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In vitro maturation of contractile skeletal muscle fibrils in 3D polyacrylamide hydrogel microchannels



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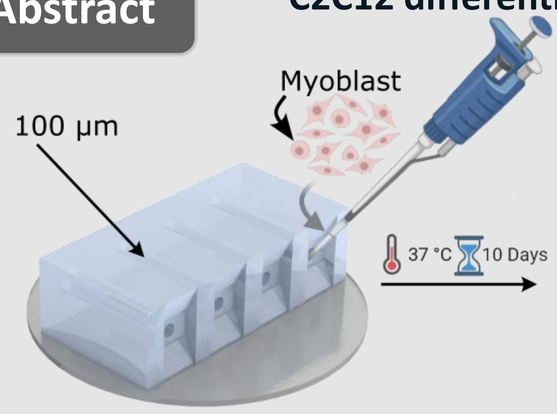
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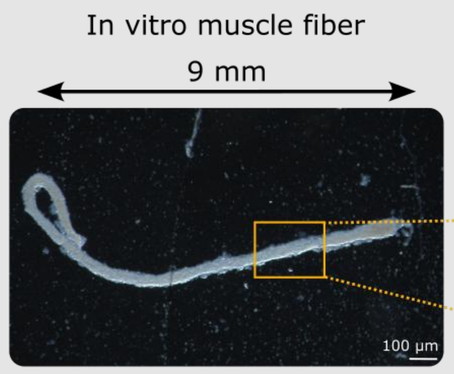
Abstract

Maturation of muscle fibers *in vitro* is highly attractive for diverse applications, such as reducing of animal testing or soft-robotic actuators. We performed myoblast maturation to a 3D environment promoting differentiation into contractile muscle fibers using 3D microchannels inside polyacrylamide hydrogels.

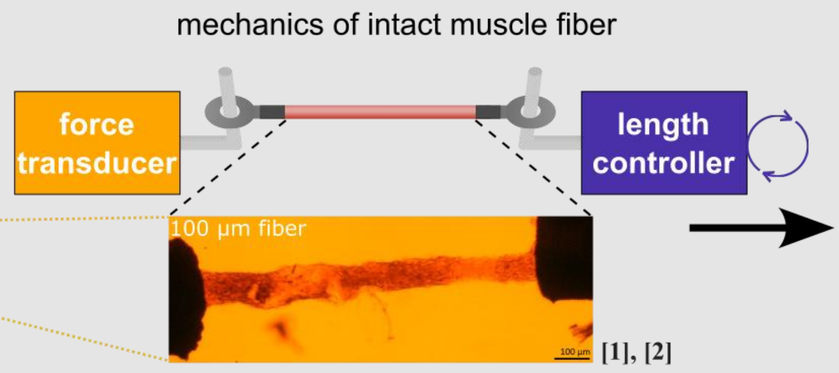
After 10 days matured myoblasts form muscle fibers, with an average diameter of 69.4 µm +/- 19.7 µm and a length of 9 mm. Tested fiber segments of 1 mm of these in vitro muscle fibers reached contractile force responses of 0.04 to 0.6 kPa under calcium activation in a custom tensile strength device.

C2C12 differentiation in micro-channels

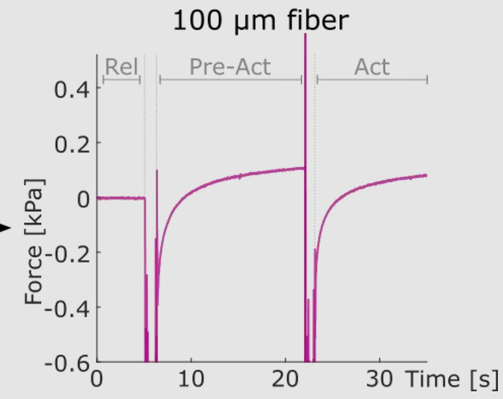




Mechanical measurements

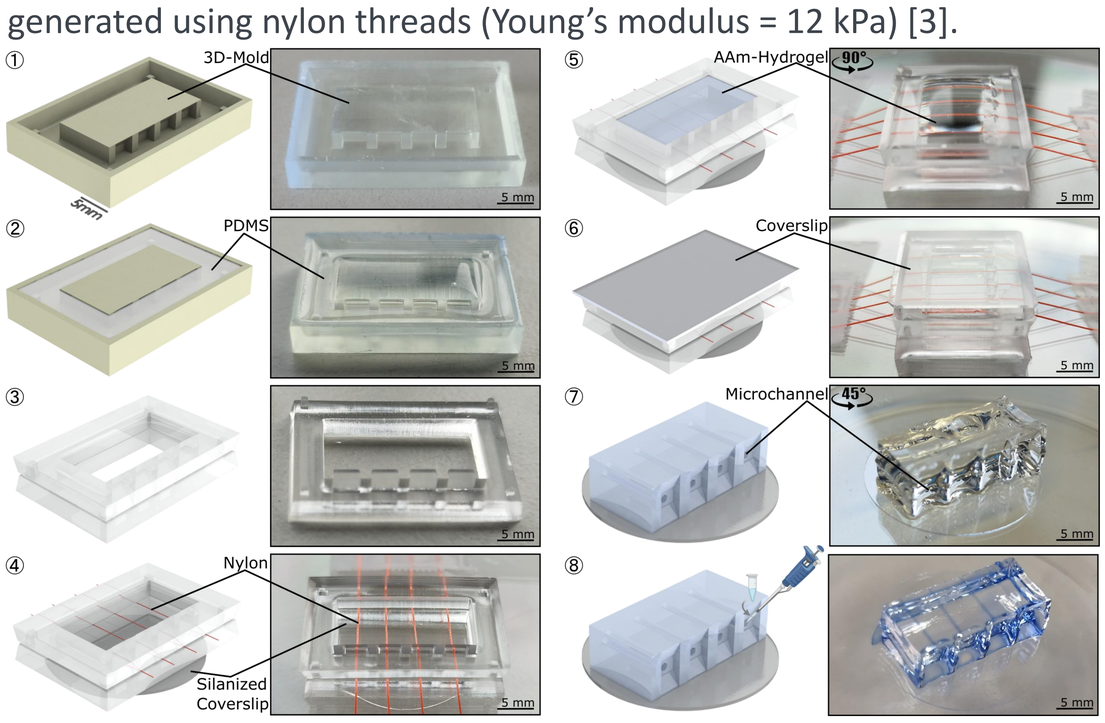


Force production



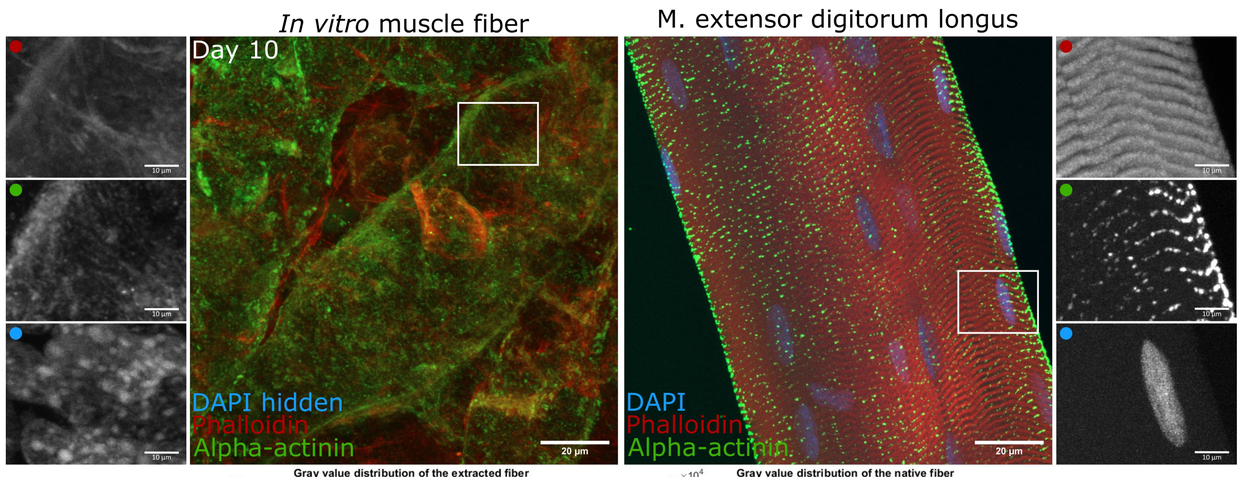
Production of hydrogel microchannels

Microscale channels (100–400 µm) embedded in a hydrogel were generated using nylon threads (Young's modulus = 12 kPa) [3].

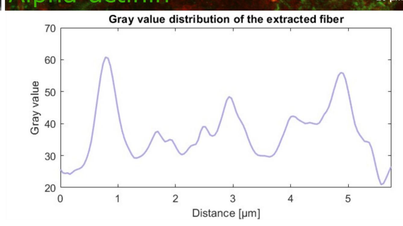


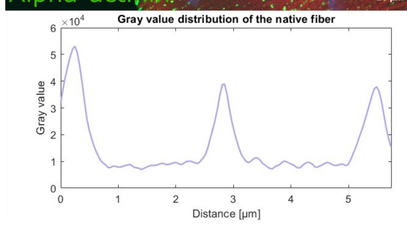
Sarcomeres in *in vitro* muscle fibers

Immunofluorescence staining of lab-grown muscle fibers, compared to native fibers, reveals sarcomere development during maturation.



100 µm fiber Gray values Day 10

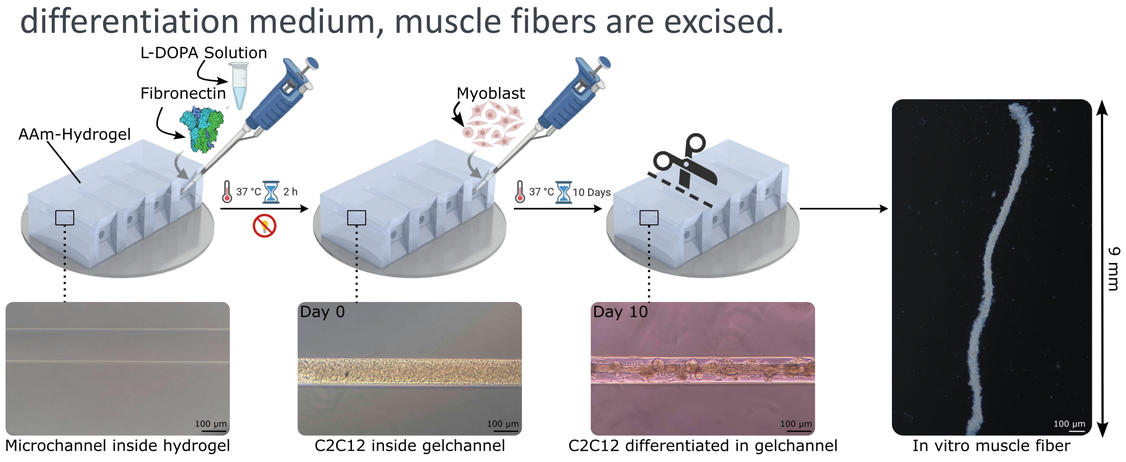




Native fiber Gray values

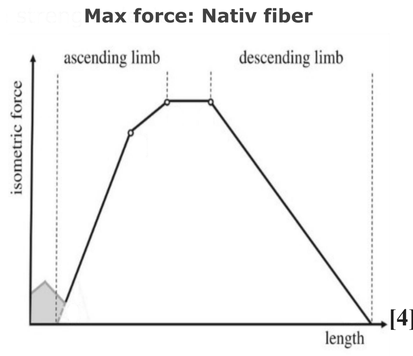
Differentiation of C2C12 to muscle fibers

Myoblasts are seeded into gel channels. After 10 days of maturation in differentiation medium, muscle fibers are excised.

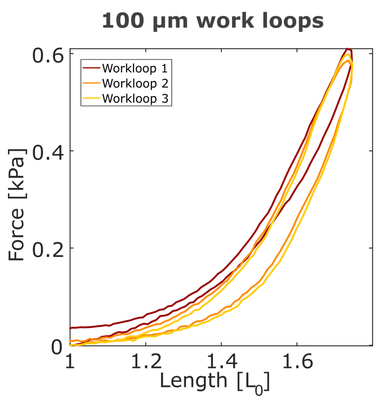


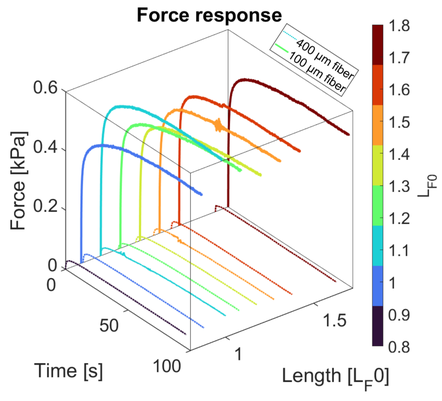
In vitro muscle fibers have contractile properties

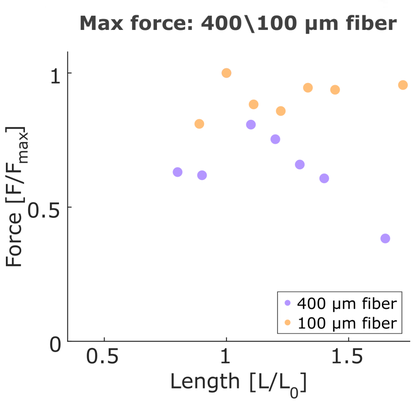
Calcium induced contractile force is significantly lower compared to native muscle fibers (67–94 kPa). In vitro muscle fibers exhibit force properties during work loops and a force–length relationship similar to native fibers.



[4]

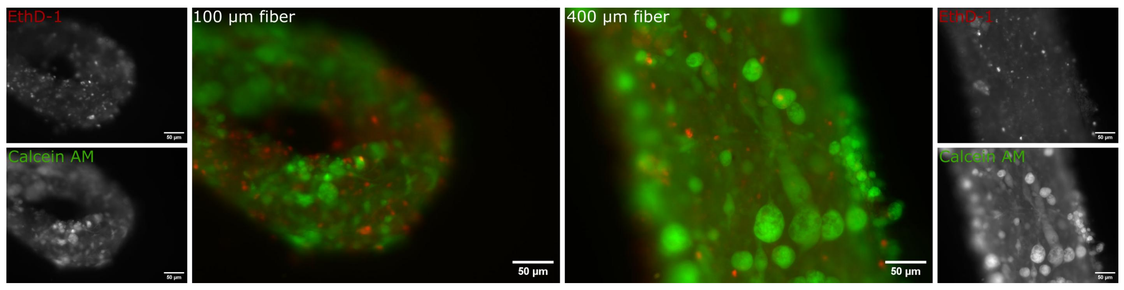






Live/Dead staining

Calcein AM is taken up by viable cells, resulting in green fluorescence.
EthD-1 stains dead cells, producing red fluorescence.



Outlook:

Improving the maturation of myoblasts by optogenetic stimulation strategies.

[1] Tomalka A, Weidner S, Hahn D, Seiberl W, Siebert T. Power Amplification Increases With Contraction Velocity During Stretch-Shortening Cycles of Skinned Muscle Fibers. Frontiers in Physiology. 2021 Mar;12.

[2] Kühn T, Tomalka A, Siebert T, Heymann M. Two-photon 3D-printed BSA hydrogel fibers resemble natice muscle contraction dynamics. Biofabrication. 2026

[3] Urciuolo A, Serena E, Ghua R, Zatti S, Giomo M, Mattei N, et al. Engineering a 3D in Vitro Model of Human Skeletal Muscle at the Single Fiber Scale. PLOS ONE. 2020 May;15(5):e0232081.

[4] Tomalka A, Rode C, Schumacher J, Siebert T. The Active Force–Length Relationship Is Invisible during Extensive EccentricContractions in Skinned Skeletal Muscle Fibres. Proceedings of the Royal Society B: Biological Sciences. 2017 May;284(1854):20162497.

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