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Characterization of crocodile teeth: Correlation of composition, microstructure, and hardness

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ABSTRACT

Structure and composition of teeth of the saltwater crocodile *Crocodylus porosus* were characterized by several high-resolution analytical techniques. X-ray diffraction in combination with elemental analysis and infrared spectroscopy showed that the mineral phase of the teeth is a carbonated calcium-deficient nanocrystalline hydroxyapatite in all three tooth-constituting tissues: Dentin, enamel, and cementum. The fluoride content in the three tissues is very low (<0.1 wt.%) and comparable to that in human teeth. The mineral content of dentin, enamel, and cementum as determined by thermogravimetry is 71.3, 80.5, and 66.8 wt.%, respectively. Synchrotron X-ray microtomography showed the internal structure and allowed to visualize the degree of mineralization in dentin, enamel, and cementum. Virtual sections through the tooth and scanning electron micrographs showed that the enamel layer is comparably thin (100–200 μm). The crystallites in the enamel are oriented perpendicularly to the tooth surface. At the dentin–enamel-junction, the packing density of crystallites decreases, and the crystallites do not display an ordered structure as in the enamel. The microhardness was 0.60 ± 0.05 GPa for dentin, 3.15 ± 0.15 GPa for enamel, 0.26 ± 0.08 GPa for cementum close to the crown, and 0.31 ± 0.04 GPa for cementum close to the root margin. This can be explained with the different degree of mineralization of the different tissue types and is comparable with human teeth.

1. Introduction

Crocodiles belong to a very old phylogenetic group that has prevailed for millions of years (Erickson et al., 2012; Janke et al., 2005). Unlike human teeth, reptile teeth including crocodile teeth are continuously replaced (Kieser et al., 1993; Osborn, 1974; Poole, 1961). For an approximately 4 m long crocodile (13 ft), it was estimated that each tooth was replaced 45 times during the lifetime of the animal (Poole, 1961). Crocodiles possess so-called thecodont teeth which are attached in sockets in the jaw (Dauphin and Williams, 2008). Compared with other animals, crocodiles exert extraordinarily high bite-forces and tooth pressures (Erickson et al., 2012). Like all vertebrate teeth, crocodile teeth consist of a crown and a root.

In general, the interior bulk of the tooth crown consists of softer, less mineralized bone-like dentin covered by an external layer of harder, highly mineralized enamel. The root, however, consists of dentin (interior) that is covered by an external layer of cementum. The inorganic mineral of human enamel is a calcium-deficient carbonated hydroxyapatite, simplified: $Ca_5(PO_4)_3(OH)$, with small amounts of an organic matrix (Busch et al., 2001; Dorozhkin and Epple, 2002; Fincham et al., 1999; Lowenstam and Weiner, 1989). For details on teeth in general see, *e.g.*, Teaford et al. (2000).

In contrast to human teeth and shark teeth (which are fully replaced upon loss) (Marks and Schroeder, 1996; Smith et al., 2012), the root of a crocodile tooth is hollow. Each mature functional tooth is accompanied by a small initial replacement tooth on the lingual side of the root that grows from a bud formed by a specialized dental lamina. Together, they form a tooth family unit. Crocodylian teeth cycle continuously. While the new tooth grows, it is moving outward and induces the resorption of the root of the old tooth which is then shed (Wu et al., 2013). The human tooth eruption follows a similar pattern. During the change from deciduous tooth to permanent tooth, the root of the old tooth is resorbed by osteoclasts and the crown erupts as a compact object (Marks and Schroeder, 1996).

Studies of the structure of reptile enamel were reported by Dauphin (1987), Sahni (1987), and Sander (1999). In general, reptile teeth have not been as thoroughly investigated as the teeth of other large animals; one reason is that their enamel does not consist of defined prisms such as mammalian teeth which can be more easily analyzed (Sander, 2000). Because reptile enamel is

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lacking prisms, it is typically denoted as "prismless enamel", a feature which is common to most non-mammalian amniotes (reptiles) (Sander, 2000). Currently, there are only a few reports about the structures of crocodile and alligator teeth (Dauphin and Williams, 2007, 2008; Erickson et al., 2012; Osborn, 1998; Sander, 1999; Sato et al., 1993, 1990; Shimada et al., 1992).

To close this gap, we have analyzed the chemical and crystallographic composition, the ultrastructure, and the microhardness of dentin, enamel, and cementum, of teeth of the saltwater crocodile *Crocodylus porosus* with the aim to correlate all parameters, namely, structure, hardness, and biological function, to gain an integral view. Additionally, we compare these properties with human teeth.

2. Materials and methods

2.1. Sample preparation and analytical methods

Teeth of the recent crocodile species *C. porosus* were stored in dry state at room temperature. We used five different teeth to produce fine powders of dentin, enamel, and cementum (several mg per sample) by mechanical abrasion with a Proxxon fine drilling and polishing tool FBS 230/E, equipped with a diamond-coated drill. The mineral phase and the size of the crystalline domains of these powder samples were determined by infrared (IR) spectroscopy and X-ray powder diffraction (XRD) measurements. Fourier-transform infrared spectroscopy (FTIR) was carried out with a Bruker Vertex 70 instrument in potassium bromide (KBr) pellets (range 400–4000 cm^{-1} and 2 cm^{-1} resolution). XRD measurements were carried out with a Panalytical Empyrean diffractometer equipped with a furnace (XRK 900, Anton Paar) using a silicon single crystal as sample holder to minimize scattering. First, a diffractogram was measured at 30 °C. Then, the sample was heated to 750 °C and held at this temperature for 2 h before another diffractogram was measured. The measurements at 750 °C were performed to identify the conversion products of the mineral phase after thermal treatment. Rietveld refinement for the calculation of the lattice parameters and the size of the crystalline domains was performed using the Bruker software TOPAS 4.2. The correction for instrumental peak broadening as determined with an LaB_6 powder sample, National Institute of Standards and Technology (NIST), as standard reference material (SRM 660b), was included. As reference, we used the pattern of hydroxyapatite (#9-0432) from the International Centre for Diffraction Data (ICDD) database.

A part of the remaining powdered sample material was used to perform elemental analysis to determine the overall chemical composition and to confirm the identity of the mineral phases. Calcium, magnesium, and sodium were determined with atomic absorption spectroscopy (AAS), fluoride with ion-selective potentiometry, and phosphate with ultraviolet (UV) spectroscopy. All measurements were carried out using several mg of powdered dentin, enamel, and cementum which were dissolved in concentrated hydrochloric acid. For fluoride analysis we used ion-selective potentiometry (ion-selective electrode, ISE; pH/ION 735, WTW; the measurements were performed by Analytische Laboratorien GmbH, Lindlar, Germany). Atomic absorption spectroscopy was performed with a Thermo Electron M-Series instrument. Phosphate was determined with a Varian Cary 300 UV–Vis spectrophotometer as phosphate-molybdenum blue complex.

Thermogravimetry (TG) was used to determine the contents of water, organic matrix, and carbonated apatite in the remaining powder samples of dentin, enamel, and cementum from five different teeth. The experiments were carried out in a Netzsch STA 449 F3 Jupiter instrument in dynamic oxygen atmosphere at a heating rate of 2 K min^{-1} from 25 to 1200 °C in open alumina crucibles. For Vicker's microhardness tests, the teeth were axially cut with a jeweler's saw (for the convention of axial and transversal denomination see Fig. 1). Subsequently, the samples were embedded in one-component UV-curable methyl methacrylate CEM 4000 Lightfix resin (Cloeren Technology GmbH, Wegburg) that was cured in a Struers UV-Box using the bottom source only for 3 min and with bottom and top source together for 6 min. The surfaces of interest were polished using successively abrasive papers with decreasing grit sizes (120, 220, 400, 600, 1000, 2500, and 4000; Hermes) followed by polishing with a 3 μm diamond suspension (Struers), and finally with a 0.1 μm silica suspension (Buehler; Saphir 320/330 instrument, ATM). In addition to polished samples, parts of teeth fractured to expose either cross sections or axial sections were also prepared for scanning electron microscopy (SEM). All SEM samples were mounted on standard aluminum holders, rotary shadowed with 4 nm of platinum using a Gatan PECS 682 sputter coater, and observed in a high resolution scanning electron microscope (Zeiss Gemini 1540XB) at acceleration voltages of 5–10 kV using a small aperture (30 μm) and either an in-lens secondary electron (SE) detector or a backscattered electron (BSE) detector for compositional contrast. For a clearer view on the microstructure, selected samples were superficially etched using aqueous EDTA solution (0.15 M and 2.5% glutaraldehyde for 20 min) followed by a quick rinse by double-distilled H_2O and 100% methanol for 1 s each. Where necessary, contrast and brightness of the digital images were adjusted using Adobe Photoshop CS3 (Adobe Inc.).

Synchrotron X-ray microtomography (SRμCT) is a very useful technique for the visualization of microstructures because it provides 3D data sets in a widely non-destructive manner. This technique was already successfully used to study biological materials, *e.g.*, bone microstructures (Bonse et al., 1994; Larrue et al., 2011; Sanchez et al., 2012) and human teeth (Dowker et al., 2004; Neues et al., 2009; Sanchez et al., 2012).

SRμCT analysis was used to evaluate the gray values as indication of the local density of the material and thus the degree of mineralization as well as to create virtual 3D sections of the tooth. SRμCT analyses were carried out at beamline ID19 of the European Synchrotron Radiation Facility (ESRF), Grenoble, France. Experimental details of the beamline and on the evaluation procedure can be found in Weitkamp (2010). The 3D images and virtual sections were rendered with the software VGStudio MAX 2.1. The gray values were identified by the graphic software ImageJ 1.45s (Schneider et al., 2012). For the measurements, the sample was

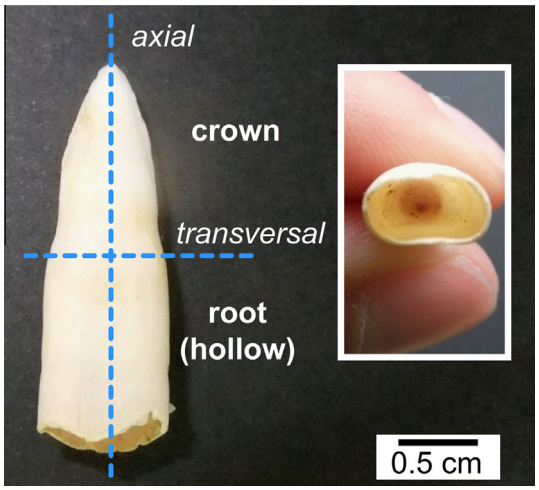

Fig. 1. Image of a crocodile tooth (*C. porosus*), including the convention of axial and transversal directions, with an additional view into the hollow root (insert).