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 1 mg anti-CD154 (POD 0), 0.5 mg 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 cyometry. 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 ($GATA\text{-}3^+$, T-bet+ and $Helios^+$) and cytokines ($IL\text{-}10^+$, $TGF\text{-}\beta^+$, and $IL\text{-}35^+$) 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.

54

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[4], 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 bioresorbable scaffolds for craniomaxillofacial 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

construct was placed. Post-operatively, animals were followed for 6 months, at which time CT imaging and micro-CT/histology of regenerated bone across the defects was evaluated.

RESULTS: The four control animals underwent surgery and achieved the 6-month post-operative study endpoint with no complications or disturbance of masticatory function. 3D printed osteotomy and plating guides facilitated surgical precision and minimized operative times. CT and gross evaluation of zygomatic and mandibular defects was consistent with incomplete heterotopic ossification. µCT confirmed the presence of dystrophic bone formation at the ostomy sites with disruption of normal bone architecture. Trichrome histologic evaluation of the experimental zygoma showed disorganized, porous bone compared to contralateral controls. Study results in these animals supported ongoing work in the experimental arm (n=8 animals), in which beta-tricalcium phosphate (βTCP) defect-specific bone scaffolds (KLS Martin, Mulheim, Germany) were 3-D printed from preoperative CT images and placed into the zygoma and mandible defects.

CONCLUSION: The described model of craniofacial bone reconstruction utilizing 3D printed, defect-specific bone regeneration templates has broad clinical applicability. Ultimately, insights from this model may realize the possibility of reconstructing bony defects of any size, shape, and thickness by harnessing the power of 3D printing and autologous bone stem cell seeding. Additionally, the described technique may enable critical scale-up capability of autologous cell populations across tissue types other than bone for use in post-traumatic and oncologic reconstruction.

55

Trans-facial Minimal-Dissection External Mandibular Distraction Osteogenesis for Neonatal Airway Obstruction from Pierre Robin Sequence

***Jeremie D. Oliver, MD, MS Candidate*[1], *Christopher M. Runyan, MD, PhD*[2], *Dominic Massary, MD*[2], *Lyfong Lor, BS*[2], *Shayan Jahanpanah, BS*[3], *Brian S. Pan, MD*[3], *Christopher B. Gordon, MD*[4], *Haithem Elhadi Babiker, MD, DMD*[3]**

[1]Mayo Clinic, Rochester, MN, USA, [2]Wake Forest Baptist Medical Center, Winston-Salem, NC, USA, [3]University of Cincinnati, Cincinnati, OH, USA, [4]Dayton Children's Hospital, Dayton, OH, USA

PURPOSE: Infants with Pierre Robin Sequence (PRS) often suffer from feeding difficulties, growth impairment and apnea of varying severity secondary to tongue-based airway obstruction (TBAO). Surgical management in those with moderate to severe obstruction includes tongue-lip adhesion, tracheostomy, and/or mandibular distraction osteogenesis (MDO). MDO has shown superior outcomes in many studies, but there is currently no standardized technique for distraction. This study describes a novel minimal-dissection trans-facial two-pin technique for MDO with external distractor.

METHODS: A retrospective review of medical record data and dental images was performed for all consecutive neonates (<1 year old) treated with this technique at a single institution from 2004–2014.

RESULTS: A total of 100 consecutive patients (male = 50, 50%) treated by two-pin trans-facial mandibular distraction were identified, including both those treated primarily (n = 68), and secondarily with MDO following initial tracheostomy (n = 32). Peri-operative complications requiring unplanned surgical revision (n = 14, 14%) included hardware failure (n = 8) and early consolidation (n = 6). Significant long-term complications included limited mouth opening (n=2), marginal mandibular nerve weakness (n=1), and TMJ ankylosis (n=2, both of whom were syndromic, Catel-Manzke and Cornelia de Lange). No patients required scar revisions.

CONCLUSION: The two-pin trans-facial technique for minimal-dissection external MDO is an effective tool for correction of severe airway obstruction in neonates with PRS, with favorable long-term outcomes and complication profile compared to traditional MDO. This technique has favorable or equivocal results for feeding and airway obstruction, measured by improved sleep studies or by tracheostomy avoidance.

56

Construction of Ear Frameworks For Microtia Repair Using AuryzoN™, A Novel Investigational Medical Device.