ABSTRACT

Novel biomimetic polymer networks were developed that are entirely synthetic and tailored to have various properties and function. These artificial networks have numerous applications such as sensing elements in biosensors, intelligent drug delivery devices, and immunoassays. In comparison to biological entities, biomimetic polymer networks are advantageous because they can be designed to mimic biological recognition pathways and at the same time exhibit other abiotic properties that are more favorable, such as greater stability in harsh environments. For many applications, it is necessary to integrate these polymeric networks at the micro-/nano-scale. In our laboratory, procedures have been developed to facilitate this micro-/nano-scale application. A mask aligner was utilized to enable precise micropatterning of ultra-thin polymer films via UV free-radical polymerization. For the case where these organic polymer networks were patterned onto inorganic silicon substrates, an organosilane coupling agent was utilized to gain covalent adhesion between the dissimilar polymer network and the silicon surface.

As an example application, a glucose microsensor was developed based on a patterned biomimetic polymer network designed to selectively recognize D-glucose among similar molecules via non-covalent complexation. Novel copolymer networks containing poly(ethylene glycol) dimethacrylate and functional monomers such as acrylic acid, methacrylic acid, and acrylamide were synthesized in polar, aprotic solvent (dimethyl sulfoxide). Results qualitatively and quantitatively demonstrate that these recognitive macromolecular networks are specific for the target molecule and can be effectively micropatterned in fine dimensions. These results are encouraging for the further development of functionalized micro-biosensors and diagnostic devices and are applicable to other biologically significant molecules and biomimetic polymer networks, in which hydrogen bonding, hydrophobic, or ionic contributions will direct recognition.

KEY WORDS
Biomimetic, imprinting, microarray, micropatterning, microsensor, molecular recognition

INTRODUCTION

This paper describes a fundamental approach to preparation, design and synthesis of polymeric networks that can exhibit unique configurational biomimetic properties. The term configurational biomimesis has been coined by our group to describe macromolecular networks with specific configurations that aid in molecular and, more specifically, biological recognition. Engineering the molecular design of biomaterials by controlling recognition and specificity is the first step in coordinating and duplicating complex biological and physiological processes. The design of a precise macromolecular chemical architecture that can recognize target molecules from an ensemble of closely related molecules has a large number of potential applications.1-3

The particular arrangement of chemical groups can be stabilized by forming a polymer network around the binding site chemistry. In general, this typically involves forming a pre-polymerization complex between the template molecule and functional monomers or functional oligomers with specific chemical groups designed to interact with the template molecule either by covalent or non-covalent forces. Once the pre-polymerization complex is formed, the polymerization reaction occurs in the presence of a crosslinking monomer and an
appropriate solvent, which controls the overall polymer morphology and macroporous structure. Once the original template molecule is removed, a heteropolymer matrix with specific recognition elements for the template molecule remains.

In particular, this work builds on previous work in development of intelligent polymer materials and their application in novel biosensors. Typically, biosensors utilize proteins and/or other biological compounds to selectively recognize and sense a specific target. The major limitations of these natural receptors are their high cost, potential antigenicity, and low stability. An alternative to these techniques is to use synthetic biomimetic networks to create hydrogels that will bind and respond to specific analytes. Biomimetic polymer networks are advantageous because they can be tailored to bind any molecule with controlled selectivity and affinity, provided that certain interactions exist. In a recent review, several methods utilizing template-mediated polymerization were proposed to design analyte responsive hydrogels that can respond to a desired analyte.1

EXPERIMENTAL PROCEDURE

The monomers studied were acrylamide (Aam) and poly(ethylene glycol) dimethacrylate (designated as PEGnDMA, where n is the average molecular weight of the PEG chain). The solvent used for the polymerization was dimethylsulfoxide (DMSO), and the analyte used to create the biomimetic polymer networks was D-glucose. The initiator used for the UV free radical polymerization was Irgracure® 184, and adhesion was gained between the silicon substrate and the polymer using an organosilane coupling agent, γ-methacryloxypropyl trimethoxysilane (γ-MPS). The Aam, γ-MPS, DMSO, and D-glucose were purchased from Aldrich (Milwaukee, WI). PEGnDMA, with n = 200, was obtained from Polysciences, Inc. (Warri ngton, PA). The Irgracure® 184 was purchased from Ciba Specialty Chemicals (Tarrytown, NY). Fluorescent D-glucose analogue, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG), was purchased from Molecular Probes, Inc. All chemicals were analytical grade.

Preparation of micropatterned biomimetic polymer networks

Crosslinked acrylamide (Aam) networks were prepared by reacting Aam with substantial amounts of PEG200DMA. A novel patterning technique based on photolithography was utilized to pattern these networks onto silicon wafers. A schematic of the technique is included as Figure 1. This micro-reactor photolithography used a Teflon® spacer with a thickness of approximately 12.5 µm to create the micro-reactor chamber. Silicon wafers were cleaved into pieces that were approximately 2 cm by 2 cm, and then cleaned utilizing a standard Piranha clean. To promote covalent adhesion between the silicon surface and the polymer, the silicon pieces were soaked in a 10 wt% solution of γ-MPS in acetone for more than 2 hours. Then, these were rinsed in acetone followed by ethanol, and then air-dried. The organosilane coupling agent formed a self-assembled monolayer on the native silicon dioxide surface and presented methacrylate pendant groups that reacted and covalently bonded the silicon surface with the polymer film.
UV400 mask aligner, enabling for alignment accuracies of 0.1 microns. After bringing the sample into contact with the mask, it was exposed to UV light with intensity of 23.0 mW/cm² for exposure times of 30 seconds. The pieces were then removed and allowed to soak in deionized distilled water for greater than 24 hours to remove any unreacted monomer and the template molecule.

Evaluation of the biomimetic polymer network micropatterns

The binding results of the biomimetic polymer micropatterns were visualized using a fluorescent glucose analogue, 2-NBDG. The analogue, 2-NBDG, (maximum absorption 466 nm; maximum emission 542 nm) was added to vials (concentration of 4.7x10⁻⁷ mole/liter) containing the samples with the polymer micropatterns and allowed to equilibrate for approximately 1 hour before imaging. Confocal analysis was performed using a Bio-Rad MRC 1024 Confocal Microscope with an MRC 1024 system. Images, z-sections, etc. were collected using LaserSharp software and image analysis was conducted using Confocal Assistant software.

RESULTS AND DISCUSSION

Using the micro-reactor photolithography technique, sharp micropatterns of biomimetic polymer networks were created on silicon substrates. Representative 3D projection images of micropatterned biomimetic polymer networks are shown in Figure 2 and 3. The thickness of the patterned polymer films was determined to be approximately 13 µm using profilometry.

![Figure 2. 3D Projection of micropatterned rectangular array of a biomimetic polymer networks obtained utilizing a confocal microscope.](image)

In Figure 4, microcantilever patterns of a control polymer network and a biomimetic polymer network are compared. In 4(b) and 4(c), z-slices at approximately 5 microns below the surface of the patterns are shown. Qualitatively comparing 4(b) and 4(c), it is clear that the biomimetic polymer network has bound more of the fluorescent analogue. These results compare favorably with previous studies within our laboratory and illustrate the potential power of applying these polymer systems for microarray and microsensing applications.

![Figure 3. In A), 3D Projection a micropatterned square array of biomimetic polymer networks obtained utilizing a confocal microscope. In B), a slice of the square array is demonstrated.](image)

SUMMARY

Novel biomimetic polymer networks were developed that are entirely synthetic and tailored to have various properties and function. In this study, a biomimetic polymer network designed to selectively recognize D-
glucose among similar molecules via non-covalent complexation was micropatterned. Specifically, a biomimetic polymer network was prepared with a Aam:PEG200DMA mole ratio of 1:2. Micropatterning results qualitatively and quantitatively demonstrate that these recognitive macromolecular networks are specific for the target molecule, D-glucose, and can be effectively micropatterned in fine dimensions. These results are encouraging for the further development of functionalized micro-biosensors and diagnostic devices and are applicable to other biologically significant molecules and biomimetic polymer networks, in which hydrogen bonding, hydrophobic, or ionic contributions will direct recognition.

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