ANTIBODY FUNCTIONALIZED MICROPATTERNED HYDROGELS

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ABSTRACT

This paper demonstrates the ability to create highly customizable structures using microfluidics, hydrogels, and micropatterning. Projection lithography was used to create structures of varying sizes, geometries, and spacing. Photodegradable poly(ethylene glycol) was used to create posts within microchannels. Encapsulated fluorescent proteins were released upon degradation, demonstrating the creation of on-demand chemical gradients within microsystems. Antibody functionalized surfaces were also created with these materials for capturing and selectively releasing circulating tumor cells. The versatile and highly tunable hydrogel structures introduced in this paper provide a route to achieving spatiotemporal control over a range of physiochemical properties within microfluidic systems.

KEYWORDS: Hydrogel, Micropatterning, Microfluidic, Photolithography, Circulating Tumor Cells

INTRODUCTION

The popularization of lab on a chip devices for clinical and biological applications has created a need for precise geometric, temporal, spatial, and chemical control over microfluidic platforms. We show that microfluidic devices, in combination with hydrogels and photopatterning, can lend spatiotemporal control over a system’s physiochemical properties. Poly(ethylene glycol) diacrylate (PEGDA) based hydrogels are commonly used in biological applications because they are biocompatible, easily modified, and do not encourage cell binding. PEG hydrogels can be covalently functionalized by adding acrylated peptides, proteins, or antibodies [1]. Additionally, larger molecules can be sterically encapsulated within PEG hydrogel meshes for delivery upon network degradation.

PEGDA hydrogels are generally formed by photopolymerization, a technique that is widely used to create drug delivery platforms and tissue scaffolds. Projection lithography can be used to provide spacial control over PEG polymerization. Recently, microstructured post were created in this fashion for bioassay platforms [2]. We use this method to create custom structures varying in size, shape, and spacing.

We employ a photolabile PEG to achieve spatiotemporal control that is used for the release of macromolecules and cells on sub-cellular length scales [3]. Photodegradable PEG posts, loaded with fluorescent macromolecules, are used to demonstrate the controlled formation of gradients upon degradation. Controlled release of compounds within microfluidic systems is potentially useful for on-chip cell culturing and testing of drug cytocompatibility and toxicity in pharmacokinetic applications. Photodegradable PEG can also be used as selective capture and release surfaces for circulating tumor cells (CTCs) by immobilizing antibodies within the hydrogel structure. This system allows for the individual release of cells for better capture purity and efficiency.

EXPERIMENTAL

Different arrays of posts were photopatterned by placing a shadow mask in the rear aperture of an inverted microscope (IX71, Olympus). All features were polymerized through a 20x objective, but the scale factor between mask and features is objective-dependent. Varying exposure times were used to control feature polymerization. Photodegradable PEG posts were created containing acrylated rhodamine and BSA-488. Posts were degraded under UV light and fluorescent images of the gradients released were taken for 600 seconds after degradation. Photodegradable PEG channel surfaces containing acryl-NeutrAvidin were created for CTC capture. Gels were functionalized with biotinylated antiEPCAM and exposed to PC3 prostate cancer cells under flow (2 µL/min) then rinsed (20 µL/min). The gel was placed under laminar flow and exposed to UV light until cells were released from degraded gel.
RESULTS AND DISCUSSION

Features of varying sizes and geometries were created using projection lithography with varied the channel depths, mask feature sizes, and exposure times (Figure 1). Cylindrical posts with diameters as small as 2.5 µm were fabricated within 10 µm thick channels with an exposure time of 500 ms.

Photodegradable PEG posts containing acrylated rhodamine and BSA-488 were degraded by exposure to UV light for 3 minutes. Acrylated rhodamine and BSA-488 decreased in fluorescent intensity after degradation. The diffusive loss of BSA-488 produced a gradient between the original gel structure and the reservoir, demonstrating the reconfiguration of the hydrogel network upon degradation (Figure 2). The ability to control the degradation rate of hydrogels provides versatile control over system properties that can be used in numerous biological applications such as pharmacokinetics, on-chip cell culture, and bioassays.

PC3 cells were captured on an antibody functionalized surface under laminar flow. A selective portion of the gel was eroded and all cells in the region were released from the gel within 30 seconds. The part of the gel not exposed to UV light remained intact. (Figure 3). This cell capture/release system results in higher capture efficiencies and purities.

CONCLUSION

We have demonstrated a facile technique with which highly customized microstructures can be created. With photodegradable PEG hydrogels we demonstrated the ability to customize release gradients upon structure degradation and the ability to capture and release CTCs from capture surfaces. These methods provide a sophisticated level of spatiotemporal control within microsystems, leading to increased experimental flexibility for a large number of biological applications.

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