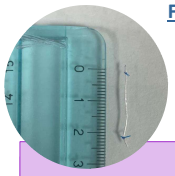
TERMIS-EU 2026
Palma de Mallorca, Spain
21st - 24th April 2026

Bioengineered collagen-coated scaffolds for advanced tendon regeneration



Francesca Romano [1,2], Francesco Lopresti [2], Chiara Di Marco [2], Denise Murgia [1], Albert Comelli [1], Vincenzo La Carrubba [2], Roberto Di Gesù [1]

[1] Musculoskeletal Tissue Engineering (MsTE) Laboratory, Ri.MED Foundation, Palermo, Italy.
[2] Bio and Tissue Engineering Laboratory, Department of Engineering, University of Palermo, Palermo, Italy

INTRODUCTION

Tendon injuries represent approximately 50% of all musculoskeletal injuries worldwide [1] and are expected to increase due to the rising participation in sports and the progressive aging of the population [2]. Conventional surgical interventions often yield suboptimal outocomes, primarily because of limited biocompatibility, inadequate mechanical strength of grafts or implants, and frequent development id fibrotic tissue following surgery. As a result, there is a growing need for alternative strategies capable of better replicating the structural, biological and mechanical features od native tendons. Tissue engineering represent a promising approach through the development of biocompatible scaffolds that can mimic the extracellular matrix (ECM) and the hierarchical structure of tendons [3]. In this study, a tendon-like scaffold scaffold was engineered using aligned electrospun poly-lactic acid (PLA) microfibers, designed to emulate the anisotropic structure and tensile strength of natural tendons. The scaffold also functioned as a drug delivery platform **Rolipram**, an antifibrotic agent [4]. **Type I Collagen**, the major ECM component in tendons [5], was applied as a surface coating to promote cell adhesion and proliferation. The combination od biomimicry, drug release, and bioactivity makes this system a valuable candidate for tendon regeneration.

MATERIALS AND METHODS

Tendon-like scaffolds were fabricated via vertical **electrospinning** using a semi-industrial apparatus. A 10% w/v poly(lactic acid) (PLA) solution was prepared in a 2:1 v/v mixture of trichloromethane and acetone. Optimized electrospinning parameters included a flow rate of **0.7 mL/h**, a **17 cm** needle-to-collector distance, an applied voltage of **17 kV**, and a 19-G stainless steel needle.
Aligned fibers were collected on a rotating drum (20 cm length, 10 cm diameter) operating at 3000 rpm. The process was carried out for 180 minutes, producing membranes approximately 40 µm thick.
For drug loading, 500 nmol of Rolipram was incorporated into the PLA solution, maintaining all other parameters constant (PLA/Rol).
Type I Collagen was extracted from **rat tails**, purified, and applied to the electrospun mats to improve bioactivity (PLA/Coll and PLA/Rol/Coll).
To mimic the hierarchical structure of tendons, 1 × 2.5 cm rectangular scaffolds were twisted into bundles using a custom 3D-printed device and secured at both ends with polypropylene suture threads (Figure 1). **Human tenocytes** (hTCs, passage 5) were used to assess cell attachment and viability on the scaffolds. Cells were cultured in low-glucose DMEM supplemented with 10 % v/v FBS, 1 % v/v penicillin/streptomycin, 1 % v/v sodium pyruvate, 1 % v/v GlutaMAX®, PDGF-BB, and TGF-β3. A 30 µL cell suspension containing 2.5 × 10^5 cells was seeded onto each scaffold (n = 3 per condition). Samples were incubated at 37 °C in a humidified atmosphere with 5 % CO_2, and culture medium was refreshed every other day for 14 days.

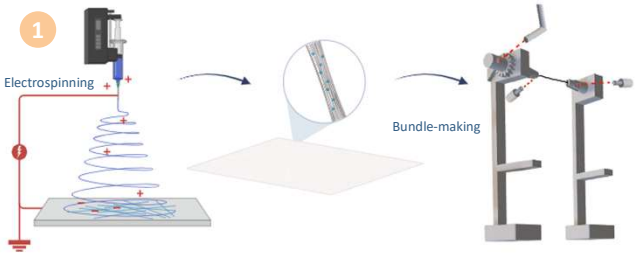


Figure 1. Schematic illustration of the vertical electrospinning system and the subsequent fabrication of tendon-like scaffolds. The process involves electrospinning, collagen coating, and bundle assembly using a custom 3D-printed bundle-maker.

RESULTS AND DISCUSSION

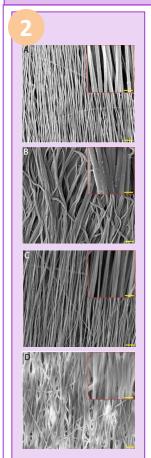


Figure 2. Scanning electron microscopy (SEM) showing fiber morphology of PLA (A), PLA/Coll (B), PLA/Rol (C), and PLA/Rol/Coll (D) electrospun mats. Main scale bar = 10 µm insert scale bar = 2.5 µm.

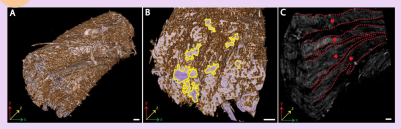


Figure 3. Rendering of µ-CT scans of the bundle-shaped scaffold captured in false colors at different angled views (A, B). X-ray images of a cross-sectional view (C). Yellow areas = fascicular suborganization in transversal view. Red dashed-lines, and red asterisks = extension of fascicular suborganization through longitudinal axis. Scale bar = 100 µm.

SCAFFOLD MORPHOLOGY

The collagen coating significantly influenced the scaffold microarchitecture by promoting the self-assembly of electrospun microfibers into **fascicle-like substructures**. This effect was evident when comparing SEM images of uncoated scaffolds with collagen coated ones. The coating likely facilitated the random aggregation of neighboring microfibers, stabilizing them into fascicle-like domains. These substructures were also retained in the 3D tendon-like scaffolds, designed to replicate the native hierarchical tendon architecture using a custom 3D-printed bundle-making device. This fabrication approach enabled the formation of a helicoidal quaternary organization, as confirmed by µCT digital renderings of the scaffold inner structure.

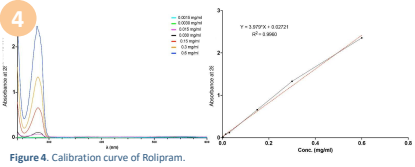


Figure 4. Calibration curve of Rolipram.

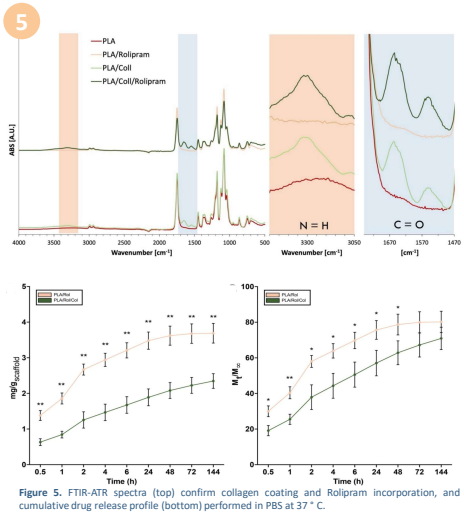
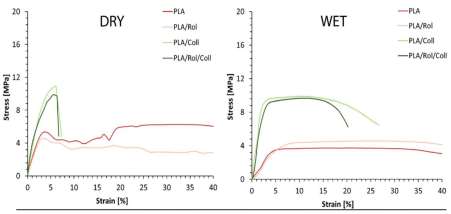


Figure 5. FTIR-ATR spectra (top) confirm collagen coating and Rolipram incorporation, and cumulative drug release profile (bottom) performed in PBS at 37 ° C.



	E [MPa]	TS [MPa]	ε, [%]
Dry condition			
PLA	102.93 ± 12.35	5.36 ± 0.42	103.82 ± 7.26
PLA/Rol	103.3 ± 5.16	4.58 ± 0.32	114.27 ± 13.71
PLA/Coll	140.09 ± 14	10.54 ± 1.26	7.32 ± 0.87
PLA/Rol/Coll	144.23 ± 15.86	9.84 ± 0.59	6.73 ± 0.47
Wet condition			
PLA	54.93 ± 4.39	3.14 ± 0.34	82.46 ± 5.77
PLA/Rol	45.91 ± 3.67	2.93 ± 0.23	80.56 ± 7.25
PLA/Coll	132.15 ± 7.92	8.9 ± 0.89	27.58 ± 3.03
PLA/Rol/Coll	130.89 ± 6.54	9.74 ± 0.48	31.32 ± 1.87

Figure 6. Stress–strain curves (top) show the tensile properties of scaffolds under dry (air, RT) and wet (PBS, 37 °C) conditions. The table (bottom) reports Young's modulus, tensile strength, and elongation at break.

BIOLOGICAL CHARACTERIZATION

To investigate the biological performance of the tendon-like scaffolds, preliminary 2D studies were conducted to identify optimal culture conditions for human tenocytes (hTCs). Since monolayer cultures are prone to tenocyte dedifferentiation and loss of tendon-specific phenotype, five different media formulations (#1–#5) were tested over 14 days. Each condition was evaluated based on cell density, morphology, and ECM production efficiency, and further analyzed through immunocytochemical staining for tenocyte positive (TNSc) and negative (SOX9) markers. Both morphological and immunocytochemical analyses identified condition #2 as the optimal formulation, supporting hTCs growth while preserving their native phenotype. Quantitative fluorescence analyses on 3D scaffolds confirmed a protective effect toward resident hTCs. After 14 days of culture, cells within Rolipram-loaded scaffolds (PLA/Rol/Coll) produced significantly higher amounts of **type I collagen** compared to PLA and PLA/Coll samples. **TNMD** expression analysis further indicated the maintenance of tenocyte functionality, showing a characteristic pericellular distribution around cell nuclei. Histological evaluation (H&E staining) demonstrated viable hTCs distributed across both inner and outer scaffold regions. The eosinophilic staining surrounding nuclei reflected active ECM deposition up to 14 days, particularly evident in collagen-coated scaffolds. Overall, fluorescence and histological results confirmed that the scaffolds preserved tenocyte viability, phenotype, and ECM-producing capacity.

ATR CHARACTERIZATION AND DRUG RELEASE ANALYSIS

The presence of collagen on the microfiber surface was verified by FTIR-ATR analysis, confirming the success of the coating procedure. The spectra of collagen-coated samples exhibited characteristic Amide I and Amide II bands at 1650 cm^{-1} and 1550 cm^{-1}, respectively. The Amide I band, associated with C=O stretching vibrations of peptide bonds, and the Amide II band, corresponding to N–H bending and C–N stretching, are indicative of the ordered triple-helix structure. The collagen coating significantly influenced Rolipram release from the scaffolds. PLA/Rol showed a faster and higher cumulative release compared to PLA/Rol/Coll, mainly due to the lower drug content per scaffold weight and the diffusion barrier effect of the collagen layer. Normalized release profiles (M_t/M_∞) confirmed that uncoated scaffolds reached ~60% release within 4 h, while coated ones required ~48 h. According to the Peppas model, both systems followed **Fickian diffusion** ($n < 0.5$). The collagen coating reduced surface porosity and increased fiber diameter, resulting in a slower, more controlled drug release.

MECHANICAL PROPERTIES UNDER DRY AND WET CONDITIONS

Collagen coating significantly enhanced the mechanical properties of the scaffolds. Under dry conditions, Young's modulus increased from 102.9 to 140.1 MPa for drug-free scaffolds and from 103.3 to 144.2 MPa for drug-loaded ones, reflecting reduced fiber sliding and **increased stiffness**. Rolipram incorporation did not affect mechanical behavior.
Under wet, in vivo-mimicking conditions, uncoated scaffolds exhibited approximately 50% reduction in Young's modulus, whereas collagen-functionalized mats largely preserved their mechanical properties, with only minor softening attributed to collagen hydration and increased PLA backbone mobility at 37 °C. The mechanical profile of collagen-coated scaffolds resembles that of the human tibialis posterior tendon, indicating potential suitability for load-bearing applications in vivo.

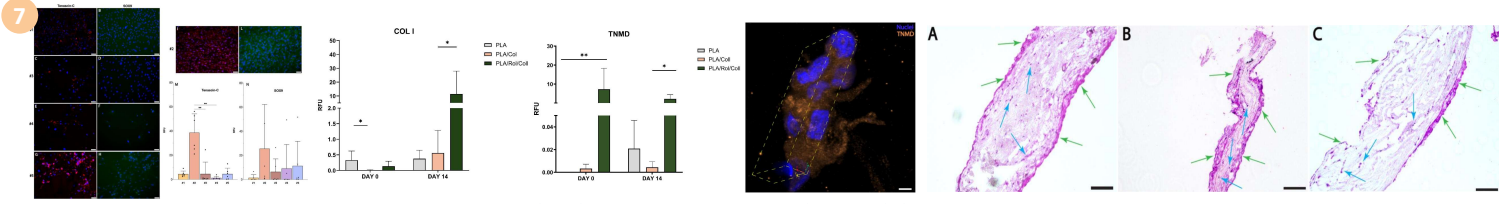


Figure 7. 2D immunofluorescence for TNC and SOX9, and 3D staining for TNMD and type I collagen. Hematoxylin/Eosin (H/E) staining of longitudinal sections of PLA (A), PLA/Coll (B), and PLA/Rol/Coll (C) electrospun scaffolds seeded with hTCs at day 14 of culture.

CONCLUSIONS

This study demonstrated the feasibility of fabricating bioactive, drug-loaded tendon-like scaffolds with promising structural and functional features for tendon regeneration. The aligned electrospun fibers successfully replicated the native collagen architecture, while the collagen coating enhanced both mechanical performance and biological response. In particular, the PLA/Rol/Coll scaffolds exhibited **sustained Rolipram release** for up to 14 days and improved cell adhesion and distribution.
The combined effects of biomimicry, controlled drug release, and enhanced bioactivity highlight the potential of these scaffolds as candidates for tendon repair applications.

REFERENCES:
1. Kane SF, Olewinski LH, Tamminga KS. Management of Chronic Tendon Injuries. Am Fam Physician. 2019 Aug 1;100(3):147-157. PMID: 31361101.
2. Leong NL, Kator JL, Clemens TL, James A, Enamoto-Iwamoto M, Jiang J. Tendon and Ligament Healing and Current Approaches to Tendon and Ligament Regeneration. J Orthop Res. 2020 Jan;38(1):7-12. doi: 10.1002/jor.24475. Epub 2019 Sep 30. PMID: 31529731; PMCID: PMC7307866.
3. Ruiz-Alonso S, Lafuente-Merchan M, Ciriza J, Saenz-Del-Burgo L, Pedraz JL. Tendon tissue engineering: Cells, growth factors, scaffolds and production techniques. J Control Release. 2021 May 10;333:448-486. doi: 10.1016/j.jconrel.2021.03.040. Epub 2021 Mar 31. PMID: 33811983.
4. Wu L, Xu L, Chen Y, Xu G, Guo Q, Meng D, Fan J, Song G, Xu P. Rolipram plays an anti-fibrotic effect in ligamentum flavum fibroblasts by inhibiting the activation of ERK1/2. BMC Musculoskelet Disord. 2021 Sep 23;22(1):818. doi: 10.1186/s12891-021-04712-9. PMID: 34556093; PMCID: PMC8461931.
5. Ryan, C.N.M. and Zeugolis, D.I. (2020), Engineering the Tenogenic Niche In Vitro with Microenvironmental Tools. Adv Therap., 3: 1900072. https:// doi.org/10.1002/adtp.201900072

Full article available here