CELLULAR MICROPATTERNS WITH BUILT-IN BIOSENSORS FOR DETECTION OF EXTRACELLULAR METABOLITES

J. Yan, J. Y. Lee, S. Shah, A. Revzin

Department of Biomedical Engineering, University of California, Davis, U.S.A.

ABSTRACT

We developed a strategy for integrating cells and biosensors based on non-fouling poly (ethylene glycol) (PEG) hydrogel microstructures serving a dual role of cell culture chambers and matrices for encapsulation of sensing elements. We demonstrated incorporation of biosensors into micropatterned mono and co-cultures, and sucessfully detected release of endogenous hydrogen peroxide (H_2O_2) by macrophages. This strategy for integrating cells and miniature sensing elements may be used in the future for detection of multiple metabolites from a small group of cells.

KEYWORDS: microfabrication, hydrogel microstructures, cell micropatterning, biosensors

INTRODUCTION

Microfabrication technology has been used extensively for cell manipulation and analysis [1, 2], however, integration of small groups of cells with sensors remains a challenge. Our previous studies demonstrated the use of photolithographically fabricated PEG hydrogel microstructures for biosensing [3] and cell patterning purposes [4, 5]. In the present work, we describe the use of non-fouling hydrogel microstructures with entrapped enzymes that serve a dual purpose of cell sequestration and detection of extracellular metabolites.

EXPERIMENTAL

In order to integrate the sensing elements with cells, non-fouling PEG hygrogel elements were micropatterned onto silane-modified glass surfaces following protocols reported previously [4, 5]. Cells were then seeded onto the silane-treated surface. To incorporate sensing elements into the micropatterned co-cultures, photopatterning of PEG was combined with photolithographic patterning of collagen (I) to enable positioning of biosensors in precise locations within the co-cultures, creating cell-adhesive domains in registration with sensing hydrogel microstructures (Figure 1). Briefly, photoresist was patterned onto the silane-modified surface, leaving desired region open for collagen deposition. PEG hydrogel microstructures containing enzyme and/or fluorophores were then aligned and registered onto pre-existing collagen patterns followed by the photoresist lift-off. Co-cultures were assembled by sequential seeding of model hepatocytes (HepG2 cells) that preferentially attached to collagen regions and then fibroblasts that attached elsewhere on the glass substrate. Enzyme-carrying PEG hydrogel microstructures remained free of cells after the co-culture assembly. In both mono-culture and co-culture cases, horseradish peroxidase (HRP) was entrapped in the PEG hydrogel microstructures and Amplex Red

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was added into the cell culture media for fluorescent H_2O_2 detection. Fluorescence signals were recorded by epi-fluorescence/confocol microscope.

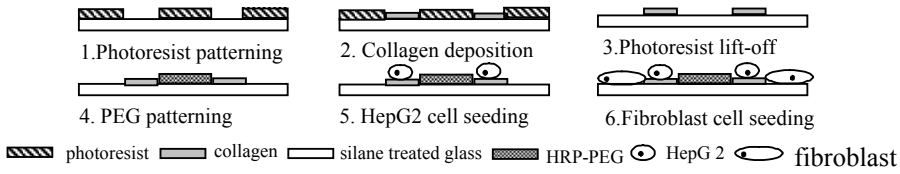

Figure 1. Diagram describing integration of sensing elements with co-culture of hepatic (HepG2) cells and nonparenchymal cells.

RESULTS AND DISCUSSION

We have developed a PEG hydrogel photolithography-based strategy for juxtaposing small groups of cells and optical biosensors for metabolite detection. As shown in Figure 2A, HRP-containing PEG hydrogel micropatterns allowed to guide cell attachment to the desired locations on the surface and to position cells next to sensing elements. Addition of exogenous H_2O_2 into the culture dish containing Amplex Red resulted in enzyme-catalyzed oxidation of Amplex Red and appearance of red fluorescence in the hydorgel sensing elements (Figure 2A). Moreover, detection of endogenous H_2O_2 in hydrogel microstructures was demonstrated after mitogenic activation of macrophages cultured alongside HRP-containing PEG elements. Figure 2B and 2C show sensing hydrogel structures before and after stimulation of macrophages and highlight appearance of fluorescence signal the latter scenario.

The top-down nature of the hydrogel photolithography approach allowed to register PEG hydrogel elements with existing microfabricated layers in order to create surface with complex physicochemical properties. Figure 1 described the combined use of traditional photoresist and PEG hydrogel photolithography to align cell-adhesive collagen (I) domains with non-fouling hydrogen regions. This alignment is demonstrated in Figure 3A, where red regions represent collagen (I) domains. As shown in Figure 3B, hepatic cells (HepG2) selectively attached onto col lagen (I) domains and did not adhere on PEG hydrogel elements or elsewhere on a glass substrate. Incuba tion of the same surface with fibroblasts resulted in the construction

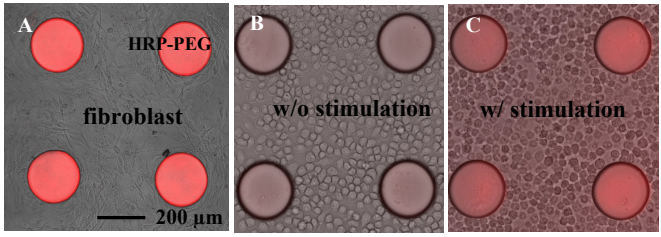

Figure 2. (A) HRP-containing PEG microstructure incubated with fibroblasts in the presence of H_2O_2. (B-C) Macrophages before (B) and after (C) exposure to PMA. Fluorescence appears on PEG gel areas on (A) & (C) but not (B).

of co-culture with integrated PEG hydrogel microstructures (Figure 3C). Importantly, incorporating biorecognition elements such as HRP into the hydrogel allowed to place sensing elements into a micropatterned co-culture. Figure 3D shows a hepatocyte-fibroblast co-culture after addition of 10 μM H_2O_2 and Amplex Red. The appearance of fluorescence in Figure 3D points to the possible future applications of sensing hydrogel microstructures for detecting local interactions between multiple micropatterned cell types.

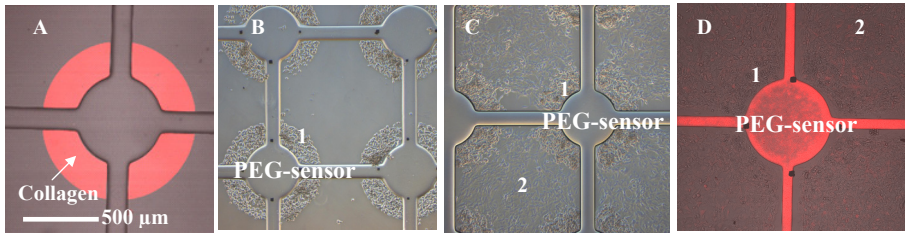

Figure 3. (A) Photopattern of PEG hydrogel in registration with collagen patterns(protein shown in red). (B,C) Seeding of HepG2cells (B) and fibroblasts (C). (D) Appearance of fluorescence inside PEG hydrogel after adding H_2O_2 in a co-culture. 1:HepG2; 2:Fibroblast.

CONCLUSIONS

In conclusion, we developed a surface engineering approach based on PEG photolithography for integration of miniature non-fouling hydrogel sensors with cellular micropatterns. This novel strategy is envisioned to have future application for multianalyte detection of cellular metabolites in small volumes based on small groups of cells.

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