U937 macrophage adhesion and TNF- α and IL-1 β mRNA expression on gelatin-based interpenetrating network (IPN) grafted with PEGylated fibronectin (FN)-derived peptides

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Statement of Purpose: IPN systems were designed for a variety of tissue-engineering applications. We have synthesized gelatin-based interpenetrating networks (IPN) grafted with PEGylated FN-derived peptides for drug delivery matrices and tissue scaffolds. The characterization of interaction of IPN and monocyte/macrophage is of importance as monocytes/macrophages play a crucial role in inflammation, immunoreaction and tissue wounding healing. In this study, we investigated the adhesion and TNF- α and IL-1 β mRNA expression of U937 cell, a human monocyte-like cell line, on gelatin-based IPN. We hypothesized that gelatin-based IPN and immobilized peptide influenced the cell behavior and gene regulation of monocytes/macrophages.

Methods: Synthesis of gelatin-based IPN involved PEG derivations, peptide conjugation to PEG-bis-N-hydroxysuccinimide (NSu)-COOH and gelatin modification with NSu-PEG-peptide. HPLC, NMR, GPC, and TNBS methods were used for characterization. Gelatin-based IPN was conjugated with following peptides: RGD, PHSRNG_6RGD, PHSRN, G_3 or no peptide. In the presence of 50 ng/ml PMA, U937 cells were seeded onto IPNs, tissue culture polystyrene (TCPS) as control. TCPS were also preadsorbed with RGD, PHSRNG_6RGD, PHSRN, G_3, FN or albumin, PBS as control. At 4 and 24hr, adherent U937 cells were quantified, and lysed for evaluation TNF- α and IL-1 β mRNA expression by RT-PCR. Results were compared to that of primary monocytes.

Results / Discussion: At 4hr cell density on RGD or PHSRNG_6RGD conjugated IPNs was significantly higher than that on IPN grafted with PHSRN or G_3. By 24hr, cell density was significantly decreased in all IPNs, but, cell numbers were still greater on RGD- or PHSRNG_6RGD-IPNs than G_3-IPN or IPN without peptide. On TCPS treated with ligands, cell densities were comparable between different samples at each time point. At 4hr TNF- α mRNA expression in adherent cells on IPNs was generally downregulated compared to that from cells on TCPS. At 24hr TNF- α mRNA levels for all IPNs were similar to the sample on TCPS (Figure 1). For cells on TCPS treated with ligands TNF- α mRNA levels were also similar at both 4 and 24hr except for those on preadsorbed FN, which had a 1.6-fold increase compared to that of the control. By 4hr IL-1 β mRNA expression of cells in all IPNs was strongly inhibited in comparison with cells on TCPS surface. At 24hr the IL-1 β mRNA in the samples on all IPNs were still at a low level (Figure 2). IL-1 β mRNA levels in samples on TCPS treated with ligands were slightly upregulated (1.8- to 2.3-fold) at 4hr,

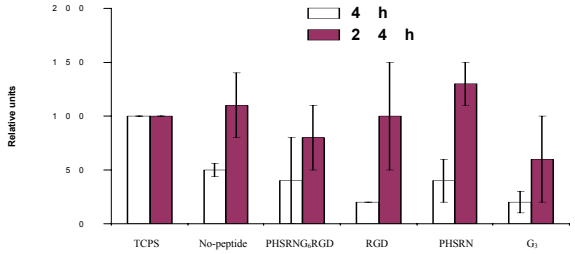

Figure 1. TNF- α mRNA levels in U937 cells on IPNs (mean ± SD)

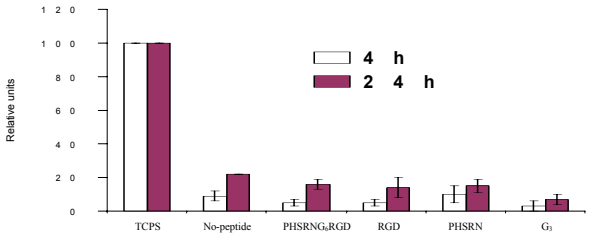

Figure 2. IL-1 β mRNA levels in U937 cells on IPNs (mean ± SD)

with the exception of RGD preadsorbed surface. At 24hr the preadsorbed ligands did not show to influence the expression of IL-1 β mRNA in U937 cells. Results from on going primary blood-derived monocytes study also suggested that gelatin-based IPN may influence cell adhesion and gene expression. Our studies will provide insight on the use of transformed cells on assessing material-host interaction.

Conclusions: The highest adherent cell numbers on RGD- and PHSRNG_6RGD-IPNs indicated that RGD was involved in promoting of cell adhesion in the IPN system. The decrease in TNF- α and IL-1 β mRNA in cells on IPNs, regardless of peptides grafted on IPNs, suggested that specificity of substrates on which the cells adhered may affect the cell gene expression.

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