Within the conduit, CSPGs incorporated into a nanofiber hydrogel form an interpenetrating network. Using a TMR rodent hindlimb model, we tested the effects of this device with and without CSPGs on neuroma formation.

**RESULTS:** The significant size mismatch at the coaptation site between the sciatic nerve and tibial branch to the lateral gastrocnemius muscle resulted in neuroma formation in the TMR group, while the use of the conduit resulted in tapered reinnervation of the sciatic nerve, demonstrating the effectiveness of this device in mechanically guiding axonal growth. Pain scores elicited by mechanical stimulation at the coaptation site were significantly lower in the CSPG-conduit group as compared to the Neuroma, TMR, and Empty Conduit groups (p<0.0001), suggesting successful prevention of neuroma formation.

**CONCLUSION:** This novel bioengineered conduit presents a biologically compatible, readily translatable means by which we could optimize TMR postoperative outcomes.

**P19. INHIBITION OF MICRORNA 126 LEADS TO COMPLETE REGRESSION OF HEMANGIOENDOTHELIOMA TUMORS IN MICE**

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**PURPOSE:** We have previously shown that urinary microRNA126 (miR126) levels are a biomarker for hemangioma growth based on a prospective longitudinal study of children with hemangiomas and healthy age matched controls. We sought to determine the significance of miR126 on the regulation of hemangioma growth using a validated mouse model.

**METHODS:** qPCR measurement of miR126 levels was done in EOMA cells and non-tumor forming endothelial cells. 6 weeks old female 129P/3 mice received a subcutaneous injection of 5x10⁶ EOMA cells. 4 days post injection tumors were visible and topical administration of control antagoniR (n=8) or miR126 antagoniR (n=15) delivered transcutaneously using tissue nanotransfection electroporation technique. Treatments were given twice weekly

**RESULTS:** EOMA cells had 8,000-fold increase in miR126 levels compared to non-tumor forming endothelial cells. Control mice had significantly larger tumors with 100% mortality by 17 days post EOMA cell injection. miR126 antagoniR treated mice were significantly different from controls with decreased mortality (40%), smaller tumor size, prolonged survival with the last mouse death at 29 days post-injection, complete tumor regression by 6 weeks (n=9) and no observed recurrence at 90 days.

**CONCLUSION:** These are the first results to demonstrate complete regression of hemangioendothelioma in response to miR26 inhibition. Elevation of miR126 levels in human hemangioma and murine hemangioendothelioma indicate shared mechanisms of growth. These findings demonstrate that miR126 is necessary for growth of both tumor types and represents a valid therapeutic target to treat hemangiomas.

**P20. CAN EXTENT OF ADIPOSE TISSUE WASHING IMPACT THE THERAPEUTIC EFFICACY OF AUTOLOGOUS SVF CELL THERAPIES?**

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**PURPOSE:** Adipose-derived SVF cell therapies have entered Phase 3 FDA testing. A major challenge for point-of-care autologous cell strategies is the inherent variability that exists when using primary cells derived from a variety of patients. Our central hypothesis is that 1) the extent of adipose tissue washing influences the number of blood derived cells (BDCs) in SVF preparations, and 2) BDCs within SVF preparations confer modifiable effects on SVF bioactivity.

**METHODS:** Lipo-harvested adipose tissue was divided into equal volumes. One sample was ‘washed’ by gravity decantation, one by agitation and sump drain irrigation. SVF cells were then isolated using identical methods and the resulting types and numbers of BDCs were characterized by flow cytometry (n=5). Separately, freshly isolated SVF cells and second passage ASCs were cultured in media containing RBCs or lysed RBCs for up to 7 days (n=4). Cell proliferation was evaluated and conditioned medium was collected on days 0, 3, and 7 and evaluated for RBC toxins using ELISA.
RESULTS: Tissue washing reduced RBCs nearly four-fold (p=0.004) and leukocytes nearly twofold (p=0.001) compared to decanted samples, while yielding higher Type 2 macrophages (p=0.01). SVF and ASC cell proliferation was higher (2.5-4x) in media with intact RBCs (p=0.001) and was inversely related to levels of free hemoglobin and hemin.

CONCLUSION: The extent of washing of adipose tissue prior to cell isolation can significantly impact the final cell composition of SVF therapies. These differences in composition can impact biological activity and may influence therapeutic efficacy.

P21. INCORPORATION OF DECELLULARIZED EXTRACELLULAR MATRIX IN 3D-PRINTED GRAPHENE-BASED SCAFFOLDS FOR TREATMENT OF VOLUMETRIC MUSCLE LOSS

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PURPOSE: Large scale injuries resulting in volumetric muscle loss (VML) reduce peripheral nerve functionality. 3D additive manufacturing processes provide an opportunity to develop patient-specific implants. Graphene has gained popularity in regenerating excitable tissues due to its conductive nature and suitable biocompatibility. The low bioactivity associated with graphene can be overcome using extracellular matrix (ECM) derived from decellularized tissue. For VML, decellularized muscle ECM (dECM) contains muscle-specific proteins and growth factors beneficial for tissue regeneration.

METHODS: Here, we present a 3D composite with mouse-pup dECM fabricated using bioink consisting of graphene and poly(lactide-co-glycolide) (PLGA). Using an extrusion-based system, graphene structures with and without dECM were printed under ambient conditions in four-layered stacks with strut sizes ranging between 125 - 250 µm in width.

RESULTS: A reduction in electrical conductivity from 286.4 S/m to 74.4 S/m was observed in graphene scaffolds containing 2.5% dECM (gECM). Zeta potential values were $-17.9 \pm 5.14$ mV in graphene scaffolds and $-22.7 \pm 5.76$ mV in gECM scaffolds. SEM images showed no differences in surface topography between the scaffold types. In vitro experiments showed graphene and gECM scaffolds capable of supporting glia and motor neurons. Both scaffold variants were effective in supporting muscle myoblast adhesion, alignment, viability, proliferation, and differentiation. Confocal imaging showed neural network interconnectivity in motor neurons seeded onto graphene scaffolds containing dECM.

CONCLUSION: These findings suggest graphene scaffolds containing dECM are capable of enhancing functional recovery following VML by promoting a neurogenic environment conducive to myoblast differentiation and myofiber maturation.

P22. SELECTIVE ACTIVATION OF RETINOID-X-RECEPTOR (RXR) ACTIVATES PROLIFERATION AND MIGRATION OF PRIMARY HUMAN LYMPHATIC ENDOTHELIAL CELLS

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PURPOSE: The lymphatic system facilitates interstitial fluid homeostasis while insufficiency triggered by infection or surgery leads to lymphedema. Previously, we showed that 9-cis retinoic acid treatment improves lymphatic regeneration. While we hypothesize that RXR is the critical receptor in lymphatic endothelial cells (LECs) responsible for pro-lymphangiogenic responses, this has not been proven. We show that RXR activation is critical in pro-lymphangiogenic responses in vitro by using bexarotene, an FDA approved 3rd generation retinoid that selectively binds RXR.

METHODS: Human primary lymphatic endothelial cells were treated with DMSO, 9-cis retinoic acid (positive control), or bexarotene in a low serum media (1% FBS) for 48 hours. Proliferation was measured with Cell Proliferation Reagent