CONCLUSIONS: SNMP has the potential to both actively preserve and enhance overall preservation of forelimbs in a swine model. It may provide the crucial enabling technology for tissue preservation, transport, and eventual transplantation of VCAs.

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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.

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Radiological and Histological Assessment in Perforator Flap Microvasculature Following Pretreatment with Topical Negative Pressure Therapy: An Experimental Rat Model

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BACKGROUND: Surgical delay and ischemic preconditioning have been traditionally used to precondition flaps to render flaps more vascular and resistant to changes in their microcirculation. Negative pressure wound therapy (NPWT) has been extensively in clinical

practice and associated with angiogenesis and accelerated wound healing. This experimental model evaluated topical negative pressure as a mechanism for non-invasive preconditioning for perforator flap microvascularity and perfusion.

METHODS: Two gluteal perforator flaps (matched control and intervention) were designed on sixteen 400g Sprague-Dawley male rats. NPWT was applied to the flap area directly continuously at -125mmHg for 7 days, after which the rats were divided into two principal groups. Group A (n=8) underwent 4D computed tomographic and angiography (CTA) with a body volume perfusion protocol after NPWT and euthanized. Group B (n=8), control and intervention flaps were raised, isolated on a single pedicle and laid back down and monitored for a further 7 days. Group B flaps were assessed using laser-assisted indocyanine fluorescence angiography before surgery, following flap harvest and at 7 days prior to euthanasia. Half of all rats in each group were analyzed with Micro-CT to assess the microvasculature. All paired specimens were assessed histologically with H&E and immunohistochemistry (IHC).

RESULTS: There was a 17% increase in CT tissue perfusion in skin treated with NPWT versus matched controls (P=0.001). LA-ICGFA demonstrated higher perfusion following NPWT treatment (P=0.006), however no significant difference immediate post flap harvest (P=0.19) but a difference was seen 7 days postoperatively (P=0.03). Micro-CT evaluation showed an increase in average vessel volume (%) from 0.005 in control to 0.009 in the NPWT flaps (P=0.04).H&E analysis showed significant difference in the epidermal thickness (P<0.001), but comparable dermal thickness (P=0.34).Quantitative analysis of CD31 IHC demonstrated a mean area fraction percentage of 4.30 and 2.68 in the NPWT and control flaps respectively (P=0.002).There was partial necrosis in the control (n=3) and NPWT flaps (N=1), however this was <5% in the NPWT flap.

CONCLUSION: We present novel multimodal approaches using static and dynamic imaging and histological assessment to provide a proof of concept on the use of NPWT for non-invasive conditioning of flaps. The study provides the basis for further investigation and clinical studies with potential for direct translation into clinical practice.

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Ex Vivo Normothermic Limb Perfusion and Limb Specific Monitoring Evaluation of Perfusion Quality

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PURPOSE: Ischemia time represents a significant limitation for successful extremity replantation and transplantation because of the rapid deterioration of ischemic muscle. Static cold storage (SCS) of the limb is the standard clinical practice. Normothermic ex vivo perfusion system has the potential to prolong viability providing oxygen and metabolites after limb amputation. The aim of our study was to establish a perfusion protocol with limb specific diagnostic tools to evaluate the quality and uniformity of perfusion in an ex vivo model.

METHODS: A total of 18 swine limbs were perfused, five of them followed the final, optimized protocol. Limbs were perfused at 39^{O}C for twelve hours using an oxygenated colloid solution with packed red blood cells. Glucose and electrolytes were kept within physiologic range by the addition of hypertonic solution or by partial hypotonic perfusate exchanges. Limb specific perfusion quality was assessed by muscle contractility upon electrical nerve stimulation, compartment pressure, creatine kinase(CK) and myoglobin concentrations, tissue oxygen saturation (near infrared spectroscopy), indocyanine green (ICG) angiography, and infrared radiation emission by thermographic imaging.

RESULTS: All five limbs reached the 12 hour perfusion target maintaining normal compartment pressure (16.23 ± 7.94 mmHg), minimal weight increase (0.54%±0.07), mean muscle temperature of 33.54 ± 1.5^{o}C, and tissue oximetry readings of 59.67%±10.21. Average values of final myoglobin and CK were 875 ± 291.4 ng/mL, and 53344 ± 14850.34 U/L, respectively. Muscle movement was present in all limbs until cessation of perfusion. Differences in uniformity and quality of distal perfusion were demonstrated using thermography and angiography imaging after 12 hours of perfusion. Colder areas on Thermographic imaging correlated to mal perfused areas on ICG angiography.