Impact of Peritoneal Pre-conditioning on Tissue Engineered Vascular Graft Intimal Hyperplasia and Inflammation

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Statement of Purpose: Tissue engineered vascular grafts (TEVGs) are a promising alternative to current surgical options. However, the viability of small diameter TEVGs is limited by the lack of a functional endothelium and by intimal hyperplasia and stenosis. In our approach, we used pre-implantation in the rat peritoneal cavity as a source for recruiting autologous cells with the goal of improving graft structural stability, patency, and endothelialization. We blend collagen with a synthetic material to modulate the post-grafting inflammatory response while avoiding aneurysmal-like dilation and failure that would occur with a pure collagen graft.[1] Interestingly, our previous analysis indicates there is a complicated, and non-systematic, impact of conduit collagen ratio on the response in the peritoneal cavity.[2] Thus, the goals of this study are to determine the impact of initial conduit collagen percentage on post-grafting response and to compare peritoneal pre-implantation with directly-grafted conduits.

Methods: Conduits were electrospun from blends of PCL and collagen. Average electrospun fiber diameters were 1.07±0.2, 0.95±0.17, and 1.14±0.78 µm for 100, 90 and 75% (w/w) PCL/collagen, respectively (SEM). Collagen content was confirmed with the XPS nitrogen 1s peak. Conduit dynamic compliance was determined. Conduits in PTFE porous pouches were implanted in the rat peritoneal cavity for 4 weeks to act as an "in vivo bioreactor." Constructs were then harvested and grafted into abdominal aorta of the same rat for 6 weeks to assess remodeling and endothelialization after grafting (**Fig. 1**). TEVG patency, changes in luminal diameter, and percent expansion with the cardiac cycle were evaluated using ultrasound. The grafts were analyzed with histology, including H&E. Morphology and phenotype of the cells that are present was determined via PCR and immunofluorescence (IF). HPLC / mass spectrometry was used to quantify levels of specific lipids and their oxidation products. Significance was determined using one-way ANOVA with Tukey comparisons ($p < 0.05$).

Results: The conduits had mechanical properties needed for grafting (e.g., burst pressure > 2,000 mmHg for PCL grafts). After grafting, preliminary ultrasound results suggest that the grafts reproducibly expanded with systole and diastole. The percent expansion, as calculated from the min and max diameters, increased with increasing collagen content and decreased with initial peritoneal implantation. Importantly, H&E stained sections suggested that pre-implantation in the peritoneal cavity reduced the thickness of the intimal layer for both 100% and 90% PCL (**Fig. 1C and 2**). The 75% PCL conduits resulted in a thicker intimal layer compared to 100% and 90% PCL samples implanted in the peritoneal cavity (p= 0.006 and p=0.057, respectively). Cellular results included that cell infiltration systematically decrease with percent collagen, with the highest infiltration within PCL samples. According to IF images, PCL samples had the highest expression of the contractile marker α-SMA and M1 macrophages marker CD80 by the cells in the graft wall compared to other compositions, and the level of expression was lower with pre-implantation (**Fig. 3**). The aorta control showed expression of α-SMA but no CD80 expression was present (not shown). We have also analyzed vWF for endothelial cells function. Lipid oxidation results indicated that peritoneal pre-implantation reduced the overall lipid oxidation after aortal grafting with the most noticeable reduction for 9-HETE, an oxidation product of arachidonic acid (p= 0.045, by t-test). Longer grafting time points are also being studied. Currently, there is a rat at 3 months post-surgery, and analysis will be performed at 4 months.

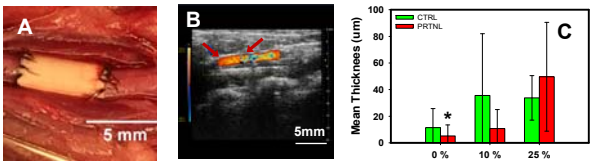

Fig. 1. 90% PCL graft (A). Ultrasound color Doppler image of the 90% condition (B). Intimal hyperplasia layer thickness (C) (* statistical difference from 75% with peritoneal pre-implantation.)

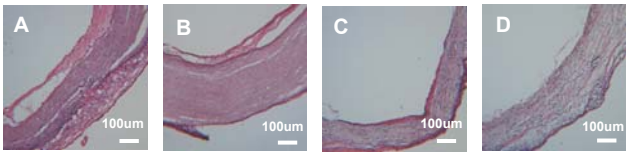

Fig. 2. H&E images of 90% PCL grafts without (A) and with (B) initial peritoneal implantation, and 100% PCL grafts without (C) and with initial peritoneal implantation (D).

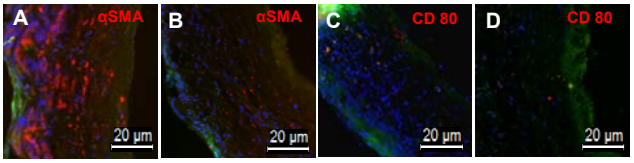

Fig. 3. Representative images of α-SMA and CD80 expression in grafted PCL constructs without (A and C) and with (B and D) pre-implantation.

Conclusions: This study suggests that there is a benefit of peritoneal pre-implantation (e.g. less intimal hyperplasia), although grafts both with and without peritoneal pre-implantation remained patent after 6 weeks in our model. A similar benefit has been shown in the literature when pre-seeding TEVGs with stem cells.[3] Our results provides more support for a potential mechanism for the reduced thickness of intimal hyperplasia with peritoneal pre-implantation, particularly by reducing long-term inflammation. Further, the percentages of collagen that we have tested here provides a unique response compared to the higher percentages that have been tested elsewhere.[3]

References **1.** Weinberg et al. Science, 1986. **2.** Birthare et al. Biomed. Materials, 2016. **3.** Roh et al. PNAS, 2010. **Funding:** American Heart Association 14BGIA18480031