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Nanoscale deformation mechanisms and yield properties of hydrated bone extracellular matrix

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ABSTRACT

Bone features a hierarchical architecture combining antagonistic properties like toughness and strength. In order to better understand the mechanisms leading to this advantageous combination, its postyield and failure behaviour was analyzed on the length scale of a single lamella. Micropillars were compressed to large strains under hydrated conditions to measure their anisotropic yield and post-yield behaviour. An increase in strength compared to the macroscale by a factor of 1.55 and a strong influence of hydration with a decrease by 60% in yield stress compared to vacuum conditions were observed. Post-compression transmission electron microscopic analysis revealed anisotropic deformation mechanisms. In axial pillars, where fibrils were oriented along the loading axis, kink bands were observed and shear cracks emerged at the interface of ordered and disordered regions. Micromechanical analysis of fibril kinking allowed an estimate of the extrafibrillar matrix shear strength to be made: 120 ± 40 MPa. When two opposing shear planes met a wedge was formed, splitting the micropillar axially in a mode 1 crack. Making use of an analytical solution, the mode 1 fracture toughness of bone extracellular matrix for splitting along the fibril direction was estimated to be 0.07 MPa$\sqrt{m}$. This is 1–2 orders of magnitude smaller than on the macroscale, which may be explained by the absence of extrinsic toughening mechanisms. In transverse pillars, where fibrils were oriented perpendicular to the loading axis, cracks formed in regions where adverse fibril orientation reduced the local fracture resistance. This study underlines the importance of bone's hierarchical microstructure for its macroscopic strength and fracture resistance and the need to study structure-property relationships as well as failure mechanisms under hydrated conditions on all length scales.

Statement of Significance

Bone's hierarchical architecture combines toughness and strength. To understand the governing deformation mechanisms, its postyield behaviour was analyzed at the microscale. Micropillars were compressed in physiological solution; an increased strength compared to macroscale and an influence of hydration was found. Transmission electron microscopy revealed cracks forming in regions with adverse fibril orientation in transverse pillars. In axial pillars kink bands were observed and shear cracks emerged at the interface of ordered and disordered regions. It was estimated that bone's fracture toughness for splitting between fibrils is significantly smaller than on the macroscale. This study underlines the importance of bone's hierarchical microstructure and the need to study structure-property relationships on all length scales.

1. Introduction

Bone is a biological material featuring a hierarchical architecture combining antagonistic properties like toughness and strength with a low specific weight. In order to better understand the mechanisms leading to this advantageous combination of

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attributes, its mechanical behaviour has to be assessed on all length scales.

Bone provides mechanical support to the musculoskeletal system, metabolizes calcium and produces bone marrow [1,2]. On the nanoscale, it is made up of parallel mineralised collagen fibrils (MCF) embedded in an extrafibrillar mineral matrix (EFM) consisting of mineral, non-collageneous proteins and water [3,1,2]. The MCF line up in fibril arrays, which form lamellae in a rotated plywood pattern [4]. Osteocytes and their processes reside in the lacuno-canalicular system, which makes up for about 1% of bone porosity. Compact or cortical bone consists of lamellae arranged concentrically around blood vessels forming osteons surrounded by a cement layer with a porosity of around 5–15% [2]. In large, fast growing animals fibrolamellar or plexiform bone is laid out first before it is converted to osteonal bone through a remodeling process [5,2].

Macroscopic mechanical tests on bone have been performed for more than a century [5,6]. However, several challenges remain, mostly due to spatial, inter-subject, disease or age variation. Considering bone as a hierarchical material and probing its properties on several length scales is of high relevance as its inelastic behaviour at a lower level of tissue organisation influences the mechanical behaviour of the whole organ.

On the macroscale, bone is known to react to overloading mainly by formation and coalescence of families of microcracks, whose orientation depend on the loading mode [7,8,6], leading to a quasi-brittle response. On the nanoscale, *in situ* small angle X-ray scattering (SAXS) and wide angle X-ray diffraction (WAXD) measurements under tensile loading showed that mineral platelets carry a significant portion of the load and are insensitive to internal flaws due to size effects [9]. The organic phase transfers the load to the mineral platelets; it was shown that apparent yielding in parallel-fibred bone was caused by inelastic deformation of the extrafibrillar matrix [10]. Interpretation of measured yield stresses at variable temperature and strain rate using an *Arrhenius*-type rate equation revealed the activation energy and volume of the underlying deformation mechanism to be around 1 eV and 1 nm^3, respectively, corresponding to charge interactions between molecules in the extrafibrillar matrix [11]. Furthermore, it was suggested that organic networks featuring sacrificial bonds and hidden length are a major determinant for bone toughness [12]. It was shown that toughness increased significantly in the presence of Ca^{2+} ions, which also hints at the presence of an ion-mediated organic glue between mineralised collagen fibrils [13].

These experiments shed light on the possible deformation mechanisms of bone on the nanoscale. However, when testing specimens of hundreds of micrometres to millimetres in size, the measured data is integrated over the response of several structural units including inhomogeneities like cement interfaces, pores, changes in fibril orientation and mineral content, as well as extrinsic toughening mechanisms caused by the multiscale microarchitecture [14–17]. As a consequence, interpretation in terms of the local deformation mechanisms or yield properties on the length scale of a single lamella is not easily extracted. Therefore, micromechanical testing methods are an attractive alternative for answering this question.

One of the most common micromechanical testing techniques for bone is microindentation [18–21]. A diamond probe with a known geometry, e.g. a three sided pyramid, is pressed into a polished sample surface and force and tip displacement are recorded simultaneously. Elastic properties may be extracted from the elastic unloading part of the indentation curve using contact mechanics [22]. The mean indentation pressure, or hardness, is related to the yield and post-yield characteristics of the studied material [23]. However, it is very difficult to uniquely interpret indentation curves in terms of nonlinear material behaviour unless the dissipative mechanisms are well understood [24,25]. Therefore, there is a need for independent experiments assessing the yield and failure properties as well as deformation mechanisms of bone on the extracellular matrix (ECM) level.

Such an alternative experimental setup is micropillar compression [26–28]. Micron sized pillars are produced by erosion of material using a focussed ion beam (FIB) and compressed with a flat punch diamond indenter. Due to the mostly uniaxial loading conditions [29], the interpretation of the resulting force-displacement curves in terms of stress-strain behaviour is relatively straightforward. This is a clear advantage when assessing yield properties compared to microindentation, which results in a heterogeneous and multiaxial stress state under the indenter [23]. Recently, this method has been applied to lamellar bone tissue [28,30,31] in the dry condition. An increased strength and ductility was found in all studies compared to macroscopic properties. Also, an absence of damage was noted at strain levels up to 8% [28]. However, several studies have shown that the hydration state of the sample is an important factor influencing the properties on several length scales [32,33,20,34,35]. Therefore, it is desirable to perform micropillar compression experiments on bone extracellular matrix under rehydrated conditions in order to obtain its physiologically relevant yield and failure properties. Finally, as failure is a highly localised phenomenon, probing small pieces of bone with a minimum of interfaces or flaws under controlled boundary conditions allows large inelastic strains in the sample to accumulate at known stress levels. This in turn can be used to reveal further insights into the nanoscale deformation mechanisms when combined with post-test analytical methods like transmission electron microscopy (TEM).

The aims of this study were to a) develop a setup and protocol for micropillar compression under hydrated conditions, b) measure the anisotropic compressive yield and failure properties, c) visualize the anisotropic deformation mechanisms under uniaxial loading, and d) interpret the findings by combining models of the observed failure mechanisms with a continuum micromechanical description of the extracellular matrix. Microindentations were performed in the axial and transverse directions in ovine osteonal bone hydrated in Hank's buffered saline solution (HBSS) [28]. Micropillars were prepared in the axial and transverse directions, rehydrated in HBSS and compressed under liquid immersion. The results were compared to micropillar compression in vacuum [28], dry and hydrated nanoindentation [28], as well as macroscopic data from the literature [36]. Approximately 200 nm thin lamellae were prepared from tested micropillars in the axial and transverse directions as well as unloaded controls and analysed using Scanning Transmission Electron Microscopy (STEM) and Selected Area Diffraction (SAD) to determine the governing deformation mechanism of hydrated bone extracellular matrix under uniaxial compression as a function of microstructure. Finally, micromechanical modeling was combined with analytical solutions for the observed failure mechanisms in order estimate the mode 1 fracture toughness of ECM for splitting along the fibre direction as well as the shear strength of the EFM.

2. Materials & methods

2.1. Sample preparation

Two ovine tibiae were acquired from a local abattoir. Axial and transverse specimens were cut from the diaphyses with a diamond-coated band saw (Exact, Germany) and embedded in but not infiltrated by Cu-filled polymethyl methacrylate (PMMA, Technovit 5000, Heraeus, Germany). The embedded specimens were glued onto scanning electron microscope (SEM) stubs with

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