3D STRAIN DISTRIBUTION VIA DVC IN ELECTROSPUN HIERARCHICAL SCAFFOLDS FOR TENDON/LIGAMENT REGENERATION

Alberto Sensini (1,2), Andrea Zucchelli (1,3), Olga Stamati (4), Gregorio Marchiori (5), Nicola Sancisi (1), Carlo Gotti (3), Milena Fini (5), Maria Letizia Focarete (6), Luca Cristofolini (1), Gianluca Tozzi (7)

1. Dept. of Industrial Engineering, University of Bologna, Italy; 2. CTR and cBITE Departments, MERLN Institute, Maastricht University, the Netherlands; 3. CIRI-MAM, University of Bologna, Italy; 4. ESRF The European Synchrotron, France; 5. IRCCS Rizzoli Orthopaedic Institute, Italy; 6. Dept. Chemistry, University of Bologna, Italy, 7. School of Engineering, University of Greenwich, United Kingdom.

Introduction

Tendons/ligaments (T/L) regeneration is complex since scaffolds must have a nanometric fibrous structure and ensure appropriate biomimetic mechanical properties [1]. Electrospinning can replicate T/L from the fibrillar-level up to the whole tissue [1,2]. Recently, Poly-L-lactic acid/Collagen (PLLA/Coll) based, electrospun bundles and hierarchical scaffolds (EHS) have demonstrated to drive fibroblasts morphology and alignment both in static and dynamic cultures [3,4]. However, the full-field strain distribution, which drives the cellular fate, morphology and extracellular-matrix production on these structures at work, is totally unexplored so far. To achieve this goal, digital volume correlation (DVC) has proven to be a suitable technique [5]. This study aims at developing the first micro-CT in situ protocol in literature, to investigate the multiscale full-field strain distribution of electrospun scaffolds using DVC.

Methods

T/L fascicle-inspired electrospun bundles and T/L-inspired EHS of PLLA/Coll 75/25 nanofibers were electrospun following a consolidated procedure [3,4]. The morphology of scaffolds was investigated via SEM and micro-CT. The mechanical properties of scaffolds were defined with a tensile test using a monotonic ramp to break in displacement control, with a strain-rate of 0.33 %/sec and dedicated capstan grips (n=5 bundles and EHS, hydrated for 2 minutes in saline before the test). The specimens (n=3 bundles and EHS) underwent a stepwise in situ micro-CT tensile tests. After two initial scans with specimen pre-loaded at the minimum strain allowed by the load cell sensitivity (bundles: 2%; EHS: 0%), they were progressively step-wise strained (bundles: 3%, 4%, 5%, 7%; EHS: 1.5%, 3%, 5%, 7%) and tomograms acquired (bundles: voxel size =13 μm; EHS: voxel size = 9 μm) after 15 minutes of waiting at each strain step, to allow for relaxation. To investigate the full-field strain distribution in scaffolds, a DVC analysis (SPAM software) [6] was carried out. For the analysis sub-volumes of 36 (bundles) and 40 (EHS) voxels were used with a 50% overlap (spatial resolution of respectively 468 and 360 μm). The uncertainty of the strain measurements (at the minimum strain step) was two orders of magnitude lower, compared to the in situ test values, for all samples.

Results & Discussion

Scaffolds resulted morphologically and mechanically similar to the T/L counterpart (whole tissue, fascicles and fibrils) [1]. Bundles and EHS showed a ductile behavior with large deformations. The micro-CT in situ workflow combined with DVC successfully allowed the study of the local mechanics of scaffolds. The DVC analysis on bundles and EHS revealed a progressive increment of the internal principal axial strains (ε_{p1}) during the test. Strains were mainly associated to the rearrangement of nanofibers and internal bundles during the initial loading regime and gradually distributed in the material with the progressive load. Strain peaks were also found close to clamps due to material constraint. The strain fields were consistent to those measured in natural tendon tissue [7]. These results will help to better understand the full-field mechanics of these electrospun scaffolds and their interaction with T/L cells.

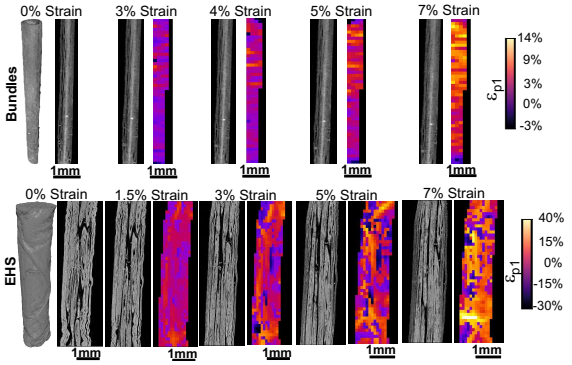

Figure 1: In situ micro-CT image and DVC-computed strain distribution (ε_{p1}) from DVC for bundles and EHS at the different strain steps.

References

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