

Dielectrophoresis (DEP) of Cells and Microparticle in PDMS Microfluidic Channels

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ABSTRACT

Electromanipulation of microparticles utilizing microelectrodes has demonstrated considerable promise for the characterization, separation and handling of biological cells. Presently considerable research and development effort is directed towards the development of miniaturized fluidic systems with integrated dielectrophoresis (DEP) electrodes. The design, development and fabrication of a DEP microfluidic assembly with an in-built interdigitated microelectrode array is presented. Continuous fractionation of microparticles in a PDMS microfluidic channel is described. Experimental verification of positive and negative DEP of yeast cells and polystyrene latex beads is demonstrated.

Keywords: dielectrophoresis, pdms, microfluidics, continuous fractionation, microfabrication

1 INTRODUCTION

New and improved methods to characterize and sort cells and micron sized particles are in high demand for a wide range of applications in the areas of biomedical research, clinical diagnosis and environment analysis. With the use of technologies from the microelectronics process industry, fabrication of low cost microfluidic devices [1] has been made possible and conventional methods of fabricating microfluidic devices by etching glass or silicon are fast being replaced by soft lithography techniques [2,3]. Fabrication of microfluidic devices in Poly(dimethylsiloxane) [PDMS] is rapid and cost effective compared to conventional methods. The utilization of this soft lithography technique [3,4,9] for the implementation of DEP in a microfluidic system is demonstrated.

2 THEORY

Dielectric particles such as cells are electrically polarized when subjected to an alternating electric (A.C.) field. If this field is furthermore inhomogeneous, then the cells will experience a dielectrophoretic (DEP) force [5] that can act

to convey them toward strong or weak field regions, depending on the dielectric polarization of the cell and that of the suspending medium [6,7,13].

The time averaged DEP force $\langle \vec{F}_{DEP} \rangle$ exerted by a non-uniform field of peak strength E acting on a homogenous spherical particle of radius a , immersed in a medium is given by [7]:

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[K_e] \nabla E_{rms}^2 \vec{r} \quad (1)$$

Where ∇E_{rms}^2 is the square of the electric field gradient.

The DEP force may attract (positive DEP) or repel (negative DEP) particles from the regions of higher field. The DEP force determined by the sign of K_e , the real part of complex Clausius-Mosotti factor, which is dependent on the complex permittivity of the particle and medium respectively [5,6].

3 FABRICATION OF MICROFLUIDIC DEVICE

Poly(dimethylsiloxane) has been one of the most actively developed polymers for microfluidics, as it reduces the time, complexity and cost of prototyping and manufacturing [8].

Patterning a gold film on an insulating glass substrate forms the microelectrode array responsible for the synthesis of a periodic nonuniform electric field. Borofloat glass wafer [Micalyne Inc., Edmonton] with a chrome-gold layer of thickness 150nm was coated with HPR 504 [Microchem Co.,] and soft baked at 110° C for 30 min. After exposure to a 365 nm UV light source through a chrome mask [ABM Mask Aligner], the exposed wafer was developed [Dev-354 Microchem Co.,] The gold-chrome metallization layer in the exposed regions were etched by a wet chemical process. The resulting electrode structure is shown in figure 1, where the width and the spacing between the successive electrode fingers is 20 μm .

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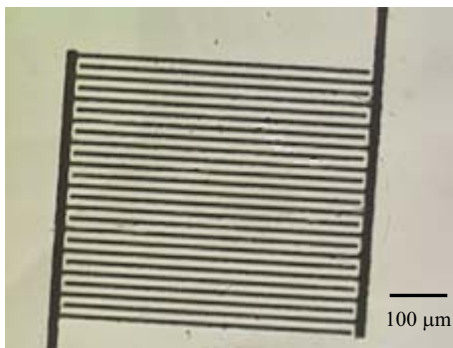


Figure 1. Interdigitated electrode structure

3.1 Molding PDMS microchannel

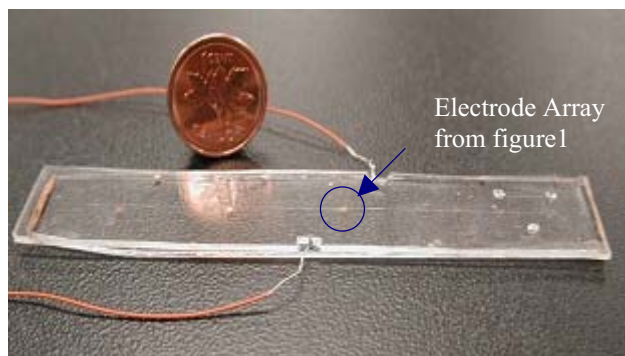


Figure 2. PDMS microfluidic assembly

Figure 2 shows the complete microfluidic device embedded with an electrode array as indicated by the arrow. The three walls of the channels are formed in PDMS and the cover plate, necessary for an enclosed channel, is formed by the glass substrate housing the interdigitated electrode array. The Si wafer with a sacrificial oxide layer of thickness 0.6 μm was coated with hexamethyldisiloxane (HMDS) at 150°C and a layer of photoresist, HPR504 [Microchem Co.] was spin coated on top and soft baked for 110°C. The wafer was then exposed to UV light through a chrome contact mask. The exposed areas were dissolved in Dev-354 [Microchem Co.,] and the sacrificial oxide layer removed using Buffer oxide Etch. Deep Reactive Ion Etching (DRIE) [Oxford Plasmalab-System100] of the exposed Si wafer resulted in a negative replica of the desired channel with a vertical sidewall as shown in Figure 3B.

PDMS prepolymer was prepared by mixing two commercially available components [Sylgard 184 Elastomer & Sylgard 184 Curing Agent, Dow Corning]. The prepolymer was mixed at 10:1 ratio by weight and subsequently poured onto the Si wafer and cured at 60° C for 1 hr [3].

The PDMS replica was then peeled from the master as shown in figure 3C. Access holes for reservoirs can be made by placing posts on the masks or punched out of the cured layer.

3.2 Surface Treatment

PDMS is hydrophobic due to the presence of negatively charges silanol groups on the surface which results in the absorption of hydrophobic species and can easily nucleate air bubbles. Exposing the cured PDMS layer to oxygen plasma at a pressure of 0.15 torr renders the surface hydrophilic [3]. This process creates ozonation on the surface and enables an irreversible bonding of the PDMS to the glass substrate. The glass substrate and the cured PDMS layer were exposed face-up to 80% oxygen plasma at a power of 45 watts for 90 secs. They are then sealed and placed on a hot plate at 60° C for 45 secs. This forms a permanent seal, attempting to break the seal can result in the failure of the bulk PDMS. The seal can withstand pressures ranging from 30-50 psi [8,9].

4 EXPERIMENTAL

4.1 Polystyrene Latex Beads

Polystyrene latex beads 6 μm in diameter with carboxyl (COOH) or plain surface (NH⁺) were purchased from Interfacial Dynamics (Interfacial Dynamics Corp., USA). The beads were washed and suspended in deionised water. Dilute samples were injected into the fluidic device using a microfluidic syringe pump [Cole-Parmer Co., Cambridge]. A 5ml plastic syringe provides a continuous flow in the range of 0.001 $\mu\text{l/h}$ – 14.33 ml/min [10]. Teflon tubing, tube end-fitting [Fisher Scientific Co.,] facilitates connection to the channel reservoir.

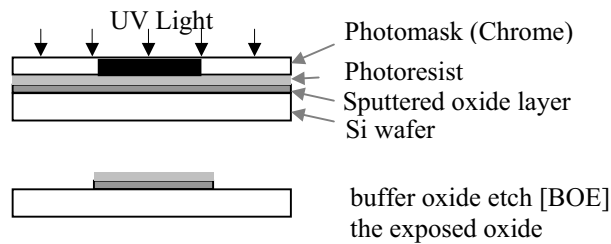
4.2 Yeast Cells

Yeast cells (*Saccharomyces Cerevisiae*) were cultured for 2 days at 30° C in a growth medium [1% yeast extract, 2% Glucose]. The samples are washed repeatedly with deionised water by centrifugation, the supernatant liquid decanted and the residual cells resuspended in fresh liquid. The final cells collected at the bottom after three successive centrifugations were diluted 1000-fold with deionised water prior to experimentation [11,12].

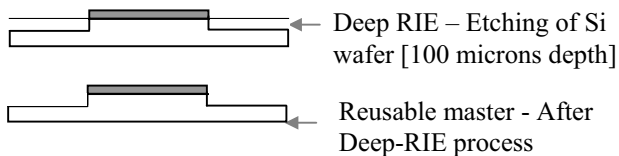
The dilute samples were injected into the microfluidic assembly as described and a function generator [Hewlett Packard, Model - 33120A] was used to supply a sinusoidal voltage required for the electrode array. The DEP induced cell motion was observed utilizing a optical microscope (Olympus, BH2) and the images captured by a video camera [Hitachi, VK-C350] coupled to the microscope station.

Fabrication of PDMS Master

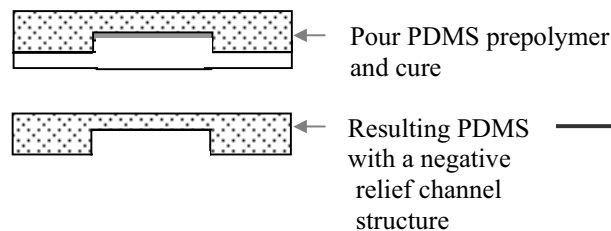
A. Photolithography



B. Deep RIE – Anisotropic Si Etching

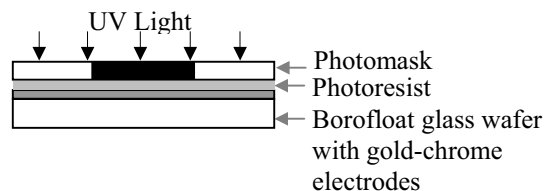


C. PDMS Molding



Patterning Interdigitated Electrode on Glass

a. Photolithography



b. Developing PR



c. Etching Au-Cr



D. Bonding

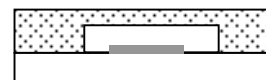


Figure 3: Fabrication Process Sequence

5 RESULTS AND DISCUSSION

Figures 4A and 4B shows negative DEP of the polystyrene latex beads, observed when a voltage of $3.8 V_{p-p}$ of field frequency 480 kHz was applied to the chamber electrodes. The microbeads were observed to be levitated and formed ‘pearl-chains’ above the electrode. The difference in focus of the electrode array and the beads in figures 4A and 4B confirm levitation and ‘pearl-chain’ phenomena.

In contrast, yeast cells when subjected to a similar A.C voltage ($3.7 V_{p-p}$) at a field frequency of 580 kHz exhibited positive DEP and hence were attracted towards the region of maximum field intensity and collect at the electrode surface as shown in figures 4C and 4D. Figure 4D shows a higher concentration of yeast cells collected near the electrode edges at regions of field maxima and formed ‘pearl-chains’.

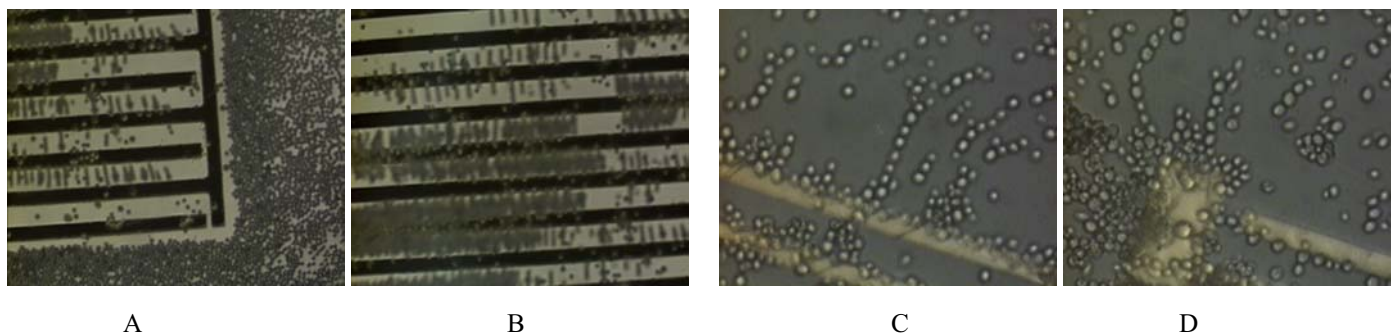


Figure 4: A - Negative DEP of polystyrene latex beads, cells were repelled from the electrodes and were levitated above them.

B - levitated beads forming ‘pearl-chains’ above the electrode surface.

C - Positive DEP of yeast cells; cells attached to the electrodes and formed ‘pearl-chains’.

D – Higher population of yeast cells collected near the electrode edges at regions of field maxima.

6 CONCLUSION

A novel polymeric-glass microfluidic system with an integrated interdigitated microelectrode array has been described. Fabrication of such low cost, reusable microsystems capable of electro-manipulation of cells and experimental verification of positive and negative DEP has been demonstrated. The polystyrene beads levitated and confined above the electrode array were continuously removed by fluid flow. Thus, this non-invasive, easy to fabricate technique could be employed for the continuous fractionation of heterogenous mixture of cells. Since PDMS can be molded at low temperatures without elaborate fabrication requirements, the microfluidic device can be readily fabricated in a normal laboratory setting. Further, integration of this technology with on-chip imaging and control will provide a microsystem capable of quantitative and sensitive analysis of DEP signatures of various cancerous cells.

7 ACKNOWLEDGEMENTS

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