

Physiological insult or the burial environment: Differentiating developmental defects from post-mortem stained enamel in deciduous dentition from the Chieftom Period of Tonga, Polynesia

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Abstract

Developmental dental defects, usually manifested as enamel discolouration, are commonly used as indicators of physiological stress in human bioarchaeological research. It is sometimes difficult to ascertain whether discolouration in enamel is a developmental defect or the result of a taphonomic process. Previous research on discolouration of dental enamel has shown that X-ray microtomography is a valid and reliable method for differentiation between hypomineralised enamel discolouration and taphonomic discolouration. The aim of this research was to use X-ray microtomography for the investigation of macroscopic deciduous enamel discolouration in a Chieftom Period skeletal sample from 'Atele, Tonga, and to assess whether these discolourations are true enamel hypomineralised defects or not. This assessment can aid in the interpretation of stress responses in the infants and children during this time of increasing hierarchy in Polynesia. Our results found evidence of reduced mineral density in discoloured teeth (Fig. 1A). These findings indicate that discolourations in deciduous dentition from 'Atele are more likely the result of developmental enamel defects rather than post-mortem staining. We interpret this evidence of developmental defects in the environmental context of infection and nutritional stress and previous palaeopathological work that has been undertaken on this sample.

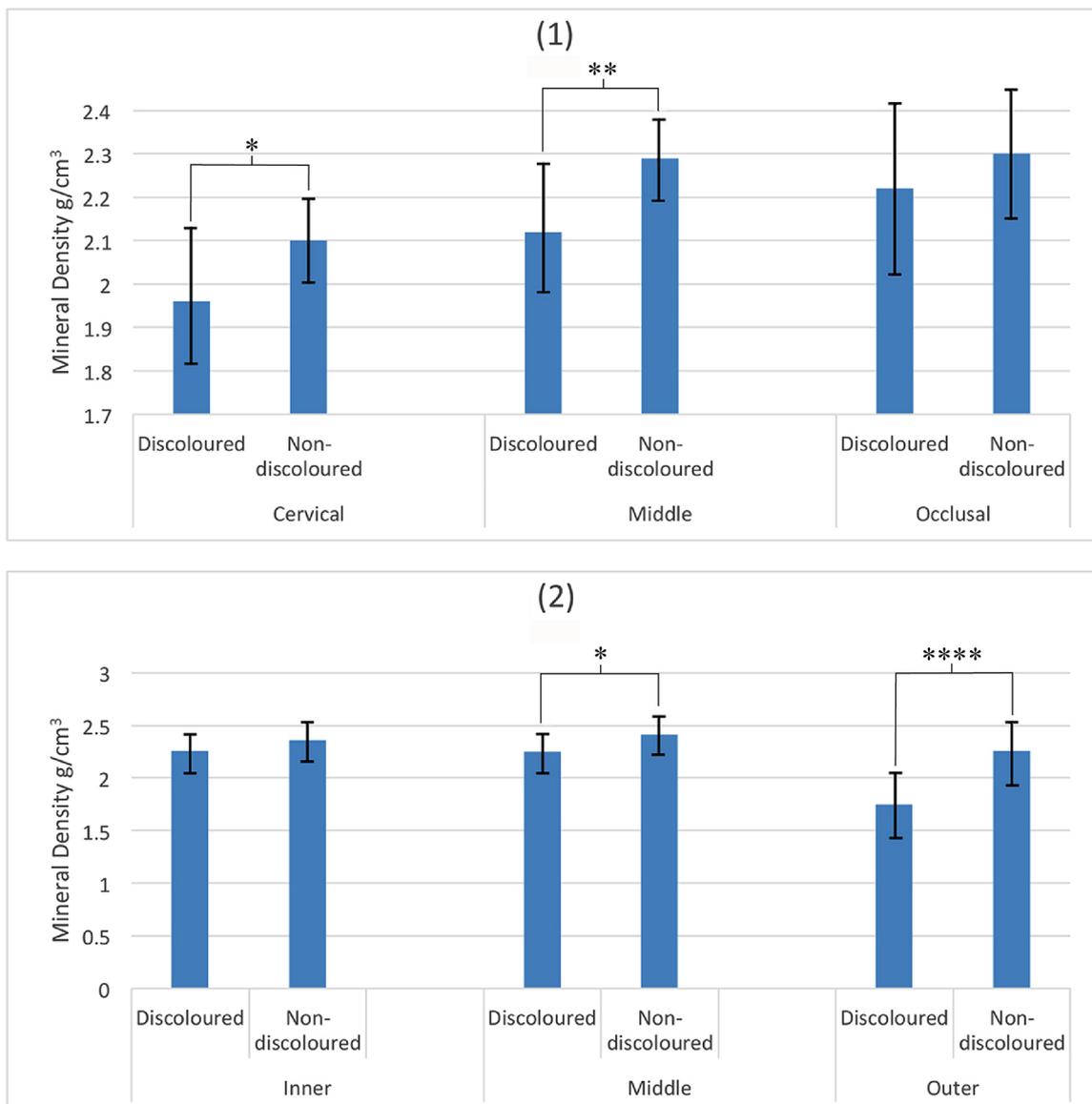
Introduction

Ameloblasts, the cells responsible for enamel formation, are very sensitive to physiological perturbations, which can result in the formation of defective enamel. The type of enamel defect depends mainly on the timing of the insult in relation to the stage of enamel formation [2]. If disruption occurs early on during enamel formation, it may result in hypoplastic enamel. But if the insult occurs later, it results in hypomineralised defect(s), with reduced mineral density (MD) [3]. These hypomineralised defects appear macroscopically as enamel opacities or discolourations. A wide range of physiological insults can affect the sensitive ameloblasts and result in enamel defects. These factors include nutritional deficiencies, infectious diseases, inborn errors of metabolism, endocrinopathies, kidney and liver diseases, and certain hereditary conditions [2] [4]. Dental enamel defects in the form of hypoplastic defects are commonly used as indicators of physiological disruption in the archaeological context [2]. However, hypomineralised defects are not as often used in archaeological samples as teeth are subject to taphonomic effects that may result in discolouration resembling hypomineralised defects [5]. Unlike hypomineralised defects, taphonomic discolourations do not display reduction in the MD of the enamel.

Objective

X-ray microtomography (XMT) is an imaging system used in the measurement of the MD of enamel, thus allowing for differentiating between truly hypomineralised enamel discolourations and non-hypomineralised taphonomic discolourations [6]. Our current study uses XMT to determine whether discoloured teeth from the prehistoric sample from 'Atele, Tonga, are the result of post-mortem staining or a developmental defect caused by physiological disruption. This archaeological sample of infants and children is

the biggest in Polynesia and presents an opportunity to address archaeological questions related to the Chieftain Period in Tonga, a time of increasing hierarchy and interactions through trade networks [7].



a

Figure Legend

Figure 1.

(A) Mineral densities of discoloured and non-discoloured enamel.

(1) Mineral density (MD) was traced (and averaged) from the cervical to the occlusal thirds. In non-discoloured teeth ($n = 10$), the average MD for the cervical third was 2.10 g/cm³ (SD = 0.09), 2.29 g/cm³ (SD = 0.12) for the middle third, and 2.30 g/cm³ (SD = 0.15) for the occlusal/incisal third. In discoloured teeth ($n = 11$), the average MD for the cervical third was 1.96 g/cm³ (SD = 0.17), 2.12 g/cm³ (SD = 0.15) for the middle third and 2.22 g/cm³ (SD = 0.20) for the occlusal/incisal third.

(2) Mineral density (MD) was traced (and averaged) from the inner to the outer thirds. In non-discoloured teeth, the average MD for the inner third was 2.36 g/cm³ (SD = 0.16), 2.41 g/cm³ (SD = 0.17) for the middle third, and 2.26 g/cm³ (SD = 0.31) for the outer third.

In discoloured teeth, the average MD for the inner third was 2.26 g/cm³ (SD = 0.14), 2.25 g/cm³ (SD = 0.13) for the middle third, and 1.75 g/cm³ (SD = 0.31) for the outer third.

Key: **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001, *****p* ≤ 0.0001.

Supplementary Figure 1. Location of 'Atele on the island of Tongatapu, Kingdom of Tonga. © OpenStreetMap contributors, adapted from openstreetmap.org and published under the CC BY-SA license, <http://www.openstreetmap.org/copyright>).

Supplementary Figure 2. Tooth 52 from burial number To-At 1/3, 'Atele, showing discolouration of enamel.

Supplementary Figure 3. Typical projection of a sample tooth with the four standard discs.

Supplementary Figure 4. Typical scatter-plot of the four standard discs with the generated regression equation.

Supplementary Table 1. Characteristics of the sample teeth. The nomenclature used for recording teeth was the two-digit system of the Fédération Dentaire Internationale (FDI) [1]. Based on the DDE index for type: 'demarcated' defect = code 1, 'diffuse' defect = code 2, and based on the extent: defect is less than 1/3 of the tooth surface = code 1, at least 1/3 but less than 2/3 = code 2, at least 2/3 = code 3.

Tooth sample

The sample of deciduous teeth analysed in this study was collected from infants and children from the prehistoric site of 'Atele on the island of Tongatapu in the Kingdom of Tonga, Western Polynesia [7] (Suppl. Fig. 1). The human skeletal sample is derived from two burial mounds (To-At 1 and To-At 2), which were excavated by archaeologist Janet Davidson in 1964 [7]. Recent Accelerator Mass Spectrometry radiocarbon dates obtained from human bone from the site yielded dates between c. 500–200 BP, which corresponds with the Chiefdom Period in Tonga [17]. The sample is curated by the Department of Anatomy, University of Otago, New Zealand. All necessary permissions to assess the human skeletal remains were received from the Kingdom of Tonga. A sample of 21 deciduous teeth (1 tooth from 21 separate individuals as identified during excavation) was collected consisting of incisors, canines, and molars. The teeth were assigned into two groups according to whether or not there was macroscopic evidence of enamel discolouration; discoloured (*n* = 11) (Suppl. Fig. 2) and non-discoloured (*n* = 10). The discoloured teeth were further classified into subcategories based on the Developmental Defects of Enamel (DDE) Index [18]. The DDE Index is a method for objectively describing/coding enamel defects at the tooth level on the basis of the appearance, extent, distribution, and location of defects. The defects were sub-classified as 'demarcated' (code 1) or 'diffuse' (code 2) on the basis of macroscopic appearance. The extent of each defect was categorised as less than 1/3 of the tooth surface (code 1), at least 1/3 but less than 2/3 (code 2), and at least 2/3 (code 3) (Suppl. Table 1). All teeth were photographed and no treatment of the enamel was carried out prior to XMT.

X-ray microtomography

XMT is an imaging system used to reconstruct a radiographic model of a small object through multiple X-ray projections. The resultant radiographic model can be virtually sectioned horizontally or sagittally. The MD of enamel at any specific site can be calculated by comparing its opacity (grey level reading) to that of four 'standard' hydroxyapatite (HA) discs (of known MDs) that are scanned along with each sample tooth (Suppl. Fig. 3). Using Microsoft Excel, the grey levels of the discs (as seen on the resultant X-ray scans) are plotted against their pre-measured MDs, thus generating a scatter plot with regression equation and a typical linear regression coefficient of 0.99 (Suppl. Fig. 4). The generated regression equation of each plot is then used to calculate the MD of the enamel at specific regions of interest. For example, from the equation in supplementary figure 4, if a certain region of interest in enamel had a grey level reading of 182, the MD can be calculated from the equation: MD = 0.016 × 182 - 0.8491 = 2.0629 g/cm³. The XMT system used in this study was SkyScan 1172 (SkyScan N.V., Aartselaar, Belgium). Images were reconstructed using a modified Feldkamp cone-beam algorithm (NRecon, Version 1.5.1.4), and subsequently analysed using the software package CT Analyser (Version 1.5.0.0, SkyScan N.V., Aartselaar, Belgium).

Mapping the mineral density

The methodology developed by McKay et al. [6] was used to map the MD of discoloured

and non-discoloured teeth. In summary, the MD of each tooth was measured at horizontal slices in increments of 1 mm from the cemento-enamel junction (CEJ) to the cusp/incisal tip. After that, one representative section was chosen for measuring the MD from the dentine-enamel junction (DEJ) to the outer surface in increments of 100 μm (see McKay et al. [6]). The representative section from discoloured teeth was chosen at the most severely discoloured part of the tooth, while in non-discoloured teeth, the representative section was chosen at the middle part of the tooth. Additional MD readings were recorded at non-discoloured areas within the discoloured teeth. A horizontal cross-sectional slice was selected for each discoloured tooth where enamel discolouration did not appear macroscopically. Readings were then taken from the DEJ to the outer surface of enamel in increments of 100 μm . This was carried out to assess whether discoloured teeth were more hypomineralised in general compared to non-discoloured teeth, or only within the affected discoloured areas.

Statistical analysis

Data were analysed in Intercooled Stata Version 13 (StataCorp, Texas). Differences in MD between discoloured and non-discoloured teeth were tested for statistical significance using unpaired t-tests. Paired t-tests were used to assess the affected and unaffected sites within the same tooth. **Results & Discussion**

Measurement of mineral density from the cemento-enamel junction to occlusal/incisal edge

MD readings were averaged for each third for the discoloured and the non-discoloured teeth. Non-discoloured teeth displayed a gradient of increasing MD from the cervical third to the occlusal/incisal third. The discoloured teeth also showed a similar gradient (Fig. 1A(1)). The differences between the discoloured and non-discoloured teeth were statistically significant for the cervical third ($t_{(19)} = 2.422$, $p = 0.0128$ (one-sided)) and the middle third ($t_{(19)} = 2.8172$, $p = 0.0055$), but not for the occlusal/incisal third ($t_{(19)} = 1.0677$, $p = 0.1495$).

Measurement of mineral density from dentino-enamel junction to external surface

The MDs were calculated from a representative cross-sectional slice for each tooth. The difference in MD between discoloured and non-discoloured enamel was statistically significant for only the middle ($t_{(18)} = 2.5293$, $p = 0.0105$) and outer thirds ($t_{(18)} = 4.5253$, $p \leq 0.0001$) (Fig. 1A(2)). For both the discoloured and non-discoloured groups, the least mineralised part was the outer third. The MD values sampled from unaffected areas within the discoloured teeth were similar to the MDs recorded from the non-discoloured teeth, with no statistically significant differences. This suggests that in the discoloured teeth, the reduction in MD is restricted to discoloured areas. There was a statistically significant difference in MD between the affected/discoloured and unaffected regions within the discoloured teeth for the middle and outer thirds of enamel. The average MD for unaffected areas at the inner third was 2.27 g/cm^3 (SD = 0.20), 2.38 g/cm^3 (SD = 0.14) for the middle third and 2.21 g/cm^3 (SD = 0.16) for the outer third.

Research using XMT to assess the MD of enamel report the range of densities for sound enamel at between 2.3 and 3.1 g/cm^3 [8] [9] [10] [11] [12]. The MD of non-discoloured enamel in the present study is towards the lower end of the normal reported range. The MD of discoloured enamel in this study had a range of 1.96–2.22 g/cm^3 , thus reflecting a true hypomineralisation.

Our findings show that the MD was not constant throughout the enamel. When traced horizontally, the non-discoloured teeth showed a positive gradient of MD from the CEJ to the cuspal/incisal edge, a finding consistent with previous studies [9] [13]. The higher MD at the cuspal/incisal edge may reflect the gnarled nature of enamel in that area, where the enamel rods are twisted over each other. The lower MD of the cervical third may be an artefact of the partial volume effect. Partial volume effect occurs when a region of interest adjacent to the outer surface incorporates part of the dark background, making the MD reading appear lower [14]. This is a possibility particularly for cervical area readings because the enamel at this point is very thin, making it difficult to identify a region of interest that excludes the dark background. The MD readings of the

discoloured teeth showed the same gradient from the CEJ to the cuspal/incisal region, although the average MDs were lower. The most severely affected area is the middle third, with an average of 7.4% reduction in MD compared with non-discoloured enamel. In sagittal sections of discoloured and non-discoloured teeth, the MD decreased from the middle third to the outer third. For both discoloured and non-discoloured samples, the lowest MD was located within the outer third, although the discoloured teeth had significantly lower MD in the outer enamel. There is some disagreement in the literature regarding MD trends when traced from the DEJ to the external enamel surface. Some studies reported an increasing gradient from the DEJ to the external surface [9] [15], whereas others documented similar findings to our study [6] [16]. The lower MD of the outer third of both discoloured and non-discoloured samples found in the current investigation may be, in part, attributed to the partial volume effect. Alternatively, low MD at the outer surface may indicate that the teeth were unerupted or newly erupted [6]. Finally, ion exchange may have occurred between the burial environment and the outer enamel of the teeth in this archaeological sample, causing the low MD reading at the outer surface [6].

To further confirm the hypomineralised nature of the affected areas in the discoloured teeth, the MD was measured for non-discoloured areas in the discoloured teeth. Those non-discoloured areas were found to have normal MD, which confirms that only the discoloured areas were truly hypomineralised. The significant difference in the MDs between the discoloured and the non-discoloured teeth from the 'Atele burial sites is in contrast to previous findings for teeth collected from the prehistoric site of Ban Non Wat, Thailand [6]. This strengthens the argument that identifying teeth as hypomineralised based solely on their macroscopic appearance is not possible.

This paper is the first step in providing a fuller understanding of the life histories of these infants and children in Chiefdom Period in Tonga, a period of increasing hierarchy and interactions through trade networks [7]. In establishing that these discoloured teeth are evidence for physiological disruption, our future work will quantify macroscopically all deciduous dental enamel defects in the sample. The analyses of the prevalence, timing of these defects during dental development, and the relationship between these defects and dental caries will be informative to the epidemiology of stress and oral pathology of the infants and children, and childhood diets. In establishing the prevalence of these defects, this may be interpreted in the wider context of disease from previous palaeopathological research [16] and dietary information that is being published. Infectious diseases such as treponematosis and hookworm were likely significant contributors to ill health in prehistoric Pacific populations [16], and there is palaeopathological evidence for infectious and metabolic disease in a high proportion of infants and children from 'Atele [16]. A hypothesis can be proposed that infection and periodic undernutrition were causative agents in the development of the observed hypomineralisation.

Further research of alternative methods to identify hypomineralisation in bioarchaeology studies could include DIAGNOdent (DIAGNOdent, KaVo, Biberach, Germany), a small hand-held laser fluorescence device used in dentistry for diagnosing dental caries.

Limitations

When mapping the MD from the dentino-enamel junction to the external surface, the representative sections from the discoloured teeth were chosen at the most severely discoloured part of each tooth, while for the non-discoloured teeth, the representative sections were chosen from the middle part of each tooth. It may have been more meaningful to use matched sections instead of only the middle parts of non-discoloured teeth, but that was not possible since the sections were chosen from different types of teeth (incisors, canines, molars). However, we compensated for this limitation by recording MD readings at various non-discoloured areas within the discoloured teeth. A horizontal cross-sectional slice was selected for each discoloured tooth where enamel discolouration did not appear macroscopically. The MD readings from the non-discoloured areas of discoloured teeth were similar to those of non-discoloured teeth.

A useful control could have been freshly shed/extracted primary teeth. However, the MD for enamel of primary teeth had been repeatedly established before [9], and the use of this control was not necessary.

Additional Information

Methods

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Supplementary Material

Please see <https://sciencematters.io/articles/201605000005>.

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Ethics Statement

The archaeological collection from 'Atele in Tonga is currently held at the Department of Anatomy, University of Otago, and all permissions were obtained from the University and the Kingdom of Tonga. No ethical approval was necessary.

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