Macrophage Phenotype as a Determinant of Remodeling Outcome with the use of Biologic Scaffolds

Stephen F. Badylak[1], Kathryn A. Kukla[1,3], Li Zhang[1], Neill J. Turner[1], Bryan N. Brown[1,2]
Corresponding Author: Stephen F. Badylak
[1]McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Biomedical Engineering, Cornell University, Ithaca, NY; and [3]Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA

Statement of Purpose: The ultimate determination of clinical success for an implanted biomaterial is the response of the host tissue following implantation. The innate immune mechanisms that participate in and modulate the host response include effector cells such as macrophages. Macrophages have been classified as having either an M1 or an M2 phenotype, depending upon gene expression, effector molecule production, and cell function. To date, the causes and the effects of macrophage polarization towards an M1 or M2 phenotype have been studied largely in the context of the host response to pathogens and in cancer biology. Recently, M1 and M2 macrophages have been shown to play distinct roles in the remodeling process following tissue injury and the host response to biologic scaffold materials (1). The goals of the present study were three-fold: (1) to determine the phenotype of macrophages which respond following the implantation of several biologic scaffold materials composed of extracellular matrix (ECM) and to relate the observed macrophage phenotype to the outcome of the tissue remodeling process associated with each scaffold type; (2) to determine whether local macrophage polarization in response to the implantation of one ECM material affects local macrophage polarization and tissue remodeling of a second ECM material implanted concurrently in the same animal; and (3) to investigate the chemoattractant ability of M1 and M2 macrophages for multipotent progenitor cells *in vitro*.

Methods: Bilateral defects of the abdominal wall musculature were repaired with urinary bladder matrix (UBM), UBM crosslinked with carbodiimide (CDI-UBM), or autograft tissue in a rat model. Materials were explanted at 1, 3, 7, 14, and 28 days. Evaluation included assessment of patterns of cellular infiltration, scaffold degradation, angiogenesis, matrix deposition, gene expression, and surface marker expression. In a separate experiment, RAW 264.7 macrophages were polarized towards either an M1 or an M2 phenotype in vitro and allowed to condition media for up to 24 hours.The ability of the conditioned media to promote the chemotaxis of well characterized perivascular progenitor cells was then investigated using Boyden chamber assay.

Results: Each test article was associated with a distinct remodeling response which did not affect the response of other test articles in the same animal. Remodeling of the UBM was characterized by scaffold degradation, angiogenesis, organized mature matrix deposition, and signs of new muscle tissue formation. Remodeling of the CDI-UBM was characterized by little scaffold degradation, a classic foreign body response and deposition of dense and disorganized connective tissue consistent with encapsulation. Remodeling of the autograft was characterized by necrosis of the muscular component of the tissue and deposition of dense mature connective tissue and adipose tissue consistent with scarring.

Despite differences in the tissue remodeling outcome, a morphologically indistinguishable population of macrophages was observed following implantation of each scaffold material. However, immunolabeling techniques showed that scaffolds which resulted in constructive remodeling (i.e., UBM) were associated with a predominant M2 (regulatory, pro-wound healing) macrophage population, while scaffolds which resulted in the deposition of dense collagenous connective tissue or encapsulation (i.e., autograft and CDI-UBM, respectively) were associated with a predominantly M1 (pro-inflammatory) macrophage profile.

In vitro assays showed that M1 and M2 macrophages had distinct chemotactic paracrine effects upon perivasular progenitor cells. M2 cells promoted the chemotaxis of these progenitor cells, while M1 cells inhibited the chemotaxis of these cells as compared to the effect of unpolarized macrophages.

Conclusions: Each test article was associated with a distinctive tissue remodeling response and a distinct macrophage polarization profile. These results suggest that different macrophage populations (i.e., different phenotypes) are associated with different mechanisms of tissue remodeling. Importantly, it was shown that the macrophage response to individual test articles did not affect (or was not affected by) the response to other test articles in the same animal, suggesting that the macrophage polarization effect on scaffold remodeling is a local and not systemic phenomenon. Finally, M1 and M2 macrophages showed distinct paracrine effects upon a perivascular stem cell population; consistent with the distinct roles for M1 and M2 macrophages in tissue remodeling. These findings provide the opportunity for novel strategies in biomaterials design and highlight a new aspect of the host: biomaterial interface that can dramatically affect downstream biocompatibility.

Acknowledgments: Funding for this study was provided by the NIH (R01 AR054940 and F31 EB007914).

Disclosures: The authors have nothing to disclose.

References:

1. Badylak SF. *Tissue Eng Part A.* 2008. (14: 1835-42)