Guided Endothelial Cell Growth in Three-dimensional Hydrogels

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Abstract: When engineered tissue constructs are transplanted in injured tissues, a vascular network is required to provide the cells and tissues with the oxygen and nutrients needed for survival. Moreover, endothelial cells define the niche in many systems and thus their role in regenerative strategies goes beyond the vascular system. To address both of these issues, we designed a three-dimensional (3D) culture system in which to guide endothelial cells with the view of both enhancing regenerative strategies and laying the foundation to investigate the vascular microenvironment. This study focuses on the guidance of endothelial cells (ECs) in a 3D agarose hydrogel using an immobilized gradient of $VEGF_{165}$. To promote cell adhesion, agarose was also modified with the ubiquitous cell adhesion peptide, GRGDS. ECs formed tubular structures in these cell-adhesive, VEGF-gradient 3D hydrogels. This 3D model of EC growth may be useful for engineered tissues and to study the vascular niche.

Methods: To immobilize cell-adhesive peptides (GRGDS) and bioactive $VEGF_{165}$ to agarose hydrogels, agarose was modified with a photolabile thiol protected-6-bromo-7-hydroxycoumarin as previously described [1,2]. A gradient of $VEGF_{165}$ was created within the GRGDS-agarose hydrogels using a multiphoton Ti/sapphire laser. Upon exposure to the laser light, the coumarin groups were photocleaved, leaving a gradient of reactive thiols within the agarose hydrogels. These were in turn reacted with maleimide-modified $VEGF_{165}$ creating a gradient pattern of immobilized $VEGF_{165}$. The gradient pattern was quantified using the Leica software based on the intensity of fluorescence.

Endothelial cells, isolated from the mouse brain [3], were plated on the agarose hydrogels. EC spheroids were formed in suspension culture, plated on GRGDS immobilized agarose hydrogels and then characterized for tubule formation and by immunohistochemistry.

Results: We successfully created a 3D $VEGF_{165}$ gradient within GRGDS agarose hydrogels. The VEGF gradient was determined to be 1 ng/ml/μm (Figure 1). After culturing spheroid-forming ECs on GRGDS agarose hydrogels for two days, ECs formed tubular structures in the gels, with stalk and tip cells identified based on their morphology (Figure 2). When ECs were seeded on top of the gradient gel, ECs were guided into the hydrogels following the gradient of immobilized $VEGF_{165}$ to more than 200 μm. However, when $VEGF_{165}$ was immobilized, but not in a gradient, ECs grew into GRGDS agarose to only a depth of 30-50μm.

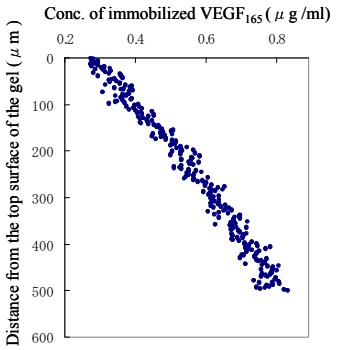

Figure 1. Quantification of a gradient of immobilized $VEGF_{165}$ within the hydrogels

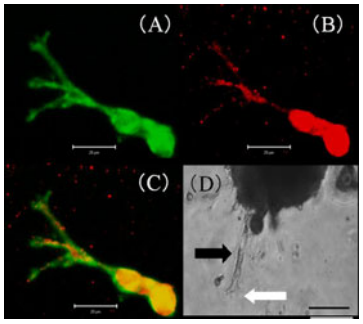

Figure 2. Tubular formation of ECs in the GRGDS hydrogels Confocal images of ECs stained by antibodies, (A) *beta*-catenin (B) laminin (C) combined. (scale bar = 20 μm) (D) Phase-contrast microscopy image. Stalk cells (black arrow) and tip cells (white arrow) were observed. (scale bar = 100 μm)

Conclusions: To gain a greater understanding of the vascular systems, we created a hydrogel to guide endothelial cells in 3D. We demonstrated that $VEGF_{165}$ was successfully immobilized to agarose hydrogels with a gradient. Cell-adhesive GRGDS agarose supported tubular formation of ECs and an immobilized gradient of $VEGF_{165}$ allowed ECs to grow into the hydrogels. This engineered matrix may provide a tool to elucidate the biological mechanisms of vascular systems.

References: [1] Wosnick JH. Chemistry of Materials, 2008, 20,55-60. [2] Aizawa Y. Biomaterials, 2008, 35, 4676-53 [3] Song LI. In Vitro Cell. Dev. Biol., 2003, 39, 313-320