Biological cell separation using dielectrophoresis in a microfluidic device

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Abstract

Basic IC fabrication techniques are employed in this paper with the purpose of designing a microfluidic device with a 3D electrode arrangement to separate live and dead biological cells. This is done using the concept of dielectrophoresis, which describes the transnational motion of particles due to the application of a non-uniform electrical field. The simulations where carried out using the protoplast model for mammalian spherical cells [4] in a wide range of electric field frequencies. Analytically, we have found that the Clausius-Mossoti Factor is negligible for live mammalian cells over a frequency range of 50 – 70 KHz whereas is maximum for the dead cells. Since the Clausius-Mossoti factor is the important term in the dielectrophoretic force formulae, we can envision our microfluidic design as a feasible tool to separate live and dead mammalian cells.

1. Introduction

In the past few years, there has been an extensive research in the manipulation and analysis of biological cells at the micro scale. There is an increase interest in applying microelectromechanical systems (MEMS) for selective trapping, manipulation and separation of bioparticles. Although there is a huge demand of automated single-cell manipulation and analysis in immunology, developmental biology and tumor biology calling for the development of suitable microsystems, the approaches currently available to meet those needs are limited [7].

The term dielectrophoresis (DEP) was first introduced by Pohl [9] to describe the transnational motion of particles due to the application of non-uniform electrical fields. The dielectrophoretic motion is determined by the magnitude and polarity of the charges induced in a particle by the applied field [8]. Usually, dielectrophoresis is performed under an alternating current (AC) field over a wide range of frequencies.

The DEP force is dependent on several parameters: the dielectric properties and size of the particle, the frequency of the applied field and the electrical properties (conductivity and permittivity) of the medium. Therefore, if is desired to achieve a good particle manipulation say cell separation, detailed analysis and careful selection need to be done in order to obtain the desired results.

In this paper, we are going to propose and analyze a microelectrode system incorporated in a microfluidic device, designed for the separation of live and dead biological cells using the dielectrophoretic force. As an application, it is desired to separate the cells to selectively apply medicine or for gene therapy using electroporation techniques [1,2].

2. Theory of Dielectrophoresis

Electrophoresis and dielectrophoresis describe the movement of particles under the influence of applied electric fields. Whereas electrophoresis is the movement of charged particles in direct current (DC) or low-frequency alternating current fields, dielectrophoresis is the movement of particles in non-uniform electric fields. The dipole moment m induced in the particle can be represented by the generation of equal and opposite charges (+q and –q) at the particle boundary. The magnitude of the induced charge q is small, equivalent to around 0.1 % of the net surface charge normally carried by cells and microorganisms, and can be generated within about a microsecond. The important fact is that this induced charge is not uniformly distributed over the bioparticle surface, but creates a macroscopic dipole.

If the applied field is non-uniform, the local electric field E and resulting force (E.δq) on each side of the particle will be different. Thus, depending on the relative polarizability of the particle with respect to the surrounding medium, it will be induced to move either towards the inner electrode and the high-electric-field region (positive DEP) or towards the outer electrode, where the field is weaker (negative DEP).

Following established theory, the DEP force F_{DEP} acting on a spherical particle of radius r suspended in a fluid of absolute dielectric permittivity ε_m is given by:

$$F_{DEP} = 2\pi R^3 \varepsilon_1 \{\mathrm{Re}[K(w)]\} \nabla E^2, \qquad (*)$$

where Re[K(w)] is the Clausius-Mossoti function and determines the effective polarizability of the particle and the factor ∇E^2 is proportional to the gradient and the strength of the applied electric field. The polarizability parameter Re[K(w)] varies as a function of the frequency of the applied field and, depending on the dielectric properties of the particle and the surrounding medium, can theoretically have a value between +1.0 and –0.5. The value for Re[K(w)] at frequencies below 1kHz is determined largely by polarizations associated with particle surface charge. While increasing frequency, first the effective conductivity and second the effective permeability are the dominant contributing factors. A positive value for Re[K(w)] leads to an induced dipole moment aligned with the applied field and to a positive DEP force. A negative value for Re[K(w)] results in an induced dipole moment aligned against the field and produces a negative DEP. The fact that the field appears as ∇E^2 in the equation of the DEP force indicates that reversing the polarity of the applied voltage does not reverse the DEP force. AC voltages can therefore be employed and, for a wide range of applied frequencies (typically 500Hz to 50MHz), the dielectric properties of the particle, as embodied in the parameter Re[K(w)], can be fully exploited.

The advantages of using microelectrodes

The advantages to be gained by reducing the scale of the electrode design can be illustrated using the example of the spherical electrodes for the case of a particle located one-tenth of the distance from the inner to the outer electrode [12]. For a 100-fold reduction of electrode size, a 1000-fold reduction of operating voltage will therefore produce the same DEP force on a particle in the same relative location.

In addition to the practical advantage of being able to use lower operating voltages for a given desired DEP force, there is also a significant reduction in electrical heating and electrochemical effects. The energy deposition form the field is proportional to σE^2, where σ is the conductivity of the suspending fluid. In the example just given, for a 100-fold reduction of electrode scale, because there is a ten-fold reduction in the applied electric field strength, the electrical heating is reduced 100-fold. In addition, as the surface area of the electrodes in contact with the fluid is decreased, surface electrochemical processes are reduced.

3. Test Structure

The test structure will be composed of two layers (bottom layer and top layer) that are going to be bonded together to form a microfluidic channel with a 3D electrode structure. The fabrication cross sections are depicted on figure 1. Both layers are going to undergo almost the same fabrication process with one difference, for the bottom layer we are going to use a single crystal silicon wafer (SCS) and for the top layer we are going to use a glass wafer. The glass wafer for the top layer was chosen to gain visual contact while the micro device is in operation.

The bottom layer fabrication process consists on the deposition and patterning of a 4 μm layer of silicon nitride (Si_3N_4) on the silicon wafer. The pattern on the Si_3N_4 is going to be 2 μm deep and it will be used to deposit the 3 μm wide platinum (Pt) electrodes by employing the liftoff technique (see Fig. 1a). The arrangement of the electrodes is going to be identical on both layers to achieve the desired electric field around the cells.

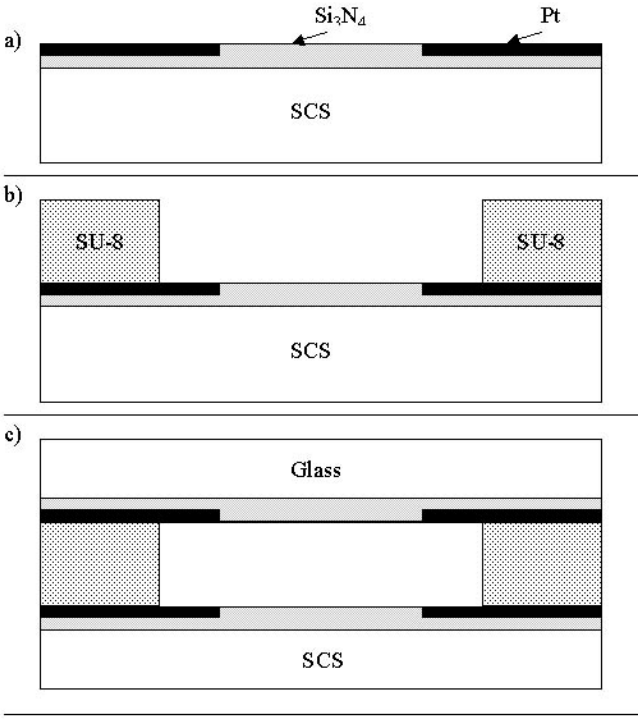

Figure 1: Micro fabrication cross-sections.

Subsequently, a 25 μm layer of negative photoresist (SU-8) is going to be deposited and patterned to form the walls of the microchannel, which are going to be 200 μm apart (see fig, 1b). SU-8 was chosen because of the ease to deal with and because of the good isolation properties. The top layer is going to undergo the same process as the bottom layer but with a glass wafer and without the SU-8 walls. The two layers are going to be bonded together carefully to achieve symmetry using NEA 121, Norland Products (see fig. 1c). Wells will be strategically etched on the SU-8 to simplify the gluing process (not shown above).

The operation of the microfluidic device is simple. We are going to employ figure 2 to explain the details of the test structure. The cells will be injected from a syringe pump gauge 18 using a 1.5 mm diameter plastic tube that is going to be glued to the wafer in section 1. The cells are going to flow through section A until they arrive at section B where the electrodes are going to centralize the cells to the middle of the microchannel [7]. The dielectrophoretic forces caused by the gradient of the electric field in the out of plane direction are canceled because of the electrode symmetry arrangement. The actual cell separation is occurring

at section C. A non-uniform electric field is chosen such that the dead cells are affected by a high positive dielectrophoretic force while the live cells are not affected or partially affected by a weak negative dielectrophoretic force. The differences in dielectric properties of the cells allow us to perform this. The live cells are going to continue an unaffected straight path through section D and the dead cells are going to be forced to take the path towards section E where they are being collected.

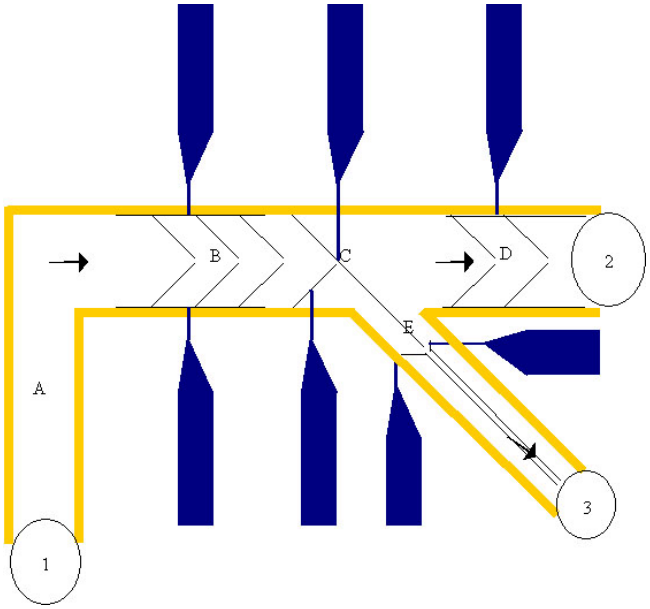

Figure 2: Top view of the test structure.

4. Numerical calculations and results

In order for the system to work adequately, some numerical calculations are needed to find the corresponding frequency that is going to influence the cells in a desirable way.
The mammalian cell model, which is going to be used, is the protoplast model [4]. The protoplasts are spherical particles where it is possible to identify a cytoplasm and a loss-less membrane (see figure 3).

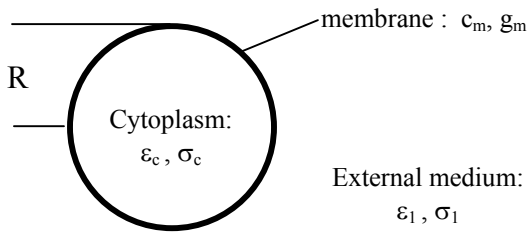

Figure 3: Protoplast model parameters

From [4], we found that the formula for the Clausius-Mossotti function is the following:

$$K(w) = -\frac{w^2(\tau_1\tau_m - \tau_c\tau_m^*) + jw(\tau_m^* - \tau_1 - \tau_m) - 1}{w^2(2\tau_1\tau_m + \tau_c\tau_m^*) - jw(\tau_m^* + 2\tau_1 + \tau_m) - 2}$$

The quantities c_m and R are the effective capacitance of the membrane and the radius of the cell respectively, while $\tau_m = c_m R/\sigma_c$ and $\tau_c = \varepsilon_c/\sigma_c$ are the time constants where σ_c is the electrical conductivity and ε_c is the electrical permitivity of the cytoplasm. We suppose that the conductance g_m is negligible (loss-less membrane). Finally the other constants are $\tau_1 = \varepsilon_1/\sigma_1$ and $\tau_m^* = c_m R/\sigma_1$.
To model a dead cell, we supposed that its membrane became irreversibly permeable. For this case the Clausius-Mossotti function is as follows:

$$K(w) = \frac{\varepsilon_c - \varepsilon_1 - j(\sigma_2 - \sigma_1)/w}{\varepsilon_c + 2\varepsilon_1 - j(\sigma_2 + 2\sigma_1)/w}$$

We choose DI water as very resistive suspension medium. Its parameters are $\varepsilon_1 = 78 * \varepsilon_0$, $\sigma_1 = 10^{-3}$ S/m.
The other model parameters are:
- Cytoplasm: $\varepsilon_c = 60 * \varepsilon_0$, $\sigma_c = 0.5$ S/m, R=2.0 µm.
- Loss-less membrane: $c_m = 1.0$ µF/cm^2, $g_m = 0$.

Figure 4 is the result of the numerical simulation for the Clausius-Mossotti function.

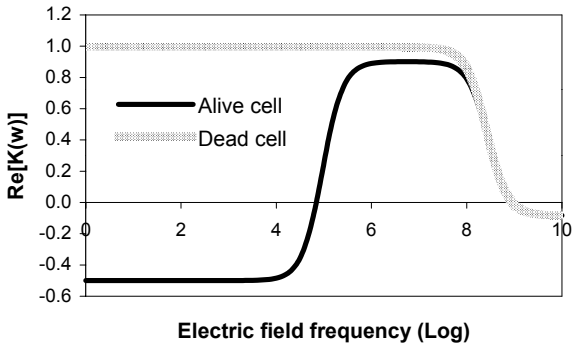

Figure 4: The Clausius-Mossotti function for dead and alive mammalian cells

It is important to notice that the dielectrophoretic force is directly proportional to the Clausius-Mossoti function. This relation is given by equation (*).

Alignment:
Sections B, D and E, from figure 3, allow us to align the cells and prevent contact between the cells and the walls of the microfluidic channels. In these sections, we can work with frequencies around 10 MHz where the Clausius-Mossoti factor is the same for dead and alive cells, therefore having similar dielectrophoretic forces.

Separation:
In section C, where the separation is occurring, we want to work in the region where Re[K(w)] is zero for live cells and

around one for dead cells. In this case, we know that the dead cells will experience a positive DEP toward the bottom channel (section E), whereas the live cells will not experience any force. We calculated that the desired frequency is around 70 kHz.
Finally, we will work with voltage around 10 volts and flows around 3500 μm/s, according to reference [7]. However it is important to know that we will need to adjust this numbers, in order to separate effectively the dead and live particles.

5. Review

The goal of this paper was to use the technique of dielectrophoresis to separate dead and live mammalian cells. The theory of DEP is well known but it is not obvious to predict how DEP will act on mammalian cells because these cells are complex: they are not simple sphere. A cell is a membrane and a cytoplasm. These two parts play a very important role in the calculation of the Clausius-Mossotti function. To simplify the problem, we used the model of protoplasts for live cells and simple sphere for dead cells. These assumptions allowed us to graphically represent Re[K(w)] and then find frequencies where we can expect to separate alive and dead cells. However, there is always the uncertainty of the theoretical analysis versus the real world. That is why we need the experimentation of the test structure, to make sure that our assumptions and simplifications are valid. If they are not valid, a reevaluation of the theory and the system is required.

If it works, what can we expect from this device?
If the test structure experimentally works, is evident that optimization is the next step. There are certain variables that must be clarified, like how fast can we deliver the separation of cells. Moreover, with what kind of cells this process can be performed. The separation speed is directly proportional to the size of the microfluidic device as well as the electrodes dimensions and dielectrophoretic force. The variety of cells in which this process can be performed depends on the dielectric properties of the particles as well as its size and shape. It is safe to say that a more thorough analysis should be carried out from a biological and fluid mechanics point of view.
However, it is possible to foresee a possible application for this system when coupled with electroporation. Electroporation employs electrical pulses applied across a cell for cell membrane permeabilization [2]. This technique is commonly used in biotechnology for genetic engineering or medicine application for cells in a batch. Our system will be of use for this particular application when the electroporation is performed only on the viable cells and not the dead cells. This will save a big amount of time when this process is performed in a continuous basis. In addition, the latest electroporation techniques are being carried out in chips fully compatible with the IC fabrication process, making it favorable for adaptation with our microfluidic system.

6. References

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