Skeletal Muscle Regeneration Utilizing Muscle Derived Stem Cell Augmented Scaffolds: Preliminary Studies to Create an Optimized in vitro Construct

Howard D. Wang, MD, Qiongyu Guo, PhD, Joseph Lopez, MD, MBA, Amy Quan, BS, Jose C. Alonso-Escalante, MD, Denver M. Lough, MD, PhD, Edward W. Swanson, MD, WP Andrew Lee, MD, Gerald Brandacher, MD and Anand R. Kumar, MD

INTRODUCTION: Skeletal muscle loss can result from trauma or oncologic resection, leading to severe cosmetic and functional deficits. Muscle derived stem cells (MDSCs) can be isolated by using a serial preplating technique and have inherent myogenic potential.1 Since their identification, previous research on MDSCs has mostly focused on ex-vivo gene therapy applications aimed at treating muscular dystrophy, and little is known about the optimal population for skeletal muscle tissue engineering.2, 3 The aim of this study is to determine which preplate population is better suited for muscle regeneration and optimize the in vitro conditions for creating an implantable skeletal muscle construct.

MATERIAL AND METHODS: Murine MDSCs were isolated from transgenic B6 mice expressing red fluorescent protein (RFP) under a ubiquitin C promoter. Proliferation studies of various preplate populations were performed by utilizing bright-field microscopy to determine time to confluence. Myogenic potential was assessed by immunostaining for myosin-heavy chain (MyHC) to evaluate myotube formation. In vitro skeletal muscle constructs were created using various concentrations of RFP-expressing MDSCs (5x10^5, 1x10^6, 2x10^6 cells/construct) seeded onto decellularized muscle scaffolds, and cellular viability and scaffold repopulation were assessed using confocal microscopy. After 14 days in culture, constructs were further analyzed by histology and immunofluorescence.

RESULTS: Preplates 3, 4 and 5 demonstrated faster expansion rates from time of isolation compared to prelates 1, 2 and 6 (p<0.05). In terms of their myogenic potential, preplates 3, 4, and 5 had comparable fusion indices (2.53 ± 0.51, 3.22 ± 0.80, 3.10 ± 1.46, p=0.316). Confocal imaging of MDSC-seeded construct demonstrated cellular viability at 48 hours and 14 days with progressive concentric scaffold repopulation at all three cellular concentrations. Immunostaining for MyHC demonstrated myotube formation within the constructs after 14 days in culture. Further histologic analysis by H&E stain confirmed successful scaffold repopulation and higher cellular density in the constructs seeded with 2x10^6 cells.

CONCLUSION: The results of this study indicate that preplates 3, 4 and 5 possess high expansion rates and myogenic potentials. Furthermore, MDSCs are capable of successful repopulation of decellularized muscle scaffolds and myotube formation in a 3-dimensional construct. Future experiments will assess the muscle regeneration capability of the MDSC-enriched scaffolds in vivo.

DISCLOSURE/FINANCIAL SUPPORT: None of the authors has a financial interest in any of the products mentioned in this manuscript.

REFERENCES:
1. Gharaibeh, B., Lu, A., Tebbets, J., et al. Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique. Nat Protoc 2008;3:1501–1509.
2. Usas, A., Huard, J. Muscle-derived stem cells for tissue engineering and regenerative therapy. Biomaterials 2007;28:5401–5406.
3. Wu, X., Wang, S., Chen, B., An, X. Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy. Cell Tissue Res 2010;340:549–567.
MATERIALS AND METHODS: The American Council of Academic Plastic Surgeons (ACAPS) website was used to generate a list of all plastic surgery divisions/departments with residency programs. Scholarly metrics were determined for 955 faculty at the 88 ACGME plastic surgery departments and divisions with residency programs. The database was binned into tertiles by numbers of citations per department/division (high, H, medium, M, low, L). Characteristics were compared between these groups to identify the traits that set these programs apart.

RESULTS: Median numbers of faculty per program were 9. The mean publications per department/division were 479, citations; 9984, publications per faculty; 38, citations per faculty; 742. Programs in H had higher numbers of publications even after adjusting for departmental size (H:59, M:33, L:21, p<0.05). Programs in the H group also had higher numbers of mean PhDs and MD-PhDs per division, and higher total numbers of NIH grants (H:7.5, M:1.2, L:0.1, p<0.05), and R01/P01/U01 grants (H:2.5, M:0.5, L:0, p<0.05). There were no differences in gender distribution across these groups. Programs in H had significantly more total residents H:11.9 vs. M:7.6 and L:6.1, p<0.05 which was mainly driven by higher numbers of integrated residents.

CONCLUSIONS: The strongest determinants of academic productivity among plastic surgery programs appear to be effective utilization of faculty with advanced degrees, emphasis on NIH funding, and the presence of integrated residency programs. A recent study suggested that the presence of an integrated residency as well as subspecialty fellowships increases the productivity of academic faculty in plastic surgery. A focus on NIH funding and the incorporation of integrated residency programs may be the optimal way to increase academic productivity in plastic surgery.

REFERENCES:
1. Mann M, Tendulkar A, Birger N, et al. National institutes of health funding for surgical research. Ann Surg. 2008;247:217–221.
2. Beasley BW, Wright SM, Cofrancesco J, Jr, et al. Promotion criteria for clinician-educators in the United States and Canada. A survey of promotion committee chairpersons. JAMA. 1997;278:723–728.
3. Duquette S, Valsangkar N, Sood R, et al. Do plastic surgery programs with integrated residencies or subspecialty fellowships have increased academic productivity? Plastic and Reconstructive Surgery Global Open. 2016;4(2):e614. doi:10.1097/GOX.0000000000000005

Contributions of Plastic Surgery to Hospital Based, Pediatric Care in the United States, 2000–2012

Jason Wink, MD, MTR; Patrick Gerety, MD; Justin Fox, MD, MHS; Jesse Taylor, MD

INTRODUCTION: Plastic surgery to address congenital or acquired conditions can have a profound impact on children’s well-being and psychosocial development. We conducted this study to estimate trends in plastic surgery procedures among United States population.

METHODS: Using the 2000–2012 Kids’ Inpatient Databases, we identified hospital discharges associated with a primary diagnosis or procedure within the pediatric plastic surgeon’s scope of practice: cleft lip and palate, craniosynostosis, congenital hand or chest anomalies, vascular malformations, and soft tissue flap or facial fracture procedures. The primary outcomes were the frequency of discharges and associated hospital charges. Regression models were used to test for significance in trends while accounting for changes in the U.S. pediatric population.

RESULTS: The final sample included 310,513 discharges between 2000 and 2012. During the study, there was a 16% increase in pediatric plastic surgery-related discharges (2000=56,389 vs 2012=67,054, p <0.001) and a 2.83-fold increase in mean, hospital charges per encounter (2000=$27,216 vs 2012=$77,032, p <0.001). The greatest growth was for vascular malformations (+76%; p <0.001), congenital chest anomalies (+61%; p <0.001), and craniosynostosis (+38%; p <0.001), while the greatest decline was for facial fracture procedures (-41%; p <0.001). The greatest growth in charges were for vascular malformations (+3.48-fold; p <0.001) and congenital hand (+3.22-fold; p <0.001). Dedicated children’s hospitals experienced a 2.1-fold increase in discharges overall with growth noted in all areas.

CONCLUSION: Plastic surgeons are playing a growing role in the care of pediatric patients across the United States, especially at dedicated children’s hospitals.