ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

Fibrous bioinks for bioprinting anisotropic micro- and nanoscale scaffolds: A novel approach for *in vitro* skeletal muscle engineering

Supplementary file

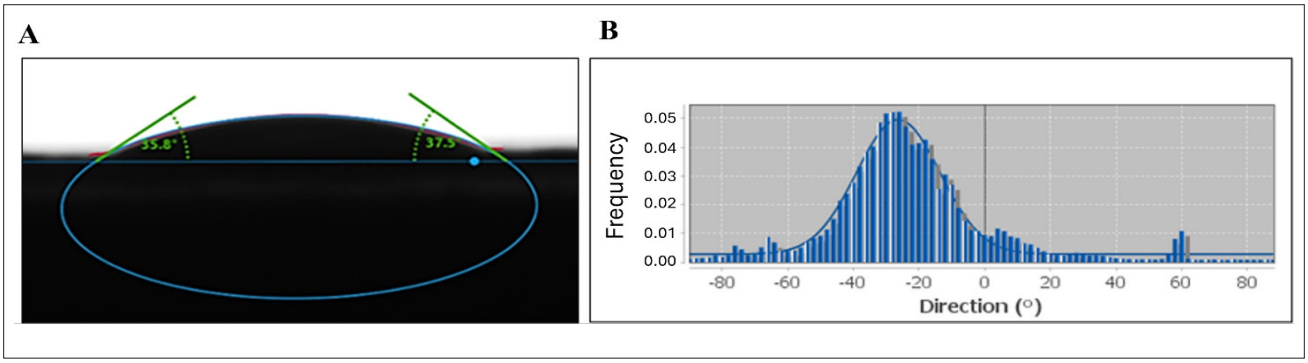

Figure S1. Characterization of aligned gelatin fiber (GF). (A) Measurement of the static contact angle on GFs, showing average angles of 35.8° and 37.5° on the left and right sides, respectively. (B) Directionality histogram for GFs obtained using the Fourier transform analysis in ImageJ (Directionality tool).

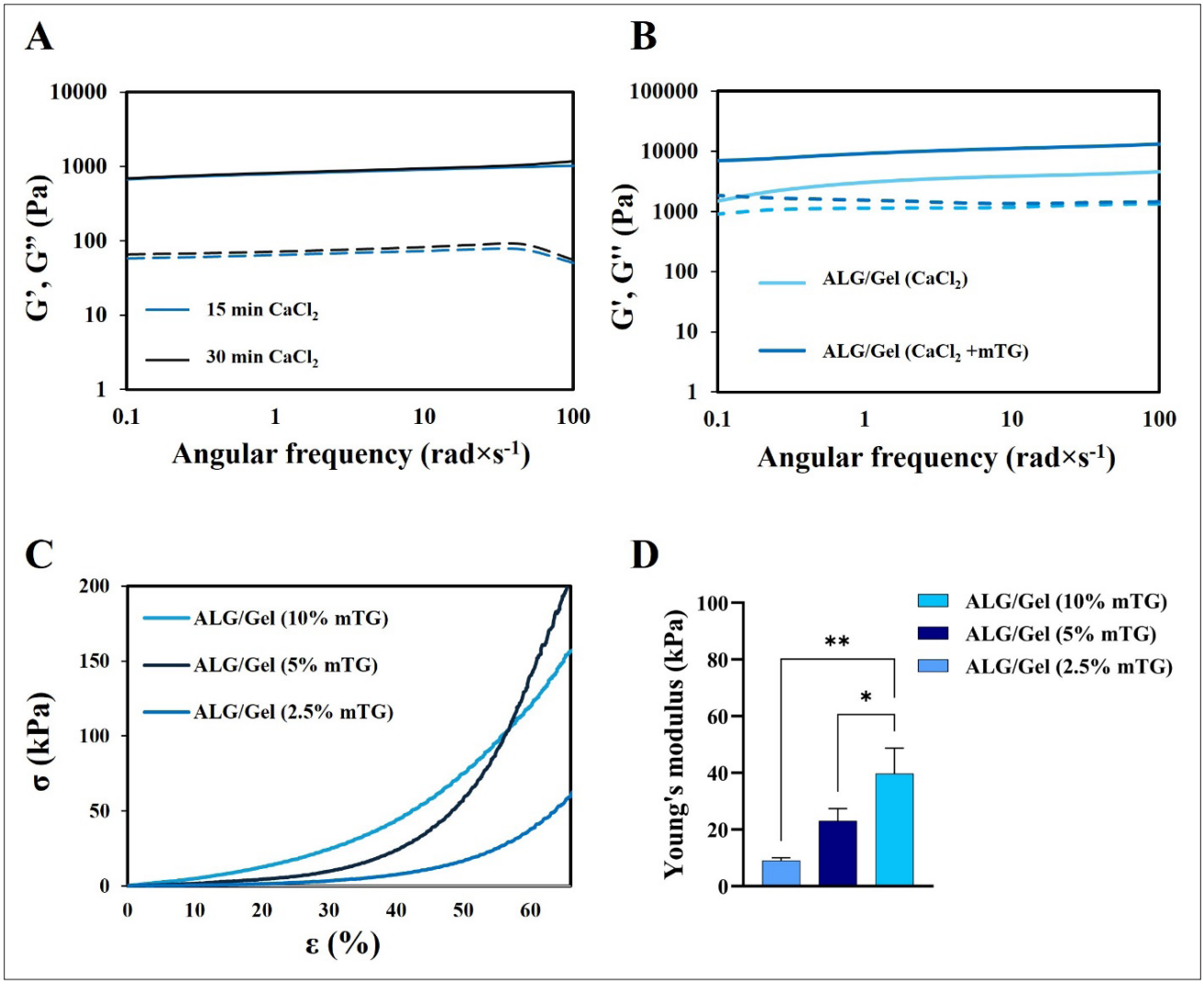

Figure S2. Evaluation of crosslinking parameters for ALG/Gel hydrogels. (A) Frequency sweep tests comparing ionic crosslinking with 0.1 M $CaCl_2$ for 15 and 30 min. A longer exposure time led to increased but not significant storage modulus (G'). (B) Frequency sweep analysis of dual-crosslinked hydrogels, comparing ionic ($CaCl_2$) versus combined ionic and enzymatic crosslinking ($CaCl_2$ + 2.5% mTG). The addition of mTG significantly increased both G' and G", confirming the reinforcing effect of enzymatic crosslinking. (C) Stress–strain curves of ALG/Gel hydrogels crosslinked with increasing mTG concentrations (2.5%, 5%, and 10% w/v). (D) Young's modulus values derived from uniaxial compression tests. Hydrogels crosslinked with 5% and 10% mTG showed excessive stiffening, compromising biomimetic compliance. Therefore, 2.5% mTG was selected as the optimal concentration for subsequent experiments. Notes: *$p < 0.05$; **$p < 0.01$. Abbreviations: ALG: Alginate; Gel: Gelatin; mTG: Microbial transglutaminase.

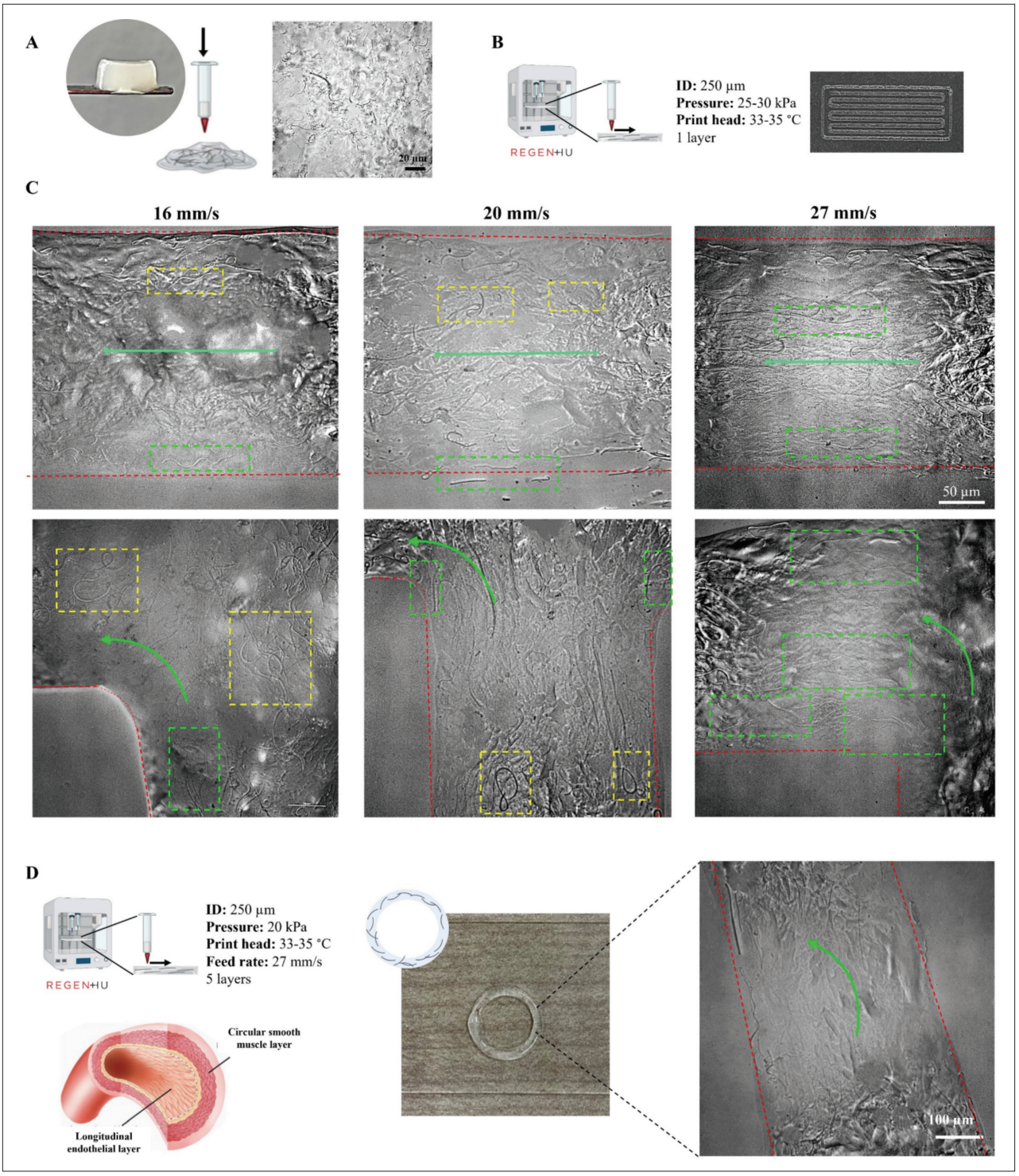

Figure S3. Characterization of shear-induced anisotropy. (A) F-ALG/Gel hydrogel obtained through manual casting and corresponding brightfield microscopy image displaying randomly distributed fibers in the absence of controlled alignment. Scale bar: 20 µm; magnification: 20×. (B) Printing parameters utilized to fabricate a single layer with aligned fiber structures from f-ALG/Gel ink, optimized to achieve controlled micro- and nanoscale anisotropy. (C) Qualitative assessment of f-GF alignment under different feed rates during the printing process, highlighting the impact of printing speed on f-GF arrangement. Scale bar: 50 µm; magnification: 20×. (D) Proof of concept illustrating the versatility of the approach in generating anisotropic constructs. Specifically, a circumferential alignment pattern was successfully reproduced, demonstrating potential applications in mimicking vascular tissue architecture with a circular smooth muscle layer and a longitudinal endothelial layer. Scale bar: 100 µm; magnification: 20×. Abbreviations: ALG: Alginate; f-GF: Fragmented gelatin fibers; Gel: Gelatin; ID: Internal diameter.