

Stimulating 3D Skeletal Muscle Microtissues in a Novel Perfusable Microphysiological System with Integrated Electrodes

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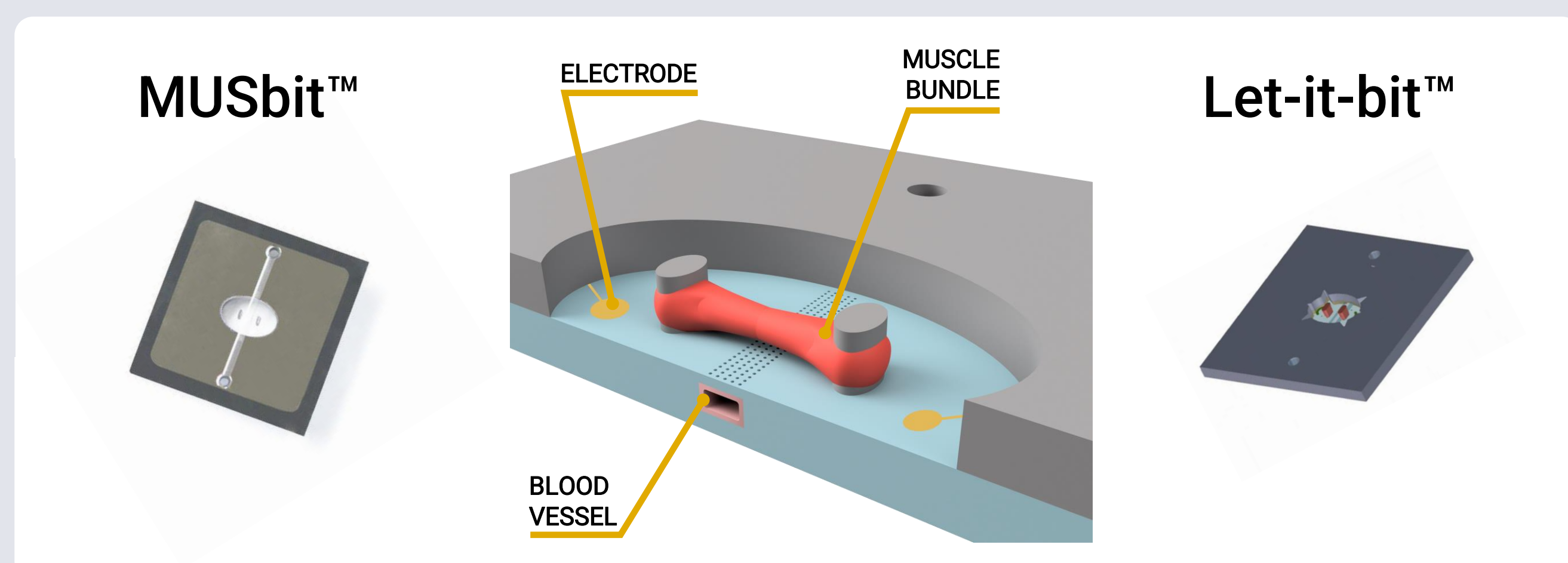
Introduction

Current OoC models for skeletal muscle tissues often require external electrodes to stimulate muscle tissue and lack perfusion for a steady influx of nutrients or pharmacological treatments.

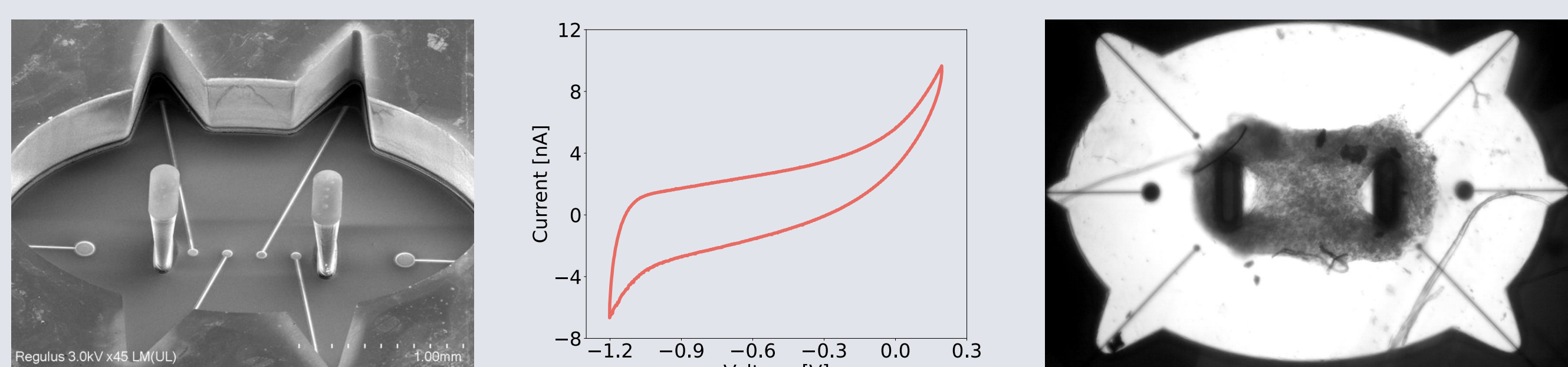
Here, we present two microphysiological systems for culturing 3D Engineered Muscle Microtissues: MUSbit™ and Let-it-bit™. Both microchips have pillars designed for anchoring myofibers and a microfluidic channel for perfusion. The Let-it-bit™ has an integrated microelectrode array for superior stimulation of 3D skeletal muscle tissue contraction *in situ*.

Microfluidic 3D Culture Solutions for Muscle Tissue

	MUSbit™	Let-it-bit™
• Pillar Design for 3D Engineered Muscle Tissue	✓	✓
• Microfluidic Channel for consistent and adjustable perfusion	✓	✓
• Integrated Electrodes for real-time stimulation and monitoring of cell contraction		✓



Functional Integrated Electrodes



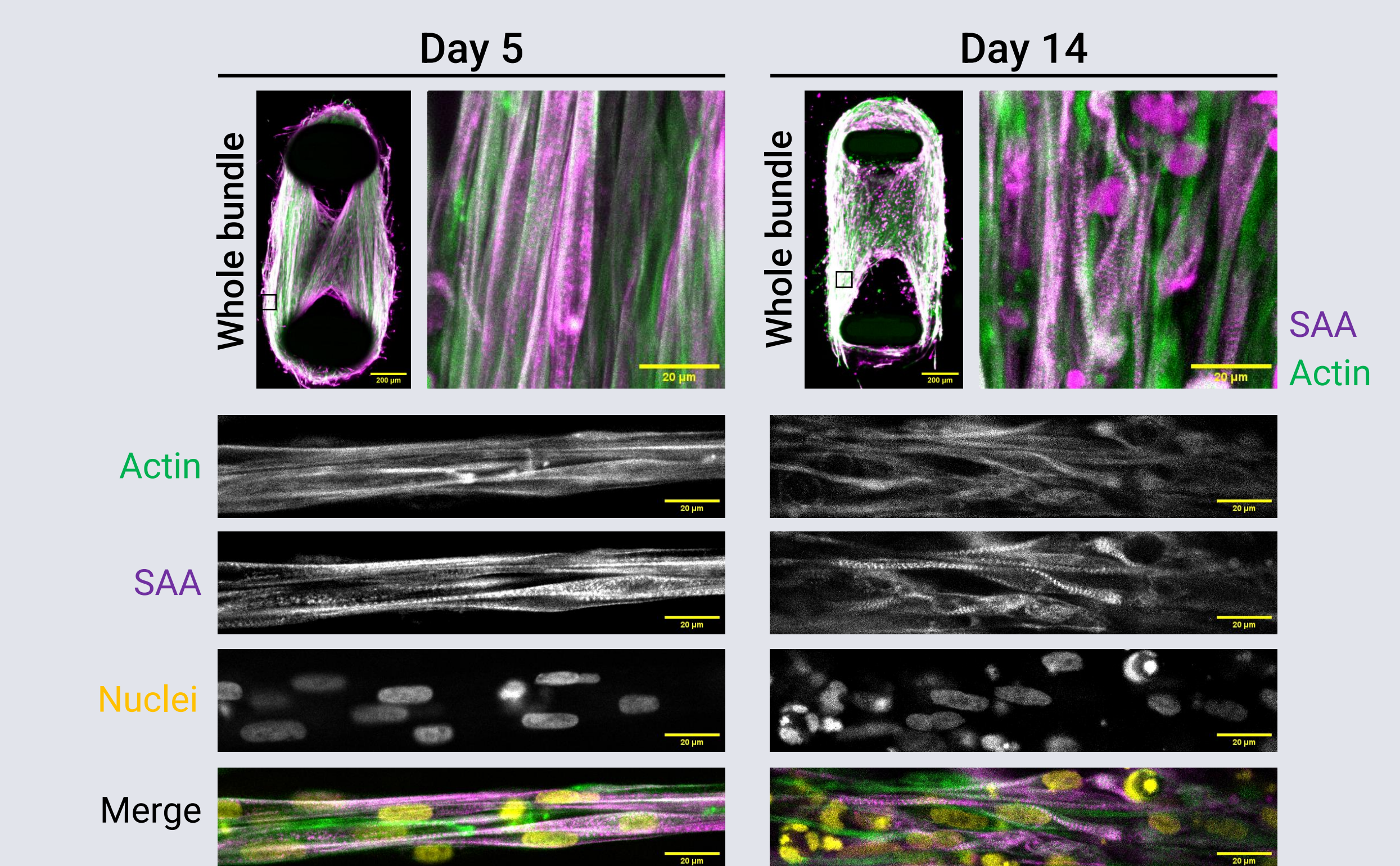
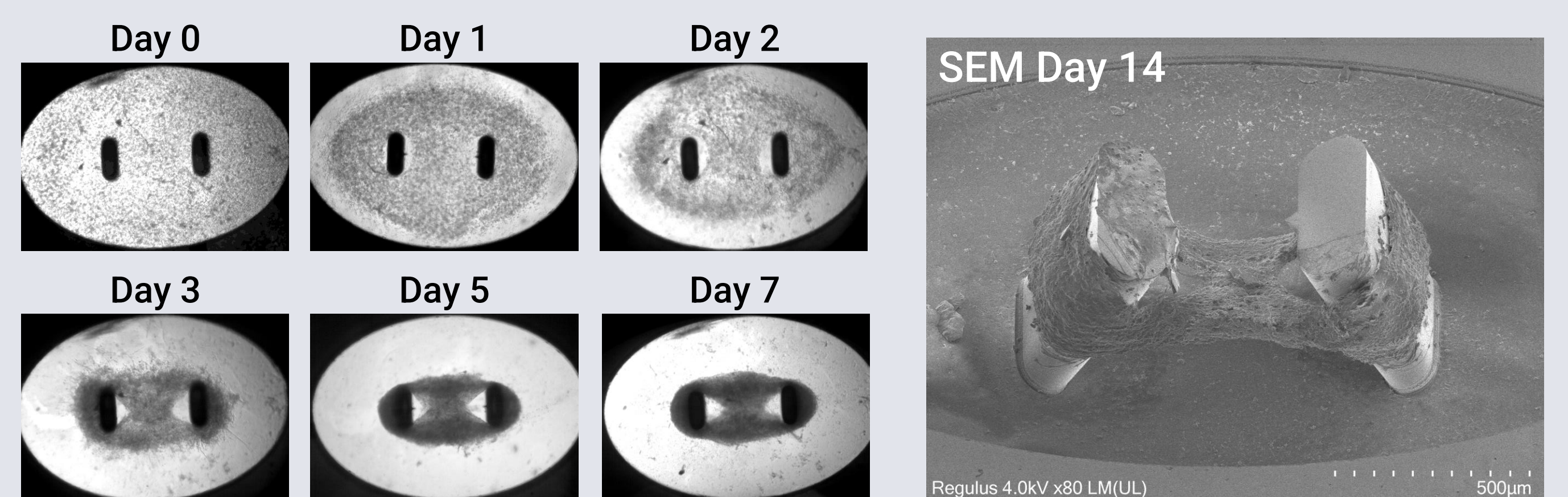
- Successful fabrication of integrated electrodes on chip
- Functional electrodes with continuity in CV measurement
- Working region sufficient for typical muscle stimulation
- Seeding of 3D Muscle Microtissue without breaking metal lines

Conclusions

- Integration of functional electrodes on chip
- Generation of Engineered 3D Skeletal Muscle Microtissues using ioSkeletal Myocytes™ on chip
- Capability for perfusion of muscle tissues
- Perfect for measuring muscle tissue contractile performance after pharmacological interventions
- Ideal for implementation of disease models

Mature 3D Skeletal Muscle on Chip

Successful muscle bundle formation in MUSbit™ microchip using ioSkeletal Myocytes™ (bit.bio)



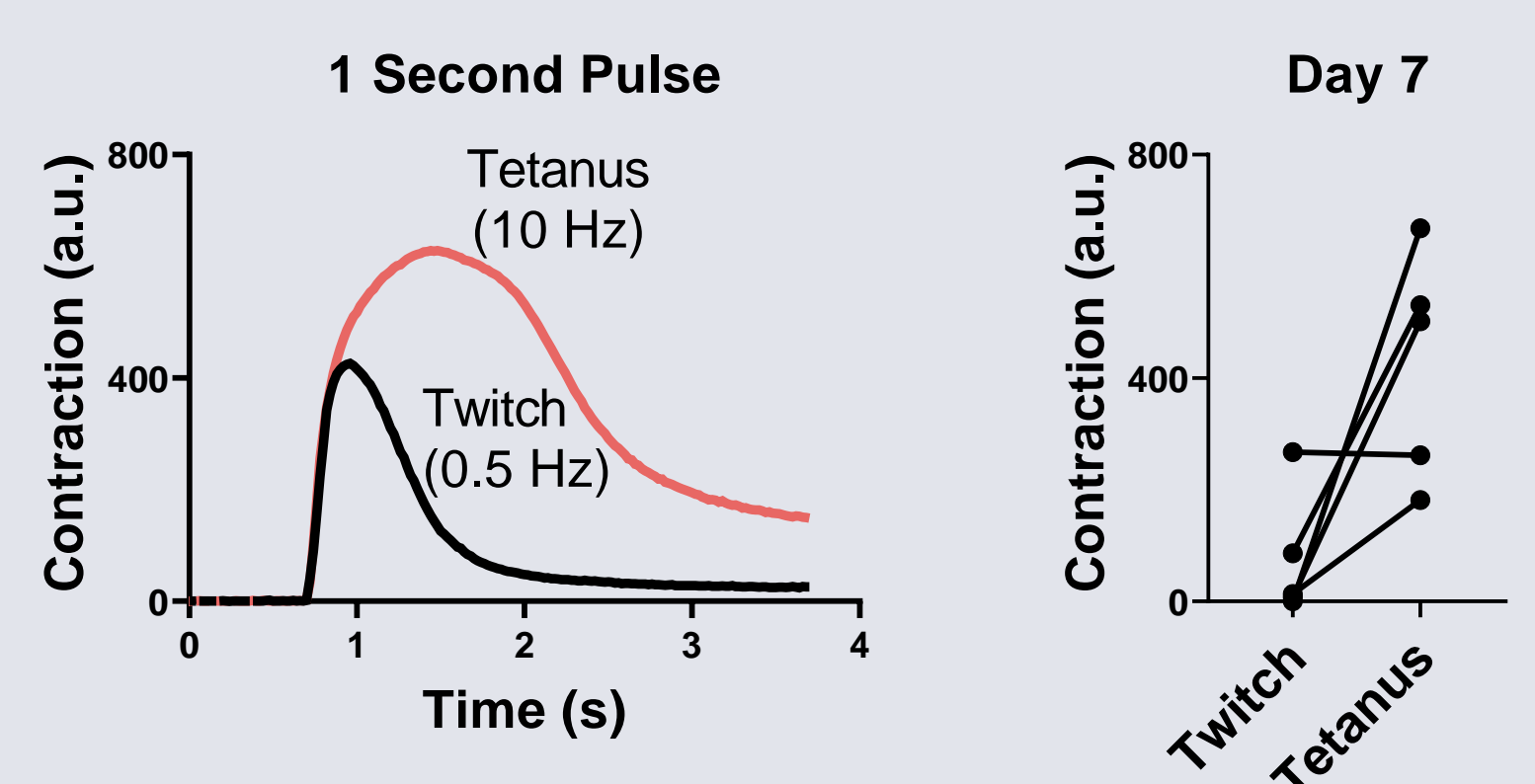
- Immunofluorescence staining of whole bundle at Day 5 and 14
- Robust expression of skeletal muscle marker sarcomeric alpha-actinin (SAA) and Actin
- More mature cross-striated SAA⁺ myofibers at Day 14 versus Day 5

Muscle Microtissue Contraction after Electrical Stimulation

Proof-of-concept: Stimulation of Engineered 3D Muscle Microtissues with external electrodes

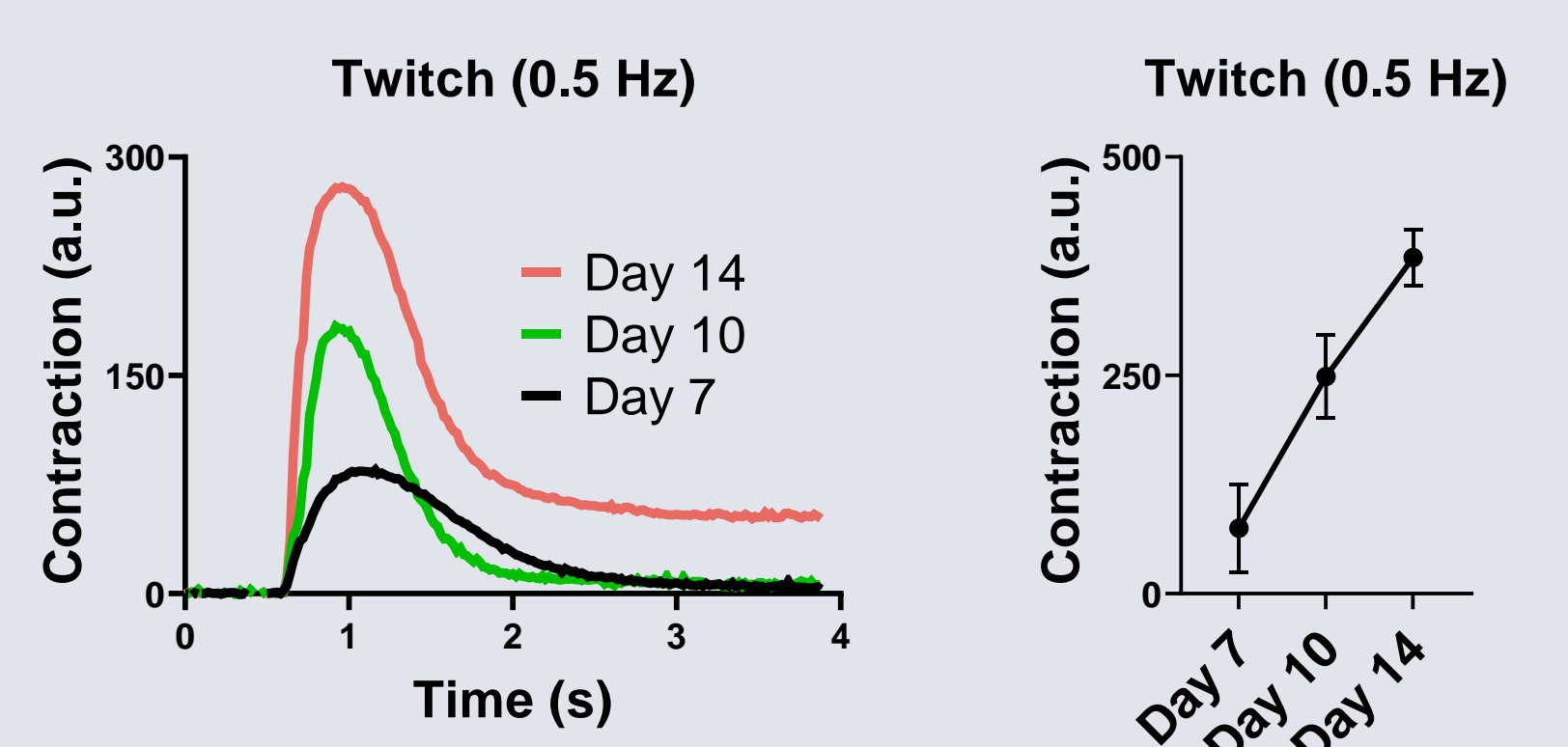
- Internally developed electrical stimulation device and software

Robust force-frequency relationship



- Microscopy-based contraction analysis (MUSCLEMOTION)*

Increased muscle performance over time



*Sala et al Circ Res. 2018 Feb 2;122(3):e5-e16

Acknowledgements

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