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Abstract

The teeth of polycotylid plesiosaurs are generally simple, cone shaped, non-serrated and only slightly recurved without distinct carinae. The surface of crowns are characterized by a series of vertical enamel wrinkles that are more highly developed on the lingual surface of the crown, and decrease in width and number toward the apex. Some of the most promising research related to fossil dentition, involves the analysis of surface and internal dental microstructure. This study, is an attempt to examine and describe polycotylid dental microstructure. It gives an overview of polycotylid plesiosaur enamel and dentine microstructures using a scanning electron microscope. Enamel type and structures vary, based on its position on the surface of the crown, and its perceived strength requirements. The dentition layer is "honeycombed" with tubular structure, possibly to provide nourishment to fast growing crowns. The study of crown microstructures may lead to a better understanding of polycotylid niche preference in the late Cretaceous oceans.

Introduction

The study of enamel microstructure of both fossil and extant amniote taxa has been extensively studied (Koenigswald and Sander 1997; Sander 2000; Hwang 2005; Stokosa 2005). According to Hwang (2005), mammalian taxa have received preferential study due to distinctive prismatic enamel, easily seen in thin section under polarized light. In comparison, most reptile taxa have nonprismatic enamel; individual crystallites can only be differentiated using the scanning electron microscopy (Sander 2000; Hwang 2005).

Polycotylids are a group of short-necked plesiosaurs known mainly from the late Cretaceous, (Sato and Storrs 2000; O'Keefe 2004). Plesiosaurs are traditionally divided into two groups, the long-necked, small-headed elasmosaurids and the short-necked, and large headed pliosaurids (Everhart, 2005). Polycotylid plesiosaurs have short necks with large heads, and have been commonly lumped with the pliosaurids (O'Keefe 2004). However, Carpenter (1996) determined these late Cretaceous plesiosaurs are more closely related to the long-necked elasmosaurids, placing Polycotylidae as a sister taxa to

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Elasmosauridae. It is hypothesized (Everhart 2005) that the short-necked, long beaked polycotylids may have evolved to fill the nice left behind by the extinction of pliosaurid plesiosaurs and ichthyosaurs earlier in the Late Cretaceous. Along with other marine taxa, polycotylid fossil skeletons are recovered from the rocks deposited by the late Cretaceous Western Interior Seaway of central North America (Carpenter 1996) as well as similarly aged deposits in Japan (Sato and Storrs 2000), Australia (Kear 2003) and Russia (Arkhangelsky *et al* 2007).

The teeth of polycotylid plesiosaurs are cone shaped, un-serrated and slightly recurved. The crowns are covered by a series of vertical enamel wrinkles that are more highly developed on the lingual surface of the crown, and decrease in width as they move toward the apex (Figures 2 and 3). Polycotylid crowns are, in all respects other than size, homodont, with little change in shape noticeable between teeth in the anterior vs. posterior portion of the jaw.

Materials and Methods

The specimen (SDSM 86604) used in this project is located in the collections of the Museum of Geology at the South Dakota School of Mines and Technology, and consists of an individual from the sedimentary strata of the Western Interior Seaway deposits of the central United States. SDSM 86604 consists of cranial and tooth material from an unknown species of plesiosaur cf. *Polycotylus*, collected from the upper part of the Boyer Bay Member of the Sharon Springs Fm., Pierre Shale Group of South Dakota (Martin *et al* 2007). A second specimen (AMM 98.1.1) was observed for comparison and is identified as *Pahasapasaurus haas* (Schumacher 2007). AMM 98.1.1 is on display at the Adam's Memorial Museum in Deadwood, South Dakota. Crowns from this specimen were measured and described for morphology, but were not available for Scanning Electron Microscope (SEM) analysis. In general, both specimens are in fair to poor condition, with few intact crowns. It is a shed crown from SDSM 86604, with the field number JEM03-1, that was analyzed for this study.

Scanning Electron Microscope

The Scanning Electron Microscope (SEM) used was the South Dakota School of Mines and Technologies' Zeiss Supra40 Variable-Pressure Field-Emission Scanning Electron Microscope. The SEM was set to High Pressure mode with an aperture of 30.00 μm, and a voltage of 10 kV. All images were taken using the Secondary Electron Emission detector.

Sample Preparation

The base of the best-preserved crown was polished transversely, by hand to provide a surface for examining the enamel structure in the SEM. The base of the crown was initially dipped in acetone to remove any adhesive residue on the base of the crown. Grinding the base of the crown in a circular pattern the surface was polished using 120 grit sandpaper, followed by a wet stone of 600 grit, then a 1 μm wet cloth with 1 μm Aluminum oxide powder, 0.3 μm cloth and powder, and finally 0.05 μm cloth and powder. The last step in the polishing process consisted of a 10-second acid etching using 2 N HCl.

Following the polishing procedure, the sample was first coated in a thin layer of carbon. The carbon was found to not coat the specimens adequately, so an additional gold coat was added; both materials are used in the SEM to improve conduction of the electron beam, and prevent electrical charging of the samples. The sample were taped to a glass slide, and then taped down to the SEM sample holder using carbon-based tape.

Initially, attempts were made to examine the base of the crown using the High Pressure mode, at low voltage (1 kV) with the standard aperture of 30.00 μm and a short working distance. After taking a few measurements, we increased the voltage to 10 kV to use the Microwave detector and do a chemical analysis of the enamel. After increasing the voltage to 10 kV, the bulk of the images of both the base structure and enamel surface structures were obtained.

Results and Discussion

Results

The enamel of reptiles, both extent and fossil, with a few exception, has been found to be prism-less (Frank *et al* 1984). Most reptiles share the basic amniote condition of possessing columnar enamel; this remains true for the polycotylid sp. in this study. The enamel of SDSM 86604 using terminology set forth by Koenigswald and Sander (1997) and Sander (1999, 2000) can be summarized as columnar, possessing convergence at the crystallite level. Individual columnar unites are challenging to identify (Figure 1) however zones of crystallite (enamel units) convergence are interpreted along with incremental lines. The converging crystallite form roughly columnar features that are perpendicular to the enamel-denting junction (EDJ). Figure 1 also shows some evidence of incremental lines in the enamel that are parallel to the EDJ and perpendicular to the crystallite columns. The convergent enamel crystallites that make up the majority of the crowns enamel structure, with the exception of the enamel wrinkles. According to Sanders (1999), the incremental line structures are the traces of intermittent growth in two-dimensional enamel segments.

Figure 2 shows the relative thickness of the enamel (~167 μm) near the base of the crown, as well as the approximate wavelength of 215 μm for the enamel wrinkles. This image was taken before the base of the sample was polished down and acid etched, allowing for the observation of structures associated with a broken surface. Structures visible in this image that are obscured in later images, post polishing, include the fine-grained nature of the dentine layer, and the presence of round nerve canals structures in the dentine (labeled α in Figure 2 and shown in more detail in Figure 7).

Polycotylid crowns are heavily striated on the lingual surface only; Figure 3 shows how the size of theses striations, more commonly referred to as enamel wrinkles. Enamel wrinkle size varies along the surface of the tooth basal to apical. The image on the left (A), shows the enamel wrinkles close to the base of the crown, where the striations have a wavelength of ~250 µm. Image B, on the right, shows the same crowns lingual surface, near the apex, where the distance between striations in reduced to ~40 µm.

Figure 4 shows a section of the lingual surface of SDSM 86604 viewed from the basal surface, after the polishing procedure. The enamel-dentine junction is very distinct in the image, as a dark line between the crystalline enamel and the more homologous dentine. It is evident from the image that the enamel wrinkles are a function of varying thickness within the enamel layer, and not an external expression of any internal dentine structures.

The enamel that makes up the wrinkles may differ from the enamel between the wrinkles in terms of schmelzmuster. Schmelzmuster is defined as the three-dimensional arrangement of enamel kinds present in a single crown (Koenigswald and Sander 1997). Figure 5 shows a more detailed view of the structure within an enamel wrinkle, incremental lines are preset, visible near the EDJ. The enamel below the wrinkles reflects the surface structure, convergent enamel columns forming where the wrinkle edges meet.

Figure 6 shows the enamel-dentine junction on the labial side of the crown, where enamel wrinkles are not present. Where enamel consists of as much as 99% inorganic material (hydroxyapatite), dentine is made of as much as 75% organic matrix, collagen (Glimcher, *et. al.*, 1990). This basic difference in their chemical structures highlights the striking differentiation between the crystalline enamel and the non-crystalline dentine seen in Figure 6.

As briefly mentioned in the discussion of Figure 2, Figure 7 is a close-up of the almost perfectly round opening seen in the dentine. These structures may represent nerve canals in the dentine layer, or the dentinal tubules. Near the enamel-dentine junction, these tubules are much less densely packed then at the center of the crown. Figure 8 shows the dentinal tubule structure near the center of the crown. These tubules form a honeycomb structure (A) through the center of the crown. Dentin tubules are responsible for the porousness of dentine and allow for uninterrupted communication between the dentine and the pulp layers (Lima *et al* 2009). In life, these dentinal tubules would have been filed with dentinal fluid and possibly innervated. These structures would have connected the main nerve of the crown, in the internal pulp cavity, with the dentine layer of the tooth, stopping just short of the EDJ.

Figure 9 shows the incidental growth rings in the dentine of the crown. Like tree rings, these structures can be used to better understand the growth of the crown, as successive layer were laid down during the growth of the crown. Since polycotylid lost and re-grew hundreds of crowns in a lifetime the rings have no relationship to the age of the animal.

Conclusions

The high concentration of dentinal tubules closer to the center, pulp structure, of the crown supports the idea that polycotylid crowns are growing quickly. The large number of dentinal tubules may be connected to the greater need for innervation in polyphyodontic animals that were constantly shedding and re-growing new crowns. These crowns would have needed high blood and nerve supply to grow quickly to replace crowns lost naturally through the feeding process. Each tubule contains a rod-like structure (Figure 8); the origin of these structures is still unclear; however, they may represent a non-mineralized material that had filled in the voids during the fossilization process. Mithiborwala et al (2012) reported similar structures where they are reported to be resin tags, remnants of the adhesive used to stabilize the fossil. Another possible that the rods represent the remnants of collagen structures that once filled the tubules, the acid etching process would have removed the surround hydroxyapatite minerals leaving the collagen to stick out above the surface. “Fossilized” collagen has recently been reported in fossil reptiles, including a Cretaceous hadrosaur and *Tyrannosaurs rex* (Schweitzer, *et. al.* 2007 and 2009).

Parallel and columnar are the two most common crystallite forms of enamel in reptile dentition. In parallel enamel, the hydroxyapatite crystals are parallel to one another and perpendicular to the enamel-dentine junction; on the other hand, columnar enamel is more organized, making up units and bundles (Stokosa 2005). SDSM 86604 shows the later of these enamel types. Columnar enamel is generally considered the more robust enamel type, and in a study with theropod dentition (Stokosa 2005) has suggested columnar enamel is more prevalent in organisms that ingest bone along with soft tissue. Enamel structure may indicate that shell was an important part of polycotylid diets, as the teeth would be more capable of withstanding the stress. Although columnar enamel structure does not directly confirm such a diet, it presence might suggest polycotylids took advantage of the numerous ammonite taxa present in their habit.

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Figures:

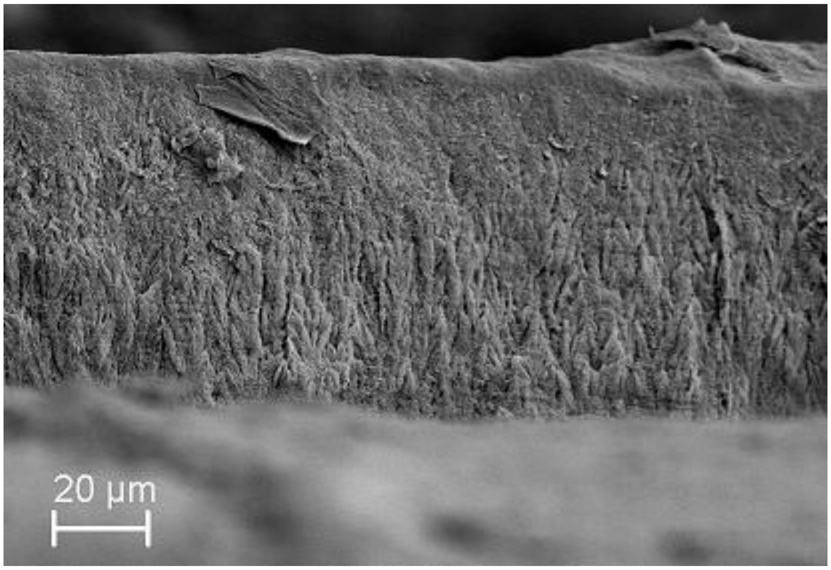

Figure 1: Scanning electron microscope image of SDSM 86604, enamel microstructure in transverse section.

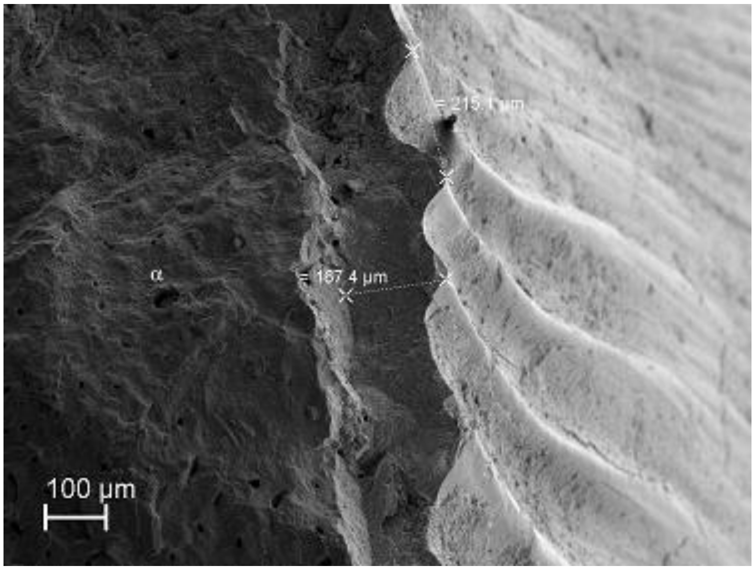

Figure 2: Enamel wrinkle structure near crown base

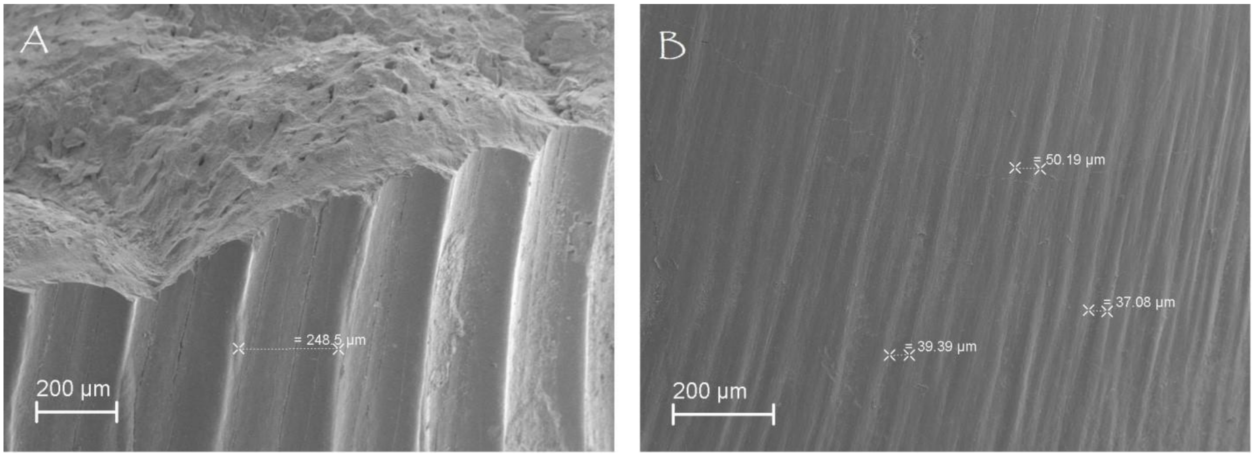

Figure 3: Enamel wrinkles on the surface of SDSM 86604, near base (A) and near apex of crown (B)

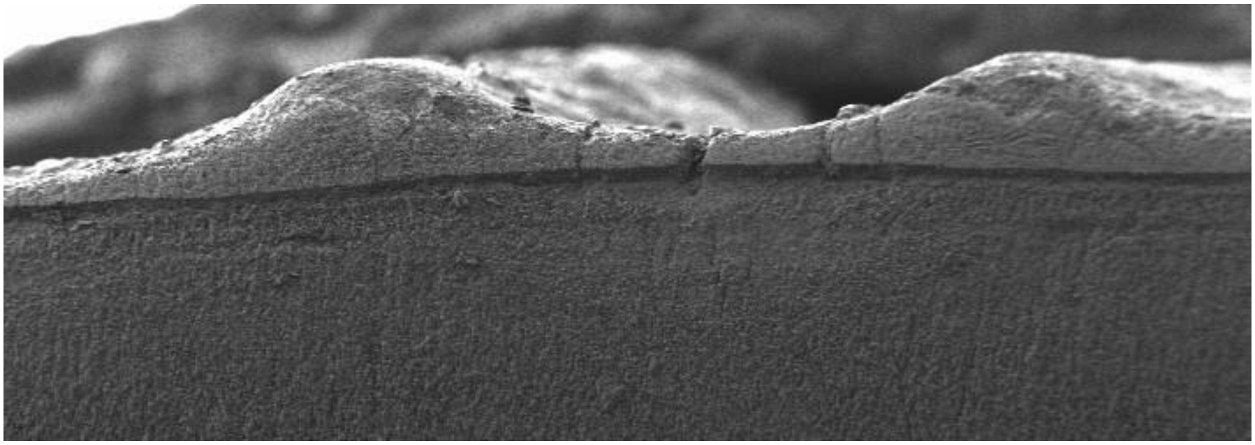

Figure 4: Enamel wrinkle structure (cross-section)

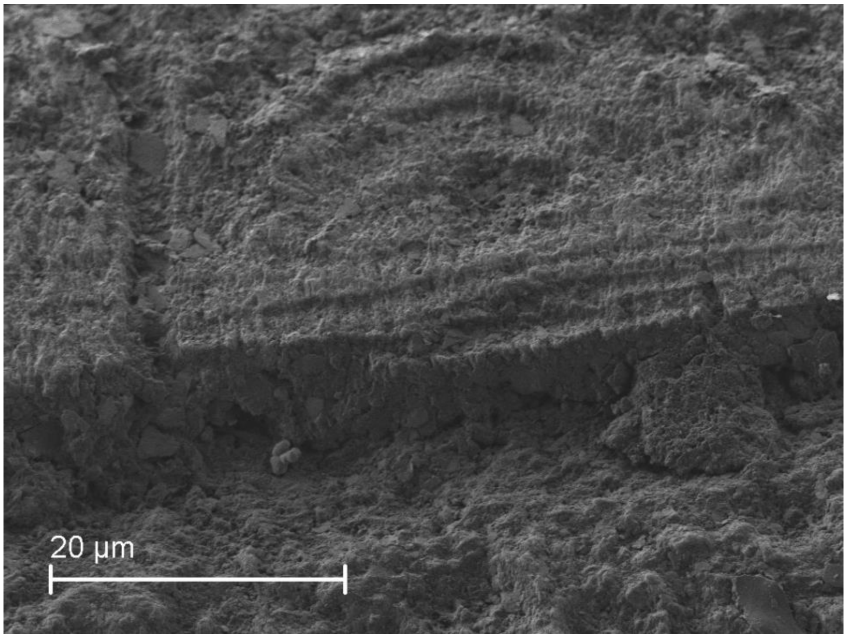

Figure 5: Close-up of enamel wrinkle structure

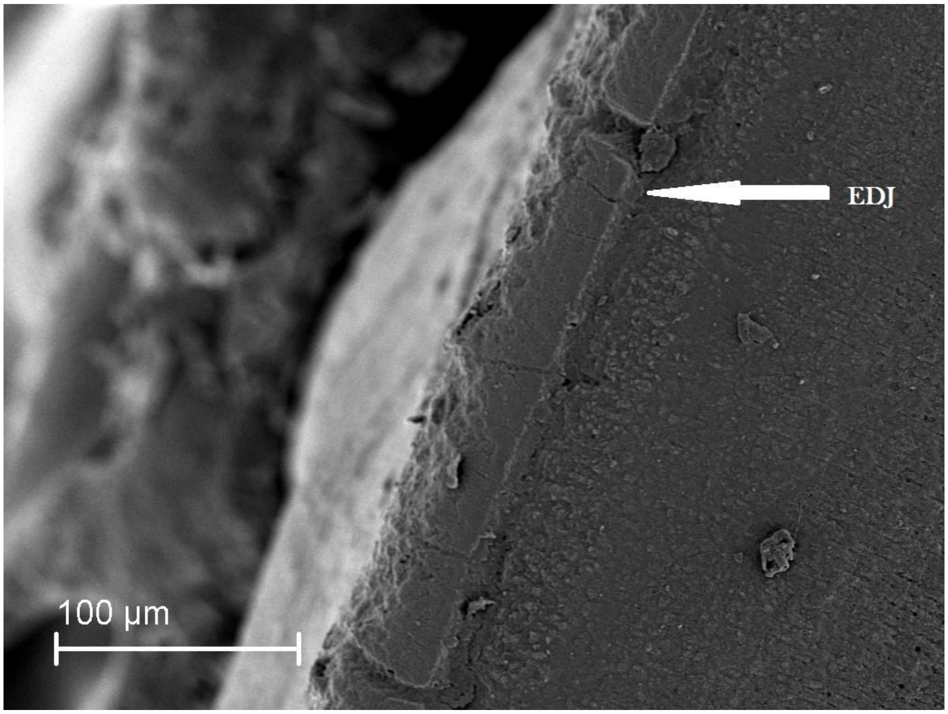

Figure 6: Enamel-dentine junction

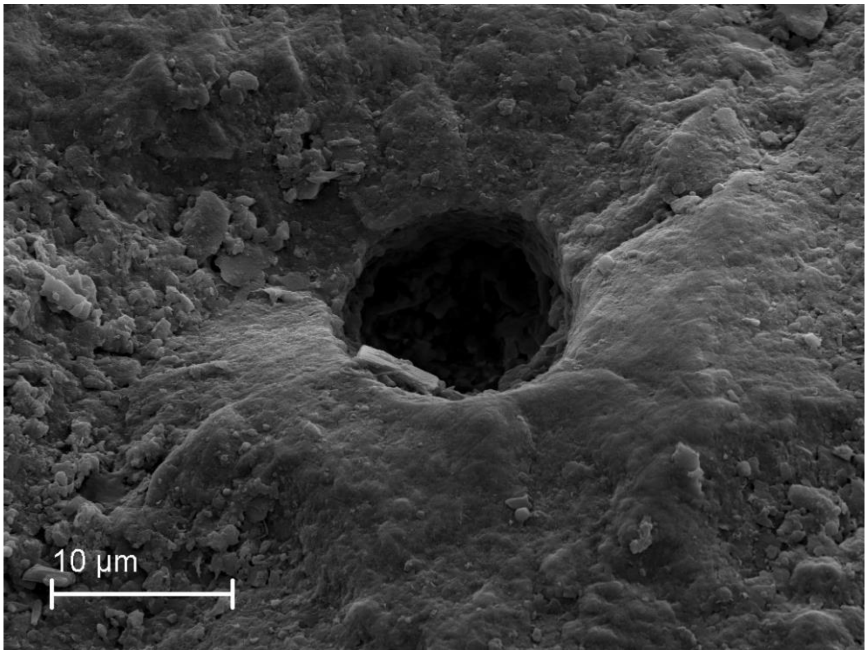

Figure 7: Opening of dentine tubule canal in dentin layer

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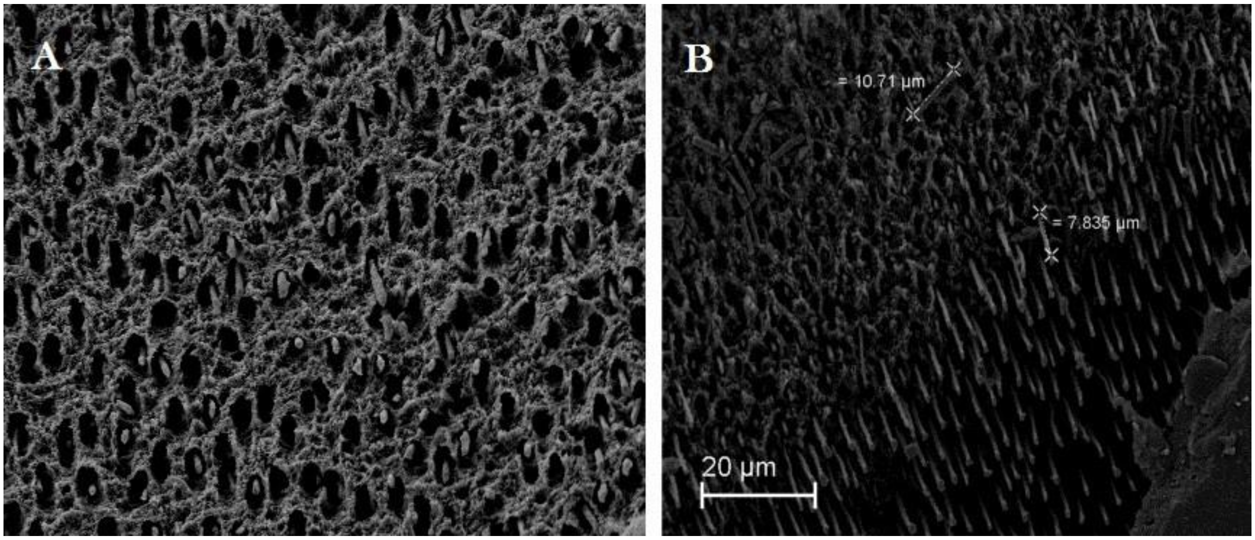

Figure 8: Dentine structures (A), rod structure within the dentine (B)

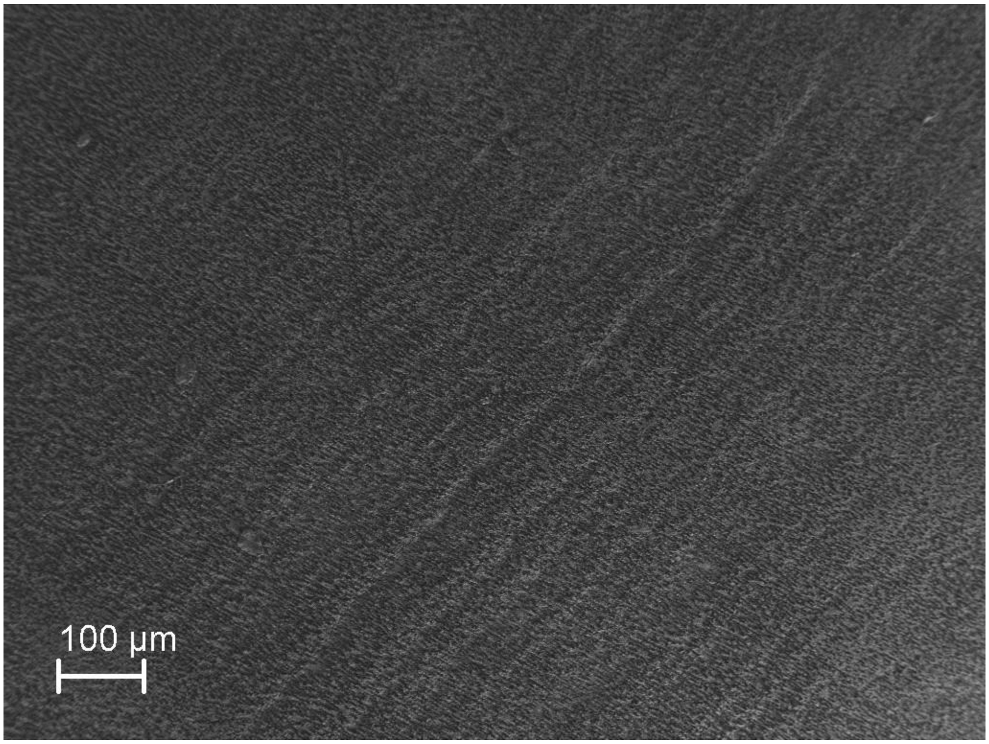

Figure 9: Incidental growth rings in dentin layer

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