Fabrication of a Tissue Engineered Living Valve for Treatment of Pulmonary Valve Pathologies in Young Adult Patients

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Introduction: The pulmonary valve, while commonly overlooked in cardiac engineering, is crucial to maintaining proper blood flow throughout the body.[1]Similar to all other valves, common pathologies include stenosis, regurgitation, and atresia. Notably, the pulmonary valve requires repair and/or replacement in pediatric patients and young adults. Even with technological advances in valve replacement limitations still present themselves for use with younger patients as mechanical valves require lifetime anticoagulation medication and bioprosthetic valves are prone to degeneration and calcification. Both options have a relatively short lifespan and would require multiple surgeries in young patients associated with high morbidity and mortality.[2] The **primary objective** of this project is to design a proof of concept for a living tissue pulmonary valve replacement, by seeding the patients' own stem cells into acellular scaffolds, thus extending the durability of implantable valves to last the patient's lifetime while limiting the risk of immunological response, degeneration and calcification.

Materials and Methods: Whole hearts were harvested from adult pigs. Pulmonary valves (PV) were dissected to obtain target tissue dimensions and thickness measurements, then further dissected into individual leaflets. Leaflets were characterized through mechanical biaxial testing, histology, and DNA quantification to serve as target values for scaffolds. Adult bovine pericardium (BP) and inferior vena cava (IVC), fetal BP and IVC, and adult porcine pericardium were then obtained and characterized as above before decellularizing by standard decellularization protocols.[3] Post-decell characteristics were then evaluated for comparison with pre-decell and the target tissue values. Two tissues with properties similar to PV leaflets, BP and IVC, were chosen for scaffold seeding from these comparisons. BP was seeded with Human Dermal Fibroblasts (HDFBs) under 2 conditions: manual needle injections or Cytoseeder injection (multi-needle platform, patent pending). After incubation in static conditions for 72 hours, the scaffolds were analyzed using a Live/Dead stain, PrestoBlue cell viability, histology, and EthBr agarose gel electrophoresis of extracted DNA.

Results and Discussion: Fresh PV leaflet Hematoxylin and Eosin (H&E) staining showed consistent cell distribution and density (Fig 1A). The seeded scaffolds showed nuclei present with H&E staining (Fig 1B and 1C) however in lower quantities and mostly limited to sites of injection at scaffold surface. Some cell migration was noted within the seeded BP scaffolds, also confirmed by Presto Blue assay and Live/Dead viability stain.

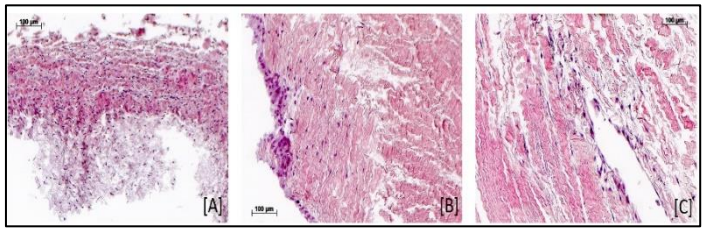

Figure 1. 10x magnification of Hematoxylin and Eosin for staining nuclei in (A) Native PV (B) Manual injection of BP scaffold and (C) Cytoseeder injection of BP scaffold

Conclusions: From comparison of biaxial mechanical testing and thickness mapping of all tissues, we were able to determine the most favorable scaffold tissues to be the adult BP and adult BIVC. Seeding with the Cytoseeder device generated significant cell repopulation of the scaffolds. Based on our previous work on bioreactor-conditioned valves, we anticipate that incubating the current cell seeded scaffolds in dynamic conditions with pulsatile flow, will induce further cell migration throughout the scaffold and maintain cell density and viability for longer time increments. Additional seeding studies of both tissues, assembly of the seeded tissues into valve stents, and hemodynamic evaluation and bioreactor testing of the living valves will be pursued in future work to reach our primary objective.

References:

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