Biological Characterization of Molecularly Imprinted Polysiloxane Scaffolds

K. Lee, R. Itharaju, and D.A. Puleo
Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40506

Introduction

Non-specific and undesired responses often occur at the interface between tissues and implants because of inappropriate surface properties of the biomaterials. The technique of molecular imprinting has the potential for improving biomaterial surfaces by making them able to specifically interact with particular biomolecules. By selectively binding certain biomolecules, such as those involved in cell adhesion or differentiation, specific cellular responses could be induced at the tissue-implant interface. The objective of this study was to develop imprinted polysiloxane materials that have the ability to selectively bind particular biomolecules and to assess the initial cytocompatibility of the surfaces developed.

Materials and Methods

1. Fabrication of scaffolds

Scaffolds were fabricated using tetraethoxysilane (TEOS) as crosslinker and γ-aminopropyltriethoxysilane (APS) as functional monomer in a two-step sol-gel process. After acid-catalyzed hydrolysis of TEOS, a solution containing APS, sodiumdocecylsulfate (to introduce macroporosity), and protein was added. The gels were aged at room temperature for 24 hr and then dried at 40˚C for 48 hr.

2. Protein imprinting

Lysozyme labeled with Alexa Fluor 350 was used as a model template molecule. Loading varied from 0-6mg. In order to determine the amount of protein available on the surface of the scaffolds, protein-imprinted scaffolds were digested in a 0.4 mg/mL solution of protease. Fluorescence of the digestate solution was then measured.

3. Protein selectivity

Blank and imprinted scaffolds were exposed to solutions of template protein in the presence of a competing molecule. Rebinding solutions containing template (lysozyme, MW 14.3 kD, labeled with Alexa Fluor 488) and competitor (RNase A, MW 13.7 kD, labeled with Alexa Fluor 594) were prepared at template to competitor ratios of 1:0, 1:1 and 0:1. After digestion, the amount bound was determined by measuring fluorescence.

4. Cytocompatibility

In order to study *in vitro* cytocompatibility of the materials, SaOS-2 osteoblastic cells were seeded on blank (non-imprinted) scaffolds as controls and on scaffolds imprinted with 3 mg lysozyme. DNA contents and alkaline phosphatase activity were measured after 1, 3, and 7 days of culture.

Results and Discussion

Porous scaffolds were successfully fabricated. The amount of lysozyme digested from the surface of scaffolds increased with increasing amount of protein added to the sol. Approximately 80% of the protein added to the samples was accessible on the surface. Figure 1 shows that the template protein was preferentially bound to the surface, even in the presence of a similarly sized competitor protein ($p<0.001$). Although some nonspecific binding of the competitor was observed, still almost twice as much of the template bound ($p<0.001$).

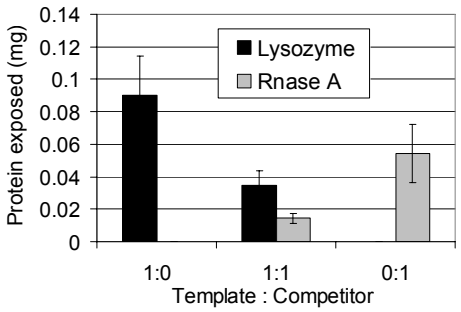

Fig. 1. Competitive rebinding of protein to 0.1 mg lysozyme-imprinted scaffolds.

Figure 2 indicates the growth of SaOS-2 osteoblastic cells on the scaffolds during the one week period of culture. There were no statistically significant differences in either DNA content or alkaline phosphatase activity ($p>0.05$).

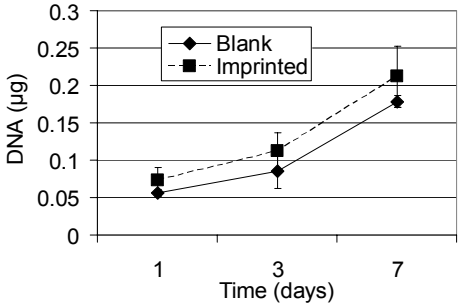

Fig. 2. DNA content of SaOS-2 cells cultured on blank and imprinted scaffolds.

Conclusions

Molecularly imprinted polysiloxanes showed the ability to preferentially bind template molecules on their surface. Initial cytocompatibility studies confirm that the scaffolds support the adhesion, growth, and activity of cells. Overall, these selectivity and cytocompatibility studies show the potential for molecularly imprinted polysiloxane scaffolds to be used as tissue engineering materials.

Acknowledgement

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