AFM-BASED MICROBEAM BENDING OF HUMAN CORTICAL BONE AT THE LAMELLAR LEVEL

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INTRODUCTION

Bone is a biological material with unique mechanical properties, owing to a complex hierarchical structure from the nanoscale up to the macroscale. To better understand bone mechanics, investigation of mechanical properties of structural elements on all hierarchical levels and how they interact is a promising approach. In this context, mechanical testing of individual structural elements at the microscale, such as individual lamellae, remains a challenge. Focused ion beam (FIB) milling is an attractive technique for machining microscopic bone samples. So far, animal bone microbeams[1][2][3] and micropillars[4][5][6] have been mechanically tested in bending via atomic force microscopy (AFM), or compression via conventional nanoindentation, respectively, mostly in dehydrated state except for one study. Similar experiments on human bone have, to the best of our knowledge not been reported. Here we present an AFM-based microbeam bending method applied for the micromechanical assessment of human cortical bone in both dehydrated and rehydrated state.

METHODS

Bone samples from the femoral midshaft of 4 male donors, aged 65-94y, were used and 4 microbeams per donor were produced. The FIB-machined microbeams (in cooperation with E057) were milled from a single bone lamella and were bent at multiple positions along the beam length[7] with an AFM tip furnished with a glass microsphere of 5 µm diameter for minimizing indentation effects (Fig.1A). The same microbeams were tested, first dehydrated in air, and, second rehydrated in Hank's Balanced Salt Solution (HBSS) after rehydration time of 2h. The measurement setup was previously calibrated by bending FIB-milled Si microbeams of known stiffness (in cooperation with E164) utilising an *in-situ* picoindenter situated within a scanning electron microscope (SEM). From the measured force vs. deflection curves apparent stiffness data along the microbeam length was obtained (Fig. 1B) and plotted against beam position (Fig.1C). The data was then fitted to obtain a value for the bending modulus as a fit parameter (Fig.1C). Additionally, the dissipated energy during bending of the rehydrated bone microbeams was calculated as the difference between the areas under the loading and unloading curve (Fig. 1B).

RESULTS AND DISCUSSION

Microbeams of length, width and height (25 x 4 x 1) μm^3 were made by FIB milling (Fig.1A) from a single bone lamella. Precise positioning of the microbeam within the AFM was achieved by imaging the base of the microbeam in force mapping mode (Fig. 1A). Bending modulus values obtained from bending measurements along the beam length ranged (25.1-48.7) GPa in air and (7.3-19.1) GPa in HBSS. Decrease of bending modulus up to 5 times was observed for microbeams upon rehydration. No significant change of bending modulus was observed with respect to age

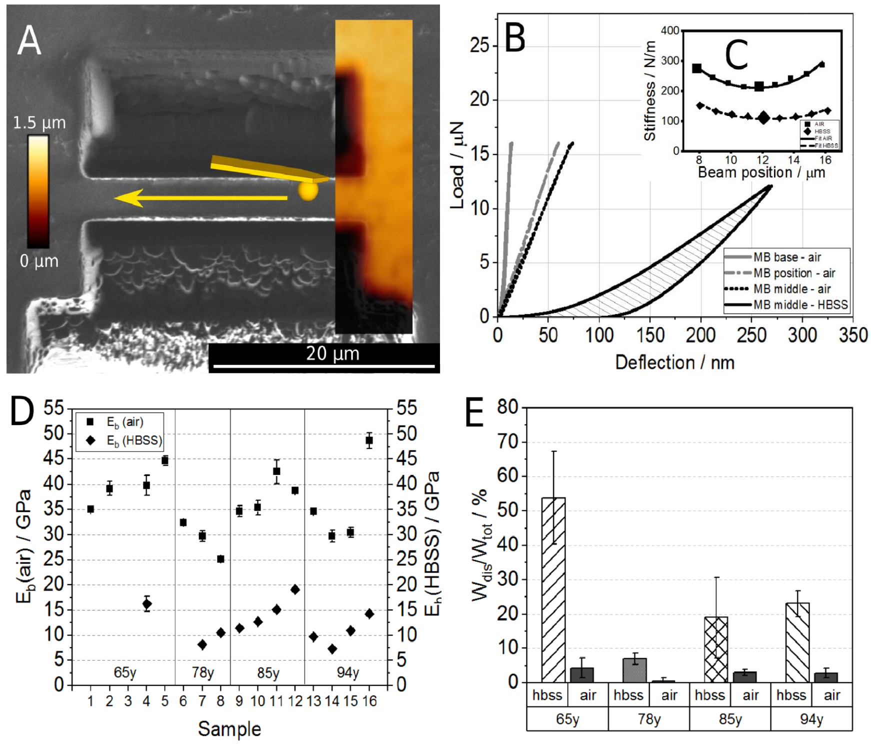

Figure 1: (A) SEM image of a bone microbeam with overlayed AFM force map (setpoint height) of the beam base. (B) Load vs. deflection bending curves at different beam positions in air (grey solid, grey dashed & black dotted) and HBSS (black solid). (C) Apparent stiffness vs. beam position from measurement (points) and fit (lines). (D) Bending moduli in air (squares) and HBSS (diamonds) of all tested microbeams from all donors. (E) Relative dissipated energy during bending vs. donor age in air and HBSS.

(Fig.1D). Moreover, bending in air exhibited linear elastic behaviour, with same apparent loading and unloading stiffness (grey solid, grey dashed and black dotted curves in Fig.1B), whereas in HBSS stiffness was higher during unloading compared to loading, and mechanical hysteresis was observed (black solid curve in Fig.1B). The dissipated energy in rehydrated samples (area between loading-unloading curves) ranged from 0.093 to 0.655 pJ (i.e. 5.8-64.5% of the total energy) and showed a trend to decrease with age (Fig.1E).

The findings show the importance of water for the micromechanical properties of bone. In air, beams appear linear elastic, whereas addition of water and salts leads to decrease of stiffness, and inelastic behaviour with significant energy dissipation. The data suggests there could be an effect of age, with possibly lower energy dissipation in bone from older donors. This in turn may play a role in decrease of fracture toughness and increased fragility of bone with age[8][9].

CONCLUSION

In conclusion, microbeam bending of single bone lamellae microbeams within an AFM was developed, both in dry and rehydrated conditions. No significant change in the elastic properties was observed with respect to age. However, our data indicates there could be a change in the viscoelastic properties and the ability to dissipate energy in bone from older donors. To further investigate these effects, the number of donors and age range should be increased and mechanical response at different loading rates should be obtained in further studies. Finally, as bone is hydrated in its physiological state and water plays a significant role in the mechanical properties of the tissue, experiments should preferentially be performed in hydrated conditions.

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