Composition of Intraperitoneal Electrospun Conduits Influence Recruited Cell Phenotype and Matrix Synthesis

Chris A. Bashur,[1] Matthew J. Eagleton,[1,2] and Anand Ramamurthi.[1]
Department of [1]Biomedical Engineering and [2]Vascular Surgery, Cleveland Clinic, Cleveland, OH

Statement of Purpose:
Long-term viability of current tissue engineered small-diameter vascular grafts is limited by poor generation of mature elastic matrix by post-neonatal cells.[1] Our strategy is to recruit an alternative autologous cell source by implanting scaffolds into the peritoneal cavity. We hypothesize that the recruited cells can differentiate into a pro-elastogenic and anti-inflammatory phenotype with appropriate biochemical cues – e.g. elastogenic hyaluronan tetramers (HA-o). Here, we investigated how intraperitoneal electrospun scaffolds incorporating collagen and modified with HA-o influence wound healing, and the phenotype and elastogenicity of recruited cells.

Methods: Electrospun poly(ε-caprolactone) (PCL) and 25% w/w collagen/PCL (blend) conduits were prepared. Fiber diameters and orientations were characterized using SEM / EDS. HA-o was tethered to the scaffolds using a carbodiimide crosslinker, and verified with HA binding protein and a fluorescent probe. Conduits were enclosed in PTFE porous pouches and then implanted within rat peritoneal cavities for 2, 4, and 6 weeks, with IACUC approval. After harvesting, the constructs were analyzed for mRNA expression and production of phenotypic markers and matrix proteins. Cells were also isolated from peritoneal fluid and conduits. Statistical significance was determined using one-way ANOVA with Tukey comparisons ($p < 0.05$, for $n = 6$ samples/ condition). Values reported as mean ± std dev, or for PCR std error.

Results: PCL and blend conduits exhibited a low degree of orientation, and average fiber diameters of 2.19 ± 0.243 and 1.62 ± 0.867 µm, respectively. (**Fig. 1**) The N1s peak verifies the presence of collagen. This study demonstrated that the incorporation of as little as 25% w/w collagen with PCL in electrospun conduits promoted a quicker progression of the wound healing response. Unlike PCL conduits, PCR showed that expression of general (*Cd68*) and pro-inflammatory M1 (*Cd80*) macrophage markers decreased from 4-6 weeks post-implantation (**Fig. 2**). However, smooth muscle cell (SMC) markers, such as myosin heavy chain (*Myh11*), remained elevated (not shown). The addition of collagen also reduced the total matrix accumulation by as early as 2 weeks post-implantation (**Fig. 2**, histology not shown). Within the blended scaffolds, a decrease in collagen (OH-Pro assay) and an increase in elastin (Fastin® assay) were observed with increasing implantation time (**Fig. 3**). Elastic matrix production was greatest with blend conduits (p=0.002). Unexpectedly, HA-o tethering provided only a limited improvement in phenotypic marker & elastin production.

In ongoing experiments, cells from both the peritoneal fluid and constructs after different implantation times have been cultured to better characterize their phenotypes and responsiveness to elastogenic cues. The peritoneal fluid cells were a mixed population that appeared to differentiate on culture polystyrene over time (**Fig. 3**), and were smoothelin$^+$ (i.e. a late-stage SMC marker) (not shown). Further characterization is ongoing.

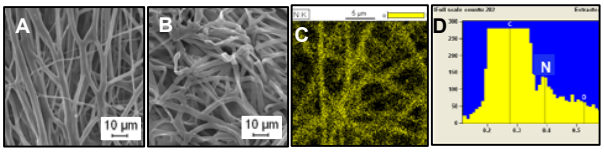

Fig. 1. PCL (A) and blend (B) meshes. EDS N1s map (C) and spectrum (D) for blend meshes.

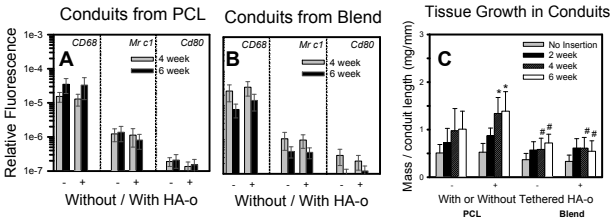

Fig. 2. Macrophage marker expression for PCL (A) and blend (B) conduits. Normalized mass post-insertion (C). Differences from no insertion (*) and HA-o PCL (#).

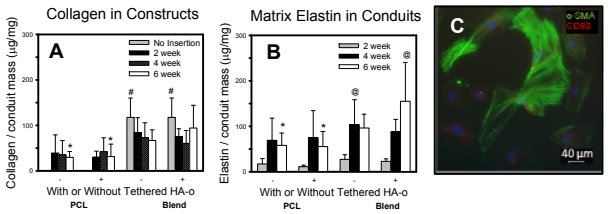

Fig. 3. Collagenous (A) and elastic (B) matrix in constructs *in vivo*. Immunofluorescent markers for cells cultured from peritoneal fluid. Differences from HA-o blend (*), PCL all times (#), and 2-week insertion (@).

Conclusions: This study demonstrates that recruited peritoneal cells exhibit greater elastogenicity when collagen is incorporated into scaffolds. Also, the reduced wound healing time with collagen-containing scaffolds is important since acute, but not chronic, inflammation tends to promote a positive wound healing response.[2] Limited impact was observed from HA-o modification, unlike *in vitro* studies.[3] It is likely that better delivery methods will be required to prevent the HA-o from being overwhelmed by HA produced during inflammation.[4] In conclusion, the peritoneal constructs have potential for use as small-diameter grafts, but further study into how recruited cell phenotype and matrix production may be modulated by scaffold composition is necessary.

Funding and References: RO1HL092051 (Ramamurthi) and F32HL108548 (Bashur)

1. Johnson e t al. Circ Res, 1995, 77: 1107; **2**. Badylak et al., Tiss Eng Part A, 2008, 14:1835; **3**. Joddar et al. Biomaterials, 2006, 27:2994; **4.** Park et al. Biomaterials, 2003, 24:1631.