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Technical notes

Conceptual design of integrated microfluidic system for magnetic cell separation, electroporation, and transfection

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ABSTRACT

For the purposes of a successful *ex vivo* gene therapy we have proposed and analyzed a new concept of an integrated microfluidic system for combined magnetic cell separation, electroporation, and magnetofection. For the analysis of magnetic and electric field distribution (given by Maxwell equations) as well as dynamics of magnetically labeled cell and transfection complex, we have used finite element method directly interfaced to the Matlab routine solving Newton dynamical equations of motion. Microfluidic chamber has been modeled as a channel with height and length 1 mm and 1 cm, respectively. Bottom electrode consisted of 100 parallel ferromagnetic straps and the upper electrode was plate of diamagnetic copper. From the dynamics of magnetic particle motion we have found that the characteristic timescales for the motion of cells (mean capture time ~ 4 s) and gene complexes (mean capture time ~ 3 min), when permanent magnets are used, are in the range suitable for efficient cell separation and gene delivery. The largest electric field intensity (~10 kV/m) was observed at the edges of the microelectrodes, in the close proximity of magnetically separated cells, which is optimal for subsequent cell electroporation.
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Introduction

One of the most promising fields of research lying on the border of medicine, biology, physics, chemistry, and engineering – "nanomedicine," is based on the applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems. In the forefront of this field is research into the rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents. The oldest and still most vivid subfield is applications of magnetic nanoparticles (MNs) in biology and medicine [1–4]. Multifunctional MNs have diverse potential applications in many biological and medical applications such as cell separation [5,6], drug targeting [7–9], electromagnetic hyperthermia [10], or magnetic resonance contrast enhancement [11]. Magnetic nanoparticle-based gene transfection (magnetofection™) has also been shown to be effective in combination with both viral vectors and with non-viral agents [12–15]. In these systems, therapeutic or reporter genes are attached to magnetic nanoparticles which are then focused to the target cells via high-gradient magnets. The technique has been shown to be efficient and rapid for *in vitro* transfection and compares well with cationic lipid-based reagents, producing good overall transfection levels with lower doses and shorter transfection times.

Electroporation, or electropermeabilization, is a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field. As has been shown the use of a two-pulse technique allows separating two effects provided by a pulsed electric field: membrane electroporation and DNA electrophoresis. The first pulse (e.g. 6 kV/cm, 10 μs) creates pores efficiently, whereas transfection efficiency is low. The second pulse of much lower amplitude, but substantially longer (e.g. 0.2 kV/cm, 10 ms), does not cause poration and transfection by itself, but enhances transfection efficiency by about one order of magnitude [16]. In two-pulse experiments, transfection efficiency rises monotonously with the increase of the second pulse duration. In another study, the transport of propidium iodide into electropermeabilized Chinese hamster ovary cells was monitored with a photomultiplier tube during and after the electric pulse. The transport became detectable as early as 60 μs after the start of the pulse, continued for tens of seconds after the pulse [17].

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The principle of our approach is used as a driving force instead of a second pulse inducing DNA electrophoresis into the cells, the magnetophoresis of DNA attached to magnetic particle. The electrophoresis last just for 10 ms, and magnetic movement of the DNA can be effective about 1000 times longer. The diameter of the pore in electropermeabilized cell membrane is of the order 100 nm, which is sufficient for the translocation of the DNA-nanoparticle complex. As has been already shown, electroporation can be used also for internalization of magnetic nanoparticles [18].

Another modification of this approach is to combine it also with magnetic separation. Cells with attached magnetic microparticles would be easily attracted to magnetic electrode, which is analogous to situation when cells form confluent monolayer at the bottom of Petri dish. At the standard magnetofection protocol, the magnet is placed under these cells and nanoparticles with attached gene added to solution are attracted to the cell layer and translocated through the membrane into the cell interior. Now we have analogous situation, the nanoparticles with DNA are also attracted to electrode, and therefore to bounded cells, although with smaller force compared to microparticles, and magnetofection take place. At this moment electric pulses can be applied. Because the separation between the electrodes in microfluidic systems is ~0.1–1 mm [19], and typical value of electric intensity in electroporation is ~100–1000 V/cm only low voltage of 10–100 V would be needed.

If we consider simple diffusion of a solute and denote T and L characteristic time and length scale, respectively, from a simple dimensional analysis follows:

$$L = \sqrt{TD},$$

where D is diffusion constant. Typical values of D are $D_1 = 2 \times 10^{-9}$ m^2/s for small ions, $D_2 = 4 \times 10^{-11}$ m^2/s for 30-base pair DNA, and $D_3 = 1 \times 10^{-12}$ m^2/s for 30-kilobase DNA [20]. During the time $T = 10$ s (when electrically induced pores are opened) these solutes are diffusing the distance $L_1 = 141$ μm, $L_2 = 20$ μm, and $L_3 = 3.2$ μm. These results are in an agreement with experimental observation that 99% DNA uptake into cell corresponds to membrane bound DNA [21]. That the diffusion of plasmid in the cytoplasm is very slow, especially for DNA molecules larger than 2000 base pairs was also shown using HeLa cells, where only very few DNA molecules were able to diffuse away from the microinjection site 1 h after delivery into the cytoplasm [22].

Our aim in this study is to design microfluidic flow-through system integrating magnetic cell separation with two transfection techniques – electric field for creation of transient pores in the cell membrane, where both magnetophoresis as well as electrophoresis would pull nanoparticles carrying DNA into cell interior.

Material and methods

Forces on magnetic particles

Magnetic force

Magnetic force acting on magnetic moment **m** in external static magnetic field with flux density **B** is defined as [23–25]

$$\mathbf{F_m} = (\mathbf{m} \cdot \nabla)\mathbf{B} \tag{1}$$

and drive magnetic moment in direction of external flux density gradient to the regions where the external field is homogeneous and there is zero gradient of magnetic field. This magnetic dipole is also affected by external field with a torque

$$\mathbf{M_m} = \mathbf{m} \times \mathbf{B} \tag{2}$$

which has tendency rotate dipole to the direction of external magnetic field, so that vectors **m** and **B** will have the same direction. Then the torque equals zero and moment is in the stable position.

Fluid drag force

A spherical particle moving relative to the fluid media in laminar flow regime (i.e. Reynolds number, $Re < 1.0$) in which it is submerged will experience drag forces. The drag force can be expressed as

$$\mathbf{F_d} = 3\pi\eta_f d\mathbf{v} \tag{3}$$

where η_f is the dynamic viscosity of the carried fluid, d is the diameter of the spherical particle, and **v** is the relative velocity of the particle to flowing media and can be expressed as $\mathbf{v} = \mathbf{v_p} - \mathbf{v_f}$, where $\mathbf{v_p}$ and $\mathbf{v_f}$ are the velocity of particle and fluid media, respectively.

Although spherical shape of the particle with attached active compound or cell on the surface is only approximation, Eq. (3) is frequently used for calculation of the fluid drag force on magnetic beads and other small particles.

FEM model of magnetic and electric field distribution

For the computational design of model of flowing through separator combined with transfection we need firstly know external magnetic field.

We have it numerically modeled [26] by finite element method (FEM) [27], using software FEMM (D. Meeker, http://femm.foster-miller.net). FEMM allows communicate as ActiveX client with MATLAB (The MathWorks), used for simulations.

In both cases of external magnetic field sources was FEM model simple approximation to the plane. In the case of permanent magnets as planar problem and in the case of Maxwell coils as axisymmetric one. We have labeled projection plane as x–z plane (in axisymmetric problem as: $x \equiv r$, $z \equiv z$). This simplification suppose, that y-dimension of the channel separator is several times smaller than y-dimension of source of external field, therefore channel can be represented as infinite parallel-plate channel instead of capillary.

To find distribution of electric field in the channel we have to solve two equations, one for the electric field intensity E:

$$\nabla \times E = 0 \tag{4}$$

and the second for electric flux density D, known as a Gauss' law, which says that the flux out of any closed volume is equal to the charge contained within the volume:

$$\nabla \cdot D = \rho \tag{5}$$

Electric flux density D and field intensity are also related to one another via the constitutive relationship:

$$D = \varepsilon E \tag{6}$$

where ε is the electrical permittivity.

To simplify the computation of fields which satisfy these conditions, electric scalar potential V defined by its relation to E as:

$$E = -\nabla \cdot V \tag{7}$$

is used.

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