Evaluation of a Degradable Polar Hydrophobic Ionic Polyurethane Designed for Vascular Graft Generation in an *in vivo* Mouse Model

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Introduction:
Tissue regeneration alternatives for peripheral vascular disease are actively being investigated as these complications affect millions of people worldwide (1). Polymeric films of a degradable-polar/hydrophobic/ ionic polyurethane (D-PHI) have been tested *in vitro* and found to promote a wound healing macrophage phenotype with suitable mechanical properties for a vascular graft (2,3). In this study, the goal was to test a three dimensional porous "disk" scaffold structure of this material to observe its acute biocompatibility *in vivo*. The tissue response to the material will determine whether a wound healing or a pro-inflammatory foreign body response will be initiated following implantation. D-PHI was synthesized such that the end product contained a balance of hydrophilic/hydrophobic/ionic characteristics optimal for cell adhesion and proliferation while producing non-toxic breakdown products (3). It is hypothesized that the carefully designed chemical structure of D-PHI, which possesses the appropriate mechanical properties for a peripheral vascular graft (3), will also possess suitable biocompatibility.

Methods:
A divinyl oligomer (DVO) was synthesized by mixing 2-hydroxyethylmethacrylate, poly (hexamethylene carbonate) diol and lysine diisocyanate in a 2:1:2 ratio with dibutyltin dilaurate catalyst at 50°C overnight. The D-PHI scaffolds consisted of DVO, methacrylic acid and methyl methacrylate at a 1:5:15 ratio with benzoyl peroxide initiator in the presence of sodium bicarbonate salt and polyethylene glycol to generate 75-80% porous scaffolds (3). Following sterilization of the scaffolds with 70% ethanol, followed by incubation for 72hrs with pen/strep in phosphate buffered saline, BALB/c mice were anaesthetized and 3 scaffolds/mouse were placed in subcutaneous pockets on the dorsal surface of the animal. Explanted scaffolds were either fixed in 4% paraformaldehyde for 25 minutes and stained using Trichrome or May-Grunwald Giemsa, prepared for a live/dead assay using carboxy fluorescein diacetate (CFDA), followed by fixing and staining with propidium iodide or the adherent tissue lysed for a cytokine antibody array (RayBiotech Inc).

Results:
There were no complications with the implantation and tissue recovered very well as can be seen when examining the tissue prior to explantation (Fig 1). The live/dead stain (Fig 2 (A-D)) showed that almost all the cells within the scaffold were alive while histological analysis showed good tissue integration throughout the scaffold with extracellular matrix formation (Fig 2 (E & G) (I & L). After 1 week of implantation a profile of inflammatory cytokines in lysates from the explanted scaffolds showed a significantly increased level of MCP-1 (monocyte chemotactic protein) compared to any pro-inflammatory cytokine ($p<0.0001$) (Data not shown). MCP-1 has been shown to play a role in reperfusion, inflammation, and skeletal muscle regeneration following ischemic injury, proving to be of importance in the process of tissue repair (4).

Fig. 1: Images of the scaffold in a subcutaneous implant following a 6 week time period. Wound has healed (left panel insert); no visible inflammation present, with tissue integration (right panel) and visible blood vessels (right panel insert (arrow)).

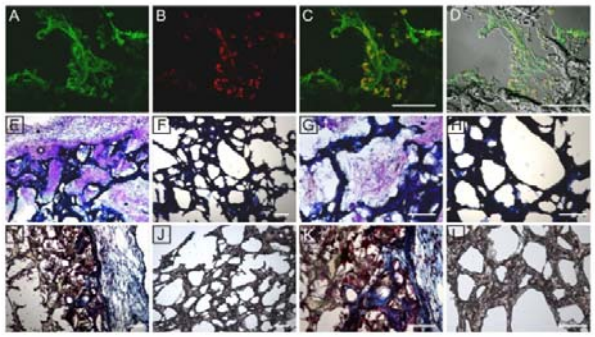

Fig. 2: Live dead assay (A) green-live cytoplasm (B) red-nuclei) (C) overlay (D) phase contrast; May Grunwald – Giemsa (E) and (G) blue/pink-cytoplasm, purple - nuclei; Trichrome (I) and K) nuclei- black, muscle fibers-red, collagen-blue (F, H, J, L were scaffolds that were not implanted). Scale bar=100µm

Conclusion:
Scaffolds synthesized using D-PHI did not invoke a pro-inflammatory response in a subcutaneous mouse model as demonstrated by the appearance of the explanted scaffold, and through the measurement of inflammatory cytokines. Within the two week implant period tissue was able to migrate into the scaffold and receive adequate nutrients for survival. These data indicate that D-PHI scaffolds show promise for tissue engineering a vascular graft.

References:
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