Application of β1 Integrin Deficient Cells in Evaluating Cytokine-Mediated Response to Networks Conjugated with Fibronectin-Derived Peptides

Heather Waldeck[1] & Weiyuan John Kao, Ph.D.[1,2]
[1] Department of Biomedical Engineering, College of Engineering, University of Wisconsin-Madison, WI USA
[2] School of Pharmacy, University of Wisconsin-Madison, WI USA

Statement of Purpose: Integrins play a central role in regulating the cellular response to biomaterials including mediating cell adhesion and outside-in signaling which, in turn, can stimulate the release of cytokines which control various aspects of inflammation. We have developed a binary photopolymerizable interpenetrating polymer network (IPN) system composed of poly(ethylene glycol)(PEG)-ylated immobilized factors[1] such as RGD. The RGD motif, as presented on fibronectin, binds primarily to the $\alpha_5\beta_1$ integrin in conjunction with the synergy sequence PHSRN. In this study, we employed the murine fibroblastic cell line GD25 which lacks the β1 integrin family. Previous work using this cell line does not address the cellular response to biomaterials. This study evaluates the role of β1 integrins in the inflammatory cytokine-mediated response to differentially conjugated IPNs.

Materials and Methods:

IPN construction: IPNs were composed of PEGdA and unmodified, methoxy-modified, or peptide-modified gelatin at a 6:4 ratio. Peptides used include GGG, PHSRN, and RGD. The method for the modification of gelatin with PEGylated-peptide follows previously established and characterized procedures[1]. Characterization of intermediate and final products was done using HPLC, GPC, ^{1}H-NMR, and a method based on trinitrobenzenesulfonic acid and spectrophotometry

Cell types: GD25 cells contain a null mutation in the β1 integrin gene which prevents expression of the β1 integrin family. GD25β1A cells were derived through transfection of GD25 cells with wild-type β1A integrin subunit cDNA.

Culture condition optimization: GD25 and GD25β1A cells were seeded at various concentrations between 10^4 and 10^6 cells/ml in both serum-free and DMEM supplemented with 10% FBS. GD25 and GD25β1A cells were then seeded at 10^5 cells/ml with 0.1-1% FBS for 168 hrs in either DMEM (with or without 24 hrs of serum-free incubation) or 50/50 DMEM/F12 media. Samples were stained and adherent cells were quantified.

Cytokine/mRNA analysis: GD25 and GD25β1A cells were seeded on seven different surfaces: TCPS, fibronectin absorbed TCPS, unmodified gelatin IPN, and IPNs containing gelatin conjugated with four different ligands: methoxy, GGG, PHSRN, and RGD. Supernatant collection and mRNA isolation was preformed at 2, 24, 96, 168 hrs. IL-1α, IL-1β, IL-6, GM-CSF, RANTES, TNF-α, and MCP-1 levels were examined using the Bio-Plex™ Cytokine Assay (Bio-Rad, Inc.) which is a bead-based assay that employs a suspension array system to examine multiple cytokines at once . Relative quantification to GAPDH of selected mRNA was preformed using RT-PCR.

Results/Discussion:

Culture condition optimization: Previous studies using GD25 cells lasted a maximum of 24 hrs in serum-free media. Culture conditions were optimized to allow for more physiological relevant analysis over 7 days (Fig. 1). We found that seeding cells at a density of 10^5 cells/ml (667 cells/mm^2) in DMEM supplemented with 0.4% FBS allowed the cells to survive for 168 hrs without becoming confluent while providing enough material for mRNA isolation and cytokine analysis based on previous work.

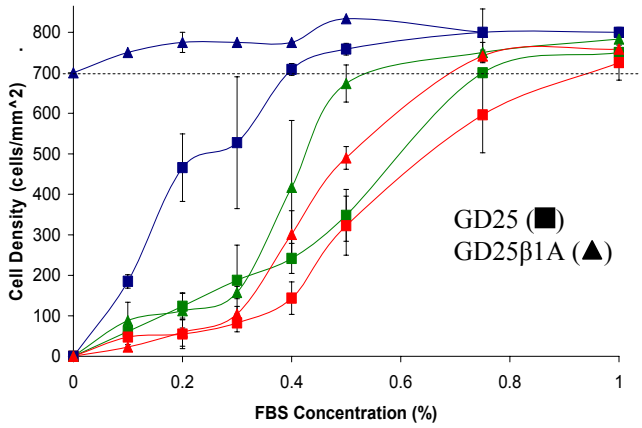

Figure 1: Cell density at 168 hr. at various FBS conc. when cultured in DMEM (red), DMEM with 24 hrs serum-free incubation (green), and 50/50 DMEM/F12 media (blue). Dotted line indicates confluence.

Cell Adhesion: On-going study is to quantitate adhesion on each surface. Qualitative data from mRNA isolation has confirmed the presence of cells on each type of surface.

Cytokine/mRNA analysis: Selected cytokines were chosen for their role in the inflammatory response of fibroblast-type cells and/or their regulation when the β1 integrin is blocked. On-going study is to quantify cytokine levels using described method. Highly up- or down-regulated cytokines' expression will then be examined through RT-PCR along with fibronectin and IL-1R1 expression.

Conclusions: The use of β1 integrin deficient cells is a useful method in which to elucidate the ways that adherence to biomaterials regulates the inflammatory pathway and how modification of biomaterials influences such signaling pathways.

References: 1. Waldeck H, Chung AS, Kao WJ. Interpenetrating polymer networks containing gelatin modified with PEGylated RGD and soluble KGF: Synthesis, characterization, application in in vivo critical dermal wound. *J Biomed Mat Res.* (2006, in press).

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