Optimization of Vasculogenesis within Naturally-Derived, Biodegradable Hybrid Hydrogel Scaffolds

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Abstract

Background: Cellular ingrowth and neovascularization of acellular tissue-regeneration scaffolds represent the rate-limiting steps of permanent integration.[1] In previous work, we have demonstrated that naturally-derived, biodegradable hybrid hydrogel scaffolds fabricated from a 10:1 w/w combination of alginate and type I collagen allow for maximal human umbilical vein endothelial cell (HUVEC) adherence and invasion in an *in vitro* wound healing model. We next sought to stimulate endothelial tubule formation within these scaffolds.

Methods: Hybrid hydrogel scaffolds were fabricated as before. Scaffolds were seeded with 3.0×10^5 HUVECs and maintained under standard cell culture conditions. After 3 days, culture media was supplemented with basic fibroblast growth factor (bFGF) at varying physiologic concentrations. Additional scaffolds were seeded with human aortic smooth muscle cells (HASMCs), followed by HUVECs 3 days later. After 2 weeks of HUVEC culture, all scaffolds were labeled with DiI-Ac-LDL and DAPI, and imaged using confocal microscopy.

Results: HUVECs were maximally confluent on hybrid scaffolds when cultured with media supplemented with bFGF 20ng/mL (372.5±37.4) compared with 0ng/mL (250.7±18.2, $p=0.005$), 1ng/mL (179.0±17.4, $p<1.0 \times 10^{-2}$), 5ng/mL (198.5±17.2, $p<1.0 \times 10^{-3}$), and 10ng/mL (273.3±22.9, $p=0.030$). However, the addition of growth factor stimulated neither an increase in HUVEC invasion nor tubule formation within the scaffold substrate. In contrast, a “dose-dependent” increase in tubule content and network-like organization was observed as the co-culture ratio of HASMCs to HUVECS from increased from 1:10 to 1:1 (Figures 1-2).

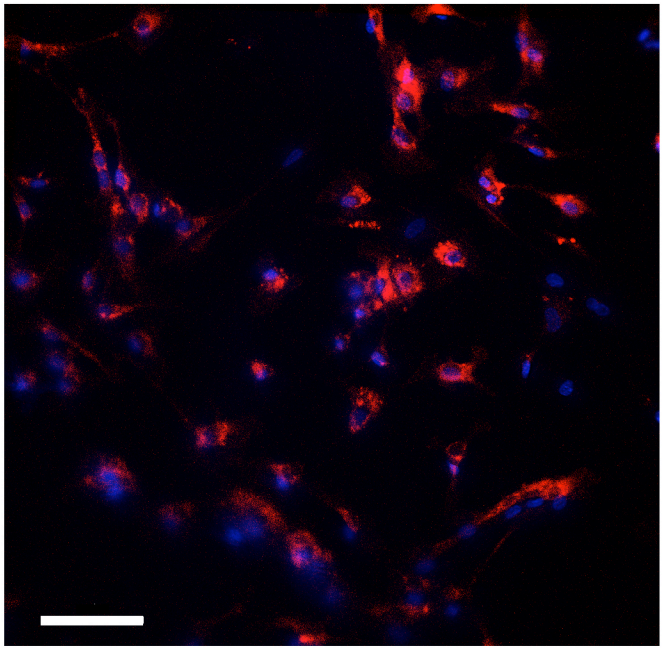

Figure 1: Co-culture of human aortic smooth muscle cells (HASMCs) and human umbilical vein endothelial cells (HUVECs) in a 1:10 ratio within hybrid hydrogel scaffolds resulted in clusters of cells without an overlying structure or pattern. *Scale bar = 100um*

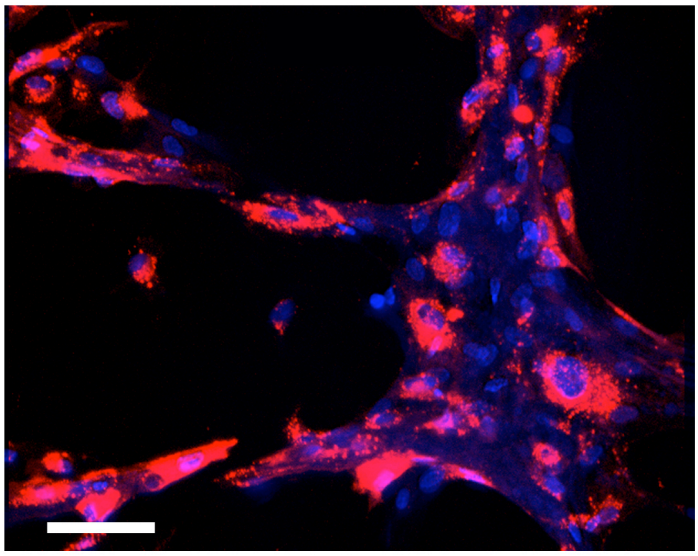

Figure 2: Co-culture of human aortic smooth muscle cells (HASMCs) and human umbilical vein endothelial cells (HUVECs) in a 1:1 ratio within hybrid hydrogel scaffolds resulted in an increase in tubule formationand network organization. *Scale bar = 100um*

Conclusions: Even at high concentrations, the presence of bFGF alone is insufficient to stimulate HUVECs to form tubules within hydrogel scaffolds. Instead, the complex chemical and possibly contextual cues offered by vascular smooth muscle cells are required to instruct endothelial cells to assemble into vasculogenic configurations. These findings provide important insights into the instruction of endothelial tubule and network formation and bring us one step closer to the fabrication of pre-vascularized artificial tissue.

References

1. Zheng, Y., Henderson, P.W., Choi, N.W., Bonassar, L.J., Spector, J.A., Stroock, A.D. Microstructured templates for directed growth and vascularization of soft tissue in vivo. Biomaterials 32: 5391-5401, 2011.

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