A two-step mechanical stimulation approach for ECM-derived hydrogels: integrating strain-induced crosslinking and dynamic 3D culture to enhance skeletal muscle tissue properties

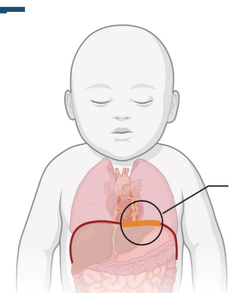
Elena Merotto[1,2], **Matteo Nifosì**[1,2], **Edoardo Maghin**[2,3], **Lucrezia Bettella**[1,2], **Silvia Todros**[1,2], **Martina Piccoli**[4] , **Piero Pavan**[1,2]

1. Department of Industrial Engineering, University of Padova, Italy. **2.** Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy. **3.** Department of Chemical and Farmaceutical Sciences, University of Trieste, Italy. **4.** Advanced Cellular Therapy Laboratory, Haematology Unit, AULSS 8 "Berica", Vicenza, Italy



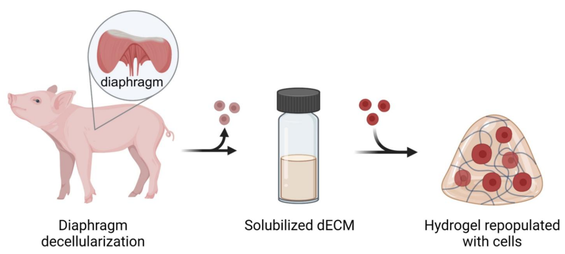
01 BACKGROUND

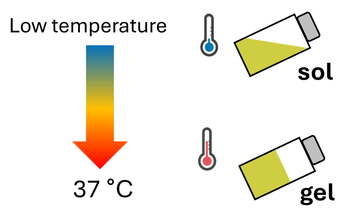
To overcome the failure of synthetic muscle repairs (for example in case of congenital diaphragmatic hernia), tissue engineering uses **natural hydrogels** derived from decellularized extracellular matrix (**dECM**). Since matching native muscle biomechanics is difficult, this research investigates how external **mechanical stimuli** affect hydrogel-based implants. By **stimulating the constructs during both reticulation and a ten-day culture period**, we aim to bridge the gap between artificial substitutes and physiological tissue. This method could promote maturation and build a scaffold that accurately reflects the mechanical and structural needs of skeletal muscle.



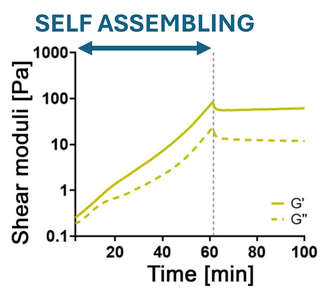
Congenital Diaphragmatic Hernia application example

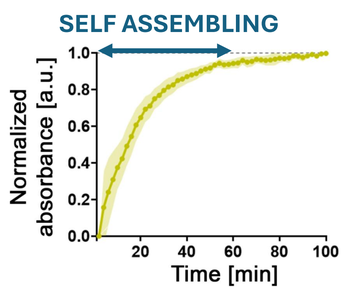
02 MATERIALS

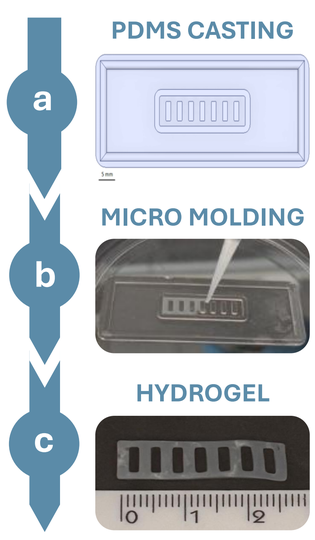




The material is completely and homogeneously **crosslinked and stable** after **90 minutes**



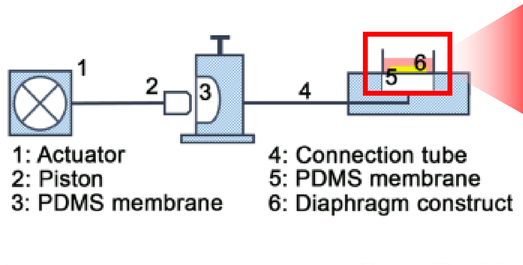


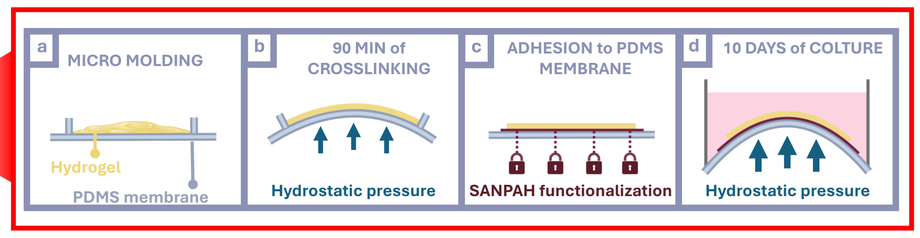


The dECM-derived hydrogel was prepared as previously described [1] and characterized via **rheometry and turbidimetry**. Rectangular patches were fabricated by casting the cell-seeded dECM-derived solution into CAD-designed polydimethylsiloxane (PDMS) moulds. The final constructs were then obtained through a **90-minute temperature-induced self-assembly process**, using a **micro-molding** technique to achieve the desired geometry.

03 METHODS

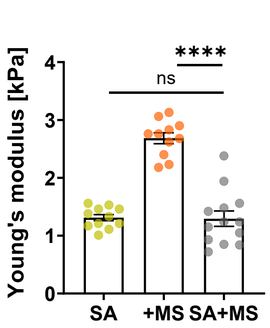
To enhance structural maturation, a **nearly-uniaxial mechanical strain** was applied during the 90-minute self-assembly phase **using a custom-developed bioreactor** [2], reaching a 7% of strain.

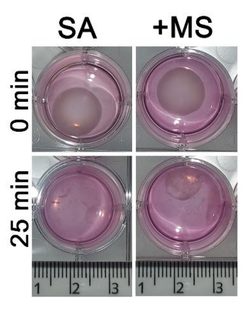


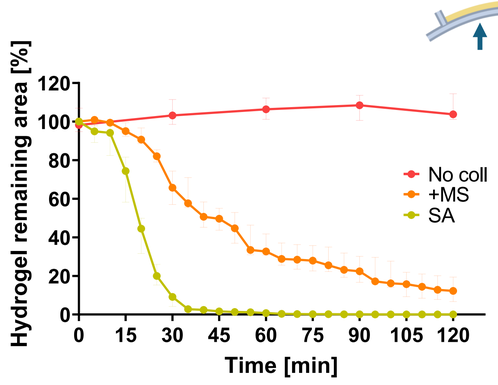


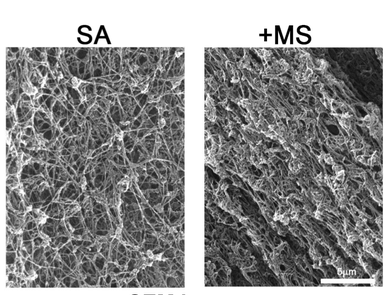
To ensure stability during culture, the PDMS surface was **functionalized with SANPAH**, preventing the constructs from floating. The patches then underwent **10 days of mechanical stimulation**, with an incremental daily strain of 3% to promote functional development and mimic a continuous stretching condition.

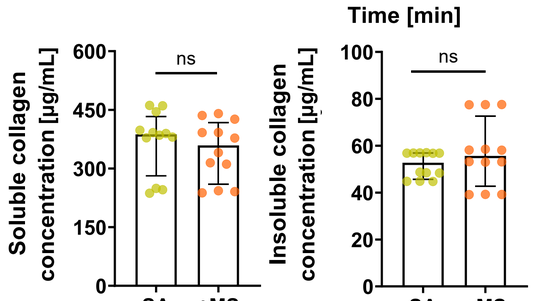
04 EFFECTS AFTER CROSSLINKING











Cell-enriched hydrogels were successfully fabricated, proving that mechanical strain during reticulation (**+MS**) is essential for **achieving muscle-like stiffness**. Compared to non-stimulated samples (**SA**), the results showed:

- **Timing**: stimulation after crosslinking (**SA+MS**) yielded no stiffness increase, confirming that **properties are set during the initial 90-minute assembly**.
- **Structure**: early strain **reduced enzymatic degradation** and **improved fiber alignment**, promoting a possible subsequent cell orientation.
- **Chemistry**: no significant differences in soluble and insoluble collagen quantification were found, indicating that conditioning improves **organization without altering the dECM's chemical nature** [3].

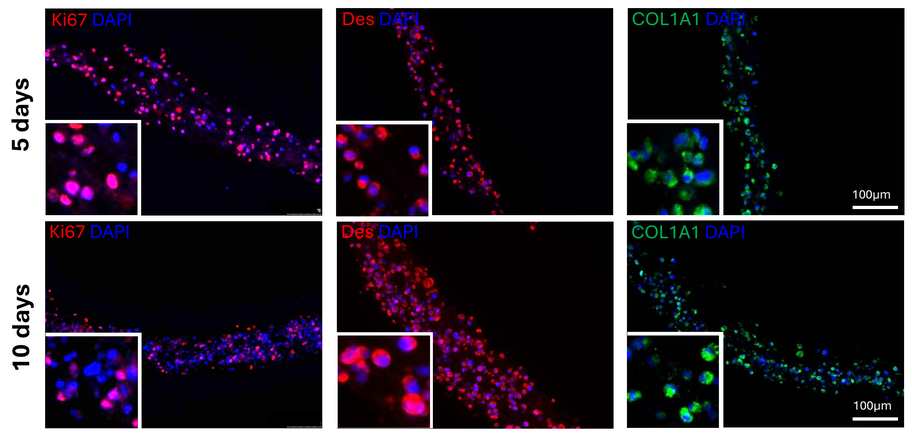
05 EFFECTS AFTER LONG COLTURE

Following the 90-minute stimulation, patches were bonded to PDMS and cultured under strain for 10 days, switching to fusion medium at day 5.

Findings include:

- **Active remodeling**: immunofluorescence showed decreased Ki67-positive cells and **increased Desmin (Des) and human Collagen I (COL1A1)**.
- **Alignment potential**: while nuclei are not yet elongated, active collagen synthesis suggests that extended culture could further enhance alignment.

This confirms cells are successfully maturing and remodeling the scaffold.



Contact me: elena.merotto@phd.unipd.it

REFERENCES

[1] [2] [3]