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On the systematic documentation of the structural characteristics of bovine enamel: A critic to the protein sheath concept

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ABSTRACT

The common structural description of bovine enamel used in materials science studies – nano-sized hydroxyapatite crystallites form micron-sized prisms surrounded by protein sheaths, which in turn build a complex decussation pattern – overlook many important morphological information. This hampers the correct interpretation of the data determined by mechanical analysis. For a profound structural description of enamel morphology, the visualization of its building blocks by high-resolution electron microscopy and focused-ion beam tomography technique, which reveals their form, orientation and configuration at different regions of a tooth (cut in different directions), is undertaken in this work. We adapted here the paleontological classification system and terminology developed for the description of enamel microstructures seen in different species, and accordingly documented the morphological singularities of bovine incisor enamel. The appearance of the boundary regions between crystallites and prisms contradicts to the well-known protein sheath concept. Neighboring crystallites and prisms are not separated by prominent gap zones but they are largely in contact with each other. Proteins might exist within the pores of 20–30 nm in size, which are distributed inhomogeneously through the boundary regions, rather than as protein sheaths covering each crystallite and prism.

1. Introduction

Dental enamel is known to be the hardest tissue of all vertebrate body, due to its high mineral content (>95 vol%, the rest is water and remnant proteins [1]), yet it can sustain cracks without catastrophic fracture under millions of chewing cycles. In order to gain more insight into the origin of its damage-tolerance, a number of experimental and numerical studies have been conducted [2–22], which base their understanding primarily to the following structural description. Enamel has a hierarchical assembly of long nm-sized hydroxyapatite-HA ($Ca_5(PO_4)_3(OH)$) crystallites form into μm-sized prisms, which in turn build Hunter-Schreger Bands (a well-ordered decussation pattern formed by prism bundles) in the inner part of the structure [23]. Moreover, it is commonly stated in the – primarily materials science – literature that each crystallite

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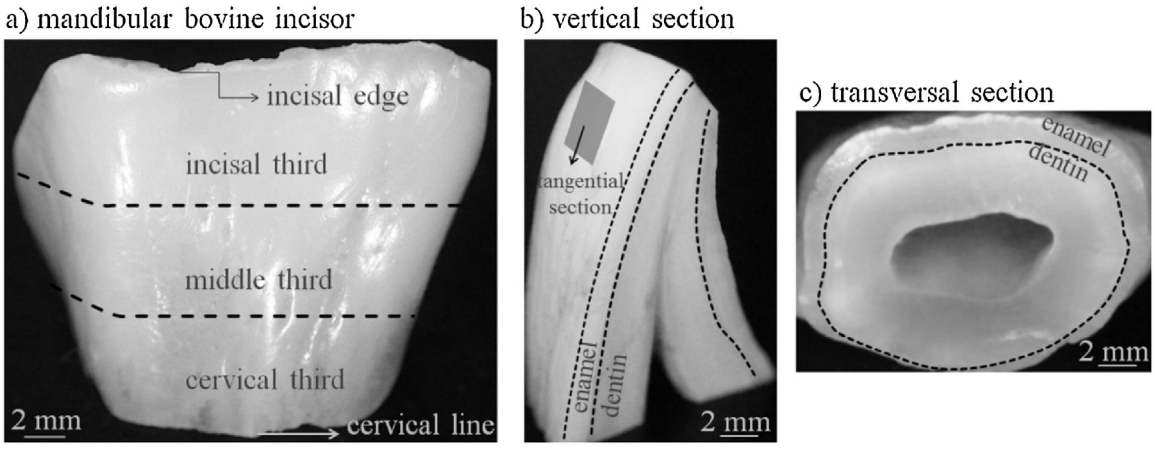

Fig. 1 – Bovine incisor tooth and cutting sections.

and prism is surrounded with gap zones filled by proteins forming the so-called "protein sheath" [5,7,12,13,23–29], by which the structure is held together and gained deformability [2,11,20,24,30–34].

This conception that the enamel prisms are surrounded by a continuous sheath was derived from electron microscopy studies that were carried out several decades ago. These studies investigated developing enamel [35–44] where a more abundant amount of proteins is present. During maturation most of the proteins are degraded, leaving only a remnant in the mature enamel. However, the remnant proteins are nevertheless often allocated to a sheath surrounding the prisms [25,26,45].

Because of this structural information, studies on the prediction of the deformation behavior of enamel implemented composite models for their analysis. However, there are inconsistencies between the modelling results and experimental ones. For instance, according to the hierarchical composite models [46,47] the failure strength and elastic modulus of enamel should increase as a function of hierarchy (from higher to lower levels), because each additional level adds more protein to the structure. Experimental outcomes, on the other hand, revealed that there is no significant difference in the elastic modulus of enamel among different hierarchical levels and also testing orientation [48,49]. Moreover, in the deformation behavior of bulk enamel (encompassing all levels), there is no non-linear region that can be associated with the plastic deformation of proteins but are successive load drops (resembling the progressive failure observed in artificial fiber-reinforced composites [50]) being a reflection of the operation of crack-stopping mechanisms [51,52]. And these mechanisms can be explained solely from the assembly of minerals without the contribution of protein deformation [53]. Moreover, although there are indirect evidences for the existence of proteins at the boundaries between the crystallites and prisms of developing enamel (enamel constitutes >%50 of proteins during the developmental stage) [23,54], somehow micrographs showing directly the protein sheaths in mature enamel are missing, leading to question whether protein sheath is a misconception or not. One argument for that is during sample preparation (cutting, polishing, etching), they are washed away and cannot be detected by microscopic techniques. Thus, another method to approach this question is to reveal the nature of the boundaries between the crystallites and prisms in mature enamel and analyze whether protein might exist here in the form of continuous sheaths or not. This is one of the main aspects of this work. In addition, enamel is not a self-similar homogenous hierarchical material but shows a high diversification among different species and different location of a single tooth in many aspects [55].

The above-given description of enamel's structure adopted in the materials science community lacks many important morphological singularities, which are decisive for the understanding of its mechanical response. Paleontological community uses a more comprehensive approach to be able to specify the morphological details of enamel in the given species [55,56–62]. However, they primarily deal with extinct species and surprisingly the systematic documentation of bovine, human and mouse enamel – the most frequently used specimens in dental and material science research – is lacking in the literature. We performed a thorough investigation to reveal the morphological characteristics of bovine incisor enamel with the aim of bridging the gap that apparently exists between these two disciplines.

2. Materials and methods

Permanent mandibular bovine incisors were used in this study. The teeth were extracted at a local slaughterhouse (Lippeck & Richter GmbH, Hamburg). Roots were cut off, the pulp interior was removed, after which they were disinfected within a 0.1 wt% thymol solution for 24 h. Teeth were then rinsed and further stored in Hank's Balanced Salt Solution (HBSS, Invitrogen, USA) until specimen preparation to avoid demineralization. The research protocol employed here was approved by the Ethics Committee of the Medical Association.

For the structural characterization, vertical and transversal slices out of mandibular bovine incisor were cut using a Buehler Isomet 4000 precision saw under water irrigation (Fig. 1). Two vertical slices were fragmented to examine the fracture surface. Other enamel slices were ground with 1200 grit silicon carbide abrasive paper and were further polished with 1 μm, 0.25 μm and 0.05 μm diamond suspensions. Some

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