The effect RGD ligand density on macrophage inflammatory and matrix remodeling response

Amy Chung[1], Qiang Gao[1] and W. John Kao, Ph.D[1,2]
University of Wisconsin-Madison, [1]School of Pharmacy and [2]Department of Biomedical Engineering

Statement of Purpose: Understanding how engineered extracellular environments influence cell adhesion and subsequent protein and genetic regulation remains critical to the study of biomaterials and the dynamic host response. Varying densities of PEGylated RGD grafted on the gelatin backbone were incorporated with PEGdiacrylate (PEGdA) to form interpenetrating networks (IPNs). Human blood-derived macrophage adhesion, matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) and interleukin-1β (IL-1β) production, and mRNA expression of these cytokines in the presence of this established IPN system were investigated[2]. We hypothesized that varying densities of the RGD-PEG grafted gelatin in the IPN modulate macrophage adhesion and subsequent MMP-2/-9 and IL-1β protein and mRNA expression.

Methods: PEGylated-ligand modified gelatin was synthesized by an established procedure[1]. HPLC, MS, NMR, GPC and trinitrobenzenesulfonic acid method were used for characterization. The ligands grafted on IPNs were methoxy, GGG, RGD, or PHSRN. IPNs were made by photo-crosslinking the modified gelatin with PEGdA. IPNs of three varying ratios of modified gelatin to PEGdA (in weight percent) were made: 3:7, 4:6 and 5:5 (37IPN, 46IPN and 55IPN, respectively) representing increasing ligand density. RGD density of IPN surfaces was determined using ELISA[3]. TCPS was preadsorbed with no ligand (PBS), GGG, RGD, PHSRN, or fibronectin (FN) for comparison. Human blood-derived macrophages were seeded onto IPNs and TCPS. At 2, 24, 96, and 168hr, samples were stained and adherent macrophages were quantified. MMP-2/-9 and IL-1β concentrations in the supernatant of each sample were analyzed with ELISA and mRNA levels from the cells were characterized by RT-PCR.

Results/Discussion:

Cell Adhesion: Adherent macrophage density on all IPNs and TCPS decreased with time. Though adherent cell density did not significantly differ ($p<0.05$) across ligands, cell density on RGD-grafted IPNs was significantly higher for compared to IPNs grafted with other ligands (within IPN formulation and time point) up to 96hr. For all formulations, adherent cell density on MPEG-grafted IPN was nominal, but RGD-grafted IPNs showed adherent density closest to that of TCPS surfaces. RGD grafted 37IPNs exhibited lower cell density compared to RGD grafted 46IPN and 55IPN at 2 and 24hr. 46 and 55IPNs did not yield significant differences in adherent cell density within ligand and time point (Figure 1).

Cytokine and mRNA expression: Cytokine expression was not ligand but substrate dependent for all IPNs (Figure 2). 37IPNs yielded significantly different MMP-2 and IL-1β expression patterns compared to 46 and 55IPN samples. High concentrations of MMP-2 at 2hr and of IL-1β at 24hr observed for 46 and 55IPNs were not observed for 37IPN samples. MMP-9 protein expression, however, generally did not significantly differ across IPN formulations. The data suggests that macrophages may be sensitive to the gelatin concentrations in IPNs with respect to MMP-2/-9 and IL-1β protein secretion into the microenvironment. RT-PCR results showed no detectable levels of MMP-2 for any sample at any time point. MMP-9 mRNA levels were nominal for 46IPN samples except at 96hr, but not detectable at other time points, or for 37 and 55IPNs at any time point. IL-1β mRNA levels decreased from 2 to 96hr, supporting the decreasing IL-1β protein levels from 24 to 168hr detected through ELISA.

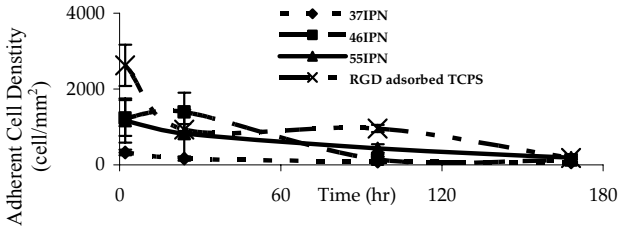

Figure 1. Adherent macrophage density over time on IPN or TCPS grafted with or preadsorbed with RGD

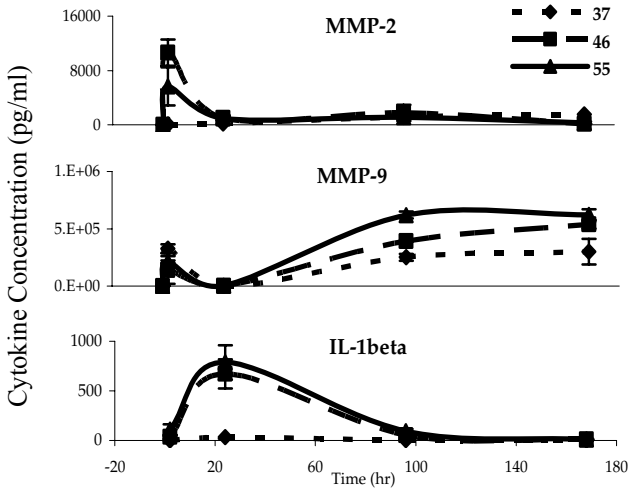

Figure 2. Cytokine levels from RGD-grafted IPN samples as representative for all ligands for all three IPN formulations across time

Conclusions: Decreased RGD density grafted onto IPNs led to ligand identity dependent decrease in adherent cell density. Cytokine expression was ligand independent and MMP-2 and IL-1β expression changed with varied modified gelatin content in the IPN.

References: 1. Phillips J and Kao WJ. Tissue Eng. 2005;11:964-973. 2. Chung AS, Gao Q, Kao WJ. Biomaterials. 2007;28:285-298 3. Benoit DS and Anseth KS. 2005;26:5209-5220.

Acknowledgements: NIH Grant R01HL077825 and R01 EB006613, NSF Predoctoral Fellowship 2006043753