Cell Injection Initiates the Recellularization Process in Decellularized Porcine Aortic Valve Scaffolds

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Statement of Purpose: Significant recellularization into the matrix of decellularized tissues is difficult to accomplish in vitro. Multiple methods of decellularization have been shown to successfully remove cells and DNA; however, the recellularization of these scaffolds often consists of seeding cells on the surface with the hope that they will migrate into the matrix over time either in a large animal model or bioreactor system. We set out to identify a method that would allow us to significantly increase the number of cells *within* the matrix prior to conditioning in a physiologic bioreactor system or implantation into large animals.

Methods: Three detergent-based decellularization methods were performed on fresh porcine aortic valves to compare the porosity of the matrix (by scanning electron microscopy) as well as to compare the ability of cells to migrate into the matrix in vitro (by histology). The decellularization procedures were as follows: A) 1% SDS and DNAse (40uL/ml) agitated at room temperature for 4 days with solution changed daily; B) Tris as described elsewhere[1], and C) Tricol as described elsewhere[2]. In order to increase porosity following decellularization, physical methods (multiple freeze/thaw cycles, lyophilisation) and an enzymatic method (collagenase treatments) were evaluated. Recellularization of the decellularized scaffolds using porcine valvular interstitial cells (passage 2-5) was attempted under atmospheric pressure (static culture), negative pressure (vacuum), and positive pressure (1 Hz pulsation between 0 and 120 mmHg). Finally, cell injection was evaluated using an automated, low-volume syringe pump system with a 27 gauge needle (Harvard Apparatus, Holliston MA).

Results: Scanning electron microscopy was used to evaluate the matrix structure of aortic valve cusps following the three decellularization protocols described above (Fig. 1). There was no difference seen in the matrix porosity following the decellularization protocols and the pore size on the surface of the cusps was consistently on the order of 1-2 um in diameter. Furthermore, we were unable to increase porosity by using the physical methods of multiple freeze/thaw cycles or lyophilisation. Collagenase treatment was also not effective for increasing pore size and prolonged exposure at multiple concentrations resulted in destruction of the valve cusp matrix. Three weeks of static culture following recellularization resulted in no cellular migration into the matrix in any of the samples tested by histological analysis with Hochest staining (Fig. 2A). Cusps receullarized under negative pressure showed results similar to Figure 2A, whereas positive pressure yielded a layer of fragmented cells on the surface of the cusp. Static culture following injection of porcine valvular interstitial cells into the matrix consistently resulted in heterogeneous cellular infiltration of the matrix (Fig. 2B).

Conclusions: We have demonstrated the feasibility of cell injection to initiate the recellularization process of decellularized porcine aortic valve cusps. This method has allowed us to overcome the poor cellular migration we have observed following surface seeding and static culture. Future work will involve testing of our recellularized scaffolds in a physiologic bioreactor system.

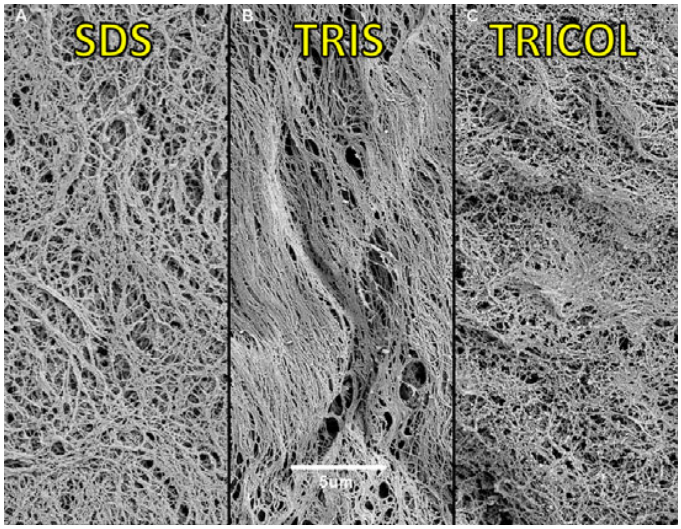

Figure 1

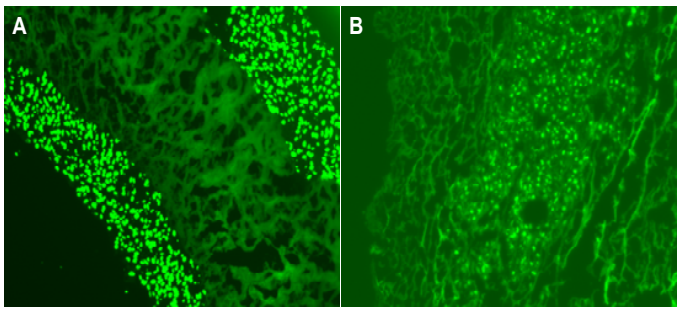

Figure 2

References:

1. Godier-Furnémont AF. Proc Natl Acad Sci USA. 2011 May 10;108(19):7974-9.
2. M. Spina. J Biomed Mater Res A. 2003 Dec 15;67(4):1338-50.