INSTANTANEOUS TRAPPING AND LONG TERM CELL SURVIVAL UNDER DIELECTROPHORETIC CONDITIONS USING A HYBRID CELL ADHESIVE SURFACE

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ABSTRACT

A dielectrophoresis (DEP) microfluidic device that allows instantaneous trapping of cells for rapid return to cell growth conditions and long-term cell experiments (cell proliferation, induction, and differentiation) of embryonic P19 cells is presented. A hybrid cell adhesive material (hCAM) comprised of a positively charged polyelectrolyte, polyallylamine hydrochloride (PAH), and fibronectin (FN), is deposited onto DEP electrodes to serve as an adhesive material for DEP-trapped cells. Cells that adhere onto the hCAM under flow conditions do not detach from the surface when DEP forces are removed. Viable neuron-like cells were obtained after 6 days in culture.

KEYWORDS: Dielectrophoresis, P19 cells, instantaneous cell trapping, fibronectin, polyelectrolyte multilayers

INTRODUCTION

A critical issue in the development of DEP cell positioning devices is the ability to retain cells in a fluid flow field at a selected position after the DEP field is removed [1-3]. Furthermore, after the cells are adhered to the surface at selected positions, the surface must be biocompatible to allow for long term cell function (e.g., proliferation, motility, differentiation). For the most part, positive DEP (pDEP) cell studies have focused on the separation of groups of cells based on their intrinsic electrical properties [4], short term trapping and assessment of single cells [5], and on single cell trapping with cell adhesive materials at slow flow rates [2]. In the latter of these studies, the control over the flow was of paramount importance since it had to be decreased to a level where the shear stress would not remove the cells while they were allowed to attach for at least 20 minutes. These cells were monitored for two days to assess the effectiveness of the DEP trapping and the cells' health. However, other studies using negative DEP (nDEP) instead have shown longer periods of cell viability [6, 7]. Cells that are positioned by nDEP are not exposed to the low conductivity media (e.g., sucrose) needed for pDEP that is thought to diminish cell health after the manipulation process. The challenge to using nDEP is that the trapping forces are weaker and the system is harder to design due to the fact that the cells are trapped at the electric field minima.

It has been shown previously that cells can be anchored onto polyelectrolyte multilayers (PEMs), an adhesive synthetic biocompatible material, when seeded in cell growth media [8, 9] and when trapped under DEP conditions (suspended in sucrose and applied electric fields) [3]. However, in the case where the cells were trapped using DEP conditions, cell viability has not been shown for extended periods of time. In this study, we examined the effects of different cell adhesive surface coatings on cell attachment and function immediately after pDEP trapping and removal of the electric field. Our work demonstrates that a hybrid surface prepared from fibronectin and PAH layers allowed for immediate cell attachment after pDEP trapping and long term cell viability.

EXPERIMENTAL

The device we developed was fabricated with Indium tin oxide (ITO) electrodes that were patterned using standard photolithographic methods and later passivated by spin coating a solution of polystyrene dissolved in toluene. Microfluidic channels were molded in polydimethyl siloxane (PDMS) using an SU8 master. The device was coated with the hCAM by non-specifically binding FN on a polyelectrolyte multilayer (PEM) with a negatively charged outermost layer, polystyrene sulfonate (PSS), and subsequently depositing a layer of PAH on top of the FN (Fig. 1). The hCAM was deposited on top of the DEP electrodes to serve as an adhesive material for the cells once they are electronically brought to the surface of the device. Fluorescent fibronectin (FN-ROX) was prepared by incubating carboxy-X-rhodamine (ROX)-NHS ester (10 ug) with fibronectin (1 mg) that was exchanged into phosphate buffered saline with a PD10 desalting column for several hours. Excess unreactive fluorphore was quenched with an aliquot of Tris buffer.

The hCAM components were imaged using fluorescence microscopy (Fig. 2). P19 cells were cultured using Alpha Minimum Essential Media (alfa MEM) supplemented with bovine calf serum and fetal bovine serum to final concentrations of 7.5 % and 2.5 %, respectively. The cells were trypsinized once they reached up to 80 % confluency and passed to a new flask at a dilution of 1/10. For the DEP experiments, the cells were resuspended in sucrose media, and a sine wave was applied of up to 10 Vp-p at a frequency of 30 MHz.

To test long term viability and cell function on the hCAM materials, P19 cells were directly seeded on glass coverslips that were prepared with a protocol identical to the electrodes. The cells were differentiated by first inducing them by exposure to retinoic acid and a low concentration of serum. After 4 days of cell induction the media is replaced by normal cell growth media (described above). Two (2) days later, projections from differentiated P19 cells are observed. To confirm that cell differentiation had occurred, immunofluorescence staining against a neuronal cell marker was performed. The immunofluorescence staining was carried out by first fixing the cells with 4 % paraformaldehyde and then incubating with an antineurofilament antibody for 2 h. After rinsing with phosphate buffer saline

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(PBS), the cells were incubated for 1 h with a secondary antibody tagged with fluorescein isothiocyanate (FITC). The neurofilaments were then visualized with fluorescence microscopy.

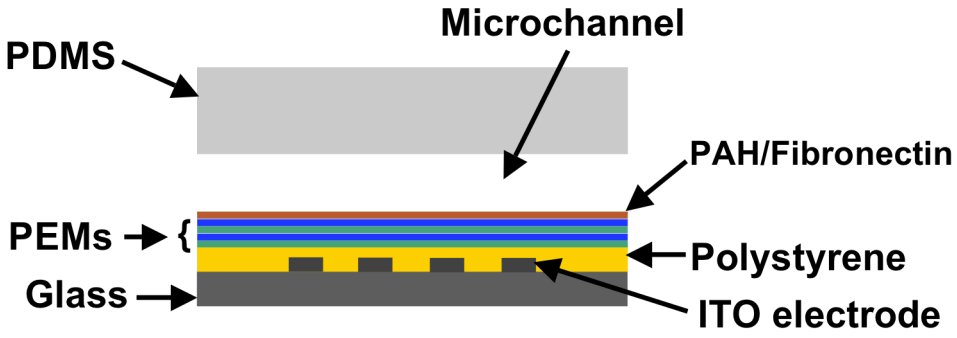

Figure 1. Side view of the DEP device for instantaneous trapping of cells. The hybrid cell adhesive material (hCAM) is deposited on top of polyelectrolyte multilayers (PEMs), which in turn are deposited onto the ITO electrodes covered by polystyrene. The microchannel is molded in PDMS.

RESULTS AND DISCUSSION

Figures 2a and 2b show the PAH-FITC labeled and the FN-ROX labeled, respectively, where each is bound to a PEM composed of four alternating layers of PAH and PSS, or $(PAH/PSS)_2$. Figure 2c shows the labeled hCAM deposited on $(PAH/PSS)_2$, where the yellow/orange color denotes the merge of the two components, FN-Rox and PAH-FITC, at the surface. This merge suggests an overlap/intermingle of PAH and FN and the absence of specific sub millimeter regions that are covered by just FN or PAH. This suggests that the cells could interact with both materials.

P19 cells were resuspended in sucrose, for cell trapping experiments, and immediately introduced into the microfluidic channel. Cells flowing into the microchannel at a linear velocity of approximately 200 µm/s were attracted towards the hCAM-coated ITO electrodes (Fig. 3a and 3b) when the electric field was applied. The cells remained anchored when the DEP electrodes were turned off (Fig. 3c). After cells were anchored onto the hCAM, two different experiments were carried out: cell proliferation and cell differentiation. Cells that were allowed to proliferate did so and were viable for up to 4 days. Cells that were induced, and in turn differentiated, remained viable for 6 days.

Figure 2. Micrographs of fluorescently labeled components of hCAM. A) PAH-FITC deposited on top of $(PAH/PSS)_2$. B) Fibronectin-ROX deposited onto $(PAH/PSS)_2$. C) PAH-FITC deposited onto fibronectin-ROX, and all onto $(PAH/PSS)_2$. Scale bar: 200 µm.

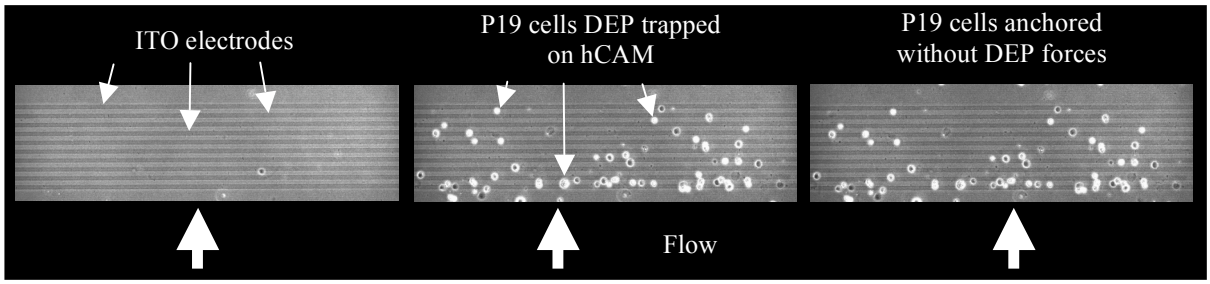

Figure 3. Micrographs of DEP ITO electrodes before, during, and after the trapping of P19 cells using DEP forces along with the hCAM surface. A) ITO electrodes covered by hCAM enclosed in a PDMS microchannel. B) DEP trapping occurs while a constant flow of cells passes over the electrodes. C) P19 cells anchored on the hCAM after DEP forces were removed and the flow still remains.

Induction of the P19 cells into neuron-like cells was initiated by replacing the sucrose with cell growth media containing retinoic acid and a low concentration of serum. Cells were induced and grew on the hCAM for 4 days. After the induction period, differentiation was achieved by replacing the induction media with cell growth media. Neurofilament formation was observed after 2 days of differentiation. Figure 4 shows the immunofluorescence staining of P19 cells after differentiation occurred. Figures 4a, 4b, and 4c show immunostained P19 cells (against neurofilament) when differentiated on a polystyrene culture flask dish, $(PAH/PSS)_2$/fibronectin, and on $(PAH/PSS)_2$/fibronectin/PAH, respectively. The arrowheads indicate neurofilaments formed on all the surfaces.

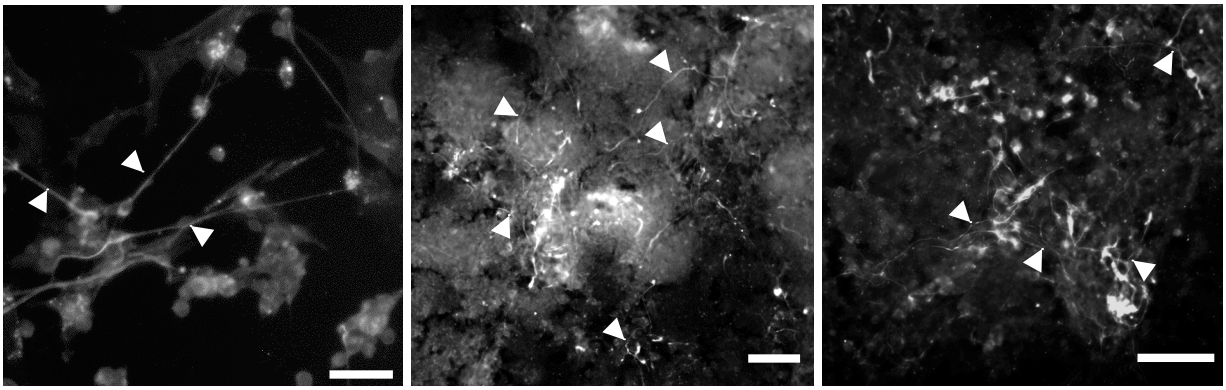

Figure 4. Differentiated P19 cells on A) polystyrene culture dishes, B) $(PAH/PSS)_2$/fibronectin, and C) $(PAH/PSS)_2$/fibronectin/PAH. Immunostained P19 cells show the neurofilaments (see arrowheads), which is a marker for neuronal differentiation. Scale bars: A) 50 µm, B) 100 µm, C)100 µm.

CONCLUSION

The system described here, using DEP combined with the hCAM examined, demonstrated the ability of the surface to provide a biocompatible material where long term cell experiments can be performed. Cell proliferation and differentiation were carried out with this system for up to 6 days. Results of P19 cell differentiation into neuron-like cells showed that the hCAM behaved similarly to the controls (polystyrene and fibronectin). These results provide evidence that this system will be applicable to studying how cell density, cell positiong and intercellular communication play a role in neuronal cell development.

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