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Modeling electroporation of the non-treated and vacuum impregnated heterogeneous tissue of spinach leaves

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ABSTRACT

Uniform electroporation of the heterogeneous structure of spinach leaf cross section is a technological challenge that is addressed in this investigation. Three dimensional models were created with cells arranged in specific tissue types, considering a leaf with its air fraction and a leaf where the air fraction was replaced by a solution of known properties using vacuum impregnation. The models were validated before electroporation, in the frequency domain, where alternating voltage and current signal at frequencies from 20 Hz to 1 MHz were used to measure conductivity of the tissue. They were also validated through measurements of current during electroporation when a single 250 μs rectangular pulse with amplitudes ranging from 50 to 500 V was applied. Model validations show that both the frequency dependent conductivity and electroporation are well predicted. The importance of the wax layer and stomata in the model is thoroughly discussed.

Industrial relevance: Our aim was to investigate electroporation of the spinach leaf by developing a model which would enable us to meet the technological challenge of achieving uniform electroporation in a highly heterogeneous structure in the context of a process aimed at improving freezing stability of plant foods. Pulsed electric field treatment may be used to introduce the cryoprotectant molecules into the cells, and hence improve the structure and properties of frozen food plants.

1. Introduction

Electroporation of a cell membrane is defined as an increase in its permeability due to exposure of the cell to a sufficiently strong, external electric field (Kotnik, Kramar, Pucihar, Miklavcic, & Tarek, 2012; Neumann & Rosenheck, 1972; Neumann, Schaefer-Ridder, Wang, & Hofschneider, 1982; Zimmermann, Pilwat, & Riemann, 1974; Zimmermann & Vienken, 1982). Depending on the amplitude and duration of the applied electric field, electroporation may occur in two forms: irreversible (Phillips, Maor, & Rubinsky, 2011; Rowan, MacGregor, Anderson, Fouracre, & Farish, 2000) or reversible (Glaser, Leikin, Chernomordik, Pastushenko, & Sokirko, 1988; Miklavčič, Serša, Brecelj, Gehl, Soden, Bianchi, et al., 2012). For either purpose, theoretical studies coupled with experimental investigation contribute to the understanding of events taking place in cells and tissues during electroporation.

Theoretical studies of single cell electroporation have been conducted from different perspectives. Aspects such as the dynamics of pore formation and the transmembrane voltage distribution (DeBruin & Krassowska, 1999), the electroporation caused by bipolar pulses and dynamic pore radii (Talele, Gaynor, Cree, & van Ekeran, 2010), the conductance of electroporated cell membrane (Suzuki, Ramos, Ribeiro, Cazarolli, Silva, Leite, et al., 2011), the number of pores and pore radii in the cell membrane (Talele & Gaynor, 2010), the process of pore disappearance in the cell membrane (Saulis, 1997) and the electroporation of intracellular membranes (Gowrishankar, Esser, Vasilkoski, Smith, & Weaver, 2006; Retelj, Pucihar, & Miklavcic, 2013) have been widely studied in the literature using single cell models. Electroporation was also studied by using a model of dense cell suspension (Mezeme, Pucihar, Pavlin, Brosseau, & Miklavčič, 2012).

Electroporation of tissues has attracted great attention. Electroporation is an important technology used for medical purposes such as cancer treatment (electrochemotherapy) (Yarmush, Golberg, Serša, Kotnik, & Miklavčič, 2014), where the modeling of the electroporation process in the tissue becomes essential for treatment planning (Pavliha, Kos, Zupanič, Marčan, Serša & Miklavčič, 2012; Pavšelj & Miklavčič, 2008). In these models describing tissue electroporation, the representation of the heterogeneity and anisotropy of the tissues becomes a challenge. There are number of models describing electroporation in different types of tissues such as tumors, muscle, liver and skin, where the heterogeneous tissue layers are considered and the thermal effects of electroporation and transdermal drug delivery are studied (Pavšelj & Miklavčič, 2011; Zorec, Becker, Reberšek, Miklavčič, & Pavšelj, 2013). Electroporation of skin was also studied in terms of the effect of bipolar pulses (Arena, Sano, Rylander, & Davalos, 2011). Three dimensional models were used to evaluate the local electric field created

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in the anisotropic skeletal muscle during the application of electric pulses (Corovic, Zupanic, Kranjc, Al Sakere, Leroy-Willig, Mir, et al., 2010) and two dimensional models of nerves, blood vessels and ducts have been used to theoretically analyze irreversible electroporation (Daniels & Rubinsky, 2009).

The models describing electroporation are however mostly focused on a simplified geometry which shows the tissues as stacks of layers with specific bulk properties. In this paper we use a three dimensional model, containing a well-defined structure built from individual cells arranged in specific tissue types. Cells belonging to a certain tissue possess shape, dimensions and location mimicking the tissues in the real spinach leaf. The entire cross section of the leaf was considered including elements such as the cuticular wax and stomata.

Our aim was to investigate electroporation of the spinach leaf cross section by developing a model which would enable us to meet the technological challenge of achieving uniform electroporation in a highly heterogeneous structure in the context of a process aimed at improving freezing stability of plant foods (Phoon, Gómez Galindo, Vicente, & Dejmek, 2008; Shayanfar, Chauhan, Toepfl, & Heinz, 2013). The influence of specific elements such as cell size, cell arrangement, cuticular wax layer and stomata on the creation of pores (i.e. electroporation of the cell membranes under different applied pulse parameters), was investigated. The effects of the connections between cells and the air fraction in the tissue are also discussed. The model was first analyzed in the frequency domain, where alternating voltage and current signal at frequencies from 20 Hz to 1 MHz were used to measure conductivity in the tissue and validate the model.

2. Theoretical considerations

2.1. Spinach leaf structure

Spinach leaf has a typical thickness of 0.4 ± 0.1 mm. The cross section of the leaf consists of different tissues arranged in four layers. The spinach cross section is shown in Fig. 1A, where leaves were incubated with fluorescein diacetate as described by Dymek, Dejmek, and Gómez Galindo (2014) and examined under a microscope (Eclipse Ti-U, Nikon, Japan). At the top of the leaf cross section there is an upper epidermal tissue, which consists of a single layer of star-shaped cells. Epidermal cells are covered by a cuticular wax layer. Underneath the upper epidermis the palisade mesophyll is built from elongated cells which are formed by two cell layers. Below the palisade mesophyll, the spongy mesophyll is located. It has a multi-cell layer structure characterized by round cells distributed in a loose and apparently random structure. The majority of the intercellular air fraction is located in the spongy mesophyll (Warmbrodt & Woude, 1990). The air fraction accounts for approximately 30% of the leaf volume (Winter, Robinson, & Heldt, 1994). At the bottom, the lower epidermis is located.

2.2. Simplifications used to model the leaf

The model represents the internal tissues of a spinach leaf, restricting the elements included in the model to the essential ones. The leaf tissue possesses an extremely complex structure; therefore, the following simplifications were introduced into the model.

Veins were not included in the model. Since obtaining a time-dependent, finite-element solution for a 3D model with a complex structure consisting of multiple cells is relatively demanding with respect to computational time and random-access memory requirement, only a small part of the leaf with an area 107 μm × 107 μm was modeled. In the spinach leaf the minor veins are located within the distance from 49 to 231 μm (Warmbrodt & Woude, 1990) and were neglected in the model.

In the leaf the majority of the air fraction is located in the spongy mesophyll. Therefore, in the model, air fraction was neglected in the epidermal tissue and palisade mesophyll. In these tissues the cells were completely surrounded by extracellular liquid. However, in the spongy mesophyll the cells were surrounded by a thin layer of

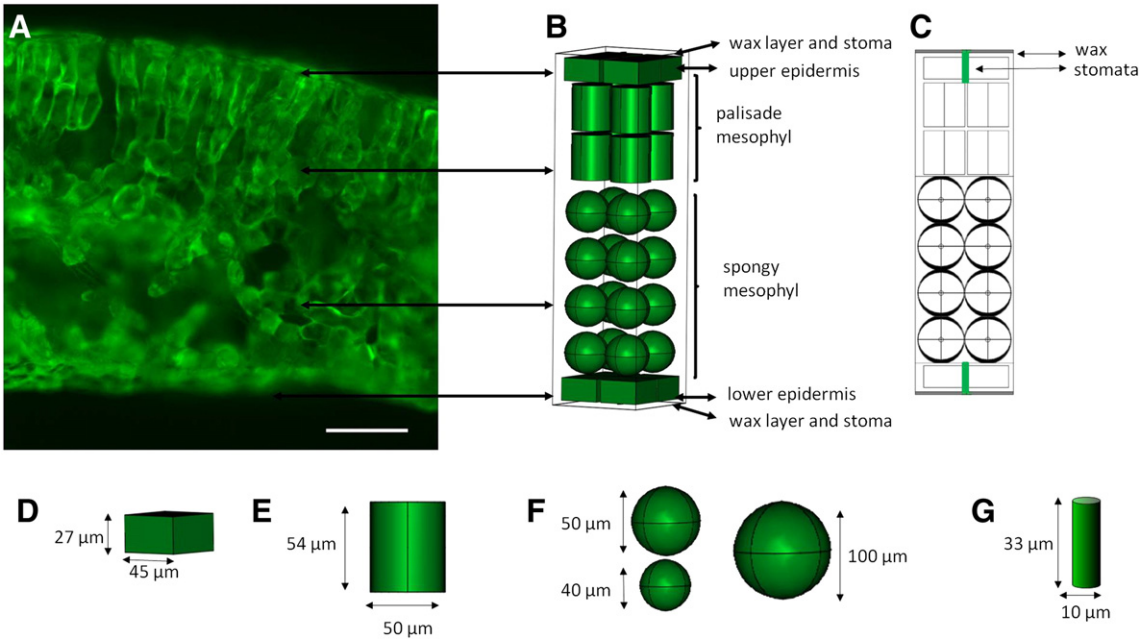

Fig. 1. The geometry of the model. A, B. The geometry of the model representing the whole spinach cross section is shown in relation with the microscopic picture. The scale bar represents 100 μm. C. Side view of the model with connections between cells marked with black. Stomata are marked in green and the cuticular wax layer in black. The dimensions of the different elements included in the model are shown in: D. epidermal cell, E. palisade mesophyll cell, F. spongy mesophyll cells, G. stoma.