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Cellular Immobilization within Microfluidic Microenvironments: Dielectrophoresis with Polyelectrolyte Multilayers

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The development of biomimetic microenvironments will improve cell culture techniques by enabling *in vitro* cell cultures that mimic *in vivo* behavior;[1−4] however, experimental control over attachment, cellular position, or intercellular distances within such microenvironments remains challenging. We report here the rapid, controllable immobilization of suspended mammalian cells within microfabricated environments using a combination of electronic (dielectrophoresis, DEP) and chemical (polyelectrolyte multilayers, PEMS) forces. While cellular position within the microsystem is rapidly patterned via intermittent DEP trapping, persistent adhesion upon removal of electronic forces is enabled by surface treatment with PEMS that are amenable to cellular attachment.[5,6] In contrast to DEP trapping alone, surface treatment enables the soluble microenvironment to be varied systematically, allowing the use of soluble probes of cell state and enabling cellular characterization in response to soluble stimuli.

Biomimetic microenvironments that have characteristic dimensions on the order of individual cells and are fluidically addressable compare favorably with bulk cultures[1,7−9] but enable improved control of environmental variables that are poorly defined with traditional culture methods,[1−4,9−14] allowing solutions to be administered chronically (e.g., media), acutely for short periods (e.g., cellular insult), or in a spatially or time-varying manner to mimic chemical gradients and oscillations commonly found in vivo (e.g., inflammation response, cellular signaling, hormone cycles).[14,15] Additionally, chemical analysis can be integrated with cell culture on a single, disposable device.[16,17] However, it remains necessary to develop facile methods for introducing and immobilizing suspended cells in a rapid, simple, and defined manner within the microenvironment to facilitate cellular assays. Patterned surface treatments have been used, but cellular attachment is slow (hours) and the resulting position within the microenvironment is poorly defined.[18] Some of the strategies explored to rapidly immobilize cells include optical,[19] hydrodynamic,[20] acoustic,[21] geometric,[8,16] and DEP[22,23] trapping.

The spatially non-uniform electric fields that immobilize suspended particles (e.g., cells) in DEP trapping scale readily to cellular dimensions[24] do not harm biological samples[24,25] and have been utilized to concentrate cells from dilute suspensions,[26] isolate cell types from mixtures,[27] move cells about surfaces,[23,28] and align cells on planar substrates.[18] However, the strength of DEP forces is particularly sensitive to the polarizabilities of cells and media, with changes to the soluble microenvironment generally weakening DEP forces considerably.[29] In this work, PEMS surface treatment produced cellular attachment that persisted after removal of DEP forces, enabling changes to the soluble microenvironment without loss of surface attachment.

In the absence of DEP forces to immobilize them, suspended mammalian cells introduced by hydrodynamic flow (~0.1 mm/s linear velocity) are carried through microfluidic channels too rapidly to adhere to the surface. However, when a DEP electrode array was energized, a potent trapping field was generated, and cells flowing past the array (≤1 s residence time) were immobilized against the floor of the microfluidic channel (Figure 1). The electrodes were insulated by a thin layer of SiO_2, preventing detrimental DC current[25] but allowing electric fields generation. With adequate electric field strengths, all cells were immobilized at the first electrode encountered and the arrangement of cells was determined by electrode geometry (perpendicular to solution flow in Figure 1). At lower electric field strengths or higher solution conductivities, weaker DEP forces yielded cellular immobilization that was distributed across the electrode array. At all field strengths, 5−10% of cells exhibited no DEP response (immobilization or observable course deflection), suggesting similar intra- and extracellular solution compositions for a small population that correlates well with the fraction of nonviable cells in bulk cultures. By operating the DEP electrode array continuously, high cell densities (5×10^{10} cells/L) were rapidly achieved (~30 s) in the electrode vicinity by immobilizing cells out of more dilute suspensions (10^9 cells/L). The frequency and solution composition utilized here were chosen to indiscriminately immobilize any mammalian cells, and trapping efficiency was identical for the R28 retinal and P19 embryonal carcinoma cell lines.

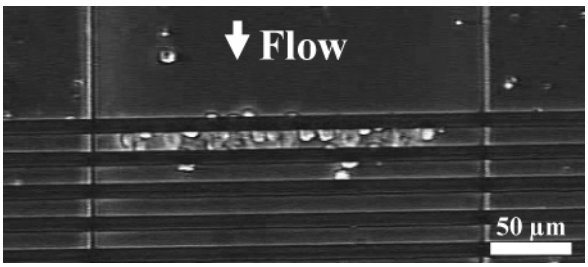

Figure 1. DEP immobilization in a PEMS-treated microenvironment. Cells were immobilized by DEP at electrodes (opaque horizontal lines). Continuous DEP for 75 s produced a dense arrangement of trapped cells from a flowing dilute cellular suspension (100 μm/s linear velocity). The DEP electrodes were de-energized without cellular detachment. Movies of this experiment are available online (Supporting Information movies 2 and 3).

When the channel floor was bare glass (SiO_2 without PEMS surface treatment), DEP immobilization was repeatable and reversible, with most of the cells being rapidly detached from the surface upon removal of DEP trapping forces (Figure 2, black). Similar results arose from changing solution conductivity for a constant applied voltage due to weakened DEP trapping forces, as expected.[29] Rapid (>1 Hz) and repetitive immobilization and release of cells was possible. Significantly different results (Figure 2, gray) were attained by coating the initial substrate (SiO_2) with PEMS known to be amenable to neuronal attachment and growth.[5,6] PEMS-treated surfaces improved DEP efficiency since favorable surface interactions assisted immobilization during DEP trapping. Upon removal

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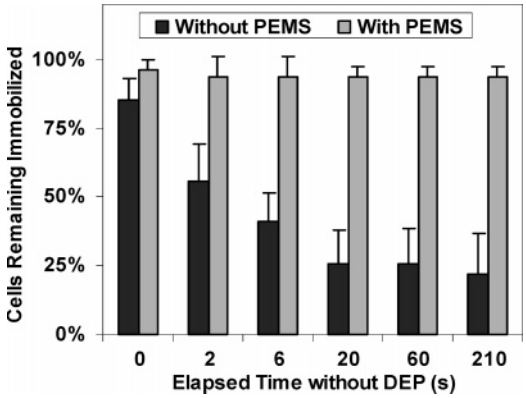

Figure 2. Persistent cellular immobilization. After removal of DEP trapping forces ($t = 0$ s), immobilized cells rapidly detach from untreated SiO_2 surfaces (black) while PEMS-treated surfaces (gray) yield persistent adherence. In both cases, the number of cells remaining was normalized by the total number of suspended cells introduced during active DEP trapping, so the 0 s time point indicates the overall DEP trapping efficiency. Standard deviations were determined from nine trials with and four trials without PEMS surface treatment. Real time videos of representative trials are available online (Supporting Information movies 1, 2, and 3).

of DEP forces, the trapped cells were not removed by solution flow, but exhibited surface attachment that was persistent and irreversible. Cells immobilized against PEMS-treated surfaces remained adherent for many hours after the electrode array was de-energized, though they could be intentionally detached with high shear forces (flow >3 mm/s linear velocity). Persistent attachment was established quickly with cells remaining adherent when only immobilized via DEP against PEMS surfaces for a few seconds. Much longer incubation times (4−12 h in bulk cultures) are required for cellular attachment in the absence of DEP forces. Accelerated adherence may be attributed to the DEP force orientation normal to the surface that deforms immobilized cells to yield larger contact areas than for suspended cells that sediment but remain quite rounded. This rapid interaction between the cells and a PEMS-treated surface is currently under investigation and is probably rooted in electrostatic attraction between the terminating polycation of the PEMS and extracellular matrix proteins or negatively charged phospholipids that are present in the cellular membrane. Rapid surface attachment was not previously reported for DEP trapping onto surfaces treated with biomolecules.[18]

Persistent immobilization following DEP trapping was useful since it allowed the solution conditions within the microenvironment to be altered without dislodging immobilized cells. Following trapping of cells against the PEMS-treated surface, the electrode array was de-energized and the solution within the microenvironment was changed systematically using multiple syringe pumps connected to convergent microchannels to allow exact control of solution composition and flow rate. For example, cell growth media was introduced to replace the isotonic sucrose solution required for DEP trapping and facilitate sustained culture; addition of Trypan Blue, an absorbance-based marker for membrane integrity, confirmed that immobilized cells remained viable, even after prolonged exposure (15−20 min) to DEP trapping forces at high electric field strengths (0.8 kV/mm). When a surfactant, Triton X, was delivered to the microenvironment, affected cells were lysed, releasing cytosol into the flow stream. Concurrent delivery of Trypan Blue enabled real time visualization of lipid membrane poration. The addition of chemicals during these experiments had no deleterious effects on the microenvironment, which could be used subsequently to immobilize a fresh batch of cells without further modification.

In conclusion, DEP trapping combined with PEMS surface modification provides a useful and reliable strategy to manipulate cellular position and adhesion. When combined with PEMS surface treatment, cell immobilization initiated by DEP is retained through substrate interactions, enabling removal of DEP forces and changes to solution composition without dislodging immobilized cells. Microfluidic delivery of chemicals to the trapping region facilitates nutrient delivery or cell monitoring and characterization, continuously or after delivery of soluble stimuli. Since dielectrophoresis can discriminate between cell types,[27,30] it may be possible to systematically pattern discrete cell populations to create intricate co-cultures. Demonstrated here for mammalian neural cells and pluripotent cells able to differentiate along, among others, neuronal pathways, this approach is applicable to assays within microenvironments for a variety of cell types.

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Supporting Information Available: Movies (3) of mammalian cell immobilization during and after DEP trapping for PEMS-treated and untreated surfaces. Methods for cell culture, device fabrication, DEP trapping, and simulations of the electric field. This material is available free of charge via the Internet at http://pubs.acs.org.

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