Steering Structural Remodelling in 3D-Engineered Skeletal Muscles *In Vitro* using Electro-Mechanical Stimuli Derived *In Vivo*

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Summary

Understanding skeletal muscle structural remodelling is key for developing personalized physical training and rehabilitation interventions. To complement this, we engineer skeletal muscle tissues (ESMT), from human-induced pluripotent stem cells, using a pillar-based system, integrating mechanical and electrical cues to mimic *in vivo* muscle behavior. This combined approach provides a platform for studying adaptation mechanisms and developing personalized rehabilitation and therapeutic strategies.

Introduction

Skeletal muscle loss in neuromuscular disorders severely impacts quality of life, highlighting the need for effective interventions. Studying muscle remodeling *in vivo* is challenging due to the need for lengthy experiments and the difficulty of obtaining data from a large number of subjects. As an alternative, ESMT offer a functional platform to test interventions and adaptation mechanisms, enabling the simultaneous analysis of multiple muscle tissues in shorter timeframes. By integrating *in vivo* and *in vitro* models, we aim to translate *in vivo* derived stimuli to the in vitro platform.

Methods

We assessed calf muscle adaptation using 3D ultrasonography to measure muscle volume and length. Muscle force and fatigability were evaluated using dynamometry and HD-electromyography based on a previous protocol [2].

ESMT were engineered as described [3] and cultured using a pillar-based platform originally designed for heart tissue [4]. This system enables functional analysis, as muscle force is derived from pillar deflection during contraction (Figure 1). Live-cell imaging was used to capture real-time tissue dynamics.

Results and Discussion

Preliminary results show that eccentric training significantly increased peak force of the gastrocnemius medialis ($p < 0.05$). Muscle fatigue resistance showed a subject-dependent trend, with some improving while others declined. Additionally, muscle volume and fascicle length increased.

We successfully formed tissues around the pillar-based platform, allowing functional testing and morphological assessment. We tracked passive force over two weeks and quantified compaction and growth phases (Figure 2). Ongoing work includes electrical pacing, with active force measurements to follow soon. The use of *in vivo* activation and strain data can further refine tissue stimulation protocols.

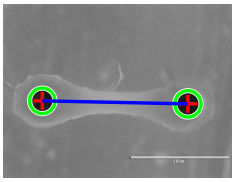

Figure 1: Representative tissue force analysis using bright-field imaging, where pillar deflection is measured by tracking black markers on the pillar tips.

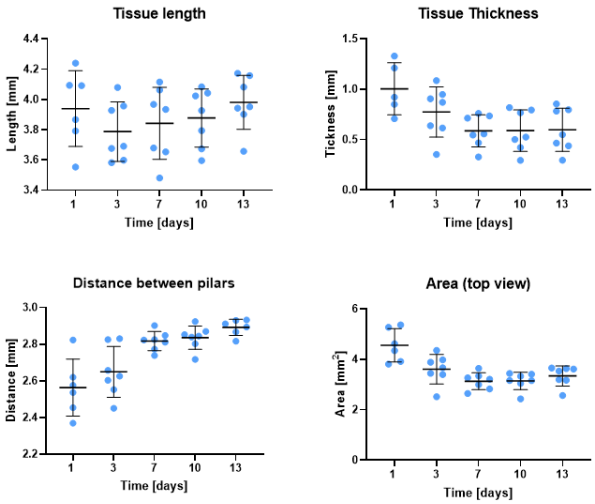

Figure 2: Preliminary pilot study results showing tissue compaction during the first week, plateauing thereafter due to the absence of electrical pacing.

Conclusions

Our findings highlight individual variability in muscle adaptation and the potential of ESMT for studying remodeling. Integrating *in vivo*-derived muscle activation and strain data into *in vitro* models can improve biomimetic tissue pacing and advance rehabilitation strategies.

Acknowledgments

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