Modeling Duchenne muscular dystrophy: development of a 3D printed pathological muscle-on-chip

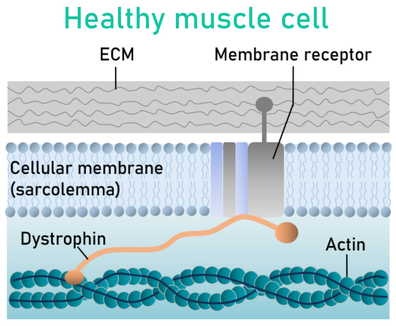
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Introduction

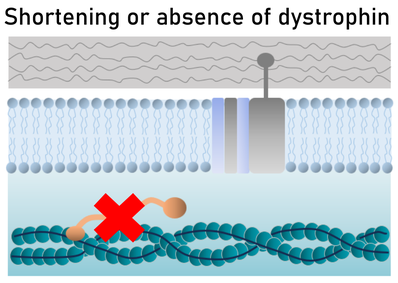
Duchenne muscular dystrophy (DMD) is a genetic disease causing muscle degeneration due to mutations on the gene coding for dystrophin. Consequently, the extracellular matrix (ECM) becomes fibrotic, with excessive type I collagen replacing functional muscle tissue.

Healthy muscle cell

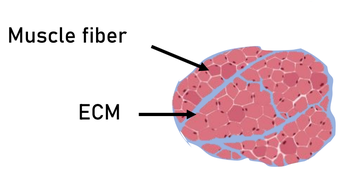


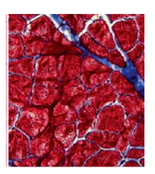
DMD muscle cell

Shortening or absence of dystrophin



Healthy ECM

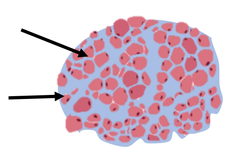


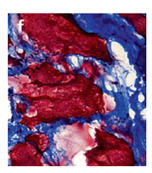


Duan et al., (2021)

Stiffness	Anisotropy	Porosity
10-20 kPa	+	Good diffusion of nutrients and oxygen

Fibrotic ECM





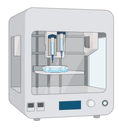
Duan et al., (2021)

Stiffness	Anisotropy	Porosity
30-100 kPa	++	Impaired diffusion of nutrients and oxygen

Existing models limitations

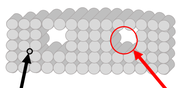
2D models	Do not recapitulate the 3D structure of ECM.
	No cells-ECM interactions.
Animal models	Species differences.
3D biomimetic models	Do not recapitulate the properties of fibrotic ECM.

Development of a model of fibrotic muscle ECM



3D printing of highly concentrated collagen in a 5× PBS bath

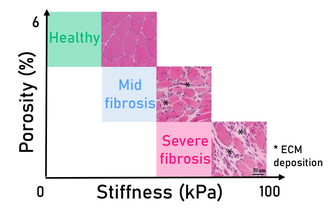
3D printed dense collagen hydrogel (30 mg/mL)



From healthy to fibrotic conditions → 2 parameters

1. Mimicking the altered perfusion by decreasing the intrinsic porosity
2. Mimicking the increased stiffness by chemical crosslinking of collagen

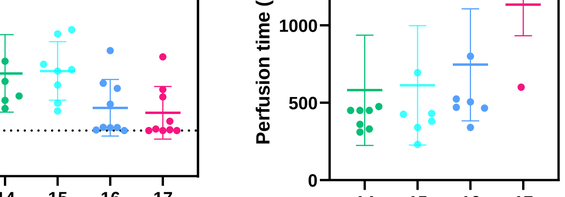
Independent control of stiffness and porosity allows to mimick different stages of fibrosis



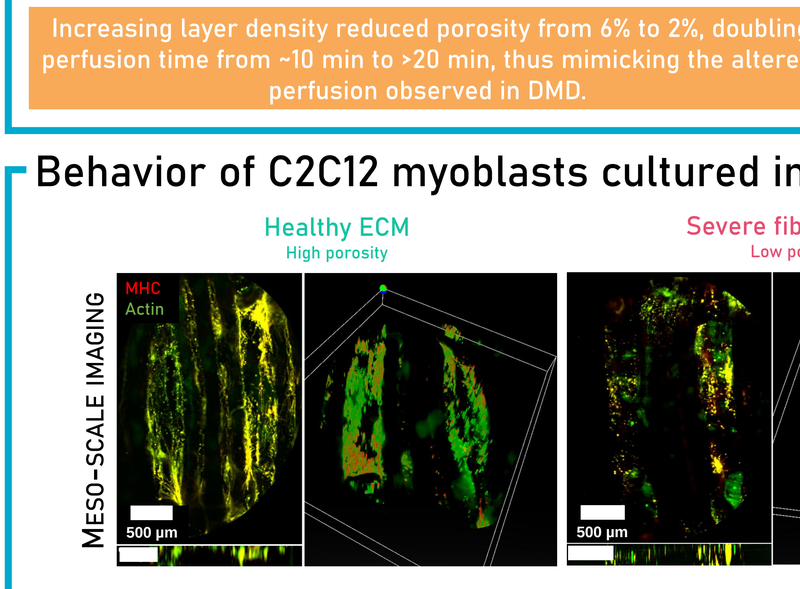
Mahdy at al., (2019)

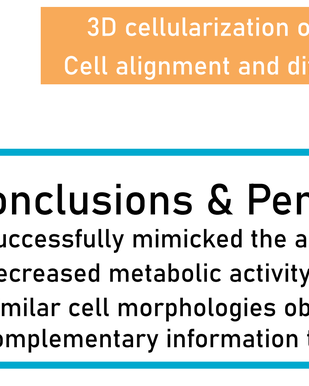
Mimicking the altered perfusion

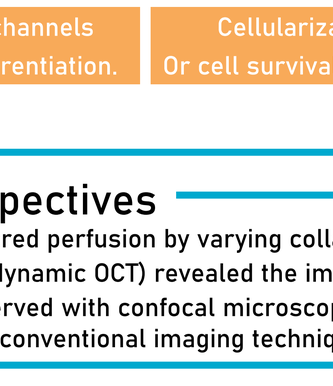
Increasing the number of collagen filaments per layer during 3D printing to decrease the intrinsic porosity



Correlation between porosity and perfusion using Optical Coherence Tomography (OCT)



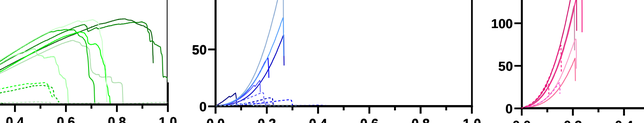




Increasing layer density reduced porosity from 6% to 2%, doubling perfusion time from ~10 min to >20 min, thus mimicking the altered perfusion observed in DMD.

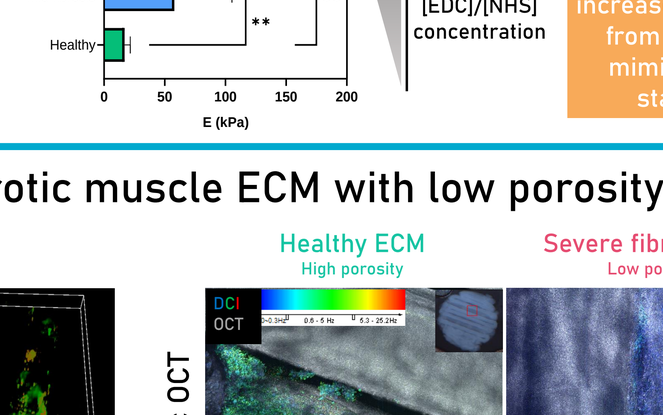
Mimicking the increased stiffness

Chemical cross-linking of collagen using EDC/NHS



Cytotoxicity – Live/Dead assay

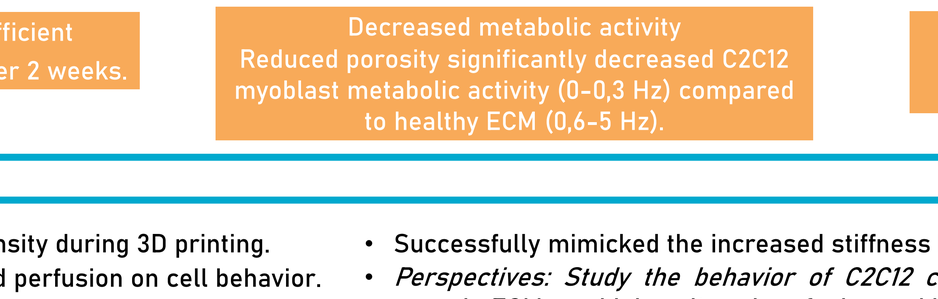
After 3 days of proliferation

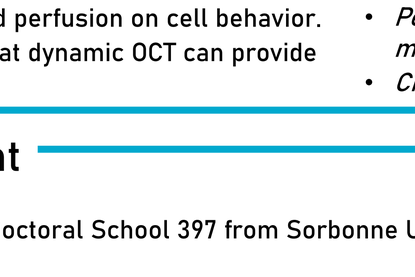


No cytotoxicity observed for C2C12 myoblasts.

Evaluation of mechanical properties by tensile testing

Matrix	Healthy	Mid fibrosis	Severe fibrosis
[EDC]/[NHS] (mg/mL)	0/0	2.5/0.6	5/1.2





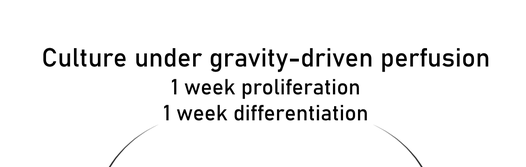
Increase of [EDC]/[NHS] concentration

By varying [EDC]/[NHS] concentrations, progressive increase of Young's modulus from 20 kPa to 130 kPa, mimicking progressive stages of fibrosis.

Cellularization setup

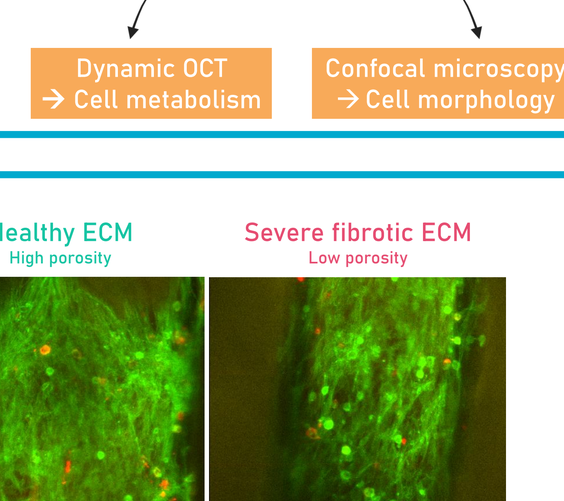
Cellularization channels – Sacrificial ink

Co-extrusion of collagen and a sacrificial ink (Laponite®) during 3D printing to create 600 µm channels.

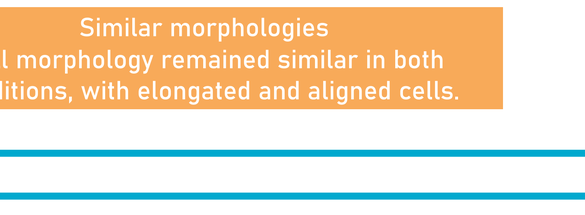


Laponite® can be eliminated by washing with distilled water.

Seeding of C2C12 into the channels



Microfluidic setup



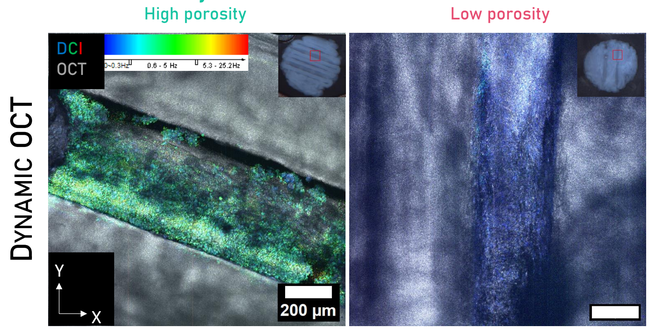
Culture under gravity-driven perfusion
1 week proliferation
1 week differentiation

- Dynamic OCT → Cell metabolism
- Confocal microscopy → Cell morphology

Behavior of C2C12 myoblasts cultured in a fibrotic muscle ECM with low porosity

3D cellularization of channels
Cell alignment and differentiation.

Cellularization is less efficient
Or cell survival impacted after 2 weeks.



Decreased metabolic activity
Reduced porosity significantly decreased C2C12 myoblast metabolic activity (0-0,3 Hz) compared to healthy ECM (0,6-5 Hz).

Similar morphologies
Cell morphology remained similar in both conditions, with elongated and aligned cells.

Conclusions & Perspectives

- Successfully mimicked the altered perfusion by varying collagen layer density during 3D printing.
- Decreased metabolic activity (dynamic OCT) revealed the impact of altered perfusion on cell behavior.
- Similar cell morphologies observed with confocal microscopy highlight that dynamic OCT can provide complementary information to conventional imaging techniques.
- Successfully mimicked the increased stiffness by chemical crosslinking of collagen with EDC/NHS.
- *Perspectives: Study the behavior of C2C12 cultured in muscle ECM with increased stiffness and muscle ECM combining altered perfusion and increased stiffness.*
- *Change the cell line for human myoblasts from healthy and DMD patients.*

Acknowledgement

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