Protein Recognitive Hydrogel Systems for Biosensor/Biodiagnostic Applications

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Statement of Purpose: The objective of this research is to develop biomimetic recognitive hydrogel polymer networks that can be applied to biosensing or other nanoscale applications when integrated with a nanodevice. 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.

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 due to the inherent properties of proteins, which include size, complexity, conformation, and solubility.

Methods: Protein imprinted polymers were synthesized via a free radical polymerization of functional monomers methacrylic acid (MAA), acrylamide (Aam), and 2-(dimethylamino)ethyl methacrylate (DMAEMA) grafted with polyethylene glycol (400) dimethacrylate (2 mol%) as the cross-linking monomer. Bovine serum albumin (BSA) was used as the model protein template. Electrostatic interactions were exploited between the charged functional monomers MAA and DMAEMA and the oppositely charged amino acids present in BSA. In addition, BSA contains polar, uncharged amino acids that can undergo hydrogen bonding with Aam. These components were dissolved in water 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. After purging with N_2 to remove the free radical scavenger O_2, Ammonium Persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) were added as the initiator and catalyst. The reaction proceeded overnight at room temperature. Control polymers were synthesized under exactly the same conditions, without the addition of BSA. The resultant polymers were wet sieved to produce sub-150 μm particles and washed with 5 rinses of DI water followed by 5 rinses of SDS/DI water (10% w/v) to remove the template. Swelling studies were performed as previously published [Spizzirri UG. Chem. Mater. 2005;17:6719-6727]. Recognition studies were conducted to assess the recognitive abilities of the imprinted polymer in relation to the control. In contrast to the majority of imprinting literature, this procedure accounts for non-specific binding via a wash phase [Hawkins DM. Anal. Chim. Acta. 2005;542:61-65]. Briefly, the procedure includes a load, wash, and elution phase. In the load phase, a solution of known BSA concentration was pipetted into previously washed particles and placed on a rotary mixer overnight for incubation. Detection of a protein in the load phase suggests that protein has not been bound within the binding sites. The polymer particles are rinsed 5 times with DI water in the wash phase. If protein is detected in these washes, non-specific binding has occurred (i.e. the protein template was physically entrapped in the network). Lastly, the particles are rinsed 5 times with SDS/DI water (10% w/v) in the elution phase. Detection of the protein template in this phase indicates specific binding between the template and polymer has occurred. Therefore, evidence of larger amounts of protein in the imprinted polymer versus the control in this phase is desired.

Results: A mesh size of 48.1 ± 9.0 nm for the molecularly imprinted polymer (MIP) and 44.6 ± 11.5 nm for the non-imprinted polymer (NIP) was determined using the Peppas-Merrill equation. These values clearly show the pore sizes of the hydrogel networks are large enough for diffusion of the 14 nm diameter BSA molecule. Figure 1, below illustrates a clear imprinting effect, as 8.8 times more BSA was present in the elution phase of the MIP sample (13.8 ± 4.5 mg) compared to that of the NIP (1.6 ± 1.1mg).

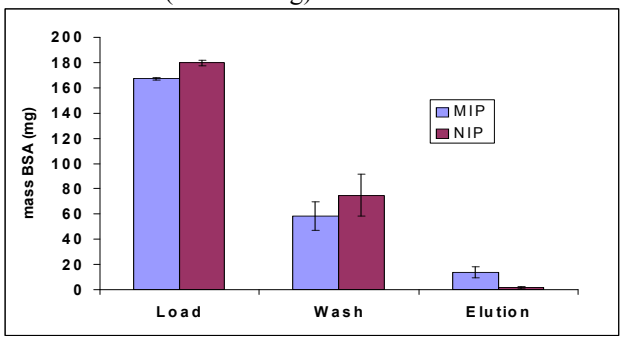

Figure 1: Recognition study (n=3, ± 1 S.D.)

Conclusions: Initial studies have shown that the synthesized protein imprinted polymers have BSA recognition capabilities. Future studies include optimization of the polymer composition in terms of template to monomer ratio, cross-linker length and amount, and functional monomer amounts. Once optimized, selectivity of the MIP to the template versus structurally similar proteins will be examined. Also, other templates will be studied, including actual protein biomarkers before integrating the MIP with a silicon substrate as a potential biosensor.