Scaffold Composition, Rather Than Delivered Growth Factor, Dramatically Altered The Chick Chorioallantoic Membrane Blood Vessel Response to Fibrin-based Constructs

[1]Jason D. Smith, [1]Molly E. Melhem, [2]Greg W. Fisher, [2]Alan S. Waggoner, [1]Phil G. Campbell
[1]Institute for Complex Engineered Systems, [2]Molecular Biosensor and Imaging Center, Carnegie Mellon University, Pittsburgh, PA

Statement of Purpose: Our laboratory is interested in developing fibrin-based constructs for bone tissue engineering applications. A major aspect of bone regeneration is the establishment of neovasculature. We study the angiogenic nature of our fibrin-based materials using the chick chorioallantoic membrane (CAM) assay, a commonly used in vivo blood vessel model. Early results using only fibrin and fibroblast growth factor 2 (FGF-2) showed that angiogenesis did occur however the blood vessels did not invade the construct. The CAM degraded the fibrin as a front, replaced it with new CAM tissue, and the blood vessels followed the invading CAM front. We reasoned that to get blood vessel invasion we needed a more "natural" and also more complex scaffold. Therefore, we began adding extracellular matrix (ECM) molecules to the fibrin that are naturally found during regeneration such as hyaluronic acid, fibronectin, and type I collagen. We examined the qualitative effect on angiogenesis using a histology and fluorescence based screening assay as well as standard histological analysis.

Methods: Fibrin gels were formulated to contain approximately 18 mg/mL fibrinogen, 5 U/mL thrombin, 10 ng/mL FGF-2 or vascular endothelial growth factor ($VEGF_{165}$), 1 μg/mL aprotinin, 1 U/mL Factor XIII, and any of the following additives: 0.01-0.1 mg/mL fibronectin, 0.1-2 mg/mL hyaluronic acid, and 0.1-3 mg/mL rat tail type I collagen. Fibrin gels were placed on the CAM of 10 day old White Leghorn Chick Embryos, which were cracked into Petri dishes at day 3 and incubated at 37 degrees C. After 48-72 hours fluorescent quantum dots were intravitally injected into a chick CAM vein. Blood vessels surrounding the construct were imaged by fluorescence followed by excision and fixation of the construct and connected CAM for 1 hour in 1% glutaraldehyde and 3% paraformaldehyde in PBS. A vibratome was used to cross-sectionally cut the sample into 200-400 μm thick sections. Sections were mounted on microscope slides and quantum dot labeled blood vessels were imaged by epifluorescence. Sections of interest were further processed and analyzed for electron microscopy, H&E staining, and/or immunohistochemistry.

Results / Discussion: Fibrin constructs containing either $VEGF_{165}$ or FGF-2 produced qualitatively the same result. A chaotic, dense network of blood vessels clustered near the fibrin/CAM interface but blood vessel invasion was not seen. Fibrin contraction was more apparent with FGF-2, presumably due to higher proliferation of myofibroblasts in response to the growth factor. The addition of fibronectin produced a more organized network of vasculature but also resulted in a lack of blood vessel invasion. Unlike the fibrin only case, fibronectin addition also resulted in large avascular CAM protrusions into the fibrin. Fibrin/type I collagen constructs showed fewer new vessels away from the construct but a high concentration of vessels making intimate contact with the construct edges, some of which appeared to make small inroads into the fibrin. Additionally, H&E staining confirmed a large infiltrate of what appeared to be fibroblasts into the construct itself. Finally, a large number of vessels tightly associated with the construct as well as some significant vessel penetration were found with fibrin/hyaluronic acid constructs. Overall, the results indicate that the various ECM proteins are providing different cues that are just as important as the growth factors used to signal the angiogenic response itself. Although the importance of scaffold design is found throughout literature this is the first evidence of the dramatic effect of scaffold composition on angiogenesis for tissue engineering application. In the future we plan to test other ECM additives as well as investigate combinations of these additives. We are also developing computer-based techniques to better quantitatively assess the angiogenic response seen in our histological sections.

Conclusions: The use of FGF-2 or $VEGF_{165}$ did not produce significant qualitative differences on the angiogenic response of the chick CAM. Through modulation of the scaffold design, with ECM proteins naturally found during the wound healing process, we saw a dramatic effect on the angiogenic response. Fibronectin appeared to create a more organized blood vessel network, type I collagen allowed for cell invasion as well as intimate blood vessel contact with the material, and hyaluronic acid appeared to allow significant blood vessel invasion. The short time of the chick CAM assay and our fluorescence-based histological screen will allow us to rapidly test a myriad of formulations to further investigate the effect of ECM molecule levels and combinations within fibrin-based constructs.