Abstract #41 (0346_0513_000053)

OPTIMIZATION OF THE PHYSICAL STRUCTURE AND CONTRACTILE PHENOTYPE OF A 3D BIOPRINTED MODEL OF SKELETAL MUSCLE

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Background:
For over 100 years, 2D cell culture has been used to study diseases despite poorly replicating *in vivo* pathologies. 3D bioprinted tissue promises to better replicate health and disease states, providing superior models for developing treatments. We have created a 3D bioprinted model of skeletal muscle for disease research, however the muscle develops enough force to tear individual fibers.

Objective:
We aimed to improve the physical stability and contractile phenotype of 3D bioprinted skeletal muscle by trialing fibroblast inclusion and fiber stiffening with fibrinogen.

Methods:
Control tissues comprised of C2C12 skeletal muscle myoblasts were bioprinted at 2.5×10^7 cells/mL in bioink containing 0.25% alginate, 1 mg/mL collagen and 5 mg/mL fibrinogen. GFP-3T3 fibroblasts were included at 0% to 20% of total cells. Fibrinogen was titrated across a range of 5 to 20 mg/mL. After bioprinting, tissues were treated with thrombin to crosslink fibrinogen, switched to growth media for three days, then differentiated for seven days. Tissue morphology was monitored by live-cell microscopy. On day ten, tissues were histologically stained for filamentous actin, and RNA isolated to determine gene expression of skeletal muscle phenotypic markers.

Results:
Fibroblast inclusion had no detrimental effect on tissue morphology, and fibroblast content remained qualitatively constant over ten days. However, fibroblast inclusion did not improve tissue structure nor contractile phenotype (NS). In contrast, fibrinogen content was positively correlated with improved myoblast differentiation. Tissues with 20mg/mL fibrinogen had visibly superior myotube formation and a two-fold increase in relative myosin heavy chain mRNA abundance ($p<0.05$).

Conclusion:
Stiffening of 3D bioprinted muscle with additional fibrinogen enhanced tissue structure and phenotype. Moving forward, antifibrinolytic treatments will be tested to determine whether additional tissue stabilization offers further phenotypic improvements. These developments are the first step towards developing a model of skeletal muscle that can better replicate health and disease states.