Additional studies are needed to determine whether this innovation is cost-effective and generalizable to the entire autologous breast reconstruction population.

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PEG Fusion Significantly Improves Return of Function in a Median/Ulnar Nerve Denervation Model

Christopher M. Frost, MD¹, Cameron Ghergherechit², George Bittner, PhD², Gerald Brandacher, MD¹, Jaimie Shores, MD¹

¹Johns Hopkins Hospital, Baltimore, MD, USA, ²University of Texas, Austin, TX, USA

PURPOSE: Peripheral nerve injury is a challenging clinical problem due to slow, incomplete regeneration that results in the poor return of function. Polyethylene glycol (PEG) has been studied in the rat sciatic nerve model to immediately restore nerve continuity and prevent Wallerian degeneration. PEG-fusion reestablishes axoplasmic continuity by nonspecifically fusing closely apposed severed axons at the lesion site. In this study we examine if PEG-fusion can improve nerve repair in a novel denervation model using serial grip strength testing (GST).

METHODS: Sprague-dawley rats (n=10) were utilized comparing suture repair alone (n=5) to PEG fusion with suture repair (n=5). In both control and PEG-fusion groups baseline grip strength was first calculated using percutaneous electrical stimulation to the rat forearm followed by GST of the rat paw using a force transducer. Next, bilateral ulnar and median nerves were exposed and divided. In both groups the proximal ulnar nerve was sutured into adjacent bicep muscle. Total nerve discontinuity was confirmed with nerve conduction testing (NCT). In both control and PEG fusion groups the median nerve was repaired using 10-0 nylon sutures. In the PEG-fusion group the nerve ends were first washed with hypotonic Plasmalyte followed by methylene blue prior to neurorrhaphy. PEG was applied for 90 seconds followed by Lactated Ringers solution. Successful PEG-fusion was confirmed using NCT. Follow up GST testing was performed at 7, 14, 21, 28, 35, 42 days under sedation using percutaneous stimulation. Statistics were performed using 2-tailed t-test with Bonferroni correction for multiple comparisons.

RESULTS: Nerve continuity was reestablished in all PEG-fusion animals with NCT demonstrating both restored compound action potentials (CAP) and compound muscle action potentials (CMAP). There was no measurable CAP or CMAP after suture repair alone. The PEG fusion group demonstrated significant (p<0.05) increase in grip strength compared to control at all time points. PEG fusion group showed an average of 54% return to baseline grip strength as early as POD 7. As expected, suture repair alone had minimal return of function at POD 7. Further histologic examination pending.

CONCLUSION: PEG fusion significantly improved return of function compared to standard neurorrhaphy in this ulnar and median nerve denervation model.

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Regulatory T Cells Promote Tolerance during the Early Post-Operative Period in Murine Osteomyocutaneous Vascularized Composite Allotransplantation

Madonna R. Anggelia¹², Hui-Yun Cheng¹, Wen-Yu Chuang³, Chih-Hung Lin¹, Gerald Brandacher⁵, Fu-Chan Wei¹, Nicholas Do¹, WP Andrew Lee⁵, Cheng-Hung Lin¹

¹Center for Vascularized Composite Allotransplantation, Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Chang Gung Medical College and Chang Gung University, Taoyuan, Taiwan, ²Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ³Department of Pathology, Chang Gung Memorial Hospital, Chang Gung Medical College and Chang Gung University, Taoyuan, Taiwan, ⁴Department of Plastic and Reconstructive Surgery, Chiayi Chang Gung Memorial Hospital, Taoyuan, Taiwan, ⁵Department of Plastic and Reconstructive Surgery, Vascularized Composite Allotransplantation (VCA) Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, USA
PURPOSE: Regulatory/suppressive immune cells, such as CD4\(^+\)CD25\(^+\)FoxP3\(^+\)regulatory T cells (Tregs), have been demonstrated to mediate allograft tolerance in various transplant models. However, their role in vascularized composite allotransplantation (VCA) has not been specifically defined. This study determines the relevant molecular mechanisms, origin (donor vs recipient), location, and activity time frame of Tregs in mediating the induction of allograft tolerance.

METHODS: Osteomyocutaneous (OMC) allografts from Balb/c were transplanted into 34 C57BL/6 mice. Immunosuppressive protocol consisted of 1mg anti-CD154 (POD 0), 0.5mg CTLA4Ig (POD 2), and 3mg/kg/day rapamycin for 7 days then reduced to 3mg/kg every other day for 3 weeks. Recipients were organized into 5 groups based on time point of Tregs depletion using anti-CD25 antibody: Group 1 (control), no Tregs depletion (n=10); Group 2, depletion on POD 0 (n=7); Group 3, POD 30 (n=7); Group 4, POD 90 (n=7); and Group 5, with anti-CD25 and Tregs isolated from tolerant mice at POD 30 (n=3). Intracellular markers and cytokines associated with Tregs activation were measured. Ratios of Tregs to rejection mediating T cell subpopulations (Th1, Th2, & Th17) were assessed by flow cytometry. To observe Tregs origins, Balb/c -Tg(FoxP3-GFP) mice were used as donor and the presence of FoxP3\(^+\)GFP\(^+\)cells were then determined by flow cytometry and immunohistochemistry. To confirm function of Tregs in the allograft, skin from naïve Balb/c or tolerated OMC allografts were grafted onto Rag2-/- mice in the presence of adoptive-transferred effector T(Teff) cells. Tolerance/rejection was assessed clinically and median survival time (MST) recorded.

RESULTS: Intracellular markers (GATA-3\(^+\), T-bet\(^+\) and Helios\(^+\)) and cytokines (IL-10\(^+\), TGF-\(\beta\)^+), and IL-35\(^+\)) associated with Tregs activation were elevated in tolerant animals vs animals experiencing rejection. Tolerant animals showed increased ratios of Tregs/Th1 cells and Tregs/Th17 cells but not of Tregs/Th2 cells. Tregs in the circulation, secondary lymphoid organs, and OMC allograft skin were of recipient origin in all animals though a higher amount was found in tolerated allografts. Tregs from the tolerant grafts circulated in Rag2\(^+\) recipient mice and delayed adoptive transferred Teff-mediated rejection (MST=37 vs 52 days). Allograft survival was significantly shortened in the groups with Treg-depletion on POD0 (3 of 7, MST=90) and POD 30 (5 of 7, MST=104, p<0.05) compared to the un-depleted control. However, allograft survival was unaffected with Tregs depletion on POD 90. Tregs depletion-mediated rejection was rescued with adoptive transfer of Tregs from allograft tolerant animals (Group5)(MST > 200).

CONCLUSION: Recipient Tregs are crucial for VCA tolerance in the early post-operative period and could be utilized as a cellular therapy to improve VCA outcomes.

Reconstruction of Craniofacial Structural Defects Through Patient-specific 3-D Printed Custom Scaffolds: Development of A Porcine Model

Richard F. Guidry, BS\(^1\), Silpa Sharma, MPH\(^2,3\), Adam Prevot, BS\(^1\), Ian R. Wisecarver, MD\(^1\), Luis Marrero, PhD\(^2\), Mandi J. Lopez, DVM, PhD\(^1\), Gerhard S. Mundinger, MD\(^2\)

\(^1\)Louisiana State University School of Medicine, New Orleans, LA, USA, \(^2\)Louisiana State University Health Sciences Center, New Orleans, LA, USA, \(^3\)Children’s Hospital of New Orleans, New Orleans, LA, USA, \(^4\)Louisiana State University Laboratory for Equine and Comparative Orthopedic Research, Baton Rouge, LA, USA

PURPOSE: 3-D printed bioreorable scaffolds for cranio-maxillofacial bone regeneration can be custom-made to fill specific defects, and can be commercially printed based on CT scans within days. Additional seeding of scaffolds with autologous stem cell populations may enable improved regeneration of normal bony architecture, minimization of donor site morbidity, enhanced ability to restore complicated three-dimensional shapes, and improved functional outcomes. However, the ability of such scaffolds to regenerate load-bearing bone is untested in large animal models.

METHODS: We developed a craniofacial porcine model of bone regeneration suitable for testing bioengineered custom 3-D printed bone scaffolds to heal non-critical (<6cm) and critical (>6cm) bone defects. Full-thickness defects were made in the body of the right zygoma and angle of the left mandible using 3-D printed custom cutting guides. In the control arm of the study reported here (n=4), no