The Influence of Fibroblast Growth Factor in a Biomimetic Hydrogel System on Endothelial Cell Proliferation

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Statement of Purpose:

A biomatrix material to be used as a tissue engineered scaffold must initiate and sustain vascularization by mimicking the cellular and molecular processes of angiogenesis and vasculogenesis. Poly (ethylene glycol) (PEG) hydrogel is selected to be a biomimetic material due to its hydrophilicity, biocompatibility, and intrinsic resistance to protein adsorption and cell adhesion [1]. Bioactive factors are incorporated to improve biofunctionality. Arg-Gly-Asp-Ser (RGDS) peptide, a ubiquitous adhesion ligand, increases the adhesion of endothelial cells (ECs) [2]. Basic Fibroblast Growth Factor (bFGF) stimulates ECs proliferation and is essential to initiate the formation of vessels [3]. RGDS and FGF both are covalently grafted into the PEG hydrogel to provide an environment in which cells are exposed to biochemical signals from both the initial and intermediate phases of angiogenesis. In this study, FGF was first conjugated to PEG to form a modified FGF (M-FGF) and then M-FGF was grafted to PEG hydrogel to assess the effects on modification on cell behavior. Cell growth was examined over several days using a proliferation bioassay.

Methods:

PEG diacrylate (PEGDA) was prepared by combining 0.1 mmol/mL dry PEG, 0.4 mmol/mL acryoyl chloride and 0.2 mmol/mL triethylamine in anhydrous dichloromethane under argon overnight. The resulting PEGDA was then precipitated with ether, filtered, and dried in vacuum. FGF (PromoKine, Heidelberg, Germany) was first covalently conjugated to PEG spacer, Acrylate-PEG-SVA (Succinimidyl Valerate, 3400 Da: Laysan, Arab, LA) (1:5 molar ratio FGF:PEG) to form M-FGF. Bicinchoninic acid (BCA: Pierce Endogen. Rockford, IL) protein assays were used to determine the concentration of M-FGF. M-FGF was grafted to PEGDA to form grafted FGF (G-FGF) hydrogel by photopolymerization when exposed to UV light (365nm, 10mW/cm2). Human Umbilical Vein Endothelial Cells (HUVECs) (PromoKine, Heidelberg, Germany) were maintained in Media 200 (Cascade Biologics) supplemented with PSG antibiotic mix and Low Serum Growth Supplement. Passage 3-5 cells were used. Cells were made quiescent before the start of the study by removal of FBS for 4 hours, to ensure cells all start from the same growth cycle. At t = 0 hrs, media with 2% FBS and 50 ng/mL of FGFs and M-FGFs were added. At the specific day, Celltiter One solution cell proliferation assay and DNA measurement were used to assay cell proliferation on plate and hydrogel respectively. The percent increase in cell number as compared with that in 2% FBS media was determined. Studies were performed in quadruplicate and one factor ANOVA tests were used for statistical analysis. *P* values less than 0.05 is considered to be significant difference.

Results:

FGF was first conjugated to PEG at different ratios to assess the effects on modification on cell behavior. Cell growth was examined over several days and it was observed that growth significantly increased as compared to when FGF was not present in 3 days. Figure 1 shows that when FGF is modified with PEG there is no significant difference in its proliferative activity.

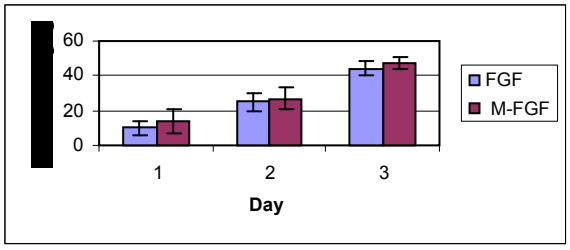

Figure 1. Proliferative activity of FGF and M-FGF on HUVECs on tissue culture plate surfaces.

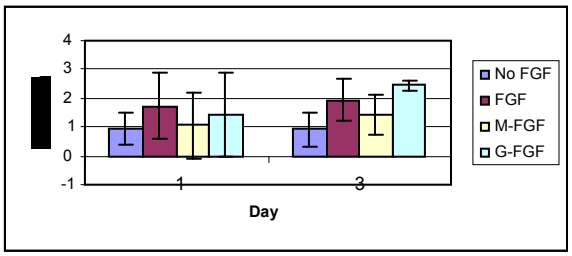

Figure 2. Proliferative activity of FGF or M-FGF suspended in media and G-FGF covalently grafted into PEGDA hydrogels on HUVECs on PEGDA surfaces. On day 3, there is significant difference between No FGF and G-FGF.

Similar results are seen when HUVECs are attached to PEGDA hydrogels (Figure 2). In addition, when FGF was covalently attached to the hydrogel, the same HUVEC growth was observed. The influence of FGF, M-FGF and G-FGF on Human Dermal Microvascular Endothelial Cells (HDMECs) had similar results

Conclusions:

It has been shown that FGF can be chemical modified with PEG and still have proliferative activity similar to that of unmodified FGF. In addition, FGF can be incorporated into PEG hydrogels to induce cell proliferation for longer periods of time. Hence, this provides us a unique platform to develop a tissue engineered scaffold to investigate angiogenesis. To further mimic the natural environment, the influence of FGF on cell migration and apoptosis rescue will be observed.

References:

1. West JL. Hubbell JA. Macromolecules. 1999; 32,241-244.
2. Mann BK, Gobin AS, Tsai AT, Schmedlen RH and West JL. Biomaterials. 2001b, 22,2045-3051.
3. DeLong SA, Moon JJ and West JL. Biomaterials. 2005, 26, 3227-3234.