Enhancing drug assessment for Duchenne muscular dystrophy using organ-on-a-chip technology and nanoplasmonic biosensing of myotube integrity

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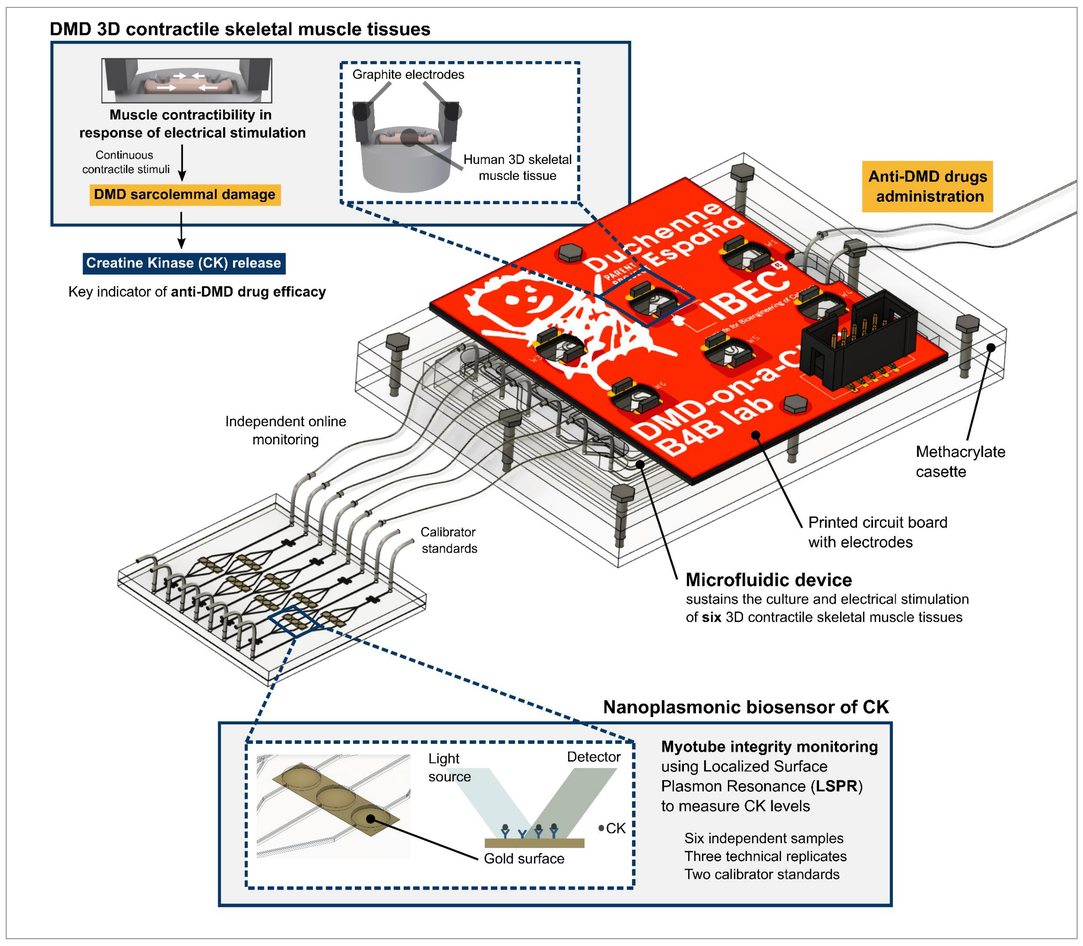
BACKGROUND

Duchenne muscular dystrophy (DMD) is characterized by a progressive degeneration of skeletal and cardiac muscles, caused by the lack of dystrophin protein. To date, there is no cure available for patients, even though there are several molecules in drug development. In this work, intending to accelerate drug development for DMD, we developed an innovative organ-on-a-chip (OOC) platform to faster evaluate anti-DMD treatment candidates. This OOC consists of a microfluidic device that sustains the culture and electrical stimulation of up to six patient-derived 3D functional skeletal muscle tissues. Moreover, it is connected to a plasmonic sensing device that allows the monitorization of myotube integrity, closely related with anti-DMD drugs effectiveness.

THE DMD-ON-A-CHIP PROJECT



Microfluidic platform

OOCs are microfluidic devices that combine bioengineered *in vitro* models on **continuously perfused, micrometer-sized chambers**. This muscle-on-a-chip device allows the electrical stimulation of up to six 3D skeletal muscle samples.

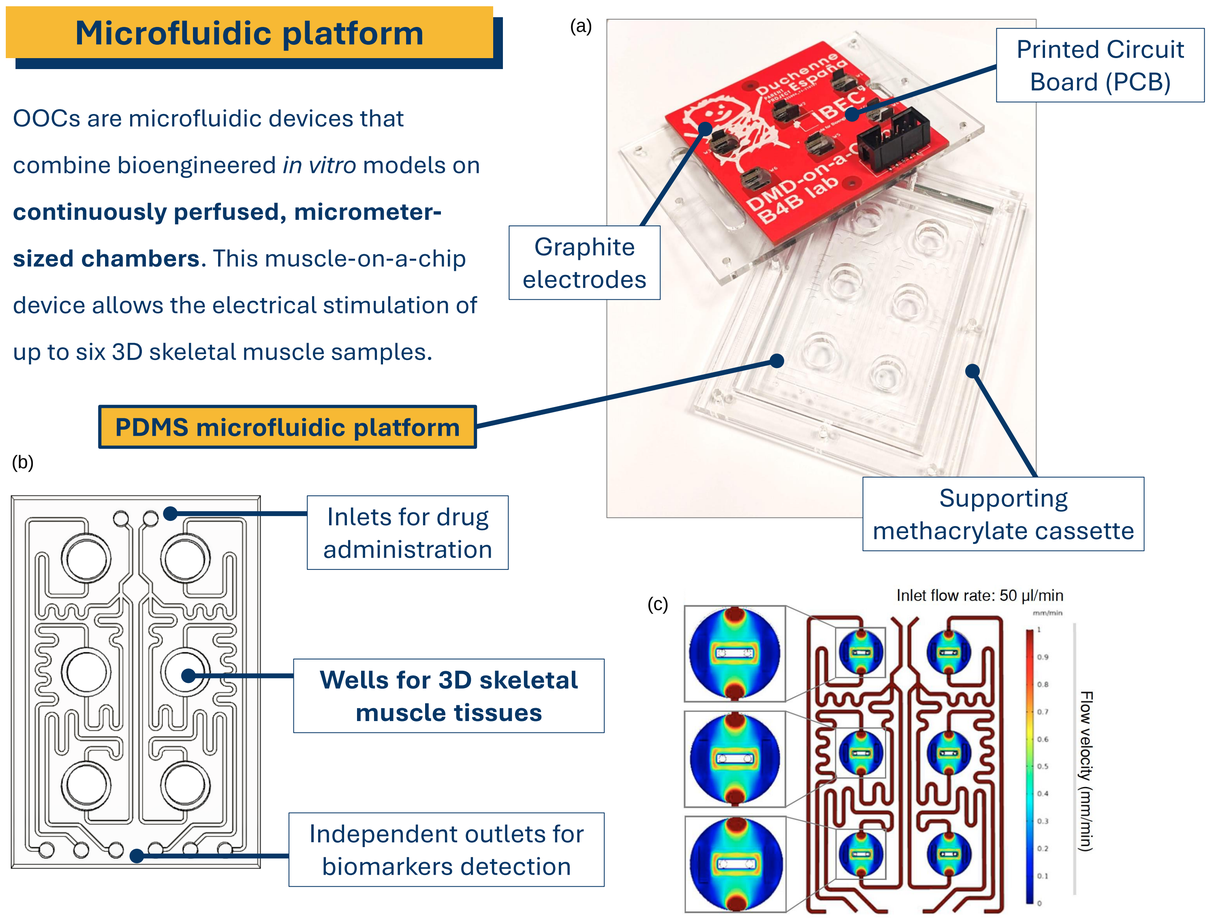
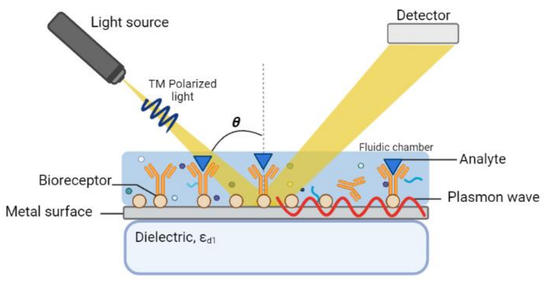


Figure 1. Design of the microfluidic culture platform. (a) Image of the muscle-on-a-chip (MoC) device. **(b)** Schematic of the microfluidic circuit. **(c)** Flow rate simulation within the wells.

Nanoplasmonic damage biosensor

Nanoplasmonic biosensors are optical sensor devices that explore light-matter interactions on metal structures. We use **localized surface plasmon resonance (SPR)** phenomena to fabricate **label-free** biosensors for **real-time detection** of **Creatine Kinase (CK)**, a muscle damage marker.



Nanoplasmonic biosensors perform **higher sensitivity** than standard sensing techniques

Limit of detection

ELISA	SPR
3.175 ng/ml	**0.153** ng/ml

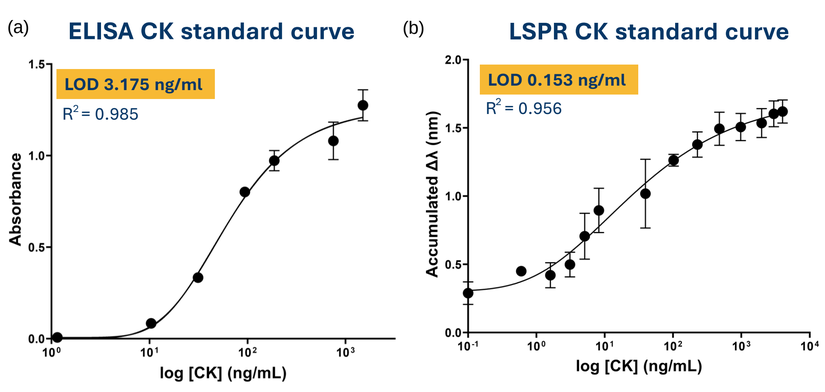
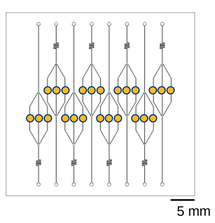


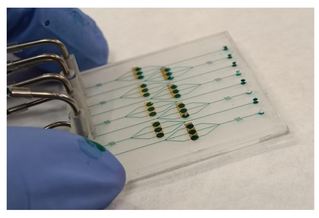
Figure 3. Comparison of traditional and novel techniques for Creatin kinase (CK) detection. Standard curve using **(a)** Enzyme-linked immunosorbent assay (ELISA) and **(b)** surface plasmon resonance (SPR).

Sensing microfluidic device



- **Inlets** connected to PDMS microfluidic device containing DMD muscle samples
- **24 sensing areas**
  - 6 muscle samples
  - 2 calibration standards

  x 3 technical replicates
- **Outlets** for waste



Real time measurements

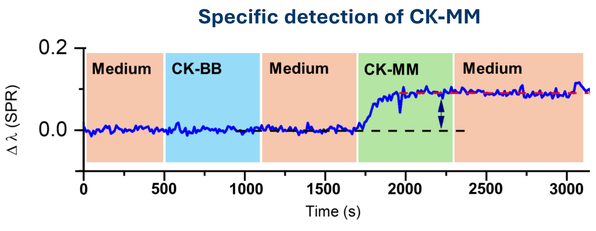


Figure 4. Sensogram showing real-time measurement of CK-MM isoform. Specific detection of skeletal muscle CK isoform CK-MM. Brain CK isoform CK-BB was not detected by the nanoplasmonic sensor

Contractile 3D DMD model

The use of human-based 3D cell culture methods aims to mimic the complex architecture of skeletal muscle. As a result, we achieve **functional** bioengineered 3D skeletal muscle cell cultures that can be **electrically stimulated** and that respond by contracting. This was essential to **generate sarcolemmal damage in DMD tissues**, showing functional fatigue-like phenotypes.

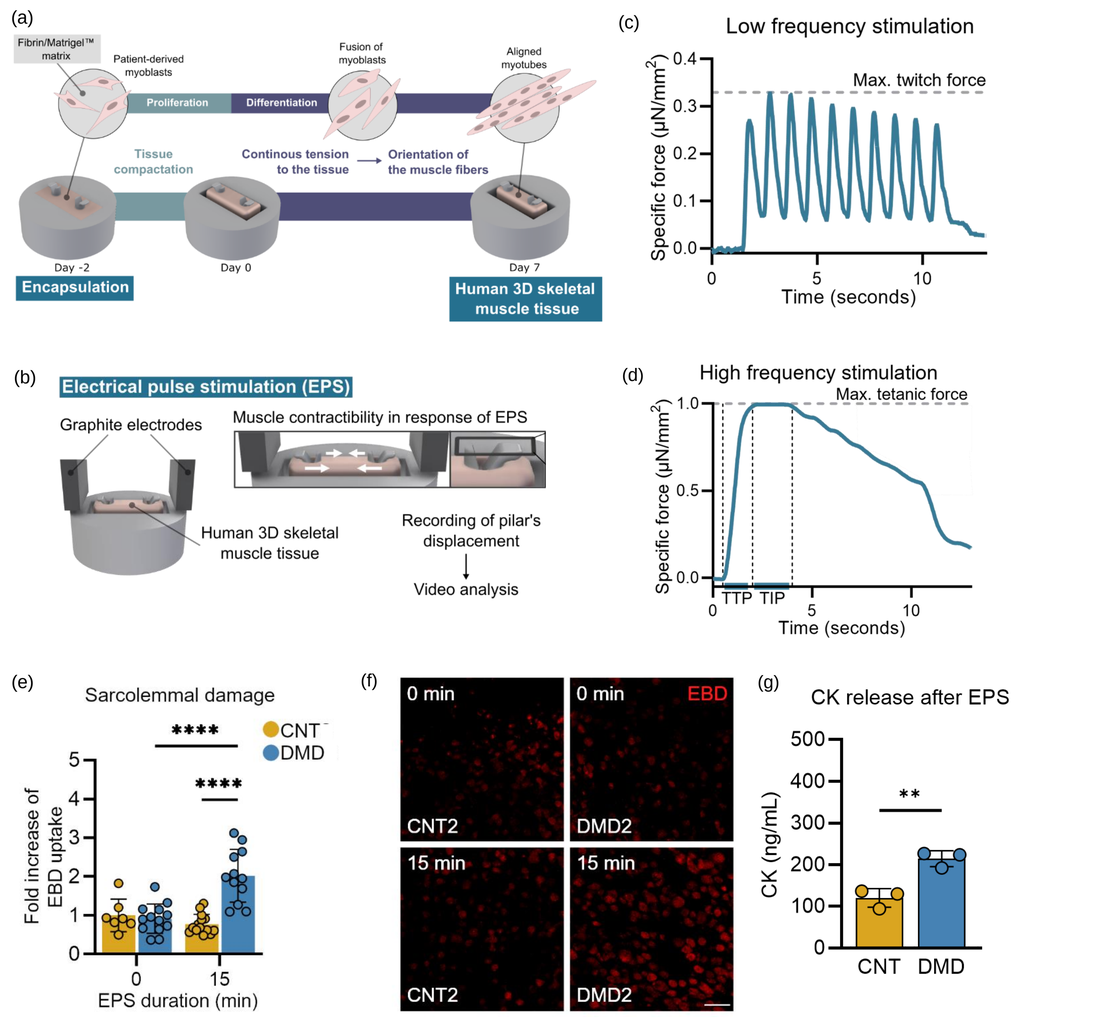


Figure 2. Functional DMD muscles 3D tissues are damaged after contraction. (a) Skeletal muscle tissues fabrication. **(b)** Electrical pulse stimulation (EPS) process. **(c, d)** Twitch and tetanic functional responses of 3D muscles after EPS. **(e, f)** Evans Blue Dye (EBD) uptake levels after 1 Hz EPS. **(g)** Creatine Kinase (CK) levels after EPS.

Check our article about the 3D DMD model!

Tejedera-Villafranca A, Montolio M, Ramón-Azcón J, Fernández-Costa JM. Mimicking sarcolemmal damage *in vitro*: a contractile 3D model of skeletal muscle for drug testing in Duchenne muscular dystrophy. Biofabrication. 2023 Sep 27;15(4).

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