yielding best decellularization outcome was selected for further assessment for the preservation of ECM content and vasculature and was transplanted orthotopically to assess the ability to reperfuse these decellularized limbs in vivo. RESULTS - Gross examination and DNA quantification of less than 50 ng/mg tissue demonstrated successful decellularization only in group 2 while preserving tissue matrix architecture, including vessels. The arterial network was found to be perfusable and intact during in vivo orthotopic transplantation of the decellularized limb. No outflow was observed, however, after 115 minutes of arterial reperfusion.

Conclusions: We describe a successful decellularization protocol for rat hindlimb VCAs by perfusion ex-vivo of 1% SDS. During its in vivo transplantation, decellularization alone allows for a perfusable arterial network and with further optimization, we aim to achieve venous perfusion as well.

THURSDAY, JUNE 10, 2021: TRANSPLANT/MICROSURGERY TOP SCORED ABSTRACTS

1

MicroRNAs: Potential For Molecular Modulation Of Mechanically-induced Skin Growth During TE

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Purpose: Tissue expansion (TE) is commonly utilized to promote skin growth prior to reconstructing a defect or deformity. In spite of its ubiquitous use, the role of molecular modulators during TE, such as microRNAs (miRNAs), has not previously been studied. MiRNAs are small endogenous molecules that regulate many biological processes, including cell proliferation, differentiation, and inflammatory response. Here, we investigate genome-wide changes in miRNA expression in skin during TE.

Methods: Changes in miRNA expression were evaluated in a porcine TE model. Full-thickness skin biopsies were collected after 1 hour, 24 hours, 3 days, and 7 days of expansion, as well as from unexpanded skin (control). RNA extracted from biopsies was analyzed with next-generation sequencing (NGS). Differential expression analysis was performed using R software with the Bioconductor-DESeq2 package. Results were corrected for multiple testing using the Benjamini-Hochberg method. A combination of adjusted p-value < 0.05 and |log2 (fold change)| > 1 were used as the threshold to determine the significance of differentially expressed (DE) miRNAs. Potential target genes for DE miRNAs were identified by in silico analysis using three data bases: miRDB, miRTarBase, and DIANA Tools with Tarbase. Functional enrichment analysis of target genes was performed using the g:GOSf functional profiling tool, and results were visualized using R and Cytoscape software.

Results: We identified 52 miRNAs that were differentially upregulated (n = 18) or downregulated (n = 34) during at least one of the tested timepoints during TE. At the four time points (1 hour, 24 hours, 3 days and 7 days), there were 15, 6, 22, and 20 DE miRNAs, respectively. Eight miRNAs (ssc-miR-193a-5p, ssc-miR-21, ssc-miR-9-1, ssc-miR-708-3p, ssc-miR-212, ssc-miR-196a, ssc-miR-15b, ssc-miR-184) were DE at more than one timepoint. The comparative analysis showed 9, 4, 16 and 15 unique miRNAs after 1 hour, 24, hours, 3 days and 7 days of TE, respectively. Gene Ontology and KEGG pathway analyses for predicted target genes demonstrated enrichment in cellular processes related to metabolism, transcription, translation, signal transduction, cell differentiation, migration, and angiogenesis.

2

Ex-vivo Normothermic Preservation Of Amputated Limbs With A Hemoglobin-based Oxygen Carrier (HBOC-201) Perfusate

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Background: Ex-vivo normothermic perfusion (EVNP) has been used as an alternative to static cold storage (SCS)
to improve allograft quality in solid organ and vascularized composite allotransplantation (VCA). Perfusates containing red blood cells (RBCs) have shown to improve outcomes during ex vivo normothermic organ perfusion when compared to acellular perfusates. However, the use of blood products is challenging due to limited availability, the need for cross-matching, and potential blood-borne infection transmission. To avoid limitations associated with the use of blood-based products, we evaluated the feasibility of EVNLP utilizing a polymerized Hemoglobin-Based Oxygen Carrier-201 (HBOC-201).

**Methods:** Twenty-four porcine forelimbs were procured from Yorkshire pigs following euthanasia. Six forelimbs underwent EVNLP with an HBOC-201 based perfusate, six with an RBC-based perfusate, and twelve served as static cold storage (SCS) controls. EVNLP termination criteria included systolic arterial pressure ≥115 mmHg, fullness of compartments, or tissue oxygen saturation drop by 20%. Limb contractility, weight change, compartment pressure, tissue oxygen saturation, oxygen uptake rates (OUR) were assessed. Perfusate fluid-dynamics, gases, electrolytes, metabolites, methemoglobin (MetHb), creatine kinase (CK), and myoglobin concentration were measured. Limb viability was assessed with indocyanine green (ICG) angiography, infrared thermography (IRT), and muscle histology.

**Results:** Warm ischemia time before EVNLP was 35.50±8.62 min in HBOC-201 perfused limbs and 30.17±8.03 min in RBC-perfused limbs (p=0.07). EVNLP duration in HBOC-201 and RBC-perfused limbs was 22.5±1.7 and 28.2±7.3 hours, respectively (p=0.04). Vascular flow (325±25 vs. 444.7±50.6 ml/min; p=0.39), OUR (2.0±1.45 vs. 1.3±0.92 mlO₂/min*g of tissue; p=0.80), lactate (14.66±4.26 vs. 13.11±6.68 mmol/L; p=0.32), and perfusate pH (7.53±0.25 HBOC-201; 7.50±0.23 RBC; p=0.82) were not significantly different between treatment groups. Additionally, flexor (28.3±22.0 vs. 27.5±10.6; p=0.99) and extensor (31.5±22.9 vs. 28.8±14.5; p=0.82) compartment pressures, contractility (3±2 vs. 4±1; p=0.57), and percent weight change (23.1±3.0% vs. 13.2±22.7%; p=0.07) were not significantly different between HBOC-201 and RBC groups. In HBOC-201 perfused limbs, MetHb levels increased, reaching 47.8±12.1% at endpoint. Methemoglobin saturation did not affect OUR (ρ = -0.15, r² = 0.022; p=0.45). Distal tissue preservation was confirmed by IRT and ICG angiography in both EVNLP groups. Hypoxic cell clusters were identified in the SCS control group at endpoint and were absent in both treatment arms.

**Conclusion:** HBOC-201- and RBC-based perfusates similarly support isolated limb physiology, metabolism, and function. Optimization of modifiable factors, including HBOC-201 oxidation, may extend EVNLP durations employing HBOC-201 and overcome logistical constraints of the utilization of traditional blood products.

**ACTA2+ Cells Activation and Dermal Changes During Skin Adaptation to Mechanical Forces**

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**Purpose:** Tissue expansion (TE) is based on the skin’s exceptional ability to regenerate under mechanical stress and is widely used to repair skin defects. However, knowledge about molecular mechanisms involved in maintaining skin integrity and homeostasis is limited. The present study aims 1) to elucidate the role of myofibroblasts in the mechanism of adaptation to mechanical stress exerted by an inflated tissue expander, and 2) to describe morphological changes in collagen structure that could lead to the re-establishment of dermal tension.

**Methods:** TE was performed on a porcine model. Each expander was placed subcutaneously over the ribs and two weeks later inflated with 30 cc of saline to induce subtle tension. After 1 day (acute stretch) and 7 days (sub-acute stretch) of expansion, the full-thickness skin biopsies were collected from the apex of the expander and control unexpanded skin (contralateral sites). Skin samples were fixed in formalin and embedded in paraffin for histological evaluation (Russell-Movat Pentachrome staining) or fixed in 4% PFA and embedded in OCT for immunohistochemistry staining (IF) of α-smooth muscle actin (α-SMA), a marker of myofibroblast. Area of fluorescent signal from α-SMA was calculated using ImageJ while collagen morphology was evaluated optically after staining.

**Results:** We compared the presence of α-SMA fluorescence between control biopsies and expanded biopsies after 1-day and 7-days of stretch. The immunofluorescence