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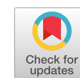
In vitro electroporation detection methods – An overview



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ABSTRACT

Exposing cells to an electric field leads to electroporation of the cell membrane which has already been explored and used in a number of applications in medicine and food biotechnology (e.g. electrochemotherapy, gene electrotransfer, extraction of biomolecules). The extent of electroporation depends on several conditions, including pulse parameters, types of cells and tissues, surrounding media, temperature etc. Each application requires a specific level of electroporation, so it must be explored in advance by employing methods for detecting electroporation. Electroporation detection is most often done by measuring increased transport of molecules across the membrane, into or out of the cell. We review here various methods of electroporation detection, together with their advantages and disadvantages. Electroporation detection can be carried out by using dyes (fluorophores or colour stains) or functional molecules, by measuring the efflux of biomolecules, by impedance measurements and voltage clamp techniques as well as by monitoring cell swelling. This review describes methods of detecting cell membrane electroporation in order to help researchers choose the most suitable ones for their specific experiments, considering available equipment and experimental conditions.

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1. Introduction

When biologic cells are exposed to a pulsed electric field of sufficient amplitude, their plasma membrane permeability increases. During this increased membrane permeability, molecules that otherwise cannot enter cells can be introduced to the cell interior or, on the other hand, cellular components can leak out of the cells. This phenomenon is termed electroporation. Electroporation can be reversible or irreversible (if the electric field is too intense for the cells to recover their membrane and cell functions) [1]. From its discovery in the late fifties of the past century [2], electroporation has been the subject of decades of extensive research and investigations,

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which has led to numerous applications in medicine (such as electrochemotherapy, gene electrotransfer, cell fusion and tissue ablation) [1] and food biotechnology (such as microbial inactivation and extraction of biomolecules) [3–5].

Although electric pulses act on all the membranes in the same way – making them more permeable – the extent of electroporation is very different. This depends on various conditions: pulse parameters (amplitude, duration, pulse number and repetition rate), membrane composition, surrounding media, the orientation of cells in the tissue, temperature etc. [6]. Each application requires a specific level of electroporation (e.g., for gene transfer: enough to introduce an active compound but, at the same time, without cell death) to be fully applicable [4]. For this purpose, the extent of electroporation must be explored in advance by using at least one of the methods for detecting electroporation. Moreover, these methods enable exploration of the basics of electroporation: the spatial and temporal dynamics of membrane permeabilization [7,8], the effects of electric pulse parameters and conditions (bathing media, temperature etc.) [9], species, cell type and tissue variations [10], to estimate membrane permeabilization [11,12] and determine thresholds for reversible and irreversible electroporation [13].

Electroporation and its extent is most often determined by detecting/measuring the increased transport of molecules across the membrane [14], either import of exogenous substances into the cell [15] or leaking of cellular components out of the cell [16]. Exogenous substances must fulfil two conditions to become successful detectors of plasma membrane permeabilization: 1. they must be non-permeant for an intact cell membrane and can enter the cells only after the plasma membrane is electroporated and 2. they have to possess an intrinsic characteristic that, in combination with a specific detection method, can give information about a molecule's transport into the cell. There are numerous substances that serve as electroporation indicators: from fluorescent dyes, which are most frequently used [17–22], colour stains [23], magnetic nanoparticles [24], functional molecules such as cytotoxic bleomycin [25,26], to the largest, nucleic acids [27]. In addition to exogenous molecules and cell leakage, electroporation can also be detected by physical and chemical methods, such as conductivity and impedance measurements [28], voltage clamp methods [29] or cell swelling [30].

We review here and briefly describe different methods of electroporation detection (Fig. 1) in order to help researchers choose the most suitable ones for their particular experiments. We also highlight the advantages and disadvantages of specific method and provide references to original reports.

2. Methods of detection of plasma membrane electroporation

2.1. Transport of non-permeant exogenous substances

A plasma membrane functions as a selective barrier between the cell interior and the environment and enables a cell to maintain concentrations of solutes in the cell different from those in its environment, i.e., extracellular media. Small non-polar and uncharged polar molecules can diffuse across a lipid bilayer. On the other hand, due to the hydrophobic interior of the lipid bilayer, a plasma membrane is non-permeable for most large uncharged polar molecules and charged molecules, including ions. Transfer of these molecules across the membrane is achieved with various transport mechanisms using membrane transport proteins (carriers and channels) [31]. Some molecules enter cells by different ways of endocytosis: they are internalized by invaginations of the plasma membrane, whereby a portion of the extracellular medium containing these molecules is enclosed in endocytotic vesicles. However, for further use of these molecules, a cell has to be able to process them into a form that can escape endocytotic vesicles or be transferred to other cellular compartments [32].

Most exogenous molecules, however, lack such transport mechanisms and thus cannot cross a plasma membrane: they are either too hydrophilic or too large for simple diffusion through the lipid bilayer and are also not transported via any membrane transport proteins [33]. Such non-permeant molecules are good candidates for the detection of plasma membrane electroporation, since the application of an electric field creates hydrophilic pores in the lipid bilayer and, during electroporation, membrane permeability for these molecules is at least temporarily increased [34]. In fact, quite a number of these molecules (e.g., propidium iodide, trypan blue), which were originally widely used to determine viability (to test whether the plasma membrane has been compromised) later served as a tool for detecting membrane electroporation. However, in the case of membrane electroporation, it must be taken into account that the plasma membrane is only temporarily permeabilized and can reseal [35].

Some of these non-permeant exogenous molecules have special properties that lead to the development of detection methods that

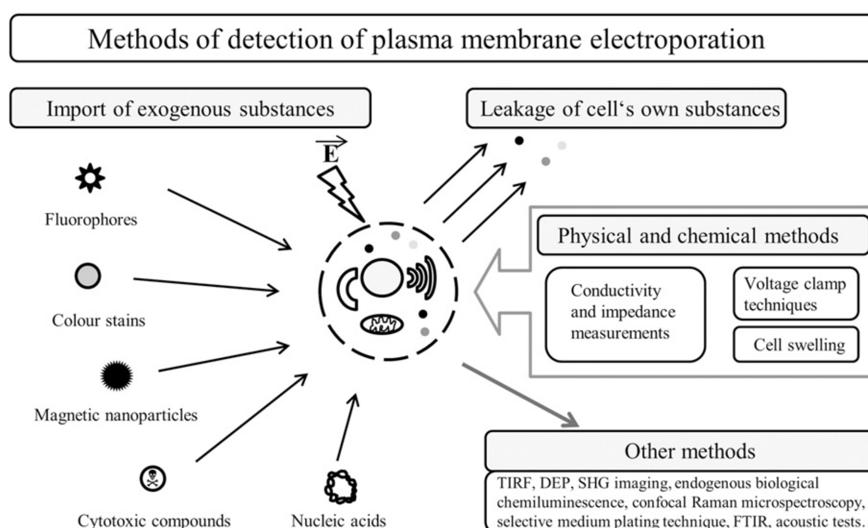

Fig. 1. Graphic outline of methods used for plasma membrane electroporation detection. Abbreviations: TIRF – total internal reflection fluorescence microscopy, DEP – dielectrophoresis, SHG – second harmonic generation, FTIR – Fourier transform infrared spectroscopy.

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