Archives of Oral Biology 89 (2018) 1–8

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Structure, property, and function of sheepshead (*Archosargus probatocephalus*) teeth

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ARTICLE INFO

Keywords:
Enameloid
Dentin
Structure-property
Nanoindentation

ABSTRACT

Objectives: This paper studies *A. probatocephalus* teeth and investigates the mechanical properties and chemical composition of the enameloid and dentin.

Design: Nanoindentation tests with a max load of 1000 μN and X-ray Energy Dispersive Spectroscopy (EDS) were performed along the diameter of the polished sample. Microstructural analysis of the dentin tubules was performed from SEM images.

Results: From nanoindentation testing, the dentin of the sheepshead teeth has a nanoindentation hardness of 0.89 ± 0.21 (mean ± S.D.) GPa and a reduced Young's modulus of 23.29 ± 5.30 GPa. The enameloid of *A. probatocephalus* has a hardness of 4.36 ± 0.44 GPa and a mean reduced Young's modulus of 98.14 ± 6.91 GPa. Additionally, nanoindentation tests showed that the enameloid's hardness and modulus increased closer to the surface of the tooth. X-ray Energy Dispersive Spectroscopy (EDS) data further suggests that the gradient may be a result of the wt% fluoride within the enameloid, where an increase in fluoride results in an increase in reduced Young's modulus and hardness.

Conclusion: The microstructural characterization of the number density and area of the dentin tubules were used to address the porosity effect in the dentin to achieve the experimentally validated microhardness. The mechanical properties of the sheepshead teeth were also compared with previous nanoindentation tests from other aquatic species. The sheepshead teeth exhibit a greater reduced Young's modulus and hardness compared to shark and piranha teeth.

1. Introduction

Archosargus probatocephalus, commonly referred to as the sheepshead fish, live along the coasts of the Atlantic and the Gulf of Mexico. They can grow to a length of about 75 cm and to a weight of 10 kg and are known for its bizarre, almost human-like in appearance, teeth (Bigelow & Shroeder, 1953). Previous studies have focused on the bones, scales, diet, and oral jaw strength of *A. probatocephalus* (Fernandez & Motta, 1997; Ogawa et al., 2004); however, the biomechanics and the material properties of the teeth have not been examined. In general, teeth can be subjected to large loads during feeding, producing a stress and strain that may result in the failure of teeth, which is even more of a danger for the sheepshead with its dietary regimen ranging from small invertebrate species (copepods, amphipods, and mysids) to hard-shelled animals such as barnacles, crabs, oysters, and clams (Bigelow & Shroeder, 1953; Overstreet & Heard, 1982; Sedberry, 1987).

Teeth are considered one of the hardest substances found in animals (He & Swain, 2008). They exhibit exceptional mechanical properties that allow them to bear various imposed loads while retaining their shape and are ideal for biting and chewing (Braly, Darnell, Mann, Teaford, & Weihs, 2007). Teeth are composed of highly mineralized enamel and dentin. For example, human enamel is 85 vol% mineralized and is the hardest tissue in the human body (Cate, 1994). The function of enamel is to provide a hard surface for crushing and slicing food and, for some species, wounding its prey (Currey, 2002). Conversely, human dentin is 50 vol% mineralized, 30 vol% organic components, and 20 vol% fluids with a microstructure characterized by distinct tubules (Habelitz, Marshall, Balooch, & Marshall, 2002). Fish teeth are made of enameloid and dentin. Enameloid is analogous to mammalian enamel, even though the developmental process is different. The individual properties of both enameloid and dentin were studied to understand the function and strength of the teeth of the sheepshead.

The chemical composition of teeth can provide insight into its

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https://doi.org/10.1016/j.archoralbio.2018.01.013
Received 3 August 2017; Received in revised form 14 December 2017; Accepted 17 January 2018

mechanical strength, particularly the wt% of calcium and phosphorus (Cuy, Mann, Livi, Teaford, & Weihs, 2002; Jeng, Lin, Hsu, Chang, & Shieh, 2011). Calcium and phosphorus are present in hydroxyapatite, $Ca_5(PO_4)_3(OH)$, the mineral strengthening human teeth; however, unlike human teeth, some fish teeth are strengthened by fluorapatite, $Ca_3(PO_4)F$ (Enax, Prymak, Raabe, & Epple, 2012). Compared to hydroxyapatite, fluorapatite has a higher bulk modulus, stiffness constants, and elastic modulus (Brunet et al., 1999; Gardner, Elliott, Sklar, & Briggs, 1992). In this paper, the chemical composition of the sheepshead enameloid and dentin are investigated through X-ray Energy Dispersive Spectroscopy (EDS). The micromechanical properties of the enameloid and dentin were captured using nanoindentation, which is a common technique used to determine the mechanical properties of hard biological tissues (Kinney, Balooch, Marshall, Marshall, & Weihs, 1996; Rho, Tsui, & Pharr, 1997). Previous research on the mechanical properties of fish teeth have focused on sharp teeth whose primary function is to tear through the flesh of its prey. In contrast, the function of *A. probatocephalus* teeth is to crush the shells of its prey. Experimental results from this study will be compared to previous nanoindentation tests done on the bonnethead shark, sand tiger shark, great white shark, and piranha (Chen et al., 2012; Whitenack, Simkins, Motta, Hirai, & Kumar, 2010). The goal of this paper is to investigate the microstructure, chemical composition, and material properties of *A. probatocephalus* teeth to gain insight into the structure's integrity.

2. Materials and methods

2.1. Sample preparation

Specimens of *A. probatocephalus* were acquired from the Mississippi Gulf Coast. Sheepshead teeth were kept in an ambient dry condition before testing, and all samples were extracted from one fish. Fig. 1 shows the extracted teeth used for this study. Molar teeth were removed, cold mounted in epoxy resin, and polished transversely approximately halfway down to create the surface for analysis. Samples were polished with a Struers TegraPol-11 (Struers Inc., Cleveland, OH) using interchangeable silicon carbide grinding discs of decreasing grit size (e.g. 320, 500, 1200, 4000). Diamond and silica suspensions with grit size 1 μm and 0.04 μm, respectively, were used for the final polishing.

2.2. Microstructural analysis

Optical micrographs of polished samples were taken using a ZEISS Axiovert 200 m. The microstructure of the dentin and enameloid were characterized by Scanning Electron Microscopy (SEM) at 20 kV using a ZEISS SUPRA 40 FESEM (Carl Zeiss Inc., Thornwood, NY). Fig. 2 diagrams the surface area used for nanoindentation, optical microscope, and SEM. The dentin tubule number density, dentin tubule diameter size, and nearest neighbor distances of the dentin tubules were quantified using ImageJ analysis software (Schneider, Rasband, & Eliceiri, 2012). The results are shown in Fig. 3.

2.3. Nanoindentation

Specimens were tested using a Triboindenter TI-900 (Hysitron Inc., Minneapolis, MN) with a diamond Berkovich tip of radius of 150 nm. A total number of 75 indentations with a max load of 1000 μN was performed on both the enameloid and dentin sections. Specimens were indented at a load rate and unload rate of 200 μN/s. The max load was held for 5 s before unloading. Load versus displacement was continuously recorded throughout the testing. Additionally, 100 indentations spaced 26 μm apart with a max load of 1000 μN were performed along the entire diameter of the polished sample to observe the changes in material properties based on the test location of the tooth. The data was analyzed using standard routines (Oliver & Pharr, 1992) to determine the hardness, *H*. The hardness for each test is defined by Eq. (1):

$$H = \frac{P_{\max}}{A} \tag{1}$$

where $P_{\max}$ is the peak load, and *A* is the projected indentation area indenter. The reduced Young's modulus is calculated during the unloading phase and is described by Eq. (2):

$$E_r = \frac{1}{2}\sqrt{\frac{\pi}{A}}\frac{dP}{dh} \tag{2}$$

where dP/dh is the slope of the unloading curve. For the sake of this convenience, the reduced Young's modulus will be referred to as modulus for the remainder of this paper.

2.4. Chemical analysis

After nanoindentation, the chemical composition of both the enameloid and dentin was explored by X-ray Energy Dispersive Spectroscopy (EDS). Testing was performed using a ZEISS SUPRA 40 FESEM equipped with an EDAX PV7715/89 ME analyzer (EDAX, Mahwah, NJ). The local variations in chemical composition were

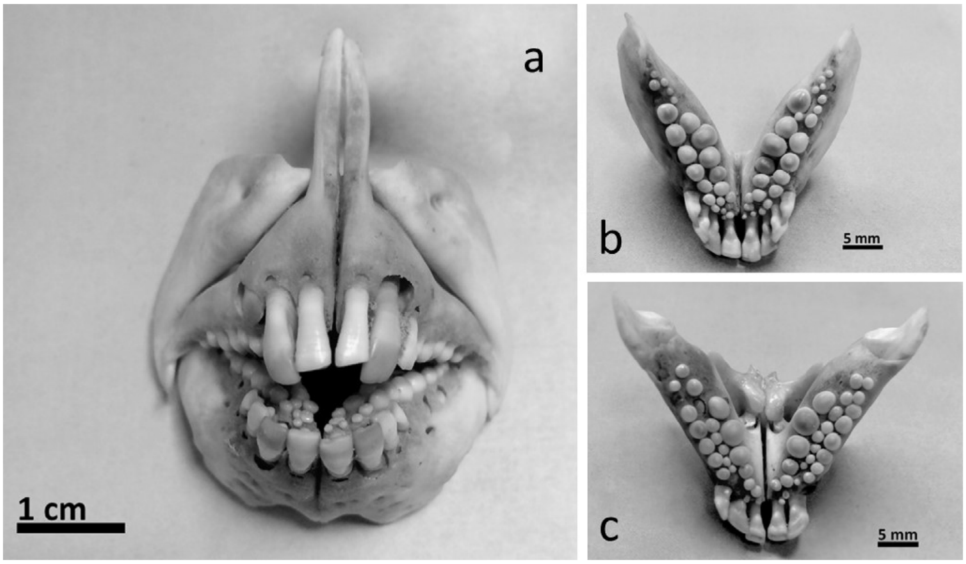

Fig. 1. (a) *A. probatocephalus* teeth. (b) Upper section. (c) Lower section.