was scaled with pulse amplitude and duration. For example, 10 kV/cm x 200 ns x 10 pulses delivered at 10 kHz, resulting in ˜50% is being permeabilized, while for 300 ns pulses, the same level of permeabilization is reached already with 7 kV/cm electric field. When 10 kHz pulses were used, permeabilization higher than 75% was achievable only with 8 – 10 kV/cm pulses and pulse durations exceeding 300 ns. Similar response was acquired with 100 kHz pulses. However, a further increase of the repetition frequency to 1 MHz significantly improved the efficiency of electroporation. High permeabilization rates could be already reached with 200 ns pulses or PEF amplitudes in the 6–8 kV/cm range. The MHz protocols were the most effective, showing high cell death rates for both drugs. The efficiency of nsECT was saturated already when pulses longer than 200 ns were used in the whole range of evaluated PEF amplitudes. The 100 and 200 ns pulses returned a -dependent response, indicating a scaling of the ECT efficiency with cell membrane permeabilization. The identical pulses delivered at a lower frequency resulted in a significantly lower cell viability inhibition rate.

Our study confirmed that high-frequency nano-electrochemotherapy with bleomycin and doxorubicin could be an effective alternative for already established ESOPE procedures. The proposed MHz range pulse repetition protocols trigger better or equivalent to ESOPE electrochemotherapy efficiencies. Although, further in vivo studies need to be performed.

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P38 - Electroporation for cardiac ablation

Wednesday afternoon Track D

Oct 12, 14:00 - 15:00

OR-180

Swine Coronary Lumen Contractures Following Irreversible Electroporation as Observed by Optical Coherence Tomography

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Epicardial ablation is gaining popularity as a viable approach for treatment of ventricular tachycardia. In order to minimize collateral damage to surrounding tissues irreversible electroporation has been explored as an emerging modality for cardiac tissues. This study demonstrates the worst-case scenario effects that irreversible electroporation has on the coronary arteries when exposed directly to the ablative field.

Electroporation therapies were delivered across the left anterior descending arteries (LADs) of reanimated swine hearts on the Visible Heart® apparatus. The electroporation energies were delivered utilizing two needle probes inserted 1cm into the myocardium. The electrodes are 1cm apart with the LAD vessel placed squarely between. For translational impact, the generator used was the clinically approved NanoKnife electroporation system. Three different energy levels were selected, 500V, 700V, and 900V to observe a dose response of the tissue. For each energy level, 70 pulses were delivered, each with 90µs pulse width. In order to prevent cardiac fibrillation, IRE pulses were triggered in sync with an ECG R-wave monitor. A Dragonfly (TM) OCT catheter was inserted into the LAD using a 0.014" coronary guidewire. Prior to ablation, the baseline measurement of the lumen is obtained as the OCT catheter pulls back such that a cross-sectional OCT image is taken every 0.1 mm over 54 mm of the artery using the OPTIS (TM) System. Up to three locations along the LAD are chosen for ablation. The guidewire was kept in place during the ablation, but the OCT catheter was pulled back to prevent damaging it. The OCT catheter was re-advanced to the same location, and a measurement was taken immediately after each ablation. Additional measurements were taken 15, 30, and 60 minutes following each ablation to evaluate the coronary recovery.

At every energy level of electroporation, there was an initial acute contraction event. The effective cross section area of the LAD was reduced to an average of 60% from its baseline level regardless of voltage delivered. At 15 minutes the average lumen recovered to 70-80% from baseline and at subsequent time points there was full recovery of lumen size for both the 500V and 700V IRE treatments. The 900V treatment remained at an average of 75% lumen area when compared to its pre-ablation size. Mean diameter of the LAD also observed a similar trend. Optical Coherence Tomography is a useful tool to measure and indicate to what extent electroporation affects cardiac coronary function. In this study, it was observed that while there is an acute contractile response of the coronary arteries to electroporation, recovery does take place soon after treatment. Higher levels of energy delivered may increase the recovery period. However, even when the ablation is delivered directly on the coronary there is less than 15% reduction of vessel size after 15 minutes of IRE delivery.

OR-183

Differences in endocardial lesion morphology between Radiofrequency Ablation (RFA) and Pulsed Field Ablation (PFA): a computational modelling study

Mario Gómez[1], Tomas Garcia-Sanchez[2], Antoni Ivorra[1]

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Radiofrequency ablation (RFA) is commonly used in the treatment of atrial fibrillation (AF). RFA applies alternating currents to induce thermal damage by ohmic heating in living tissues. It can cause blood clots and steam-pops increasing the risk of strokes or tamponade. Besides, heating of adjacent structures increases the risk of atrioesophagial fistulas or internal bleeding. To minimize these risks, a novel technique, based on irreversible electroporation, has emerged: Pulsed Field Ablation (PFA). This study offers a comparison of lesion morphology for monopolar and bipolar catheter configurations, evaluating the impact of blood velocity and catheter orientation in the lesions inside the cardiac chamber. A computational model was implemented for both techniques using the same geometry: a slab of heart cavity; and three tissues: blood (in motion), cardiac and skeletal muscles. Non-irrigated catheters were considered. Both models include three physics (electric currents, thermal and fluid dynamics) with a double-coupled problem between electric and thermal solution to model tissue Joule heating, and between thermal and fluid dynamics to model the cooling effect of blood in motion. The temperature dependence of the electrical conductivity of the living tissues was included to account for the changes in conductivity of ionic solutions with temperature. In PFA, the electric field dependence of the electrical conductivity (modelling the electroporation) was also included. The thermal problem was modelled using the Pennes' Bioheat equation and the blood flow was solved as a laminar flow using the Navier-Stokes' equation. The electrical and thermal properties of the model materials were set according to the frequency of each technique. A temperature-controlled RFA strategy was used maintaining a constant temperature (55 ºC) at the electrode sensor (application of 30 seconds). In PFA, the delivery protocol included 20 bursts of biphasic pulses (150 kHz) of 100 µs per burst repeated at 1 Hz. The applied PFA voltages were adjusted to get the same lesion depth than in RFA, using a reference blood velocity (6 cm/s). In RFA tissues were considered ablated if above 50 ºC. In PFA tissues were considered ablated if the local electric field was above 1000 V/cm. This PFA threshold was arbitrarily set as a wort-scenario case under some assumptions from other studies. Also, the Arrhenius' equation was used to compute possible thermal damage in PFA. Simulations predict lesion differences in terms of morphology between RFA and PFA for both catheter configurations. For the same lesion depth, PFA lesions are wider, more symmetrical and with a bigger volume than those obtained in RFA. Blood velocity has high impact only on RFA: lesions being shallower, narrower and more asymmetric for low velocities. Perpendicular catheter orientations with respect heart surface generate higher volume lesions than sideways angles in RFA. The opposite effect is predicted for PFA.

OR-184

Experimental and numerical evaluation of effect of tissue-electrode proximity during cardiac pulsed field ablation

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Introduction: Pulsed field ablation (PFA) for the treatment of cardiac arrhythmias is a method that has recently been the subject of intense research. Thermal catheter ablation is currently the cornerstone of treatment for drug-resistant AF [1], but these technologies rely on electrode-tissue contact to achieve optimal results [2,3]. Because PFA relies on irreversible electroporation as a method to induce cell death, it has been hypothesized that PFA does not require direct contact with tissue to maintain its efficacy.

Methods: Isolated hearts were prepared from male Yorkshire pigs (n=8). PFA was applied to the epicardial surface of the ventricle with a 4-electrode linear catheter immersed in heparinized blood, using an offset tool to precisely control the distance between the electrodes and the epicardium. After a 2-hour period, the tissue was stained with triphenyl tetrazolium chloride (TTC), and the cross sections were imaged and analyzed using ImageJ. Two numerical models were created using COMSOL Multiphysics: with the offset tool and with unlimited blood domain. The numerical model was validated with the measured values of the current during the treatment. Different values of electric field thresholds were used to

extract measurements of lesion depth. These values were used to train an explicit numerical model of lesion depth as a function of electrode-tissue distance. The model was then used to determine the electric field strength threshold that best fit the experimental data.
Results: Average lesion depth was 4.3 ± 0.4 mm, 2.7 ± 0.4 mm, and 1.3 ± 0.4 mm for the 0, 2, and 4-mm electrode-tissue distances, respectively. The resulting slope of the linear regression was -0.74 (R2=0.91). The best fitting electric field strength in the numerical model was 490 V/cm. The slope of the numerical model was -0.72. The numerical model with unlimited blood domain had a slope of -0.95.
Discussion: The numerical model agrees very well with the experimental results and even manages to reproduce some effects at the edges of the experimental offset tool setup. Since the offset tool had a limited extension around the electrodes, the electric current density at the edges of the offset tool was larger than in the model with unlimited blood pool. This resulted in a lesion depth that decreased less than the distance between the electrodes and the tissue. However, in the model where the current was not restricted with the offset tool, the depth of the lesion decreased at approximately the same rate as the distance between the electrodes and tissue increased. These results indicate that PFA does not critically depend on contact between electrodes and tissue.

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OR-185

Effect of Contact Force on Pulsed Field Ablation Lesions in Porcine Cardiac Tissue

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Background: Thermal based cardiac ablation technologies require tissue contact to enable heat transfer to the tissue to form a lesion. Due to this, contact force has been used as a tool to ensure lesion formation clinically. Pulsed Field Ablation (PFA) is a field-based ablation technology for which limited evidence of the importance of contact force on lesion size is available. Initial work has been done to establish the role of contact force with a unipolar, biphasic system, but a bipolar, biphasic system has yet to be evaluated.1
Objective: Determine the relationship between contact force and lesion size using a focal PFA Catheter with a bipolar, biphasic PFA system in an isolated porcine heart model.
Methods: Isolated porcine hearts (n=7) were perfused using a modified Langendorff set-up using a Krebs-Henseleit buffer. The hearts were submerged in 0.45% saline to mimic the conductivity of blood. Mechanical motion of the heart was arrested by delivering blebbistatin (25 – 50 g) retrograde to the aortic root. A prototype focal PFA catheter was held perpendicular to the epicardium and lowered until contact was made while attached to a force gauge (Mark-10, Copiague, NY). Once the catheter was in place, 1500 V PFA waveforms were applied eight times in a bipolar, biphasic manner. Contact force was automatically recorded during each waveform delivery using a LabView program and averaged. Multiple locations on the epicardium of the right and left ventricle were ablated, avoiding coronary arteries and epicardial fat. Lesions were allowed to mature for 90 minutes, cross sectioned, and stained with triphenyl tetrazolium chloride to evaluate tissue viability. The lesions were photographed, and the widths and depths were measured using ImageJ (NIH, Bethesda, MD).
Results: A total of 83 lesions were evaluated with contact forces between 1.3 g and 48.6 g. Lesion depth ranged from 2.1 mm to 7.4 mm with a mean depth of 4.8 ± 0.9 mm. Lesion width ranged from 5.3 mm to 13.5 mm with an average depth of 9.1 ± 1.3 mm. A linear model showed an increase of 0.02 mm in depth (Depth = 0.02*Contact Force + 4.37) and 0.03 mm in width (Width = 0.03*Contact Force + 8.32) for each additional gram of contact force. When evaluating low (0-8g, n=13)), medium (8-30g, n=37), and high (>30g, n=32) contact force, lesion depths were 4.3 ± 1.1 mm, 4.7 ± 0.9 mm, and 5.0 ± 0.8 mm respectively. Lesion widths for low, medium, and high contact forces were 8.5 ± 1.4 mm, 8.9 ± 1.0 mm, and 9.6 ± 1.4 mm. No statistical difference was observed between lesion depth, but lesion width with high contact force was significant ($p<0.05$) compared to the low and medium contact force groups.
Conclusion: Increasing contact force using a bipolar, biphasic focal PFA system has minimal effects on acute lesion depths or widths in an isolated porcine heart model.

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P27 - In memoriam of Justin Teissié - N'espérez pas, mesurez (don't expect, measure)

Wednesday late afternoon Track A

Oct 12, 16:00 - 17:30

OR-219

N'ESPÉREZ PAS, MESUREZ (DON'T EXPECT, MEASURE)

Marie-Pierre Rols

Justin Teissié, a biophysicist, pioneer in the study of biological membranes and the basic processes of electroporation, passed away on September 2020. After his post-doctorate in the laboratory of Prof. T.Y. Tsong, at the Johns Hopkins University, USA, he created and developed his research team in Toulouse, France. He has dedicated his life to the study of biological membranes and the fundamental processes leading to membrane permeabilisation, and has played a major role in the development of its applications in the medical field for electrochemotherapy and in industry for sterilization. He has been equally aware of the need and importance of developing fundamental knowledge and understanding of cell membrane electropermeabilisation as well as of developing and promoting use of electroporation in medicine and biotechnology. One of his favorite sentence was « Go back to the bench ». While being a researcher at the CNRS, he has constantly paid great attention to the training of young people, trainees, doctoral and post-doctoral students. He was one of the funding faculty member of the Electroproation Based Technologies and Treatments Postgraduate Course and International Scientific Workshop in Ljubljana, Slovenia.

Justin Teissié has been always asking questions reminding us that our knowledge is still incomplete. We, his students, colleagues and friends will never forget him, and he will remain as a model capable of enlightening our future choices.

OR-72

Fourty Years of Electroporation (1982-2022) – New View on the Poration-Resealing Hysteresis

Eberhard Neumann

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The term „Electroporation", short for „electric pore formation", has been coined in 1982 in the context of the first electro-reprogramming of biological cells with added foreign DNA by trains of electric pulses [1]. Prior to functional electro-uptake, it had been found in 1972 that electric pulsing causes release of intra-organellular components without impairing cell viability [2]. The concept of electroporation (EP) qualifies the very structural basis of this electric field effect. The applied field (pulse) causes rapid nucleation of electropores at „weak membrane sites" (e.g., short-chain lipids). In particular structural electro-optic and ion-conductivity relaxation spectrometry reveal that „two types of electropores" are apparent [3]. At low field intensity, small (Type I) pores are dominant; caused by changes in the (non-covalent) hydrophobic lipid-water interactions. At higher field intensities the „new interpretation" suggests that pore nucleation is preceded by chemical bond breaking. Field-induced radicals cause lipid fragments (evidenced by Mir et al.) that, as a part of the pore wall, constitute the larger Type II-pores as the origin for release of intracellular components under Maxwell stress (cell elongation). After pulse termination, the kinetic data indicate pore resealing back to the closed membrane. It is the slow post-field responses that indicate „structural longevity" of the induced porous states causing permeability for molecules (electropermeabilisation) that usually do not penetrate the membrane like DNA. Long-lasting porosity underlies transient endocytotic encapsulation of plasmid DNA. Another long-lived post-field effect refers to electroporated cells in close contact slowly fusing to large multinuclear syncytia (electrofusion) [4]. Long-lived pore states also rationalize the accumulation of the effect of consecutive pulses in a pulse train. Physical chemical thermodynamics rationalizes both rapid in-field poration and slow post-field pore annealing and chemical repairing as the two branches of the hysteresis in the degree of poration f(E) as a function of the field intensity (E). Oscillations in small-ion conductivity and in global shape of vesicles at constant applied field both represent the cycling around the hysteresis loop of structural changes [5,6].

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OR-222

Information about the ISEBTT « Justin Teissié » Award

Muriel Golzio

CNRS, Institut de Pharmacologie et Biologie Structurale (IPBS), France

This award is endorsed by the ISEBTT, and the nominees have to be members of our Society in good standing. It is the first price of our Society and we expect that other awards will enlarge the list of the ISEBTT meritorious members that our Society will recognize though these awards. The « Justin Teissié » Award is funded by our members (and non-members, they are also welcome) through donations to a specific account in the Society, which was initiated by Justin's colleagues and that is fully dedicated to perpetuate the attribution of the Award. Contributions are welcome!

OR-122

Non-canonical biological targets of intense pulsed electric field: proteins

Michal Cifra

Institute of Photonics and Electronics of the Czech Academy of Sciences, Czech Republic

Pulsed electric field research and technology primarily focus on the membranes, and for good reasons. However, the growing accessibility of technology, which provides higher electric field strength enables triggering the response in the nanoscopic electromechanical machines which power life: proteins. Huge diversity in the proteome opens new horizons for PEF in both fundamental biophysics research, bionanotechnology, and applications. In this talk, I present an overview of electric field effects on proteins, focusing on proteins that build up and interact with the cellular skeleton – a crucial system enabling intracellular transport and cell division. On an example of proteins constituting one type of cytoskeleton fibers - microtubules, I will explain the primary mechanisms of coupling of an electric field to proteins. Then I will highlight selected technologies combining advanced microscopy techniques and chips that enable us to explore the real-time effects of PEF. These results deepen our understanding of molecular level effects of electric field on proteins and open new possibilities in biomedical and bionanotechnological applications.

MC acknowledges support from Czech Science Foundation GX20-06873X.

P33 - Imaging and treatment planning in clinical trials

Wednesday late afternoon Track B

Oct 12, 16:00 - 17:30

OR-39

Finite Element evaluation of the electric field distribution in a non-homogeneous environment

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In this work, the effect of the electrical characteristics of the extracellular environment on the electric field distribution in the electroporation conditions was studied by means of the Finite Element (FE) simulation and experiments. In particular, the simulation results, exploiting experimentally acquired electromagnetic properties of the materials, showed how using the same boundary conditions the inhomogeneities of the electrical characteristic can modify the electric field distribution around the cells respect to that happens by using a homogeneous media. This is the case of in vitro experiments that include cells electroporated in suspension compared to cells in 3D cultures where production of fibrous extracellular matrix occurs. In particular, it was evaluated how an accurate simulation of the extracellular environment can predict more accurately the electric field around the cell membrane. Since the electric field distribution is correlated to the cell permeabilization, the electric field intensity using different suspension media (e.g. RPMI or electroporation buffer with low conductivity) was assessed. The electroporation of cell was verified by means of cell suspension experiments.

It is well known that the distribution of the electric field intensity in a material with non-homogeneous electrical characteristics is different respect to the one obtained in homogeneous electrical properties. In particular, the electric field in presence of cells was simulated by FE Model considering a parallelepiped and applying a voltage to two parallel faces to obtain an electric field with intensity equal to 1000 V/cm. In both models, homogeneous and non-homogeneous, cells were represented as circular objects. In the non-homogeneous model, the simulated region included fibrous collagen areas immersed in the studied media to generate a discontinuity in electric conductivity. The electrical properties were experimentally determined and compared with those in literature. The numerical model assessed the intensity of the field in the examined region containing areas of non-homogeneity and compared it with the field obtained in the same areas under conditions of homogeneity.

The local discontinuity could improve the electroporation efficiency for the same applied electric field. The data obtained showed that the cellular organization and the presence of extracellular matrix can modulate the local electrical properties influencing the efficiency of electroporation.

Simulation results were validated by means of suitable experiments with HCC1954 breast cancer cells in suspension and cultured on a hyaluronic acid (HA) hydrogels enriched with self-assembling peptides carrying IKVAV motifs. Cell culture and suspensions was electroporated in a parallelepiped chamber slide using EPS-01 (Igea S.p.A, Carpi, MO, Italy) and a plate electrode. Electroporation was evaluated using Propidium Iodide.

OR-34

Monitoring of current density and electric field distribution during electroporation of heterogeneous tissues using MR techniques

Matej Kranjc[1], Marko Strucic[1], Jessica Genovese[2], Rok Šmerc[1], Vitalij Novickij[3], Samo Mahnič-Kalamiza[1], Igor Serša[4], Damijan Miklavčič[1]

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Electrical properties of biological tissues have been of interest for over a century as they determine the pathways of current flow through the body, and are therefore important in the analysis of a wide range of biomedical applications. Numerous factors determine electrical proper-

ties of biological tissues, such as cellular structure, amount of intra- and extra-cellular fluids, concentration and mobility of ions in these fluids, temperature of the tissue, and their pathological conditions, to name only a few. Tissue is a heterogeneous material with important interfacial processes and cells of uneven size and different functions, and so from the electrical point of view, it is impossible to consider tissue as a homogeneous material.
Measuring and evaluating electrical changes in real time is important for electroporation-based treatments and technologies. Therefore, an efficient approach is needed to monitor the electric field in studied tissue during the delivery of electroporation pulses. Electric field can be reconstructed from current density distribution data by magnetic resonance electric impedance tomography (MREIT). MREIT is enabled by Current Density Imaging (CDI), an MRI modality designed to detect electric currents via the temporal change of magnetic field that is induced by the currents. Since monitoring is performed during pulse delivery, the determined electric field distribution considers all heterogeneities and changes that occur in the treated tissue.
This work will give an overview of monitoring of electric field distribution in tissues of different levels of structural complexity. Experiments were performed in apple fruit, potato tuber, and carrot tissue since these vegetable matrices are – in this order – of ever increasing complexity. Additionally, muscle tissue samples were taken from the neck and thigh region of a domestic pig (Sus domesticus) in order to evaluate anisotropy. Electroporation protocol consisted of two sequences of 4 pulses with a duration of 100 µs per pulse and with a repetition rate of 5 kHz. The voltage amplitude was adjusted to obtain a sufficient signal-to-noise ratio of the electric field in the sample. Application of electric pulses was triggered by an MRI control unit and synchronised with the CDI pulse sequence.
MREIT demonstrated to be suitable for monitoring of the electric field distribution inside structures of plant tissues, whereas in muscle tissues we were able to detect different current density behaviour based on application of electric pulses relative to the muscle fibre orientation. These findings provide useful insights into the evaluation of electroporation and suggest that magnetic resonance techniques could be used as an efficient tool to improve the effectiveness of electroporation-based treatments.

OR-35

Discrete Dual Finite Volumes (DDFV) for electroporation modeling

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Numerous experimental studies have been carried out. In this context, the trans-membrane voltage is a data of importance, it is generally determined by computation. However, few accurate computational models have been developed. Modeling is challenging because of the thin thickness of the membrane compared to the overall dimensions, the electric field jump conditions at interfaces, and the dispersion of the media composing the biological cell.
Most of the computational schemes rely on the finite-element method or the transport lattice method. A Finite Volume method is proposed here because it is expected, first to ensure flux consistency at each mesh cell, second to preserve the basic physical properties of the Poisson equation, and third to provide accuracy on a coarse mesh with high heterogeneities. The Discrete Dual Finite Volume (DDFV) scheme is a numerical method that has been originally developed to solve the Poisson equation on coarse and conforming mesh without the orthogonality conditions.
A complete electromagnetic model of a biological cell is proposed. The Poisson equation is solved by a DDFV method. Time-varying fields are computed by assuming that the quasi-static approximation is valid. Conductive media are modeled by a metal-dielectric equivalence. The cell model is oversimplified. It consists of a sphere separated from the biological solution by a dielectric layer. The problem is clearly 2.5D (axisymmetric 2D). The cell is described by three media: the inner of the cell, the membrane, and the biological solution. The electric transition conditions between each of them are accounted for. All of this leads to a generalized Poisson equation resolution with appropriate jump conditions. Note that the membrane is meshed so that the transmembrane potential is calculated by a simple difference of potential. DDFV unknowns are located at each center of the mesh cell and also on vertices. A new discrete balance equation is then associated with each vertex. Two dual meshes are then introduced. The trans-membrane potential is computed without any interpolation or refinement. Numerical experiments are proposed. The time variation of the membrane potential when a nanosecond voltage pulse is applied to the biological solution is given as an example. The dispersion properties of each medium are modeled by the classical Debye formulation. The results are compared to those obtained in the literature.

OR-37

Coarse-to-fine segmentation of needles on CBCT scans for the evaluation of electroporation ablation

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Electroporation ablation is a promising technique for the removal of deep-seated tumours as it can be used near vital structures without affecting them. Indeed, it is minimally invasive and produces little thermal effects unlike more traditional techniques such as radio-frequency ablation. However, it remains very complex and thus requires cautious planning and evaluation. Our goal is to enable the surgeons to evaluate the electroporation ablation procedure online by providing a visualisation of the electric field distribution along with the tumour localisation.

A crucial step in this matter is the segmentation of the electric probes delivering the electric pulses that leads to

the irreversible electroporation of tumorous cells. From the localisation of the probes, the electric field distribution can be modelled to determine the area where the treatment is effective. However, challenges arise from the nature of the scans (CBCT scans have low contrast and low signal-to-noise ratio but low radiation exposure) and the nature of the objects to segment (the probes are metallic and thin, so artefacts are produced during capture and the dataset is highly unbalanced).

To tackle this task, we propose a coarse-to-fine segmentation algorithm combining deep learning for the coarse segmentation of the probes and the Hough transform for their precise localisation. A modified U-Net with a patch optimisation learning strategy and a loss based on the Dice coefficient and the cross entropy provides a rough segmentation mask then converted into a point cloud. The Hough transform, completed with a voting procedure, is applied to provide an analytical representation of the needles. Finally, a standard linear model is used to compute the electric field from the probes localisation and the pulses information. It is known to underestimate the electric field and thus leads to a suitable first approximation from the medical point of view.

The algorithm is evaluated on 8 of the 16 patients from our database, the rest of the data being used in the training of the U-Net. The artificial neural network is assessed by comparing the predicted segmentation and the ground truth with the Dice coefficient. The overall approach is assessed by computing the distance between the needle tips as segmented and their real coordinates. Our approach is shown to be more robust and precise than the previously used segmentation based on thresholding and the Hough transform. It is also suitable for an online evaluation during the procedure as it provides the probes location under two minutes on a commodity hardware.

OR-38

PIRET: a Software Platform for Treatment Planning in Electroporation-based Therapies

Enric Perera-Bel[1], Kenneth N. Aycock[2], Zaid S. Salameh[2], Mario Gómez[1], Rafael V. Davalos[2], Miguel A. González Ballester[1], *Antoni Ivorra*[1]

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Tissue electroporation is the basis of several therapies. Electroporation is performed by briefly exposing the tissues to high electric fields. These fields are typically generated by delivering short high-voltage waveforms across electrodes in tissue. It is generally accepted that electroporation is effective where the electric field magnitude threshold is overreached. In therapies based on electroporation, it must be ensured that the whole target volume is covered by suprathreshold fields. However, it is difficult to preoperatively estimate the field distribution because it is highly dependent on patient anatomy and several treatment parameters. Thus, there is a need for treatment planning tools for therapies based on electroporation. In response, we have created PIRET, a platform to preoperatively predict the treatment volume in electroporation-based therapies that is user-friendly and fast. The platform seamlessly integrates tools to plan patient-specific electroporation interventions. First, patient anatomy is segmented from medical images and 3D reconstruction aids the user in placing the electrodes and setting up treatment parameters. The electric field is then simulated considering tissue non-linearities typically observed during electroporation therapies, that is, the dependency of the conductivity on the electric field and the dependency of the conductivity on the temperature. Finally, thanks to the included postprocessing tools, the user can easily evaluate treatment feasibility and fine-tune the parameters to ensure an optimal outcome. To illustrate the use of the platform, four canine patients that had been treated with irreversible electroporation were retrospectively planned with PIRET and with a workflow commonly used in previous studies, which uses different general-purpose software platforms for each of the planning steps. We found that PIRET was the fastest, outperforming the other method by 65 minutes on average ($\times$1.7 faster). Both approaches computed similarly accurate electric field distributions, with Dice scores across a sweep of the electric field higher than 0.93. The platform here presented is, to the best of our knowledge, the most complete software for treatment planning in electroporation-based therapies, not only for solid tumor treatment, as it was illustrated here, but potentially for other electroporation applications. Remarkably, PIRET is an offline platform. This avoids transferring personal sensitive data regarding patients. PIRET stands for "Planning Irreversible and Reversible Electroporation Treatments".

OR-36

Electric Field Assisted Volumetric Tumor Profiling

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Introduction: Tumor sampling by Fine Needle Aspiration (FNA), a common technique for molecular cancer diagnosis, is restricted to the immediate vicinity of the needle, with considerable variability in contents. Reversible electroporation (RE) is known to release intracellular materials from permeabilized cells. We investigated RE for enhancing FNA sample extraction and whether computational models can map the tumor volume being sampled.

Materials & Methods: Mouse breast (4T1) and bladder cancer (MB49) cell lines underwent EP in a 4 mm gap cuvette or in a 3D agarose hydrogel tumor mimic (2 pin electrodes, 1cm gap). EP parameters were screened (1000-2000 V/cm, 8-15 pulses, 80 µs length) to determine RNA (Nanodrop) and protein (BCA Assay) levels in the sample and were adjusted for cell viability and proliferation (Trypan blue stain and CCK-8 assay). Agilent Bioanalyzer and Coomassie brilliant blue staining were used to compare the molecular weight spectrum of RNA and proteins eluted by EP treated cells and whole cell lysate. A Comsol model of the in-vitro tumor mimic was used to map the electric field strength and energy delivered with the volume sampled by using two different reporters (cells with GFP and Cell Tracker dye). Fluorescent microscopy

with nucleus (DAPI) and viability stains (Propidium Iodide/Calcein AM) were used to establish spatial extent of EP. In-vivo validation was done in mice with bilateral subcutaneous tumors, comparing FNA +/- EP for total RNA and protein yield. Immunohistochemistry was performed to identify damage or alterations in the tumors from EP.
Results & Discussion: Increasing EP energy dose correlated to the quantity of RNA and protein in samples and a reduction in cell viability and proliferation. Pulse parameters with optimal viability (1500 V/cm, 8 pulses at 80 µs) had over 70?ll viability. At this EP condition, protein levels were 5:1 and 3:1 and RNA levels were 3:1 and 2:1 for 4T1 and MB49 cells respectively when compared to sham. Bioanalyzer results showed comparable levels for small and medium sized RNA, with whole cell lysate having slightly higher levels of large RNA. In the 3D tumor mimic gel, levels of material extraction correlated to the region showing PI staining and could be controlled to extract a predictable ratio of GFP and cell track dye. Simulation models calibrated to the 3D tumor mimic could be used to plan region of dye extraction. Mouse studies demonstrated that EP+FNA yielded more protein and RNA from both 4T1 (4:1 and 10:1, respectively) and MB49 (17:1 and 6:1, respectively) tumors versus FNA alone. T cell/macrophage stains showed that EP did not cause inflammation and TUNEL stains revealed that regions of necrosis were no different from samples that underwent FNA alone.
Conclusion: EP can enhance FNA sampling without injury to the tissue being sampled. Computational models can assist pulse parameter optimization and estimate volumetric region being sampled.

P36 - New Technologies for Cells and Tissues Electropermeabilization-II

Wednesday late afternoon Track C
Oct 12, 16:00 - 17:30

OR-147

Membrane permeabilization by high-intensity pulsed electromagnetic fields – a non-contact electroporation?

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Electroporation of cell membrane leading to increased membrane permeability that is exploited in electrochemotherapy and gene electrotransfer is usually achieved by exposing cells in vitro or in vivo to high voltage electric pulses. Although the value of electric field achieving membrane peremabilization will vary and will depend on cell size, orientation, density in vitro and tissue types in vivo as well as on pulse duration and number of pulses applied the electric fields to which cells need to be exposed are in the range of hundreds of V/cm. In all these applications electrodes in contact with the medium/tissue are used.
We have used electric field induced by time varying high-intensity pulsed electromagnetic fields (HI-PEMF) to achieve membrane permeabilization. Even though the induced electric fields in our experiments were significantly lower (up to 20 V/cm and 0.23 V/cm in experiments in vitro and in vivo, respectively) we have observed membrane permeabilzation as detected by fluorescent dyes such as Propidium Iodide and YO-PRO in vitro; we then compared and achieved successful electrochemotherapy in vivo in experimental murine tumor model which yielded similar tumor growth as obtained by classical electrode mediated electroporation both with cisplatin and bleomycin. Using HI-PEMF we also achieved electrotransfer of siRNA and plasmid DNA in vivo, and recently also successfully achieved gene electrotransfer in vitro by HI-PEMF.
Although the results obtained by HI-PEMF are comparable (albeit sometimes inferior) to electroporation using direct contact of electrodes with target cells and tissue, the benefits of using non-contact electroporation are appealing. In the presentation the evidence available in the literature will be presented and possible mechanisms discussed.

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OR-23

Ultrashort high-intensity pulse generators for simultaneous cellular permeabilization and endoscopic imaging for future biomedical applications

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High-intensity (˜50–100 kV/cm) ultrashort (duration $<$ 1 µs) pulses can be used as a powerful tool for intracellular manipulation. Emerging technologies use ultrashort pulses, for example, for cardiac defibrillation, neurostimulation, or cancer therapy [1]. Indeed, pulses with a duration of the order of the ns (nsPEF) and field strengths of a tenth of kV/cm, may affect internal cellular structures inducing numerous bioeffects such as membrane permeabilization through the formation of nanopores, loss of mitochondrial activity, or modulation of ion channels [1]. However, the delivery of strong electric fields of very short duration complicates the engineering of the devices that require specific advanced technologies to produce pulses with controllable amplitude, energy, power, duration, and shape. In the last 20 years, numerous generators have been developed paving the way for bioelectric studies [2]. To contribute to the understanding of biological effects induced by ultrashort pulses, we designed and characterized –in the frequency and time domains– novel versatile generators able to provide unipolar, bipolar, and paired pulses of about 10 ns, 1 ns, and a few hundred ps. The main

novelty of our generators consists in obtaining a very short delay between the pulse polarities in the ns range (˜5 to 350 ns) which seems to play an important role in cellular response [3]. The proposed systems allow pulse generation and delivery through a mechanism based on a "slow charging and fast discharging" of the energy also known as the frozen wave principle. This means that the energy of a high voltage power supply is stored into a pulse-forming line and then released to a load (e.g., cells) through a specific delivery system (i.e., electrodes) using high-power ultrafast switching elements. By exploiting the propagation of the electromagnetic waves in the line, we generated pulses with different duration, amplitude, shape (unipolar, bipolar, and paired), and interphase delays. In addition, the characterization of a generator providing pulses of the order of a few hundred ps is ongoing. An optoelectronic delivery system based on a pair of electrodes embedded in a polyethersulfone (PES) fiber that also encloses an optical fiber will be used for the simultaneous pulse application and acquisition of endoscopic images. Bioelectric in vitro investigations are envisaged aiming to the future development of medical applications using ultrashort pulses that may selectively target specific intracellular structures.

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OR-158

Effect of the electric field vector change on the efficacy of nanosecond pulse trains

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A phenomenon of bipolar cancellation stands for the suppression of bioeffects of nanosecond electric pulses (nsEP) when the electric field polarity is reversed. In most studies, bipolar electric field is generated by applying bipolar voltage to one electrode, while the second electrode serves for current return (ground). Bipolar electric field can also be created by applying unipolar pulses to two electrodes in alternation, when the electrode not energized becomes ground. In both cases, the electric field vector turns by 180o. To change this angle, we added a third electrode forming an isosceles triangle. This electrode at the apex was always connected to the ground, while the other two electrodes were energized in alternation. This way, the electric field lines from either active electrode converged on the apex electrode, merging into a single long pulse. In other words, the electric field direction between the electrodes at the base of the triangle changed by 180o, but this angle gradually decreased to 0o closer to the apex electrode. As a result, cells situated near and between the two alternately energized electrodes experience "true" bipolar electric field oscillations, while cells close to the apex see only a small change of the electric field vector. A train of unipolar pulses applied alternately to the base electrodes generates a long unipolar pulse near the apex, with duration equal to the train duration.
We have employed this 3-electrode configuration in two different experiments. In the 1st one, we studied the effect of the vector change angle on the efficiency of electroporation by 600-ns pulses in BPAE cell monolayers. The maximum bipolar cancellation (13-fold vs a unipolar pulse) was observed with 180o vector change. Cancellation tapered out towards the apex electrode because of the reduction of the vector change angle. At angles less than 90-120o, cancellation was replaced by summation, i.e., the effects became stronger (3-fold at 22o). In the 2nd experiment, we utilized this effect to evoke electroporation and excitation (measured by Ca2+ mobilization) in smooth muscle cell monolayers. We achieved the effect at the apex electrode while avoiding it at two base electrodes, despite the stronger electric field there. Indeed, bipolar nsEP oscillations between alternately energized electrodes had low biological efficiency (because of both the ns duration and bipolar pulse shape). The same pulses merged into a long unipolar pulse near the apex electrode. The duration of this created unipolar pulse can be made in µs or ms range, making it far more efficient than the bipolar ns oscillations. This effect can be utilized for enhancing focal stimulation or electroporation at one of the electrodes while fully avoiding it at the other electrodes. We are further exploring the utility of this method for targeted focal brain stimulation and for directed "unipolar" tumor ablation.

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OR-146

Targeted excitation of murine hippocampal neurons by spatiotemporal summation of nanosecond electric pulses

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Nanosecond electric pulses (nsEPs) are inherently less efficient than conventional "long" pulses for neurostimulation without cell damage. However, nsEPs can be utilized to create novel and unique stimulation protocols. We took advantage of the bipolar cancellation phenomenon when nsEP effects are inhibited by switching the pulse polarity. We used the bipolar cancellation phenomenon in a 3-electrode array to trigger action potentials (APs) in dissociated hippocampal neurons by spatiotemporal summation of nsEPs at a chosen location.

Neurons were enzymatically isolated from newborn mice and incubated in glass-bottomed culture dishes for 7-10 days until they started producing APs. To visualize plasma membrane (PM) electric potential changes, neurons were loaded with a FluoVolt voltage-sensitive dye. Time-lapse image stacks (3048 frames/s) were acquired using a CCD Camera on an inverted microscope.
The electrode array for neuron excitation constituted three needles in the isosceles right triangle positioned 50 µm above a culture dish perpendicular to the bottom. A bipolar electric field was created by alternating twenty-four 300 ns unipolar electric pulses between two electrodes where one electrode served as a ground at the moment when the other was energized. Both active electrodes were distanced 1mm apart from the third, permanently a ground one where electric field lines converged, combining into a single long pulse. Such exposure aimed to use bipolar cancellation to suppress neuron excitation anywhere within the array except for the targeted region at the ground electrode.
To provide experimental validation of electric field bipolar oscillations, we used a custom-made strobe imaging system to observe changes in the PM electric potential of CHO-K1 cells reflecting the electric field vector alterations upon exposure. Closer to active electrodes, PM potential at the side of the cell facing either electrode alternated from de- to hyperpolarization at 300 ns intervals. Exposure of the cell in a targeted region resulted in a 7 µs long continuous PM depolarization at the side facing the ground. If above the excitation threshold, this PM depolarization would be sufficient to excite neurons.
Neurons were exposed at various distances from the ground toward active electrodes to analyze how their position within the electrode array affects AP initiation. The amplitude of nsEPs in the trains was increased until a threshold of AP initiation. We found that AP can be triggered at the same threshold within 100 µm from the ground. In the range from 100 to 300 µm, the AP threshold increased twofold. Farther than 300 µm from the ground, there was no successful AP initiation regardless of pulse amplitude. If neurons were exposed to a 7 µs pulse instead, AP was initiated anywhere within the array. This result shows how the bipolar cancellation phenomenon in the 3-electrode array can be utilized for targeted stimulation at a single electrode.

Support: AFOSR MURI

P12 - Electroporation and Cellular Pathways

Thursday morning Track A
Oct 13, 10:30 - 12:10

OR-216

Cold atmospheric plasma does not stimulate dermal collagen remodeling at tissue scale

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Under physiological conditions, reactive oxygen species (ROS) are known to play a role in cell signaling, regulating a wide variety of functions, especially those implicated in extracellular matrix (ECM) remodeling [1]. Thus, the use of ROS-modulating technologies is becoming a relevant and fruitful strategy to locally modulate ECM [2]. Cold atmospheric plasma (CAP) is an emerging technology that generates a unique cocktail of short- and long-lasting reactive oxygen and nitrogen species (RONS). Main medical applications of CAP are antitumor strategy via multiple mechanisms of action and wound treatment [3]. In antitumor context, it is nowadays largely unknown how CAP therapy outcome is affected by tumor microenvironment and more particularly ECM [4].
In our study [5], we assessed the potential of direct cold atmospheric helium plasma jet to remodel collagen at dermal tissue scale. Four distinct conditions were studied: no exposure (control), 30 sec exposure to helium plasma jet, 2 min exposure to helium plasma jet and control condition of 2 min exposure to helium gas alone. First, we chemically quantified major RONS generated in our experimental set up. Secondly, we checked the antitumor properties of this plasma treatment onto 3D tumor spheroid model composed of human colorectal tumor HCT-116 cells. Finally, using an original tissue-engineered human dermal substitute model rich in endogenous extracellular matrix, we analyzed collagen remodeling after CAP treatment through quantification of pro-collagen I secretion (ELISA), dosage of global metalloproteases MMPs activity (fluorimetry), MMP1 quantification (ELISA), and chemical quantification of hydroxyproline (amino acid specific to collagens family) of the whole tissue.
Our results indicated that H2O2, NO3- and NO2- were produced during exposure to helium plasma jet. In our experimental condition, we confirmed that 2 min exposure to helium plasma jet was the only condition to induce tumor cell apoptosis and spheroid growth delay. At human dermal tissue scale, the conditions applied did not 1) alter cell viability after 24h, 2) alter pro-collagen I secretion over 48h after treatment, 3) modify global MMPs activity over 48h after treatment, and 4) change hydroxyproline content over 5 days after treatment. The only modification observed was a transient increase in MMP-1 level 6h after treatment in all conditions, which disappeared at 24 post-treatment.
In conclusion, our results indicate that helium-based cold atmospheric plasma revealed to be efficient in inhibiting tumor growth in in vitro 3D spheroid model while not inducing dermal extracellular matrix remodeling in the same exposure conditions.

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OR-196

Necroptosis and Pyroptosis Contribute to Cell Death of NTIRE Treatment

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Recently, non-thermal irreversible electroporation (NTIRE) has been employed as a new medical modality to ablate cancer. While the cell death mechanism of NTIRE is yet unclear. Nowadays, according to TUNEL stain experiments apoptosis is widely recognized as a noninflammatory reason of cell death after NTIRE[1, 2].
However, in our previous study, we found the TUNEL stain after NTIRE treatment is spread throughout the treated region, which is different from typical TUNEL stains of apoptotic cells, in which the nucleus is stained[3]. Moreover, cleaved Caspase 3 was not upregulated at any time after NTIRE, which is a key marker of apoptosis. So, we presumed that apoptosis, a non-inflammatory mechanism of cell death, did not contribute to the cell death from NTIRE.
The study of the mechanisms that lead to cell death has evolved well beyond the concepts of necrosis and apoptosis[4]. A variety of other molecular mechanisms can lead to various modes of programed necrosis characterized by the breaching of the cell membrane, including pyroptosis and necroptosis. Pyroptosis is an inflammatory caspase-dependent form of programmed necrosis[5]. Morphologically, pyroptotic cells display cell swelling and rapid plasma membrane lysis. This form of cell death is driven by the inflammatory caspases: caspase 1, 4, 5 and 11. Activation of caspase 1 and failure to activate caspase 3 are considered a key marker of pyroptosis[6]. Once caspases 1, 11, 4 or 5 have been activated by either the canonical or non-canonical inflammasome pathway, they trigger pyroptosis by cleaving gasdermin D between Asp276 and Gly277[7]. Necroptosis is a programmed form of necrosis that is dependent on activation of receptor-interacting kinase (RIPK3) and the mixed lineage kinase domain-like (MLKL) psuedokinase[8, 9]. This form of cell death involves membrane rupture and release of cytoplasmic contents and is also distinct from apoptosis.
In our study, we found that both caspase-1 and GSDMD were up-regulated at 6 and 24 hours after NTIRE treatment, which confirmed that pyroptosis type signaling was activated after NTIRE treatment. Execution of necroptosis, occurs through MLKL introducing ion channels in the plasma membrane, resulting in the loss of integrity of the cell membrane[10], the RIP1/RIP3 and MLKL are also activated 6 hours after NTIRE[3], which adds complexity of understanding NTIRE cell death. The fact that caspase 1 and RIP1/RIP 3 are activated and induce the activation of GSDMD and MLKL, tentatively suggests that NTIRE cell death is associated with a molecular path which combines both elements of pyroptosis and necroptosis.
However, our findings are interesting and different from conventional point, substantially more research is needed to elucidate the mechanism of cell death from NTIRE. The findings and the possibilities we raise may be of interest to researchers studying the interaction of electric fields with biological cells.

OR-197

Pulsed electric fields with calcium ions stimulate oxidative alternations and lipid peroxidation in human non-small cell lung cancer

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Background. Pulsed electric fields (PEFs) are commonly used to facilitate the delivery of various molecules, including pharmaceuticals, into living cells [1]. However, the applied protocols still require optimization regarding the conditions of the permeabilization process, i.e., pulse waveform, voltage, duration, and the number of pulses in a burst. This study highlights the importance of the electroporation buffers in the electropermeabilization and anticancer effects in the lung cancer model.
Material and methods. This research investigated the effects of electroporation on human non-small cell lung cancer cells (A549) in potassium (SKM) and HEPES-based buffers (SHM) using sub-microsecond and microsecond range pulses. The experiments were performed using 100 ns – 100 μs (0.6–15 kV/cm) bursts with 8 pulses in a sequence. Cell membrane permeabilization rate was measured by flow cytometry using an impermeant dye Yo-Pro-1. Cell viability was analyzed after 24 and 72h by MTT and SRB assay. The confocal microscopy method was used for CellROX® and HCS CellMask™ imaging. ROS level after PEF alone and with calcium ions was detected by ROS-Glo™ H2O2 luminescent assay. The lipid peroxidation process and neutral lipids were assessed by confocal microscopy (Click-iTTM Lipid Peroxidation Imaging Kit and HCS LipidTOX™ Deep Red Neutral Lipid Stain).
Results. It was shown that depending on the buffer composition, the susceptibility of cells to PEF varies, while calcium enhances the cytotoxic effects of PEF, if high cell membrane permeabilization is triggered. It was also determined that electroporation with calcium ions induces oxidative stress in cells, including lipid peroxidation, generation of reactive oxygen species, and neutral lipid droplets.
Conclusions. The model of lung cancer used in the study is not typical for the electroporation studies. Here, we

demonstrated that calcium ions and optimized pulse parameters could potentiate PEF efficacy and oxidative alternations in lung cancer cells. Thus, the anticancer efficacy of PEF in lung cancers in combination with standard cytostatic drugs or calcium ions should be considered, but this issue still requires in-depth detailed studies with in vivo models.

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OR-198

Ultrastructural analysis on normal astrocytes and medulloblastoma cancer stem cells after microsecond pulsed electric field exposure to dissect the cell response specificity

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Pulsed electric fields (PEFs) have become an important clinical tool for the treatment of tumors. In this contest, our preview study showed that a specific pulse protocol (PEF-5: 0.3 MV/m, 40 us, 5 pulses) was able to induce irreversible membrane permeabilization and activate apoptosis and senescence in D283, a model of medulloblastoma (MB) cancer stem cells (CSCs). PEF-5 exposure further promoted a radiosensitizing process enabling the complete inhibition of engrafted tumor growth.
To verify if PEF-5 exposure could be selective for CSCs, Normal Human Astrocytes (NHA), representing cells located in the immediate surroundings of the tumor space, were also exposed to the same protocol. NHA were characterized by a higher threshold for irreversible electroporation than MB CSCs resulting more resistant to the electric pulses application maintaining a high viability.
To attempt understanding the mechanism of such selective response (at morphological, functional and molecular level) and considering that the cell membrane seems the main PEFs target, in this work, we performed an electron microscopy analysis on both cell types to highlight cell morphological changing in relation to the specific plasma membrane composition and complexity. To achieve this aim, Transmission Electron Microscopy (TEM) analysis was performed to explore inner and outer ultrastructural cell changes occurring one hour after PEF-5 exposure. Results showed alterations of the internal organelles and membrane protrusions in both cell lines.
Scanning Electron Microscopy (SEM) analysis was used to study cell surface changes occurring at the same time point after PEF-5 exposure and results confirmed retraction of filipodia and extra-cellular budding formation related to plasma membrane damage repair.
These observations seem to not indicate a clear difference in morphological changes induced by the exposure in both cell lines.
Therefore, an infrastructural approach by immunogold staining in pre-embedding was performed to evaluate the distribution of CD133 protein in exposed cells. CD133 is a trans-membrane protein involved in cell stemness process. It is highly expressed in D283 cells and absent in NHA so it could be the molecular mediator of the observed differential reaction on the two analyzed cell types. First immunogold results showed a qualitative reduction of this protein after PEF-5 exposure in D283. Indeed, CD133 proteins are mainly positioned on cell protrusions highly affected by the pulses exposure. In D283, CD133 dysfunction could explain the evasion in reactive oxygen species defense, and the consequent radio-sensitivity induction.
In conclusion, this study investigates differences in morphological structure of two different cell lines after pulses exposure suggesting the role of membrane protrusions in the activation of specific cell signaling and molecular responses.

OR-195

Finding an effective MRI sequence to visualise the electroporated area in plant-based models by quantitative mapping

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MRI provides a means of visualizing the electroporated region in 3D. Identifying an effective MRI sequence for visualising electroporated area plays an essential role in the evaluation and follow-up of in vivo electroporation treatments. For research purposes, plant-based electroporation models have emerged as an alternative to animal experiments. In the past, the FLAIR MRI sequence was found to have high contrast between electroporated and non-electroporated areas in potatoes (Hjouj et al. 2010). This study aims to find an optimised MRI sequence for analysing electroporated tissue of plant-based models by employing both qualitative and quantitative MRI techniques.
Potatoes and apples were electroporated with 800, 1000, 1500 V/cm and 800, 1000 V/cm and imaged with T1-weighted, T2- weighted and FLAIR MRI sequences at three different time points (3, 24, 48 hours) after electroporation. The sequence for best visualization of the electroporated area with high contrast in apple and potato was identified. To better understand the contrast behaviour, quantitative T1 maps of the central ablated slice were obtained based on five inversion recovery measurements and voxel-wise fitting of the function M(TI) = M0 * (1 - 2 * exp(-TI/T1)), where M0, TI and TR are the proton density, the inversion time and the T1 relaxation time.
All T1 weighted images showed a well-defined contrast between the electroporated and non-electroporated re-

gions compared to the T2 weighted images of potatoes after electroporation, which did not visualize the ablation zone. This implies that differences in the T1 relaxation time between the electroporated and non-electroporated regions explain the contrast behaviour.
In conclusion, morphologic and quantitative imaging showed that T1 changes are the driver for contrast in plant-based (potato and apple) models for electroporation. The potato was found to be the more suitable model for MRI-based visualization of the ablation zone. Importantly, knowledge of T1 values for ablated and non-ablated regions will enable fine-tuning of the MRI sequence parameters towards optimal visualisations of the electroporated zone.

OR-199

Flexible electronics integrated into increasingly complex glioblastoma models for the study of pulsed electric fields effect in tumor and its microenvironment

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Glioblastoma multiforme is a brain cancer that is highly resistant and show high recurrence probability [1]. For this reason, bioelectric therapies based on the delivery of pulsed electric fields (PEF) are more and more investigated as an alternative to standard therapies. Indeed, PEFs have a direct effect on membrane permeability of tumors and their microenvironment. However, most of these treatments are delivered by stiff metal electrodes resulting in a mechanical mismatch with the brain and causing acute and chronic injuries [2].
Our approach is based on the development of flexible and biocompatible devices as a means of delivering PEFs, that can be implanted onto glioblastoma tumors without damaging surrounding tissues. In this purpose, gold interdigitated electrodes were patterned onto a thin layer of the transparent organic parylene-c and coated with the conductive polymer PEDOT:PSS. The effect of PEFs on tumors and their microenvironment was studied at different levels. First, experiments were performed on glioblastoma cells stably expressing a genetically encoded calcium indicator directly cultured on our in vitro devices. Mechanistic studies demonstrated that the delivery of electric pulses induces changes in intracellular calcium and allowed to investigate on the role of ATP signaling in this calcium response. Despite being simple and inexpensive, 2D in vitro experiments are less robust than in vivo models as they fail to mimic the microenvironment of the tumors. In order to minimize the use of animal models that raises ethical issues, we developed an intermediate model combining a living organism, a quail embryo, and an in vitro three-dimensional (3D) model of vascularized tumor [3]. 3D engineered glioblastoma spheroids were grafted in the chorioallantoic membrane of a quail embryo and were vascularized by the embryo's vasculature over time. Indeed, at early embryonic stage, tumors are not recognized as foreign bodies due to the lack of immune system [4]. Using this model, we demonstrated that PEFs induced intracellular calcium elevation, electropermeabilization of cellular membranes and vasoconstriction of tumors' vasculature. Also, that implanted flexible electrode devices could be used to dysregulate intracellular calcium homeostasis. This showed that flexible devices can be used as a less invasive alternative to rigid metal electrodes for the delivery of PEF and that our model is suitable for first experiments under intravital conditions, before using in vivo animal models. Finally, preclinical studies were performed with the same devices in a syngenic, orthograft immunocompetent mouse model to study the effect of PEFs on tumors and the triggered immune response.

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P25 - Electrochemotherapy for Cutaneous Metastases

Thursday morning Track B
Oct 13, 10:30 - 12:10

OR-103

Efficacy of electrochemotherapy in breast cancer patients of different hormonal status: The INSPECT experience

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Introduction. Electrochemotherapy has proven to be an efficient treatment for cutaneous metastases of various cancers including breast cancer (BC). The large number of patients collected within INSPECT database provide the possibility of a differentiated analysis on BC with different receptor status (estrogen receptor and HER2 receptor).
Materials and methods. Data on Breast Cancer patients with cutaneous metastases have been retrieved from the INSPECT database. Patients have been divided into 3 groups: HER2+ = patients with HER2 positive, Hormone receptor positive (HR+) = patients with either ER or PG positive (HER2 negative), Triple negative (TN) = patients with ER, PG and HER2 negative. The groups comprised of 43, 94 and 34 patients, respectively.
Results. Groups were similar for: histological subtype (ductal carcinoma was present in 72% HER2+, 82% HR+ and 91% TN), location of the cutaneous nodules at chest level (92%, 89% and 95% respectively). Most of patients

in all groups have been pre-treated with surgery/systemic therapy/radiotherapy. Preirradiation of treated lesions was observed in 76% of lesions in the TN group, 45% in HER2+ and 52% in HR+ group. Some patients (70% in HER2+, 55% in HR+, 38% in TN) were under concomitant systemic treatment at ECT session. Half of patients were treated with ECT for multiple lesions. Multiple lesions were significantly smaller than single lesions: mean size of single lesions was 12.4±12.9 cm, whilst mean size of multiple lesions was 2.6±3.6 cm (p=0.003).
Response to ECT has been evaluated in terms of objective response (OR) per patient and per nodule. OR per patient is 86% in HER2+, 80% in HR+, 76% in TN (p=0.8664); OR per nodule is 89% in HER2+, 86% in HR+, 83% in TN (p=0.3846). Factors affecting response to ECT are different among groups, except for lesions' size which significantly affects the response in all groups (higher response rate for sizes < 3cm, p=0.0105, p=0.0001, p=0.0266 respectively). Furthermore, in HER2+ group response is affected by metastatic condition (higher response rate in non-metastatic patients p=0.0143); in HR+ is affected by concomitant systemic therapy (higher response rate under concomitant treatment p=0.0044) and lesion numerosity (higher response rate in multiple lesions p=0.0065); in TN is affected by lesions numerosity (higher response rate in multiple lesions p=0.0011). Local tumor control is higher for HER2+ and HR+ groups (1 year local progression free survival or 78% and 81% respectively) with respect to TN group (61%).
ECT treatment is equally effective among groups, despite different conditions, age, time since, diagnosis, previous or concomitant treatments, treatment characteristics. Response and local tumor control seems to be better in small multiple lesions than in big armour-like lesions, suggesting that treating smaller, even multiple, lesions at the time of occurrence is much more effective than treating bigger long lasting armour-like cutaneous lesions.

OR-104

Health-related quality of life trajectories in melanoma patients after electrochemotherapy: real-world insights from the InspECT register

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Introduction: Electrochemotherapy (ECT) effectively controls skin metastases from cutaneous melanoma. The objective of this study was to evaluate health-related quality of life (HRQoL) in melanoma patients pre-/post-ECT and its influence on treatment outcome.
Materials and methods: The analysis included prospective data from the International Network for Sharing Practices of ECT (InspECT) register. Following the Standard Operating Procedures, patients received intravenous or intratumoural bleomycin (15,000 IU/m2; 1000 IU mL/cm3) followed by 100-microsecond, 1000-V/cm electric pulses. Endpoints included response (RECIST v3.0), local progression-free survival (LPFS), toxicity (CTCAE v5.0), and patient-reported HRQoL at baseline, one, two, four and ten months (EuroQol [EQ-5D-3L] questionnaire, including 5-item utility score [EQ-5D] and visual analogue scale for self-reported health state [EQ-VAS]). Comparisons were made for statistical and minimal important differences (MID). Scores and clinical covariates were analysed to identify predictors of response in multivariate analysis.
Results: Complete response rate, G3 toxicity and one-year LPFS in 378 patients (76% of the InspECT melanoma cohort) were 47%, 5%, and 78%. At baseline, age-paired HRQoL did not differ from the general European population. Following ECT, both EQ-5D and EQ-VAS scores remained within MID boundaries, particularly among complete responders. A subanalysis of the EQ-5D items revealed a statistically significant worsening in pain/discomfort and mobility (at one-two months), and self-care and usual activities (throughout the follow-up). Concomitant checkpoint inhibition was associated with better EQ-5D and EQ-VAS trajectories. Baseline EQ-5D was the exclusive independent predictor of response (RR 14.76, p=0.001).
Conclusion: HRQoL of melanoma patients candidates to ECT is similar to the general population and preserved in complete responders. Transient deterioration in pain/discomfort and mobility domains and persistent decline in self-care and usual activities may warrant targeted support interventions. Combination with checkpoint inhibitors is associated with superior QoL outcomes. Baseline HRQoL provides predictive information which can help identify patients most likely to respond.

OR-105

High Frequency Electroporation and Chemotherapy for the treatment of Cutaneous Malignancies; Evaluation of Early Clinical Utility and Response

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Introduction: There has been increasing interest in manipulating the pulse parameters used to deliver successful electroporation in order to reduce muscle contractions and the pain associated with treatment as well as potentially ensuring a more uniform tumour exposure. The ePORE device, Mirai Medical, Galway is utilising a higher frequency electroporation delivery to successfully permeabilise the tumour cell membrane and enable patients to be treated under local anaesthesia. This allows for an increased group of patients to be treated with the anaesthetic risk reduced allowing for a safer treatment. The pre-clinical data on the efficacy of the new high frequency electroporation parameters has been published which reduces the pulse length from 100 microseconds unipolar to packets of 2 microsecond bipolar pulses. These high frequency parameters have been successfully validated in-vitro on melanoma cells to induce cell death when combined with a chemothera-

peutic. This case series examines early clinical utility and outcomes.
Method: Between July 2019 and March 2021, 20 patients were treated at Cork University Hospital, Ireland with high frequency electroporation and chemotherapy. 10 were treated as part of the initial evaluation of efficacy and a further 10 for therapeutic indications. Up to 11 lesions per patient were included in the analysis, evaluated for response (CR, PR, NR, DP) over time as well as any complications. Patients included those who had disease progression on current treatment regimes with no suitable alternative options and those who could not tolerate or declined general anaesthesia thus removing the option of lower frequency electroporation.
Results: Of the 20 patients treated, 16 are available for follow up. 2 were unavailable for follow up and 2 were recently treated and outcome data is pending. All successfully underwent treatment with no procedural complications and objectively successful electroporation. 6 patients underwent treatment under local anaesthesia with no sedation.
In total 278 lesions were treated and 89 of these included in the analysis. Lesions analysed included a variety of histiotypes including malignant melanoma (52), Basal Cell Carcinoma (29), Breast Carcinoma (7) and Squamous cell carcinoma (1). At 12 weeks post treatment, Complete response (CR) was observed in 62/78 (79%,) Partial response in 13% (10/78), Disease progression in 5/78(6.5%) and unable to assess in 1/78 (1 %).
Conclusions: High frequency electroporation and chemotherapy is showing optimistic early response rates comparable to the rates seen with electrochemotherapy using the standard procedures. No procedural issues were encountered. As such, treatment with these novel parameters represent an alternative treatment modality that may expand the treatment envelope for patients who could benefit form electroporation based treatment for cutaneous malignancies especially under local anaesthesia.

OR-106

Outcomes of patients with metastatic melanoma treated with electrochemotherapy, pembrolizumab or their combination: a retrospective matched cohort analysis from InspECT and Slovenian Cancer Registry

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Background: Pembrolizumab is the standard of care for patients with metastatic melanoma. Electrochemotherapy has been shown to provide durable local control on superficial metastases. The combination of systemic immunotherapy with pembrolizumab and local treatment with ECT has not been investigated.
Methods: We compared three groups of melanoma patients with stage IIIC-IV disease and skin metastases. The groups were matched for age, disease stage, performance status (ECOG) and size of skin metastases. The first group (n=41 patients) received only ECT (one or more sessions); the second group (n=44) received only pembrolizumab; the third group (n=45) received ECT in concomitance with pembrolizumab. The percentage of patients who had previous systemic treatment was 54% in the ECT alone group 32% in the pembrolizumab alone group, 67% in the ECT plus pembrolizumab group (p=0.004). Local objective response (OR) was evaluated on skin metastases at six months.
Results: The OR rate was higher in patients who underwent ECT and ECT+pembrolizumab (80.5% and 77.8% respectively) than those who received pembrolizumab alone (38.6%), p<0.001. The percentage of patients who experienced local progression was 26.7% in the ECT + pembrolizumab group (after a mean time of 20±12 months) and 56.1% in the pembrolizumab group (after a mean time of 7±8 months). Cox regression analysis corrected for previous systemic treatment revealed a significantly higher risk of local progression in the pembrolizumab compared with the ECT+pembrolizumab group (RR 5.76, C.I. [2.41-13.77], p<0.001). One-year local progression free survival was 86% in the ECT+pembrolizumab group and 51% in pembrolizumab group (p=0.0002).
Systemic OR was similar between pembrolizumab and ECT+pembrolizumab groups (25.5% vs 24.4%, p=1). In the pembrolizumab group 61.4% of patients experienced systemic progression and 53.3% in ECT + pembrolizumab group (p=0.522); the time to progression was significantly shorter in the pembrolizumab compared with the ECT+pembrolizumab group (8±9 vs 17±15 months, p<0.001). Cox regression analysis corrected for previous systemic treatment showed a significantly higher risk of systemic progression in the pembrolizumab group compared with ECT+pembrolizumab group (RR 1.96, C.I. [1.07-3.60], p=0.0305). One-year systemic progression free survival was 63% in the ECT+pembrolizumab group and 39% in the pembrolizumab group (p=0.0344).
One-year overall survival was 88% and 64% in the ECT+pembrolizumab and pembrolizumab group, respectively (p=0.0062). Cox regression analysis corrected for previous systemic treatment showed a significantly higher risk of death in the pembrolizumab compared with respect to the ECT+pembrolizumab group (RR 2.02, C.I. [1.01-4.03], p=0.045).
Conclusions: Our results suggest that the combination of pembrolizumab and ECT provides durable local disease control on superficial metastases, which may beneficially impacts on the systemic progression of the disease.

OR-107

Electrochemotherapy with intravenous bleomycin for patients with cutaneous malignancies, across tumour histology: A systematic review

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Background: Electrochemotherapy (ECT) is an established treatment for primary and secondary skin tumours. The method combines chemotherapy with electroporation. Bleomycin is the drug of choice for ECT, as it is already

well established as a treatment for several cancer types and has the largest increase in efficacy when combined with electroporation, enhancing the toxic effect several hundred fold. The response rates of ECT have been high and consistent over the past 30 years. However, some patients are excluded from treatment due to possible side effects. Case based reports point out that the efficacy possibly can be maintained even when the dose of bleomycin is reduced. Consequently, in 2018, studies began investigating reducing the bleomycin dose. ECT is often performed as a palliative treatment, which is why quality of life is an important parameter to include in evaluation.
Aim: The purpose of this review is to summarize all data published using intravenous bleomycin for cutaneous malignancies and is to our knowledge the first review to examine the use of a reduced bleomycin dose in ECT. Methods: This study is a systematic review. Fifty-five clinical studies investigating ECT with intravenous bleomycin for patients with any cutaneous malignancies (basal cell carcinoma, squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma and breast cancer metastases) were included.
Results: Studies published from 1993-2021 investigating the effect of ECT include 3729 patients and indicate a consistent and high response with a mean objective response rate (ORR) of 81.5%. Interestingly, studies using lower doses of bleomycin observe a similar ORR (85.5%), opening the possibility that a lower dose may not be inferior. Eight studies have performed a quality of life assessment using EORTC questionnaires, concluding an improved quality of life after ECT.
Conclusion: This study gives an overview of published studies on ECT with intravenous bleomycin for patients with cutaneous malignancies, including the use of a reduced bleomycin dose, as preparation for a randomised study.

OR-108

Hybrid ECT – a different approach

Michael Rice, *Giulia Colavitti*, Antonio Orlando
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Electro-Chemo-Therapy(ECT) is a combination treatment based on the simultaneous administration of chemotherapy and the application of an electric field to the area to be treated in order to produce reversible electroporation to the cancer cells. The electroporation increases the intracellular migration of the cytotoxic drug locally enhancing its efficacy. This technique has been a valuable tool in the treatment of various tumours for the last 15 years since the publication of the ESOPE (European Standard Operating Procedure for Electrochemotherapy)in 2006.
According to the ESOPE the bleomycin can be administered intravenously (bolus of 15,000 IU/Sqm) or intralesionally (1000 IU/Ml 0.25-1 ml/cm3).
In our practice, based mainly on skin deposits from MM, SCC and Breast cancer, we frequently encounter extensive skin involvement suitable only for IV treatment.
Published data shows that intra-lesional treatment (IL) is more effective than intra-venous (IV) but it is mainly used for localised disease. There is also clear evidence that ECT is more effective in small volume deposits proportionally reducing its efficacy as the lesions increase in volume.
Keeping in mind the above considerations we have developed our own protocol which has shown to be improve our results.
Technique: Patients are managed under General Anaesthetic as Day case
1- All patients receive systemic Bleomycin via intravenous administration with dose in line with ESOPE (15,000 IU/Sqm in 100cc of N.Saline),
2- A small amount (5-10mls) is taken from the IV bolus (leaving the total dose administered unchanged and it is used to infiltrate intra-lesionally the bigger cancer deposits. (10-20 mm in diameter)
3- If there are nodules bigger than 2 cm they are surgically excised with needle cutting diathermy with 1mm margin and after achieving full haemostasis the wound is treated with electroporation
4- The area treated with electroporation is extended to at least 3 cm margin around the clinically visible nodules or/and to any area where we would expect development of further recurrences
Our pre-ECT clinical experience, when multiple skin deposit from melanoma and breast cancer where treated with narrow margin surgical excision, was characterised by a rapid early relapse. We have noticed a much longer disease free interval since we have started treating a much larger area of skin around the clinically visible nodules and we believe that this is due to clearance of microscopic disease which is very susceptible to ECT.
The treatment of the bigger lesions with a combination of local and systemic therapy has improved our complete response. The surgical excision of bulky disease provides a clean wound which is more manageable, less symptomatic and heals quicker than the ones following partial or complete tumour necrosis improving quality of life.
Conclusions: We believe that our algorithm improves response rate, disease free interval and reduce post treatment symptoms avoiding long term management of necrotic wounds. It is simple to implement without increase of risks as respects ESOPE recommendations of maximum doses.

P35 - Modelling

Thursday morning Track C
Oct 13, 10:30 - 12:10

OR-25

Mean field model of single cell electroporation

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Electroporation is a phenomenon that takes place when a cell is immersed in a strong enough electric field. The cell membrane which is mainly made up of a lipid bilayer behaves like a dielectric separating two conductive media (the interior and exterior of the cell). Charge accumulates on either side of the membrane creating a transmembrane voltage (TMV). When the TMV

surpasses a certain threshold the membrane permeability to the exterior environment drastically increases. This induces an exchange between the cell and its exterior medium and in particular enables certain molecules to enter the cell that could not do so before [4].
Various models have been proposed over the years to explain this phenomenon at the scale of a single cell. By far the most successful one was presented by Neu and Krassowska in [2]. More recently [1], Leguebe et al have proposed a more accurate model however it is phenomenological in nature and so it cannot explain the phenomenon. On the other hand molecular dynamics simulations have enabled us to represent in extreme detail small patches of cell membrane under the effect of an external electric field [3]. However these simulations seem to contradict what one would expect according to the model from Neu and Krassowska. Moreover, the patches of cell membrane that are computationally feasible to simulate are too small to compare results with both models. Lastly, even molecular dynamics seem to agree with experiments but only qualitatively. In practice they consider electric pulse that are about two orders of magnitude stronger than the ones used in experiments.
Our goal is to present a physically based, single cell electroporation model which is also coherent when applied to a small patch of the cell membrane and which takes into account pore interactions and dynamics. This is important as it enables us to make a link current molecular dynamics simulations of a small patches of cell membrane and a global description of the cell membrane. The main idea is to use a phase ordering kinetics model to describe the state of the cell membrane. The evolution of the membrane will then be determined by its free energy also known as the mean field Ginzburg-Landau excess free energy.
We conclude this work with some simulations in the same setting as in the molecular dynamics simulations using physically relevant coefficients in our model and compare our results with current dynamical simulations.

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[3] M. Breton, L. Delemotte, A. Silve, L. M. Mir, and M. Tarek. Transport of siRNA through lipid membranes driven by nanosecond electric pulses: an experimental and computational study. J. Am. Chem. Soc., 134(34):13938–13941, 2012.
[4] A. Gothelf, L. M. Mir, and J. Gehl. ...

OR-26

Water Pores in Planar Lipid Bilayers with an addition of cholesterol

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A fundamental understanding of the barrier properties of biological membranes can be obtained by studying model systems, such as planar lipid bilayers. The planar lipid bilayer mimics a fragment of the cell membrane when it separates two compartments filled with electrolytes, i. e. when it is formed on a small aperture that separates two compartments. Electrodes immersed in each compartment allow measurements of electric parameters of the planar lipid bilayer, which can be considered electrically as a non-perfect capacitor - parallel connection of an ideal capacitor and resistor. The changes in planar lipid bilayer electrical parameters can reflect structural changes of planar lipid bilayer.

Using linearly increasing transmembrane voltage we measured the capacitance, breakdown voltage, and time required for rupture of planar lipid bilayers composed of 1-pamitoyl 2-oleoyl phosphatidylcholine (POPC), 1-pamitoyl 2-oleoyl phosphatidylserine (POPS), mixture of POPC and POPS lipids in a 1:1 ratio, and POPC lipids with an addition of 20, 30, 50 and 80 mol% of cholesterol. We evaluated the change in the capacitance of the planar lipid bilayer corresponding to the formation of water pores, the radius of water pores at membrane rupture, and the fraction of the area of the planar lipid bilayer occupied by water pores. The estimated pore radii of the planar lipid bilayer are 0.101 nm, 0.110 nm, and 0.106 nm for membranes composed of POPC, POPS, and POPC:POPS, respectively. For POPC lipids with 20, 30, 50, and 80 mol% cholesterol added, the estimated pore radii are 0.097 nm, 0.071 nm, 0.067 nm, and 0.083 nm, respectively. The fraction of the surface occupied by water pores at the time of rupture of the planar lipid bilayer is in the range of 0.1–1.8% for POPC, POPS and their mixture. For POPC lipids with an addition of 20, 30, 50, and 80 mol% cholesterol, the fraction of the surface occupied by water pores is estimated to be 12.55%, 22.85%, 0.4%, and 0.9%, respectively.

Interactions between lipids are important determinants of membrane organization and physical properties. It is known that cholesterol and unsaturated PC molecules are not easily miscible, leading to the formation of cholesterol-rich domains. These domains are small and have short lifetime; 1 to 100 ns. It was stipulated that the incorporation of cholesterol (30 mol%) increases the penetration of water into the headgroup region and into the near-surface region of the hydrocarbon chains. The results of our study indicate that smaller water pores form in planar lipid bilayers composed of POPC lipids with an addition of 30 mol% cholesterol molecules in an electric field than in planar lipid bilayers composed of POPC lipids alone. However, it is possible that the water pores occupy a large part (approx. 23%) of the planar lipid bilayer, which contributes to its greater stability in the electric field.

OR-29

Build me a skeletal muscle in silico: Insights into tissue electroporation from an experimentally-validated multiscale numerical model

Rok Šmerc[1], David A. Ramirez[2], Samo Mahnič-Kalamiza[1], Janja Dermol-Černe[1], Daniel C. Sigg[3], Lars M. Mattison[3], Paul A. Iaizzo[2], Damijan Miklavčič[1]

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Gene electrotransfer is an important application of electroporation, with skeletal muscle the most used target tissue due to its ability to express genes and secrete proteins into the bloodstream. The specific architecture of skeletal muscle, consisting of long fibres, imposes an anisotropic electrical conductivity, leading to fibre-orientation dependent electric field distribution at pulse application. The electrical conductivity of skeletal muscle in the direction of fibres is higher than in the direction perpendicular to the fibres. The anisotropic electrical conductivity was also observed in cardiac muscle, a tissue of interest in another electroporation application of increasing importance: the treatment of cardiac arrhythmias, particularly of atrial fibrillation, by means of pulmonary vein tissue ablation using irreversible electroporation. Due to a variety of tissue types in electroporation-based treatments, further research on muscle anisotropy and its significance is of great importance. The objective of our study was to gain better insight into muscle anisotropy, with the goal of determining the importance of field orientation in an anisotropic tissue to the extent of irreversible damage by means of an experimentally validated mathematical model.

Our study consisted of an experimental and a numerical part. In the experimental part, we delivered electrical pulses into the skeletal muscle tissue of pigs in vivo, inserting the needle electrodes in two different orientations with respect to the muscle fibres: the first, in which the direction of the applied electric field was parallel to the direction of the muscle fibres, and the second, in which the electric field was perpendicular to the muscle fibres. We then used triphenyl tetrazolium chloride (TTC) staining to determine the shape of the lesion for every application. In the numerical part of the study, we built a multiscale numerical model of the skeletal muscle. We used a single-cell model to determine cell-level conductivity during electroporation, and then generalised the calculated conductivity changes to the bulk tissue. In this way, we were able to determine the electric field strength distribution in skeletal muscle tissue during electroporation. Finally, we compared the experimentally determined lesions with the calculated field strength distributions using the Sørensen-Dice similarity coefficient to find the contours of the electric field strength threshold beyond which irreversible damage is believed to occur.

The study leads to two conclusions important for our understanding of muscle electroporation and guiding further research; firstly, muscle anisotropy is of significant importance when considering electric field application, and secondly, we were able to extend the electroporated single cell in situ properties to bulk tissue properties, illustrating the power of such a multiscale approach in understanding of the electroporation phenomenon from the mechanistic perspective.

OR-27

Real-Time Conductivity Distribution Characterization for Electroporation using Plant Tissue

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This paper is focused on electroporation for medical applications. Classical application of electroporation is carried out using fixed protocols based on preplanning or monitoring the treatment by means of global impedance measurements. Consequently, it is often preferred to apply the protocol repeatedly or to ensure a low enough final global impedance to ensure complete treatment and prevent tumour relapses. These methods, although partially effective, do not allow to focus and control the treatment effects and therefore healthy tissue can be overheated and damaged. In this context, new multi-output electroporation systems with real-time monitoring tools have been proposed to improve control and targeting of current treatments.

In this work, a multi-output electroporation generator has been used together with a multi-electrode structure to propose a real-time conductivity distribution estimation method. The goal of this method is to estimate the electric conductivity distribution inside the tissue before each electroporation pulse, i.e. in real-time. This will allow the system to target the treatment effect, improve its control, and increase the preserved healthy tissue. The proposed method combines a small-signal impedance monitoring block with a multi-electrode structure. Firstly, the monitoring block allows to study the conductivity dynamics by means of the generation of measurement constant-voltage pulse trains and a subsequent accurate current measurement. Then, the multi-output structure allows applying the measuring pulses in different areas of the tissue which allows to analyze the impedance in diverse volumes of the tissue. Finally, up to 81 impedance measurements are processed by means of Least-Mean-Square algorithm to estimate a conductivity map composed of 18 voxels.

The proposed method has been developed by means of a FEA model in COMSOL Multiphysics, which allows to simulate the impedance measurements taken by the monitoring system, which are then normalized and processed by MATLAB. The proposed approach has been tested on potato tissue combined with phantom gel. These elements are selected because the potato is a vegetal tissue with homogeneous electrical properties that allows to study the electroporation effects, and phantom gel allows to create volumes with controlled and constant properties. These are easy-to-use tissues for intensive experimentation, and they have been widely used for similar purposes in multiple electroporation studies. The tissue samples have a thickness of 1 cm, and square multi-electrode based on parallel-plates electrodes that it is composed of isolated

square cells of 1 cm of side were used.
In conclusion, a method to estimate the electric conductivity distribution in real-time was developed and validated by means of vegetal tissue model and finite element analysis. The final version of this paper will include experimental results and future insights demonstrating the feasibility of this proposal.

OR-96

Characterization of an experimental setup for recording fluorescence in real-time from a cell membrane exposed to electric pulses

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Carol Davila University of Medicine and Pharmacy, Biophysics and Cellular Biotechnology, Romania

Understanding the molecular mechanisms of the main processes involved in electroporation continues to be of significant interest and requires experimental systems designed to record in real time the evolution of pertinent cell parameters. Here we present a setup which can be used to evaluate by fluorescence various membrane properties before, during and after application of the electroporation pulses to cells in suspension.
The system is based on the design of adequate electroporation electrodes, compatible with a standard spectrofluorometer cuvette housing (to allow the excitation beam to travel into the sample a window in the electrode plate was done). Four different window geometries and sizes were considered: two circular and two rectangular; these geometries were 3D modeled in AutoDesk Fusion 360; for all geometries, the spatial electric field distribution was simulated in COMSOL Multiphysics 5.3a.
The electrodes ensuring the greatest homogeneity of the field in combination with the best possible illumination of the sample, were then built using a computer-controlled cutting machine from a non-magnetizing stainless steel plate (to allow magnetic bar stirring of cells in suspension).
As an example of the setup reliability, fluorescence spectra of laurdan molecules labelling cells in suspension are presented together with the kinetics of the parameter called "generalized polarization" for a varying number of electroporation pulses. This parameter is strongly correlated to water presence in the hydrophobic core of cell membrane. The system may be employed for many other fluorescence measurements (fluorescence depolarization, Förster resonance energy transfer, etc.) useful to the characterization of the electroporation process.

[1] Tivig I. et al., European Biophysics Journal, DOI : 10.1007/s00249-019-01417-9

P17 - Using Nanosecond Pulsed Electric Fields (nsPEF) to Treat Cancer

Thursday afternoon Track A
Oct 13, 13:30 - 15:00

OR-116

Nanosecond Pulsed Electric Field in Tumor Ablation, From Lab Experiment to Clinical Practice

Xinhua Chen

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Nanosecond pulsed electric field (nsPEF) utilizes high-power short electric pulses to disrupt cellular membranes and intracellular structures, e.g. nucleus and mitochondria, and consequently induces the apoptosis of tumor cells. It was approved in the laboratory by the different solid tumor models, indicating it is an ideal option for treating tumor nodules close to aforementioned delicate structures. A prospective clinical trial (clinicaltrials.gov identifier: NCT04039747) was conducted to evaluate the safety and efficacy of nsPEF ablation in 195 HCC patients with unresectable liver cancer that were ineligible for thermal ablation. The initial results suggest nsPEF is an effective loco-regional treatment modality for liver carcinoma located near gallbladder, hepatic vessels, portal vessels or gastrointestinal tract. After a follow-up of 6 months. nsPEF has shown promising efficacy. Notably, the non-thermal ablation mechanism of nsPEF could spare vital structures from collateral thermal damage as observed in conventional thermal ablation. The concomitant activation of anti-tumor immunity by nsPEF can also potentially prevent tumor recurrence. However, the specific mechanism of nsPEF ablation inhibited tumor recurrence needs further immune activation investigation from a clinical perspective. The long term follow up of the above 195 unresectable HCC patients who had the nsPEF ablation is still ongoing.

OR-14

Tissue-specific clearance thresholds using high repetition rate nanosecond pulsed electric fields

Richard Nuccitelli, Amanda McDaniel, Kristin Von Rothstein, Dacia Gonzalez, Esin Sozer

Pulse Biosciences, Biology, United States

The proprietary Nano-Pulse Stimulation™ (NPS™) treatment triggers regulated cell death and subsequent clearance of tissues by applying nanosecond pulses at high amplitude electric fields (˜ 30 kV/cm) with low repetition rates (<10 Hz). The short, nanosecond pulse duration is significantly shorter than the typical tissue capacitive charging time constant, resulting in a clearance requirement of a very high electric field amplitude and the linear charging evolution of capacitive tissue components similarly across many tissue types.
Here we report that, in contrast to the proprietary NPS™ treatments, the high repetition rate (1 -3 MHz) NPS™ treatments can achieve tissue clearance in packets of nanosecond pulses (high-repetition-rate packets of up to 200

nanosecond pulses) at a lower electric field amplitude (e.g. 8 kV/cm). Our results show successful clearance across multiple tumor types indicating a capacitive charging/discharging that takes much longer than the pulse duration and the off-time between adjacent pulses in the high repetition rate packet. The longer time constant of charging/discharging together with the lower electric field application results in additive charging of the capacitive components of the tissue during the whole duration of the packet. Moreover, we show that the consistently successful clearance with MHz repetition rate NPS™ (MHz-NPS™) treatment can be achieved with tissue-specific packet sizes. At the same and even higher energy levels, tissue clearance is not possible unless the packet size is above a tissue-specific threshold in length. This unique ability of MHz-NPS™ therapy to be able to deliver a fine-tuned electrical exposure that targets a specific tissue type can be helpful when a pathology creates a localized electrically-distinct environment with respect to the surrounding healthy tissue. We compare MHz-NPS™ exposures to equivalent microsecond (µs)-long pulses that provide an electrically-equivalent plasma membrane charging. We find the equivalent µs-pulse exposures are not able to provide the same tissue clearance effectiveness as MHz-NPS™ therapy. We discuss the intracellular membrane charging potentially contributing to the final biological effect since it is a result of the high-frequency components of the NPS exposures that are absent in µs-pulse exposures.

OR-11

Multicellular spheroids as three-dimensional in vitro models for bipolar cancellation assessment

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Unipolar high-intensity nanosecond pulsed electric fields (nsPEF) induce numerous bioeffects including regulated cell death [1] and cellular permeabilization through the formation of nm-sized pores [2]. However, when a second phase of opposite polarity is applied immediately after the first polarity (e.g., bipolar pulse), the effects induced by unipolar pulses are highly reduced [3]. This phenomenon, called bipolar cancellation, is typical for nsPEF and it has been reported on numerous two-dimensional (2D) cellular models for several pulse durations between 2 to 900 ns. Despite several hypotheses formulated, the mechanism behind is still unknown. However, it is of great interest to the bioelectric community because it would allow to potentially tailor electric pulses to modulate specific biological responses. In order to contribute to the understanding of this newly discovered phenomenon, we studied for the first time, the occurrence of bipolar cancellation in realistic three-dimensional (3D) multicellular spheroids. These in vitro models made of cancerous cells, allowed us to evaluate cellular permeabilization and viability upon nsPEF exposure. Spheroid tumor models, made with HCT-116 human colorectal carcinoma cells, were exposed to 10 ns (50 kV/cm) pulses. Different shapes (unipolar and bipolar), number of pulses (up to 500), and interphase delay of 100 ns between the pulse polarities were investigated using a high-voltage nsPEF generator that we have previously designed and characterized [4]. We showed that cell membrane permeabilization, as well as cellular death, occur when at least 100 pulses are applied. Both effects increased as a function of the number of pulses. Similarly, spheroid growth decreased with the increase of the number of pulses, with a complete growth inhibition observed following the application of 500 pulses. The application of bipolar pulses resulted in a significant reduction of the effects induced by unipolar pulses. However, the introduction of a delay of 100 ns between the two pulses' phases, resulted in a complete restoration of the unipolar effect. The results of this study suggest that bipolar cancellation might occur also within in vivo models. Further investigations with different pulses' shapes and interphase delays are needed to understand the mechanism behind nsPEF bipolar cancellation.

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OR-12

Negative Effects of Cancellation During Bipolar Nanosecond Electrochemotherapy

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Electrochemotherapy (ECT) with nanosecond pulses seems to be a logical evolution of currently established procedures based on microsecond pulse bursts. In this work, we studied the feasibility of high frequency nanosecond bipolar pulses for bleomycin and calcium electrochemotherapy.

As a model, A549 (human adenocarcinoma alveolar basal epithelial cells) and a multidrug-resistant H69AR human lung cancer cell lines were used. For electroporation, the bursts of 200 and 400 ns pulses (5, 7, 9 kV/cm) were delivered (n=50) with a repetition frequency of 1 MHz. Alternatively, the pulses were accompanied by a bipolar component (200, 400 ns) with or without delay (200 ns) between them. For detection of cell permeabilization, Yo-Pro-1 (YP) and flow cytometry were employed. Electrochemotherapy was performed with the same pulsing

conditions but with the addition of bleomycin (100 nM) or 2 mM CaCl2.
It was shown that the application of unipolar nanosecond bursts triggers low to high permeabilization depending on the pulse duration and amplitude, which is an expected result. The introduction of a bipolar component (i.e., symmetrical pulse 200 + 200 ns), results in the cancellation effect. Basically, even though the delivered energy is doubled, the cells become impermeable to YP due to rapid membrane depolarization by the opposite polarity pulse. If the pulse is asymmetrical (i.e., 400 ns + 200 ns), the cell permeabilization is still detectable but is significantly lower ($P<0.05$) compared to the unipolar procedure. A total of 13 unipolar/bipolar protocols with various combinations of pulse durations and delays were tested with three different amplitudes (5, 7, 9 kV/cm). In all cases when a bipolar pulse component was introduced, the same phenomena of bipolar cancellation were detectable. Further, the permeabilization results were supported by electrochemotherapy data with bleomycin or calcium in vitro. It was shown that the effects of electrochemotherapy are also canceled out when a bipolar component is present. The cell viability remains the same as in control samples, which were not treated by the pulsed electric field.
Our study shows that high-frequency unipolar nanosecond pulses result in successful electrochemotherapy and can be an excellent alternative to ESOPE procedures. However, the symmetrical bipolar nanosecond pulses have limited to no applicability due to the cancellation effect. In order to overcome the problem, the modulation of the time delay between the unipolar and bipolar pulse should be performed.

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OR-15

Electrochemotherapy Using Anticancer Drug Cocktail for Treatment of Drug-resistant Cancer Cells

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Electrochemotherapy (ECT) is the combination of electroporation and drugs with inhibited or limited transport. In this work, we study the electrochemotherapy with an anticancer cocktail containing a combination of bleomycin (BLM) with other drugs such as cisplatin (CIS), metformin (MET). The electroporation phenomenon's dependence on pulse amplitude and pulse repetition frequency was investigated. As a model, drug-resitant human lung adenocarcinoma cell line (H69AR) was used. For electroporation, 4 and 8 kV/cm, 500 ns square wave high voltage pulses were delivered to cells in 1 mm gap electroporation cuvette. Bursts of 100 pulses were delivered with a varied frequency of 1 kHz and 1 MHz. For detection of cell permeabilization, Yo-Pro-1 and flow cytometry were employed. Cell viability was evaluated 48-, 72- and 96-hours post-electroporation. As a reference, ESOPE (1.25 kV/cm x 100 µs x 8, 1 Hz) protocol was used.
Without electroporation, all three drugs involved in the study (or the drug cocktail) did not result in any significant cell viability changes. However, combination with electroporation triggered detectable electrochemotherapy. The ESOPE protocol resulted in 75%, 52% and 53% viability after 72 h for CIS, BLM and MET, respectively. The drug cocktail induced higher cell death ($P<0.05$) with up to 10% improvement. Further, the ESOPE protocol was compared with nanosecond protocols. MET was not effective as a chemotherapeutic agent when nanosecond sequences were involved. Only the highest intensity 8 kV/cm x 500 ns x 100, 1 MHz protocol showed statistically significant changes versus control. Bleomycin with the same protocol induced comparable cell death rate (43%) as the ESOPE protocol. Respectively, more than 60% of cells remained viable after nano-electrochmotherapy with CIS. The 8 kV/cm x 500 ns x 100 protocol but delivered with 1 kHz frequency significantly hindered the treatment even though the permeabilization rate was still above 90%. Similar results were observed for 4 kV/cm protocols indicating a dose and a frequency dependent tendency. Finally, the drug cocktail was used. With the best protocol (8 kV/cm, 1 MHz) the viability of the cells was 35%, which is a considerable improvement taking into account the multi-drug resistance of the selected cell line.
Our results show that application of drug cocktails in the context of electrochemotherapy can improve the response of the drug-resistant cancer lines. Also, high-frequency nanosecond electrochemotherapy can be as effective as ESOPE protocols, however, it's also dependent on the chemotherapeutic agent involved.

Acknowledgment: The research was funded by the Polish National Centre of Science of DAINA 2 (2020/38/L/NZ7/00342; PI: J. Kulbacka), and also supported by the Research Council of Lithuania grant (Nr. S-LL-21-4, PI: V. Novickij).

P26 - Electrochemotherapy – Internal Tumors

Thursday afternoon Track B
Oct 13, 13:30 - 15:00

OR-152

Intraoperative electrochemotherapy of colorectal liver metastases: Long term results of a prospective phase II study

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Background: A previous pilot study proved the feasibility, safety and efficacy of electrochemotherapy in the treatment of colorectal liver metastases. The aim of this study was to evaluate long-term results and safety of electrochemotherapy in the treatment of unresectable colorectal liver metastases.

Patients and Methods: In this prospective phase II study, patients with metachronous colorectal liver metastases were included. In all patients, at least one metastasis was unresectable due to its central location or a too-small future remnant liver volume. Patients were treated by electrochemotherapy using intravenously administered bleomycin during open surgery.

Results: 84 metastases from 39 patients were treated. The objective response was 75% (63% CR, 12% PR). The median duration of the response was 20.8 months for metastases in CR and 9.8 months for metastases in PR. The therapy was significantly more effective for metastases smaller than 3 cm in diameter than for larger ones. There was no difference in response according to the metastatic location, i.e., metastases in central vs. peripheral locations. Progression-free survival was better in patients who responded well to electrochemotherapy compared to those metastases that had a partial response or progressive disease. However, there was no difference in overall survival, with a median of 29.0 months.

Conclusions: Electrochemotherapy has proven to be safe and effective in the treatment of colorectal liver metastases, with a durable response. It provides local tumor control that enables patients with unresectable metastases to receive further treatments.

OR-153

Long term results of a prospective phase II study evaluating intraoperative electrochemotherapy of hepatocellular carcinoma

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The aim of this clinical study was to investigate the effectiveness and long-term safety as well as long-term results of electrochemotherapy as an emerging treatment for HCC in patients not suitable for other treatment options. A prospective phase II clinical study was conducted in patients with primary HCC who were not suitable for other treatment options according to the Barcelona Clinic Liver Cancer classification. A total of 24 patients with 32 tumors were treated by electrochemotherapy. The procedure was effective, feasible, and safe with some procedure-related side effects. The responses of the 32 treated nodules were: 84.4% complete response (CR), 12.5% partial response (PR), and 3.1% stable disease (SD). The treatment was equally effective for nodules located centrally and peripherally. Electrochemotherapy provided a durable response with local tumor control over 60 months of observation in 78.0% of nodules. The patient responses were: 79.2% CR and 16.6% PR. The median progression-free survival was 15 months (range 2.7–60), and the overall survival over 5 years of observation was 72.0%. This prospective phase II clinical study showed that electrochemotherapy was an effective, feasible, and safe option for treating HCC in patients not suitable for other treatment options.

OR-154

Bleomycin based electrochemotherapy using variable electrode geometry electrodes for the treatment of deep-seated soft tissue sarcomas

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Introduction: Bleomycin based electrochemotherapy (ECT) poses as an emerging technique not only in the treatment of superficial skin tumours, but also in case of advanced, metastatic and surgically inoperable deep-seated soft tissue sarcomas.

Patients and Methods: During a 2-year period (February 2019- February 2021) 7 patients (5 male/2 female) with median age of 54 years (49-88) were treated with deep seated, inoperable soft tissue sarcomas (STS) through bleomycin based electrochemotherapy at the University of Szeged Department of Surgery. Tumour histology included fibromyxoid sarcoma (n=2), epitheloid sarcoma (n=3), liposarcoma (n=1) and myofibroblastic sarcoma (n=1).

All treatments were performed under general anesthaesia, with the use of long needle VEG (variable electrode geometry) electrodes. Treatment planning for electrode placement was preoperatively carried out in each case by Pulsar software and confirmed with intraoperative ultrasound during the procedure. Each procedure was started 8 minutes after intravenous bleomycin administration (15000 IU/m2) and lasted for a maximum of 40 minutes. Prior to- and after treatment (1 week, 1-2-4-6 months) prospective data collection was carried out. Patient health status and QoL was assessed at each follow-up visit. Tumour response was evaluated through imaging (CT/PET CT/MRI) 2 months after ECT treatment as per RECIST 1.1 guidelines, adverse events were evaluated and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [3]. Previous

treatments included surgical resection (7/7 cases), chemoradiation (4/7 cases), radiation therapy (2/7 cases) and immunotherapy (1/7 case).
Results: Median operative time was 75 min (35-180), median hospital stay 2 days (2-20). Treatments were well tolerated with minimal side effects. Grade 2 ulceration was experienced in four cases, and a transient left musculus quadriceps femoris plegia occured in one patient. Median tumour diameter, tumour volume and tumour depth was 5.9 cm (3.7-22.5), 131.13 cm3 (35.6-2456.22) and 6.18 cm (3.74-18.18), respectively. Two month follow-up confirmed partial response in 5 patients (71.42%), while stable disease in 1 patient (14.28%), and progressive disease in 1 case (14.28%) as per RECIST v.1.1.
Conclusion: Local control of deep-seated STSs with BLM-based VEG ECT combined with other treatment options hold a promising perspective and our results may serve as a useful guide for further investigation and treatment planning.

OR-155

Calcium electroporation, an experimental cancer treatment – results from a pilot trial within advanced esophageal cancer

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Background: Calcium electroporation (CaEP) is a novel anti-cancer treatment where a high influx of calcium, induced by local injection of a calcium solution and locally applied reversible electroporation, leads to tumor necrosis. In this first-in-man trial, CaEP was administered to patients with advanced esophageal cancer. The primary aim of this exploratory study was to establish safety and feasibility.
Materials and Methods: Patients with advanced esophageal cancer, without other available treatment options, could be included. In an outpatient setting, all patients were put in general anesthesia. For the endoscopic procedure, we used a pulse generator (ePORE®, Mirai Medical) and a single-use probe with two parallel electrodes (EndoVE®, Mirai Medical) connected to a gastroscope. The calcium solution (Calcium gluconate, B.Braun 0.23 mmol/ml) was injected into the tumor followed by locally applied electrical pulses (bipolar pulses of 5000 kilohertz/1000 Volt). After treatment, adverse events, pain, and dysphagia were registered and all patients were followed with computed tomography scans and upper endoscopies for up to three months.
Results: Eight patients were treated. One serious adverse event (one-day hospitalization due to anemia, requiring a single blood transfusion) and four adverse events were registered including anemia, local pain, and oral thrush. Initially, five patients suffered from dysphagia, two reported dysphagia relief and three reported no change. From the imaging evaluation, one patient had a partial response, three patients had no response, and four patients had tumor progression during the follow-up period.
Conclusion: CaEP in patients with advanced esophageal cancer was conducted without major safety concerns. This study opens the way for larger studies evaluating tumor regression and symptom palliation.

OR-156

Electrochemotherapy of peri-hilar primary liver tumors

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Purpose: To evaluate long-term efficacy of Electrochemopherapy (ECT), of Hepatocellular Carcinoma (HCC) or Cholangiocarcinoma (CCC) infiltrating hepatic hilum (pH).
Material and Methods: We retrospectively reviewed data of 30 consecutive patients (20 M, 10 F; 43-85 year, mean: 62 year)with pH-HCC (n=19) or pH-CCC (n=11)treated with ECT. Tumors' diameter ranged: 2.5-7.5 cm (mean:4.1 cm). 63% (n=12/19) HCC had portal vein tumor thrombosis (PVTT). 66% (n=6/9) CCC with Klatskin tumor had preliminar percutaneous biliary drainage. 25 patients received US-guided percutaneous ECT ablation, 5 patients had ECT in open laparatomy, and were followed-up with contrast-enhanced-MDCT 4 weeks after treatment and every 6 months thereafter.
Results: No perioperative major complication occurred. 2/30(6.7 %) HCC patients dropped out follow-up because of experienced fatal hemorrhage from gastroaesophageal varices 4-5 weeks after ECT. 2 patients underwent Liver transplantation after successful ablation of portal vein tumor thrombus with ECT. Post-treatment CT showed an overall complete ablation in 17/28 cases (60%). Complete ablation was achieved in 14/17%(83%) HCC patients and in 3/11(27%) CCC patients. The follow-up ranged from 6 to 66 months (median: 24 months,). Follow-up CT showed local tumor recurrence in 15/17(88%) HCC patients within 6 - 18 months, and in 9/11 (81%) CCC patients within 3–12 months. After 6–66 months follow-up, 9/17 (53%) HCC patients and 2/11 (18%) CCC patients are still alive. Patients died for tumor progression , liver failure, gastrointestinal hemorrhage or cardiovascular failure
Conclusion: In our retrospective cohort, ECT showed encouraging efficacy for the local control of pH-HCC and was moderately effective for pH-CCC.

OR-157

Histologic changes of porcine portal vein anostomosis after electrochemotherapy with bleomycin

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Introduction: Locally advanced pancreatic cancer frequently invades portal vein and it has to be resected and anastomosed. We currently do not know the effect of ECT on vein anastomoses, therefore it cannot be used in this setting to potentially reduce local recidives. The aim of this study is to assess histologic changes of portal vein anastomosis after ECT.
Methods: Porcine model was chosen for the study as they have similar hepatobiliary anatomy and function as humans. In total 12 pigs were enrolled in the experiment. Study was approved by the national Ethics Commission for experiments on animals. After induction to anaesthesia, laparotomy was performed and inferior v. cava, v. portae and v. lienalis were identified. Then the anastomosis of portal vein (treated with ECT) and lienal vein (control vein) were made. Inferior v. cava was treated with ECT. We will observe histological changes over 7, 14 and 28 days after ECT.
Pigs were divided in 4 groups of three. First group was control group (electroporation only) and was sacrificed 28 days after ECT. Group 2, 3 and 4 were treated with ECT with bleomycin and sacrificed at 7, 14 and 28 days respectively. Electroporation was made with plate electrodes. We used 12 electric pulses with amplitude of 1040 V, 20 µs long and 0,5 Hz in frequency (IGEA S.p.A, Carpi, Italia). Eight minutes before electroporation, groups 2, 3 and 4 recived 15.000 IE/m2 of bleomycin.
Results: Out of 12 pigs, 3 of them had to be euthanized 1-3 days earlier because of laparotomy dehiscence and major postoperative hernias. It did not have impact on the results as 2 of the animals were in the 28 day group. Histological results were exceedingly homogenous. In 7 day group, we observed endothelial loss with signs of regeneration. Also, severe muscle cell loss and fibrosis was seen in tunica media. Thrombosed minor blood vessels were seen in tunica adventitia. In the 14 day group endothelium regenerated completely. More and more fibrosis organization were seen in tunica media on 14 and 28 day groups. Histological changes were comparable on intact vena kava and anastomosis of v. lienalis which was not treated with ECT. No thrombosis was evident in all animals. Histological findings did not differ from electroporation only and ECT groups.
Discussion: Our study showed that ECT of vein anastomosis is safe and feasible. Although ECT significantly diminished the muscular layer of veins, findings were comparable with untreated anastomosis which are made on a daily basis in operating rooms for variable reasons. Veins are a low-pressure system, therefore, any pseudoaneurysm formation is not likely. On the other hand, loss of muscular layer could possibly predispose in pseudoaneurysm formation if treating arteries. In our opinion, the risk of thrombosis should not be higher than in normal vein anastomoses, because of complete endothelial regeneration.
Conclusion: ECT of vein anastomosis is safe and feasible. Care should be taken if treating arteries.

P31 - Gene Electrotransfer for Antibody Production

Thursday afternoon Track C
Oct 13, 13:30 - 15:00

OR-78

Combined treatment of intratumoral DNA-based anti-CTLA4 antibody gene electrotransfer and irradiation for treatment of solid tumors

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Recombinant monoclonal antibodies (mAbs) are among the most promising classes of cancer immunotherapeutics. Since FDA's approval of the anti-CTLA-4 (cytotoxic T-lymphocyte–associated protein 4) antibody Ipilimumab for the treatment of melanoma, the field dramatically expanded and mAbs targeting several other immune checkpoints were developed. Immune checkpoint inhibitors (ICIs) are now widely used for treatment of different cancer types. However, mAbs have several drawbacks including high production costs, frequent high dosing, limited efficacy as single agents, and systemic toxicity following i.v. infusion. Systemic immune-related toxicity can be circumvented with intratumoral injection of mAbs, which, while retaining similar efficacy, still require frequent dosing. On the other hand, plasmid DNA (pDNA) encoding CI antibodies presents a promising alternative to the conventional mAb proteins. Gene electrotransfer (GET) which utilizes electroporation, a clinically-established technique, can be used to efficiently deliver pDNA to cells and tissues, including tumors. ICIs are especially effective in treatment of tumors with pre-existing anti-tumor immunity ('hot tumors'), with limited efficacy in tumors lacking anti-tumor immunity ('cold tumors'). Radiotherapy can be used to trigger the beginning of transition from cold to hot tumors, thus facilitating ICI action. To examine the therapeutic potential of combining intratumoral GET of ICIs and radiotherapy we performed GET of plasmid DNA encoding murine anti-mouse CTLA-4 mAb (p(aCTLA4)) and combine it with two irradiation regimes, a single dose of 10 Gy and fractionated dose of 3x 5 Gy in murine MC38 colon cancer. We first compared two previously established sets of GET pulse parameters: one for pDNA delivery to tumors (8 pulses, 600 V/cm, 5 ms, 1 Hz) and one for electrochemotherapy ((ECT), 8 pulses, 1300 V/cm, 100 µs, 1Hz). GET of p(aCTLA4) to tumors resulted in similar anti-CTLA4 mAb plasma levels regardless of the pulse parameters, however; due to a pronounced anti-tumor effect of control plasmid DNA (pCtrl) when delivered with pulse parameters for tumors, ECT pulse parameters were used in the rest of experiments. When GET of p(aCTLA4) was combined with irradiation, the combination with fractionated dose of 3x 5 Gy, which induces more immunostimulatory effects, had a more pronounced anti-tumor effect than the combination with single dose irradiation of 10 Gy. Moreover, only

the combination of fractionated irradiation with GET of p(aCTLA-4) outperformed better than the combination with pCtrl.

We confirmed that GET of p(aCTLA4) could be a potential alternative to systemic infusion of ICIs and when combined with irradiation, an anti-tumor effect can be achieved. Future research will focus on determining the optimal schedule for a "booster" GET of p(aCTLA4) after irradiation and also exploring the use of pDNA encoding other ICIs, to achieve a better anti-tumor effect.

OR-79

Building a genetic medicine platform for DNA-encoded antibody therapeutics

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DNA-based delivery aims to administer the antibody-encoding nucleotides using a non-viral plasmid DNA (pDNA) vector. Such genetic shortcut allows for prolonged antibody production in vivo, and addresses some of the bottlenecks of conventional antibody protein therapeutics. This can allow for a broader antibody accessibility and e.g. facilitate more effective cocktails. We previously presented preclinical proof of concept for various DNA-encoded full-length monoclonal antibodies (mAbs) and nanobodies, delivered to muscle or tumor.

To advance our DNA platform, we evaluated examples of product development. First, we focused on COVID-19. Using B cell mining in a convalescent patient, we identified a panel of human mAbs that potently neutralized the main SARS-CoV-2 variants of concern. The lead mAb 3B8 also retains its activity against omicron. In hamsters, intramuscular electroporation of DNA-encoded 3B8 resulted in median mAb serum levels up to 90 μg/ml ten days after pDNA delivery, and protected animals against SARS-CoV-2 infection. Second, we focused on immunotherapy by delivering a cocktail of DNA-encoded immunomodulators directly into the tumor. In a murine cancer model, intratumoral electroporation of plasmids encoding IL-12 and an anti-PD-1 and anti-CTLA-4 antibody significantly delayed tumor growth compared to IL-12 alone and the combination of anti-PD-1 and anti-CTLA-4 antibodies. The triple combination also enabled significant abscopal effects, which was not the case for the other treatments.

To assess translational feasibility, we previously reported on intramuscular DNA-based antibody delivery in 40-70 kg sheep, a clinically relevant model. Here, we further aligned and affirmed the electroporation setup to clinical practice. In groups of eight sheep each, escalating doses of pDNA (1 and 4 mg) gave serum mAb levels up to the μg/ml range, without inducing anti-drug-antibodies during the six week follow-up.

While the currently attained mAb titers are effective in various rodent disease models, we explored further improvements. In vitro, mAb levels consistently improved with decreasing sizes of plasmid backbone. In vivo, following intramuscular pDNA electrotransfer in mice, the correlation was less consistent. While improving biosafety, a reduction in size beyond a standard conventional plasmid backbone did not improve mAb levels in vivo. Cassette modifications, such as swapping antibody chain order or use of two versus a single encoding plasmid, significantly increased antibody expression in vitro, but failed to translate in vivo. Conversely, a significant improvement in vivo but not in vitro was found with a set of muscle-specific promoters compared to the ubiquitous CAG promoter. Despite the limited translation between in vitro and in vivo, improvements in potency and biosafety were identified.

In conclusion, our new findings contribute to a broadly and clinically applicable genetic medicine platform for DNA-based antibody therapeutics.

OR-80

Advancing DNA-based antibody therapeutics through evaluation and characterization of in vivo expression

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DNA-based antibody therapy seeks to administer the encoding nucleotide sequence, rather than the antibody protein. This innovative approach can allow the patient's body to produce its own medicine, and presents an alternative to the complex production and frequent administration of antibody protein. We previously obtained therapeutic proof of concept in mice for several plasmid-based (pDNA) antibody formats delivered via intramuscular electroporation, and demonstrated clinical translation in sheep. To further improve the in vivo monoclonal antibody (mAb) expression, a better understanding of what happens after the administration of the plasmids is required. The current PK profile of mAbs expressed in vivo in mice is typically characterized by a steady increase in the first week(s) reaching peak levels in plasma of 1-10 μg/mL. Thereafter though, in most cases, a steep decline of around 5 to 10-fold is observed. Ideally, plasma levels need to be maintained above 10 μg/mL for a longer time, in line with trough levels for most therapeutic mAbs. We aim to gain insight in the factors that affect mAb expression after intramuscular pDNA electroporation at DNA, mRNA, protein, and cellular level.

To evaluate the pDNA and RNA expression levels over time, BALB/c mice were treated with pDNA encoding the murine 4D5 mAb targeting HER2, delivered via intramuscular injection followed by electroporation. Following pDNA electrotransfer, biopsies and blood samples were taken at different timepoints (up to 3 months). pDNA and RNA was quantified using (reverse transcription) quantitative PCR. The RNA expression levels were on average stable, but there was a high inter-animal variation. In contrast, a fast decline of pDNA was observed, i.e. 24h after treatment the pDNA levels were <1% of the injected amount. However, the fast and substantial loss of

pDNA does not correspond with the typically observed continuing protein detection, up to one year after pDNA injection. We hypothesize that only a very small amount of the administered pDNA is responsible for the observed protein expression and that the pDNA that is lost within the first 24h did not enter the cell and/or nucleus. To test this hypothesis, muscles isolated from treated animals were sliced and used in an RNA scope assay to localize the pDNA in the muscle cells. Preliminary results indicate that the pDNA is localized mainly at the injection site. Therefore, only a small amount of muscle cells will be part of the protein factory.
In conclusion, this study investigates some fundamental aspects providing more insights about the efficiency of the DNA-based antibody delivery and the factors that can play a role in the obtained protein levels. All methods generated in this study can be used as a toolbox to evaluate other plasmid constructs or delivery methods with the ultimate goal to achieve a robust and prolonged antibody expression.

OR-81

MYO Technology for DNA-Based Delivery of Next-Generation Antibody Therapeutics

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As a class of drugs, antibodies have soared in both clinical development and use in the last decade. There are now more than 100 approved antibodies in the United States and Europe, for use in diverse disease areas from arthritis and breast cancer to osteoporosis and migraines. In addition, though long considered to have great potential within the infectious disease research community, the larger world has now been exposed to the utility of antibodies for the treatment and prevention of viral diseases as part of the efforts to combat the COVID-19 pandemic. Despite the broad applicability of antibody-based drugs, their more widespread use is limited by high manufacturing costs and administration hurdles. Many antibodies are delivered via time-consuming intravenous infusions and others, regardless of administration route, need to be dosed at regular 2-to-4-week intervals. Another drawback of many biologics, also highlighted by the distribution and storage of antibodies and vaccines for COVID-19, is the requirement for maintenance in a low temperature environment prior to use. Such cold-chain dependencies can significantly hinder the ability to get therapeutics to large numbers of people, a problem that is amplified in resource poor settings. MYO Technology™, a DNA-based platform for the delivery of therapeutic proteins, was developed to overcome these barriers to increased antibody use. DNA is easier to manufacture than antibodies and lacks most cold chain requirements. Additionally, administration using MYO Technology takes just a few minutes, and therapeutic levels of antibody can potentially be maintained for months, in contrast to the weeks-long durability when administered by standard methods. The MYO Technology platform consists of antibody-encoding plasmid DNA (pDNA) and a proprietary medical device for the intramuscular injection of pDNA, followed by the delivery of very short electrical pulses to the muscle tissue surrounding the injection site. These pulses promote the in vivo transfection of muscle cells, resulting in antibody production and secretion, and ultimately uptake into peripheral circulation. Animal proof-of-concept studies demonstrate that these in vivo-produced antibodies are functional and demonstrate efficacy in a diverse array of disease models, including cancer, inflammatory disease, and infectious disease. Through significant improvements in manufacturing, distribution and storage, administration time, and administration frequency, MYO technology has the potential to dramatically improve the accessibility and usage of antibody drugs.

OR-169

Mouse, canine and human interleukin-12 antibiotic resistance gene-free plasmids: bacterial maintenance and gene electrotransfer efficiency

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Background: Gene electrotransfer (GET) of plasmids encoding the cytokine interleukin 12 (IL-12) is currently approaching clinical use for treatment of various superficial solid tumors. In this study, we present the preparation and testing of antibiotic resistance gene-free plasmids encoding mouse, canine and human IL-12, which comply with the EU regulatory requirements that recommend against the use of antibiotics during the production of clinical grade plasmids. The mouse orthologue was prepared to facilitate preclinical evaluation in mouse tumor models, canine for cancer treatment of client-owned dogs, and human for translation into human clinical testing. Our aim was to evaluate the maintenance of these plasmids in a bacterial culture and test their transfection efficiency after GET to melanoma cells.
Methods: Plasmids encoding mouse (pORF-mIL-12-ORT), canine (pORF-caIL-12-ORT) and human IL-12 (pORF-hIL-12-ORT) were prepared using the antibiotic-free selection strategy operator-repressor titration (ORT®, Cobra Biologics), and all have the same backbone without an antibiotic resistance gene. A commercially available plasmid encoding a mouse IL-12 (pORF-mIL-12 (p40p35), Invivogen), with an ampicillin resistance gene in the backbone was used as a control. Plasmid maintenance was evaluated by determining plasmid yields and topologies after sub-culturing of transformed bacteria. Transfection efficiency was evaluated by determining the plasmid copy number, expression and cytotoxicity after GET to mouse (B16-F10), canine (CMeC-1) and human (SK-Mel-28) melanoma cell lines.
Results: Plasmid isolation yields of antibiotic resistance gene-free plasmids were on average lower than that of the commercial IL-12 plasmid. However, the yields remained constant after sub-culturing, confirming that these plasmids are stably maintained in transformed bacteria during cell division, and are not lost over generations. After GET to melanoma cells, the number of plasmids detected

in transfected cell was relatively low independently of the plasmid used (from 2 to 36 copies per cell). Nevertheless, IL-12 expression from all four plasmids was detected in all three cell lines, confirming that even 2 copies per cell are sufficient for transgene expression. The IL-12 mRNA expression levels did not correlate with the plasmid copy number.
Conclusions: We showed that the tested antibiotic resistance gene-free IL-12 plasmids are stably maintained in bacteria and support sufficient IL-12 expression after GET in vitro. Therefore, these plasmids have the potential to proceed to in vivo evaluation and, ultimately, translation to clinical studies in Europe enabled by the absence of the antibiotic resistance gene. However, before translation, plasmid production still needs to be optimized to ensure improved plasmid yield, quality and, above all, standardization of the final antibiotic-free product in good manufacturing practice conditions.

P5 - Mechanisms and Applications of PEF in the Food Industry

Thursday afternoon Track D
Oct 13, 13:30 - 15:00

OR-194
Modelling and validation of the electrochemical phenomena at the electrode-solution interface of a PEF treatment chamber

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In PEF processing the food matrix is typically placed in direct contact with charged metal electrodes of either batch or continuous treatment chamber and exposed to repetitive (up to kHz) short duration (s-ms) electric field pulses of low (0.1-1 kV/cm), moderate (1-5 kV/cm) or high intensity (10-40 kV/cm), supplied by a high voltage pulse generator.
However, when these typical conditions for PEF processing are applied, undesired electrochemical reactions, especially those involving metal release from the electrodes, unavoidably occur at the electrode-solution interface of a PEF treatment chamber. The occurrence of these electrode reactions is a very complex phenomena, which is affected by several factors, such as PEF chamber design and electrode material, PEF electrical parameters, as well as composition and chemical-physical properties of the treated products.
For this reason, numerical simulations could significantly help to improve the understanding of the electrochemical phenomena occurring at the electrode-food interface of a PEF chamber, by clarifying the effects of the main electrical parameters and food constituents on electrode corrosion or release of electrode's materials.
In this work, a Multiphysics model based on the Finite Element Method (FEM), was developed to predict the effect of different electrical parameters as well as treatment medium characteristics on the metal release from the stainless steel (type AISI 316L) electrodes of a PEF chamber. Different processing conditions obtained by coupling different field strength (12–31 kV/cm) and total specific energy input (20–100 kJ/kg) were simulated with two model liquid food solutions with different values of pH (3.5 and 7) and electrical conductivities (2 and 3.5 mS/cm) and different halides concentration chloride concentration (0 - 0.0201 mol/L). A validation of the predicted results was achieved by using experimental data on metal release measured with ICP-MS technique.
The results showed that the developed model was able to accurately predict the extent of metal release from the electrodes of a PEF chamber. The concentration of metals released from the electrodes depends on the process parameters of PEF treatment (field strength, energy input) with a key role being played by the pulse repetition frequency. Moreover, regarding the treatment medium characteristics, the presence of halides, high conductivity, and low pH, significantly affect the release of metals. Specifically, it was shown that higher electrical conductivity lead to higher current passing through the chamber promoting the metal release. On the other hand, lower pH values increase the faradaic current density, thus increasing build-up of charge at the double layer, leading to higher release of metals.

OR-94
Effect of post-electroporation recovery of Thai basil leaves prior convective and vacuum drying

Grant Thamkaew, Allan G. Rasmusson, Dmytro Orlov, *Federico Gómez Galindo*
Lund University, Sweden

Pretreatment by reversible electroporation followed by resting (storage under saturated moisture at 21 ± 2 °C) was evaluated for modification of the properties of dried and rehydrated Thai basil leaves. The treated leaves were dried by convection at 40 °C or in a vacuum at room temperature. The results showed that vacuum drying provoked more cell damage and tissue collapse than convective air drying at a moisture ratio (MR) of 0.2 and 0.1. Under this level of MR, the pulsed electric field (PEF) and resting pretreatment exerts a protective effect of the tissue for both drying methods. However, under complete dehydration (water activity, aw = 0.05) damage seems to be similar for both drying methods despite the PEF pretreatment. Remarkably, reversible electroporation followed by resting resulted in higher trichome preservation. At MR of 0.05, the area of trichomes on the surface of convective-dried, PEF-rested and fresh samples were not statistically different at 2267 ± 89 μm2 and 2218 ± 65 μm2, respectively, showing that this pretreatment still exerts a protective effect on trichomes when complete dehydration is achieved.

OR-92
Effect of pulsed electric field (PEF) intensity on separated cream yield, physico-chemical properties and stability

Markus Walkling-Ribeiro, Thomas Jacob, Lilia Ahrné
University of Copenhagen, Food Science, Denmark

Pulsed electric field (PEF) technology has the potential to be an effective emerging technology for the modification of structural components in dairy food, thereby, opening the door to new applications and, typically, retaining the native character of the latter. In this study the impact of PEF intensity on fat separation from raw milk after centrifugation was investigated in terms of yield, physico-chemical properties and physical stability of the produced cream.
Raw milk was kept at 4°C prior to PEF treatment. The latter was carried out with a continuous-flow co-current treatment chamber using electric field strengths ranging from 9 to 27 kV/cm for a treatment time of 66 µs. The PEF-treated milk was then heated to 40°C before being skimmed, allowing for subsequent cream analyses using a Milkoscan (yield and fat content), Mastersizer (particle size), and high-shear mixer (overrun after 20 and 60 s) and centrifugation (stability after 20 and 60 s).
Yield of cream increased significantly ($P<0.05$), for milk PEF-treated at 21 kV/cm and below considering the total amount of fat removed from the skimmed milk. However, no significant differences were observed for the fat content when comparing cream from untreated and PEF-treated milk and for the particle size of its milk fat globules (P 0.05). The PEF treatments also influenced the overrun of the produced cream, with the most intense treatment at 27 kV/cm decreasing the overrun capacity, whereas the mildest treatment at 9 kV/cm increased it by 30% compared to cream that was not treated with PEF ($P<0.05$) at 20 s. At 60 s an increased overrun was observed from 9 to 21 kV/cm, achieving an overrun up to 65% greater for the least intense PEF treatment conditions than for untreated cream ($P<0.05$). Moreover, the stability analyses of the cream showed a stronger cohesion induced by the most intense exposure to PEF.
PEF processing of raw milk at different intensities and subsequent milk separation for cream production, led to a higher yield in cream, while not impacting fat content and particle size. Based on the different PEF intensities applied, cream overrun and stability could be tailored to industrial applications.

OR-193

Pulse electric fields-assisted steam peeling of different fruits and vegetables

Gianpiero Pataro, Oscar Mauricio Pulgarin Lopez, Giovanna Ferrari
University of Salerno, Italy

Peeling is the first unit operation performed during the industrial transformation of fruit and vegetables, whose performance is crucial for maximizing the efficiency of overall process, while preserving the quality of the fresh product.
Current industrial methods of tomato peeling involve the use of hot lye (sodium hydroxide) solution or pressurized steam, which provide high quality peeled tomato fruits. However, both these methods suffer from various disadvantages such as disposal of caustic, high pH waste solution, and excessive water and energy consumption.
For these reasons, research is focusing towards new sustainable peeling alternatives that can effectively peel fruits and vegetables with minimum peeling losses and a better quality of the product, while providing less environmental problems and reducing water and energy consumption. In the recent years, the application of unconventional technologies as a pre-treatment for the peeling, such as infrared radiation heating, ohmic heating, ultrasounds-assisted lye peeling, pulse electric fields-assisted steam peeling, as well as the use of enzymes, is intensively investigated.
The aim of this study, which was in part carried out in the frame of the European project AccelWater (Project ID: 958266), was to assess the potential of PEF technology to facilitate the steam peeling of different fruits, including tomatoes, peaches and apples. Samples were exposed to PEF treatment of different intensity in terms of field strength (0.25-1 kV/cm) and energy input (0.25-1 kJ/kg), before steam peeling (1-3 bar). The impact of PEF on the peelability of fruits was assessed by measuring the change in the textural properties (hardness, peel strength) of the fruits, as well as evaluating the peeling index, peeling loss, and weight.
Results obtained from this study demonstrate the potential of PEF pre-treatment to reduce the peel strength and to induce high score of peeling ability for of all the fruits that led to less product loss. In particular, regardless the type of processed fruit, the best results were obtained for intermediate values of PEF treatment intensity (0.5 kV/cm, 0.5 kJ/kg). Moreover, as compared to the conventional steam peeling method, the application of PEF pre-treatment enabled to reduce the steam pressure during the thermophysical peeling phase by about 20% for tomatoes, 33% for apples, and 25% for peaches, thus reducing the consumption of energy and water.

OR-93

Combination of different techniques for assessing PEF-treatment in plant and animal tissues

Jessica Genovese[1], Matej Kranjc[2], Igor Serša[3], Pietro Rocculi[1], Damijan Miklavčič[2], Samo Mahnič-Kalamiza[2]
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[2]University of Ljubljana, Faculty of Electrical Engineering, Slovenia
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In the field of food innovation, pulsed electric field (PEF) technology has attracted increasing interest in recent years and has become one of the most attractive new non-thermal technologies due to its lower energy consumption and short treatment times. Although there is a considerable body of scientific work available, detailed information on detection and quantification of the effects of electroporation in complex and inhomogeneous multicellular systems, such as real food systems, is still limited. The effectiveness of PEF treatment depends on both process parameters and properties of the biological material, and the appropriate choice of methods to detect and assess changes caused by electroporation in food matrices is needed. A deeper insight into the changes that occur after electroporation and their relation to the complexity of the food matrices tested is of great value to develop new ideas for electroporation-based treatments in the food industry.

Therefore, different characterization techniques were combined, namely electrical impedance spectroscopy, current-voltage measurements, and magnetic resonance imaging to evaluate the physical changes caused by PEF treatment in raw plant and skeletal muscles of interest for food and/or feed. Experiments were performed on potato tuber and apple fruit, as these plant tissues are most commonly used for industrial PEF applications, and on the chicken broiler Pectoralis major that was selected as reference skeletal muscle. Electrical impedance spectroscopy was used to measure the dielectric properties of the biological tissue before and after the application of pulses, as this is a common technique in food PEF applications to determine the degree of cell disruption. Another possible but rarely used technique is to analyse the voltage and current waveforms recorded during the application of electrical pulses. Analysis of electric current signals allowed us to detect changes in electrical properties of the cell membrane in real time. Advanced MR techniques were the third technique used to monitor the spatially-dependent effect of PEF treatment. In particular, the transverse relaxation time T2 provided evidence of the redistribution of water and solutes in the tissue as a function of the applied electric field during PEF treatment. The results of this study contribute to a better understanding of the effect of electroporation in complex and inhomogeneous multicellular systems, and provide important insights and calls for critical choice of electroporation assessment methods to optimize PEF treatment conditions.

POSTER PRESENTATIONS' ABSTRACTS

Poster Session (and Coffee break)

Monday Poster Session Track
Oct 10, 14:45 - 16:00

PO-001

A Review of Electronic Technology for Medical Applications of Electroporation

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Electroporation is based on the permeability increment of cell membranes by means of the application of electric field pulses to induce controlled potential in the cell membranes. For this purpose, it is necessary to develop electronic technology capable of applying and controlling electric field pulses. In the area of electroporation for medical applications the requirements are challenging, since it is necessary to apply electric field pulses in a wide range of intensities, and these must be controlled precisely to induce the desired effect.

This paper presents the basic concepts and a review of the state-of-the-art of electronic technologies involved in medical applications of electroporation, including electric-field pulse generators for the delivery of high-voltage electric pulses, electroporation electrodes to control electric field, and electroporation monitoring and control systems to optimize treatment delivery.

Firstly, in the field of electroporation generators, it is essential to design technology that allows to apply voltage waves in the most controlled and precise way. Nowadays, the voltage pulse is the most widely used waveform in medical electroporation application and, there are already a few commercially certified available generators. Despite this, requirements of electroporation treatments are becoming increasingly challenging, and a wide range of experimental electroporation generators have been proposed. These are based on multilevel electronic structures, transformer topologies, resonant circuits, and multi-output systems, among others.

Secondly, the electrodes are key elements in the electroporation process to generate the required electric field. They may also have built-in sensors to send information to the control system and optimized geometries to create special electric field patterns. Currently the most widely used are those based on needles or parallel plates, but there are many novel proposals including mono-electrode devices and multi-electrode implementations.

Finally, medical applications of electroporation require control systems that allow to carry out effective and safe treatments. Ideally, such systems allow to control the applied electric field to achieve the desired effects, depending on the electroporation medical application. For these reasons, it is necessary to develop monitoring and control technologies that allow to know and control the treatment status. Nowadays, the most widespread methods of control are based on the preplanning of treatments by means of finite element models and on-line monitoring of impedance and temperature, although more innovative proposals are being proposed in this field.

The final version of this paper will include the references that allow to study the current electronic technology for medical applications.

PO-016

Membrane extracellular vesicles released after electroporation as mediators for melanoma-fibroblasts communication

Anna Choromańska, Urszula Szwedowicz, Jolanta Saczko, Julita Kulbacka

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Analyzing the electroporation (EP) phenomenon and the objective complexity of the effects at the cell level associated with it, we came across a problem that has not yet been investigated to increase electrochemotherapy's therapeutic effectiveness (ECT). In the context of ECT optimization, we aim to isolate the extracellular vesicles (EVs) released during the different reversible EP procedures. Up to now, the exact nature of that EVs is unknown. It is also not clear how the profile of the released EVs depends on the pulse duration (nano-, micro-and millisecond pulses). Today, we know that EVs are essential mediators of intercellular communication, enabling the functional transfer of bioactive molecules from one cell to another. Melanoma-derived exosomes stimulated cancer cell proliferation, mediated the epithelial-mesenchymal transition, and induced pre-metastatic niche formation [Hatanaka et al. 2014].

This study investigated the EVs-mediated transformation of normal fibroblasts to tumor-associated fibroblasts, focusing on the functional regulation of vascular cell adhesion molecule-1 (VCAM-1) expression, cell viability, and migration capacity. The experiments were performed on human primary fibroblasts and two commercial malignant melanoma cell lines: A375 - primary human skin malignant melanoma cell line with endogenous BRAFV600E mutation, and Me45 – human metastatic malignant melanoma cell line. Electroporation with a low-intensity electric field (with a range of 800-1600 V/cm) was delivered by a BTX square wave generator. The Presto Blue test was applied to assess cell viability. Cellular migration was analyzed with the wound healing assay, and the VCAM-1 expression in fibroblast cells was determined by Real-Time PCR after 72 hours of incubation.

We observed that after incubation with melanoma-derived EVs, the primary human fibroblasts presented differences in cell viability, migration capacity, and VCAM-1 expression, dependent on EP parameters and the degree of melanoma cells malignancy.

Financing: This study was supported by funding from the Department of Molecular and Cellular Biology, Wroclaw Medical University grant no.SUBZ.D260.22.016.

References: Hatanaka M, et al. Cleaved CD147 shed from the surface of malignant melanoma cells activates MMP2 produced by fibroblasts. Anticancer Res. 2014,

34, 7091-7096.

PO-019

Non-invasive real time analysis of electroporation phenomenon of individual cells

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Background: Electropermeabilization (EP) is a promising approach for the targeted treatment of diseases such as cancer. This technique allows the transient pores formation in the cell membranes, facilitating the access of therapeutic agents such as bleomycin and cisplatin in the case of electrochemotherapy (ECT).
However, while the efficacy and safety of EP have been demonstrated, questions remain and hinder its use. The differences in response between healthy and cancerous cells and the non-response of certain cancerous cells are still unknown.
The current study aims at proposing an early detection of responses of healthy and cancerous cells, untreated or treated by ECT or by different types of EP (reversible, irreversible, calcium), using the microwave dielectric spectroscopy (MDS) technique for cellular and tissue analysis.
Methods: To carry out this study, we perform both on-chip EP experiments and dielectric sensing in miniature devices. This method has the advantages of non-invasive testing, with fast test times and rapid diagnosis, while requiring small amounts of reagents and limited applied voltages to reach high electric fields.
Among the different existing analysis methods, MDS proves to be an innovative approach to discriminate different cell types and states, while meeting the on chip requirements. Indeed, for the microwave range, the cell membrane becomes transparent, allowing the waves to reach the cell contents. The MDS method, adaptable from single cell to tissue scales, allows to obtain the dielectric properties of the sample in a non-invasive and non-destructive way, in real time and directly in the culture medium [1].
Results: Single cell analysis is of paramount importance for a better understanding of the effects of EP at larger scales. We measure the electrical signature of single cells isolated using a microfluidic device allowing to co-integrate EP and MDS. First, we calibrate the applied field for in-situ EP at the single cell level. We evaluate the different types of EP in a static manner. Second, in order to investigate the dynamics of EP within individual cells, we carry out real-time analyses.

References:
[1] A. Tamra, "Spectroscopie diélectrique HyperFréquence des cellules biologiques soumises à l'électroporation," Theses, Université Toulouse 3 Paul Sabatier, Mar. 2017. [Online]. Available: https://hal.laas.fr/tel-01499406

PO-023

The efficacy of microsecond electric pulses with calcium ions is related to the expression of drug resistance genes and proteins

Nina Rembiałkowska[1], Vitalij Novickij[2], Dagmara Baczyńska[1], Magda Dubińska-Magiera[3], Wojciech Szlasa[1], Jolanta Saczko[1], Julita Kulbacka[1]

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Drug and multidrug resistance (MDR) in cancers is still the unsolved problem of anticancer protocols. Various MDR inhibitors, modulators, or nanocarriers are used to omit this phenomenon. Here we propose a physical method that employs short electric pulsed to increase the efficacy of cytostatics in drug resistant cancers. The main purpose of the study was to investigate whether various electroporation parameters can modulate the expression of drug resistance-related genes. We tested different parameters of electroporation (1 and 1.5 kV/cm, 8 pulses, 60 and 100 us square wave voltage, 1Hz) without and with Ca2+ (CaEP – calcium electroporation). The human cancer cell lines: sensitive and resistant, were tested. For detection of cell permeabilization, Yo-Pro-1 and flow cytometry were employed. Cell viability was evaluated 72-hours post-electroporation. The confocal laser scanning microscope (CLSM) method was used for Cadherin® and CellMask™ Deep Red imaging. Western blotting was used for the analysis of multidrug resistance (MDR) protein expressed after electroporation without and with Ca2+. MDR genes were analyzed by RT-PCR method. The obtained results confirmed that electroporation permeabilizes the cell membrane and enables the effective delivery of molecules. Moreover,CaEP originates a significant reduction in cells viability, and changes the expression of genes related to drug resistance. It was also determined that electroporation with calcium ions induces significant reorganization of cadherin after exposure to electroporation with Ca2+.
Concluding, we can state that CaEP can be an alternative tool to treat cancers demonstrating drug resistance. However, further investigation is required.

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PO-026

Electroporation of excitable cells studied with genetically engineered HEK cells

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Most electroporation-based treatments directly or indirectly target excitable cells such as muscle and nerve cells. This may lead to undesirable side effects (muscle twitching, adverse effects on tissues adjacent to the electroporation area). Therefore, it is of great importance to

study how excitable cells, especially their voltage-gated ion channels, respond to electric pulses and how this affects membrane permeability, excitability and function of excitable cells. We used a genetically engineered human embryonic kidney cell line HEK-293T for the study. This cell line is characterized by stable expression of voltage-gated sodium channels (NaV1.5) and has a Tet-on system for doxycycline-induced expression of inwardly rectifying potassium channels (Kir2.1). This makes these cells a simple model for excitable cells with minimal complement of sodium and potassium channels required for cellular excitability. We were able to trigger single or multiple action potentials in excitable HEK cells with single 100 µs electric pulses. We were also able to detect depolarization in nonexcitable HEK cells (cells lacking Kir2.1 channels) as a result of electroporation.
Depolarization and action potentials were monitored optically under a fluorescence microscope using a fluorescent potentiometric probe Di-4-ANEQ(F)PTEA with a sampling time of 36 ms. To quantify how pulses of increasing amplitude influence the transmembrane voltage changes and the ability of cells to trigger action potentials, we analyzed the fluorescence images with a custom code in Matlab. Most representative membrane regions were selected by thresholding the images, then the fluorescence signal was corrected for photobleaching. This allowed automatic extraction of several time-dependent parameters from the experimental conditions, such as the time from pulse delivery to action potential and the time to recovery of the fluorescence signal toward baseline fluorescence. Overall, we find excitable and nonexcitable engineered HEK cells a valuable tool for studying the effects of electric pulses on excitability and electroporation of excitable cells as well as ion channels.

PO-030

Efficacy of shock waves and expansion waves generated by nanosecond pulsed electric discharges for permeabilizing cells and delivering drugs

Ryuichi Nakajo, Nushin Hosano, Ryosuke Inoue, Takashi Sakugawa, Hamid Hosano
Kumamoto University, Japan

Clinical application of shock waves, as a completely non-invasive procedure, has been expanded during the past decades. Recent investigations have revealed further interesting therapeutic and diagnostic potentials of shock waves. While the effects of shock waves on cells and tissue have been extensively studied, little is known about the effects of pulse (short duration, high intensity) expansion waves. Furthermore, it is imperative to have knowledge about effects of pulse expansion waves on tissue, as focused shock waves are always followed by an expansion wave, and shock wave reflects as an expansion wave from low acoustic impedance tissue, like fat. Meanwhile, tensile stress is more effective than compression to permeabilize/porate cells, which makes expansion waves further interesting. In this study, pulse expansion waves were produced by reflection of shock waves from a thin air layer in an innovative setup. Shock waves were produced by nanosecond pulsed electric discharge. Shock waves, expansion waves, and cavitation behind expansion waves were visualized by schlieren and shadowgraph techniques using an ultra-high-speed framing camera. The pressure of shock and expansion waves was measured by a fiber optic probe hydrophone (FOPH) pressure transducer. U937 cells, human histiocytic lymphoma cell line, were used in an in-vitro setup to evaluate the survival rate, number of viable cells, cell growth, cells diameter and morphology, and propidium iodide uptake, after exposure to shock waves and expansion waves. The results show enhanced permeabilization/poration effects of expansion compare to shock waves.

PO-007

New high frequency electroporation protocols for human and veterinary medicine applications

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For more than 20 years, electroporation has been developing in the field of cancer and is becoming increasingly common in the treatment of skin cancers. By increasing the cytotoxic effect of anti-tumor drugs (bleomycin, cisplatin), electrochemotherapy has already proven its effectiveness on tumors in human medicine but also in veterinary practice. However, this treatment requires locoregional or even general anesthesia, as electrical pulses can be painful and cause muscle contractions. Several publications proved that application of high frequency pulses (5,000 Hz) resulted in much less discomfort to the patient than the 1 Hz protocol used in the clinical practice in the 90's and 2000's.
In order to maintain the effectiveness of the treatment and reduce the pain associated with contractions, we are developing new protocols using a high-frequency generator, and new multipolar electrodes. Our ongoing results obtained on a colorectal cancer cells line (HCT-116) cultured both 2D and 3D (spheroids) showed a clear effect on cell permeability assessed by propidium iodide uptake. Using our new protocols, the permeability rate was around 90 % and the viability rate of 95 ?tween 1 kV/cm and 1.5 kV/cm on cell suspensions with isoenergy tests. These results were compared to ESOPE protocol (8 pulses of 1,000 V/cm lasting 100 µs at 1 to 5,000 Hz) showing a permeability rate of 97% and a cell viability around 90%. Therefore, our high frequency electroporation protocols appear promising for an effective cell permeabilization with a low mortality rate with similar temperature increase than ESOPE protocol and without noticeable muscular contraction.

PO-038

Pulsed electric field pasteurisation of orange juice: Inactivation of Escherichia coli and native microflora and impact on product quality

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In the last years, consumer preferences have shifted towards high-quality minimally processed foods with an extended shelf life and prioritizing food products with a clean label and without the addition of preservatives. In order to obtain such products, the thermal stress on the food has to be kept low, to preserve its nutrients and sensory attributes. For this purpose, pulsed electric field (PEF) treatment has emerged as a non-thermal preservation method, especially due to its suitability for food pasteurisation. Therefore, the aim of this study was to investigate the efficacy of PEF for orange juice pasteurisation by assessing microbial inactivation and quality retention. For this purpose, the inactivation of Escherichia coli DSM 1116 (E. coli) and the natural microflora (predominantly different yeast strains) by PEF was evaluated. For the further trials, process parameters were selected that caused a 5 log reduction of E. coli (11.3 kV/cm, 250 Hz, 403 kJ/kg). A second series of PEF experiments with lower pulse repetition frequencies and specific energy input levels (120 Hz, 182 kJ/kg) was carried out, aiming to inactivate the native microbial flora in the juice under more gentle process conditions. After the treatments, the orange juice was stored in plastic bottles at 4 °C and 20 °C for eight weeks. Additionally, thermal pasteurisation (80°C, 1 min) and high pressure processing (HPP; up to 600 MPa, 2 min) were chosen as reference processes, representing established thermal and non-thermal preservation methods. Both PEF process designs resulted in an extension of shelf-life at 4 °C (up to 21 days) compared to the native juice (7 days) but did not remain microbiologically stable throughout the entire storage period. At storage conditions of 20 °C, both PEF treated juices and the native juice showed a comparable shelf-life of less than 7 days. PEF treatment conditions featuring lower pulse repetition frequencies (120 Hz) resulted in a comparable product quality (soluble solids, cloud stability, vitamin C, colour, pH) to conventional thermal pasteurisation and HPP. However, higher pulse repetition frequencies (250 Hz) resulted in higher energy input levels (up to 403 kJ/kg) and treatment temperatures (up to 68 °C) which led to a significant loss of juice quality, mainly degradation of vitamin C and changes in colour. Therefore, it can be stated that the pasteurisation of orange juice by means of PEF has distinct potential as a non-thermal pasteurisation method for orange juice. However, further research is still needed for the product-related optimization of the process design, in order to be able to guarantee microbial stability as well as quality retention throughout the whole desired storage period.

PO-041

Effects of pulsed electric fields on technological properties and dietary fiber content of carrot pomace

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Large amounts of vegetable wastes are generated in juice production industry. Among them, carrot pomace is an excellent source of dietary fiber (DF). However, the high content of insoluble dietary fiber (IDF) of carrot pomace makes difficult its incorporation into food products. Therefore, there is a great interest in modifying its structure for increasing the content of soluble dietary fiber (SDF) and improving the technological properties. Pulsed electric fields (PEF) is a non-thermal processing technology, which is able to modify the structure and properties of different biomolecules. The aim of this study was to evaluate the effect of PEF on the technological properties and DF content (SDF and IDF) of carrot pomace.
PEF treatments with different electric field strengths (5-15 kV cm-1), and different number of pulses (5-75) were applied on carrot pomace (Daucus carota cv. Nantes). The PEF-treated pomace was freeze-dried, crushed and sieved (0.3 µm-mesh sieve). Then, water retention capacity (WRC), oil retention capacity (ORC), cation exchange capacity (CEC), water swelling capacity (WSC), stabilizing and emulsifying capacity, solubility and DF content (SDF and IDF) were evaluated.
The pomace treated with 5 pulses of 15 kV cm-1 (1.17 kJ kg-1) showed a significant increase ($p<0.05$) in the SDF content (20.9%), WRC (8.0%), ORC (8.1%) and solubility (59.8%), while the IDF content was lower (15.4%) than that of the untreated pomace. Stabilizing and emulsifying capacities, CEC and WSC were not affected, regardless the treatment applied. Obtained results demonstrate that PEF treatments could induce modifications in the configuration and structure of the DF molecular chains. These changes could cause an increase in the content of the soluble fraction and, consequently, an improvement in its solubility and its ability to retain water and oil. The reduction in IDF content by PEF treatment was likely due to modification of the cell-wall structure of pomace where degradation of the insoluble fraction generally occurs.
PEF could be considered a promising technology to enhance the technological properties and to increase the SDF content of carrot pomace, thus facilitating its incorporation as a functional ingredient in processed foods.

PO-043

Comparison of extraction of bio-molecules assisted by pulsed electric energy and ultrasonication: Efficiencies for different microalgal species

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The effects of three physical treatments (pulsed electrical fields (PEF), high voltage electrical discharges (HVED) and ultrasonication (US)) on cell disruption and release of intracellular bio-molecules from different microalgal species (P. tricornutum, Nannochloropsis sp. and P. kessleri) were investigated. The extraction kinetic behaviours of hydrophilic (carbohydrates and proteins) and hydrophobic (chlorophyll a) bio-molecules and selectivity

indexes were evaluated. At equivalent energy consumption, the extraction efficiency arranged in the rows of HVED > US > PEF (for carbohydrates) and US > HVED > PEF (for proteins) was observed for all tested microalgal species. Among them, the PEF treatment demonstrated the smallest efficiency for extraction of carbohydrates and proteins due to its mechanism only cause cell membrane damage. However, for all tested physical treatments, the extraction degree of carbohydrates was 40%, while the extraction degree of proteins was 10%. They allowed selective extraction more carbohydrates than proteins. The relative mild pulsed electric energy treatments (PEF and HVED) have the higher extraction selectivity than US treatment. Moreover, three physical treatments presented different extraction behaviours of chlorophyll a. The extraction of chlorophyll a using PEF and HVED treatments occurs in one stage (diffusion). By contrast, the extraction using US treatment occurs in two stages (convection and diffusion), the first stage with a fast chlorophyll a transfer from the inside of microalgal cell, and the second stage corresponds to the prolonged chlorophyll a transfer by molecular diffusion from interior of the microalgal cell. Furthermore, based on the observation of the different behaviors between green microalgae and diatom, the cell wall of P. tricornutum was more fragile than Nannochloropsissp. or P. kessleri. These obtained results will help for understanding the correlations between selected methods and efficiency of extracted target bio-molecules. The appropriated cell disruption methods should be selected and used on tune the desired target molecules. However, in order to obtain higher extraction efficiencies of intracellular molecules, combined process will be needed.

PO-046

Red wine vinification on a pilot-plant winery based on Pulsed Electric Fields (PEF)

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The increasing concern of food industry regarding sustainability and the public demand for food with higher nutritional value and quality led to the development of several novel food processing techniques. One of these is the application of Pulsed Electric Fields (PEF) in order to enhance the mass extraction of valued food components from cells through the electroporation effect, limiting the thermal effect of conventional techniques. In winemaking, PEF treatment of red grape musts improves mass transfer phenomenon, translating in the reduction of maceration times and enhancing the economic value and logistic capacity of wineries.
Although there are numerous studies of PEF in laboratory and batch condition, the reduced number of studies regarding PEF application in industrial winemaking facilities limits the capability of obtaining robust results that can be extrapolated to the daily functioning of a winery.
This work was performed with the objective of further understand the impact of PEF on Tinta Roriz (Tempranillo), a red wine variety in a medium scale Pilot-Plant Winery, assessing various physico-chemical parameters such as phenolic compounds, native microflora, fermentation, maceration dynamics and also to determine factors that can impact the applicability of PEF in a winery. This specific variety was selected considering its availability in the Dão wine region of Portugal and representativity in the viticultural international panorama, e.g. in Spain.
Two batches of 1 ton of grapes each were acquired and separated in two similar fractions to allow the comparison of vinification resorting to PEF vs Control. It was also possible to assess the impact of grape sanity on PEF effectiveness. Grapes were treated on a continuous treatment co-axial chamber, working at a flow of 4 T/h, with a specific energy of 2 to 2.8 kJ/kg and electric field strength of 2.0 kV/cm. Positive results were most visible in grapes with lower sanity conditions for which PEF ended the 4-day maceration period with +18.69% TPI, +44.74% IFC, +51.5?techins, and +73.1% Anthocyanins than Control. With PEF processing the ability to inactivate microorganisms, it was also important to assess the impact of low field energy treatments on native flora of musts since anecdotal evidence shows an increased interest of the producers and consumers on the use of spontaneous alcoholic fermentation. Results show, also, that PEF did not have a significant impact on the native microflora of musts at the used treatment field intensity.

PO-010

IREC study- electroporation (IRE), calcium electroporation (CaEP) and electrochemotherapy (ECT) in pancreatic cancer patient's treatment: From science to practice

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Pancreatic cancer still has an inferior prognosis. Despite the improvement in cancer biology knowledge and major developments in radiotherapy procedures, pancreatic cancer has the same high index for mortality to morbidity for many years. According to the estimates, by 2030, pancreatic cancer will become the second cause of death in the USA and Germany. In Poland morbidity is about 3000 patients/year and mortality is almost the same. 80 % of the cases are non-resectable pancreatic cancers. For this patients only chemotherapy is the way of the treatment. Electroporation, as non- thermal technique, may be another possibility for better patient's overral survival (OS), progression free survival (PFS) or quality of life (QQL).
IREC (Effect of electroporation, calcium electroporation, eletrochemotherapy (IRE, CaEP, ECT) on quality of life and progression free survival in pancreatic cancer patients) was created and developed in cooperation of scientists and surgeons in Medical University of Wrocław, Poland on the basis of pilot study "Personalization of pancreatic cancer treatment". Inclusion criteria are non- resectable, histopathologically confirmed pancreatic cancer adult patients (stage III), with lesions to 6 cm in CT not older than 30 days, patient in 0,1,2 in ECOG

scale. Exclusion criteria are severe cardiac arrythmia, pacemaker, lung fibrosis, bleomycin allergy.
Patients are randomized to three groups. Group A- receive only electroporation (IRE), B- calcium electroporation (CaEP), C- electrochemotherapy (ECT) based on bleomycin with intratumoral and intravenous administration. Patients are operated with open or transcutaneous technique in general anesthesia. Time of observation is 12 months. CT or MR, laboratory tests and physical examination are done. Also tests of quality of life (EORTC QLQ- PAN 26, QQL-WHO).
The primary end- points are patient's disease free survival (DFS) and quality of life (QQL). Secondary end-points are overral survival, the best "therapeutic moment" for the procedure- personalization of treatment, safety of the procedure, comparison of effectiveness between A, B, C groups, immunological effects.
Project started on march 2022 and till this moment several patients were included.

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PO-048

Biological characterization of pulsed electric field-obtained extra virgin olive oil

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Background and aim: Pulsed electric fields (PEF) is a non-thermal process characterized by the electroporation of cell membranes which is used for several applications including improving extraction of intracellular compounds of interest from plant-based foods. PEF technology applied to extra virgin olive oil (EVOO) extraction slightly improves the extraction efficiency, without apparently affecting physic-chemical characteristics of EVOO. However, it has never been studied possible effects at biological level. The objective of the work is to characterize EVOO biological properties regarding atherosclerosis and fatty liver disease by using Apoe-deficient mice as spontaneous model of both pathologies.
Methods and results: EVOO oils from Empeltre variety were prepared by standard (STD) and PEF procedures (2 kV/cm; 3.9 kJ/kg) and characterized. Male and female Apoe-deficient mice were fed for 12 weeks with 2 purified Western diets differing on the type of used EVOO. After the dietary intervention, blood, aorta, heart and liver samples were taken for analysis. As expected, PEF technology increased of the oil yield from 10.2% to 12.2% with a malaxing time of 30 minutes, and reduced the malaxing time required to obtain the maximal yield to 30 minutes instead of 60 minutes for the STD EVOO. Compared with the STD EVOO, PEF EVOO showed a slightly increase in the peroxide index from 6.9 to 8.2 meq O2/kg of fat, in free fatty acids from 0.11 to 0.14, in the phytosterol content which rose from 1267 to 1372 mg/kg and in total phenolic compounds which increased from 115 to 121 expressed as mg/kg of tyrosol. The PEF EVOO also displayed a significant increase (log2 fold change of 5.43 between both EVOO) in the microRNA, oeu-mir-31 5p. Mice consuming both oils did not show significant differences in feed consumption, body weight, atherosclerotic lesion or hepatic fat content expressed as lipid droplet area. However, females consuming the PEF EVOO showed a decrease in plasma total cholesterol (498 $\pm$ 47 mg/dL) compared to the group receiving STD EVOO diet (588 $\pm$ 68 mg/dL). In addition, in both sexes there were slight differences in the ROS content of the different isolated lipoproteins.
Conclusions: Our results showed that the PEF applied to EVOO extraction improves the oil yield percentage, and reduces the malaxing time maintaining the same quality of EVOO. The observed increase in phytosterols, total phenolic compounds and oeu-MIR-31 5p with the use of PEF technology may be responsible for the reduction in total cholesterol observed in females, and a slight change in the oxidation profile of the different lipoproteins. No changes were observed on pathological outcomes such as atherosclerosis or development of fatty liver disease.

PO-051

Effect of Pulse Electric Fields on the Growth Stimulation of Lactobacillus plantarum CCDM 181

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The pulsed electric fields (PEF) is a very promising method in the food industry. Nowadays, it is already used in industrial scale for non-thermal pasteurization and to streamline other processes during food production such as extraction, drying, freezing, etc. In terms of its impact on microorganisms, the inactivation effect of the PEF has been examined so far. In some cases, it is desirable to stimulate microorganisms, for example, during fermentation processes. By exposing microorganisms to the pulsed electric field at the sublethal level, reparation and protection mechanisms are triggered. Thus, the microorganism is able to accumulate nutrients, increase biomass production and adapt to stressful conditions. As part of this experimental work, the influence of the set parameters (electric field strength, pulse length) on the bacterium Lactobacillus plantarum CCDM181 was observed in order to achieve reversible electroporation leading to stimulation of the growth of this bacterium. The impact of the PEF on the ability of this bacterium to grow under anaerobic and aerobic conditions was also examined. Within the monitored parameters, stimulation of growth of 0.8 log was achieved with a PEF in unipolar mode with an intensity of 2 kV/cm, a frequency of 100 Hz, a pulse length of 5 μs (the specific energy input 2 kJ/kg) and subsequent aerobic cultivation. During anaerobic cultivation, stimulation of 0.6 log was achieved by treatment with a PEF in unipolar mode with an intensity of 3 kV/cm, a frequency of 100 Hz and a pulse length of 5 μs (the specific energy in-

put 3 kJ/kg). It has been shown that with increasing pulse length, the stimulating effects of the pulse electric field increase while maintaining the same electric field strength (3 kV/cm) and pulse frequency (100 Hz), when the greatest stimulation was achieved using unipolar pulses of 8 μs. In terms of growth properties in the aerobic environment, an improvement in growth was achieved after Lactobacillus plantarum CCDM 181 was exposed to PEF.

PO-054

Influence of pulsed electric fields on carotenoids content of carrot purees during storage and changes in their bioaccessibility

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Introduction. Carrots are an excellent source of carotenoids. However, within the food structure, these health-related compounds are surrounded by cellular barriers that hinder their release during digestion. Pulsed electric fields (PEF) is a nonthermal technology that may facilitate the release of carotenoids through the disruption of cell membranes, thus leading to improve their bioaccessibility. In general, carotenoids bioaccessibility is quite low. Therefore, developing functional products that maintain a high content during storage is essential to preserve their beneficial health-promoting properties. Objective. The aim of this work was to evaluate the effect of PEF on the carotenoid content of carrot purees during refrigerated storage and to study their bioaccessibility just after treatments. Methodology. Purees were produced by blending carrot pieces with water [1:1 (w/w)] and adding olive oil [5% (w/w)]. The resulting purees were subjected to a PEF pre-treatment (5 pulses of 3.5 kV/cm) and then thermally-treated (T) (70 ºC for 10 min) to achieve shelf-stability. Carotenoid content was evaluated for 21 days at 4 ºC by HPLC-DAD. In addition, purees were submitted to in vitro digestion to assess carotenoid bioaccessibility just after treatments. Obtained results were compared to untreated purees (without PEF and/or thermal treatments). Results. During storage, a carotenoid reduction of 33 % in thermally-treated purees was observed, whereas carotenoid content of PEF-treated purees just decreased by 16?ter 21 days. The highest carotenoid bioaccessibility was obtained in purees subjected to PEF or PEF/T (18.7-20.4 %). These results suggest that electropermeabilization may allow a better release of carotenoids from cells and facilitated their micellarization. PEF-treated purees had lower content than those thermally-treated. Electroporation likely allows the release of intracellular compounds, which will be more available to be degraded by free radicals formed during PEF treatment application. Conclusions. Results suggest that combining PEF and heat application is the most effective treatment to promote carotenoids bioaccessibility. Furthermore, it stands also as a suitable technology to better keep the health-promoting properties of carrot puree during storage.

PO-056

Visualization of local thermalization of conductive fluids in a continuous flow PEF treatment cell

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The use of pulsed electric fields (PEF) is a promising method to sterilize conductive liquid foods at a relatively low temperature that does not damage the ingredients (1). Since Sale & Hamilton's experiment (2), numerous batch-type experiments and small flow-rate experiments have been reported. However, at the large flow-rate treatment of 10 L/h or more, several unfavored phenomena, such as reduced sterilization effectiveness, lower dielectric strength and the electrode contamination are obstacles to the practical use. Some steady-state multi-physics simulations (3) have indicated that a significant local thermalization occurs near the wall of treatment cell because of an intensive energy deposition to the reduced velocity layer near the wall. Here, we discuss the local thermalization of the fluids by visualizing the flow state and temperature distribution of conductive fluids exposed to repetitive high-power pulses in using time-resolved Schlieren imaging and interferogram methods.

The PEF exposure chamber used to observe the fluid flowing between the parallel electrodes consists of stainless-steel electrodes 2 mm thick and 30 mm high, 4 mm apart and facing each other, sandwiched between two glass windows. The cross section of the flow channel is H-shaped. Fluid was circulated using a peristaltic pump (IWAKI, PST-550H) with an average velocity of 1 m/s between electrodes. The fluid temperature was adjusted to 26°C at the inlet of the PEF exposure section. An Nd:YAG laser (532 ns, Minilite, Continuum) with a pulse width of 7 ns was used as the light source for observation. By synchronizing the pulsed laser and the pulsed power supply, the phenomena were observed at arbitrary timing relative to the PEF exposure.

High repetition pulses were applied to three different fluids with different viscosities. For the high viscosity temperature increased by 25°C only near the electrode surface and by only 2°C at the center of the flow. This local thermalization is attributed to the velocity distribution of the fluid in the tube. Fluid near the tube wall flows very slowly and therefore receives more electrical energy from the repetitive pulses. This localized thermalization causes a variety of problems in high-speed PEF pasteurization of temperature-sensitive liquid foods, such as milk and liquid whole eggs. On the other hand, it was observed that local thermalization near the wall surface is suppressed in low viscosity fluids. The Schlieren image of the fluid in this case shows turbulent flow. It is considered that local thermalization near the wall surface is suppressed by lateral thermal diffusion in turbulent flow.

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PO-059

Automated Irreversible electroporated region prediction in different electrode type with deep learning approach

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The main purpose of cancer treatment with irreversible electroporation (IRE) is maximize tumor damage and minimize surrounding healthy tissue damage. Finite element analysis is one of the popular ways to calculate electric field and cell kill probability in IRE. However, this method also has limitations. This paper will focus on use of deep neural network (DNN) in IRE with different electrode types for prediction of irreversible electroporated regions for treatment planning purposes.

COMSOL Multiphysics was used to simulate the IRE. To create accurate data sets of electric field distribution and cell kill probability distributions, the electric conductivity change during IRE was considered. We used eight pulses with a pulse width of 100 μs, frequency of 1 Hz, and pulse voltage of 2500 V. Different electrode type such as needle pairs (1, 2, 3), plate, and bipolar electrodes were used in the current study. To create masks for DNN training, 90?ll kill probability contour was used. After data set creation, U-Net architecture was trained for predicting of irreversible electroporated regions.

In this study, average U-Net DICE coefficient on test data were 0.96, 0.94, 0.91, 0.89, 0.96 with 1 pair needle electrode, 2 pair needle electrode, 3 pair needle electrode, plate electrode, and bipolar electrode. Also, the average accuracy of U-Net for predicting irreversible electroporated region with all electrode types was 0.98.

The present study provides important evidence for use of U-Net for predicting of irreversible electroporated region in treatment planning with different electrode type.

PO-061

Electrodeformation study of Giant Unilamellar vesicles (GUVs) and Multi Vesicular Vesicles (MVVs) under DC and AC electric field Pulses

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The GUVs are micrometers sized vesicles made up of lipid bilayers. The exterior and interior of the GUVs are filled with the liquid solution. The micrometers size and formation with lipid bilayers, makes a GUVs right choice as a bio mimic cell. To explore the fundamentals mechanisms of electroporation and electrodeformation of cells membrane, the GUVs are best choice to know the physics behind all these mechanisms.

When a GUV is subjected to electric field it deforms. The shape deformation of the GUVs depends on whether the applied field is AC or a DC field. Apart from this, factors such as ratio of the internal and outer conductivity of the solution (α), electric pulse duration, electric pulse amplitude, types of GUVs (charged and uncharged) also affect the deformation. Our experiments on GUVs under pulsed DC fields indicates that when the value of the conductivity ratio of the inner to outer fluid, α =1, the GUVs prefers the prolate shape and shapes deformation shown more at higher electric field amplitude. However, when α >1, the GUVs gets deformed more into prolate shape and almost spindle types shapes are also formed. On the other hand, oblate shapes are seen when α<1, means. The oblate shapes formation is due to compressive electric forces applied at the equator of the GUVs. The range of electric field amplitude and pulse duration is investigated to know not only the shape deformation but study the reversible and irreversible electroporation of the GUVs. The reduction in the volume of the GUVs after PEF treatment shows loss of liquid from inside of the GUV, indicating reversible electroporation. On the other hand, GUVs that burst show irreversible electroporation. Fluorescence microscopy studies indicate that tubules could be formed either on the inner or on the outer side of the vesicle. Our analysis indicates that the formation of microscopic structures in such vesicles, are long lived, even after the pulse is swtiched off, and depend sensitively on the size and strength of the pulse. In this particular study, the pulse width was changed from 100 microseconds to 5 ms and electric field strength was altered from a value of 0.2 kV/cm to 2 kV/cm. The amount and variety of microstructures generated in the vesicle increases when the pulse width and the strength of the electric field is increased.

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PO-064

Engineering the tumor environments in vitro using peptide-enriched, hyaluronic acid-based hydrogels

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Electrochemotherapy is a well-assessed therapy used for some skin-related tumors like melanoma or breast cancer recurrences after mastectomy. In recent years it was evaluated in the treatment of some other types of tumors, such as liver, head and neck, colon carcinomas, and soft

tissue sarcomas.
The electroporation (EP) efficiency of the cell membrane depends on cell size and cell density as well as on the conductivity and composition of the medium where cells are suspended during the EP. It is well known that in the tissue the cells experience cell-cell connection and are surrounded by the extra-cellular matrix that is a component of the tumor microenvironment. In recent years, several three-dimensional (3D) in vitro models have been used in EP experiments: the cell-cell interactions have been simulated using spheroids, whereas the cell-ECM interactions have been considered in cultures grown in hydrogels. Herein, hyaluronic (HA) acid-based scaffolds showed higher potentiality with respect to just formulated solutions. These scaffolds promote spheroids formation where cells experiment cell-cell interactions, but also improve the deposition of ECM increasing cell-ECM interactions. HA-based scaffolds were enriched with a self-assembly peptide named EAbuK. Liver cancer (HepG2) cell lines were cultured on the scaffolds above mentioned. Both the cultures were characterized by cell viability assessment and hematoxylin/eosin and Masson's stainings. Then after, cultures were electroporated varying the electric field amplitude in order to find the optimal EP condition.
The EP experiments were performed using EPS02 EP equipment (manufactured by Igea SpA Carpi (MO), Italy) varying the amplitude of the applied voltage in the range 0-1200 V/cm. In these conditions, the EP efficiency was evaluated by propidium iodide staining. Three-D cultures on HA without functionalization and cells in adhesion electroporated using culture medium or phosphate-based EP buffer were taken as controls. Three-D cultures were electroporated using a cell medium.
Our data showed that HepG2 cells cultured on HA-EAbuK scaffolds were already completely electroporated at 800 V/cm, whereas cells cultured on HA started to be electroporated at 1200 V/cm. In 2D cultures, HEPG2 cells were electroporated at 1000 V/cm and 1200 V/cm in EP buffer and in culture medium, respectively.
Collectively, our preliminary results suggest that HepG2 cell cultures on HA-EAbuK hydrogels may represent a promising tool for in vitro evaluation of EP efficiency.

PO-067

Time-dependent numerical model of electroporation comparing prolate spheroid and real-shaped geometry of a cardiomyocyte

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Cardiac arrhythmias are currently treated using two thermal energy methods: radiofrequency and cryoablation to ablate the targeted heart tissue. However, the two methods cause potential damage to the esophagus, phrenic nerve injury, and pulmonary vein stenosis. Consequently, a new promising non-thermal ablation method to treat cardiac arrhythmias in particular for atrial fibrillation called pulsed-field ablation (PFA), is being developed. The PFA treatment involves the application of electric pulses to arrhythmogenic regions in the heart enabling complete and durable isolation of pulmonary veins avoiding damage to critical nearby structures. In the case of cardiac ablation, the elongated and oriented cardiac cells might affect the distribution of the electric field and, consequently, the PFA treatment efficiency. As several different treatment parameters can be varied, a quick and efficient option to determine the optimal pulse parameters of the treatment (amplitude, duration, number, repetition frequency) are numerical models.
The shape of a cardiomyocyte is complex, being rod-shaped with a non-smooth membrane surface and a complex array of microtubules. However, it is mostly approximated to a simple prolate spheroid which was shown to be a good-enough approximation in stationary conditions. The aim of our research work was to establish if the prolate spheroid geometry is a good-enough approximation of a cardiomyocyte also in the time domain.
We developed a time-dependent numerical model of pore formation on a cardiomyocyte. To represent a cardiomyocyte, we used: 1) commonly used prolate spheroid and 2) real-shaped cardiomyocyte. The electric field was applied parallel and perpendicular to the long axis of the cardiomyocyte at different electric fields, from 100 V/cm to 10000 V/cm using different pulse lengths from 100 ns to 1 ms. The initial value of pore density when no electric field is applied is 10^9 pores/m^2. By increasing the electric field, pore density increases. The value of pores density usually used as threshold of electroporation is 10^{13} pores/m^2.
The prolate spheroid geometry is a good approximation of a cardiomyocyte for very high and very low electric fields. 1) When low electric fields are applied and pores just start forming with pore density between 10^9 - 10^{11} pores/m^2, i.e., probably before electroporation occurs. 2) When high electric fields are applied with pore density above 10^{15} pores/m^2, i.e. when probably most of the cells are irreversibly electroporated. The prolate spheroid is a too simplified approximation of a cardiomyocyte when medium electric fields are applied and the density of the pores is between 10^{11} - 10^{15} pores/m^2, i.e. when cells are probably reversibly electroporated.
In conclusion, we suggest using realistic shapes of cardiomyocytes at cell level numerical calculations when studying the effects of the application of a medium electric field on a cardiomyocyte.

PO-070

Tumor location method based on multi-electrode structures and machine learning

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Electroporation is a promising technique for cancer treatment that involves the application of high intensity electric fields. To achieve the optimum results, it is important to focus the treatment on the tumor tissue. In this work, a machine learning algorithm is proposed to locate tumoral tissue by means the impedance measurements taken by a multi-output electroporation generator in conjunction with a multi-electrode structure. This can

also be used to apply a controlled and targeted electric field, consequently improving the effectiveness of the local treatment. It can also simplify the electrode placement procedure, often the most time-consuming task.
Several studies have determined that tumor tissue is more conductive than healthy tissue, usually at least 3 times more. Consequently, the purpose of this system is to locate the tumor tissue between electrodes by means of the impedance measurements on every part of the tissue. To detect the tumor tissue inside the volume between the electrodes, 81 impedance measurements are taken applying low electric field pulses in different locations and directions.
To find the tumors in the tissue, the volume between the electrodes is discretized into 18 voxels, in two square 3-by-3 parallel planes, so that a matrix of 18 neural networks can determine if there is tumor tissue or not in each cell. The 81 impedance measurements compose the input layer of the 18 feedforward neural networks, each of them with 10 neurons in a single hidden layer. The output of each neural network is a single true or false statement, indicating if there is or not tumor tissue in each voxel. Having a neural network per cell allows to design a simpler architecture of the neural networks, which is faster and easier to train. The neural networks have been designed and trained in Matlab with data obtained from a model developed in COMSOL Multiphysics.
The multi-electrode electroporation system consists of by two differential square parallel-plate electrodes, each one of them is divided into a 3 by 3 matrix of plate electrodes individually driven by an 18-output generator. It can deliver up to 1250 V unipolar or bipolar pulses and apply low voltage pulses to measure tissue impedance. Each electrode cell has a side length of 8.5 mm, and 1.5mm between cells guarantees isolation.
An experimental validation has been carried out using 10 mm thick phantom gel and potato tissue samples. The gel has inserts of other more conductive gel in several areas, with conductivity ratios of 1:2, 1:3 and 1:5, emulating tumors of known location, conductivity, and size. Processing data with the neural networks shows the voxels with the more conductive gel effectively.
In conclusion a method to locate tumor tissue between electrodes has been developed and validated. The final version of this paper will include experimental results and future insights demonstrating the feasibility of this proposal.

PO-073

Modeling of cardiac electrophysiology of a tissue containing an electroporated area

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In healthy hearts, the propagation of the electrical waves follows a predictable pattern described by a so-called bidomain model [1]. When people suffer from a cardiac rhythm disorder, the electrical wave can become chaotic and directly affects the pumping function of the heart. One of the main treatments for these arrhythmias is catheter ablation, which destroys small areas of heart tissue to isolate or eliminate the cause of the rapid and irregular heartbeats. Most of the catheter ablations are thermal by delivering a radiofrequency electromagnetic field (RFA). In this numerical study, we focus on the study of a novel and mainly non-thermal ablation technique: the pulsed electric field ablation (PFA), which takes advantage of irreversible electroporation, a complex phenomenon of cell membrane rupture that occurs when tissues are subjected to very intense electric pulses. This technique has been used in oncology for more than a decade, but it is still in its infancy in cardiology. Preclinical evaluations of PFA in atrial fibrillation [2] and ventricular ablation in large animal studies [3] show successful results with possible transmural lesions, sparing vulnerable adjacent structures.
In this context, the objective of this work is to derive a model considering an electroporated area in the geometry to better understand how PFA works over the long term and in particular why it appears to be more stable than RFA. We propose a specific model of electroporated cardiac tissue, in order to couple the classical bidomain system in the healthy parts of the ventricles with the electroporated region. Our numerical study enables to quantify on synthetic data the degree of electroporation needed to isolate the ill region of the hearts which generate the arrhythmias. In order to accelerate the simulation we propose a well adapted Schwarz algorithm to transmit the electric wave from the heart to the electroporated region.

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PO-033

The bystander effect after Ca electroporation, BLM electrotransfer and irreversible electroporation in multiple cell lines

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Introduction: Novel electroporation-based cancer treatments utilize the phenomena of increased cell membrane permeability to the exogenous molecules because of increased transmembrane potential induced by the application of external electric fields. Electrochemotherapy and calcium electroporation anticancer therapies rely on higher specific death-causing molecule entrance to the cell (Calcium ions, or chemotherapeutical drugs: bleomycin, cisplatin). However, the utilization of electroablation is based on irreversible electroporation phenomena causing the irreversibly affected cell membrane permeability to in-

crease, hence cell death.
In a previous study, we showed the presence of the bystander effect on CHO cells after bleomycin electrotransfer. In this study, we aimed to extend the investigation of the bystander effect using 3 different cell lines, namely CHO, A-549, and 4T1 cell lines. In addition, we implemented not only bleomycin electrotransfer but also calcium electroporation and irreversible electroporation in vitro.
Methodology: Chinese hamster ovary (CHO), adenocarcinomic human alveolar basal epithelial (A-549), and mice breast cancer (4T1) cell lines were used for experiments. One electric pulse (1400 V/cm 100 us) was used for bleomycin (20 nM) and calcium (1 mM) electrotransfer into cells. Irreversible electroporation was triggered with one 1400 V/cm 100 us pulse. We used laboratory-made phosphate buffer and sucrose-based electroporation media at pH 7, and conductivity at 0.1 S/m. The bystander effect was measured by taking the media from the affected cells 24-72 hours post electroporation and applying it to the bystander cells for clonogenic assay or Alamar blue assay.
Results and discussion: The presence of the bystander effect was confirmed in all cell lines. However, depending on the collection medium time after cell electroporation, the bystander effect after irreversible electroporation had a positive impact on cell viability. We have also obtained the difference in viability changes over time when comparing cell lines. Similar results were obtained by using the Alamar blue assay (metabolic activity of the cells) and clonogenic assay (ability to form colonies).

PO-035

Pulsed electric field assisted rehydration of dry-salted cod (Gadus morhua)

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Salted cod (G. morhua) is a highly appreciated product, traditionally imported by Mediterranean countries and commercialised with different moisture content depending on the extension of the dehydration process. Dry-salted cod must be rehydrated before consumption and this step can take up to five days.Desalting of cod on an industrial scale is usually carried out immersing the product in stagnant water, resulting not only in sample rehydration but also in the loss of salt. It therefore poses many problems, mainly related to the long processing times and the quality of the final product. For this reason, many researchers have focused on finding new desalting methods to improve mass transfer. The application of pulsed electric field (PEF) has been proposed as an alternative method to improve mass transfer in many food processes. However, there is no previous literature on the use of PEF to improve animal tissue rehydration. Therefore, the aim of this work was to investigate the influence of two PEF pre-treatments (PEF (1) -500 V · cm-1 and PEF (2) -1000 V · cm-1) on mass transport kinetics during the rehydration process of salted cod. Samples were treated at room temperature in tap water, with an initial electrical conductivity of 396 ± 5 µS · cm-1 at 25 °C (EC-meter Mod. Basic 30, Crison, Spain). Trials were conducted filling the treatment chamber with a product-to-water ratio of around 1:5 (w/w) and delivering n=1000 pulses at fixed amplitude (10 ± 1 µs) and frequency (100 Hz). The rehydration process was carried out under static conditions for 6 days, immersing dry-salted cod samples in tap water (5 ± 0.5 °C) using a ratio of cod:water of 1:10 (w/v). Mass transfer parameters were determined at 0, 4, 6, 24, 48, 72, 96,120 and 144 h of the rehydration process. The results show that the use of PEF technology increases the rate of the rehydration process of dry-salted cod and influences the redistribution of salt. In general, the samples pre-treated with PEF showed higher weight gain and lower salt loss than the control samples during the rehydration process. Nevertheless, the calculated salt content in both pre-treated sample was found to be in the range of commercial rehydrated cod products, namely about 10- 20 g · kg-1 NaCl (10.0 ± 7.0 g · kg-1 NaCl control; 20.0 ± 6.0 g · kg-1 NaCl PEF (1); 10.5 ± 6.0 g · kg-1 NaCl PEF (2)). The application of PEF prior to rehydration of salted cod samples could be of interest to the food industry due to higher process yield (higher weight gain) and the possibility to reduce the water renewal, as less NaCl is lost in the wastewater.

PO-075

Low pulsed electrical fields for inducing transient BBB disruption - Mechanism of action

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The blood-brain barrier (BBB) limits transcellular and paracellular passage of molecules from the blood system into brain tissues, turning it into a major hurdle for treating brain diseases. High pulsed electrical fields (PEFs) can disrupt the BBB by inducing electroporation (EP), resulting in permeability of the transcellular route. We have discovered that low PEFs (L-PEFs), well below EP threshold, can induce transient BBB disruption (BBBd). Here we studied the mechanisms involved in L-PEFs induced BBBd.
10 pulses (5-800 V, 0.68 cm electrodes gap, 50 µs, 1 Hz) were applied to a human in vitro BBB model composed of brain-like endothelial cells and brain pericytes. Changes in permeability to different size molecules were studied. Viability and membrane permeabilization (EP) were evaluated by Presto-Blue, Lactate dehydrogenase release assays and Propidium iodide. The effects on cytoskeleton and tight junction/adherent junction (TJ/AJ) proteins were studied using immunohistochemistry. Finally, phosphoproteomic analysis was conducted to identify molecular signaling pathways.
EP was undetected below 100 V, though max membrane permeabilization occurred at 500 V. Cell death was detected at 1400 V. Starting at 10 V, BBBd increased for small (NaF 376 Da) and large (IgG, 150 kDa) molecules in a dose dependent manner: At 20 V permeability increased by 28.9±4.9/56 ±11.9% for NaF and IgG, while at 50 V it increased by 135±12/212.94±33%, and permeability to an 8 kDa negatively charged antisense oligonucleotide in-

creased by 420±126%.
There were no changes in expression of ZO-1 or Beta Catenin following L-PEFs or EP. Decreased expression of VE-cadherin was observed 30 min after EP only. There was no significant change in the appearance of the cells following L-PEFs (50 V), while at 800 V the cells seemed more elongated, swollen and electrofusion was clearly seen as early as 1 min after EP. At 24 hrs giant cells with multinuclei were observed only after EP.
1 min after L-PEFs at 50 V, the expression pattern of F actin, a key cytoskeleton protein, changed extensively. Decreased density of the stress fibers across the cell body and increase in misaligned fibers were observed. At 800 V, honeycomb-like peripheral actin and fragmented filaments were seen in abundance. Stress fibers across the cell body disappeared, the actin cortex became thicker and many misaligned fibers were seen. 24 hrs after treatment at 50 V, full recovery of the cytoskeleton was observed, while for 800 V recovery attempt was observed, reflected by longer fragments of stress fibers across the cell body. This was supported by phosphoproteomic analysis showing strong activation of Rho GTPases signaling cascade which is a major regulator of cytoskeleton.
Our results suggest that L-PEFs induce transient BBBd via the paracellular pathway and that cytoskeleton reorganization mediated by Rho GTPases signaling is a possible mechanism of action. These findings may lead the way to a new/non-invasive approach for drug delivery into the brain.

PO-080

Microelectrode array-based electroporation for use in multifunctional retinal implants

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Retinal implants are intended to enable artificial vision in patients suffering from degenerative diseases. In the past, we developed several types of epiretinal implants using microelectrode arrays (MEAs) to evoke a phosphene-based vision. However, all former concepts did not take the retinal pathology and remodeling into account. Upon disease progression, the photoreceptor cells degenerate and a functional reorganization of the neural network occurs. This reorganization directly affects the stimulation efficiency of the implants. In our next-generation approaches, we aim to incorporate additional functionalities into the retinal implants. One hypothesis is that the remaining retinal cells could be protected by genetic modification, which could improve the stimulation efficiency of the implants. While viral transduction is considered the gold standard in gene therapy, transfection by electroporation (electrofection) is an immune-friendly alternative. In the presented work, we investigate the possibility of using MEAs to generate efficient electric field strengths for cell electrofection.

In a conventional electroporator, the electric field between the electrodes is typically approximated to that of a parallel plate capacitor with perpendicular electric field lines. Therefore, the cell suspension experiences a reasonably homogeneous electric field at the center distance between the electrodes. However, in an MEA all electrodes are embedded in a planar substrate. Therefore, the electric field distribution becomes more complex and is quite inhomogeneous in the region of the attached cells.

In this study, we present first results of an MEA-based electroporation, using a FITC-labeled dextran as a fluorescent probe (2 MDa, Sigma-Aldrich) and an MEA-based electrofection, using pMAX GFP (Cell Line Nucleofector™ Kit V, Lonza). For this, we applied electrical pulses to adherent HEK293T cells via our custom-made MEAs comprising 60 individually addressable electrodes. In our experiments, we varied the electrical pulse parameters (square pulses, sinusoidal pulses with different voltage levels, frequencies, and durations) and the electrode material (gold or iridium oxide). We also compared two different electrode configurations, in which we either applied a checkerboard arrangement of stimulation and counter electrodes on the MEA or short-circuited all electrodes against a platinum wire as the counter electrode. We found successful transfection of HEK293T cells with both electrode arrangements and both materials. The highest transfection efficiency was observed when applying a sinusoidal 5V voltage pulse at 40kHz for 200ms to gold electrodes in the electrode arrangement with the platinum wire.

In the future, we plan to further optimize the MEA fabrication process and pulse protocol to achieve high efficiency with the checkerboard stimulation arrangement eliminating the need for a large counter electrode. Once successful, this concept should be further optimized for the ex vivo electrofection of retina explants and later implemented into the next generation of epiretinal implants.

PO-015

Electrical Impedance changes as a possible real-time indicator of Pulsed Field Ablation (PFA) efficacy

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In recent years, the use of irreversible electroporation (IRE) as a method for ablation of cardiac tissue in the treatment of cardiac arrhythmias, also known as Pulsed Field Ablation (PFA), has rapidly moved from preclinical studies to clinic. Although safety, efficacy and lesion durability of the treatments have been recently demonstrated in limited groups of patients, studies in larger groups of individuals are still under development to show comparative evidence of its superiority against radiofrequency ablation and cryoablation.
One of the many open questions of this new technology is how the local electrical activity of electroporated myocar-

dial tissue is modified during treatment and the differences between the reversibly and irreversibly damaged areas. This leads to question if the measurement of local electrograms, which are usually registered during ablation with thermal methods, can accurately reflect the treatment outcome and motivates the research on new real-time lesion assessment methods.
In this in vivo study we performed a parametric study using bursts of high frequency biphasic square waveforms of different amplitudes and frequencies leading to different levels of ablation efficacy. They consisted on square biphasic bursts (10 bursts of 100 µs separated 1 s) at three different frequencies (90, 260 and 450 kHz) and two fixed voltages (500 and 800 Vp), additional a group of monophasic 100 µs square pulses at 500 V was also studied. PFA in vivo treatments were performed in Sprague-Dawley rats following a protocol approved by the Sant Pau Hospital Animal Experimentation Ethical Committee. Under general anesthesia, a left thoracotomy was made to create a surgical window. A monopolar epicardial electrode was carefully positioned over the left ventricle epicardium and a return electrode patch was attached to the back of the animals. During the procedure we continuously recorded the temperature at the electrode tip, the ECG and muscle contractions. In-burst electrical impedance was calculated from the acquired voltage and electric current waveforms. Once the catheter was in position, baseline electrical impedance was recorded using 4 low voltage (50 Vp) bursts identical to the treatment waveforms, subsequently, high voltage treatment bursts were applied. Twenty-one days later, animals were euthanized and the hearts were fixed for histological analysis with Masson's trichrome staining to assess the extent of fibrotic lesions.
Our results show a clear dependence of ablation lesion size with frequency and amplitude of the applied waveform. This demonstrates how the IRE lethal threshold value increases with the increase in the frequency of the applied waveform. The analysis of the impedance changes suggests that the impedance magnitude drop measured during PFA applications correlates with the chronic ablation lesions observed. These preliminary results suggest the possible use of electrical impedance as a real-time indicator of PFA efficacy.

Poster Session (and Coffee break)

Tuesday Poster Session Track
Oct 11, 14:45 - 16:00

PO-002

Successful Treatment with Electrochemotherapy for Multiple Non-Melanoma Skin Cancers in Kidney Transplant Recipients

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Introduction: The risk of cutaneous malignancies is significantly higher in immunosuppressed patients compared to the general population. These high-risk skin tumors tend to be aggressive, multiplex, rapidly growing lesions. The standard treatment is surgery, which can be annoying regarding the number of lesions, and the left scars, or RT with limited possibilities of repetition. Our clinical case report aims to highlight the possibility of electrochemotherapy (ECT) as a successful treatment of the multiple non-melanoma tumors of renal transplant patients nevertheless that raising evidence shows that immune system crucially contributes to ECT efficiency.
Case presentations: Two 70-year-old male patients were referred to the Department of Dermatology, University of Szeged, with multiple non-melanoma skin tumors (n=15, n=11) located in the head-neck area (n=11, n=10), on the limbs (n=2, n=1) and trunk (n=2, n=0). Both patients underwent renal transplantation 7, and 4 years before referral, and have been treated with immunosuppressive medication since then. Over the years, they developed multiple non-melanoma skin tumors, which were previously removed surgically (n=27, n=3). Our multidisciplinary tumor board decided to perform ECT. The patients were treated according to the Standard Operating Procedures, under general anesthesia, with intravenous administration of Bleomycin, because of the high number of lesions. The treated lesions were in complete remission in both patient thirty-one and three months after ECT. One patient received another session of ECT due to novel tumor lesions outside the treated area.
Conclusion: Organ transplant recipients with immunosuppressive therapy are a unique group regarding high-risk, multiple skin tumor development. ECT can be a suitable treatment for immunosuppressed patients because of its high response rate, good cosmetic result, and only minimal, local, low-grade side-effects.

PO-005

Calcium electroporation in cancer treatment – design, test and evaluation of an evidence-based curriculum

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Calcium-electroporation has proven to be effective but yet gentle in treatment of skin cancer.
Following the new treatment oncology must be updated to support the quality of treatment in order for the patients to achieve a professional, equal and gentle way through the treatment and care. It is noted that in oncology nurses take over more tasks and skilled, up-to-date qualified nurses are needed in order to provide the patients with the best and less stressful experience during treatment. With participation from researchers in Denmark and Germany, the EU-based Interreg programme funded the project "Changing Cancer Care". One of its objective was to develop, pilot and evaluate an evidence-based curriculum for continuing education of nurses working in oncology.
Methods: A multiple step approach was used: (1) Mixed-methods studies and evidence syntheses were performed (2) A curriculum was developed for a 6-week (10 ETCS) course targeting nurses working in oncology in Denmark. (3) This curriculum was tested in a training course. (4)

A similar curriculum is being developed in Germany and tested. The final prototype of the curriculum and scope-review will be part of the deliverance.
Curriculum content: The curriculum addresses: calcium electroporation treatment, geronto-oncology clinical leadership in nursing, dermatology, pain management, body image, family dialogue, and technology. The course was finalised with an oral examen.
Results: Nurses working in oncological care have gained a robust theoretical background in caring for patients treated using calcium electroporation. The evaluation was positive, however insights into nutrition may enhance the course.
Perspectives: This research demonstrates that investing in an evidence-based curriculum lift the competences. It will benefit the patients to experience a safer, more reliable pathway through the hospital system and eventually a higher quality of life.

PO-008

Electroporation in liver cancer multiphysics simulation

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Introduction: Minimally invasive cancer therapies are a popular area of research. Among these minimally invasive cancer therapies, electroporation-based therapies are promising new methods, mainly due to their non-thermal effect and tumor specific approach. Electroporation uses short electrical field pulses (in current clinical therapies about 70-100 µs) to create so called nanopores in the plasma membrane. There are two types of therapies: In irreversible electroporation, the cell membrane can't repair the induced nanopores because of their size and amount which causes the cell to undergo apoptosis, the natural cell death. In reversible electroporation, the cell can repair their phospholipid bilayer and continue on with their normal cell functions. In tumor therapy, those hydrophilic pores are used to highly induce the diffusion of a chemotherapeutic drug, and is known as Electrochemotherapy[1].
This study aimed to predict the electric field lines around the hepatic cancer tissue after electroporation, as well as define the distribution of Temperature on the probe and the Tumor using computer simulations.
Methods: In order to predict the results, different simulations were done in COMSOL-Multiphysics simulation software with different voltages to see how the electric field was spreading across the cells. In order to obtain the strength of the Electric Field and the rise in Temperature, the following fixed parameters (70 pulses / 100 µs pulse length / 100 µs interval) and variable parameters (200 V/600 V/1500 V) were applied on the model for both Electric field and Temperature Simulations.
Results: The simulation that were done on COMSOL software showed that by increasing the voltage, and thereby the electric field strength, the fraction of irreversible electroporated cells over reversible electroporated cells will increase also the rise in Temperature will increase.
Discussion: The electroporation results of both Electrical and Thermal Simulations are in line with literature, an increasing electrical field will cause more permanent nanopores in the cell membrane, leading to more irreversible cell damage and thereby apoptotic cell death [2]. The different values of electric field and Temperature was due to the different geometry and scales that were used in both simulations.
Conclusion: To conclude, this study found an increasing fraction of irreversible electroporated cells over reversible electroporated cells at increasing field strengths alongside with an increase in Temperature through simulation.ir

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PO-011

Anti-inflammatory response characterization of microsecond electric pulses stimulation for spinal cord injuries application

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Spinal cord injury (SCI) is one of the most devastating and debilitating conditions that an individual can sustain. It leads to temporary or permanent changes in the spinal cord's normal motor, sensory, and autonomic functions.
SCI is composed by two different phases: a primary and secondary injury. The first is due to the initial traumatic insult which damages or destroys the tissue and is due to the hemostatic response and acute cell death. This first phase is followed by the progressive secondary injury cascade characterized by ischemia, proapoptotic signaling, and peripheral inflammatory cell infiltration. Over the subsequent hours, the release of proinflammatory cytokines and cytotoxic debris (DNA, ATP, reactive oxygen species) cyclically adds to the harsh postinjury microenvironment, making this environment totally hostile to any kind of therapeutic treatments. For this reason, it is extremely important, in case of SCI, to act against the inflammatory cascade, to have the possibility of intervening therapeutically.
Here we present the results obtained by applying differ-

ent exposure protocols in terms of repetition frequency, pulses number, amplitude, duration, and polarity on human microglia and macrophages cell lines, HMC3 and U937, to evaluate the anti-inflammatory effect of these stimulations. These experiments are a specific task of RISEUP project (fully described in another abstract submitted to WC2022), funded by the European community in the H2020 FET-OPEN program, whose aim is to set an innovative approach for SCI regeneration based on micro pulses electric stimulation.

PO-014

Treatment Planning under Uncertainty for Electroporation-Based Cancer Therapies

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Electroporation-based treatment and therapies (EBT) are a novel advancement in the fight against cancer. These methods leverage the phenomenon of electroporation to ablate the tumor through a minimally invasive procedure. The procedure involves inducing a voltage difference across the tumor through needle-like electrodes to create pores in the cell membrane. The pores can be utilized as temporary gateways to deliver chemo drugs (called electro chemotherapy (ECT)) or to damage the structural stability (called irreversible electroporation (IRE)). The delivery of ECT and IRE treatments is primarily based on the electric field strength in the tumor cells. Hence, optimizing the positioning and driving parameters of the electrodes is an essential component of treatment planning.

In this work, we investigate model-based treatment planning algorithms that identify the optimal position and voltage parameters of the electrodes for a successful treatment outcome. The optimal parameters are calculated by solving an optimization problem constrained by a biophysical model for the EBT. The numerical solution of the model is used to predict the electric field distribution and consequently the tumor ablation. However, the electric field can depend on various factors (like the electrode position, electrical conductivity values, etc.) and this can present difficulties in the planning. Firstly, the tissue properties are commonly patient specific (based on water-fat content) and are not routinely measured prior to or during interventions. Secondly, even if the measurements were available, the measurement errors should be taken into account. Finally, the inaccuracies in the electrode positioning due to the lack of precise guiding tool, resolution of the medical imaging, image registration errors and breathing motion may also affect the treatment outcome. Hence, it is important to consider the effect of these uncertainties in the planning for EBT interventions. To account for such stochastic effects, the uncertainties are incorporated into the predictive biophysical model. This results in a stochastic computational model of the EBT, comprising of a set of partial differential equations with random model parameters and inputs. Thus, we define the treatment planning algorithm as a stochastic optimization problem where the optimal treatment plan accounts for the uncertainties in the treatment outcome predicted by the stochastic model. To ensure usage during the treatment intervention, we propose a computationally efficient approach involving a surrogate estimator of the propagation of the uncertainties from the input parameters to the success measure of the treatment. This surrogate is a probabilistic representation of the treatment outcome and is validated against the predictive model for accuracy. The surrogate is ultimately used in a global optimization routine to come up with optimally planned treatment strategies that are robust against uncertainties.

PO-017

Construction of three-dimensional structure and research of folding-analogues of human kinetochore-microtubule used in transmembrane drug delivery

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Introduction: It is obvious, that cellular and organismal fitness requires proper partitioning of genetic material during cell division. Furthermore, failure to accurately segregate chromosomes causes aneuploidy, the most prevalent genetic alteration in tumor cells and a potential factor in the evolution of cancer. Chromosome segregation is driven by microtubule-based forces, which are generated at kinetochores.

Basically, kinetochore-microtubule of human has important role in drug and protein delivery in human cells during electroporation. But the structure of Kinetochore-microtubule has been unfortunately still undescribed.

The aim of this work was to modulate the three-dimensional (3D) structure and to research the folding-analogues of human kinetochore-microtubule.

Material and methods: Template search with Blast and HHBlits has been performed by the SWISS-MODEL template library. Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodelled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II.

Results and Discussion: Three-dimensional (3D) structure of human kinetochore-microtubule has been built.

As folding-analogues of human kinetochore-microtubule there have been found the anaphase-promoting complex/Cyclosome, in complex with the Mitotic checkpoint complex and Cryo-EM unit of the Anaphase-promoting complex/Cyclosome, in complex with the Mitotic checkpoint complex.

Information about three-dimensional (3D) structure and partial analogy with anaphase-promoting com-

plex/Cyclosome may help to understand physical properties as well as spatial configuration of human kinetochore-microtubule, and to examine its ability in drug and protein delivery in human cells during electroporation.

PO-020

Structure and folding-analogues of mutant form of human mitotic serine/threonine kinase B delivered into cells by electroporation

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Introduction: Initially, mitotic serine/threonine kinase B is located to the kinetochore and plays a role in the inhibition of the anaphase-promoting complex/cyclosome (APC/C), delaying the onset of anaphase and ensuring proper chromosome segregation. The interesting fact is that the impaired spindle checkpoint function has been found in many forms of cancer. Mutant forms of mitotic serine/threonine kinase B are leading to cancer cell mitosis depression and may be delivered into the cell by electroporation. But currently the structure of mutant form of mitotic serine/threonine kinase B is still uncharacterized. The aim of this work was to modulate the three-dimensional (3D) structure and to study the folding-analogues of mutant form of human mitotic serine/threonine kinase B.

Material and methods: Template search with Blast and HHBlits has been performed by the SWISS-MODEL template library. Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodeled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II.

Results and Discussion: Three-dimensional (3D) structure of mutant form of human mitotic serine/threonine kinase B has been built.

As folding-analogues of mutant form of human mitotic serine/threonine kinase B there have been detected: Mitotic serine/threonine-protein kinase BUB1, beta, Mitotic serine/threonine-protein kinase BUB1 beta, and Fibroblast growth factor receptor 2.

Information about three-dimensional (3D) structure and partial analogy with the detected folding-analogues may help to understand physical properties, furthermore spatial configuration of mutant form of human mitotic serine/threonine kinase B, and to research the role of electroporation in delivering of mutant form of mitotic serine/threonine kinase B into human cells.

PO-024

Development of 3D melanoma cultures on a hyaluronic acid-based scaffold with synthetic self-assembling peptides

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Electrochemotherapy (ECT) with bleomycin is an established and effective treatment in the care of patients with superficially metastatic tumors. Cutaneous melanoma is among the most frequent indications, with a complete response rate close to 50%, according to the most recent meta-analyses. Nonetheless, novel drugs and electroporation (EP) conditions are being investigated to improve patient outcome further.

In general, ECT efficacy is evaluated in vitro on cell suspensions or using cells in adhesion, and in vivo on animal models. Nevertheless, the first two mentioned approaches completely lack extracellular matrix components, leading to unreliable results because they do not reflect the natural tissue architecture. To overcome this problem, several three-dimensional (3D) in vitro models, such as spheroids and hydrogel-based cultures, have been proposed to mimic the complex tumour microenvironment. These in vitro models always require a suitable low-conductive medium to allow EP of the cell membranes through a sequence of voltage pulses. In this frame, a new synthetic scaffold based on hyaluronic acid (HA) and self-assembling peptides is proposed as an advantageous alternative. Self-assembling peptides (SAPs) are a class of ionic-complementary peptides (EAbuK) able to aggregate, forming a hydrogel scaffold with a fibrous structure. The self-assembling sequence was condensed with an adhesive motif mapped on Laminin (IKVAV). SKMEL28 cells, a malignant melanoma cell line, were seeded on these two-components scaffolds and cultures were characterized at 3 and 5 days by cell viability assessment and Masson's trichrome staining.

Electroporation was performed using EPS02 EP equipment (Igea SpA, Carpi, Modena, Italy). Several voltage amplitudes and strengths of the applied electric field (0, 400, 600, 800, and 1000 V/cm) were tested. The EP efficiency was evaluated at different electric field strengths using propidium iodide. Three-D cultures seeded on HA without SAPs and cells in adhesion electroporated using the culture medium or the electroporation buffer were taken as controls. The 3D cultures were electroporated in the culture medium.

Our data demonstrated that cells cultured on HA-EAbuK-IKVAV scaffolds start to be electroporated at 400 V/cm (50% of electroporated cells). In contrast, the cells cultured on HA are not efficiently electroporated at the same electric field strength. The 2D experiments showed that EP starts at 600 V/cm using electroporation buffer and at 800 V/cm in culture medium but with very low efficiency (<50% of electroporated cells). In conclusion,

the HA-SAP 3D cultures allowed the simulation of a more tumor-tissue-like environment and may represent a tool to study cell electroporation conditions. EP is greatly influenced by the local conductivity of the extracellular medium and cell-cell connections.

PO-027

CaCl2 influence on pDNA electrotransfer efficiency and cell viability

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Calcium (Ca2+) is one of the most important second messengers that is associated with a variety of different processes including development, cell proliferation, and cellular motility. Intracellular free Ca2+ concentration is tightly regulated and varies depending on cellular location. Experiments already revealed that using electroporation – a physical delivery method that increases the permeability of cell membranes – in combination with calcium necrotic cell death can be induced.
Although gene electrotransfer has been researched extensively in both in vitro and in vivo systems, the exact mechanisms by which DNA enters and navigates through cells are still not fully understood. It has been proposed that the negative charge of DNA induces its electrophoretic movement near the cell membrane after an electric field is applied. DNA interaction with the permeabilized cell membrane, followed by endocytosis results in DNA transfer across the cell membrane and nuclear import. Experimental data also revealed that divalent ions like Ca2+ which abolish electrostatic repulsion between DNA and the cell membrane might enhance DNA absorption on the cell membrane. However, less is known about how exactly calcium can influence the electrotransfer efficacy of pDNA or how this simultaneous electrotransfer of DNA and Ca2+ affects cell viability patterns. Thereby, the aim of this study was to evaluate the effect of different extracellular CaCl2 concentrations (0 – 1mM) on the pDNA transfection efficiency and cell viability tendencies. Experiments were performed using two sets of electroporation parameters: 1400V/cm, 100 μs, and 1200V/cm, 250 μs. pDNA transfection efficiency and fluorescence intensity were evaluated using flow cytometry 24h after electroporation. Fluorescence microscopy was used for the evaluation of labeled pDNA electrotransfer tendencies. For the evaluation of cell viability patterns, flow cytometry assay and MTT methods were used. Results revealed that the higher Ca2+ (> 0.25mM) concentrations are associated with significantly decreased plasmid electrotransfer efficiency and fluorescence intensity. What is more, a combination of pDNA and calcium electroporation resulted in a significant decrease in cell viability. The reason for this phenomenon is still under investigation.

PO-028

Effects of calcium electroporation and irreversible electroporation on HPAF II cells in vitro study

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Pancreatic cancer (PC) was the seventh leading cause of cancer death in 2020, and the 5-year survival rate is less than 10%. This fatal disease is predicted to be the third leading cause of cancer death in 2025. Pancreatic cancer has no symptoms until the disease has advanced and is aggressive cancer with early metastasis. Up to now, the only curative treatment is surgical resection, and it is possible in the early stages of the disease. Irreversible electroporation treatment offers new hope for patients with unresectable tumors. Our study aims to determine the efficacy of calcium electroporation (CaEP), in treating patients with pancreatic cancer, which is expected to improve treatment outcomes and bring measurable benefits to patients. This innovative method combines a non-pharmacological approach (electroporation) with calcium ion administration upon IRE procedure beyond medicine specifications in anticancer therapy in patients diagnosed "de novo" or who do not improve with standard treatment methods.

Here we used the HPAF II cell line as a model for pancreatic cancer. The following EP protocols were used: 3.5kV/cm×900ns×100, 1kHZ; 5.7kV/cm×600ns×100, 1kHz; 5.7kV/cm×900ns×100, 1kHz; compared to clinical standard ESOPE (European Standard Operating Procedures of Electrochemotherapy) 1.3kV/cm×100µs×8. All protocols were combined with 2.5mM calcium chloride (CaCl2) in a HEPES-based buffer, and cell viability was determined using the MTT assay after 48 and 72 hours. The cell membrane permeabilization rate was measured by flow cytometry using a cell-impermeant dye Yo-Pro-1. Additionally, proinflammatory cytokines (IL-6 - Hs00174131_m1, IL-8 -Hs00174103_m1) and anti-inflammatory cytokine (IL-10 - Hs00174086_m1) gene expression was quantitatively assessed in HPAF II cells, 72h after EP protocols using real-time RT-PCR. The obtained results indicate a significant anticancer effect after calcium electroporation. In relation to EP alone, the addition of calcium ions caused cell viability to decrease to 20% of control cells after 48h and about 30 percent after 72h. The most effective protocol occurred at 5.7kV/cm×900ns×100, which was comparable to ESOPE. Cell permeabilization increased with the increasing electric field intensity. We observed changes in cytokine mRNA expression levels, but additional research is needed.

Our research is entirely innovative, and according to our current knowledge, there are limited reports about calcium ion electroporation (CaCl2) in pancreatic cancer.

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PO-031

Measuring cell membrane charging limits in current clamp mode

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When electrical stimuli increase the transmembrane membrane potential (TMP) to 200-500 mV, they damage the membrane by an electroporation. The first and arguably the most sensitive manifestation of electroporation is the decrease in the membrane electrical resistance (Rm), suggesting that measuring the electric current across the membrane is the best way to detect electroporation. Indeed, several studies utilized the whole-cell voltage clamp configuration of patch clamp to explore the limits to which cell membrane can be charged safely without electroporation. The same approach was employed for calibration of voltage-sensitive dyes such as FluoVolt.

The inherent problem of voltage clamp measurements is the impact of the series resistance (Rs) at the tip of the measuring pipette. The command voltage (Vc) from the patch clamp amplifier is divided between Rs and Rm proportionally to their values. When Rm»Rs, TMP is approximately equal to Vc. However, electroporation decreases Rm to values comparable with Rs. Hence, the voltage drop at Rs has to be calculated from the measured current (I) and subtracted from Vc to obtain the actual TMP value as: TMP=Vc - Rs x I. This calculation works for slow Vc changes and for steady-state Vc. However, cell capacitance (Cm) slows membrane charging to the intended TMP proportionally to the charging time constant τ = Cm x Rm. When Rm is changing due to electroporation, the value of τ is not known and also changes in time. As a result, the actual TMP reached by fast Vc steps or ramps is difficult or impossible to calculate.

We propose to address this problem by measuring TMP in the current clamp mode, when we apply current steps or ramps and measure the voltage induced. This voltage V equals to TMP when Rm»Rs. If Rm is decreased by electroporation, TMP = V - Rs x I, where I is clamped current value. Most important, this calculation does not require the knowledge of Rm or τ, and can be employed with the fastest current steps or ramps. For example, current clamp can be employed to test if the breakdown voltage depends on how fast the membrane is polarized. Current clamp can also be employed to calibrate voltage-sensitive dyes in intact and already electroporated membranes, to compare if electroporation affects dye sensitivity.

We employed fast current ramps (0 to +/-1nA in 50ms) to measure membrane breakdown voltage in CHO-K1 cells. De-and hyperpolarization caused a distinct breakdown at 229±11mV and -230±16mV, respectively. Further increase in current beyond this voltage neither increased nor increased TMP, indicating that Rm is changing dynamically to maintain the TMP at the breakdown value. We employed current steps (50ms/step, in 100 pA increments down to -1 nA or up to +1 nA) to calibrate voltage sensitivity of the FluoVolt dye in intact and electroporated cells. Electroporation had no significant impact on the dye sensitivity, which averaged 17.1±1.3% per 100mV.

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PO-034

Combination of cold plasma and pulsed electric field for microalgae treatment

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The potential of microalgae helps to tackle the challenges such as the growing demand for energy resources, food and bioactive compounds is well known. However, solutions are still being investigated to efficiently reduce the cost of algae cell disruption while increasing the quantitative and qualitative yield of the resulting products. In contrast to conventional chemical and mechanical methods of algae treatment, pulsed electric field (PEF) and non-thermal plasma treatment have the greatest potential to address the above-mentioned problems.

Studies have already been conducted showing that mammalian and bacterial membranes oxidised in plasma are more sensitive to the PEF treatment [1,2]. However, there are no studies on the treatment of microalgae. Therefore, this work aims to investigate the effect of a combination of PEF and plasma treatment on microalgae.

The gliding arc discharge and PEF technologies were applied on Chlorella vulgaris cultivated in the BG-11 medium. The plasma treatment was performed using these parameters: compressed air flow rate ˜22.8 l/min, the distance between the "knife-edge" type electrodes and surface of the algae suspension was 30 mm, treatment duration 300 s, power generator voltage 50–250 V and frequency 270 kHz. PEF treatment consisted of 10 μs long 1-10 pulses at a frequency of 1 Hz, where applied electric field strength varied from 23-25 kV/cm. Afterwards, changes in the electrical conductivity, extracted chlorophyll-a and protein content of untreated and treated algae suspension were determined.

First, the effects of treatment using PEF or plasma were evaluated separately. The highest concentrations of excreted chlorophyll-a concentration were obtained after 10 pulses of PEF (180 µg/mL) or 210-250 V plasma treatment (190 µg/mL). The combination of plasma and PEF treatment did not significantly increase pigment levels (200 ± 10 µg/mL) compared to the PEF effect only.

From a protein extraction perspective, the PEF treatment yielded 700 µg/ml of protein and the plasma treatment (50-130 V) was 600 ± 100 µg/ml. In contrast, plasma treatment with a discharge voltage of 210 V or more resulted in a lower concentration of protein extracted from C. vulgaris compared to untreated algae. Meanwhile, the combined effect of plasma and PEF did not lead to an increase in the amount of proteins released, but rather to a significant decrease.

This unexpected decrease in protein extraction effi-

ciency suggests that further studies are needed to understand the effects of combined plasma and PEF treatment.

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PO-036

Mass transfer modulation during salting of PEF pre-treated salmon fillets

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Salting is one of the oldest and simplest methods of preserving large quantities of fish for long periods of time; it is often used by the industry in combination with other traditional processing techniques, such as smoking, drying and cooking. This results in safer products with a better sensory appearance, but the salting kinetics require long times for the salt to diffuse into the product and to work properly. Among existing emerging technologies, pulsed electric fields (PEF) are a non-thermal treatment that has been shown to be effective in increasing mass transfer in both plant and animal tissues, without affecting the nutritional value, flavour, colour and texture of products. The aim of the present study was to study the application of PEF to Atlantic salmon filets before subjecting them to dry salting in order to improve the process effectiveness. The experimental design included 5 salmon (Salmo salar) sample groups for each salting time (3 and 6 hours): control (NT) and 4 types of PEF pre-treatment (PEF1, PEF2, PEF3, PEF 4). At the end of the salting times, the samples were rinsed in running water, dried and then subjected to the following analytical determinations: weight change, water activity, NaCl content change, water content change, texture, colour and the level of thiobarbituric acid reactive substances (tBars). The results shown that pulsed electric fields of 0.64 kV/cm (detected by PEF3), applied prior to the 3-hour salting of salmon, promote the diffusion of salt into the tissues, leading to increased NaCl retention by the muscle, thanks to the PEF permeabilization effect on cell membranes. These process parameters in fact generated a reversible electroporation capable of favoring a more homogeneous distribution of salt within the product, also allowing for a lower percentage weight variation compared to untreated samples. PEF did not provide any advantages in terms of reducing the water activity of the samples, especially during the shorter salting times, but it did improve the water retention properties of the salmon with 3-hour salting, probably because of a more permeable structure that allowed retention of more liquid within the tissue. It is also possible that the results obtained were the consequence of a conformational change in the proteins induced by the applied treatment, which allowed greater NaCl absorption and less water loss from the samples. Regarding texture, color and lipid oxidation, the treatment did not provide any difference in the treated samples compared to the control.

The result obtained may be of great importance to the salmon processing industry because the achievement of higher salt levels, in a product that simultaneously loses less water, provides a technological advantage. In fact, the salting process is thus more efficient as processing times are significantly reduced (to only 3 hours) and higher processing yields are achieved with attractive cost savings for companies, improving the performance of industrial processes.

PO-039

Developing and optimizing ohmic heating for cake

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Conventional baking process is time and energy-consuming process mainly due to heat transfer is limited by internal conduction within the product. Ohmic heating is potentially solving this problem by allowing volumetric heating of the product and thereby, uniform and rapid heating and consequently, leads to a more energy-efficient and sustainable process, however, its potential is not utilized yet. Thus, the presentation will focus on recent development in our understanding of ohmic heating for baking processes.

The aim of current work focused on the investigation of the potential use of ohmic heating for cake baking and the influence of the process on cake quality changes.

An experimental design based on a Central Composite Design carried out to evaluate the effects of four key process parameters: applied voltage, salt, butter and baking powder content. The studied responses are process time, expansion, moisture content, hardness and springiness of the cake. The results showed that voltage is the parameter that affects all the responses significantly. Combination of high voltage and high salt content led to decreased baking times (up to 80%), increased moisture loss and increased hardness. Baking powder affected positively the expansion and springiness of the cake, but negatively the hardness and the process time. Butter did not influence significantly the textural properties; however, it enhanced the expansion and reduced the final moisture content.

A mathematical model was established and used to identify the optimum settings for ohmic baking process. The quality properties of two commercial cakes and a pound cake baked in the convection oven were used as target values. The cake baked at obtained optimum condition resulted in similar textural properties as commercial products. An assessment of the energy requirements showed that energy losses increase slightly with higher electric fields, but ohmic heating can use up to 47% less energy than the industrial ovens. The results are encouraging and ohmic heating is a promising technology for cake baking.

PO-042

Bioactive properties of air-dried organic apples pre-treated by pulsed electric field

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Pulsed electric field (PEF) was demonstrated to enhance the drying kinetics, reduce drying time and specific energy consumption of the process. However, the literature on the quality aspects of such processed materials and especially chemical properties, is not very abundant.

The aim of this research was to evaluate the effect of PEF treatment and air temperature on selected chemical properties of convective dried organic apples. PEF application was carried out using batch system (PEFPilot™, ELEA, Germany) and apple slices (thickness of 5 mm) were hot air dried (Promis, Poland). Response Surface Methodology with central composite face centred plan (CCF) was used to design the experiment. The energy input of PEF (1 kV/cm) varied from 1 to 6 kJ/kg and hot air temperature ranged between 60-80°C. Following chemical properties were evaluated in dried apple slices: vitamin C, total phenolics (TPC) and total flavonoids (TFC), antioxidant activity with DPPH radical (AA) and reducing power (RP).

The vitamin C concentration of investigated samples varied between 6.5 and 130.6 mg/100 g d.m. In all cases, untreated dried apples exhibited higher concentration of vitamin C – it was equal 130.6, 112.2 and 82.2 mg/100 g d.m., when temperature of 60, 70 and 80°C was used, respectively. In turn, PEF treated samples exhibited vitamin C content of 6.5-12.7, 7.3-19.8 and 8.0-10.1 mg/100 g d.m, for the same temperatures, respectively. Such tremendous decrease may be related to liberation of both vitamin C and enzymes (e.g. ascorbic acid oxidase) which remained active during drying. Similar results were found for phenolics and flavonoids. In these cases, alike all PEF evaluated variants were characterized by lower TPC and TFC. Moreover, at temperature of 60 and 70°C the negative relationship between energy input and phenolics and flavonoids content was found. For instance, apples dried at 70°C were characterized by TPC od 1482 mg/100 g d.m whereas materials pre-treated by 1, 3.5 and 6 kJ/kg exhibited values of 1201, 796 and 720 mg/100 g d.m., respectively. Antioxidant activity of dried slices pre-treated by PEF was smaller in comparison to control apples. The smallest difference was found when energy input was 1 kJ/kg and drying was carried out at 70°C. Similar observations can be withdrawn for reducing power.

Performed analysis showed that PEF treatment in the range of 1-6 kJ/kg applied before air-drying decreased concentration of analysed bioactive compounds and antioxidant activity. In most cases the reduction stayed in relation with the energy input which indicates that PEF conditions aimed towards drying enhancement should be selected carefully, especially when chemical properties conservation is required.

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PO-044

The impact of matrix on pulsed electric field preceded food drying

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Pulsed electric field is one of the most promising technologies used for drying enhancement. The literature of the subjects shows that the effect of PEF on kinetics and quality strongly depends not only on parameters of pre-treatment, drying technique but also on matrix.

This aim of this research was to investigate the effect of PEF pre-treatment on air drying kinetics and selected properties of apple, strawberry, carrot, and mushrooms. The experiment was designed using response surface methodology with central composite face cantered plan. Drying was performed at temperature of 55, 70 and 85°C whereas energy input of PEF was selected based on electroporation efficiency measurement and it ranged from 1-6 kJ/kg depending on the raw material. Kinetics of the process and drying time was evaluated based on the water evaporation curves. Following quality parameters were evaluated: total phenolics, total colour difference and antioxidant activity (DPPH assay, EC50).

Obtained results showed that the role of matrix is of paramount importance when it comes to the effect of PEF on drying improvement. For instance, in the case of apple tissue, drying time was reduced (by 5-20%) no matter the parameters of drying and PEF treatment were whereas in the case of mushrooms the reduction was found mainly when the highest drying temperature was used (1-12%). The ambiguous results were also found when strawberry was used. In the case of carrot samples, the reduction of 4-30% was found but only at 55 and 70°C while no reduction or even extension of drying time was stated when drying was performed at 85°C. PEF pre-treatment reduced total phenolics concentration of most investigated variants of apples and strawberries. Similar observations were withdrawn for antioxidant activity. In turn, total phenolics content of mushrooms increased no matter the treatment parameters were. Total colour difference of PEF pre-treated materials was usually higher in comparison to untreated materials, however the direction of colour change was in some cases rather desirable – for instance, carrot tissue was characterized by higher share of red (a*) and yellow (b*) than control material.

Research that was carried out shows that the effect of PEF depends strongly on matrix and no standard, universal, optimal protocol can be generated for all raw materials. Thus, the process requires optimization considering the technological aim of drying and pre-treatmetn.

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PO-047

Plant-based model for the optical evaluation of electroporated area after irreversible electroporation and its comparison to in-vivo animal data

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Electroporation is widely used in medicine, such as cancer treatment, in form of electrochemotherapy or irreversible electroporation. For electroporation device testing, living cells or tissue inside living organism (including animals) are needed. Plant-based models seem to be a promising alternative to substitute animal models. The aim of this study is to find a suitable plant-based model for optical evaluation of IRE, and to compare the geometry of electroporated area with in-vivo animal data.
For this purpose, a variety of fruit and vegetables were selected and optically evaluated after 0/5/22 hours after electroporation. Apple, fresh and old potato were found to be suitable models as they enabled an optical evaluation of the electroporated area. For these models, the electroporated area was calculated after 0/5/22 hours. The electroporated area of apple, which showed the fastest optical results, was then compared to a retrospectively evaluated swine liver IRE dataset which had been obtained for similar conditions. The electroporated area of the apple and swine liver both showed a spherical geometry of comparable size. For all experiments, the standard protocol for human liver IRE was followed.
To conclude, potato and apple were found to be suitable plant-based models for the optical evaluation of electroporated area after irreversible electroporation, with apple being the best choice for fast optical results. Given the comparable range, the size of the electroporated area of the apple may be promising as a quantitative predictor in animal tissue. Even if plant-based models cannot completely replace animal experiments, they can be used in the early stages of electroporation device development and testing, decreasing animal experiments to the necessary minimum.

PO-049

Study of the impact of Pulsed electric fields pretreatment on yellow mealworm insects in a biorefinery concept

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With the world's population predicted to reach 9 billion by 2050, it is essential to find new sustainable agricultural practices and protein sources for the feed and food sectors. Edible insects are promising alternative biomass that can meet the growing demand for proteins, presenting many advantages over conventional farming, such as minimal resource needs, low environmental impact (less GHG and NH3 emissions), low feed conversion ratio (around 1.7 for insects compared with 10 for cattle) and above all their integration into a logic of circular economy.
This research work is part of the RAFINSECT project funded by the HDF region (France) and aims to set up an insect biorefinery. The objective is to fractionate and valorize all biomass fractions such as proteins, lipids and chitin by incorporating them into a variety of food and feed matrices, and into cosmetic products and packaging as well.
PEF treatment was applied to living mealworms, with an electric field strength between 200 and 2000 V/cm and a treatment time between 20 and 200 ms, under different treatment conditions (in distilled water, tap water, compacted or pre-rinsed). Treated larvae were then pressed as an energy-efficient defatting and dewatering method or dried in a ventilated oven. The impacts of the PEF treatment on cell membrane electroporation, larval mortality, pressing ability, drying kinetics and product quality were studied. The treatment processes were compared and optimized.
PEF pretreatment successfully killed the larvae with the mortality being linked to the treatment's electric field strength, and pre-rinsing was found to improve the killing efficiency. The SEM images show visible damage and changes in the larvae's structure. The treatment has significantly increased the hydraulic press extraction yield from 65% in blanched larvae to 78% in PEF-treated larvae, which might be caused by the enhanced membrane permeability due to electroporation, as well as accelerated the drying kinetics and water diffusivity (an improvement of water diffusivity by 470%).

PO-052

Eradication of Saccharomyces cerevisiae by PulsedElectric Field Treatments

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One of the promising technologies that can inactivate microorganisms without heat is pulsed electric field (PEF) treatment. The aim of this study was to examine the influence of PEF treatment (2.9 kV cm−1, 100 Hz, 5000 pulses in trains mode of 500 pulses with a pulse duration of 10 µs) on Saccharomyces cerevisiae eradication and resealing in dierent conditions, such as current density (which is influenced by the medium conductivity), the sort of medium (phosphate buered saline (PBS) vs. yeast malt broth (YMB) and a combined treatment of PEF with the addition of preservatives. When the S. cerevisiae were suspended in PBS, increasing the current density from 0.02 to 3.3 A cm−2 (corresponding to a total specific energy of 22.04 to 614.59 kJ kg^{-1}) led to an increase of S. cerevisiae eradication. At 3.3 A cm−2 , a total S. cerevisiae eradication was observed. However, when the S. cerevisiae in PBS was treated with the highest current density of 3.3 A cm−2 , followed by dilution in a rich YMB medium, a phe-

nomenon of cell membrane resealing was observed by flow cytometry (FCM) and CFU analysis. The viability of S. cerevisiae was also examined when the culture was exposed to repeating PEF treatments (up to four cycles) with and without the addition of preservatives. This experiment was performed when the S. cerevisiae were suspended in YMB containing tartaric acid (pH 3.4) and ethanol to a final concentration of 10% (v/v), which mimics wine. It was shown that one PEF treatment cycle led to a reduction of 1.35 log10, compared to 2.24 log10 when four cycles were applied. However, no synergic eect was observed when the preservatives, free SO2, and sorbic acid were added. This study shows the important and necessary knowledge about yeast eradication and membrane recovery processes after PEF treatment, in particular for application in the liquid food industry.

PO-055

Influence of pulsed electric field-assisted dehydration on the volatile compounds of Genovese basil (Ocinum basilicum L.)

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Pulsed electric field (PEF) was applied to basil leaves (Ocinum basilicum L.) prior air drying at 40 °C. The parameters of the electric treatment were designed in such a way that (i) electroporated the tissue reversibly, provoking a permanent opening of the stomatal guard cells and (ii) electroporated the tissue irreversibly, damaging the cells.

Treated leaves lost some volatile compounds due to both PEF treatments, probably related with the direct effect of permeabilization on the secretory cells of glandular trichomes. Upon drying, the irreversible permeabilization treatment showed the highest influence on the profile of volatiles in the dried leaves showing better retention of some terpenoids than the control. The performed statistical analysis allowed to select six compounds that can be used as markers both for the effect of pre-treatments prior dehydration and for the effects of dehydration itself on the volatile compounds of basil leaves.

PO-057

Mathematical model of biliary metal stent occlusion treatment using irreversible electroporation

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Electroporation finds its place in a wide range of scientific and industrial fields. This abstract focuses on a novel application of irreversible electroporation (IRE) to recanalize occluded biliary metal stents used in cholangiocarcinoma treatment. The occlusion, or blockage, of the stent, is usually caused by epithelial hyperplasia, tumour ingrowth or overgrowth into the stent mesh, as well as clot accumulation. The survival of patients has improved to exceed 12 months however, the metal stent patency rate is reportedly between 14 and 321 days. Standard recanalization techniques such as mechanical cleaning with balloon catheter are not effective in case of hyperplasia or tumour ingrowth. Endoluminal radiofrequency ablation could be used but there is a certain risk of thermal damage that could be caused by the stent becoming an active electrode. These factors suggest that IRE could be a convenient choice.

In this study, we present the analysis of the electric field and temperature distribution inside the occluding tissue that was modelled as liver parenchyma. Simulations are based on a 3D FEM model. Electroporation is induced using a specialised 3-electrode catheter where two plate electrodes are in contact with the undesired tissue blocking metal biliary stent. Boundary conditions were set to drive the electrodes to deliver 100 of one hundred microseconds long pulses with a frequency of 1 Hz. The tissue was exposed to four different amplitudes of voltage (300 V, 650 V, 1000 V and 1300 V). The model parameters were based on real experiments.

The simulations show that the distribution of the electric field in the occluding tissue is enclosed in the metal stent if the stent mesh is dense enough. This is caused by the faraday cage-like behaviour which works to our advantage and protects the healthy surrounding tissue from the IRE effect. The ablation volumes are affected by the geometry and position of the electrodes and stent but generally increase with higher voltage amplitudes. In our model, it ranges approximately from 76 mm3 to 184 mm3. The temperature rise is acceptable in most cases. Only when using an amplitude of 1300 V does the temperature reach values above 50 °C.

Based on the results of FEM simulations and the generally accepted threshold for IRE of 700 V/cm the electroporation effect should occur in all simulated cases. Yet based on the pilot experiments it seems that the threshold for the IRE is higher and corresponds with the intensity of the electric field above 1000 V/cm. According to our findings, the optimal voltage for IRE in occluded biliary stents ranges between 650 – 1000 V. If we would increase the voltage, the ablation volume would be higher but we could induce thermal damage. The results suggest that this IRE application is feasible with certain parameters of the procedure. It has great potential, and if validated, this form of recanalization could be used in other metallic stent occlusions.

PO-060

An optimal dose-response in terms of pulse length in EP-based treatment protocols

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An optimal EP-based treatment, i.e., a treatment based on electroporation (EP), such as electrochemotherapy (ECT), Gene Electrotransfer (GET), or irreversible electroporation (IRE), is a function of pulse amplitude,

length, number, and frequency, among other variables. Finding an optimal EP-based treatment in a space of 4 parameters is a difficult task. In previous works [1-2] optimal GET was studied in terms of pulse number, for a range of pulse intensities, with all other variables kept constant; unwanted damage due to pH fronts and the concept of the dose-response relationship was discussed. Here we extend those results by analyzing an optimal EP-based treatment in terms of pulse length considering damage due to temperature and/or irreversible electroporation effects. An optimal dose-response relationship in an EP-based treatment, such as IRE, for the range of pulse intensities with fixed pulse number and frequency, tested here, is predicted as the critical pulse length dosage yielding maximum irreversibly electroporated tissue with minimal damage due to temperature.

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[2] Marino M. et al, OpenEP: an open-source simulator for electroporation-based tumor
Treatments, Scientific Reports (2021) 11:1423

PO-062

Electric Field Fabrication: A method for 3D printing electroporation microdevices

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Microfluidic devices have been used extensively in the field of electroporation for characterization, transfection, and pasteurization and also provide a variety of advantages when compared to macro-sized systems. In these devices, spacing between the electrodes is small, ultimately requiring low applied potentials to electroporate the cell for its intended application. In turn, these low potentials allow for a wide range of generators and signals, not adequate for traditional setups, to be used. A microdevice offers features comparable to cell size for high-resolution characterization, small required sample size for rare cell-types, compatibility with other microfluidic processes upstream and downstream, and the ability to monitor the process in real-time. The design and complexity of these devices, while they offer unique advantages for in vitro studies, are limited by current soft-lithography techniques and have yet to extensively benefit from the possible geometries of additive manufacturing (AM).

Few additive manufacturing processes are appropriate for the fabrication of microfluidic electroporation devices. The available high-resolution methods have limited biocompatible material options and do not permit component integration between layers; thus, requiring assembly and electrode integration after the part is complete. Here, we introduce Electric Field Fabrication (EFF), an additive manufacturing method that manipulates liquid build material using dielectrophoresis (DEP). Dielectrophoresis, or polarization of a dielectric particle or fluid in the presence of a non-uniform electric field is used to form the liquid build material in the desired shape of each layer. This shape is defined by an interdigitated electrode array– combining the resolution of integrated circuit fabrication and soft lithography with the complexity of AM in the third dimension. After a layer is shaped, it is subsequently cured; this process is repeated until a 3D structure is formed. Because EFF does not take place in a vat of resin, components such as electrodes or membranes can be included mid-build to create lab-on-a-chip systems that are biologically relevant for treatment planning and characterization. A unique advantage of EFF is also the material selection. Proof-of-concept parts printed on this platform include microdevices made with uncommon AM materials but very common microfluidic materials such as polydimethylsiloxane (PDMS), a biocompatible polymer with sufficient transparency for optical monitoring. The devices printed on this platform include a millimeter-scaled gear, an enclosed microfluidic tapered channel, and a layer of PDMS for interfacing with copper electrodes.

Electric field fabrication is an additive manufacturing method that combines high resolution fabrication techniques with the complexity of additive manufacturing for faster, easier, and more innovative designs for electroporation microdevices.

PO-065

Electrical parameters for (ir)reversible electroporation on hepatocellular carcinoma cells in vitro

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Hepatocellular carcinoma (HCC), which accounts for >80% of primary liver cancers worldwide, has a heavy disease burden and is a leading cause of cancer-related death in many parts of the world. Traditional treatment options are not always efficient in eradicating the tumor, hence the need for new treatment methods. During the promising minimally invasive electroporation-based therapies, biological cell membranes are exposed to an external, sufficiently high, pulsed electric field, which can lead to a rapid and large increase in electric conductivity and permeability, creating so called nanopores into the lipid bilayer of the cell membrane. During irreversible electroporation (IRE), the cell membrane cannot repair the induced nanopores because of their size and amount, which causes the cell to undergo apoptosis. During reversible electroporation (RE), the cells can repair their phospholipid bilayer and continue with their normal cell functions. In tumor therapy, those hydrophilic pores are used to increase the diffusion of a chemotherapeutic drug which is known as electrochemotherapy (ECT). For both IRE and RE, the success of the treatment is dependent on application of the appropriate electric field. Therefore, this study aims to define the (electrical) parameters for IRE and RE on hepatocellular carcinoma (HepG2) cells in-vitro.
In a custom-made in-vitro setup, HepG2 cell viability (at 0, 5, 10 and 15 minutes), and the peak temperature were measured after electroporation with the different IRE and RE pulsing protocols, to determine the most successful

settings for IRE and RE. In addition, A CAM/PI flow cytometric assay was performed to confirm cell permeabilization for the RE pulsing protocols with the highest cell viability.
The results indicated that an IRE pulsing protocol (70 pulses, 100 µs pulse length, 100 ms interval) with an electric field strength of 4000 V/cm was needed as threshold for almost complete cell death of HepG2 cells. A RE pulsing protocol (8 pulses, 100 µs pulse length, 1000 ms interval) with an electric field strength of 1000 V/cm was needed as threshold for viable and permeabilized HepG2 cells. The low peak temperatures (max 30.1°C for IRE, max 23.1°C for RE) within this study indicated that the reduction in HepG2 cell viability was caused by the applied electric field and was not a result of Joule heating.

PO-068

Potentiating the efficacy of clinical antibiotics by electroporation

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Electroporation is a promising complementary technique for bacterial inactivation. Exposing bacteria to short electric pulses of sufficient strength permeabilizes their envelope, thereby facilitating the uptake of molecules, including antibiotics. Electroporation is effective against a broad range of bacteria and as it is based on pore formation, it is largely impervious to development of resistance. So far, most studies investigating electroporation to potentiate the efficacy of antibacterials used substances permissible in food industry, and only few used clinical antibiotics, as addition of these is problematic, which limits acceptable applications to treatment of wastewaters inherently contaminated with such antibiotics. Moreover, even the studies utilizing clinical antibiotics have mostly focused on achieving the maximal effect, and less on underlying mechanisms and on the possible dependence of the potentiation on the antibiotic's target site or mode of action.
Our aim was to determine the effect of antibiotics mode of action and concentration, electric field amplitude and incubation time after treatment on Escherichia coli inactivation.
Three antibiotics with different modes of action were used: ampicillin (inhibition of cell wall synthesis in the peptidoglycan layer of the cell wall), tetracycline (intracellular inhibition of protein synthesis) and ciprofloxacin (intracellular inhibition of DNA replication). Concentrations used in the study were determined based on their previously determined minimum inhibitory concentrations. For electroporation treatment, one pulse of 1 ms duration and electric field amplitude of 5, 10, 15, or 20 kV/cm was used. Level of inactivation was determined after different post-treatment incubation times at room temperature.
Our results show that with increasing pulse amplitude, antibiotic concentration, and/or incubation time, the potentiation of inactivation consistently increases. Ampicillin only needs to permeate the outer membrane of bacterial cell wall, which can explain the highest potentiation of inactivation achieved with it. Although considerable inactivation (3-4 log) with combined treatment was achieved, this may not be achievable for antibiotic concentrations usually present in wastewater, which are typically much lower. Also, to reach considerable potentiation, some incubation with antibiotic is required even for ampicillin.

PO-071

Efficient recycling of electronic-waste by nanosecond pulsed electric discharge

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Kumamoto University, Japan

Recycling of valuable materials such as rare metals is becoming an imperative topic from the perspective of resource conservation and environmental sustainability. The existing techniques for electronic waste recycling and metal separation, e.g., chemical or mechanical crashing methods, are not cost effective and can cause further environmental burden. In this regard, pulsed power technology has attracted considerable attention for green recycling. In this study, metal coated electronics were used as a separation processing model. A magnetic pulse compression pulsed power generator (MPC-PPG) was used for providing positive nanosecond pulses. By applying underwater electric discharge, the metallic parts were separated. To understand the separation mechanisms an ultra-high-speed framing camera with 5 ns exposure time, equipped with schlieren and shadowgraph optical setups, were employed. The whole process of plasma generation, shock waves production, propagation, and interface interactions, and afterward flow fields were observed. A fiber optic probe hydrophone (FOPH) pressure transducer with 3 ns rise time was used for incident and transmitted shock waves pressure measurements. The results clearly confirm that the proposed method is highly efficient for green recycling. The technique can be applied to electronic waste recycling on a commercial scale.

PO-074

Study of permittivity change in the cervical cell membrane 3D realistic model due to application of subnanosecond electric field using SRD based pulse generator

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Indian institute of Information Technology Allahabaad, India

The paper represents a change in permittivity of the dielectric layer in the cell membrane of the cervical cell using a high voltage output generated by the diffused step recovery diode producing a Gaussian pulse of 30ns and an electric field of 10KV/cm . The high-frequency electric field allows the permittivity of the cell membrane to lower down efficiently which can be easily studied with help of realistic geometry of a single cervical cell model generated using image processing techniques and CAD tools and introduced into a multi-physics tool. The realistic pulse generated by the diffused step recovery diode-based generator is introduced in the multi-physics area. The Debye dispersion relation is used to model the dielectric relaxation in the cell membrane which is a bi-lipid layer

of 5nm thickness. The study of dielectric in the cervical cells is important as the second-order time-domain equation helps the trans-membrane potential to easily reach the potential of 1-1.5V. This transmembrane voltage generated can cause pores formation on the nano-meter thin layer which is important in the case of drug and dye delivery. The dye delivery can easily detect the morphological changes in the cell structure of the cervical cells which is important in cancer detection. Drug delivery can be used for electrochemotherapy applications. Thus the change in permittivity of the bi lipid layer considerably reduces the amount of electric field required to facilitate the pore formation.

Poster Session (and Coffee break)

Wednesday Poster Session Track
Oct 12, 15:00 - 16:00

PO-003

Electrochemotherapy in Oncodermatology, current uses in Argentina

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Introduction: Electrochemotherapy (ECT) is a standard treatment modality in Europe since 2006, and now also in Argentina since 2020. In this work, we present the results of three patients treated.
Patients and methods: The procedures were performed in an operating theater with heart monitoring. The patients were treated using IV bleomycin 15,000 IU/m2 in bolus. The electric pulses were delivered using the electroporator OncoPore (BIOTEX, Buenos Aires, Argentina), a medical-grade device. Needle electrodes were used in all cases, as well as a single-dose of intramuscular NSAID and corticosteroids for pain management after the procedure.
Squamous cell carcinoma in the forehead. The patient was 80 years old and presented a tumor surrounded by satellite lesions (total size was 3.5x2.8 cm). The mild cognitive impairment of the patient precluded the use of general anesthesia, rendering the surgical procedures impossible. Only lidocaine without epinephrine was used as a local anesthetic.
Basal cell carcinoma at the back of the ear. The patient was 60 years old and in good clinical shape. Smoker, with peripheral vascular disease. The tumor had a size of 1x1.5 cm. Mild sedation with propofol plus local lidocaine without epinephrine was used for the procedure. The patient reported having no pain during or after the procedure.
Angiosarcoma in the trunk. The patient was 85 years old and in good clinical shape. He was in optimal cognitive status. Due to previous radiotherapy treatment for a sarcoma in the armpit, he developed two angiosarcomas (the first one was surgically removed). For treating the second one, he rejected a new surgical procedure because of the slow recovery time. The size of the remaining tumor was 12x6.5 cm. For the ECT procedure, the patient received mild sedation with intravenous ketamine and midazolam. No local anesthetics were used.
Results: Total treatment time ranged from 18 to 30 minutes. No cardiac rhythm alterations occurred during or after the procedure. A progressive reduction of the size of the lesion until its complete disappearance was seen with no necrosis. All cases obtained a complete response, free of relapse during the follow-up time. Tolerance was excellent, even with local anesthesia. No systemic side effects or post-procedural pain were observed. Hyperpigmentation due to bleomycin developed in one patient, but it started to fade away progressively. The cosmetic results in the other patients were very good.

PO-006

The best treatment and care for patients suffering from skin cancer - lifting competencies for nurses working in advanced cancer care with calcium electroporation

Christina Louise Lindhardt

University of Southern Denmark, Clinical Institute, Denmark

Purpose: The patients come first and need the best treatment and care is the objective of the project 'Changing Cancer Care'. We identified how a tailored evidence-based training course for oncology nurses could improve qualifications in the care and treatment of patients treated with calcium electroporation (EP) and, subsequently, quality of life. Initially, we describe the development test and evaluation of the intervention, and implementation strategy, followed by the evaluation. The research, test and evaluation were conducted in 2019-2020.
Methods: The intervention was developed using a literature review and individual- and focus group interviews with healthcare professionals experienced in the field and patients. A prototype eight-day training course for nurses working in advanced oncology where patients treated with calcium EP was developed, tested, and evaluated with mixed methods, including oral, written evaluation and an online assessment tool concerning content and relevance.
Results: The participating nurses evaluated the training course as relevant and appropriate for their practice in advanced cancer care. Knowledge of skin cancer focused on body image, skin and pain management, family dialogue, person-centred approaches, clinical leadership, and shared decision making was evaluated as indispensable. The participant felt confident in using the newly acquired skills immediately after the course and being resourceful for healthcare professionals on their ward.
Conclusion: This research demonstrates that investing in an evidence-based curriculum shifts nursing skills and care. These skills will ultimately benefit patients by providing a safer, more reliable transition through the hospital system and improving patient quality of life. The curriculum based on theory and novel knowledge regarding

calcium EP treatment for patients with cancer enhanced the nurses' qualifications and supported them to provide optimal and effective oncological care. Further curriculum developments may include patient involvement and co-operation, e.g., patient and relatives' boards and organisations.

PO-009

Randomised controlled trial investigating the effect of reduced bleomycin in electrochemotherapy on patients with cutaneous malignancies: A protocol

Freya Bastrup[1], Mille Vissing[2], Volker-Jürgen Schmidt[2], Mohammad Farooq Nassari[2], Taiba Alrasheed[2], Anni Linnet Nielsen[3], Camilla Kjær Lønkvist[3], Lisbeth Rosenkrantz Hølmich[3], Michael Prangsgaard Møller[3], Biljana Mojsoska[4], Elizabeth Emilie Rosted[2], Julie Gehl[2]

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Background: Response rates of electrochemotherapy (ECT) with bleomycin have been high and consistent when applied for the treatment of skin cancer. However, due to possible side effects e.g. lung fibrosis, some patient groups are excluded from treatment. Consequently, in 2018, studies began to investigate the possibilities of reducing the dose, finding a similar objective overall response (ORR), thus indicating that a lower dose may not be inferior.

Aim: This protocol for a randomized controlled trial (RCT) aims to investigate whether the bleomycin dose can be reduced with 50% and retain the ORR in patients with cutaneous malignancies.

Methods: The RCT will include 55 patients, as calculated in a statistical power analysis for non-inferiority studies using categorical data. The patients will be randomised 1:1 in two groups; one group receiving the standard bleomycin dose of 15,000 IU/m2 and the other group receiving 7,500 IU/m2. In order to be included the patients must be over 18 years old, have a histologically verified cutaneous or subcutaneous, primary or secondary cancer of any histology, with a life expectancy over 3 months and have a creatinine within normal upper limit. Patients will be excluded if they are pregnant, allergic to bleomycin or with impaired lung function. The primary endpoint is to evaluate the ORR after three months. The trial will also include qualitative interviews for analysing quality of life before and after ECT, and biopsies and blood samples to investigate bleomycin pharmacokinetics and drug distribution. The trial is planned to start in January 2023.

Conclusions: Reducing the bleomycin dose appears to be promising. After searching the literature of clinical trials, no RCT study has to our knowledge, considered lowering the bleomycin dose for ECT treatment. This is necessary to properly investigate the issue.

This project is funded by the Danish Cancer Society.

PO-012

Electrochemotherapy combined with immunotherapy as a facial nerve preserving treatment modality of the late intraparotideal metastasis of a non-melanoma skin cancer

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University of Szeged, Hungary

Authors present a case of a late intraparotideal metastasis of a non-melanoma skin cancer treated successfully with a combined treatment of electrochemotherapy (ECT) and immunotherapy.

The 72-year-old male patient was operated with a planocellular carcinoma of the skin in the right temporal region. Due to R1 resection and ipsilateral lymph node metastases in the parotid gland and on the neck, reresection, partial parotidectomy and neck dissection was carried out. After negative staging examinations the patient received definitive dose postoperative radiotherapy according to oncoteam decision.

4 years later the patient was presented with a growing mass in the remnant of the right parotid gland. The MRI suspected malignancy, the recurrent planocellular carcinoma was proven by open biopsy. Following the negative staging examinations, the oncoteam decision was immunotherapy (cemiplimab) in combination with ECT, because surgical removal of the tumor would have destroyed the facial nerve.

After 2 regimens of cemiplimab ECT was carried out according to the Standard Operating Procedure (SOP) with intravenous bleomycin, standard adjustable linear electrodes, Cliniporator Vitae by Igea S.p.A. The third cemiplimab was administered 2 weeks after ECT. 1 month after ECT complete remission was visible, which was proved by MRI as well, with intact facial nerve functions. The patient is tumor-free 8 months after ECT and is receiving regular immunotherapies.

Our case demonstrates the organ and nerve function sparing ability of ECT in combination with immunotherapy.

PO-018

Evaluation of TNF alpha production due to electrochemotherapy applied on glioblastoma spheroids co-cultured with monocytes

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There is an increased interest in the evaluation of immune response involvement in the clinical outcome of electrochemotherapy (ECT)(1, 2). In the present study glioblastoma spheroids were obtained, treated with ECT and co-cultured with monocytes. We evaluated the pro-inflammatory TNF-α and anti-inflammatory IL 10 levels as a response of naïve resident monocytes to the exposure of the spheroids to ECT with either temozolomide (TMZ) or cisplatin (Cis).

Spheroids were prepared from U87 human glioblastoma cell line (ATCC) at a seeding of 10k cells/well, grown for 7 days in 96 well plates, U bottom type, agarose coated wells. Spheroid growth rate was monitored every 2 days

via optical microscopy at 5x. ECT was applied on spheroids using an Electro Cell B10 (β-Tech) generator (following the ESOPE protocol (3). After treatment, the co-culture was done by pipetting the spheroids in new wells, previously covered with a monocyte layer (seeding 5k cells/well). Monocytes were freshly separated from the same human healthy donor on the day of ECT, using negative immunomagnetic selection assay (MojoSort Human Pan Monocyte isolation kit). Multiple control groups were considered as follows: U87 spheroids in culture medium; U87 spheroids with monocytes; U87 spheroids exposed to chemotherapy alone; U87 spheroids exposed to chemotherapy and monocytes (without electric pulses).
TNF-α was evaluated from co-culture growth medium 5 hours after ECT and again at 7 days post treatment using ELISA (TNF alpha Abcam 181421). IL-10 was also measured (IL-10 Abcam 185986).
The measurements showed that after ECT with Cis (at 833uM) or TMZ (at 100uM) applied on spheroids, there was a significant increase of TNF alpha levels in the co-culture medium at both 5 hours and 7 days with respect to the co-cultures not exposed to the electric pulses. The levels of TNF alpha were generally lower in case of ECT – TMZ group than in that of ECT – Cis. The levels of IL-10 were not significantly modified by ECT.
The spheroids growth rate was computed as an area-based index using Organoseg software(4). The index was decreased at 7 days in the case of ECT + monocytes exposure for both TMZ and Cis when compared to chemotherapy alone.
It is expected that after electroporation intracytoplasmic molecules are released and they could play the role of immunogenic signals as classical DAMPs (ATP, calreticulin). Considering that our study showed increased levels of pro-inflammatory TNF-alpha but not for anti-inflammatory IL-10, we may speculate that ECT recruits naïve monocytes towards the M1 pro-inflammatory type, with known anti-tumoral activity, avoiding in the same type their transformation into tumor associated macrophages (TAMs, or M2 anti-inflammatory type) known for their pro-tumoral activity(5).

PO-021

Electroporation-based modalities fused with 17β-estradiol in ovarian cancer therapy in vitro

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Ovarian cancer (OC) is an estrogen-dependent malignancy and the most fatality among all gynecological carcinomas [1]. Estrogens stimulate the proliferation of OC; however, depending on their concentration, they also can have a cytotoxic effect on cancer cells [2,3]. So far, this has not been thoroughly investigated. Calcium electroporation (CaEP) is a modification of electrochemotherapy (ECT), which enables the introduction of supraphysiological doses of calcium ions (Ca2+) into the cytosol using pulsed electric fields (PEFs). Unfortunately, the available literature lacks data focusing on the efficiency of CaEP as the potential OC treatment method.
The presented research aimed to investigate the impact of ECT (with cisplatin or calcium chloride (CaCl2) fused with a high dose of 17β-estradiol (E2) preincubation on OC cells (OvBH-1). Furthermore, the influence of the analyzed treatment on normal cells (CHO-K1) has been investigated. In the study, standard ESOPE protocol (100 µs × 100 Hz × 8 pulses) with variable voltage [kV/cm] has been applied. Subsequently, the impact on cells' viability was examined by MTT assay. For each parameter, the influence of ECT on cells' mobility was studied using a wound-healing assay. The correlation between preincubation with E2 and plasma membrane permeabilization was examined by measuring the uptake of the Yo Pro™-1 dye using flow cytometry. After treatment, morphological changes occurring in cells have been visualized using fluorescent staining of the actin cytoskeleton and observed by confocal laser scanning and holotomographic microscopy. Furthermore, the authors determined the type of cell death that occurred post-treatment using Annexin V Apoptosis Necrosis Assay. Then, the BAX, Bcl-2, and Caspase-12 levels were analyzed on protein (Western-Blotting) and transcript levels (Real-Time qRT-PCR). Also, a sensitive luminescent assay was used to assess the Caspase-3/-7 activity.
The obtained results revealed that preincubation with E2 enhanced the cytotoxic effect of CT and ECT on OC cells. Concurrently, preincubation decreased the plasma membrane's permeabilization. It may suggest that the enhanced efficiency of ECT after preincubation is not related to plasma membrane permeability.
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PO-022

Effect of electrochemotherapy on myogenesis of mouse skeletal muscle C2C12 cells in vitro: "side-effects"

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Electrochemotherapy (ECT) is a local ablative therapy for the treatment of different skin and subcutaneous tumors as well as for certain tumors in internal organs.

The use of electroporation enables the application of small doses of bleomycin or cisplatin with high therapeutic efficiency, resulting in minimal systemic toxicity. Skeletal muscle, in addition to cutaneous, vascular, endothelial, and neural tissue, represents a major tumor-surrounding tissue, which is exposed to side effects of ECT. The aim of this study was to investigate the effects of ECT on the myogenesis of C2C12 cells in vitro, as the effects of ECT on the different stages of myogenesis are not known.
The effect of ECT was determined in mouse skeletal muscle C2C12 cell line. First, the electroporation efficiency of differentiated C2C12 myotubes was determined at increasing voltages from 200 to 1300 V/cm using propidium iodide (PI). Viability was measured using the Presto Blue® assay. Further, the effect of ECT with bleomycin or cisplatin on the survival of C2C12 myoblasts and myotubes using optimized electric pulse protocol was evaluated. Increasing doses of bleomycin (from 14000 nM to 0.14 nM) and cisplatin (from 200 µM to 5 µM) were tested and cell survival was determined at 3, 5 and 7 days after ECT. In addition, the effect of ECT on myoblast differentiation including IL-6 secretion was evaluated using ELISA.
Permeabilization of C2C12 membranes by the intake of PI was voltage-dependent with app. 42?ficiency at 500 V/cm and 74% to almost 100?ficiency at higher voltages from 900 V/cm to 1300 V/cm. At these tested voltages the high cell survival rate and myotube integrity has been maintained until day 5 after electroporation. The decrease of cell viability in myoblast or myotubes has been observed after ECT with high doses of bleomycin or cisplatin, whereas low doses (up to 1.4 nM for bleomycin and 15 µM for cisplatin) have shown no effect on cell survival. However, myotubes were less sensitive to ECT with bleomycin or cisplatin compared to myoblast, resulting in lower IC50 values for bleomycin and cisplatin, up to 11.9-times and up to 7.3-times, respectively. Moreover, ECT affected myoblast differentiation capability, indicated by a significant reduction of myotube formation, and a decrease in IL-6 expression compared to control. These preliminary findings contribute to the safety profile of ECT for tumor treatment.

PO-025

Molecular insight into denaturation of plasma membrane ion channels by pulsed electric fields

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Formation of aqueous pores in the plasma membrane lipid bilayer is a well-known mechanism of increased membrane permeability associated with electroporation. However, much less is known whether and how membrane proteins are affected by intense pulsed electric fields. In our previous study (Rems et al., Biophys. J. 119:190-205, 2020) we have shown through molecular dynamics simulations that electric fields can induce pores in the voltage-sensor domains (VSDs) of voltage gated ion channels. Specifically, we studied three ion channels, including a bacterial sodium channel NavMs, an eucaryotic (cockroach) channel NavPaS, and a human hyperpolarization-activated cyclic nucleotide-gated channel HCN1. We have demonstrated that pores form more easily in VSDs that are more hydrated and are electrostatically more favourable for the entry of ions. In addition, we demonstrated that pores in VSDs can expand into so-called complex pores, which become stabilized by lipid headgroups and can lead to severe unfolding of VSDs from the channel. Corroborated by previous electrophysiological measurements, we predicted that ion channels with such unfolded VSDs become dysfunctional. Here we extend out study to various other ion channels, including members of potassium channels, voltage-gated sodium channels, voltage-gated calcium channels, chloride channels, and a member of the transient receptor potential channel. We show that complex pores can form in VSDs of many different ion channels but not all channels. Interestingly, we show that complex pores in VSDs can form more easily than in a pure phosphatidylcholine lipid bilayer. We discuss our findings in the light of how electric field-induced alteration of membrane ion channels could affect the biological outcome of electroporation.

PO-029

Nanoparticle targeted drug delivery with nanosecond pulsed electric discharge induced shock waves

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Underwater shock wave, in medical range, causes discontinuous change in the tissue's pressure and density. Shock waves are produced when stored energy is instantaneously released in the fluid. Shock waves are applied as a non-surgical treatment method, as converging shock waves can be generated outside the body, coupled with the skin, and focused on the targeted tumor/tissue, without damaging the intervening tissue. In the meantime, nanomedicine is rapidly developing for diagnostic and therapy to increase the bioavailability of drugs to tissue and tumors. Nanoparticles, with their small size and large surface area to volume ratio, are effective to bind, absorb and carry drugs, DNA, RNA, and proteins, along with imaging agents, to tumors with high efficiency. In this paper, we used a solid-state magnetic pulse compression circuit (MPC) to generate nanosecond pulsed electric discharges and to produce reliable and controllable underwater shock waves. Shock pressure histories were measured by a fiber optic probe hydrophone (FOPH) pressure transducer. Variety of shock wave overpressures from 20 to 150 MPa were applied and tested. Silica nanoparticles were used as drug carriers and shock waves exposures were used to enhance the effectiveness of the targeted drug delivery. Suspension of human lymphoma cell line U937 were utilized for in-vitro experiments. The results are promising and will be further developed for effective target drug delivery.

PO-032

Role of resting membrane potential in Ca2+ influx following exposure to intense electrical pulse

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Introduction: Applying pulsed electric field (PEF) to a living cell permeabilizes the cell membrane and subsequently leads to the transmembrane substance transportation. This phenomenon, known as the electropermeabilization (EPB), is used for the introduction of drugs and genes into cells and the extraction of intracellular components [1-2]. In particular, calcium ion (Ca2+) influx following the PEF exposure is about to be used for cancer treatment [3]. While typical pulse width of PEF is hundreds of microseconds or less, the subsequent Ca2+ influx lasts for from milliseconds to minutes. Although there are some hypotheses for mechanism of the Ca2+ influx, such as a diffusion, an electrophoresis driven by the resting membrane potential (RMP), or biochemical reactions including ion transporters, it is not clear. We have studied the ion influx using artificial cells with RMP. Artificial cells allow us to exclude the biochemical processes associated to the ion influx and outflux for discussing the mechanism of Ca2+ influx. This paper mainly describes the production of RMP in artificial cells and the role of RMP in the Ca2+ influx.

Materials and Method: We used giant unilamellar vesicles (GUVs) as artificial cells. The w/o emulsion method was used to prepare GUVs, which contains potassium (K+) ions inside. The membrane potential was generated for simulating RMP in living cells by using the K+ ionophore valinomycin to drain internal K+ by the concentration gradient. The membrane potential was controlled by the initial intracellular K+ concentration. Fluorescent probe for Ca2+, Fluo-8, contained only inside GUVs enables us to detect Ca2+ influx following the PEF exposure. A single PEF (10 μs, 5 kV/cm) was applied to GUVs with or without the membrane potential, and the amount of Ca2+ uptake was evaluated by the brightness of fluorescent intensity of Fluo-8.

Result and Discussion: The observed rate of change of fluorescence brightness values in the GUVs before and after pulse application showed that the rate of change was 2% for the GUVs without membrane potential, while 18% was observed for the GUVs with membrane potential. Only a slight increase in the intracellular Ca2+ concentration was detected in the GUVs without membrane potential. This slight increase is considered being owing to the diffusion through the electropermeabilized membrane. On the other hands, significant Ca2+ increase occurred in the GUVs with membrane potential. Besides, the increase in the intracellular Ca2+ concentration was proportional to the membrane potential. These results clearly indicate the membrane potential plays a significant role in the Ca2+ influx rather than the diffusion.

These results will provide with the knowledge to understand better the substance mobilization through the cell membrane following the PEF exposure and to find the optimum parameters for the present various PEF applications.

PO-037

Pulsed electric field treatment application to improve product yield and waste valorization in kiwifruit processing

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The present contribution focuses on the effect of Pulsed Electric Field (PEF) and the related impact of different treatment levels on the peeling characteristics of kiwifruit and consequently on the physicochemical properties. Kiwifruit is considered as a very healthy product due to its high concentration in vitamin C and high natural antioxidative capacity correlating with a favorable aroma. Because of the health promoting ingredients, it is of high interest to use kiwi as an ingredient in various fruit products, such as smoothies. An important process step required for the use in juice production is peeling. Mostly, abrasive peeling is used, but as it is related to a high waste percentage and massive yield loss the industry is looking for alternatives. Moreover, the ripening status and harvesting time are very important parameters to consider, which makes the peeling process even more complex. A promising technology for peeling is the use of PEF technology.

In this work, the influence of different PEF settings, determined as specific energy, were studied on peeling effectiveness and chemical properties of the kiwi using statistical analysis. Experimental results showed that the specific energy input has a significantly influence on kiwifruit specific peeling force. For applied PEF energy Wspec about 0.5 kJ/kg, the kiwifruit skin can be removed with a low force indicating an improved peeling. A further increase in PEF specific energy did not change the peeling ability significantly. The results demonstrated that the proposed PEF treatment of kiwifruit had less peeling loss compared to untreated (control) sample. Low energy level of PEF treatment (0.3 kJ/kg) did not show an effect on the peeling. Increasing the specific PEF energy, thereby increasing the number of pulses, improved peelability and decrease mass loss for achieving the desired peeling performance and product quality.

PEF treatment also influenced the physical properties of the kiwifruit at a specific energy of 1 kJ/kg. The pH values of the kiwi flour were significantly different to the untreated control, as the pH of the PEF kiwi skin and PEF bagasse were lower. Moreover, the water activity of the PEF treated skin was significantly higher than the untreated control skin samples. No significant differences were observed in the L*a*b*values. The influence of the PEF treatment on the phenolic compounds was analyzed and the PEF treated samples tended to have higher amount of phenolic compound than the untreated samples. The antioxidant capacity of the PEF treated bagasse was significantly higher compared to the not treated bagasse. PEF treatment can positively influence the peeling and chemical properties of kiwifruit. Depending on the applied energy, different benefits can be generated and can have an influence on the peeling process and on the health

promoting substances in kiwifruit juices.

PO-040

Pulsed Electric Field Treatment of Seeds with Improvement of Seed Vigour

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Crop productions with the use of chemical additives have intensified in order to satisfy the rapidly growing human population. Chemicals help to boost agricultural yields; however they cause environmental pollution and health problems in agriculture workers as well as human consume these products. Seeds not only pass on genetic materials but also transmit different pathogens such as bacteria, fungi and viruses. Seed-borne pathogens have been controlled by chemicals, or by such treatments such as heat, electrons, natural antifungal products or biological control. Although these methods appear to be effective in controlling seed-borne fungal diseases, there are drawbacks such as fungicide resistance and incomplete efficiency. Technological improvements and alternative methodologies for efficient disease control are therefore needed. Efficacy of pulsed electric fields on inactivation of endogenous bacteria and mold and yeast in addition to germination rate (GR), normal seedling rate (NSR), and resistance to cold and salt stresses. It was revealed that increased energy application provided more inactivation on endogenous microflora of vegetable seeds and grains with increased GR and NSR. PEF treatment provided stronger root formation with more developed seedling. Seedlings developed significant resistance to cold and salt stresses. It is concluded that applied electric fields improved seed vigour being as an viable alternative to chemical seed treatment.

PO-045

Application of pulse electric fields (PEF) on drying and frying processes of vegetables

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This work focuses on the effect of pulsed electric field (PEF) treatment on various drying and frying processes from vegetables (potatoes and carrots). Interactions between different drying modes and pretreatment have been studied. The impact of PEF treatment and pre-drying by hot air or pre-drying by vacuum drying on frying kinetics and the quality of fried products were also analyzed. PEF pretreatment results in electro-permeabilization of the cell membranes, which favors the acceleration of mass transfer processes and physical/chemical changes of the product during drying and frying. The results showed that the drying time was significantly reduced in all processes (hot air drying, microwave drying, vacuum drying). The advantage of the PEF treatment was also manifested by a decrease of the internal temperature of the product during drying. This lower temperature has a significant advantage in the preservation of heat-sensitive compounds (carotene, polyphenol,etc.). The dried sample pretreated by PEF could better retain the initial product color and had a reduced color deviation after rehydration. In regards to the frying process, the application of the PEF treatment showed not only an advantage in terms of the frying time but also in terms of oil content absorbed and acrylamide content produced. The oil and acrylamide content of PEF treated sample was lower compared to untreated ones. Moreover, the combination of the PEF pretreatment and hot air pre-drying (or vacuum pre-drying) showed a synergistic efficiency on frying time and also in terms of oil content absorbed.

PO-050

Frequency Domain Dielectric Spectroscopy of Gram-negative Bacteria Exposed to Pulsed Electric Fields

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Pulsed high electric field (PEF) has been studied as a non-thermal sterilization method for liquid foods. PEF causes significant morphological and functional changes in cells and tissues. The effect of PEF on bacteria is dependent on the conditions of PEF, such as field strength, pulse width, and frequency of application, as well as the environment, such as temperature and conductivity.

Previously, the effectiveness of PEF was determined empirically from the results of sterilization experiments and experiments using fluorescence microscopes, making it very difficult to optimize conditions. Therefore, it is important to analyze the physical properties of the effect of PEF on bacteria and to investigate the optimal conditions for each application. We focused on a technique called impedance spectroscopy, which measures the reflected signal against the incident signal and measures how the amplitude and phase change on measured objects. In particular, frequency-domain analysis is characterized by high information accuracy and a wide range of frequencies that can be measured, since the information density is uniform with respect to frequency. In this study, we have established a method for analyzing the physical properties of PEF-applied bacteria using impedance spectroscopy.

We proposed to use impedance spectroscopy to measure the dielectric properties of bacteria, and investigated its effectiveness and applicable conditions.

It was found that a measurement frequency of 1 MHz, a bacterial concentration of 1010 CFU/mL or higher, and a conductivity of 0.73 mS/cm or lower were required for accurate measurements.

Then, the impedance method was then used to measure the dielectric properties of the bacteria when the number of times PEF was applied was changed.

Capacitance decreased and conductance increased before and after PEF application. This is thought to be due to the decrease in the ability to store electric charge due to the formation of pores in the bacterial membrane and to the leakage of the bacterial contents.

The electromagnetic field simulation was used to estimate

the physical properties of the bacterial membrane, and the conductivity increased significantly with the increase in the number of applications, while the relative permittivity did not change significantly. This is thought to be because the change in conductivity was so dominant that the change in permittivity was almost negligible.

PO-053

Effect of pulsed electric fields and ultrasound on protein extraction, yield and techno-functionality from duckweed (L. minor and L. gibba) in an indoor farming cultivation system

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An interdisciplinary Team of scientists from food technology, horticulture and biotechnology from HSWT are establishing the basis for optimal cultivation conditions for duckweed in an indoor farming cultivation system. In corporation with industry partner Elea Technology GmbH, gentle extraction methods will be established for optimal protein extraction assisted by physical processes like pulsed electric fields (PEF) and ultrasound to observe their implications on cell disruption to release protein from duckweed (L. minor and L. gibba). The project aims to investigate the effect of cultivation parameters (light spectra/intensities and nutrient solutions), innovative extraction methods and their implications on techno-functional properties of duckweed protein. As new alternative protein source for food applications, duckweed has an increasing potential with its protein content, protein density and amino acid composition.

Different light spectra and intensities as well as compositions of macro- and micronutrients of the nutrient solution are tested for their effect on protein composition in duckweed species L. minor and L. gibba, with the aim of optimizing protein content, yield and characteristics of the final protein extract.

As the main component of "leaf proteins" RuBisCo protein, more specifically, an enzyme that plays an important role in carbon fixation in all green plants, the extraction process is divided into three processing steps: pre-extraction, separation of pigments/antioxidants and protein isolation. Pre-extraction of freeze-dried and subsequently ground duckweed powder as well as fresh material is carried out using PEF to break down plant cell membrane by gentle cell disintegration. Ultrasound is used alternatively to support pre-extraction.

PEF will be applied directly on the cell system and compared in terms of its performance on increased protein extraction yield and effects on techno-functional properties. While ultrasound subsequently assists in the disruption of the particles and in the case of ground duckweed, resulted in smaller particles than 100 µm for 97% of the particles size distribution. This indicates cell disruption of plant cells, which are normally 100-300 µm in size and resulted in about 10-15% increased protein solubility compared to the untreated reference. Isolation of proteins will then be performed based on pre-defined optimum pre-extraction procedure. Isolation of the proteins will be carried out by mild heat and ultrafiltration/diafiltration. Isolation conditions will be defined according to digestibility and flavor profile of duckweed protein isolate.

PO-058

The induction of cell electrosensitization or electrodesensitization with application microsecond pulsed electric fields

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Introduction: Nowadays the phenomenon of electroporation is being widely applied. However, it still narrows down to the transient (reversible electroporation) or permanent (irreversible electroporation) cell membrane permeability increase as a result of electric field application. This phenomenon is fundamentally researched for a few decades yet still some additionally triggered phenomena are still not fully understood among researchers. One of such phenomena is electrosensitization. This phenomenon indicates the change of cell sensitivity to the electric field when cells were priorly affected by electric field. Usually, the effect of electrosensitization is being researched when the time lag in between nanosecond pulsed electric fields is in the sub-millisecond range. However, the electrosensitization has been discussed in microsecond pulsed electric field range with a time lag ranging from seconds to minutes. However, the term of "cell memory" phase was used rather than electrosensitization. Here we investigate the process of the electrosensitization phenomena in the time lag range that could be considered as "cell memory". We have found that depending on the number of applied electric pulses one can gain electrosensitization as well as desensitization. Indeed, we have found a statistically significant changes (negative and positive) in the percentage of electroporated cells when minutes post-application of sensitizing electric fields.

Methodology: Electrosensitization experiments were done with Chinese hamster ovary cells. Electroporation has been performed with laboratory-made stainless steel plate electrodes with 2 cm gap. All used pulsed electric fields that were used here were in between 1200 and 1400 V/cm. Electroporation was done with sucrose and glucose-based phosphate electroporation media at osmolarity at 270 mOsm, pH 7.1 and conductivity at 0,1 S/m. The electrosensitization treatment experiment setup was to apply electroporating electric field to incubate cells at a particular time and electroporate them again. Electroporation was evaluated by propidium iodide electrotransfer (40 M).

Results and discussion: We have found that after sensitizing electric pulse and incubation in a range of minutes one can change cell response to electric fields by changing their size. Depending on the applied electric field cells shrink or increase in size. Under these conditions, viability was not significantly affected. Nevertheless, such change in cell size greatly influenced electroporation

efficiency, hence cells get desensitized or sensitized to the electroporation.

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PO-063

Application catheter parameters affecting PFA outcome based on simulation

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This abstract focuses on pulsed-field ablation (PFA), which is a form of irreversible electroporation (IRE). In contrast to other ablation techniques, e.g. radiofrequency ablation (RFA), innovative PFA has a big potential for cardiac ablation since it is tissue-selective and with minimal danger to patients.
In this work, we present 3D FEM simulations of ablation inside the heart with a focus on various parametric analyses, including electric field and temperature distribution in the tissue. The simulation is based on the usage of standard RFA catheters because, at this time, the commercial availability of PFA cardiac catheters is very limited.
The 3D FEM model includes a simple cylindrical catheter with a tip electrode which is in contact with heart tissue in a form of a cube. The electroporation pulses were set to be a 100 us log with a frequency of 1 Hz. We were changing blood velocity on a Y-axis, the angle of the catheter-tissue contact, catheter pressure (electrode surface in contact with blood), as well as pulse parameters such as voltage, pulse length and the time gap between pulse bursts.
The simulations show the biggest Joule losses near the active electrode in the blood, where is the highest current density. It shows that blood velocity in the heart is sufficient for cooling the electrode if it is not surrounded by the tissue when pressed downward (no contact with blood).
However, the higher the voltage higher the temperature and the contact area must be cooled down.
On the other hand, we show that with larger contact with blood, the electric current flow is bigger. That means a greater danger for patients. There is a need to find a good compromise between the value of electric current and temperature where both of them are sufficiently low.
In real experiments, the position of the catheter may not be exactly perpendicular to the tissue but maybe at some angle. We show that with the perpendicular catheter-tissue position, there is the lowest temperature rise. Further, when the electrode is tilted in the direction of blood flow the cooling is better than when tilted in the opposite direction. The temperature during experiments is measured with a thermocouple on the tip of the catheter. The simulations show that this kind of measurement is not optimal and needs improvement. The size of created lesions in the myocardium is also discussed and compared to those from real experiments. The results show that the final ablation outcome is affected by many variables which need to be taken into account for a more precise and reliable solution.
Our interest is also the development and testing of new PFA catheter geometries. Two new designs show that it is theoretically possible to create lesions of the same size but with lesser current flow thus more suitable for a safe PFA procedure. Parametric analyses revealed the impact of electrode distance on lesion size and temperature distribution.

PO-066

Study of surface oxides modifications on nitinol electrodes during electroporation protocols and possible consequences

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The purpose of this study is to evaluate the efficiency and the possible risk of the utilization of nitinol as an active electrode for electroporation (EPN).
Nitinol is widely used in medical devices for its particular ultra-elasticity and shape-memory properties. It is also considered as bio-compatible due to the TiO_2 layer generated on the surface during processing. Different surface modification procedures are known to improve the thickness of this layer in order to ensure the long term bio-compatibility of passive implants [1].
However, as this alloy is now commonly used as active electrode for different purposes, knowledge of the behavior of nitinol in the body must be updated. It has been proven safe and efficient to use as micro-electrodes in neural prosthesis [2], but its suitability as an electrode for EPN needs to be ensured. Indeed, the delivery of high-intensity square pulses (typically 1ms and 1000V/cm) causes electrochemical reactions at the surface of the electrodes, in particular metal releases due to the oxidation of the anode. This phenomenon has been observed in vitro with aluminum [3] and stainless steel electrodes [4], with the release of aluminum and iron ions respectively. In this case, the possible release of nickel ions into the body might be a problem for clinical use. Furthermore, as the TiO_2 layer on the nitinol is less conductive than stainless steel, a drop in efficiency could be expected.
We have developed a device enabling us to test the use of different electrodes on a spheroid population. The experimental comparison between nitinol and stainless steel did not exhibit any significant difference in terms of EPN efficiency in this configuration. We then studied the surface condition of differently processed nitinol wires after their use in EPN protocols in a conductive solution. We compared qualitatively with SEM images a heat-treated nitinol wire and a nitinol electrode, the latter being previously cleaned of its surface oxide and then oxidized through an electroporation protocol. This confirmed that the surface oxide thus formed is significantly different from the one formed through heat-treatment. We are currently extending this comparison to wires

protected by an oxide layer and used for EPN. We are also using EDX and Raman spectroscopy to compare quantitatively the exact surface composition of the samples. The presence of nickel releases in the solution after EPN will be investigated with Hach Lang test kits. In the future, different EPN protocols will be tested, as the use of shorter pulses seems to reduce metal releases [3].

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PO-069

Persistent membrane depolarization following conventional electroporation depends on temperature and is influenced by ion channel blockers

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All cells maintain an electric potential difference across their plasma membranes, which results from the differences in membrane permeabilities for potassium, sodium, calcium, and chloride ions. This potential difference is called the resting voltage or resting membrane potential and is maintained by a system of ion channels and pumps. By convention the resting voltage is negative, meaning that the cell interior is electrically more negative compared to its exterior, and the membrane is considered hyperpolarized. The value of the resting voltage changes dynamically with the cell cycle and has an important biological function by controlling the activity of various membrane proteins. When cells are electroporated, their membrane voltage becomes disrupted and remains depolarized for several minutes after pulse exposure. In this study we aim to gain a better understanding on the mechanisms of prolonged membrane depolarization upon electroporation. By studying different cell lines, including Chinese hamster ovary cells and human glioblastoma cells, stained with a potentiometric dye, we show that depolarization markedly depends on the ambient temperature (room temperature vs. 37°C). Not only do the cells remain depolarized for a longer period of time when electroporated at room temperature, they are often slightly depolarized even before electroporation compared to conditions under 37°C. Furthermore, by using different ion channel blockers we show how membrane depolarization in not purely the consequence of ion leakage through nonselective pores in the permeabilized membrane, but involves the opening and closing of membrane ion channels. We discuss the potential biological consequences of these findings, from the aspect of cell handling for basic studies of cell electroporation, towards the possibility of controlling cells' susceptibility to electroporation by pharmacological agents.

PO-072

Could the presence of a coronary stent provoke distortion of the electric field and any thermal side effect during epicardial pulsed field ablation? Insights from an in-silico computational modelling

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Background and objectives: Pulsed Field Ablation (PFA) has been proposed as a non-thermal energy to treat atrial fibrillation by an epicardial approach, for ablation of ganglionated plexi. This energy provokes permeabilization of the cell membrane (creation of pores), leading to cellular homeostasis disruption and cell death. We recently checked the safety of this energy, which is totally delivered at the target epicardial sites without damaging the underlying myocardium and the adjacent organs (lungs and oesophagus). However, there is still no assessment on how the electric field in the target zone could be affected by the presence of pre-existing implants, such as coronary stents – neither is there an understanding of possible undesirable thermal effects. The aim of this work is to develop computational models to assess the electric field and temperature distribution on the epicardium (target), upon application of high-intensity electric field pulses (PEFs).
Methods: Coupled electrical-thermal computer models based on the heart anatomy including epicardial fat with the left circumflex coronary artery, myocardium and blood were built. The stent was placed within the coronary artery. Different positions of the stent with respect to the ablation device were also assessed. A period of latency (i.e. a period of time without applying PEF energy) was taken into account after the application of PEFs to assess if there was any collateral heating in the target site. PEF parameters such as voltage profile, pulse width, inter-pulse interval and number of pulses were those already used preclinically and clinically to ablate epicardial ganglionated plexi.
Results: The electric field distribution on the epicardium was practically the same with and without having a stent, even at a very close distance from the ablation device (0.25 mm). The presence of a stent has not provoked any increased heating after the latency period, with the temperature being 37.5°C in the zone around the coronary artery with and without stent.
Conclusions: Computational results showed that the presence of a coronary stent near the ablation site does not cause any distortion of the electric field nor any thermal side effect in the target.

PO-077

Downstaging of portal vein tumor thrombus from Hepatocellular Carcinoma with Electrochemotherapy as bridge to liver transplantation

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S.R. 53 years. January 2009 : HCV-related cirrhosis, Child-Pugh A5 class, EGDS no aesophageal Varices. No important comorbidities. Treated with PEG-IFN+Ribavirin (march-november 2009) with subsequent sustained virologic response . HCVRNA absent overtime . October 2016 :CT detected small HCC nodule in the VIII segment (diam.=12 mm). Treated with US guided RF-ablation. November 2016 CT: complete necrosis. Unfortunately, the patient dropped out US and CT follow-up controls.September 2018: asthenia and weight loss. CT showed a large tumor infiltrating V-VII-VI segments and complete PVTT of right portal vein and its branches . Surgical Consultation excluded indication to Liver resection and OLT . 23 october 2018: ECT of a large peri-hilar area of the tumor including the PVTT. 1 and 3 months post-treatment CT showed complete necrosis and retraction of the thrombus and residual viable tumor in the peripheral portion of the right lobe . Therefor, the patient was re-evaluated for OLT and considered eligible in waiting list . March 2019: CT showed no perihilar or portal vein recurrence and distant progression in the right lobe . March 2019 : Trans-arterial-Radio-therapy (TARE) of the right lobe. Post-treatment CT demonstraded no perihilar or portal vein recurrence and extensive necrosis of the residual tumor . December 2019: CT demonstrated several recurrences of HCC infiltrating the VI and VII segment . Howewer no recurrence was observed at hepatic hilum and in portal vessels . Therefore, on February 2020 the patient received OLT. At 2 years follow-up, no complication or recurrence or liver disfunction have been observed.

PO-078

Intense electric field effects on microtubule systems

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Understanding electromagnetic field-biomatter interaction is crucial for the development of novel diagnostic and therapeutic methods as well as for novel procedures in bio-nanotechnology. Tubulin proteins, the building blocks of microtubules (MTs) have exceptionally high structural electric charges and dipole moments. Consequently, it is probable that MTs could be susceptible targets to electric field through which one could modulate the function of biological systems. Our molecular dynamics simulations showed that intense nanosecond pulsed electric field (nsPEF) opens the MT lattice on the nanosecond time scale and this depends on the electric field strength and temperature.
We present follow-up experimental work which aims to verify these computational predictions. The work is centered around protocol development and systematic testing of a new experimental system which delivers ˜ MV/m field strength nsPEF to MTs in vitro while enabling measurement of MT stability via absorbance in real time. Moreover, we follow the morphological changes of MTs after nsPEF treatment with several imaging techniques such as phase contrast microscopy, differential interference contrast microscopy, holographic microscopy and atomic force microscopy. These techniques are applicable also for the exploration of the effects of intense subTHz electric field on tubulin and MTs. Our results contribute to development of novel electromagnetic methods for modulating function of biomolecular matter at nanoscale.

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PO-076

Nanosecond Pulse Water Surface Discharge for Water and Wastewater Treatments

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Underwater discharge and its dynamics have been studied with great interest over the past few decades. In this regard, one of their most noteworthy applications has been drinking and wastewater treatments, as they can directly affect our health and environment. While underwater electric discharge has been extensively studied, the required knowledge regarding the phenomenon of water surface discharge is inadequate yet. This paper reports on the physical characteristics of water surface discharge, including plasma production and propagation, and simultaneous generation of shock waves in water and air. Nanosecond pulsed electric fields on the surface of water were generated using a magnetic pulse compression (MPC) unit, comprising of a charger and a control unit. A point to plane electrode technique along with positive pulse was employed for water surface discharge. The discharge phenomenon and shock waves propagations were visualized using a time-resolved shadowgraph scheme by employing an ultra-high-speed framing camera. Different gap distances between the positive electrode and the surface of water as well as different solution conductivities were considered for the experiments, to confirm and understand the physical mechanisms at different environmental conditions of water and wastewater treatments. The results confirm that nanosecond water surface discharge is an effective and highly efficient method for decontaminating water.

PO-004

Endoscopic calcium electroporation as a palliative treatment for inoperable colorectal cancer

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Background: Colorectal cancer is one of the most common malignancies worldwide cancer. Despite advances within the field of colorectal cancer, a number of patients

can only be offered palliative treatment due to metastatic or inoperable colorectal cancer. Health-related quality of life is of great importance in this fragile group. In some cases, treatment options are limited as the patient may not be suitable for chemotherapy or radiotherapy due to comorbidities, making tumor-related symptoms very challenging in these cases.
Calcium electroporation is a promising anticancer treatment, which has been shown to induce tumor cell necrosis. Studies show that cancer cells are vulnerable to the treatment, whereas normal cells are more resistant to calcium electroporation. An advantage is the simplicity of the procedure, facilitating use in a palliative setting.
Method: In this case report, patients were unfit for standard palliative treatment and were offered endoscopic calcium electroporation as an experimental treatment.
Treatments were performed under propofol sedation and patients were discharged within a few hours after the procedure. Calcium chloride was injected intratumorally, followed by electrical pulses delivered through the EndoVE device. The device was connected to the ePORE generator.
Results : Case 1 was an 80-year old male with non-metastatic rectal cancer. The patient rejected surgical resection and chemotherapy. The patient received CaEP as an experimental treatment in May 2020 and additional treatments were performed in August 2020 and February 2021. Endoscopic assessment showed visual tumor regression after CaEP.
Case 2 was an 88-year old male with sigmoid colon cancer. The patient suffered from anemia due to bleeding from the tumor. The patient was offered CaEP in July 2020 with repeated treatments in February 2021 and August 2021. Currently, the patient has been followed for 20 month and has stable hemoglobin levels.
Case 3 was a 70-year old women with recurrent rectal cancer. The patient rejected further surgical interventions. CaEP was performed in February 2022 followed by electrochemotherapy in March 2022. Follow-up MRI showed tumor regression.
Case 4 was a 76-year old male with sigmoid colon cancer. The patient had limited treatment options due to several comorbidities. He was treated with CaEP to limit further progression of stenosis. The patient reported relief of symptoms.
Conclusion: Endoscopic calcium electroporation is a safe and feasible procedure for colorectal cancer and may be a valuable treatment modality for patients with limited treatment options.

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