Testing the *in vivo* compatibility and biodegradation of a degradable-polar/hydrophobic/ionic polyurethane for vascular tissue engineering applications

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Statement of Purpose: There is a market for elastomeric degradable scaffolds for tissue engineering (TE) applications such as vascular grafts. Poly lactic glycolic acid (PLGA) is a biocompatible, degradable polymer that has been used in nanotechnology and drug delivery systems[1]; however, its mechanical properties have limited its applications in TE. PLGA scaffolds degraded 5-6% after 8 weeks, decreased 12% in molecular weight and released acidic products that could lead to inflammation[2]. A recent study described a degradable-polar/ hydrophobic/ionic polyurethane (D-PHI) that demonstrated appropriate mechanical properties for blood vessel TE[3] while eliciting a more wound healing phenotype macrophage[4]. Recently it was shown that D-PHI had negligible degradation after 8 wks and 12% mass loss after 4 months in PBS (unpublished data); however *in vivo* degradation data have yet to be reported on for D-PHI. The present study compared porous D-PHI and PLGA scaffolds in an *in vivo* rat model, looking at degradation up to 21d. It is hypothesized that D-PHI will show low toxicity, reduced inflammation, and a rate of degradation suited to blood vessel TE requirements[3].

Methods: ***Scaffold preparation*:** PLGA (75:25 poly lactic:glycolic acid; Sigma) and D-PHI scaffolds were prepared in Teflon molds (6mm diameter, 2mm high disks). **PLGA** was dissolved 1:10 in chloroform and sodium bicarbonate porogen (SBP; ~140μm-250μm; Sigma) was added to a weight ratio 25%:75% polymer:porogen. The mixture was packed into the Teflon mold and the chloroform was evaporated off.

D-PHI synthesis: Divinyl oligomer (DVO) was prepared with lysine diisocyanate as previously described[3]. D-PHI scaffolds were prepared by mixing DVO, methacrylic acid, and methyl methacrylate (1:5:15) with benzoyl peroxide initiator overnight. SBP particles (65 wt%) and polyethylene glycol (~40μm; 10wt%) were added as porogens to the D-PHI (25wt%) mixture and cured at 110°C for 24h. The disks (PLGA and D-PHI) were desalted by sonicating in H_2O. ***Porosity measurements:*** Dry, porous D-PHI and PLGA disks (n=3) were analyzed by X-ray microtomography (μCT) to evaluate porosity. ***Rat in vivo protocol*:** Prior to implantation, dry, weighed scaffolds were gamma irradiated (2.5Mrad) using a Gammacell 220. Wistar rats (Protocol# 20007839, ex.06/30/10) (3 months old) were anesthetized prior to the subcutaneous implantation of porous PLGA or D-PHI scaffold disks (6 disks /rat) for up to 21d. ***Scanning electron* microscopy (*SEM*):** 7d disks were fixed in 3% glutaraldehyde as previously described[4]. ***Histological staining:*** 7d disks were fixed in 4% paraformaldehyde and stained with May-Grunwald Giemsa or Trichrome (Sigma). ***Tissue removal:*** The remaining disks were incubated with trypsin/EDTA, proteinase K, collagenase A, and SDS-lysis buffer to remove cells and protein prior to weighing.

Results: Fig.1 shows porous D-PHI (1a) and PLGA (1b) scaffolds prior to implantation. D-PHI scaffolds were 79.4% and PLGA scaffolds 82.7% porous as determined by μCT. D-PHI explants showed that cells infiltrated the scaffold and extracellular matrix was deposited within the pores (Fig.1c; arrow). PLGA scaffolds showed tissue around the border of the scaffold (Fig.1d; arrow shows border of scaffold) but less tissue in the pores of the scaffold. However, May-Grunwald Giemsa staining (Fig.2a,b) showed good cell infiltration (blue nuclei) and Trichrome staining matrix deposition in and around the pores for both D-PHI and PLGA surfaces (Fig.2c,d; purple). Both surfaces showed that 21d degradation was greater than 14d degradation (Tab.I). It was noted that the non-implanted blank PLGA lost 4.78% mass due to the tissue extraction protocol whereas D-PHI showed no mass loss. Mass loss reported in Tab.I has the blank mass loss subtracted. D-PHI scaffolds maintained their shape better than PLGA scaffolds after 21d suggesting D-PHI could retain vascular form in an implant.

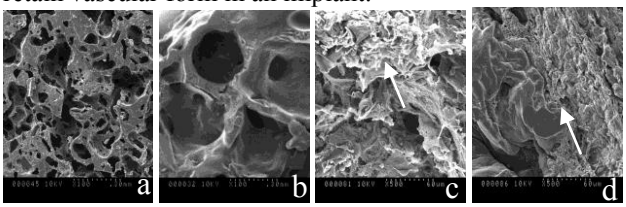

Fig. 1: SEMs of control D-PHI(a) or PLGA(b) scaffolds and D-PHI(c) or PLGA(d) 7d *in vivo* explants.

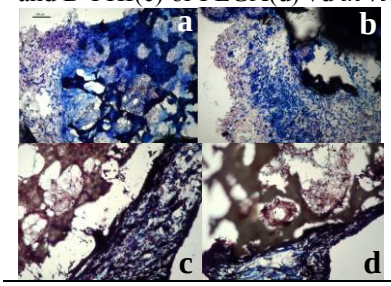

Fig.2: May-Grunwald Giemsa (a, b) or Trichrome staining (c,d) of 7d explanted D-PHI (a, c, respectively) or PLGA (b, d respectively) scaffolds.

Surface	7d (%)	14d (%)	21d (%)
D-PHI	**7.44+/-3.48**	**9.75+/-0.51**	**15.2+/-3.70 ***
PLGA	**16.8 +/-4.35**	**8.81+/-1.56**	**19.4+/-3.03 ***

Table I: *In vivo* degradation of D-PHI and PLGA scaffolds (avg mass loss, less blank +/-std error). N=3 for 7d, N=8 for 14d, 21d. *Higher than 14d, $p<0.002$.

Conclusions: The elastic D-PHI scaffold demonstrated good biocompatibility with the *in vivo* environment (cell infiltration and tissue matrix development) and a controlled rate of degradation. Three month implant degradation studies are currently on-going to further confirm suitability of D-PHI for TE applications.

References: [1]Lu JM, *et al.* Expert Rev Mol Diagn. 2009;9(4):325-41. [2]Shin HJ, *et al.* J Biomater Sci Polym Ed. 2006;17(1-2):103-19. [3]Sharifpoor S, *et al.* Biomac. 2009;10(10):2729-39. [4]McBane JE, *et al.* Biomater. 2009;30(29):5497-504. **Funding**: HSF PDF scholarship, CIHR/NSERC CHRP 337246/83459.