

Sunday, January 12, 2014 - Basic Science/CVA Scientific Paper Session - 1:00pm - 2:45pm

1:00pm - 1:04pm

Single Perforator Harvest of the Deep Inferior Epigastric Artery Perforator Flap Results in Tissue Hyperperfusion

Chrisovalantis Lakhiani, MD¹; Angela Cheng, MD²; Michael Mangum¹; Karel Zuzak¹; Sumeet Teotia, MD¹; Michel Saint-Cyr, MD³; (1)University of Texas Southwestern Medical Center, (2)UT Southwestern Medical Center, (3)Mayo Institution where the work was prepared: University of Texas Southwestern Medical Center, Dallas, TX, USA

Background: Knowledge of hemodynamic changes that occur during perforator flap harvest is necessary for preoperative planning and minimizing complications, such as flap loss and fat necrosis. It has previously been shown that hemodynamic changes after deep inferior epigastric artery perforator (DIEP) flap harvest include increased flow through the pedicle in the short- and long-term. However, it is unknown if flap harvest on a single perforator leads to increased perfusion in the cutaneous microvasculature postoperatively.

Methods: Fifteen patients (23 flaps) underwent DIEP flap harvest using a prospectively designed protocol. Perforators were marked and imaged with a novel system for quantitatively measuring surface flap tissue oxygenation, the Digital Light Hyperspectral Imager (DLHsI®). Images were taken preoperatively and after raising the flap on a single perforator. These were then analyzed and quantitative perfusion changes were compared using a Wilcoxon signed-rank test.

Results: Mean flap tissue oxygenation was found to be significantly different ($p=0.01$) between preoperatively imaged flap tissue (%HbO₂ = 65.6) compared to post-harvest (%HbO₂ = 67.5%). Increased tissue oxygenation was observed after flap harvest in all cases. Figures 1 and 2 illustrate observed differences in flap perfusion using the Hyperspectral Imager.

Conclusion: Perforator flap harvest results in hyperperfusion and greater tissue oxygenation compared to preoperatively. These results validate mathematical models of DIEP flap arterial flow and may help to explain the large flap territory that may be safely raised on a single perforator.

Figure 1. Preoperative View of Flap Perfusion

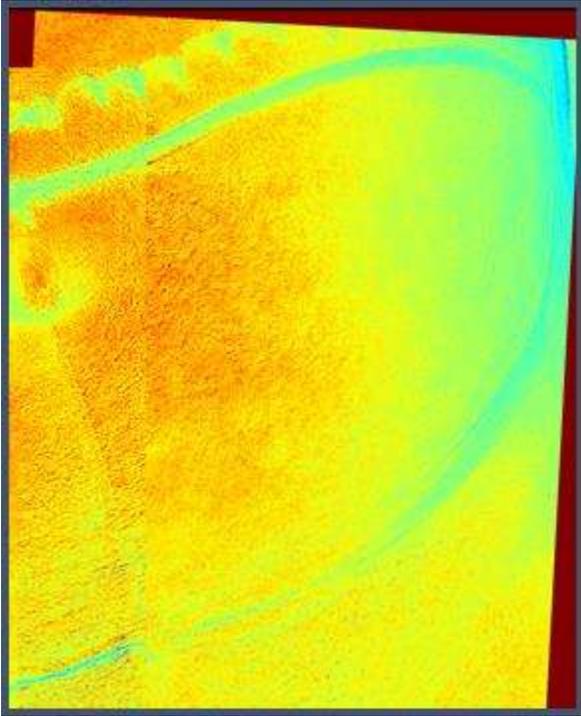
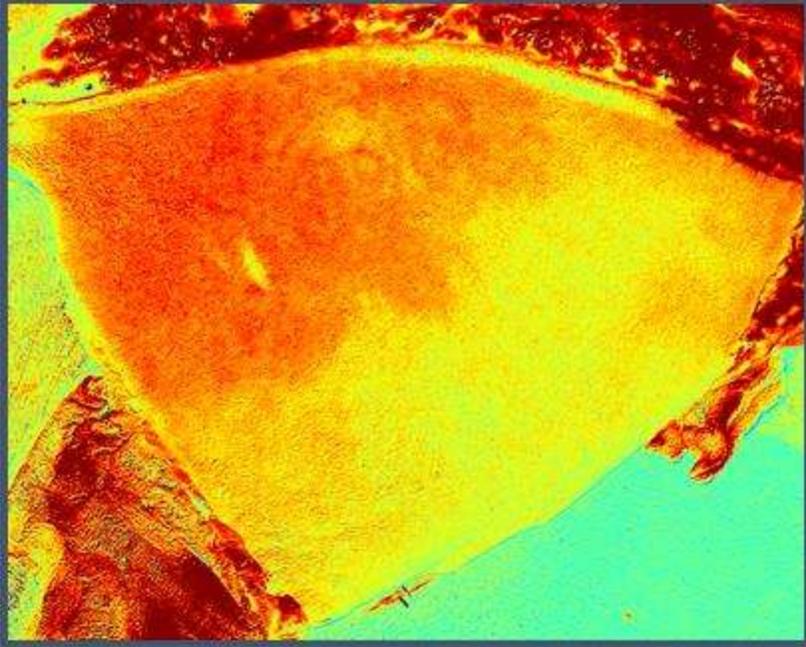


Figure 2. The DIEP Flap is Hyperperfused after Harvest on a Single Perforator



1:04pm – 1:08pm

Suprascarpal Fat Pad Thickness May Predict Venous Drainage Patterns in Abdominal Wall Flaps

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Institution where the work was prepared: Weill Cornell Medical Center, New York, NY, USA

Background:

Abdominal wall flaps are routinely used in reconstructive procedures. The predictability of the medial and lateral row perforators and the large caliber of the deep inferior epigastric vessels lend these flaps to good outcomes. Occasionally these deep system perforators are of limited caliber, which may lead to poor flap perfusion or venous congestion from inadequate drainage, often leading to fat necrosis or flap failure. The superficial inferior epigastric vessels (SIEV) are occasionally of sufficient size to allow for microvascular revascularization, however they have a higher rate of fat necrosis. We designed this study to look at the ratio of the sub- and suprascarpal fat layers, the number of deep system perforators, and the SIEV diameter to determine any correlation of the fat topography and the SIEV system.

Methods:

50 abdominal/pelvic CT angiograms (100 hemi-abdomens) were examined of women between the ages of 34 and 70. The CTAs were examined for number of perforators, SIEV diameter at 10cm below the umbilicus, and sub- and suprascarpal fat pad thickness at 10cm below the umbilicus at the edge of rectus fascia. Data were analyzed using a multivariate model to analyze the relationship between supra- and subscarpal fascial thickness with number of perforators and SIEV caliber.

Results:

The average suprafascial height was 18.6 mm with a range of 3.6 to 62.2mm. Average subfascial height was 6.2mm with a range of 0 to 38.9mm. The average ratio of fat pads was 3.1. The average SIEV diameter was 2.06 (range 0.81 to 4.0mm), and the average number of perforators was 2.09, (range 0 to 4) per hemiabdomen.

Hemi-abdomens with suprafascial fat greater than 23 mm in height had greater vein diameter 2.69 v 1.80mm ($p < 0.0001$). The fat layer thickness did not significantly correlate with the number of perforators. Furthermore, neither the thickness of the subfascial fat layer nor the ratio of superscarpal-to-subscarpal fat layer thickness correlated significantly with SIEV caliber or number of perforators in a multivariate model.

Conclusion:

We identified that a suprascarpal fat layer thicker than 23mm has a larger SIEV irrespective of the number of deep system perforators. This may indicate a cohort of patients who could be at risk for venous congestion and fat necrosis due to poor venous drainage if only the deep system is revascularized. We recommend harvesting the superficial vein in all patients with suprascarpal fat pads thicker than 23mm to potentially drain the superficial system if clinically needed.

1:08pm - 1:12pm

Annexin V-6L15, A Novel Injury-Site Directed Anticoagulant Ameliorates Ischemic-Reperfusion Injury and Promotes Survival of Rat Inguinal Island Skin Flaps

Victor Bong-Hang Shyu, MD¹; Chung En Hsu¹; Chih-Jen Wen, PhD¹; Hui-Yun Cheng, PhD¹; Tze-Chein Wun, PhD²; Fu-Chan Wei, MD, FACS¹; (1)Chang Gung Memorial Hospital, (2)EVAS Therapeutics
Institution where the work was prepared: Chang Gung Memorial Hospital, Taoyuan County, Taiwan

Background:

Reconstructive and microsurgical procedures involving free tissue transfer are often lengthy in duration. Extended ischemia time and ischemic-reperfusion injury occurring after successful vessel repair are major reasons for microcirculatory derangements and flap failure. Successful outcomes also depend on appropriate antithrombotic therapy, which is usually achieved by systemic or local antithrombotic interventions, flap perfusion washout, or systemic dextran use. However, there are no existing site-targeted methods of promoting flap survival that tackle both anticoagulation and ischemic-reperfusion injury. The Annexin V-6L15 (ANV-6L15) fusion protein was developed for this purpose. Annexin V is a protein that specifically targets exposed phosphatidylserine binding sites on apoptotic or injured endothelial cells, and prevents prothrombin-directed coagulation and neutrophil adhesion. 6L15 is an aprotinin mutant belonging to the Kunitz protease inhibitor group that functions as an inhibitor of tissue factor-VIIa pathway within the coagulatory cascade. We sought to evaluate the treatment effects of this fusion protein on rat island muscle and inguinal fasciocutaneous flaps.

Materials and Methods

The rat cremaster muscle island flap was used to evaluate the effect of ANV-6L15 on ischemia-reperfusion injury related leukocyte-endothelial interactions via intravital microscopy. TTC assay was used to determine live versus dead tissue in treatment versus non-treatment groups. Serum cytokine assay was used to evaluate changes in leukocyte chemoattractants. Rat inguinal island skin flaps subjected to critical ischemia times were treated with ANV-6L15 after reperfusion and survival was assessed on day 7.

Results

Intravital microscopy demonstrated a significant difference between the number of increased adhesive leukocytes for treatment group versus no treatment (1.5 ± 1.7 vs. 10.75 ± 7.0 , $p < 0.05$), and decreased number of functional capillaries (0.75 ± 1.0 vs. 3 ± 0.8 , $p < 0.05$). Treatment group showed a significant decrease in dead tissue via 2,3,5 TTC assay ($14.68 \pm 4.35\%$ vs. $28.92 \pm 9.17\%$, $p < 0.05$). Serum cytokine levels of GRO/KC, a neutrophil chemoattractant also known as neutrophil-activating protein 3, exhibited a significant decrease after treatment (1850.22 ± 547.72 vs. 884.16 ± 324.60 pg/ml, $p < 0.05$). Preliminary studies demonstrated that rat inguinal island skin flaps subjected to ischemia showed early recovery between treatment and no treatment groups.

Conclusions

The novel fusion protein ANV-6L15 successfully ameliorated leukocyte-related ischemic-reperfusion injury and promoted survival of rat cremaster muscle and inguinal fasciocutaneous island flaps. The use of ANV-6L15 in reconstructive and microsurgical procedures should be evaluated for potential clinical use.

1:12pm - 1:16pm

Negative Pressure Wound Therapy (NPWT) Salvage of Muscle Flaps with Venous Compromise

Douglas Corey Campbell, MD; Michael Morykwas; Louis Argenta, MD; Ivo Pestana; Wake Forest Baptist Medical Center

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Negative Pressure Wound Therapy (NPWT) Salvage of Muscle Flaps with Venous Compromise Douglas Campbell MD, Michael Morykwas PhD, Louis Argenta MD, Ivo A. Pestana MD Abstract BACKGROUND: Pedicled flaps remain an important component of the reconstructive surgeon's armamentarium. Despite their high success rate, venous congestion is a common cause for partial or complete flap loss. Etiology identification and correction of venous compromise is critical for flap salvage. Anticoagulation initiation or operative re-exploration and implementation of microsurgical techniques for outflow augmentation are frequently employed during attempted flap salvage. If these interventions fail, there are few other options for tissue preservation. NPWT has been utilized to improve random pattern flap survival. Decrease in interstitial fluid pressure and induction of neovascularization are proposed mechanism for improved flap survival. Our aim was to investigate the effect of NPWT application on flap outcomes after complete transection of the pedicle vein in a pedicled latissimus dorsi muscle flap model. METHODS: Bilateral latissimus flaps were dissected in pigs. One side served as the control while the other side was treated with external application of sub-atmospheric pressure employing the VAC device. NPWT was applied at increasing time periods after vein ligation. Data on flap survival, complete or partial flap loss, NPWT aspirate volumes, and NPWT duration were collected. RESULTS: Thirteen pigs with 26 latissimus dorsi flaps were included in the study. Total flap loss was observed in all of the 13 controls. NPWT at continuous 125 mm Hg was applied to the study flaps at 1 hour increments (0h to 8h). If topical sub-atmospheric pressure was applied within 5 hours or less after vein ligation, complete flap salvage was observed in 8 of 10 flaps. Of the flaps treated with NPWT within 5 hours of vein ligation, one flap was noted to have distal flap necrosis and another flap was lost due to NPWT air leak. If the onset of treatment was 6 hours or later after vein transection (n=3), all flaps were lost. CONCLUSIONS: The application of NPWT can be a salvage procedure in cases where sufficient venous outflow cannot be established after the initial procedure, or after major venous occlusion occurs. Topical sub-atmospheric pressure may be an effective treatment in such conditions without the risk of drug complications or protracted surgical re-intervention.

1:26pm – 1:30pm

Determination of the Optimal Sacrificial Material for Fabrication of Three-Dimensional Vascular Networks within Tissue Engineered Hydrogel Constructs

Jeremiah Joyce; Rachel Campbell, MD; Remco Bleeker; Adam Jacoby; Ryan Walters; Jason A. Spector, MD, FACS

Institution where the work was prepared: Weill Cornell Medical College, New York, NY, USA

Introduction: Fabrication of microvascular networks remains a critical challenge in the creation of tissue-engineered constructs. Ideally, engineered microvascular networks should be suitable for perfusion, mimic capillary density and be surgically relevant. We have previously demonstrated the creation of microchannels within hydrogel constructs using sacrificial techniques. Here we investigate various polymers to determine the optimal sacrificial material for the synthesis of microvessels within collagen and alginate hydrogels.

Methods: Pluronic F127, "Carbohydrate Glass" (a mixture of sucrose, glucose, and dextran), and Shellac (a natural polymer extracted from beetles - SSB 55 Astra FL) were used to create macrofibers 1.5mm in diameter using pre-formed polydimethylsiloxane (PDMS) molds with longitudinal or "loop" channel features. Additionally, three-dimensional (3D) networks were created using three techniques: 1) manual extrusion of a 100-500 μm diameter Pluronic F127 microfiber "mesh" 2) melt-spun 10-400 μm diameter Shellac microfiber "fluff" and 3) melt-fusion of micro- and macrofibers of varying diameters. All networks coalesced into macro inlets and outlets for perfusion and evaluation of patency. Networks were embedded in alginate or type I collagen and sacrificed in the appropriate solvent. Microchannel architecture was confirmed via histology and imaging.

Results: Single longitudinal and "loop" microchannels as well as 3D networks of interconnected microfibers were successfully embedded and sacrificed in type I collagen and alginate. Patency was confirmed via successful perfusion with colored buffer solution and gadolinium contrast during microMRI imaging. Histology confirmed intact, interconnected microchannels similar to the size and density of microvascular networks *in vivo*.

Conclusions: We have developed several novel techniques for the fabrication of both simple and complex microvascular networks utilizing sacrificial microfibers within biodegradable, biocompatible hydrogels. These

findings represent a significant advancement towards the creation of surgically relevant engineered tissue constructs, ready for endothelialization, with architecture that recapitulates that found *in vivo*.

1:30pm - 1:34pm

The Vascularized Periosteum Flap as New Tissue Engineering Model for Repair of Cartilage

Leila Kolios, MD¹; Jung-Ju Huang, MD²; Shu-Wei Kao, BS²; Yen-Lin Wu, BS²; Ming-Huei Cheng, MD²; Chao-Min Cheng, PhD³; (1)BG Trauma Center Ludwigshafen, Dpt. of Plastic Surgery of the University of Heidelberg, (2)Chang Gung Memorial Hospital, (3)National Tsing-Hua University Institution where the work was prepared: Chang Gung Memorial Hospital, Taoyuan, Taiwan

Since articular cartilage possesses neither a blood supply nor a source of mesenchymal stem cells, it has a limited potential to repair itself when damaged or diseased. Mesenchymal stem cells in periosteum have been shown to differentiate into neochondrocytes with the potential to form cartilage. A new vascularized periosteal flap model was designed as a key technique to solve the difficulties recently existing in the field of cartilage engineering.

In six 3-month-old New Zealand white rabbits bilateral full-thickness critical-size cartilage defects of the medial and lateral femur condyles were created. The medial condyles were covered with an axial pattern tibia periosteal flap based on the saphenous artery and its venae comitantes, the lateral condyles served as negative controls. The dorsal aspect of the medial condyles was taken as positive control. After 4 weeks, gait analyses were performed and after sacrifice the condyles were evaluated in macroscopical, histological and biomechanical terms.

In gait analysis, all rabbits showed an intact walking pattern. In macroscopic and histological analyses the medial condyles showed a stable attachment of the periosteal flaps. The flaps changed to real cartilage tissue with a smooth joint surface macroscopically and typical cartilage matrix and vital chondrocytes in the histologic sections. The lateral negative control defects showed no healing signs. In biomechanical testing the properties of the neo-cartilage in terms of stiffness, yield load and maximum load reached almost the level of the positive control.

The vascularized periosteum flap as autologous biomaterial has for the first time shown to be able to change to cartilage tissue and to repair cartilage defects of the knee joint. These results are promising for treatment of joint cartilage defects with autologous material where today still joint replacements are state of the art. Further studies are necessary to proof these flaps to be also successful first in big animals and later possibly in humans.

1:34pm – 1:38pm

Treatment of Large Calvarial Defects with Vascularized Bone Transport Osteogenesis: An Alternative to Conventional Calvarial Reconstruction in a Pre-Clinical Sheep Model

Patrick A. Gerety, MD; Jason D. Wink, BA; Rami D. Sherif; Nadya Clarke, MD; Gregory Heuer, MD; James T. Paliga, BA; Hyun-Duck Nah, DMD, PhD; Jesse A. Taylor, MD; University of Pennsylvania and Children's Hospital of Philadelphia

Institution where the work was prepared: University of Pennsylvania, Philadelphia, PA, USA

Background:

Bone transport osteogenesis (BTO), distraction of a free portion of bone across a defect, offers a vascularized, autologous solution to large cranial defects that may allow treatment without permanent hardware implantation. Critical variables that have not been previously investigated include rate of distraction and the use of complex, curvilinear distraction vectors that mimic native calvarial morphology. This study establishes a sheep model to evaluate a) feasibility, b) distraction kinetics, and c) efficacy of a curvilinear transport vector to more closely mimic the complex geometry of the native calvarium.

Methods:

Sub-total cranial defects (3.5 x 3.5cm) were created in 12 young adult male Dorset sheep. (Figure 1) A transport segment (3.5 x 2cm) traversed the defect at varying distraction rates (N=8) (0, 0.5, 1.0, 1.5 mm/day) using semi-buried linear (8 sheep) and curvilinear (4 sheep) (1mm/day) cranial distractors. Periodic x-ray was used to monitor progress of the transport segment across the defect. After a 6 week consolidation period, sheep were euthanized and the resultant bone was analyzed by CT, histology, and mechanical testing.

Results:

Gross examination, histology, and 3D-CT revealed that control animals had fibrous nonunion while distraction animals had ossified defects with fibrous non-union at the distal docking site (Figure 2 (L-Control, M-0.5mm/day, R-1.5mm/day)). In the linear distraction groups the volume of bony

regenerate in the 0.5, 1.0, 1.5 mm/day distraction rate groups was statistically indistinct ($p=0.56$). The flexural modulus (MPa) of non-decalcified samples from the control cranium, transport segment, and bone regenerate was found to be 4.50 ± 4.9 , 6.17 ± 2.1 , and 4.14 ± 4.8 respectively ($p=0.24$). During linear distraction, the transport segment exited the plane of the native calvarium and docked 2-3mm cephalad to native bone, consistently with a fibrous union. In contrast, curvilinear distraction resulted in similar bone volume, improved contour, and appropriate docking geometry. (Figure 3) Complications included one premature consolidation in the 0.5mm/day group, and three wound infections that were treated with antibiotics.

Conclusions:

This experiment provides proof of concept for BTO for large calvarial defects in a sheep model. While differences in distraction rate have significant effects on experimental duration they do not appear to significantly affect regenerate quantity or quality. Future work will include the use of increasingly complex curvilinear distraction devices for three-dimensional contour and investigation of the physical limitations of distraction.

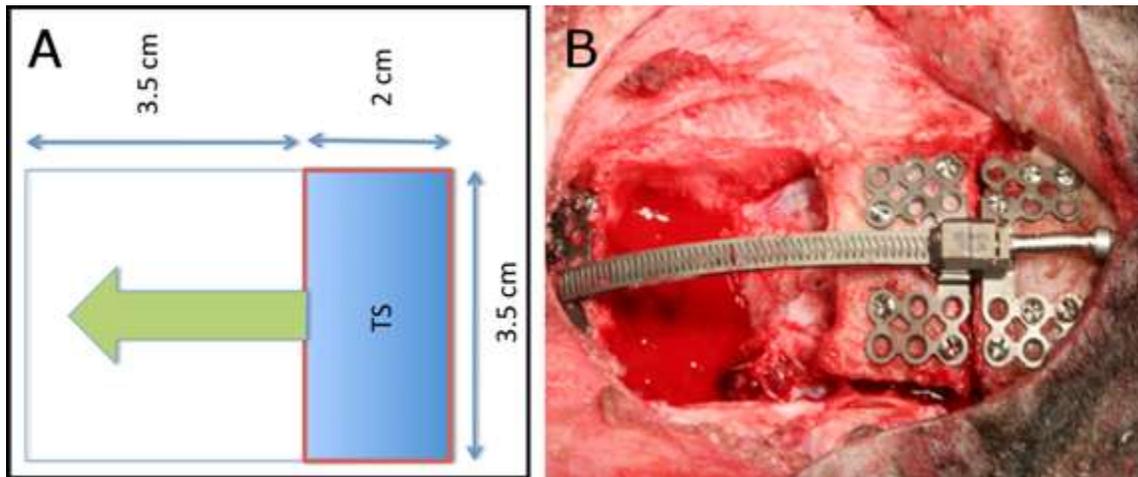


Figure 1.

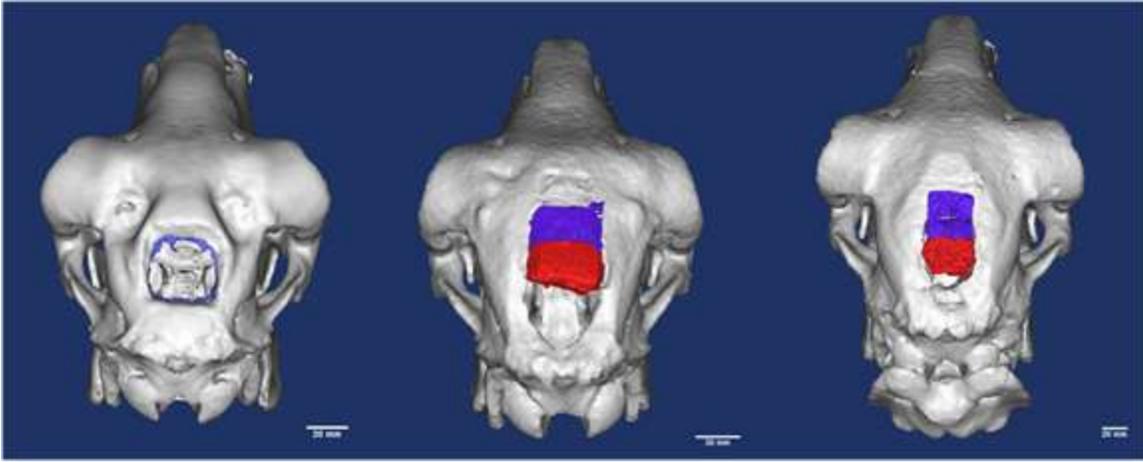


Figure 2.

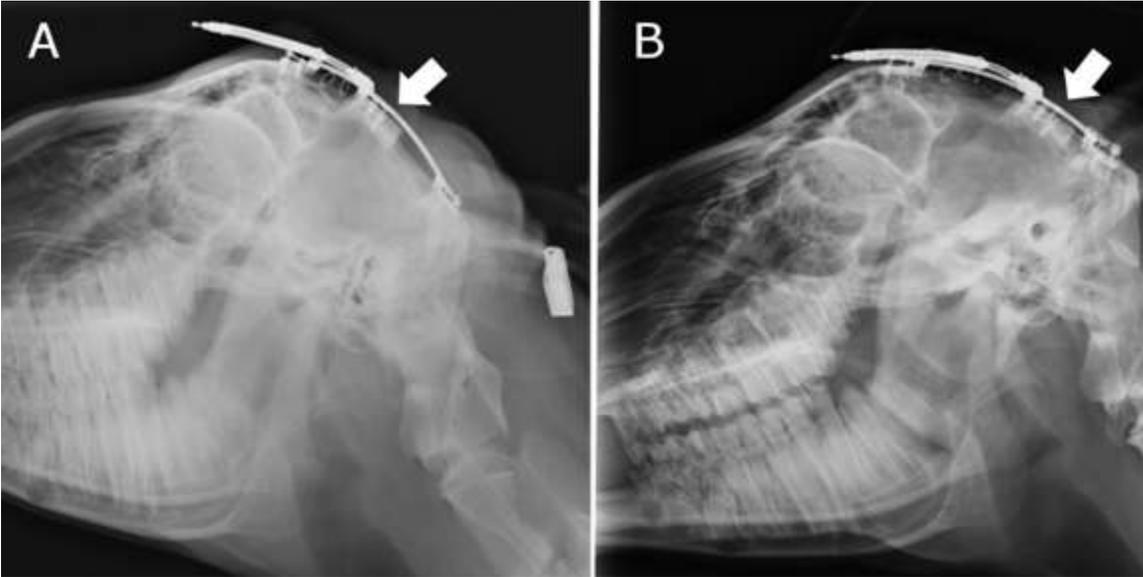


Figure 3.

1:38pm - 1:42pm

Multiple Finger Allotransplantation: Shifting Paradigms in Reconstructive Surgery

Antonio Rampazzo, MD¹; Bahar Bassiri Gharb, MD²; Christina Kaufman, PhD²; Linda Bright, RT, CT³; Yorell Manon-Matos, MD²; Joseph Kutz, MD²;

(1)Christine M. Kleinert Institute, (2)Christine Marie Kleinert Institute for Hand and Microsurgery, (3)Jewish Hospital

Institution where the work was prepared: Christine Marie Kleinert Institute for Hand and Microsurgery, Louisville, KY, USA

Introduction-Bilateral amputation of all fingers is a severely disabling injury that is functionally equivalent to bilateral hand amputation. Although vascularized toe transfer can recover a primitive grip, it falls short of restoring specialized abilities. Vascularized composite allotransplantation can benefit a unique subset of patients requiring all fingers to return to the previous occupation. Contrarily to the replantation surgery where the fingers need to be replanted individually, in a transplant procedure the availability of tissues makes the transfer of the fingers as a single flap feasible, decreasing the surgery length and increasing the chances of a successful outcome.

The aim of this study was to investigate the feasibility of the transfer of the long fingers and the thumb as a single allograft.

Materials and Methods- Eighteen hands were used in this study. In 14 hands the long fingers and thumb were harvested as a single flap vascularized by the superficial and deep palmar arches. The dorsal digital veins were dissected until confluent in two or more main dorsal veins on the dorsum of the wrist. The digital nerves were dissected to their origin from the median and ulnar nerves. The flexor tendons and extensor tendons were transected respectively in zone V and VI. The osteotomies were performed at the base of proximal phalanx. After harvest, ulnar and radial arteries were injected with red and blue india ink, respectively, followed by injection of lead gel in the ulnar artery to study the perfusion of the fingers. The allografts were x-rayed and CT scanned to document the presence of the contrast. The transplant procedure was simulated harvesting two allografts from one cadaver and transferring them to a recipient cadaver.

Results-It was always possible to harvest the long fingers and thumb based on the superficial palmar arch. The ulnar artery consistently perfused the small, ring, long and ulnar half of the index finger, while the radial artery was the principal blood supply to the thumb. The index finger represented a watershed area between the two vascular territories. Multiple anatomical

variations were documented. Ct scan confirmed the presence of contrast in the long fingers with decreasing staining toward the radial fingers.

Conclusion-Multiple finger transplantation as a single allograft is an anatomically feasible procedure. Although the ulnar artery can supply the whole flap, the variable anatomy of the palmar arches should be taken into consideration and the flap harvested based on both ulnar and radial artery.

1:54pm - 1:58pm

Significance and Management of Low Grade Rejection in Hand Transplantation

Bahar Bassiri Gharb, MD¹; Antonio Rampazzo, MD²; Joseph Kutz, MD¹; Brenda Blair, RN¹; Rosemary Ouseph, MD³; Michael Marvin, MD³; Christina Kaufman, PhD¹; (1)Christine Marie Kleinert Institute for Hand and Microsurgery, (2)Christine M. Kleinert Institute, (3)University of Louisville Institution where the work was prepared: Christine Marie Kleinert Institute for Hand and Microsurgery, Louisville, KY, USA

Introduction

Episodes of mild cellular rejection represent the majority of the acute rejection events in vascularized tissue allotransplantation. Management of low grade rejection still represents a therapeutic challenge as an aggressive treatment can accelerate the occurrence of side effects of the systemic immunosuppressive therapy. In absence of clinical signs, the natural evolution of histologically diagnosed grade I rejection is not well understood, further complicating the clinical decision making. The aim of this study was to evaluate the significance and outcome of the low grade rejection episodes in hand transplant patients.

Materials and methods

A retrospective study was conducted on 8 patients who received 9 hand transplants between 1999 and 2012. Patients' data concerning clinical course (rejection episodes, complications, functional outcome), immunosuppressive treatment, biopsy reports (Banff classification), high resolution ultrasound biomicroscopy were reviewed. The outcomes of the different treatments in the first year post-transplant were compared to the outcomes in the subsequent years with a Fisher's exact test.

Results

Three hundred and ten biopsies were reviewed (147 grade 0, 118 grade I, 33 grade II and 12 grade III). When clinical and pathological criteria were combined a total of 141 rejection episodes were identified. Forty eight episodes were considered grade I⁻ and 24 episodes were grade I⁺ where (+) indicates presence and (-) indicates the absence of clinical signs of rejection. When treated conservatively (observation or topical immunosuppressants) 63% of grade I⁻ episodes resolved in the first year as opposed to 100% in the subsequent years (p=0.005). Eighty four percent of grade I⁺ episodes resolved in the first year with a conservative approach as opposed to 100%

in the subsequent years ($p=1$). All of I^+ episodes presenting with less than 10% involvement resolved. There was no statistical difference between the outcome of the conservative management in I^+ versus I^- .

Conclusions

Grade I rejection events, when the through levels of the maintenance immunosuppressants are adequate, could be safely observed or treated with topical immunosuppressants, after the first year post-transplant. In the first year post transplantation, a conservative approach can be justified for grade I^+ episodes if the rejection is clinically mild (<10%).

1:58pm – 2:02pm

Peripheral Blood Profiles of Cytokines in The Recipients That Have Developed Tolerance to Vascularized Composite Allografts

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Institution where the work was prepared: Chang Gung Memorial Hospital, Gueishan, Taiwan

Background:

Over the past several years, great efforts from various institutes have made vascularized composite allotransplantation (VCA) a clinical reality. However, chronic application of immunosuppressants is still essential for long-term survival of the allografts. To circumvent this, major research efforts have been focused on establishing the allograft-specific tolerance. Although tolerance can be successfully induced in various animal models, the knowledge on the induction mechanism as well as molecular markers is still lacking. In this study, cytokine profile of peripheral blood derived from VCA recipient rats that have developed allograft tolerance was compared to naïve rats to gain more insights into the cytokine signature that is specific for VCA tolerance

Methods:

The hind limb vascularized osteomyocutaneous flap allotransplantation was performed on a rat model with Brown-Norway donors and Lewis recipients. The immunosuppression protocol included anti-lymphocyte serum administration at one day before and 10 days after surgery (POD -1 and 10), cyclosporine administration at 16 mg/kg from POD 0-to-10, and syngeneic ADSC infusion at POD 1 with 2×10^6 cells/dose. Serum was collected weekly starting from POD21. Serum from recipients that have allografts survived longer than 120 days as well as that collected from naïve Lewis rats were subjected to Bio-Plex Pro Rat cytokine assay (Bio-rad). Quantitative analysis of 26 cytokines can be performed from each serum sample.

Results:

Serum from 11 tolerant recipient and 6 naïve rats were analyzed. Out of 26 cytokines analyzed, the levels of EPO, GCSF, IFN-g, GRO/KC, IL-6, IL-7, IL-

18, M-CSF, RANTES, TNF-a and TGF-b1 in serum collected from tolerant recipients at POD 120 were significantly different from those in naïve rats. Each cytokines showed different patterns of evolution between POD 21 and POD 120, which may provide insights into significant time points for developing tolerance.

Conclusion:

Using Bio-plex cytokine assay, a specific panel of cytokines in VCA recipients that have developed tolerance showed significant differences compared to naïve rats. Such panel provides clue about mechanisms for developing VCA tolerance in addition to potentially serving as a marker for developed VCA tolerance.

2:02pm - 2:06pm

Migratory Pathways of Human Ex-vivo Created Di- Chimeric Cells in the Athymic Nude Rat Model: A Preliminary Report

Maria Siemionow, MD, PhD, DSc; Joanna Cwykiel, MSc; Grzegorz Kwiecien, MD; Medhat Askar, MD, PhD; Cleveland Clinic

Institution where the work was prepared: Cleveland Clinic, Cleveland, OH, USA

Introduction: Cellular therapies can significantly benefit vascular composite allograft (VCA) survival by modulation of immune responses of the recipient and elimination of the use of life-long immunosuppression. We propose a cellular therapy based on the ex vivo created di-chimeric cells (DCC) as an alternative approach to BM based therapies in support of VCA. The aim of this study was to evaluate the survival and migratory pathways of ex-vivo fused human DCC delivered into the bone of the nude rat recipient.

Methods: Twelve ex vivo fusions of human umbilical cord blood (UCB) cells were performed. UCB mononuclear cells from 2 different donors were stained separately with PKH26 and PKH67 fluorescent membrane dyes. Fusion procedure was performed using polyethylene glycol (PEG) technique. Double PKH26 and PKH67 stained human (DCC) were sorted and subjected to viability assessments by Trypan Blue and LIVE/DEAD Cell Stain staining as well as lymphocytotoxicity (LCT) test for class I and II antigens. DCC ($4-7 \times 10^6$) were injected to the bone of athymic nude rat recipients (Hsd:RH-Foxn1^{nu}). The presence of DCC was assessed at the following time points in 6 animal groups: Group 1 - 24 hours, Group 2 - 72 hours, Group 3 - 7 days, Group 4 - 21 days, Group 5 - 1 month and Group 6 - 2 months. At given time points, blood, bone marrow (injected and contralateral bone), lymph nodes, spleen, lung, liver, skin and brain samples were harvested. The presence of DCC in the nude rat tissues was assessed using flow cytometry (FC) and immunofluorescent staining.

Results: Successful creation of DCC was confirmed by FC and fluorescent microscopy using PKH staining. On the surface of fused DCC's, LCT analysis confirmed presence of HLA class I and II specific for both UCB donors used for cell fusion.. Viability of cells ranged between 94-99%. The presence of cells was detected by anti-human HLA class I ABC staining. FC and fluorescent microscopy assessment of nude rat tissues confirmed presence of DCC up to 2 months after cell injection. DCC migration from the injected to the contralateral bone and to the lymphoid organs (spleen and lymph nodes) was detected within 24 -72 hours after cell injection.

Conclusion: The survival of DCC and their migratory potential was confirmed. Characterization of migratory pathways of DCC will contribute to the better understanding of DCC mechanism of action. The unique concept of DCC therapy introduces new applications in VCA transplantation.

2:18pm - 2:22pm

The Final Frontier: Microanastomosis and Perfusion of Vascularized Tissue-Engineered Constructs

Rachel Campbell, MD; Karina A. Hernandez, DO; Jeremiah Joyce, BA; Adam Jacoby, BA; Alice Harper, BA; Alyssa J. Reiffel, MD; Jason A. Spector, MD, FACS; Weill Cornell Medical College

Institution where the work was prepared: Weill Cornell Medical College, New York, NY, USA

Purpose: Our knowledge of vascular anatomy gives reconstructive surgeons the ability to harvest and transfer composite autologous tissues to treat complex wounds. However, such procedures produce obligatory donor site morbidity including pain, functional loss, paresthesias, dyesthesia, and scarring. Tissue engineering offers an appealing alternative, allowing for the synthesis of tissues and organs while obviating donor site morbidity. In previous work we synthesized and performed an *in vivo* microvascular anastomosis of a collagen construct containing an unseeded internal longitudinal microchannel. Here we fabricate and microsurgically anastomose collagen constructs containing an internal endothelialized microchannel.

Methods: Pluronic F127 microfibers were embedded in neutralized type I collagen, then sacrificed leaving a central "loop" microchannel, 1.5 mm in diameter. Constructs contained an inlet and outlet and were reinforced with polyglactone mesh for tensile strength at the anastomotic site. Microchannels were seeded with 5×10^6 cells/mL human umbilical vein endothelial cells (HUVEC) and constructs placed in static culture for 7 days with daily media changes. Seeded and unseeded constructs were microsurgically anastomosed to the femoral artery and vein of nude rats. Following completion of anastomoses, patency was evaluated via venous strip tests and *in vivo* microdoppler assessment. Following perfusion, all constructs were fixed in 10% formalin, embedded, stained and analyzed.

Results: Loop microchannel-containing constructs were successfully anastomosed to the femoral artery and vein of nude rats and perfused. *In vivo* gross inspection and H&E staining of seeded and unseeded constructs following harvest revealed intact microchannels, which tolerated physiologic perfusion pressures with minimal microchannel deformation. Anterograde flow was confirmed via venous strip tests. Auscultation over the perfused microchannel revealed continuous pulsatile blood flow. Post-perfusion analysis of unseeded constructs demonstrated significant aggregation of leukocytes along the microchannel wall. Histological analysis following perfusion of seeded channels revealed an intact HUVEC lining along the

endoluminal surface of the microchannel, with minimal leukocyte aggregation. Immunohistochemical analysis of seeded microchannels demonstrated CD31 expressing endothelial cells.

Conclusions: We have successfully created vascularized biocompatible, biodegradable constructs that support microchannel endothelialization and microsurgical anastomosis *in vivo*. Constructs with their own inherent vascular network lined by a healthy layer of endothelium can be directly anastomosed to host vasculature providing immediate perfusion, increasing the survival of cellular constituents within the scaffold as well as the rate of incorporation into the host. This represents a major leap forward in tissue engineering and opens the door to the creation and application of larger, more complex surgically relevant constructs.

2:22pm – 2:26pm

Aesthetic and Functional Facial Transplantation: A Classification System and Treatment Algorithm

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Purpose

As of April 2013, 26 facial vascularized composite allotransplantations (VCA) have been performed. We sought to develop a classification system and treatment algorithm that is practical and surgically applicable.

Methods

The majority of the transplants have been described in the surgical literature and media, and a review of the data was performed. A classification system (Figure 1) and treatment algorithm was designed. Skeletal defects were defined by craniofacial osteotomies and soft tissue defects by aesthetic facial subunits. The soft tissue defect was sub-divided into the following subunits: oral-nasal [1], oro-nasal-orbital [2], and full facial [3]. The bony defects were sub-divided into Le Fort 1 [A], Le Fort 3 [B], and Monobloc [C].

Results

Of the face transplants performed to date, the mechanisms of injury included trauma (n=12), burns (n=8), congenital (n=3), oncology (n=1), and unreported (n=2). According to the proposed classification system (Table 1): 1 was Type 1X-; 1 was Type 1X+; 1 was Type 1B+; 2 were Type 2X-; 2 were Type 2B-; 1 was Type 2B+; 6 were Type 3X-; 1 was Type 3B-; 3 were Type 3B+; 8 could not be fully classified due to lack of data. The treatment algorithm (Figure 2) designed a VCA that addressed the bony and soft tissue components.

Conclusions

Patient selection for these limited but complicated procedures, currently dependent on life-long immunosuppression, is crucial to its success. We herein describe a classification system and treatment algorithm for facial defects that may be ideally suited for facial transplantation. The proposed

classification and treatment summary may assist centers define indications and ideally improve patient outcomes.

Diagram of Classification System for Facial Transplantation

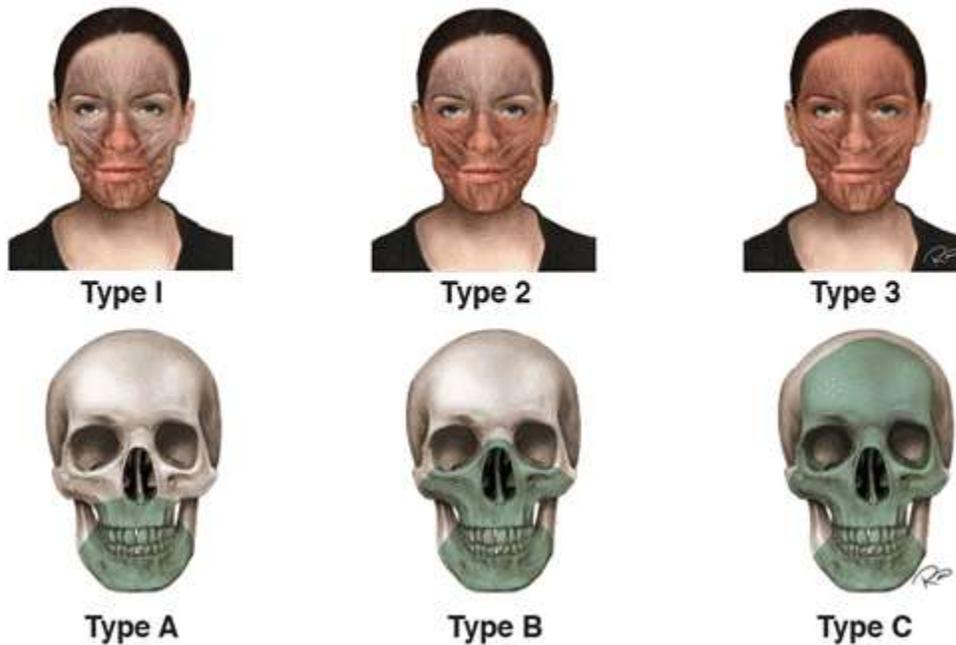


Figure 1. Diagram of the classification system for facial transplantation illustrates the extent of each soft tissue (shaded in red) and bony defect (shaded in green).

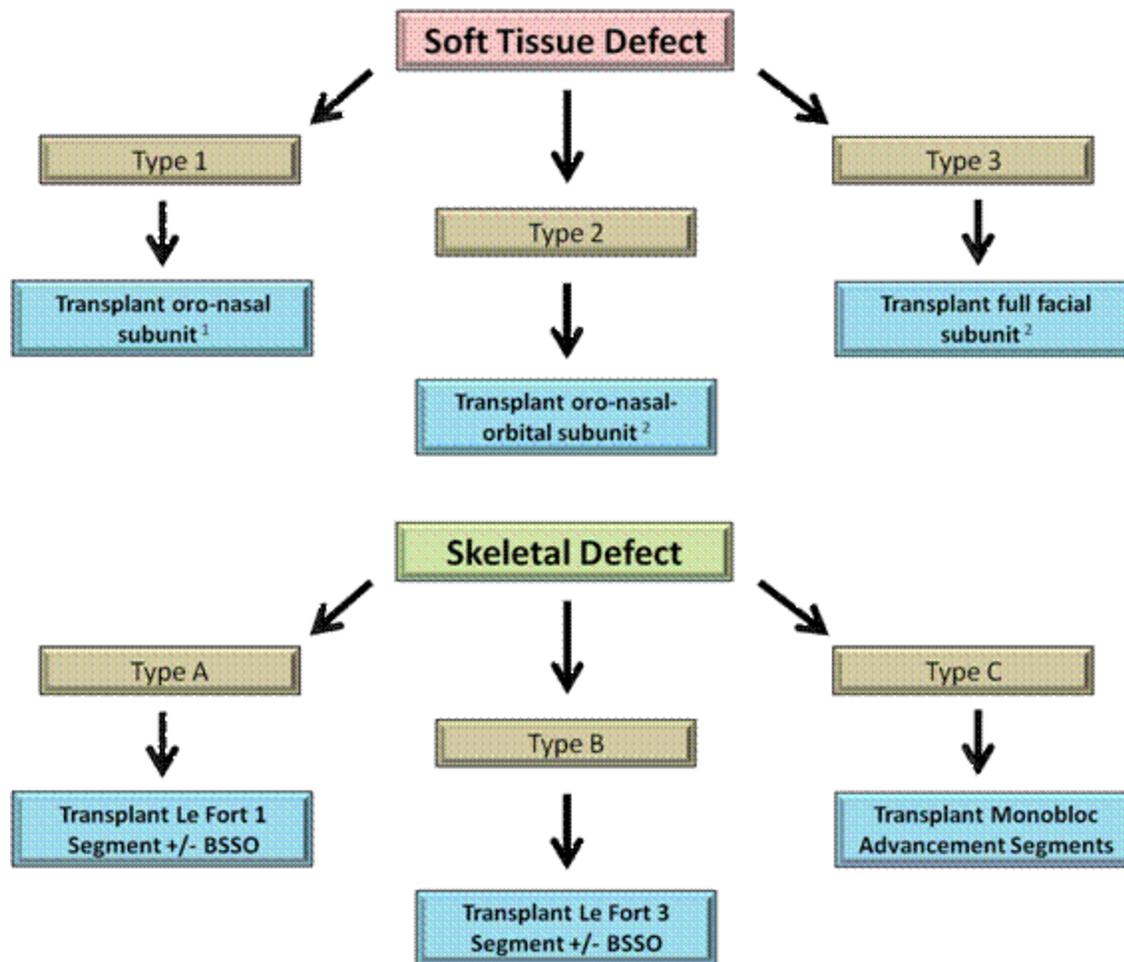


Figure 2. This flowchart algorithm addresses each type of bony or soft tissue defect within the classification system. The majority of face transplants performed followed the treatment algorithm shown. As stated in the text, for any classification with a large mandibular defect (designated as +), transplantation of the mandible should be considered.¹ In the event of isolated oral subunit loss, Type 0, only the oral subunit should be transplanted.² For these soft tissue types, a naso-orbito-ethmoid osteotomy with the medial canthal bearing segments and nasolacrimal apparatus should be included in the VCA.

Table 1. Classification system for facial transplantation

Soft tissue

Type	Functional subunit	Anatomic defects
0	oral subunit	Upper lip, lower lip, and oral commissures
1	oral-nasal subunit	Septum, nasal cartilage and nasal soft tissues, +/- defects in type 0
2	oral-nasal-orbital subunit	Lower eyelids and malar soft tissues, +/- defects in type 1
3	full facial subunit	Palpebral and frontal regions, can include all soft tissues of face

Bone

Type	Defect
-	No bony defect or bony defect not involving mandible or dental alveolus
+	Presence of mandibular defect
A	(Le Fort I segment) Maxillary alveolus, +/- mandible
B	(Le Fort III segment) Nasal bones, maxilla, zygoma, vomer, ethmoid, inferomedial orbital bones, +/- mandible
C	(Monobloc Advancement segments) Frontal bone, supraorbital rims and defects in type B, +/- mandible

2:26pm - 2:30pm

Vascularization of the Facial Bones by Facial Artery: Implications for Full Face Allotransplantation

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Introduction

With growing consensus on vascularized composite allotransplantation for reconstruction of massive facial defects, the research has focused on optimizing the technique to achieve the best functional outcome.

Although the maxillary artery has been recognized as the main vascular supply of the facial bones, clinical experience supports a co-dominant role for the facial artery. The aim of this study was to define the extent of the facial skeleton within the allograft that can be harvested based on the facial artery.

Methods

A total of 23 cadaver heads were used. In 12 heads, the right facial (6), superficial temporal (3) and maxillary (3) arteries were injected with lead-oxide. In 1 head, facial artery angiography was performed. Ten facial allografts containing the mandible, naso-maxillo-orbito-zygomatic complex, tongue and mouth floor were raised based on the maxillary artery (4) and facial artery (6), the latter was injected after the flap was raised.

In 19 heads, the soft tissues were dissected to show the anastomotic connections between the three arteries. Thereafter, all soft tissues were removed, and radiograms and CT scans of the mandible and the naso-maxillo-orbito-zygomatic complex were performed.

Results

Constant anastomosis between facial artery, inferior alveolar artery and infraorbital artery at mental and infraorbital foramina were found. Facial artery gave periosteal branches to the symphysis, body and ramus of the mandible and to the maxilla, zygoma and the nasal bones.

The facial artery consistently vascularized the homolateral mandibular symphysis, alveolar process, body and ramus. The condylar and coronoid processes were vascularized in 67% of the allografts. The homolateral zygomatic and frontal processes, the orbital plate of the maxilla and the maxillary bone were contrasted in all allografts, while the alveolar and the palatine processes contained the contrast in 83%. The maxillary process of the zygomatic bone was perfused in all allografts, followed by the body and frontal process (83%) and temporal process (67%). The homolateral palate, nasal lateral wall and septum were vascularized in 83% of allografts. The medial and lateral walls of the orbit and the orbital floor contained the staining in all specimens. The zygomatic process of the temporal bone was the least perfused bone.

Conclusions

With preservation of the mental and infraorbital foramina, a composite allograft containing all the facial bones with exclusion of the mandibular condyle, coronoid process, temporal process of zygomatic bone and zygomatic process of temporal bone, can be safely based on bilateral facial arteries.

2:30pm - 2:34pm

Measuring Outcomes in Vascularized Composite Allotransplantation of the Face: Toward a Unified International Approach

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BACKGROUND:

Since 2005, twenty three facial allotransplantation cases have been performed by 12 different teams in six different countries. Efforts to refine an international registry cataloging surgical and immunologic outcomes are ongoing. However, global standardization of functional and aesthetic outcome measures does not currently exist. The aim of this study is to identify current measures used by transplant teams to evaluate the form and function of their patients.

METHODS:

A literature review was performed to identify all documented face transplants. Any manuscript specifically containing functional or aesthetic clinical followup information was included. Additional internet searches were performed to identify facial allograft procedures recorded in media outlets, but not yet documented in the medical literature. Functional outcome measures were grouped into neurosensory testing and clinical observations via physical exam, photography and videography. Methods of aesthetic outcome determination were noted.

RESULTS:

Twelve publications from 7 transplant teams contained functional or aesthetic clinical follow-up information for 14 patients. Additional information was gleaned from hospital sanctioned media press releases for the remaining 9 transplant recipients.

Seven transplant teams used clinical observation by physical exam to document sensory and motor recovery. All of these teams used photography, 2 used videography. Ability to eat, speak, and smile was noted in all cases but specific temporal followup information was not specified.

Seven of twelve transplant teams documented neurosensory recovery using specific testing modalities. EMG was most commonly used to analyze motor nerve regeneration. Semmes-Weinstein monofilament, two-point discrimination, and calorimetric testing were most commonly used to evaluate sensory reinnervation of the allograft. MRI and Doppler ultrasound were performed in 3 patients to evaluate the structural aspects of transplanted tissues.

Aesthetic evaluation was performed in all documented cases by physical exam and was primarily identified issues of skin color match and flap bulk. Photodocumentation was used in 50% cases to make aesthetic determinations about facial symmetry and morphology.

CONCLUSIONS:

As surgical approaches to facial allotransplantation become increasingly standardized, so too should the metrics used to evaluate motor, sensory and aesthetic outcomes. Herein, we highlight the current nonuniformity in postoperative evaluation techniques amongst international facial transplant teams. We believe that global consensus on, and routine recording of functional and aesthetic measures in an international registry will enhance the quality of facial transplant outcomes.