Design and Fabrication of a 3D Printed Auxetic-Electrospun Scaffold for Tendon Structure Modelling

Giacomo Cortella[1], Alessandro Velletri[2,3], Erwin Pavel Lamparelli[1], Adamo Lancellotti[1], Aurora De Acutis[2,3], Carmelo De Maria[2,3], Nicola Maffulli[4], Giovanni Vozzi[2,3], Giovanna Della Porta[1,5]

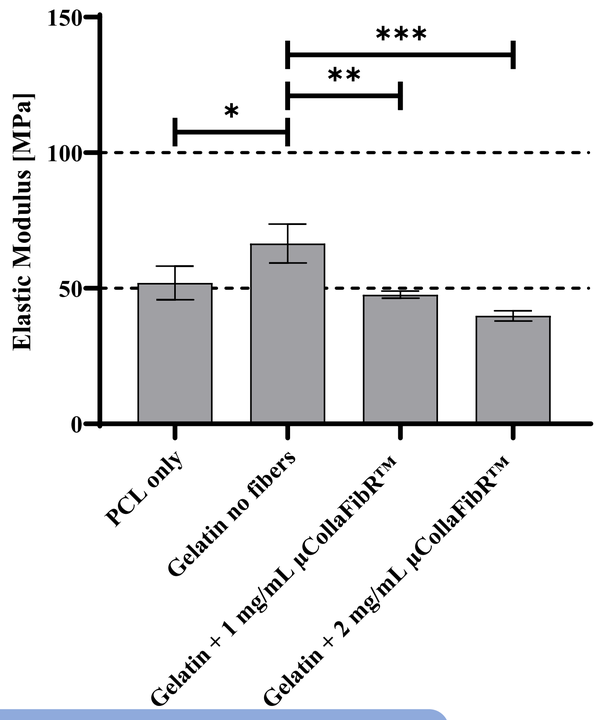
[1] Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi, Italy
[2] Research Centre "E. Piaggio", University of Pisa, Pisa, Italy
[3] Department of Information Engineering, University of Pisa, Pisa, Italy
4 Department of Trauma and Orthopaedics, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy
5 Research Centre for Biomaterials (BIONAM), University of Salerno, Fisciano, Italy

1 Introduction:

Tendon tissue engineering requires scaffolds that combine mechanical robustness with biomimetic features to support cell alignment and function under cyclic strain. While electrospun matrices offer extracellular matrix (ECM)–like topography, they lack load-bearing capacity, whereas thermoplastic scaffolds provide strength but limited biological instruction. Auxetic architectures, characterized by a negative apparent Poisson's ratio, enable enhanced stress distribution and resilience under tensile loading. In this study, we developed a hybrid scaffold integrating a 3D-printed auxetic polycaprolactone (PCL) core with electrospun gelatin-GPTMS layers functionalized with collagen microfibers, aiming to reproduce tendon-relevant mechanics, hierarchical fibrous architecture, and cell-instructive cues for advanced in vitro tendon modeling.

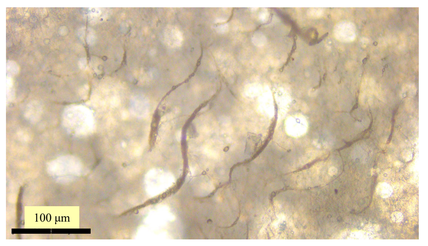
2 Methods:

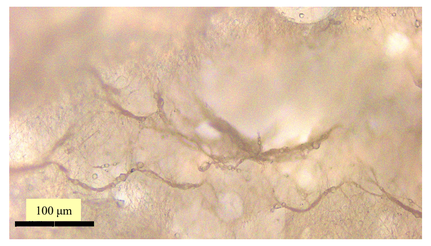
Auxetic scaffolds were designed as re-entrant honeycomb lattices to impart tendon-relevant anisotropy and negative apparent Poisson's ratio, and fabricated by pneumatic melt extrusion of polycaprolactone (PCL) using a multi-technology bioprinting platform. Electrospun gelatin crosslinked with GPTMS was deposited on both sides of the PCL core to provide extracellular matrix (ECM)–mimetic topography, with dispersed collagen microfibers incorporated to generate a hierarchical fibrous architecture. Scaffold geometry and morphology were assessed by optical microscopy and field emission scanning electron microscopy (FE-SEM). Mechanical performance was evaluated by uniaxial tensile testing to determine elastic modulus and ultimate tensile strain. Human tendon stem/progenitor cells (hTSPCs) were seeded onto preconditioned scaffolds, and cytocompatibility was assessed by Live/Dead staining and fluorescence microscopy.

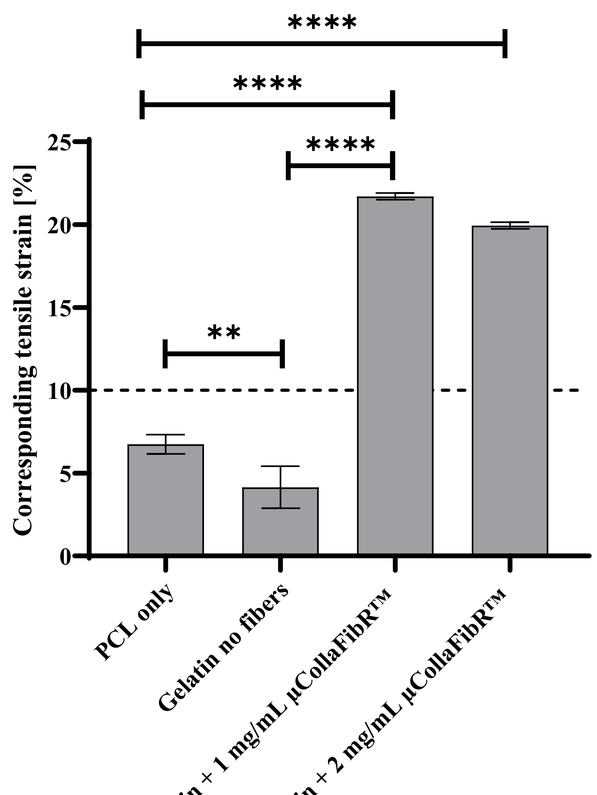


Elastic modulus of hybrid scaffolds.
Gelatin coating increased stiffness, while collagen microfiber incorporation reduced elastic modulus to tendon-relevant values. Data shown as mean ± SD (N = 3); *p < 0.05, **p < 0.01, ***p < 0.001.

Optical microscopy of gelatin-based layers with collagen microfibers.
Electrospun gelatin-GPTMS incorporating 1 mg/mL µCollaFibR™ (**left**) and 2 mg/mL µCollaFibR™ (**right**), showing increased microfiber density at higher collagen concentration. Scale bars: 100 µm.

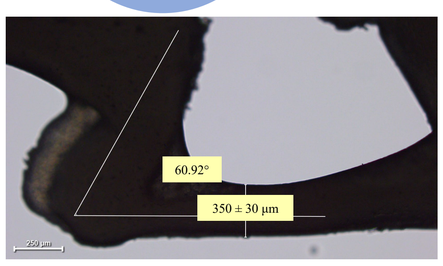


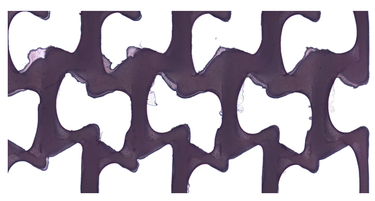




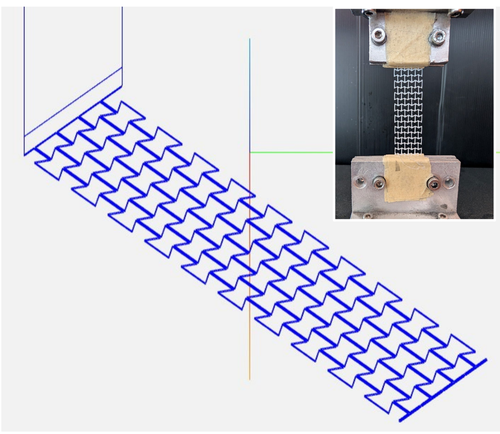
Ultimate tensile strain of hybrid scaffolds.
Comparison of extensibility for PCL-only, gelatin-coated scaffolds without collagen, and hybrid scaffolds incorporating 1 mg/mL and 2 mg/mL µCollaFibR™. Collagen microfiber supplementation significantly increased ultimate tensile strain, reaching tendon-relevant values, while gelatin coating alone reduced extensibility. Data shown as mean ± SD (N = 3); **p < 0.01, ****p < 0.0001.

3 Geometric Characterization and Mechanical Tests:



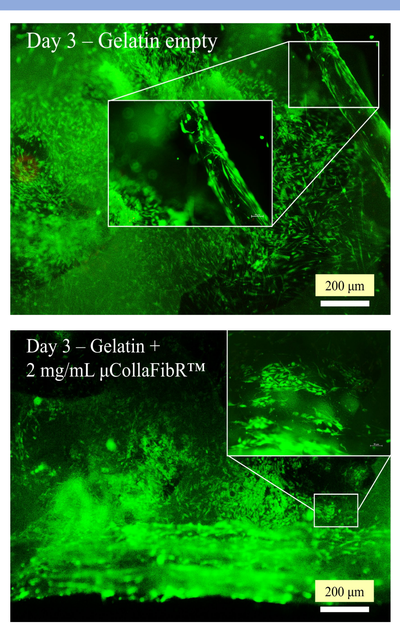


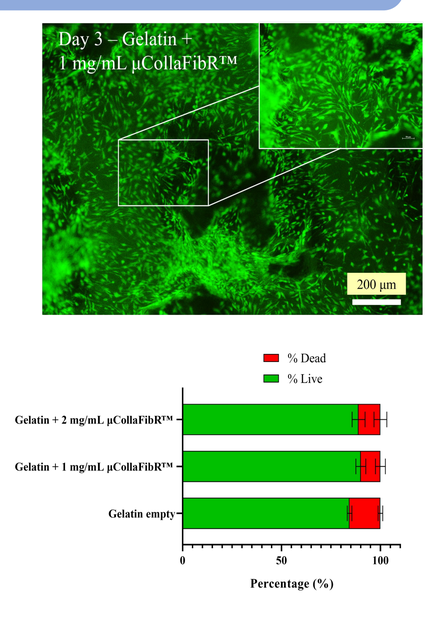
The auxetic polycaprolactone (PCL) core was fabricated via pneumatic melt extrusion, achieving high geometric fidelity to the designed re-entrant honeycomb architecture. Optical microscopy confirmed a re-entrant angle of 60.9 ± 2.1° and a strut thickness of 350 ± 30 µm, consistent with the targeted auxetic design and supporting a negative apparent Poisson's ratio. Uniaxial tensile testing revealed that PCL scaffolds printed at 1000 mbar exhibited an elastic modulus of 51.9 ± 6.2 MPa and an ultimate tensile strain of 6.8 ± 0.6%, selected as an optimal balance between stiffness and extensibility. Integration of electrospun gelatin-GPTMS layers increased scaffold stiffness but reduced extensibility, whereas supplementation with dispersed collagen microfibers significantly enhanced ultimate tensile strain (up to ≈21%) while maintaining tendon-relevant elastic moduli (≈40–48 MPa). Multiscale optical and FE-SEM analyses confirmed a hierarchical architecture, with collagen microfibers (1–5 µm diameter) uniformly embedded within the nanofibrous gelatin matrix, providing both mechanical tunability and structural biomimicry of native tendon tissue.

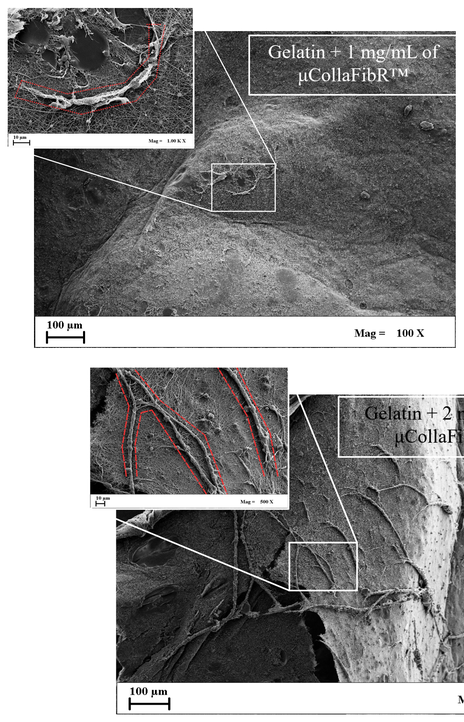


4 Cytocompatibility:

Human tendon stem/progenitor cells (hTSPCs) seeded onto the hybrid scaffolds exhibited high viability across all formulations, with Live/Dead staining showing more than 85% viable cells after 3 days of culture. Fluorescence microscopy revealed an elongated and spread cell morphology, with cells preferentially aligning along the underlying fibrous architecture of the electrospun layers. The incorporation of gelatin-GPTMS coatings supported effective cell adhesion, while the presence of dispersed collagen microfibers preserved cytocompatibility and contributed to a biomimetic microenvironment. These results confirm that the auxetic-electrospun scaffold provides a cell-friendly surface suitable for tendon-relevant in vitro modeling.







Cytocompatibility of gelatin-based hybrid scaffolds.
Live/Dead fluorescence microscopy of hTSPCs cultured for 3 days on gelatin-GPTMS scaffolds without collagen (Gelatin empty) and with 1 mg/mL or 2 mg/mL µCollaFibR™. Predominant green fluorescence indicates high cell viability and elongated cell morphology across all conditions. Quantitative analysis confirms >85% viable cells, demonstrating preserved cytocompatibility following collagen microfiber incorporation. Scale bars: 200 µm.

5 Conclusions:

The 3D-printed auxetic PCL core provides tunable, tendon-relevant mechanical properties, while electrospun gelatin-GPTMS layers functionalized with collagen microfibers introduce hierarchical fibrous cues and enhance extensibility without compromising cytocompatibility. High geometric fidelity, favorable mechanical performance, and strong hTSPC viability and alignment confirm the scaffold's suitability as a biomimetic platform for advanced in vitro tendon modeling and future dynamic culture under cyclic strain.

References

- E.P. Lamparelli, et al. Int. Journal of Pharm. 624:122007, 2022.
- M.C. Ciardulli, et al. Cells, 11(16):2545, 2022.
- G. Cortella, et al., MDPI, 10.1002/btm2.10723
- E.P. Lamparelli, et al. Biofabrication, 10.1088/1748-605X/ae574c

For further information, please contact: gcortella@unisa.it