The structure-function relationships of human cortical bone are strain rate dependent: Insight from synchrotron X-ray imaging combined with micromechanical testing

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I. INTRODUCTION

Strain rates applied to body tissues can vary enormously, from that seen during normal walking, estimated to be in the range of 0.001s-1, rising to 0.1s-1 during downhill running [1-2]. The strain rates experienced during blast can be orders of magnitude higher, with an associated increase in the damage sustained. There have been very few attempts to examine the behaviour of bone at quasi-static to extremely fast strain rates and they only showed in a limited way that increasing the strain rate affected the mechanical properties of cortical bone [3]. However, the exact mechanism for these alterations in mechanical properties during different strain rates has not been explained due to the lack of experimental data from the hierarchical levels in biological tissues. The aim of this study was to determine the effects of strain rates on structure-function relationship of cortical bone, using in-situ mechanical testing combined with time-resolved microfocus small angle X-ray diffraction (SAXD) and wide angle X-ray diffraction (WAXD). The high brilliance of synchrotron X-ray beams enables time-resolution of the order of seconds, enabling a series of scattering or diffraction spectra to be measured concurrently during macroscopic deformation. This approach thus measures directly the deformation of the mineralised collagen fibrils [4] and mineral-platelets [5] in the bone matrix during the application of physiological loading rates, and may be considered a form of nanomechanical imaging.

II. METHODS

In an attempt to understand the strain-rate sensitivity better at a fibrillar and molecular level we conducted mechanical testing utilizing synchrotron SAXD and WAXD on human cortical bone specimens to obtain fibrillar and mineral strain, respectively. We used the same experimental protocol to previous studies [4-6] to measure fibrillar deformation at 0.001, 0.005, 0.01 and 0.05/s. Twenty cortical bone specimens were prepared (5 per strain rate) and gripped in a micro-tensile tester [4] that was mounted on a 2-axis motorised stage beam-line I22 at Diamond Light Source, UK. A synchrotron X-ray beam (wavelength 0.886 Å, beam cross section 10 × 12 μm) was used to measure the SAXD and WAXD patterns, which were collected by a Pilatus detector system. The sample-to-detector distance was 1 m for SAXD and 0.3 m for WAXD. For each strain rate a SAXD and WAXD pattern with 1 s exposure time was collected at every 1% applied external (grip-to-grip) strain up to failure. The fibril strain was measured as described elsewhere [4] by tracking the change in the D-periodicity (~ 67 nm) of the meridional banding patterns in the collagen fibrils arising from the intrafibrillar tropocollagen packing. The strain in the mineral phase was measured by percentage shifts in the wide angle diffraction spectrum [5]. The tester was inclined to the X-ray beam at half the Bragg angle for the c-axis (002) hcp apatite reflection to ensure that strain measurements are only from mineral particles with c-axis along the loading direction. Continuous stream of images are acquired by the CCD camera (Basler Vision Technologies, Ahrensreide, Germany) at a maximum rate of 100 frames per second, while the edge detection is applied and the tissue strain is calculated. The load is read out by the load cell (SLC31/00005, RDP electronics, West Midlands, United Kingdom) and transmitted to the computer where the applied stress on the sample is calculated.

III. INITIAL FINDINGS

Stress vs tissue strain curves are shown in Figure 2 (a) as functions of strain rate. Tensile modulus and failure stress of the cortical bone increased by an average of approximately 3- and 2-fold, respectively, over the strain rates tested. Representative results showing increase of fibril strain with tissue strain are shown in Figure 2 (b) for different strain rates. It was observed that fibril-to-tissue strain ratio deceases from quasi-static strain rates

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to dynamic strain rates (Figure 2 (b)). Furthermore an elastic fibrillar response can be inferred from the linearity of the stress/fibril strain plots (leading to an effective fibril modulus) (data not shown here). Increased collagen effective fibril modulus with increasing strain rates was observed from our in-situ synchrotron experiments.

IV. DISCUSSION

In human cortical bone, the slope of stress vs tissue strain curves increase with strain rates has been established by previous studies [1-3] and confirmed by this study. But our nanoscale results demonstrate deformation mechanism for this strain rate dependency which was inaccessible before. The stiffening of the collagen showed by reduction of fibril-to-tissue ratio at dynamic strain rates associates with changes in the organic matrix of the bone. This mechanism could make the bone stiffer and more predisposed to catastrophic fracture simply by overloading the mineral phase. Since the collagen fibrils are surrounded by extrafibrillar matrix, a possible mechanism could be that at higher strain rates critical interfacial shear strength between fibrils and matrix exceeds rapidly. When this occurs, due to frictional loss matrix flows past the fibrils hence reduced fibrillar strain. This information will be directly used to improve non-fatal injury mitigation through the development of physical and computational models that will allow us to understand, simulate and predict fractures during different injuries.

V. REFERENCES

[1] Lanyon et al, *Acta orthopaedica Scandinavica*. 1975, 46(2):256-68 [2] Burr, *Bone.* 1996, 18(5):405 [3] McElhaney JH. *J Appl Physiol,* 1966, 21(4) [4] Karunaratne A, et al. *JBMR*, 2012, 27(4) [5] Gupta HS, et al. *PNAS,* 2006, 103(47):17741-17746 [6] Karunaratne A, et al. *Bone,* 2013, 52(2).

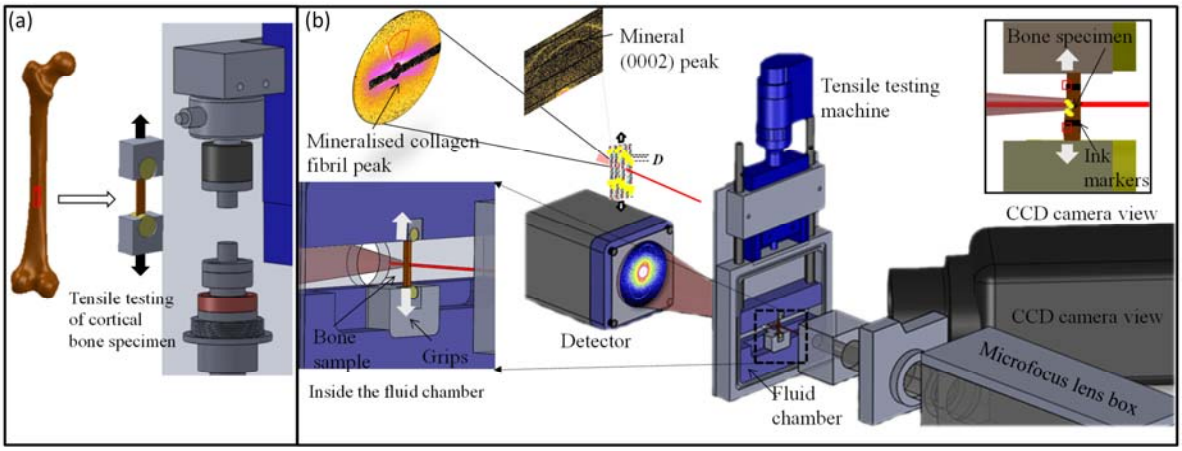

Fig. 1. (a) Tensile testing grips and specimen mounting (b) The experimental setup for the in situ tensile testing with synchrotron SAXD and WAXD. Sample is immersed in the fluid chamber

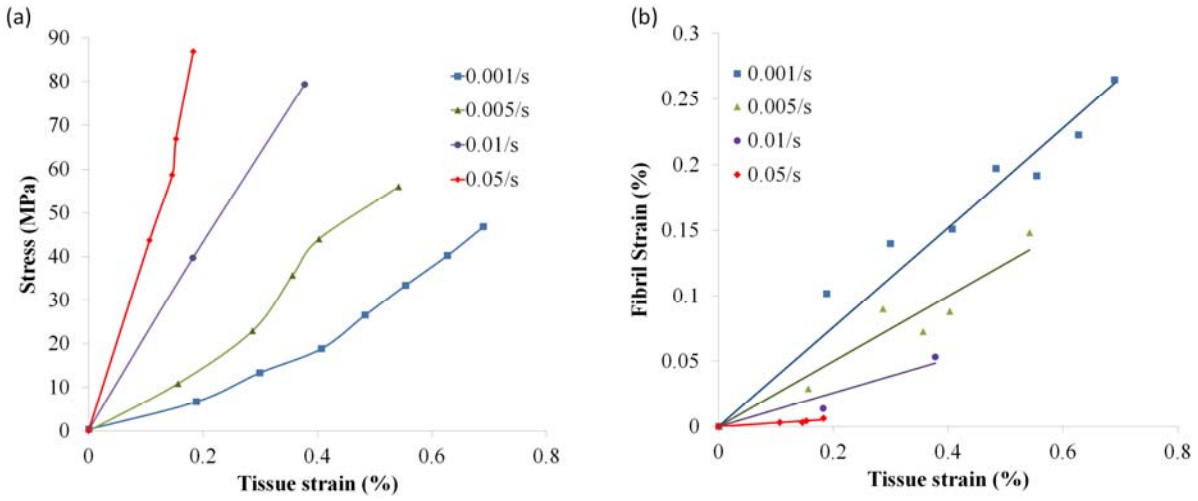

Fig. 1. (a) Typical stress – strain curves for 5 different specimens for different strain rates (b) Typical fibril strain – tissue strain curves for 5 different specimens different strain rates.