Protein Recognitive Hydrogel Systems for Biosensor Applications

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Statement of Purpose: The ability to selectively recognize a specific protein in a complex solution (such as blood) would have many applications, including serving as a biosensor. Protein imprinted polymers are ideal replacements to their biological counterparts as they can be easily tailored to a variety of templates, are inexpensive and straightforward to prepare, have greater stability in harsh conditions, and are reusable. Because of this, protein imprinting has gained a great deal of attention from the scientific community; however, efforts to do so have achieved limited success.

The objective of this research is to develop novel protein sensing devices through the integration of recognitive hydrogel polymer networks and ultra sensitive microcantilever transducers. Molecular recognition or molecular imprinting is an emerging field of interest in which a polymer network is formed with specific recognition for a desired template molecule. Briefly, functional monomers are chosen which exhibit chemical structures designed to interact with the template molecule via covalent or non-covalent chemistry. The monomers are then polymerized in the presence of the desired template, the template is subsequently removed, and the product is a polymer with binding sites specific to the template molecule. This technique has been successfully applied to small molecule templates in the areas of separations, solid-phase extractions, artificial enzymes, and chemical sensors.

Methods: Protein imprinted polymers were synthesized via free radical polymerization of functional monomers methacrylic acid (MAA), acrylamide (Aam), and 2-(dimethylamino)ethyl methacrylate (DMAEMA) grafted with polyethylene glycol (400) dimethacrylate (1.8 mol%) as the cross-linking monomer. Bovine serum albumin (BSA) was used as the model protein template. Electrostatic interactions between the charged functional monomers MAA and DMAEMA and the oppositely charged amino acids present in BSA were optimized using isothermal titration calorimetry. These components were dissolved in Phosphate Buffer (10 mM, pH=7.4) to form the pre-polymerization solution, at which time the template and monomers were allowed to complex for 30 minutes in order for these interactions to occur. Control polymers were synthesized under exactly the same conditions, without the addition of BSA. The resultant bulk polymers were wet sieved to produce sub-150 μm particles and washed with 5 rinses of Phosphate Buffer followed by 5 rinses of 3M NaCl/Phosphate Buffer for template removal and retain binding sites specific to the protein. Recognition studies have also been conducted on these bulk imprinted polymers to test the affinity (mass BSA absorbed by the imprinted polymer versus the control) of the system.

In addition, methods previously developed in the lab for coating of pH responsive hydrogels onto microcantilevers have been applied for recognitive hydrogels [Hilt JZ. Biomedical Microdevices. 2003;5:177-184]. Briefly, commercially available silicon cantilevers were soaked in a 10 wt% solution of γ-MPS in acetone to promote covalent adhesion between the SiO_2 surface and the hydrogel. The monomer mixture with the water soluble UV initiator Irgacure 2959 was spin coated onto the silicon beams at 2000 rpm for 30 seconds. Micropatterns were created using a mask aligner and then exposed to UV light for 2 minutes at an intensity of 23 mW/cm^2. The mask aligner is critical to precise patterning of the polymer onto the cantilever (which prevents the hydrogel from polymerizing on the very tip of the beam). Preliminary deflection measurements have been conducted by focusing an optical laser at the tip of the cantilever and reflecting it off the cantilever into a position sensitive diode which can detect the vertical position of the cantilever beam.

Results: Figure 1, below, illustrates typical data from the removal of the protein template from the bulk polymer networks using Phosphate Buffer (steps 1-5) and 3M NaCl (steps 6-10) as ~99% of the protein initially included in the monomer mixture is removed. 3M NaCl is used as a protein denaturant and is required to remove the remainder of the protein after the buffer washes in order to break the bonds between the protein and polymer network. In addition, a clear imprinting effect has been demonstrated with the affinity recognition studies, as 8.8 times more BSA was absorbed by the MIP sample (13.8 ± 4.5 mg) compared to that of the NIP (1.6 ± 1.1 mg).

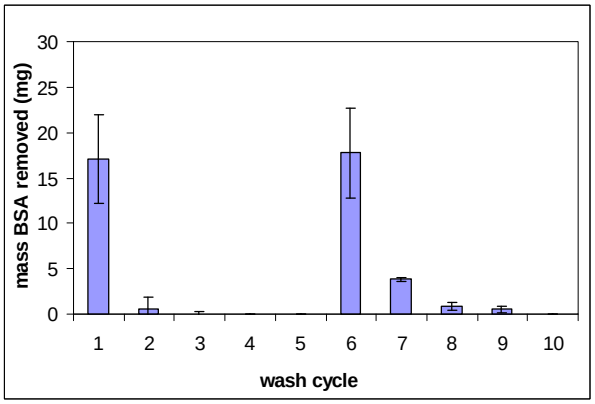

Figure 1: Template removal results (n=3, ± 1 S.D.)

Conclusions: Template removal studies demonstrate the ability to consistently remove BSA at levels near and above literature values. Initial studies have also shown that the synthesized protein imprinted polymers have BSA recognition capabilities. The preliminary cantilever deflection measurements have been promising towards indicating the sensitivity of the system to low BSA concentrations. Future studies include investigating the selectivity of the system to the template versus other structurally similar proteins as well as other protein templates, including actual protein biomarkers.