Quantitative 3D Analysis Of Microvascular Networks In Tissue Engineering

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Citation

Abstract

Tissue engineering is an exciting field that has arisen amidst projections of revolutionizing reconstructive surgery. Fabricating tissue with a patient-specific, three-dimensional geometry from a cocktail of autologous cells, growth factors, and biocompatible/biodegradable polymers would address donor site morbidity concerns, alleviate tissue shortages, and improve patient quality of life. Achieving these rather lofty goals requires the collaborative effort of scientists, engineers, and surgeons in recognizing and addressing the unique concerns of engineered tissue.

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One of the most important design constraints to providing clinically translatable engineered tissue is the development of a patent microvascular network with properly structured geometry within the tissue engineered constructs. Microvascular networks control the mass transport of nutrients, ensure tissue viability, control tissue differentiation, and allow long-term tissue maintenance. It has been shown that cells cannot survive greater then 200 µm from a blood supply. It is also recognized that angiogenesis occurring in an unregulated fashion produces undesirable results. This is most recognized in the oft-studied tumor angiogenesis; however, little is known about the formation, growth-kinetics, and architecture of microvascular networks in tissue engineered constructs.

A complete understanding of angiogenesis in any system is impossible with current assays and imaging modalities. Many techniques have been developed to study angiogenesis and microvascular architecture. These methods include in vitro gels, animal preparations, vascular casts, micro-CT, histology, and immunohistochemistry. Microvascular networks are complex, exhibiting wide variation among environments and can be greatly complicated due to various pathologies. These assays require broad assumptions about or oversimplification of the networks. Angiogenesis can be adequately understood only with a high resolution, three-dimensional, quantifiable assay. Result images allow measurement of many parameters, including vascular network density, vessel diameters, void space, and tortuosity.

We have developed a novel, sophisticated method with resolution to the endothelial cell level that allows visualization and quantitation of microvascular networks in three dimensions. Briefly, tissue is excised, cryofixed, and six micron serial sections are cut on a cryostat. Each section is immunostained for CD-31, an endothelial cell specific antigen, and stained sections are imaged in 2D using a CCD camera. Immunohistochemistry used in combination with microscopy allows high resolution images of antigenically distinct features of tissue. Although quantitative data can be obtained using other methods, only immunohistochemistry allows quantitation at the cellular level while maintaining the relationship between stained cells and overall tissue morphology. The 2D images are aligned using automated image registration techniques and volume rendered to produce a 3D, quantifiable image of the microvascular networks within the tissue.

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Currently, we are applying this technique to the study of angiogenesis in model tissue engineered constructs. The movie depicts capillary invasion into a fibrin gel following implantation of a model tissue engineered construct within a rat model. The image is 1200 x 500 x 240 μm with 1 μm resolution. Vessels and endothelial cells are pseudo-colored red and the fibrin white. Vessels can be seen growing within the fibrin and endothelial cells along the periphery of the fibrin indicate the leading edge of angiogenesis.

The inosculation mechanisms by which surgical free flaps (the tissue engineering gold standard) establish and maintain a patent vascular network are also being studied. This work will lead to a better understanding of angiogenesis in tissue engineering and will contribute to the development of clinically useful tissue engineered products.

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References

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