

Protein-imprinted polysiloxane scaffolds

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Abstract

Molecular imprinting is a technique used to create specific recognition sites on the surface of materials. Although widely developed for chromatographic separation of small molecules, this approach has not been adequately investigated for biomaterial applications. Thus, the objective of these experiments was to explore the potential of molecular imprinting for creating biomaterials that preferentially bind specific proteins. Macroporous polysiloxane (silica) scaffolds were imprinted with either lysozyme or RNase A using sol–gel processing. The quantity of surface-accessible protein, which was related to the number of potential binding sites, was varied by changing the amount of protein loaded into the sol. Up to 62% of loaded protein was accessible. The amount of protein per unit surface area ranged from $0.3 \mu\text{g m}^{-2}$ for low loading of RNase to $152 \mu\text{g m}^{-2}$ for high loading of lysozyme. Protein-imprinted scaffolds were then evaluated for their ability to preferentially recognize the template biomolecule when incubated in mixtures containing both the imprinted protein and a competitor protein of comparable size (approximately 14 kD). In solutions containing a single protein, up to 3.6 times more template bound compared with the competitor. Furthermore, in solutions containing equal amounts of both molecules, the porous scaffolds bound up to three times more template than the competitor protein, which is a level of preferential binding similar to values reported in the molecular imprinting literature for both organic and inorganic materials.

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1. Introduction

A variety of metals, ceramics, polymers and composites are used as biomaterials. It has been estimated that more than 5% of the US population have implants [1]. Although these devices have benefited millions of people, the fact remains that most of the “biomaterials” were not originally intended to be placed in the human body. Consequently, biological reactions to these substrates tend to be characterized by non-specificity, sluggish kinetics, a broad spectrum of active processes simultaneously occurring and an opportunistic, rather than designed, outcome [2]. Hundreds of biomolecules can adsorb from tissues and body fluids onto the biomaterial surfaces, and the molecules can exist in several conformational and orientational states [3]. Sub-

sequent cellular and tissue responses will depend on which particular molecules adsorb and their states.

Materials designed to promote biomolecular recognition may be able to stimulate adhesion, growth, differentiation, and activity of particular cell types. A common approach, as reviewed by Shin et al. [4], is surface or bulk modification of biomaterials to attach short peptides, fragments or whole chains of extracellular matrix molecules. Use of large molecules, however, poses problems with long-term shelf-stability of the immobilized polypeptides and can be expensive. On the other hand, use of small peptides leads to difficulties with selectively binding or stimulating only one type of cell.

Molecular imprinting is a technique for preparing substrates to selectively bind particular biomolecules. In the more common non-covalent approach, functional monomers assemble with the template biomolecule in solution. Subsequent crosslinking of monomers “locks in” the shape and chemical functionality of the template. Removal of the

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template leaves nanocavities of specific shape and with a defined arrangement of functional groups. Analogous to the way an antibody recognizes an antigen, the combination of geometry and chemistry of the cavities can make them selective for the template molecule. Most molecular imprinting studies have used methacrylic polymers imprinted with low molecular weight compounds, such as sugars, steroids and pesticides (e.g., Refs. [5–10]). Comparatively few reports describe imprinting inorganic materials [11–16].

The aims of this study were to begin development and characterization of protein-imprinted biomaterials to enable preferential binding of particular biomolecules.

2. Materials and methods

2.1. Fabrication of the polysiloxane scaffolds

Polysiloxane scaffolds were fabricated by a two-step sol–gel procedure. Tetraethoxysilane (TEOS; Fluka, Milwaukee, WI) was used as a crosslinker, γ -aminopropyltriethoxysilane (APS; Sigma, St. Louis, MO) as a functional monomer, HCl as a catalyst, ethanol as a co-solvent and sodium dodecylsulfate (SDS; Sigma) as a foaming agent to generate macroporosity for cell ingrowth. In the first step, a solution was mixed to include 1.32 ml of TEOS, 0.235 ml of deionized water, 0.33 ml of 0.1 M HCl and 0.4 ml of absolute ethanol in a cylindrical plastic vial. After prehydrolysis of TEOS at room temperature for 24 h, this solution was mixed with a solution containing 0.33 ml of APS and 1 ml of 0.1 M SDS. Vials were vortexed to mix the silane components and to foam the sol.

After gelation, the vials were capped, and the gels were aged at room temperature for 24 h and then dried at 40 °C for 48 h. To remove non-uniform glassy layers, the top and bottom surfaces of the scaffolds were ground using 600 grit silicon carbide paper on an ECOMET 3 grinder–polisher (Buehler). After grinding, all samples were thoroughly washed with phosphate-buffered saline (PBS), pH 7.4, to remove debris, and the scaffolds were stored in the dark until used.

2.2. Protein loading

The model template/competitor molecules lysozyme (chicken egg white; Sigma) and RNase A (Sigma) were fluorescently labeled with Alexa Fluor 350 (Molecular Probes; Eugene, OR) according to the manufacturer's protocol before imprinting. Specific fluorescence of the labeled proteins was determined through construction of standard curves relating fluorescence (Spectra MAX Gemini XS, Sunnyvale, CA; $\lambda_{\text{ex}} = 346$ nm and $\lambda_{\text{em}} = 442$ nm) to protein concentration as quantified using the BCA protein assay (Pierce, Rockford, IL). The labeled template molecules were incorporated in the scaffolds by addition to the APS-containing solution prior to mixing with the TEOS solution. To determine the amount of protein avail-

able on the surface of scaffolds after loading, samples were gently shaken in a solution of 0.4 mg protease (Pronase E; Sigma) per ml of 0.1 M carbonate–bicarbonate buffer, pH 8.5, for up to 24 h. The amount of protein released from the scaffold surfaces was quantified by measuring fluorescence at 3 and 24 h.

2.3. Characterization of scaffolds

Blank scaffolds and scaffolds loaded with 1 or 10 mg of lysozyme were examined using scanning electron microscopy (SEM). Samples were sputter-coated with gold in argon gas using an Emscope sc 400 before visualization with a Hitachi S-3200 (Tokyo, Japan) at an accelerating voltage of 20 kV.

The surface area of scaffolds was measured by nitrogen gas adsorption. Scaffolds (blanks and imprinted with 0.1, 1 and 6 mg of lysozyme or RNase A) were placed in an oven at 40 °C for three days and then kept under vacuum for 24 h. The weight of each scaffold was measured after desiccation in a sample tube at 120 °C for 24 h. The surface area was then measured with a TriStar 3000 (Micromeritics, Norcross, GA) instrument and calculated using the BET (Brunauer–Emmett–Teller) method.

Porosity of scaffolds was measured with an Autopore IV 9500 mercury intrusion porosimeter (Micromeritics). Blank scaffolds and scaffolds imprinted with 0.05 mg of lysozyme or RNase A were completely dried at 40 °C for three days before testing. Three samples were placed in a 5 cc penetrometer. After testing, total intrusion volume, average pore diameter, density and porosity were calculated.

2.4. Preferential protein binding

In order to examine the ability of imprinted scaffolds to preferentially bind their template proteins, scaffolds were immersed in single or double protein solutions following digestion of the attached biomolecules. The proteins in the solutions were labeled with fluorophores that have different spectra compared with the labels used for protein loading. Lysozyme was labeled with Alexa Fluor 488 (molecular probes; $\lambda_{\text{ex}} = 495$ nm and $\lambda_{\text{em}} = 519$ nm), and RNase A was labeled with Alexa Fluor 594 (molecular probes; $\lambda_{\text{ex}} = 590$ nm and $\lambda_{\text{em}} = 617$ nm). For the lysozyme-imprinted scaffolds, lysozyme was used as template protein and RNase A was used as competitor protein in the rebinding solution. The total amount of protein in the rebinding solutions was kept constant at the maximum amount of protein released from each type of sample. For example, if 100 μg of template protein was digested from the scaffold, the total amount of template plus competitor in the rebinding solutions was 100 μg . The ratios of template to competitor protein were 1:0, 1:1 and 0:1. In order to check the selectivity of scaffolds for another protein, RNase A-imprinted scaffolds were exposed to rebinding solutions containing template protein (RNase A) and competitor protein (lysozyme). The template to competitor

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