Augmentation of *in vitro* Angiogenesis Induced by the R136K Thrombin Resistant FGF-1 Mutant within Fibrin Hydrogels

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Statement of Purpose: Atherosclerotic disease is a leading cause of death worldwide and necessitates a large number of vascular interventions each year. Many of these procedures result in damage to the existing vascular intima leading to thrombosis and concomitant myointmal hyperplasia limiting the longevity of these interventions. Evidence suggests that the formation of an endothelial cell monolayer could limit events leading to interventional failure. Previous research established that localized delivery of potent angiogens can result in capillary sprout formation in vitro and in vivo[1] and that this strategy can be exploited to induce endothelialization. Fibrin gels have been explored extensively as biomaterials for application in regenerative medicine. The purpose of this study was to quantify the angiogenic activity of a fibroblast growth factor (FGF-1) derived mutant R136K as compared to wild-type FGF-1when delivered in a fibrin glue matrix in a 3-D angiogenesis assay. This mutant was designed for prolonged bioactivity by resisting thrombin-induced proteolysis and was created by substituting an arginine for lysine on the 136 residue, the primary site of thrombin cleavage for wild-type FGF-1.[2] We previously reported enhanced molecular stability of this mutant vs. wtFGF-1 following thrombin exposure. This approach demonstrates the potential utility of fibrin glue for delivery of therapeutic angiogens to ischemic tissue beds such as those seen in diabetes and diffuse atherosclerosis as well as for promoting microvascularization in engineered tissues including vascular grafts.

Methods: 20,000 human umbilical vein endothelial cells (HUVECs) were suspended upside down in a methylcellulose suspension solution containing media for 2 days to form endothelial cell pellets. After careful removal of the media, the pellets were isolated and four pellets per group were embedded into 3-D fibrin glue (FG) matrices supported by a nylon mesh ring. Each FG matrix contained 6 ηmol concentrations of FGF-1 or R136K. A negative control group lacking the angiogen was established. The assay disc was cultured in a 24-well plate containing assay media with serum and heparin. The 3-D cultures were digitally photographed each day and average length of sprouts (ALS) was quantified using imaging software (Axiovision) and Adobe Photoshop. One way analysis of variance with a post-hoc Tukey test was used to analyze the data.

Results/Discussion: The FG containing either growth factor demonstrated a statistically significant increase in ALS on Day 1 when compared to the negative control group. R136K matched FGF-1 in capillary sprout induction at Day 1 and exceeded FGF-1 in the days to follow. At 2 days, R136K had significantly greater ALS than FGF-1 and the untreated group (R136K ALS 287±44 µm, FGF-1 ALS 247±68µm, untreated 188±57µm – p values respectively $p=0.007$ and $p<0.001$). R136K displayed statistically significant increases compared to untreated groups until day 6 whereas the FGF-1 ALS increases stopped after day 4.

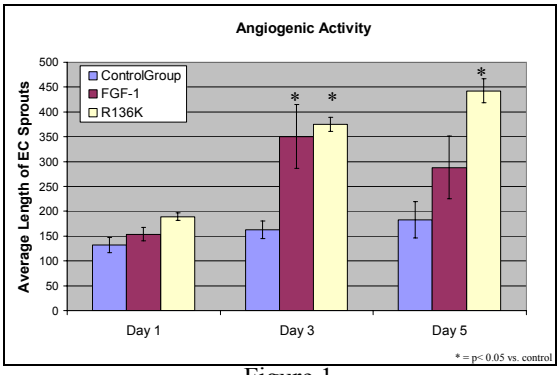

Figure 1.

Conclusions: The mutant growth factor R136K is more effective than FGF-1 in the establishment of microvascular networks within a fibrin glue matrix as demonstrated by its earlier and more persistent angiogenic activity as compared to FGF-1. This likely results from the resistance of R136K to thrombin proteolysis allowing for the increased duration of molecular stability and bioactivity within FG. The combinatorial application of FG with R136K may induce endothelization of prosthetic vascular grafts or microvascularization of ischemic tissue beds via angiogenic induction from surrounding tissue. Our data suggest that the thrombin resistance and increased bioavailability of R136K allows for sustained growth factor activity in FG and therefore represents an exciting development in the formation of novel growth factor delivery systems for in vivo implantation.

References:

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