Macrophage polarity in the foreign body reaction is substrate-specific.

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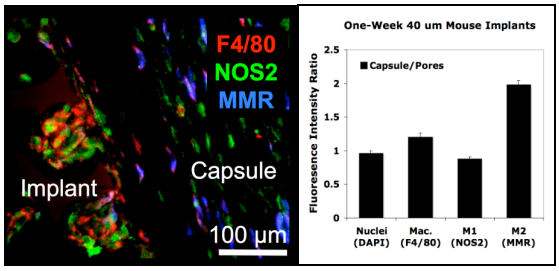
Purpose: Medical devices and artificial organs must address the natural biological response to implanted materials, the foreign body reaction (FBR). Improved response is often measured as an increase in vascularization or a decrease in the collagenous foreign body capsule (FBC). A class of materials that achieves these outcomes is sphere-templated porous scaffolds developed in our lab **[1]**. The macrophage (MØ) is the putative orchestrator cell of the FBR and correlation has been demonstrated between implant healing and MØ polarity, where M1 and M2 are pro-inflammatory and pro-healing polarized MØs [2]. The present work entails identification of MØ populations *in vivo* within the FBR and engineering an *in vitro* model of the FBR in which MØs can be programmed to behave similarly to their *in vivo* counterparts. This model will then be used to study specific MØ effects on the FBR.

Methods: Sphere-templated scaffolds of poly(2-hydroxyethyl methacrylate) (pHEMA) were fabricated using techniques reported previously **[1]**. Discs 3 mm diameter and 1 mm thick were implanted subcutaneously in BAT-gal mice for one week. Implants were zinc-fixed and processed for immunohistochemistry. Antibodies used were MØ marker F4/80 (clone BM8, Accurate Chemical), nitric oxide synthase 2 (NOS2, SantaCruz), and MØ mannose receptor (MMR, RnD Systems). Fluorophore-labeled secondary antibodies (Invitrogen) were used to detect primary antibody binding.

To create models of the FBR, scaffolds were placed in 1 mg/mL collagen type I (from rat tail, BD Biosciences) and polymerized in the presence of bone marrow-derived MØs (BMDM) and media. BMDM were generated from the bone marrow of BAT-gal mice according to standard protocols **[3]**. After one day in culture, samples were fixed in 4% paraformaldehyde in PBS, frozen-sectioned and stained similarly as described above.

Results: MØs in the foreign body reaction are uniquely polarized based on their location within the tissue at the site of an implant. In this study, after one week FBRs developed as expected, comprising a large MØ infiltrate and initiation of FBC formation. Visually, MØs are elongated in the FBC adjacent to the implant while more glubular in shape within implant pores. Most MØs (F4/80+) also appear NOS2+ and MMR+ (Figure 1a). However, quantitative morphometry revealed that the average fluoresence intensity of cells stained MMR+ is about 2X in capsule tissue compared to cells within scaffold pores (Figure 1b). Conversely, F4/80 and NOS2 staining did not vary based on MØ location. Control DAPI nuclear staining also did not vary based on cell location. Therefore, MØs in the FBC are M2-polarized compared to those within the pores of scaffolds.



Figures 1a and 1b. MØ polarity differences in the FBR. 1a) Immunohistochemical analysis for expression of MØ markers. 1b) Comparison of marker expression in implant pores and FBC tissue.

Given that we have shown that MØ phenotype is dependent on the location of the MØ within the FBR, we want to determine whether this difference in phenotype is responsible for the improved healing observed for implanted materials with specific sphere-templated architectures. To achieve this, a model for MØ-substrate interaction in the FBR is needed. The two major MØ-substrate interactions in the FBR are MØ-implant and MØ-collagen. By combining these components into a single system, we can directly compare MØ phenotypes. In Figure 2, we demonstrate the feasibility of engineering such a system. A collagen gel encapsulates a sphere-templated scaffold and F4/80-positve BMDMs are present. MØs appear to adhere to both collagen and scaffold surfaces. These MØs, however, do not appear to endogenously express NOS2 or MMR.

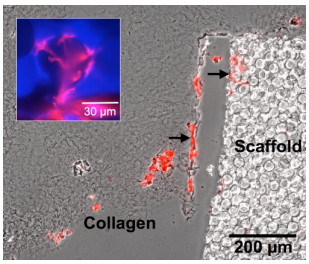


Figure 2. Model of MØ growth in the FBR. MØ (F4/80+, red, arrows) attached to collagen matrix and porous scaffold (inset).

Conclusions: MØs in the FBR express multiple markers of polarization, and appear to have greater pro-healing M2 expression when located in the FBC. To determine the substrate effects on MØ polarization, a model has been created where MØs may be simultaneously cultured on collagen or porous scaffolds. We have shown that the BMDM seeded in these cultures do not express detectable levels of NOS2 or MMR, thus providing a naïve population of cells which we can stimulate as desired. This model system will be used investigate how MØ location, density, and polarization can affect vascularization and FBC formation.

References:

[1] Madden LR. PNAS 2010;107:15211-15216.
[2] Brown BN. Biomaterials 2009;30:1482-1491.
[3] Osathanon T. Biomaterials 2008;29:4091-4099.