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Uniaxial compressive behavior of micro-pillars of dental enamel characterized in multiple directions

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ABSTRACT

In this work, the compressive elastic modulus and failure strength values of bovine enamel at the first hierarchical level formed by hydroxyapatite (HA) nanofibers and organic matter are identified in longitudinal, transverse and oblique direction with the uniaxial micro-compression method. The elastic modulus values (~70 GPa) measured here are within the range of results reported in the literature but these values were found surprisingly uniform in all orientations as opposed to the previous nanoindentation findings revealing anisotropic elastic properties in enamel. Failure strengths were recorded up to ~1.7 GPa and different failure modes (such as shear, microbuckling, fiber fracture) governed by the orientation of the HA nanofibers were visualized. Structural irregularities leading to mineral contacts between the nanofibers are postulated as the main reason for the high compressive strength and direction-independent elastic behavior on enamels first hierarchical level.

1. Introduction

Biomineralized materials are composites of stiff and organic matter that are mostly arranged in different structural motifs from nanometer to micrometer length scales with increasing complexity. The composition and unique hierarchical design of these natural materials are believed to be selected over millions of years of evolution to fulfill their mechanical functions, which require a combination of high strength, stiffness and resistance to catastrophic failure (damage-tolerance). Enamel, for example, being the most highly mineralized tissue of all vertebrates demonstrated its load-bearing ability even after the initiation of failure [1–3], which is uncommon for ceramics and hence widely attributed to its hierarchical and highly directional microstructure.

Enamel is made predominantly of crystalline calcium phosphates in the form of extremely long hydroxyapatite (HA) fibers (Fig. 1c). They are nanometer-sized in width and thickness and their length continuously spans the whole enamel thickness from dentin-enamel junction (DEJ) to the outer surface (OS) (Fig. 1a). These HA nanofibers bundle together and form micrometer-sized rods and interrod (ir) layers (called also as "matrix" or "sheet") that are either surrounding or sandwiching the rods. The latter is illustrated in Fig. 1c. Depending on the spatial location with respect to the DEJ, rods may arrange parallel to each other or as observed in most mammals, they bundle together into bands and these bands change their orientations periodically forming a complex decussation pattern (Fig. 1b). The hierarchical levels of enamel can be counted simply: HA nanofibers (intra-rod) (level-1), multiple rods (level-2), and decussating bundles of rods (also known as Hunter–Schreger bands-HSB) (level-3). Each level of hierarchy in enamel has a composite nature. HA nanofibers and rods are delimited with less mineralized porous space, which are often postulated to be filled by remnant organics and water [4]. Moreover, the relative amounts of the mineral, organic and water contents vary in different teeth and also spatially in a single tooth from OS to DEJ [5–8]. Despite this structural information, stress–strain curves of individual structural elements and their direction-dependency are still lacking in the literature. However, for multiscale modeling studies seeking to model the overall mechanical performance of hierarchical structured materials as a function of the properties of their basic building blocks [9,10], the mechanical information obtained at lower length scales is essential and therefore needs to be characterized experimentally.

The aim of this study is to determine the compressive stress–strain curve of bovine enamel at the first hierarchical level in multiple directions and deriving from it the failure strength, elastic modulus, failure mechanisms. Small-scale mechanical characterization of enamel has been largely based on the nanoindentation technique up to date [6–8,11–15,15–21]. However, the indentation

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method quantifies material properties under a multi-axial stress-state and highly constrained conditions that average the properties of the underlying bulk material. Therefore it is difficult to identify anisotropic mechanical properties. A more adequate but challenging method has been established over the last decade for small-scale characterization of materials using micro-fabricated specimens via focused ion beam (FIB) [22,23]. The advantage of this method is to be able to isolate structures corresponding to different hierarchical units in desired shapes and sizes, obtain their stress–strain curves under uniaxial loading and also capture their failure modes. Considering the fact that enamel operates predominantly under compressive stresses, it is of great interest to assess these properties under compression, which is the subject of this work. Micro-pillars consisting of isolated nanofibers from the bulk enamel were fabricated by FIB milling and subjected to the micro-compression test, which has been previously used by Han et al. [24] to assess the mechanical anisotropy in a HA-based nanocomposite exoskeleton. To evaluate enamel's direction-dependence, nanofibers were examined here in different orientations. The deformation curves and failure modes were captured. Moreover, specific features of the microstructure were identified and their relevance to registered results was discussed.

2. Materials and methods

Bovine enamel was investigated in this study and its specific structure is displayed in Fig. 1. It is important to mention here that bovine incisors are considered as an appropriate substitute of human teeth in dental research due to their chemical and structural similarity [25,26]. Permanent mandibular incisors were extracted at a local slaughterhouse (Lippeck & Richter GmbH, Hamburg). The research procedure was approved by the Ethics Committee of the Medical Association of Hamburg. Roots were cut off, the pulp interior was removed and teeth were disinfected in 0,1 wt.% Thymol solution for 24 h. The teeth were then rinsed and further stored in Hank's Balanced Salt Solution (HBSS, Invitrogen, USA) till the FIB specimen fabrication. A total of four teeth samples were used, from which slices of around 2 mm thickness (Fig. 1a) were cut out from the middle using a Buehler Isomet 4000 precision saw under water irrigation. Smaller pieces from those slices were cut subsequently and ground with 1200 grit SiC paper and further polished with 1 μm, 0.25 μm and 0.05 μm diamond suspensions. The polished surfaces were subsequently etched for 1 s using 36% hydrochloric acid and again polished with a 1 μm diamond suspension until the enamel rod boundaries were barely visible under a light microscope in order to set the FIB milling location correctly. Then all samples were bonded to electron microscope stubs using conductive silver and were further vacuum dried. Finally they were sputter-coated with a thin gold layer (a few nanometers).

Micro-pillar specimens were machined using a CrossBeam Zeiss Auriga Canion FIB system using gallium ions at 30 kV. Annular milling technique [22], where a high-beam current of 2 nA for quickly removing material from around the volume of interest and then fine milling currents (50–200 pA) for fine-tuning the specimen into the desired shape, was employed to cut cylindrical micro-pillars in this study. The nominal orientation of the rods and consequently the nanofibers within the rods, out of which the micro-pillars were milled out, was judged from the shape of the rod boundaries (Fig. 2a and b). The micro-pillars milled out within these rods (marked with a pink spot in Fig. 2b) are assigned as "transverse (T)" specimens, where the compressive loading direction was perpendicular to the nanofiber long axis. Fig. 2c illustrates the milling location of the specimen groups; "longitudinal (L)" (nanofibers are parallel to the loading direction), "oblique (O)" (nanofibers are inclined with respect to the loading direction at ~23°). Tapering of the micro-pillars produced by FIB milling is inevitable and it leads to a gradually increasing diameter along the micro-pillar from top to bottom. The diameter of the top (D_1), the diameter of the bottom (D_2), and the height (H) of the micro-pillars were measured using scanning electron microscopy (SEM) (Fig. 2d) and the values are listed in Table 1.

As prepared micro-pillars were loaded in an Agilent Nano Indenter G200 system by using a diamond flat-ended punch (with a diameter of 10 μm) in ambient conditions. Using the nanoindentation system's optical microscope, the nanoindenter tip was positioned with an accuracy of 1 μm at the micro-pillar's top surfaces. Trenches with a diameter of ~20 μm and a height of ~2 μm were milled around the micro-pillars to ensure that the nanoindenter tip loads only the micro-pillar itself and not to the surrounding material. Tests were set in general to a single load/hold/unload cycle to prescribed displacements with a loading segment imposing a constant strain rate target of 0.05 s^{-1}. The unloading rates were almost twice as fast as the loading rates. Typically a test takes 100–200 s depending on the given compression depth. Automatic correction for the frame compliance and thermal drift were implemented on the load–displacement data. The micro-pillars were not loaded till fracture in a single step but with successive cycles of gradually increasing displacement targets, which lie between 50 and 200 nm. After each loading cycle, the micro-pillars were imaged by using Zeiss Supra 55 VP scanning electron microscope (3 kV, 10^{-6} mbar).

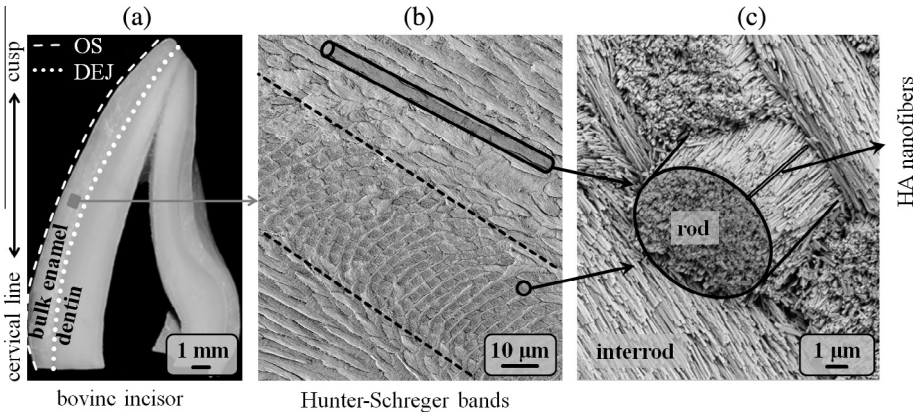

Fig. 1. Hierarchical organization of bovine enamel: The outermost layer of bovine tooth cross-section is occupied by bulk enamel (a). In the inner part of bulk enamel, groups of rods decussating with each other form Hunter–Schreger bands (b). Each rod is made of bundles of HA nanofibers (c). The HA nanofibers lying outside the rods form interrod layers.