Design and Analysis of the Cell Microinjection Device

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Abstract. The orientation of the cell has a significant impact on the survival rate of the cell after it is injected. A novel microinjection device with the function of adjusting the position and orientation of the cell is designed based on the technology of PDMS microfluidic, the operation of the cell’s microinjection is accomplished by controlling the moving of the cell. The injection device consists of micro channels and microelectrodes. The injection microneedle is fixed on the bottom center of a cylindrical area in this device, which joints several micro channels. The micro fluid ejected from the microchannel is employed to drive the cell rotate to adjust its orientation. The frequency of the alternating current signal applied on the electrode can be adjusted so that the dielectrophoresis force can be controllably varied. Therefore the motion of the cell can be spatially controlled, and the cell can be pierced by microneedle. The variation of dielectrophoresis force on cells with the variation of frequency in different conductivity solutions is also analyzed, the optimal frequency range of controlling cell’s motion is obtained. The result of analysis show that the position and orientation of the cell can be adjusted by using this microinjection device and the cell can also be injected in it.

Introduction

Microinjection is used to deliver RNAs, neutralizing antibodies, dominant negative mutants, sperms and nanoparticles. It is also used to produce transgenic animals and in in vitro fertilization in clinics\cite{1}. Microinjection as the existing technique that allows efficient introduction of a wide variety of molecules or structures in a quantitative fashion into single cell \cite{2}. It had been widely used in biomedical engineering and life sciences\cite{3}. The existing microinjection operating system is based on automatic control and robot technology, the micro needle and pipette which are fixed on the electromechanical device(robotic arm) with micron level displacement resolution respectively. Using the robotic arm, a trained operator holds a single cell with a pipette and penetrate the cell membrane with a pulled glass microneedle, then the materials can be introduced into the cell through the microneedle. Many robotic injection device were reported in the past years. Gabriele et.al developed microinjection system, its characteristic is that it can identify and locate the cell through machine visual servoing method, it can realize automatic manipulation with the help of robot control technology\cite{4}. Kenji et al invented a two-finger manipulator, each finger has three degrees of freedom, it can fetch, move, rotate and release the cells. Not only the cell microinjection can be done by using this, the manipulator can also be used to extract intracellular organelles or chromosome\cite{5}. A new design of the piezo-driven cell injector is developed and introduced in cell injection of zebra fish embryos by Haibo et.al, the damage to the cells is greatly reduced and this technique results in comparably high survival rate of cell \cite{6}. Xie addresses two visual servoing issues in a
robot-assist zebrafish embryo microinjection system. The first one is the microscope autofocusg method with the aim of reducing the sophistication of the whole manipulation system. The other issue is the implementation of the visual processing algorithm for cell detection and location [7]. Unfortunately, the microinjection technique described above are costly and time consuming, some microinjection systems are complex and difficult to operate. What is more, the cell position and orientation was not adjusted before it is treated with a microinjection. Some researches show that, the injection position on the membrane of the cell has a significant impact on the survival rate of the injected cell. In the process of intracytoplasmic sperm injection, the different orientation of the egg first polar body has an important effect on the quality of the fertilized egg and the development of the embryo [8-9].

In this paper, a low cost microinjection device base on the technology of PDMS microfluidic is designed. The cell in it can be adjusted to the appropriate orientation before injection. The microneedle is fixed in this device. The operation of the cell’s microinjection is accomplished by controlling the moving of the cell, instead of moving a microneedle towards an immobilized cell, as done in conventional. The exogenous substance is introduced into cells via microneedle after the cell is pierced.

Design and working principle

The cell microinjection system is composed of a microinjection device, signal generator, micro-fluidic pumps, microscope and computer real time image system, among those the microinjection device is the execution devise. The signal generator is connected with an external connecting terminal of the device through a wire, the microfluidic pump and the petri dish are connected with the wells of the PDMS microinjection device through silicone tube. By adjusting parameters of micro-fluidic pump, the flow velocity in the micro channels of this device can be controlled. The state of cells in the microinjection device will be presented on the monitor with the help of microscope and computer real time image system. The operator adjust the parameters of micro-fluidic pump to drive the cell to a appropriate orientation according to the current state of the cell, and then adjust the parameters of the signal generator, so that the operation of cell microinjection can be accomplished.

Figure 1. Design model of microinjection device.

Figure 2. Physical model of microinjection device.

The device, shown in Fig.1 and Fig.2, consists of micro channels and microelectrodes. The micro channels are fabricated by PDMS and the microelectrodes are made on a piece of glass. The microelectrodes are made up of five concentric rings whose widths are both 100 micron. The injection microneedle is fixed on the bottom center of a cylindrical area with a diameter of 500 microns, which joints several micro channels.

Cell injection is achieved by moving cells onto a stationary microneedle instead of moving the needle into a immobilized cell. In the operation of this device, several suspended cells coming from a supply reservoir is lined up in the micro channel(channel A shown in Fig.3.a )
and then they are transported towards the channel B by the fluidic stream. When the first cell is in the channel B, the fluidic stream in channel A (shown in Fig. 3.b) can be stopped. The fluidic stream in channel B is driven to cylindrical area F, the cell in channel B will be also moved toward there, shown in Fig. 3.c). The cell will be suspended in solution when it is above the microelectrodes because of the dielectrophoresis force. The micro fluid ejected from the microchannel is employed to drive the cell rotate to adjust its orientation when the cell is above the injection needle (shown in Fig. 4.a). At this time, channel A and channel B is closed. Then the parameters of the signal generator is adjusted to change alternating current applied on the microelectrodes in order to change the dielectrophoresis force. So the cell can be moved vertical downward and pierced by micro needle. The exogenous substance will be introduced into the cell by micro needle (shown in Fig. 4.b). After this, adjust the parameters of the signal generator to drive the cell suspend again (shown in Fig. 4.c). Finally, the injected cell will be driven out of the microinjection device (shown in Fig. 3.d).

The analysis of the device

After the electrically neutral particles are polarized in the electric field, the particles will be turned into electric dipoles. Oriented movement of those electric dipoles in non-uniform electric field is called dielectric electrophoresis (DEP). Pohl had been made the in-depth theoretical and applied study based on the DEP, and then the computational formula of dielectrophoresis force ($F_{DEP}$) is obtained as follows [10]:

$$ F_{DEP} = 2\pi r^3 \varepsilon_m \Re \left[ K(\omega) \right] \nabla |E|^2 $$

(1)

$$ K(\omega) = \frac{\varepsilon'_p - \varepsilon'_m}{\varepsilon'_p + 2\varepsilon'_m} $$

(2)

$$ \varepsilon^* = \varepsilon - \frac{\sigma}{\omega} j $$

(3)
Where, \( r \) is the particle radius, \( \varepsilon_m \) is dielectric constant of medium solution, \( K(\omega) \) is Clausius-Mossotti factor which is short for CM factor, \( \text{Re}[K(\omega)] \) is the real part of CM factor. \( \nabla|E|^2 \) is gradient of the square of the electric field strength, \( \varepsilon_m^* \) and \( \varepsilon_p^* \) is composite dielectric constant of medium solution and particles, \( \omega \) is the angular frequency of external electric field signal, \( \sigma \) is the conductivity. Particles will be moved toward high electric field region in the non-uniform electric field when \( \text{Re}[K(\omega)] > 0 \), which is positive dielectrophoresis (pDEP). Conversely, particles would be excluded from region with high electric field strength to low one, which is negative dielectrophoresis (nDEP).

If the phase of alternating current applied on the microelectrodes is changed, the travelling wave dielectrophoresis (twDEP) will appear. The computational formula of travelling wave dielectrophoresis force \( (F_{\text{twDEP}}) \) can be expressed as follows:

\[
F_{\text{twDEP}} = -2\pi r^3 \varepsilon_m \text{Im}[K(\omega)](\nabla \times (\nabla \Phi_\parallel \times \nabla \Phi_\parallel))
\] (4)

Where, \( \text{Im}[K(\omega)] \) is imaginary part of CM factor, \( \Phi_\parallel \) and \( \Phi_\perp \) are the real part and imaginary part of the electric potential.

From the formula (1) and (4), we can know that \( F_{\text{DEP}} \) and \( F_{\text{twDEP}} \) are both related to the parameter of particle radius, electric field intensity, dielectric constant, conductivity and CM factor. For this microinjection device, particle radius, electric field intensity and dielectric constant are available and affirmative. Conductivity of medium solution and angular frequency of external electric field signal is the ideal controllable parameters if we want to change the value and direction of dielectrophoresis force. The angular frequency is the important parameters to CM factor, the real part of CM factor is in proportion to dielectrophoresis force. So the selection strategy of medium solution conductivity and angular frequency should be analyzed.

According to the multilayer shell theory of Jones[11], the cell can be equivalent to a continuous spherical particle, the complex permittivity of the equivalent cell model is:

\[
\varepsilon_p^* = \varepsilon_1^* \left( \frac{R_i / R_j} + 2(\varepsilon_2^* - \varepsilon_1^*) / (\varepsilon_2^* + 2\varepsilon_1^*) \left( \frac{R_i / R_j} - (\varepsilon_2^* - \varepsilon_1^*) (\varepsilon_2^* + 2\varepsilon_1^*) \right) \right)
\] (5)

Where, \( R_i \) and \( R_j \) are the radiiuses of the cell and the cytoplasm, \( \varepsilon_1^* \) and \( \varepsilon_2^* \) are the complex dielectric constant of the cell and the cytoplasm. Based on the cell two shells mode, dielectric constant and conductivity of cell and the cytoplasm obtained by Zhang Li [12], CM factor spectrum curve is gained by using MATLAB, the curve of CM factor’s real and imaginary parts are as follows:
It should be pointed out that the value of $\sigma_p$ can be obtained from formula (5) and $\sigma_p = 1.61 \text{mS/m}$. From Fig.3 we know that $\text{Re}[K(\omega)] > 0$ under the condition of $\sigma_m < \sigma_p$, so the dielectrophoresis force is always positive. From Fig.3, when $\sigma_m > \sigma_p$, the $\text{Re}[K(\omega)] < 0$ (nDEP) under the condition of that the frequency is less than $\text{Re}[K(\omega)] < 0$ and $\text{Re}[K(\omega)] > 0$ (pDEP) under the condition of that the frequency is greater than $3 \times 10^5 \text{Hz}$. No matter what is the value relationship between $\sigma_m$ and $\sigma_p$, $\text{Im}[K(\omega)] > 0$ when the frequency is less than a specific frequency value and $\text{Im}[K(\omega)] < 0$ when greater than that frequency.

In this microinjection device, the cell microinjection is accomplished in the microinjection area which is a cylindrical area with a diameter of 500 microns and 350 microns depth, as shown in Fig.1 and Fig.2. The cell suspend above the micro needle under the action of negative dielectrophoresis force. After the cell orientation is adjusted to appropriate, it will be moved vertical downward and pierced by micro needle under the positive dielectrophoresis force. Then the exogenous substance was injected into the cell. The cell suspend above the micro needle again, finally the cell will be driven out of the microinjection device by the micro fluidic stream. In the process of adjusting the frequency to let the cell is subjected to the downward movement under the dielectrophoresis force, the traveling wave dielectrophoretic force applied on cell will be pointing towards the center of the cylindrical area under the condition of $\text{Im}[K(\omega)] < 0$. In view of the requirement of microinjection operation in this device, we can conclude that, when $\sigma_m > \sigma_p$ it will meet the experimental requirements. In the condition of $\sigma_m = 7.5 \text{mS/m}$, shown in Fig.4, The optimal frequency range to rise the cells to the suspension state is $10^4 \text{Hz} \sim 10^5 \text{Hz}$ and the best frequency range of the cell's vertical downward movement is $2 \times 10^4 \text{Hz} \sim 3 \times 10^5 \text{Hz}$.

**Summary**

In this paper, a novel microinjection device with the function of adjusting the position and orientation of the cell is designed based on the technology of PDMS microfluidic, the operation of the cell’s microinjection and working principle of this device are introduced above. The micro fluid ejected from the microchannel is employed to drive the cell rotate to adjust its orientation. The dielectrophoresis force applied on the cell is used to control the cell move up and down in order to accomplish the cell microinjection. The selection strategy of medium solution conductivity and angular frequency is analyzed. Only when $\sigma_m > \sigma_p$, it will meet the
experimental requirements. Particularly, in the condition of $\sigma_{\text{m}}=7.5\text{mS/m}$, the optimal frequency range to rise the cells to the suspension state is $10^3\text{Hz} \sim 10^5\text{Hz}$ and the best frequency range of the cell's vertical downward movement is $2 \times 10^6\text{Hz} \sim 3 \times 10^6\text{Hz}$.

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