Development of a Small Caliber Vascular Graft with Decellularized Arteries

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Statement of Purpose: Small caliber vascular grafts (inner diameter < 5mm) to bypass blocked vessels due to atherosclerosis are of significant research and clinical interest. To date, small diameter prosthetic vascular grafts suffer from low patency rates due to thrombosis and intimal hyperplasia.[1] We aim to engineer biologically functional vascular grafts that can overcome these problems through modification of the extracellular matrix.

Methods: Rat aortas were harvested and decellularized using a sequential combination of weak detergents (1% Triton X-100 and 1.5% sodium dodecyl sulfate) followed by a nuclease treatment. The decellualrized aortas (DA) were characterized to confirm the removal of the cellular component and the retention of critical elements of the extracellular matrix. Poly(1,8 octanediol citrate) (POC) pre-polymer was synthesized as previously described [2] and coated onto DA at 37°C or 45°C. Heparin sodium were then conjugated to POC coated DA with standard carbodiimide chemistry, using diaminohexane as intermediate.[2] The POC-heparin modified DA were then evaluated for their swelling ratios and diameter change. Platelet adhesion assay and re-calcified whole blood clotting assay were performed to evaluate the anti-thrombotic effect of heparin on DA. Cell interaction with DA were evaluated in vitro by seeding vascular cells to the grafts.

Results: Removal of cells from the scaffold was confirmed with both H&E and DAPI staining (Figure 1). DNA quantification revealed approximately 95% of DNA elimination. Masson's trichrome stain clearly shows retention of the dense collagen fibrils of the artery (blue) (Figure 2, left), while Weigert's elastin staining revealed that elastin is maintained (red) after decellularization (Figure 2, right).

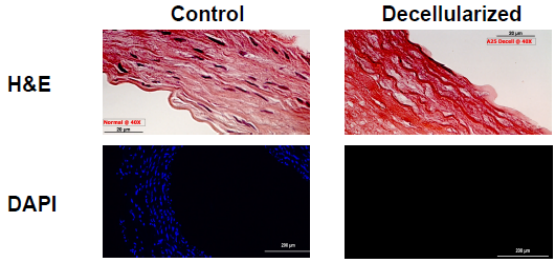

Figure 1. H&E and DAPI staining of native (left) and decellularized (right) aorta showed removal of nuclei after decellularization process.

After POC coating and heparin conjugation, the grafts showed no significant change in diameter or swelling ratio. (Table 1) POC-heparin modified DA showed significant anti-thrombotic properties, as tested by platelet adhesion assay (Figure 3. A) and whole blood clotting assay (Figure 3.B). Vascular cell interaction with DA are currently under investigation. A rat abdominal aorta interposition model are also used to compare the modified and control DA in vivo for signs of inflammation, endothelialization, thrombosis and intimal hyperplasia.

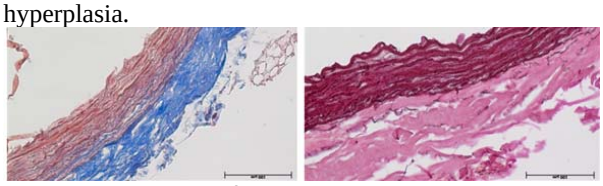

Figure 2. Retention of ECM with Masson's trichrome (left) and Weigert's elastin (right) staining.

Table 1. Inner diameter and swelling ratios of DA.

		Inner Diameter	Swelling Ratio
Control		1.24±0.33mm	3.13±0.04
POC	37°C	1.20±0.12mm	3.48±0.38
	45°C	1.16±0.14mm	4.21±1.10
POC +Hep	37°C	1.20±0.05mm	3.03±0.49
	45°C	1.00±0.26mm	3.76±0.42

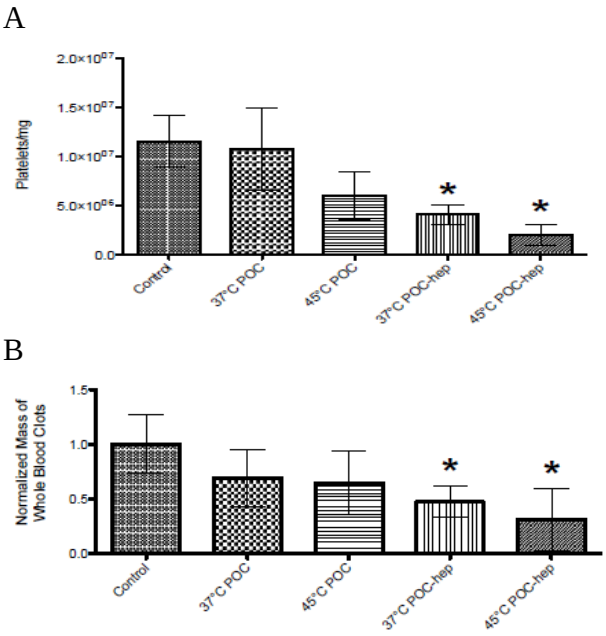

Figure 3. Platelet adhesion (A) and whole blood clotting (B) assays showed significantly lower (* $p<0.05$) number of platelets adherent and clotting on POC-heparin modified DA relative to not coated DA (control).

Conclusions: We have developed a vascular tissue engineering scaffold based on a decellularized arterial conduit with biopolymer modification. The complete decellularization eliminates potential immune rejection, while the ECM structure is well preserved. The modification with POC and heparin gave rise to significant improvement in graft anti-thrombotic properties. Therefore, the modified DA exhibited great potential as small caliber vascular graft scaffold.

References:

1. Zilla P et al. Biomaterials, 2007. 28 (34), 5009-27.
2. Hoshi R et al. Biomaterials. 2013; 34 (1): 30-41.