Reducing Ambient Oxygen Tension Optimizes the Fabrication and Maturation of Pre-Vascularized Tissue Engineered Flaps

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INTRODUCTION: Oxygen is a potent modulator of cell function and wound repair *in vivo*. Hypoxia can enhance the production of specific extracellular matrix components and increase angiogenesis through the hypoxia-inducible factor-1 pathway. However, *in vivo*, very few cells within the body experience ambient (21%) oxygen tension. Thus, for clinically relevant tissue-engineered pre-vascularized skin flaps, hypoxic conditions can be exploited for promoting angiogenesis. We sought to identify the ideal oxygen tension in which to fabricate our novel, pre-vascularized tissue constructs containing a vascularized 1 mm diameter microchannel lined with human cells.

MATERIALS AND METHODS: Vascular networks were fabricated by sacrificing Pluronic F127 macrofibers in type I collagen with encapsulated human foreskin fibroblasts (HFF1) and human placental pericytes (HPPL) at a density of 1 x 10^6 cells/mL, respectively. Twenty-four hours following fiber sacrifice, 5 x 10^6 cells/mL of human aortic smooth muscle cells (HASMC) and 5 x 10^6 cells/mL of human umbilical vein endothelial cells (HUVEC) were seeded sequentially into the patent luminal space. Subsequently, 48 hours after fiber sacrifice, 1 x 10^6 cells/mL of human epidermal keratinocytes (HEK) were topically seeded onto scaffolds. Scaffolds were incubated at 1.5%, 5.0%, or 20.0% oxygen, underwent daily media changes, and were analyzed after 7 and 14 days in culture.

RESULTS: Macrochannels were successfully lined with HUVEC and HASMC, generating anatomically appropriate neointimal and neomedial layers by as early as day 7. The most robust cellular linings were seen in constructs incubated in 5.0% oxygen. Immunohistochemical analysis revealed CD31+ HUVEC along the luminal surface of the macrochannel, and α-SMA expressing HASMC in the subendothelial plane. Furthermore, proliferation of HFF1 was evident as early as 7 days after seeding. HEK proliferated leading to the formation of a stratified epidermal layer along the construct surface and fibroblast specific-1-expressing fibroblasts within the "neo dermis."

CONCLUSION: Hypoxic conditions promote increased angiogenesis and vascular stability in our tissue engineered, pre-vascularized skin flaps without detrimental effects on other flap cellular constituents. With a built-in vascular network, vital epidermal (HEK) and dermal (HFF1, collagen) components, these full-thickness, tissue engineered skin scaffolds hold tremendous promise as a platform to aid in evaluating cellular responses to changing oxygen concentrations in parallel to generating tissue-engineered flaps of clinically relevant sizes.