accumulates increasing epigenetic changes from e18 and p30 (significant peaks = 88 vs. 545). Lastly, reciprocal transplantation of e16 fibroblasts into a p1 host and vice versa reveal a significant difference in collagen overlap (2.13% versus 24.18%) and morphologic changes suggestive of quiescence versus reactivity.

CONCLUSIONS: Our data suggest that fibroblast phenotype is highly cell intrinsic and based on the accumulation of epigenetic change. Epigenetic change correlates with the transition in healing phenotype, and localizes to promoter sequences. By using the CRISPR-Cas9 system in future experiments, we will delineate which genes associated with e18 open promoters are the master regulators of fibrosis. Intervention at these genes may allow for scarless healing in adults.

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35

Integrating Tissue Engineering Principles for Skeletal Regeneration of the Radius and Mandible in Translational Models

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PURPOSE: Vascularized bone flaps can successfully reconstruct large bony defects of the upper extremity and mandible, but these interventions have several limitations. Tissue engineering may offer alternative solutions but there is a paucity of translational work investigating large bony defect healing. Furthermore, several osteogenic biomolecules exist, but well-investigated molecules such as rhBMP-2 have concerning effects, including: exuberant bone formation, osteolysis and malignant degeneration. We recently reported that 3D-printed bioceramic scaffolds designed with osteoconductive geometries1 can regenerate vascularized bone at critical-sized mandibulectomies.2 We have also recently reported that adenosine A2A receptor ligation can facilitate robust osteogenesis as well as to BMP-2 by upregulating osteoblast proliferation and attenuating osteoclast activity.3 This study investigates the combined regenerative capacity of 3D-printed bioceramic scaffolds locally delivering Dipyridamole (DIPY), an adenosine A2A receptor indirect agonist, at critical-sized bony defects of the rabbit radius and mandible.

METHODS: Experimental group design is described. Critical-sized (~11mm) full-thickness bony defects were created in rabbit radii (n=20) and rabbit mandibular rami (n=15). Defects were replaced with 3D-printed bioceramic scaffolds designed through microCT imaging to fit and fill defects. Within the ramus defect, devices differed only in DIPY concentration. No activity restriction occurred post-operatively. At t=8 weeks, animals were euthanized. Bone regeneration was assessed within scaffold interstices with microCT/AMIRA 3D reconstruction software and non-decalcified histology. Mechanical properties assessed included reduced elastic modulus through nanoidentation. One-way ANOVA analysis was performed, significance at α=0.05.

RESULTS: Highly cellular and vascularized intramembranous-like bone healing was observed irrespective of anatomic site or scaffold treatment. Bone generation was seen only within scaffold porosity and no exuberant or ectopic bone formation was observed. Radii critical-sized defect negative controls failed to regenerate to any significant degree & was quantified at 12.12 ± 4.73% (significantly less than all groups, p<0.05). Bone regeneration were quantified at 23.87 ± 7.31% (uncoated), 30.21 ± 6.75% (100μM DIPY), and 41.81 ± 4.6% (1,000μM DIPY) of scaffold interstices. 1,000μM DIPY bone formation was significantly greater than uncoated group (p=0.001) and 100μM-coated group (p=0.02). Reduced elastic modulus values were not statistically different from native bone for all groups. Mandibular rami bone regeneration were quantified at 12.3 ± 8.3% (uncoated), 6.9 ± 8.3% (COL1) and 26.9 ± 10.7% (100μM-DIPY) (p<0.03 DIPY vs. control and p<0.01 DIPY vs. COL1).

CONCLUSIONS: Rapid, controlled, and defect-specific bone regeneration using 3D-printed bioceramic scaffolds
is feasible and enhanced with local A2AR activation. Dipyridamole and β-tricalcium phosphate have well-established safety profiles, making this regenerative approach highly translatable. Further studies are warranted. Acknowledgements: This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases 5R01AR068593-02 & 3R01AR068593-02S1 References:

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36

Neurotization Of A Tissue Engineered Muscle Repair Construct, Potential For Improved Functional Outcomes

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BACKGROUND: The traumatic loss of a large volume of skeletal muscle and associated functional debilitation is a significant clinical problem and currently available treatments to restore the functional deficit of volumetric muscle loss (VML) remains the transfer of pedicled or innervated free muscle flaps into the defect in conjunction with physical therapy. This approach leads to significant donor site morbidity by sacrificing existing neuro-muscular flaps instead of promoting regeneration of lost skeletal muscle. Engineered tissue constructs hold tremendous potential to achieve the muscle bulk and function needed for recovery. However, such approaches have been limited to the implantation of engineered muscle grafts with myocyte regeneration limited to the interface of the host skeletal muscle in animal models. Innervation and vascularization of regenerated tissue remains a significant hurdle in creating functional repairs of VML.

METHODS: Using a small animal model of neurotization, we investigated the ability of maintaining viability of a large donor motor nerve within a tissue engineered muscle repair (TEMR) construct that has previously shown the ability to promote functional regeneration in a skeletal muscle defect. TEMR constructs (muscle-derived myoblasts seeded on a bladder acellular matrix (BAM)) were fabricated, preconditioned with uniaxial mechanical strain and anchored to the gracilis muscles of Lewis rats. At the time of placement, the TEMR constructs were folded around the dissected and isolated femoral vascular pedicle. Additionally, the motor branch of the femoral nerve was sharply divided and the proximal stump was embedded into the construct. Animals were recovered and observed for 12 weeks post injury when the constructs were carefully excised, fixed and stained with hemotoxin and eosin (H&E) and markers for neural and vascular tissue.

RESULTS: The animals recovered fully from the procedure without a noticeable gait deficit. Histological and immunohistological analysis showed that the femoral nerve placed within the TEMR maintained its original diameter with organized NF200+ filaments and axons, in addition to neural filaments throughout the constructs. These filaments were closely associated with support cells. Additionally, the samples exhibited regions of skeletal muscle within the constructs. A robust cellular integration was seen, including the presence of regenerative M2 macrophages throughout the constructs, confirmed through macrophage staining targeting CD68 and CD163. There was also evidence that the vascular pedicle maintained patency and the construct was able to integrate around it with a robust microcirculation throughout the tissue. This was confirmed through CD31 (endothelial cell marker) and alpha smooth muscle actin (αSMA) staining.

CONCLUSIONS: This is the first study to evaluate the potential of neurotization of a TEMR construct as a means to promote more complete integration and functional recovery from VML. By demonstrating that a motor nerve used to neurotize the construct was able to maintain its size and architect along with newly forming neural and muscle tissue, provides the foundation for full neurotization of the TEMR through embedding the terminal end of a nerve, including the motor end plates. Subsequently, placing the neurotized TEMR into a volumetric muscle defect will provide the regenerative signaling needed for improved functional outcomes.

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