Fibroblasts Regulate Monocyte Response to ECM-derived Matrix: The Effects on Monocyte Adhesion and the Production of Inflammatory, Matrix Remodeling and Growth Factor Proteins

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Statement of Purpose: Characterization of inflammatory, remodeling and critical wound healing factors provides insight into the dynamic host response to biomaterials. A monocyte/fibroblast co-culture in the presence of a semi-interpenetrating network (sIPN) composed of PEGylated RGD-modified gelatin and PEGdiacrylate (PEGdA) was constructed as shown in Figure 1. Fibroblast perturbation upon monocyte interactions with the extracellular matrix (ECM)-derived scaffold was evaluated. We hypothesized that fibroblast decrease monocyte adhesion by either affecting monocyte viability or by downregulating key focal adhesion proteins such as vinculin in monocytes. Preliminary data revealed a drastic increase in granulocyte-macrophage colony-stimulating factor (GM-CSF) and matrix metalloproteinase-2 (MMP-2) only in co-culture and not in monoculture and thus we investigated the factors involved in inducing the drastic upregulation of these proteins in co-culture. We hypothesized that GM-CSF induces an upregulation of GM-CSF and MMP-2 in a monocyte and fibroblast co-culture in the presence of the ECM-derived sIPN.

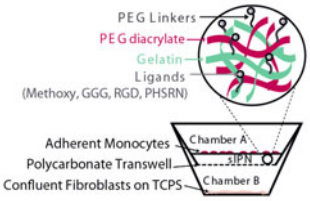

Figure 1. Co-culture Schematic

Methods: PEGylated-ligand modified gelatin was synthesized by an established procedure[1]. HPLC, MS, ^{1}H-NMR, GPC and trinitrobenzenesulfonic acid method were used for characterization. The ligand-PEG grafted on the gelatin backbone were GGG-, RGD-, and PHSRN-PEG. sIPNs were made by photo-crosslinking the ligand-PEG grafted gelatin with PEGdA. Density of peptide-PEG grafted was analyzed on RGD-PEG grafted sIPNs via ELISA using RGD directed antibody[2]. Human blood-derived macrophages were seeded on the sIPNs cast in the polycarbonate transwell insert and then placed above a confluent layer of fibroblasts. Over time, live adhered monocytes were quantified, and nonadhered monocyte viability was examined. Adhered monocytes were also lysed for vinculin characterization with immunoblotting. Supernatant was collected for ELISA or protein multiplex immunoassay over time. Interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), MMP-2, matrix metalloproteinase-9 (MMP-9), vascular endothelial growth factor (VEGF) and GM-CSF were analyzed. Monocyte and fibroblast monocultures were challenged with rhGM-CSF to identify GM-CSF as the potential GM-CSF and MMP-2 inducing factor in the presence of the ECM scaffold.

Results/Discussion:

Cell Adhesion: Adherent cell density on RGD-grafted sIPNs was significantly higher compared to sIPNs grafted with other ligands (within time point) from 2 to 96h. However, enhanced monocyte adhesion onto RGD-PEG grafted sIPN was hindered by the presence of fibroblasts, shown in Table 1 below.

Live Cells Adhered (cells/mm^2)

sIPN Surface	Time (h) 2	24	96
Monocyte Monoculture			
GGG-PEG	158 ± 38	142 ± 18	53 ± 21
RGD-PEG	609 ± 90	296 ± 43	174 ± 27
PHSRN-PEG	121 ± 35	126 ± 12	76 ± 22
Monocyte/Fibroblast Co-Culture			
GGG-PEG	95 ± 23	114 ± 11	30 ± 13
RGD-PEG	273 ± 24	340 ± 48	99 ± 18
PHSRN-PEG	116 ± 23	141 ± 35	24 ± 9

Table 1. Adherent monocyte density over time on ligand-PEG grafted sIPN

Protein expression: The ECM-derived sIPNs led to increased monocyte expression of inflammatory proteins IL-1β and TNF-α initially but levels decreased over time. The sIPN also led to increased monocyte MMP-9, VEGF and GM-CSF expression over time. Fibroblasts drastically increased monocyte GM-CSF except on RGD and PHSRN grafted sIPNs at 96 h (Figure 2). Monocytes also led to drastically increased fibroblast GM-CSF and MMP-2 at later time points.

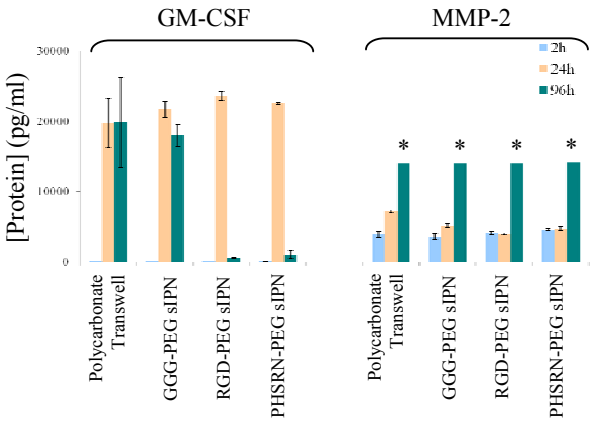

Figure 2. GM-CSF and MMP-2 concentrations over time in co-culture. * Over Detection (>18000 pg/ml)

Characterization of nonadherent monocyte viability and monocyte vinculin regulation to investigate monocytes' active versus passive detachment from the sIPN are currently in progress. rhGM-CSF induction of monocyte and fibroblast GM-CSF and MMP-2 is also in progress.

Conclusions: RGD-PEG grafted sIPNs enhanced monocyte adhesion but the presence of fibroblasts inhibited this effect. In both mono and co-culture, MMP-9, VEGF and GM-CSF were increased over time due to the presence of the ECM-derived sIPN. Drastically increased GM-CSF and MMP-2 were observed under co-culture but not monocultures.

References: 1. Phillips J and Kao WJ. Tissue Eng. 2005;11:964-973. 2. Benoit DS and Anseth KS. 2005;26:5209-5220.

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