BIOMIMETIC POLYMERS IN DRUG DELIVERY AND SENSING APPLICATIONS: EFFECT OF NETWORK MOLECULAR STRUCTURE ON RECOGNITION PROPERTIES

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INTRODUCTION

Biomimetic polymer networks have been developed with tailored affinities and transport properties for a given target molecule, and these networks promise to significantly impact a wide variety of biomedical fields, including controlled drug delivery and biosensing. Biosensors typically employ biological compounds as recognition elements, and biomimetic artificial networks would be advantageous alternatives since they can be designed to mimic biological recognition pathways, while being more robust and cost effective. In the field of controlled drug delivery, the ability to precisely tailor the transport properties of a given therapeutic molecule is fundamental to the success of a delivery device.

Methods were developed to integrate biomimetic networks with silicon substrates at the micro-/nanoscale, enabling the fabrication of microdevice platforms that are based on silicon technologies. Biomimetic polymer networks specific for a target biomolecule were micropatterned onto silicon substrates and characterized by single and competitive fluorescent and confocal microscopy studies, SEM, and profilometry. Specifically, polymer networks that selectively recognize D-glucose among similar molecules via non-covalent complexation were micropatterned in fine dimensions. Novel copolymer networks containing poly(ethylene glycol) n dimethacrylate (where the molecular weight of the PEG, n = 44, 200, and 600) at various crosslinking percentages (30%, 67%, and 80%) and acrylamide as a functional monomer were synthesized in polar, aprotic solvent (dimethyl sulfoxide). These biomimetic polymer networks were effectively micropatterned and demonstrated to be specific for the target molecule. In particular, the binding and release kinetics were examined, and the relative binding affinities were analyzed.

EXPERIMENTAL

D-Glucose Recognitive Network Synthesis

Acrylamide (Aam), 2,2-dimethoxy-2-phenyl acetophenone (DMPA), dimethylsulfoxide (DMSO), ethylene glycol dimethacrylate (EGDMA), and D-glucose, were purchased from Aldrich (Milwaukee, WI). PEGnDMA, with n = 200, 400, and 600, was obtained from Polysciences, Inc. (Warrington, PA). Irgacure® 184, 1-hydroxycyclohexyl phenyl ketone, 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-deoxy-glucose (2-NBDG), was purchased from Molecular Probes, Inc.

Novel heteropolymer films of differing composition and percentage of crosslinking monomer were synthesized in a aprotic, polar solvent via UV free-radical polymerization in a nitrogen atmosphere. In a typical experiment (e.g. PEG200DMA as crosslinking monomer), 15

mmoles of Aam and 60, 30, or 10 mmoles of PEG200DMA (e.g., 80, 67, or 30% mole crosslinking monomer/mole all monomers, respectively) were allowed to complex with 3 mmoles of D-glucose mixed with 6 mL DMSO. After mixing and checking mutual solubilities, Irgacure® 184 initiator was added in the amount of 1-2 wt%. Control polymers were made with exactly the same composition except D-glucose was not added.

After preparation, the solution was placed in a nitrogen atmosphere and nitrogen was bubbled for 30 minutes to remove oxygen, which is a free-radical scavenger and inhibits the free-radical polymerization. The monomer mixtures were pipetted between two clamped 6" by 6" glass plates with a Teflon® spacer that was 790 microns thick. Next, the glass plate assembly was placed under a UV source (Dymax Ultraviolet Flood Cure System) and exposed to UV light with an intensity of 10.0-15.0 mW/cm^2 for 15 minutes to initiate the free-radical polymerization. Polymers were placed in deionized water immediately after preparation, were carefully separated from the slides, and then were cut into various diameter discs using a cork borer. Discs were then placed in 50 mL centrifuge tubes and placed on a rotating mixer (25 RPM, 70 degree angle, Glas-Col, Terre Haute, IN) and resuspended within multiple 24 hour wash steps to remove glucose and excess monomer. The resulting discs were then dried in air at ambient conditions and placed in a vacuum oven (T=26 ˚C, 28 mm Hg vacuum) until a constant weight was obtained (less than 0.1 wt% difference). The discs were then stored in a dessicator until testing.

Analysis of Kinetic Binding and Release via Fluorescent Microscopy

Kinetic binding and release experiments were conducted to examine the relative rates of uptake and release from the polymer networks. The effect of the network structure on the diffusion coefficient of the D-glucose fluorescent analogue, 2-NBDG, was examined. By analyzing fluorescent intensity values from polymer discs of equal thickness, quantitative analysis of relative amount bound in network can be made. An aliquot of known concentration of 2-NBDG (1×10^{-4} mg/ml (2.9×10^{-4} mM)) was added to washed, solution-swollen polymer discs (preparation described above) in 50mL of solution within centrifuge tubes. The tubes were covered with aluminum foil and placed on a rotating mixer (Glas-Col., Terre Haute, IN; 70 degree angle, 25 RPM). At various time points, kinetic analysis of the binding was carried out. For the release studies, polymer films that had reached equilibrium binding were placed in 50 ml centrifuge tubes filled with DI water. The water was frequently replaced with fresh DI water, allowing for an infinite sink condition to be assumed. Analysis of fluorescent intensity values from polymer discs of equal thickness provided quantitative analysis of binding and release.

A Nikon Eclipse ME600L fluorescent microscope with a FITC filter set was used and images were acquired with Coolsnap digital camera. Meta-View software from Universal Imaging was utilized to analyze a large amount of pixels within these images for calculation of an average fluorescent intensity and standard deviation across the image.

RESULTS

In Figures 1, a representative kinetic binding result is presented for imprinted and control acrylamide-based polymer systems with 67% crosslinking percentage and PEG200DMA as the crosslinker. It was observed that, as the length of the crosslinker is increased (EGDMA ➔ PEG200DMA ➔ PEG600DMA), the time to reach equilibrium binding is decreased. Therefore, the longer crosslinkers result in looser networks, which allow for faster diffusion of the target analyte. This same trend was observed for the systems (EGDMA, PEG200DMA, and PEG600DMA) and 30% crosslinking percentage.

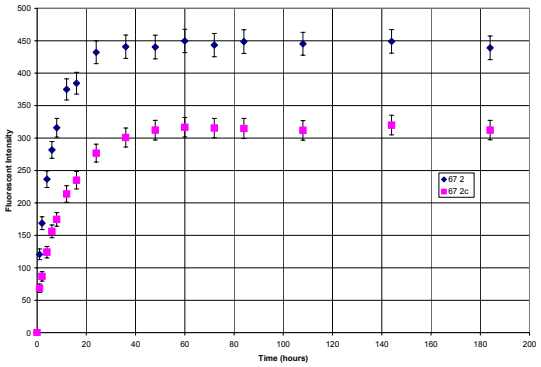

Figure 1 – Kinetic binding results for imprinted and control acrylamide-based polymer systems with 67% PEG200DMA crosslinking of various crosslinker lengths.

For the 67% systems, the power law fit and early time fit for Fickian diffusion in a slab geometry were applied to determine the power law exponent, n, and the diffusion constant of 2-NBDG in these polymer networks. All systems exhibited n values of approximately 0.5, and therefore, can be described by Fickian diffusion. The calculated diffusion constants for the 2-NBDG decreased with decreasing crosslinker length, with the diffusion constant of PEG600DMA network (5.4×10^{-7} cm^2/s) being 5 times larger than for the EGDMA network (1×10^{-7} cm^2/s). For the networks crosslinked with EGDMA and PEG200DMA, the 2-NBDG diffusion constant of the control networks were approximately half of the imprinted networks. This enhancement of the diffusion constants in the imprinted networks is caused by the increased porosity resulting from polymerization in the presence of a template molecule, which acts as a porogen to certain degree. This effect was not observed in the PEG600DMA networks.

DISCUSSION

Novel glucose-binding gels were synthesized via biomimetic molecular imprinting techniques using non-covalent complexation interactions, and fluorescent microscopy was utilized as a novel method to characterize the kinetic and equilibrium binding properties of the polymer systems. Specifically, networks based on acrylamide were prepared and characterized using a novel fluorescent microscopy technique, which allowed for microscale observation of the binding and for the direct observation of the analyte uptake within the polymer film. The equilibrium binding characteristics and the kinetic binding and release characteristics of the fluorescent glucose analogue was analyzed.

The relative binding affinity and amount of fluorescent analogue, 2-NBDG, bound within the network was demonstrated to be controlled by the structure and properties of the polymer network. In general, the more rigid networks (shorter crosslinkers and high crosslinking percentages) exhibited higher affinities for 2-NBDG. As crosslinking percentage of the network decreased, the macromolecular recognition of the network chains decreased. Also, as the length of the crosslinker increased, the bound ratio decreased for a given concentration, indicating less imparted affinity. It is important to note that the relative amount of PEG or Aam within the polymer networks did not have a discernible effect on the binding affinity. In addition, 2-NBDG was an exceptional model compound to illustrate the applicability of imprinted polymers in novel drug loading and release applications.