MAGNETO-ELECTROPORATION: ENHANCEMENT AND TARGETING OF GENE TRANSFECTION USING MAGNETIC NANOPARTICLES AND MICROCHIPS

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ABSTRACT
This study demonstrated that DNA associated with magnetic nanoparticles can be attracted to specific areas of cell surface under magnetic fields, which highly increased the DNA concentration at specific areas and further enhanced the gene transfection in an electroporation (EP) method. Simulations and experimental verifications were conducted and showed a good correlation. This report focused on enhancement and targeting of gene transfection by using 6 nm dispersed superparamagnetic iron oxide (Fe₃O₄) and electroporation microchips.

Keywords: Electroporation, Magnetic Nanoparticle, Transfection

1. INTRODUCTION
Gene therapy is a potential therapeutic modality, which requires effective gene delivery into cells. Different types of gene delivery systems have been developed. Electroporation has been widely applied on gene delivery. The Microchip used to gene transfection had been reported [1]. For enhancing the transfection rate, nanoparticles have shown their potential on this domain. Using silica nanoparticles (diameter ~225nm) together with sedimentation methodology were employed to deliver genes. The calculated sedimentation rate is 8.3 mm/hr [2]. Magnetofection utilizing magnetic nanoparticles, the diameters of 400 nm-1µm, have significant progress in delivering genes by means of magnetic forces [3]. In this study, DNA plasmids associated with 6 nm dispersed superparamagnetic iron oxide (γ-Fe₃O₄) nanoparticles were attracted to specific cell surface areas using strong magnetic fields, followed by electroporation to deliver genes into cells, further providing enhancement and site-specific gene transfection.

2. EXPERIMENTAL
2.1. Microchip and Magnetic Nanoparticles Preparation
The EP microchips were designed in two components, the well-defined cavity for cell accommodation as well as the interdigitated electrodes for providing electroporation electric power, as shown in Fig. 1. The interdigitated Au/Ti thin film electrodes were fabricated on the glass slide by MEMS technologies [1]. The microelectrodes are 100 µm
wide with a 100-μm spacing. The cavity made of PDMS helps to get the desired cell density, and save culture cell process. The iron oxides particles have the diameter of 5.8 ± 0.5nm (Fig. 2). Experiment used 1×10³mM Fe₂O₃ particles in sucrose solution as the electroporation buffer.

2.2. Gene and Cell Preparation

The pEGFP-N1 plasmids coding for green fluorescent protein (GFP) and the mouse clonal Osteoblast-like cells line (MC3T3E1) were used in this study. For cell culture, the microchip was coated with 0.01% poly-lysine in phosphate-buffered saline for cell adhesion and then washed with PBS. The cells, at a concentration of 10⁵ cell/ml in a total volume of 50 μl, were seeded onto the pretreated microchip. The cells were maintained at 37°C, under 5% CO₂, in culture medium for 24 hrs. The mixture of GFP plasmids associated with Fe₂O₃ nanoparticles were added into the culture cavity prior to applying magnetic fields and electroporation process. The mixture of GFP plasmids and Fe₂O₃ nanoparticles was washed out by medium after electroporation process. After electroporation the cells were maintained in culture medium for 36 hr before detection.

2.3. Magnetic Field Simulation and Magneto-Electroporation

The transfection process was under a magnetic field combining with electroporation process using 1–3 V applied voltages. The asymmetrically magnetic field was created to generate a non-uniform density profile of the DNA-magnetic particle complex. Fig. 3 shows the schematic diagram of the DNA associated with magnetic nanoparticles attracted to the stronger field under an asymmetrically magnetic field.

To investigate the strengths of the magnetic fields in the cell-culture cavity, 3-D magnetic field simulations were performed using ANSYS 5.5 (Swanson Analysis System Inc., USA) run on a personal computer. The magnetic field distributions of different

Figure 1. 3D expanded view of the electroporation microchip (both the width and space of the interdigitated electrodes are 100 μm).

Figure 2. TEM photo of γ-Fe₂O₃ nanoparticles.
positions of magnet placed under the EP microchip were simulated and shown in Fig. 4. The positions of the magnet edge (L') related to the wall (L) of the cell-culture cavity were at boundary, quarter, half, or full of the cavity width (0.6 cm). To further evaluate the magnetic field effect to the enhancement of gene transfection, the position of the magnet was placed at the half of the cell-culture cavity to have a significantly asymmetric magnetic field.

Figure 3. Schematic drawing of the

Figure 4. Magnetic field distribution of an EP chip. (a) magnetic field distribution of the dashed line region in Fig. 3, (b) magnetic density along the center-line from the edge L of a cell-culture cavity toward the center, magnet located at the half width of cell-culture cavity exhibiting highest asymmetric magnetic density.

3. RESULTS AND DISCUSSION

A strong magnet (3k Gauss) was placed under the cavity at the position 0.3 mm from the left side of the cavity wall (Fig. 3) for 5 minutes to attract the magnetic nanoparticles-DNA complex to the cell surface. An asymmetric magnetic field was created and the maximum magnetic flux density was 2.5-fold higher than the minimum. Fig. 5 shows that the transfection rate under a stronger field is much higher than that under a weaker field.
To further prove the concept that the gene-superparamagnetic-nanoparticle complex had a strong correlation with the magnetic field distribution, an electromagnet was used instead of magnet. The electromagnet to generate a high magnetic field in an annular region, the large amount of the transfected-cells, expressing green fluorescence, occurred in the annularity with high magnetic field, as illustrated in Fig. 6. The transfection rate is highly dependent on the intensity of the magnetic field. The performance of the magnetic particles carrying the genes into the targeted cells could be attributed to the occurrence of the interaction, such as hydrogen bonding, between the nucleic acids and the –OH groups on the iron oxide surfaces.

4. CONCLUSIONS

This study used 6 nm superparamagnetic iron oxide nanoparticles to carry the DNA plasmids to the area with high magnetic field. Simulations and experimental verifications were conducted. This study successfully demonstrates the magnetic nanoparticles associated with DNA can help gene delivery and target a specific region utilizing a guiding magnetic field.

REFERENCES