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Electroporation – Basic Principles and Applications

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Abstract: Electroporation is a widely used method for the delivery of large and charged molecules like DNA into the cells. Although the precise mechanism is not clear, formation of transient pores in the cellular membrane enables the transfer into both, prokaryotic and eukaryotic cells. A wide range of protocols with varying voltage, pulse duration and other parameters suits to virtually all conditions. Recent advances in the field include the use of high-throughput in vitro electroporation with cuvettes in a 96-well microplate format and in vivo applications. Non-viral gene therapy vectors applied in vivo with electroporation into muscle cells are now even moving to the clinical use.

Keywords: Electroporation, DNA, Cells, Voltage, Pulse duration, Review

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1. Introduction

Transfer of substances through the plasma membrane can be achieved by the cell via active or passive transport with the use of pumps, channels or simple diffusion. In contrast to the variety of possibilities a cell possess, it proved quite difficult to get exogenous molecules into cells that do not take them on their own. In general, chemical and physical methods can be distinguished. Liposomes, polymers like polyethyleneimine or polyethylene glycol and other chemicals have been proved as effective in enabling lipofection or other forms of transfection with therapeutic molecules or markers [1].

However, in several applications, the most commonly used approach is based on the effects of the electro-magnetic field on the cellular membrane called electroporation [2]. Principle steps in the electroporation process are shown in Fig. 1.

2. Principles and applications of electroporation

The discovery of the electric effect at cells opens new opportunities of transfer of substances through the plasma membrane. First experiments were focused only on small molecules e.g. sucrose, dyes. It was found out later that, with the right voltage and duration, it is possible to transport such huge molecules as DNA into cells. A new way for gene therapy was opened up. That was possible because of the discovery of the square wave pulse generator, which replaces the exponential wave pulse. Pulse amplitude and length could be set separately at the new square wave pulse generators.

Firstly, what should be understood under the term electroporation? Approximation needs some imagination. A balloon full of helium has a special ability – it can repair its surface. So if something goes through it, the surface repairs itself in a given time. If we want to get small objects into the balloon, we can use a bow and an arrow. If the bow is strong enough, the arrow will fly through the balloon and make two holes in the surface. For a short time it is possible now to get small objects inside. The balloon is a cell, its surface is the membrane and the bow is the voltage between electrodes of the pulse generator. The arrow represents the electromagnetic pulse that generates the holes – pores in the plasma membrane. Time needed to repair the surface is the time of membrane recovery. Nowadays, protocols are available to introduce large molecules and complexes into cells [3].

These four parameters are important for in vitro electroporation and are included in protocols. Cell size – larger radius of a cell means a higher potential needed. Temperature – membrane recovery is strongly affected by temperature. Manipulation with cell after pulse – it is not recommended to manipulate with the sample during the first 15 minutes after electroporation. Composition of electrodes and medium – these affect the electrical capacity, and, thus, the current flow. In addition, during long pulses parts of the electrodes can break down and damage the sample.

Although the range of applications of electroporation is wide, the transfer of DNA is the most common use of this technique. DNA molecules, usually plasmids, are large and charged, what makes the transfer challenging. Increase in the pulse time and voltage can overcome this hurdle. Interestingly, calcium ions seem to decrease the efficiency of electroporation-mediated DNA delivery in vivo [4]. The electrophoretic movement of DNA with a negative charge in the electromagnetic field can further improve the transfer efficiency both, in vitro and in vivo. Currently used protocols for in vivo electroporation suggest a 3-times change of the current direction by swapping the electrodes (Fig. 2).

It is crucial for some in vitro applications to have the possibility to upscale the process of transfection to a high-throughput level. Preparation of sequence libraries, in vitro evolution and selection, transfection of bacterial collections or transfection under variable conditions, all these techniques can be done with the use of microplate-based cuvettes [5, 6]. The 96-well format became available on the market few years ago and it saves a lot of time and hand-work in labs using the previously mentioned methods. In combination with automatic pipetting stations and centrifuge rotors capable of microplates experiments that took weeks, it can now be done within one working day.

It has several advantages in comparison to alternatives like lipofection or the use of biological vectors electro-poration. It is simple, safe and requires virtually no consumables except for the electrodes that can be recycled in case of need. An important factor for choosing electroporation is the flexibility, as there is a wide range of protocols available for any application. Last but not least, the use of viral vectors or chemical transfection includes administration of foreign proteins, chemicals that induce a specific immune response if applied in vivo. Immunogenicity, however, means difficulties with repeated application, which is not the case with electroporation, as it induces very little inflammation even when targeting sensitive organs like the lung [7]. On the other hand, electro-poration is relatively harsh to cells and tissues. Despite optimization of the used parameters, in vitro and in vivo the price for high transfection efficiency is a high cytotoxicity. Depending on the application, this might be an issue and a reason for using an alternative approach.

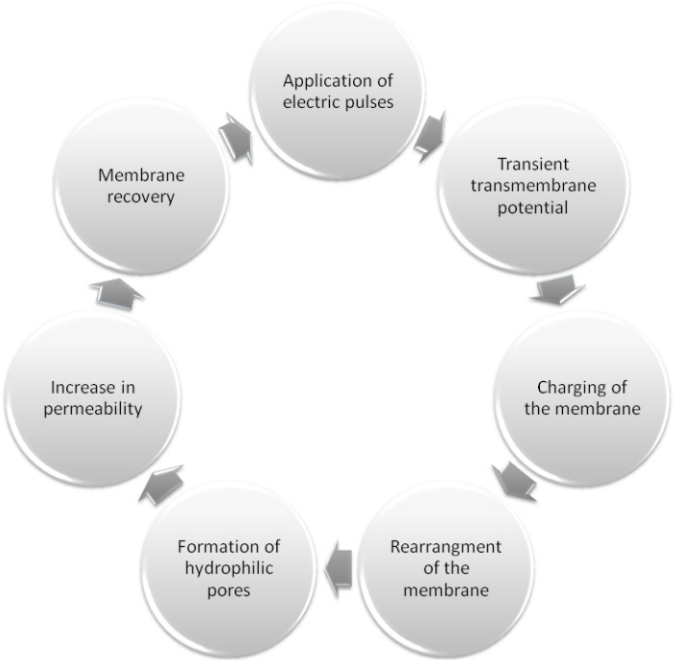

Fig. 1. Step-by-step diagram of the processes on the cell membrane during electroporation.

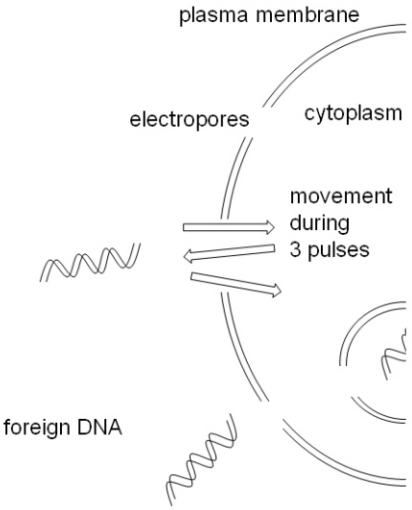

Fig. 2. Transfection of a cell using exogenous DNA with electrophoretic movements during 3 electroporation pulses with changing the current direction by swapping electrodes.

Electroporation is a method of choice for the transfection of bacteria with plasmid DNA [8]. However, the use of non-viral vectors with low transfection efficiency per se, stimulated the development of numerous in vivo protocols. Most of the protocols and electrodes are designed for electroporation of muscle tissue. Specific organs like the kidney can also be electroporated [9]. Interestingly, for the treatment of renal fibrosis, even adenoviral vectors were electroporated into the renal cortex [10]. Although electroporation in vivo seems to be rather regionally selective, it has been shown in the kidney that tissue selectivity can also be achieved with glomerular expression of electroporated transgene applied intravenously [11]. An important application of in vivo electroporation is the DNA vaccination with several clinical trials currently ongoing [12]. In DNA vaccination, but also in gene therapy approaches, low amount of administered DNA is preferable and electroporation-based in vivo experiments have shown, that the need for DNA is much lower in comparison to other non-viral approaches [13].

The future of electroporation is difficult to predict. The current increase in interest in gene therapy suggests that this technique might find its way to routine clinical medicine. The main field of applications will surely be in the experimental research with novel approaches being discovered like the postnatal creation of knock-outs using electroporation of the respective constructs very early in life [14].

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