

Microstructure, Interfaces, Composition - Towards Better Microscale Experimentation and Models of Bone

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ABSTRACT

The evaluation of fracture risk in osteoporotic patients is still mostly based on Bone Mineral Density (BMD) measurements. During the past decades the research community has identified that not only bone mass, i.e. BMD, but also bone quality should be evaluated in order to achieve a reliable diagnosis of bone fracture risk for the individual. Bone quality includes among many other parameters the matrix material properties, which are dependent on the ultra- and microstructural arrangement of components that make up bone tissue. These components are non-stoichiometric carbonated apatite, collagen type I, water and noncollagenous proteins (NCPs). While the relative amount of NCPs is small they seem to accumulate in interfaces i.e. inter-lamellar areas and cement lines and densely populate fracture surfaces, which are mostly located within those areas [1, 2]. Our past research has shown that NCPs, such as osteopontin, have the capability to form strong networks, especially in the presence of metal ions with more than one positive charge [3]. These networks have a molecular self-healing mechanism and are able to repeatedly dissipate large amounts of energy as well as a capability to store energy upon compression. Interestingly, we could further show that the deletion of osteopontin in a knockout mouse model leads to a significant reduction in fracture toughness, which cannot be explained by changes in porosity, bone mineral density, ultrastructure or collagen scaffold mechanics [4]. This underpins our hypothesis that osteopontin and other NCPs strengthen interfaces, perhaps at several hierarchical levels, toughening bone and impeding crack propagation. To further investigate this hypothesis nanoscale mechanical experiments are necessary. However, given the limitations of size it currently seems highly challenging to investigate the post-yield and fracture behavior of bone at the smallest building block, i.e. the mineralized collagen fibril. For this reason we investigated bone fracture and post-yield behavior rather at the microstructural than the nanostructural level, i.e. within multiple lamellae focusing on inter-lamellar areas (interfaces) and cement lines, which are enriched in NCPs.

Atomic force microscopy (AFM) combined with in situ micromechanical testing of bovine bone nanostructure enabled to image and monitor the stepwise progression of cracks in bone the microstructure of transverse surfaces of femoral bone samples. Firstly, these experiments confirmed that cracks in bone preferably propagate along interfaces, i.e. inter-lamellar areas and cement lines as shown in Figure 1a.

Cantilever-based nanoindentation on lamellae and inter-lamellar areas showed that the latter are significantly less stiff. Upon application of a transverse tensile load the inter-lamellar areas selectively stiffen. An explanation of the different behavior of lamellae and inter-lamellar areas can be

formulated from further characterization experiments: μ -Raman imaging showed that inter-lamellar areas are enriched in NCPs, whereas the collagen concentration is lowered compared to lamellae. Further, AFM imaging did show that also the collagen orientation changes in the inter-lamellar areas to “lying” (transverse) fibrils rather than “standing” (longitudinal) in the lamellae. This would mean that strains in inter-lamellar areas and cement lines are elevated upon loading in bone and that these microstructural features do guide cracks, providing toughening mechanisms via crack deflection, energy-dissipation in the NCP moiety, ligament bridging. In addition, inter-lamellar areas and cement lines seem to provide a mechanism similar to elastic bearing pads, i.e. allowing for micromotion and energy dissipation within bone tissue without the generation of cracks. Finite element models guided by microstructural investigations as well as using literature values for lamellar as well as inter-lamellar and cement line stiffness agrees well with this explanation. Strains are largely amplified in the soft interfaces as expected as shown in Figure 1b.

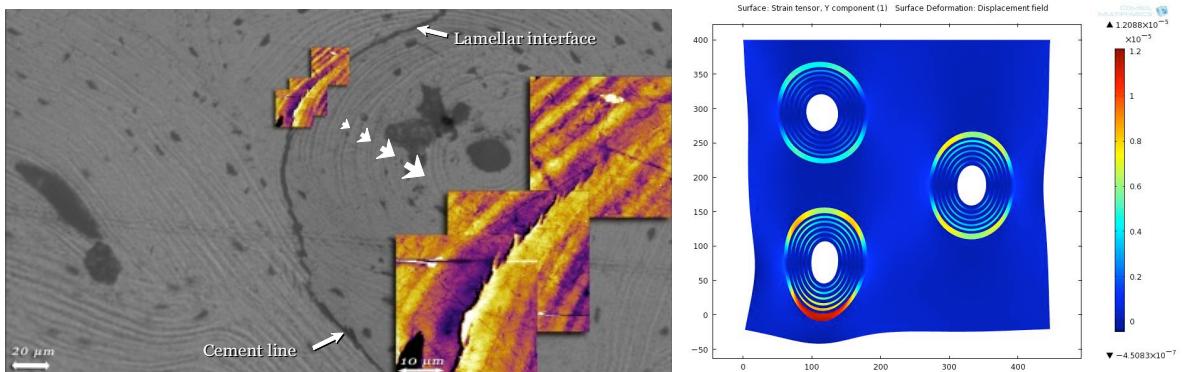


Figure 1: a) AFM and light microscopy composite image showing crack propagation in bovine femoral bone (transverse surface) (left) b) (right) Local vertical strains of a 2D bone structure subjected to tension in the vertical direction in silico, strains are notably amplified in the “soft” interfaces

Overall, our research points to the fact that soft interfaces and in part composed of NCPs are important for modulating the properties of the hierarchical composite bone to achieve both high stiffness and toughness and could also be important for mechanobiological processes. Changes in bone composition and alteration of micromechanical properties could therefore impair the mechanical competence. From this perspective there is also potential for future diagnostics and therapy targeting bone composition and compact bone microstructure in addition to bone mass.

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