The Phenomenon of Electroporation

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Abbreviations

CHO	Chinese hamster ovarian
DAMP	Damage-associated molecular pattern
EIT	Electric impedance tomography
HPEF	High pulsed electric field
MD	Molecular dynamics
MEF	Moderate electric field
MREIT	Magnetic resonance electric impedance tomography
PEF	Pulsed electric fields
PFA	Pulsed field ablation
ROS	Reactive oxygen species
TMV	Transmembrane voltage

The Phenomenon and Its Applications

Exposure of biological cells and tissues to short electric pulses of sufficient amplitude to increase the permeability of the membrane was "discovered" at the end of 1950s (Stampfli 1958). Though still a subject of some controversy and debate as to whom the laurel for this discovery should go (Sitzmann et al. 2016), it is by now a well-known and extensively investigated phenomenon at the heart of a broadly applicable set of techniques. The phenomenon facilitates techniques of increasing importance in biomedicine (Yarmush et al. 2014; Bradley and Haines 2020; Geboers et al. 2020), in food science and technology (Mahnič-Kalamiza et al. 2014), as well as in biotechnology and environmental science (Kotnik et al. 2015). In different application fields, the process or technique is referred to either as electropermeabilization, electroporation, electropulsation, PEF (pulsed electric field) treatment, or PFA (pulsed field ablation). This terminology, although it may seem often arbitrary,

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reflects the emphasis on a particular property or mechanism of action of the treatment within its respective application field. Electroporation emphasizes that the phenomenon is believed to be responsible for electrically induced formation of aqueous pores in the lipid bilayer under the influence of the induced transmembrane voltage (Neumann et al. 1982; Weaver 1993; Weaver and Chizmadzhev 1996). Electropermeabilization somewhat more reservedly places the emphasis on the electrically induced increase in the membrane permeability for molecules devoid of physiological mechanisms of transmembrane transport that is observed following application of electric pulses, without alluring to any mechanisms themselves that might or might not be involved (Teissié 2014). The link and the limit between the two are being established (Mir 2020). Electropulsation, pulsed electric field treatment, and pulsed field ablation are perhaps the most generic or neutral of the terms and explicitly stand only for the process of exposing cells to electric pulses itself, with only implied consequences of this treatment leading to membranes' structural alteration and increased conductivity and/or permeability.

The underlying phenomenon is termed either electroporation or electropermeabilization. The terms are often used interchangeably and can be considered synonyms, although the former term refers only to the contribution of aqueous pores formed in the lipid bilayer to the increased permeability of the membrane (see Fig. 1), while the latter term is more general. Electropermeabilization ascribes the increased permeability of the membrane to a broader range of biophysical and biochemical mechanisms potentially involving structures other than the bare lipid bilayer (Kotnik et al. 2019). Formation of (transient) hydrophilic pores in the lipid bilayer – termed electroporation – is now a widely recognized mechanism of membrane permeabilization governed by thermodynamics. The mechanism has been

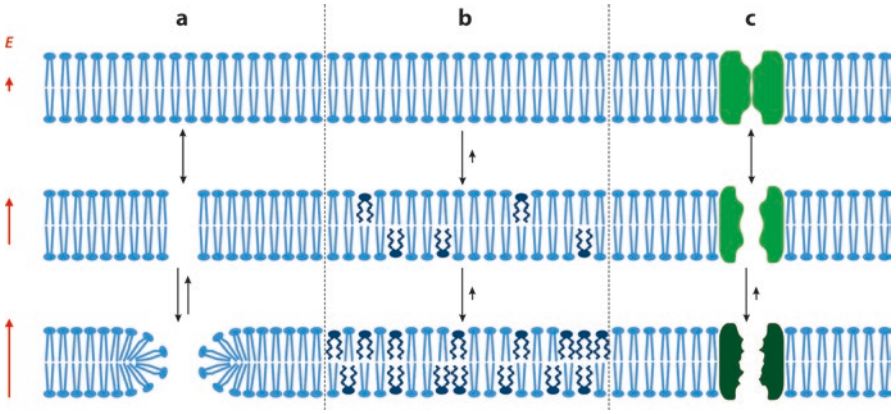

Fig. 1 The phenomenon of electroporation on a molecular level as a simplified conceptual scheme. (**a**) Aqueous pores are formed under the influence of the electric field in the lipid bilayer, shown in two stages; (**b**) electrically induced chemical changes to lipids constituting the membrane – peroxidation deforms their tails and increases the bilayer's permeability to water, ions, and small molecules; (**c**) electrically induced modulation of membrane proteins' function, shown for a voltage-gated channel. (Reproduced with permission from Kotnik et al. 2019)

corroborated by molecular dynamics (MD) simulations; however, there is increasing evidence that exposure to electric pulses also causes chemical changes to the lipids as well as alteration of the membrane proteins' function, both contributing factors to the membrane's increased permeability (Breton and Mir 2018; Kotnik et al. 2019).

Throughout the past several decades, the phenomenon of electroporation has been greatly elucidated through extensive research, in the process of which many fascinating features were revealed about its nature and mechanisms. Among other discoveries, it was found that electroporation can be used to nonselectively increase cell uptake of drugs and genetic material (Mir et al. 1988; Escoffre et al. 2012; Miklavčič et al. 2014) and extract molecules from cells (Knorr et al. 1994; Mahnič-Kalamiza et al. 2014), for membrane protein insertion (El Ouagari et al. 1993; Raffy et al. 2004), to induce cell-to-cell fusion and cell-vesicle fusion (with viable hybrids as a result) (Zimmermann 1982; Saito et al. 2014), to fuse individual cells with tissue (Heller and Grasso 1990), to initiate targeted necrotic or apoptotic cell death (Miller et al. 2005; Pakhomova et al. 2013), to induce intracellular effects (e.g., release of intracellular calcium) or indeed introduction of calcium into cells for purposes of triggering cell death (Vernier et al. 2003; Frandsen et al. 2020), and to modify the viscoelastic properties and texture of plant tissues (Mahnič-Kalamiza and Vorobiev 2015; Botero-Uribe et al. 2017).

The most appealing feature of electroporation is that it seems to be universal to the lipid bilayer and consequently is ubiquitous throughout the spectrum of various life forms, i.e., the phenomenon can be observed in all cell types (eukaryotes, bacteria, and archaea (Polak et al. 2014)), in all cell arrangements (be it the cells are in suspension, grow adhered to surfaces, clustered, or in tissue). Apart from cells, electroporation can also be observed in any other membrane bilayer systems, e.g., in planar lipid bilayers, lipid vesicles, and polymeric vesicles (Benz et al. 1979; Teissie and Tsong 1981; Aranda-Espinoza et al. 2001). This has led to the development of a considerable number of applications in diverse fields as mentioned in the first paragraph of the section; some of these applications have already reached patients and consumers. At present, the most developed and promising biomedical applications include cardiac muscle ablation for the treatment of arrhythmias (Stewart et al. 2019; Maor et al. 2019; Reddy et al. 2019; Sugrue et al. 2019), electrochemotherapy (Campana et al. 2019a, b), gene electrotransfer (Broderick and Humeau 2015; Lambricht et al. 2016; Rosazza et al. 2016b), and tumor tissue ablation by means of irreversible electroporation (Scheffer et al. 2014; Geboers et al. 2020). In food processing existing industrial applications range from pasteurization of liquid foods (Min et al. 2007; Jin 2017) to modifying structural and textural properties of raw materials (e.g., potato tubers (Fincan and Dejmek 2003; Chalermchat and Dejmek 2005)) and enhancing mass transport processes, thus facilitating extraction of valuable compounds from raw materials or by-products (biological waste materials). Electroporation for enhancing mass transport is important in, e.g., recovering sugars from sugar beet root (Mhemdi et al. 2016; Vorobiev and Lebovka 2017), improving the maceration process in winemaking (Sack et al. 2010), and enhancement of drying (Thamkaew and Gómez Galindo 2020), with additional applications ranging

into the field of biorefinery where electroporation can be and has been used for valorization of various by-products (e.g., extraction of polyphenols from grape pomace (Saldaña et al. 2017), polyphenol and protein extraction from rapeseed stems and leaves (Yu et al. 2015), etc.). There is also growing interest in using electroporation for cooking (Blahovec et al. 2017).

In many of the applications listed above, electroporation is performed on cells comprising tissues. Understanding the mechanisms by which electric pulses act upon cells in such a complex environment as the biological tissue requires a multiscale approach, where we combine the insights gained from molecular dynamics models of simple lipid systems with those gained from in vitro and in vivo experiments (Rems and Miklavčič 2016). The in vitro data come primarily from mammalian cell cultures, for which the literature is most abundant and since studying electroporation at the level of single cells allows for the most efficient and straightforward approach to understanding the basic aspects of the phenomenon. Results obtained at that level can then be translated to increasingly complex levels up to the level of biological tissue in vivo. In continuation of this chapter, we present the current understanding of the electroporation phenomenon at scales that are orders of magnitude apart. Assembling and reconciling findings obtained at such disparate levels of examination remains a daunting challenge, as is briefly discussed in the concluding section on current challenges facing basic electroporation research.

Applying an electric field to the cell can have, in general, three possible outcomes. The outcome depends on the local field strength, the duration of cell's exposure to the electric field, and the membrane recovery rate. Provided that the field strength and exposure time are completely insufficient, electroporation is not achieved, and cell's permeability and consequently viability are left unaffected. If, however, the field strength exceeds the so-called reversible threshold and exposure to the sufficiently high field strength is for an adequate amount of time, *reversible electroporation* occurs (Rols and Teissié 1990; Čorović et al. 2012). In reversible electroporation, the membrane is permeabilized and remains in a state of increased permeability for a period. The phenomenon is reversible as the term would imply, and the membrane eventually returns to its original state by a process of membrane resealing. Membrane resealing entails pore closure and restoration of the cell's normal (called *resting*) transmembrane potential (see sect. 2 in continuation). This process, as is the process of pore formation and evolution, is still a subject of experimental research and modelling endeavors (molecular- and cell-scale) to determine its exact underlying mechanisms and kinetics (Saulis et al. 1991; Demiryurek et al. 2015; Kotnik et al. 2019). It should be noted that reversibility of the phenomenon of pore formation and re-establishing of the transmembrane potential are only possible if the environmental conditions are conducive to cell survival and function.

In case field strength and/or energy is too high, *irreversible electroporation* occurs (Rubinsky et al. 2007). Irreversible electroporation results in loss of cell homeostasis, effectively killing the cell. See Fig. 2 for a simplified diagram of some of the applications of reversible and irreversible electroporation. Note that though the figure gives multiple application examples for reversible electroporation, and only cell death as resulting from irreversible electroporation, the latter as a modality

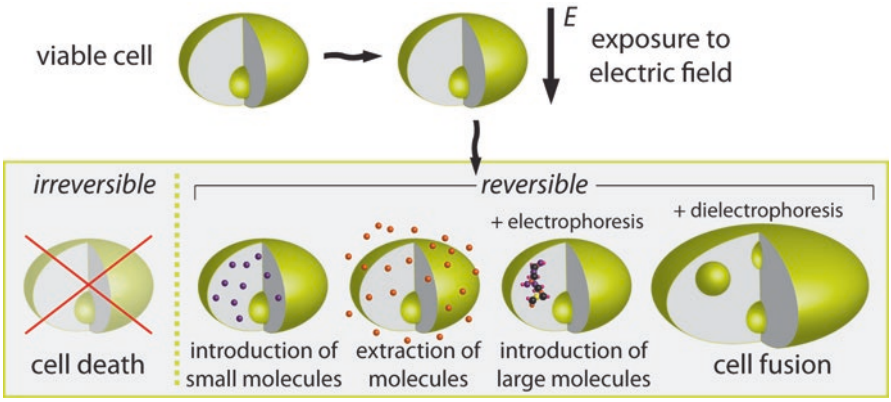

Fig. 2 Some of the possible outcomes/target processes of exposing a viable biological cell to an electric field of appropriate strength. (Redrawn based on a figure first published in Mahnič-Kalamiza et al. 2014)

of tissue disintegration and ablation does have its own broad set of uses. The reader should be aware that it proves difficult to achieve exclusively reversible electroporation without any irreversible damage and vice versa, especially when delivering electroporation to tissues. This is mainly due to finite electrode geometry and material inhomogeneities (Hjouj et al. 2012; Blumrosen et al. 2014; Zmuc et al. 2019; Polajžer et al. 2020) resulting in a heterogeneous field strength distribution and coverage. If reversible electroporation pulses are delivered to target cells/tissues in combination with other fields of appropriate form and strength, other phenomena can be facilitated, such as electrophoresis and dielectrophoresis, used for introduction of large molecules such as DNA in gene delivery applications and for cell fusion, respectively (Hu et al. 2013; Dean 2013; Rems et al. 2013). As previously evidenced by the review of electroporation applications, both reversible and irreversible electroporation have found their applications in such disparate fields such as biomedicine, food technology, and biotechnology (Toepfl et al. 2014; Yarmush et al. 2014; Kotnik et al. 2015; Geboers et al. 2020).

In line with the subject matter this book is devoted to, i.e., electroporation applications in the food industry, we dedicate the remainder of this section to developing the list of applications in this field a bit further, to give the reader a broader overview of what can be achieved when applying pulsed electric fields to food matrices.

In food processing, electroporation has been applied to either enhance or facilitate extraction of juices and other valuable compounds from tissues and microorganisms (e.g., microalgae) (Fincan et al. 2004; Donsi et al. 2010; Vorobiev and Lebovka 2010; Vanthoor-Koopmans et al. 2013), tissue dehydration (Ade-Omowaye et al. 2001; Lebovka et al. 2007; Shynkaryk et al. 2008; Thamkaew and Gómez Galindo 2020), and nonthermal preservation (of mainly liquid foods) by microbial inactivation (Wesierska and Trziszka 2007; Mosqueda-Melgar et al. 2008; Bermudez-Aguirre et al. 2012; Marsellés-Fontanet et al. 2012). In food engineering, reversible electroporation may help in preventing biological tissues from sustaining

damage due to ice crystal formation during freezing (Phoon et al. 2008; Shayanfar et al. 2013; Dymek 2015) and serve as a method of cell metabolism stimulation (Ye et al. 2004; Dymek et al. 2012; Straessner et al. 2013).

Regardless whether the objective is to introduce into or extract out of tissue either water or solutes, electroporation entails applying a series of trains of electric pulses of moderate or high field strength. The reader should note that though no formal definitions exist, the electric field strengths are often, in literature, referred to per their defined ranges of field strength or by abbreviations. These fields do not necessarily reflect the actual local electric field strength to which the cells are exposed. Moderate electric field, abbr. MEF, is most often considered to be in the range of several 100 V/cm up to 1–2 kV/cm (see Table 1 in (Puertolas et al. 2012)), which is a broad range of field strength values. Note that arbitrary categorizations by field strength or any other pulse parameter are best avoided, since the augmenting effect of electroporation to mass transport has already been observed in case of low-intensity electric fields on the order of less than 100 V/cm (Asavasanti et al. 2010). It is therefore desirable to respect the general recommendation guidelines whenever reporting on research studies of electroporation technology in food and biotechnological processes (Raso et al. 2016) so as to avoid any ambiguity. Higher-intensity electric pulses (abbr. HPEF (high pulsed electric field)), i.e., on the order of about 5–50 kV/cm, are associated with irreversible damage to plant and animal cells and can possibly result in irreversible electroporation of bacterial and yeast cells and can therefore be used as a method of preserving material by inactivating and destroying these microorganisms of spoilage.

The objective of microbial inactivation by electroporation is mainly to pasteurize biological liquids: foods or sludge (Álvarez et al. 2006; Mosqueda-Melgar et al. 2008; Guerrero-Beltran et al. 2010; Bermudez-Aguirre et al. 2012). During the past two or three decades, electroporation as means of food preservation has found numerous applications, e.g., a number of microorganism species have effectively been inactivated in different liquid foods (e.g., cider, milk, beer, soups, miscellaneous fruit, and vegetable juices) and semisolid/solid food products. Moreover, synergistic effects between electroporation and other treatments, e.g., nisin, acid, low-temperature/low-energy heating, or high pressure, have been demonstrated (Kotnik et al. 2015). The combined approach of electroporation in tandem with high pressure and ultraviolet light is important as it seems to hold promise in the inactivation of bacterial spores, for which other, traditional mechanisms normally fail to achieve inactivation on their own. In fact, as many harmful microorganisms prove hard to destroy by electroporation alone, electroporation is almost exclusively applied as combined treatment with traditional or other novel techniques of food preservation, such as thermal, enzymatic, ultraviolet, chemical, or pressure-based (Shin et al. 2010; Sobrino-Lopez and Martin-Belloso 2010). Inactivation of bacterial spores is generally considered as one of the hurdles in pasteurization and sterilization processes due to the spores' capability of tolerating high field strengths, temperatures, and pressures (Pol et al. 2001; Siemer et al. 2014a, b). Note the higher field requirements and specific energy consumption in microbial inactivation applications as compared to other objectives of electroporation application in Fig. 3.

Table 1 The phenomenon of electroporation, its time evolution, and principal characteristics of its stages. *DAMPs (damage-associated molecular patterns) molecules are biomolecules associated with an inflammatory response, released from damaged and/or dying cells

Stage	Characterized by	Illustrated by	Timescale
1- Initiation of the state of elevated permeability (cell is exposed to an electric field)	• Charged carriers in the electrolyte are ions/charged molecules • Charging of the membrane • Induced membrane potential difference ≈ 500 mV over membrane thickness ≈ 5 nm • Establishing huge electric field within the membrane, V/d = 500 mV/5 nm = 100 MV/m (!); note that electrical breakdown in air occurs at E > 3 MV/m	Figure 5a	ns-ms
2- Formation and expansion of the transmembrane transport area	• Pores are formed in the lipid domain of the membrane • Phosphatidylserine externalization • ROS* generation and access to lipids in the membrane • Lipid oxidative damage • Electro-conformational change of membrane proteins	Figure 5b	ns-ms
3- Stabilization (with partial recovery); the cell remains in a state of increased membrane permeability	• Leakage of ions/ionic and osmotic imbalance • Loss of cell homeostasis • DAMP* molecule release (ATP, HMGB1, nucleic acids, etc.) • Membrane protein structure and function alteration • Cytoskeleton disassembly	Figure 5c	ms-min
4- Cell membrane repair/ resealing and cell recovery	• Exocytosis/patching/membrane repair • Reassembling cytoskeleton • Recovering protein function • Exocytosis/patching/membrane repair • Reassembling cytoskeleton • Recovering protein function • Cell response to stress(stress-related gene expression) • Re-establishing homeostasis	Figure 5dandFig. 5e	min-hrs
5- Cessation of the cells' altered physiological processes; complete cell recovery, or loss of homeostasis and cell death	• Cell response to stress(stress related gene expression) • Loss of cell homeostasis • Cell death	Figure 5f	min-hrs

To understand why significantly higher field strengths and energies are generally required for microbial inactivation, in comparison to, for instance, extraction applications, we must understand the mechanism of action of electroporation, i.e., the mechanism by which inactivation of microorganisms is achieved by pulsed electric

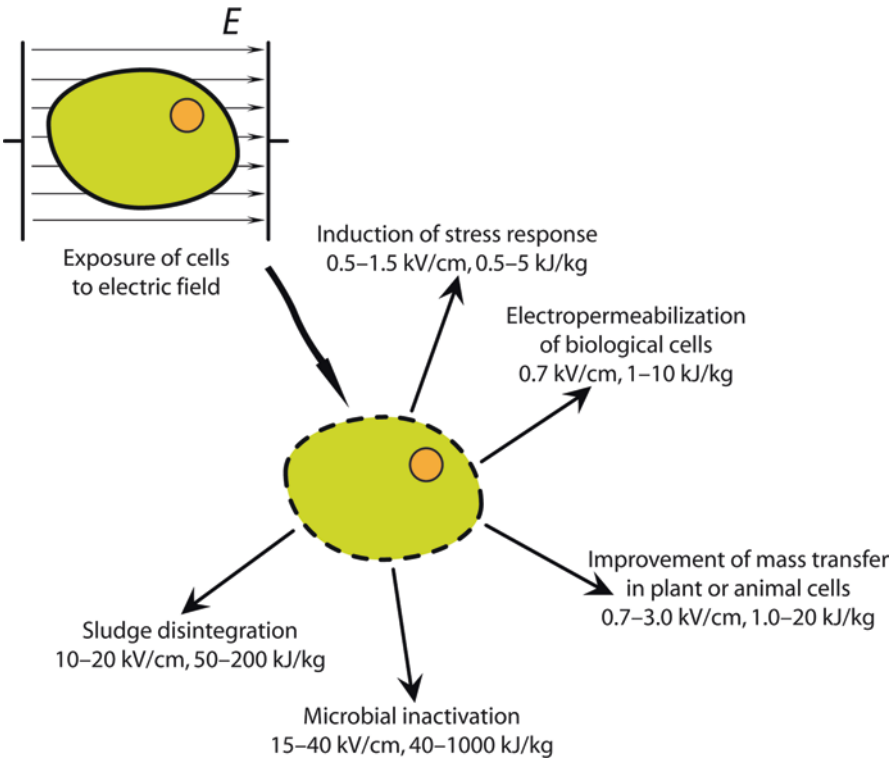

Fig. 3 Target mechanisms of action and applications of electroporation by field strength and delivered energy; a schematic representation of exposing a biological cell to an external electric field with various corresponding applications. (Redrawn based on a figure first published in Toepfl et al. 2006)

fields. If we follow the paradigm presented by Saulis (2010), the process of exposing a living and viable cell, capable of reproduction, to an external electric field can be divided into stages. The first stage involves an increase in transmembrane potential in time, as the plasma membrane is charged by the externally superimposed electric field. The final amplitude of this transmembrane potential that is reached is determined by material and geometrical properties, namely, cell size and shape, membrane, and medium ionic composition that is affecting the conductivity (intra- and extracellular), and by the pulse parameters (e.g., shape, duration, amplitude). When the critical transmembrane potential is reached, the pore initiation stage begins, and small metastable hydrophilic pores appear in the plasma membrane. Pores evolve in time in both size and number. Following the cessation of pulse application is the posttreatment stage, which can last from milliseconds to hours. During this stage leakage of intracellular compounds to the cell exterior, influx of substances from the extracellular space into the cell, and finally either pore resealing and membrane repair or cell death will occur. The final result of the treatment is then either a viable or a dead cell; the outcome is determined by the relevant

conditions and processes taking place during the mentioned stages of electroporation. These conditions vary considerably for different applications (see, e.g., Figure 3 for the field strength and delivered energy aspect).

As an example, consider a pathogenic bacterial species representing a typical prokaryotic microorganism several micrometers in length. For this prokaryotic cell, the induced transmembrane voltage will be an order of magnitude lower given the same external electric field strength, compared to a small eukaryotic cell with a diameter of tens of micrometers. The presence and structure of any cell wall must likewise be considered (Aronsson et al. 2005; Golberg et al. 2012). For a more thorough treatment of microbial inactivation and considerations relevant to electroporation, refer to chapters 5, "Microbial inactivation by PEF," and 6, "Liquid food pasteurization by PEF" in the book.

In addition to the enhanced mass transport or irreversible cell damage effects of electroporation and applications facilitated by these effects, electroporation can be used at lower intensities (below 100 V/cm) for inducing a stress response in plant and yeast cells. At these low field strengths, electroporation may be achieved without affecting cell viability by carefully controlling the electric pulse parameters, resulting in predominantly reversible electroporation of cells. However, even though the electroporated cells survive the electric field treatment, they are still stressed due to the opening of pores in the membrane, triggered repair mechanisms, and various biochemical processes, as they struggle to recover their normal functionality (re-establishing homeostasis). The exact details of recovering from reversible electroporation at the level of the cell and its membranes on the molecular level, as well as physiological responses to electroporation-induced stress, remain largely unknown (Gómez Galindo 2016).

One of the challenges when electroporating plant tissue with intent of reversibly electroporating them and thereby inducing cell stress is posed by the cell's heterogeneous structures. Since cells vary in shape, size, and cell wall structure, these heterogeneities influence the effect of electroporation protocols on cells of a particular size, shape, or within a particular spatial region. If cells are successfully reversibly permeabilized, physiological responses to electroporation-induced stress will include, among others, the production of ROS (reactive oxygen species), release of stored energy, activation of regulatory genes in charge of the cell's stress response, as well as the production of secondary metabolites. Applying reversible electroporation has also been shown, as a few examples, to influence barley seed germination, increase the strength of the cell wall in potatoes, and, by consequence, alter the potato's textural properties. Reversible electroporation can also have an impact on protoplasts (i.e., plant cells with cell walls having been removed) and consequentially the regeneration of new plants. Reversible permeabilization of plant cells and tissue is not often reported upon; however, foundations have been laid for a fascinating area of academic research and industrial innovation (Gómez Galindo 2016).

Electroporation at the Membrane and Molecular Level

Researchers have endeavored to provide a theoretical description of the events underlying the phenomenon of electroporation since its discovery. A number of competing theories have emerged over the years (Pavlin et al. 2008; Yarmush et al. 2014). The set of possible explanations includes assuming a certain type of lipid deformation (Crowley 1973), lipid phase transition (Sugár 1979), breakdown of interfaces adjoining domains of different lipid compositions (Cruzeiro-Hansson and Mouritsen 1988), and/or membrane protein denaturation (Tsong 1991). These descriptions, however, by themselves, fail to provide an adequate explanation for observed phenomena associated with electroporation, and the state-of-the-art consensus is that electroporation can be best described as formation of aqueous pores in the lipid bilayer (Freeman et al. 1994; Kotnik et al. 2012; Casciola and Tarek 2016). This description is supportive of the continued use of the term that has become prevalent – *electroporation*. Another, broader term for the phenomenon is *electropermeabilization*. The latter describes the consequence, i.e., observed increased membrane permeability, rather than the underlying mechanism; as such, it is applicable to other, alternative explanations for the phenomenon in a broader sense.

The theory of aqueous pore formation is based on consideration of thermodynamic relations (Neumann et al. 1982); according to the theory, the formation of aqueous pores is initiated by water molecules penetrating the membrane's lipid bilayer. This promotes reorientation of the adjacent lipids and their polar head groups, which, being hydrophilic, begin pointing toward these penetrating water molecules (see Fig. 4). Unstable, short-lived pores (of nanosecond lifetime) can form even in the absence of an external electric field, but these rare events are of a stochastic nature. They are also forming after or when an externally imposed electric field is present, with a higher probability of occurrence. Contrary to these sporadic spontaneous pores, exposure of the membrane to an electroporating field strength reduces the energy barrier experienced by water molecules preventing

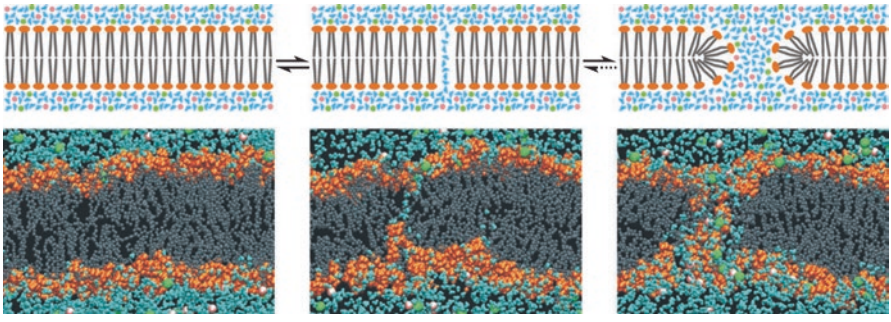

Fig. 4 An idealized molecular-level scheme (top row) and an atomic-level MD simulation (bottom row) of electroporation. The electric field is perpendicular to the bilayer plane. (Reproduced with permission from Yarmush et al. 2014)

them to penetrate the bilayer. Within nanoseconds, penetration results from the transfer of the external field to the membrane. This is followed, within a microsecond, by the transmembrane field being amplified by polarization (positive and negative charge carriers accumulating either side of the membrane), resulting in the buildup of an induced transmembrane voltage (Kotnik and Miklavčič 2006). The intrusion of water into the bilayer increases the statistical probability of a pore forming, resulting in a bulk increase in the number of pores formed in the bilayer per unit of time and unit area. Field-induced pores are more stable than the spontaneous, sporadic, and short-lived pores mentioned above. For transmembrane voltages of several hundred mV, pores become sufficiently numerous and long-lasting (up to milliseconds (Saulis and Saule 2012)) to produce a detectable increase in membrane permeability to those molecules that would otherwise not be able to cross the membrane.

These aqueous pores with radii of at most a few nm are too small for optical microscopy. Moreover, sample preparation for electron microscopy of soft matter is too detrimental to the structures in question (semi-stable nanopores in the bilayer), so much that pores cannot be distinguished from imaging artifacts. Still, there is evidence in favor of the theory of aqueous pore formation, and it comes in the form of molecular dynamics (MD) modelling and simulation. MD simulations by and large agree with the hypothesized sequence of events on the molecular-scale and also demonstrate a clear correlation between the rate of pore formation and the strength of the electric field to which the membrane is exposed (Delemotte and Tarek 2012; Ho et al. 2013; Casciola et al. 2014; Casciola and Tarek 2016).

MD simulations enable two possible disparate approaches to modelling electroporation conditions (Tarek et al. 2010; Rems and Miklavčič 2016). The first option is to model for an electric field strength $\boldsymbol{E}$, acting on charged atoms with force $\boldsymbol{F}_e = q_i\boldsymbol{E}$ (q_i is the charge of the i-th atom). Note that this electric field is not the same one as reported in experimental studies, which the bulk of material is exposed to. This imposed electric field causes the water dipoles to reorient in the electric field, an effect most pronounced at the water-lipid interface. This increases the electric field inside the bilayer, thus increasing the voltage across it (Böckmann et al. 2008; Vernier et al. 2013). The second approach in order to increase the transmembrane voltage is by imposing a charge imbalance. This can be achieved via placement of an excess number of monovalent cations on one side of the bilayer and a corresponding excess number of monovalent anions on the other side of the bilayer (Delemotte and Tarek 2012). The first approach is ordinarily simulated in the absence of ions, modelling only the dielectric response. This scenario represents ps or ns pulses, pulses too short for ion redistribution to occur, and thus there is no charging of the membrane (Vernier 2020). The alternative, second approach is thought to be more relevant for situations employing longer pulses and does allow for full charging of the membrane.

Regardless of which of the two methods is employed, evolution of pore formation and closure is similar to both (Delemotte and Tarek 2012). When the imposed electric field (or the charge imbalance) is sufficient, a conical structure composed of water molecules (a so-called water finger) penetrates into the bilayer's hydrophobic

core. Eventually, a water-spanning column is formed across the bilayer as water from one side connects with water from the other side (Levine and Vernier 2010) (see Fig. 4, center column). Because this configuration exposes the hydrophobic lipid tails to water, it is known as a hydrophobic pore (Abidor et al. 1979). If the bilayer were composed of specific lipids, e.g., lipids with large head groups or negatively charged lipids, the head group reorientation energy barrier would be very high. This would cause the hydrophobic pore to expand, allowing passing of ions (Dehez et al. 2014; Polak et al. 2014). In the alternative case of a typical zwitterionic phospholipid comprising the bilayer, the lipid head group migration is in direction along the water column and establishes a so-called hydrophilic pore (Ziegler and Vernier 2008; Levine and Vernier 2010). This pore then stabilizes, increases in size, and is capable of conducting ions (Ho et al. 2013; Dehez et al. 2014). Upon removal or cessation of the external energy source (either superimposed electric field or a charge imbalance), the pore evolution then follows the formation sequence of events in reverse order and eventually closes (Gurtovenko and Vattulainen 2005; Ziegler and Vernier 2008; Levine and Vernier 2010).

Electroporation at the Level of a Single Cell and Cell Suspension

The majority of cells continue to constantly maintain an electric potential difference between their inner and outer side of their plasma membrane. This electric potential difference, called the resting transmembrane voltage (TMV), is generated and regulated by a system of ion pumps and channels in the membrane. For a typical eukaryotic cell, the resting TMV ranges from about −40 to about −70 mV. The minus sign indicates that inner potential (the potential within the cell at the membrane's inner surface) is lower than the one on the outer side of the membrane. This can be considered the natural state of a biological membrane, and both lipid and protein components of the membrane are well adapted (by evolution) to function under voltages in this range (Kotnik et al. 2019).

Upon exposure of a cell to an external electric field, an additional component is superimposed to the resting TMV and is termed the induced TMV, as previously introduced in the section on molecular dynamics (see sect. 2). Induced TMV is only sustained for the duration of the externally imposed electric field and is proportional to its strength (Ehrenberg et al. 1987; Pucihar et al. 2006). For a single spherical cell with a nonconductive plasma membrane, the induced TMV is calculated according to the steady-state Schwan equation (Pauly and Schwan 1959): induced $\mathrm{TMV} = 1.5 \times E \times R \times \cos\theta$, where E is the electric field, to which the cell is exposed, R is the cell radius, and θ is the angle measured from the center of the cell with respect to the direction of the field (Kotnik et al. 2010). Thus, exposure to a sufficiently strong field can induce transmembrane voltages that are far exceeding their resting values, which causes both structural changes to the membrane and changes

to the constituent molecules. These changes generally would not occur under normal physiological conditions and lead to among others, membrane electropermeabilization. As previously explained, electropermeabilization is the rapid onset of a substantial increase in membrane permeability, indicated to the observer by transmembrane transport of molecules for which an intact membrane is practically impermeable (Kennedy et al. 2008; Kotnik et al. 2010). In the preceding section, we attributed this effect of increased permeability to the formation of aqueous conduits or pores in the lipid bilayer.

A number of studies, both experimental and theoretical, imply that the molecular flow across the permeabilized membrane or, in other words, the heightened degree of permeability of the membrane seems to be limited to the regions of the membrane that were exposed to sufficiently high TMV (Hibino et al. 1993; Towhidi et al. 2008). This is repeatedly shown experimentally for single cells as well as clusters of cells, by monitoring both the TMV and the transmembrane transport on the same cells upon their exposure to electric pulses (Kotnik et al. 2010).

The elevated state of membrane permeability facilitates the inflow of membrane-impermeant molecules into the cell and the outflow of biomolecules from the cell. The kinetics of this transmembrane transport that is enabled by electroporation have been studied extensively, showing that membrane electrical conductivity and permeability increase considerably and measurably within less than a microsecond after the onset of the electric pulse, provided that the TMV locally (for the cell under observation) exceeds a certain critical value. This critical value is not a universal constant but rather a variable. Nevertheless, to summarize some general observations, the kinetics of transmembrane transport can roughly be divided into five stages: (1) the initiation of the state of elevated permeability, (2) the formation and expansion of the transmembrane transport area, (3) stabilization (with partial recovery), (4) the resealing/repair of the membrane, and finally (5) gradual cessation of the cells' altered physiological processes, followed by any reactions to various stressors, possibly resulting in complete cell recovery but possibly also resulting in cell death. Table 1 and Fig. 5 are in aid of illustrating these stages.

Electroporation of the cell membrane, from a theoretical perspective, is not strictly a threshold event. By a threshold event, we would mean that these processes would occur only in an electric field exceeding a certain value. On the contrary, we observe at most that the rates of these processes increase nonlinearly with the increase in the field amplitude which the cells are exposed to. Empirically, however, there is a critical value of the electric field to which a cell must be exposed for electroporation-mediated transport to become detectable. This critical value (the reversible electroporation field strength) depends on the type of cell, the type of molecule being transported/observed, the duration of the exposure, and on the particular set of environmental conditions, e.g., temperature. There is also another, higher critical value of the field (the irreversible electroporation field strength) that must not be exceeded if membrane restabilization, recovery, and resealing are to be expected. As a consequence of this thresholding nature of the phenomenon as observed in experiments, experimentalists often treat electroporation as a quasi-threshold phenomenon. These thresholds are however so ambiguous and dependent

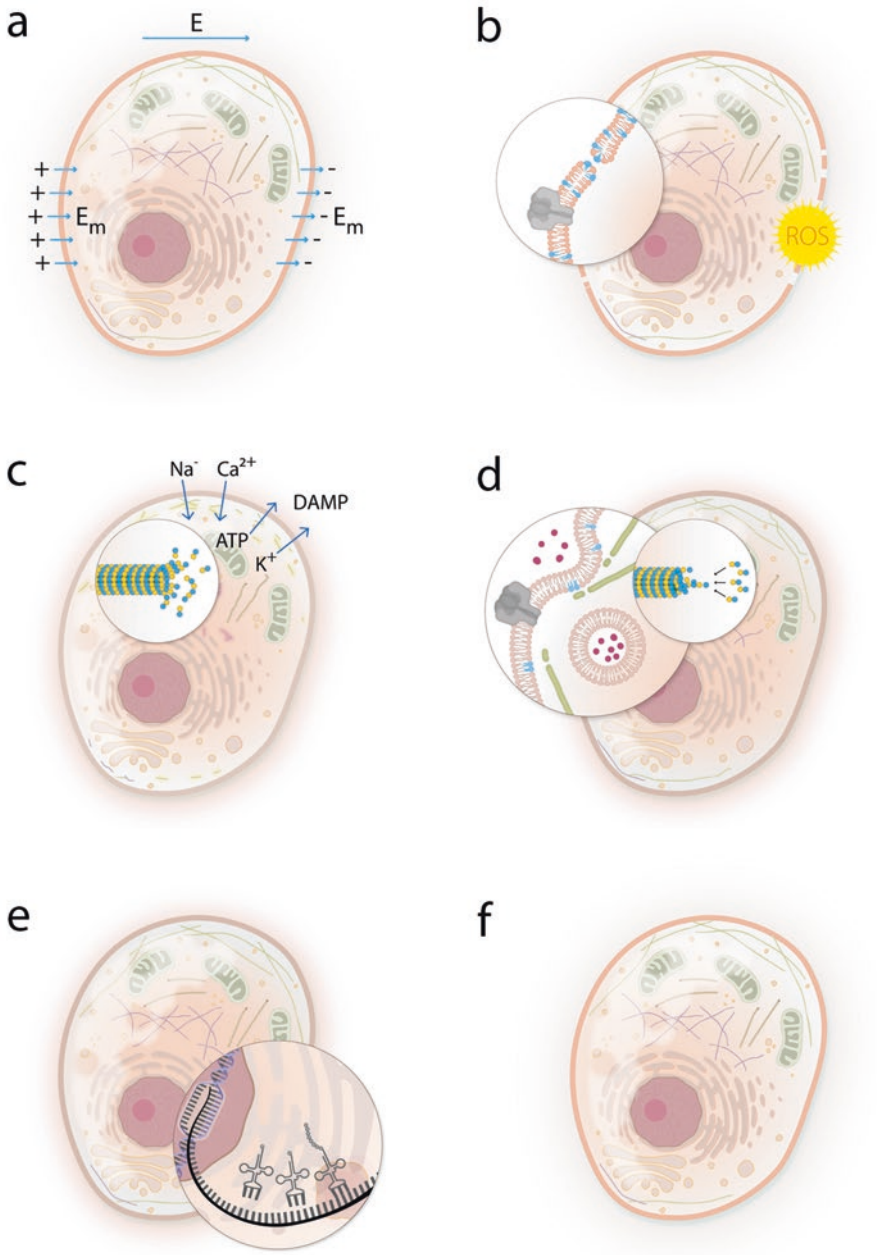

Fig. 5 Different stages of single-cell electroporation: (**a**) initiation of the state of elevated permeability (the cell is exposed to an electric field); (**b**) formation and expansion of the transmembrane transport area, transmembrane transport; (**c**) stabilization (with partial recovery) – cell remains in a state of increased membrane permeability; (**d**) and (**e**) cell membrane repair/resealing and recovery; (**f**) recovery of cell functions or loss of cell homeostasis and subsequent cell death. *ROS stands for reactive oxygen species

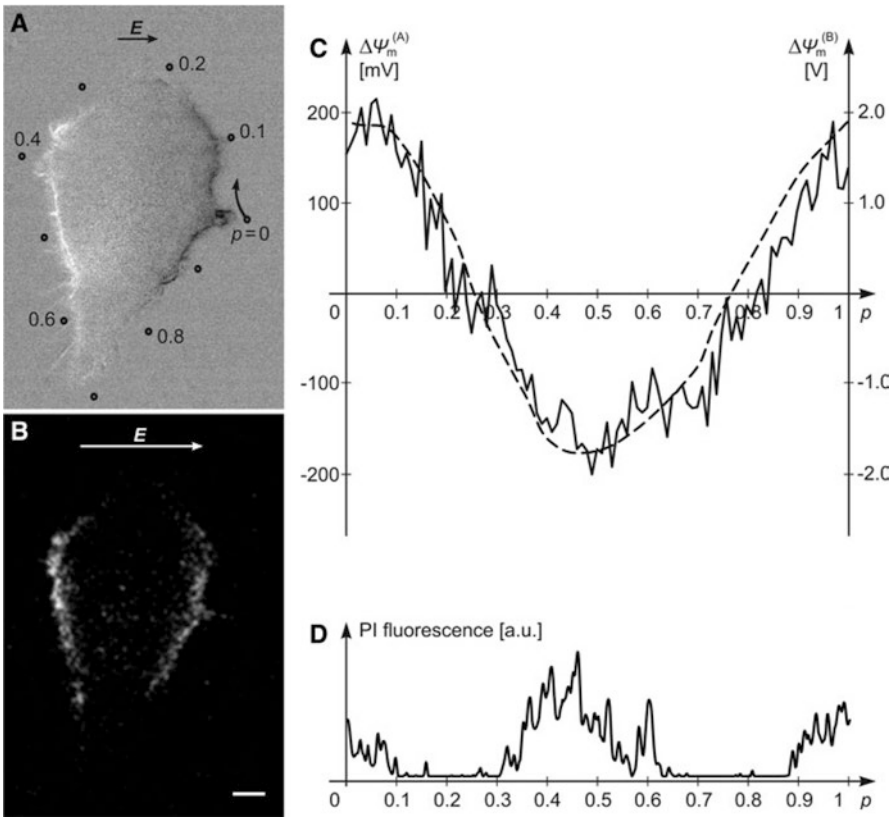

Fig. 6 Steady-state induced transmembrane voltage and electroporation of an irregular CHO (Chinese hamster ovarian) cell; (**a**) changes in fluorescence of di-8-ANEPPS (a fluorescent dye) caused by a non-porating 50 ms, 100 V/cm pulse; (**b**) transport of PI into the same cell caused by a porating 200 μs, 1000 V/cm pulse, visualized 200 ms after exposure; (**c**) steady-state induced transmembrane voltage along the path shown in (**a**) as measured (solid) and as predicted in a numerical model (dashed), the left ordinate axis scale corresponds to the 100 V/cm pulse amplitude used in (**a**), and the right ordinate axis scale to the 1000 V/cm used in (**b**); (**d**) propidium iodide fluorescence measured along the path shown in (**a**). (Reproduced with permission from Kotnik et al. 2010)

on so many factors that only the order of magnitude for their values can confidently be determined in general for a particular cell type and set of experimental conditions. In example, for a eukaryotic cell, detection occurs for electric fields resulting in TMV in hundreds of mV and irreversible damage for electric fields about three to five times higher than the minimum for detection (Teissié and Rols 1993; Towhidi et al. 2008; Kotnik et al. 2010) (Fig. 6).

As just mentioned, the critical electric field strength and the corresponding TMV for detectable electroporation depend on the cell type (Čemažar et al. 1998), transported molecule (Rols and Teissié 1998; Puc et al. 2003), and duration of exposure

(Hibino et al. 1993; Rols and Teissié 1998; Puc et al. 2003). These critical strengths are also influenced by cell size and local membrane curvature (Kakorin et al. 2003; Towhidi et al. 2008; Henslee et al. 2011), temperature (Kandušer et al. 2008; Polak et al. 2014), and osmotic pressure (Golzio et al. 1998). Our ability to accurately measure them is also subject to the sensitivity of the detection technique (Kotnik et al. 2000; Pakhomov et al. 2015; Wegner et al. 2015).

Direct microscopic observations reveal that electroporation-facilitated transport is strongly dependent on the size and charge of the molecules traversing the membrane through the electropores. Small molecules can enter the cell both during and after the pulse through the membrane regions with sufficiently high (positive or negative) TMV (Saulis and Saule 2012). For charged species, the entry is mostly electrophoretic (during the pulse as electrophoresis is field-driven) and proceeds, for the given net charge, from the side with the opposite polarity of the TMV. The transport after the pulse is mainly diffusive and takes place from both sides of the membrane (Gabriel and Teissié 1998; Gabriel and Teissie 1999; Pucihar et al. 2008). Experiments also suggest a non-negligible contribution of post-pulse TMV recovery in the transport of small charged species (Sözer et al. 2018a). Larger molecules and/or molecules exhibiting multiple charges enter only while the pulse is on and that only from the side with the opposite polarity of the TMV (e.g., negatively charged oligonucleotides enter from the side with positive TMV) (Paganin-Gioanni et al. 2011). For still larger molecules, such as plasmid DNA, electroporation initializes the transport, with longer (approx. ms duration) pulses required for sufficient electrophoretic drag on DNA to form a so-called DNA-membrane complex (Wolf et al. 1994; Rols and Teissié 1998; Mir et al. 1999). The subsequent DNA uptake is a slow process (as compared to the timescales of pore formation) and involves endocytosis for DNA uptake into the cytosol and its intracellular trafficking to the nucleus (Golzio et al. 2002; Rosazza et al. 2016a). A much more detailed account of the molecule-dependent specifics of electroporation-facilitated transport is provided by Rems and Miklavčič (2016).

Electroporation at the Level of Cell Suspensions and Tissues

This section focuses on cell electroporation in a multicellular environment. In particular, we consider cell suspensions of increasing densities and tissues, mainly from the perspective of the distribution of the electric field and the induced TMV (transmembrane voltage). The TMV is, in a multicellular environment, affected by the proximity of neighboring cells. If a cell is forcibly charged (e.g., when exposed to an electric pulse), redistribution of ions around the membrane will alter the electric field in the cell's immediate vicinity. This means that, provided the cells are in close proximity, the cells will *feel* the electric field perturbation caused by their neighboring cells, and that field will be superimposed to the already present externally applied electric field (Rems and Miklavčič 2016). The first time this has been described was in the study by Susil et al. (1998).

First, let us consider a cell suspension of ever-increasing density. When in a dilute suspension, cells can be considered sufficiently distant from one another not to, on average, sense the alteration of the electric field resulting from their neighboring cells. With increasing density of the suspension however, the induced TMV on a cell is more and more impacted by the field generated by other cells in its proximity. If spherical cells are in close contact, the maximum induced TMV will equal the product of the electric field strength and the cell's radius (TMV = E × R). This corresponds to a 1.5 times reduction with respect to the TMV on isolated cells (Susil et al. 1998; Pavlin et al. 2002). This means that cells in dense suspensions have to be electroporated at higher electric field strength than those in dilute suspensions, and that is due to the reduction in the induced TMV. This has been corroborated by both numerical calculations (Essone Mezeme et al. 2012b) and experiments (Canatella and Prausnitz 2001; Pucihar et al. 2007). Additionally, in experiments, the number of molecules that entered densely packed cells was determined to be lower, possibly due to a limited number of dye molecules available for transmembrane transport in the extracellular space. This is further potentiated by the phenomenon of cell swelling, following the cell exposure to electric pulses in a low-conductivity medium (Pavlin et al. 2005; Pucihar et al. 2007).

To elaborate a bit further on the phenomenon of cell swelling, note that electroporation of cells is accompanied by cytosolic solutes leaking into the extracellular medium and the (transient) inability of the cell to regulate homeostasis. In dense suspensions (when the volume fraction of cells is on par with the volume of the extracellular space), this leakage results in an increase in the suspension conductivity. In a very low-conductivity medium, the cell's inability to regulate cross-membrane transport results in a considerable amount of water to be taken up by the cell via the permeable membrane, thus disrupting the osmotic balance of the cell. This disruption in osmotic balance and water uptake cause the cell to swell up, while the release of intracellular ions to the cell's exterior medium results in medium conductivity changes. These changes and cell swelling were extensively studied by Pavlin et al. (Pavlin et al. 2005, 2007; Pavlin and Miklavcic 2008) during electroporation in dense suspensions of B16-F1 cells. They found a two-part increase in suspension conductivity: a large increase during the pulse cause by an increase in the conductance of the cell membranes and the second, gradual increase between the pulses caused by efflux of ions from the cells (Rems and Miklavčič 2016).

The contribution to bulk conductivity due to the increase in conductance of the individual cell membranes diminishes rapidly after the pulse during fast membrane recovery (pore closure). But since the cells are electroporated by the electric field imposed from multiple directions, this affects the conductivity of the cell suspension in an anisotropic fashion. During the pulse, the conductivity of the suspension is higher in the direction parallel to the electric field than in the direction perpendicular to it. Similar increase in conductivity anisotropic in nature can be observed in tissues (Essone Mezeme et al. 2012a).

Cells in suspensions can be brought into contact by manipulation (e.g., using electrophoresis, dielectrophoresis). The quality of thusly achieved contact is lacking as compared to the level of contact when cells are growing in monolayers or

clusters. Neighboring cells in cell cultures form spontaneous contacts by connecting via different membrane structures. These contacts are formed rapidly (within 20 min) of cell plating (Ušaj et al. 2013). Kotnik et al. (Kotnik et al. 2010) studied the effect of cell connections on the induced TMV by means of an in vitro model of CHO cell clusters. Cells in such clusters are interconnected by gap junctions. These junctions form conductive pathways between the cytoplasm of one and another interconnected cell. By consequence, if the connected cells are exposed to a non-electroporative pulse, the cluster will act as a single large cell with a single cytoplasm. On the other hand, an electroporative pulse will result in a higher induced TMV across the membranes, impacting the dynamics of the opening and closing of gap junctions. As gap junctions become affected by electroporation, cells in the cluster start behaving as individual cells. This allows for molecular transport at membrane areas where the cells form connections with each other.

Although clustered cells behave as though they are individual entities with regard to electroporation, their shape and orientation in a plated cluster can vary. Cells are spread over the surface to which they are adhered and irregularly shaped, thereby increasing their size in comparison to cells in suspension. Electroporation can, in surface-adhered clusters of cells, be detected at lower electric field strength than in suspensions (Rols and Teissie 1989). But as we gradually increase the electric field strength, we notice that larger cells whose orientation is longitudinally aligned with the electric field (i.e., their long axis is parallel to the electric field vector) get electroporated at lower field strengths (Valič et al. 2003). Note that this holds for longer pulses (e.g., tens or hundreds of μs), but not for short (Dermol-Černe et al. 2020). Still, the amount of transmembrane molecular transport is lower for monolayers as compared to suspensions (Pucihar et al. 2008). Partially the reason is the effective reduction in the electric field by the neighboring cells (field shading) and partially the hindered diffusion of molecules (for both cell-to-cell and cell-to-surface contacts).

Overall, we can conclude that cells in an assembly will respond to electric pulses similarly as will single cells in dilute suspensions of nearly perfect spherical shape, provided we account for the local electric field distribution. With the latter, it is important to determine the actual field strength an individual cell is exposed to. The knowledge of basic characteristics of membrane electroporation as deduced from experiments on single cells is transferable, in a large degree, to cells in assemblies. However, the experimental studies also highlighted the importance of cell assembly heterogeneity (shape, size, orientation, and sensitivity to electric pulses), cell clustering (hindering inter-cell diffusion), and possible implications of other structures (e.g., the extracellular matrix, which is absent in suspended cells). The theoretical analysis of reversible and irreversible electroporation as correlated with the electric field distribution in tissue is therefore indispensable if we are to understand how different factors affect cell electroporation in assemblies and how to transfer (or "upscale") the knowledge obtained in vitro on cell suspensions to structures such as adhered cell monolayers or even tissues (Miklavcic et al. 1998; Miklavčič et al. 2000; Kranjc et al. 2016).

When theoretically modelling tissue in relation to studies of the phenomenon of electroporation, we most often work under the assumption that the tissue is a homogeneous structure. This homogeneity is meant in the sense of some hypothetical bulk electrical properties, i.e., conductivity and permittivity, which we can measure. By such analysis we of course completely neglect the cellular structure of tissue. Such simplifications can be justified by their benefits, chief among them being the computational cost which arises from modelling cells individually in a large volume. The second benefit stems from incomplete knowledge on how structures in tissue manifest themselves as bulk tissue properties.

In the bulk tissue model, the heterogeneity of the tissue structure is reflected in the functional dependence of these electrical properties on the frequency (Čorović et al. 2010) and on the applied field (Gabriel et al. 1996; Sel et al. 2005). The frequency dependency is tissue-specific, since tissues differ in their microscopic structure (shape, size, orientation, and cell density). Also, some tissues exhibit anisotropy due to cells' orientation in one preferential direction. Skeletal muscle tissue is a prime example of an anisotropic structure, where the long muscle fibers conduct the electric current more readily in the direction parallel than perpendicular to the fibers (Miklavčič et al. 2006).

If bulk properties of a tissue are known, it is possible to calculate the macroscopic electric field distribution inside with relative ease for an arbitrary electrode configuration. As tissues are also subject to a threshold phenomenon for electroporation with respect to the electric field strength, it is possible to do a comparison between numerically determined field strength distribution and the experimentally determined reversibly and irreversibly porated tissue area/volume, in order to determine the reversible and irreversible electroporation threshold, respectively (Miklavčič et al. 2000; Šel et al. 2007).

The daunting challenge here is, once having determined the "bulk" tissue electroporation thresholds of reversible and irreversible electroporation, how to then relate these bulk thresholds back with the micro-level, local electric field as experienced by individual cells. One simple possibility is to model a simplified, "average" representative cell in tissue and then treat the problem as though the cells are in a dense suspension. Thus it has been shown (Miklavčič et al. 2000) that the behavior of cells in tissue is not unlike the behavior of cells in dense suspensions, at least when considering the electroporation threshold.

The simplified treatment just described does however not account for local tissue conductivity changes as resulting from electroporation. If a square-wave pulse is applied by means of needle electrodes inserted into tissue, the electric field distribution will be inhomogeneous. Do note however that this heterogeneous current density is still present, although less pronounced, in other electrode geometries, including a seemingly homogenous field-producing configuration of plate electrodes. In those regions exposed to above reversible electroporation threshold field strengths, tissue conductivity will increase due to increase in cell membrane conductivity. These localized changes in conductivity consequently change the electric field distribution both during the pulse and between individual pulses (provided several are applied in a train with inter-pulse pause short enough not to permit

membrane resealing). The electric field strength thus becomes higher in those regions which have not yet experienced noticeable electroporation and remain less conductive. This results in a gradual propagation (though self-limited) of the electroporated tissue area. These conductivity changes have profound and significant implications for determining thresholds for tissue electroporation based on comparison of experimental results and numerical modelling. The importance of accounting for conductivity changes and the local field strength distribution in tissue has been highlighted in numerous studies (Miklavčič et al. 2000; Sel et al. 2005; Neal 2nd et al. 2012; Neal et al. 2015; Corovic et al. 2013; Bhonsle et al. 2015). One of the difficulties in estimating tissue conductivity changes is that they form an inhomogeneous area within the target tissue and cannot be directly resolved (spatially) by measuring the electric current between the electrodes. Important progress in this direction has nevertheless been achieved recently by a novel technique permitting imaging of local tissue conductivity during pulse application using electric impedance tomography (EIT) (Davalos et al. 2002, 2004; Granot et al. 2009). This technique was then further advanced and developed into a complementary technique of magnetic resonance electric impedance tomography (MREIT) (Kranjc et al. 2015, 2016), permitting imaging of electric current density distributions in tissue during electroporation.

If considering electroporation in connection with food processing applications, by far the most common target tissue for the treatment will be plant tissue. As shown by the schematic representation of plant tissue in Fig. 7 (left-hand side), the most distinguishing feature of plant tissue compared to animal tissues is the structure of the extracellular matrix, i.e., the cell wall. The cell wall is there not only to support the plasma membrane and give the cell its shape but also as a warrant of structural integrity, enabling the cell to withstand pressure differences across the membrane that can be enormous (the cell would otherwise burst in an instance due to turgor). It acts as a selective filter, allowing water and ions to freely diffuse, while posing a limiting factor to transmembrane transport of large molecules (over approx. 20 kDa in size). However, smaller molecules (up to 10 kDa) can pass between cells of some

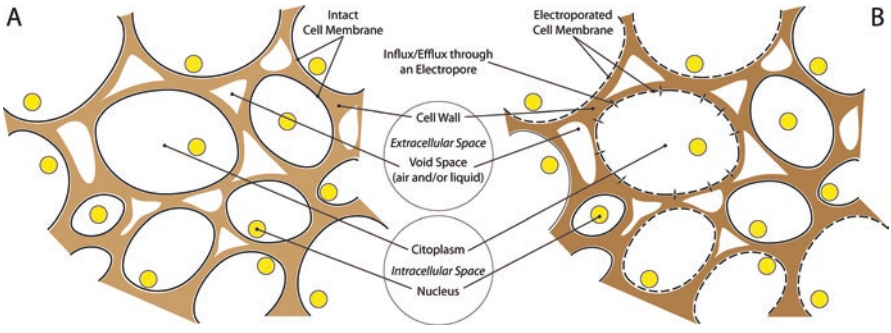

Fig. 7 A considerably simplified schematic representation of tissue comprising cells with intact membrane (left) and electroporated membrane (right). The cell membrane delineates the intracellular and the extracellular space

higher plants through structures known as plasmodesmata. Plasmodesmata are specialized cell-to-cell junctions extending through the cell wall (Lodish et al. 2008). Note that these junctions are not shown in Fig. 7.

If plant tissue is intact, its plasma membrane represents the greatest resistance to cell-to-cell transport and to (transmembrane) transport between the intracellular and the extracellular space. Moreover, there is the additional hindrance and filtering behavior of the cell wall. Both of these limiting structures must be considered when studying mass transport in plant tissues (Buttersack and Basler 1991). This must be contrasted to the problem of animal tissues (Dermol-Černe et al. 2018; Dermol-Černe and Miklavčič 2018), where, although an extracellular matrix is present and to a degree is a hinderance to transport, cells are not embedded in an extracellular structure akin to a cell wall. The cell wall in this sense presents an additional level of complexity (Dymek et al. 2015) that must be considered when studying electroporation-mediated mass transport to, from, and within plant tissues (Janositz and Knorr 2010; Janositz et al. 2011).

Figure 7 (right-hand side) is a simplified representation of electroporated plant tissue. Plasma membrane's permeability is illustrated as increased by the treatment and has lost the ability to selectively control the influx/efflux of water and of solutes that are able to pass through the membrane (subject to their hydrodynamic size). According to current understanding (Galindo et al. 2008; Ganeva et al. 2014; Stirke et al. 2014), electroporation may also affect the cell wall by either decreasing or increasing its permeability. Recent studies' results are contradictory, highlighting the need for further studies in the fundamentals of electroporation-based phenomena, something that has historically not been receiving due attention in the field of food processing applications of electroporation (this trend has recently seen a turn for the better). Nevertheless, according to recent literature, we can summarize that the limited electroporation effect on the cell wall is dependent on cell configuration (tissues, monolayers, cells in suspension), the organism (yeast or plant species), and the treatment protocol. Observed increase in permeability of electroporation-treated (plant) tissue remains attributable in the largest extent to the increase in permeability of the cell membrane.

As mentioned in preceding sections of this chapter, electroporation can irreversibly affect a cell membrane, causing it to ultimately break down. Plant cells are in no lesser extent subject to this phenomenon than animal cells and tissues. However, in case of plant tissue, the cytosol, parts of the disintegrated membrane, and the intracellular material will most likely remain entrapped in the extracellular matrix following irreversible electroporation. Given the enormous turgor pressures in some of the constituent cells in plant tissues, it is questionable whether we can speak of reversible electroporation, given that once the osmotic balance of a cell is disrupted, the cell is no longer able to control the influx or efflux of water.

Conclusions

Even though the mechanisms of electroporation have been investigated for at least four decades (and in food processing, applying electric fields to food matrices has an even longer tradition), there are still open questions remaining to be answered. One of the main reasons why understanding electroporation is posing a challenge is the wide range of spatial scales (from nm-thick membranes through μm-sized cells and up to cm-scale tissue segments) and temporal scales involved (from ns to hrs, or even days, as evident from Table 1). Therefore, investigation of electroporation necessitates a multi-scale modelling approach, ranging from MD simulations to large-scale continuum models of cells and tissues, coupled with systematic experiments at every stage of the modelling way. In recent years, such an approach has indeed resulted in significant progress (Kotnik et al. 2019).

The importance of quantifying transport, both the number of transported solutes into the cell and its temporal dynamics, is increasingly gaining recognition (Pakhomov et al. 2015; Sözer et al. 2017, 2018b). While there is a general consensus that the TMV induced by an electric field promotes formation of pores in the lipid bilayer, the contribution of other mechanisms to cell membrane permeabilization remains to be elucidated. Recently, the long-standing assumption that the pores formed due to the pulse application are also the primary conduits of transport for seconds and up to minutes after the pulse is increasingly being challenged (Smith et al. 2004; Son et al. 2014), since MD simulations failed to indicate that pores retain any stability once the induced TMV vanishes or drops to a very low level. One possible explanation could be that these "primary" pores evolve into more complex, stable pores that involve both lipids and other molecules. However, the molecular organization of these secondary, complex pores, remains elusive (Weaver and Vernier 2017). Another hypothesis is that lipid oxidation caused by exposure to the imposed electric field results in spontaneous formation of pores in areas of oxidized membrane lesions. Both possible explanations just mentioned imply that we may have to distinguish between at least two different types of pores. This has previously been proposed (Neumann et al. 1998; Pavlin and Miklavcic 2008), but a description of the underlying pore structure is lacking. Another possibility still would be that the non-transient (i.e., longer-lived) permeability following the pulse does not involve pores at all but is instead facilitated by leaky peroxidized membrane lesions and modified membrane proteins (Teissié et al. 2005; Mir 2020).

Answering these important and at least partially open questions is prerequisite to optimizing existing and developing new electroporation-based technologies and treatments, food processing applications included.

We conclude with a brief mention of the problems and challenges related to electroporation as applied to food processing (Lebovka and Vorobiev 2016). The nature of electroporation when applied to food tissues can be complicated, as it includes different scales, ranging all the way from single cells to suspensions and tissues with elaborate structure and profoundly expressed heterogeneity. What effects electroporation will have on plant material depends not only on the size of the

constituent cells but also on their orientation and spatial distribution. Also important are electrophysical parameters of cells, the pH of media, and the presence of osmotic agents. This means that optimization of electroporation treatment and electroporation-assisted processing of plant tissues entails, among other, precise adaptation of electroporation protocols, selection of the optimal electric field strength, optimal pulse duration, pulse repetition time, and the most suitable temperature. Mathematical modelling can provide valuable information when optimizing electroporation protocols in terms of both optimal power consumption and preservation of the food material quality. Additionally, numerical simulations can be useful when describing electroporation effects in the presence of complex transport processes and changes in temperature, electrical conductivity, electric field strength, and spatial distribution of the electrolytes in tissue during electroporation. In recent decades, various models and simulations were performed, aiming at optimizing electroporation for treatment of liquid foods, geometrical optimization of the treatment chambers, and prediction of the electric field distribution, flow velocity, and temperature inside the treatment chamber. Figure 8 illustrates electric field and temperature distributions in a parallel-plate (a–d) and a colinear (e–f) treatment chamber at the end of a single pulse of 100 μs with the fluid flow field taken into account. Notice the inhomogeneity that is particularly present in the colinear treatment chamber and is less pronounced in the parallel-plate flow chamber, though some heterogeneity is present there as well, in particular at the electrode edges (see the zoomed-in pair of subfigures, Fig. 8b, d). Since the available computing power available to researchers for modelling and simulation has greatly increased over the last couple of decades, it is now possible to construct and analyze using very detailed mechanistic models of equipment and processes, historically considered too demanding for computation. New, multi-physics approaches (i.e., modelling of highly interdependent, coupled quantities such as flow, temperature, electric field/current and conductivity, etc.) can now be employed for studying spatial distributions in systems of interest also in the temporal domain (dynamics!) in an efficient manner, as opposed to computing only the steady state or relying on experiment-based empirical models.

In preparing such a broad overview chapter on the fundamental principles underpinning electroporation as a phenomenon, one is faced with difficulties in summarizing existing findings, as experimental detail is lacking in many reports, making comparison of results from different studies difficult, if not impossible. To address this, it is extremely important that further studies published in future articles follow the recently published recommendation papers (Campana et al. 2016; Raso et al. 2016; Cemazar et al. 2018). This holds in particular for the problem of evaluating the local electric field strength, more often than it should be simply estimated as the voltage-to-distance ratio, in spite of diverse electrode geometries for many of which such estimation is an oversimplification.

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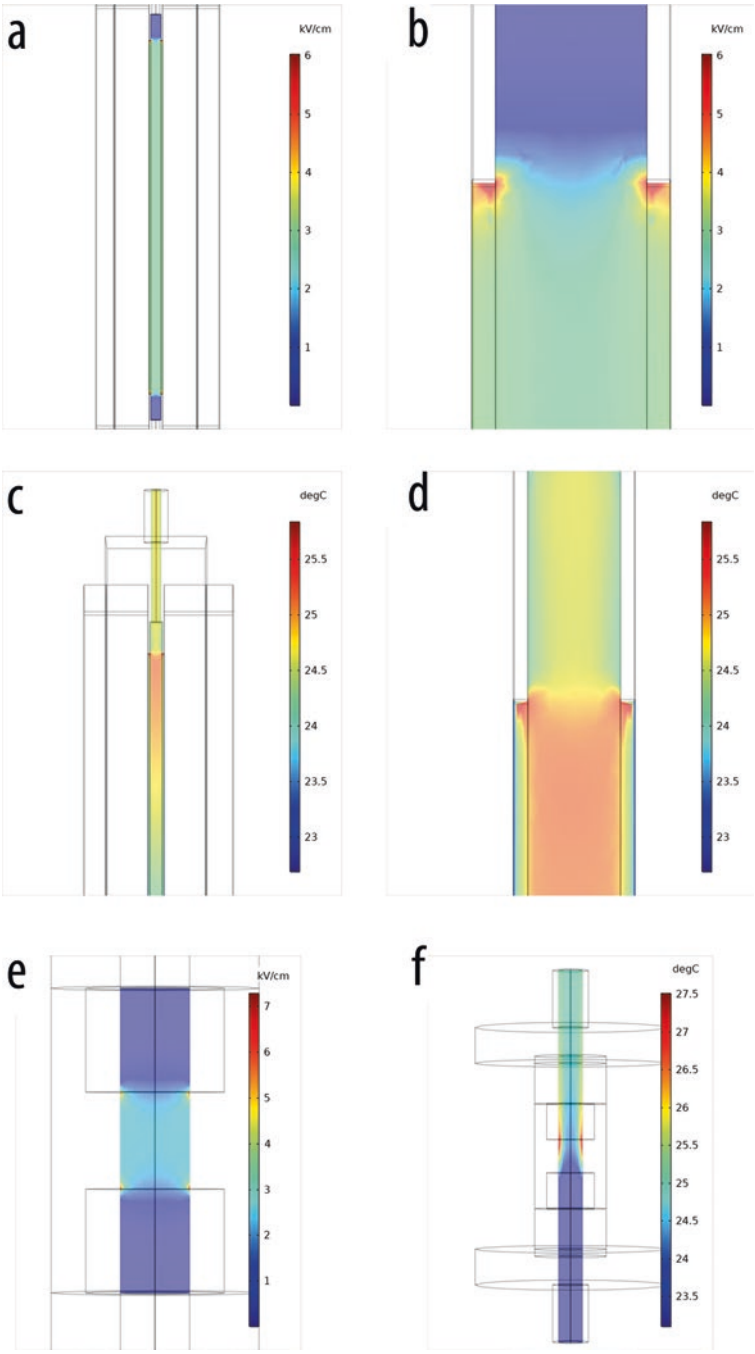

Fig. 8 Electric field strength and temperature distribution in a parallel-plate and colinear flow-through treatment chamber, in aid of illustrating one of the remaining challenges in electroporation research in the domain of food processing. The electrical, thermal, and flow relations in continuous flow treatment chambers are complex, and necessitate advanced multi-physics computational models for accurate description of conditions impacting treatment efficiency

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