CREATING CELLULAR MICROPATTERNS BY SWITCHING FOULING PROPERTIES OF ELECTROACTIVE ITO SURFACES

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ABSTRACT

This paper describes a novel approach of forming micropatterned co-cultures using a combination of oxygen plasma ashing and electrochemical removal of poly(ethylene glycol) (PEG) silane from indium tin oxide (ITO) substrates. After assembling a layer of PEG silane on ITO, photolithography and oxygen plasma cleaning were used to form cell-adhesive domains within the non-fouling PEG silane layer. After patterning hepatocytes in these domains, the surrounding PEG silane layer was desorbed by applying negative voltage (-1.4 V vs. Ag/AgCl) to the underlying ITO substrate. This switched the fouling properties of the ITO substrate and allowed the adsorption of another cell type creating a micropatterned co-culture.

KEYWORDS: micropatterned co-culture, switchable biointerface, photolithography

INTRODUCTION

A number of approaches for creating micropatterned co-cultures have been proposed to understand the cellular complexities of native tissues [1, 2]. In this paper, we describe a surface engineering strategy that allows exercising spatial and temporal control over attachment of two distinct cell types onto the same surface. The strategy, described in Figure 1, entailed modifying ITO substrates with non-fouling PEG silane layer, photopatterning positive tone resist on the surface and exposing the surface to oxygen plasma treatment. Removal of the photoresist revealed cell-adhesive domains within the non-fouling PEG-modified ITO substrate. Incubation of such surfaces with cells led to organization of cell clusters in the regions void of PEG silane (Figure 1, Step 3). Importantly, applying negative potential to the ITO substrate resulted in removal of the non-fouling PEG silane

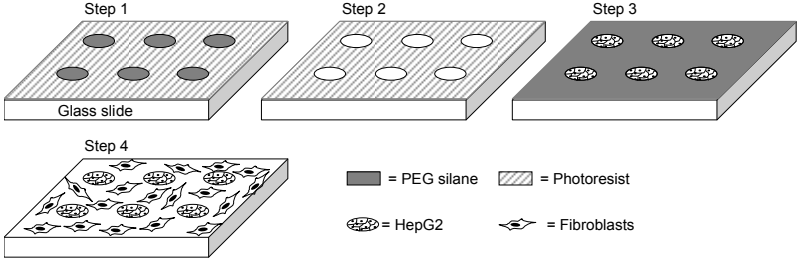

Figure 1: Step 1: Photoresist patterning on PEG silane. Step 2: Removal of exposed PEG silane using oxygen plasma ashing. Step 3: Lift-off of photoresist followed by seeding of HepG2 cells. Step 4: Electrochemical removal of surrounding PEG silane followed by attachment of fibroblasts to complete the co-culture.

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layer and was followed by the addition of another cell type to the surface to create the micropatterned co-culture. The method described here allows flexibility in choosing the sequence of cell seeding steps and may be used to organize cell types of similar "adhesiveness". In the future, this technique will be used to co-culture embryonic stem cells with mature hepatocytes to induce liver-specific differentiation.

MATERIALS AND METHODS

Modification of ITO substrates with PEG silane and photolithographic patterning of these substrates were discussed by us previously [3]. PEG silane modified ITO substrates containing patterned photoresist were exposed to oxygen plasma at 300W for 10 minutes resulting in removal of the silane layer from the regions not protected by photoresist. iElli2000 imaging null ellipsometer was used to create thickness maps after the plasma removal of PEG silane. Ellipsometric measurements were made using an optical model and a refractive index of 1.45. Deposition of fibronectin on PEG silane desorbed regions was confirmed using standard immunostaining protocols [4].

To assemble the co-cultures, surfaces were incubated with HepG2 cells ($1x10^6$ cells/mL) for 30 minutes. Unattached cells were washed off using 1x PBS solution. Prior to fibroblast seeding, surrounding PEG silane regions were desorbed by applying a voltage of -1.4V for 60 seconds vs. Ag/AgCl reference electrode. Fibroblasts were seeded onto the same surface forming a micropatterned co-culture.

RESULTS AND DISCUSSION

Figure 2A,B shows ellipsometric results of patterns formed in PEG silane after oxygen plasma ashing. The average thickness of PEG silane at three different locations was measured to be 2.25 nm with RMS roughness of 0.15 nm. The PEG silane desorbed regions had an average thickness of 0.23 nm with a roughness of 0.07 nm. The thickness values for PEG silane closely corresponded to our previously reported measurement of 2.03 nm [3]. Figure 2C shows immunofluorescent staining of fibronectin deposited on the surface highlighting the lack of protein attachment on the PEG silane-modified regions of ITO. Figure 3A shows the attachment of HepG2 cells in the regions affected by oxygen plasma ashing (analogous to Step 3 of Figure 1). Stellate cells, primary hepatocytes and stem cells have also been patterned

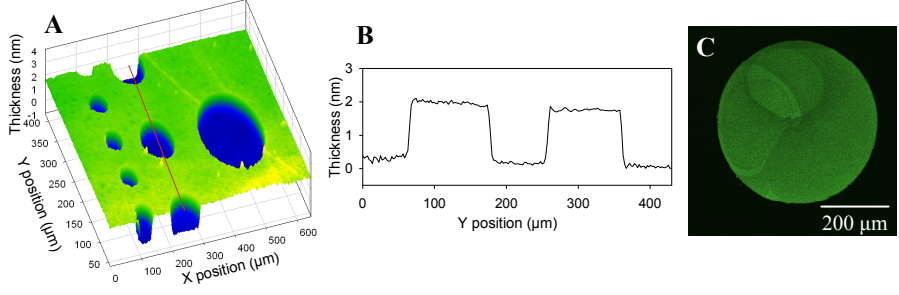

Figure 2: (A) Imaging ellipsometer results of PEG silane patterns formed from oxygen plasma removal with (B) corresponding cursor profile. (C) Immunostaining of fibronectin deposited in PEG silane desorbed regions

in a similar fashion (data not shown). To promote HepG2 cell binding the surface was pre-coated with collagen (I) prior to cell seeding. Application of reductive potential (-1.4V) to the ITO substrate containing micropatterned mono-culture led to desorption of the PEG silane layer and was followed by seeding of the second cell type to create a co-culture. Figure 3B shows the attachment of fibroblasts around islands of hepatocytes to create a micropatterned co-culture.

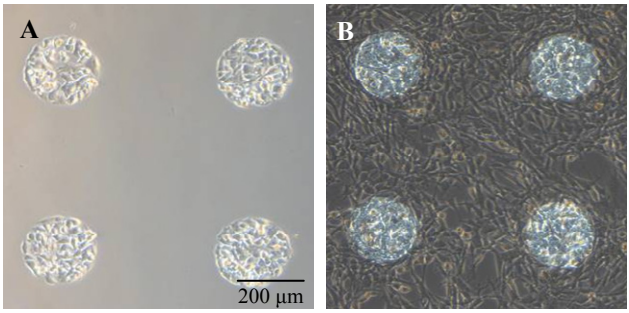

Figure 3: (A) HepG2 arrays formed on ITO substrate. (B) Attachment of fibroblasts and creation of a co-culture after electrochemical release of PEG silane.

CONCLUSION

A combination of oxygen-plasma induced and electrochemical removal of non-fouling PEG silane layer was used to pattern two cell types (hepatocytes and fibroblasts) on the same substrate. In the future, this technique will be used to pattern embryonic stem cells with mature liver cells in an effort to differentiate the stem cells.

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