Simultaneous Micropillar Compression and X-ray Scattering or Diffraction to Investigate Scale Effects of Strains in Mineralised Collagen Fibres

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Introduction

Skeletal diseases such as osteoporosis affect millions of patients world-wide. In order to engineer patient specific treatment solutions, it is necessary to understand the multiscale structure-mechanics hierarchy and the irreversible behaviour of the fundamental building blocks of those mineralised tissues. While bone's mechanical behaviour is well characterised at the organ level [1], the underlying non-linear properties of mineralised collagen fibre (MCF) assemblies remain unclear. Thus, the aim of this study is to investigate the post-yield behaviour of individual MCF assemblies by simultaneous micropillar strength tests and structural measurements using small-angle X-ray scattering (SAXS) and diffraction (XRD).

Methods

Mineralised turkey leg tendon (MTLT) was used as a model system. Turkey legs were obtained from a local butcher and kept frozen at -22°C until dissection. After thawing, an approximately 10 mm piece of MTLT was prepared, dried for 24 h and glued into an aluminium endcap. Sixteen cylindrical micropillars of approximately 6x12 µm were centred on a single MCF from the interstitial tendon compartment using ultra-milling (Reichert-Jung), laser ablation (Trumpf) [2] and focus ion beam (FIB) (FEI) milling [3]. A custom made microindenter (Alemnis AG) was implemented in the microfocus beamline ID13 of the ESRF. Samples were loaded with 5 nm/s to 1.5 µm deformation including 50 nm partial unloading steps every 150 nm (**Error! Reference source not found.**).

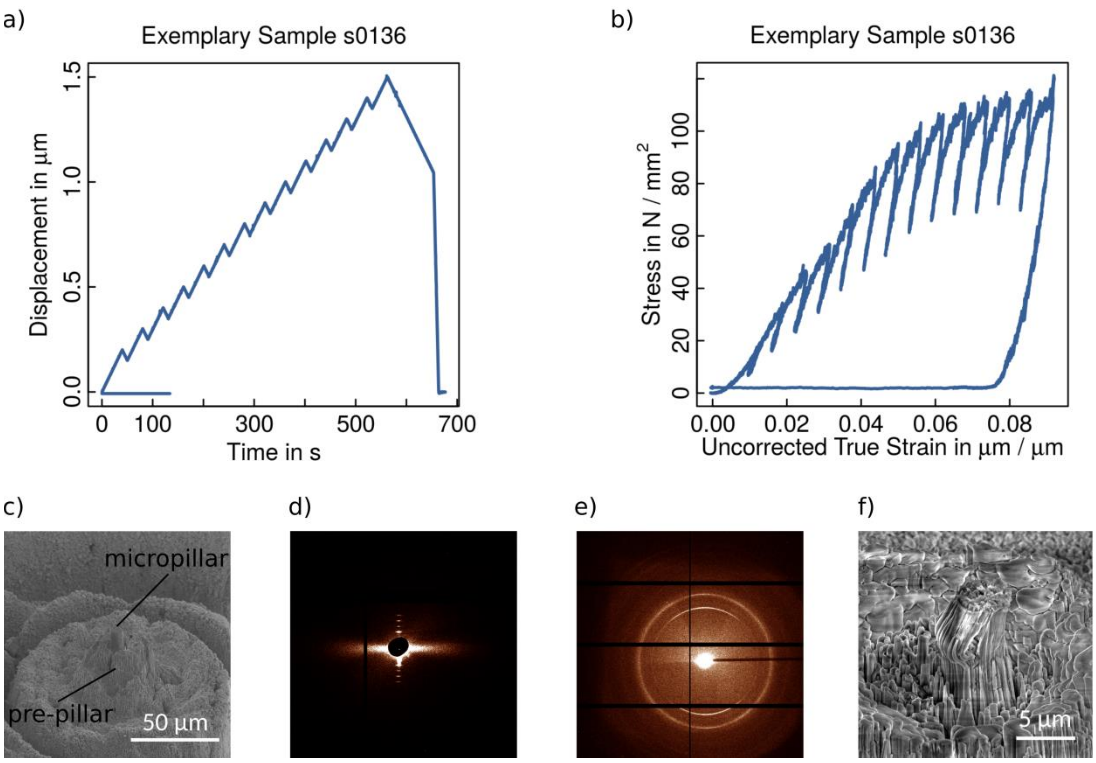

Figure 1: Results of an exemplary sample. (a) shows the monotonic loading with partial unloading steps of 50 nm every 150 nm loading and (b) the corresponding loading path. (c) shows a SEM image of the pre-test micropillar and (d) and (e) SAXS and WAXD patterns under load. (f) depicts a post-test SEM image revealing fibre kinking.

Samples were scanned in a SAXS and an XRD group to obtain the deformation in the collagen and mineral phase [4], respectively and exposed to radiation every 5 s. One sample per group was used for creep tests to investigate the influence of irradiation. Displacements and forces were base [5] and frame compliance corrected and converted into strains and stresses to determine yield point (0.2% offset), strength, and partial unloading moduli. Yield strains were toe-region corrected and plastic strains calculated according to [3]. The final unloading modulus served as substrate modulus and was used for normalisation. SAXS data were analysed via radial integration of azimuthal sectors to calculate changes in the meridional reflections [5]. Statistical tests were performed in R using a significance threshold p = 0.05. Quantile-quantile plots were used to verified normal distribution and mean ± standard deviation are reported.

Results

Two micropillars were lost due to operator failure. The mechanical data in the SAXS and XRD group were not significantly different. Yield stress and strain, strength and substrate modulus were 0.15 ± 0.05 GPa, 0.03 ± 0.01 µm/µm, 0.18 ± 0.04 GPa, and 16.47 ± 3.40 GPa. No significant increase in damage, measured as stiffness reduction, with accumulating plastic strain up to 11.9% was detectable (Figure 2). Analysis of the SAXS specimen showed a maximum fibril strain of 0.7% yielding a fibril to tissue strain ratio of 0.07. The relaxation tests confirmed no influence of irradiation.

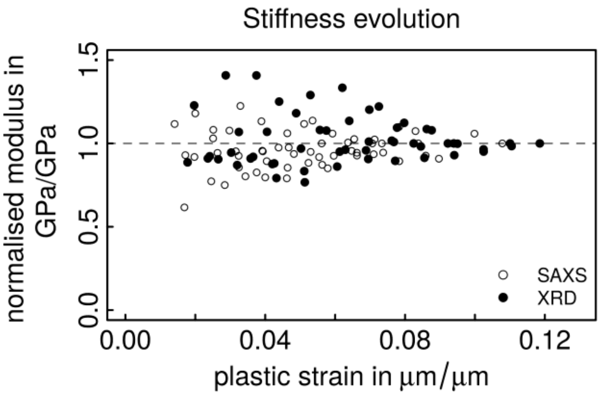

Figure 2: Normalised modulus vs plastic strain reveals an absence of damage as measured by stiffness reduction after passing the yield point.

Discussion and Conclusion

The study demonstrated the simultaneous mechanical and structural testing of individual MCF assemblies. Mechanical test results comply with tests on ovine lamellar bone, especially regarding the absence of damage [3]. Interestingly, maximum fibril strain in compression as well as the fibril to tissue strain ratio was small in comparison to findings in tension [5]. The uniaxial setup of MTLT allows to assume a system of parallel springs so that the apparent stress can be related to the fibrils whose structural arrangement are currently investigated with post-test SEM and synchrotron radiation computed-tomography at 20 nm spatial resolution. The unique test setup provides a powerful tool to gain insight into the mechanical behaviour of nanometre constituents under controlled boundary conditions. The presented data may help to unravel the non-linear properties of MCF assemblies which can inform rheological models [6] to predict the elasto-plastic behaviour of mineralised tissues. This, in turn, may then be of help to engineer personalised treatment solutions for skeletal diseases.

References

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