Fabrication of a Tissue-Engineered Pre-Vascularized Perfusable Skin Flap

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INTRODUCTION: Currently, there remains no clinically translatable tissue engineered skin flap with the ability to provide whole tissue perfusion. Herein, we fabricate a pre-vascularized full-thickness cellularized skin equivalent containing a three-dimensional vascularized network of interconnected macro and microchannels lined with vascular cells, within a collagen neodermis containing encapsulated fibroblasts, and an epidermis comprised of human keratinocytes capable of providing whole tissue perfusion.

METHODS: Pluronic F127 was used for network preparation: 1.5 mm diameter “U” shaped macrofibers and 100-500 μm-interwoven microfibers were heat extruded and then embedded within Type I collagen into which CFP-tagged human placental pericytes (HPLP-CFP) and human foreskin fibroblasts (HFF1) at a density of 1x10^6 cells/mL, respectively had been encapsulated. After pluronic sacrifice, channels were intraluminally seeded with 5x10^5 RFP-tagged human aortic smooth muscle cells (HASMC-RFP) and 5x10^5 GFP-tagged human umbilical vein endothelial cells (HUVEC-GFP). The construct was then topically seeded with 1x10^6 human epidermal keratinocytes (HEK). Constructs were incubated for 7,14 and 28 days and subsequently live flaps were analyzed using multiphoton microscopy (MPM) or fixed and processed for histology. Flaps were microsurgically anastomosed to rat femoral artery and vein and perfused, *in vivo.*

RESULTS: MPM imaging demonstrated a hierarchical vascular network containing macro and microvessels lined by endothelial and smooth muscle cells, supported by perivascular pericytes, all in appropriate microanatomic arrangement. Neodermal HFF1 proliferated throughout the observation period and the HEK neoepidermis developed into a stratified epithelium along the superficial aspect of the construct. MPM images indicated angiogenic sprouting from the nascent vascular network into neovessel like structures. Skin flaps were successfully anastomosed and perfused while withstanding the physiological pressures and maintaining their inherent vascular network architecture.

CONCLUSION: We have successfully fabricated and microsurgically anastomosed the first ever tissue-engineered pre-vascularized full thickness skin flap, which recapitulates the inherent hierarchical vasculature found within the human skin and is suitable to provide whole tissue perfusion. We provide the platform for an on-demand, geometrically tunable tissue engineered skin equivalent with an anastomosable vascular network which will transform reconstructive surgical practice by eliminating the consequences of donor site morbidity and enabling rationally designed, patient specific flaps for each unique wound environment and anatomic location.