Natural collagen scaffolds for blood vessel engineering

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Statement of Purpose: Mechanical characteristics of vascular grafts can significantly alter their ability to remain patent. It is therefore important that tissue engineered blood vessels (TEBV) possess mechanical properties similar to the native tissue. Here we describe the physical properties of a small-caliber TEBV developed from porcine acellular arterial tissue. Porcine carotid arteries were decellularized using multiple steps. The results of the mechanical properties illustrated that the TEBV performed similar to native artery with regards to compliance, stress, strain, and burst pressure. Moreover, chemical staining (Movat) of the decellularized vessels showed that the native matrix architecture, including several collagen layers within the internal and external elastin layers were preserved. Biocompatibility properties of the matrix were also demonstrated by endothelialization of the scaffold. These results demonstrate that acellular porcine arteries can provide suitable mechanical properties for development of small-caliber TEBV.

Methods: Carotid arterial segments were obtained from large pigs. The blood vessels had an internal luminal size of 3 to 4 mm and were cut into segments of approximately 50 mm in length. Vessels were incubated in a decellularization solution for 5 days and secured onto luer fittings, freeze-dried, and sterilized via ethylene oxide. To measure the stress-strain response of the acellular scaffolds, rectangular segments were tested. To evaluate compliance, the vessels were immersed in a water bath and cannulated at either end. One cannula was connected to a column of water and the other to a drainage tube. The column of water was high enough to create a pressure of 120 mmHg within the vessels. Water was drained through the scaffold in order to lower the pressure in increments of 10 mmHg. At each increment, the diameter of the scaffold was recorded using a digital camera. This process was repeated until the pressure was 0 mmHg. The burst pressure for vascular scaffolds was found by monitoring increasing pressures within the vessel until failure occurred. A pressure catheter was inserted through a cannulating fixture at one end of the vessel. A 60 cc pressure syringe was inserted through a custom cannula at the other end of the vessel. The pressure was increased until failure or leakage occurred and the pressure change was recorded. For biocompatibility testing, Bovine endothelial cells (EC) were seeded at a density of $1x10^6$ cells/ml and preconditioned via a bioreactor.

Results / Discussion: The porcine arterial segments that were decellularized and sterilized maintained their size and shape. Samples of decellularized vessel were processed for H&E and Movat staining. No cellular components could be detected and only layers of collagen and elastin were observed. Scanning Electron Microscope images illustrated the structure and the porosity remained intact. Collagen layers were visible throughout the vessel wall with large spaces between the layers. Compliance results showed the pressure-diameter curve for the decellularized vessels behaved similarly to native vessel (figure 1a). The diameter change was approximately 5% for native and 10% for decellularized scaffolds. Figure 1b illustrates the stress-strain behavior of the decellularized vessel in both the circumferential and longitudinal directions. As can be seen, the mechanical behavior of the decellularized vessel is comparable to the native artery. The burst pressure for the decellularized construct was measured at 1,960 mmHg, or approximately 16 times the systolic pressure. An EC monolayer was achieved in the lumen after 1 week of preconditioning via a bioreactor.

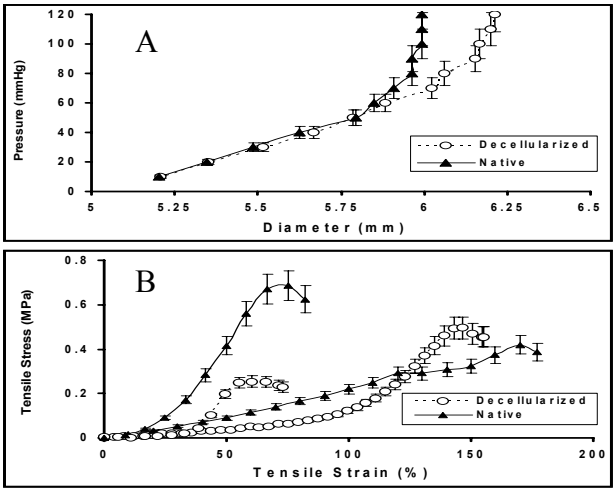

Figure 1. A) Pressure versus diameter measurements of the decellularized vessel. B) Stress versus strain measurements in the longitudinal and circumferential direction.

Conclusions: There exists a specific need to replace small diameter synthetic bypass grafts due to their poor patency. Tissue engineered small-caliber blood vessels represents one solution. As demonstrated in this study, aceullular porcine arterial segments mimic the mechanical behavior of native tissue. Moreover, the conduit possesses good biocompatibility properties and supports cell adhesion. Thus, acellular scaffolds show great promise in development of small-caliber TEBV. Long term studies are needed to observe any change in mechanical properties in-vivo.