TUESDAY BASIC SCIENCE

10:15 AM - 10:20 AM

RM128 Biodegradable Wireless Sensor for Continuous Monitoring Vascular Flow

Stanford University, Palo Alto

Presenter: **Yukitoshi Kaizawa, MD, PhD**

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Background

Postoperative monitoring of tissue perfusion after reconstructive microsurgery is critical. Early recognition of vascular compromise and prompt surgical intervention improve chances for flap salvage. We developed a novel wireless, biodegradable sensor for continuous postoperative monitoring. We hypothesized our sensor would provide biocompatible accurate detection of vascular flow. We evaluated the sensor's biocompatibility in comparison with other FDA approved biomaterials. Sensor function and influence on the arterial patency and wall thickness were evaluated using a rat femoral artery model.

Methods For evaluation of biocompatibility, the sensor packaging material, poly(octamethylene maleate (anhydride) citrate) (POMaC), and FDA approved biodegradable biomaterials, poly glycolic acid (PGA) and poly L-lactic acid (PLLA), were implanted in subcutaneous pockets of on the rat dorsum. Three weeks after implantation, the width of fibrous capsular formation and foreign body reaction around the materials were evaluated histologically. To test sensor function and evaluate arterial wall changes, the sensor was implanted around rat femoral arteries. Sensor function was assessed immediately at implantation and 1 week later. The sensor and surrounding tissues were harvested 6 weeks after implantation. Inner and outer diameters of the arteries were measured on cross sections of the arteries. Inner diameter divided by outer diameter (ID/OD) was calculated and compared to a sham operative group.

Results H&E staining and immunohistochemistry for CD68 3 weeks after implantation showed fibrous capsular formation and a significant number of CD68 positive cells around all materials. Quantification of the width of fibrous capsule (POMaC:60.9±15.6um, PGA:88.1±26.3um, and PLLA:90.0±5.5um) and CD68 positive cell density (POMaC:385±143/mm^2, PGA:715±105/mm^2, and PLLA:565±74/mm^2) revealed that POMaC had comparable biocompatibility to PGA and PLLA. Accurate vascular flow was successfully detected 1 week after implantation. At 6 weeks, the arterial lumen was patent without a significant increase in wall thickness (ID/OD: 0.70±0.04 (sensor group) vs 0.66±0.13(sham group), p=0.6).

Conclusion Our novel biodegradable sensor demonstrated no harmful effects after implantation. The sensor maintained accurate detection of changes in vascular flow for 1 week after implantation. This sensor can be applied clinically as a useful monitoring tool after reconstructive microsurgery. It is superior to current available devices due to its wireless design

that eliminates wires tugging on the fragile anastomosis. Additionally, the wireless design and remote communication allows for flap monitoring outside of the hospital setting by both the patient and physician.

10:20 AM - 10:25 AM

RM129 The Effects of Growth Hormone on Nerve Regeneration, Functional Recovery and the Immune Response in Vascularized Composite Allotransplantation

Johns Hopkins University School of Medicine, Baltimore

Presenter: **Jennifer Rath, BS**

Jennifer Rath, BS(1), Xianyu Zhou, MD(2), Philip J Hanwright, MD(3), Neha Amin, BS(4), Nicholas von Guionneau, MBBS(3), Chia Na Min, MS(3), Thomas G.W. Harris, BSc(3), Sai Pinni, .(5), WP Andrew Lee, MD(3), Gerald Brandacher, MD(3) and Sami Tuffaha, MD(6)

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Background: Functional recovery following upper extremity transplantation remains poor, primarily as a result of prolonged denervation and resultant muscle atrophy. Growth hormone (GH) has well-established trophic effects on neurons, myocytes, and Schwann cells and represents a promising therapeutic strategy to accelerate axonal regeneration and also maintain muscle and Schwann cells prior to reinnervation. The aims of this study were to confirm the positive effects of GH on nerve regeneration and functional recovery and to evaluate the effects of GH treatment on the immune response in the setting of vascularized composite allotransplantation. **Methods:** Rats underwent orthotopic forelimb transplantation with full MHC-mismatch (Brown Norway to Lewis) and were randomly assigned to receive either porcine-derived growth hormone (0.6 mg/kg/day) or no treatment (n=10 per group). All animals received tacrolimus (2 mg/kg/day) for graft maintenance. Animals underwent functional assessments every four weeks using electrically-stimulated grip strength testing. Animals were monitored for clinical signs of rejection. Skin biopsies and serum cytokine levels were obtained at the mid- and end-point to evaluate for subclinical rejection. Animals were sacrificed at 16 weeks or if they demonstrated advanced rejection (grades III/IV). Quantitative histological assessments of axonal regeneration, neuromuscular junction reinnervation, muscle atrophy, and Schwann cell proliferation were performed on muscle and nerve specimens upon sacrifice. **Results:** Grip strength was improved in the growth hormone-treated animals as compared to the control group at weeks 4 and 16 ($p=0.03$ and $p=0.05$, respectively; Figure 1). Preliminary quantitative analysis of axon histomorphometry revealed trends towards a greater degree of myelination and increased axon counts in the GH-treated group ($p>0.05$). Rates of clinical rejection did not significantly differ among groups. Remaining data is pending. **Conclusion:** Growth hormone treatment improved functional recovery in rats undergoing allogenic orthotopic

forelimb transplantation. GH therapy did not significantly affect rates of clinical rejection.

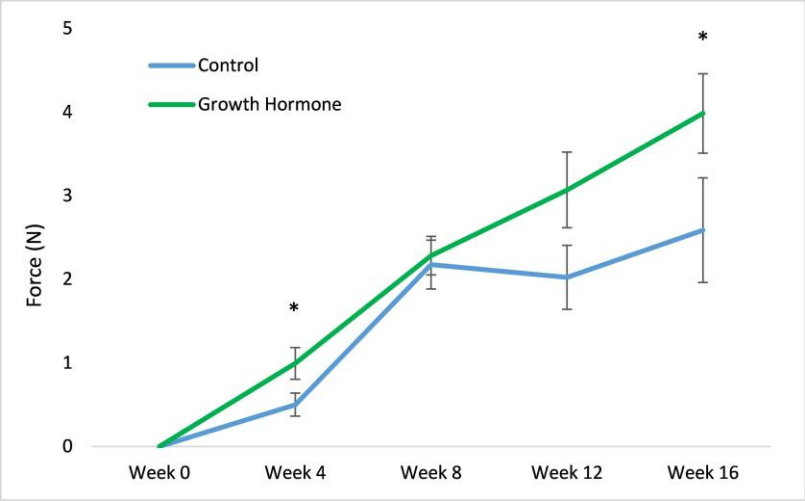

Figure 1: Post-transplantation funtional recovery as measured by grip strength. Error bars depict standard error.

10:25 AM - 10:30 AM

RM130 First Successful 24 Hours Preservation in Vascularized Composite Allograft Using a Subzero Non-Freezing Protocol

Massachusetts General Hospital/Harvard Medical School, Boston

Presenter: **Laura C Burlage, MD**

Laura C Burlage, MD(1,2), Alexandre G Lellouch, MD(3,4), Olivia N Mamane, N/A(1), Casie A Pendexter, MSc(1), Mark A Randolph, MSc(4), Laurent Lantieri, MD, PhD(3), Robert J Porte, Md, PhD(2), Shannon N Tessier, PhD(1), Curtis L Cetrulo, Jr., MD, FACS(4) and Korkut Uygun, PhD(1)

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Background: Vascularized composite allotransplantation (VCA) remains the most advanced treatment option to restore motor function and aesthetics in patients living with devastating disfigurements. The current gold standard of organ preservation is static cold storage allowing 5-8 hours of ischemia in VCA. This duration constitutes a major limiting factor for the matching process and drastically reduces the donor pool. Subzero non-freezing (SZNF) is a novel technique recently demonstrated on liver for long-term preservation of organs below the freezing point without the induction of ice formation. The aim of this study was to optimize an extended preservation protocol using SZNF in a rodent hind limb model.

Methods: Seven rodent hind limbs were procured after systemic heparinization (300 IU). All limbs were perfused (21 degrees Celsius) for 2 hours through the femoral artery with a mixture of muscle media, bovine serum albumin, polyethylene glycol (PEG) and a non-metabolizable glucose analogue (3-OMG). Limbs were then cooled until graft temperature reached 4-7 degrees Celsius and subsequently flushed with a storage solution/PEG mixture. All limbs were placed in a temperature controlled chiller and preserved for 24 hours at minus 5 degrees Celsius. After SZNF, 3 limbs were recovered using 1 hour of machine perfusion (21 degrees Celsius) with the loading phase solution less 3-OMG. During machine perfusion, arterial flow and vascular resistance were monitored. Lactate levels and oxygen consumption were evaluated as markers of viability of the muscle tissue.

Results: During the loading phase, arterial outflow and vascular resistance remained stable, between 1.0-1.7 mL/min and 25-40 mmHg/mL/min respectively. Lactate levels decreased and oxygen consumption remained stable. Average weight gain during the loading phase was 6.1%. During 24 hours of SZNF, one limb froze as a result of vibrations of the chiller. After optimization of the SZNF technique, 6 out of 7 limbs did not freeze during SNZF. During the recovery phase, arterial outflow and vascular resistance remained stable, between 0.5-0.7 mL/min and 25-40 mmHg/mL/min respectively. Lactate levels decreased and oxygen consumption remained stable. However, average weight gain during recovery was 31.8%.

Conclusion: This proof of concept study demonstrates that 24 hours of subzero preservation in rodent hind limbs is feasible. SZNF has the potential to elongate the maximum preservation time

of vascularized composite allografts up to 3-4 times longer than standard cold preservation, which will greatly benefit the clinical application of VCA. Current studies are investigating the optimization of the recovery phase prior to transplantation.

10:35 AM - 10:40 AM

RM131 Anorectal Transplantation: The First Long-Term Successful Report in a Preclinical Canine Model

Shizuoka cancer center, Shizuoka

Presenter: **Jun Araki, MD, PhD**

Jun Araki, MD, PhD(1,2), Yuji Nishizawa, MD, PhD(3), Naoki Fujita, PhD(4), Naito Munekazu, MD, PhD(2), Flavio H Galvão, MD, PhD(5) and Masahiro Nakagawa, MD, PhD(6)

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Background

Although anorectal transplantation is a challenging procedure, it is a promising option for patients who have completely lost or failed to develop their anorectum. In this study, we utilized a canine model of anorectal transplantation, evaluated the long-term outcomes, and controlled the rejection and infection in allotransplantation.

Methods

In the pudendal nerve functional study, six dogs were randomly divided into two groups: cut and anastomosis, and compared with a sham operated dog. In anorectal transplantation model, four dogs were assigned to four particulars: autotransplant, allotransplant with immunosuppression, allotransplant without immunosuppression, and normal control. In the both studies, macroscopic findings, anorectal monometry, and microscopic findings of the pudendal nerve and the sphincter muscle were evaluated.

Results

In the pudendal nerve functional study, anorectal manometry indicated the mean pressure of anastomosis group and resection group were significantly dropped compared to sham group after operation. Anastomosis group showed partial recovery from postoperative six months onward and resection group never showed recovery to end. The pudendal nerve and the sphincter muscle were microscopically regenerated and well-maintained in anastomosis group at the end point. Anorectal transplantation was technically successful with three-staged operative procedure; preparative colostomy, anorectal transplantation, and stoma closure. Anorectal manometry of transplanted dogs showed partial recovery from postoperative six months onward as well as the pudendal nerve functional study. Dog in allotransplant with immunosuppression was given tacrolimus and methylprednisolone after transplantation. He had two episodes of mild rejection, which were all reserved by methylprednisolone and tacrolimus treatment. Dog in allotransplant without immunosuppression had an acute rejection, which resulted in graft necrosis and was sacrificed on the next day of transplantation. Survived dogs took effective control of their defecation and the pudendal nerve and the sphincter muscle were microscopically regenerated and well-maintained at the end point.

Conclusion

We described the first long term outcome after anorectal transplantation in a canine model. This report is a proof-of-concept for anorectal transplantation as a treatment for ostomy patients due to anorectal dysfunction. Furthermore, the results show the feasibility of the first human trial in the near future.

10:40 AM - 10:45 AM

RM132 Loss of HIF-1α in CD4 T Cell Improve Outcome of Mouse Vascularized Osteomyocutaneous Allotransplantation

Center for VCA Chang Gung Memorial Hospital, Taoyuan

Presenter: **Cheng-Hung Lin, MD**

Cheng-Hung Lin, MD(1), Madonna Rica Anggelia, MSc(1), Huang-Yu Yang, MD(2), Wen-Yu Chuang, MD(3), Hui-Yun Cheng, PhD(4) and Gerald Brandacher, MD(5)

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Background: Allograft survival depends on the balance of effector T cells (Teff) and regulatory T cells (Tregs). Previous study suggested that the balance is controlled by HIF-1α. In this study, we investigated the role of HIF-1α-deficient CD4 T-cell in promoting acceptance of vascularized composite allografts.

Methods: Thirty-eight osteomyocutaneous allografts from Balb/c were transplanted to wild type (WT) and HIF-1$\alpha^{fl/fl}$CD4^{Cre} C57BL/6 mice. Animals received combined costimulation blockade (1 mg anti-CD154 at POD 0, 0.5 mg CTLA4Ig at POD 2) with or without rapamycin (3mg/kg/day for 7 days then every other day for 3 weeks). Allograft survival, ratio of Teff/Tregs subpopulation in the periphery and allograft were assessed.

Results: Twelve of 15 WT recipients under costimulation blockade and 1-month rapamycin achieved long-term allograft survival (>120 days). In the tolerant animals, ratio of Tregs/Th1 cells and Tregs/Th17 cells in periphery at POD 30 and in allograft were greater than those in rejected animals. In MLR study, conditional HIF-1α deficiency in CD4 resulted CD4 T-cell hyporesponsiveness. HIF-1$\alpha^{fl/fl}$CD4^{Cre} mice generated CD4^{+}cells with greater expression of FoxP3^{+} and IL-10 but less expression of IL17^{+} and RORγ^{+} than WT in response to allografts. Without the use of rapamycin, improved allograft survival rate was achieved in HIF-1$\alpha^{fl/fl}$CD4^{Cre} mice compared to WT mice (100 vs 36.5 days). Animals with long-term allograft survival showed donor-specific T cell hyporesponsiveness.

Conclusion: Loss of HIF-1α in CD4 T cells improves allograft survival in the absence of long-term immunosuppressant drugs. Targeting potential mechanisms involved in the differentiation of CD4 T cells may play an important role in improving allograft survival.

10:45 AM - 10:50 AM

RM133 Lentivirally-Delivered shRNA Knockdown of CXCL12 Prevents Fibrosis in a Rodent Model of Radiation Late Adverse Effects

Institute of Cancer Research/The Royal Marsden Hospital, London

Presenter: **Aadil A Khan, MPH, FRCS (Plast), PhD**

James T. Paget, BM, BCh, MRCS(1), Martin McLaughlin, PhD(2), Joan Kyula, PhD(2), David Mansfield, BSc(3), Henry Smith, MBBS, MRCS(3), Victoria Roulstone, BSc(3), Alan Melcher, MRCP, PhD(3), Navita Somaiah, MRCP, FRCR, PhD(3), Kevin J. Harrington, FRCP, FRCR, PhD(4) and **Aadil A Khan, MPH, FRCS (Plast), PhD**(5,6)

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Background

Late Adverse Effects (LAEs) following radiotherapy (RT) present difficult clinical problems during cancer survivorship. However, free tissue transfer also offers a novel therapeutic window for the delivery of viral gene therapy vectors, *ex vivo*, selectively into flap tissues with exquisite anatomical control.

Methods

We hypothesized that knockdown of CXCL12 would ameliorate the LAE phenotype. Lentiviral vectors for both CXCL12 silencing (shRNA) and over-expression were developed and validated *in vitro.* A rodent model of SIEA flap irradiation was used to evaluate this radioprotective strategy. Flaps were infected with lentiviral vectors encoding shRNA against CXCL12 (LVshCXCL12), over-expression of CXCL12 (LVoeCXCL12), scrambled sequences (LVSCR) or saline only prior to RT. A tumor recurrence model was used to evaluate the effect of this strategy on the tumor response to RT *in vivo*.

Results

We observed increases in CXCL12 expression post-RT ($p<0.001$) associated with increases in CXCR4 ($p<0.05$) and CD68 ($p<0.01$) expression *in vivo*. This was associated with significant increases in macrophage ingress and fibroblast activation ($p<0.001$). These findings were validated further in human tissues taken 14-days post-RT where we observed increased CXCL12 and CD68 expression compared to matched, non-irradiated controls.

LVshCXCL12 flaps demonstrated reduced acute toxicities, whilst CXCL12 over-expression resulted in exaggerated acute toxicities. LVshCXCL12-infected flaps exhibited almost complete reversal of flap contracture and were not different significantly from un-irradiated flaps. Radiation Therapy Oncology Group LAE scores were also significantly improved compared

with LVoeCXCL12 ($p<0.0001$), LvSCR ($p<0.0001$) and PBS groups ($p=0.0011$). LVoeCXCL12 animals developed late toxicities more rapidly than other groups. Tumor recurrence experiments *in vivo* demonstrated that tumors that grew in flaps infected with LVshCXCL12 exhibited a better response to RT compared to those growing in control flaps.

Flow-assisted cytometry analysis of flap tissues at 7 and 21 days post-RT and demonstrated that LVshCXCL12-infection resulted in reduced macrophage trafficking into irradiated tissues ($p=0.014$). This was associated with reduced fibroblast migration ($p=0.015$), collagen deposition ($p=0.019$) and *Acta2* gene expression ($p=0.045$).

Conclusion

In summary, we postulate that CXCL12 over-expression in normal tissues post-RT signals an innate immune response mediated primarily by macrophages. Our data would suggest that modulation of the CXCL12/CXCR4, with therapeutic intent, can prevent the development of LAEs without compromising the cytotoxic efficacy of RT. Further work will aim to understand the mechanism of immune-fibroblast cross-talk in the context of radiation fibrosis.