Primary macrophage gelatinase A and B expression on interpenetrating networks containing gelatin modified with PEGylated RGD

Amy Gustafson[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: Characterization of cytokines that impact inflammation and rearrangement of the extracellular matrix provides insights in understanding the dynamic host response to biomaterials. Interpenetrating networks (IPNs), composed of PEGylated RGD-modified gelatin and polyethylene glycol diacrylate, were synthesized and human blood-derived macrophage adhesion, gelatinase (A and B) and interleukin-1β (IL-1β) production, and mRNA expression of these cytokines in the presence of the IPNs were investigated. We hypothesized that immobilized RGD modulates macrophage adhesion and gelatinase A and B release and that IPNs with greater gelatin concentration will induce more gelatinase release.

Methods: PEGylated-peptide modified gelatin was synthesized by converting PEG-diol to bis-ethyl acetate-PEG, cleaving the ethyl groups to make bis-COOH-PEG, activating the COOH groups with N-hydroxysuccinimide, and conjugating peptide to the bis-NSu-acetate-PEG to form NSu-PEG-peptide[1]. PEG derivation steps were characterized with HPLC, MS, and NMR. The NSu-PEG-peptide was conjugated onto the gelatin backbone and analyzed with GPC, NMR and trinitrobenzenesulfonic acid method. IPNs were made by photo-crosslinking the modified gelatin with PEG diacrylate (PEGdA) in either 4:6 or 5:5 (gelatin:PEGdA) ratio. Gelatin hydrogels crosslinked with 0.1% glutaraldehyde were used for comparison. Human blood-derived macrophages were seeded onto TCPS surfaces preadsorbed with no ligands (PBS), GGG, RGD, PHSRN, and fibronectin (FN), and onto IPNs with the following immobilized ligands: methyl capped PEG (MPEG), GGG, RGD, and PHSRN. At 2, 24, 96, and 168hr, samples were stained and adherent macrophages were quantified. Gelatinase A/B and IL-1β concentration in the supernatant of each sample was analyzed with ELISA and mRNA levels were characterized by RT-PCR.

Results / Discussion:

Cell Adhesion: Adherent monocyte density on all substrates decreased with time. Cell density was significantly higher in the presence of RGD-IPN than that of all other IPNs and was similar to that of TCPS control with no preadsorbed ligands. Cell density in the presence of MPEG-IPN was nominal and cell density increased with GGG-IPN and even further with PHSRN-IPN, but all cell densities were lower than observed with RGD-IPN. Macrophages on gelatin hydrogels exhibited similar adhesion density to macrophages in the presence of GGG-IPN.

Cytokine secretion (see Table below): Monocytes in the presence of all TCPS substrates and of gelatin hydrogel produced gelatinase A at a non-detectable level (below 140pg/ml). High concentrations of gelatinase A was observed at 2hr in the presence of all IPNs, followed by a ten-fold increase by 24hr, then a slight increase by 168hr. A similar fluctuation in concentrations was observed with gelatinase B at the same culture periods, but a more drastic increase in gelatinase B concentration was observed between 24 and 96hr. For TCPS and gelatin hydrogel, macrophages steadily increased gelatinase B production until 96hr then decreased production thereafter, while IL-1β production for TCPS remained constant from 2 to 168hr. The data suggests that MPEG-IPNs induced the greatest cytokine production per cell, followed by GGG, PHSRN, and RGD-IPN. Simultaneous decrease in IL-1β with increase in gelatinase A concentrations at coinciding culture periods suggest that the gelatinases and IL-1β may be involved in regulatory signaling. Ongoing RT-PCR results complemented the cytokine concentrations detected with ELISA.

Conclusions: No significant difference in cell adherent density was observed with varying gelatin:PEGdA ratio within the same ligands. Higher PEGdA concentration did not induce a higher level of secretion of both gelatinases. Gelatin hydrogels did not induce cytokine secretion higher than that of IPNs, suggesting that ligands, rather than the presence of gelatin, may have greater impact on macrophage secretion of inflammatory agents.

References: 1. Phillips, J and W.J Kao. Macrophage adhesion on gelatin –based interpenetrating networks grafted with PEGylated RGD. Tissue Engineering (2005), 11(5/6), 964-973.

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Macrophage cytokine concentration (x100 pg/ml) at various culture periods

	Gelatinase A			Gelatinase B			IL-1β		
	2	24	96	2	24	96	2	24	96
4:6 MPEG	139	13	14	>60	19	>60	0.2	5	8
4:6 GGG	123	12	22	>60	11	>60	0.6	8	7
4:6 RGD	106	10	18	>60	20	>60	0.4	7	5
4:6 PHSRN	110	7	17	>60	24	>60	0.4	5	8
5:5 MPEG	104	13	29	>60	21	>60	1.3	9	6
5:5 GGG	79	12	23	>60	21	>60	0.6	8	9
5:5 RGD	57	10	11	>60	27	>60	1.0	8	9
5:5 PHSRN	46	10	12	>60	16	>60	2.0	5	7
Gelatin Hydrogel	<14	<14	<14	14	>60	>60	0.2	5	1

n=3, SEM omitted for clarity <14 indicates below and >60 indicates above detectable concentration