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REVIEW

Electroporation: an arsenal of application

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Abstract Electroporation is a way to induce nanometersized membrane pore for exogenous substances delivery into cytoplasm using an artificial electric field. Now it was widely used for molecules transfer especially in molecular experiments and genetic aspects. In recent years, modern electroporation on the embryo was developed, whose most important point is that it adopts low energy and rectangular pulse that could obtain high transfection efficiency and low damage to the embryo. This paper reviewed on the pool of application: from lab works to human clinical treatments.

Keywords Electroporation · Gene transfer · Electrotransfection · Electrochemotherapy

Introduction

Electric field was found to induce diameter-voltage-dependent pore on cell membrane that will reseal rapidly. The hydrophilic pore was induced by electric pulses on human erythrocyte (Kinosita et al. 1977a, b, c), and successful plasmid delivery to mammalian cells was achieved at 1982 (Neumann et al. 1982; Wong et al. 1982). After that, electroporation models were built on both monocot and dicot plant cells (Fromm et al. 1985, 1986; Ou-Lee et al. 1986), bacteria cells (Schivarova et al. 1983), yeasts (Hashimoto et al. 1985; Karube et al. 1985) and eventually embryos (Muramatsu et al. 1996; Akamatsu et al. 1999). The advantages of this method include precise transfection, higher efficiency when a smaller volume of materials was used, the fast detection of reporter genes and the less toxicity to tissues (Momose et al. 1999). It is also possible to perform electroporation on a part of the cell (Lovell et al. 2006) or introduce substances into organelles. In modern electroporation, the application of low-voltage protects organs or embryos from severe hurt, implying a promising future in human clinical treatments.

Electroporation on cell or cells: electrode manipulation to electroporation chips

The batch mode for large treatment of cells has already become a common laboratory tool for gene transfer and nonpermeant molecules delivery into cells (Neumann et al. 2000a). Besides nucleic acids, successes on different compounds of biological and medical interest such as antibodies (Chakrabarti et al. 1989; Berglund et al. 1991; Baron et al. 2000), peptides (Traas et al. 1987; Hashimoto et al. 1989.)

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and enzymes (Winegar et al. 1989; Yorifuji T et al. 1990) were also achieved.

Different electric pulses have been used to induce membrane pore formation, varying from Direct currents (high field strength) (Kinostia et al. 1977a, b, c), exponentially attenuating currents (low field strength compared to DC) (Sowers 1984), to continuous Alternating currents such as oscillating electric field (Chang 1989a,b) and rotating electric fields (Arnold et al. 1982; Schwan 1989). Alternating currents and other multi-pulses, efficiently using the compressive stress of the electric field, were reported to show high efficiencies compared to DC pulses (Wang et al. 2000). However, more theoretical works are needed to seek for the reasons of different efficiencies, which was not included in this review.

The first electroporation experiment on single cell was carried out using carbon fiber microelectrodes (Lundqvist et al. 1998), and fluorescein was introduced into the cultured neuronal progenitor cells from adult rat. This indicates the possibility to perform electroporation without affecting adjacent cells and thus provides the means for biochemical manipulation of single cells in populations (Olofsson et al. 2003). There are also works of microelectroporations by the facilities of capillaries and micropettes (Nolkrantz et al. 2001; Haas et al. 2001). As to the electrolyte-filled capillary technique, the electric field is of lower strength nevertheless the duration of the electric field is longer. However, high voltage generators have to be used as the major potential was lost in the capillary considering its great resistance (Olofsson et al. 2003).

The idea of using microarrays emerged in 2000 (Neumann et al. 2000b; Huang and Rubinsky 2000). With the integration of several steps, continuous electroporation using flow-through microchips or other microfluidic devices were reported (Lin et al. 2002; Fox et al. 2006). At present these electroporation devices were roughly divided into three categories: analyzing cellular properties or intracellular content, transfecting cells and inactivating cells (Fox et al. 2006). Chip methods help to manipulate cells in a single manner under different parameters at the same time, which makes comparison of different settings on the same chip possible; nevertheless most of the chips so far have focused on studies of the electroporation process, and the in situ detection and live screening might add further value to these devices.

Electroporations in embryos, larvae and adult tissues: modern low-voltage manipulation

Currently electroporation was widely applied to in vivo gene transfer experiments, especially on embryos. Firstly, this approach offers ways of genetic manipulation in animals that lack the methods for targeted transgenic studies (Swartz et al. 2001). One of the most successful models was built with chicken embryos, which was also called In Ovo Electroporation and the first reported studies of in ovo electroporation used the firefly luciferase gene as the live reporter (Muramatsu et al. 1996). Moreover, this method could provide a quick way of gaining or loss of function. Compared to the traditional methods of knock-out/knock-in strategy that might take several months to get one transgenic mouse, the expression was narrowed to hours long. It is also suggest that the expression of mRNA could last from embryo time to postnatal days (Saito and Nakatsuji 2001). Thirdly, embryo electroporation uses the nontransfected side as the control, which is preponderant in samples that differ greatly with each other. It is also a facility to perform electroporation of different materials on several embryos at a time in the same pregnant mother.

In ovo electroporation has been used most successfully in investigations on genetic basis of brain regionalization and pattern formation (Swartz et al. 2001). The neural tube is an ideal place for ectopic material injection and retention because of its usable capacity and the ability to differentiate into neural systems. Misexpression of diverse transcription factors and signal molecules via electroporation have been used to study the isthmic organizing center located at the junction of midbrain and hindbrain (Araki et al. 1999; Katahira et al. 2000; Matsunaga et al. 2000), and molecules that participate in the dorsal-ventral polarity forming mechanism (Briscoe et al. 2000; Watanabe and Nakamura 2000); these experiments elicit series of molecular functional analysis in patterning the rostrocaudal and the dorsoventral polarity (Swartz et al. 2001). Overexpression of genes in specific group of neurons such as Purkinje cells was also reported via in vivo electroporation (Luo and Reides 2004). Additionally, a new way called ex ovo electroporation was developed to meet the need for investigation on the later stages of development without hurt of the vitelline blood vessels (Luo and Reides 2005).

At the same time, electroporation on mice embryos was also developed however most of them were performed on the brains considering that later closed neural tube will be less available. The first reported success was to demonstrate the inducing function of HuB and HuC that are ELAV-like RNA-binding protein (Akamatsu et al. 1999). Then fluorescent protein and LacZ reporter was adopted to establish the optimal parameters (Itasaki et al. 1999; Saito and Nakatsuji 2001; Tabata and Nakajima 2001). Gene transfer into single neurons in mouse brain slices (Haas et al. 2001) was reported and the gene deliver into adult brain was performed firstly in 2003 to seek for the role of calmodulin on synaptic plasticity in the anterior cingulate cortex (Wei et al. 2003). Electroporation in adult tissues largely expanded transfection works on mature animals and elicited the possibility for drug delivery and tumor regression via this method.

There are also applications of electroporation on other animals, for instance, *Xenopus* tailbud stage embryos (Eide et al. 2000) and Zebra fish (Buono and Pierani 1992; Muller et al. 1993). Additionally, there are reports of electrotransfer in insects. Electroporation on *Drosophila melanogaster* was firstly reported by Kamdar et al. (1992), then in *Helicoverpa zea* embryos (Leopold et al. 1996) and *Bombyx mori* (Moto et al. 1999; Thomas 2003). These results suggest the possible application of electroporation in invertebrate genetic manipulation and genome function analysis.

Adjust parameters

Electroporation could serve as a stable and speeded method in many cells and diverse organisms. The establishing of a successful electroporation model should consider the resistance, endurance of the tissue or the solution in which cells are suspended and electric currents that influence your voltage setting, the material of the electrodes that you use, the concentration and the amount of the substances that you would like to deliver. It is a need to note that embryo electroporation adopts low energy and rectangular pulses in order to obtain high transfection efficiency and low damage to the embryo. This is different with the settings for cells.

In the electroporations of chick embryos, HH10-15 neural tubes were manipulated under 20–25 V at five pulse times each of which lasted for 50 ms with a 4 mm electrode distance (Gould et al. 1998; Funahashi et al. 1999; Itasaki et al. 1999). The lower level of voltage significantly improved embryo viability, though a higher voltage resulted in the higher efficiency of gene transfer (Osumi and Inuone 2001). In the manipulation of mouse embryos, the adopted voltage varied from E8.5 at about 30V at the distance of 5 mm to E15.5 at 60 V with a electrode gap of 9 mm (Itasaki et al. 1999; Saito and Nakatsuji 2001; Osumi and Inoue 2001; Swartz et al. 2001); these electric fields were computed to be around 700 V/m to several thousands. Moreover, optimal parameters for insect embryos were reported to be at 250 V/cm with duration of 50 ms for 5–10 times. It was noted that compared with 5 pulses, the use of 10 pulses significantly attenuated the polarized spreading of the spots but with no dramatic increase of the number of X-gal spots (Thomas 2003).

Cell electroporation was still widely used to test different kinds of electric fields. For instance, it was shown that an extension at low voltage after the higher former pulse could, significantly, increase the electroporation efficiency (Sowers 1984). This might be explained as that the lower lasting electric field could inhibit the reseal, thus more substances were delivered into the cytoplasm (Sugar et al. 1987; Dimitrov and Jain 1984). Some electric fields have shown their advantages in both the cell viability and the transfer efficiency (Chang 1989a,b; Arnold and Zimmermann 1982; Schwan et al. 1989), but they were not broadly used for in tissue electroporation at present.

More applications in the future: from cells to human?

One of the most important points that increases the application of electroporation is the diversity of substances to be delivered, which includes dyes (often used to test the efficiency as well as the parameters) (Shin et al. 2004), heavy metal ions (Baker and Knight 1978), gene segments varying from plasmid DNA (Dityateva et al. 2003; Leclere et al. 2005) to small interfering RNA (siRNA) (Prechtel et al. 2006; Ghartey-Tagoe et al. 2006), antibodies (Lukas et al. 1994; Baron et al. 2000; Rui et al. 2002), enzymes (Dagher et al. 1992), drugs (Whelan 2002; Mori et al. 2003) and so on.

At present many of the electroporations were used for testing the function of a gene with gain or loss strategies spatially and temporally using plasmid DNA, siRNA (Ghartey-Tagoe et al. 2006), dsRNA and morpholinos (Mellitzer et al. 2002) and so on. Ectopic expression was also performed to test the function of target protein (Xiang et al. 2004). It is also possible to remove Loxp-flanked genes by introducing Cre-recombinase mRNA into the cell (den Plas et al. 2003), which provides a better way of regionally gene removal compared to the diffusion of tamoxifen induction in Cre-ER mice. Overexpression using region-specific enhancer to monitor gene expression patterns over time in living embryos is also feasible (Itasaki et al. 1999; Luo and Reides 2004). There are also reports using electroporation to analyze the mRNA stability (Hilgers et al. 2005) and genome regulatory sequences (Uchikawa et al. 2004).

Furthermore, this method holds exceptional potential for gene therapy approaches to tumor, muscle or vasculature disorders (Mir et al. 1999; Swartz et al. 2001). A work that introduces DNA and small chemotherapeutic molecules (Rols et al. 1998) showed that mRNA could be detected after the plasmid DNA delivery in a long follow up time (Saito and Nakatsuji 2001), which is the key component for long-term clinical use (Vicat et al. 2000). Electroporation success in adult brain (Wei et al. 2003) raises the possibility of gene therapy in adult human brains. Recently, an innovation for gene delivery injecting DNA during insertion (Tjelle et al. 2005) provides efficient ways for transfection in large animals, which is significative for clinical use. Moreover, we can expect further clinical treatments for human disorders in many organs, especially into organs such as brains and testis which has the drug-rejected blood-barrier. The low-energy manipulation is also of little suffering, providing a promising future into a non- or part-anaesthetic use.

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