Interfacial Molecular Imprinting for the Production of Immunoresponsive Materials

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Abstract

Traditionally, molecular imprinting of proteins has proved challenging due to solvent limitations, recovery of the protein template, and most importantly, lack of specificity due to the large number of possible interaction sites. In this work, a surface imprinting technique has been developed to template hydrogel materials for specific protein recognition. Our model protein, the immunoglobulin IgG, was a unique choice due to its conserved/variable structure. Use of this 150 kDa molecule allowed for experiments to separate imprinting effects due to shape, surface chemistry, and molecular orientation.

A template mask was produced whereby protein (monoclonal IgG) was immobilized in a controlled orientation through a hydrazide reaction such that the antigen binding domains were fully exposed. A monomer mixture was brought in contact with this mask and polymerized to produce a surface imprinted hydrogel specific to the target protein. Non-imprinted, control materials were produced in the absence of protein. Imprinted hydrogels formed from the monomer 2-hydroxyethylmethacrylate and crosslinker ethylene glycol dimethacrylate bound significantly more IgG (monoclonal anti-c-myc) than the non-imprinted control materials. In addition, no significant binding was seen of a second immunoglobulin (anti-pig-IgG) by the imprinted gel, indicating specificity for the target protein.

1. Introduction

Molecular imprinted polymers (MIPs) are materials with well-defined nanostructures which give them the ability to recognize specific compounds [1]. MIP formation involves polymerization in the presence of a template molecule, with some recently used templates including glucose [2,3], metals [4], and proteins [5]. The development of MIPs capable of recognizing proteins is very important, since they would find use in separations, biosensors and in the development of biomedical materials [5]. However, the formation of protein recognitive MIPs is relatively difficult because of challenges associated with these large molecules, including solvent limitations, the need to recover an often expensive template molecule, and interaction with multiple epitopes (leading to binding site heterogeneity). In this work, we investigate a surface imprinting technique where protein is first immobilized on a solid support before imprinting. Controlling the molecular orientation of the template allows for the imprinting of specific epitopes. To study the imprinting of various epitopes, we use the immunoglobulin IgG as template due to its conserved / variable structure.

2. Experimental

2.1 Materials

Anti-c-myc coated microplates and alkaline phosphatase conjugates of anti-c-myc and rabbit anti-pig IgG were purchased from Sigma (St. Louis). Polystyrene microplates (Costar 3370) and non binding polystyrene microplates (Corning 3990) were used as supplied. The crosslinker ethylene glycol dimethacrylate (EGDMA) and photoinitiator, 1-hydroxyclohyxyl phenyl ketone, were purchased from Sigma (St. Louis). The monomer 2-hydroxyethylmethacrylate (HEMA) was purchased from VWR and vacuum distilled prior to use. All other reagents were used as received.

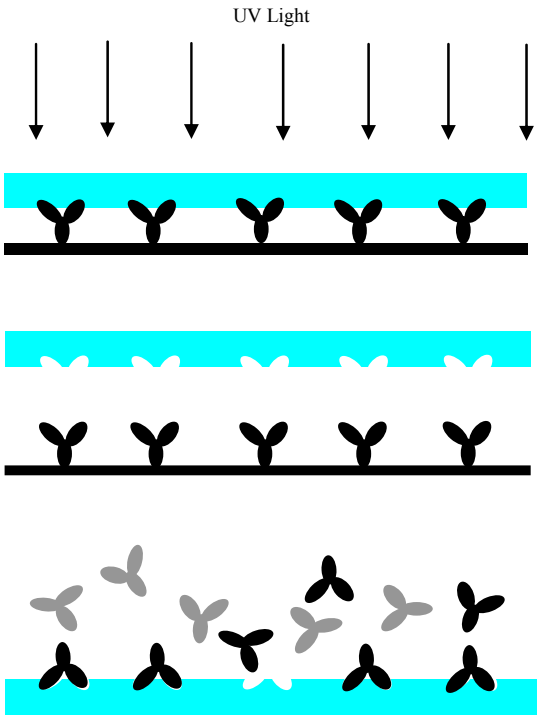

Figure 1. Surface imprinting procedure.
A templating mask is produced by immobilizing, in a controlled orientation, the target protein on a solid substrate.
This mask is brought in contact with monomer, crosslinker, and photoinitiator, and subsequently polymerized with UV irradiation.

Separation of the newly formed protein responsive material.

Material is tested for binding with the target molecule as well as competitive analogues.

2.2 Formation of immunoglobulin recognitive materials

Creation of the imprinted materials, detailed in Figure 1, was as follows. After optimization, a molar ratio of 4:1::EGDMA:HEMA was found to be the optimal prepolymerization formulation. A photoinitiator, 1-hydroxyclohyxyl phenyl ketone, was added to 2% (w/w). Just before polymerization, 45µl of monomer solution was mixed with 30µl of dimethyl silfoxide in a polystyrene 96-well microplate well. The microplates were placed in a nitrogen filled glove box in order to remove any free radical scavenging oxygen molecules. After 30 minutes in the inert atmosphere, UV initiated polymerization occurred when the wells were exposed with light at 375 nm (UV Shark LED, Optotech, Wheeling, IL). Total reaction dose was 3 minutes at 300 mW/cm^2. Non-imprinted (control) samples were fashioned using polystyrene 96-well plates, while imprinted materials were created using anti-c-myc coated microplates. Following reaction, the cured samples were removed from the templating microplate wells and placed in clean microplates functionalized for low protein binding (Corning Non Binding Surface). Tris buffer (200µl, pH 7.5) was then added to allow the materials to swell and release any unreacted material. The templating microplates were then used for a second polymerization (same procedures as listed above) in order to gauge the possibility of reuse.

2.3 Evaluation of MIP protein binding and specificity

Following polymerization, the newly formed materials were placed in clean microplate wells with the imprinted side facing up. Following one hour of dialysis in Tris buffer, the buffer was removed and the materials were incubated with test IgG solutions to evaluate binding. Briefly, an antibody-enzyme conjugate was dissolved in Tris-buffered saline at a concentration of 1/1000 mg/ml. An aliquot of 200µl antibody test solution was added to the materials and allowed to incubate for 40 minutes at 37°C. Protein recognition was tested with the use of anti-c-myc antibody conjugated with alkaline phosphatase. A second immunoglobulin-enzyme conjugate, rabbit anti-pig IgG-alkaline phosphatase, was also used in order to evaluate the material's specificity. Following incubation, the antibody solution was discarded and the materials washed ten times with a PBS-Tween 20 buffer in order to remove any unbound protein.

Substrate for the alkaline phosphatase enzyme, 1-step PNPP (Pierce, Rockford, IL), was added (100µl) and allowed to react for 20 minutes at room temperature. Conversion was monitored at 405 nm.

3. Results

The efficacy of the surface imprinting technique was tested using equilibrium binding studies with the antibody-enzyme conjugate. Incubation of the materials with this protein was done in a low protein binding microplate in order to reduce any contributions of non-specific binding by the microplate. This technique allowed for a relative comparison of binding abilities between the imprinted and non-imprinted materials. Control materials (formed in blank polystyrene microplates) were first tested to determine baseline levels of protein adsorption (Figure 2). These non-imprinted surfaces bound similar amounts of both anti-c-myc and anti-pig IgG. Next, anti-c-myc imprinted materials were tested for protein adsorption by either target or structural analogue proteins. The imprinted polymer showed a 73% increase in protein binding for the target protein, with no significant increase in anti-pig IgG adsorption.

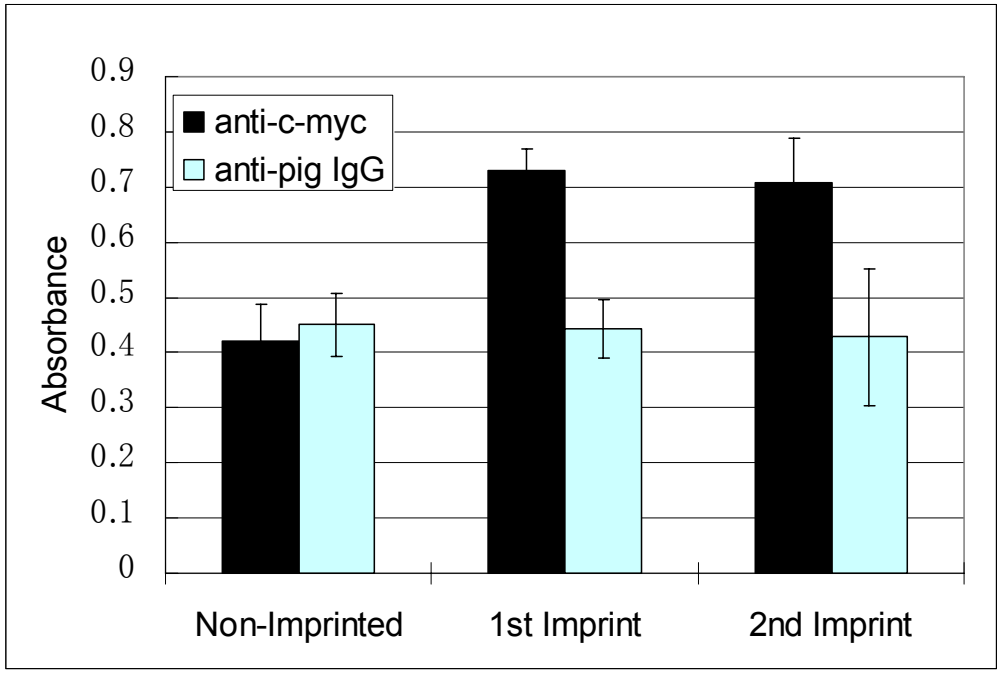

Figure 2. Equilibrium protein binding studies. Comparison of immunoglobulin binding by anti-c-myc imprinted and non-imprinted (control) materials with both anti-c-myc (target) and anti-pig IgG (structural analogue) proteins.

A major concern in the production of protein recognitive MIPs is the recovery of the often expensive protein template. Since these molecules are covalently linked to the templating mask, recovery is simply a matter of mask removal. However, mask degradation, through either polymer deposition or protein abstraction, is possible and warrants study. A second imprint cycle using the imprinting masks was attempted. There was no significant decrease in imprinting ability between the first and second imprinting cycles; however, variability in the experimental data did increase (Figure 2). This trend was seen in experiments with the target molecule as well as with the structural analogue. Surface analysis of the mask was done using attenuated total reflectance FTIR (ATR-FTIR), as shown in Figure 3. FTIR scans were done of the templating mask before use (3a), after a single imprint cycle (3b), and after two

cycles (3c). In addition, FTIR scans were done of the bulk polymer (3d) and polystyrene microplate material (3e) for comparison. As can be seen, HEMA/EGDMA polymer is deposited on the mask after the first imprinting cycle (peaks centered at 1150 and 1710 cm^{-1}). After the second imprinting cycle, additional polymer residue may be observed on the templating mask.

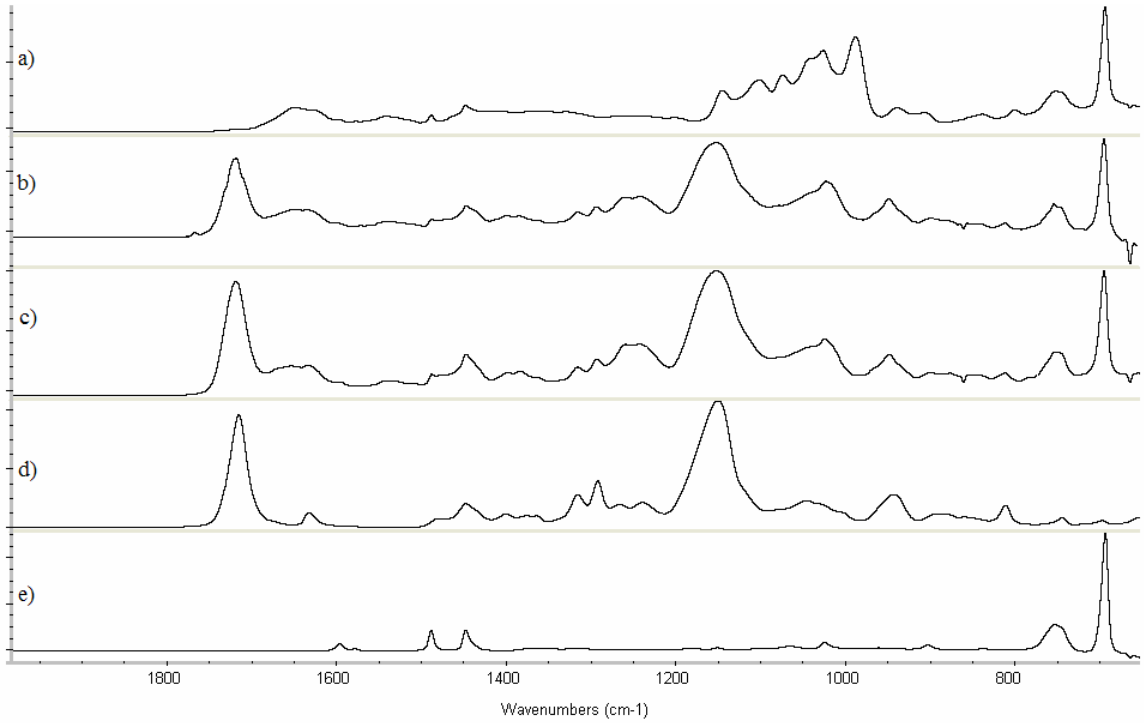

Figure 3. FTIR spectra of templating substrate. a) Spectrum of IgG coated polystyrene microplate (templating surface), b) templating surface after first imprint, c) templating surface after second imprinting, d) reference spectrum of 80% EGDMA/20% HEMA polymer, e) reference spectrum of polystyrene substrate material.

4. Conclusions

A surface imprinting procedure was developed to produce synthetic, protein recognitive materials. This method allows researchers to control the orientation of the template protein so as to promote the imprinting of specific epitopes. Molecules from the IgG family were chosen as model proteins due to their conserved/variable nature. Templating masks that promoted interaction with the antigen binding domains were used to produce imprinted materials. Equilibrium binding studies showed that these materials bind 73% more protein than non-imprinted, control materials. A second immunoglobulin, anti-pig IgG, was used to study binding of a structural analogue, with no significant increase recorded. Investigations into whether the templating mask was reusable demonstrated no significant decrease in imprinting efficacy after a single use. FTIR spectroscopy was used to study the surface chemistry of the mask after imprinting. Deposition of polymer was noted on the mask after the first and second imprinting cycles.

References:

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