# Patterns of Tissue Remodeling and Macrophage Polarization Following Implantation of Non-Crosslinked and Crosslinked Extracellular Matrix Scaffolds in a Model of Bilateral Rat Body Wall Defect Repair

Bryan N. Brown[1,3], Kathryn A. Kukla[3,4], Neill J. Turner[3], Stephen F. Badylak[1,2,3]

[1]University of Pittsburgh Department of Bioengineering, [2] University of Pittsburgh Department of Surgery, [3]McGowan Institute for Regenerative Medicine, [4] Carnegie Mellon University Department of Chemical Engineering

**Statement of Purpose:** Activated macrophages have been described as having either an M1 or M2 phenotype based upon patterns of cell surface marker and gene expression, effector molecule production, and function. Recently, the importance of macrophage phenotype has been investigated in the context of regenerative medicine approaches to tissue reconstruction. It has been shown that certain biomaterial implants are capable of modulating the default host response following injury, and that phenotypic differences in the macrophage population that participates in the host response to implanted biomaterials are predictive of downstream tissue remodeling outcomes. In brief, a predominance of M1 macrophages has been associated with encapsulation and/or the formation of scar tissue within the implant site. A predominance of M2 macrophages has been associated with a more constructive remodeling type response; specifically, the formation of new host tissue that is site appropriate from both a structural and functional perspective. The present study utilized a bilateral abdominal wall defect repair model to investigate and compare the host remodeling and macrophage responses associated with biomaterials known to elicit an M1 or an M2 type macrophage response. The goals of the study were twofold: (1) to perform a comparative analysis of tissue remodeling, matrix metalloproteinase (MMP) expression, and macrophage polarization following the implantation of two different materials in the same animal; and (2) to determine whether macrophage polarization in response to one implanted biomaterial affects the macrophage polarization and tissue remodeling outcome of a second material in the same animal.

**Methods:** Bilateral defects were created in the ventral abdominal wall musculature of Sprague-Dawley rats (one on each side of the midline) and included the internal and external oblique muscles leaving the transversalus fascia and peritoneum intact. Defects were repaired using one of two extracellular matrix (ECM) scaffold test articles or replacement of the excised autologous tissue. Test articles included urinary bladder matrix (UBM) and urinary bladder matrix crosslinked with 10 mM carbodiimide (CDI-UBM). The implanted materials were explanted at time points of 1, 3, 7, 14, and 28 days. Tissue explants were subjected to histologic staining, gene expression analysis, and immunofluorescent labeling to determine the host tissue remodeling response and macrophage polarization profile. Histologic evaluation included assessment of spatial and temporal patterns of cellular infiltration, scaffold degradation, angiogenesis, and neo-matrix deposition. Gene expression analysis included markers of extracellular matrix remodeling (MMP2, 3, 7, 9, 10) as well as markers of M1 (iNOS, CXCL10, IL12) and M2 (Arginase, CD36, IL10) macrophage polarization. Finally, labeling with 4 immunofluorescent markers (DRAQ5: nuclear stain, CD68: pan-macrophage, CCR7: M1, CD206: M2) was used to quantitatively assess macrophage polarization within the remodeling tissue.

**Results:** Results showed that each test article was associated with a distinct host tissue remodeling response which did not appear to affect (or be affected by) the remodeling response observed for other test articles implanted in the same animal. UBM was associated with dense neutrophil infiltration surrounding and within the test article at 1 and 3 days post implantation changing to a predominantly mononuclear cell population thereafter. The remodeling response to UBM was characterized by rapid scaffold degradation, angiogenesis, and organized neo-matrix deposition. Some signs of new muscle tissue formation were also observed. CDI-UBM was associated with a dense neutrophil population at 1 and 3 days post implantation changing to a mixed mononuclear and foreign body giant cell population thereafter. The cells responding to the CDI-UBM test article were found only peripheral to the implanted material and a response consisting predominantly of foreign body giant cells was observed at the material-tissue interface. The remodeling response to CDI-UBM was characterized by angiogenesis and progressive deposition of dense connective tissue indicative of encapsulation in the surrounding tissue, and little scaffold degradation. The autograft was associated with a predominantly mononuclear cell infiltrate with little to no neutrophil involvement. The remodeling response to the autograft consisted of progressive necrosis of the muscle tissue component of the graft and deposition of dense host connective tissue consistent with scarring. Each test article was also associated with a distinct profile of ECM remodeling (MMPs), M1/M2 gene expression, and cell surface markers.

**Conclusions:** The results of this study showed that each test article was associated with a distinct host tissue remodeling response that was related to the composition of the implant and methods used to prepare the test article (i.e. crosslinking). Each test article was also associated with differences in markers of ECM remodeling as well as macrophage polarization suggesting that different macrophage populations are associated with different mechanisms of tissue remodeling. Further, the host macrophage response to individual test articles was not observed to affect (or be affected by) the response to other test articles suggesting that macrophage polarization occurs locally at the remodeling site and is, therefore, likely a function of macrophage-scaffold interactions. A more in depth understanding of the causative factors of the macrophage phenotypes observed in this study may lead to the design of biomaterials that promote the restoration of functional, site appropriate tissue as opposed to inflammation and scar tissue formation.