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 EVNL duration 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
staining revealed 2.32 times more \(\alpha\)-SMA fluorescent staining in skin expanded for 1 day \((p = 0.0065)\) and 2.19 times more \(\alpha\)-SMA fluorescence in skin expanded for 7 days \((p = 0.0047)\). The increase in number of \(\alpha\)-SMA positive cells were mostly observed in the outermost 400µm of papillary dermis. Histological staining showed minimal collagen morphological changes in both the papillary and reticular dermis after 1-day of expansion. However, shortening of fibril length, increased density, and increased disorder were observed in the papillary dermis collagen after 7-days of subtle expansion.

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Correlation Of Face Transplant Smile Excursion Measurements With Emotional Evaluation By Artificial Intelligence

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**Purpose:** Examining the outcomes and progression of face transplants has expanded to include the recent advancements in artificial intelligence. This novel methodology has allowed for the objective exploration of emotional expression. To our knowledge, this examined emotional expression has yet to be correlated to the hypothesized functioning of the allograft. We have combined the result of the AI software with a software analyzing smile excursion; demonstrating the progression of the allograft in our face transplant patient. This study helps illuminate the software possibilities for objective measures in transplant progression.

**Methods:** Images were analyzed from our face transplant patient post-operatively at approximately 6-month intervals for four years. The still images for each time interval include both a neutral facial expression and a full-face smile with teeth. The images were taken from video clips of the patient and then analyzed using the FACEgram software (Hadlock & Urban, 2012). The measurements of the smile excursion were standardized based off of the pupil diameter and excursion was measured relative to the neutral expression of each time period. The emotional expression data was acquired using FaceReader AI software from Noldus Information Technology (Wageningen, The Netherlands). The emotional analysis was done on 2 second clips from clinical videos of the patient, the same videos used for the still images. This data was correlated with the smile excursion measurements.

**Results:** The post-operative smile excursion analysis from 6 to 49 months demonstrated a near double in smile excursion from 3.58mm to 6.04mm. The smile excursion data has a squared correlation of 0.705; a strong upward linear trend of excursion. From 6 to 35 months, the happy emotion increased gradually from 0% to 15.8% (49-month data unavailable). Performed correlative analysis showed a positive correlation coefficient of 0.63. This moderate to strong correlation demonstrates the intuitive relationship of happy emotion and lip excursion during full face smiling. The findings also substantiate the AI software’s detection of increasing activity in the lip corner puller action unit from zero to 3/5 intensity over the same period.

**Conclusion:** This study demonstrates the correlation of smile excursion to happy emotional expression in our face transplant patient. The correlation square of the lip excursion shows a trend of increased functioning of the allograft over time; substantiating the AI’s finding. This data provides evidence for the use of artificial intelligence as a measure of transplant strength progression. The conjoined analysis of patient images and videos has supported proof for the ability to analyze transplant healing progression and efficacy in an objective form. Hadlock, T. A., & Urban, L. S. (2012). Toward a Universal, Automated Facial Measurement Tool in Facial Reanimation. *Archives of Facial Plastic Surgery, 14*(4). doi:10.1001/archfacial.2012.111

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ICG-Fluorescence Lymphography After Immediate Lymphatic Reconstruction

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**Purpose:** Immediate lymphatic reconstruction (ILR), performed at the time of axillary lymph node dissection (ALND), has demonstrated promising reductions in the development of breast-cancer associated lymphedema. However, questions remain regarding the effects of adjuvant therapies on the continued patency of the lymphaticovenous anastomosis. The aim of our study is to assess