10:15 AM - 10:20 AM  
RM 146. **Perfusion of Tissue-Engineered Pre-Vascularized Skin Flap**  
*Weill Cornell Medical College, New York*  
Presenter: Justin S. Buro BS  
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**Introduction**

Fabrication of tissues with an inherent hierarchical vascular network remains the holy grail of tissue engineering. 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.

**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 5x10^6 cells/mL RFP-tagged human aortic smooth muscle cells (HASMC-RFP), 5x10^6 cells/mL GFP-tagged human umbilical vein endothelial cells (HUVEC-GFP), and the construct was then topically seeded with 1x10^6 cells/mL human epidermal keratinocytes (HEK). Multiphoton microscopy (MPM) and histology were conducted after 7 and 14 days of culture.

**Results**

MPM imaging demonstrates a hierarchical vascular network containing macro and microvessels lined by endothelial and smooth muscle cells and supported by perivascular pericytes, all in appropriate microanatomic arrangement. Neodermal HFF1 proliferated throughout the observation period and the HEK neoeipidermis remained stable along the superficial aspect of the construct.

**Conclusion**

We have successfully fabricated a tissue-engineered pre-vascularized full thickness skin flap construct with stable and anatomically appropriate vascularity. Damage to the skin due to trauma, burns, infection or disease represents a significant danger to the patient given that such injuries span large areas and leave few available sites for autologous tissue harvest. In this context, our platform provides tremendous promise in furthering the development of tissue-engineered skin and other types of pre-vascularized flaps.
Background

A singular challenge in Tissue Engineering and reconstructive microsurgery is growing fully vascularized tissue replacements in vitro for reconstructing open deep tissue wounds. Lack of a complete toolset to control the physical and biological parameters of these complex cultures and make them compatible with microsurgery, has prevented its realization. We have pioneered approaches to form microvascular networks within 3-D tissue scaffolds and present here an autonomous tissue cartridge (ATC) for growing vascularized tissues which solves these problems threefold: 1) Precise flow control within vessels, perfusing microvascular networks with nanoliter-precision. 2) Hardware enabling fluidic, thermal and atmospheric control of cultures in a compact, portable and versatile benchtop platform. Importantly, it eliminates conventional incubators and provides live, multi-wavelength fluorescence imaging 3) Scaffolding that enables microsurgical anastomosis of fully cellularized vessels within 3-D matrices in animal models. Experimental results from the system show the effect of hemodynamic forces on vascular cells and the use of adipose-derived mesenchymal stem cells (ADSC) as a cell source for building vascularized tissue constructs.

Methods Microvessel tissues were constructed in collagen and seeded with fluorescently labeled endothelial and Smooth-muscle/perivascular cells, combined with ADSC in the surrounding bulk tissue. The fully assembled microfluidic tissue culture device with enclosed microvessels was cultured in the benchtop system with live imaging for 7-14 days under pump-driven flow using a range of flow rates to achieve physiologic shear stress against the vessel walls, (0.5 Pa, 1.5 Pa, 2.5 Pa). Cellular identity, morphology, alignment and migration were analyzed via live and endpoint immunohistochemistry.

Results The ATC provided consistently stable environmental and temperature control throughout extended cultures. Live imaging revealed healthy confluent vessels with contiguous cell-cell junctions, intact cytoskeletons, pericyte coverage and dynamic endothelial cells migrating throughout the vessel walls, both downstream (with) and upstream (against) the flow direction. Microvessel cross-sectional areas expanded and changed profile from original lithographically defined squares toward elliptical cross-sections with larger dimensions. ADSC incorporated into the vasculature, influenced by density and proximity. Migrating cell displacement correlated positively with the applied shear stress, with maximum displacement at the highest shear 2.5 Pa. Similarly, cells elongated and aligned in the direction of flow with net alignment increasing with the magnitude of shear.

Conclusion This breakthrough sets the stage for future clinical translation of pre-vascularized tissues, provides new insights into relationships between hemodynamic forces, cell morphology and dynamics, and further advances studies of ADSC fate determination and mechano-biological molecular mechanisms by which cells sense shear stress.
Background: Free jejunal flap is one of the most commonly used flaps for esophageal reconstruction. However, ischemia-reperfusion (I/R) injury due to warm ischemia seen during the transfer limits its use. Because jejunum is one of the organs that are least tolerant to ischemia. Iloprost, a prostacyclin analogue, was shown to be a promising agent in reducing I/R injury in different organs including liver, heart, skeletal muscle and kidney. Favorable results obtained from animal and clinical case-control studies led researchers to initiate a prospective randomized clinical study on the effect of iloprost in liver transplantation. The goal of this study was to evaluate the effects of iloprost (ILO) and ischemic pre-conditioning (IPC) on intestinal I/R injury and to compare the effectiveness of these two modalities.

Methods: A 5-7 cm long jejunal segment was isolated with its supplying vessels under the microscope. Thirty-four Sprague-Dawley rats were randomized into 5 groups: Sham, I/R only (control), ischemic preconditioning (IPC), iloprost(ILO) and IPC+ILO. Jejunal flaps in all groups, except sham group, underwent ischemia for 60 min and reperfusion for 2 hours by occluding the flap pedicle. Before the ischemia, 1 mcg/kg normal saline or iloprost was injected as an intravenous bolus through the internal jugular vein in each group. Ischemic preconditioning was done by 3 cycles of 3 min ischemia and 3 min reperfusion in IPC and IPC+ILO groups. Flap perfusion was assessed by laser Doppler flowmetry throughout the study. The histologic sections were scored using Chiu scoring system. Superoxide dismutase (SOD) and myeloperoxidase (MPO) levels were measured via spectrophotometry.

Results: Animals administered iloprost or underwent IPC had significantly better post-ischemic recovery of mesenteric perfusion and histologic scores compared with control group. (Figure-1 and 2) Iloprost group had improved perfusion and histology of jejunal flaps more than IPC group following I/R injury. In treatment groups (ILO, PC, ILO+PC) the levels of the antioxidant enzyme, SOD, were increased and that of MPO, one of inflammatory markers, were reduced.

Conclusion: We showed that iloprost and ischemic pre-conditioning increased the mucosal perfusion and reduced the mucosal injury of jejunal flap following I/R injury. Particularly, iloprost might be considered as a novel treatment agent in the future to reduce the I/R injury of jejunal flaps.
Figure-1: Change in blood flow to the jejunal flap at different time points. IPC: Ischemic pre-conditioning; ILO: Iloprost.

Figure-2: Histological examination of ischemia-reperfusion injury in jejunal flap model. The lower scores correlate with less injury. IPC: Ischemic pre-conditioning; ILO: Iloprost.
Background: The goal of this study was to determine whether intraoperative treatment of facial nerves with polyethylene glycol (PEG) fusion technology improves facial nerve regeneration. PEG fusion technology has been shown to seal axonal membranes, restore both electrophysiological and morphological nerve continuity, and dramatically improve recovery times in rat sciatic nerve injury. This technology thus has a large potential impact in improving facial reanimation recovery time and effectiveness.

Methods: 30 rats were utilized in this study. 15 underwent facial nerve regeneration without PEG fusion, and 15 with PEG fusion. The facial nerve branches of the left side were exposed and facial paralysis initiated by transection of the buccal and marginal mandibular branches. The buccal branch was then repaired through standard microsuture technique. Following repair, the neurorrhaphy sites of rats in the PEG group were exposed to calcium free saline, methylene blue, and polyethylene glycol. Sites were washed with calcium-containing saline and incisions were closed. Nerve continuity was immediately assessed following surgery in five animals in each group through hematoxylin-eosin staining and electron microscopy. Electrophysiology and functional whisking movement was assessed in the remaining 10 in each group by EMG and motion analysis immediately after surgery, and then at weekly intervals for eight weeks. At 8 weeks, nerves and distal muscles were collected and histologically analyzed.

Results: PEG fusion technology immediately restored axonal continuity following surgery, demonstrated by electron microscopy. Electrophysiology was also similarly restored across the site immediately, determined through intraoperative nerve stimulation, in the PEG fusion group. The nonintervention group showed dramatically reduced functional recovery than the PEG fusion group following surgery, shown by lower whisking activity and poor electrophysiology outcomes. Furthermore, the PEG fusion group showed statistically significant higher fascicle counts, myelination diameter, axonal diameter, and distal muscle fibers histologically.

Conclusion: This study demonstrated the potential of polyethylene fusion technology in improving facial reanimation outcomes. PEG is already a FDA-approved drug, and thus the promise of this compound for other clinical applications is promising and can now be investigated further in the context of facial reanimation. We demonstrate promising clinical implications in improving function and recovery time in facial nerve regeneration for facial paralysis patients by utilizing this technology.
Methylene blue is first administered to prevent the cut ends of the axon membranes from sealing over. Polyethylene glycol is then introduced, which effectively “fuses” the severed axon ends, drastically decreasing the amount of time required to restore nerve function.
Usefulness of Transit Time Ultrasound Flowmetry to predict Long-Term Postoperative Microvascular Patency: Preclinical Study

Jesús Usón Minimally Invasive Surgery Center, Cáceres
Presenter: Alberto Ballestin, DVM, MSc
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Background

The value of the historical double forceps patency test is limited. It can only tell the surgeon if there is flow or not thorough an anastomosis, but it does not describe how is the blood flow.

Quantification of blood flow using flowmeters is accepted as the standard method for intraoperative patency assessment of coronary vascular anastomoses.

In reconstructive procedures, an intraoperative objective patency assessment of microsurgical anastomoses may be critical to the success of these procedures and it could be a key to avoid failure in anastomoses, flaps and vascular grafts. This will enable the surgeon to correct flow restrictions while the patient is still anesthetized.

The goal of this preclinical study was to evaluate microsurgical arterial and venous anastomoses using transit-time ultrasound technology to predict blood flow patency one week after the procedure.

Methods

Surgical end-to-end anastomoses were performed on 35 Wistar rat femoral arteries and veins.

Blood flow was assessed in the vessels prior to the microsurgical anastomosis, immediately after the anastomosis and one week postoperatively.

It was used transit-time ultrasound technology to quantify blood flow (in milliliters per minute).

We used as standard a novel published minimal cutoff flow value of 0.30ml/minute for vessels ranging from 0.6 to 1.2mm diameter to predict long-term postoperative vascular patency.

Results

The 35 arteries ranged from 0.6 to 1.0 mm diameter while the 35 veins ranged from 0.8 to 1.2 mm.

The mean postoperative blood flow for arteries was 0.52 ml/min ±0.19SD.

The mean postoperative blood flow for veins was 0.486 ml/min ±0.14SD.

Any postoperative blood flow measurement was lower than 0.3ml/min.
All 70 anastomoses were patent after one week. The mean value for arteries was 1.11ml/min ±0.71SD and for veins 1.032ml/min ±0.67SD.

Conclusion

The published cutoff value has reliably predicted in our study the patency of the anastomoses performed.

Using this cutoff value it seems reasonable to expect flowmetry will help to assess objectively the anastomoses intra-operatively and predict clinically both outcomes and the necessity for on table revision, thus improving the clinical outcomes of free-tissue transfer.
151. Ex-Situ Extended Limb Preservation: A Protocol for Porcine Limb Perfusion

Cleveland Clinic Foundation, Cleveland

Presenter: Edoardo Dalla Pozza, MD

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Background

Ischemia and reperfusion injury remains a significant limiting factor for the success of vascularized composite allotransplantation. Compared to the cold storage of the procured organs, normothermic ex-situ perfusion is a novel approach to prolong viability of the limb by maintaining physiologic cellular metabolism avoiding the deleterious effects of both hypoxia and cooling. This study aims to develop an ex-situ normothermic limb perfusion system to preserve the viability and function of amputated limbs for over 24 hours.

Methods

A total of 22 swine limbs were perfused. Limbs were perfused using an oxygenated colloid solution at 39°C containing washed RBCs. The first 13 limbs were used to optimize the perfusion protocol. The subsequent 5 limbs (Group A) were perfused for 12 hours and the following 4 limbs (Group B) as long as muscle contractility/peripheral perfusion was present. Electrolytes were kept within physiologic ranges by partial perfusate exchanges. Limb viability was assessed and compared in the 2 groups by muscle contractility, compartment pressure, tissue oxygen saturation, indocyanine green (ICG)angiography and thermography.

Results

Perfused limbs were able to retain physiological parameters and function for 12 hours in group A and up to 44 hours (2644) in group B. The final weight increase (0.54%±0.07 VS 16.25%±17.86) (p=0.15) and compartment pressure (16.23±7.10 VS 24.75±7.79) (p=0.175) were lower in group A compared to group B. The average muscle temperature was 33.54±1.5°C in group A and 35.15±1.40°C in group B (p=0.072). Final myoglobin and CK mean values were lower in group A compared with group B (875±291.4 ng/mL VS 1010.6±323.6 ng/mL and 53344±14850.34 U/L VS 71881±20475 ng/mL). Thermography and ICG angiography showed minimal variations of peripheral limb perfusion overtime in both groups.

Conclusion

This protocol has shown the potential to significantly impact outcomes in limb transplantation. Our results suggest that limbs with shorter ex-situ perfusion retain better physiologic parameters and muscular contraction. However, the continuous optimization of the protocol allowed to maintain function and peripheral perfusion of perfused limbs for up to 44 hours.
Background: Quality of life following surgical or traumatic injury is dependent on restoring form and function. As robust vascular supply and sensory-motor reinnervation are essential for wound healing and tissue regeneration, revascularizing and re-innervating soft tissues are critical aspects of reconstructive microsurgery. The ability to create autologous human neurovascular bundles (NVBs), from xenogeneic sources, is therefore a novel tool for a wide range of applications. For example: (i) rehabilitating massive soft-tissue scarring of the chest wall and enabling true erogenous sensation in the reconstructed nipple-areola complex post-irradiation therapy for breast cancer; (ii) creating healthy arteries for coronary artery bypass grafting or arterio-venous fistulae for dialysis; (iii) vascularizing and innervating synthetic scaffolds such as neurovascularized hybrid-synthetic bone scaffolds for jaw reconstruction; (iv) functionalizing engineered tissues, such as muscle, at a clinically relevant scale. We have developed bioreactors and protocols to create human neurovascular bundles using xenogeneic extracellular matrix (ECM) scaffolds from 4 progressively larger species (rat, rabbit, pig, and non-human primate) to demonstrate scalability. Based on the similarity of porcine vascular structures to human ones, this source has been selected as our primary ECM of choice for in vitro recellularization with human endothelial cells (ECs) and Schwann cells (SCs).

Methods: Femoral NVBs have been harvested from rat (2.0 cm), rabbit (3.5 cm), and pig (7 cm); and brachial NVBs have been harvested from non-human primates (10.0 cm). Tissues were decellularized (SDS, Triton-X) and characterized (histology, immunohistochemistry, PicoGreen® DNA assay, collagenase assay, and differential scanning calorimetry). Samples were recellularized with human ECs and SCs, for 5 days in a bioreactor. Final endothelial integrity is verified by SEM and vascular casting.

Results: Regenerated NVBs demonstrated vessels with intact human vascular endothelium, and nerves with viable human SCs. For decellularization, perfusion demonstrated no advantages over immersion; but for revascularization, pulsatile flow leads to a highly aligned and contiguous EC luminal lining. Specimens are currently being prepared for in vivo re-implantation in the NIH nude rat model (RNU Rat, Charles River Strain 316, Crl:NIH-Foxn1nu).

Conclusion: We have developed techniques for harvesting, decellularizing and recellularizing NVBs in 4 species. These novel "autologous" off-the-shelf NVBs aim to play an important role in reinnervation and revascularization for soft and hard tissue reconstruction, rehabilitation of massive scarring, and engineered tissues; and as free vascularized nerve grafts; while avoiding donor site morbidity and the need for immunosuppression.