Monocyte adhesion and secretion in response to PEG hydrogels grafted with RGD and PHSRN separated by interpositional spacers of various lengths

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Statement of Purpose: Polyethylene glycol (PEG) is often cited as a "stealth" polymer, capable of resisting both protein adsorption and cell adhesion.[1-2] However, increases in monocyte and neutrophil adhesion to PEG have been observed under certain circumstances.[3] PHSRN is often cited as a synergistic molecule to promote cell adhesion alongside RGD; however, no previous research has consistently found a synergistic increase in monocyte adhesion and secretion patterns following interaction with these peptides.[4] Research with other cell types has indicated that the spacing between RGD and PHSRN plays an important role.[5] To investigate both the potential for pro-inflammatory responses to PEG and the effect of varied spacing between RGD and PHSRN, primary human monocytes were seeded on tissue culture polystyrene (TCPS), a PEG-only hydrogel surface, and PEG conjugated with the following peptides: RGD, PHSRN, $PHSRNG_6RGD$, and $PHSRNG_{13}RGD$. Monocyte adhesion and secretion of the pro-inflammatory molecules interleukin 1 beta (IL-1β), tumor necrosis factor – alpha (TNF-α), and granulocyte macrophage-colony stimulating factor (GM-CSF) were measured in addition to three molecules involved in tissue repair: matrixmetalloprotease-2 (MMP-2), matrixmetalloprotease-9 (MMP-9), and fibronectin (FN).

Methods: Acrylated heterodifunctional PEG linkers were synthesized from a well-established method using acryloyl-PEG-N-hydroxysuccinimide and peptide dissolved in sodium bicarbonate buffer.[5-6] Materials were characterized via HPLC. 10% hydrogel discs were formed in a Teflon™ mold under UV light and the presence of grafted peptide was characterized via a previously reported RGD ELISA assay.[5] Monocytes were isolated from human donors and statically seeded on the hydrogel films. Adherent monocyte density was determined via light microscope and molecular concentrations were measured using ELISA kits. Results were analyzed via two-way ANOVA.

Results/Discussion: Monocyte cell density was similar for both the PEG-only hydrogel and TCPS; however, secretion of the pro-inflammatory molecules IL-1β, TNF-α, and GM-CSF increased dramatically following interaction with PEG compared to TCPS (Figure 1). MMP-9 and FN concentrations were consistently higher than the range of the ELISA assays for all surfaces. The cell density was higher on PEG surfaces grafted with $PHSRNG_{13}RGD$ compared to $PHSRNG_6RGD$, but neither sequence increased cell density compared to RGD alone. There were several cases of variation in molecular secretion levels amongst different peptides, although this variation was minimal in comparison to the surface effects between TCPS and PEG.

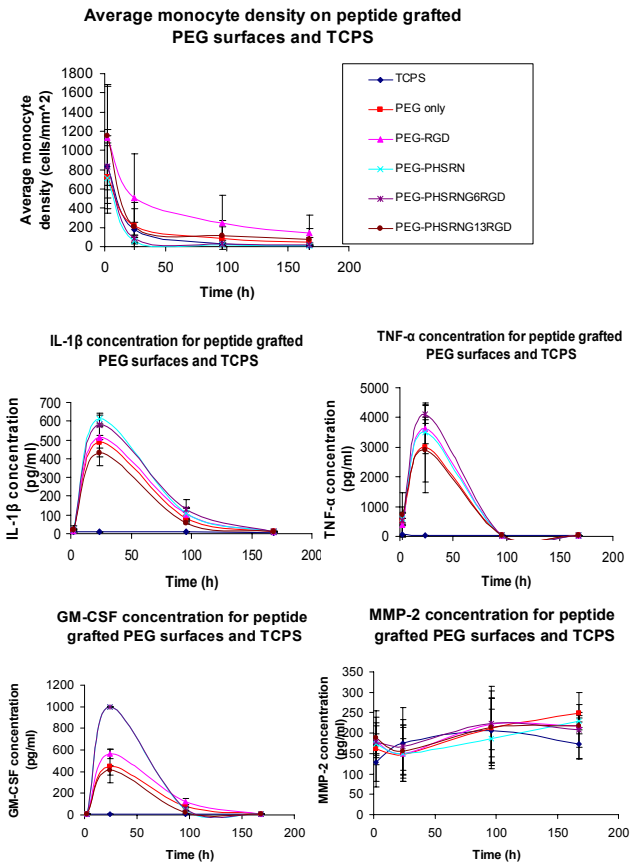

Figure 1: Monocyte adhesion and protein concentrations for peptide-grafted PEG surfaces and TCPS

Conclusions: PEG did not inhibit monocyte adhesion as compared to TCPS, and monocyte adhesion to PEG hydrogels grafted with $PHSRNG_{13}RGD$ was comparable to RGD alone and greater than $PHSRNG_6RGD$. The similarities in cell adhesion between $PHSRNG_{13}RGD$ and RGD as compared to $PHSRNG_6RGD$ are likely due to increased accessibility of RGD with the longer spacer. The PEG surfaces induced a significant and sustained pro-inflammatory response as compared to TCPS, indicating a potential for macrophage inflammatory reactions to certain forms of PEG.

References:

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