

Porous Elastin Scaffolds with Controlled Degradation Rate for Vascular Grafts

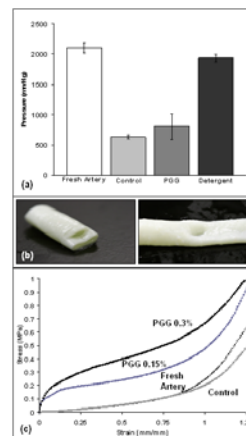
Chuang, Ting-Hsien; Dan, Simionescu PhD

Dept. of Bioengineering, Clemson University, SC 29634

Statement of Purpose Cardiovascular diseases are the major health killer to human health in developed countries. There is no pharmaceutical cure available: diseased or damaged blood vessels need to be replaced eventually. Tissue-engineered vascular grafts have been proposed to serve as promising source of replacements, especially for small-diameter applications. Scaffolds undergo remodeling and integrate with host body in the long run. For those load-bearing grafts, a moderate degradation rate is desired. Here we propose a novel method using a plant-derived polyphenol to stabilize elastin scaffolds. **Methods:** Fresh porcine carotid after manual cleaning, were processed for elastin scaffold preparation by incubation in 0.1M NaOH solution at 37°C for 24 hours and then rinsing extensively with deionized water until pH dropped to neutral. Scaffolds were finally rinsed with 70% ethanol and sterile saline and then stored in sterile saline supplemented with 0.02% NaN₃ at 4°C. Elastin scaffolds were treated with 0.3% PGG in 50 mM dibasic sodium phosphate buffer in saline with 20% isopropanol, pH 5.5. To test the effectiveness of PGG stabilization, scaffolds were tested for resistance to elastase. The groups under investigation were control elastin scaffolds, elastin scaffolds treated with PGG and carotid arteries decellularized by detergent method. Samples (n=6) were lyophilized, weighed and treated with 10 U/ml elastase in 50mM Tris buffer, 1 mM calcium chloride, 0.02% sodium azide at 37°C for 48 hours, then rinsed exhaustively and lyophilized to record dry weight after elastase. The difference in dry weight was used to calculate percent mass loss. For the PGG concentration study, samples from each fixation group (n=6) were rinsed, lyophilized and treated with elastase as described above. Burst pressures of treated scaffolds were determined using a peristaltic pump and a piezoelectric pressure transducer connected to a PC via an USB-interfaced data acquisition. The tubular scaffolds were mounted using plastic ties onto polyethylene connectors and physiologic saline pressure build up was created by gradually increasing the speed of the peristaltic pump. Burst pressures were recorded for vascular elastin scaffolds and compared to fresh carotid arteries and detergent-extracted arteries as controls. For tensile testing, fresh carotid arteries and elastin scaffolds were cut into 5 mm x 50 mm dumbbell shapes in the longitudinal direction of the blood vessel and analyzed at a constant uniaxial velocity of 0.1 mm/sec till failure using a 10-Newton load cell. **Results:** PGG concentration study showed that tissue mass loss decreased with increasing PGG concentration. This demonstrates that PGG fixation endows tissue with resistance to elastase. In vitro burst pressures analysis showed of fresh carotid arteries at about 2000 mmHg, values which were not significantly different from detergent-decellularized arteries (p>0.05). Vascular elastin scaffolds obtained by alkaline extraction exhibited mean burst pressures values of 630 mmHg

(p<0.05 compared to fresh artery). After PGG treatment burst pressure values increased to more than 800 mmHg, but these values were not statistically different from controls. Most elastin scaffolds exhibited similar burst patterns, with circumferential ruptures predominating over longitudinal tears. When compared to fresh arteries, vascular elastin scaffolds showed similar distensibility at low strains and a reduction in ultimate stress at higher strains. PGG fixation changed the shape of the curve and showed stiffening of the scaffolds at low strains, while maintaining similar slopes at higher strain levels.

Figure 1. Burst pressure & tensile stretch test



Conclusions: Alkali-purified, tubular arterial elastin scaffolds exhibit many desirable properties to be recommended for clinical applications as vascular grafts. The scaffolds are fully acellular, very manageable, exhibit high resistance to burst pressures but also are biodegradable. Treatment with PGG reduces the rate of elastin biodegradation in vitro and thus may allow for control of in vivo biodegradation. We plan to seed vascular smooth muscle cells (SMCs) within the media layer of grafts and re-endothelialize the vessel lumen. Two different methods of cell seeding, either applying electrical field or vacuum suction, will be applied. Primary porcine endothelial cells could grow on PGG-stabilized elastin scaffolds at static culture condition (data not shown); further test under dynamic shear flow will be performed.

References:

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