Development of a Model Surface for Bioprosthetic Heart Valves

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Statement of Purpose: There is a great need for non-thrombogenic, non-immunogenic heart valve replacement options, especially for pediatric patients. Coating existing bioprosthetic valve replacements with molecules to prevent unwanted blood interactions is an approach to this problem that has only been explored to a limited extent.[1,2] A difficulty in developing such beneficial biocompatible and bioactive coatings is the rough topography and opacity of the heart valve surface, making analysis of the surface difficult. The goal in this work was to develop a model surface to facilitate the optimization of such a coating strategy. Specifically, glutaraldehyde-fixed bovine collagen gels were investigated as a model surface for bioprosthetic heart valves. Collagen gels were chosen because collagen is the primary matrix component of the heart valve, and is the primary component cross-linked by glutaraldehyde in bioprosthetic valves. Furthermore, amine groups on collagen can serve as anchors for various coating methods. Finally, crosslinking time of the gel can be modulated to match valve surface mechanics, therefore facilitating similar cellular interactions. The properties of the model surface were compared to glutaraldehyde-fixed leaflet samples to determine the appropriateness of collagen gels as a model surface and to provide guidance toward improving the model surface for future testing.

Methods: Collagen gels were synthesized by neutralizing Vitrogen 100 (Advanced BioMatrix, San Diego, CA) and gelling at 37°C. Valve leaflet samples were obtained from biopsies of fresh porcine leaflets. Both gels and fresh leaflets were fixed by submersion in 0.2% glutaraldehyde for 3 days. Additionally, surface endothelial cells were removed from some native leaflets to expose the underlying matrix. X-ray photoelectron spectroscopy (XPS) was performed with a PHI Quantera instrument (PHI, Chanhassen, MN) to analyze the atomic composition and functional group availability on valve and gel surfaces. Atomic percentages of C and N were determined, and the calculated N/C ratios were analyzed statistically by one-way ANOVA. To determine toxicity of valves and gels before and after treatment with detoxification methods, human dermal fibroblasts were seeded in a 24-well plate, and placed in indirect contact with valves and gels via a transwell membrane. A LIVE/DEAD assay (Invitrogen, Grand Island, NY) was performed to determine toxicity qualitatively.

Results: XPS data revealed significantly increased nitrogen content in fixed and unfixed collagen gels as compared to all leaflet samples (Figure 1). Even enzymatically debriding the leaflet surface to remove endothelial cells did not have a significant effect on the N/C ratio. Qualitatively, unfixed gels were extraordinarily weak and difficult to manipulate; however, fixation with glutaraldehyde enabled easy manipulation of the gel with mechanics similar to unfixed leaflets. Leaflets fixed with glutaraldehyde contracted slightly, and were noticeably stiffer than native valves.

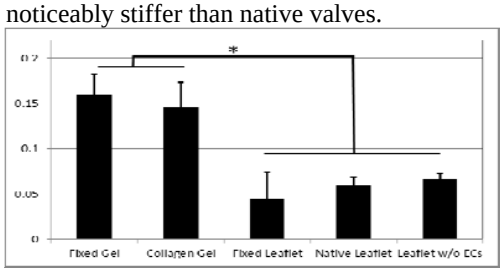

Figure 1. Graph of N/C ratio determined by XPS analysis. Data given as mean ± standard deviation. ($p \leq 0.01$)*

Cells grown in contact with unfixed gels were alive and healthy, as shown by LIVE/DEAD staining, whereas fixed gels were mostly dead after 24h in culture (Figure 2A-B, respectively). These results were similar to fixed leaflets, which resulted in mostly dead, rounded cell populations after just 24h in culture (Figure 2D), as compared to cells alone (Figure 2C).The LIVE/DEAD assay qualitatively showed that treatment with 0.8% L-glutamine or 10% citric acid for 1 – 3 days, two methods common in the literature, enabled fibroblasts to survive in the presence of a glutaraldehyde-fixed leaflet.

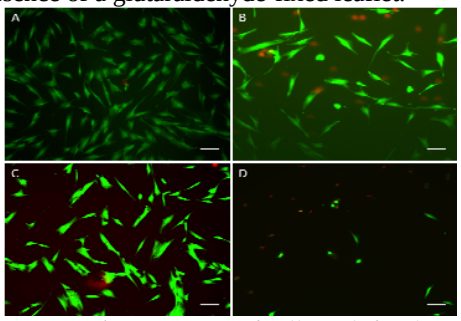

Figure 2. LIVE/DEAD stain of cells with fixed or unfixed gels (A,B), or without or with fixed leaflets (C, D). Scale bar = 100 μm

Conclusions: Increased nitrogen content in fixed and unfixed gels suggest that more amine functional groups are available on both of these surfaces for attachment of biocompatible coatings. Therefore, attachment of such coatings to the gel surface will represent a best-case scenario of attachment to fixed leaflets. Additional matrix components or polymers could be added to the gel to modulate amine availability and also increase the stiffness of gels to match the mechanical properties of fixed valves. Work is underway to test gels and leaflets via micropipette aspiration, to quantitatively match the mechanics that cells will experience on both surfaces. The fixed gels accurately mimic the toxicity of fixed leaflets, and it is expected that detoxification methods will be equally effective in reducing cellular toxicity.

References:

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