L9 Modelling, visualizing, and tracking pH front formation in tissue phantoms

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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) and Electroporation hardware safety (S2). No prior experience with laboratory work is required. Basic skills in using chemical/biological lab equipment (e.g., pipettes, analytical balances) are helpful but not mandatory.

The aim of this laboratory exercise is to demonstrate how numerical modelling using finite element software (COMSOL Multiphysics) can be applied to study complex electrochemical processes occurring at the interface between metallic electrodes and electrolytic solutions. These phenomena include the formation and propagation of pH fronts caused by electromigration and diffusion during pulse delivery.

Through hands-on experiments using agarose-based tissue phantoms and pH-sensitive dyes, participants will validate the simulation results and gain insight into the electrochemical activity that accompanies typical electroporation protocols, i.e. such as those used in gene electrotransfer. The exercise emphasizes how seemingly simple pulsed-field treatments involve rich underlying chemistry, particularly at the tissue–electrode interface.

THEORETICAL BACKGROUND

In electroporation applications, the delivery of electrical energy via metallic electrodes into biological tissue inherently gives rise to electrochemical reactions. At the interface between the electrode and the electrolyte (the tissue or tissue-mimicking medium), a double layer forms in which complex chemical processes facilitate current flow. These reactions often produce by-products that can be damaging to both tissue and electrodes [1,2].

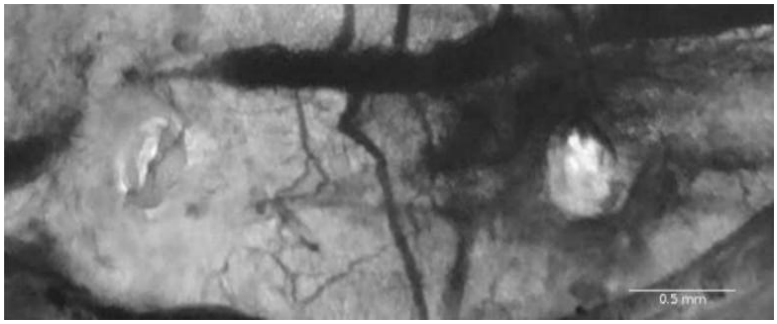

Figure 1: pH changes in the skin due to pulse delivery, surrounding the two needle electrodes (anode-left, cathode-right). Dorsal skin flap of a mouse, observed through a window chamber, in vivo. From [5].

In monopolar pulse protocols, pH fronts develop as a direct result of these reactions (see Figure 1): an acidic front forms near the anode, while a basic front develops near the cathode, both of which propagate during and after pulse delivery [3,4].

A well-established method for observing these pH changes in model systems involves the incorporation of pH-sensitive dyes. These dyes exhibit distinct color changes in response to shifts

in local pH, enabling real-time visualization of pH gradients in tissue phantoms. When incorporated into materials such as agarose or collagen gels, these dyes allow researchers to observe and analyze the effects of electrical pulse delivery on local chemical environments [6-8].

This approach offers a practical and non-invasive way to study electrochemical interactions, validate numerical models, and optimize protocols for electroporation-based therapies. The dyes act as reliable visual indicators, making it possible to track the spatial and temporal dynamics of pH shifts induced by electrical stimulation.

EXPERIMENT

Part 1: Simulation review

We begin with a review of numerical simulations (COMSOL Multiphysics) modelling pH front formation during a standard **gene electrotransfer (GET)** [9] pulse protocol applied with needle electrodes. Two media are compared: an **unbuffered solution** (0.9% saline) and a **buffered medium** (bicarbonate buffer, pH 7.4). These results demonstrate the effects of buffer capacity on the formation and propagation of acidic (anode) and basic (cathode) fronts, and serve as a basis for comparison with physical experiments using **monophasic GET** and **biphasic HFIRE** protocols.

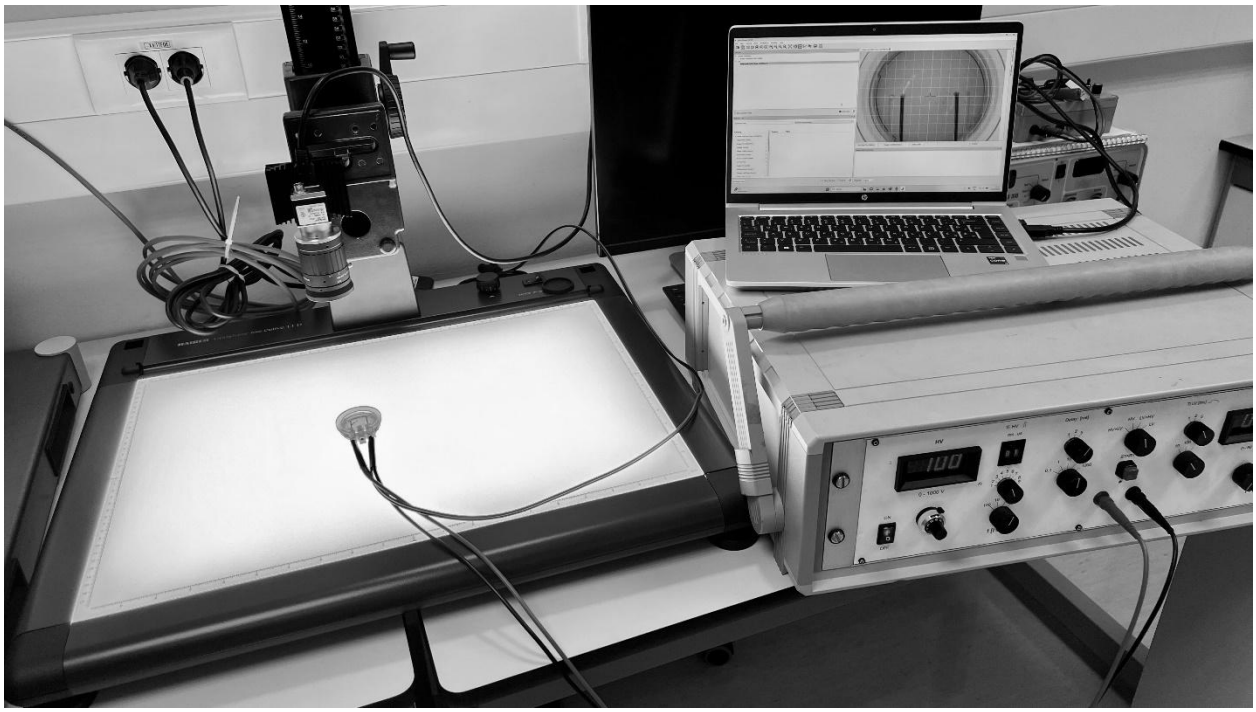

Figure 2: Experimental setup showing the camera rig and the agarose sample under treatment in a petri dish (diameter of 34 mm) with a custom-made cover/electrode holding guide, pulse generator, and laptop computer for controlling the camera and recording video data.

Part 2: Experimental materials and setup for model validation

Following the simulation review, we will move on to the experimental phase of the lab, where we replicate and validate the predicted formation of pH fronts using simple, observable model systems. This portion of the lab is designed not only to demonstrate the real-world electrochemical processes occurring during pulse delivery but also to serve as a **practical validation of the numerical models** discussed earlier.

Preparation of indicator solution and agarose phantoms

We begin by preparing a **pH indicator solution** to visualize the propagation of acidic and basic fronts during pulse delivery. This solution consists of:

- 150 mL of purified water
- 0.012 g methyl red
- 0.060 g bromothymol blue
- 0.050 g phenolphthalein disodium salt

These three dyes span a broad pH range and will provide clear colorimetric feedback on local pH changes within the tissue phantoms.

Next, we prepare a **0.60% agarose solution** using two types of electrolyte media:

1. **Unbuffered medium**: 0.9% NaCl (saline)
2. **Buffered medium**: 1 M phosphate-buffered saline (PBS), containing NaCl, KCl, Na_2HPO_4, and KH_2PO_4, with a pH of 7.4

To mimic the **electrical conductivity of skeletal muscle**[1] (approximately 0.25 S/m), both media are diluted with purified water in a **5:1 ratio** (water to solution). This ensures that the agarose phantoms provide a physiologically relevant electrical environment for pulse propagation.

Each diluted agarose solution is then **heated in a microwave** to just below boiling. While still hot—but before gelation begins—each is mixed with the previously prepared **indicator solution in a 9:1 ratio** (medium to indicator). After thorough mixing, the warm agarose mixture is pipetted into **small petri dishes** (3 mL per dish). The gels will solidify at room temperature in approximately 10–15 minutes.

Pulse delivery and recording

Once the agarose phantoms have set, electrical pulses will be delivered using **needle electrodes** connected to a **pulse generator**, applying one of the following protocols:

- **Monophasic GET protocol**: 4 pulses, each 5 ms in duration, delivered at 1 s^{-1}
- **Biphasic HFIRE protocol**: 5 µs positive phase + 5 µs interphase delay + 5 µs negative phase; 200 pulses per train; 10 trains at 1 s^{-1}

Both protocols are designed to deliver the **same total "on-time" (20 ms)**, allowing for a fair comparison of electrochemical effects independent of total energy delivered. You are encouraged to test a range of **pulse amplitudes**, as the phantoms' conductivity is constant and changes in current will directly influence the extent of observed pH front development.

The pH front dynamics will be **captured using a high-speed digital camera** equipped with a macro lens and mounted on a lighting-equipped stand. A connected laptop will be used to record and store video footage of the pH front formation and progression in real time. The complete experimental setup is shown in Figure 2.

Analysis and model comparison

After recording, you will qualitatively assess the **color changes and spatial movement of pH fronts** in both the buffered and unbuffered phantoms. Focus your analysis on a **single chosen voltage** to streamline comparisons. Your observations should be evaluated in two contexts:

- Against the **simulation results** shown earlier for the GET protocol in both media (serving as a model validation).

- In comparison between the two **pulse protocols** (monophasic GET vs. biphasic HFIRE), each applied in both buffered and unbuffered phantoms.

This experimental setup allows you to assess how well the model predictions hold in practice and provides insight into how **pulse configuration and medium composition** influence pH dynamics at the electrode interface. The clear visual changes produced by the pH indicators offer a highly accessible way to explore otherwise invisible electrochemical processes, deepening your understanding of electroporation beyond electric field distribution alone.

[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 [10].

REFERENCES:

[1] Miklavčič D., Serša G., Kryžanowski M., Novaković S., Bobanović F., Golouh R. Tumor treatment by direct electric current tumor temperature y pH, electrode material and configuration. *Bioelectroch Bioenerg,* 30:209-20, 1993.

[2] Phillips M., Krishnan H., Raju N., Rubinsky B. Tissue ablation by a synergistic combination of electroporation and electrolysis delivered by a single pulse. *Ann Biomed Eng,* 44:3144-3154, 2016.

[3] Nilsson E., Berendson J., Fontes E. Development of a dosage method for electrochemical treatment of tumours: a simplified mathematical model. *Bioelectrochem Bioenerget,* 47:11-18, 1998.

[4] Nilsson E., Fontes E. Mathematical modelling of physicochemical reactions and transport processes occurring around a platinum cathode during the electrochemical treatment of tumours. *Bioelectrochemistry,* 53:213-224, 2001.

[5] Marino M., Olaiz N., Signori E., Maglietti F., Suárez C., Michinski S., Marshall G. pH fronts and tissue natural buffer interaction in gene electrotransfer protocols. *Electrochim Acta,* 255:463-471, 2017.

[6] Šmerc R., Miklavčič D., Mahnič-Kalamiza S. An experimentally validated numerical model of pH changes in surrogate tissue induced by electroporation pulses. *Electrochim Acta*, 511:145363, 2025.

[7] Rubinsky L., Guenther E., Mikus P., Stehling M., Rubinsky B. Electrolytic effects during tissue ablation by electroporation. *Technol Cancer Res Treat,* 15:95-103, 2016.

[8] Kim K.H., An J., Park Y.J., Park J.H., Kim H.B., Yi J.H., Kim H.S. Tissue ablation using irreversible electrolytic electroporation with reduced voltage. *Electronics,* 12:2916, 2023.

[9] Potočnik T., Maček Lebar A., Kos B., Reberšek M., Pirc E., Serša G., Miklavčič D. Effect of experimental electrical and biological parameters on gene transfer by electroporation: A systematic review and meta-analysis. *Pharmaceutics,* 14:2700, 2022.

[10] Miklavčič D., Pavšelj N., Hart F.X. Electric properties of tissues, in: Akay, M. (Ed.), *Wiley Encyclopedia of Biomedical Engineering*. John Wiley & Sons, New York, NY, 3578-3589, 2006.

EXPECTED RESULTS

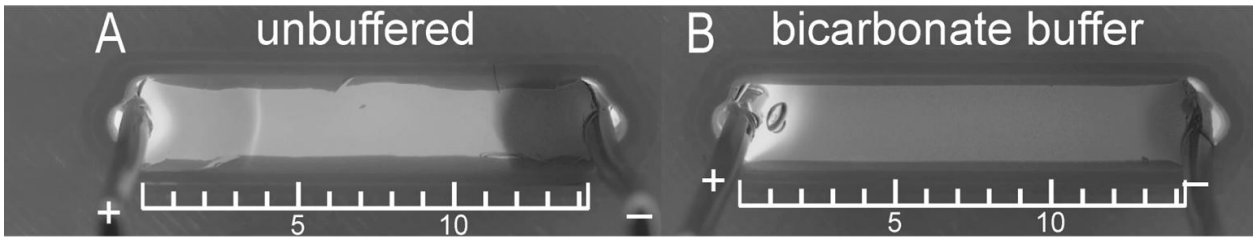

Figure 3: Changes in agarose gel pH following 8 rectangular pulses (10 ms duration, 400 V amplitude, 1 s^{-1} repetition rate), performed in (A) unbuffered medium and (B) bicarbonate buffer medium. In order to observe also the effects of the diffusion, gels were photographed 60 seconds from the start of the delivery of the first pulse. Note that the scale bar dimensions are expressed in mm. Adapted from [6].

NOTES & RESULTS