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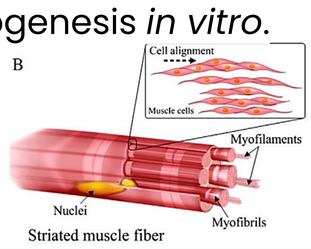
Fibrous hydrogels as bioinks for the 3D bioprinting of anisotropic scaffolds in hierarchically skeletal muscle engineering

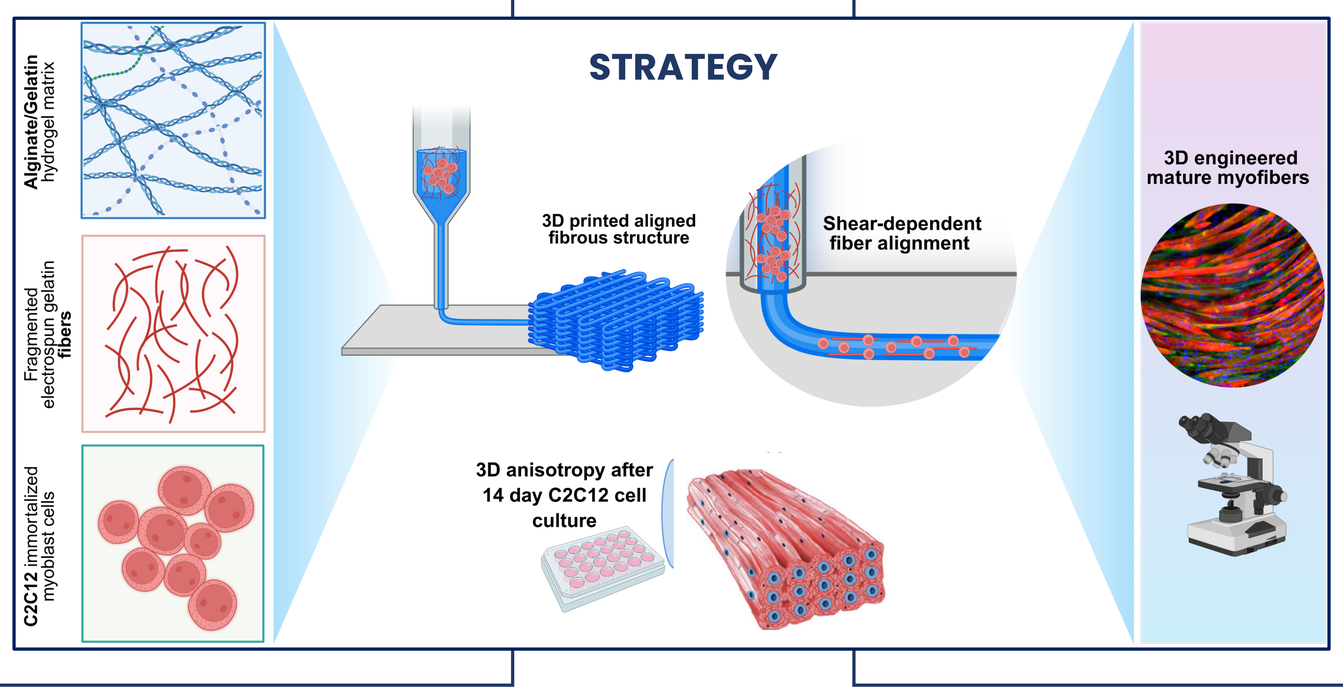
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BACKGROUND

- **Skeletal muscle tissue (SMT)** depends on a well-organized hierarchical microstructure. It consists of **uniaxially oriented** and packed myofibers.
- Conventional bioinks are **isotropic** hydrogels.
- **Lack of structural cues** limits myogenesis *in vitro*.





AIM OF THE WORK

Engineering a **3D *in vitro* platform to model SMT** anisotropy, via combination of **electrospinning with 3D micro-extrusion bioprinting** (fibrous bioink)

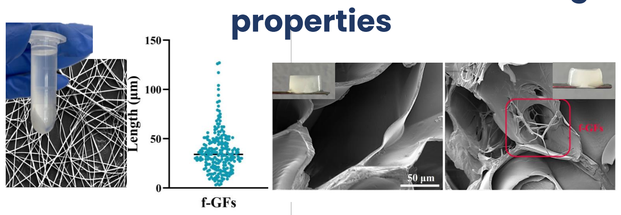
MATERIALS & METHODS

Electrospun gelatin fibers (diameter ≈ 425 nm) fragmented by mechanical homogenization.

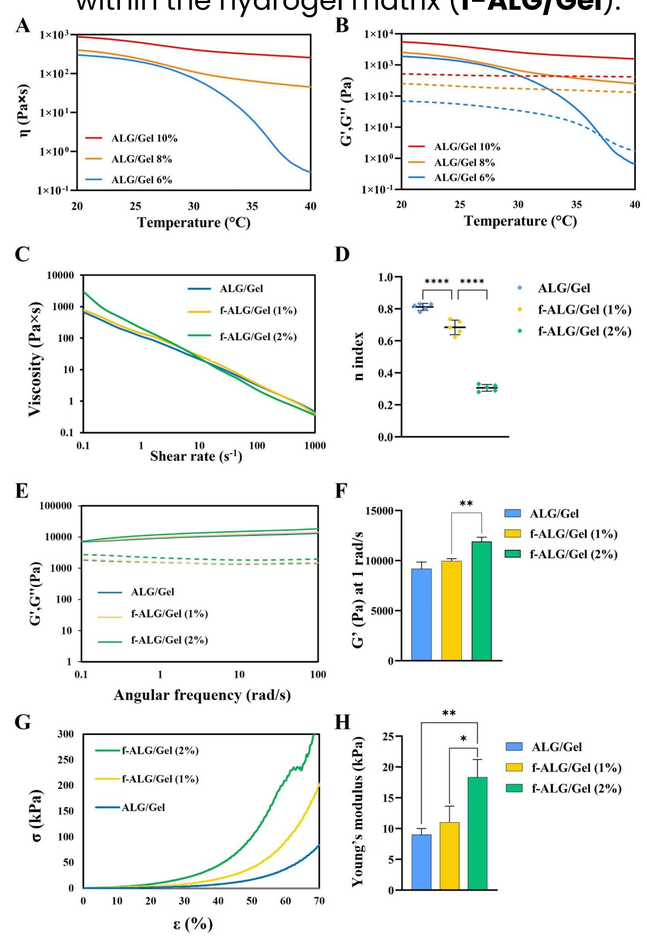
Bioink formulation:
2% (w/v) fragmented gelatin fibers (**f-GFs**)
6% (w/v) alginate/gelatin hydrogel (**ALG/Gel**, 30:70)
Dual crosslinking: **$CaCl_2$ + mTG**

RESULTS

Fiber fragmentation: matrix reinforcement and enhanced mechanical and rheological properties

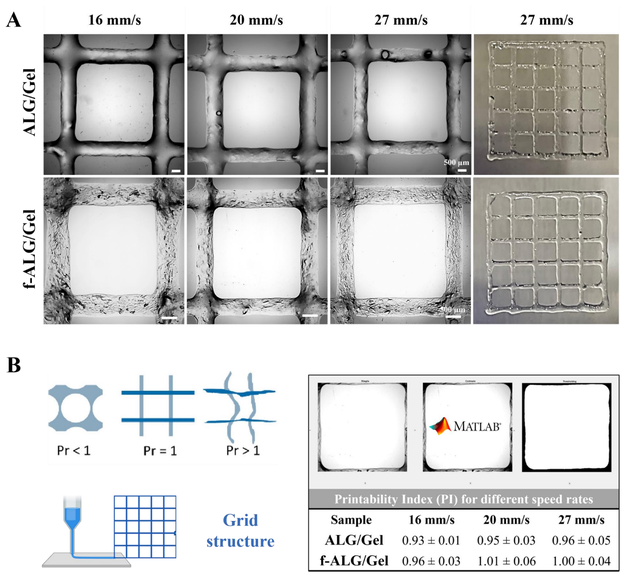


Electrospun gelatin fibers were fragmented into **short f-GFs** (≈30-40 µm), enabling homogeneous dispersion within the hydrogel matrix (**f-ALG/Gel**).



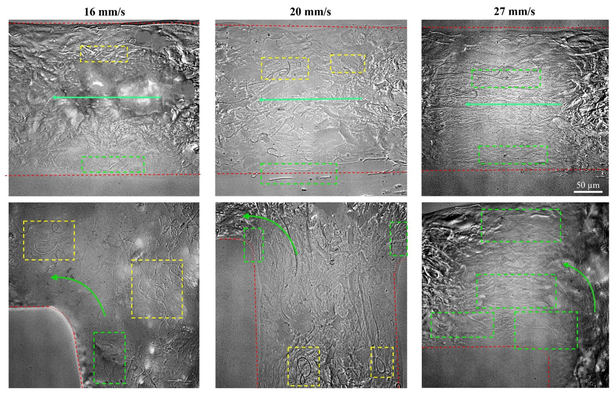
Rheological and mechanical characterization. (A–B) Temperature sweep tests. (C–D) Shear-rate–dependent viscosity and shear-thinning index. (E–F) Frequency sweep of dual-crosslinked hydrogels (Ca^{2+}/mTG). (G–H) Stress–strain curves and Young's modulus from compression tests.

Fiber-embedded micro-extrusion printing: enhanced printability and shape fidelity



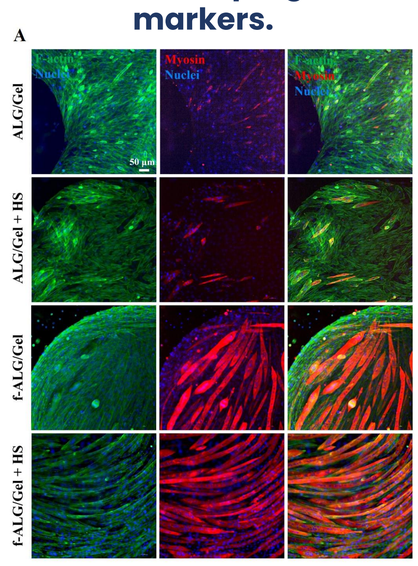
Sample	16 mm/s	20 mm/s	27 mm/s
ALG/Gel	0.93 ± 0.01	0.95 ± 0.03	0.96 ± 0.05
f-ALG/Gel	0.96 ± 0.03	1.01 ± 0.06	1.00 ± 0.04

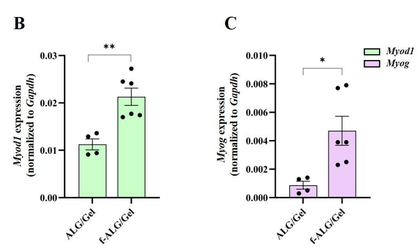
Printability assessment of ALG/Gel and f-ALG/Gel bioinks. (A) Grid structures at increasing speeds (16–27 mm/s). (B) Printability index (PI) based on pore geometry. (C) MATLAB-based image processing for pore segmentation. (D) PI quantification showing improved shape fidelity for f-ALG/Gel bioinks.

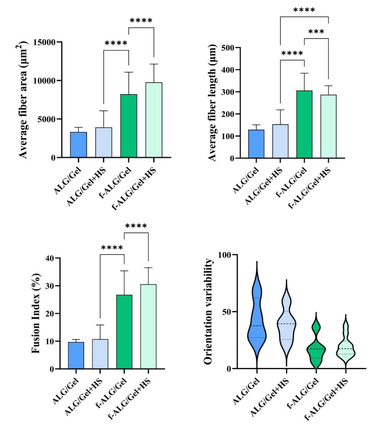


Shear-induced fiber alignment during extrusion bioprinting. Microscopy images of printed filaments at increasing speeds (16–27 mm/s). Dashed boxes highlight representative fiber regions, while arrows indicate the preferential alignment along the printing direction.

3D anisotropy: enhanced myotube alignment, fusion, maturation, and upregulated myogenic markers.







Fibrous scaffolds promote skeletal muscle differentiation. (A) Immunofluorescence images of myosin expression. (B–C) Gene expression (MyoD1 and Myog)

Fragmented electrospun fibers enable shear-induced anisotropy in printable bioinks, promoting skeletal muscle formation.

CONCLUSIONS

- A **fibrous bioink (f-ALG/Gel)** with fragmented gelatin fibers was developed for extrusion bioprinting.
- **Shear-induced fiber alignment** generated scaffolds with intrinsic anisotropy.
- Fiber reinforcement improved **mechanical properties and printability**.
- The anisotropic architecture enhanced **C2C12 alignment, fusion, and myotube formation**.

FUTURE DEVELOPMENTS

- Validation with **human myoblasts or hiPSC-derived muscle cells**.
- Integration of **electrical or mechanical stimulation** to improve maturation.

REFERENCES

Ruocco G, Marcello E, Paoletti C, *et al.* Fibrous bioinks for bioprinting anisotropic micro-and nanoscale scaffolds: A novel strategy for *in vitro* skeletal muscle engineering. *Int J Bioprint*. doi: 10.36922/IJB025440455.

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