ECM Components Recruited with Peritoneal Preimplantation and Correlation with Vascular Graft Outcomes

Valentina I Ochoa Mendoza[1], Mahyar Sameti[1] Lisa K. Moore[2] Chris A Bashur[1]
[1]Department of Biomedical, Chemical Engineering and Science, Florida Institute of Technology, Melbourne, FL, 32901

Statement of Purpose: Tissue engineered vascular grafts (TEVGs) are a promising substitute to autologous grafts due to their potential to remodel over time and integrate with surrounding artery. Yet, there are no FDA-approved TEVGs for high pressure arteries with a diameter <4 mm. In previous studies, we developed conduits that showed unique properties after pre-implanting in rat peritoneal cavities for four weeks and then grafting in rat aortae for six weeks. This included reduced platelet adhesion, reduced long term inflammation, and lower intimal hyperplasia [1]. However, our previous grafts are not ready on demand. Thus, our goal of this study is to identify new targets for graft modification based upon extracellular matrix (ECM) components that are recruited in the pre-implantation step.

Methods: Conduits were electrospun from both a blend of 10% w/w collagen type I/PCL and pure PCL. The conduits were enclosed within pouches composed of either poly tetrafluoroethylene (PTFE) or polyethylene glycol diacrylate (PEGDA) to avoid peritoneal adhesions. Pouches were implanted within the peritoneal cavity of Sprague-Dawley rats. After four weeks, they were removed and the conduits were either analyzed or grafted in the aorta of the same rat. A group of constructs were immersed in RIPA buffer for analyzing protein content via BCA assay and then MS/MS mass spectroscopy. Another group were sectioned and stained for histology and immunofluorescence (IF). Tissues (e.g., aorta, spleen) were also harvested for staining controls.

Results: Collagen/PCL and pure PCL conduits were prepared in different pouches and implanted in the rat peritoneal cavity for four weeks (**Fig. 1A-C**). Tissue deposition occurred during this pre-implantation step with a range of protein bands as visualized with coomassie blue stained gels (**Fig. 1D**). The response varied with the type of pouch material implanted, with lower tissue deposition and cell infiltration on PEGDA than PTFE pouches (n=3/condition), even though we have previously found benefits of pre-implantation with both pouch materials. Further protein analysis with mass spectroscopy found >1500 proteins for each condition. We focused on ECM proteins because an analysis of our data showed a low correlation between overall cellularity and intimal thickness within our constructs (**Fig. 2A).** These results suggest that ECM components may be what is providing the benefit from pre-implantation. A cluster analysis of the most prevalent ECM components appeared to show that some ECM proteins and proteoglycans (e.g., biglycan, decorin, fibrinogen, and thrombospondin) are found in higher abundance in the PTFE pouches conditions (**Fig 2B**). This agrees with the H&E images that show more tissue deposition with PTFE pouches. Further, IF analysis confirms a higher decorin content in the wall of conduits from PTFE pouches (**Fig 3A-B**). Alcian blue staining does not show a clear difference between conduits from the two pouch materials (**Fig 3C-D**), suggesting that the differences may be proteoglycan specific. There also appeared to be some proteins more prevalent with pure PCL conduits vs. collagen/PCL conduits (e.g., collagen V, fibrillin, fibulin, and versican). However, one protein, collagen (XII), was found in high abundance in all conditions. We are currently evaluating the impact of potential target ECM components with cell culture.

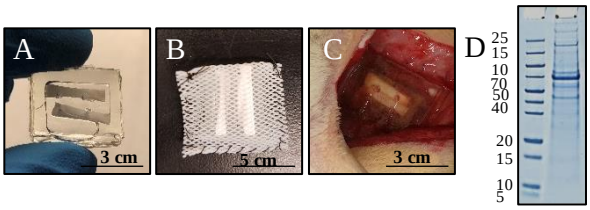

Fig. 1 Representative PEGDA (A) and PTFE (B) pouches with enclosed conduits. PEGDA pouch after four weeks of pre-implantation (C). SDS-Page gel of PEGDA pouch sample after pre-implantation with corresponding ladder (D)

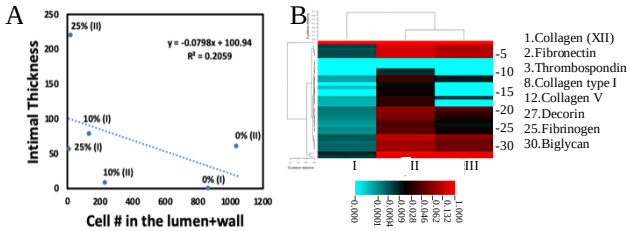

Fig. 2 Correlation showing graft intimal thickness vs overall cellularity of the constructs after pre-implantation (A). Cluster Analysis showing the prevalence of ECM components within preimplanted conduits among the different conditions: PEGDA 90%(I), PTFE 100%(II), and PTFE 90%(III) (B)

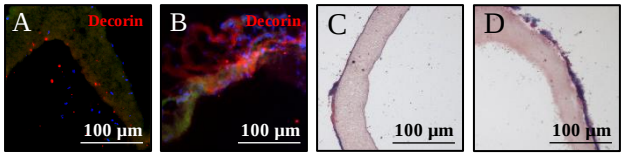

Fig. 3 Representative IF images for decorin with conduits after implantation in PEGDA (A) and PTFE (B) pouches. Nuclei are stained with DAPI (blue). The green channel is autofluorescence with increased brightness / contrast to visualize the scaffold. Alcian blue staining for conduits from PEGDA (C) and PTFE (D).

Conclusions: Some of the ECM components found within our PTFE pouch (e.g., decorin, biglycan), are abundant in the peritoneal fluid that our pouches are exposed to. These proteoglycans are involved in collagen fiber assembly and could be potential targets for inclusion in our graft. Further, collagen XII was shown to be a promising target for graft modification as it was the most prevalent ECM component for all conditions. Overall, these results provide insight into ECM components recruited with peritoneal pre-implantation that can potentially lead to the development of TEVGs that can be used on demand with lower intimal hyperplasia and other benefits over control acellular grafts.

References: [1] Shojaee et. al, Acta Biomaterialia, 2017.
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