

# PATTERNING RETINAL CELLS ON POLYELECTROLYTE MULTILAYERS

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## ABSTRACT

The patterning of retinal epithelial cells on polyelectrolyte multilayer (PEM) lines is presented. PEMs were deposited in discrete lines using a poly(dimethylsiloxane) (PDMS) microfluidic network on top of a flat PDMS slab. The layers were formed by sequentially flowing the polyions throughout the microfluidic network. Retinal cells, seeded on flat PEMs/PDMS surfaces adhered and grew on the PEM areas preferentially. Cells were allowed to grow for up to two weeks showing the pattern delineated by the PEMs. Poly(ethyleneimine) and poly(allylamine hydrochloride) showed better cell adhesion and growth properties for these type of cells than poly(diallyldimethylammonium chloride).

Keywords: cell patterning, polyelectrolyte multilayers (PEMs), retinal cells

## INTRODUCTION

Existing methods for surface micropatterning of cells include the use of a microcontact stamp [1] to imprint the adhesive molecules, the use of micropattern masks [2] to expose only the areas in the substrate where the cells or adhesive proteins will be anchored, and photolithographic patterning of siloxanes with functional groups that promote selective adhesion of various types of cells [3]. All of these methods employ adhesive proteins that can degrade with time causing instability in the surface adhesion. The use of non-biological polymers as adhesive elements will avoid the presence of biological material, minimizing the degradation of the bonds linking the cell to the surface. Polyelectrolyte multilayers (PEMs), which consist of alternating layers of polyanions and polycations adsorbed on a charged surface, can be used to modify a particular surface region. By using microfluidic networks, polyelectrolytes can be transported and patterned over discrete surface areas, while the rest of the surface is masked and consequently remains unchanged. This work presents the use of poly(ethyleneimine) (PEI), poly(allylamine hydrochloride) (PAH), poly(diallyldimethylammonium chloride) (PDADMAC), and polystyrene sulfonic acid (PSS) to form PEM films by this technique, to generate micropatterned positively charged areas and to use these as adhesion sites for retinal cells for further usage in cell-based assays.

## EXPERIMENTAL

Microfluidic channels molded in PDMS were used to pattern the PEMs onto a substrate. Standard silicon microfabrication procedures [4] were used to produce the

masters for molding the PDMS microfluidic channels. The PDMS channel network was produced by bringing into contact the molded PDMS with a flat plasma oxidized PDMS surface (Figure 1). The PEMs were formed by flowing polycation and polyanion solutions through the channels in a sequential fashion. After the PEMs were patterned the molded PDMS was removed thus exposing a top positive layer on the flat PDMS surface. The surface will ultimately exhibit positive lines of PEMs surrounded by a neutral PDMS surface, due to the reversible nature of plasma oxidized PDMS surfaces when exposed to air [5]. Three polycations poly(allylamine hydrochloride), (PAH); poly(diallyl-dimethyl ammonium chloride), (PDADMAC); and poly(ethyleneimine), (PEI) were tested for adhesion and as growing surfaces for the cells. In all cases, polystyrene sulfonate was used as the polyanion.

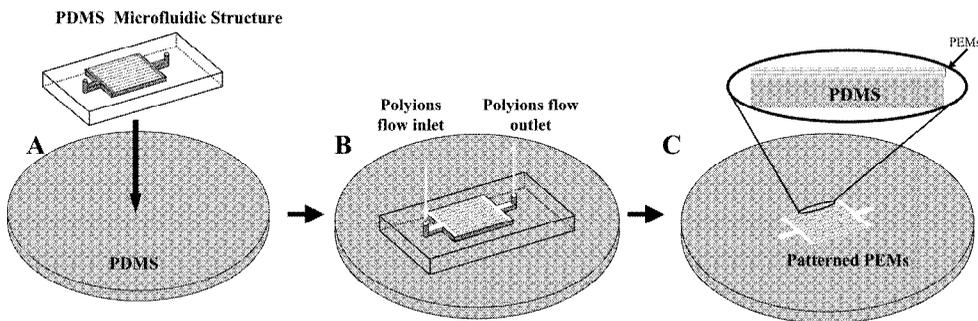


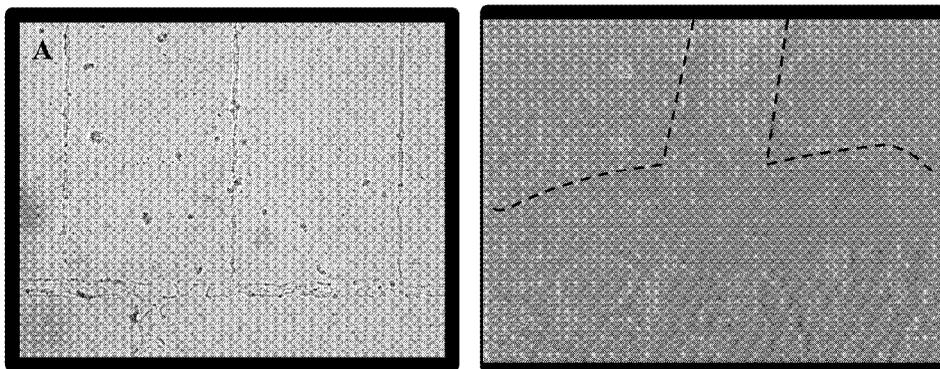
Figure 1. A) The molded PDMS is brought into contact with a flat PDMS surface. B) Polyions are introduced through the reservoirs sequentially (the dark lines show the solution of a polyion filling the channels) and are left for at least 15 minutes inside the microfluidic structure to allow the formation of the polyion layer pattern. The channel is rinsed with water and filled again with the next polyion. This cycle is repeated until the desired number of layers is obtained. C) The molded PDMS is removed and the stacked layers of polyelectrolytes are exposed along with the surrounding PDMS surface.

Immortalized R28 rat retinal cells were cultured for two to three days on polystyrene culture flasks until confluent. DME medium (DMEMF12) was changed every other day. Once the culture was confluent, the cells were trypsinized, resuspended in media and seeded (diluted 1/10) onto the culture dishes containing the PEMs. Some of the cell patterns were fixed using 4 % paraformaldehyde in 0.1 M phosphate.

## RESULTS AND DISCUSSION

Immortalized rat retinal cells were incubated overnight on micropatterned PEMs surfaces. Figure 2A shows adhesion and growth on PAH patterns after 18 h. Only a few

cells agglomerated on the PDMS surface. After 52 h, the cells completely covered the PEM lines, also extending a small distance from the PEM lines towards the PDMS surface (Figure 2B). Cells adhesion and growth were also attempted on PEI surfaces. As observed in figure 3A, the cells remain patterned on the PEM lines with only a few cells agglomerated on the PDMS surface. Figure 3B shows cells that were allowed to adhere and grow for up to two weeks on PEI. The picture shows the microfluidic structure (outlined) when the cells grew on the completed PEMs structure. The insert photo in Figure 3B shows the growth of cells occurred mostly in the area under the PEMs, although growth towards the PDMS surface is also observed. PDADMAC was the least likely to promote cell adhesion and growth on such patterns, whereas the PAH and PEI exhibited better adhesion and cell growth.



*Figure 2. A) Overnight growth (~18 h) of retinal cells on poly(allylamine hydrochloride). The cell growth is observed in patterned lines. B) Cells growing on poly(allylamine hydrochloride) after 52 h (PDMS surface, upper left, and upper right corners of the image).*

Preliminary studies cultivating these cells in culture flasks after detachment from the PEMs, have shown that cells can grow again on such surfaces, and morphological differences are not observed.

## CONCLUSIONS

These polymers proved useful for the selective patterning of retinal cells. PEI and PAH exhibited good adhesion properties for this line of cells, whereas PDADMAC showed adhesion properties to a lesser extent. PEI proved to be a good material for longer growth time (at least two weeks). We believe this approach could provide a useful and simple tool to pattern single cells or cell networks in specific locations to monitor neural activity of retinal cells as they are exposed to different stimuli.

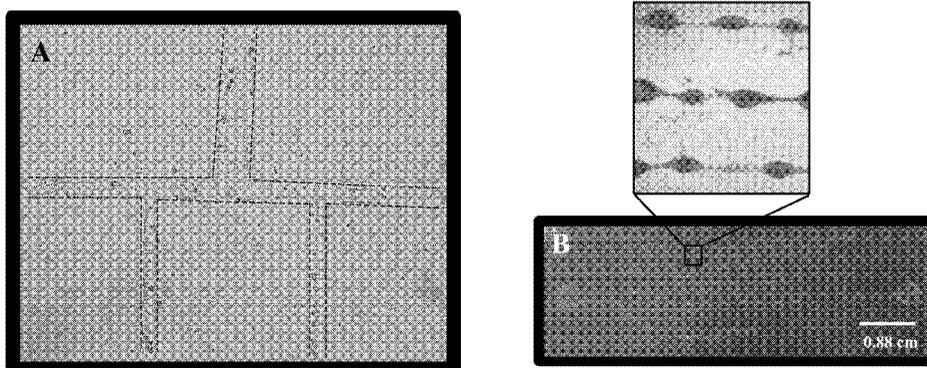


Figure 3. A) Overnight growth (~18h) of retinal cells on poly(ethyleneimine). Cell growth is observed on the polycation surface areas (within the dotted black lines). B) Two weeks cell growth on poly(ethyleneimine). Cells covered all the polycation region.

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