transection without repair. First, we evaluated the extensor digitorum longus (EDL) muscles of 15 adult wildtype C57BL/6 mice (n=3 per time point) at days 1, 3, 5, 7, and 14 after sciatic nerve injury. The uninjured EDL muscles served as the experimental controls. These muscles were harvested for immunostaining with CD68 (monocytes/macrophages) and DAPI (nuclear) staining. Next, using the same injury and mouse model, flow cytometry was utilized to evaluate total cells present in EDL muscle after sciatic nerve injury. Animals were sacrificed at days 1 and 5 after nerve injury, and all muscles of the hindlimb innervated by the sciatic nerve were harvested from the right injured and left uninjured legs. Cells were analyzed following muscle digestion.

RESULTS: At all timepoints after nerve injury, there were significantly more CD68+ cells recruited to denervated EDL muscles than to uninjured controls in our immunocytochemistry analysis. On flow cytometry, there was a higher number of CD45+ hematopoietic cells isolated from denervated muscle than uninjured controls. Moreover, data demonstrate significantly more $Ly6C^{+}F480^{-}$ monocytes and $CD206^{+}MerTK^{+}$ macrophages recruited to the muscle following acute nerve injury. At postoperative day 5, $CD206^{+}MerTK^{+}$ macrophages had decreased CD11c expression, suggesting activation of these immune cells.

CONCLUSIONS: Our studies demonstrate the novel finding that acute nerve injury induces macrophage recruitment to the distal target muscle. Moreover, recruited macrophages have an altered phenotype, which may suggest a functional transformation of these important inflammatory and regenerative immune cells. Further studies are ongoing to determine the functional impact of this macrophage phenotypic change on reinnervation of the muscle following acute nerve injury. Knowledge of this process may provide new therapeutic targets to improve functional recovery following nerve injury.

P28

Nanofiber-Hydrogel Composite with Human Adipose-Derived Stem Cells to Enable Soft Tissue Regeneration

Brian H. Cho, MD[1,2], Xiaowei Li, PhD[2,3], Sashank Reddy, MD, PhD[1], Russell Martin, PhD[2,3], Michelle Seu, BA[1,2], Gurjot Walia, BS[1], Hai-Quan Mao, PhD[2,3], Justin M. Sacks, MD, MBA, FACS[1]

[1]Department of Plastic and Reconstructive Surgery, Johns Hopkins School of Medicine, Baltimore, MD, [2]Translational Tissue Engineering Center, Johns Hopkins School of Medicine, Baltimore, MD, [3]Department of Materials Science & Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, MD

PURPOSE: Develop a mechanically-tunable nanofiber-hydrogel composite to promote vascular ingrowth, survival, and migration of transplanted human adipose-derived stem cells (hASCs) for soft-tissue regeneration. This composite material directly addresses the limited utility of current soft-tissue repair paradigms, including fat grafting and dermal fillers, which are limited to small-volume defects and transient-volume restoration, respectively.

METHODS: We developed a unique composite scaffold by interfacially bonding biodegradable poly (caprolactone) fibers with hyaluronic acid hydrogel, forming an integrated structure resembling the architecture and mechanical properties of adipose tissue. We optimized our composite for ability to promote hASC migration and vascularization *in vitro*. Using the optimized composite as a carrier, we subcutaneously delivered hASCs into rats to assess the effect of composite-mediated delivery on survival, adipose differentiation, and host-tissue integration of the transplanted cells.

RESULTS: Human ASCs migrated the longest distance within the composite compared to soft and medium hydrogel controls (203 vs. 122, and 0 μm; $P<0.05$). Within the soft hydrogel control and the composite, cultured ASCs exhibited vascular morphogenesis and organized to form multicellular tubular structures with branches and open luminal spaces. As shown in, composite exhibited the highest network density (total length of interconnected branches divided by total area, 16.4 vs. 12.4, and 2.1 mm/mm^2). In the rat model, we observed a significantly higher density of $RECA\text{-}1^{+}$ endothelial cells within our composite compared with controls. Additionally, composite-mediated hASC delivery yielded the highest degree of cell survival, spreading, and differentiation.

CONCLUSION: Our composite scaffold promotes angiogenesis and enables delivery of hASCs and tissue regeneration for treatment of soft tissue defects. This composite scaffold has the potential for wide application to improve soft-tissue restoration in the clinical setting.