Modulating the Biological Milieu Within Cardiovascular Biomaterials using Novel Constructs Derived from Fibronectin

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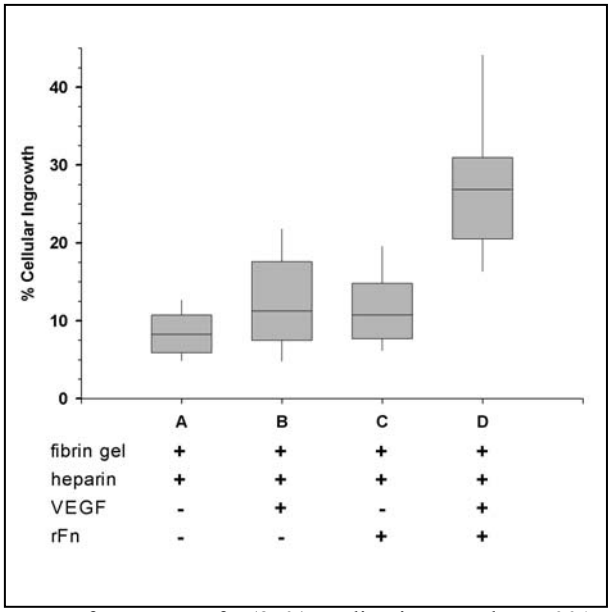
Statement of Purpose: Cell and capillary in-growth into cardiovascular biomaterials has been a critical goal for successful implants. While the use of RGD ligands and growth factors have helped to enhance cell attachment and tissue in-growth experimentally, there still is no prosthetic vascular graft that will heal naturally with an endothelial lining in humans. We discovered that the heparin-II domain of fibronectin (Fn) is a unique binding site for VEGF[1]. When linked with the Fn cell-binding domain, VEGF-binding domains of Fn synergistically enhance VEGF-induced endothelial and stem cell responses, significantly more than native Fn or other matrix proteins[2,3]. We wished to test the hypothesis that a unique recombinant Fn construct, when combined with VEGF, would enhance cell and capillary ingrowth into expanded polytetrafluoroethylene (ePTFE) in vivo.

Methods: A recombinant protein (rFn) based on the cell-binding (Type III repeats 9-10) and VEGF-binding (III 12-14) domains of Fn was devised, expressed in the P*ichia pastoris* expression system (Invitrogen), and purified. A subcutaneous implant model in the rat used ePTFE discs measuring 4 mm in diameter, 0.6 mm wall thickness, with a porosity of 60 micron internodal distance (Atrium Medical). Using a fibrin glue, the discs were sterilely impregnated with unfractioned heparin (25ug/ml) and different combinations of VEGF (100 ug/ml) and/or $rFnIII_{9\text{-}10/12\text{-}14}$ (25ug/ml). Sets of 4 were implanted in the dorsal subcutaneous space, and explanted at 2 and 4 weeks. Sections were stained with H&E, GSL-1 (*Griffonia simplicifolia* lectin) to identify endothelial cells, anti-smooth muscle cell actin, and fluorescent DAPI for quantifying explant cellularity. Quantitative histologic analyses were performed to compare biological treatments.

Results: In preliminary studies with 4 rats (16 implants), the addition of the complete rFn/VEGF/heparin mixture to the fibrin glue dramatically increased cellular in-growth within the ePTFE (mean 26% ± 2% sem vs. controls 10.6% ± 2.3% sem). The degree of cellular in-growth at four weeks was not significantly different than two weeks, and pilot samples placed in an infrascapular intramuscular location were no different than an adjacent subcutaneous site.

Subsequent experiments compared 4 different biological milieux in 8 rats (32 subcutaneous implants studied at 2 wks): A. control - fibrin gel and heparin alone; B. fibrin gel, heparin, VEGF; C. fibrin gel, heparin, rFn; and D. fibrin gel, heparin, VEGF and rFn. Figure 1 summarizes the quantitative analysis of cellular in-growth. The inclusion of either VEGF (11% median in-growth) or rFn (8.25% median in-growth) caused a modest but significant increase in cellular in-growth compared to the controls (fibrin gel plus heparin, $p < .004$). The combination of rFn/VEGF/heparin dramatically increased cellular in-growth of the interstitial



space of ePTFE grafts (27% median in-growth, $p<.001$ compared to all other treatments). Quantitation of neocapillary formation revealed the same pattern, with significantly more capillaries in the implants treated with rFn+VEGF compared to controls (fibrin glue + heparin): 25 capillaries/mm^2 ± 1.6 vs. 7.7 ± .9.

Conclusions: These studies of ePTFE implants in rats confirm the potency of an engineered protein construct designed to significantly enhance VEGF-induced cellular and capillary in-growth. In preliminary implants, the main determinant of cell and capillary in-growth was the presence or absence of the ternary mixture of rFn/VEGF/heparin.. More detailed studies examining the independent roles of VEGF and the rFn construct showed that maximal in-growth and capillarity was achieved with the ternary complex. The binary combinations of VEGF plus heparin, and rFn plus heparin also significantly enhanced in-growth compared to controls, but less so. Researchers have taken many different approaches to reprise a more natural biological milieu within implants, including decellularized tissues and bioreactors. By capitalizing on new insights into growth factor-matrix protein biology and rational protein design, this current approach may advance the design of a ready-made, off the shelf, small diameter vascular prosthesis that possesses improved capacities for healing and endothelialization.

References:

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