Teaching electroporation

The University of Ljubljana's Department for Biomedical Engineering is esteemed for its excellence in electroporation and its annual EBTT event. **Professor Damijan Miklavčič** and **Dr Lluis M Mir** reveal all...

PROFESSOR DAMIJAN MIKLAVČIČ

What is the history of the annual school workshop and what is its significance within the research field?

DM: The school was first organised in 2003. We brought our students and invited others to join us; it was quite primitive, but full of enthusiasm. From the beginning we had a vision of organising the event periodically, complementing lectures with hands-on lab work. It was then and still remains an officially-accredited University of Ljubljana course – after passing the exam at the end of the week, students receive credits so the course forms part of their formal education.

LM: The first bilaterally-supported exchange projects on electroporation between France and Slovenia started in 1997, and in 2000 I was granted EU funding for the first in a series of three projects which gathered, among others, the groups of the initial Faculty of this school. It was clear that the research being conducted, as well as its biomedical and biotechnological applications, had to be disseminated in an organised way.

Can you outline some of the successes and highlights from workshops you have organised at the University?

DM: Where else in the world can you do experiments fusing cells, transfecting them, observing and calculating induced transmembrane potential and simulating the molecular dynamics of membrane electroporation? The morning lectures by world-leading experts from prominent universities such as Berkeley, MIT, Paris XI and KIT are followed by dinner with famous names like Neumann, Teissie, Weaver and Mir.

LM: The attraction the school exerts on students from all over the world is extraordinary. All of the places are booked every year and the satisfaction level, gauged using a questionnaire distributed to students at the end of the course, is always high. The workshops closely follow progress in this rapidly-evolving field, with the lecture topics being modified to relay fundamentals as well as the latest innovations from the field.

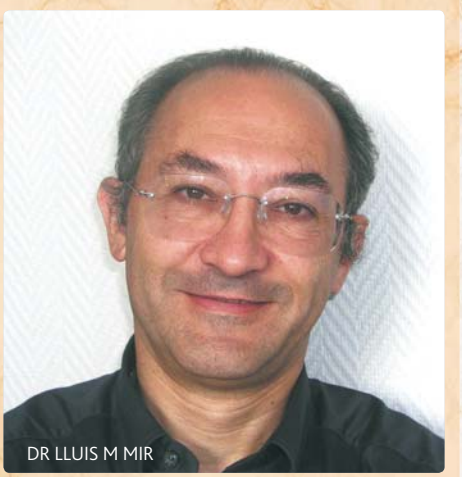
Can you expand on how you explore current applications and future prospects of electroporation, both at the Electroporation-based Technologies and Treatments (EBTT) conference and in your day-to-day research activities?

DM: We are currently witnessing a number of applications being introduced into clinics and the food-processing industry. In medicine, we see electrochemotherapy as a local and highly-effective treatment for solid tumours, and non-thermal irreversible electroporation as an ablation method. The next application in medicine seems to be gene electrotransfer for DNA vaccination and gene therapy. Current applications in food technology mainly deal with the extraction of sugar from sugar beet at lower energetic cost, oil from olives, or low temperature pasteurisation through microbial deactivation.

We are currently interested in developing planning procedures for treating deep-seated solid tumours, and are also very much involved in understanding gene electrotransfer and cell fusion. However, our main focus remains the combination of experimentation with modelling to enhance our fundamental understanding of the phenomena of electroporation at membrane, cell and tissue levels.

What are the highlights of the workshop in terms of research proposals and lectures?

DM: This year we have invited David A Dean from the University of Rochester, US, to



DR LLUIS M MIR

discuss cell-specific targeting strategies for electroporation-mediated gene delivery in the lung, and Dr Julie Gehl, a clinician at University Hospital, Herlev, Denmark, who will discuss electrochemotherapy and safety and toxicity of electroporation. Our third lecturer is Rafael Davalos from Virginia Tech, US, who will lecture on non-thermal irreversible electroporation as a tissue ablation therapy. Finally, Stefan Toepfel from the German Institute of Food Technologies will talk about electroporation in food processing.

Do you believe that outreach and education are important factors in research programmes and if so, what benefits do you believe they bring both to the field and to the students who participate in them?

DM: I think we now recognise how important it is to speak to the public about our work and to organise schools like this – too often, money is spent more than once for the same research and we reinvent the wheel. It's a pleasure to see former students become heads of labs or take up teaching positions around the world, developing new applications and treatments, and contributing new knowledge.

LM: Electroporation is a simple and robust procedure, but understanding the fundamentals when developing a new application is crucial. The students of today will be the developers of tomorrow's new applications. It is therefore important that those preparing their PhD theses integrate courses like EBTT into their research.

A one stop knowledge shop

Now in its ninth year, **Electroporation-based Technologies and Treatments** is an international workshop and postgraduate course bringing together the world's leading experts in the field of electroporation

ELECTROPORATION INVOLVES THE application of an electric field to a cell's plasma membrane – a process which leads to the creation of 'nanopores' in the membrane and consequently increases its permeability. It can be used as a method of introducing a substance into a cell, eg. a drug or a segment of coding DNA which would not naturally diffuse across the cell membrane. Assuming appropriate voltage thresholds are maintained, it is a temporary phenomenon, with the cell membranes returning to their previous state once the application of the electric field is discontinued. At higher voltages, however, electroporation can create permanent damage which a cell is unable to repair – a phenomenon which can be used to cause a range of target cells to lose homeostasis and die, while leaving neighbouring cells unaffected. This makes it a promising treatment for certain diseases, such as cancer, which require selective tissue removal.

At the Department for Biomedical Engineering at the University of Ljubljana in Slovenia, Professor Damijan Miklavčič and Dr Luis Mir coordinate Electroporation-based Technologies and Treatments (EBTT) – an annual, week-long event which has been running for nine years. The course has two primary objectives: firstly, to offer a comprehensive learning experience for PhD students and newcomers to the field through lectures from leading experts and lab work, and secondly, to gather individuals with expertise to discuss new developments and applications, thereby building common knowledge. Together with faculty members, four guest lecturers are invited each year, selected for novel and groundbreaking achievements either in applications or their understanding of the phenomenon.

DIFFERENT PERSPECTIVES

"The key objectives of EBTT are the diffusion of the fundamentals and the most recent data on electroporation and its applications, through lectures dedicated to PhD students, postdocs and young researchers in the field," outlines Mir. The social component is clearly important: networking and getting to know one another is facilitated not only on the course but through extracurricular events such as dinners, trips to the coast or sightseeing, and Miklavčič is enthusiastic about the resultant potential for knowledge-sharing. "Lectures, discussions and student presentations all help to spark new ideas and different perspectives, and many projects and experiments are planned during or soon after the course," he explains. "The workshops introduce you to others who have equipment, knowledge and experience you need, and when so many people from the same field spend all day together, it is easy to start discussions or arrange lab visits. The attending lecturers leave with a much clearer understanding of students' interests, enabling them to better fulfil the students' expectations."

PIONEERS

The Faculty members at Ljubljana are pre-eminent members of the community of researchers on electroporation fundamentals and applications. In 1982, Eberhard Neumann published the first paper showing that nucleic acids can be transferred into living cells by electric pulses while preserving the viability of a cell, despite the fact that large molecules cannot normally cross its membrane. Justin Teissié pioneered the electrofusion of cells using electric pulses, while Luis Mir established conditions under which almost all of the exposed cells were permeabilised while remaining alive. Mir also conceived electrochemotherapy using bleomycin, conducting the first preclinical and clinical trials in 1991 and today, cancer centres across Europe routinely treat patients with electrochemotherapy. Gregor Serša, meanwhile introduced cisplatin – an already efficient and widely used cancer drug – into the concept of electrochemotherapy. He was also the first to demonstrate the importance of the effect that electrochemotherapy has on tumour vasculature. Véronique Préat pioneered the use of electroporation of the skin to facilitate transcutaneous drug delivery and more recently, P Tom Vernier has made seminal contributions in nanosecond-duration electric pulses.

HOT TOPICS

"Our laboratory has always been internationally active and well-connected," Miklavčič reveals. "Slovenia has a programme of bilateral collaboration agreements with more than 80 countries around the world, including the US, Korea, France, Poland, Argentina, Romania and many others. Small grants cover travel and subsistence from Slovenia to the partner country and vice-

LAB EXPERIMENT ON TRANSFECTING BACTERIAL CELLS WITH PLASMID DNA BY MEANS OF ELECTROPORATION

EBTT

ELECTROPORATION BASED TECHNOLOGIES AND TREATMENTS INTERNATIONAL SCIENTIFIC WORKSHOP AND POSTGRADUATE COURSE

OBJECTIVES

To disseminate recent research results and understanding on electroporation and its applications, through lectures dedicated to PhD students, postdocs and young researchers in the field.

KEY COLLABORATORS

Eberhard Neumann, University of Bielefeld, Germany • **Véronique Préat**, Catholic University of Louvain, Brussels, Belgium • **Gregor Serša**, Institute of Oncology, Ljubljana, Slovenia • **Justin Teissié**, CNRS and IPBS, Toulouse, France • **P Thomas Vernier**, University of Southern California, USA • **Tadej Kotnik**, University of Ljubljana, Ljubljana, Slovenia • **Marie-Pierre Rols**, CNRS and IPBS, Toulouse, France • **Mounir Tarek**, Université Henri-Poincaré, Nancy, France • **Maja Čemažar**, Insitute of Oncology, Ljubljana, Slovenia • **Peter Kramar**, University of Ljubljana, Faculty of Electrical Engineering • **Maša Kandušer**, University of Ljubljana, Faculty of Electrical Engineering

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DAMIJAN MIKLAVČIČ has been a Professor of Electrical Engineering at the University of Ljubljana since 2002. He is the Head of the Laboratory of Biocybernetics and the Chair of the Department of Biomedical Engineering.

LLUIS M MIR received his DSc degree from University of Toulouse. He is Director of Research at the CNRS, Director of the Laboratory of Vectorology and Anticancer Therapies of the CNRS, Institute Gustave-Roussy and University Paris-Sud in Villejuif, France.

LEA EBAM
European Laboratory of Pulsed Electric Fields Applications in Biology and Medicine

versa, which we find invaluable in fostering an international network of collaborators."

Miklavčič and Mir are co-directors of the European Associated Laboratory (LEA) on the Electroporation in Biology and Medicine (EBAM) and at EU level, Miklavčič chairs a COST TD1104 action comprising national groups working on electroporation from 22 EU countries and six others from around the world. Mir's group is one of the 10 members of the International Bioelectrics Consortium, and he is also President of the European BioElectromagnetics Association (EBEA) which organises specialised courses in Sicily as well as annual scientific meetings. All of these contacts, both in the field of electroporation and related fields of the interactions of electric and electromagnetic fields with living organisms, help to broaden knowledge of electroporation-based technologies and treatments.

"After nearly 10 years of experience we have struck the perfect balance, with basic lectures from faculty members in the morning focusing on membranes, cells and tissues, as well as medical applications such as gene transfer, electrochemotherapy and biotechnology," enthuses Miklavčič. "The early afternoon guest lectures are highlights; always hot topics, and extremely well received. Later in the afternoon, attendees split into groups of four and go to the labs to work on numerical modelling, electroporating bacteria and eukaryotic cells, fusing or transfecting them, and building planar lipid bilayers."

EMERGING APPLICATIONS

The number of applications of electroporation technologies is increasing, and the team at Ljubljana's EBTT school is responsible for several key findings, particularly with regard to medical treatments. Perhaps the most prominent of these achievements was bringing electrochemotherapy from the lab to the bedside.

"Electrochemotherapy has been successfully introduced to clinics and is now used successfully for treating tumours and metastasis in the skin, irrespective of histological origin, in more than 85 clinical centres around Europe," Mir reveals. Several researchers are developing procedures for treating deep-seated tumours such as bone metastasis, liver tumours, soft tissue sarcoma and brain tumours. Elsewhere, endoscopic treatment of tumours in the colon and oesophagus is also being explored, and Mir believes that the next medical applications will involve gene electrotransfer for DNA vaccination and gene therapy, with several clinical trials already underway.

Last year, the team submitted a proposal to the EC – one of only three selected from a possible 100 – to fund a COST action on electroporation. The start of this Action earlier this year is already allowing for better collaboration and coordination of research efforts in the field of electroporation and electroporation-based technologies and treatments in Europe and worldwide, providing funds for meetings, the exchange of researchers between labs and organising schools similar to the one at Ljubljana. Over the next four years, in addition to those from 22 EU countries, researchers from Argentina, Australia, US, New Zealand, Russia and Ukraine will have the opportunity to meet and discuss electroporation and its emerging applications, such as plant cryopreservation, 'cooking' with electroporation, extracting substances from cells, tissue decellularisation, water disinfection and many more.

"I would like to highlight the extraordinary and unexpected progress in the field over the last 20 years, which is particularly important in the context of biotechnology in the food industry and in medical applications," Mir concludes. "Electrochemotherapy is now evolving to include imaging-guided treatment of deep-seated tumours, currently only in a palliative capacity but in the future, most likely in a curative one. The field is still young and we can expect many more important and exciting developments."

LAB EXPERIMENT OF SKIN ELECTROPORATION FOR TRANSDERMAL DRUG DELIVERY ENHANCEMENT