RM37 **Outcomes of Ex-Vivo Normothermic Limb Perfusion (EVNLP) with a Hemoglobin Based Oxygen Carrier (HBOC-201)**

Cleveland Clinic Foundation , Cleveland
Presenter: **Brian A Figueroa, MD**
Brian A Figueroa, MD(1), Carlos Xavier Ordenana, MD(1), Majid Rezaei, DDS, MSc(1), Sayf Said, MD(1), Lynn M Orfahli, BM(1), Henri Brunengraber, MD, PhD(2), Srinivasan Dasarathy, MD(1), Francis Papay, MD(1), Antonio Rampazzo, MD, PhD(3) and Bahar Bassiri Gharb, MD, PhD(4)
(1)Cleveland Clinic Foundation, Cleveland, OH, (2)Case Western Reserve University, Cleveland, OH, (3)Plastic Surgery, Cleveland Clinic Foundation, Cleveland, OH, (4)Plastic Surgery, Cleveland Clinic, Cleveland, OH

Background

Ex-vivo normothermic limb perfusion (EVNLP) has been proven to preserve limb viability and function for a longer duration than cold storage. Perfusates containing red blood cells (RBCs) as an oxygen carrier improve outcomes when compared to acellular perfusates. However, the use of blood products is challenging due to limited availability, need for cross-matching and potential transmission of blood-borne infections. Hemoglobin-Based Oxygen Carrier-201 (HBOC-201), a polymerized, highly purified bovine hemoglobin (Hb), has a room-temperature shelf life of 3-years, low immunogenicity, and an oxygen-carrying capacity similar to that of RBCs. The aim of this study was to compare outcomes of EVNLP with HBOC-201 to EVNLP with an RBC-based perfusate.

Methods

Eighteen porcine forelimbs were procured from Yorkshire pigs following euthanasia. Twelve limbs underwent EVNLP until the perfusate pressure reached 115 mmHg. Six limbs were perfused with an HBOC-201-based perfusate, 6 with an RBC-based perfusate and 6 were stored at 4°C (cold storage control). Hemodynamics (flow and pressure), perfusate electrolytes, arterial and venous gases, O_2 uptake rates (OUR), methemoglobin (MetHb), glucose, and lactate were measured. Limb temperature, weight, compartment pressure, and peripheral perfusion, assessed by indocyanine green (ICG) angiography and Infra-Red (IR) thermography, were recorded. Histologic evaluation of skin and muscle was performed with H&E.

Results

EVNLP duration in HBOC-201 and RBC-perfused limbs was 22.5 ± 1.7 and 28.2 ± 7.3 hours, respectively (p=0.04). Throughout perfusion, HBOC-201 and RBC forelimbs exhibited comparable results regarding vascular flow (325±25 vs. 444.7±50.6 ml/min; p = 0.39), OUR (2.0 ± 1.45 vs. 1.3 ± 0.92 mlO_2/min*g of tissue; p = 0.80), and weight change (23.1 ± 3.0% vs. 13.2 ± 22.7; p= 0.07). In the HBOC-201 group, MetHb levels increased reaching a total of 47.85 ± 12.1% at end-point. Throughout EVNLP, electrolytes increased within groups. Differences in flexor (28.3 ± 22.0 vs. 27.5 ± 10.6) and extensor (31.5 ± 22.9 vs. 28.8 ± 14.5) compartment pressures did not reach statistical significance (flexor p=1; extensor p=0.82). At the end of perfusion, HBOC-201 limbs had a significant increase in lactate concentrations (20.2 ± 2.82 vs.

14.6 ± 3.9; p= 0.02). The preservation of peripheral tissue perfusion was confirmed by IR thermography and ICG angiography in both groups.

Conclusion

The results of this study suggest that there is a trend toward better EVNLP outcomes with an RBC-based perfusate. However, for shorter durations of EVNLP, HBOC-201 could be effective as an oxygen carrier in EVNLP, overcoming logistical constraints imposed by cross-matching and blood availability.

RM70 **Development and Evaluation of a Machine Learning Prediction Model for Flap Failure in Microvascular Breast Reconstruction**

Division of Plastic Surgery, University of Toronto, Toronto
Presenter: **Anne C O'Neill, MD PHD**
Anne C O'Neill, MD PHD(1), Dongyang Yang, PhD(2), Melissa Roy, mD PHD(1), Stefan O.P. Hofer, MD, Ph.D., FRCS(C)(3) and Wei Xu, PhD(4)
(1)University Health Network, Toronto, ON, Canada, (2)Princess Margaret cancer Center, Toronto, ON, Canada, (3)Division of Plastic & Reconstructive Surgery, University Health Network, Toronto, ON, Canada, (4)Princess Margaret Cancer Center, Toronto, ON, Canada

Background In spite of high success rates, flap failure remains an inherent risk in microvascular breast reconstruction. Identifying patients who are at high risk for flap failure would enable us to recommend alternative reconstructive techniques. However, as flap failure is a rare event, identification of risk factors is statistically challenging. Machine learning is a form of artificial intelligence that automates analytical model building. It has been proposed that machine learning can build superior prediction models when the outcome of interest is rare.

Methods In this study we evaluate machine learning resampling and decision tree classification models for the prediction of flap failure in a large retrospective cohort of microvascular breast reconstructions.

Results One thousand and twelve patients were included in the study. Twelve patients (1.1%) experienced flap failure. Multivariate logistic regression failed to identify significant predictors of flap failure. Informed oversampling strategies created synthetic events for model training, resulting in the highest predictive accuracy. The ROSE informed oversampling technique and decision tree classification resulted in a strong prediction model (AUC 0.95) with high sensitivity and specificity. In the testing cohort the model maintained acceptable specificity and predictive power (AUC 0.67) but sensitivity was reduced. The model identified four high-risk patient groups. Obesity, comorbidities and smoking were found to contribute to flap loss. The flap failure rate in high -risk patients was 7.8% compared to 0.44% in the low risk cohort (p=0.001).

Conclusion This machine learning risk prediction model suggests that flap failure may not be a random event. The algorithm indicates that flap failure is multifactorial and identifies a number of potential contributing factors that warrant further investigation.

RM39 **The Effect of Topical Tacrolimus on Pedicled Flap Survival: The Secret Is in the Sauce**

Van, NY
Presenter: **Y-vu Robert Van, MD**
Y-vu Robert Van, MD(1), Gal Wald, BS(2), Connie Lu, BS(3), Arash Samadi, BS(2) and David Otterburn, MD(4)
(1)New York Presbyterian, NY, NY, (2)Weill Cornell Medical College, NY, NY, (3)Weill Cornell Medicine, New York, NY, (4)New York Presbyterian Hospital - Weill Cornell Medical Center, New York, NY

Background
Skin necrosis is a known postoperative complication of mastectomies. The pathophysiology of tissue necrosis involves lymphatic congestion, followed by venous congestion and ultimately arterial insufficiency. Recent mouse model studies have shown topical tacrolimus to increase growth of lymphatic collateral vessels and decrease lymphedema, potentially obviating the cycle of necrosis and increasing skin survival. The purpose of this study is to investigate the effect of topical tacrolimus on skin flap necrosis in a rat model.

Methods
A 3x10cm cranially based dorsal skin flap was raised and re-inset on twenty-two Sprague Dawley rats. They were then randomized to either the control (topical petroleum jelly) or the treatment (topical 0.1% tacrolimus) arm. 0.2 grams of either ointment was spread over the flap and then covered with an occlusive dressing. Dressings were changed daily with reapplication of both the topical ointment and the occlusive dressing. The rats were sacrificed 7 days post-op; areas of viable tissue, reversible ischemia and full thickness necrosis were measured with Fiji software and comparative analysis was performed with GraphPad statistical software.

Results
The average area of the dorsal flaps in the control and tacrolimus groups was 22.5 and 23.9 cm^2, respectively. In the control cohort, the average viable area was 42.4%, the average reversible ischemia area was 43.6%, and the average necrotic area was 13.9%. In the tacrolimus cohort, the average viable area was 31.5%, the average reversible ischemia area was 59.3%, and the average necrotic area was 9.2%. Total necrotic area was significantly lower in rats receiving topical tacrolimus as compared to controls ($p=0.015$). Furthermore, the ratios of necrotic to reversible ischemia and necrotic to viable tissue were significantly lower in the tacrolimus group as compared to controls ($p=0.003$, $p=0.015$). Of note, there was one incidence of wound dehiscence secondary to rodent self-removal of dressings and suture that required re-operation and re-inset. There were no other complications.

Conclusion
Topical tacrolimus was associated with significantly less full thickness necrosis as compared to topical petroleum jelly in this model. Future animal studies investigating dosages, differences in pre and post-operative application, and histological examination will further delineate the use of tacrolimus in flap healing, survival and eventual clinical use.

RM40 **Variations in Axillary Artery Branching: Anatomy and Relevance in Microvascular Reconstruction**

Mayo Clinic Arizona, Phoenix
Presenter: **Danielle A Thornburg, MD**
Danielle A Thornburg, MD(1), Nikita Gupta, BS(2), Cameron M Thiele, BS(2), William J. Casey III, MD(3), Sonya Van Nuland, PhD(4) and Natalie Langley, PhD(4)
(1)Mayo Clinic, Phoenix, AZ, (2)Mayo Clinic Alix School of Medicine, Scottsdale, AZ, (3)Division of Plastic and Reconstructive Surgery, Mayo Clinic Arizona, Phoenix, AZ, (4)Mayo Clinic College of Medicine and Science, Scottsdale, AZ

Background
Variable branching patterns of the axillary artery have been documented in the literature, primarily as case reports. Microvascular and pedicled flap reconstruction frequently utilizes branches of the axillary artery and requires in-depth knowledge of the anatomy of this artery. Few studies to date have comprehensively examined the anatomical variations in the axillary artery within a large cohort of cadaveric limbs.

Methods
The axillae of 34 cadaveric limbs were dissected for in the Mayo Clinic Arizona Department of Anatomy to examine the branching patterns of the axillary artery. The branching patterns of the subscapular system, lateral thoracic, anterior and posterior circumflex arteries were cataloged and digitally rendered. IRB approval was obtained from the Mayo Clinic Biospecimens Subcommittee.

Results
The most common anatomical variations were observed in the lateral thoracic artery, anterior and posterior circumflex humeral arteries, and the subscapular artery tree. The lateral thoracic artery was found in its most commonly recorded location in 44% of dissected limbs. The anterior and posterior circumflex humeral arteries were found in their expected locations 74% and 56% of the time, respectively. The most common variations included aberrant subscapular tree branches, including a posterior circumflex humeral artery arising from the subscapular artery (18%) and lateral thoracic artery arising from the subscapular artery (26%). In 21% of limbs, the anterior and posterior circumflex humeral arteries were found arising from a common trunk. Furthermore, anatomical variations were identified that have not previously been recorded in the literature including the lateral thoracic artery originating from the thoracodorsal artery rather than directly from the axillary artery (26.4%). Two cadaveric thoracodorsal arteries which originated from the circumflex scapular artery (5.9%) and one cadaveric superior thoracic artery (2.9%) gave off the thoracodorsal artery.

Conclusion
Knowledge of the common anatomical variations and branching patterns of the axillary artery is critical during surgical and other interventional procedures. Flap reconstruction relies heavily upon vascular anatomy and knowledge of these possible branching patterns is paramount during procedures that rely upon the axillary artery for a flap's blood supply. We identified variations that may alter intraoperative dissection and the ultimate operative plan.

RM41 **Mitigating Ischemia Reperfusion Injury (IRI) and Vascular Thrombosis in Vascularized Composite Allotransplantation (VCA)**

Medical University of South Carolina, Charleston
Presenter: **Jerec Ricci, MD**
Jerec Ricci, MD, Caroline Wallace, PhD, Fernando Herrera, MD, Stephen Tomlinson, PhD, Satish Nadig, MD, PhD and Carl Atkinson, PhD
Medical University of South Carolina, Charleston, SC

Background: Vascularized composite allotransplantation (VCA) provides an avenue to restore both aesthetic appearance and function of lost or devitalized tissues. Ischemia reperfusion injury (IRI) is an unavoidable event in the transplantation processes which is characterized by the upregulation of adhesion molecules, such as P-selection (PSel), and the complement system. These immune events prime the graft for immune cell infiltration and graft injury. We have designed a dual functioning complement inhibitor which targets to the graft by utilizing a PSel single chain variable fragment (scFv), which blocks PSel functions, and that is linked to a C3 convertase inhibitor (Crry), to inhibit complement activity. Here we demonstrate that administration of PSel-Crry reduces IRI and targets specifically to the grafted tissues. **Methods:** The efficacy of PSel-Crry construct was investigated utilizing two murine model systems; 1. C57Bl/6 Hindlimb IRI model, and 2. Syngeneic C57Bl/6-C57Bl/6 orthotopic vascularized composite limb transplant model (VCA). Mice were randomized into two groups; Control or Psel-Crry treated with inhibitors injected i.v immediately post reperfusion at either 0.25 or 0.5 mg. PSel-Crry targeting was determined using maestro in-vivo imaging of Dynalite labeled protein. Efficacy was determined by histological, serological and limb perfusion measurements at 6 and 24 hrs post reperfusion. **Results:** In-vivo maestro imaging demonstrated preferential biodistribution of labeled P-sel-Crry to the ischemic limb in hindlimb IRI studies. In therapeutic studies, PSel-Crry dose dependently decreased leukocyte infiltration, reduced edema and skeletal muscle cell injury resulting in an overall reduction in IRI. Measurement of limb perfusion in the VCA mouse model was performed, with limb perfusion and edema significantly improved following administration of the Psel-Crry inhibitor in a dose response fashion. **Conclusion:** Taken together these data demonstrate that these novel bi-functional inhibitors exhibit site-specific action and significantly reduce IRI associated limb injury. Future studies are needed to determine whether inhibition of IRI with these novel inhibitors impacts the severity and tempo of acute rejection.

RM42 **Effects of Breast Reconstruction on Cutaneous Gene Expression in Irradiated Breast Cancer Patients**

University of Wisconsin , Madison
Presenter: **Rebecca L. Farmer, MD, PhD**
Rebecca L. Farmer, MD, PhD(1), Kirsten A. Gunderson, BS(2), Sarah M. Lyon, MD(2), Jacqueline S. Israel, MD(1), Sandra Splinter BonDurant, MS(3), Katherine M. Gast, MD, MS(2), Samuel O. Poore, MD, PhD(1) and John W. Siebert, MD(2)
(1)University of Wisconsin - Madison, Division of Plastic Surgery, Madison, WI, (2)University of Wisconsin, Division of Plastic and Reconstructive Surgery, Madison, WI, (3)University of Wisconsin Gene Expression Center, Madison, WI

Background: Radiation-induced skin changes are a notorious adverse effect of adjuvant radiation therapy for breast cancer. While the acute changes of radiation therapy eventually resolve, the quality of the skin is forever altered, leading to wound healing complications and possible failure of breast reconstruction. The molecular mechanisms by which radiation alters the clinical behavior of breast tissue are not well understood. Furthermore, while anecdotal evidence indicates that certain reconstruction techniques can improve the quality of irradiated skin, the genetic alterations underlying these phenotypic changes are unknown. We utilized RNA-Seq technology to analyze the whole genome expression of irradiated and non-irradiated breast tissue to identify the major pathways that are affected by radiation therapy. We also used this technology to determine if any of the dysregulated genetic pathways in irradiated patients were altered by autologous reconstruction techniques.

Methods: Skin samples were collected from irradiated and non-irradiated breast cancer patients at the time of mastectomy and reconstruction. Patients undergoing delayed or immediate autologous reconstruction were included, and additional samples were collected 3-6 months later at the time of secondary revision. Breast reduction patients were included as controls. All samples were analyzed using RNA-Seq to determine the cellular transcriptome for irradiated versus non-irradiated breast skin, both before and after reconstruction. Gene expression was then analyzed via hierarchical clustering to determine the biologic pathways that were altered by radiation and reconstruction.

Results: Thirty patients were enrolled for analysis, including 10 irradiated and 10 non-irradiated autologous reconstruction patients. Whole genome analysis of these patient samples demonstrated multiple dysregulated biologic pathways in irradiated tissue, primarily in the areas of tissue inflammation, hypoxia and fibrosis. In irradiated patients who underwent reconstruction with autologous free tissue transfer, a significant number of these dysregulated genetic pathways were reversed to more normal levels following reconstruction.

Conclusion: Through whole genome analysis of breast cancer patients, we identified several of the main biologic pathways that are altered by radiation and demonstrated that many are corrected by autologous reconstruction efforts. Plastic surgeons can use these findings to specifically tailor reconstructive surgery in irradiated patients in an attempt to reverse radiation-induced tissue changes and improve overall reconstructive outcomes.

RM43 **Systemic-Intraosseous Delivery of Dystrophin Expressing Chimeric (DEC) Cell Therapy As a Novel Approach for Muscle Regeneration and Restoration of Function**

University of Illinois at Chicago, Chicago
Presenter: **Maria Siemionow, MD, PhD, DSc**
Maria Siemionow, MD, PhD, DSc, Michal Harasymczuk, MD, Paulina Langa, PhD and Ahlke Heydemann, PhD
University of Illinois at Chicago, Chicago, IL

Background

Chimeric cells, created *via ex-vivo* fusion of donor and recipient cells, represent a promising therapeutic option for tissues regeneration. We previously confirmed that local delivery of Dystrophin Expressing Chimeric (DEC) cells strengthens gastrocnemius muscle (GM) function 90 days after intramuscular injection. This study assessed therapeutic effect of systemic-intraosseous delivery of human DEC lines on restoration of muscle strength and function tested in the *mdx/scid* mouse model of Duchenne muscular dystrophy (DMD), offering a novel universal approach for muscle regeneration.

Methods

DEC lines were created *via ex-vivo* fusion of normal human myoblasts (MB^{N1}/MB^{N2}), and normal and DMD-affected myoblasts (MB^{N}/MB^{DMD}). The phenotype of DEC was assessed by flow cytometry ($CD56^{+}$, $CD90^{+}$, $CD45^{-}$, $CD34^{-}$) and DNA stability by COMET assay. Therapeutic effect of DEC was tested in *mdx/scid* mice after systemic-intraosseous injection: Group 1 – vehicle (control), Group 2 – MB^{N1}/MB^{N2} ($0.5x10^{6}$) and Group 3 – MB^{N}/MB^{DMD} ($0.5x10^{6}$). At 90 days after injection, DEC engraftment was assessed by Immunofluorescence in GM, diaphragm and heart muscle for dystrophin expression and by H&E for presence of inflammation and fibrosis. Functional tests included grip strength, *ex vivo* muscle strength and plethysmography. MRI was performed to exclude tumorogenicity of DEC.

Results

We confirmed feasibility of DEC creation by *ex vivo* fusion. COMET assay on DEC culture confirmed fusion safety by lack of DNA damage. DEC engraftment was confirmed by restoration of dystrophin expression in GM, diaphragm and heart at 90 days after intraosseous delivery. This correlated with improved functional outcomes of: grip strength increase (Group 2 – 36.8% and Group 3 – 38.2 % compared to Group 1 vehicle control revealing 18.9% decrease). *Ex vivo* muscle force test confirmed reduced skeletal muscle disease by increased muscle force (Group 2 – 19.4g±3.36 and Group 3 – 27.32g±1.63 compared to Group 1 vehicle controls – 8.6g±2.04), and decreased muscle fatigue (Group 3 – 29.14%±3.51 compared to Group 1 controls – 45.55%±1.24) and increased muscle weight in Groups 2 and 3. Systemic effect of DEC was confirmed by significant improvement of respiratory function in Group 3 by plethysmography. MRI confirmed lack of tumor-like changes at 90 days after DEC injection.

Conclusion

This study confirmed efficacy and safety of DEC in restoration of muscle function following systemic-intraosseous injection. DEC therapy offers novel, universal approach for restoration of muscle function in patients suffering from muscle atrophy or traumatic muscle tissue loss and introduces promising solution for regeneration of muscle components of the VCA.

RM44 **Effect of Silicone Implant Shells on Proliferation of Triple Negative Breast Cancer Cells (TNBC-468) in Engineered Biomimetic Patient-Derived Breast Tissue**

Weill Cornell Medicine, New York
Presenter: **Ishani D Premaratne, BA**
Ishani D Premaratne, BA(1), Matthew A Wright, BA(2), Runlei Zhao, MD(1), Mariam Gadjiko, BA(1), Giorgio Inghirami, MD(1), Kristy A Brown, PhD(1) and Jason A Spector, MD(3)
(1)Weill Cornell Medicine, New York, NY, (2)Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, (3)Division of Plastic and Reconstructive Surgery, New York Presbyterian Hospital, Weill-Cornell Medical Center, New York, NY

Background

As its name implies, breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is associated with, and in fact caused by the presence of silicone breast implants. Although our lab has previously demonstrated that the presence of silicone implant shells increase BIA-ALCL cell proliferation in a high-fidelity *ex vivo* biomimetic, three-dimensional model, the effect of silicone in proximity to breast cancer cells remains largely unknown. In this study we use this powerful model system to study the effects of implant shells on triple negative breast cancer cells (TNBCs) within the breast microenvironment.

Methods

Patient-derived breast tissue was processed for its component adipocytes, organoids, and stromal vascular fraction. These were suspended within 50 µl of 0.3% type I collagen matrix with TNBC-468 cells at a density of 200,000 cells/mL before being plated into 6mm wells. As a control, TNBC-468 cells were suspended within type I collagen without breast components. Before plating, wells were lined with either textured, smooth, or no implant shells. These were 1cm by 2cm pieces of implant shell dissected from the whole implant, cleaned of silicone gel, and autoclaved before being placed into the wells with the superficial aspect of the shell facing inward. Eight wells were plated per implant shell type: four biomimetic platform wells and four collagen-only controls. All groups started at an equal density of 1000 TNBC-468s cells per confocal snapshot. Wells were imaged immediately and every other day using confocal microscopy.

Results

TNBC-468 cell proliferation was significantly more robust in the biomimetic platform relative to the collagen-only groups regardless of implant shell type (p-value less than 0.01). However, there was no statistically significant difference in TNBC-468 proliferation between smooth, textured, and no implant groups. After ten days in culture, mean cell counts in the textured and smooth shell biomimetic groups were 3120±523 and 3368±233, respectively, compared with 2811±120 in the biomimetic group lacking implant shell **(Figure 1).**

Conclusion

Within a tissue-engineered, three-dimensional *ex vivo* model of the breast microenvironment that incorporates different silicone shell types, we have demonstrated that TNBC-468 cells proliferate more robustly within the biomimetic platform when compared to collagen matrix alone. However, neither textured nor smooth silicone implant shells promote increased proliferation of breast cancer cells. Although further study is warranted, these data indicate that silicone shells themselves do not directly promote TNBC proliferation and growth.

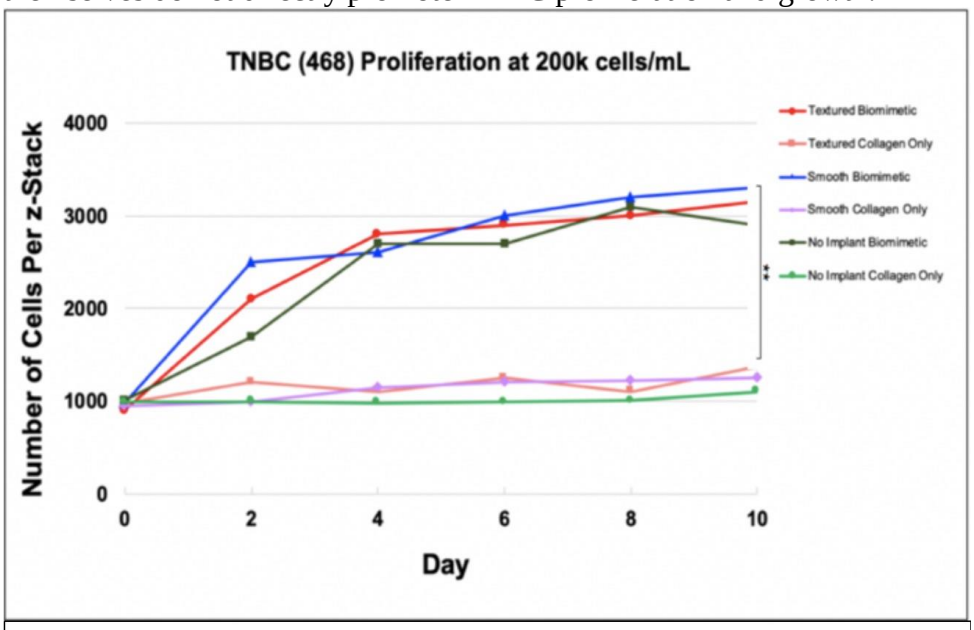


Figure 1. TNBC-468 cell proliferation over 10 days in 6 different groups, with groups differing in terms of biomimetic platform versus collagen-only controls and in terms of implant shell type (textured, smooth, or no implant).

RM45 **Combined Antagonism of Early Inflammatory Pathways Does Not Prevent Acute Rejection in Vascularized Composite Allotransplantation (VCA)**

Cleveland Clinic Foundation, Cleveland
Presenter: **Majid Rezaei, DDS, MSc**
Majid Rezaei, DDS, MSc(1), Carlos Xavier Ordenana, MD(1), Vahe Fahradyan, MD(1), Brian Alexander Figueroa, MD(1), Robert Fairchild, PhD(1), William Baldwin, MD PhD(1), Danielle Kish, PhD(1), Francis A. Papay, MD(2), Antonio Rampazzo, MD, PhD(3) and Bahar Bassiri Gharb, MD, PhD(4)
(1)Cleveland Clinic Foundation, Cleveland, OH, (2)Cleveland Clinic, Cleveland, OH, (3)Plastic Surgery, Cleveland Clinic Foundation, Cleveland, OH, (4)Plastic Surgery, Cleveland Clinic, Cleveland, OH

Background: Long-term immunosuppression, required to prevent rejection of vascularized composite allografts, is associated with systemic side effects, such as drug toxicity, opportunistic infections, and malignancy. Antagonism of early inflammation following transplantation through blockade of three key mediators, Tumor Necrosis ? (TNF-?), CD40 and Lymphocyte Function Associated Antigen 1 (LFA-1) has shown to prolong the survival of heart allografts up to 100 days. TNF- ? is a mediator of ischemia-reperfusion injury and its increased systemic levels has been detected during acute rejection episodes. CD-40/CD40L pathway mediates activation of cytotoxic lymphocyte response, B-cell pathways and production of TNF-?. LFA-1 is critical for T cell arrest on the vascular endothelium and activation. The goal of this study was to characterize the efficacy of combined suppression of early inflammatory pathways in preventing acute rejection in a rat hindlimb transplant model. **Methods:** Twelve hindlimb transplants were performed from ACI donors to Lewis recipients. Animals in the control group (n=6) received Tacrolimus (1mg/kg), Mycophenolate (20mg/kg) and Methylprednisolone (0.5mg/kg) daily for 100 days. In the experimental group (n=6), Anti-LFA1 (10mg/kg) monoclonal antibody was given on the day before surgery. On transplantation day, anti-TNF-? (10mg/kg) and anti-CD40L (16mg/kg) monoclonal antibodies were administered. On post-operative day 1, all three antibodies were given. **Results:** The average survival rate for the control group was 81±27 days. On average, the animals lost 23% of their initial weights. Three animals survived until the end point of the study. Three animals died for failure to thrive and development of gastrointestinal tumors. In the experimental group, all animals presented acute rejection with complete necrosis of the allograft within an average of 8±2 days. The survival rates of the 2 groups were significantly different ($p<0.001$). Histology of the skin and muscle samples in the control group showed normal epidermis, dermis and muscular tissue, while the experimental group displayed necrosis of the epidermis and massive inflammation of underlying dermis and muscle. **Conclusion:** Our results showed that the triple antibody regimen alone would not prevent acute rejection in this model of VCA. This strategy does not seem strong enough to prevent the immediate response of the immune system. The efficacy of the combined antibody treatment following induction with conventional agents has to be investigated yet.

RM46 **Potential of Polyethylene Glycol Fusion to Improve Cross-Face Nerve Graft Outcomes**

Mayo Clinic, Rochester
Presenter: **Marissa Suchyta, BA**
Marissa Suchyta, BA(1), Huan Wang, MD, PhD(2), Karim Bakri, MBBS(3) and Samir Mardini, MD(4)
(1)Mayo Clinic, Rochester, MN, (2)Neurologic Surgery, Mayo Clinic, Rochester, MN, (3)Plastic Surgery, Mayo Clinic, Rochester, MN, (4)Division of Plastic Surgery, Mayo Clinic, Rochester, MN

Background: The goal of this study was to determine whether intraoperative treatment of facial nerves with polyethylene glycol (PEG) fusion technology improves outcomes in a rat cross-face nerve graft model. 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 cross-face nerve graft recovery time and effectiveness.

Methods: 30 male Lewis rats were utilized in this study. 20 rats underwent cross-face nerve grafting with donor common peroneal nerve grafts harvested from the remaining 10 rats. Immunosuppression was not necessary due to extensive inbreeding of the rat strain. In recipient rats, both buccal and marginal mandibular branches of the facial nerve were cut on both sides of the face. The proximal buccal branch was coapted to the graft, which was tunneled over the skull and coapted to the distal buccal branch of the left side of the face. In 10 rats, polyethylene glycol was applied intraoperatively, whereas the other 10 received saline control. Electrophysiology and functional whisking movement was assessed 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 significantly less atrophy of distal muscle fibers histologically.

Conclusion: This study demonstrated the potential of polyethylene fusion technology in improving facial reanimation outcomes in cross-face nerve grafting. 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.

RM47 **Outcomes of Vascularized Bone Allotransplantation with Surgical Induced Autogenous Angiogenesis and Short-Term Immunosuppression in a Large Animal Model: Bone Healing, Pedicle Patency, Viability and Remodeling Due to Recipient Derived Neo-Angiogenesis**

Mayo Clinic , Rochester
Presenter: **Rudolph H. Houben, MD**
Rudolph H. Houben, MD(1), Patricia F. Friedrich, AAS(2), Alexander Y. Shin, MD(2) and Allen T. Bishop, MD(2)
(1)Mayo Clinic, Rochester, MN, (2)Department of Orthopedic Surgery, Microvascular Research Laboratory, Mayo Clinic, Rochester, MN

Background: Current treatment options for large bone defects are associated with significant problems and complications. Transplantation of living allogenic bone, a form of vascularized composite allotransplantation (VCA) would potentially combine the benefits of living bone and the ability to closely match the specific defect morphology without donor site complications. In this study we evaluate bone VCA reconstruction in a large animal model with surgical induced angiogenesis and short-term immunosuppression.

Materials and Methods: In this study, we assess bone healing, pedicle patency, formation of a neoangenic circulation, transplant viability, and bone remodeling after bone allotransplantation. We microsurgically transplanted 14 vascularized tibia segments for the reconstruction of a size-matched tibial defect in 14 Yucatan miniature swine with 20 weeks' survival time. All animals received two weeks of immunosuppressive therapy. Two groups were used- one with a patent implanted AV bundle, and another (ligated AV-bundle control). Radiographic evaluation of the allotransplant and vascular pedicle at 0, 2, 4, 6, 10 and 20 weeks after transplantation provided information on bone healing and pedicle patency. With the use of a micro-angiography, we were able to quantify the neoangenic circulation. Quantitative histomorphometry of the allotransplant at 20 weeks, provided information on transplant viability and remodeling.

Results: We successfully transplanted 12 bone only VCA's in a porcine model across a major mismatch in histocompatibility. Serial ultrasounds showed vascular pedicle patency was maintained until 4-6 weeks. Quantification of the micro-angiography, osteocyte viability and remodeling parameters showed significant increased transplant viability due to the formation of a neoangenic autogenous circulation. The implantation of a patent AV-bundle did not have an effect on bone healing scores over time.

Conclusion s: We demonstrate a technique useful for the experimental evaluation of bone only VCA with results consistent with an earlier preliminary report. Two weeks' immunosuppression is sufficient to maintain transplant blood flow for 4-6 weeks. Significant periosteal new bone formed by the allotransplant seen in both groups and the lack of significant differences in bone healing scores between groups suggests that this initial blood flow is responsible for much of the observed bone healing. The implantation of patent AV-bundle shows, significant better-maintained allotransplant viability and remodeling after withdraw of the immunosuppressive therapy. Bone VCA can safely be performed in a porcine animal model with reproducible results. More research should be performed to further evaluate the systemic and local immune response and repopulation of the allotransplant.

RM48 **Microsurgical Oxygen Biosensors for the Novel Monitoring of Surgical Flaps and Soft Tissue Manipulation**

Duke University School of Medicine, Durham
Presenter: **Preet S Patel, B.S.**
Preet S Patel, B.S.(1), Suhail Kamrudin K. Mithani, MD(2), Fraser J. Leversedge, MD(3), Steven M. Koehler, M.D.(4), David Kerr, M.D.(5), Bruce Klitzman, PhD(1) and Mohamed Ibrahim, M.D.(1)
(1)Duke University School of Medicine, Durham, NC, (2)Department of Orthopaedic Surgery, Duke University Medical Center, Durham, NC, (3)Department of Orthopaedic Surgery, DUKE UNIVERSITY, Durham, NC, (4)State University of New York (SUNY), Downstate Medical Center, Brooklyn, NY, (5)Duke University, Durham, NC

Background:

Current flap and replantation perfusion monitoring techniques have limitations in detection of tissue compromise, calibration, and cost. To address these limitations, we have designed an implantable sensor that can provide an accurate, cost-effective, and reliable real-time monitoring of tissue oxygenation.

Methods:

Experimental sensors of an oxygen-dependent fluorescence emitting benzo-porphyrin dye microporous platform were dermally implanted in a rat model within three distinct regions of tissue perfusion of a Superficial Inferior Epigastric Artery (SIEA) based flap and adjacent controls. (Fig. 1)

Blood flow was greatest at the base of the flap, diminishing towards the tip, thus creating a perfusion gradient. Changes in tissue oxygen tension (PO_2) were estimated by measuring the steady-state fluorescence of the optical sensors using an in vivo fluorescent (IVIS) imaging system. The sensors were used to collect data of tissue oxygen tension (TOT) during episodes of decreased environmental oxygen, induced acute vascular compromise, and longitudinally over a 7-day period of relative zones of tissue perfusion.

Additionally, sensors placed in human skin across a range of pigmentation with TOT readings taken and IVIS imaging performed.

Results:

Inspired FiO2 was decreased from 100% to 12% with a corresponding change in the TOT readings from all sensors. (Fig. 2)

Acute vascular compromise of the feeding blood vessels in the pedicle was immediately detected within 70 seconds (*$p<0.05$). (Fig. 3)

The tip portion of the flap demonstrated the most profound detection of decreased tissue perfusion and the base demonstrating the least with correlating TOT sensor readings. (Fig. 4)

Conclusion:

This study introduces and validates a novel technique to monitor acute vascular occlusion and flap viability in the immediate postoperative period in a validated rodent model. Sensor readings were taken from human tissue and demonstrated efficacy across a range of skin pigmentation. Future directions will be aimed toward clinical feasibility studies.

Fig. 1

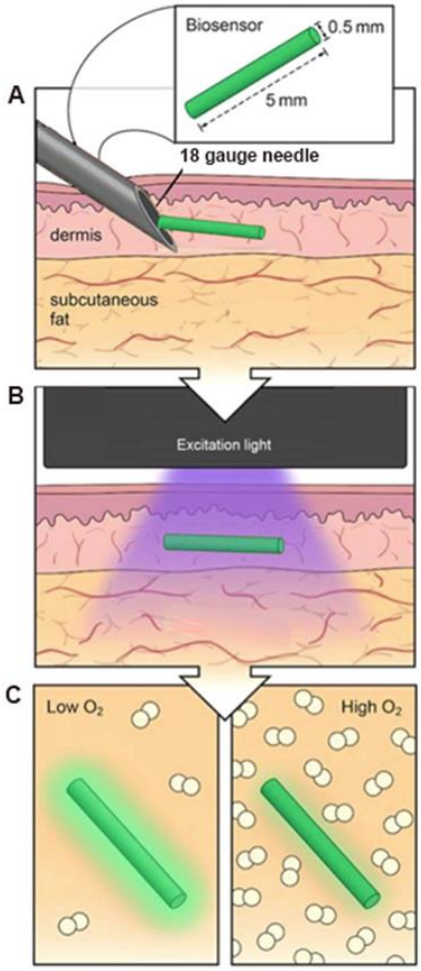


Fig. 2

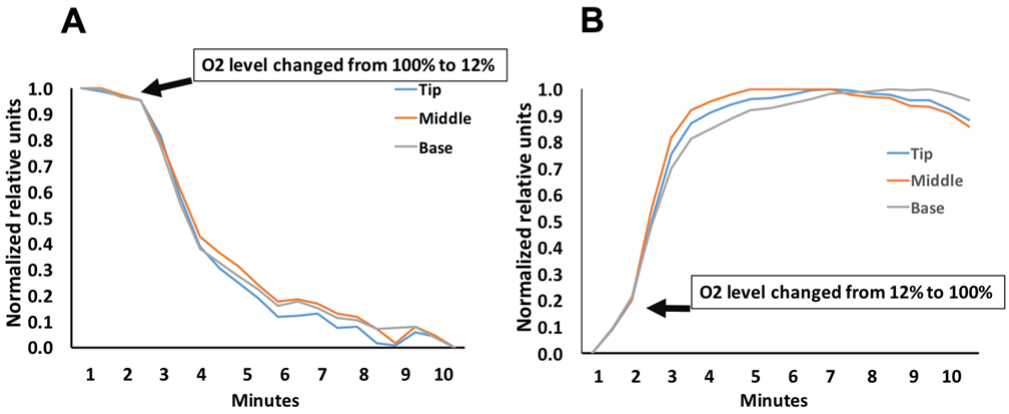

A
O2 level changed from 100% to 12%
Tip
Middle
Base
Normalized relative units
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 4 5 6 7 8 9 10
Minutes
B
Tip
Middle
Base
O2 level changed from 12% to 100%
Normalized relative units
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 4 5 6 7 8 9 10
Minutes

Fig. 3

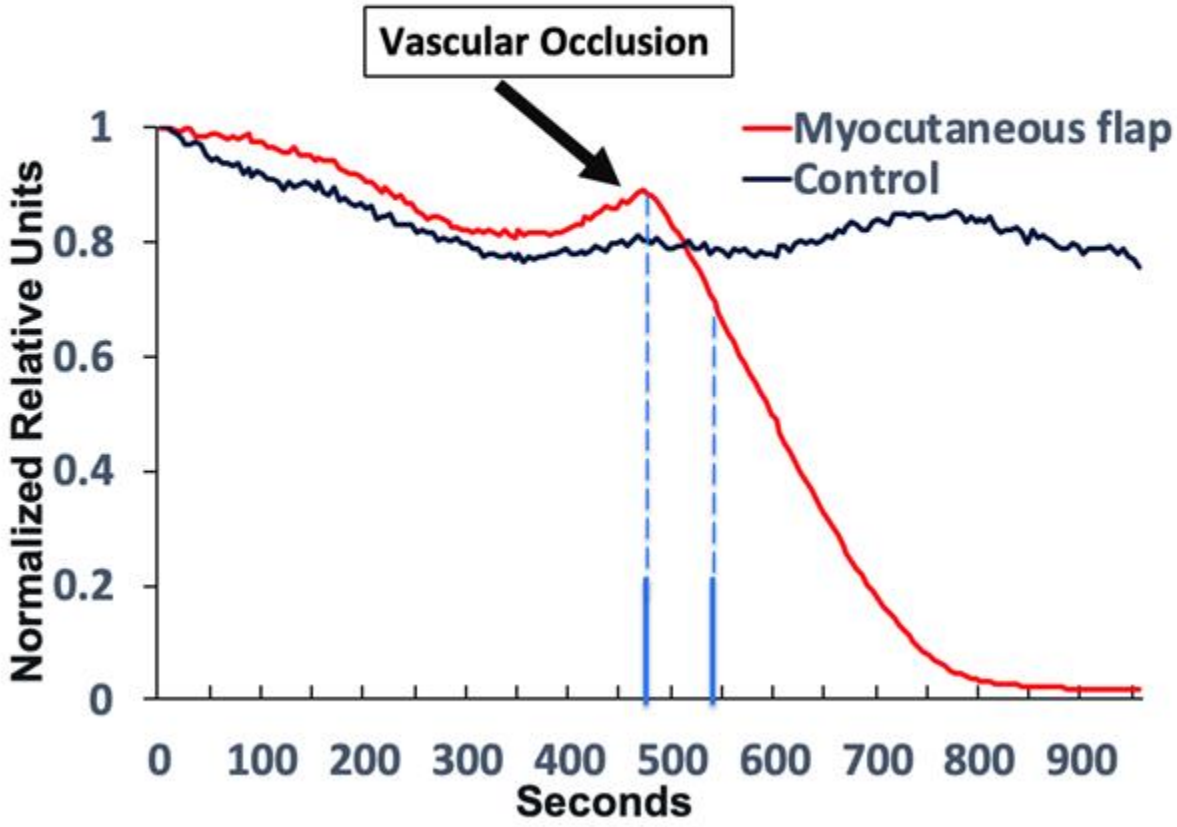
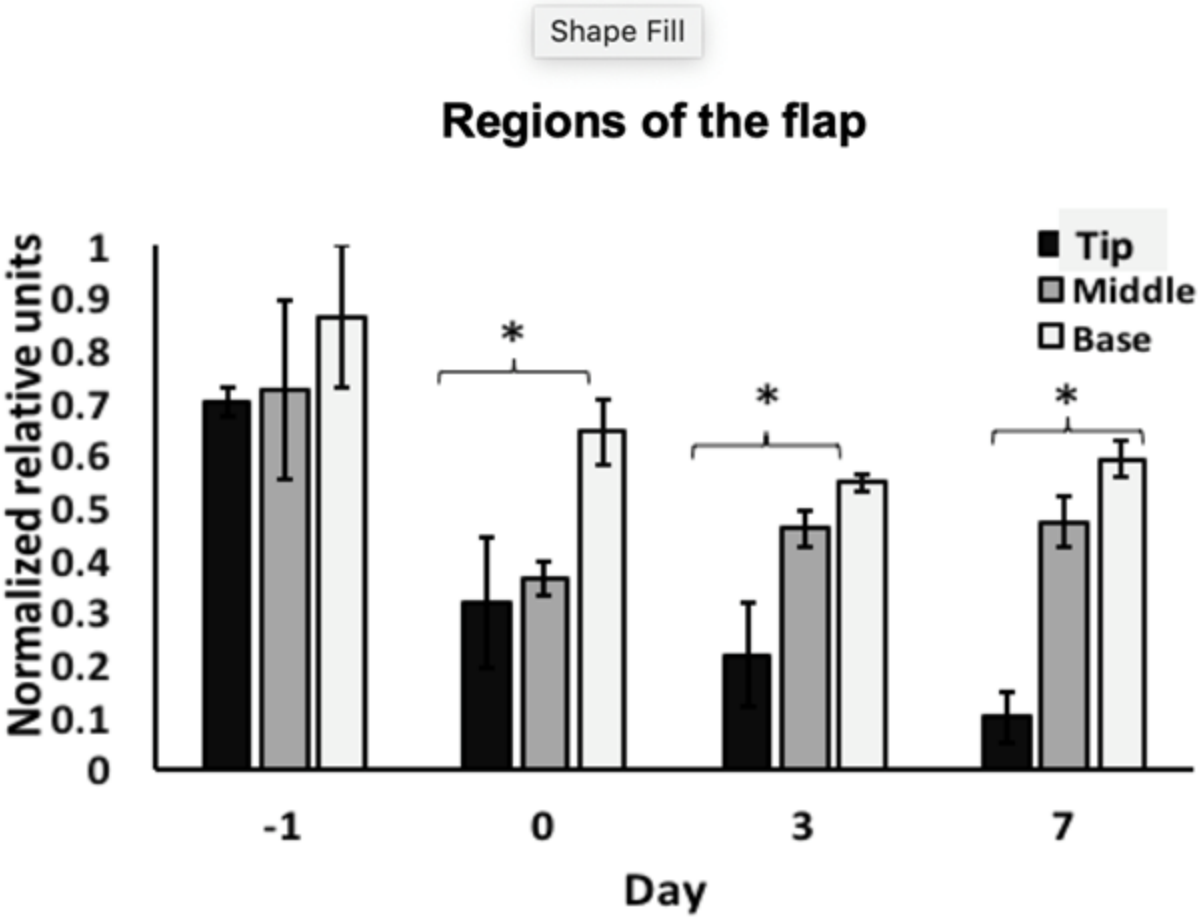

Vascular Occlusion
Myocutaneous flap
Control
Normalized Relative Units
1
0.8
0.6
0.4
0.2
0
0 100 200 300 400 500 600 700 800 900
Seconds

Fig. 4


Shape Fill
Regions of the flap
Normalized relative units
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Tip
Middle
Base
*
*
*
-1
0
3
7
Day

RM49 **Early Outcomes of Limb Transplantation Following Ex Vivo Normothermic Perfusion in an Orthotopic Forelimb Transplantation Model**

Cleveland Clinic, Plastic Surgery Department, Cleveland
Presenter: **Carlos Xavier Ordenana, MD**
Carlos Xavier Ordenana, MD(1), Majid Rezaei, DDS, MSc(1), Vahe Fahradyan, MD(1), Sayf Al-deen Said, MD(2), Brian Alexander Figueroa, MD(1), Lynn M Orfahli, BM(1), Edoardo Dalla Pozza, MD(2), Maria Madajka, PhD(3), Francis A. Papay, MD(2), Antonio Rampazzo, MD, PhD(4) and Bahar Bassiri Gharb, MD, PhD(3)
(1)Cleveland Clinic Foundation, Cleveland, OH, (2)Cleveland Clinic, Cleveland, OH, (3)Plastic Surgery, Cleveland Clinic, Cleveland, OH, (4)Plastic Surgery, Cleveland Clinic Foundation, Cleveland, OH

Background Ischemia-reperfusion injury remains one of the major limiting factors for limbs replantation and transplantation. Ex-vivo normothermic limb perfusion (EVNLP) has been proven to preserve viability and function of amputated limbs longer than cold storage, by maintaining the physiologic metabolism and avoiding the deleterious effects of hypoxia and cooling. This study aimed to assess the early (15 days) outcomes of limb transplantation following EVNLP. **Methods** Eight Yucatan miniature pigs (4 donors, 4 recipients) were used. Donor forelimbs were amputated at mid-humeral level and preserved with EVNLP with an oxygenated colloid solution containing HBOC-201 (HbO2 Therapeutics, Souderton, PA) at 38¡C during preparation of the recipient. Perfusion quality was assessed hourly by monitoring the perfusate electrolytes and gases, tissue O2 saturation, muscle temperature, surface infrared thermography and muscle contraction. The humerus was fixed with a 3.5 mm 5-hole DCP plate (Synthes, West Chester, PA); microsurgical anastomosis of the brachial artery, cephalic vein, and repair of radial, ulnar, and median nerves were performed. Tendons of the biceps and triceps were repaired with a pulvertaft weave technique. Brachioradialis was repaired at the muscle belly level. Peripheral perfusion was confirmed with indocyanine green angiography. The first animal was genetically matched to the donor and did not receive immunosuppressive medications. Systemic immunosuppression for the remaining animals included: antithymoglobulin (induction), cyclosporine, mycophenolate mofetil and methylprednisolone. Limbs were monitored clinically for signs of rejection and biopsies were taken per cause. The endpoint of the study was 90 days. **Results** Warm ischemia time before EVNLP was 20.6±9 minutes. EVNLP lasted 4.3±0.52 hours. Warm ischemia time after EVNLP was 2.2±0.25 hours. Total time to revascularize was 6.8±0.5 hours. Perfusate flow was 240±50 ml/min maintaining the MAP at 87±11 mmHg. Blood gases values for PO2, PCO2, pH and Lactate where 557±72mmHg, 6.5±2.6 mmHg, 7.5±0.1, 5.6±0.9mmol/L respectively. Muscle temperature and contractions at the end of EVNLP were 34.7±0.6 and 4/5 respectively. Infrared thermography confirmed uniform perfusion of the limb during EVNLP. All forelimbs were successfully transplanted. The first limb showed evidence of acute rejection on POD 4 and the animal was euthanized on day 6. All the remaining animals developed a seroma in the axilla that required drainage between 4-7 days. All incisions healed and initial edema resolved by day 14. There were no infections. **Conclusion** s This study confirmed that extremity transplantation can be successfully performed following EVNLP. EVNLP does not increase risk of vascular, early infectious complications.

RM50 **The Ocular Immune Response in a Rodent Model of Whole Eye Transplantation**

University of Colorado Denver Anschutz Medical Campus, Aurora
Presenter: **Bing Li, MD**
Bing Li, MD(1,2), Yong Wang, MD(1,2), Charles Owens, BS(1), Wensheng Zhang, PhD(2), Touka Banaee, MD(2), Wendy Chen, MD, MS(2), Chiaki Komatsu, MD(2), Ariel C Johnson, BS(1), Mario Solari, MD(2), David Mathes, MD(1), An-Jey Su, PhD(1,2), Christene Huang, PhD(1) and Kia M Washington, MD(1,2)
(1)University of Colorado Anschutz Medical Campus, Aurora, CO, (2)University of Pittsburgh, Pittsburgh, PA

Background
Approximately 36 million people throughout the world suffer from blindness. Whole eye transplantation (WET) may offer a potential solution to restore lost vision, in particular in the setting of ocular trauma. The possibility of immune rejection is a major barrier to successful transplantation. We used an orthotropic rodent whole eye transplant model with MHC class I and II mismatched donor/recipient, without immunosuppression, to elucidate for the features of the immune reaction following WET.

Methods
14-wk old male Brown Norway (BN) rats served as donor and recipient in the syngeneic group (S-group). 14-wk old male BN rats served as donors with age/sex matched Lewis rats used as recipient in the allogeneic group (A-group). 6 naïve rats were used as a control group (C-group). After A and S-groups received WET (n=18, respectively), transplanted eye blood supply and structure were evaluated on post-operative day (POD) 2 by optical coherence tomography (OCT). Skin and cornea rejection at POD 2, 4, 5, 6, 8 were assessed by the Banff based scoring system. Gene expression associated with rejection was measured by qPCR of eye tissue for all groups on POD5. Serum cytokine/ chemokine levels were tested by ELISA.

Results
Allogeneic corneas of WET animals began to reject at POD 5, displaying a change in transparency. Skin the histology showed signs of rejection at POD4. Despite the earlier onset of skin rejection, the rate of total rejection was faster for corneas *vs.* skin (1 day). qPCR revealed significant upregulation of genes associated with classic T-cell mediated adaptive immunity such as: Ccl5, Psmb9, Cd28, Stat1, Stat4, Cxcl11, IFN-γ. Peripheral serum IFN-γ of naïve and syngeneic rats remained low both before and after WET while, serum IFN-γ increased rapidly on POD5 then returned to basal levels by POD6 in allogeneic non-immunosuppressed animals.

Conclusion
Our results suggest a T-cell mediated acute rejection in allogeneic WET models without immunosuppressive treatment. The IFN-γ levels showed that WET immune response is an early rapidly developing process as measured by corneal transparency change and serum IFN-γ levels. The rapid rise and fall of IFN-γ levels might provide a non-invasive method to monitor irreversible rejection after WET.

RM51 **Can Ultrasound Surface Wave Elastography be Used to Assess Limb Lymphedema? a Clinical Pilot Study**

Mayo Clinic, Rochester
Presenter: **Samyd Said Bustos, MD**
Samyd Said Bustos, MD, Tony Chieh-Ting Huang, MD, MSc, Juntao Shao, MBBS, Boran Zhou, PhD, Xiaoming Zhang, PhD and Oscar J Manrique, MD
Mayo Clinic, Rochester, MN

Background: Ultrasound surface wave elastography (USWE) has been used to study the viscoelasticity of the skin in patients with systemic sclerosis and the extent of lung fibrosis in patients with interstitial lung diseases.[1] One of its advantages is its capability to measure subcutaneous tissue up to 45 mm. Hence, USWE may be used to assess skin, subcutaneous connective tissue, and muscle. Herein, we conducted a study to determine if USWE produces different recording patterns in healthy and lymphedematous limbs, which could potentiate its use as a non-invasive diagnostic tool.

Methods: We performed a prospective study in adult patients with limb lymphedema between March and April 2019. We used three anatomic landmarks for measurement: 10 cm above ankle/wrist, and 10 cm above and below knee/elbow. Both normal and affected limbs were measured. A 0.1-s harmonic vibration was generated by the indenter of the handheld shaker on the limb. The vibration was generated at 3 frequencies: 100, 150 and 200 Hz. Ultrasound probe was positioned adjacent to the indenter and used for detecting the tissue motion. Particle velocity in the axial direction was measured using autocorrelation technique. Shear wave speed (SWS) of the tissue was measured using 2D processing window technique.[2] A region of interest was selected to measure superficial and deep limb tissues.

Results: Eleven patients with secondary lymphedema (6 with upper limb and 5 with lower limb lymphedema) were included. Based on the International Society of Lymphology staging system, 3 were stage I, whereas 8 had stage II-III. Mean time with lymphedema-associated symptoms was 70.7 months (range, 3 – 362). The magnitudes of the wave speeds of the superficial fat tissue at 100, 150 and 200 Hz at lymphedema sites (3.97 ± 0.06, 4.89 ± 0.82, 5.95 ± 0.49 m/s) were statistically higher than those of the control sites (2.35 ± 0.32, 2.81 ± 0.46, 3.12 ± 0.61 m/s), respectively. The magnitudes of the wave speeds of the deep fat tissue at 100, 150 and 200 Hz at lymphedema sites (4.24 ± 0.43, 5.12 ± 0.76, 6.06 ± 0.66 m/s) were statistically higher than those of the control sites (2.55 ± 0.07, 2.94 ± 0.43, 3.24 ± 0.4 m/s), respectively.

Conclusion: Based on this pilot prospective study, we believe USWE may be a promising tool to evaluate patients with limb lymphedema. In addition, this none-invasive method can potentially assess and compare the surgical outcomes before and after functional lymphatic surgery.