The Immune Response to Xenogeneic Acellular Biologic Scaffold Materials

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Statement of Purpose: The host innate immune response to acellular biologic scaffolds composed of extracellular matrix (ECM) is not fully understood[1]. The normal wound healing response involves macrophages that express a predominantly M1 phenotype immediately after tissue injury[2]. Transition to the M2 phenotype occurs concurrently with resolution of the inflammatory process, deposition of scar tissue, and the initiation of the remodeling phase of the wound healing process[2]. Certain biologic materials composed of ECM have been shown to modulate this default response and facilitate the formation of site-appropriate functional tissue[3,4]; a phenomenon associated with a notable shift to the M2 phenotype in the macrophage population. Macrophage phenotype has been shown to respond to degradation products of the extracellular matrix, cellular remnants[5], and other cells in the microenvironment[3]. **Hypothesis:** Patterns of macrophage gene expression *in vitro* following exposure to ECM materials correlate to *in vivo* performance in a model of abdominal wall reconstruction in the rat.

Methods: Cell culture: ATCC RAW mouse macrophage cell line was used for the *in vitro* experiments. Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin / streptomycin. Gene profile *in vitro*: Passage 12 cells were exposed for 24 hrs to either urinary bladder matrix (UBM) or chemically cross linked (UBM) in 12-well plates at a density of $5x10^6$ cells per well. Polarization was assessed using gene expression analysis with qRT-PCR of IL12, IL1b, and iNOS for the M1 phenotype profile and IL-10, IL-1ra, and ARG for the M2 phenotype profile.
In vivo remodeling: UBM or cross linked UBM scaffolds were used to repair a 1cm by 1cm partial thickness abdominal wall defect in a rat model. The animals were euthanized at 14 and 35 days post op, and the explanted samples were fixed in formalin for 24hrs and processed for histology. Histomorphologic assessment: Samples were stained with H&E and Masson's Trichrome, and submitted for semi-quantitative histomorphologic scoring that included categories relevant to tissue repair such as neovascularization, cellular infiltration, multinucleate giant cells, capsule formation, and scaffold degradation. In vivo M1/M2 phenotype: Tissue samples were exposed to antibodies to a pan-macrophage marker (CD68), an M1 macrophage phenotype marker (CCR7), and an M2 macrophage phenotype marker (CD206). The number of cells labeled positively for each marker within the three boxes was then counted and expressed as a ratio of the number of M2 (CD206+) cells to M1 (CCR7+) cells.

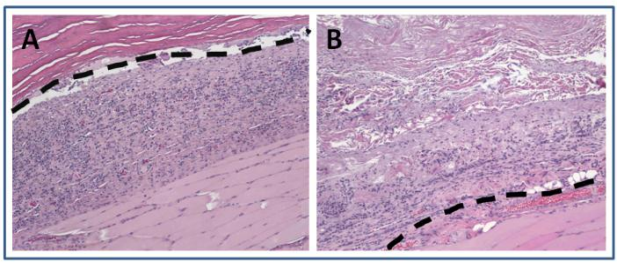

Figure 1. 14-Day explants of cross linked-UBM (A) and UBM (B). Segmented black line denotes interface between biomaterial (top) and native tissue (bottom)

Results: Histomorphology: The *in vivo* host response to cross linked UBM (Figure 1A) was characterized by little to no cellular infiltration or neovascularization within the implant, a dense population of macrophages at the implant interface, and multinucleate giant cells. Deposition of disorganized connective tissues surrounding the implant, and little to no degradation of the material at 14 and 35 days were observed. In contrast, the host response to UBM (Figure 1B) was characterized by an infiltrate of mononuclear cells at early time points and deposition of organized connective tissue, the presence of vasculature throughout the materials, and rapid scaffold degradation.
In vivo macrophage phenotype: Cross linked UBM was characterized by an accumulation of primarily M1 macrophages at the surface of the material. Few macrophages were observed within the materials, and those that were observed were primarily of an M1 phenotype. UBM implants were characterized by lower number of macrophages present at the interface and throughout the materials. A population consisting of predominantly M2 macrophages was observed at the periphery of the site of implantation.

Conclusions: The present study shows that there is a strong correlation between the short term *in vitro* macrophage gene profiles and remodeling outcome in response to implanted ECM scaffold materials.

References:

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