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Optimized biological tools: ultrastructure of rodent and bat teeth compared to human teeth

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Enamel, the outer layer of the teeth of vertebrates, is the hardest tissue in their body. In general, enamel is highly mineralized and has a special ultrastructure that directs the mechanical properties of teeth and thereby their biological functions. However, there are differences between the enamel ultrastructures of different species. The authors compare the teeth of the Eurasian beaver *Castor fiber*, the African mole rat *Fukomys kafuensis* and the common pipistrelle bat *Pipistrellus pipistrellus* by high-resolution analytical methods, including scanning electron microscopy, quantitative wavelength-dispersive X-ray spectroscopy and X-ray powder diffraction. The enamel of all animals consists of long, thin and differently oriented calcium phosphate crystallites (length > 1 µm; thickness about 50 nm). On the outer surface of the beaver tooth, a thin layer of brown iron oxide was found. The diameter of the dentin tubuli was between 1 and 3 µm for all species – that is, comparable to that for human teeth.

Notation

R_{wp} weighted profile R -factor

1. Introduction

Teeth represent the hardest tissue in vertebrates. As in several different hard tissues in nature, their increased hardness arises not only from their material alone, but also from their delicate ultrastructure.^{1–3} In most taxa, including mammals, fish and sauropsids, bone and teeth consist of carbonated hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$), the mineral dahllite.^{4–7} In contrast to dentin, which contains ~30 wt% organic matrix, tooth enamel is much more highly mineralized and contains only ~1 wt% organic matrix.^{5,8,9} In mammalian tooth enamel, the mineral phase hydroxyapatite forms needle-like crystallites. Over the course of evolution, these crystallites have developed into distinct types of arrangements in different taxa that range from relatively uniform parallel orientation (parallel crystallite enamel) over the formation of columnar structures, microunits and compound units of various shapes to regular bundles termed ‘enamel prisms’.¹⁰ The crystallite architecture in the enamel is denoted by the German word ‘schmelzmuster’, which is also used in English.¹¹ The ultrastructure is closely related to the function of the tooth – that is, the diet of the respective organisms – which is supported by the fact that similar schmelzmusters have appeared convergently across different taxa during evolution.¹⁰

In general, enamel crystallites originate at the dentin–enamel junction (DEJ) where the forming enameloblasts are located, and

their orientation is more or less perpendicular to the tooth surface.^{10,12–16} The hierarchical structure and the resulting mechanical properties of biological hard tissues, including teeth, serve as inspiration to create biomimetic and bioinspired materials.^{17,18} Here, the authors describe the structural organization and mineral composition of the teeth of two rodents (*Castor fiber* and *Fukomys kafuensis*) and one bat (*Pipistrellus pipistrellus*) in comparison to those of a human (deciduous) tooth.

2. Materials and methods

Teeth of *C. fiber* were provided by O. Zink, Museum der Westlausitz, Kamenz, Germany. From all skulls, incisivi had been excavated from the jaw. Teeth (incisive) of *F. kafuensis* were provided by Professor Dr H. Burda/Dr P. Dammann, Department of General Zoology, University of Duisburg-Essen. Teeth of *P. pipistrellus* were provided by Professor Dr. M. Eppler and taxonomically determined by Dr. H. Fabritius. The deciduous human tooth was obtained from a 12-year-old male. The samples were separated into the individual tissues (dentin and enamel), as described later. Scanning electron microscopy (SEM) was used to visualize the internal structure of enamel, dentin and the DEJ.

The teeth were immersed in liquid nitrogen for 2 min and mechanically broken into two pieces for axial freeze fracture. Secondary electron microscopy and qualitative energy-dispersive X-ray spectroscopy (EDX) were both carried out with an ESEM

Figure 1. (a) SEM image of an incisor of the bat *P. pipistrellus* and images of incisors of (b) the mole rat *F. kafuensis* and (c) the beaver *C. fiber*

Quanta 400 FEG instrument after sputtering with gold and palladium (80:20). Fragments of a tooth of *C. fiber* were also analyzed by wavelength-dispersive X-ray spectroscopy (WDS) with a Zeiss Gemini 300 scanning electron microscope, equipped with an Oxford Wave 500 detector.

For X-ray powder diffraction (XRD), the samples were ground to a fine powder as follows: the teeth were transversally cut with a Proxxon FBS 230/E fine drilling and polishing tool equipped with a diamond-coated cutting disk. Fine powders of enamel or dentin (a few milligrams per sample), were obtained from corresponding areas of the cut teeth with the same instrument using a diamond-coated drill. The obtained powder was used for XRD with a Bruker D8 Advance diffractometer (copper (Cu) $K\alpha$ radiation, $\lambda = 1.54 \text{ \AA}$) on a silicon single-crystal-sample holder to minimize background scattering. For the qualitative phase analysis with the Bruker software Diffrac.Suite Eva V1, the pattern of hydroxyapatite from the International Centre for Diffraction Data (ICDD) database (number 09-0432) was used as reference. Rietveld refinement for the calculation of the lattice parameters and the crystallite sizes was performed with the Bruker software Topas 5.0. For Rietveld refinement, the instrumental correction was included as determined with a standard powder sample of lanthanum hexaboride (LaB_6) (from the National Institute of Standards and Technology, standard reference material SRM 660b). The size of the crystallites was calculated by Rietveld refinement, based on the Scherrer equation after correction for instrumental peak broadening.¹⁹

3. Results and discussion

The investigated samples are shown in Figure 1. The incisors of *P. pipistrellus* had a size of about 1 mm, those of *F. kafuensis* had a curved length of about 3–4 cm and those of *C. fiber* had a curved length of about 8 cm. All teeth were white, except for those of *C. fiber*, which had a distinct reddish-brown color on the convex part, representing the fully mature enamel surface of the distal part, including the cutting edge.

Figure 2 shows SEM images of the cross-section of a human deciduous tooth (Figure 2(a)). Starting from the DEJ, the hydroxyapatite prisms are arranged perpendicular to the outer enamel surface (Figure 2(b)).²⁰ Within the prismatic enamel, the crystallites are oriented with their long axes in a similar fashion (Figure 2(c), arrows). The dentin consists of rather disordered crystallites, embedded in an organic matrix that is pervaded by parallel dentin tubuli with a diameter of about 1 µm (Figure 2(d), arrows).

Figure 3 shows SEM images of a fractured tooth of *C. fiber*. The surface of the tooth is covered with a brown iron-rich layer on the highly oriented enamel crystals below (Figure 3(a)). The entire

Figure 2. SEM images of a human deciduous tooth: (a) overview; (b) magnification of the DEJ; (c) magnification of the enamel; (d) magnification of the dentin

Figure 3. SEM images of a tooth of *C. fiber*: (a, b) cross-sections of the tooth surface and the enamel, showing the iron-rich enamel part (convex) and the white enamel part (concave), respectively; (c–e) the enamel at higher magnification; (f) the dentin microstructure

enamel layer is ~1 mm thick. In the cross-section, the first ~200 μm thick outermost layer below the surface appears distinctly separated from the underlying bulk of the enamel, indicating a structural difference (Figure 3(a)). At higher magnification, this layer consists of parallel crystallites that form flat layers oriented perpendicularly to the tooth surface (Figure 3(b)). The EDX analysis (Figure 4) of the enamel confirms the presence of iron in the outer part of this layer besides the major phase calcium phosphate. The uncolored enamel consists of long prisms that either form an interwoven pattern close to the surface with angles of about 90° between them (Figure 3(c)) or proceed in a very regular parallel arrangement (close to the DEJ) (Figure 3(d)). Their thickness is relatively uniform and amounts to 30–50 nm. The dentin resembles that of human teeth as it is being pervaded with long tubuli with similar diameters. The diameter of the tubuli is 2–3 μm.

Figure 5 shows a line scan across the iron-rich part of the *C. fiber* tooth recorded by WDS for the elements iron, calcium (Ca), phosphorus and oxygen, including an SEM image of the sampled region. The thickness of the iron-rich layer is estimated to be around

Figure 4. EDX analysis of the enamel layer of an incisor of *C. fiber* close to the cutting edge: (a) iron-rich enamel and (b) white enamel. Carbon (C), oxygen (O), iron (Fe), magnesium (Mg), phosphorus (P) and calcium (Ca) signals originate from the tooth. The silicon (Si) and palladium (Pd) signals are caused by the sample holder and sputtering, respectively. The tooth mineral consists of calcium phosphate. Due to the irregular nature of the samples, EDX did not give quantitative results. However, the calcium–phosphorus molar ratio was around 1.5:1, as expected for a biological apatite

10 μm. Iron is located only in the outermost layer of the enamel, which otherwise consists of calcium phosphate. Earlier, Gordon *et al.*²¹ had identified the mineral in the brown iron-containing layer in *C. fiber* as a mixture of ferrihydrite, $\text{Fe}_2\text{O}_3 \cdot x\text{H}_2\text{O}$, and amorphous iron-calcium phosphate. It was not possible to record an EDX map due to the lower sensitivity of EDX compared to WDS.

SEM images of a tooth of *F. kafuensis* are shown in Figure 6. The enamel is ~250 μm thick, in good agreement with the result of Teleky *et al.*,²² with a uniform structure. The enamel consists of alternating layers of crystallite prisms tilted against each other at

Figure 5. (a) SEM image and (b) WDS line scans of iron, phosphorus, calcium and oxygen across an incisor of *C. fiber* with a brown surface (outer convex part)

an angle of less than 90° . The prisms are equally densely arranged as in the tooth of *C. fiber*. The dentin appears less organized and contains thin and long tubuli with a thickness of less than $1\ \mu\text{m}$. EDX mapping of the tooth cross-section (Figure 7) shows the calcium phosphate biomineral with a stronger calcium signal in enamel compared to dentin, indicating an increasing degree of mineralization from dentin to enamel. The molar ratio of calcium to phosphorus was about 1.46:1 across the scan, indicating the presence of calcium-deficient biological apatite.^{5,9}

Figure 8 shows a representative XRD pattern of the dentin of *F. kafuensis*. The mineral phase consists of hydroxyapatite, with a crystallite size of $9 \pm 1\ \text{nm}$ and lattice parameters of $9.47 \pm 0.01\ \text{\AA}$ (a axis) and $6.88 \pm 0.01\ \text{\AA}$ (c axis), as determined by Rietveld refinement. The increased lattice parameters a compared to reference hydroxyapatite ($a = 9.42\ \text{\AA}$ from ICDD number 09-0432) point to an atomic substitution in the apatite structure.

Figure 6. SEM images of a tooth of *F. kafuensis*: (a) overview with enamel and dentin; (b, c) enamel; (d) dentin

Figure 7. EDX mapping of a tooth of *F. kafuensis*, showing the distribution of the elements across the tooth (enamel and dentin). The enamel is higher mineralized (calcium, phosphorus) and contains less organic matrix (carbon)

Figure 8. Representative X-ray powder diffractogram of *F. kafuensis* dentin with a fit to hydroxyapatite as the only crystalline phase by Rietveld refinement ($R_{wp} = 7.2$)

It was not possible to analyze the enamel of *F. kafuensis* teeth by XRD because not enough material could be obtained from the very thin enamel layer.

Figure 9 shows SEM images of a tooth of *P. pipistrellus*. The tooth is very thin with a large pulpa cavity. Interestingly, the enamel takes up almost one-third of the thickness, while the rest is dentin (Figure 9(a)). Compared to the other species, the

enamel–dentin ratio is much larger and thus the tooth can be expected to be harder than the three other examples, which is in good agreement with its function as a sharp piercing tool. Although the dentin is very thin, the tubuli have the same basic dimensions as in human teeth, but contain more ramifications close to the DEJ (Figure 9(b)). A higher magnification of the enamel shows needle-shaped prisms that are oriented parallel to each other and to the tooth surface (Figure 9(c)).

Figure 9. SEM images of the fracture surface of a tooth of *P. pipistrellus*: (a) overview (enamel: top; dentin: bottom); (b) dentin layer; (c, d) enamel layer

The long axes of the prisms are oriented longitudinally from the base of the tooth toward the sharp tip. Such an arrangement will increase the stability of the tooth in the direction where the bat stabs its prey, which consists of insects whose exoskeletons can be quite hard and tough. This arrangement differs from human and rodent enamel and is reminiscent of the structural organization of shark teeth, which have a similar function.²³ The prisms are very densely distributed with very little interprismatic enamel (Figure 9(d)). Figure 10 shows that the mineral phase consists exclusively of calcium phosphate. XRD was not possible due to the very small amount of material in the bat teeth.

Human enamel has a highly regulated structure. The needle-like hydroxyapatite crystallites, which are roughly rectangular in cross-section,²⁴ form bundles that are held together by organic molecules into larger structures, called enamel prisms. These prisms are arranged perpendicular to the DEJ toward the outer enamel surface. They can be twisted together or change their direction in some areas in order to increase the hardness of the overall structure.²⁵ The hardness of human enamel is 3.5 ± 0.5 GPa, and the reduced elastic modulus is 77 ± 10 GPa.²⁶ Human third molar dentin shows a hardness of 1.0 ± 0.15 GPa and a reduced elastic modulus of 23.7 ± 2.6 GPa.²⁶ The structure

Figure 10. EDX-measurement of a tooth of *P. pipistrellus*, showing the presence of calcium phosphate mineral. Due to the irregular nature of the sample, EDX did not give quantitative results. However, the calcium–phosphorus molar ratio was around 1.5:1, as expected for biological apatite

of human molar teeth arises from the diet: their main functions are to chew, to tear and to cut.

While humans can be considered as omnivorous (and hence chew food of variable hardness), echolocating bats such as *Pipistrellus* use their incisors primarily to catch and kill their insect prey.²⁷ In contrast, incisors of the two rodent species in this study are confronted with more demanding tasks: besides gnawing all kinds of plant material for food, beavers use their incisors extensively to cut trees for building dams, lodges and canals^{28–30} and African mole rats, including *Fukomys*, dig extensive burrow systems in soils of varying hardness with their incisors.^{31–33} In contrast to many other mammals, most rodents show monophyodonty – that is, they do not shed their primary teeth.

Rodent incisors are characterized by a sophisticated structure. The enamel is present in only one small, lamellar film on the outer part of the tooth. Furthermore, as an adaptation to the requirements described earlier, rodent incisors grow continuously and their length is controlled by continuous abrasion caused by their comparably hard and fibrous diet – for example, wood, nuts or other plant materials – or even digging through soils like in the case of *Fukomys*.^{32,33} Their particular structure with an enamel layer located on the labial (outer) surface is supported only by the roughly cylindrical rest of the tooth that consists of dentin. This is responsible for the rodents' ability to maintain a sharp cutting edge always. During chewing, the softer inner dentin layer is eroded by the outer, harder enamel, leaving a sharp edge both on the upper and lower jaw incisors. Beaver enamel can be divided into three enamel types: the pigmented iron-containing outer enamel, the underlying non-pigmented enamel and the inner

enamel. The hardness decreases from the outside to the inside: 5–6 GPa for the pigmented iron-containing enamel and 4–5 GPa for the non-pigmented white enamel.²¹ The hardness correlates with the function of the teeth and the diet of the vertebrate.³⁰

4. Conclusions

Teeth of two rodent species, namely the European beaver *C. fiber* and the African mole rat *F. kafuensis*, and of the bat *P. pipistrellus* all consist of calcium phosphate with a high similarity to human teeth. A highly mineralized outer enamel layer surrounds the less mineralized inner dentin. In comparison to human teeth, the enamel layer in the two rodents and in the bat is much thinner. The dentin tubuli in the three species have a diameter (between 1 and 3 µm) comparable to that of human teeth, making them relatively large in the small bat teeth. The beaver teeth contain a thin layer (~10 µm) of iron-containing mineral (most likely iron oxide) on their outer surface, probably to increase their hardness.

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