Online bioimpedance feedback for \textit{in vivo} electroporated tissues

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\textbf{Abstract}. Electroporation \textit{in vivo} is a biotechnology method that uses short-duration high intensity electric fields to enhance plasma membrane permeability in living cells in order to facilitate the uptake of drugs, DNA, genes and proteins into the cytoplasm. The degree of permeability is related to the tissue’s bioimpedance; hence, accurate impedance evaluation throughout electroporation treatment is essential to 1) avoid over-treating tissues resulting in excessive cell death and 2) under-treating tissues resulting in poor permeability. Cell viability and membrane permeability is based on a number of factors, including: time elapsed after electroporation, electroporation pulse amplitude, tissue type, and so on; thus, efficient feedback protocols must minimize delays between treatment and impedance readings. Current methods of bioimpedance feedback are often cumbersome and impedance analysis devices can be expensive, bulky, and immobile. Emerging technologies facilitate economical methods, fast protocols, and portability to realize bioimpedance measurement and feedback online (i.e. real-time). Consequently, this research uses automation software, logic-biased protocols, an inexpensive commercially available impedance analyzer microchip, and a custom-built hexagonal electrode probe to measure dynamic bioimpedance changes. This work demonstrates how this novel system measures tissue bioimpedance instantly and efficiently before and after electroporation. Additionally this system allows for the comparison of electrode geometries as well as electric field magnitudes and distributions. Follow up work will pursue the optimization of plasma membrane permeability for several tissue/cell types.

1. Introduction

Bioimpedance frequency spectra of many biological tissues have been widely reported in terms of complex permittivity components (e.g. conductivity). To date, however, there is little data on \textit{in vivo} bioimpedance measurements taken immediately before and after electroporation (EP) to measure cell membrane permeability [1, 2]. This current work presents a new approach to performing online bioimpedance feedback for \textit{in vivo} (and \textit{ex vivo}) electroporated tissues by using inexpensive and portable equipment, faster protocols, and modular components that allow for segregating pulsing and measuring electrodes, as well as the application of unipolar pulsing over two paths to provide extended electric field coverage. Pre and post electroporation resistivity feedback data is immediately analyzed for \textit{ex vivo} bovine kidney tissue and \textit{in vivo} on rodent skin using 150/cm and 200V/cm electric fields in 4 unipolar square pulses of 20ms duration and 1 second intervals form 5kHz to 100 KHz, and two electrode geometries -a needle electrodes and a hexagonal type multielectrode array.
2. Methods and materials

2.1 Electrical impedance spectroscopy

Tissue bioimpedance was measured using the instrumentation setup shown in Figure 1. Prior to experimentation, the accuracy of the Analog Devices AD5933 Impedance Analyzer (IA) chip was validated with an Agilent 4294A Precision Impedance Analyzer and calibrated using known RC analog circuits. A validation impedance magnitude of $|Z| = 5,636\,\Omega$ resulted in an error of less than 1% as specified by the manufacturer [3].

The equivalent circuit model used for tissue was a series resistor followed by a parallel RC combination known as the Debye single-dispersion electrical model [4]. Given this model, the IA was calibrated for an impedance magnitude of $|Z| = 5.5\,\text{k}\Omega$ at a midrange frequency of 40 kHz as shown by Gabriel [5] and in Figure 3b. This skin tissue resistivity data from Gabriel was also used for comparative and validation of the results. The IA was controlled by a PC to set the frequency sweep parameters from 5 kHz to 100 kHz at 1kHz increments with a sample speed of one million samples per second (MSPS), set excitation voltage at 1Vpp, select circuit modeling and calibration gain, measure tissue temperature, and collect data. A BTX EMC 830 Square Pulse Generator was set for 60V or 80V (i.e., 150V/cm or 200V/cm electric fields) and a 20 ms pulse duration with four repetitions every second.

2.2 Electrode geometries

Two geometries of surface electrodes were used for impedance probing. The first probe was comprised of two needle electrodes of 1mm diameter at a distance of 5mm apart. The second probe contained six needle electrodes of 0.38 mm diameter, spaced 2mm apart and a 4mm diameter as shown in Figure 1. This second electrode configuration allowed for different electrodes to be used for applying pulses and measuring impedance. For skin measurements, conductive gel (Spectra 360 electrode gel) was placed onto each electrode before they were contacted with the stratum corneum. Tissue treatment area was held constant by virtue of the fixed spacing between the electrodes. The K ratio use to convert the impedance measurement to resistivity was calculated as 2.5 cm. However, it should be noted that the focus of this work is on the analysis of the differential resistivity before and after EP and not necessarily as a database for tissue impedances itself.

2.3 Ex vivo tissue

Bovine tissue for ex vivo electroporation was obtained ‘fresh’ and allowed to equilibrate to room temperature. Measurements were taken with a 150V/cm field for a total of 12 measurements and 1200 data points were analyzed.
2.4 *In vivo* mouse skin tissue

Mouse skin measurements were obtained on female C57Bl/6 mice (NIH, Bethesda, MD) that were eight weeks of age and weighed 18 grams. Impedance measurements and EP were performed on animals anesthetized with 2% isoflurane in oxygen. Core temperatures were maintained by placing the animal’s ventral-side-down on a 32°C heating pad during anesthesia exposure. Measurements were taken on the left and right flank skin, which maintained a constant temperature of 32°C. EP was carried out by exposing the skin to a field strength of 150 V/cm or 200 V/cm applied by one set of electrodes on the applicator. A total of 18 measurements and 1800 data points were acquired and analyzed over nine separate skin areas. Following data acquisition, the animals were humanely euthanized in accordance with an approved Institutional Animal Care and Use Committee protocol.

2.5 *Rat skin tissue*

Measurements were taken from freshly harvested skin tissue from male Sprague-Dawley rats that were 18 weeks of age and weighed between 450 and 550g. Measurements were made at room temperature for EP electric field parameters of 150 and 200 V/cm. 100 data points were sampled during nine measurements over nine rat skin areas of three rats for a total of 8,100 measurements.

3. Results and discussion

The resistivity of bovine liver *ex vivo* before and after EP is shown in Figure 2. Twelve liver samples were measured using the hexagonal type electrode with an electrical field of 150V/cm for a total of 1,200 data points. Bioimpedance after EP was consistently lower than the pre-EP values for the entire spectrum. However, this difference was not statistically significant at any of the points shown in figure 2 (95% level of significance Student’s T-test). This result is consistent with previous published data on swine liver electrical resistivity postmortem. It has been noted that *ex-vivo* resistivity decreases when compared to *in vivo* tissue resistivity due to a progressive increase in extracellular fluid due to membrane rupture that begins about 2 hours postmortem [6]. This result suggests that a possible application of bioimpedance probing could be the estimation of the degree of ‘freshness’ of edible meats.

The resistivity of mouse skin *in vivo* before and after EP is shown in Figure 3a. The same electrodes were used for pulsing and measuring with 150V/cm and 200V/cm electric fields. The pulse duration was 20 ms with four repetitions every second over nine differentiated skin sections on the back of mice. A studentized T-test found a statistically significant reduction in skin impedance following EP for both 150 V/cm and 200 V/cm pulses. For 150 V/cm, the impedance was significantly different for frequencies ranging from 5 to 35 kHz. For 200 V/cm, the impedance drop was only significant from 5 to 100 kHz. These results were from a single set of electrodes used for both pulsing and measuring impedance. Employing this electrode configuration causes a bias in the samples, because measurements obtained with this applicator include those from dead cells caused by electrode polarization.

The resistivity of rat skin *in vivo* before and after EP is shown in Figure 3b. A 150 V/cm electric field and the hexagonal electrode configuration were used over nine separate skin sections on three different mice for a total of 5,400 data points as shown with corresponding standard deviations.
A studentized T-test demonstrated with 95% confidence that EP resulted in a significantly lower bioimpedance in rat skin at frequencies of 5 to 100 kHz.

**4. Conclusions**

This work provides a very cost effective and novel means to accurately measure bioimpedance changes during and after electroporation of tissues *in vivo*. The system proved capable of obtaining accurate measurements at sweep frequencies from 5 to 100 kHz within milliseconds using an inexpensive microchip in parallel with a pulse generator. A custom-built measurement probe consisting of an array of three sets of electrodes was designed and built in order to separate electrode pairs for pulsing and measurement in order to avoid the influence of electrolytic products and tissue damage on the electrodes used for impedance measurements.

As expected, electroporation yielded lower bioimpedance drops for *ex vivo* tissue when compared to *in vivo* tissue. Thus, this experiment suggests that a possible application for bioimpedance measurements could be the estimation of the ‘degree of freshness and leanness’ of market edible meats. *In vivo* mouse skin impedance measured after EP using two electrical fields (150 and 200 V/cm) showed a larger drop at the lower electric field which may be explained by electrode effects and/or cell death. Similarly, an impedance drop was determined in rat skin as a result of EP at 150 V/cm.

**5. References**

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