Surface Modification of a Perfluorinated Ionomer Using a Glow Discharge Deposition Method to Control Protein Adsorption and Fibroblast Inflammatory Response

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Purpose: Implantable glucose biosensors are dependant on the transport of analytes to and from their surface. Upon implantation, the formation of a fibrous capsule is quite detrimental to the device, thereby limiting its utility in an *in vivo* environment (*1*). To limit the formation of a fibrous capsule, it becomes important to modulate the biological response of surrounding cells. One potentially effective method is to alter the surface characteristics of an implant to present desired properties. In this work, the surface of an existing polymer, Nafion™ (Dupont Chemical), currently used for implantable biosensors, is modified via new surface coatings. Our intent is to prevent the random adsorption of proteins at the surface while at the same time, provide enough functional sites (hydroxyl groups) to hold a biologically active peptide known to induce cellular adhesion (YRGDS). It is the goal of this work to engineer a glucose permeable membrane with a surface that cells actively interact with while preventing the foreign body response.

Materials and Methods:

1. RF glow discharge deposition (RFGDD): Advantages of RFGDD have been described elsewhere (*2*). Several variations of surface coatings were created using two base monomers individually or by combining the two at different concentrations. They were *(1)* tetraethylene glycol dimethyl ether (tetraglyme) (*3*) and (2) 2-hydroxyethyl methacrylate (HEMA) (*4*). Combinations used were: pure tetraglyme, 2.5% HEMA with 97.5% tetraglyme; 5% HEMA with 95% tetraglyme, 10% HEMA with 90% tetraglyme and pure HEMA. Nafion disks were cut to shape (5x5 cm^2 or 6 mm diameter punch), cleaned , and placed on a rack. Monomer(s) were degassed by freezing-thawing cycles, and Nafion was etched in Argon plasma (40W, 0.175 mmHg) for 2 min. Monomer(s) were vaporized by heating to 105˚ C and RF maintained at 80 W for 1 min, 40 W for 30 sec, and 10 W for 17 min.

2. Investigation of hydroxyl content: To more clearly investigate the presence of hydroxyl groups at the surface of the tetraglyme, tetraglyme-HEMA and pure HEMA surfaces were derivatized with a trifluoroacetic anhydride (TFAA) reaction. This reaction substitutes hydroxyl groups with fluorine and can be semi-quantified via ESCA techniques.

3. ESCA Analysis: Electron spectroscopy for chemical analysis (ESCA) was used to analyze the newly synthesized surfaces for their elemental composition using an SSX-100 surface analysis instrument (NESAC/BIO, University of Washington) with a take off angle of 55˚, corresponding to 50 Å sampling depth.

4. Hydroxyl activation with Carbonyldiimidazole: Surfaces containing hydroxyl groups were reacted with 1,1 Carbonyldiimidazole (CDI) in anhydrous acetone at 20 mM in order to covalently attach the YRGDS peptide.

5. Radiolabeling of proteins and peptide: In order to test the ability of the newly synthesized surfaces to repel proteins and/or attach YRGDS peptide, albumin, IgG, fibronectin, or the YRGDS peptide were radiolabeled with ^{125}I using the Iodobead ™ method.

6. Fibroblast cell culture: To test the *in vitro* response to modified surfaces, normal human dermal fibroblasts were cultured in low glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 3.7g/l $NaHCO_3$. Cells were plated on the various surfaces and using Live/Dead™ stain (Molecular Probes) cell numbers, viability, cell spreading (Image J, NIH), and collagen synthesis (via Metra™ ELISA, Quidel Corp.) were quantified. Collagen synthesis was also tested in response to biosensor bi-products and inflammatory mediators (IL-1, Glucose oxidase, H_2O_2, Ascorbic acid).

Results/Discussion:

1. Plasma deposited films: Plasma deposited films of various chemical compositions containing pure monomers or a mixture, were successfully deposited on Nafion. High resolution C*1s* ESCA shows a dominant C-O peak (286.5 eV) for all modified surfaces. The C-O peak was indicative of ether carbon or hydroxyl content. Upon further examination with TFAA, it was discerned that increasing concentrations of HEMA yielded a decrease in hydroxyl content.

2. Protein Adsorption and peptide binding: Surfaces exhibiting ether carbons (tetraglyme) were the most effective at repelling proteins. Maximum protein adsorption was seen on surfaces containing an optimal amount of hydroxyl groups. Surfaces modified with 2.5% HEMA exhibited the most protein adsorption, followed by 5% HEMA surfaces. Surfaces modified with 10% HEMA exhibited a limited amount of protein adsorption, similar to ether carbon rich surfaces (non-fouling, i.e. tetraglyme), and were chosen for subsequent cellular experiments. An increase in hydroxyl groups correlated directly with an increase in YRGDS peptide.

3. Cell Response: In vitro responses were quantified on unmodified and modified surfaces for cell adhesion numbers, viability, area of cell spreading and collagen synthesis. An optimal density of peptide was found to induce a maximum amount of cell spreading. When cultured on the different surfaces, collagen synthesis was reduced at least 4-fold on surfaces modified with 10% HEMA, as Figure 1 below shows.

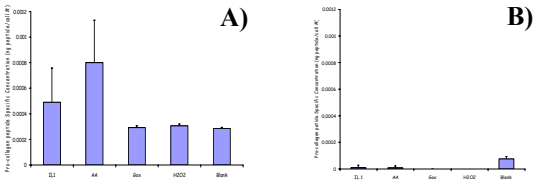

Figure 1. Type I collagen ELISA on A) Unmodified B) Modified Nafion surfaces in response to potential stimulants.

Conclusions:

Specific changes to surface chemistry can render a biomaterial surfaces non-fouling to minimize random protein adsorption. In addition, sufficient functional sites can be deposited in order to bind biologically active entities, which can specifically attach cells and dictate cell behavior. Surfaces modified with 10% HEMA accomplished the goal of maintaining a non-fouling surface decorated with the YRGDS peptide and decreased the production of Type I collagen in an *in vitro* model. Optimizing conditions for surface chemical groups, protein adsorbed, peptide bound, cell spreading can dictate the synthesis of extracellular matrix, which may in turn modulate the thickness of a fibrotic capsule surrounding an implant.

References:

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