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

Joachim Enax\textsuperscript{a}, Helge-Otto Fabritius\textsuperscript{b}, Alexander Rack\textsuperscript{c}, Oleg Prymak\textsuperscript{a}, Dierk Raabe\textsuperscript{b}, Matthias Eppe\textsuperscript{a,*}

\textsuperscript{a} Institute of Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen, Universit"{a}tsstr. 5-7, 45117 Essen, Germany

\textsuperscript{b} Microstructure Physics and Alloy Design, Max-Planck-Institut f"{u}r Eisenforschung, Max-Planck-Str. 1, 40237 D"{u}sseldorf, Germany

\textsuperscript{c} European Synchrotron Radiation Facility (ESRF), 6 Rue Jules Horowitz, 38000 Grenoble Cedex, France

* Correspondence to:
Matthias Eppe, Institute of Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen, Universit"{a}tsstr. 5-7, 45117 Essen, Germany
Telephone: + 49 (0) 201 183-2413
Fax: + 49 (0) 201 183-2621
E-mail: matthias.eppe@uni-due.de
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.

Keywords

Biomineralization; Teeth; Calcium phosphate; Mechanical properties; Synchrotron X-ray microtomography; Crocodiles
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: \( \text{Ca}_5(\text{PO}_4)_3(\text{OH}) \), 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 Jr. 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 Jr. 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 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; Dauphin and Williams, 2008; Erickson et al., 2012; Osborn, 1998; Sander, 1999; Sato et al., 1993; Sato et al., 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 Figure 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 H₂O 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 et al. (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 placed 935 mm upstream of an indirect X-ray imaging detector operating with an effective pixel size of 5 μm (ESRF inhouse CCD camera FReLoN type F_7899 in combination with a 125 μm-thick LuAG:Ce single-crystal scintillator and a 2.8× magnifying optical system, 2048×2048 pixels). Polychromatic radiation was chosen, i.e. the beamline U17.6
undulator was combined with only 2.8 mm Al and 0.4 mm Cu absorbers at a gap of 15 mm in order to gain a sufficient photon flux density (the ESRF operated in 4-bunch mode). The resulting spectrum was dominated by the 2nd harmonic of the undulator around an X-ray photon energy of 35 keV. 2000 projection images were acquired over a tomographic scan range of 180 degrees with an exposure time of 2 s each. Single-distance phase retrieval was applied in order to establish a direct correlation between the gray levels of the voxels and the materials associated with them before the tomographic reconstruction by means of filtered-back projection was performed (Weitkamp et al., 2011). The chosen approach is robust and allows to work with a single propagation distance only but as well only for a single component: The dentin-enamel junction (dej) was fringe-free after this phase-retrieval while other interfaces such as dentin-air remained with fringes (Paganin et al., 2002).

Vicker’s microhardness tests were carried out on the exposed axial sections of two teeth in four designated areas of interest: The crown enamel, crown dentin, the distal cementum layer close to the crown and the cementum layer close to the proximal margin of the tooth. In every area of interest on each sample, 20 indentations were made with a Leco M-400-H1 microhardness testing device. The location of each indentation was manually chosen and a weight of 10 g (HV0.01) was applied for 15 s. These experiments, including the distance between two indentations, were performed according to DIN EN ISO 6507-1 and DIN EN ISO 6507-4 and the indentations were manually controlled and evaluated. Vicker’s hardness HV0.01 was converted into Berkovich hardness $H$ according to $HV0.01/\text{kg mm}^2 = 92.65 \text{s}^2 \text{m}^{-1} \cdot H/\text{GPa}$.

3. Results
A typical tooth of the crocodile species *C. porosus* has a cone-shaped crown and a hollow, roughly cylindrical root (Figure 1). The absolute size of each tooth varies depending on the age and size of individual animals.

The transition between dentin and enamel is marked by the so-called dentin-enamel-junction (dej) and is clearly visible on scanning electron micrographs of axially polished cross sections through the teeth (Figures 2A, 2B, and 3A). The crocodile enamel has a thickness of about 100-200 µm at the crown (Figure 3A), becomes thinner towards the root and disappears completely just before the onset of the hollow part (Figure 2A). In this region, an enamel-cementum interface can be observed where a thin cementum layer is present that covers the enamel layer (Figure 2A). The uniform contrasts observed in composition-sensitive backscattered electron (BSE) contrast micrographs (Figure 2) indicate a homogeneously distributed mineral content in all three layers. Surfaces of fractured enamel show that it consists of small elongated mineral crystallites that are mainly oriented with their long axes perpendicular to the tooth surface (Figures 3C and 4). These crystallites are very densely packed and therefore the shape and dimensions of individual crystallites are difficult to determine. Superficial etching of polished enamel surfaces revealed the needle-like shape of the mineral structures. The crystallites in the enamel have a length of a few µm. No defined crystallite bundles as they occur in prismatic enamel of *e.g.*, mammalians were visible (Figure 4). Throughout the enamel, a fine horizontal (parallel to the tooth surface) striation can be observed (Figure 4A). At the dentin-enamel-junction (dej) the packing density of the crystallites decreases and their orientation becomes more random (Figures 3B, 4B). The dej is further characterized by a relatively high content of randomly arranged organic fibers (Figures 2B, 3B). The mineral phase of the cementum is not as clearly structured as in
the enamel in terms of regularly arranged crystallites. The cementum contains a loose
network of randomly arranged organic fibers (Figures 2C, 3D). These fibers disappear in
the transition between cementum and dentin (dentin-cementum-junction, dcj), which is
not as sharp as the dej (Figures 2C, 3D). The dentin of crocodile teeth is pervaded by
numerous µm-sized dentin tubuli (Figure 2D). The SEM-micrographs of fractured teeth
indicate that the mineral phase of dentin also consists of needle-shaped crystallites
(Figure 2B). However, a well-defined structural organization as found in enamel could
not be observed with the techniques used in our study.
Elemental analysis of the crocodile teeth shows that the enamel contains more calcium,
phosphate, and sodium but less magnesium than dentin (Table 1). Sodium is present in
dentin and enamel but absent in the cementum. The fluoride concentration in crocodile
teeth altogether is very low (<0.1 wt%). The cementum contains significantly more
fluoride than dentin and enamel. The magnesium content is very high, especially in
dentin, and it was included to compute the (Ca+Mg)/P molar ratio in the apatite mineral
phase. In all cases, the stoichiometry of a calcium-deficient hydroxyapatite was found,
\textit{i.e.} around \( n(Ca+Mg):n(P)=1.67:1 \) (Dorozhkin and Epple, 2002).
The mineral phase of the teeth was analyzed by X-ray powder diffraction in combination
with Rietveld analyses (Figure 5 and Table 2). The diffractograms of dentin, enamel, and
cementum show the typical peak pattern of hydroxyapatite. While the diffractograms of
dentin and cementum show broad diffraction peaks, those of the enamel are slightly
sharper. The average size of the crystalline domains is 8-9 nm, and the lattice
parameters and cell volumes differ just a little between dentin, enamel, and cementum.
Heating of the samples to 750 °C in vacuum resulted in a mixture of hydroxyapatite and
\( \beta \)-tricalcium phosphate, \( \beta-Ca_3(PO_4)_2 \), in comparable amounts indicating the presence of
a calcium-deficient hydroxyapatite in the initial phase (i.e. before calcination) (Dorozhkin and Epple, 2002).

The IR-spectra recorded for dentin, enamel, and cementum (Figure 6) show the absorption bands specific for phosphate (490-640 cm\(^{-1}\) and 900-1220 cm\(^{-1}\)), carbonate (875 cm\(^{-1}\) and 1360-1590 cm\(^{-1}\)), and water (3010-3660 cm\(^{-1}\)). Additional bands that indicate the organic matrix (mainly proteins) appear in all three layers at 2940 cm\(^{-1}\) (C-H) and 1600-1700 cm\(^{-1}\) (amide I band of proteins) (Preston et al., 2011).

Thermogravimetry of dentin, enamel, and cementum shows three main regions of mass loss: Release of water (<200 °C), the combustion of the organic matrix (200-500 °C), and finally the release of CO\(_2\) from carbonated apatite (>500 °C) (LeGeros, 1981; Peters et al., 2000) (Figure 7). The residue is the decarboxylated mineral phase, i.e. pure calcium phosphate. Enamel has a much higher mineral content than dentin and cementum with a low content of organic matrix (Table 3). Cementum has a lower mineral content than dentin. The content of water is comparable in all three tissues. Note that the content of water may be variable, depending on the storage conditions.

SR\(\mu\)CT analyses show the exact geometry of the crocodile teeth and the spatial arrangement of the constituting layers (Figure 8). The enamel layer (whitish color) is confined to the crown and generally very thin with the thickest regions located at the tooth tip. From the 3D images, it is difficult to distinguish between dentin and the very thin cementum layer that covers the root due to a very similar X-ray absorption contrast. In axial sections, it becomes obvious that the hollow root takes up over two thirds of the total tooth size, and that the crown is comparatively small. Moreover, the cavity formed by the root has the shape of a distally pointed cone, and the root's wall thickness is decreasing in proximal direction.
In the SRµCT-3D images, the enamel has a white color, and the dentin has a gray color. Evaluation of the gray values using line scans through the tooth crown (Figures 9A and 10A) shows that the enamel has a much higher gray value (~2.8) than the dentin (~1.8). The root (Figures 9B and 10B), however, shows a lower gray value for the dentin (~1.3) compared to the crown dentin. Cementum has a slightly higher gray value than the dentin in the root.

Representative Vicker’s microhardness tests were performed at different positions of polished tooth samples of *C. porosus*. For better comparability, the results were converted into Berkovich hardness. We found 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 margin of the root (Table 4).

**4. Discussion**

The teeth of the saltwater crocodile *C. porosus* are mainly characterized by their specific shape, a small crown with a comparably large root, and the three constituting tissues dentin, enamel, and cementum that differ in microstructure, composition, and resulting mechanical properties. The major part of both the crown and the root consists of dentin which contains dentin tubuli that are also present in human teeth (Marten et al., 2010). Both exterior layers, the cementum covering the root and the enamel covering the crown, are separated from the dentin by a structurally distinct layer, the dentin-cementum-junction (dcj) and the dentin-enamel-junction (dej), respectively. The dentin-enamel-junction is also present in mammalian teeth (Line and Novaes, 2005; Walker and Fricke, 2006). Within the enamel, the mineral crystallites are all oriented parallel,
hence, this type of enamel is typically denoted as "parallel crystallite enamel" (Sander, 2000). A similar enamel microstructure was found for teeth of Alligator mississippiensis (Sato et al., 1990). The horizontal striation observed on etched enamel surfaces indicates the presence of incremental growth lines that have also been described in teeth of other ectotherm animals (Line and Novaes, 2005).

X-ray diffractograms of dentin and enamel showed broad diffraction peaks which indicate a comparable size of crystalline domains between these tissues. That is remarkable because in mammalian enamel and shark enameloid the apatite rods have a larger size of crystalline domains than the apatite nanocrystals in dentin (LeGeros, 1994; Xue et al., 2008). The lattice parameters of the mineral in crocodile teeth are very similar to those of human teeth and of geological hydroxyapatite and confirm the presence of apatite. The small differences in the lattice parameters of dentin, enamel, and cementum can be ascribed to different amounts of incorporated ions, e.g., magnesium, sodium, and carbonate into the apatite lattice, as it is well known for biological apatite (Dauphin and Williams, 2007; LeGeros, 1981). Magnesium as substituent is known to reduce the crystallinity of apatites while sodium has no significant influence (Elstnerova et al., 2010; LeGeros, 1994). Especially magnesium strongly influences the lattice parameters. Apatites with high contents of magnesium become amorphous (Masayuki et al., 1986). The fluoride content of crocodile teeth is very low like in human teeth (Aoba, 1997; LeGeros, 1981). Overall, the results of elemental analysis agree well with microprobe analyses of recent reptile teeth (Dauphin and Williams, 2007; Dauphin and Williams, 2008). Our results indicate that dentin, enamel, and cementum all contain nanocrystalline hydroxyapatite. The size of the crystalline domains of the different tooth layers is comparable.
The amide I band of proteins in the IR-spectra in enamel is less intense compared to dentin and cementum, probably due to the lower content of organic matrix. This is supported by thermogravimetry. Human dentin and cementum are known to have a high content of organic matrix which consists mainly of collagen (Wiesmann et al., 2005). The thermogravimetric analysis of human dentin showed similar results as found here for crocodile dentin (Lim and Liboff, 1972). In general, the IR spectra of dentin, enamel, and cementum are all very similar to each other and confirm the results of X-ray diffraction. They are also very similar to human teeth (LeGeros, 1981), synthetic hydroxyapatite (Zhou et al., 1993), and also shark teeth which do not consist of hydroxyapatite but of fluoroapatite, $\text{Ca}_5(\text{PO}_4)_3\text{F}$ (Enax et al., 2012).

The results of X-ray diffraction correspond well with the microstructure analysis by scanning electron microscopy. The prismless enamel of crocodile teeth contains no crystallite bundles and no complex microstructures but only needle-like mineral structures which are ordered perpendicularly to the tooth surface. Human teeth show a especially high fracture toughness (Padmanabhan et al., 2010; Yilmaz et al., 2013) due to the presence of crystallite bundles (enamel prisms) which are hierarchically ordered in a mm-thick enamel layer (Ang et al., 2010; Dunlop and Fratzl, 2010; He and Swain, 2008). In contrast, the enamel of crocodile teeth is very thin compared to mammalian teeth, which is consistent with their specific function. Crocodiles do not use their teeth for cutting and chewing, but only for gripping and securing their prey, which is then pulled into the water and killed by drowning. Generally, their teeth are not used to dismember prey animals; instead crocodiles use violent body movements to tear pieces off which are then swallowed. The enamel cap on mammalian teeth can be up to 5 mm thick (Lucas et al., 2008). Here, SRµCT showed clearly that the enamel thickness of crocodile
teeth reaches its maximum at the tooth tip and is rapidly thinning towards the root. This indicates that crocodile teeth must be very resistant at the tip area, probably optimized for snapping their prey. Crocodiles are ambush hunters which attack with a very fast bite where they exert extraordinarily high bite-forces and tooth pressures (Erickson et al., 2012). The thick enamel at the tip of the teeth might help to prevent damage upon impact on hard parts of the prey, e.g. bones, during the attack. Interestingly, the arrangement of teeth in the upper and lower jaw is such that opposing teeth will not get in contact if the bite misses the target. This is also corroborated by the fact that the root dentin shows a lower gray value than the crown dentin, corresponding to a lower mineral content and thus a higher potential for deformation without suffering a brittle fracture. The comparably higher deformability and lower hardness together with the large size of the root and thus large contact area with the jawbone via the teeth-ridge further helps to dissipate the pressure and thus the kinetic energy acting on individual teeth during the impact on prey animals.

Thus, the structural and compositional organization of the crocodile tooth is ideally suited for its function. Nevertheless, the hunting mode using very fast and powerful bites presumably increases the risk of teeth getting damaged compared to other predatory animals like mammalian carnivores that use similarly shaped canine teeth for killing their prey. However, these animals use their teeth in a much more controlled, almost surgical mode by biting into specific neuralgic points of their prey. The constant replacement of crocodile teeth may thus represent an additional adaptation to their predation technique that was necessary to compensate the higher risk of damage. Vicker’s microhardness tests of crocodile teeth gave a hardness close to the hardness of human teeth and shark teeth. This is surprising because crocodile teeth do not have hierarchically organized
crystallite bundles like human teeth. The different hardness values of the different tissues found in crocodile teeth can be explained by different degrees of mineralization and thus by different mineral contents which was shown by SRµCT, SEM, elemental analysis, and thermogravimetry. This is in good agreement with results for human teeth. It was shown by spatially resolved studies that an increasing calcium content (and thus mineral content) leads to a higher hardness in human enamel (Cuy et al., 2002; Jeng et al., 2011). Note that the microhardness of teeth is lower than that of pure (geological) hydroxyapatite which amounts to 5.4±1.3 GPa (White et al., 2001). This is because a geological hydroxyapatite crystal does not contain an organic matrix and is therefore much more brittle (and less elastic).

5. Conclusions
The hardness of human teeth and crocodile teeth is comparable, although their microstructures are significantly different. The prismless enamel layer of crocodile teeth is very thin, with a maximum thickness at tooth tip and consists of nanocrystalline hydroxyapatite, in striking contrast to mammalian enamel and shark tooth enameloïd. Structure and composition of crocodile teeth are well suited for their biological function. In contrast to most mammals, crocodiles do no not need their teeth for cutting but mainly for holding their prey. Crocodiles are changing their teeth continuously during their lifetime, possibly because of their way of hunting, i.e. very fast and powerful bites. As consequence, crocodiles have a higher risk of tooth-damage than other animals like mammalian carnivores. The construction of their teeth is well adapted to their biological function.
Acknowledgements

We thank C. Fischer, Essen, Germany, for help with the tooth preparation, M. Ruiz, Grenoble, France, for support during the beamtime at the ESRF, and A. Gillis, Halifax, Canada, for helpful discussions. We thank the Deutsche Forschungsgemeinschaft for support within the priority program SPP 1420.
References


Figure captions

Figure 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).

Figure 2: Scanning electron micrographs in back-scattered electron mode (BSE) of a polished tooth surface of *C. porosus*, showing the dentin-enamel-junction and also the dentin-cementum-junction (A), the dentin-enamel-junction in higher magnification (B), the dentin-cementum-junction in higher magnification (C), and the dentin with its tubuli (D). (d dentin, e enamel, c cementum, dej dentin-enamel-junction, dcj dentin-cementum-junction).

Figure 3: Scanning electron micrographs of fractured tooth samples of *C. porosus*. (A) The overview of an axially fractured tooth tip shows a clear border between dentin and enamel. (B) The organization of the dentin-enamel-junction at higher magnification. (C) Area at the tooth surface where the outermost layer of enamel was chipped off, revealing the constituting small elongated mineral crystallites that are mainly oriented with their long axes perpendicular to the tooth surface. (D) The organization of the dentin-cementum-junction at higher magnification. (d dentin, e enamel, c cementum, dej dentin-enamel-junction, dcj dentin-cementum-junction).

Figure 4: Scanning electron micrographs recorded in BSE contrast of the exposed axial surface of enamel from teeth of *C. porosus* that was gently etched using EDTA to expose the shapes and arrangement of the mineral crystallites. (A) The outermost
enamel consists of horizontal layers of parallel needle-shaped crystallites that are all oriented with their long axes perpendicular to the tooth surface. The different contrasts, especially of the horizontal bands, originates from slight differences in composition. (B) Close to the dentin-enamel-junction, the horizontal layers of needle-shaped crystallites are still visible, but frequently interrupted by the cavities of small canaliculae. (e enamel, dej dentin-enamel-junction).

**Figure 5:** X-ray powder diffractograms of dentin (A), enamel (B), and cementum (C) of teeth of *C. porosus* in comparison to pure hydroxyapatite (#9-0432 from the ICDD database; computed as nanocrystalline phase) (D).

**Figure 6:** IR spectra obtained from dentin, enamel, and cementum of teeth of *C. porosus*.

**Figure 7:** Thermogravimetric analysis of dentin, enamel, and cementum from teeth of *C. porosus*: <200 °C: Release of water (1), 200-500 °C: Combustion of the organic matrix (2), and >500 °C: Release of CO₂ from carbonated apatite (3).

**Figure 8:** SRµCT-3D images of the complete tooth (A) and of two virtual axial sections (B, C) through a tooth of *C. porosus*.

**Figure 9:** Line scans on transverse sections through the crocodile tooth obtained by SRµCT to generate the gray value profiles shown in Figure 10. A representative virtual section of the crown (A) and a section through the root just below the altitude where the
enamel has been replaced by cementum (B) are shown. Interference fringes between enamel and air as well as dentin and air remain due to the phase-retrieval chosen algorithm (Paganin et al., 2002).

Figure 10: Comparison of the gray values of two virtual transversal sections through a tooth of *C. porosus*. Linescan of the tooth crown (A) and linescan of the tooth root (B).
Table 1: The chemical composition of dentin, enamel, and cementum of teeth of *C. porosus* (in wt%), in comparison to human teeth.

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<th>Human teeth</th>
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<td>Ca/P molar ratio</td>
<td>1.26:1</td>
<td>1.55:1</td>
<td>1.65:1</td>
</tr>
<tr>
<td>(Ca+Mg)/P molar ratio</td>
<td>1.51:1</td>
<td>1.63:1</td>
<td>1.74:1</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>0.78</td>
<td>1.01</td>
<td>--</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>2.47</td>
<td>1.10</td>
<td>0.75</td>
</tr>
<tr>
<td>F$^-$</td>
<td>0.06</td>
<td>0.09</td>
<td>0.21</td>
</tr>
</tbody>
</table>

* Calculated from the content of phosphorus.
**Table 2:** Crystallographic properties of the mineral phase in teeth of *C. porosus* compared to geological hydroxyapatite crystals and to human teeth. \(^a\) Saenger and Kuhs (1992), \(^b\) LeGeros (1981) and \(^c\) Enax et al. (2012). The standard deviation is given in parentheses.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(a)-axis / Å</th>
<th>(c)-axis / Å</th>
<th>(V) / Å(^3)</th>
<th>Size of crystalline domains / nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crocodile dentin</td>
<td>9.43(1)</td>
<td>6.857(9)</td>
<td>528(2)</td>
<td>9</td>
</tr>
<tr>
<td>Crocodile enamel</td>
<td>9.451(8)</td>
<td>6.883(7)</td>
<td>532(1)</td>
<td>8</td>
</tr>
<tr>
<td>Crocodile cementum</td>
<td>9.409(8)</td>
<td>6.873(6)</td>
<td>527(1)</td>
<td>8</td>
</tr>
<tr>
<td>Geological hydroxyapatite (^a)</td>
<td>9.4249(4)</td>
<td>6.8838(4)</td>
<td>529.56</td>
<td>-</td>
</tr>
<tr>
<td>Human dentin (^b)</td>
<td>9.421(3)</td>
<td>6.887(3)</td>
<td>529.3</td>
<td>-</td>
</tr>
<tr>
<td>Human enamel (^b)</td>
<td>9.441(3)</td>
<td>6.880(3)</td>
<td>531.1</td>
<td>-</td>
</tr>
<tr>
<td>Shark dentin (^c)</td>
<td>9.404(5)</td>
<td>6.842(10)</td>
<td>524.0(7)</td>
<td>2.13</td>
</tr>
<tr>
<td>Shark enameloid (fluoroapatite) (^c)</td>
<td>9.385(2)</td>
<td>6.883(2)</td>
<td>525.1(1)</td>
<td>34.75</td>
</tr>
</tbody>
</table>
Table 3: Results of the thermogravimetric analysis of dentin, enamel, and cementum of teeth of *C. porosus*.

<table>
<thead>
<tr>
<th></th>
<th>Dentin (wt%)</th>
<th>Enamel (wt%)</th>
<th>Cementum (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Release of water (&lt;200 °C)</td>
<td>2.7</td>
<td>3.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Combustion of the organic matrix (200-500 °C)</td>
<td>24.2</td>
<td>13.8</td>
<td>27.7</td>
</tr>
<tr>
<td>Release of CO₂ from carbonated apatite (&gt;500 °C)</td>
<td>1.8</td>
<td>2.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Mineral content (residual)</td>
<td>71.3</td>
<td>80.5</td>
<td>66.8</td>
</tr>
</tbody>
</table>
**Table 4:** Results of Vicker’s microhardness tests (HV0.01) of *C. porosus* teeth compared to values obtained for human teeth and shark teeth from literature. The values are averages of all tested specimens including the standard deviation. They were converted into Berkovich hardness in GPa. \(^a\) del Pilar Gutierrez-Salazar and Reyes-Gasga (2003), \(^b\) Malek et al. (2001), and \(^c\) Enax et al. (2012).

<table>
<thead>
<tr>
<th></th>
<th>Crocodile teeth / GPa</th>
<th>Human teeth / GPa</th>
<th>Shark teeth / GPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentin</td>
<td>0.60±0.05</td>
<td>0.5..0.6(^a)</td>
<td>0.5..0.7(^c)</td>
</tr>
<tr>
<td>Enamel</td>
<td>3.15±0.15</td>
<td>2.9..3.9(^a)</td>
<td>3..4(^c)</td>
</tr>
<tr>
<td>Cementum (distal, close to crown)</td>
<td>0.26±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cementum (proximal, close to root margin)</td>
<td></td>
<td>0.2..0.6(^b)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>