The Use of PEG Hydrogels to Analyze Angiogenic Processes *In Vitro*

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Statement of Purpose: Angiogenesis is the process by which new blood vessels arise from the pre-existing vasculature[1]. Endothelial cells are known to be involved in three cellular processes during angiogenesis: Cell proliferation, survival of apoptosis, and degradation of the extracellular matrix during cell migration[1]. Vascular endothelial growth factor (VEGF) is a cytokine which has been shown to fascilitate angiogenesis[2]. VEGF upregulates the three above-stated processes during angiogenesis, thus increasing the amount of new blood vessel formation[2]. Although these processes are well known in the literature, a synthetic system with exact control over the signals of angiogenesis has yet to be developed. Our goal is to develop a polyethylene glycol based hydrogel system, which can be tailored to contain covalently grafted VEGF and adhesion peptides for precise assessment of VEGF-induced angiogenic processes on human umbelical vein (HUVEC) and human microvascular (HMEC) endothelial cells *in vitro*.

Methods: Endothelial cell proliferation was compared after 24 and 72 hrs, between cells on hydrogels with modified VEGF (50 ng/ml) or with VEGF added to the media. Cell number was counted via hemacytometer. Endothelial cell apoptosis survival was using a DeadEndfluorometric TUNEL assay (Promega; Madison, WI). Extrinsic apoptosis was induced by TRAIL (50ng/ml) in the media for 6 hours. Intrinsic apoptosis was induced by serum starvation for 24 hours. Cells were administered VEGF (50ng/ml) for a subsequent 24 hour incubation.

Endothelial cell 2-D migration was studied using a fence assay and fluorescent imaging for quantification. Cells were seeded on hydrogels with grafted VEGF (50 ng/ml) (or similar amount within the media as a control) near confluence behind a fence, which when removed the cells were allowed to migrate randomly for 48 hrs. After which time the cells were fixed with 4% formaldehyde and stained with DAPI to assess total distance migrated from start point. The study was performed in the presence of mitomycin C (0.5 µg/ml), which inhibits DNA synthesis and permits observation of migration without influence of proliferating cells.

Results: In Figure 1, results indicate that VEGF which has been grafted in the hydrogel is capable of inducing the same increases in cell proliferation as compared to VEGF in the media at equal concentrations for HMEC and HUVEC, respectively. These findings suggest that covalently grafting VEGF into the hydrogel does not lower its biological activity in terms of cell proliferation.

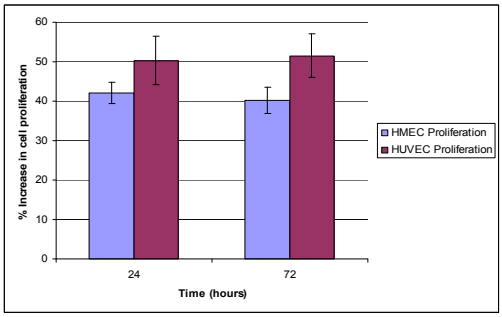

Fig.1: HMEC and HUVEC Proliferation

Figure 2 illustrates the ability of VEGF to induce increased cell survival when cells undergo intrinsic (mitochondria-mediated) and extrinsic (extracellular ligand/receptor-mediated) apoptosis, respectively. In each case, VEGF which has been grafted into the hydrogels is as effective as VEGF which has been suspended in the media at inducing apoptosis survival.

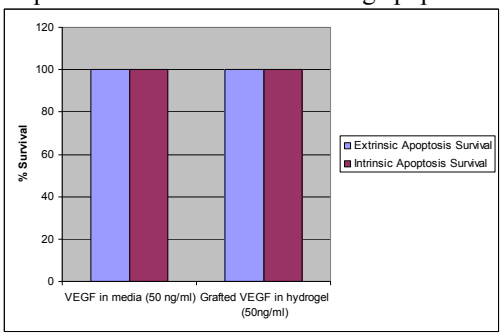

Fig.2: HUVEC Apoptosis Survival

Figure 3 represents 2-D HUVEC migration on hydrogels. The data suggest VEGF which has been grafted in the hydrogel is as effective as VEGF suspended in the media at inducing increases in cell migration.

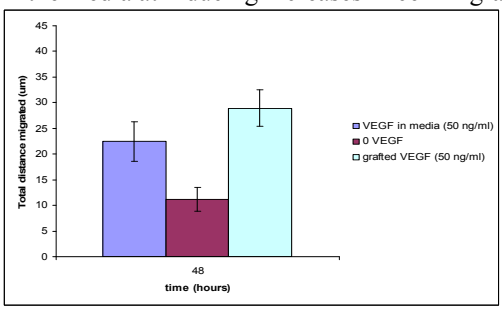

Fig.3: HUVEC Migration

Conclusions: Based on our findings, VEGF which has been covalently grafted into a PEG hydrogel system is shown to be as effective as free VEGF in inducing key angiogenic processes in human endothelial cells *in vitro*. Future studies will include analysis of key proteins involved in the afore-mentioned processes.

References:

1. Shibuya, M. Journal of Biochemistry and Mol. Biology. 2006; 39: 469-478.
2. Distler, O, et al. QJ Nucl Medicine. 2003; 47: 149-161.