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Dual-porosity model of mass transport in electroporated biological tissue: Simulations and experimental work for model validation

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ABSTRACT

Electroporation or pulsed electric field treatment is an important technique for facilitating mass transport in biological tissues with proven benefits for the food processing industry. One of the challenges in understanding its basic mechanisms and effects is mass transport processes in treated tissue. We recently presented a mathematical model called dual-porosity model to describe post-electroporation diffusion in biological tissue and filtration–consolidation behavior of electroporated tissue during pressing. In this work we bring the two analogues together and study the model's applicability and performance by comparing experimental and simulated kinetics. We use two kinds of plant tissue of dissimilar properties (sugar beet taproot and apple fruit), but employ the same methodology to evaluate the validity of basic assumptions. We show that the model describes experimental data and provides more insight into the mass transport processes during post-pulse extraction/pressing. We comment on treatment conditions that expose limitations and indicate possibilities for future development.

Industrial relevance: In order to study and optimize extraction processes following treatment of biological material with electroporation (pulsed electric fields), good knowledge on mass transport processes in electroporated tissue is of essential importance. Development, final form and application of a new mathematical model are presented that will aid in understanding of mass transport by solute diffusion and filtration–consolidation behavior of electroporated tissue under external pressure. It is foreseen that such a model could be used for predictive purposes and optimization of treatment parameters in industrial applications of electroporation, where *in silico* modeling can thus help find new or improved protocols to increase efficiency and efficacy in pulsed electric field applications.

1. Introduction

The terms electroporation, electropermeabilization, and pulsed electric field treatment, are commonly used to refer to the application of short, high-intensity electrical impulses to biological material and consequences that such electric pulses have on biological material (Kotnik, Kramar, Pucihar, Miklavcic, & Tarek, 2012; Raso & Heinz, 2010; Yarmush, Golberg, Serša, Kotnik, & Miklavčič, 2014). We prefer to and will use the term electroporation throughout the remainder of this article. In electroporation, the electric pulses of specific parameters are applied to the target tissue or cell suspension, the parameters depending on the intended goal of application. Often the objectives are to induce a transient increase in cell membrane permeability (Gehl, 2003; Haberl, Miklavcic, Sersa, Frey, & Rubinsky, 2013; Marty et al., 2006; Zorec, Préat, Miklavčič, & Pavšelj, 2013), or to permanently damage and ultimately destroy the cells (Goettel, Eing, Gusbeth, Straessner, & Frey, 2013; Golberg & Yarmush, 2013; Jiang, Qin, & Bischof, 2014; Morales-de la Pena, Elez-Martinez, & Martin-Belloso, 2011; Saulis, 2010). To achieve selective extraction of bio-compounds, complete destruction of the cells is an undesirable effect leading to impure solutions.

A closer look at the electroporation processes on the biochemical level reveals that treatment outcome and efficacy are largely governed by electrical and (related) chemical properties of the treated material, and mass transport that occurs during and after application of electric pulses (Kotnik et al., 2012; Li & Lin, 2011a; Li, Tan, Yu, & Lin, 2013b; Pucihar, Kotnik, Miklavcic, & Teissie, 2008; Sel et al., 2005). These properties and transport phenomena influence the development of the electropermeabilized state of the cell membrane during electroporation, and continue to be important in the post-pulsation period of pore shrinkage, resealing, cell lysis, etc. (Reigada, 2014; Sridhara & Joshi, 2014).

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Mass transport is also of significance in light of the intended purpose of electroporation treatment application. If electroporation is applied to facilitate solute extraction by diffusion (increasing rate, yield, etc.) or to change the permeability of the cell membrane and overall tissue for improving juice expression or tissue dehydration, the mass transport processes (both of solutes and liquid) are of primary importance and should be the focus of study. Moreover, since much the same processes of transport in electroporated cells and tissues are of interest in other fields of electroporation applications, such as biomedicine—in e.g. electrochemotherapy (Cadossi, Ronchetti, & Cadossi, 2014; Miklavcic, Mali, Kos, Heller, & Sersa, 2014), gene transfection (Dean, 2013), trans- and intradermal drug delivery (Becker, 2012), etc.—the study of mass transport phenomena in electroporated biological material is a trans-domain research field. Within this expanded field incorporating biology, medicine, pharmacology, electrical engineering, process and food engineering, chemistry and chemical physics, as well as several other domains, there is an abundance of published theoretical and experimental approaches employed to identify and describe the basic mechanisms of electroporation. These developments and theoretical advances add pieces to the greater puzzle that is the theory of electroporation. Mass transport phenomena represent an important integral part of this work in progress. The significance of the trans-domain span in research on electroporation is in the analogies that appear throughout the various domains of electroporation applications that can complement to form a more complete and complex picture of the effects of short-duration, high-intensity electric fields on biological material. To illustrate with a particular example; if passive diffusion of solute through an electroporated membrane is the predominant mechanism of mass transport in electroporated tissue in the post-pulse period (Pucihar et al., 2008), a study of the process is of greatest importance to biomedicine in e.g. electrochemotherapy (introduction of molecules of a chemotherapeutic drug into tumor cells—see sources on electrochemotherapy cited above), and for food processing industry in e.g. industrial extraction of valuable compounds from their primary biological sources (extraction of carbohydrates, polyphenols, lipids, etc.) (see e.g. Boussetta, Soichi, Lanoiselle, & Vorobiev, 2014; Donsi, Ferrari, & Pataro, 2010; Grimi et al., 2014; Liu, Lebovka, & Vorobiev, 2013).To give another example; liquid pressure gradients are present in many of the tissues which are of interest in electroporation. Plant cells for instance maintain their shape and plant tissue its turgidity due to turgor pressure, resulting from a solute concentration imbalance across the cell membrane that causes an osmotic pressure build-up (Campbell et al., 2008; Pereira, Galindo, Vicente, & Dejmek, 2009). In tumors, poorly formed vascular system and lacking lymphatic drainage system result in local gradients in interstitial fluid pressure, leading to a higher intratumoral pressure as compared to liquid pressure in the surrounding healthy tissue (Ariffin, Forde, Jahangeer, Soden, & Hinchion, 2014; Liu, Brown, Ewing, & Schlesinger, 2011; Pusenjak & Miklavcic, 2000; Simonsen, Gaustad, Leinaas, & Rofstad, 2012). Rendering the cell membrane semipermeable in presence of pressure gradients will, in theory, result in filtration flows in the direction opposite to that of the pressure gradient both during and after electroporation. Pressure gradients exist in untreated tissue (e.g. osmotic pressure, interstitial fluid pressure) that may be of significant importance already during application of electric pulses, and after electroporation treatment, a pressure gradient is established by the externally-applied pressure during the pressing stage. This is yet another mechanism of solid–liquid mass transport of importance in relation to electroporation in two disparate domains of electroporation application.If the same approach, from the theoretical point of view, can be used to study two very disparate goals of electroporation application, one should need to develop mathematical descriptions of the process physics (i.e. *models*) once only for the process and then apply them, with necessary modifications, to each particular application. Following this paradigm, we recently published two works on the development of a mathematical model we refer to as the dual-porosity model. The first account presents the model as describing extraction or introduction of solute by diffusion out of or into electroporated biological tissue (Mahnič-Kalamiza, Miklavčič, & Vorobiev, 2014). We used sucrose extraction from sugar beets for the model study, but also suggested two possible applications of the model for introduction of compounds into animal or plant cells. Furthermore, the analogy between diffusion and liquid flow laws allows for a rapid adaptation of the model for the problem of filtration–consolidation behavior of electroporated tissue, and is the subject of the second account (Mahnič-Kalamiza & Vorobiev, 2014). In the present work, we bring the two analogues together and examine the model validity and its performance by comparing experimental data with diffusion/expression kinetics, which result from simulations done using the model proposed. We use two kinds of vegetable tissue as model material with markedly different properties (sugar beet taproot and apple fruit), but employ the same methodology to determine if the postulates and simplifications during model development are justifiable, i.e. can results of model simulations be reconciled with those obtained via experiments. We also show under what treatment conditions it is expected the model will be insufficient to describe experimental kinetics, since the model has been simplified in order to preserve the ability to work with its analytical solution. This analysis enables us to indicate how the model needs to be expanded and to point towards the possible improvements that will need to be accomplished during future development.

2. Materials and methods

2.1. Disintegration index Z

The disintegration index Z is a conductivity-based measure that can be used to estimate the degree of tissue damage during or after treatment—in our case electrical—and is defined as $Z = (\sigma - \sigma_i)/(\sigma_d - \sigma_i)$, where σ is the material conductivity (during or at the end of treatment protocol application), σ_i is the conductivity of the intact sample tissue (prior to treatment), and σ_d corresponds to the conductivity of a tissue sample considered to be destroyed by the treatment, i.e. fully treated. The value of Z increases during electrical treatment, beginning at 0 (intact tissue) and approaches 1 (completely permeabilized cells—i.e. maximally damaged tissue) as the conductivity of the treated sample increases. Various methods can be used to determine σ_d; e.g. freezing–thawing or high-intensity long-duration electroporation have been proposed (Vorobiev & Lebovka, 2008). For the purposes of the present study, an average σ_d was obtained by averaging the measured conductivity of several tissue samples, each of which was treated with 50 trains of 10 pulses of amplitude 400 V (per 5 mm electrode distance), pulse width 100 μs and 5 s pause in between the trains to allow for cooling of the sample and thus avoiding thermal damage to the tissue.

2.2. Diffusion experiments

Cylindrical samples (disks) of sugar beet taproot and apple fruit tissue (skin removed) were obtained from 5 mm thick sugar beet taproot or apple fruit slices.[1] All samples measured 25 mm in diameter. Each sample was subjected to electroporation treatment by applying 150, 200, 300, or 400 V between two parallel plate stainless-steel electrodes at 5 mm inter-electrode distance (sample thickness). Our intent was to subject the treated tissue to field strengths of 300, 400, 600, and 800 V/cm, respectively. Note that this would hold if the tissue were electrically homogeneous material, and only in the central area away from electrode edges. Rectangular pulses of alternating polarity (see Fig. 2) of 100 μs duration each, and pulse repetition frequency of 1 kHz, were delivered within each train of eight pulses. Two such trains

[1] In the remainder of the paper, for brevity, we refer to «apple fruit tissue» and «sugar beet taproot tissue» as «apple tissue» and «sugar beet tissue», respectively.