Engineered Skeletal Muscle Enhances Bone Regeneration in Composite Injuries

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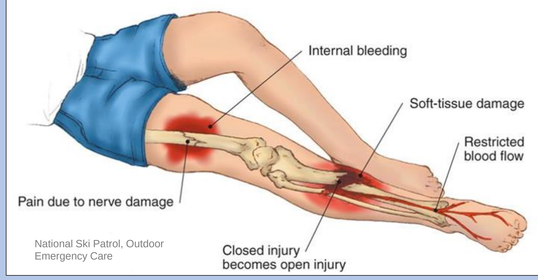
CLINICAL SIGNIFICANCE

- Composite injuries, bone fractures with parallel volumetric muscle loss (VML), are **4-5 times** more likely to result in delayed or failed bone healing.[1]
- **60%** of patients experience chronic disability[2]
- Current surgical interventions focus on bone repair rather than functional muscle recovery.



PREVIOUS WORK

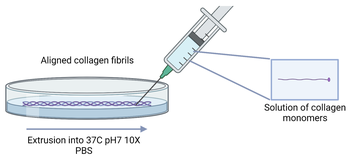


Figure 1. Fabrication of engineered scaffolds.

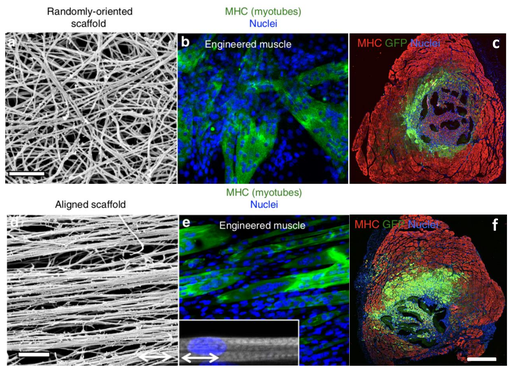


Figure 2. Scaffold topography improves skeletal muscle differentiation in vitro and in a VML mouse model. a,d Scanning electron microscopy images of scaffold topography. **b,d** Immunofluorescent images of muscle scaffolds. **c,f** Tissue cross sections of muscle transplanted with engineered muscle scaffolds. GFP (green) indicates cells originating from the scaffold.[3]

AIM

Determine if an engineered biomaterial for the regeneration of skeletal muscle will also improve bone regeneration

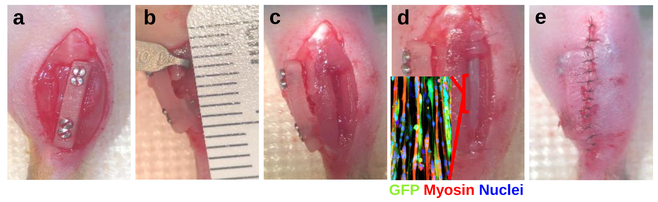


Figure 3. Mouse composite injury surgery. a. External fixation of the tibia with custom plate. **b.** 2mm tibia osteotomy **c.** 20% VML of the tibialis anterior (TA) muscle **d.** Transplantation of engineered muscle scaffold into VML with a representative immunofluorescent image of transplanted cells **e.** Muscle and skin closure**.** 56 days post-surgery bone repair was assessed using histology and microCT imaging. Muscle regeneration was evaluated using further histology and muscle physiology.

METHODS

Figure 5. Experimental workflow of engineered muscle conditioned media experiment. Skeletal muscle cell secretions were analyzed using a MILLIPLEX Mouse Myokine Magnetic Bead Panel. Bone cell maturation was assessed using a colorimetric alkaline phosphatase (ALP) assay.

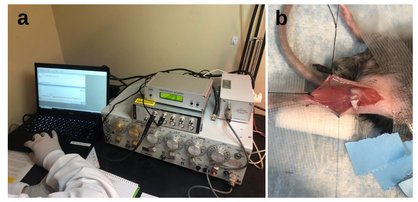
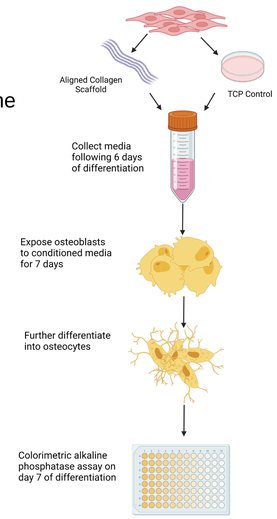
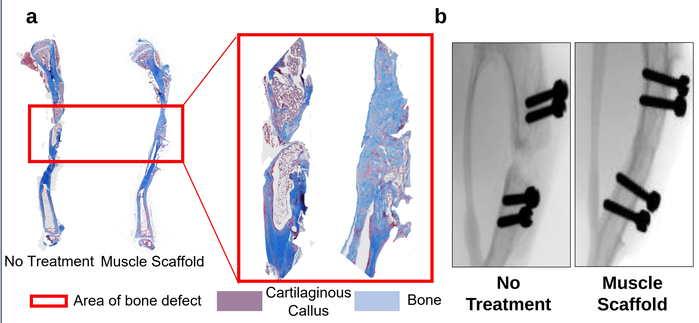


Figure 4. Muscle physiology set-up. a. The muscle stimulator and data collection using LabChart software **b.** The testing set-up in which the distal end of the TA muscle is attached to a force transducer. The muscle was stimulated using a nerve cuff attached to the stimulator.



RESULTS



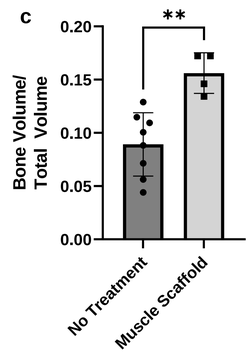


Figure 6. Transplantation of engineered muscle scaffolds into the TA muscle of a composite injury improves bone healing. a Masson's trichrome staining indicating the presence of collagen (blue) in mature bone. **b.** Representative radiographs 56 days post-injury. **c.** Ratios of bone volume to total volume calculated using microCT images.

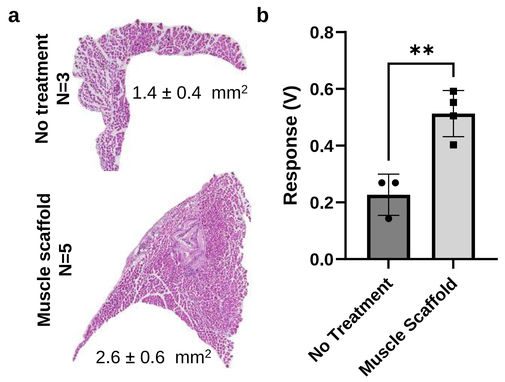


Figure 7. Treatment with an engineered muscle scaffold recovers TA size and function. a H&E staining of TA muscles. **b.** Muscle response calculated using muscle physiology.

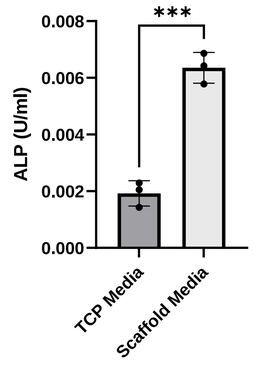


Figure 8. Conditioned media from engineered muscle scaffolds as opposed to tissue culture plastic (TCP) enhances ALP activity, an early marker of bone maturation.

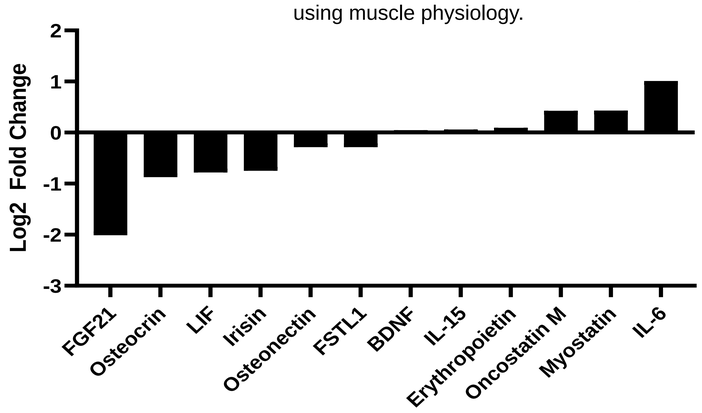


Figure 9. Engineered muscle scaffolds modulate their secretions of biological factors which support and oppose bone maturation

CONCLUSION

Engineered muscle scaffolds enhance the efficiency of skeletal muscle regeneration and through this enhancement, improve bone repair.

FUTURE WORK

Further determine a mechanism by which engineered muscle scaffolds improves bone repair through the analysis of blood serum collected from the composite injury model.

ACKNOWLEDGEMENTS

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Figures were created using Biorender.com

REFERENCES

1. Thakore et al. (2017) *Eur J Trauma Emerg Surg* **43**
2. Ruehle et al. (2019) *J. Orthop. Res* ***37***
3. Nakayama et al. (2019) *Nat Comms.* ***2***(170)