constructs that remained viable for more than 28 days in static culture. However, without exposing the vascular lining cells to flow, their functionality and in vivo stability are suboptimal. Here, we “prime” the constructs by dynamically perfusing them and determine how flow induced shear stress optimizes the endoluminal surfaces of our tissue-engineered vessels.

MATERIALS AND METHODS: Pluronic F127 fibers, 1.5 mm in diameter, were sacrificed in type I collagen, creating a central looped microchannel. 100 µL polyculture cell suspension mixture of 5 x 10^6 cells/mL of human foreskin fibroblasts and 5 x 10^6 cells/mL of human aortic smooth muscle cells was seeded into the microchannel. The following day, a 100 µL cell suspension of 5 x 10^5 cells/mL of human placental pericytes and 5 x 10^6 cells/mL of human umbilical vein endothelial cells was seeded into the microchannel. All constructs underwent daily media changes in static culture for 7 days, and then half were perfused at 10 dynes/cm² for an additional 7 days. After 14 days, scaffolds were fixed and processed.

RESULTS: After 7 and 14 days of culture, constructs formed intact endoluminal linings along the microchannel with increasing thickness over time. CD31 expressing endothelial cells were noted along the luminal surface after 7 days and throughout the endoluminal lining after 14 days, establishing a neointima. Constructs undergoing static and dynamic culture had robust, vascular linings that spanned the entire microchannel. However, dynamic constructs had a 59% thicker lining in the channel (p = 0.0057). Ki67 staining demonstrated statistically significant increased cell proliferation in constructs that experienced dynamic perfusion suggesting stimulation by the shear stress (p = 0.0429).

CONCLUSION: Shear stress through dynamic perfusion was used to optimize the development of a layer of vascular lining cells to provide a non-thrombogenic surface to allow continuous blood flow in these tissue engineered vessels. Exposing pre-vascularized engineered tissues to controlled perfusion produces vessels with architecture that more accurately recapitulates the in vivo phenotype and provides a surface for thrombosis-free blood flow, allowing for surgical implantation via microanastomosis.

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Single and Double Reinnervation of the Gastrocnemius Muscle in Rats - Experimental Model

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INTRODUCTION: Muscle contraction generated by electrical impulses originated simultaneously from two different neural sources may be an interesting alternative for facial palsy and brachial plexus injury treatment. We hypothesized that double reinnervation leads to better muscle functional recovery. Thus, an experimental model was created to assess double and single muscle reinnervation of the gastrocnemius muscle in rats.

MATERIALS AND METHODS: Fifty adult Wistar rats after having their right peroneal nerve sectioned were allocated into 5 groups: (C) control; (TS) in which the right tibial nerve was also sectioned and not repaired; (EE) where after section, the right tibial nerve was immediately repaired by primary neurorrhaphy; (ES) where after section, the right tibial nerve was immediately repaired by end-to-end neurorrhaphy associated to end-to-side neurorrhaphy of the peroneal nerve to the tibial nerve distal to the primary neurorrhaphy site; and (CEE) where after section, the right tibial nerve was immediately repaired by convergent end-to-end neurorrhaphy between the proximal stumps of the tibial and peroneal nerves to the distal stump of the tibial nerve. The outcomes were assessed 12 weeks after the experiment by walking track, electromyography, gastrocnemius muscle weight ratio and histomorphometric analysis of distal tibial nerve.

RESULTS: Compared to simple innervation group (EE), the double innervation groups had higher functional results in walking track (p < 0.05). When compared to the EE group, the CEE group showed greater amplitude (p = 0.006) and higher latency (p = 0.041) to electromyography. Regarding muscle weight index, there was no difference between groups of single and double innervation (p > 0.705). Histologic analysis revealed higher axonal density in the CEE group compared to the EE group (p = 0.001) and the ES group (p = 0.002).
CONCLUSION: Both double innervation techniques (ES and CEE) showed earlier and greater functional recovery of the gastrocnemius muscle when compared with simple innervation technique (EE). Animals of the CEE group showed higher number of regenerated axons in the distal stump of the repaired nerve than animals of the others experimental groups.

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Fluorescent Angiography Demonstrates Changes in Wound Microvasculature as a Result of Hyperbaric Oxygen Therapy

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BACKGROUND: The efficacy of hyperbaric oxygen therapy (HBOT) to facilitate wound healing in diabetic lower extremity ulcers is well established.¹ The exact mechanism of HBOT-mediated wound healing is unclear, but is thought to relate to increased reactive oxygen species and reactive nitrogen species (ROS &RNS).² ROS and RNS lead to many downstream effects that impact wound healing including increased growth factors, diminished inflammatory responses and improved neovascularization. The impact on tissue perfusion, however, is not known. The purpose of this pilot study was to ascertain the effects of HBOT on the microvasculature of chronic wounds as assessed by fluorescent angiography as compared with healthy controls.

METHODS: Patients underwent fluorescent angiography at 4 different time points: immediately prior and immediately after the first and second HBOT treatments. Photo imaging with infrared camera began concurrently with the initiation of the IC-Green™ injection and lasted for 5 minutes. All videos were analyzed via MATLAB. The wound bed and the peri-wound area were then outlined as masks for the image analysis. 2 time points were determined: the onset of inflow and the time of maximal outflow (defined as the surrogate for onset of venous outflow).

RESULTS: Immediately after HBOT, there was evidence of increased flow. The onset of arterial inflow as well as venous outflow occurred earlier after the initial HBO treatment when compared with pretreatment in the chronic wound patients, indicating an immediate HBO associated vasodilatory effect. Interestingly, in healthy controls, the opposite phenomenon was observed in that both arterial and venous flow occurred in a delayed manner as a result of HBOT, perhaps suggesting a vasoconstrictive effect, consistent with prior reports.³

CONCLUSION: This pilot study demonstrates that hyperbaric oxygen therapy appears to immediately impact the microcirculation both on an inflow (arterial) and outflow (venous) level. Interestingly, results of this study suggest inherent differences in micro-vascular physiologic responsiveness between healthy and chronic wound patients. This work offers an insight into the potential mechanism of HBOT and may direct future applications and eventually customize care.

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