Revealing the actual stress-strain behaviour of porous materials for scaffolds development

G. Marchiori[1], N. Sancisi[2], G. Tozzi[3], M. Zingales[4], G. Prezioso[5], A. Visani[1], G. Giavaresi[1], A. Zucchelli[2] and A. Sensini[2,6]

[1] *SC Scienze e Tecnologie Chirurgiche, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy*
[2] *Department of Industrial Engineering, University of Bologna, Bologna, Italy*
[3] *School of Engineering, University of Greenwich, London, UK*
[4] *Department of Engineering, University of Palermo, Palermo, Italy*
[5] *Me.Pre.C.C., University of Palermo, Palermo, Italy*
[6] *CTR and cBITE Departments, MERLN Institute, Maastricht University, Maastricht, The Netherlands*

***Abstract*—Market of artificial scaffolds for tissue regeneration or reinforcement is increasing. Specifically to musculoskeletal tissues, revealing the actual stress-strain behaviour of scaffold candidates is fundamental for their development. To give indications in the field, this study investigated the influence of porosity and of strain-dependent morphology on stress description, by a microtomography *in situ* test on novel electrospun hierarchical scaffolds biomimicking tendons and ligaments. Main indications for a procedure both practical and reliable getting close to the scaffold actual mechanical description resulted to consider initial volumetric fraction (i.e. porosity) and Poisson's ratio (for predicting cross-section evolution with strain).**

***Keywords*—Apparent/actual stress, porous, electrospinning, micro-CT.**

I. Introduction

A significant clinical scenario in the context of musculoskeletal tissues is the rupture of tendons and ligaments (T/L), a common debilitating orthopaedic injury. Damaged tendons – such as Achilles, patellar and quadriceps – and ligaments – such as anterior cruciate, posterior cruciate and collaterals – may need reconstruction by a graft. Standard procedures use biological grafts, i.e. autografts or allografts. However, they do not represent the optimal solution in many cases, such as revision after graft failure, risk of rejection for allografts, need for rapid recovery. For those cases, various synthetic grafts have been designed, but they still often have unsatisfactory outcomes at both the short and long term of clinical applications [1]. Nevertheless, the market of artificial T/L is expected to strongly increase globally [2].

For commercial success, the target product should be biocompatible, functional, and tunable for many clinical cases. Thus, scaffolds for T/L tissue engineering should be customized to replicate the hierarchical structure and overall the biomechanical properties of the various T/L of interest. Among the different biofabrication techniques, electrospinning emerged as one of the most promising ones to produce biomimetic T/L scaffolds, replicating the T/L structure from the fascicle [3] up to the whole tissue level [4].

When targeting the scaffold's mechanical characteristics, apparent stress-strain description has become a standard: few geometrical measures on the specimen are needed, taken at the beginning of the mechanical test using a caliper or a photo with scale (i.e. initial cross-section area and length). However, it may not describe the actual constitutive state of the material due to: (i) presence of voids (i.e., specimen density is not equal to material density); (ii) section changes during the mechanical test (i.e., with progressing deformation).

Therefore, working on electrospun biomimetic T/L grafts, the study aims to change the perspective in characterizing the mechanical behavior of tissue scaffolds, specifically by investigating (1) with a non-standard, complex, experimental testing, the hypothesis that porosity and strain-dependent morphology affect the stress metrics (i.e., actual stress); (ii) how to upgrade standard metrics and to implement morphological strain-dependency in a standard testing set-up integrated with few, simple solutions.

II. Materials and Methods

Single bundles (SB) and hierarchical structures (HS: 8 SB enveloped by a membrane) were produced by electrospinning as tendon/ligament scaffolds of poly-L(lactic) acid and Collagen type I [4].

These specimens underwent a micro-tomography *in situ* tensile test (Fig. 1). The initial length (L_0, mm) and section (A_0, mm^2) of the dried samples were measured using ImageJ on photographic macro images, while their mass (w) by a micro-balance. The initial volume fraction (f_0) was calculated as:

$$f_0 = \frac{w}{L_0 A_0 \rho} \quad (1)$$

where ρ is the density of the blend and is equal to 1.27 g cm^{-3}.

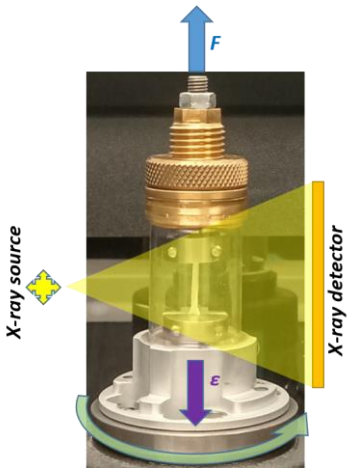

Fig. 1: material testing stage (MTS) for micro-CT *in situ* traction test; MTS rotates (green arrow) between X-ray source and detector for volumetric scan, while it tractions the sample (hierarchical structure T/L scaffold as example) imposing displacement (and thus strain, ε) and reading load (F).

Each sample was then immersed in PBS for 2 minutes before being clamped in the micro-CT testing system (MTS *in situ* mechanical tester for Skyscan 1172, Bruker, Belgium; maximum actuator displacement = 5.5 mm, displacement rate = 0.001 mm/s fixed in the loading system). The gauge length of the samples was measured as the initial clamp-clamp distance (≈ 10 mm for both SB and HS length) from a single micro-CT image at the minimum strain allowed by the load cell (0.45 N, corresponding to about 2% strain for SB and 0% strain for HS). The first micro-CT scan was acquired under these conditions. Based on the gauge length, testing system displacements were imposed to reach defined strains for SB (i.e. 3%, 4%, 5%, 7%) and for HS (i.e., 1.5%, 3%, 5%, 7%); corresponding tensile forces (F, N) were read by the load cell (max 200 N). These specific strain levels were chosen as relevant in the force-strain curves of previous *ex situ* mechanical tests on the same kind of samples [5]. A 15-minute stress-relaxation period was applied at each strain step to stabilize the sample microstructure, followed by a micro-CT acquisition [5]; in fact, by preliminary tests, we verified the time needed to obtain a stabilization of the force following a traction, identifying 15 minutes as an adequate time (on average, load relaxed of only 5% during a scan).

SB were scanned with an applied voltage of 40 kV and a current of 75 µA. The scan orbit was 180° with a rotation step of 0.8°, and 4 frames were averaged for each rotation angle; the voxel size was 13 µm, resulting in a scan duration of ~ 17 minutes. The scanning protocol for HS was the same, except for decreasing the voxel size to 9 µm to better discriminate between bundles and membranes. The image reconstruction was carried out with a modified Feldkamp algorithm using the SkyScanTM Nrecon software accelerated by GPU, applying a level-5 ring-artifact correction without beam-hardening correction and smoothing.

For each sample and strain level, the following parameters were calculated:

1. Apparent stress (MPa):

$$\sigma_{A_0} = \frac{F}{A_0} \quad (2)$$

2. Net stress (MPa):

$$\sigma_{A_0 f_0} = \frac{\sigma_{A_0}}{f_0} \quad (3)$$

3. Actual section (A, mm^2): A_0 updated by strain-dependent evolution as measured in micro-CT reconstructed images.

4. Actual stress (MPa):

$$\sigma_{Af_0} = \frac{F}{Af_0} \quad (4)$$

5. Estimated net stress (MPa): to predict cross-section area change, the second-order volume change of the specimen was considered by Poisson's ratio ν, carrying the following model for stress:

$$\sigma_{Ae2f_0} = \sigma_{A_0 f_0}/(1+\varepsilon)^{(-2\nu)} \quad (5)$$

III. Results and Discussion

Whatever the stress metric, SB showed stress values higher than HS (Fig. 2): this is due to still partially crimped bundles and membrane nanofibers not preferentially aligned with load in HS [5].

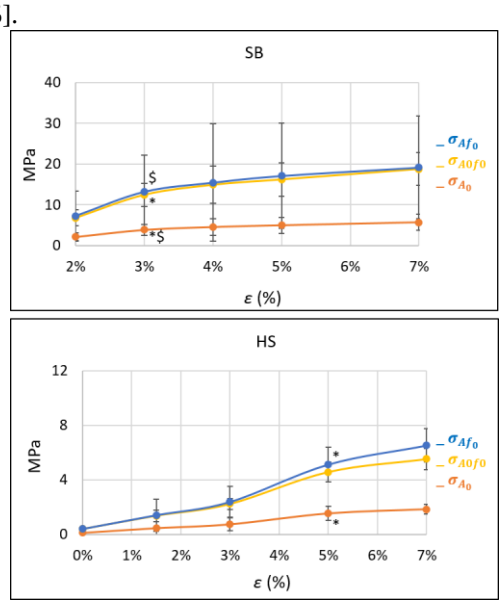

Fig. 2: curves of stress-strain (mean ± standard deviation) comparing the various samples (SB vs. HS) and the various stress calculations (σ_{A_0} vs. $\sigma_{A_0 f_0}$ vs. σ_{Af_0}). For each sample type, symbols indicate significant difference between stress-strain gradients with different stress metric, in the linear region (ε 3% for SB, 5% for HS; Kruskal-Wallis test, p-value < 0.05).

Also the gross shape of stress-strain curves was not strongly affected by the chosen stress metric: (i) at 2-3% strain, HS still appeared in toe-region, showing higher non-linearity; (ii) non-linearity near yielding occurred around 3% strain for SB and around 5% for HS; (iii) both SB and HS do not show failure until 7% strain.

However, the specific stress metric affected the computed stress, both for SB and HS (Fig. 2). The material volume fraction (f_0) – i.e. porosity (1-f_0) – had the greatest impact. To consider morphometric changes with strain – i.e. actual section (A) – had a significant role too. Indeed, for HS, stress-strain gradient in the linear region (i.e., elastic modulus) was statistically different respect to apparent (i.e. standard) stress only for actual stress. This has a functional impact: the hierarchical structures tested here show an apparent stress distant from the native T/L mechanical response, while the actual stress falls in the range of tissues with similar dimensions, making the studied scaffolds quite promising [5]. Moreover, there is a potential biological impact: σ_{Af_0} gives a measure of the actual substrate stiffness that cells will face in vivo. Our measures indicate for HS a σ_{Af_0} stress-strain gradient of 163 MPa, close to the stiffest – and most effective in terms of tenogenic differentiation – substrate studied by Islam et al. [6].

Finally, a simplification of the experimental procedure is permitted by predicting the cross-section area change with strain – i.e. the actual stress – with the model of Eq. (5). Indeed, the model accurately replicated the stress obtained by the impractical microCT *in situ* test (Fig. 3), considering equivalent Poisson's moduli of 0.3 for SB and of 1.2 for HS.

A main limitation is the lack of wet-conditions, although the samples have been immersed in PBS before testing them. However, the used material loading system does not allow either the immersion of the sample in liquid or its moistening during the test pauses. On the other hand, it permitted to maintain some humidity level inside the loading chamber, as highlighted by the X-ray attenuation levels stable between all the testing steps.

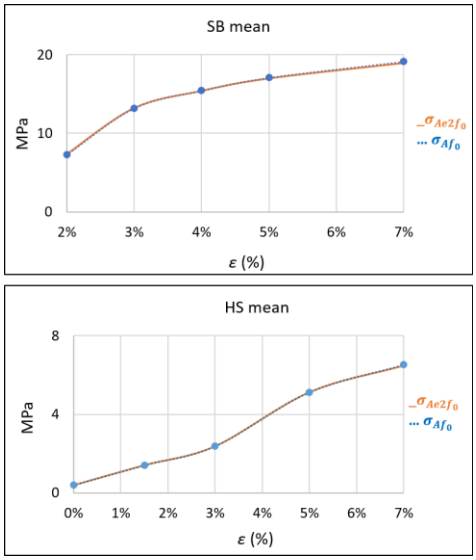

Fig. 3: curves of experimental (σ_{Af_0}) vs estimated ($\sigma_{A_{e2}f_0}$) stress-strain.

IV. Conclusion

This study gives important general indications for the reliable mechanical description and design of hierarchical, porous materials, e.g., of advanced scaffolds for tissue engineering: it suggests using strain-dependent net stress as reference metric and gives indications on how to estimate it with standard experimental testing.

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