Engineering Biomimetic 3D Skeletal Muscle Architectures Using FRESH 3D Printed Collagen Scaffolds

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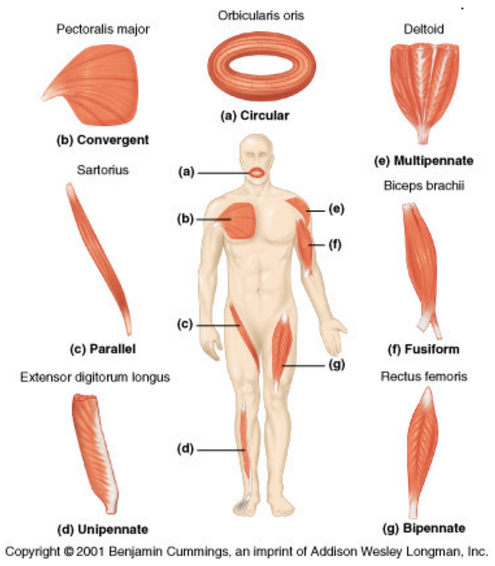
OVERVIEW

Motivation: Muscular diseases and injuries affect millions of individuals each year and can lead to significant impairment or loss of muscle function. Muscle tissue serves many critical functions, including protecting and supporting the skeleton, enabling locomotion, and maintaining homeostasis. There are a variety of muscle architectures that are present throughout the body. Muscle function is highly dependent on muscle architecture.

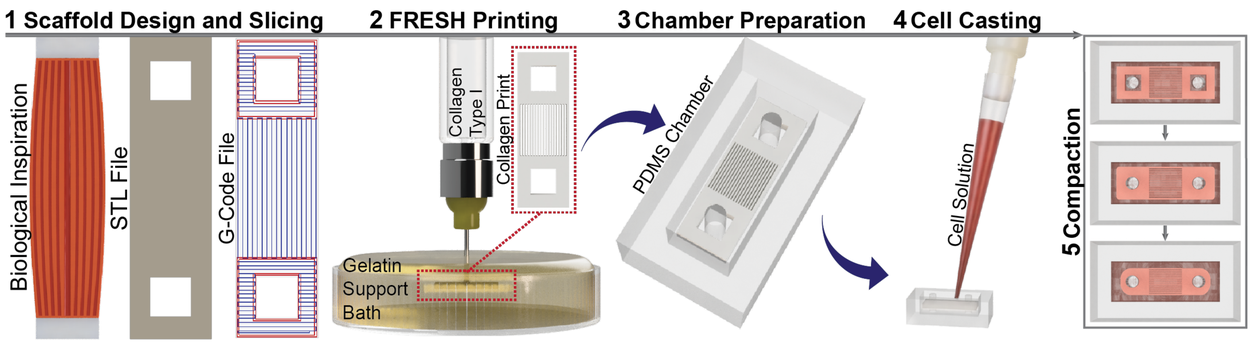


Current Therapies:

- Clinical: *autologous muscle transfer* faces complications such as donor site morbidity, infection, and/or necrosis, which can ultimately lead to complete graft failure
- Research: *transplantation of exogenous myoblasts* is only moderately successful
- **Engineered skeletal muscle tissue** is a potential solution to restore muscle function, but current tissue engineering approaches are not able to recapitulate the various native muscle architectures

Goal: Engineer skeletal muscle tissues with complex native-like architectures using Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting.

APPROACH



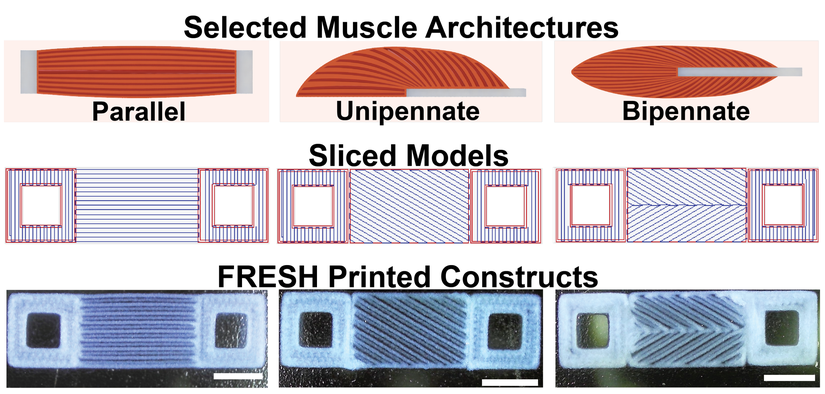
1. Print parameters (infill pattern, density, angle) were selected in slicing software to mimic native muscle architectures.
2. Acidified collagen type I was FRESH bioprinted into a pH-buffered bath to create scaffolds.
3. Scaffolds were transferred to sterile PDMS chambers, which were used to house tissues during cell culture.
4. C2C12 myoblasts were cast in a fibrinogen-thrombin solution around the collagen scaffold.
5. Over time, cell-mediated compaction drove cell infiltration into the collagen scaffold followed by differentiation into highly aligned muscle constructs.

RESULTS

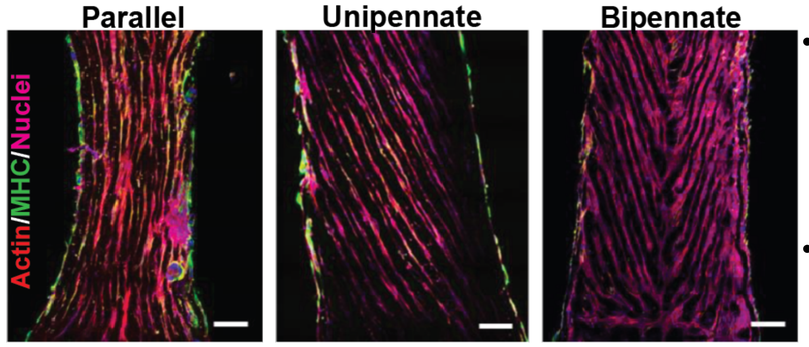
FRESH 3D Printing

Three muscle architectures were selected: **parallel**, **unipennate**, and **bipennate**.

Images demonstrate that scaffolds can be FRESH bioprinted with high fidelity (scale bars: 2 mm).



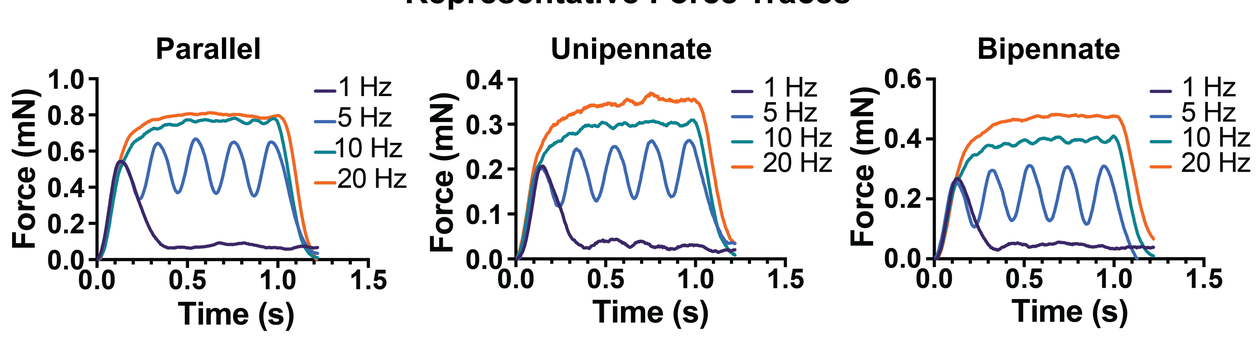
Immunofluorescent Images Reveal Cell Alignment to Scaffold Architecture



- Engineered muscle tissues were stained at day 5+14 for actin (red), myosin heavy chain (MHC) (green), and nuclei (magenta)
- Cells were aligned along the collagen filaments and fused into multinucleated myotubes

Scale bars: 500 μm

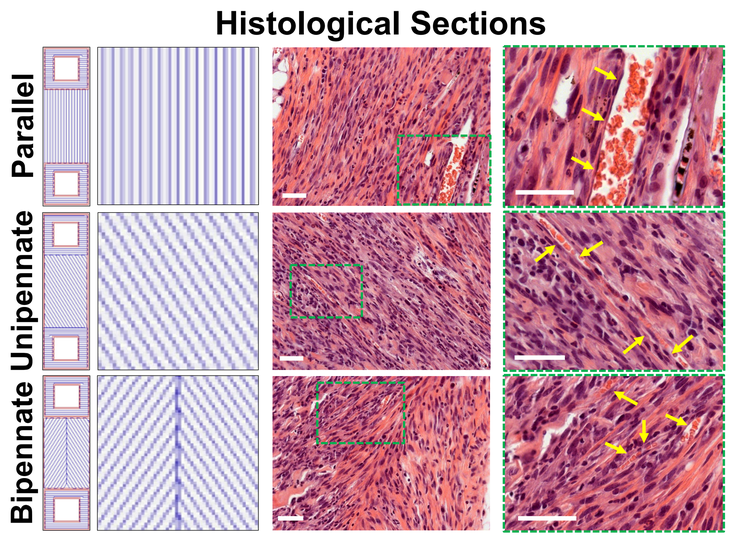
Tissues Generate Contractions with a Positive Force-Frequency Relationship



- Engineered muscle tissues under field stimulation displayed muscle contraction and demonstrated a positive force-frequency relationship
- Tissues approached tetanus at higher frequencies (~20 Hz)

Scaffold Architecture Can Direct Vascularization *In Vivo*

- Engineered muscle tissues were subcutaneously implanted into C3H mice for ten days
- Histological sections demonstrated that in select areas, vascular ingrowth occurred along printed collagen fibers (yellow arrows indicate red blood cells)
- Future work includes incorporating VEGF into the collagen ink to determine if this can induce widespread vascularization



Scale bars: 50 μm

SUMMARY

- FRESH 3D bioprinting can be utilized to create scaffolds mimicking a variety of native skeletal muscle architectures
- Scaffolds can direct myogenesis, creating highly aligned muscle tissues with parallel, unipennate, and bipennate myofiber architectures
- Tissues generate contractions with a positive force-frequency relationship and approach tetanus ~20 Hz
- Scaffold architecture may direct vascularization i*n vivo*

ACKNOWLEDGEMENTS

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