PPF Enforced Pericardium for Use in Cardiovascular Applications

Laura Bracaglia[1], Yu Li, MD[2], Vanessa Niba[1], Naru Habino MD PhD[3], John P Fisher, PhD[1]
1.Fischell Department of Bioengineering University of Maryland 2. Wuhan University, China 3. Department of Cardiothoracic Surgery, The Heart Center Nationwide Children's Hospital

Statement of Purpose: Outcomes of complex cardiovascular surgeries would be significantly improved with an innovative biomaterial for use in vascular patches and tissue valves. The current use of pericardium in these applications employs a chemical fixing agent, glutaraldehyde (GA), to crosslink collagen fibers within the tissue. The crosslinking process is efficient at blocking proteolytic enzymes from accessing and degrading the fiber network of the tissue, but is also associated with increased calcification compared to natural or synthetic material[1], a predicament that leads to eventual failure of the device. Our overall hypothesis is that applying a paintable polymer to the surface of pericardium tissue will provide the physical support and biological block to natural degradation without employing a chemical crosslinking agent. For this purpose, poly(propylene fumarate) (PPF), was selected for its demonstrated biocompatibility, biodegradability, and strength in other medical applications[2]. By diluting the polymer with its monomer, diethylene fumarate, properties of the crosslinked product can be tuned to affect viscosity and resulting mechanical properties of the product. Specifically regarding this study, it was hypothesized that a formulation of PPF suitable for cardiovascular applications can enforce pericardium for use as a material. Due to reduced immune activity of this composite when compared to the GA treated or untreated control, a lower calcification rate and a subsequent improved durability of the material can be obtained.

Methods: Upon successful bonding of the polymer to pericardium, the composite was fully evaluated to determine physical characteristics and constraints. Various polymer molecular weights, viscosity, and thicknesses of applied polymer to pericardium were defined. These variables were compared in crosslinking percent of the polymer, and compliance, elastic modulus and yield strength of the composite using an Instron mechanical tester. Various composites were then challenged in an in vitro degradation and calcification model, and resistance to degradation and amount of calcium deposition of the material was measured using mechanical testing, quantification assay and histology. Each combination of PPF and pericardium was then implanted subdermally in an animal model to evaluate the activity of immune signatures in vivo. Implants will be explanted and examined at set time points over 45 days, using histological methods to check for the presence of macrophages and other lymphatic cells, and an ELISA to check for recruiting cytokines and adhesion molecules (TNFa and IL1) on or near the material. Material will be studied for calcium presence using histology a quantification assay, and material will be mechanically tested to detect loss of material integrity. In each experiment, GA treated tissue and natural untreated tissue served as the controls.

Results: A set of feasible compositions of PPF and pericardium were identified from the mechanical properties identified. Sol fraction of thin sheets of PPF was calculated as 13.3 % ± 2.7%, (n=9). Results from the in vitro degradation test are shown in Figure 1.

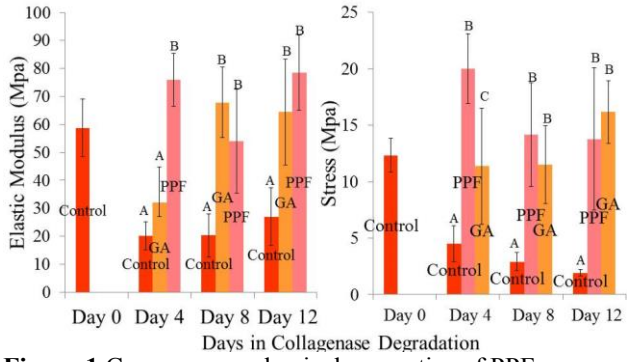

Figure 1 Compares mechanical properties of PPF enforced pericardium to the gold standard, GA treated pericardium, and to an untreated tissue after enzymatic exposure. PPF enforced pericardium was not significantly different than GA treated, considered within time points. Degradation is shown to have a severe result on untreated pericardium, indicated in the significantly lower elastic modulus and yield strength of untreated pericardium from the treated and enforced tissue within each time point, as well as from the untreated control (day 0).

These results are supported in histological photographs of the pericardium samples, which contrast a highly disordered fresh pericardium fiber network with a more ordered and dense GA treated and PPF enforced pericardium sample. In the calcification model, pericardium enforced with PPF is observed to collect significantly lower calcium when compared at different time points, with a final difference of 1.2 µg/mg of tissue after 45 days. It is further expected that a lower concentration of immune associated cells and recruitment molecules will be seen in response to the PPF enforced pericardium than the GA control, which will result in lower calcium deposits and prolonged mechanical integrity of the PPF enforced tissue.

Conclusions: The unique environment in the cardiovascular system demands special considerations to withstand severe environmental stress and active biological components. This study demonstrates the a PPF enforced pericardium can withstand degradation as well as the GA alternative, but without matching the immune response and calcification

References: 1.Vasudev, S.C. et al., J Biomed Mater Res, 1997. **35**(3): p. 357-69. 2. Fisher, J.P., et al., J Biomed Mater Res, 2002. **59**(3): p. 547-56.

Acknowledgments: This work is supported by Sheikh Zayed Institute for Pediatric Surgical Innovation at Children's National Medical Center and the Clark School of Engineering Seed Grant