

## **LOP01: PREOPERATIVE PERFORATOR MAPPING WITH DYNAMIC INFRARED THERMOGRAPHY IN BREAST RECONSTRUCTION WITH A DIEP FLAPS**

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**INTRODUCTION:** Multidetector computed tomography (MDCT) angiography is "the gold standard" for preoperative perforator selection. DIRT is based on the relationship between dermal perfusion and the change in rate and pattern of skin rewarming following a cold challenge. Can DIRT be an alternative to MDCT?

**METHODS AND MATERIALS:** 23 patients scheduled for breast reconstruction with DIEP flap were included. Preoperatively arterial perforator sounds were located with a hand held Doppler. Afterwards a desktop fan was used to deliver a mild cold challenge to the lower abdomen. The rate and pattern of rewarming of the skin was registered with an infrared camera. The locations of first appearing hot spots on the skin were registered. Eight patients had an additional MDCT scan.

**RESULTS:** A rapid appearing "hot spot" was associated with an arterial Doppler sound and a suitable perforator intraoperatively. All DIEP flaps survived and were based on the perforator as selected from DIRT. The selected hot spot could easily be related to a perforator on the MDCT scan.

**CONCLUSION:** Perforator selection of DIEP flaps is facilitated with the use of DIRT. The technique is non-invasive, provides real-time information and does not require exposure to ionizing radiation or intravenous contrast medium. DIRT could be an alternative for MDCT in perforator selection.

## **LOP02: MicroRNA-155 MODULATES AND AGGRAVATES THE INFLAMMATORY RESPONSE IN ISCHEMIA REPERFUSION INJURY FOLLOWING FREE TISSUE TRANSFER**

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**OBJECTIVE:** Ischemia-reperfusion injury (IRI) is a major causal factor of tissue injury and transplant failure in free flap surgery and composite tissue allotransplantation in reconstructive surgery. It is also a crucial pathogenic factor in various clinical settings including myocardial infarction and stroke. Previous work has identified a role for microRNA-155 (miR-155) in the modulation of the innate and adaptive immune response. Therefore we hypothesized that it fulfils a modulating function in inflammation driven conditions, such as IRI. Here we investigate the role of miR-155 in IRI.

**METHODS:** Expression levels of miR-155 were analysed by real time stem loop PCR of human striated muscle tissue after free flap surgery and IRI at various time points. Values were correlated with expression levels of markers of inflammation (TNF- $\alpha$ , IL-1 $\beta$ ), angiogenesis (VEGF, CD105) and apoptosis (Caspase3), as well as SOCS-1 (suppressor of cytokine signalling 1), a direct target of miR-155.

The functional consequences of miR-155 expression in IRI challenged muscle tissue were evaluated by an intravital imaging model of IRI in the cremasteric muscle of miR-155 knockout mice compared to wildtype mice (wt). Rolling and adherence of leukocytes in post capillary venules were monitored as parameters of inflammation. Immunohistology of the IRI challenged murine cremasteric muscle was performed to assess the infiltration of leukocytes in the tissue.

To further analyse the involved mechanisms we evaluated the influence of miR-155 over expression on CD11b expression in THP-1 monocytes and production of reactive oxygen species (ROS) by flow cytometry.

**RESULTS:** miR-155 expression in human striated muscle tissue is increased after IRI, showing a significant positive correlation with the increased expression of inflammation (TNF- $\alpha$ ), angiogenesis (CD105) and apoptosis (Caspase3). The direct miR-155 target SOCS-1 is correspondingly down-regulated in IRI challenged human striated muscle. The intravital analysis of IRI in mice reveals attenuated rolling and adhesion of leukocytes reperfusion injury in miR-155  $-/-$  mice compared to wt mice.

The immunohistological analysis of murine cremasteric tissue shows significantly less infiltration of inflammatory cells in the miR-155  $-/-$  mice compared to wt-mice. MiR-155 overexpression leads to an overexpression of CD11b as well as increased ROS production in monocytes, thereby mediating leukocyte adhesion and tissue damage.

**CONCLUSION:** The increased expression levels of miR-155 and correlation with increased expression levels of markers of inflammation, angiogenesis and apoptosis after free flap surgery suggest a modulatory

role of miR-155 in the pathogenesis of IRI. In a miR-155 deficient mouse model we show that miR-155 aggravates the inflammatory response, leukocyte infiltration and tissue damage. We address the underlying mechanism and show that miR-155 influences the expression of integrin receptors responsible for leukocyte adhesion and extravasation as well as production of ROS. Our data suggest that miR-155 is a potential target for the treatment or prevention of IRI in various clinical settings.

## **LOP03: FREE- PERFORATOR FLAP SURGERY AND THE USEFULNESS OF INTRAOPERATIVE DYNAMIC INFRARED THERMOGRAPHY (DIRT)**

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**INTRODUCTION:** Flap failure in free perforator flap surgery is often related to thrombosis at the anastomosis site or due to thrombosis caused by pedicle problems like kinking, torsion or external compression. We evaluated the usefulness of dynamic infrared thermography (DIRT) as a method to detect intraoperatively perfusion problems of the deep inferior epigastric perforator (DIEP) flap

**MATERIAL AND METHODS:** In DIRT a mild cold challenge is applied to the area of interest and the rate and pattern of rewarming towards equilibrium are registered with an infrared camera. Measuring of skin temperature provides indirect information on skin perfusion. Twenty women underwent secondary breast reconstruction with a free DIEP flap. The perfusion of the DIEP flap was evaluated with DIRT just after dissection of the perforator at the donor site, just after opening of the anastomoses, and at the end of the operation.

**RESULTS:** The use of DIRT provided valuable information on the quality of the perforator after it was dissected free. Arterial and venous anastomotic failures were easily detected. Kinking, torsion, or external compression of the pedicle was rapidly detected as well as the need for venous superdrainage.

**CONCLUSION:** DIRT is a valuable, non-invasive tool that provides real-time information on flap perfusion during free perforator flap surgery.

## **LOP04: RAPID RECOVERY PROTOCOL IN COMPLEX HEAD & NECK FREE TISSUE TRANSFER**

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**BACKGROUND:** Patients undergoing complex head and neck oncologic reconstructions with free tissue transfer are commonly admitted to an intensive care unit with maintenance on mechanical ventilation in the early postoperative period. However for select patients, recent literature supports immediate extubation in the operating room, and admission to non-ICU settings when possible. This study reviews indications and outcomes of free tissue head and neck reconstructive patients undergoing a rapid recovery protocol (RRP) compared to continued intubation in an intensive care unit.

**METHODS:** We retrospectively reviewed a prospective database of consecutive patients who underwent head and neck microvascular reconstructions by a single surgeon between August 2001 and April 2011. Patients' records were analyzed for demographics, co-morbidities, adjunct therapies, reconstruction, operative time, complications, and length of stay.

**RESULTS:** 565 consecutive cancer patients underwent head and neck microvascular reconstructions during the study period: 529 were intubated overnight in the ICU postoperatively and 36 underwent a rapid recovery protocol. The percentage of active smokers, or those with preexisting comorbid conditions was not significantly different between the RRP and ICU groups. For all patients, 239 (42.3%) complications occurred within 90 days of surgery. Overall, there were 8 (1.4%) total flap losses, and 8 (1.4%) partial flap necrosis, all occurring within the ICU patients but neither achieved significance ( $p=0.99$ ). Complication rates for the ICU group for all complications (42.5%) and minor events (37.6%) were similar to the RRP group for all (38.9%,  $p=0.73$ ) and minor (30.5%,  $p=0.48$ ) events. Length of stay was significantly less for overall patients (5.6 days RRP versus 10.3 days ICU;  $p=0.001$ ) and for the 56-65 (5.6 versus 9.3,  $p=0.02$ ) and the 66 and over (5.5 versus 11.5,  $p=0.04$ ) age-stratified subgroups. For patients with comorbidities, the RRP cohort had a lower complication rate (32.1%) versus the ICU group (51.9%,  $p=0.04$ ).

**CONCLUSIONS:** The recovery of select patients with early extubation and mobilization is superior to maintenance on mechanical ventilation. Candidates most benefiting from an RPR protocol are the elderly and those with significant comorbidities. A rapid recovery protocol is dependent upon accurate preoperative assessment, adequate postoperative airway, and maintenance of specialized free flap floor units with adequately trained nursing staff.

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#### LOP05: NERVE REPAIR BY FRESH MUSCLE-VEIN-COMBINED NERVE GUIDES: CLINICAL RESULTS AND ACTUAL INDICATIONS

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**INTRODUCTION:** Although autogenous nerve grafting is still considered the best method for bridging nerve defects, several alternative types of conduits (biological and synthetic) have been studied. We have demonstrated in previous experimental research that a graft made using a vein (providing a guide for nerve regeneration) filled with fresh skeletal muscle (to prevent vein collapse and support axon regeneration) gave similar results to traditional nerve grafts, in the rat. On this basis, we decided to use the muscle-vein-combined grafts in clinical cases not only for sensory nerves but also for mixed nerves. Despite continuous researches and surgical innovations, the treatment of peripheral nerve injuries remains a complex problem particularly in non sharp lesions where this kind of reconstruction is a good option of treatment. We report our case series and results.

**MATERIAL AND METHODS:** Mixed nerves: we reviewed 23 patients operated from 1993 to 2004 with this technique. The mean follow up was 26 months (minimum 14 months - maximum 58 months). The mean length of conduits was 2.5 cm (0.5 to 6 cm). Case series: 4 radial nerve at the elbow level, 9 median nerve at the distal third of the forearm, 6 ulnar nerve at the forearm, 1 ulnar nerve at the wrist, 1 ulnar nerve at the arm, 2 proximal cord of the brachial plexus. Sensory nerves: we operated 13 patients for sensory nerve reconstruction at the hand and wrist level. About these patients 13 were operated in emergency for crush injuries of sensory and mixed nerves.

We evaluated our results by the criteria of the Nerve Injuries Committee of the BMRC modified by Mackinnon-Dellon. We classified the results in three groups with the grading system proposed by Sakellarides. Very Good: <sup>3</sup> M4 / <sup>3</sup> S3+; Good: M3 / S3-S2+; Poor: < M2 / < S2+.

**RESULTS:** Mixed nerves: In 12 (52%) cases we had a good and very good results. In 6 cases (26%) a good sensory restoration has been not accompanied by a good motor recovery. In 2 cases (8.5%) we had a good motor recovery and a fair sensory recovery. In the last 3 cases (13%), in gap longer than 3 cm, we had a fair results both for sensory and motor recovery.

Sensory nerves: In the muscle-vein-combined group, 10 patients (76.9%) showed Very Good results while only 3 patients (23.1%) showed Good results.

**CONCLUSIONS:** The clinical employment of tubes as an alternative to autogenous nerve grafts is mainly justified by the limited availability of donor tissue for nerve autograft and its related morbidity. Indication, in this little series of patients operated in ten years, had been very restricted: treatment in emergency, not enough nerve graft, no will of the patient on harvesting a healthy nerve. An attempt of reconstruction in emergency with muscle-vein combined graft or alternative conduits is justified considering the possible advantages offered by this kind of nerve repair.

Ref: Tos P, Battiston B, Ciclamini D, Geuna S, Artiaco S. Primary repair of crush nerve injuries by means of biological tubulization with muscle-vein-combined grafts. *Microsurgery*. 2012 Mar 16. doi: 0.1002/micr.21957.

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#### LOP06: IN VIVO SENSORY RECORDINGS USING A NOVEL SKIN-FLAP CHAMBER AND CUSTOM MECHANICAL INDENTER

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**INTRODUCTION:** Touch receptors are responsible for converting force into an electrical signal. After a mechanical stimulus is applied to the skin surface, it becomes encoded as a unique train of afferent nerve action potentials once a predetermined mechanical threshold is reached. Our

laboratory has developed a regenerative peripheral nerve interface (RPNI) to restore the sense of touch to neuroprostheses. However, precise replication of trains of afferent nerve action potentials is necessary to provide high fidelity, discriminable, graded somatosensory feedback. This work describes the transformation of a previous *ex vivo* experimental setup into an *in vivo* rat model for the purpose of capturing sensory recordings from the whole sural nerve.

**MATERIALS AND METHODS:** A depilated neurocutaneous flap (peninsula-shaped skin paddle) in the sural nerve distribution was elevated off the dorsolateral aspect of the rat's foot leaving an intact skin bridge for its neurovascular supply. The flap was rotated into a skin-flap chamber containing phosphate-buffered saline, and secured to a silicone elastomer base. The purely sensory sural nerve was then exposed in the proximal thigh where *in vivo* multi-unit recordings were obtained, while a mechanical indenter fitted with a 3-mm diameter probe tip provided ramp-and-hold stimuli at depths of 1.0, 1.5, and 2.0 mm. Outcomes of interest included action potential firing rate and force application at the probe tip versus amount of displacement.

**RESULTS:** Increasing amounts of displacement provided by the mechanical indenter resulted in increasing force detected by the load cell. As the amount of displacement increased, the action potential firing rate also increased over each stimulation period with 8.68, 42.00, and 61.10 spikes/s recorded at depths of 1.0, 1.5, and 2.0 mm, respectively. These findings indicate that the mechanical indenter is capable of producing differential action potential firing rates in the whole sural nerve by varying the amount of displacement.

**CONCLUSIONS:** *In vivo* afferent nerve action potentials were orthotopically recorded from the whole sural nerve using a novel skin-flap chamber, nerve conduction instrumentation, and a custom mechanical indenter. Varying the amount of displacement produced differential action potential firing rates demonstrating discriminable, graded somatosensory feedback. This experimental setup will serve as the foundation for future mechanical stimulation experiments to develop and validate an electrical stimulation algorithm for producing multi-unit recordings from the RPNI.

**ACKNOWLEDGEMENTS:** The views expressed in this work are those of the authors and do not necessarily reflect official Army policy. This work was sponsored by the Plastic Surgery Foundation's Research Fellowship Grant and by the Defense Advanced Research Projects Agency under grant N66001-11-C-4190.

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#### LOP07: THE ANATOMY OF THE SUBSCAPULAR NERVES - A NEW NOMENCLATURE

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**AIM:** Previous study has delineated a variation in the posterior cord nerve branch anatomy. We sought to examine any variability and provide a nomenclature, if possible, to aid surgeons in navigating the Posterior cord (PC) of the Brachial plexus.

**METHODS:** Thirty-three preserved cadaveric upper limb and shoulder girdles were dissected. The origin of all nerves arising from the PC and their target muscle were recorded.

**RESULTS:** Additional nerves not classically described were commonly found on dissection. Additional nerves were seen in 76% of specimens. These were named Upper and lower Subscapular accessory nerves dependent on their topography (aUS, aLS). Accessory upper Subscapular nerves were present in 55% (n=18) and 11% of these (n=2) had two aUS nerves. Contiguous accessory lower Subscapular nerves (aLS) were present in 9% (n=3). All upper Subscapular nerves (USN) took origin from the PC. ALS nerves were present in 30% (n=10) of specimens. Of this group 50% (n=5) took origin from the PC, 30% (n=3) from Thoracodorsal nerve (TD), 20% (n=2) from Lower Subscapular nerve (LSN). Two aLS nerves were present in 3% (n=1). The LSN took origin from the Axillary nerve (AN) in 15% of specimens and the TD in 3%.

**CONCLUSION:** We propose a new nomenclature for these common variations to aid identification during plexus surgery. The accessory upper Subscapular nerves solely innervate Subscapularis and arise exclusively between the USN and TD. Similarly the accessory lower Subscapular nerves arise distal to the TD and solely innervate the Subscapularis muscle. These accessory nerves constitute additional neural tissue distinct from the USN and LSN with definitive and consistent innervation to the Subscapularis. Recognition of these accessory nerves may provide donor nerves for neurotisation. Future treatment modalities for plexus insult are discussed such as selective denervation of Subscapularis in cases of severe internal rotation contractures.

#### **LOP08: MYELINATION AND NODES OF RANVIER FORMATION IN THE REGENERATED SCIATIC NERVE BY TRANSPLANTATION OF MYELIN-FORMING CELLS AND DIRECT COMPARISON OF SCHWANN CELLS AND OLFACTORY ENSHEATHING CELLS**

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**INTRODUCTION:** After peripheral nerve injury Schwann cells reorganize and provide a permissive environment for nerve regeneration. Moreover, it is important that these regenerated axons become myelinated. While endogenous Schwann cells can perform these functions, additional transplantation of Schwann cells or olfactory ensheathing cells (OECs) into transected nerves were shown to facilitate this complex repair process. It is unclear and controversially discussed if Schwann cell- or OEC-transplantation results in more effective regeneration. The present study was performed to determine if transplanted Schwann cells and OECs participate in axonal regeneration and remyelination. Results from both groups are directly compared.

**MATERIAL AND METHODS:** Schwann cells or OECs were prepared from GFP transgenic male rodents. Sciatic nerves of female rats were exposed and crushed to transect all axons. Immediately after nerve transection GFP-Schwann cells or OECs were injected distal to the crush site. Twenty-one days later the nerves were removed and prepared for histology. The engrafted cells were identified by GFP fluorescence and FISH for Y chromosome. Immunostaining for sodium channels (NaV 1.6) and paranodal regions (Caspr) was used to define nodes of Ranvier on regenerated axons. Axon counts were performed and the amount of remyelination was determined.

**RESULTS:** The transplantation of identified Schwann cells or olfactory ensheathing cells into an axotomy model results in axonal regeneration and remyelination enhanced by the transplanted cells. The donor myelin-forming cells could be identified by GFP fluorescence and by FISH for Y chromosome. The transplanted cells were able to survive and form myelin with mature nodes of Ranvier expressing the sodium channel NaV 1.6 on the regenerated axons.

**CONCLUSION:** These results indicate that engrafted Schwann cells or olfactory ensheathing cells into injured peripheral nerve can integrate and participate in neural repair. The comparison between OEC and Schwann cells from rodent into a peripheral nerve injury model showed that OECs transplantation results in significant better remyelination potential than Schwann cells.

#### **LOP09: ESTABLISHING THE RELATIONSHIP OF SUPERFICIAL AND DEEP WOUND MICROBIOLOGY: A 6 YEAR REVIEW OF DEEP HAND INFECTIONS**

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**INTRODUCTION:** Hand infections are challenging because of the anatomy involved and wide spectrum of microbiology. The aim of this study is to evaluate the bacteriological spectrum and the antimicrobial susceptibility patterns of patients with hand infections, in particular analysing the relationship of the superficial wound cultures to deep wound cultures.

**METHODS:** Microbiology laboratory data was accessed via the APEX database from 2006 to 2012 identifying all microbiology swabs related to hand infections. Patients with both superficial and deep hand microbiological samples were selected.

**RESULTS:** 7823 hand bacteriology specimens were generated. Patients with both superficial and deep swabs were selected leaving 123 specimens from a total of 26 patients. 24/26 (92.3%) of these patients grew matching microbiological cultures on superficial and deep swabs. The most common organism cultured was Staphylococcus aureus susceptible to Flucloxacillin. One of the two patients with discordant cultures grew Group C Streptococcus superficial culture and S.aureus on deep culture. The second grew Pseudomonas aeruginosa on superficial culture and coagulase-negative Staphylococcus on deep culture.

**CONCLUSION:** S. aureus is the most common bacterium responsible for deep hand infections in our region and we recommend Flucloxacillin as the first-line antibiotic and macrolides for penicillin-allergic patients. There is good correlation between deep and superficial swabs and empirical treatment with Flucloxacillin can be commenced on the basis of either swab culture. In this time of increasing emphasis on health economics, results of either superficial or deep swab could justify

nullifying the other. This will help save time with subsequent cost savings in the management of deep hand infections.

#### **LOP10: OPTIMIZATION OF FLEXOR TENDON TISSUE ENGINEERING: HUMAN ADIPODERIVED STEM CELL-TENOCYTE CO-CULTURES FOR SEEDING OF AN ACELLULARIZED TENDON SCAFFOLDS**

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**INTRODUCTION:** Complex hand injuries often require multiple tendon grafting. The supply of autologous grafts is limited, so that there is a demand for artificial tendon grafts. Seeding acellularized tendons with cells is an approach to provide grafts with favorable biomechanical properties. It was our aim to evaluate if human adipoderived stem cells (ASCs) could replace tenocytes for scaffold seeding.

**METHODS:** ASCs and tenocytes were co-cultured in different ratios (3:1, 1:1, 1:3) and with three different methods (1. direct co-culture, 2. tenocyte-conditioned media on ASCs and 3. and an insert system to keep both cell types in same media without contact). Proliferation, collagen production and tenogenic marker expression were measured by hematoxytometry, immunocytochemistry, ELISA and real-time PCR.

**RESULTS:** Proliferation and collagen production were similar for tenocytes and ASCs alone. Tenascin C was a reliable tenocyte marker. Proliferation was increased in direct co-culture, especially at an ASC:tenocyte ratio of 3:1, and for tenocytes in ASC-conditioned media. Direct co-culture caused significant upregulation in tenascin C expression in ASCs (4.0x, p

**CONCLUSION:** ASCs are good candidates for tendon tissue engineering because they are similar to tenocytes in proliferation and collagen production. They increase proliferation in co-culture and adapt a tenocyte-like phenotype. An ASC : tenocyte ratio of 3:1 is optimal to provide good proliferation and phenotype change by minimizing donor morbidity.

#### **LOP11: CORTICOSTEROID INJECTION THERAPY FOR TRIGGER DIGITS: A RETROSPECTIVE REVIEW OF 577 DIGITS**

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**PURPOSE:** A retrospective review (1998-2011) of patient demographics with flexor tenosynovitis or trigger digits (TDs) and the efficacy of corticosteroid injections and surgery.

**METHODS:** Gender, age, diabetes, hand dominance, and distribution of TDs were assessed. Patients received steroid injection as the initial treatment (8 mg Triamcinolone Acetonide mixed with 1% lidocaine). Recurrence, duration of efficacy, and surgery were examined.

**RESULTS:** There were 577 TDs in 362 patients (F=258 (71.3%); M=104 (28.7%); 1st injection); 300 days (2nd injection); 286 (3rd injection). Surgical intervention was required in 117 TDs with a recurrence rate of 0.85%. There was no complication from the injections.

**CONCLUSIONS:** Corticosteroid injection therapy is a highly safe and effective treatment with an 80% efficacy rate. Surgical release when required provides a definitive solution.

#### **LOP12: LOCAL PRECONDITIONING WITH IMPLANTATION OF NON-VIRAL TRANSFECTED FIBROBLASTS IN AN ISCHEMIC RAT FLAP MODEL IMPROVES REGENERATION**

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**INTRODUCTION:** Protein delivery from transfected cells can induce expression of tissue inductive factors to stimulate the cellular processes required for regeneration. In the present work, we used a cell-based, non

viral gene-transfer method using fibroblasts to temporarily produce bFGF and VEGF165 in ischemic tissue for therapeutic purposes as a form of pharmacological local preconditioning before tissue ischemia occurs. Functional evaluations were performed to detect the protein expression and resulting clinical effects.

**MATERIAL AND METHODS:** Primary skin fibroblasts were transfected with eukaryotic expression vectors harboring VEGF and bFGF cDNAs mediated by Amaxa Nucleofector. To determine an improvement in ischemically challenged tissue, a genetically modified cellspool was injected in an ischemic rat flap model. Cells were implanted into 40 rats. Gene expression and protein production were measured in vitro and in vivo by real time PCR and immunoassay (BioPlex) respectively at different time points. Clinical outcome was demonstrated by planimetric measurements and immunohistology.

**RESULTS:** After injection of genetically modified cells, transient protein expression of bFGF and VEGF165 in the target tissue of the ischemic flap model increased significantly compared to controls. A reduction in flap necrosis by one-third was detected, after two weeks if transfected cells were applied 1 week before ischemia. A highly significant improvement of endothelial cell counts was observed after administration of the transfected cells.

**CONCLUSION:** Our results indicate that transient expression of bFGF and VEGF165 induces therapeutically relevant effects after local preconditioning with non-viral transfected fibroblasts in the ischemic rat flap model. Our standardized transfection technology is now used in preclinical research.

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#### **LOP13: CRUSHED CARTILAGE WITH VICRYL MESH TO AVOID POST-RHINOPLASTY DORSUM IRREGULARITIES**

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**INTRODUCTION:** An acceptable aesthetic result of nasal dorsum is sometimes easy, simple, and secure, but in some complex and difficult cases we need to use grafts or implants to obtain a nasal dorsum with a nice contour and definition. Of the autogenous graft types, autogenous cartilages is widely accepted as ideal for dorsal augmentation and contour as it is easy to carve, has a high degree of tissue tolerance, a low infection rate, and is easily accessible. However, cartilaginous dorsal nasal grafts are associated with limitations, such as limited amounts of tissue, additional surgery time, donor site morbidity, visible graft contour, postoperative distortions, and deformities at the graft-host interface. At 1 to 2 years after surgery, crushed cartilage grafts may become perceptible through nasal skin after the resolution of tissue oedema. The irregularity post rhinoplasty is one of the complications that may have a negative impact to the patient and to the long time outcome of rhinoplasty. We present our technique of crushed cartilage with Vicryl mesh to do a single layer structure put simply below the skin to overcome the post-rhinoplasty dorsum irregularity problem.

**PATIENTS AND METHODS:** 250 patients who underwent rhinoplasty, over 10 years, the age range 19-49 year old, they were operated by the same hand. Follow-up for a period ranged between 3 months to 3 years by the same team. The technique consists of using the excised part of alar and lateral nasal cartilages and also the pieces of cartilage which are excised from the dorsum to be crushed by hammer or cartilage crusher with a Vicryl mesh (Polyglactin 910 mesh, trademark: Ethicon Vicryl 8.5X10.5 cm) to do a single homogenous layer of the mixture, then we put this layer at the dorsum of the nose subcutaneously to avoid post rhinoplasty irregularity. The using of Vicryl mesh helps to join all particles of crushed cartilages and avoid slipping some of cartilages particles from the created homogenous layer of the mixture, and keep it homogenous. The time of Vicryl absorption is the time to get enough tissue healing and fibrosis to replace it, and this guarantee a long term homogenous dorsum without any irregularities. We can apply this technique in closed or open rhinoplasty

**RESULTS:** Postoperatively, the patients have a nasal splint for 8 days. We put them on simple analgesia for the first 2-3 days. Follow-up on day 21, then after 3 months, and after a year. We did experience any case of infection, necrosis and eventually no dorsum irregularities neither in short term follow-up period nor in long duration.

**CONCLUSION:** This technique can avoid the post-rhinoplasty irregularities and "operated look" complication. This technique is easy, cheap, takes no so much extra time because we use the already excised alar and lateral nasal cartilages, with minimal risk of complications (actually we experience negligible complications) and no postoperative irregularities in all our patients. In addition to that this technique can augment the dorsum of nose in minor degree of saddle nose.

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#### **LOP14: COMPARISON OF THREE DIFFERENT HARVESTING METHODS TO OBTAIN PREADIPOCYTES: IMPACT ON VIABILITY AND DIFFERENTIATION TO ADIPOCYTES**

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**QUESTION:** Autologous fat transfer is a wide spread technique for soft tissue augmentation. Different tools for harvesting fat tissue have been established. Such devices should be easy to handle, time saving, low priced, safe and provide a high amount of viable cells in the aspirate.

Aim of this study was to compare three different methods for harvesting fat tissue for lipotransfer: Water assisted liposuction (WAL), power assisted liposuction (PAL) and manual aspiration.

**METHODS:** Fat tissue was obtained from nine donors undergoing abdominoplasty. Samples were divided into three sections. Out of each section fat was harvested using either WAL, PAL or manual aspiration. Preadipocytes were isolated using a standard protocol. The amount of extracted preadipocytes was evaluated using cell count, viability was evaluated using annexin/PI staining. The ability of isolated preadipocytes to differentiate was determined by expression of adipocyte markers adiponectin, GLUT4 and PPARG.

**RESULTS:** Our results show that there are significant differences using different harvesting methods: number and viability of extracted preadipocytes was significantly higher using PAL than WAL or manual aspiration. Furthermore their ability to differentiate into mature adipocytes differs significantly as adiponectin, GLUT4 and PPARG were significantly higher using PAL.

**CONCLUSION:** Preadipocytes play an important role in autologous fat transfer. Therefore these results should be considered when choosing the harvesting method.

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#### **LOP15: THE EFFECT OF TYPE AND DEGREE OF PRESSURE ON FAT GRAFTING**

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**INTRODUCTION:** Autologous fat grafting has many clinical applications; however, outcomes remain highly variable. To date, many factors have been implicated in fat graft survival such as harvesting, processing, and injection techniques. One critical variable within these techniques is pressure. In this study, we examined the role of pressure on human fat grafts in a nude mouse model.

**MATERIAL AND METHODS:** Negative Pressure: Tumescence liposuction was performed in the laboratory on fresh panniculectomy specimens. Suction pressure was either -0.5 atm or -0.83 atm. Lipoaspirate was centrifuged at 1200g and injected into the dorsal flanks of nude mice. Positive Pressure: Fresh operating room lipoaspirate was obtained and positive pressure was applied up to six atmospheres for up to three minutes and then injected into nude mice. Shear Stress: Lipoaspirate was centrifuged at 1200g for three minutes and then injected at different speeds: 3-5 ml/sec vs. 0.5-1 ml/sec. After four weeks, the fat lobules were analyzed for weight and histology.

**RESULTS:** Negative Pressure: There were no differences in weight or histology with high versus low suction pressures. Positive Pressure: Various pressures and time points did not demonstrate a significant difference in weight or histology. Shear Stress: In vivo, slow injections yielded a 38% increase in weight ( $p < 0.001$ ) compared to fast injection. This correlated with histology.

**CONCLUSION:** Aspiration pressures up to -0.83 atm and positive pressure up to six atmospheres did not affect fat graft viability in vivo. The degree of shear stress, which is a function of flow rate, did significantly affect fat graft viability. Fat injected slowly with low shear stress significantly outperformed fat injected fast with high shear stress. Based on these results, we suggest the use of high pressure for added harvest efficiency and the better consideration of shear force for optimal fat graft survival.

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#### **LOP16: SUBJECTIVE EVALUATIONS OF THE FEMALE BREAST IN A STUDY OF 50 PATIENTS TREATED FOR ASYMMETRY**

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**QUESTION:** Breast asymmetry represents a challenge for every Plastic Surgeon. The aim of the following study was to identify those factors, which influence long-term postoperative overall satisfaction the most.

**METHODS:** 64 patients with asymmetric breasts received surgical treatment between 2000 and 2009 at our Department of Plastic Surgery. 50 patients were seen during follow-up 2 to 11 years postoperatively. Anthropomorphic measurements were taken and subjective assessments by the patients were conducted. In this abstract, however, not all of the parameters have been analyzed so far. Until now thought has been given to the subjective parameters overall satisfaction, scarring, sensitivity, symmetry, size and shape of the breast by means of a visual analog scale (VAS) ranging from 1 (worst) to 10 (best). An analysis of correlation was performed by the Kendall-Tau-b test and those parameters were identified, which correlated most with postoperative overall satisfaction.

**RESULTS:** 50 patients were seen during follow-up after a mean time of 5 years (2-10 years). At time of operation the mean age was 32 years (16-73 years). Overall satisfaction was 8.3 (2-10) on the VAS. The older patients were at time of operation, the significantly more satisfied they were at follow-up. The time lapse until follow up and BMI had no significant impact in this regard. The analysis of correlation between overall satisfaction and subjective parameters revealed the strongest correlation between overall satisfaction and shape of the breast, followed by asymmetry and size. Scarring and sensitivity only had little impact on overall satisfaction. Shape, however, is most influenced by size.

**CONCLUSIONS:** Asymmetry of the breast can cause major functional, social and emotional problems. Surgical treatment is truly challenging, the thankfulness of the patients is represented by the high score of 8.3 on the VAS. Probably because of the longer period of suffering older patients judge postoperative results better than younger patients. Postoperative overall satisfaction is primarily dependent on the shape of the breast. Symmetry and size are secondary. Surprisingly scarring and sensitivity only play a minor role. Possible implications on the surgical procedure to correct breast asymmetry are discussed.

#### LOP17: CONGENITAL SYMMASTIA REVISITED

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**BACKGROUND:** Symmastia is defined as medial confluence of the breast. The term "Symmastia" is modified from Greek (syn meaning "together", and mastos meaning "breast") and was first presented by Spence et al in 1983. Two forms of symmastia exist: an iatrogenic and a congenital version. Congenital symmastia is a rare condition, with soft-tissue web over the sternum connecting the breasts. The literature on congenital symmastia is limited, few cases have been published and knowledge about the optimal treatment is still insufficient.

**MATERIAL AND METHODS:** Congenital symmastia was evaluated as an entity using a review of the literature and a theoretical model. We analysed the problem using the three-step principle formulated by Blondeel that describe the breast as a "footprint", "conus" and "skin-envelope". To date, few papers on congenital symmastia have been published with most focusing on the application of various surgical approaches. We examined the literature and evaluated the procedures used, and present two recent cases of congenital symmastia as examples. By combining review and analysis we offer a rational treatment practise.

**RESULTS:** The analysis showed that the treatment is to correct the "footprint" by removing the excess "conus" over sternum and reattach the "skin-envelope" to the sternum and thereby create the normal medial border of the "footprint". Two overall approaches have been used to treat congenital symmastia a reduction mammoplasty and liposuction.

**CONCLUSION:** Combining the Blondeel analysis and review of the procedures used, we developed a flowchart to offer a treatment practise.

#### LOP18: A NEW TOOL IN GUIDED-TISSUE REGENERATION: MODIFIED SILK MEMBRANES

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**INTRODUCTION:** Guided tissue regeneration (GTR) is well established for different types of barrier membranes. Here we report a novel ST-silk membrane which offers advantages compared to established collagen membranes derived from animal tissues. ST-silk membranes can be surface functionalised, are free from potentially infective animal pathogens and have excellent mechanical characteristics. In this study we achieved functionalisation of ST-silk membranes with hydroxyapatite (HA) and evaluated the proliferative effects of HA-functionalised silk membranes on osteoprogenitor cells like rat mesenchymal stromal cells (MSC) in vitro.

**METHODS:** First, native ST-silk membranes were evaluated regarding their effects on proliferation rates of L929 fibroblasts und dysplastic oral keratinocytes (DOK cell line). Possible cytotoxic effects were analysed by monitoring LDH activity. Thereafter, HA-functionalized ST-silk membranes were seeded with rat MSCs and effects on osteogenic differentiation were evaluated for 7 days. Furthermore SEM,  $\mu$ CT and digital microscopy (VHX-600;Fa. Keyence) of the membranes were performed.

**RESULTS:** ST-silk membranes demonstrated good biocompatibility as no negativ effect in terms of vitality/proliferation of L929 and DOK cells could be observed over 22 days. HA-functionalized ST-silk membranes showed osteoinductive effects (increase in alkaline phosphatase activity) on rat MSCs after seven days.

**CONCLUSIONS:** Functionalization of ST-silk membranes with hydroxyapatite seems to have an osteoinductive effect on MSCs in vitro. These innovative membranes open promising avenues for application in guided tissue regeneration and as scaffold material in tissue engineering for dental applications.

#### LOP19: PLANNING SURGICAL RECONSTRUCTION IN TREACHER - COLLINS SYNDROME USING GEOMETRIC MORPHOMETRICS

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**INTRODUCTION:** Treacher Collins syndrome (TCS) is a rare autosomal dominant condition that results in craniofacial deformities of varied phenotypic expression. The surgical correction in this syndrome is difficult; approaches vary between craniofacial departments worldwide.

We aimed to design standardized tools for planning zygomatic and mandibular reconstruction in TCS using geometric morphometrics and also define the facial morphology of TCS.

**METHODS:** The Great Ormond Street Hospital Database was retrospectively identified for patients with TCS. 6 children (2 - 12 yrs) who had suitable un-operated three-dimensional computed tomography head scans (3DCT) were included. 3DCT scans were compared using a template of 96 anatomically defined landmarks to 49 age-matched dry skulls donated from the National History Museum and the John Hopkins Bosma collection.

**RESULTS:** Principal component analysis based on the comparison of the normal landmark vector and TCS landmark vector determined the characteristic deformities of retromicrognathia, zygomatic and orbital wall hypoplasia. Thin plate spline movies identified the abnormalities of the TCS skull and demonstrated 'virtual regrowth' of sites of complete agenesis. Intra-rater reliability of the landmarks was acceptable and within a SD < 1mm on univariate analysis of 10 repeated scans.

**CONCLUSIONS:** Virtual normalisation of the TCS skull can help guide surgical reconstruction. Furthermore size matched stereolithographic templates can be designed to guide zygomatic and mandibular reconstruction in the TCS patient.

#### LOP20: ADHESION AND OSTEOGENIC DIFFERENTIATION OF NON-ADHERENT PROGENITORS FROM ADIPOSE-DERIVED STEM CELLS (napASCs) OVER INTEGRA DERMAL REGENERATION TEMPLATE

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**INTRODUCTION:** Non-adherent progenitors from adipose-derived stem cells (n.a.p.ASCs) represent an upstream lineage of multipotent stem cell progenitors capable of clonal expansion and asymmetric divisions comparable to stem cell spheres observed in breast, thyroid and colon, and, as such, confirm the stemness of cells extracted from lipospirate. The identification of napASCs confirms the stem-cell origin of the more differentiated and commonly used adherent mesenchymal stromal cells (MSCs).

NapASCs adhesion for differentiation purposes may be useful in tissue engineering. In this study we investigate the feasibility of napASCs adhesion over Integra® dermal regeneration template for cell colonization and differentiation toward the osteogenic lineage.

**MATERIAL AND METHODS:** NapASCs were extracted from adipose tissue from 18 healthy donors following patients written consent. NapASCs were expanded in suspension and plated with Integra® and

osteogenic medium containing dexamethasone, beta-glycerophosphate and ascorbic acid. Differentiation to the osteogenic lineage was assessed by presence of alkaline phosphatase (ALP), osteopontin (OP) and calcium deposits. H&E stain was used to assess pre-osteoblasts within the dermal template.

**RESULTS:** NapASCs spheres were visible in 1-2 weeks and their stemness confirmed in vitro by clonal expansion. Adhesion of napASCs to Integra® and phenotype change to fibroblast-like precursors was achieved in 48-72 hours. Phenotype change to the osteogenic lineage was obtained in 3-7 days, strong ALP activity in 14 days, OP positivity and calcium deposits on Von Kossa in 21 days. Spheres of napASCs and pre-osteoblasts were visible on H&E stain in histology sections within Integra® fibers.

**CONCLUSIONS:** NapASCs can grow and differentiate in pre-osteoblasts within a clinically available three-dimensional regenerative template such as Integra®. These promising results should encourage further in vivo studies for future clinical application.

#### **LOP21: MIDFACIAL ANTHROPOMETRIC CHANGES AFTER SURGICAL ASSISTED RAPID PALATAL EXPANSION**

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**PROBLEM:** In orthognathic surgery, bony nasal base changes after surgical assisted rapid palatal expansion are well known. The surgical assisted rapid palatal expansion effects the whole bony maxilla below the LeFort-I-layer. These bony changes could cause midfacial soft tissue changes. Aim of the study was an analysis of midfacial anthropometric changes after surgical assisted rapid palatal expansion.

**MATERIAL AND METHOD:** Between April 2005 and October 2010 77 patients (average age: 30 years (M=28; F=49; 15 - 59, Median age 30 years) underwent a surgical assisted rapid palatal expansion. In every case an orthognathic non-surgical assisted rapid palatal expansion failed. In 7 cases the surgical assisted rapid palatal expansion was performed with a palatal distractor, in 70 cases with a Hyrax expander. A standardized photo analysis took place preoperative und 6 month postoperative. The following midfacial anthropometric parameters were raised: Eye fissure, canthal tilt, upper lid sulcus height, lower iris coverage, upper iris coverage, scleral show, ectropion, position of lower eyelid to iris, intercanthal width, nasal base width, nasal length and medial diastema width.

**RESULTS:** The average palatal expansion was 10±4mms. The nasal base width was proportional for palatal expansion and medial diastema width. There were no significant changes ( $p>0,05$ ) in eye fissure, canthal tilt, upper lid sulcus height, lower iris coverage, upper iris coverage, scleral show, ectropion, position of lower eyelid to iris, intercanthal width and nasal length. There also were no significant changes ( $p>0,05$ ) in palatal expansions bigger than 10mms.

**CONCLUSION:** Except the nasal base width no midfacial anthropometric changes after surgical assisted rapid palatal expansion, also with expansions bigger than 10mms, appeared. In expansions up to 18mms the surgical assisted rapid palatal expansion seems to have no effect in the periorbital region. According to this the nasal base width seems to be the limiting aesthetical factor for the palatal expansion.

#### **LOP22: NAGER SYNDROME DENTAL PULP STEM CELLS HAVE OSTEOGENIC POTENTIAL**

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**BACKGROUND & PURPOSE:** Nager syndrome patients with severe micrognathia, malar hypoplasia, cleft palate, and radial club hand deformities require bone reconstruction for severe deformities. Novel stem cell therapies may be used in the future to decrease donor site morbidity in these patients. To study this, we isolated stem cells from dental pulp tissue of Nager syndrome patients and specifically looked at osteogenesis.

**METHODS:** Deciduous teeth in Nager and normal patients were obtained and used to isolate dental pulp stem cells using a pre-plating technique. Harvested dental pulp stem cells were confirmed with stem cell antigens (CD29, CD90, CD105, CD166) and tested for hematopoietic cell markers (CD34,CD45) and endothelial cell markers (CD31) to confirm mesenchymal origin. After 7 days in osteogenic media, osteogenesis was tested with Von Kossa staining and rt-PCR (runx2,c-fos, osteocalcin, osteonectin, and osteopontin). In addition, Nager cells were stressed in an in vivo microdistraction model and studied for osteogenic potential.

**RESULTS:** Isolated Nager dental pulp stem cells were positive for mesenchymal stem cell antigens while negative for hematopoietic cell markers, and for endothelial cell marker. Cells were capable of undergoing osteogenic differentiation, as evidenced by positive Von Kossa staining and real time PCR for osteogenic genes: runx2 (3.3 fold), c-fos (2.2 fold), osteocalcin (12 fold), osteopontin (3.1 fold). Comparison with preosteoblasts and normal dental pulp stem cells also confirmed differential osteogenic expression of Nager cells. Linear stress of Nager cells within the microdistractor showed earlier osteogenic expression compared to non-stressed cells and to controls.

**CONCLUSIONS:** Mesenchymal cells isolated from dental pulp of Nager patient have osteogenic potential that can be used to correct hypoplastic mandibular and radial club hand deformities. Exfoliation of deciduous teeth allow for a promising source of stem cells in Nager syndrome patients.

#### **LOP23: THE DIFFERENTIAL OSTEOGENIC POTENTIAL OF BMPs IN IMMORTALIZED CALVARIAL CELLS**

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**INTRODUCTION:** Bone Morphogenetic Proteins (BMPs) play a vital role in the proliferation and differentiation of osteoblasts. Multiple studies have demonstrated their therapeutic potential through stimulation of bone growth in vivo. In calvarial defect models, adenoviral gene delivery of BMPs has shown promise, but also poor transgene expression when not applied within a cell-based approach. Our objective was to quantify osteogenic stimulation in immortalized murine calvarial cells by multiple ad-BMPs to lay the groundwork for future osseous tissue engineering.

**METHODS:** Previously immortalized calvarial cells (iCALs) from juvenile murine skulls were infected with adenoviral vectors encoding BMPs with previously demonstrated osteogenic activity (adBMP-X). Adenovirus-green fluorescent protein (adGFP)-infected cells served as controls. Ad-BMP-infected iCALs were compared through alkaline phosphatase assays at varying time points. RT-PCR was used to study BMP-mediated gene upregulation, whereas Alizarin Red staining was used to analyze terminal cell differentiation (mineralization). Stem cell implantation into the quadriceps of nude mice was performed to determine in vivo ectopic bone formation by the various adBMP-infected iCals.

**RESULTS:** Statistically significant increase of alkaline phosphatase secretion was detected in ad-BMP-9, ad-BMP-2, and ad-BMP-4-treated cells compared to ad-GFP. This was corroborated qualitatively via immunohistochemical staining. Differential upregulation of various gene transcripts known to signify osteogenic differentiation (Id1-3, Smad2-4, RunX2, OCN/OPN, OsX) was elucidated by RT-PCR and pointed toward mechanism of adBMP-mediated osteogenesis. Alizarin Red staining demonstrated increased mineralized nodule formation of adBMP9- and adBMP2-infected cells compared to adBMP4-, adBMP7-, and adGFP-infected counterparts. In vivo stem cell implantation assay revealed successful and differential bone nodule formation in ad-BMP-treated cells versus controls. Micro-CT of explanted nodules revealed larger and more consolidated ectopic bone in adBMP-9-treated cells over adBMP-2, adBMP-4 and adBMP-7-treated cells. Histological staining (H&E, Trichrome, and Alcian Blue) revealed more mature bone in adBMP-9-treated iCALs.

**CONCLUSIONS:** Bone Morphogenetic Proteins (BMPs) show significant and varying stimulation of osteogenesis in immortalized murine calvarial cells (iCALs) both in vivo and in vitro. BMP-9 and BMP-2 displayed the greatest degree of osteogenic potential in this cell line as demonstrated by early, middle and late markers of differentiation.

#### **LOP24: REDUCTION OF SUTURE ASSOCIATED INFLAMMATION USING THE NOVEL BIOCOMPATIBLE POLY ESTER AMIDE PSEUDO-PROTEIN**

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**INTRODUCTION:** We have developed a novel Poly(ester amide) (PEA) with the potential to modulate the immune response to implantable medical devices. We hypothesized PEA-coating would reduce the immune response to 2 typically inflammatory suture materials.

**METHODS:** 28 C57BL/6 mice underwent silk or plain-gut suture implantation in the bilateral gluteal muscles: PEA-coated in the right and

non-coated, control in the left. Animals were sacrificed after 3, 7, 14 and 28d. Gluteal muscles were harvested and processed for histology. The area of inflammation surrounding each suture was quantified and compared between groups.

**RESULTS:** PEA-coated silk sutures resulted in significantly decreased mean areas of inflammation compared with non-coated controls after 7 and 28d ( $686,897\mu\text{m}^2 \pm 99,646\mu\text{m}^2$  v.  $2,095,447\mu\text{m}^2 \pm 385,461\mu\text{m}^2$ , p2 v.  $272,230\mu\text{m}^2 \pm 40,156\mu\text{m}^2$ , p2 v.  $2,502,000\mu\text{m}^2 \pm 462,461\mu\text{m}^2$ , p

**CONCLUSION:** PEA-coating significantly decreases the local immune response to inflammatory plain-gut and silk sutures. Although further study is warranted prior to clinical use, suture modification via PEA-coating offers a promising means to improve suture biocompatibility and minimize surgical site morbidity.

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**LOP25: ANGIOGENIC FACTORS PLACENTAL GROWTH FACTOR (PIGF) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ARE IN PART RESPONSIBLE FOR THE BENEFICIAL EFFECTS OF HUMAN BLOOD OUTGROWTH ENDOTHELIAL CELLS (hBOECs) AND DERMAL FIBROBLAST SHEETS (hDFS) ON WOUND HEALING**

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**QUESTION:** Appropriate vascularisation of skin grafts is a critical requirement for their survival and proper reconstructive function. We previously showed that integration of human blood outgrowth endothelial cells (hBOECs) in a multilayered human dermal fibroblast sheet (hDFS) was a suitable engineering approach to efficiently promote dermal and epidermal healing and reduce contraction in a full-thickness murine wound model. The key to this success was in part related to the secretion of a set of growth factors both by hBOECs and hDFS. In the current follow-up study, we intended to investigate the specific contribution of two growth factors - hBOEC-derived placental growth factor or PIGF and hDFS-derived vascular endothelial growth factor or VEGF - to the beneficial effect on wound healing.

**METHODS:** We knocked down PIGF or VEGF by short hairpin RNA (shRNA) technology and studied the effect of this intervention on the in vitro and in vivo behaviour of the cells.

**RESULTS:** PIGF and VEGF knockdown significantly reduced hBOEC and dermal fibroblast proliferation in vitro, respectively. Moreover, supernatant of PIGF or VEGF knockdown cells significantly hampered in vitro keratinocyte migration. Furthermore, while PIGF knockdown did not significantly affect hBOEC engraftment, upon transplantation of PIGF knockdown hBOECs in wounds, there was a significant decline in vessel density in the wound edges and epidermal coverage was reduced compared to mice transplanted with BOECs transduced with scrambled shRNA. Knockdown of VEGF in dermal fibroblasts significantly reduced graft survival and vascularisation, the latter shown by reduced vascular density and invasion. VEGF-knockdown-hDFS also were less efficient in reducing wound contraction, which correlated with an increase in myofibroblast appearance at the wound edges. Conditioned media of VEGF-knockdown-hDFS was less efficient in stimulating keratinocyte migration in vitro. Accordingly, keratinocyte coverage of the wound bed was significantly reduced in the absence of hDFS-derived VEGF. Finally, wounds treated with hDFS showed significant leakage of blood at the wound edges which was dramatically reduced upon VEGF knockdown in these constructs. The decreased leakage in the absence of VEGF was most likely related to a more elaborate smooth muscle cell coating of the vessels.

**CONCLUSION:** In conclusion, hBOEC-derived PIGF and hDFS-derived VEGF both have an essential role in the beneficial effect of the hBOEC-hDFS skin graft on wound healing.

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**LOP26: AUTOLOGOUS FAT GRAFTING IMPROVES WOUND HEALING**

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**QUESTION:** Autologous fat grafting, better known as Lipofilling, has shown to have regenerative effects on human skin. It is assumed that the presence of mesenchymal stem cells may be responsible for these

observations. The aim of the performed case-analysis was to assess the effect of sublesional autologous fat transplantation on healing processes of chronic wounds.

**METHODS:** We analyzed a case series of 8 patients with chronic leg ulcers, at which sublesional autologous fat transplantation was performed. The ulcers existed at least for 8 weeks and showed no tendency to heal. Possible treatments to improve peripheral tissue perfusion were excluded. After debridement of the ulcer, liposuced fat tissue from the abdomen was transplanted below the wound. For this purpose the lipoaspirate was prepared by a modification of the Colemans method, and then injected into the wound edges and sublesional in different layers. The wound coverage was made with a hydrocolloid dressing (Hydrokoll®, Hartmann, Germany), which was changed every 3 to 4 days. At regular intervals, we performed a wound assessment with determination of ulcer size, and a digital photo documentation. The observation period for each patient was 12 weeks. Based on computerized photos the wound areas were determined and analyzed statistically.

**RESULTS:** Before intervention ulcers existed between 8 weeks and 4 years. After debridement, the wound size averaged  $6.41 \pm 9$  cm<sup>2</sup>. After 6 weeks, the wounds have decreased by  $72 \pm 10\%$ . The mean duration until complete healing was 12 weeks.

**CONCLUSIONS:** It is assumed that autologous fat transplantation improves healing of chronic leg ulcers. To prove this hypothesis currently a prospective, randomized, clinical trial is conducted at our department.

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**LOP27: APPLICATIONS OF THE OMENTUM FOR LIMB SALVAGE: THE REGENERATIVE POWER OF OMENTAL STROMAL CELLS**

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**BACKGROUND:** The omentum is one of the oldest, but often overlooked options for extremity reconstruction. Due to the large amount of pliable tissue, long vascular pedicle, associated lymphoid tissue, and angiogenic properties, the omentum should be considered when reconstructing complex extremity defects. We report the largest series of free omental transfers for reconstruction of complex extremity defects.

**MATERIALS AND METHODS:** A retrospective analysis of 23 omental free tissue transfers in 21 patients with complex upper and lower extremity defects between 1999 and 2011 was performed. Indications, operative technique, and outcome were evaluated.

**RESULTS:** Patient age ranged from 12-63 years with 17 males and 4 females. Mean follow-up was 2 years. Indications included defects due to crush-degloving injuries, IIIB/IIIC fractures, pitbull mauling, or infection. Ten omental flaps were for upper and thirteen for lower extremity defects, with bilateral coverage using split omentum performed in two patients. Mean defect size was 691cm<sup>2</sup> and all patients achieved wound coverage. Complications included total flap loss (1), partial flap loss (3) partial skin graft loss (3), and donor site infection (1). Laparoscopic-assisted harvest was performed in three cases. Histologic analysis of the transferred omentum showed persistent characteristics of omental fat. Furthermore analysis of 5 separate omental tissue samples compared to subcutaneous tissue samples showed a superior growth and differentiation potential, resembling mesenchymal stem cell morphology.

**CONCLUSION:** The long vascular pedicle and large amount of pliable tissue allow the omentum to be aggressively contoured for complex defects. In addition, laparoscopic-assisted harvest may reduce donor site morbidity. By maintaining its unique physiology, including omental stromal cells and lymphoreticular bodies, the omentum may facilitate healing by reducing edema and fighting infection, thereby remaining an important option in complex extremity reconstruction.

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**LOP28: HYPOXIA ENHANCES METASTATIC EFFICIENCY IN THE HUMAN FIBROSARCOMA HT1080 XENOGRAFT MODEL**

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**INTRODUCTION:** Transplant survival remains an obstacle in metastatic soft tissue sarcoma xenograft models. Studies have suggested that exposure of tumour cells to hypoxia may have a beneficial effect on the metastatic process and enhance metastatic efficiency. We

hypothesised that metastatic sarcoma models with tumour cells exposed to hypoxia are easily reproducible. The aim of this study was to establish a metastatic xenograft model of human fibrosarcoma HT1080 cells.

**MATERIALS AND METHODS:** The HT1080 cell line was cultured in vitro and  $1 \times 10^6$  cells undergoing exponential growth were collected. To render cells hypoxic, dishes were placed in a hypoxic chamber flushed with a gas mixture of 5% CO<sub>2</sub>, 1% O<sub>2</sub> and 94% N<sub>2</sub>. It took 6 h to achieve severe hypoxia in medium. The cells remained for another 24 h under these hypoxic conditions. For reoxygenation, cells were incubated in a tissue culture incubator for 6 h. The cells were intravenously inoculated as a 0.15 ml suspension into the lateral tail vein of athymic nude mice. Lung metastases development and growth were evaluated utilizing luciferase bioluminescent imaging. The metastatic sarcoma xenografts were subsequently analysed by histological and immunohistochemical staining.

**RESULTS:** In 75% xenotransplants (n=4) of HT1080 cells engrafted and led to the development of metastatic tumours in mice lungs. Histological and immunohistochemical staining confirmed the xenografts as being fibrosarcomas.

**CONCLUSION:** This metastatic sarcoma xenograft model with tumour cells exposed to hypoxia could be of high value for studying human soft tissue sarcomas and their therapy.

#### **LOP29: A SYNGENEIC IMMUNOCOMPETENT MURINE FIBROSARCOMA (BFS-1) MODEL TO STUDY HOST DEFENSE-LIKE LYTIC PEPTIDE AND DOXORUBICIN COMBINATION THERAPY**

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**INTRODUCTION:** Convenient animal models are needed to study the treatment of soft tissue sarcomas in vivo. The aim of this study was to establish a syngeneic (immunocompetent) murine fibrosarcoma (BFS-1) model that would allow study of host defense-like lytic peptide ([D]-K3H3L9) and doxorubicin combination therapy

**MATERIALS AND METHODS:** Murine fibrosarcoma BSF-1 cells in Matrigel (BD Biosciences, San Jose, CA, USA) were injected subcutaneously ( $1 \times 10^6$  cells) into the animal's flank. Every second day mice were weighed and tumor volume was determined with the formula of length $\times$ width $\times$ depth $\times 0.5$  in mm<sup>3</sup>. When the tumours reach an average volume of 130 mm<sup>3</sup> (BFS-1) the animals will be grouped according to the principles of a randomized control trial. The Host-Defense-Like Peptide [D]-K3H3L9 alone, doxorubicin alone or combination [D]-K3H3L9 and doxorubicin therapy will be injected intratumorally or intraperitoneally for three weeks. PBS (pH 7.0-7.5) will be applied as control substance.

**RESULTS:** 75% of syngeneic mice (n=20) transplanted with BFS-1 cells engrafted and led to the development of solid tumours.

**CONCLUSION:** Successful transplantation of BFS-1 fibrosarcoma cells in syngeneic mice was established. The mice in this sarcoma model are currently being treated and this model could be of high value for studying therapy of soft tissue sarcomas.

#### **LOP30: ELECTIVE LOCALIZATION OF THE DERMATOFIBROSARCOMA PROTUBERANS IN THE LEFT CHEST**

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**INTRODUCTION:** The etiology of Dermatofibrosarcoma Protuberans (DFSP) is still under discussion. The trunk is the most common site of involvement for DFSP, accounting for almost half of all cases, but tumors may occur in any part of the body. Between 1998 and 2010 we have observed and treated 24 patients with DFSP. The most common site of occurrence has been the chest; we have observed a "hot spot" of localization in the subclavicular/ parasternal region of the left chest. The aim of our study was to first report, and then provide an explanation of, the prevalence of DFSP on the left chest, as resulted from our clinical series.

**MATERIAL AND METHODS:** Perforator arteries in 4 different anatomical regions (parasternal, sub-clavicular, upper forearm, anterior thigh) were studied by the use of hand held Doppler and Color-Doppler techniques in 7 healthy volunteers, in order to investigate differences in hemodynamic parameters between the chest and other regions and between left and right sides; we have focused our attention on the Wall Shear Stress.

**RESULTS:** From 1998 to 2011, 24 patients were identified to have RIS. 17 were female, 7 male. The mean age at time of diagnosis is 67 years (range 40-85). The average latency period is 12.8 years (range 1-50). The 2 most common primary oncological diagnosis were breast carcinoma 11 (11, 45.8%) and endometrial carcinoma (3, 12.5%). The sarcoma subtypes were 9 angiosarcomas (37.5%), 7 pleomorphic sarcomas (29.1%), 3 leiomyosarcomas (12.5%), 2 myofibroblastic sarcomas (8.4%), 1 MPNST (4.2%) and 1 myxoid liposarcoma (4.2%). At the time of this study, 7 patients were deceased, 3 undergoing active treatment, 12 under surveillance, 1 palliative and 1 discharged from follow-up.

**CONCLUSION:** The left anterior chest should be considered as one of the elective localization of the DFSP. We suggest that there could be a link between high Shear Stress in the skin perforators of a specific anatomical region and DFSP occurrence.

#### **LOP31: RADIATION INDUCED SARCOMA**

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**AIMS:** To evaluate the incidence, patient demographics, primary tumour characteristics and treatment modalities of patients with radiation induced sarcoma (RIS) presenting to the East Midlands Sarcoma Service at Nottingham City Hospital.

**METHODS:** All consecutive patients with histologically proven RIS were entered into our database. Case notes were retrospectively analysed to identify patient demographics, oncological features and treatment outcome.

**RESULTS:** From 1998 to 2011, 24 patients were identified to have RIS. 17 were female, 7 male. The mean age at time of diagnosis is 67 years (range 40-85). The average latency period is 12.8 years (range 1-50). The 2 most common primary oncological diagnosis were breast carcinoma 11 (11, 45.8%) and endometrial carcinoma (3, 12.5%). The sarcoma subtypes were 9 angiosarcomas (37.5%), 7 pleomorphic sarcomas (29.1%), 3 leiomyosarcomas (12.5%), 2 myofibroblastic sarcomas (8.4%), 1 MPNST (4.2%) and 1 myxoid liposarcoma (4.2%). At the time of this study, 7 patients were deceased, 3 undergoing active treatment, 12 under surveillance, 1 palliative and 1 discharged from follow-up.

**CONCLUSIONS:** RIS are rare and we present our 13 year experience in the management of these tumours. We plan to continue to monitor the outcome in these patients.

#### **LOP32: THE ROLE OF GAMMA-DELTA ( $\gamma\delta$ ) T LYMPHOCYTES IN MELANOMA: BASIC SCIENCE FINDINGS AND CLINICAL CORRELATIONS**

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**INTRODUCTION:** Advanced melanoma is associated with a poor prognosis. Numerous clinical trials have focused on the potential of tumor-infiltrating lymphocytes (TILs) for immunotherapy in stage III and IV melanoma, but its treatment remains challenging. Gamma-delta ( $\gamma\delta$ ) T cells are the most represented subpopulation of TILs and have shown therapeutic potentials in many solid tumors, although their role against melanoma has not been fully investigated.

**MATERIALS AND METHODS:** Seventy-four patients with cutaneous melanoma underwent peritumoral skin biopsies and blood sample collection. TILs and circulating lymphocytes phenotype and effector functions were analyzed using immunohistochemistry and flow cytometry. Blood samples from 8 healthy subjects were used as control. In vitro cytotoxicity assays on  $\gamma\delta$  T cells were performed using melanoma cell lines pre-treated and not pre-treated with zoledronate. Mortality and relapsed rates were recorded over a mean follow-up period of 29 months (range 12-48).

**RESULTS:**  $\gamma\delta$  T lymphocytes were isolated in 62% of skin biopsies and represented the predominant population of TILs, with a significant difference between stage 0-II (71.9%) and stage III-IV (29.4%). A significantly higher number of effector peripheral  $\gamma\delta$  T cells were found compared to controls (27.7% vs 20%, p<0.05), despite a similar absolute  $\gamma\delta$  T cell number. Both TILs and circulating lymphocytes showed a predominant effector memory phenotype (40%) and a strong cytotoxic capacity toward melanoma cells enhanced after in vitro stimulation with zoledronate. Isolation of peripheral  $\gamma\delta$  T cells inversely correlates with



mortality and relapse rates in metastatic melanoma (0% vs 50% and 13% vs 32.1% respectively).

**CONCLUSIONS:** Our data suggests that  $\gamma\delta$  T cells may play a pivotal role in the antitumoral response against melanoma, which significantly decreases in the advanced disease. They display a strong in vitro cytotoxicity and should be regarded as prominent target cells for enhancing antitumor response in advanced melanoma. Their striking in vitro response to stimulation with zoledronate may prompt further in vivo investigation and innovative immunotherapeutic approaches.

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#### **LOP33: A COMPLEX ECM 3D SCAFFOLD WITH VASCULAR CHANNELS BY DECELLULARIZATION OF HUMAN AND RAT OMENTUM**

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**INTRODUCTION:** lack of vascular network affects the long term survival in grafts of considerable size, while flaps have limited availability and imply co-morbidity. A bioengineered graft with organized vascular channels may overcome this limit: decellularized omentum was investigated as potential source of ECM 3D scaffold with preformed vascular network.

**MATERIALS AND METHODS:** Rat and human omentum samples were de-cellularized with a modified protocol for adipose tissue. At each step samples were collected for histological and immunohistochemical assessment of the effectiveness of the protocol.

**RESULTS:** Histological and IHC samples showed effective cell removal with preservation of ECM structure and composition. Blood vessel walls in particular appeared intact, maintaining their general architecture.

**CONCLUSION:** Omentum decellularization appears as a feasible strategy to obtain a scaffold with complex ECM and a preserved flap-like vascular channel network, which may support and stimulate guided cell growth in vitro or in vivo. It could represent an innovative option for more effective closure of soft tissue defects.

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#### **LOP34: FABRICATION OF BIOCOMPATIBLE BIODEGRADABLE ARTIFICIAL TISSUE CONSTRUCTS VIA SACRIFICIAL NONIONIC TRIBLOCK COPOLYMER NETWORKS**

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**INTRODUCTION:** Fabrication of pre-vascularized artificial tissue is currently beyond the scope of any tissue engineering approach. We present a novel technique for the creation of biodegradable, biocompatible artificial tissue constructs via the utilization of a sacrificial poloxamer network embedded within an alginate hydrogel bulk.

**MATERIAL AND METHODS:** To create a sacrificial network (the negative of which would comprise microchannels within the construct), molten poloxamer (407) was cast into a configuration recapitulating a capillary bed. This network was then embedded in alginate. The construct's internal structure was evaluated via methylene blue injection and saline-contrast 3T  $\mu$ MRI.

**RESULTS:** The poloxamer network dissolved completely when embedded in alginate, leaving in its place a network of microchannels of diameter  $\sim$ 250 $\mu$ m. Methylene blue injection demonstrated patency of the microchannel structure. 3D reconstruction of  $\mu$ MRI images illustrated the complexity of the internal network architecture.

**CONCLUSION:** We have successfully createdde novoconstructs from biodegradable, biocompatible polymers containing a network of channels effectively recapitulating a vascular bed. We believe our constructs represent a major advancement towards the creation of surgically relevant tissue-engineered constructs to use in place of autologous tissue for transfer.

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#### **LOP35: CHEMICAL AND IMMUNOLOGICAL TESTING OF SPIDER SILK AS A BIOPOLYMER**

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**INTRODUCTION:** Because of its high mechanical properties and biocompatibility, spider silk represents an interesting material for biomedical purposes. However, it is yet not known how it influences cell

culturing conditions. Here, changes of medium composition and in vitro-immune reactions to spider silk were measured.

**MATERIALS AND METHODS:** Spider silk was cultured with fibroblasts in culture medium for 3 and 7 days. Osmolarity, and changes of pH value as well as glucose and lactate concentration in medium were determined. TNF- $\alpha$ , IL1 $\beta$  and IL6 liberation in granulocyte and macrophage assays was measured after contact to spider silk and commonly used biomaterials for 4, 8 and 24 hours, LPS stimulated samples served as positive controls, assays were validated by LPS stimulation.

**RESULTS:** Spider silk samples showed no significant changes in osmolarity or pH value. Cells were viable, though less fluorescent than positive controls. Glucose or lactate amount showed a time-dependent decrease or increase, respectively. No significant immune reaction to spider silk or controls could be found.

**CONCLUSIONS:** Regarding the changes in medium composition, spider silk can be considered as inert with no influence to viability and metabolic activity of cells. As immune cells were not activated, a very low immunogenicity can be assumed. Thus, it is an appropriate biomaterial for skin, tendon and cartilage tissue engineering as well as use as microsurgical suture.

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#### **LOP36: THE COIMPLANTATION OF HUMAN ADIPOSE DERIVED STEM CELLS WITH ENDOTHELIAL PROGENITOR CELLS FOR ADIPOSE TISSUE ENGINEERING IN VIVO**

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**INTRODUCTION:** A lack of vascularization is presumed to be the main reason for the resorption of tissue-engineered adipose grafts. Therefore, the aim of this study was to provide adequate vascularisation for these grafts by co-transplantation of human adipose-derived stem cells (ASCs) with endothelial progenitor cells (EPCs) into athymic nude mice and to investigate the effects on adipogenic differentiation.

**MATERIAL AND METHODS:** ASCs were isolated from human fat tissue and EPCs were isolated from human peripheral blood. Both cell types were suspended in fibrin glue and subcutaneously injected into athymic nude mice for 1,3 and 6 months. To survey the volume of newly formed adipose tissue, intravital magnetic resonance imaging (MRI) was performed at 24 hours, 1, 3 and 6 months post transplantation. The differentiation of human ASCs to mature adipocytes was histologically evaluated by human-specific anti-vimentin staining.

**RESULTS:** Evaluating the mean volume of newly formed human adipose tissue after 6 months, MRI data revealed a 5,4 times bigger volume in the co-transplantation group compared to the group with ASCs only. Histological analysis confirmed the human origin of the newly formed adipose tissue in the corresponding regions.

**CONCLUSIONS:** The co-transplantation of human ASCs and EPCs results in increased volume of tissue engineered adipose tissue and improve long-term volume persistence. Therefore, as both cell types can be easily isolated from autologous sources, they represent interesting cell populations in tissue engineering.

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#### **LOP37: VESSEL DEVELOPMENT DURING SKIN GRAFT REVASCULARISATION - IMPLICATIONS FOR TISSUE ENGINEERING**

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**INTRODUCTION:** Despite advances in tissue engineering of human skin, the exact revascularisation processes during taking remain unclear. Therefore it was the aim of this study to investigate the vascular transformations during engraftment and to identify proteolytic activity.

**MATERIALS AND METHODS:** Preparation of the modified dorsal skinfold chamber was performed in C57BL/6J mice (n=5) and C57BL/6-Tg(ACTB-EGFP)10sb/J (n=5). Crossover transplantation was carried out. Further, the expression of proteases within wound bed and skin graft was visualized by immunohistochemistry.

**RESULTS:** After reperfusion, GFP positive wound-bed derived structures were visible in the graft finally leading to a 68% replacement of graft vasculature in the center and 100% replacement in the periphery after 10 days. MT-MMP1 was detected at the tip of in-growing vessels. Further proteolytic activity was assigned to MMP-2 associated with vascular structures in the dermis progressing into the graft.

**CONCLUSION:** These in vivo data show the connection of angiogenic bed vessels to the graft vasculature resulting in reperfusion. MT-MMP1

expression was limited to the front of ingrowing host vessels, indicating its role as sprout growth “facilitator” and potentially in “lysing” the existing graft capillaries in order to undergo anastomoses. Angiogenesis was further associated with increased levels of MMP-2.

#### **LOP38: RHINOPLASTY IN THE BURNED NOSE**

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**INTRODUCTION:** The nose is particularly exposed to facial burn accidents due to its situation and is usually accompanied with deformities of other organs of the face.

The aim of our study is the discussion of how rhinoplasty can be done safely in these victims with pleasing outcome.

**MATERIAL AND METHOD:** We present 10 cases, with complete or subtotal nasal burn. Classic aesthetic rhinoplasty operations were performed to create a better appearance and correct any internal or external deviations.

Standard view photographs were taken before and after operation. Patients and surgeon satisfaction were recorded.

**RESULTS:** Ten patients (9 F, 1 M) whose noses had burn scar or has been grafted or reconstructed, were operated. Patient age ranged from 18 to 46. We performed the classic rhinoplasty operation to repair any respiratory or aesthetic problems due to shrinkage of soft tissues.

These procedures are carried out under severely burned skins, or previously grafted and reconstructed noses. Cases were followed for about a 9 months period.

The cosmetic results, discussed by 3 surgeons and subjective patient feelings, were considered satisfactory in 90% of cases.

**CONCLUSIONS:** Nasal skin flaps should be thick enough to prevent probable necrosis in distal part, and no skin thinning procedure should be done.

The other problem is the rigid covering grafted or scarred skin, which is less pliable to take the form of the modified osteocartilagenous skeleton.

Rhinoplasty seems to complete and improve the results of the standard surgical approach in burned face.

#### **LOP39: SUB ESCHAR INFILTRATION OF EPINEPHRINE IN EARLY EXCISION AND SKIN GRAFTING TO DECREASE BLOOD LOSS IN BURN SURGERY**

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One of the problems in primary tangential excision of burn wounds is bleeding. To reduce bleeding, epinephrine solution used is composed of 1000 ml normal saline, with an added 1 ml 1/1000 aqueous epinephrine hydrochloride (1 mg epinephrine hydrochloride/1000 ml solution , 1:1000 000) has been used to infiltrate the excision area as well as donor site.

As the amount of blood loss during the operation is not known precisely, subsequent calculation depending on pre operative and post operative PCV readings, by using specific formula to calculate blood loss.

In 19 randomly selected patients, deep dermal and full-thickness burns covering 5-40% body surface were included.

Mean blood loss was 72 ml per each percent debrided and covered with skin graft, ranging from 15.6 ml for excision in the posterior trunk to 193 ml for excision of the upper limb with the hands involved.

The main advantages of this method are the reduction of blood loss, the prevention of uncontrollable profuse bleeding and decrease post operative blood transfusion.

#### **LOP40: ALLOGENEIC MESENCHYMAL STEM CELL THERAPY IMPROVES BURN WOUND HEALING VIA A PREDOMINANTLY PARACRINE MECHANISM AND IS ASSOCIATED WITH INCREASED COLLAGEN DEPOSITION**

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**INTRODUCTION:** Cell therapies can improve burn wound healing, but their use is limited due to poor availability at the time of injury. Progenitor cells are attractive targets for developing novel cell based therapies, driving intrinsic wound regeneration. Two sources of progenitor cells, Allogeneic Mesenchymal Stem Cells (MSC) and Culture

Modified Monocytes (CMM), both widely available shortly after injury were assessed for their ability to influence burn wound healing.

**METHOD:** Partial thickness contact burns were induced in pigs, before labelled cells were applied to the wounds in a fibrin hydrogel. Wounds were assessed over a two week period for rate of wound healing before being harvested and assessed histologically.

**RESULTS:** Application of MSC significantly decreased the area of burn unhealed compared to CMM after 14 days (6% MSC, 27% CMM, p<0.001). Labelled MSC and CMM were identified in the wounds in low numbers (MSC 0.33%, CMM 0.18%), mainly in the dermis with rare transdifferentiation into keratin 14 expressing cells (MSC 0.11%,CMM 0%). MSC treated wounds had increased collagen content (MSC 49%, CMM 42%, p<0.01) and dermal thickness (MSC 1108 µm, CMM 1007 µm, p<0.01).

**CONCLUSION:** Allogeneic MSC improve burn wound healing with rare evidence of keratinocyte transdifferentiation, demonstrating potential as a cell therapy for to improve burn healing that is available at time of injury.

#### **LOP41: FIRST EXPERIENCES WITH A NEW SURGICAL APPROACH FOR PATIENTS SUFFERING FROM DEEP BURNS: SINGLE STEP RECONSTRUCTION OF EPIDERMIS, DERMIS AND SUBCUTIS BY USE OF SPLIT THICKNESS SKIN GRAFTING, DERMAL REPLACEMENT AND LIPO-TRANSFER**

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**BACKGROUND:** In adult burn patients suffering from full thickness burns, deep excision down to the muscle fascia and split thickness skin grafting is state of the art in modern burn care. Aim of this study was to evaluate a new surgical approach that combines the use of a dermal matrix, autologous fat transfer and split thickness skin grafting in patients with burns treated by deep excision to the muscle fascia.

**MATERIAL AND METHODS:** Full thickness burn wounds of five consecutive patients were excised down to the muscle fascia. Parts of the excised adipose tissue were harvested for in-vitro analysis of adipocyte and preadipocyte viability and its potential for proliferation and differentiation. The excised fat tissue used for transplantation was aspirated manually with 10 ml syringes and a Coleman Liposuction needle. The harvested fat tissue was spread onto a collage- elastin scaffold (MatridermÖ). The MatridermÖ sheet was put upside down onto the wound and was covered with split thickness graft in the same operation. Skin biopsies of study areas for histological evaluation were taken 30 and 90 days postoperatively.

**RESULTS:** Preadipocytes from excised burn wounds showed physiological cell behavior. Ten days after surgery 3 patients showed more than 90% vital split thickness skin grafts. On postoperative day 30 complete wound closure of the study area was identified in all patients. All skin biopsies confirmed the presence of adipose tissue.

**DISCUSSION/CONCLUSION:** Autologous fat transfer in combination with MatridermÖ and split thickness skin grafts is a possible one-stage procedure for covering defects after deep excision down to the fascia. It could be demonstrated that adipose tissue excised during necrosectomy down to the muscle fascia is viable and therefore suitable for autologous fat transfer.

#### **LOP42: DIGITAL IMAGE SPECKLE CORRELATION (DISC) FOR PROJECTION OF CANDIDATE AREAS FOR REGRAFTING AFTER SEVERE BURN SCARRING**

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**INTRODUCTION:** Scar contraction is a major factor contributing to a patient's return to work. While many therapies are employed in an attempt to minimize or soften scars, there is no gold standard or validated technique for burn scar evaluation. Subjective evaluation criteria such as the Vancouver Scar Scale have been highlighted in numerous studies, but provide only qualitative judgment of the scar. Here we present a novel

digital image speckle correlation (DISC) algorithm to assess burn scar thickness and contraction zone. DISC tracks the movement of skin pores or other fine structures as the result of an applied force on the skin. Requiring simple digital photographs, DISC is capable of providing non-invasive quantitative measurements of scar contracture and thickness. Here we demonstrate DISC tracking of burn scars using a validated vertical progression porcine burn model.

**MATERIAL AND METHODS:** Twenty 2.5cmx2.5cm burns were created on the dorsum of four female swine using an aluminum bar. Three burn temperatures and exposure times have been shown in prior studies to create distinct burn depths - 70°C for 30 seconds, 80°C for 20 seconds, and 80°C for 30 seconds. DISC images were taken 4 weeks post burn using a high resolution camera oriented perpendicular to the wound bed. Pig respiration was used as an applied variable force, and vector maps were created using an algorithm that measures the displacement of multiple pores on the skin surface over the course of respiration.

**RESULTS:** Deep dermal wounds were found to display nearly symmetric vector forces targeting a very small diameter area in the center of the wound bed, while more superficial wounds displayed irregular vector dynamics with no trend towards a center. Flank wounds and wounds above the rib cage were found to display more symmetric forces, correlating with increased contraction as compared to paramedian and caudal wounds. These observations were confirmed by clinical measurements.

**CONCLUSIONS:** DISC illustrates that symmetric and unified forces within a wound bed can be associated with significant contraction. The diameter of the center of the force vector appears to correlate with the degree of burn contraction observed clinically. DISC presents a novel methodology for quickly and reliably highlighting zones of skin contracture, allowing for ideal aesthetic and mobility outcomes without the need for unnecessary revisions.

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#### **LOP43: EFFECTS OF ENDOTHELIAL CELLS ON PROLIFERATION AND SURVIVAL OF HUMAN PRIMARY OSTEOBLASTS**

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**INTRODUCTION:** Bone tissue engineering as well as physiological bone regeneration demand an adequate vascularization. Furthermore manifold studies reveal that cocultivation of human primary osteoblasts (hOBs) with endothelial cells (ECs) influences cell physiology of hOBs e.g. osteogenic differentiation. This study intended to enlighten the specific interactions between hOBs and ECs concerning proliferation and apoptosis.

**MATERIALS AND METHODS:** The proliferation rate was quantified after 1,3,5, and 7 days. Apoptosis was investigated by DNA fragmentation ELISA and phospho-BAD ELISA. To differentiate between effects due to heterotypic cell contacts or paracrine mediators, direct and indirect cocultures of hOBs and HUVECs were performed.

**RESULTS:** After 7 days of direct cocultivation, the proliferation rate of hOBs showed a 3.7 fold increase in comparison to the hOBs in monoculture ( $p < 0.005$ ). Interestingly proliferation of cocultured ECs remained unvaried. Beyond that EC significantly ( $p < 0.05$ ) reduced apoptosis of hOBs in direct as well as in indirect coculture. Whereas phosphorylation and consequently inactivation of proapoptotic protein BAD was only caused by direct cocultivation.

**CONCLUSION:** ECs were able to increase the proliferation rate and reduce apoptosis of cocultured hOBs. Intriguingly heterotypic cell contacts seemed to be immanent in this concern.

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#### **LOP44: SILENCING OF EphB4 TYROSINE-KINASE RECEPTOR IN SYNOVIAL SARCOMA BY RNA-INTERFERENCE INHIBITS TUMOR PROGRESSION**

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**INTRODUCTION:** Soft tissue sarcomas display a rare and heterogeneous group of tumors derived from connective tissue. They comprise less than one percent of all malignant tumors. The aim of this study was to analyse the biological role of the tyrosine-kinase receptor EphB4 in soft tissue sarcomas by RNA-interference, explicit in synovial sarcoma.

**MATERIALS AND METHODS:** ATCC listed cell lines (fibrosarcoma, synovial sarcoma, liposarcoma and malignant fibrous histiocytoma (MFH)) were examined for the expression of EphB4-mRNA by quantitative real time PCR. Additionally sections of six primary synovial sarcomas with healthy surrounding tissue were scanned for EphB4-protein expression by immunofluorescence staining. Cell lines were transfected with siRNA against EphB4 and control-siRNA. Proliferation and vitality after silencing were measured by BrdU- and MTT-assay. Furthermore the migration of synovial sarcoma cells was analyzed by a scratch-assay.

**RESULTS:** All cell lines exhibited mRNA expression of EphB4. A high level of EphB4 protein was seen in all primary synovial sarcoma sections but not in the healthy surrounding tissue. Silencing of EphB4 reduced the proliferation rate of synovial sarcoma and fibrosarcoma cells to 40% and 60% (both  $p < 0.01$ ), respectively. The proliferation of liposarcoma and MFH was not affected significantly. The MTT-assay showed a decrease of vitality up to 68% ( $p < 0.01$ ) in synovial sarcoma and to 50% ( $p = 0.018$ ) in fibrosarcoma. No significant reduction was seen in other cell lines. The migration of EphB4-silenced synovial sarcoma cells was also decreased significantly ( $p < 0.01$ ).

**CONCLUSION:** These data provide first evidence of the importance of EphB4 in the tumorigenesis of synovial sarcomas and present EphB4 as a potential target in the therapy of this malignancy. Upcoming investigations towards the impact of EphB4 in vivo are in progress.

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#### **LOP45: RECELLULARIZED ABDOMINAL WALL FREE FLAP BIOINTEGRATION: PROSPECTIVE OF AN EXPERIMENTAL MODEL**

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**INTRODUCTION:** Synthetic or semi-synthetic scaffold are often insufficient to allow a satisfactory bio integration.

In our experience a decellularized tissue from cadaver could provide a 3d scaffold with performed vascular network that could allow a long term survival of the graft once implanted in vivo.

**MATERIALS AND METHODS:** Rabbits abdominal wall samples were decellularized with a modified protocol for adipose tissue. At each step samples were collected for histological and immunohistochemical assessment of the protocol effectiveness.

**RESULTS:** Histological and IHC samples showed effective cell removal with preservation of ECM structure and composition. Integrity of vascular channels was maintained.

**CONCLUSIONS:** In the future we hope to be able to prepare different kinds of homo-autologous free flaps suitable for microsurgery anastomosis, derived from homologous tissue and recellularized with autologous stem cells, solving the problem of comorbidity, rejection and about the number (bank concept).

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#### **LOP46: COMPOSITE TISSUE XENOPRESERVATION: A NEW LIVING TISSUE BANK**

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The need to preserve composite tissue and use it when needed is a clinical necessity in modern plastic surgery practice. The amputate needs to be preserved and used at a future date, in the amputation patient whose clinical situation will not allow a long surgical procedure.

An appropriate preservation method for composite tissues has not been described up to date. This study was planned on the hypothesis composite tissues could be preserved and conserve their viability if xenotransplanted.

Two concordant species: Sprague Dawley Rats (no:6) and Mice (no:6) were used. The groin flap of the rat was used as a xenotransplant and the vessels on the neck of the Mouse used as a recipient site.

The groin flap of the rat was transported to the neck area of the Mouse and microanastomoses were performed between the femoral pedicle of the flap and carotis artery and jugularis externa vein of the Mouse. Immunosuppression was administered in order to prevent rejection. After a 7 days period of preservation on the site, samples were collected from the skin and vascular structures and the flap was carried to the donor's opposite groin area. Anastomoses were performed between the flaps pedicle and the femoral artery and vein. The flap was monitored daily. Fifteen days after the second surgical procedures the rats were euthenized

and samples were collected. All the samples were evaluated by Haematoxyline-Eosine Stain.

This is the first study the groin flap has been used as a xenotransplantation model and successfully preserved. Tissue evaluation indicated inflammation was prominent in the tissue, but these changes were reversible to some extent. We believe further studies are needed, in order to control and modify these changes.

A method for composite tissue preservation and a basic model for further investigation has been developed in this study. Additional studies are needed in order to find diverse strategies to modulate the tissue changes.

#### **LOP47: ACUTE ADMINISTRATION OF EMPTY VIRAL VECTOR CAUSES SUBSTANTIAL IMPROVEMENT IN DORSAL SKIN FLAP SURVIVAL IN RAT MODELS**

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**INTRODUCTION:** Despite the aesthetic advantages and oncologic safety of skin-sparing mastectomies, the persistent occurrence of partial thickness necrosis poses an unnecessary source of morbidity. Traumatized skin located above new acellular dermal matrix is of additional concern. While the acute administration of adenoviral vectors has been shown to have a negligible effect on skin survival, the pro-inflammatory effects caused by empty "null" adenovirus may improve wound healing outcome. Here we compare clinical and bloodflow changes caused by the acute adenoviral vector administration on surgically isolated McFarlane skin flaps.

**MATERIAL AND METHODS:** A 3x9 cm area of skin based 1 cm from the tip of the scapulae was marked on the dorsum of 25 male Sprague Dawley rats. Animal groups (n = 5) received .1 mL subdermal injections at 3x10<sup>9</sup> pu of AdVEGF-All (a blend of several large isoforms of VEGF), AdEGR-1, or AdNull vector at 1 cm increments throughout the caudal 2/3 of the flap. A cranially-based modified McFarlane flap was raised, and medical grade silicone 1mm in thickness was placed on top of the wound bed prior flap replacement. Controls (n=5) consisted of one group with no adenovirus administered, and another without silicone blocking. Clinical scores and Laser Doppler Imaging (LDI) was performed at 1, 2, 3, and 7 days post-surgery. Animals were euthanized on POD7.

**RESULTS:** Flaps blocked using medical silicone created a significantly more severe model, displaying a rapid transition from salvage - the maintenance of skin pliability and elasticity but without normal adnexal components - to full thickness necrosis. However, the viable region was equally preserved in both models. No significant improvement in flap viability was observed in animals administered either AdVEGF-All or AdEGR-1 vectors. A significant improvement (p = .004) was observed between AdNull treated animals (12.31 +/- .27) and blocked controls (8.67 +/- .71). LDI measurements demonstrated a near two-fold increase in flap perfusion in all AdNull animals during POD 1, 2, and 3. However, these differences became insignificant by POD7.

**CONCLUSIONS:** Acute administration of AdNull vector to a region of skin prior to acute ischemia creates a high perfusion state that contributes to an increase in overall flap survival. Further, using a blocking material to reduce the influence of peripheral angiogenesis creates a more severe model of ischemia, though does not impact the maintenance of viable skin.

#### **LOP48: EMERGING TRENDS IN INFECTIOUS COMPLICATIONS IN TISSUE EXPANDER BREAST RECONSTRUCTION: ARE THE CAUSATIVE BACTERIA EVOLVING?**

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**PURPOSE:** Post operative infection in tissue expander (TE) breast reconstructions that necessitates implant removal represent a devastating complication to both patient and surgeon. We evaluated culture data from infected TE explantations to identify the causative bacteria, their antibiotic susceptibilities and their sensitivity to antimicrobial therapy.

**METHODS:** All patients who underwent post-mastectomy TE reconstruction and developed postoperative infection requiring TE removal at the MD Anderson Cancer Center between 2003-2010 were evaluated. Bacteria isolated on intraoperative culture were characterized

by bacterial type: skin flora, non-skin flora and mixed flora. Perioperative prophylactic, empiric and therapeutic antibiotics were evaluated for susceptibility towards the causative pathogen.

**RESULTS:** Overall 97 patients underwent TE explantation, 75 with positive cultures. Fifty-one patients (68%) were infected with skin flora bacteria, 17 (23%) non-skin flora bacteria and 7 (9%) had mixed bacteria. Patient characteristics and risk factors including smoking status, diabetes, obesity, pre-op chemotherapy and/or radiation therapy, and time to infection were not significantly different among the three bacterial groups. Fifteen patients underwent bioprosthetic mesh reconstructions had a statistically higher incidence of infection with non skin flora (46.7% vs. 16.7%, p=0.04).

For perioperative prophylaxis Ancef/Duricef were most commonly used in 76% of patients followed by Ampicillin/Amoxicillin in 9.3%, and Clindamycin in 6.7% of patients. Empiric therapy centered around Vancomycin/Zosyn in 29.3% of patients, Vancomycin in 16% and Unasyn in 14.7% of patients. Once final culture results were available antibiotics were modified in 26 patients (34.7%), which did not differ among the three bacterial groups (p=0.17). The incidence of appropriate antibiotic therapy targeted towards the isolated microbes ranged from preoperative prophylaxis 16% of cases, postoperative prophylaxis 4% and empiric therapy 53.3% of cases. When aggregate bacterial sensitivity was evaluated by antibiotic type sensitivity in greater than 70% of cases was seen with Vancomycin, Zosyn, Bactrim, Ciprofloxacin and Rifampin and antimicrobial resistance in greater than 65% of cases was seen with Unasyn and Cephalosporins.

**CONCLUSION:** Once a periprosthetic TE infection is diagnosed targeted antimicrobial therapy beyond standard skin flora is warranted including resistant gram positive organisms and gram negative non-skin flora. Given the overall sensitivities of bacterial pathogens identified targeted empiric antimicrobial therapy towards both skin flora and non skin flora with dual drug therapy provide the best option for adequate treatment. Comprehensive multiple drug combinations are available based on oral or intravenous routes and potential patient drug allergies: Vancomycin/Zosyn, Vancomycin/Ciprofloxacin, Bactrim/Rifampin and Ciprofloxacin/Rifampin to broaden empiric therapy increasing the potential to salvage an infected tissue expander breast reconstruction.

#### **LOP49: IMMEDIATE SINGLE-STAGE BREAST IMPLANT RECONSTRUCTION WITH ABSORBABLE MESH**

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**INTRODUCTION:** Immediate direct-to-implant breast reconstruction has gained popularity in recent years. The increased prevalence of skin/nipple-sparing mastectomy techniques and the availability of acellular dermal matrix (ADM) has allowed for placement of a full-sized permanent implant at the time of mastectomy. Success in immediate single-stage implant reconstruction using an ADM sling has been demonstrated in multiple studies, including a large published series from our own institution. However, other studies have demonstrated significant complications related to the use of ADMs, particularly an increased rate of infection and seroma. Additionally, ADMs are expensive.

We hypothesize that single-stage reconstruction is possible with absorbable mesh in place of ADM, providing for a reliable and cost-effective reconstruction. Similar to ADMs, absorbable mesh (Vicryl) allows for positioning of the implant on the chest wall, definition of the IMF/lateral breast border, and prevents window-shading of the pectoralis muscle, maximizing sub-muscular coverage and decreasing skin tension. This report describes our early results from a series demonstrating that comparable aesthetic results can be achieved using a readily available, cost-effective absorbable mesh without significant complications.

**METHODS:** A retrospective review was performed of 40 consecutive single-stage direct-to-implant breast reconstructions performed by the authors at the Massachusetts General Hospital.

**RESULTS:** Sixty-one breasts in 40 consecutive patients have been reconstructed using an immediate direct-to-implant approach with an absorbable mesh sling. All implants were smooth silicone (Mentor) with a mean implant size of 465 (range 150 - 800) cubic centimeters. In cases where the mastectomy specimen weight was recorded, the mean implant size (cc)/specimen weight (gms) ratio was 90%. Mean follow-up is 4.1 months (range 13-252 days). Nineteen (47%) cases were unilateral and 21 (53%) cases were bilateral. Three patients developed complications. One patient who underwent bilateral reconstruction developed a unilateral implant infection on POD #18 ultimately requiring explantation. One patient developed diffuse medication-induced

dermatitis. The third patient who underwent bilateral reconstruction was dissatisfied with the size of her implants and underwent exchange for larger implants 6 months after her original reconstruction. No patients developed skin necrosis or seroma. Implant position and aesthetic results were acceptable in all patients. Cost analysis using Vicryl in place of Alloderm in single-stage reconstruction demonstrated a 33% overall cost reduction in unilateral cases and 41% in bilateral cases.

**CONCLUSIONS:** Analysis of our experience with immediate single-stage implant reconstruction using absorbable mesh demonstrates an excellent early aesthetic result and low complication rate. These early results are very promising and longer follow-up of a larger prospective series of patients may lead to a novel cost-effective approach to immediate implant reconstruction.

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#### **LOP50: AUTODERM: THE ULTIMATE BIOLOGIC FOR BREAST RECONSTRUCTION**

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**BACKGROUND:** The use of bioprosthetic mesh in tissue expander breast reconstruction has rapidly gained in popularity, but appears to be accompanied by an increased risk of seroma and infection, and is extremely costly. This study introduces a novel technique that obviates these shortcomings using an autologous dermal graft (Autoderm) in implant based breast reconstruction. The authors detail operative technique and analyze short term outcomes.

**METHODS:** All patients undergoing post-mastectomy tissue expander reconstruction with autologous dermal graft for lower pole support at the M.D. Anderson Cancer Center between April 2011 and December 2011 were evaluated. A retrospective review of a prospectively maintained departmental database was performed including patient demographics, indications, surgical technique, complications, and post-operative course.

**RESULTS:** Twenty-three breast reconstructions in 14 patients underwent 5 unilateral and 9 bilateral, implant-based breast reconstructions with Autoderm. Mean follow-up was 117 days (range 34-240). Overall complication rate was 28%, included mastectomy skin flap necrosis (n=3). No patients developed breast cellulitis, breast hyperemia, periprosthetic infection, seroma, or implant extrusion.

**CONCLUSIONS:** Autoderm provides the advantages of lower pole support and breast shaping, without the disadvantages of increased periprosthetic complications and high cost associated with allograft and xenograft bioprosthetic meshes.

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#### **LOP51: LYMPHATIC DRAINAGE OF MAMMARY GLAND AND UPPER EXTREMITIES: FROM ANATOMY TO SURGERY TO MICROSURGERY**

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**QUESTION:** The incidence of secondary arm lymphedema varies from 7 to 77 % in patients following axillary lymph nodal dissection (ALND). On the other hand, the incidence of arm lymphedema after sentinel lymph node biopsy (SLNB) varies from 0 to 13 %. The first objective of this study is to carry out a detailed description of the breast lymphatic drainage remarking the correlation between upper limb derivative lymphatic pathways and the onset of secondary lymphedema after ALND/SLNB. The second goal of this research is to underline the role of lymphatic microsurgery regarding the primary prevention of secondary lymphedema following breast cancer treatment.

**METHODS:** In this study 350 mammary glands and upper limbs together with 80 sections of anterior pectoral skin of deceased fetuses and of 20 adults were injected. The injection had been performed with the modified Gerota's mass. Dissection had been carried out after appropriate fixation of the specimens in 40% formaldehyde for 6 days, then immersed in a 100-volume hydrogen peroxide solution for 24 hours. In 90 fetus specimens the Spalteholz technique for diaphanization had been performed.

**RESULTS:** Breast lymph flows through the perilobular lymphatics and the interlobular spaces which initiate the lymphatic capillaries and thus give origin to secondary pedicles. These lymphatic vessels exit the

mammary gland at specific sites (external, internal and posterior), thus constituting the following draining pedicles: external or axillary pedicle (95,33 %), internal or mediastinal pedicle (36,6 %) and posterior or retromammary pedicle (17,1%). Regarding the skin lymphatic drainage of breast area, there are two main lymphatic pathways, the ipsilateral and the contralateral. In addition, we can observe three different derivative lymphatic pathways of the upper limb: anterior external superficial pathway, posterior external superficial way and anterior internal deep pathway.

**CONCLUSIONS:** Microsurgery has a key role regarding primary prevention of secondary arm lymphedema at the same time of ALND/SLNB. Planning breast cancer surgery, patients should undergo an appropriate clinical assessment together with lymphoscintigraphy in order to evaluate their lymphedema low-moderate-high risk.

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#### **LOP52: MECHANISMS OF EXTERNAL VOLUME EXPANSION (EVE) SYSTEMS FOR SITE PREPARATION TO FAT GRAFTING: EFFECTS ON TISSUE PERFUSION IN A MOUSE MODEL**

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**QUESTIONS:** External volume expansion (EVE) is used to prepare fat grafting sites in breast augmentation. Recently, we developed a murine model of EVE demonstrating adipogenesis, increased cell proliferation and angiogenesis. Hypothesizing that mechanical forces, through edema, ischemia, and inflammation, may explain these effects, we studied EVE effects on tissue perfusion.

**METHODS:** A miniaturized EVE device was applied on mice (n=18) for 6h at -25mmHg. Local oxyhemoglobin and deoxyhemoglobin were assessed by hyperspectral imaging pre-treatment and post-EVE for 2 days.

**RESULTS:** EVE induced macroscopic tissue swelling. Upon release, OxyHb increased over baseline peaking at 1'30" (p<0.01), then decreased to steady-state higher than baseline by 6' (p<0.01). A higher peak (p<0.05) was observed at 4h, and normalized by day 1. DeoxyHb quickly decreased post-EVE, stabilizing at values 40% below baseline (p<0.01); was still elevated at day 1 and returned to baseline by day 2. Total Hb, peaking 1' post treatment, maintained elevated for up to 1 day (p<0.01).

**CONCLUSIONS:** EVE, through mechanical stretch and edema, establishes persistent tissue ischemia, known as pro-proliferative and angiogenic. Upon release, elevated blood influx can sustain metabolism, while the late OxyHb peak suggests inflammatory response, potentially further stimulating proliferation and angiogenesis.

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#### **LOP53: ANTIBIOTIC USE AND INFECTION IN BREAST RECONSTRUCTION WITH ACELLULAR DERMAL MATRIX: A REVIEW OF THE LITERATURE**

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**INTRODUCTION:** Infection rates have been noted to be as high as 29% in patients undergoing breast reconstruction with overall complication rates exceeding 60%. Patients who receive reconstruction with acellular dermal matrix (ADM) have experienced infections rates of more than 31%. Antibiotic use in breast reconstruction remains controversial due to the absence of evidence-based literature. The CDC guidelines recommend a maximum of 24 hours of perioperative antibiotics while many surgeons continue antibiotics until drain removal. This recommendation does not specifically address patients with ADM reconstruction. The purpose of this study was to examine published antibiotic regimens and their associated infection rates in this specific population.

**METHODS:** Systematic electronic searches were performed in PubMed, OVID, and the Cochrane databases for studies that reported on prophylactic antibiotic use and infection in breast reconstruction patients. A combination of relevant MeSH terms used in PubMed & OVID includes: Mammoplasty, Anti-Bacterial Agents, Breast Reconstruction, Antibiotics, and Infection. Two independent authors reviewed studies between 1970 - 2011 for inclusion and data extraction. Reference lists of each included article were evaluated to complete a full circular search. For inclusion, publications had to report a specific antibiotic protocol, documented infection rates, and use of ADM. Non-ADM patients were not included in this analysis.

**RESULTS:** A total of 995 studies were identified, and after duplicate deletion a total of 834 were reviewed using their abstracts. A total of 12 articles were included in the review with a total of 714 patients with 1051 reconstructions using ADM. 5 of these studies included only patients with ADM reconstruction while the other 7 included both Non-ADM & ADM patients for comparison. An overwhelming majority of these reported on AlloDerm (LifeCell Corp., Branchburg, NJ). The reported infection rates varied between 0% and 31% with an average of 11.1%. When comparing antibiotic protocols of < 24 hours and > 24 hours, the average infection rate was 1.5% and 13.7%, respectively. The most common antibiotic regimen was continued until all drains were removed.

**CONCLUSION:** The current literature lacks consensus on the necessary duration for postoperative antibiotic prophylaxis following breast reconstruction. The potential increased risk of infection associated with ADM remains controversial. A randomized controlled trial is warranted to further evaluate the necessary duration of antibiotics in breast reconstruction patients with ADM.

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**LOP54: MICRO-TOPOGRAPHICAL CONTROL OF FIBROBLAST ADHESION AND FUNCTION ON SILICONE IMPLANTS: A NEW STRATEGY TO REDUCE CAPSULAR CONTRACTION**

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**QUESTION:** Capsular contraction remains the main problem of breast implants, with myofibroblasts playing a main role in the pathogenesis of this process. We here introduce a micro-pattern of adhesion sites for silicone implants that increases physiological cell attachment and therefore increases the biocompatibility of implant biomaterials.

**METHODS:** Silicone surfaces were either coated homogeneously (general coating=GC) or with micro patterned arrays of collagen I (2x2 microns, 4x2, 10x2, 20x2 micro pattern coating =MC) and tested in vitro for cell attachment and alpha-SMA expression. Results were compared to non-coated silicone (NC). In vivo, capsule formation around implants (GC, MC, NC) was analyzed one month after subcutaneous implantation into the dorsum of rats (n=20) for capsule thickness, alpha-SMA expression and collagen.

**RESULTS:** In vitro, myofibroblast differentiation was significantly decreased (20%), when cells were cultured on 2x4 micro pattern compared to completely coated surfaces (30%, p<0.05). In vivo, 2x4 microns arrays coating reduced alpha-SMA (p<0.01) and collagen deposition in the peri-implant capsule compared to non-coated and completely coated implants.

**CONCLUSION:** Silicone coating using micro topographical control to guide cell behavior can dramatically reduce capsule formation and the presence of myofibroblasts. Micro topographical coating may be a promising approach to reduce capsular contracture around implantable devices.

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**LOP55: COMPARISON OF DIFFERENT SEEDING STRATEGIES TO ENHANCE FIBROBLAST PENETRATION WITHIN A HUMAN ACCELLULAR DERMIS FOR SOFT TISSUE AUGMENTATION**

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**INTRODUCTION:** Effective cell seeding often determines the success of tissue-engineering products. To create a stable soft tissue replacement, it would be desirable to achieve a maximum seeding efficiency, but also a homogenous cell distribution throughout the ADM. Natural matrices such as acellular dermis have the disadvantage of low permeability, due to their dense network, compared to synthetic materials with larger pore size. The purpose of this study was to compare different cell seeding methods regarding their seeding efficiency, homogeneity, infiltration depth and proliferation within an acellular dermis.

**METHODS:** The examined methods can be divided into static, dynamic seeding techniques and a combination of both optional with PDGF as mitogen. Static seeding techniques include surface seeding, direct injection of cell suspension by a syringe, incision of the matrix to increase the surface and diffusion and application of low-pressure and ultrasonic bath to remove trapped air. Dynamic seeding methods include

an orbital shaker and the use of centrifugal force with different rotational speed and duration. After seeding, ADMs were incubated for up to 12 days and analyzed at day 0, 4, 8 and 12. At each corresponding time point, seeded ADMs were fixed, embedded vertically in paraffin, histologically sectioned and stained with propidium-iodide to analyze the cell distribution and penetration depth. Furthermore, cell proliferation, seeding efficiency and survival was evaluated by a MTT assay.

**RESULTS:** When using static methods without low- pressure pretreatment, cells were deposited on the surface as a single layer and no penetration into the matrix could be detected. However, after degassing the matrix, we were able to detect a significant improvement in penetration and proliferation. Dynamic seeding using a centrifuge increases the initial number of entrapped cells into the ADM; nevertheless we could neither demonstrate a high proliferation nor find any cells in the central areas. Whereas centrifugal force combined with low- pressure forces significantly more cells inside the ADM and increases the cell mass and homogeneity within 12 days than compared to the other methods.

**DISCUSSION:** As we have shown, the air in the pores significantly impeded the proliferation and therefore the penetration. Thus, the use of a single conventional method results in relatively inefficient colonization results when trying to colonize a dense matrix. We could archive the highest seeding efficiency, homogeneity, infiltration depth and proliferation by using low- pressure and centrifugation at 300g for 5x 1Min in addition with PDGF. Thus, we conclude that this combination is the most effective to repopulate dense natural matrices for soft- tissue augmentation.

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**LOP56: A NEW MICROSURGICAL ANASTOMOSIS TECHNIQUE USING AN ABSORBABLE STENT AND TISSUE ADHESIVE – A SUITABLE ALTERNATIVE TO CONVENTIONAL MICROVASCULAR ANASTOMOSIS?**

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**INTRODUCTION:** The creation of a microvascular anastomosis is a complex procedure. A reduction of the operation time by a faster, safer, yet qualitative equal microvascular procedure would be advantageous. N-fibroin, one of the silk proteins, is biocompatible, proteolytically degradable and causes no foreign body reactions. Stents, made of n-fibroin are flexible, dimensionally stable and provide at the same time a high primary stability and surface quality. The aim of this pilot study was to evaluate a new microvascular anastomosis technique, which is easy to handle and reduces the ischemic time of the transplant.

**MATERIAL AND METHODS:** N-fibroin stents (diameter 0,9 to 1,15mm) were implanted into the infrarenal aorta of Sprague-Dawley rats (n=6). After clamping and cutting the infrarenal aorta, the stents were inserted in both vessel ends, which were afterwards readapted again and fixed with a commercially available tissue adhesive. The animals were euthanized at different points in time (17 +/- 2 weeks) after implantation and the aortic anastomosis site was histologically (HE, EVG, van Kossa staining) examined

**RESULTS:** The n-fibroin stents showed high tissue compatibility. No foreign body reactions, rejection reactions or inflammatory reactions were seen. The vessel walls showed no pathologic reactions like aneurysm or stenosis. An inert degradation of the stents was observed.

**SUMMARY:** In contrast to the established collagen scaffolds made of animal tissue, n-fibroin offers several advantages: individual adaptations in the production are possible, the risk of infection at the implantation site is low and the mechanical properties are excellent. The microvascular anastomosis technique using n-fibroin stents is a promising method, which could reduce operation time significantly and the rate of anastomosis closures. The biological as well as the mechanical properties of n-fibroin stents seem to be of great advantage for the presented indication.

**LOP57: AN INTRALUMINAL THERMOSENSITIVE GEL (LEGOOTM) FOR CLAMPLESS MICROANASTOMOSIS: EXPERIENCES OF THE FIRST APPLICATIONS IN RECONSTRUCTIVE MICROSURGERY**

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**INTRODUCTION:** For a microvascular anastomosis microclamps are needed to provide a bloodless situation, but they may cause intimal lesions, need space in confined sites and provide the risk of backwalling due to vessel flattening. In arteriosclerotic vessels their haemostatic effect is often insufficient. A new, CE-certified, FDA-approved thermosensitive gel (LeGooTM), already clinically proven in cardiovascular surgery allows a reliable clampless microanastomosis. We present the technique and our experiences in the first applications.

**MATERIAL AND METHODS:** In a series of nine patients, aged 24 to 75 years, ten flaps for extremity reconstruction using a clampless anastomosis with LeGooTM were performed. We transplanted one fabricated chimaeric fibula plus gracilis, three gracilis muscle, three anterolateral thigh (ALT), one parascapular and one medial sural artery perforator (MSAP) flap. Pre- and postoperative protocols were alike to a 'standard' procedure with microclamps.

**RESULTS:** All flaps survived except a small area on fibula skin-island, which was attributed to suboptimal skin-island placement. The gel-assisted technique has a quick learning curve. It provides circular stenting and gentle distension of the vessels for a safe and blood-free anastomotic site. It completely dissolves by irrigating the vessel with cold saline after anastomosis. Repolymerisation in the periphery did not occur.

**CONCLUSIONS:** From the experiences of this series and other specialities the use of the thermosensitive gel LeGooTM permits a safe clampless microanastomosis technique, minimising mechanical vessel manipulation, making it an alternative to microclamps, especially for arteriosclerotic arteries and confined anastomosis sites.

**LOP58: A NEW COLLAGEN CONDUIT FOR THE REGENERATION OF THE PERIPHERAL NERVES USING TISSUE ENGINEERING - FINAL RESULT**

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An ideal conduit to treat a nerve gap has not been found. Initially, silicone conduits were employed. Later, conduits were fabricated from collagen or polyesters carbonates. The aim of this study was to develop an artificial, biocompatible, nerve guide to induce regeneration in the peripheral nervous system. The authors compared the regeneration of a sciatic nerve in a rat model through a 1 cm gap, using a new nerve conduit of reticulated collagen, filled with saline or several neurotrophic tissues

Fifty Brown Norway rats were randomized to five nerve reconstruction groups: 1) reversed sciatic nerve autograft (control group); 2) saline-filled new conduit; 3) conduit containing the morselized sciatic sectioned nerve fragment; 4) conduit containing autologous bone marrow stromal cells; or 5) conduit containing morselized adipose stem cells.

A second lot (n=10) was tested with the saline-filled new conduit for an extended period of time, 20 weeks.

The regeneration was evaluated with the functional walking test (sciatic index), muscle weight analysis (tibialis anterior muscle) and histological analysis of the regenerated axons passing through the conduit.

After 2 sets of testing, the final data suggested that nerve regeneration for the saline-filled new conduit approximates the graft control group. Overall the nerve regeneration is significantly enhanced by the inclusion of various neurotrophic and neurotropic "enhancing" tissues that serves as a "stepping stone" for nerve regeneration through the conduit. This engineered tissue potentially simulates the neurotrophic environment of a nerve graft through the contribution of the stem cells and other neural elements.

**LOP59: ABSORBABLE GLYCOLIC ACID/TRIMETHYLENE CARBONATE SYNTHETIC MESH DEMONSTRATES SUPERIOR IN-GROWTH AND COLLAGEN DEPOSITION**

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**INTRODUCTION:** GORE BIO-A® Tissue Reinforcement is a bio-absorbable synthetic mesh. This study compares tissue response to BIO-A versus biologic meshes.

**METHODS:** Twenty-four BIO-A, FlexHD, Strattice and Permacol grafts were implanted subcutaneously in rabbits and harvested at 7, 14, 30, 60, 90 and 180 days. Histology was graded semi-quantitatively.

**RESULTS:** Type of mesh and implantation duration were significant predictors of all outcomes (p<0.0001). BIO-A exhibited significant increase in cellular in-growth between 7 and 30 days (p<0.0001) reaching a grade of 5.0. Biologics did not demonstrate significant change in in-growth between 7-180 days with a max grade of 3.3 for FlexHD. BIO-A and Permacol exhibited increase in blood vessels between 7 and 14 days (p=0.007; 0.005), with BIO-A having the greatest vascular in-growth (grade=4.4, p<0.0001). FlexHD and Strattice had no significant change between 7-180 days. BIO-A had significantly greater collagen deposition between 7-90 days. Type I collagen was demonstrated in 100% of BIO-A samples at 30 days - earlier than the biologics (p=0.006). BIO-A exhibited the least inflammation over time.

**CONCLUSIONS:** BIO-A exhibited a higher degree of cellular and vascular in-growth and collagen deposition than three commonly used biologic meshes. The use of a glycolic acid/trimethylene carbonate absorbable mesh results in a favorable tissue response.

**LOP60: A PROSPECTIVE STUDY OF INTRA-LESIONAL BLEOMYCIN INJECTION FOR THE MANAGEMENT OF LOW FLOW VASCULAR MALFORMATIONS**

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**BACKGROUND:** Vascular malformations (AVMs) are challenging to manage, particularly with the propensity to grow and can lead to severe disfigurement and dysfunction. Traditional surgical excision is fraught with tedious dissection and complications, particularly in the head and neck region. Trends toward less invasive techniques, such as intralesional sclerotherapy, is proving to be a successful independent treatment or adjunct in management.

**METHODS:** This study reports the outcomes of 32 patients with radiological confirmed vascular malformations which were clinically measurable and commenced on intralesional bleomycin injection (IBI) therapy. Patient demographics, lesion characteristics, imaging findings, treatment course, radiological and clinical response to treatment was recorded.

**RESULTS:** An overall 90.7% response rate was achieved with 31.3% complete resolved. Lesions were sub-categorised into arteriovenous malformation (AVM) (n=14), venous malformation (n=13) and lymphatic malformation (LM)(n=5). 84.4% experienced no complications. Local complications included superficial skin infection (n=2), skin necrosis (n=1), hyperpigmentation and minor contour deformity. There was no recurrence and no systemic side effects to bleomycin. Mean follow up was 32 months.

**CONCLUSION:** Intralesional bleomycin intralesional injection is a promising sclerosing agent, which is effective and also safe in a pediatric population for the successful management of symptomatic or disfiguring vascular malformations. It can be used as an independent therapy or combination with surgical debulking or excision.

**LOP61: CHALLENGES BEYOND THE MIDLINE: IMPROVING OUTCOMES IN LATERAL ABDOMINAL WALL RECONSTRUCTION**

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**PURPOSE:** Lateral abdominal wall (LAW) defects, subcostal hernias, flank bulges and full-thickness resections differ from midline defects due to the paucity of LAW fascia, inability to perform component separation, obligate oblique muscle denervation and difficult access for repair. We adopted the pillar anchored repair (PAR) technique reinforcing the LAW well beyond the defect itself to address these factors. PAR anchors mesh inlay to innervated musculofascia and structural pillars of the LAW (costal margin, linea semilunaris, lineal alba and pelvis) rather than a standard mesh repair (SMR) circumferential mesh inlay. We hypothesize

that LAW reconstruction outcomes would be superior with PAR than SMR.

**METHODS:** We retrospectively evaluated the surgical outcomes of all consecutive patients who underwent LAW reconstruction over an 8-year period at a major cancer center. Patients were grouped based on type of surgical repair; PAR or SMR. Primary outcome measures included differences in surgical outcomes between patients with PAR vs. SMR, particularly recurrent hernia/bulge formation.

**RESULTS:** Eighty-six patients were included in the study; 28 PAR and 58 SMR patients. Mean follow-up was 28.2 and 30.5 months, respectively. Patient characteristics and comorbidities were similar between groups. There were no differences in surgical outcomes: infection, seroma, wound dehiscence; between groups (14% vs. 12%, respectively;  $p=0.728$ ). However, the rate of recurrent hernia/bulge formation was significantly lower in the PAR group (21% vs. 50%,  $p=0.0183$ ).

**CONCLUSIONS:** For LAW reconstruction PAR results in significantly lower rates of recurrent hernia/bulge formation than SMR without additional morbidity. PAR is the preferred method of reconstructing LAW defects.

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#### **LOP62: GRACILIS MUSCLE TRANSPOSITION TO CLOSE ANAL AND RECTAL FISTULA - LONG TERM FOLLOW UP**

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**BACKGROUND:** The aim of this study was to review the results of gracilis muscle transposition using the perineal approach.

**Methods:** A retrospective review of all patients who underwent gracilis muscle transposition between 2000 and 2011 in a single institution was undertaken. Gracilis muscle transposition was performed in 47 patients using a perineal approach. Patient outcomes were assessed after surgery in a long term follow up.

**RESULTS:** Gracilis muscle transposition was performed in 47 patients (age 29-75 years, mean: 50 years). The majority of patients were female: 37 patients (78.7%). The mean follow-up time was  $35.9 \pm 34$  months after Gracilis muscle transposition. Thirtytwo of the patients (68.0%) underwent gracilis muscle transposition for the treatment of complex perianal or rectal fistulas; 78.1% had a frequency of 2.6 prior attempts of repair (range 1 - 8). Gracilis muscle transposition was indicated in 13 patients (27.7%) with additional faecal incontinence.

The aetiologies were Crohn's disease in 18 patients (38.3%), brachytherapy and surgery after rectal, uterus, ovary and prostate cancer in 11 patients (23.3%), iatrogenic injury to the rectum in seven patients (14.9%). Fifteen patients (31.9%) experienced at least one postoperative complication. The fistula was closed in 80.9% (38 patients).

**CONCLUSION:** The gracilis muscle transposition using the perineal approach is associated with low morbidity and has a high success rate treating complex perianal/anorectal fistulas and fecal incontinence.

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#### **LOP63: PEDICLED PROPELLER PERFORATOR FLAPS (PPP FLAPS) - PERSONAL EXPERIENCE**

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**BACKGROUND:** A perforator flap originally harvested for a microsurgical free flap can be harvested as a pedicled perforator flap. The Perforator concepts in microsurgical free flap surgery can therefore also be applied in pedicled flaps for local reconstruction. With this concept a new tool is given into the hand of the reconstructive surgeon.

**PATIENTS AND METHODS:** The authors present their personal experience with local reconstruction in several anatomical regions with pedicled propeller perforator flaps arising from various source vessels. A backup plan was made preoperative with the patients in case no adequate perforator vessels were found.

**RESULTS:** Between July 2006 and May 2012 a reconstruction in various regions of the body were performed with 30 pedicled propeller perforator flaps were performed. 15 different source vessels were described. The indications varied from defects following tumour resection, decubitus ulcer and extended wound healing problems. One flap loss occurred. In 4 cases partial tip necrosis of the flap were registered.

**CONCLUSION:** The principles of perforator dissection and flap design can be applied to pedicled perforator flaps. These axially based flaps can be used in a variety of anatomical locations as described. Proper understanding of perforator anatomy increases reliability and allows greater freedom of flap design. The big advantage of this kind reconstruction is the symbiosis between maximal safety and

diminishment of donor side morbidity respecting the aesthetic point of view of reconstruction replacing "like with like". The eliminating of the microsurgical component is an additional advantage and so these applications can be widespread and the potential risk of flap loss diminished. The drawbacks is the variability of the anatomy of the region surrounding the defect and previous interventions followed by scars which sometimes render a local reconstruction impossible.

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#### **LOP64: CLINICAL AND RADIOLOGICAL CHANGES IN TERES MAJOR MUSCLE AFTER (OSTEO-) FASCIOCUTANEOUS FLAP-HARVEST**

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**INTRODUCTION:** The subscapular vessel system is a well described source of several flaps. The teres major (TM) muscle, mainly nourished by a branch of the circumflex scapular artery (CSA) clinically results in a functional Type I (Mathes/ Nahai) although very small branches of the angular artery as secondary pedicle have constantly been shown to nourish the muscles origin. The fasciocutaneous parascapular flap (FC-PSF) is supplied by the cutaneous parascapular artery, as a branch of the CSA. Or the osteocutaneous (OC) flap variant, the lateral margin of the scapular bone pedicled on the CSA often means releasing the TM-muscle including the angular branch from its origin: This background raised the question of a possible structural change and clinical impairment of the TM-muscle due to its devascularisation when harvesting a PSF.

**MATERIALS/METHODS:** A retrospective review of 20 patients, who underwent the microsurgical free tissue transfer of FC- PSF (10) and OC-PSF (10) at the BG Trauma Center Murnau between January 2005 and December 2009 was conducted. Patients were questionaired (DASH) and their charts were reviewed. They underwent a clinical donor site examination, an objective force- and function measurement of the upper extremities (Cybex/ Neutral Zero Method). Additionally, an MRI of the scapular region was performed. In blind study style defined axial T1 weighted sections were used for bilateral geometrical measurements of the TM-muscle and its signal intensity; Results were compared intra-individually.

**RESULTS:** All patients denied suffering from major limitations concerning the donor site and non of them expressed major aesthetic concerns. Force- and function measurements demonstrated no significant deficit between donor and contralateral side.

MRI analysis elucidates less TM- muscle volume at the donor site, independent from the patients' handedness. Intraindividual volume differences (left vs. right) were greater in patients after the OC-PSF. Signal intensity representing fatty degeneration were not consistently found in the donor side.

All patients mentioned a high level of satisfaction and would choose this free tissue transfer from the upper back again.

**CONCLUSION:** Overall it may be stated, that the use of a FC- or OC-PSF is associated with minor, but clinically asymptomatic atrophy of the TM-muscle, which did not resolve in clinical impairement. Still, the postoperative structural alterations of the muscle, demonstrated by MRI may have a clinical relevance: This study indicates that previous harvest of a free PSF may contraindicate rotator cuff stabilizing procedures involving the TM-muscle like the L'Episcopo procedure due to impaired vascularisation and structural changes in this muscle.

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