Modelling, visualising, and tracking pH front formation during pulse delivery to agarose-based tissue phantoms   L14

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Duration of the experiments: 120 min
Max. number of participants: 3
Location: Tissue Laboratory
Level: Basic

PREREQUISITES
Participants should be familiar with Laboratory safety (S1). No prior knowledge of laboratory work is required. Possession of basic skills of handling chemical/biological laboratory equipment (pipette, analytical scale) is an advantage, but not prerequisite.

THEORETICAL BACKGROUND
In electroporation applications we inevitably encounter chemical phenomena associated with the direct supply of electrical energy to living organisms by means of metallic electrodes. As these are inserted into an electrolyte solution, such as is tissue, a special region (layer) forms at the interface between the electrode and the tissue, where dynamic electrochemical processes occur to enable flow of an electric current. The products of these processes can be both beneficial or highly harmful to tissue (and electrodes). Assuming monopolar pulse delivery, an acidic pH front forms at the anode and a basic at the cathode, propagating away from the electrodes during, but also after pulse delivery.

While gene electrotransfer (GET, Potočnik et al., 2022) holds great potential for therapeutic applications, it is important to consider the potential effects of pH changes induced by this method in target tissues. Change of pH levels during GET can lead to tissue damage and cell death, reducing the effectiveness of the treatment by potentially damaging portions of the tissue where transfection would otherwise be successful. The presence of hydroxyl ions increases the damage and is even more fatal for the cells, as their interaction with lipids leads to disruption and breakdown of the cell membrane structure (Li et al., 2015). It is thus important to carefully optimize the electroporation parameters to minimize pH-induced damage and maintain tissue viability during GET, where often longer (several milliseconds) pulses are used as part of the treatment protocol.

There has been intense development of alternative tissue ablation therapies recently, either using high-voltage pulses such as in irreversible electroporation (IRE, Cindrič et al., 2021) or pulsed field ablation (PFA, Sugrue et al., 2019), or (side)products of electrochemical reactions in what is called electrochemical therapy (EChT, Nilsson et al., 1998, Nilsson et al., 2001), or a combination of both, called electrolytic electroporation (E^2, Phillips et al., 2016). These therapies exploit not only the effects of high field strengths, but also the altered acid-base properties of the tissue (change of pH), to successfully remove pathologically altered tissue. It is thus of importance for treatment design and success that these electro-chemical processes altering pH in tissue are well understood and, if appropriate, exploited to our advantage.

One effective approach to detect and visualize pH changes during the delivery of electrical pulses to model tissues or tissue phantoms involves the use of pH-sensitive dyes or indicators.
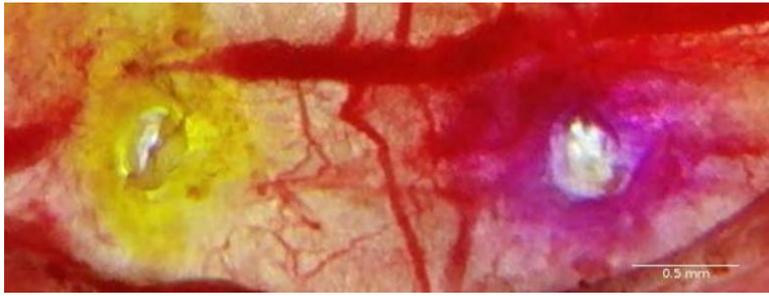
Figure 1: pH changes in the skin due to pulse delivery, surrounding the two needle electrodes (anode-left, cathode-right). Dorsal skin flap of a mouse, observed through a window chamber, *in vivo*. From Marino et al., 2017.

These dyes exhibit a colour change in response to variations in pH values, allowing for real-time monitoring of pH dynamics within the tissue or tissue phantoms. By incorporating these pH-sensitive dyes into the model tissue, which can be prepared using materials such as collagen or agarose, we can observe and analyse the electrochemical reactions and subsequent pH changes that occur at the interface between the metal electrodes and the tissue (Rubinsky et al., 2016, Kim et al., 2023). This enables a direct visualization of the impact of electrical pulse delivery on the pH environment, providing valuable insights into the underlying processes and helping to optimize the therapeutic applications of electroporation. The pH-sensitive dyes act as visual indicators, offering a convenient and non-invasive means to assess the local pH changes induced by the electrical pulses, thus enhancing our understanding of the intricate electrochemical interactions between metal electrodes and biological tissues.

The aim of this laboratory practice is to showcase how we can use numerical modelling techniques using advanced finite-element software models (COMSOL Multiphysics) to study complex electrochemical phenomena occurring at the electrode-electrolytic solution interface, and their propagation by electromigration and diffusion in a phantom tissue model. The laboratory exercise will demonstrate, through practical work, how such models can subsequently be validated using practical and comparatively simple lab experiments. The students will learn just how much electrochemistry is "going on" during a typical electroporation application.

EXPERIMENT

We will first review simulation results demonstrating pH front evolution and advancement in a typical needle-electrode delivery of pulses using a typical GET protocol to i.) an unbuffered medium, and ii.) a buffered medium of comparable buffering capabilities to those of tissue. We will then move to the wet lab portion of the lab work, where we will deliver pulses to agarose phantoms using the same pulse protocol as previously demonstrated through simulation results at various voltages (field strengths). Additionally, we will deliver pulses to the phantoms using an additional protocol employing short, biphasic pulses, to study if these do indeed result in significantly less change in pH as per theory.

We will prepare the agarose phantoms with either unbuffered or buffered saline to demonstrate the natural buffering capabilities of tissue. The two pulse protocols will be chosen to demonstrate the difference between monophasic and biphasic delivery of pulses. The experimental system for observing pH changes consists of a high-speed digital camera with a macro lens, a camera stand with lighting panel, an electroporation pulse generator, and a suitable laptop for capturing the camera's video signal.
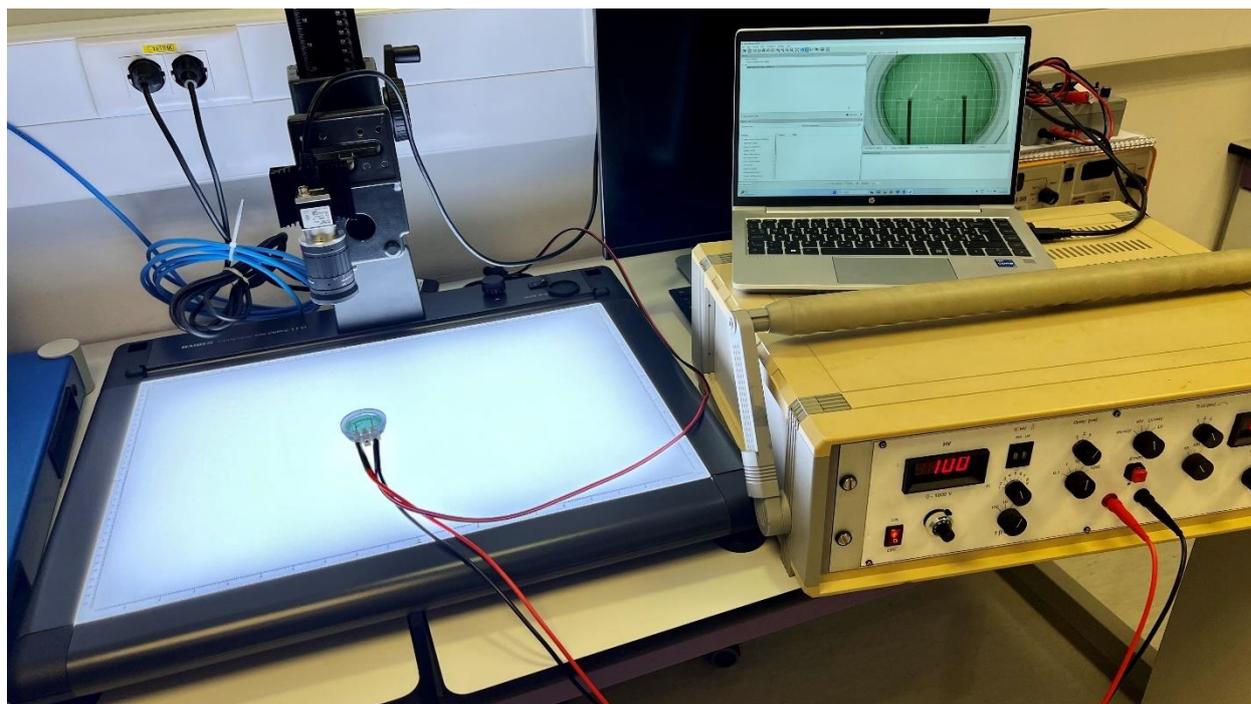
Figure 2: Experimental setup showing the camera rig and the agarose sample under treatment in a mini petri dish with a custom made cover/electrode holding guide, pulse generator, and laptop computer for controlling the camera and recording video data.

Protocol:

Following the demonstration of numerical simulation results (a slide presentation), we will move to the wet lab where we will begin by mixing an indicator solution using purified water, methyl red, bromothymol blue, and phenolphthalein disodium salt in the following quantities: 150 mL water, 0.012 g methyl red, 0.060 g bromothymol blue, and 0.050 g phenolphthalein disodium salt. We will then prepare a 0.60 % agarose solution using two different ready-made solutions; the first will be a representation of an unbuffered medium and will consist of 0.9 % saline, the second will be prepared by taking 1 molar phosphate-buffered saline that will act as an example of a buffered medium. Note that PBS consists of saline, potassium chloride, dibasic sodium hydrogen phosphate, and monobasic potassium hydrogen phosphate and has a pH of 7.4. Both solutions need to be diluted in a solution-to-purified water ratio of 5-to-1, since we would like our agarose phantoms to model *in vivo* tissue conductivity of skeletal muscle of about 0.25 S/m[1].

Having prepared the buffered and unbuffered agarose solution, the two solutions should be quickly heated using a microwave almost to the point of boiling, after which (but before pouring into petri dishes) the solutions have to be supplemented with the indicator solution in the ratio of 9-to-1 (medium-to-indicator). Following the addition of the indicator solution and agitation, agarose can be measured out into small petri dishes with a pipette (3 mL per dish) and await hardening (about 10-15 minutes should suffice).

Once hardened, you will deliver to the agarose phantoms either a typical gene electrotransfer (GET) protocol (4 x 5 ms delivered at 1 s^{-1}), or a typical short, biphasic (HFIRE) protocol (5 μs positive/negative phase, 5 μs interphase delay, 500 μs interpulse delay, 200 pulses per train, train repetition frequency 1 s^{-1}, 10 trains in total) using the pulse generator and a pair of needle electrodes. Note the total "on time" of the two protocols is the same – 20 ms. Different

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[1] Skeletal muscle conductivity exhibits a wide range of values that can fall anywhere between 0.04 and 0.8 S/m. We chose 0.25 S/m for the model and experiment as a rough midpoint off that interval (Miklavčič et al. 2006).

amplitudes of the pulses can be used to study the effects of lower/higher pulse current (the phantom conductivity is constant) – try experimenting with a few voltages!

You will record the videos of the experiments using the fast camera and subsequently analyse the pH front spatial progression and colour changes (in qualitative terms) by comparing the observed front appearance and migration (at a single chosen voltage, to save time):

- with model results seen earlier during the theoretical introductory part of the lab work for the GET protocol considering the buffered and unbuffered medium, and
- for the two different protocols, i.e., the monophasic GET and biphasic HFIRE as experimentally recorded in the buffered and unbuffered medium.

FURTHER READING:

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NOTES & RESULTS

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