# Fabrication and characterization of electrospun scaffolds for tendon reconstruction

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***Abstract*—Tendon injuries represent an unsolved clinical need. Electrospun bundles using blends of poly-L-lactic acid (PLLA) and collagen (Coll) in different percentages were prepared by an 'ad hoc' crosslinking system to increase the mechanical properties and reduce the loss of collagen after ageing in a physiological environment. The mechanical properties and cell proliferation were tested. High-resolution x-ray tomography was also performed to investigate the morphologyof the bundles.**

***Keywords*—Electrospinning, tendon tissue engineering, x-ray tomography, cell viability.**

## I. Introduction

TENDON injuries represent an unsolved clinical need. Recent preliminary studies confirmed the suitability of resorbable electrospun scaffolds (bundles), for mimicking the multiscale structure of tendon fascicles [1]-[2]. In the present work we produced resorbable electrospun crosslinked bundles made of PLLA/Coll blends at different compositions. The productions process aimed to reproduce the hierarchical structure and the mechanical properties of Achilles' tendon fascicles. The aims of this work are to: (i) test the mechanical properties and the effect of crosslinking before and after ageing in a physiological environment; (ii) produce a morphological analysis of the bundles via high-resolution x-ray tomograph; (iii) test the cell viability and proliferation on the bundles.

## II. Materials & Methods

Bundles were produced with two blends:

- PLLA/Coll 75/25 w/w.
- PLLA/Coll 50/50 w/w.

To prepare 3D bundles made of aligned fibers, a high-speed rotating drum collector was used. The electrospun layer was manually rolled along the drum and then removed (Fig.1A). The bundles were 470 mm long and 550-650 µm in diameter. The crosslinking process was performed with a solution of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) 0.02 M mixed in Ethanol/distilled water 95/5 v/v. Five specimens for each blend were mechanically tested immediately after crosslinking, and after ageing in phosphate buffer solution (PBS) for 7 and 14 days. As a reference five specimens as spun for each blend were also tested. To evaluate the mechanical properties of the bundles a tensile test was conducted (Fig.2):

- Specimens were immersed in 0.9% NaCl saline solution for two minutes before testing.
- Customized capstan grips to minimize stress concentrations.
- Gauge length: 16 mm.
- A strain rate close to physiological: 1 $s^{-1}$.
- Monotonic ramp to break detection.

Morphological characterization of the bundles was carried out via high-resolution x-ray tomography (Zeiss Versa 510, voxel size = 1 and 0.4 µm, Fig.1B). Cell viability was assessed via a resazurin reduction assay with non-tumor human fibroblasts (NTF-322) on days 1, 7, 14 and 21 (Fig. 2D). Cells were also immunostained for acetylated alpha-tubulin and counterstained with DAPI to assess cellular alignment on day 7 (Fig.1C).

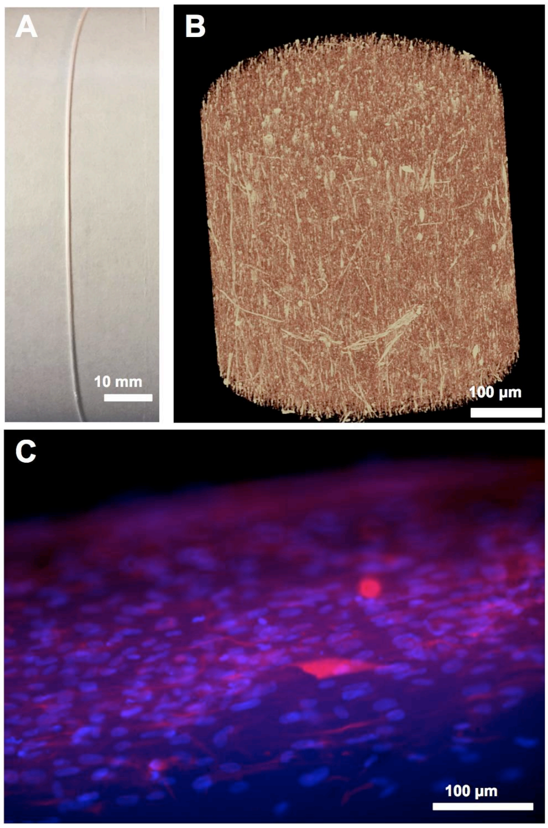


Fig.1: A) Bundle wrapped on the drum; B) x-ray tomography of bundle; C) NTF-322s aligned on a bundle (Blue-DAPI, Red-Acetylated alpha-tubulin).


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## III. Results & Discussion

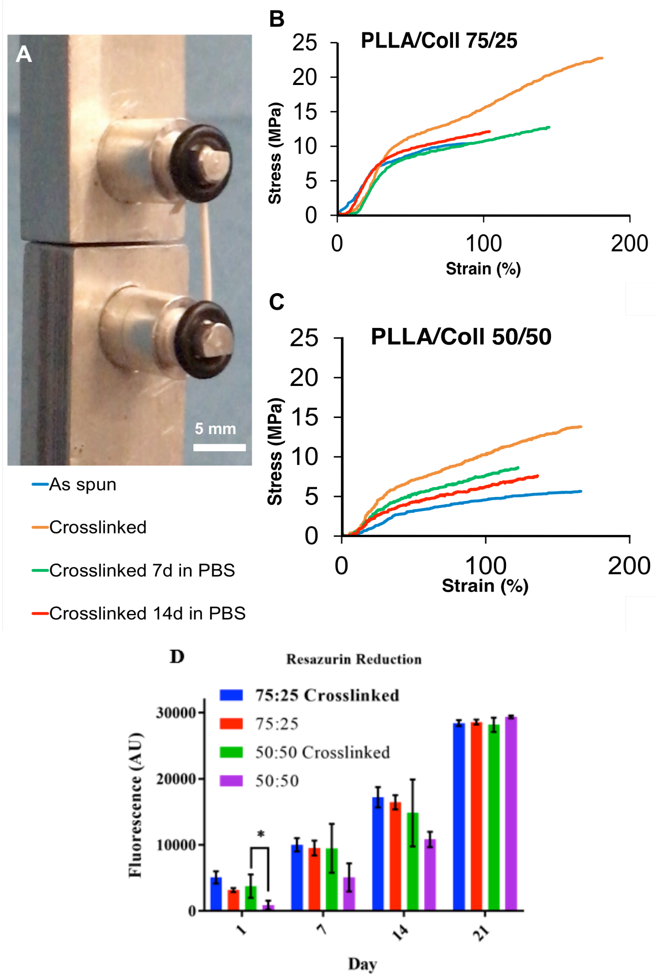


Fig.2: A) Capstan grips with a bundle; B) Stress-strain curves for PLLA/Coll 75/25; C) Stress-strain curves for PLLA/Coll 50/50. D) Cell viability of NTF-322 on bundles.

All the specimens showed a ductile behaviour with large deformations (Fig.2). For all the bundles the stress-strain curves showed an initial non-linear toe region, similar to the laxity of the tendon collagen fibers at rest. The values of failure stress for the two compositions, immediately after crosslinking (Table I-II), were higher than the as spun ones reported in previous studies [1]-[2]. In particular for the PLLA/Coll 75/25 blend, stress values were in the same order of magnitude of the human tendon fascicles [3], although decreased, after ageing in PBS. The hydration increased the failure strain and decreased the Young modulus of all specimens compared to the dry specimens [1]-[2]. The x-ray tomography on the bundles confirmed the good morphology and alignment of the nanofibers, also after the crosslinking process. The cross-linking process had no negative effect on the viability of the cells and all fibres supported cell growth over a 21 day period (Fig 2D). However the PLLA/Coll 50/50 blend did show a reduction in initial attachment.

TABLE I
PLLA/Coll 75/25 mechanical properties

| Samples | Young modulus (MPa) | Yield stress (MPa) | Failure stress (MPa) | Failure strain (%) |
|---|---|---|---|---|
| As spun | 28.0-32.5 | 7.7-8.5 | 10.3-12.3 | 76.2-97.2 |
| Crosslink | 28.0-39.4 | 5.3-9.9 | 12.6-22.8 | 139-183 |
| Crosslink 7d PBS | 25.4-33.0 | 5.8-7.9 | 10.2-14.0 | 110-145 |
| Crosslink 14d PBS | 22.1-38.8 | 5.1-6.1 | 9.1-12.1 | 72-137 |

TABLE II
PLLA/Coll 50/50 mechanical properties

| Samples | Young modulus (MPa) | Yield stress (MPa) | Failure stress (MPa) | Failure strain (%) |
|---|---|---|---|---|
| As spun | 9.0-22.6 | 2.4-3.7 | 5.0-7.3 | 141-166 |
| Crosslink | 20.5-36.9 | 3.3-5.0 | 10.6-17.0 | 151-236 |
| Crosslink 7d PBS | 8.0-18.1 | 2.8-4.2 | 2.3-9.2 | 122-161 |
| Crosslink 14d PBS | 7.1-26.1 | 1.5-2.3 | 5.4-7.7 | 62.0-150 |

## IV. Conclusion

The mechanical properties of the crosslinked bundles for the two blends suggested their suitability for tendon tissue regeneration. The high-resolution x-ray tomography confirmed the good morphology of the nanofibers before and after the crosslinking process. All fibres supported cell proliferation over a 21 day period. These promising results for the new crosslinked bundles confirm their potential for tendon tissue reconstruction.

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