Characterization of a Decorin Surface Coating for Reduction of Fibrous Encapsulation Around Implants

Marisa L. Sylvester, Buddy D. Ratner.
University of Washington, Seattle, WA.

Statement of Purpose: Fibrous encapsulation of implants often leads to device malfunction and failure. Performance degradation with time is one of the key barriers to overcome in developing long-term implantable sensors, such as glucose sensors. A promising target for prevention of fibrous encapsulation is the pro-fibrotic cytokine, transforming growth factor beta (TGF-β). The proteoglycan decorin has been shown to reduce fibrosis in several animal models of fibrotic diseases through its ability to bind and inactivate TGF-β. Since TGF-β is important for the formation of skin, cartilage and normal tissues, localized delivery of decorin would be ideal for preventing fibrous encapsulation around implants. Decorin has previously been shown to retain its ability to bind TGF-β when bound to collagen molecules. Thus by binding decorin to a collagen surface, it is likely that the molecules will bind in a specific orientation and retain their activity against TGF-β. In these studies, we have designed and characterized a decorin surface coating with potential application for medical implants.

Methods: Decorin was attached to the surface of an implant through its affinity to type I collagen. Collagen I was first covalently linked to the surface of a poly-2-hydroxyethyl methacrylate (pHEMA) surface via 1,1-carbonyldiimidazole (CDI) chemistry. This collagen surface was then incubated with decorin. Surface composition of the pHEMA substrate as well as each subsequent modification step was verified using electron spectroscopy for chemical analysis (ESCA). Total elemental composition was determined from survey scans, and more detailed composition was obtained from high resolution scans. Presence of decorin on the surface was evaluated by time-of-flight secondary ion mass spectrometry (ToF-SIMS). CDI immobilized collagen + decorin surfaces were compared to surfaces with only decorin and only collagen. Differences in ToF-SIMS spectra from the surfaces were evaluated using principal component analysis (PCA). Adsorbed collagen and pHEMA controls, and CDI immobilized collagen surfaces were incubated with ^{125}I labeled decorin to quantify binding. Initial counts were performed and quantity of decorin retained on the surface was measured throughout a four-week period. Initial cellular response was evaluated by an adhesion assay using RAW 264.7 mouse macrophage-like cell line. The same controls and treatments used in radiolabeling were used for the adhesion assay. Cells were allowed to adhere for 45 minutes and the samples were rinsed, fixed, stained, and mounted. Three 10x brightfield images were taken from each sample and total cells per image were counted.

Results/Discussion: ESCA data confirmed expected pHEMA composition as well as addition of CDI, collagen and decorin though the two proteins were not distinguished using this analysis technique. Presence of CDI activation of surfaces was indicated by the appearance of a nitrogen peak in survey scans, as well as a doublet peak in the high resolution nitrogen scan corresponding to the two distinct nitrogen environments present in the CDI molecule. Addition of protein was indicated by increased percentage of nitrogen as well as replacement of the doublet peak in the high resolution nitrogen scan with a broad peak. ESCA also confirmed that surfaces were free of contaminants. The collagen surfaces, decorin surfaces and collagen + decorin surfaces can be distinguished by PCA analysis of ToF-SIMS spectra. Key differences affecting the separation include the large number of leucine repeats in decorin and the large amount of proline contained in collagen. Radiolabeling data showed increasing amounts of decorin present as the solution concentration increased from 30-150μg/mL. At the highest two decorin concentrations used, a quantity consistent with a monolayer of decorin was observed. Results of initial counts are presented in Figure 1. Quantity of decorin on the surface dropped significantly in the first 24 hours and leveled off after approximately three days. Cell binding results showed that decorin at all concentrations used completely prevented RAW cell binding. Collagen surfaces had large numbers of cells bound consistent with previous studies on similar surfaces.

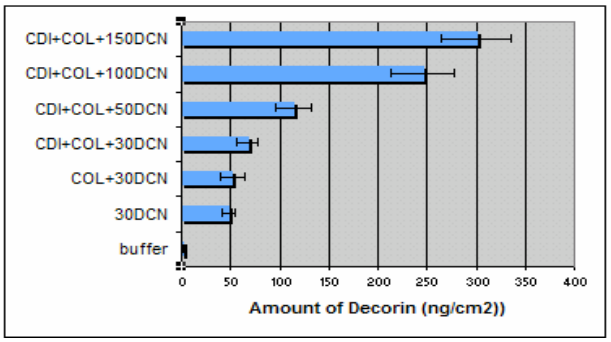

Figure 1. Quantity of Decorin on Surfaces (Conditions include CDI, Collagen (COL), and concentration of Decorin (DCN) in μg/mL)

Conclusions: This surface coating provides a method to deliver biologically active decorin locally at an implant site. Studies are in progress to verify the activity of the coating against TGF-β *in vitro*. Future work includes implantation into an animal model to evaluate the coating effects on fibrous capsule formation. Ability of the coating to prevent fibrous encapsulation of an implant would greatly contribute to the development of long-term implantable glucose sensors as well as reduce failure of current medical implants.

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