

The First Latin American Workshop on Electroporation and its Applications.

Faculty of Exact and Natural
Sciences, University Campus,
University of Buenos Aires, Argentina.
October 8 and 8, 2018.

Program and Book of Abstracts

Organized by:

the Faculty of Exact and Natural Sciences from the University of Buenos Aires with the general sponsorship of the University of Buenos Aires (UBACYT), the National Research Council of Argentina (CONICET) and the Ministry of Science and Technology (MINCYT) through the Bilateral project Slovenia-Argentina 1918.

Endorsed by:

The 1st LAWE2018 is sponsored and endorsed by the International Society of Electroporation-based Technologies and Treatments (ISEBTT).



Edited by:

Guillermo Marshall

Welcome note

Welcome to the First Latin American Workshop on Electroporation and its Applications (1st LAWE2018), the first scientific workshop organized in Latin America with the purpose of promoting its use in the region, and of reuniting academics from different disciplines, such as Biology, Medicine, Veterinary and Food Processing doing research in electroporation, as well as developers and users of electroporation-based technologies.

The 1st LAWE2018 is organized by the Faculty of Exact and Natural Sciences from the University of Buenos Aires with the general sponsorship of the University of Buenos Aires (UBACYT), the National Research Council of Argentina (CONICET) and the Ministry of Science and Technology (MINCYT) through the Bilateral project Slovenia-Argentina 2018.

The 1st LAWE2018 is also sponsored and endorsed by the International Society of Electroporation-based Technologies and Treatments (ISEBTT).

We hope that you will be enjoying the 1st LAWE2018 and your visit to Buenos Aires,

Guillermo Marshall

On behalf of the Organizing Committee

Organizing Committee

Guillermo Marshall (coordinator)

Felipe Maglietti

Matías Tellado

Nahuel Olaiz

Sebastián Michinski

Carolina Schebor

Emmanuel Luján

Program

The official languages of the Workshop will be English and Spanish. The workshop will take place at the Aula Magna, Pabellón Industrias, Ciudad Universitaria, Buenos Aires.

Monday 8

09:30 – 10:00 hs	Welcome coffee
10:00 – 10:30 hs	Dr. Honoris Causa Award to Lluís M. Mir
10:30 – 11:30 hs	Electrochemotherapy and other clinical applications of cell electroporation: history, fundamentals and recent methods of study – Lluís M. Mir (in Spanish, free access)
11:30 – 11:45 hs	Workshop opening (registered participants) – Guillermo Marshall
	Chair: Felipe Maglietti
11:45 – 12:00 hs	Brownian dynamics simulation studies of ion throughput during cellular electroporation – Juan A. González Cuevas
12:00 – 12:15 hs	Electromagnetic fields: biological effects and possible effects on health – Víctor Cruz Ornetta
12:15 – 13:30 hs	Lunch
	Chair: Guillermo Marshall
13:30 – 14:00 hs	Keynote: Potential therapeutic applications of gene electrotransfer – <i>Richard Heller</i> , Chelsea Edelblute, Cathryn Lundberg, Sezgi Arpag-McIntosh and Anna Bulysheva
14:00 – 14:30 hs	Keynote: Innate immunity induced by gene electrotransfer – <i>Loree Heller</i> , Nina Semenova, Masa Bosnjak, Bostjan Markelc, Katarina Znidar, Maja Cemazar
	Chair: Carolina Schebor
14:30 – 14:50 hs	Uses and applications of electroporation in food processing and biotechnology – Samo Mahnic-Kalamiza
14:50 – 15:10 hs	Electroporation in continuous-flow food processing (in Spanish) – Nahuel Olaiz
	Chair: Richard Heller
15:10 – 15:40 hs	Keynote: Electroporation as a new tool in cancer immunotherapy – Emanuela Signori
15:40 – 16:10 hs	Coffee break
16:10 – 16:40 hs	Keynote: High-Frequency irreversible electroporation for brain cancer therapy – Melvin F. Lorenzo, John H. Rossmeisl, Chris B. Arena, Paulo A. Garcia, Scott S. Verbridge, <i>Rafael V. Davalos</i>
	Chair: Víctor Cruz Ornetta
16:40 – 17:30 hs	Roundtable: extending implementation and applications of electroporation in Latin America

Tuesday 9

09:30 – 10:00 hs	Welcome coffee
	Chair: Daniela Suzuki
10:00 – 10:20 hs	Electrochemotherapy for treating oral malignant melanoma (in Spanish) – <i>Matías Tellado</i> , Felipe Maglietti, Sebastian Michinski, Guillermo Marshall and Emanuela Signori
10:20 – 10:40 hs	Treating bladder cancer with electrochemotherapy (in Spanish) – <i>Marcelo Monte Mór Rangel</i> , Jennifer Ostrand Freytag, Krishna Duro de Oliveira, Priscila Gil Quadros, Daniela Ota Suzuki
10:40 – 11:00 hs	Gene electrotransfer with Ca-IL-12 in veterinary medicine – <i>Urša Lampreht Tratar</i> , Nina Milevoj, Nataša Tozon, Ana Nemeč, Gregor Serša, Maja Čemažar
	Chair: Cecilia Suárez
11:00 – 11:30 hs	Electroporation: dosimetry, accuracy, and uncertainties (in Spanish) – Lluís M. Mir
11:30 – 11:50 hs	Treating nasal tumors with electrochemotherapy (in Spanish) – Felipe Maglietti
11:50 – 12:10 hs	Opening the cell membrane: veterinary experiences with electro-gene-transfer and electrochemotherapy in the United States – Joseph Impellizzeri
12:10 – 13:30 hs	Lunch
	Chair: Marcelo Mor Rangel
13:30 – 14:00 hs	Medical applications with Electrochemotherapy – Víctor Farricha
14:00 – 14:20 hs	Boron uptake with electroporation (in Spanish) – <i>M. A. Garabalino</i> , N. Olaiz, I. S. Santa Cruz, P.S. Ramos, A. Portu, G. Saint Martin, M.E. Itoiz, E.C. Pozzi, S. Thorp, P. Curotto, A. Monti Hughes, D.W. Nigg, V.A. Trivillin, G. Marshall, A.E. Schwint
	Chairs: Matías Tellado and Marcelo Mór Rangel
14:20 – 15:20 hs	Forum for the presentation of cases and discussion (in Spanish) <ul style="list-style-type: none"> • Multilobular osteochondrosarcoma treated with electrochemotherapy: Experience of two cases – <i>Javier Ojeda</i>, Erica Silva, Heine Muller, Pedro Molina, Daniela Zaror • Electrochemotherapy plus cryosurgery in feline – SCC <i>Laura Pisano</i>, Matías Tellado and Felipe Maglietti
15:20 – 15:50 hs	Coffee break
15:50 – 17:00 hs	Forum for the presentation of cases and discussion (in Spanish) <ul style="list-style-type: none"> • Conservative surgery plus electrochemotherapy on intranasal carcinoma – <i>Priscila Gil Quadros</i>, Jennifer Ostrand Freytag, Krishna Duro de Oliveira, Daniela Ota Suzuki, Marcelo Monte Mór Rangel • Computational model applied for the treatment of a bone tumor in canine patient – <i>Daniela Ota Hisayasu Suzuki</i>, Taila Becker Damiani, Jennifer Ostrand Freytag, Krishna Duro de Oliveira, Marcelo Monte Mór Rangel • Electrochemotherapy in a horse - Case Report – <i>Fernández J</i>, Tellado M, Descalzi T.S, Muriel M, Zapata G, Hernández HZ, Michinski S, Maglietti F, Marshall G.
17:00 hs	Closing remarks

ABSTRACTS

Monday, October 8

Electrochemotherapy and other clinical applications of cell electroporation: history, fundamentals and recent methods of study

Lluís M. Mir

CNRS, UMR 8121, Institut Gustave-Roussy, France

Electrochemotherapy is a new antitumor treatment that combines short intense electric pulses that transiently permeabilize the cells, with anticancer drugs that do not enter the cells in the absence of the membrane permeabilization. The first part of the presentation will report the fundamentals of the electrochemotherapy as well as the steps that had to be overpassed to move from the concept of the electrochemotherapy until its dissemination in human and veterinarian medicine. This journey included experiments with cells in culture, large preclinical tests in laboratory animals and small clinical trials, followed by multicenter trials to prepare the Standard Operating Procedures. The efficacy of electrochemotherapy in the treatment of various types of solid tumors will be shown. The effects of the electric pulses were supposed to be the generation of pores in the cell membranes and the process was called electroporation. However, its detection was almost all the time evidenced by the transport of markers across the permeabilized membrane and the term electroporeabilization was also used. New investigational tools nowadays allow to elaborate and validate new models that conciliate electroporation and electroporeabilization.

Brownian dynamics simulation studies of ion throughput during cellular electroporation

Juan A. González Cuevas

Facultad de Ingeniería, Universidad Nacional de Asunción

The motion of ions through pores formed in the plasma membrane of cells during electroporation is simulated in 3-D space using a Brownian dynamics model, which is mostly deterministic but has some stochastic properties to account for elastic scattering in water. The pore's conductance, diffusion coefficient, and mobility are estimated from the model, which aid in the design of electrical pulses and waveforms for maximizing the throughput of DNA, drugs and gene materials into cells.

Electromagnetic fields: biological effects and possible effects on health

Victor Cruz Ornetta

Universidad Nacional Mayor de San Marcos, Lima, Perú

The widespread use of electromagnetic fields for different applications in daily life is increasing in the latest years including electricity appliances and networks, telecommunication devices and networks, biomedical devices, domestic applications such as microwave ovens but the concern about possible health effects these electromagnetic fields growth parallel to the use of them. The objective of this study was to review the biological effects and possible health effects of electromagnetic fields based on very important international and national revisions, especially those who has been conducted within the frame of the International Electromagnetic Field Project of the World Health Organization. It was found that the established biological effects for Low Frequency Fields, which extends from 1 Hz to 100 kHz, depends on the internal electric fields which in turn develops currents that could cause excitation of nerve and muscle tissue and for Radiofrequency Fields (10MHz – 300 GHz) is linked to the thermal effect which in turn produces body temperature increasement. Possible health effects depends on how big these effects are and if the fields are over the limits of exposure. The exposure limits are based on established effects and the compliance of them implies that exposure to field levels lower than the limits do not produce health effects within human body.

Key words: Non- ionizing radiations, electromagnetic fields, low frequencies, radiofrequencies

Potential therapeutic applications of gene electrotransfer

Richard Heller^{1,2}, Chelsea Edelblute¹, Cathryn Lundberg¹, Sezgi Arpag-McIntosh¹ and Anna Bulysheva¹

¹ *Old Dominion University, Frank Reidy Research Center for Bioelectrics, United States*

² *Old Dominion University Institution, School of Medical Diagnostics and Translational Research. United States*

Gene therapy has held great promise for effective treatment of a variety of disorders including cancer, metabolic disorders, cardiovascular diseases, and genetic disorders as well as a prophylactic approach for infectious diseases and cancer. It is clear that a significant effort is still needed and that delivery, particularly delivery to a specific target, still remains an important hurdle. One area that has seen tremendous growth is non-viral gene delivery systems including the use of physical delivery approaches. Of the physical delivery methods, electroporation (gene electrotransfer; (GET)) has made the most progress with over 90 clinical trials. Expression of the transgene can be controlled by manipulating the GET parameters such as pulse width, applied voltage, pulse number, and electrode configuration. This control of the expression profile is a key element in developing successful therapeutic applications. We have utilized GET for delivering DNA vaccines as well as to evaluate the potential for accelerating wound healing or for inducing reperfusion of ischemic tissue. Work has also been focused on

developing an immune gene transfer approach for the treatment of cancer. Delivery of plasmids encoding cytokines directly to tumors has been shown by our lab and others to induce not only a local immune response but a systemic one as well. The positive responses were directly related to the ability to achieve the appropriate expression profile following delivery of the plasmid. This approach has been successfully tested in multiple Phase II clinical trials. GET is an effective approach for delivering plasmid DNA and the number of applications has been steadily growing.

Innate immunity induced by gene electrotransfer

Loree Heller^{1,2}, Nina Semenova¹, Masa Bosnjak³, Bostjan Markelc³, Katarina Znidar⁴, Maja Cemazar^{3,4}

¹Old Dominion University, Frank Reidy Research Center for Bioelectrics, USA

²Old Dominion University, School of Medical Diagnostic and Translational Sciences, Norfolk, USA

³Institute of Oncology Ljubljana, Department of Experimental Oncology, Slovenia

⁴University of Primorska, Faculty of Health Sciences, Slovenia

Gene therapies, whether delivered virally or by methods such as electrotransfer, induce inflammation in the targeted tissue. The root cause of this inflammation may be the activation of pattern recognition receptors (PRRs), found in all mammalian cells. PRRs represent an ancient, germline-encoded mechanism to detect molecules typical of pathogen invasion, including genomic nucleic acids. Binding and activation of these primitive PRRs induce the production of proinflammatory proteins, inflammation, and diverse forms of programmed cell death. Along with the well-known endosomal PRR TLR9, approximately 15 putative cytosolic DNA-specific PRRs have been described. Our studies demonstrate that both normal and cancer respond similarly to plasmid DNA electroporation with the production of inflammatory markers and the induction of cell-type dependent cell death. A significant upregulation of the mRNAs and proteins of the PRRs DAI/ZBP1 and DDX60 is observed after pDNA delivery. In addition, we recently demonstrated the direct binding of pDNA by DAI/ZBP1 but not by other PRRs within minutes of plasmid DNA electroporation. These initial observations support the concept that DNA-specific PRRs are activated by DNA electroporation. A better understanding of the molecular underpinnings of nucleic acid sensing may allow us to modulate these possibly unavoidable effects to enhance the efficacy of gene therapies.

Uses and applications of electroporation in food processing and biotechnology

Samo Mahnič-Kalamiza

University of Ljubljana, Faculty of Electrical Engineering, Trzaskacesta 25, SI-1000 Ljubljana, Slovenia

Electroporation, electropermeabilization, or pulsed electric field treatment, as this treatment has been variously referred to as, is a method of treatment of plant tissue that, due to its principally non-thermal nature, enables preservation of the natural quality, colour, and vitamin composition of food products.

The range of processes or applications in food processing where electroporation was shown to preserve quality, increase extract yield or optimize energy input into the process is overwhelming, though most likely not completely exhausted as yet. To name a few: electroporation is of aid in enhancing the quality and yield in the extraction of valuable compounds and juices; in the valorization of waste materials; in decreasing energy and required processing time in dehydration; it can enable us to impregnate tissue with substances that result in freeze-tolerance of the treated material, etc. Electroporation is also, due to its antimicrobial action – which only exists under appropriate conditions! (i.e. treatment parameters) – a subject of research as one stage of the pasteurization or sterilization process, or at least as a gentle treatment complementary to existing sterilization methods.

The low-energy expenditure (as compared to e.g. thermal treatment) is also of interest to biotechnology, where the treatment is being developed as the key technology that could render lipid extraction from microalgae a net energy gain process.

Last but not least, electroporation has been studied as a potential method of plant metabolism stimulation, as well as a treatment method in a perhaps surprising area – cuisine – where it has been introduced as a low-temperature cooking method!

Electroporation in continuous-flow food processing

Nahuel Olaiz

*CONICET- Universidad de Buenos Aires. Instituto de Física del Plasma (INFIP).
Buenos Aires, Argentina*

Every day more people demand fresher, healthier products with health-promoting qualities. In the industry, simple and reliable methods are required to permeabilize the cell membrane and inactivate unwanted microorganisms. Pulsed electric fields have been shown to be very effective in increasing the extraction or inactivating microorganisms. The drawback of this current technology is in reducing energy consumption without increasing temperature. Driven by this problem, we decided to solve the extraction and pasteurization processes in one of the most difficult products on the market. The "rennet": is a hypersaline substance that contains thermolabile peptidases (enzymes) and is used to curdle milk. We developed a continuous flow electroporation system that allows an increase in the enzymatic activity by at least 70% while lowering energy costs 97% relative to other systems. Results were analyzed in terms of inactivation efficiency and specific energy consumption. This new system will improve the processing of a continuous flow of food without increasing the temperature.

Electroporation as a new tool in cancer immunotherapy

Emanuela Signori

*CNR-Institute of Translational Pharmacology, Roma-Italy; Campus Bio-Medico
University of Rome, School of Medicine, Roma-Italy*

Knowledge of the immune system and how it interacts with cancer cells is rapidly increasing. Tumor development and survival is a process involving the interplay between cancer cells, normal stromal cells, and host defense mechanisms.

Oncoimmunology studies are focused on the interactions between tumors and the immune system and to possible new immunotherapy-based strategies. Nowadays, electroporation (EP) has emerged as a good tool for the delivery of chemotherapeutic molecules and genes. Many studies have highlighted the beneficial effects of EP in activating the immune system response. Recent data lead to encouraging results when electrochemotherapy is combined with immunogeneelectrotransfer. Optimization of EP protocols is mandatory to translate in human patients cancer immunotherapy treatments based on electrotransfer. In the lecture, besides discussing the basic principles of cancer immunoediting, mechanisms and strategies for a successful drug and gene delivery based on electroporation will be described.

Irreversible Electroporation for Brain Cancer Treatment

Melvin F. Lorenzo, John H. Rossmeisl, Chris B. Arena, Paulo A. Garcia, Scott S. Verbridge, *Rafael V. Davalos*

Virginia Tech-Wake Forest University, Blacksburg, VA, USA

Irreversible Electroporation (IRE) is a new focal ablation technique we invented to treat patients with unresectable tumors. IRE therapy uses small (1-2mm) surgical probes to deliver low-energy microsecond pulses for approximately 5 minutes. These pulses induce nanoscale defects within the lipid bilayers of the targeted tissue, killing the cells with sub-millimeter resolution at therapy margins. Through treatment planning algorithms, the IRE zone can accurately be predicted to optimize treatment outcome and mitigate thermal effects. Treatment planning is complicated by the fact that the field distribution depends on the electrode configuration, pulse parameters, and any tissue heterogeneities. 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 currently developing IRE for the treatment of Malignant Glioma (MG), and in particular glioblastoma multiforme (GBM), which has a patient median survival of only 15 months. Our preclinical work to date has focused on helping canine patients with naturally occurring MG, which are excellent translational models of human MG. Our results show that IRE treatment can be planned and monitored with techniques used routinely in neurosurgical practice and administered in a minimally invasive fashion. Results of our ongoing trials have been extremely positive, further supporting that IRE is effective for the treatment of MG, including tumors refractory to surgery, radio- and chemotherapies.

Tuesday, October 9

Electrochemotherapy for treating oral malignant melanoma in canine patients

Matías Tellado^{1,2}, Felipe Maglietti¹, Sebastian Michinski¹, Guillermo Marshall¹ and Emanuela Signori³

1. *Instituto de Física del Plasma, Departamento de Física, FCEyN, UBA-CONICET, Ciudad de Buenos Aires, Buenos Aires, Argentina.*
2. *Vetoncologiaclinic, Ciudad de Buenos Aires, Buenos Aires, Argentina.*
3. *CNR-Institute of Translational Pharmacology, Roma – Italy; Campus Bio-Medico University of Rome, School of Medicine, Roma-Italy*

Canine malignant melanoma is the most common cause of oral cancer in dogs which has a very aggressive behavior. It early metastasizes to lymph nodes, and lungs and greatly reduces the quality of life of the patients. Electrochemotherapy is a treatment modality which has been increasingly used in veterinary and human medicine for treating cutaneous and subcutaneous tumors. Gradually, its applications grew among other locations based on its high response rates and minimal side effects. The aim of this work was to evaluate the effectiveness and prognosis of 67 canine patients with oral malignant melanoma treated with ECT, using a single 15,000 IU/m² IV dose of bleomycin and delivering the electric pulses according to the standard operating procedures for electrochemotherapy. For the cases with tumoral invasion of the nasal cavity, the Single Needle Electrode® was used. The patients were followed-up for two years. In accordance with WHO criteria the objective response rate was 70.1%; with 20.9% complete responses, 49.3% partial responses, 16.4% stable diseases and 13.4% progressive diseases. The overall median survival was 7.5 months (2-30 months, mean 9.1 months). Average overall survival of patients in stage I was 15.3 months, in stage II was 9.6 months, in stage III 7.1 months and in stage IV 4.4 months. The average number of ECT sessions was 1.5 for every stage. A lower incidence of metastases was observed in the treated patients when compared to literature. The treatment greatly improved the quality of life of the patients. In conclusion, electrochemotherapy is a very good treatment option that allows excellent response and survival rates in canine patients who did not undergo radical surgery. obtaining better results than those reported with conservative surgery plus chemotherapy or chemotherapy alone.

Treating bladder cancer with electrochemotherapy

*Marcelo Monte Mór Rangel*¹, Jennifer Ostrand Freytag¹ Krishna Duro de Oliveira¹ Priscila Gil Quadros¹, Daniela Ota Suzuki²

1 – *VetCâncerClinic – São Paulo/Brazil*

2 – *Universidade Federal de Santa Catarina – UFSC/Brazil*

Bladder tumors are relatively common in veterinary medicine. The canine species is the most affected among domestic animals. Bladder neoplasms usually involve the region of the bladder trigone which makes the surgical approach quite limited.

Electrochemotherapy is a new modality of treatment against cancer that combines

electropermeabilization and antineoplastic agents that do not permeate the cell membrane. The high index of response and selectivity of the technique could promote a more effective control of the disease while preserving healthy tissues. This study aims to present the feasibility of the application of electrochemotherapy in bladder neoplasms in dogs. The technique was applied in 13 dogs. All dogs underwent previous staging and pre-surgical exams. Exclusion criteria were the impossibility of performing a surgical procedure and/or tumor infiltration of the serous tunic of the bladder during trans-operative histological evaluation. All patients underwent laparotomy and cystotomy for the application of electrochemotherapy. No patient died during the procedure or had dehiscence of the suture in the bladder after the procedure. No patient died because of the procedure. All patients had temporary urinary incontinence after the procedure. Of the 13 patients, 9 presented a CR and one of the nine patients presented recurrence. Of the 13 patients 6 are still alive, 5 in CR. Of the 7 that died, 4 due to other causes, 1 of metastasis and 1 was euthanized due to the tumor. The results corroborate that the bladder electrochemotherapy procedure presents a low risk of death and, although further studies are required, the number of CR indicates that the electrochemotherapy in the bladder may become an alternative treatment for bladder tumors in dogs.

Electrochemotherapy in combination with gene electrotransfer of ca-IL-12 for the treatment of skin and oral tumors in dogs

Urša Lampreht Tratar¹, Nina Milevoj², Nataša Tozon², Ana Nemec², Gregor Serša¹, Maja Čemažar^{1,3}

¹*Institute of Oncology Ljubljana, Ljubljana, Slovenia*

²*University of Ljubljana, Veterinary Faculty, Small Animal Clinic, Ljubljana, Slovenia*

³*University of Primorska, Faculty of Health Sciences, Isola, Slovenia*

Electrochemotherapy is a local treatment where bleomycin and cisplatin with the combination of electric pulses are used. Electrochemotherapy is an effective treatment of tumors in dogs, cats, and horses. However, electrochemotherapy is only a local treatment, therefore it has no or little effect on distant tumors or metastasis. In order to achieve a systemic effect, the combination of electrochemotherapy and immunotherapies was proposed. Immunotherapy such as gene electrotransfer of interleukin -12 (IL-12) showed pronounced antitumor effectiveness in preclinical studies and in studies treating human and veterinary patients. Therefore, the aim of this study was to use a combination of electrochemotherapy and ca-IL-12 gene electrotransfer for the treatment of tumors. In the study 58 client-owned dogs of different breeds whose owners refused any other type of standard treatment, were included. The tumors treated were skin tumors (40 mast cell tumors, 3 plasmacytomas, and one epitheliotropic lymphoma) and oral tumors (10 oral malignant melanoma, 3 oral fibrosarcomas, and one squamous cell carcinoma). Results of our study showed the good antitumor effectiveness of the combined therapy in skin tumors as the objective response at the end of the observation period was over 90% (median observation time 15 months). In the case of oral tumors, we achieved the median survival times similar to the reported median survival times of the standard therapies (median 7 months). In conclusion, our study showed a significant

antitumor response in the case of skin tumors and presented a valuable treatment option for dogs with oral tumors, especially when other treatment approaches are not acceptable.

Electroporation: dosimetry, accuracy, and uncertainties

Lluís M. Mir

CNRS, UMR 8121, Institut Gustave-Roussy, France

The potential interactions of the electric pulses on the cell membranes will be presented, as well as the effects on tissues. To optimally apply the electric pulses in the clinics it is important to understand the basics of these interactions. Dosimetry is mandatory to ensure a convenient application of the electric pulses. While analytical or numerical modeling is important, models need to be validated by experiments. Moreover, they do not solve all uncertainty even if currents and field amplitudes can usually be measured almost in real time. In vivo, the heterogeneity of the tissues is a source of uncertainty that can be easily apprehended. However even in vitro sources of uncertainty can be identified, that will be presented and discussed. In spite of these sources of uncertainty related to the interactions of the electric fields with the biological objects, electrochemotherapy is a very robust treatment because its bases are not only electrical but also chemical and biological.

Treating nasal tumors with electrochemotherapy

Maglietti F, Tellado M, Michinski S, Marshall G

Instituto de Física del Plasma, DF, FCEN, UBA-CONICET, Buenos Aires, Argentina

Nasal cavity tumors are a complex disease which usually is diagnosed late. They infiltrate adjacent tissues early in the course of the disease, making the treatment very difficult most of the times. Electrochemotherapy (ECT) offers a great treatment opportunity because of its high response rates and minimum side effects. Patients were treated with the Single Needle Electrode, an electrode designed to deliver the electric field in the area that surrounds it. The effects on the tissue that surrounds the electrode comes from three different sources: extreme pH changes, irreversible electroporation and reversible electroporation. This phenomenon are studied in silico, in vitro, and in vivo. Our results show that the electrode treats a volume of: 3 cm³. Of that volume 50% corresponds to reversible electroporation, 40% corresponds to extreme pH conditions plus irreversible electroporation and 10% corresponds to mechanical damage of the electrode. In vivo results show that patient stratification, and evaluation of the tumor with MRI or CT scans is mandatory for a satisfactory treatment planning. In order to achieve a complete response, the disease has to be limited to the nasal duct. If there is infiltration to the paranasal sinuses, the cribriform plate or retro orbital area, there is a great chance of relapse. Patients with tumors in the hard or soft palate are prone to have tumoral invasion to the nasal duct, and for that reasons we recommend to prophylactically treat both nasal passages. Finally, tumoral volume should be lesser than

3cm³ in order to treat it with only one application. In other cases, more than one will be required. In conclusion, ECT is a safe and effective procedure for selected cases.

Opening the Cell Membrane-Veterinary Experiences with Electro-Gene-Transfer and Electrochemotherapy in the United States

Joseph A. Impellizeri

Veterinary Oncology Services, PLLC- New York, USA, www.petcancerinformation.com

Electroporation is an emerging modality in the United States in veterinary medicine. This presentation will highlight experiences with electrochemotherapy in veterinary medicine with needle electrodes, endoscopic approaches and electrogenetransfer with immunotherapy with DNA plasmids targeting canine telomerase and canine HER2. Dr. Impellizeri is a 1994 graduate of Cornell University, College of Veterinary Medicine. In 1995, he completed a small animal medical and surgical internship at Colorado State University, before returning to the east coast to work for 5 years in small animal emergency/primary care, followed by a residency in Medical Oncology. Dr. Impellizeri became a Diplomate in the American College of Veterinary Internal Medicine, a subspecialty of Oncology in 2005. In 2011, Dr. Impellizeri created the Barrymore Center for Advanced Cancer Therapeutics with a focus on Cancer Immunotherapy and Electrogenetransfer. He is a frequent lecturer on these topics.

Medical applications with Electrochemotherapy

Victor Farricha

*Portuguese Oncological Institute of Lisbon – Melanoma & Sarcoma Unit
Faculty of Medicine of Lisbon, Portugal*

During the past four decades, advances in fundamental and experimental electroporation research have allowed for the translation of electroporation-based technologies to the clinic. Electrochemotherapy (ECT) established its position as a treatment for cutaneous primary and secondary tumours. The practice of electrochemotherapy has developed through clinical trials, standard operating procedures (SOP) as well as the clinically certified electric pulse generators, to a point where electrochemotherapy is routinely used in Europe. As electrochemotherapy is efficient with limited side effects for cutaneous tumours, the procedure is now increasingly being used, and also introduced into clinical guidelines and medical applications. ECT is now going far beyond skin tumors and many clinical trials and case reports seem to show us the usefulness in head and neck tumors, rectal and esophageal malignancies, bone metastasis, soft tissue sarcomas, brain tumors, liver metastasis or pancreatic cancer. In Europe, more than 150 centers perform ECT according to institutional protocols and European guidelines. In Lisbon since 2008, we have made more than 750 sessions of Electrochemotherapy. Our field is mainly cutaneous lesions, but we had good results in mucosal tumors (vulvar, anal and rectal) using electrodes developed for this kind of malignancies. The overall response rate in this series is very high (95%) with a complete response rate of 73%. Melanoma patients (68%), Squamous Cell Carcinoma and Breast Cancer (12%) are the

main pathologies treated. As published in the literature the complications observed were easily managed. Most of the treatment sessions were performed under sedation or general anesthesia but we think that in the future with high-speed ECT we can avoid the anesthesia obstacle. In my presentation, I will share our experience and talk about the current applications of electroporation in medicine and try to discuss some of the current challenges in new clinical applications.

Electroporation as a tool to enhance the therapeutic efficacy of Boron Neutron Capture Therapy (BNCT)

M. A. Garabalino^{1§}, N. Olaiz^{2,3 §}, I. S. Santa Cruz¹, P.S. Ramos¹, A. Portu^{1,2}, G. Saint Martin¹, M.E. Itoiz^{1,4}, E.C. Pozzi¹, S. Thorp¹, P. Curotto¹, A. Monti Hughes^{1,2}, D.W. Nigg⁵, V.A. Trivillin^{1,2}, G. Marshall^{2,3}, A.E. Schwint^{1,2}

¹National Atomic Energy Commission (CNEA), Argentina; ²National Research Council, Argentina (CONICET); ³Faculty of Exact and Natural Sciences, UBA, Argentina; ⁴Faculty of Odontology, UBA, Argentina; ⁵Idaho National Laboratory, USA.

§These authors contributed equally

Introduction: A critical aspect of the therapeutic efficacy of BNCT is the biodistribution of ¹⁰B in tumor and in the dose-limiting normal and precancerous tissues in the target volume. Given that electroporation (EP) can act as a non-specific system to administer anti-tumoral agents, the aim of the present study was to evaluate if EP could improve the targeting of ¹⁰B in BNCT thus increasing tumor response *in vivo* in an oral cancer model. **Materials and Methods:** Exophytic tumors (Squamous Cell Carcinoma) were induced in the hamster cheek pouch by topical application of the carcinogen DMBA. We performed electroporation experiments in tumors as part of 2 boron administration protocols employing boric acid (BA) or GB-10, varying the time between EP and the administration of the boron compound (Early EP or Late EP). Tissue samples were processed for boron measurements. Irradiations employing Early EP were carried in the thermal facility of the RA-3 Nuclear Reactor. Tumor response and degree of mucositis in precancerous tissue were evaluated over a period of 28 days post-irradiation. **Results:** Biodistribution studies showed an increase in mean gross ¹⁰B concentration in the tumor in both protocols (BA and GB-10) with Early EP. Early EP significantly increased selective boron uptake by tumor only in the case of GB-10. Early EP enhanced tumor response induced by BNCT mediated by BA or GB-10 vs the control animals with no EP. No severe radiotoxicity was observed. **Conclusion:** Electroporation increases the therapeutic efficacy of BNCT *in vivo* in an oral cancer model.

Multilobular osteochondrosarcoma treated with electrochemotherapy: Experience of two cases.

Javier Ojeda, Erica Silva, Heine Muller, Pedro Molina, Daniela Zaror.

Instituto de Cs. Clínicas Veterinarias, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia-Chile.

The most prevalent bone neoplasias are appendicular osteosarcoma, extra-appendicular osteosarcoma, fibrosarcoma, and chondrosarcoma. The Multilobular osteochondrosarcoma (MLO) is a uncommon bone neoplasia localized principally in skull and fingers. Clinically it is characterized by a firm and delimited swelling with/without pain to palpation. The radiographical findings are a delimited tumor with a coarse granular mineral density throughout. Histologically, these tumors are composed of multiple lobules with a core of cartilaginous or bony matrix that is surrounded by a thin layer of spindle cells. The resection of the tumor had been showed more than 50% of recurrence. The present reports describe two cases of MLO one of those located in the zygomatic arch and another in frontal bone. Both cases were diagnosed using CT and histopathological exams. Then a surgical resection with neoadjuvant electrochemotherapy (ECT) using bleomycin was performed for both dogs. The MLO located in zygomatic bone had a partial surgical resection with a delayed recurrence compared with the first surgery performed. The dog was euthanized one year after. The frontal MLO had an almost total surgical resection but the neoadjuvant ECT was effective because reached a complete remission. The neoadjuvant ECT could be an effective alternative for MLO considering the high recurrence behaviour.

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Electrochemotherapy plus cryosurgery in afeline SCC -Case Report-

Laura Pisano¹, Matías Tellado^{1,2} and Felipe Maglietti²

1. *Vetoncologiaclinic, Ciudad de Buenos Aires, Buenos Aires, Argentina.*
2. *Instituto de Física del Plasma, Departamento de Física, FCEyN, UBA-CONICET, Ciudad de Buenos Aires, Buenos Aires, Argentina.*

A case report of a feline cutaneous squamous cell carcinoma (SCC) treated with electrochemotherapy plus cryosurgery is presented. The patient is a 7-years-old, white, cross-breed female with a large lesion in the supraorbital area and two smaller lesions in lower and upper eyelids respectively. Histopathologic samples of the lesions stained

with H&E revealed the presence of an SCC. Staging studies did not show regional or distant metastases. A single dose of 15,000 IU/m² of bleomycin was administered in an IV bolus, the electric pulses were delivered 8 minutes later. Pulsing procedure consisted of 8 square pulses of 1000V/cm and 100 μ s long at a repetition frequency of 10 Hz. The lesions were treated using a 27G 4-needle electrode. After 60 days a CR was observed in the large lesion, but a PR was observed in the upper and lower eyelid lesions. A cytology of the residual lesions confirmed a malignant epithelial neoplasia. Thus, a cryosurgery session with liquid nitrogen at -196 C was performed through 3 consecutive cycles of freezing and thawing using a 21G cryotip to generate cryonecrosis of the residual lesions, and the subsequent regeneration of the frozen tissue. A CR was obtained without evidence of side effects in the remaining lesions. Cryosurgery is a promising tool to treat remaining lesions after ECT. We are currently conducting a large-scale study to verify these results and study the long-term control of the lesions without toxicities or complications associated with the combination of both techniques.

Conservative surgery plus electrochemotherapy on intranasal carcinoma

*Priscila Gil Quadros*¹, Jennifer Ostrand Freytag¹, Krishna Duro de Oliveira¹, Daniela Ota Suzuki², Marcelo Monte Mór Rangel¹

1 – VetCâncerClinic – São Paulo/Brazil

2 – Universidade Federal de Santa Catarina – UFSC/Brazil

Intranasal tumors are often diagnosed in advanced staging. Among the conventional modalities of treatment we can highlight radiotherapy and radical surgery as those that provide the best results. The first modality in Brazil is still little available and costly and radical surgery has a high rejection rate by the owners due to the comorbidities and aesthetic changes involved. The high index of response and the selectivity to the neoplastic tissue by the electrochemotherapy, in theory, could provide conservative procedures to the patients affected by neoplasias with this location when associated with conservative surgery. The present report presents a case of nasal sinus carcinoma in advanced stage, involving all the left nasal cavity. Planning consisted of conservative surgery, aiming to remove only the macroscopic part of the tumor and to perform electrochemotherapy on the surgical margins. To perform the excision of the macroscopic part of tumor in a conservative approach and to reach with the electrodes the surgical margins by the electrochemotherapy, a "window" was opened in the maxilla region, adjacent to the formation. For the application of electrochemotherapy in the deeper regions of the nasal cavity an electrode was developed where the needles are retracted in a small caliber cannula and are exposed only at the moment of the application through a trigger. After the procedures the "window" was not closed for better clinical monitoring after the procedure. Two procedures were performed and the patient presented reduction of tumor volume about 95%. The first procedure was performed 455 days ago. The patient does not present difficulties to feed or drink water and their quality of life is considered normal. In addition to the functional aspects, the patient also maintained his aesthetic appearance similar to normal. The present report corroborates the idea that more studies about this approach should be performed due to

the excellent results achieved in both the functional, aesthetic aspects, acceptance of owner and survival.

Computational model applied for the treatment of bone tumor on patient

*Daniela Ota Hisayasu Suzuki*¹, *Taila Becker Damiani*¹, *Jennifer Ostrand Freytag*², *Krishna Duro de Oliveira*², *Marcelo Monte Mor Rangel*²

1 – Universidade Federal de Santa Catarina – UFSC/Brazil

2 – Vet Câncer Clinic – São Paulo/Brazil

Electrochemotherapy (ECT) is a combination of high electric field and anticancer drugs. The treatment basis is electroporation or electroporabilization of the cell membrane. Electroporation is a threshold phenomenon and, for efficient treatment, an adequate local distribution of electric field within the treated tissue is important. When this local electric field is not enough, there is a regrown tumor cell. Cortical bones provide low electric conductivity and affects the electric field distribution. In previous work, we demonstrated the cavity bones with tumor are harmful to efficiency ECT treatment. This work presents the effect of a tooth with tumor treatment in periodontal ligament. *In vivo* case of ECT treatment with and without tooth are compared to *in silico*. *In silico* study from computational tomography of three teeth are implemented to analysis different possibilities of treatment. The results demonstrated tooth extraction before ECT is recommended.

Electrochemotherapy in a horse - Case Report

*Fernández J*¹, *Tellado M*¹, *Descalzi TS*², *Muriel M*², *Zapata G*², *Hernández HZ*², *Michinski S*¹, *Maglietti F*¹, *Marshall G*¹

1. Instituto de Física del Plasma, Departamento de Física, FCEyN, UBA-CONICET, Ciudad de Buenos Aires, Buenos Aires, Argentina.

2. Facultad de Ciencias Veterinarias, Universidad de La Plata, La Plata, Buenos Aires, Argentina.

Here we present a female, 7 years-old, light-brown horse with a sarcoid. The main lesion is round shaped, 4 cm in diameter located in the left arm, also satellite lesions were present surrounding the first one. The histopathological diagnosis was confirmed by H&E stains. For the treatment, intralesional bleomycin was administered in a dose of 125 IU/cm³ covering whole tumor surface and margins. The electric pulses were delivered using a 6-needle-electrode to the lesions and to the safety margin. Fifty days after treatment a partial response was obtained, the patient continued in follow-up with no further treatment. At 70 days after treatment, a complete response was achieved. Electrochemotherapy is a novel treatment option that is very useful in the rural setting, due to the possibility of performing the treatment at the countryside, it requires almost no care after the procedure, it enables to control diseases with no other treatment available and is virtually side effects free.