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Recellularization of Porcine Internal Thoracic Arteries as a Tissue Engineered Small-Diameter Vascular Graft Alternative

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ABSTRACT

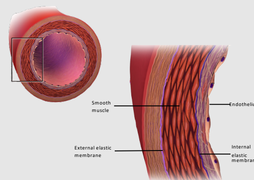
Small-diameter vascular grafts are the leading treatment for myocardial infarctions resulting from atherosclerosed coronary vasculature. A potential alternative to using patient-derived grafts is the use of tissue engineered vessels. The removal of native cells from xenogenic vessels allows for recellularization with human cells types; however, the decellularization process depends on the use of cytotoxic reagents that must be removed prior to recellularization. The recellularization process must produce a functional graft that contains the multiple cell types found within arteries. **We hypothesized that porcine internal thoracic arteries decellularized using a combination of detergents could be processed to support human endothelial and smooth muscle cell growth. We also hypothesized that in order to optimize in vitro co-culture conditions during recellularization, a mix of media types would be necessary to simultaneously support endothelial and smooth muscle cell growth.** A cytotoxicity assay was performed to assess the effects of residual detergents on endothelial cells seeded onto the scaffolds. A relationship between the degree of detergent rinsing and cellular viability was identified via a resazurin reduction assay with more extensive rinsing significantly enhancing cell viability. This same cell viability assay was used to identify media combinations that supported growth of both endothelial and smooth muscle cells. Both cell types were able to grow in a 50:50 mixture of their media types without any loss of viability or effect on morphology. Furthermore, endothelial cells grown in the mixed medium maintained their characteristic CD31 expression. Taken together, these results show that human cells native to arteries can remain viable within the extracellular matrix of porcine internal thoracic artery scaffolds after thorough scaffold detoxification. **Additionally, the co-culture conditions established can support the growth of both endothelial and smooth muscle cell types found within arteries. Future work will focus on simultaneously culturing the cells within scaffolds to build toward the goal of tissue engineering small-diameter vascular graft alternatives.**

INTRODUCTION

The internal thoracic artery has been found to be a reliable graft source in coronary artery bypass graft surgeries. A possible tissue engineered graft source and replacement to autografts are porcine internal thoracic arteries (PITAs); however the utilization of xenografts will require compatibility with recipient. Therefore, removing native cells from the specimen as well as establishing conditions for recellularization with patient-specific cells are an important step in the development of a viable graft. Anionic detergents (SDS/SDC) are commonly used to decellularize tissues, and cell viability assays are a form of detecting cytotoxic residue or incompatibility with culture conditions. In this project, cell viability was assessed via a resazurin reduction assay for establishing scaffold rinsing and cell-culturing protocols. Additionally, flow cytometry was used to confirm that co-culture conditions do not alter the phenotype of endothelial cells.

METHODS

- **Decellularization**
- **Cytotoxicity (Resazurin Reduction) Assay**
- **SEM Imaging**
- **Endothelial cell culturing within PITA Scaffolds**
- **Culture Optimization**
- **Flow Cytometry**



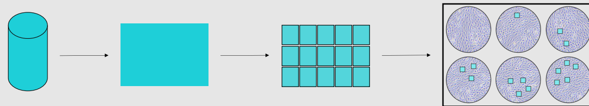


Figure 1. Obtaining sections of PITA scaffold for cytotoxicity testing through a resazurin reduction assay. Decellularized PITA scaffolds were rinsed and sectioned to be placed in their respective normal rinse or extensive rinse 6-well plate. The metabolic activity of HAECs in the presence increasing numbers of PITA sections was assessed after 24 and 48 hours.

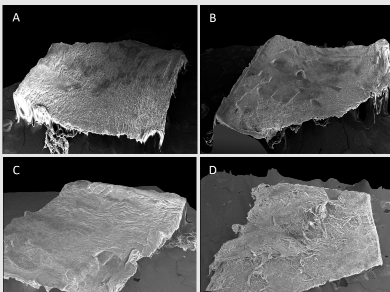


Figure 2. 3D renderings of PITA scaffolds from SEM images. Scaffolds were treated with increasing amount of SDS. The luminal surface was imaged. Concentrations of SDS used were control (A), 0% (B), 0.5% (C), and 1.5% (D)

METHODS

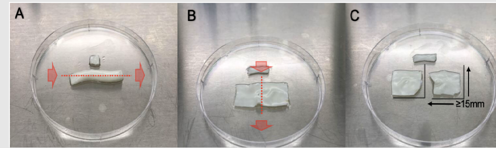


Figure 3. PITA scaffold processing. A longitudinal incision is made about the artery to expose the luminal surface. Sections are then made for installation into seeding windows.

RESULTS

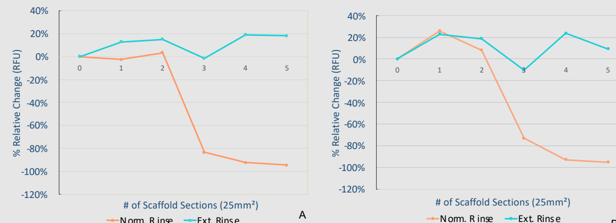


Figure 4. Extensive rinsing of decellularized PITA scaffolds eliminates cytotoxicity associated with residual detergent following decellularization. See figure 1 for experimental setup. At both 24 (A) and 48 hours (B) the viability of endothelial cells decreased as additional normally rinsed scaffold pieces were added, while viability in the presence of extensively rinsed scaffolds pieces was not affected.

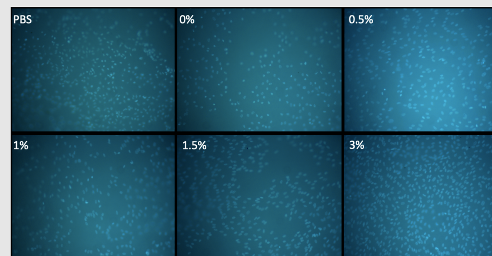


Figure 5. Recellularization of PITA Scaffolds with Endothelial Cells. Whole-mount Hoechst staining was performed to visualize the nuclei of endothelial cells seeded onto PITA scaffolds decellularized with the indicated SDS/SDC concentrations following the extensive rinsing protocol. 20X magnification.

RESULTS

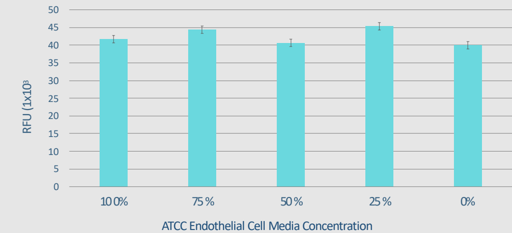


Figure 6. Endothelial cells maintain proliferative potential when cultured in up to 100% ATCC smooth muscle cell medium. Data are 72h after addition of indicated media. Results for growth in mixtures of ATCC smooth muscle and ATCC endothelial cell media. Data are means +/- standard error.

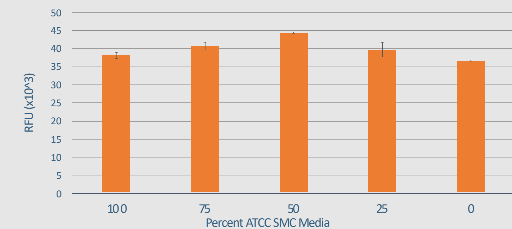
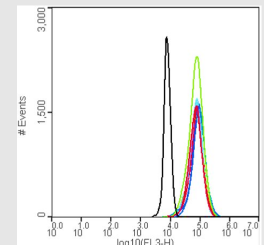


Figure 7. Smooth muscle cell proliferation is not negatively impacted by culture in ATCC endothelial cell medium relative to proliferation in ATCC smooth muscle cell medium. Results for growth in mixtures of ATCC smooth muscle and ATCC endothelial cell media after 72h incubation. Data are means +/- standard error.

Figure 8. Endothelial cells maintain CD31 expression in all 50:50 media combinations tested.

- **Black** – negative control (0% $CD31^+$)
- **Green** – 50% MSC media (97.8% $CD31^+$)
- **Cyan** – 50% ATCC SMC (98.4% $CD31^+$)
- **Blue** – 50% F-12 SMC (96.9% $CD31^+$)
- **Red** – 100% endothelial media (97.9% $CD31^+$)



CONCLUSIONS

1. **Following detergent decellularization to produce PITA scaffolds, the extensive rinsing protocol must be used to eliminate residual cytotoxicity.**
2. **Recellularization of extensively rinsed PITAs with endothelial cells was successful.**
3. **SMC and EC culture in a 50:50 mixture of their respective media does not reduce the viability of either cell type or affect CD31 expression in endothelial cells.**

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