Distinct Local Macrophage Phenotypes Are Associated With Divergent Tissue Remodeling Outcomes Following Implantation of Biologic Scaffolds

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Statement of Purpose: The ultimate determination of clinical success for an implanted biomaterial is the response of the host following implantation. The innate immune mechanisms that modulate the host response include effector cells such as macrophages, the presence of which heretofore has been considered to be detrimental with negative implications for functionality of implanted biomaterials. Recently, however, macrophages have been shown to exhibit diverse and plastic phenotypes on a spectrum between M1 (classically activated, pro-inflammatory) and M2 (alternatively activated, anti-inflammatory, regulatory). These diverse populations have been suggested to play distinct and potentially determinant roles in the outcome of tissue remodeling following injury. The present study examined the phenotype of macrophages which responded following the implantation of two extracellular matrix based biomaterials known to either elicit the formation of new, site-appropriate, functional host tissues or deposition of dense encapsulating tissue without functional recovery.

The objectives of the present study were three-fold: (1) to analyze tissue remodeling, matrix metalloproteinase, and tissue cytokine expression following implantation of two different materials concurrently in the same animal; (2) to determine the phenotype of macrophages which respond following implantation of the same ECM scaffold materials and relate the macrophage response to the observed tissue remodeling outcomes; and (3) to determine the ability of M1 and M2 polarized macrophages to promote the recruitment of perivascular progenitor cell populations which have been suggested to participate in the remodeling of extracellular matrix scaffold materials.

Methods: Bilateral defects were created in the abdominal wall musculature of Sprague-Dawley rats. Defects were repaired using one of two extracellular matrix (ECM) scaffold test articles or autograft 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 spatio-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-macropahge, CCR7: M1, CD206: M2) was used to quantitatively assess macrophage polarization within the remodeling tissue.

Results: The results of the present study show that ECM scaffold materials elicit distinct tissue remodeling responses depending upon the methods used in their production (i.e. crosslinking). These differences were both qualitative and quantitative. Differences in structure and signs of constructive remodeling were observed. Additionally, the quality and composition of the remodeled tissues were distinct. These qualitative differences in histomorphologic appearance and composition were accompanied by quantitative differences in MMP expression, particularly at late (14-28 days) time points in the remodeling process. Qualitative outcomes were not affected by implantation of a second ECM scaffold concurrently in the contralateral side of the same animal, suggesting that tissue remodeling is determined locally at the site of scaffold implantation.

The host macrophage response was examined to determine if differences in macrophage polarization was related to the observed tissue remodeling outcomes. The results showed differences in both the spatial organization and in the phenotypic profile of the cells at the scaffold surface. Briefly, autologous tissue was subject to a mixed M1 and M2 cell population within the site of tissue implantation and resulted in necrosis of the implant and formation of disorganized collagenous connective tissue within the site of remodeling. Chemically crosslinked scaffold materials were shown to elicit a response similar to a classical foreign body response. A dense layer of CCR7+ cells was observed at the interface with the material, and in particular the superficial interface at later time points in the remodeling process. The non-crosslinked UBM implants were rapidly infiltrated at 7 days post implantation by a population of macrophages expressing mixed M1/M2 phenotypes. These cells were observed to be interacting with the scaffold material as it degraded. Differences in surface marker expression were also associated with differences in expression of genes associated with M1 and M2 macrophage phenotypes throughout the course of the tissue remodeling process. In vitro work showed that M1 and M2 macrophages have distinct effects upon the recruitment of perivascular progenitor cell population, further suggesting distinct roles for M1 and M2 macrophages in tissue remodeling.

Conclusions: The results of this study showed that each test article was associated with a distinct host tissue remodeling response. Each test article was also associated with differences in macrophage polarization, suggesting that different macrophage populations are associated with different mechanisms of tissue remodeling. Further, the 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.