Incorporation of RGD Attenuates The Foreign Body Reaction to PEG Hydrogels

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Statement of Purpose: Photopolymerized poly(ethylene glycol) (PEG) hydrogels prepared from PEG di(meth)-acrylate precursors are attractive cell carriers for *in vivo* cell delivery. The cell adhesion oligopeptide, RGD (arginine, glycine, aspartic acid) has been introduced into PEG hydrogels to facilitate cell adhesion and maintain cell viability within hydrogels. For PEG-based hydrogels to be utilized *in vivo*, the foreign body reaction must be well characterized. Recently our group demonstrated that macrophages, the cells thought to orchestrate the foreign body reaction (FBR), attach to PEG hydrogels in the presence of serum proteins and are activated as evidenced by increased expression of pro-inflammatory molecules. The incorporation of RGD reduced macrophage activation *in vitro* and led to an overall attenuated foreign body reaction at 4 weeks [1]. The mechanism by which RGD modulates the FBR to PEG-based hydrogel remains unknown. 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*. The second goal of this study was to begin to elucidate the role of RGD in mediating the FBR to PEG-based hydrogels utilizing a scrambled peptide, RDG.

Methods: PEG-diacrylate (PEGDA) was synthesized by reacting acryloyl chloride with PEG (3000 Da) in the presence of triethylamine in dry toluene. PEGDA was filtered over alumina, purified in cold diethyl ether, dried, and stored at 4°C. Peptides, YRGDS or YRDGS (GenScript), were conjugated to monoacrylated-PEG_{3400} - Succinimidyl Carboxymethyl (Laysan Bio) in a 1.1:1 molar ratio in 50mM sodium bicarbonate buffer (pH 8.4) for 2 hours. The Acr-PEG-peptides were purified by dialysis and lyophilized. Hydrogels were formed from 20wt% PEGDA with or without 2.5mM Acr-PEG-RGD via photopolymerization, rinsed multiple times, sterilized, and determined to be endotoxin-free. Hydrogels were implanted subcutaneously into 6 week old, C57BL/6 (Charles River Laboratories) male mice for 28 days following IACUC approved protocol. Hydrogels were explanted and examined through immunohistochemistry and gene expression. Histological sections were stained against mac3 following standard protocols and visualized by 3,3'-diaminobenzidine. Quantitative RT-PCR was used to evaluate pro-inflammatory gene expression of the cells at the surface of the hydrogel implants relative to the housekeeping gene, L32. For *in vitro* cell adhesion studies, a macrophage cell line, RAW 264.7, was used where, macrophages were seeded at $1.02x10^6$ cells/cm^2, allowed to adhere for 4 hours, and then fixed in paraformaldehyde and stained for nuclei and actin with DAPI and rhodamine phalloidin, respectively, following standard protocols.

Results: To further characterize the FBR to PEG-based hydrogels, PEG-only and PEG-RGD hydrogels were implanted subcutaneously into C57BL/6 mice. The presence of macrophages was observed as early as day 2 post-implantation and by 21 days, a large presence of macrophages can be observed adjacent to the PEG-only hydrogels, but the presence of RGD led to a reduced presence of macrophages (Fig. 1). The inflammatory state of the cells adjacent to the implant was also evaluated by gene expression for interleukin-1β (IL-1β), a pro-inflammatory molecule. In response to PEG-only hydrogels, IL-1β relative expression increased 18-fold from day 2 to day 14 to 11.8±2.2. In response to PEG-RGD hydrogels, IL-1β expression increased by 10-fold from day 2 to day 14 to 3.6±1.9, significantly lower than the PEG-only.

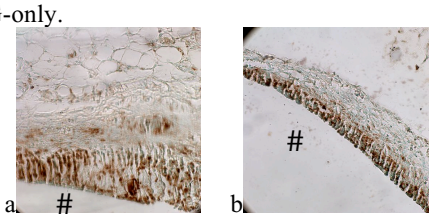

Figure 1. a)PEGDA b)PEG-RGD hydrogels explanted after 21 days. Tissue sections were stained brown with an antibody for mac3. # Denotes the location of the implant.

To begin to elucidate the mechanisms by which RGD modulates the FBR, we investigated whether macrophage adhesion to PEG-RGD was partly mediated by the presence of adsorbed proteins. Using an *in vitro* culture platform, we tested three surfaces, PEG-only, PEG containing RGD or PEG containing RDG, in the absence of serum or after pre-soaking for 2 hrs. Interestingly, macrophages attached to all surfaces with similar densities after 4 hours, although cell spreading was not yet observed. These findings indicate that macrophages are able to interact with hydrogel surfaces in the absence of cell adhesion ligands. Studies are underway to examine whether cell attachment occurs long term in the absence of serum and to elucidate the integrins involved in mediating macrophage activation.

Table 1. Macrophage density

Material	Density (cells/ cm^2)*10^5	
	without serum	**with serum**
PEG-only	19.52±6.46	19.80±5.54
PEG-RGD	27.81±8.73	22.30±6.69
PEG-RDG	21.89±7.09	17.71±4.74

Conclusions: PEG-only hydrogels elicited a strong inflammatory response, while the incorporation of RGD tethers partly attenuated this response. Interestingly, macrophages are capable of attaching to hydrogel surfaces in the absence of serum at least for short culture times. Ongoing work will explore macrophage activation on the three materials via gene expression of proinflammatory cytokines and attempt to tease out which integrins are playing a role in macrophage activation.

References: [1] Lynn *et al*. J. Biomed. Mat. Res. 2010;93(3):941-53.