Tubulogenesis of iPS Endothelial Cells Stabilized by Human Mesenchymal Stem Cells in Fibrin Gels

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Motivation: Tissue engineering aims to develop complex living tissues *de novo*, with applications for organ-on-a-chip high-throughput drug screening studies, the recapitulation of tumor microenvironments to elucidate mechanisms of cancer progression, and toward whole human organ replacement therapies. Regardless of the intended goal for tissue engineering, a major challenge remains the creation of multiscale vasculature to provide oxygen and nutrients to multicellular life and remove metabolic waste products. We seek to develop a system in which we can better understand the process by which endothelial cells can form stable capillary networks. Previous work has demonstrated HUVEC and 10T1/2 co-cultures can provide long term stability of vasculogenic capillary beds[1-3] We aim to extend this work with therapeutically relevant human cells, coupled with fluorescent genetic reporters that would allow us to monitor capillary morphogenesis over time. Here, we demonstrate that human derived iPS cells differentiated into endothelial cells (iPS-ECs) in co-culture with human mesenchymal stem cells (MSC) can form a putative capillary bed in fibrin gels over one week in 3D culture.

Methods: We transduced primary cells with second-generation lentivirus to generate stable expression of multi-color fluorescent reporters. Human mesenchymal stem cells (hMSCs, RoosterBio) were transduced with the multi-gene cassette PGK-H2B-mCherry CMV-EGFP-IRES-Puromycin to provide fluorescent GFP cytoplasm and mCherry nuclei. Separately, iPS-derived endothelial cells (iPS-ECs, Cellular Dynamics) were transduced with hEF1α-H2B-mVenus-IRES-mPlum-PGK-Puromycin to provide mPlum cytoplasm and mVenus nuclei. Transduced cells were selected with puromycin to yield pure populations of dual colored iPS-ECs and hMSCs. Labeled cells were encapsulated in fibrin gels (10 mg/mL) at a 50:1 or 100:1 ratio of iPS-ECs to hMSCs, or iPS-ECs alone (5e6 cells/mL). Cellular morphogenesis was monitored with a Nikon Eclipse Ti microscope or a Nikon A1 Confocal microscope over one week. Multispectral confocal imaging permitted the collection of multidimensional lambda-stacks which were linearly unmixed to separate each of the four proteins and facilitate discrimination of cell type and their morphologies (Figure 1).

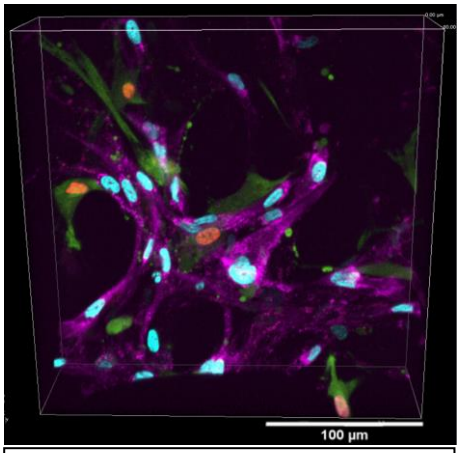

Figure 1. Multispectral confocal z-stack of iPS-ECs (cyan, H2B-mVenus nuclei; purple, mPlum cytoplasm) and hMSCs (red, H2B-mCherry nuclei; green, GFP cytoplasm) encapsulated at 100:1 ratio in a 10mg/mL fibrin gel. iPS-ECs form a tubulogenic network stabilized by hMSCs.

Results: Lentiviral transduction and selection resulted in pure dual labeled cell populations for both iPS-ECs (H2B-mVenus/mPlum) and hMSCs (H2B-mCherry/EGFP). We found that tubulation depended on the fibrin matrix concentration and cell density, as well as the ratio of hMSCs to iPS-ECs. Our unique 4-color fluorescent reporter system demonstrated multicellular iPS-EC tubules with hMSCs attaching to the networks in the pericellular region (Figure 1).

Conclusion: We describe the mechanical and cellular parameters which govern tubulogenesis and stabilization of therapeutically relevant iPS-ECs and hMSCs. Our novel multicolor genetic reporter system is enabling a new class of longitudinal studies of tubulogenesis and their integration with 3D printed vasculature[4]. Future work will link these studies with timelapse confocal imaging and more quantitative mapping of putative tubule networks of iPS-ECs and hMSCs. This system may help us visualize and control multiscale vessel architecture with patent connection between interstitial, bulk capillary formation and printed channel geometries agnostic of the printing method.

References: 1) Koike, N+. *Nature* 2004. 428:138-9. 2) Moon, +. *Biomaterials* 2010. 31(14): 3840–3847. 3) Cheng, G+. *Blood* 2011. 118(17): 4740–4749. 4) Miller, JS+ *Nature Materials*. 2012. 11(9): 768-774.