Electrokinetic Assembly of Microsphere and Cellular Arrays

Mihrimah Ozkan[1], Sadik C. Esener[1] and Sangeeta N. Bhatia[2]
[1]Department of Electrical and Computer Engineering
[2]Departments of Bioengineering and Medicine
University of California at San Diego, La Jolla CA

ABSTRACT

We have developed a novel electrochemical system for field assisted, fluidic assembly of objects on a microfabricated silicon substrate by means of electrical addressing. The principle of our technique is based on the movement of charged species in solution to oppositely charged electrodes, as seen commonly in electrophoresis. Here, charged species such as beads and cells are moved electrokinetically through an aqueous solution towards a charged electrode. Micro patterning of the electrodes allows localization of charged species. We present a theoretical framework to predict the electric potential for assembly and disassembly of spherical objects. We correlate theoretical predictions with the motion of negatively charged polystyrene beads of 20 μm diameter on 100 μm feature micro patterned substrates. In addition, we extended these results to arraying of 20-30 μm diameter live mammalian cells by means of electrical addressing. This technique has applications in creation of 'active' cellular arrays for cell biology research, drug discovery and tissue engineering.

INTRODUCTION

The potential synergy of combining MEMS (micro-electro-mechanical systems) with biological systems has become increasingly apparent in recent years. Already, the interface between biology and micro technology has led to the development of enabling tools for biological research, clinical diagnostics, and medical devices [1,2]. At the "chip" level, this confluence of technologies has led to DNA microarrays [3], catalytic RNA arrays [4], protein arrays [5], and even live cell arrays [6,7,2]. Cell arraying technology has proven to be a useful tool for studies of cell fate, cell-cell interaction, and cell-matrix interactions. Furthermore, some investigators have proposed the use of live cells as "high content sensors" for biological event [6]. Existing cell arraying technologies primarily rely on patterned surface chemistries or localization through polymeric templates [8]; however, the process of cell arraying due to cell adhesion requires on the order of 2-12 h. Here, we propose a method to rapidly array live cells based on their negative charge rather than relying on receptor-mediated adhesion. We first develop this tool by using polystyrene beads as model "cells" and then extend this work to cell lines of human and murine origin. Here we present a new electronic micro array technology that can be used for dynamic, reversible assembly of organic objects. Photolithographically patterned conductive substrates are used to transport and array charged species (micro spheres, live cells) in solution.
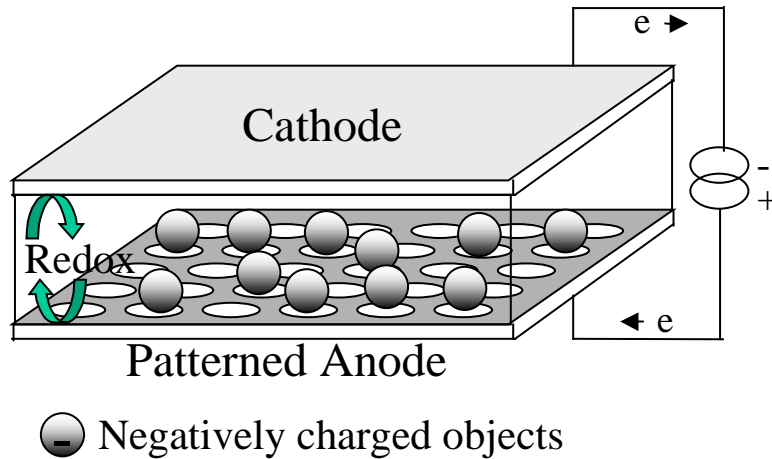
Figure 1. Schematic illustration of electrochemical micro array patterning system.

Materials and Methods

For polystyrene micro sphere array arrangement, patterned silicon electrodes have been fabricated as anodic substrates. First, a 400 nm thick silicon nitride dielectric layer was deposited on silicon wafer by using plasma enhanced chemical vapor deposition technique. Then, 100 μm diameter electrodes array were defined by photolithography. Finally, nitride layer within the 100 μm diameter field was etched away by CF_4 plasma. With this electrode configuration, the silicon electrode is in contact with electrolyte solution only at 100 μm diameter openings.

THEORY AND EXPERIMENTAL RESULTS

System Diagram

An electrochemical system consisting of two parallel plates has been used to transport charged objects in solution. A dielectric (silicon dioxide or silicon nitride) patterned semiconductor substrate has been fabricated and is used as anode. Another transparent semiconductor is used as cathode to enable in-situ observations. The details of the system are given elsewhere [9,10]. Figure 1 shows the schematic illustration of the electrochemical system. Here, spheres represent negatively charged objects inside the electrolyte solution. H^+ and OH^- ion exchange between two electrodes occurs as a result of the reduction and oxidation (REDOX) of water ions under the applied bias. The circulation of ions results in completion of the electrical circuit within the system.

Figure 2 illustrates a 2-Dimensional finite element model of the current density distribution inside the system. Arrows show the direction of the electric field. Notice the non-uniform field distribution in comparison to regular micro patterned electrode configuration. As expected, current density is larger over the electrode surface the elsewhere. Furthermore, the current density at the silicon–nitride interface is found to be larger than the center of the electrode.

<table>
<tr><td>silicon</td><td>nitride</td><td>silicon</td><td>nitride</td><td>silicon</td></tr>
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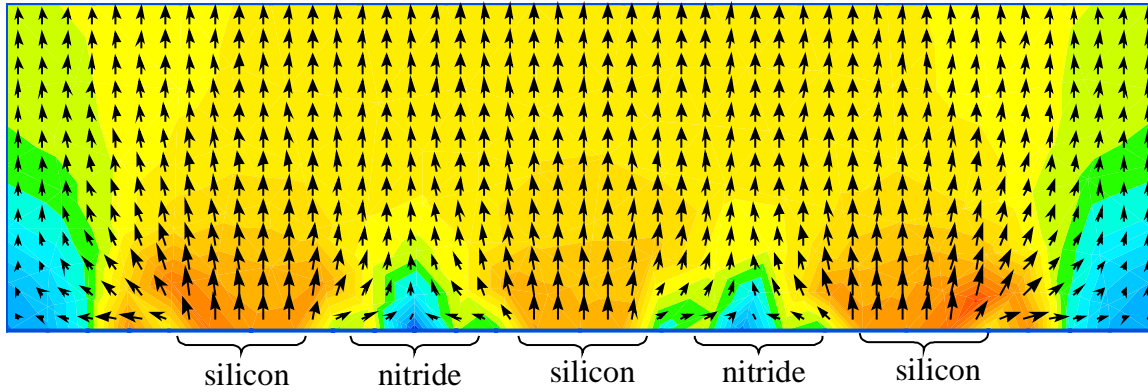
Figure 2. 2D finite element modeling of current density distribution is performed for micro patterned electrode configuration at applied potential of 3V. Arrows correlate with the field direction in electrochemical cell with a following composition: Silicon-silicon nitride/DI-water/ITO. Current density is predicted at highest near to the silicon electrode and lowest above the nitride layer.

Force balance on patterned objects

The required minimum bias to pattern objects under an applied electric field in a fluidic environment depends on the total forces acting on object. Hence, it is important to understand forces acting on the cells inside the electrolyte when a bias is applied. Figure 3a represents the forces on the sphere inside the bulk solution (e.g. during assembly), and figure 3b depicts the forces when the sphere is near the electrode surface (e.g. during disassembly). Note the inclusion of surface interactions in the latter.

Equation (1) gives the individual forces and their definitions, when the object is transported down to the anode [11]:

$$F_{drag} = F_g^{'} + F_{electrophoresis} \qquad (1)$$

where, $F_{drag} = 6\pi\eta R\upsilon$ is the fluidic drag force acting on a sphere, $F_g^{'} = F_g + F_b = V(\rho_p + \rho_l)g$ is the resultant force acting on a sphere due to gravitational and buoyancy forces , and $F_{electrophoresis} = -q\dfrac{dV}{dx}$ is the electrophoretic force acting on the object.

Equation (2) represents the forces acting on the sphere when it is near to the electrode surface. In order to dislodge the object from the electrode surface the applied bias has to overcome the sum of opposite forces acting on object.

$$F_{drag} + F_g^{'} + F_{vanderWaals} = F_{electrophoresis} \qquad (2)$$

Assembly	Disassembly

F drag

F electrophoresis

F' g   **F** electrophoresis

F' g   **F** drag   **F** van der Waals

(A)   (B)
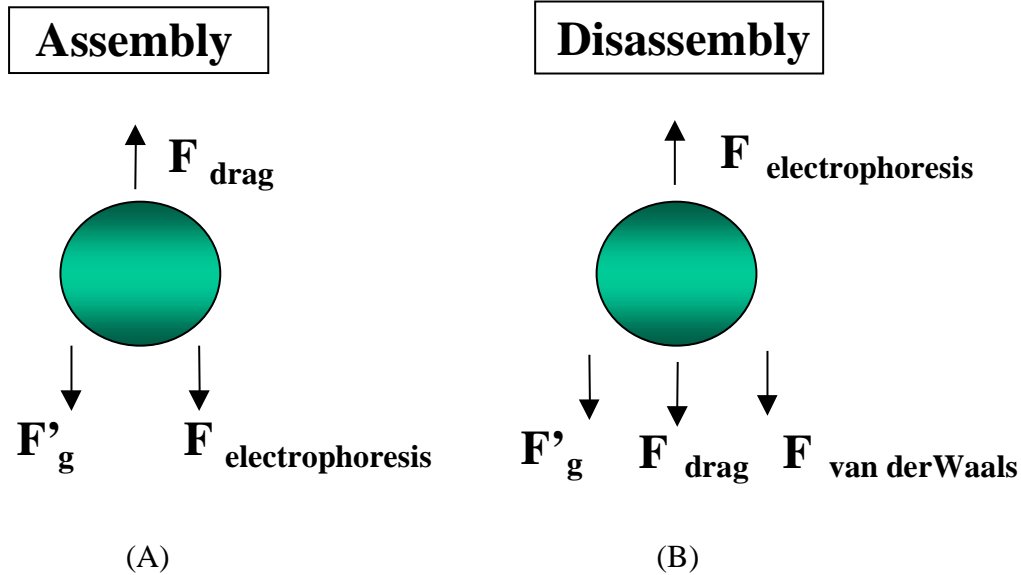
Figure 3. The forces acting on a sphere when the sphere is (a) inside the bulk solution, and (b) near to the electrode surface in an electrophoresis chamber.

where, $F_{vanderWaals} \sim \dfrac{1}{d^7}$ is the van der Waals force present between electrode and object.

Van der Waals force is inversely proportional to the seventh power of spacing between object and electrode surface.

In our preliminary experiments, in order to test our model predictions, we explored the applied repulsive potential necessary to prevent the sedimentation of a given sphere. 20 μm diameter polystyrene spheres have been used as models cell. Equation (1) gives about 0.75 mV/mm of electric field acting in the opposite direction that is needed to balance the forces acting on the sphere when the sphere moves with the speed of 10^{-4} cm/s. Otherwise, sphere will sediment in deionized water, since the density of polystyrene is 1.05 g/cc. This behavior correlates with our experimental observations for the object velocity of the same order of magnitude. On the other hand, in order to reject the pre-patterned sphere from the electrode surface, the applied electric field has to overcome all the other existing forces on the sphere as shown in figure 3b. The required electric field to dislodge a 20 μm diameter polystyrene sphere in deionized water is calculated to be approximately 4 V/mm when the sphere is about 100 nm away from the electrode surface. Experimental observations have shown that spheres are dislodged with as little as 2 V/mm. The difference could be due to variations in the spacing between sphere and electrode surface or other fluidic forces that act on the sphere (e.g. electrohydrodynamic or electrosmotic forces).

Experimental Data: Microspheres

Figure 4a and 4b summarize the results of 20 μm diameter polystyrene spheres patterning in deionized water. Figure 4a shows that spheres were patterned within the electrode areas and figure 4b illustrates that after reversing the bias spheres were dislodged from electrode surfaces.
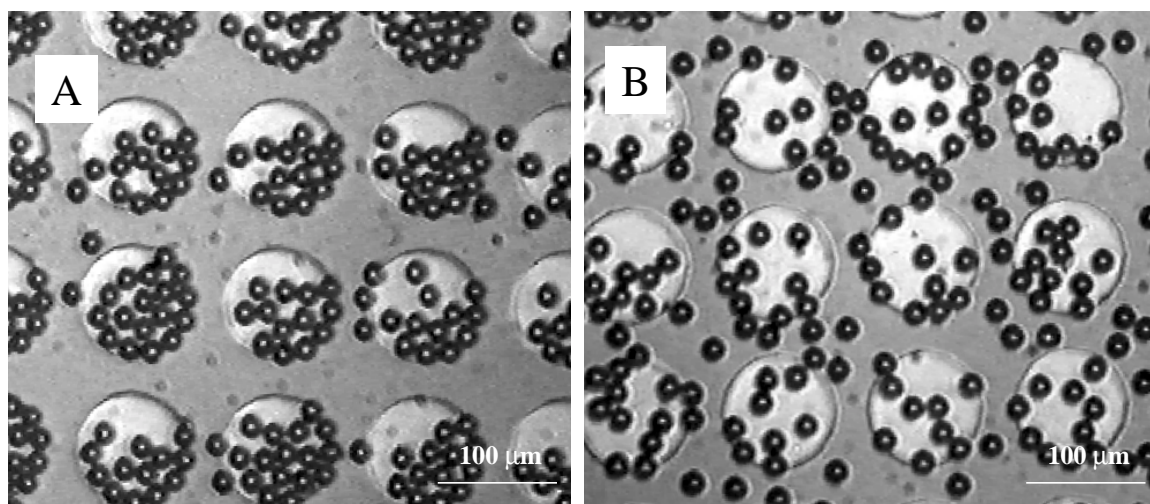
Figure 4. 20 μm diameter polystyrene sphere patterning on pre-patterned silicon substrate (a) assembly under applied +1 volt, and (b) disassembly under applied –2V.

The electrokinetic transport effect of electrophoretic, electrohydrodynamic and electrosmotic forces is discussed elsewhere [9]. As seen in figure 4a, spheres are aligned closer to the edge of the electrode surface, which agrees with our finite element model that predicted larger current density distribution near the silicon-nitride interface (figure 2.).

Cells

A major difference in between patterning of live cells, in contrast the polystyrene spheres restricts the composition of the electrolytic solution. Cells must be in osmotic equilibrium with the environment. Furthermore, ions, nutrients and pH must be tightly controlled to preserve cell viability. Classical cell culture media is rich in salt and sugar (i.e. highly conductive) which makes the electrochemical transport of the cells difficult and slow. In order to study the effect of solution conductance on electrophoretic transport, a variety of physiological solutions were screened using polystyrene spheres as model cells. These data are presented in detailed elsewhere. Some solutions, such as phosphate buffered saline were so highly conductive that object transport was reduced dramatically. Currently, we are evaluating the effects of introducing solution conductivity and object zeta potential into the theoretical model depicted in figure 3.

Finally, we have used the same technique for patterning live mammalians cells. Arraying of the human fibrosarcoma cells (20-30 μm in diameter) and mouse 3T3 fibroblast cells on gold and silicon substrates was achieved (results not shown here) [9]. Preliminary data suggests that cells exposed to applied potential are capable of attachment and spreading on polystyrene and resume cycling within 24 hours.

CONCLUSIONS

An electrochemical system with two parallel plates electrodes has been used to form microsphere and cellular micro arrays for drug screening and tissue engineering applications. We developed a simple theoretical model by computing a force balance on spherical objects. This model predicts that the voltage required for polystyrene bead disassembly is substantially higher than the voltage required for assembly. The difference arises due to the combined effects of gravity, frictional drag and van der Waals. Furthermore, we extended the system for patterning of live mammalians cells by using low conductance physiologic buffers. Finally, our preliminary data with mouse 3T3 fibroblast demonstrates that cells maintain the ability to attach, spread and divide after exposure of up to 100V/cm. This platform will serve as a powerful tool for high throughput biological screening.

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