Integrated Microfluidic System for Magnetic Cell Separation, Electroporation, and Transfection: Conceptual Design

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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 labelled cell and transfection complex, we have used finite element method (FEM) directly interfaced to the MATLAB routine solving Newton dynamical equations of motion. Microfluidic chamber has been modelled 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 time-scales 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. [1]*

Keywords: Magnetic Nanoparticles, Cell Separation, Cell Transfection, Simulation

1. Introduction

Electroporation, 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 ms) 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 [2]. In two-pulse experiments, transfection efficiency rises monotonously with the increase of the second pulse duration. In another study, was monitored transport of propidium iodide into electropermeabilized Chinese hamster ovary cells, and became detectable as early as 60 ms after the start of the pulse, continued for tens of seconds after the pulse [3].

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 electro-permeabilized 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 [4].

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, and typical value of electric intensity in electroporation is ~ 100 - 1000 V/cm only low voltage of 10 - 100 V would be needed.

2. Subject and Methods

Integrated microfluidic system – Basic description

Own separator consists of the channel (see Fig. 1), with flowing water carrying magnetically labelled objects (MLO) - cells or genes - by superparamagnetic particles (Table 1). Presence of electrodes above and below the channel, allows apply short high intensity electric pulse to generate electric field (FEM model (FEMM v4.2, D. Meeker, 2008) see in Fig. 2c) able to create membrane pores on cells localized in the chamber. Channel with electrodes are located in an external magnetic field with gradient (FEM model see in Fig. 2a, b), that allows us to use this field for separation of MLO and their targeting to the bottom surface of the chamber.

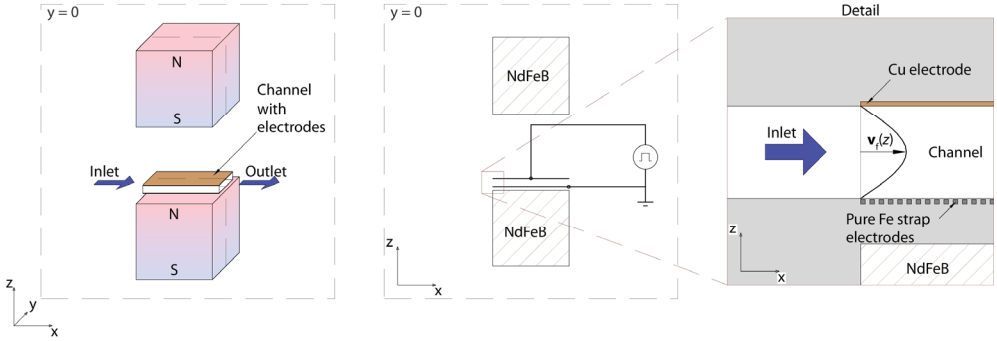

Fig. 1. A schematic illustration of a microfluidic system for combined separation, electroporation and magnetofection. The channel (treatment zone) with electrodes and two NdFeB magnets as source of magnetic field. (right) Detail of inlet of the channel with electrodes.

Table 1. Specifications of used magnetically labelled objects (MLO) [1].

Object	Diameter	Weight [kg]	Magn. moment [A m^2]
Magn. labelled CELL	10 μm	1.0×10^{-12}	1.0×10^{-11}
Magn. labelled GENE	300 nm	1.0×10^{-17}	2.0×10^{-15}

Simulation of motion

MLO motion simulation has involved a magnetic force and a Stokes viscous-drag force occurring in the Newton dynamical equation of motion for single particle problem:

$$m_p \frac{d\mathbf{v}_p}{dt} = |\boldsymbol{\mu}_p| \left(\frac{\mathbf{B}}{B} \cdot \nabla \right) \mathbf{B} - 3\pi\eta_f D_p (\mathbf{v}_p - \mathbf{v}_f) \qquad (1)$$

in which object is represented as sphere with diameter D_p, mass m_p, velocity $\mathbf{v}_p$ and has magnetic moment with magnitude $|\boldsymbol{\mu}_p|$. External magnetic field with flux density $\mathbf{B}$ has gradient $(\mathbf{B}/B \cdot \nabla)\mathbf{B} \equiv \mathbf{G}$. Water as carrying fluid media has dynamical viscosity η_f and

velocity $\mathbf{v}_f$. Its flow in the channel (with height h) is described as Poiseuille flow problem of Newtonian fluid with no-slip boundary condition on static infinite parallel plates, i.e. with parabolic profile $\mathbf{v}_f(z) = [v_{f,mean}\, 6(h-z)z/h^2, 0, 0]$ where $\mathbf{v}_{f,mean}$ is its mean velocity. As simplification we suppose planar magnetostatic problem and assume fully magnetically saturated MLO. Objects motion simulations in viscous fluid and external magnetic field described by Eq. 1 were done by using MATLAB (The MathWorks, Inc.) ordinary differential equations solver *ode45*, or *ode15s* and *ode23s* in the case if the problem was too stiff.

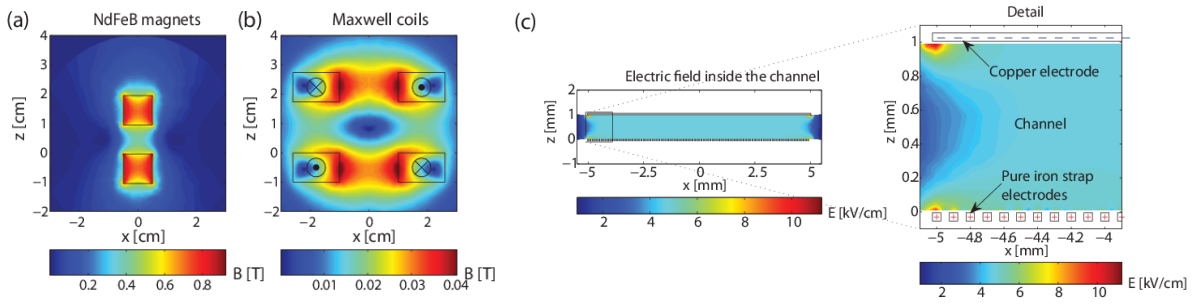

Fig. 2. (a, b) External sources of magnetic field (FEM): (a) two NdFeB N40 magnets, and (b) Maxwell coils [1]. (c) Electric field intensity distribution in the 1 mm height chamber with applied voltage difference 15 V between bottom and upper electrodes (FEM), which is sufficient for cell electropermeabilization.

3. Results and discusion

Electrodes designed so that to modulate external magnetic field by localized increasing of magnetic field gradient on bottom part of the channel (see Fig. 3), allow catch MLO to the bottom of the chamber along its whole length in suitable mean flow velocity ($v_{f,mean}$ in Fig. 4 or $v_{f,t}$ in Table 2), and prevent its next dragged movement by fluid ambient. The MLO capturing efficiency in the channel was described by time needed to move of the object from its random initial position in the channel to its bottom surface (by capturing) or out of the chamber (due to drift). As we can see from simulations results (Fig 4 and Table 2), motion of smaller MLO - genes (Fig. 4a) - in comparison with cells (Fig. 4b) is slower in about two orders of magnitude, and also for both types of objects in the case of NdFeB magnets as the source of external magnetic field is still in the range of periods for existence of membrane pores of electropermeabilized cells [3]. That shows possibility to combine magnetic separation of cells to the bottom of the chamber with their subsequent electroporation and magnetic targeting of genes to their surface, all-in-one integrated microfluidic system.

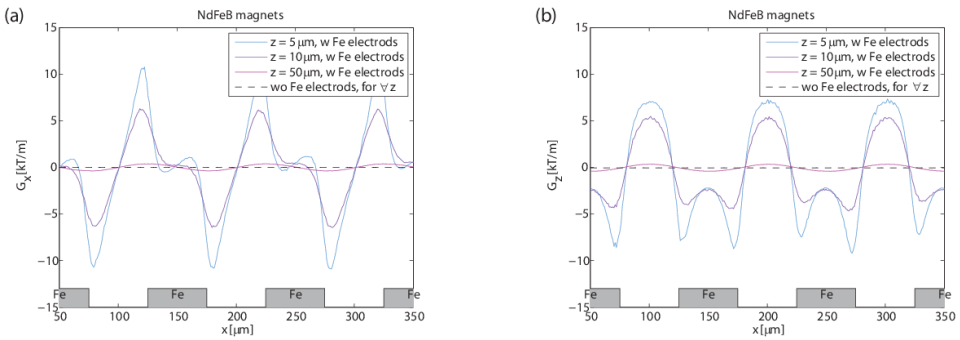

Fig. 3. Components: (a) G_x and (b) G_z of magnetic field gradient over the bottom strap Fe electrodes (z = 5, 10, 50 µm) in the middle part of the chamber in the field of NdFeB magnets (planar problem: $G_y = 0$).

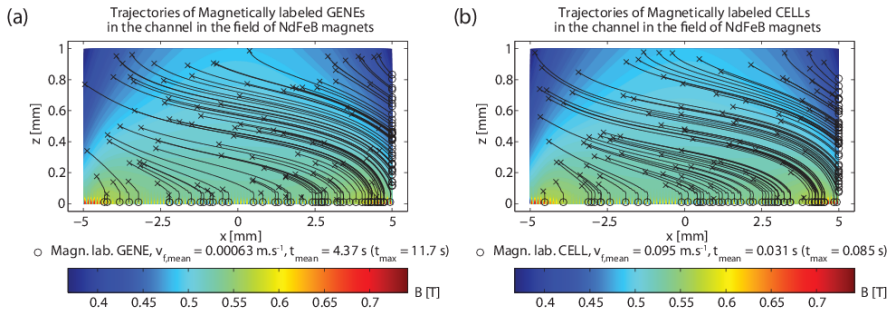

Fig. 4. Trajectories of magnetically labelled (a) genes and (b) cells in the channel with flowing water in the *x*-axis direction with mean flow velocity ($v_{f,mean}$) in the field of two NdFeB magnets. There are also shown the mean capture time of 100 objects (t_{mean}) and the movement time of the slowest one (t_{max}).

Table 2. Capturing of magnetic objects (magnetically labelled gene/cell) in the channel.

Object	External magnetic field sources					
	NdFeB N40 magnets			Maxwell coils		
	$v_{f,t}$ [a] [m/s]	t_{mean} [s]	t_{max} [s]	$v_{f,t}$ [a] [m/s]	t_{mean} [s]	t_{max} [s]
Magn. label. CELL	9.5×10^{-2}	0.031	0.085	2.6×10^{-4}	0.90	3.22
Magn. label. GENE	6.3×10^{-4}	4.37	11.7	1.7×10^{-5}	165	416

[a] Threshold velocity of fluid media flow for effective capturing of magn. label. objects along the bottom of the channel.

4. Conclusions

According to recent studies of magnetofection mechanism magnetic fields influence only the extracellular phase of lipoplex delivery but does not induce a direct entry of the lipoplex into the cytoplasm via the plasma membrane. Combination of the magnetofection with electroporation, which is able to open plasma membrane for magnetic nanoparticles bearing plasmid, as proposed in this study, is therefore very natural and able to overcome cellular barrier for gene transfer. Also, low voltages due to the close electrode spacing employed, leading to reduced heat generation and higher cell viability.

Acknowledgements

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References

[1] Durdík Š, Krafčík A, Babincová M, Babinec P. Conceptual design of integrated microfluidic system for magnetic cell separation, electroporation, and transfection. *Physica Medica*, 2012. In press. http://dx.doi.org/10.1016/j.ejmp.2012.11.003

[2] Chang DC, Chassy BM, Saunders JA. Guide to electroporation and electrofusion. San Diego, US: Academic Press, 1992.

[3] Pucihar G, Kotnik T, Miklavcic D, Teissie J. Kinetics of trans-membrane transport of small molecules into electro-permeabilized cells. *Biophys. Journal*, 95:2837-2848, 2008.

[4] Walczak P, Kedziorek DA, Gilad AA, Lin S, Bulte JW. Instant MR labeling of stem cells using magnetoelectroporation. *Magnetic Resonance in Medicine* 54 (4): 769-774, 2005.