Tissue-Engineered Muscle Therapy Enhances Functional Recovery and Survival in Composite Lower Extremity Injury

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Purpose: OOpen fractures with soft tissue damage are often result in delayed/nonunions, poor limb function, chronic pain, and amputation. Advancements in extremity trauma care are hindered by the the inability to restore both powerful muscle and stable bone. In prior work, we have demonstrated that spatial patterning cues from nanoscale extracellular matrices modulate angiogenesis and skeletal muscle myogenesis. When patterned materials are transplanted into a severe muscle injury in mice, large volumetric muscle defects can be regenerated and reinnervated comparable to native tissue. We hypothesize the regenerative dependency of bone repair on mobilized muscle cells and secreted factors, and therefore that a muscle-focused regenerative strategy would support improved bone healing. Herein we describe the extension of topography mediated myogenesis from a purely muscle injury to a composite muscle/bone mouse injury model.

Methods: Aligned nanofibrillar collagen type I scaffolds were fabricated using a shear-based extrusion method and seeded with green fluorescent protein (GFP+) primary mouse muscle cells (predifferentiated into myotubes). A critical sized tibia/tibialis anterior composite injury model was created in the mouse lower limb with a 2-mm bone defect and 30% muscle ablation. Fractured tibias were stabilized with a custom 3D-printed curved bone fixation plate; patterned scaffolds were transplanted into the muscle defect site. Healing of muscle and bone assessments were histology and functionally assessed by (1) muscle physiology, (2) fluoroscopic modified radiographic union scale for tibia fractures (mRUST) scoring, and (3) bone volume ratio (bone volume per total volume) measurements.

Results: Tibialis anterior muscles receiving scaffolds demonstrated significantly greater muscle mass ($P \leq 0.001$, $N \geq 3$) and twofold increase in maximum isometric force ($P \leq 0.05$, $N = 3$, day 56) compared to muscles that were left untreated. However, decreased bone volume ($P < 0.05$) and bone mineral density ($P < 0.05$) of the bone defect adjacent to the muscle treatment was observed, indicating possible compensatory mechanisms between muscle and bone recovery. Of significant interest was an increased survival rate (improvement from 68% to 83%) and reduced limb morbidity (tissue necrosis, bone re-breakage, scabbing, reopening of wounds, etc) of animals that received muscle treatments.

Conclusion: Functional healing in muscle, improved survival, and reduced limb morbidity were achieved by transplanting a muscle scaffold into the tibialis anterior defect of a tbia/tibialis anterior composite injury. Our findings demonstrate a regenerative relationship between muscle healing on functional outcomes and highlights the restorative potential of our approach for the treatment of composite injuries.

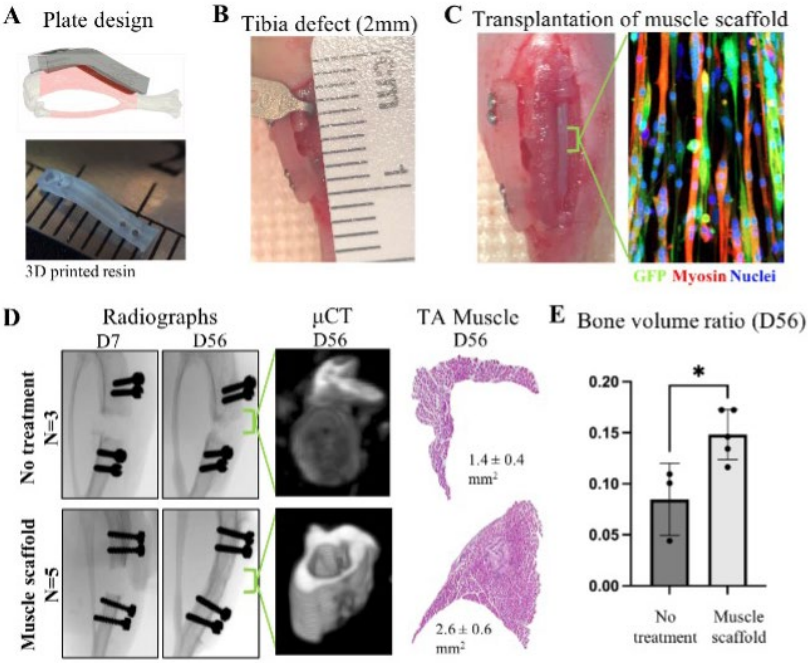

Figure 1. Bone fixation plate design and 3D printed plate (**A**); Tibia defect for the composite mouse injury (**B**); Transplantation of GFP+ primary mouse muscle cells on a patterned collagen scaffold into the muscle defect site of a composite injury (**C**); Bone and muscle healing in mice treated with the muscle scaffold versus no treatment (**D**); Bone volume ratio (BV/TV) of the defect region 56 days after initial injury of mice treated with the muscle scaffold versus no treatment (**E**).