Modifying Macrophage Activation and the Foreign Body Response to PEG-based Hydrogels

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Statement of Purpose: Poly(ethylene glycol) (PEG) hydrogels are attractive cell carriers for many tissue engineering applications, but their *in vivo* potential has not been fully characterized. We recently reported that when PEG hydrogels are implanted subcutaneously, an atypical inflammatory reaction ensues over a 4 week period, but surprisingly the incorporation of a cell adhesion moiety, RGD, attenuated this response, instead leading to a classic foreign body reaction (FBR) with capsule formation[1]. These findings demonstrate our ability to modulate the host reaction through simple changes in the gel chemistry, however the role of the hydrogel in modulating the host response remains unclear. Therefore, the first goal of this study was to better characterize the host response to PEG-based hydrogels by examining the recruitment of inflammatory cells and the evolution of the FBR *in vivo* over 4 weeks. The *in vivo* responses were then compared to *in vitro* models in which primary macrophages (MΦ) were exposed to lipopoly-saccharide (LPS) in order to establish an activated MΦ phenotype. The second goal of this study was to design new strategies to modulate the FBR to PEG-based hydrogels and we present our initial efforts in designing cytocompatible coatings targeted to interact with the host response.

Methods: Hydrogel Synthesis –Diacrylated PEG_{3000} (PEG-dA) was synthesized and hydrogels were formed by photopolymerization of the PEG-dA in PBS with a photoinitiator (Irgacure 2959, Ciba Specialty Chem.). PEG-only gels were formed from a 20% (w/w) solution. PEG-RGD gels were formed from a 20% (w/w) solution containing 5mM monoacrylated-PEG_{3400}-YRGDS.

MΦ Isolation – Bone marrow derived MΦs isolated from 6 week-old c57bl/6 males[2] were seeded onto PEG-only, PEG-RGD and silicone (SIL), as the control, as previously described[1]. LPS was added to the culture media 24 hours after seeding. Samples were lysed and processed for RT-PCR at specified time points.

Implantation Studies – Implantation studies were performed as described elsewhere[1]. Disks and surrounding tissue were explanted at specified time points, and processed for histological analysis (Hematoxylin and Eosin (H&E), Massons Trichome or MΦ specific antibodies (Mac3, F4/80)).

RT-PCR – Custom primers were created to monitor gene expression of TNF-α, IL1-β, IL-10, IL-12β, Arginase Type I, inducible Nitric Oxide Synthase, F4/80, VEGF, PDGF and L32 (housekeeping gene).

Results: After 7 days post-implantation, the PEG-only hydrogel-tissue interface (Fig1a) is characterized by a thick layer of inflammatory cells (primarily MΦ, data not shown) surrounded by two layers consisting of a transient and minimally cellularized matrix followed by a thicker and highly cellularized tissue. In contrast, PEG-RGD (Fig1b) implants are surrounded by a structurally similar, but significantly thinner capsule. SIL disks are surrounded only by a loose layer of cells. *In vitro,* the MΦs adhered to PEG-RGD show decreased expression of IL-1β (Fig1c), TNF-α and IL-12β within 24 hours of exposure to LPS compared to PEG-only samples. MΦ seeded on PEG-RGD gels show an increase in the ratio of IL-10:IL-12β (Fig1d) expression within 24 hours compared those on PEG-only disks. Finally, a highly uniform hydrogel coating of 21μm thickness was formed at the surface of a PEG gel using a redox initiating system (Fig1e).

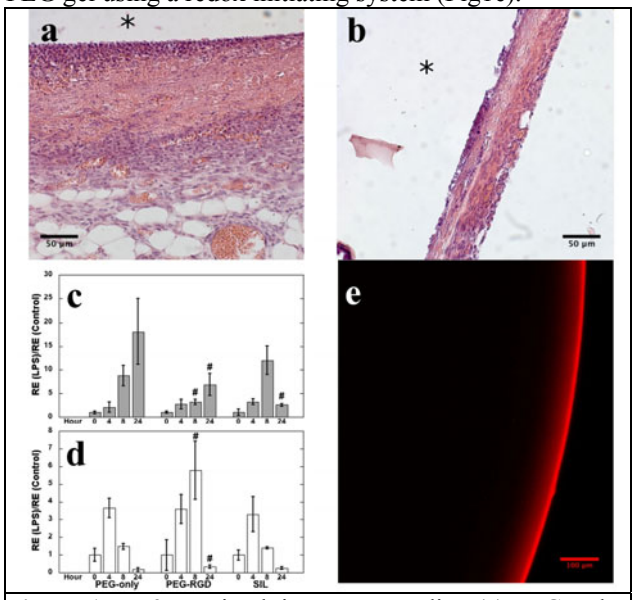

Figure 1 – H&E stained tissue surrounding (a) PEG-only and (b) PEG-RGD. Gene expression of (c) IL-1β and (d) IL-10:IL12β in MΦ exposed to LPS seeded onto different materials (normalized to control). (e) A coating created on a PEG-only disk after 90s in coating solution. Scale: a,b - 50μm e,f 100μm.*-hydrogel. #-sig. comp. to PEG $p<0.05$.

Conclusions: The host response to PEG-only hydrogels at early time points differs from the response to PEG-RGD and SIL implants. The *in vivo* data demonstrate that PEG-only gels elicit an inflammatory response upon implantation and that the presence of RGD diminishes this reaction in concordance with our long-term *in vivo* studies[1]. The *in vitro* model system confirms that PEG promotes a classically activated macrophage phenotype while RGD appears to promote a regulatory phenotype as characterized by increases in the expression of the antiinflammatory cytokine IL-10 relative to proinflammatory IL-12β even in the presence of LPS[3]. Studies are underway characterizing the MΦ phenotype *in vivo* and to develop the novel coating system to incorporate bioactive molecules such as RGD, Osteopontin or IL-4 known to modify MΦ behavior in an effort to mimize the FBR *in vivo*.

References: (1)Lynn AD. J Biomed Mater Res A 2009. (2)Jay SM. Am J of Path 2007 171(2):632-640. (3)Mosser, D.M. Nat Rev Imm, 2008. 8(12): p. 958-969.