CHARACTERIZATION OF HEPG2 CELLS BEHAVIOR IN CRITICAL FREQUENCY DOMAIN ON TIOPC-BASED OPTOELECTRONIC DIELECTROPHORESIS CHIP


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ABSTRACT

In this article we categorize frequency dependent behaviors of HepG2 cell on TiOPc-based optoelectronic dielectrophoresis chip. Previous works of optoelectronic manipulation only focus on positive or negative dielectrophoresis effect of cell manipulation. Our cell behavior calibration fills the blank of frequency domain between positive and negative dielectrophoresis on TiOPc-coated substrate. Besides, the careful investigation of critical frequency would apply to separate and select high and low permeable membrane cells. Finally, utilizing atomic force microscope to measure the TiOPc-coated surface roughness, determining cell viability on TiOPc surface for 168 hrs, and applying negative dielectrophoresis force to arrange cells array provides deeper knowledge for this organic photoconductive material application.

KEY WORDS: Optoelectronic Dielectrophoresis, Organic Ophotoconductivity, Cell Manipulation, Cell Culture.

INTRODUCTION

The applications of titanium oxide phthalocyanine (TiOPc) magnify the potential of microparticles manipulation via optoelectronic tweezers [1]. The relative investigations of HepG2 patterning, polystyrene beads or magnetic microparticles have been reported [2, 3]. Previous works of optoelectronic manipulation only focus on positive dielectrophoresis (pDEP) or negative dielectrophoresis (nDEP) cell manipulation. The proposed research work focus on the frequency dependent Hepatocellular carcinoma (HepG2) cell behaviors, cell viability and surface quality determination of TiOPc-based substrate. We characterize frequency dependent behaviors of HepG2 cells on TiOPc-based optoelectronic dielectrophoresis (DEP) chip. Besides, the careful investigation of critical frequency was applied to separate and select high and low permeable membrane cells.

SIMULATION

Fig. 1 illustrates the structure of TiOPc-based optoelectronic DEP chip, non-uniform electric field simulation, and operation principle for single cell manipulation. HepG2 cells suspended in low conductive DEP buffer are sandwiched between top ITO glass and bottom TiOPc-coated substrate. The difference between cell membrane permeability makes the low-permeable and high-permeable cell membrane experience pDEP and nDEP in same AC frequency range.

![Image](image_url)

Figure 1: Numerical simulation of virtual electrode on TiOPc surface by applying the potentials of 7 Vpp at 100 kHz. Low-permeable membrane cell is attracted toward illuminating region and high permeable membrane cell is repelled away from the light image.

EXPERIMENTAL

The surface roughness of TiOPc-coated substrate is measured by atomic force microscope depicted in Fig. 2. The thickness of TiOPc layer is 500 nm and the average surface roughness is less than 80μm which has no influence on HepG2 cell of 10μm diameter. Even after 20 hrs of DEP manipulation, HepG2 cell exhibits the cell growth, division and viability on...
TiOPc surface. We utilize Clausius Mossotti (CM) factor calculation to classify the cells behaviors under different AC frequency. When the external AC frequency is $\leq 20$ kHz, the HepG2 cell suspended in low conductivity DEP buffer experiences nDEP and is driven outside the light image. When the AC frequency is $\geq 80$ kHz, the cell is driven by pDEP force and manipulated within dynamic light pattern as represented in Fig. 3.

Figure 2: The TiOPc-coated surface scanning via atomic force microscope within $50\mu m \times 50\mu m$ region where the thickness of TiOPc layer is 500 nm. Comparing with $10\mu m$ diameter HepG2 cell size, the roughness about 100 nm is quite smooth. After DEP manipulation, HepG2 cells growth and division phenomena are observed on TiOPc-coated surface, as shown in the right-up image.

Figure 3: We characterize the operational AC frequency of positive and negative DEP phenomenon for single cell driving. On our TiOPc-based optoelectonic chip, the frequency above 80kHz provides pDEP to cell. When the AC frequency is below 20kHz, the HepG2 cell suspended in low conductive surrounding is driven by nDEP force.

These results are in line with the theoretical predictions of CM factor. Dynamic light pattern projected on the TiOPc surface is able to differentiate the cells with different membrane permeability via critical operation frequency, shown in Fig. 4. In 30k~70kHz frequency range, low-permeable and high-permeable membrane cells experience pDEP and nDEP respectively. Therefore, when the light pattern sweeps the cells, high-permeable membrane cell is separated outside the light pattern and low-permeable membrane cell is trapped within the light pattern.

The AC frequency range between 20 kHz and 100 kHz is critical frequency range where, the pDEP (nDEP) force acting on the cell would be modified as nDEP (pDEP) force immediately. In Fig. 5(a)~(c), HepG2 cells are repelled out the dark region via pDEP force at 100kHz. When the frequency reduces to 10 kHz, the cell would be attracted towards dark area via nDEP force immediately.

Figure 4: Two HepG2 cells with different membrane permeability are driven at 30kHz AC frequency. One cell with high permeable membrane experiences nDEP force outside dynamic light pattern and the other with less permeable membrane is driven by pDEP force within light projecting area.
RESULTS AND DISCUSSION

The cell velocity driven by nDEP and pDEP on TiOPc-based optoelectronic chip is plotted in Fig. 6. HepG2 cells driven by nDEP experiences four pico-Newton which is larger than pDEP force, two pico-Newton.

CONCLUSION

This article determinates the frequency dependent behavior of HepG2 cells in non-uniform electric field. A light-induced dynamic dielectrophoresis chip with TiOPc-coated substrate provides a convenient approach to manipulate and measure the cell velocity. These results supply a basic foundation for the development of organic photoconductive material in optoelectronic DEP region.

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REFERENCES


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