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ScienceDirect

Procedia Engineering 170 (2017) 410 – 415

Procedia Engineering

www.elsevier.com/locate/procedia

Engineering Physics International Conference, EPIC 2016

# Impedance Measurement System of a Biological Material Undergoing Pulsed Electric Field Exposed

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**Abstract**

This work deals with the development of an impedance measurement system for the exposure of an animal tissue (e.g. sheep muscle tissue model) to pulsed electric field (PEF). When exposed to short electric pulse, disturbances on the membrane and intra cellular components occur, modifying the behavioral response of cells exposed to foreign small materials. The phenomenon is believed to affect the electrical impedance of the tissue. While the conventional frequency based impedance measurement may reveal the electrical properties on the steady state condition, the method have been found to be insufficient for identifying impedance undergoing such very short pulse. Furthermore, this method can only take place before and after PEF. New techniques based on the pulsed voltage and current measurements have been developed for capturing the properties. The presented measurement system is composed of stainless steel electrodes that are not only designed to deliver energy to the biological tissue but also as measurement electrodes. The influence of electric field intensity of four different levels was investigated. The measured electrical impedance during pulsing was then represented to a recovery rate. The rate is defined as the changes in electrical impedance toward the electrical impedance of tissue that served as a reference of untreated tissue. The results showed that an impedance changing as a characterization of PEF level has been detected. Experiment shows that the impedance drop significantly when the PEF rise and then gradually increase regardless PEF decrease rate. A larger PEF intensity has more influence to slower down the cell membranes recovery in the tissue. The larger the PEF intensity, the slower the impedance rate.

Peer-review under responsibility of the organizing committee of the Engineering Physics International Conference 2016

*Keywords:* pulsed electric field; animal tissue; impedance; measurement

## 1. Introduction

The development of pulsed electric field (PEF) and its applications to biological materials increase fast in the recent years. Later, such PEF application is then known as electroporation or electropermeabilization. It covers the area of the subject of biology, medicine and engineering. Plasma layer of living cells constitutes the obstruction between the intracellular and extracellular media and controls the transport from or into the cell. Electroporation, is a condition when cell layers are presented to high electric field. At the point when the parameters of such electric field are suitable, a transient condition of porousness to small particle is created. The system is presently utilized as an apparatus to convey layer impermeable particles into the cells both in vitro and in vivo. The measurement of PEF effect by using a frequency based method can be used [1].

A measurement system of electrical properties is conducted before and after PEF in food industry application e.g. processing of grape fruit wine. The pulse generation device comprises of a 6-phase Marx generator charging voltage of 50 kV for every stage. The results show the successful of the measurement that the conductance of fruit liquid decrease. In other side application in animal cell need much lower electric field, usually not more than 1 kV/cm to make sure that the cells will re-seal its membranes and still alive after PEF. It is known as reversible electroporation that currently the most uses in PEF technology applications especially in medicine research e.g. electro-chemotherapy, DNA vaccine, etc. The direct effect of EP is not clearly understood due to its difficulties of in vivo measurement and also the on-going phenomenon not easy to be observed [2].

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Peer-review under responsibility of the organizing committee of the Engineering Physics International Conference 2016
doi:10.1016/j.proeng.2017.03.066

A study by [3] showed a temporary change in electrical impedance of biological cells after an application of high voltage pulse with very short duration. The result of the increase in conductivity is followed by recovery. But when the field intensity is very high, the recovery delays up to 200 ms. The increase in conductivity due to 10 pulses at intervals of 3 seconds is measured. Time domain-based impedance measurements were used to study the changes in electrical parameters of biological samples following the application of ultra-short high voltage (HV) pulses. Pulses with very short duration (300 ns) caused a significant drop in post pulse resistance of the plasma membrane only at high field strengths in excess of 20 kV/cm. The conductivity of the plasma membrane returned to almost pre-pulse values within less than 10 ms after the field was applied. Further steps of recovery, attributed to pore shrinking and resealing with an exponential decay of the conductivity, as is expected in electroporation, were not observed. An increase in medium conductance, as recorded minutes after the pulse, arises mainly from cell damage.

A wavelet-based signal processing approach for measuring treatment process utilizing PEF is established. The method is to monitor and detect abnormal voltage in PEF food processing. Contrasted with the traditional Fourier transform, direct use of wavelet transform can be utilized viably to anticipate the closeness of an arcing occasion and recognize inner and outside arcing exercises. This strategy can be utilized as a necessary part to screen the quality control of the PEF process [4].

Impedance in the form of resistance and capacitance of sheep muscle tissue has been measured in [5]. The measurement give an average resistance of 4369.75 Ohm and capacitance of 147.01 nF. Therefore the average conductivity and permittivity are calculated as 0.0022885 S/cm and 147.01 nF/cm. The tissue properties show that capacitance and permittivity are very small therefore it may not be considered during the experiment.

Two applications category develops i.e. application on plants and animal either in cell or tissue level. PEF uses high voltage that has a fatal risk if inappropriate level applied. To prevent the risk, firstly, it is necessary to know the effect of PEF by a measurement. The electrical properties are easy to be measured than others. Although measurement of electrical properties of biological tissue is already available, the observation of changes of electrical characteristic during the electric impulse has not been performed yet. In the case that the pulse is very short but not very high amplitude like in animal application, it is important to study the electrical impedance behavior during the pulse, otherwise it is missed the detection.

## 2. Device and Sample

### *2.1. Devices*

To explore the effect of PEF on the electrical impedance of the tissue, it is necessary to develop an experimental set up that consist of a pulse generator, electrodes, switch, and measuring tools. A pulse generator based on capacitor discharging circuit is used. To obtained higher and variable voltage, multiple stages is constructed. The electrodes comprises of 4 stainless steel needles with diameter of 0.45 mm and 50 mm long. Two electrodes function as pulsing electrodes. The other 2 are as voltage measurement electrodes. Despite of normal excitation, the pulse itself is used as excitations with electrodes form tetra polar configuration impedance measurement. The pulse electrode distant is 10 mm and the measurement electrode is 3.33 mm. The pulse generator output is a form of exponential waveform with charging voltage of 250, 500, 750 and 1000 V with small deviations. The concept comprises a variable high voltage power supply $V_{dc}$, a capacitor $C$, a switch $S$ and load Fig. 1. The generator operates in two phases, charge and discharge, and generates exponentially decaying pulses. During the charge phase, the switch S is in the position 1 and variable high voltage power supply V charges the capacitor C to the preset voltage. In the discharge phase, the switch is in the position 2, and the capacitor discharges through the load (tissue sample) connected to the output. Time constant of discharge $\tau$ can be approximated by product $Z_L C$, where $C$ is the capacitance of capacitor and $Z_L$ is the absolute value of the tissue impedance [6].

### *2.2. Sample preparations*

To explore the effect of PEF the experiment uses ex vivo approach which all component of bio organism already available. Large mammalian animal e.g. sheep from farm is used as sample source with age of about 2 year olds. The sample is muscle tissue which is sliced into a sheet and shaped square block with size of 20x20x2mm. Every sample is conditioned in a constant ambient temperature of 25 degree Celsius. For each treatment group 30 samples is prepared for 4 treatment groups with variation of pulse voltage so the total sample is 120 samples.