L14 Visualizing gas bubble formation during pulsed field delivery

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Duration of the experiments: 120 min
Max. number of participants: 4
Location: Tissue Laboratory
Level: Basic

PREREQUISITES

Participants should be familiar with Laboratory safety (S1) and Electroporation hardware safety (S2). Familiarity with basic laboratory equipment, such as pipettes, power supplies, or data acquisition systems, is helpful but not essential.

The aim of this lab session is to demonstrate gas bubble formation during pulsed electric field application in saline. Using a fixed-camera transillumination system, participants will observe bubble generation around the electrodes of a modified radiofrequency ablation (RFA) catheter, a clinically relevant device, as well as around custom-fabricated asymmetrical electrodes designed to impose different current densities. By delivering monophasic and biphasic pulse trains, they will explore how waveform shape, energy delivery, and timing affect bubble dynamics. The session highlights the electrochemical and thermal mechanisms of bubble formation, along with the clinical and safety implications in pulsed field ablation (PFA) [1,2] and related electroporation-based therapies [3,4], aiming to foster a solid understanding of how electrical, chemical, and thermal effects interact during electroporation.

THEORETICAL BACKGROUND

The application of electric fields in biological media is not only a biophysical process affecting cell membranes, but also an electrochemical and thermodynamic phenomenon. When electrical pulses are delivered into conductive solutions (e.g. an aqueous electrolyte solution), such as blood or saline, several concurrent processes occur at the electrode–electrolytic solution interface:

1. Electrolysis: At sufficient charge transfer, redox reactions at the electrodes lead to the release of gaseous species such as hydrogen (H_2), oxygen (O_2), and chlorine (Cl_2). This is typical in monophasic pulsing with longer pulse durations.
2. Degassing: As the solution warms due to Joule heating, its capacity to dissolve gases such as oxygen and nitrogen decreases [5]. This effect, while thermodynamically predictable, is believed to contribute relatively little to total bubble volume.
3. Boiling: In protocols delivering high power or continuous pulse trains, the local temperature may exceed 100 °C, especially near points of high current density (e.g., sharp electrode edges [6]). In such cases, bubbles of steam can rapidly form and collapse [7], often within milliseconds after the energy is no longer delivered into the system, causing (audible) cavitation events.

The morphology and persistence of these bubbles differ substantially:

- Bubbles from electrolysis tend to be small but long-lived, sometimes adhering to electrodes and accumulating.
- Steam bubbles are generally larger, but transient, i.e. short-lived.

- Bubbles formed due to degassing are sparse and typically sub-visible under modest heating.
- Any persistent bubbles formed through boiling of water or degassed from the medium due to elevated temperatures, are also quickly blown away by cavitation resulting from rapid transition of steam back into liquid phase.

Understanding these mechanisms is essential in contexts such as PFA [8], where gas formation near or inside the heart could lead to microemboli [9], posing risks of stroke or organ damage. Moreover, if they are thermal in origin, they are indicative of potential blood coagulation and protein denaturation, generating coagula that can travel within the blood stream carrying an even greater risk of an embolism. Thus, visualizing and quantifying bubble dynamics in a controlled *in vitro* setting provides both technical insights and educational value.

EXPERIMENT

Overview of setup

The experimental system consists of a transparent beaker filled with 0.45% NaCl solution, into which either (i) a modified RFA catheter or (ii) a set of custom-made asymmetric electrodes is submerged, depending on the experiment. The modified RFA catheter has been altered such that the two electrodes nearest the tip are wired together (one polarity) and the rest of the segments together (of opposing polarity). In the alternate configuration, custom-fabricated electrodes with asymmetrical surface areas (e.g., one small needle electrode and one large plate electrode) are used to intentionally create different current densities at each electrode. This facilitates the distinction between electrochemical and thermal bubble generation mechanisms.

A white LED transillumination panel is positioned under the beaker to provide strong and uniform backlighting, enhancing bubble visibility. A fixed digital camera (≈400 fps), mounted on a tripod, records the experiments. Illumination and camera focus are adjusted to clearly resolve the electrode tip and any developing bubbles. All experiments are controlled and monitored using a laboratory-built pulse generator and acquisition system.

Steps:

1. Preparation of the system:
    - Fill a 200 mL glass beaker with 0.45 % saline solution (prepared using deionised water and NaCl).
    - Depending on the experiment:
        - RFA catheter setup: Secure the modified RFA catheter in a horizontal orientation, submerged such that the tip and ring electrodes are visible.
        - Asymmetric electrode setup: Mount two custom-fabricated electrodes (one small, one large) horizontally in the solution with a separation of ≈5 mm.
    - Align the transillumination panel directly below or behind the beaker.
    - Adjust the digital camera height, focus, and frame to ensure high contrast at the electrode site.
2. Pulse delivery:
    - Apply different pulse protocols including:
        - Monophasic protocol: e.g., 100 µs pulses, 8 pulses at 1 s^{-1} repetition rate.
        - Biphasic protocol: e.g., 5–5–5–500 µs pattern (5 µs positive pulse, 5 µs inter-phase delay, 5 µs negative pulse, 500 µs inter-pulse delay) [10].
    - Use at least two voltage amplitudes for each protocol (e.g., 250 V and 750 V).

- Record each delivery with the camera; you can synchronize pulse delivery with video using a purpose-built LED that flashes with every pulse for approximately 10-15 ms.

3. Repeatability and comparison:
    - Perform multiple trials for each protocol to assess reproducibility.
    - Optional: Test at slightly elevated temperatures (~50 °C) to examine any influence on degassing. Note that you must adjust the voltage to match the current across temperatures, otherwise your observations will be skewed by differences in total charge passed, leading to more faradaic reactions and bubble formation that are not attributable to temperature effects alone.
    - Compare bubble generation between electrode configurations (RFA vs. asymmetric) to differentiate between thermal and electrochemical bubble origins based on current density, timing, and spatial onset.

DATA ANALYSIS AND INTERPRETATION

Participants will analyze recorded videos to identify and evaluate bubble formation:

- Frame-by-frame review: Determine the exact frame where bubbles first appear.
- Bubble size estimation: Use ImageJ or MATLAB to measure bubble diameters and estimate volume.
- Quantify bubble count per protocol, and correlate with:
    - Waveform shape (mono- vs. biphasic)
    - Voltage amplitude
    - Electrode configuration (RFA catheter vs. asymmetric electrode pair)

Discuss:

- Which protocols produce more bubbles? In other words, how does the pulse protocol influence gas formation?
- What types of bubbles were observed, and what is their likely origin?
- Are bubbles persistent (electrolysis, degassing) or transient (boiling)?
- How does electrode geometry (e.g. sharp vs. blunt tip, high vs. low current density) influence localization and intensity of bubble formation?
- What are the clinical implications of these findings for safe and effective pulsed field ablation (PFA)?

You are encouraged to compare your findings with observations published in [11].

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EXPECTED RESULTS

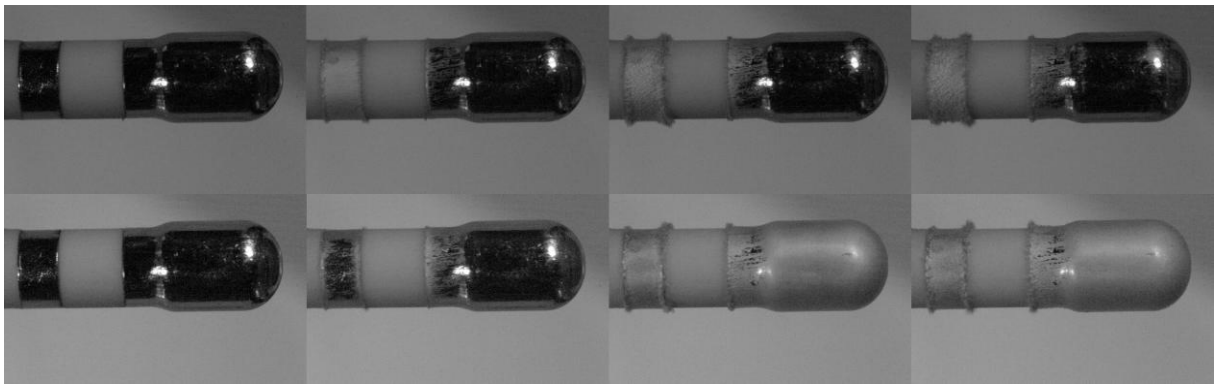

Figure 1: Microbubble formation on the catheter electrodes on and near the tip electrodes during delivery of a monophasic pulse protocol. Note the rather uniform bubble formation on the surface of electrodes due to electrochemical (faradaic) evolution of gas. Adapted from [10].

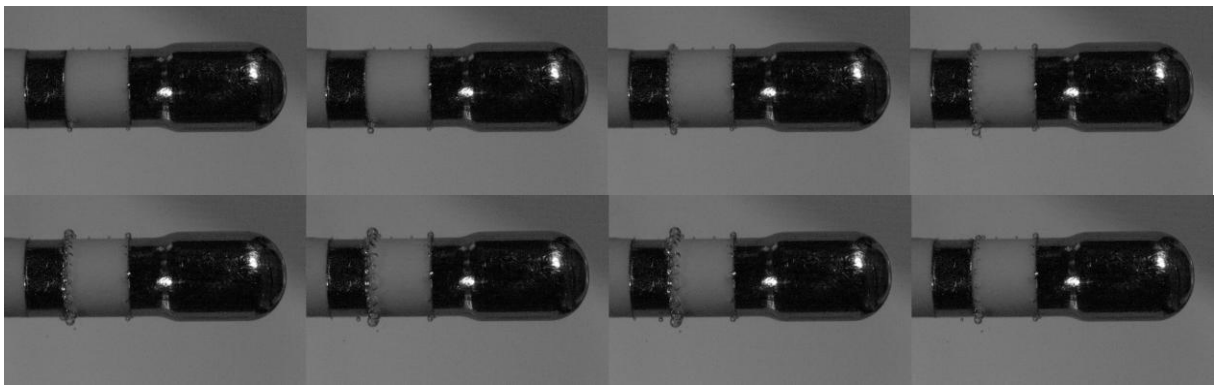

Figure 2: Microbubble formation on the edges of electrodes on or near the tip of the catheter during delivery of a biphasic, high duty cycle pulse protocol. Note the bubbles of thermal origin near the edges where the current density is the highest. Adapted from [10].

NOTES & RESULTS