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Iron deposition in modern and archaeological teeth

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ABSTRACT

Iron surface concentrations and profile maps were measured on the enamel of archaeological and modern teeth to determine how iron is deposited in tooth enamel and if it was affected by the post-mortem environment. Teeth from Australian children who died in the second half of the 19th century were compared with contemporary teeth extracted for orthodontic purposes. Surface analysis of the teeth was performed using the 3 MV Van Der Graff Accelerator at The Australian Nuclear Science and Technology Organisation (ANSTO), Sydney, Australia. A small sample of teeth were then cut in the mid sagittal plane and analysed using ANSTO High Energy Heavy Ion Microprobe. Maps and linear profiles were produced showing the distribution of iron across the enamel. Results show that both the levels and distribution of iron in archaeological teeth is quite different to contemporary teeth, raising the suggestion that iron has been significantly altered by the post-mortem environment.

1. Introduction

Trace element levels in tooth enamel have been widely used as indicators of the diet, health, migration status and environmental exposure of a person. As well as studying teeth form modern populations [2,3,13,28,45,24,23,38,17,35,41,49] it is also possible to use archaeological teeth to investigate past populations [46,20,11,15,14,45]. However, a major concern with archaeological teeth is the potential post-mortem alteration or diagenesis of the samples. In order to make conclusions about historic and pre-historic populations it is critical to demonstrate that no post-mortem alteration has taken place in the tooth enamel for the element of interest or that it is possible to remove the contamination to reveal a clean or biogenic signal.

The purpose of this paper is to use trace element surface analysis and trace element depth profiles to investigate the question of post-mortem alteration of iron in tooth enamel. Although bone has long been known to be subject to post-mortem change [25,37,10,39,40,43], tooth enamel has been considered to be more stable and therefore less likely to alter post-mortem [21,33]. However, more recently questions on the stability of tooth enamel, particularly fossil teeth, have arisen [22,26,27] therefore an assumption cannot be made that tooth enamel is stable in the post-mortem environment.

If a particular sample of tooth enamel is in fact resistant to post-mortem alteration, or unaffected areas of enamel can be selected for analysis then teeth may have value in determining the diet, health or environment of past populations. Therefore it is of great interest to answer the question whether or not enamel is subject to post-mortem alteration.

This paper focuses on iron, one of the most important dietary trace elements. Iron is widespread in the environment but with the exception of red meat, it is poorly absorbed by the body. Deficiency leads to anaemia, impaired immune function and may delay growth; optimal iron exposure can also block uptake of toxic elements such as lead [44,5]. Iron overload is seen in a number of medical conditions such as hemochromatosis and has been seen following the intake of food contaminated from cooking pots or from beer brewed in non-galvanised steel drums.

It is thought that iron is deposited in the enamel at the time of tooth formation with very little change once the enamel is fully formed [7]. Therefore iron levels in tooth enamel may have potential to determine iron exposure during childhood with the formation of deciduous tooth enamel commencing in utero with crown completion by 10–11 months of age and permanent enamel commencing at 3–4 months of age with crown completion around 12–16 years of age [48]. Lane and Peach [29] suggested that low iron can be seen in teeth from females who frequently suffer from low iron levels, however, this was not seen in a study by Nagaraj et al. [35] nor did they find a difference in iron in whole teeth in vegetarians compared to non-vegetarians. In summary little focus has been made on how low nutritional status for iron is reflected in teeth and no studies have investigated how chronic iron overload may be represented in tooth enamel.

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Iron distribution across enamel is unclear with some studies suggesting a relatively homogeneous distribution [16,3,24] whilst others suggest a degree of surface enrichment [8,2,38] and Dolphin et al. found higher iron levels in enamel deposited postnatally compared to prenatally deposited enamel [18].

Iron concentrations in whole teeth from modern donors ranged from 5 to 40 $\mu g\, g^{-1}$ [19,35,1], 10 to 50 $\mu g\, g^{-1}$ for modern whole enamel [2,3] and for modern surface enamel the range was from 10 to 140 $\mu g\, g^{-1}$ [16,2,38,41]. In contrast to most measurements Solis et al. measured iron in modern teeth at levels up to 700 $\mu g\, g^{-1}$ [45].

As previously mentioned, a problem with trace element analysis in archaeological teeth is the possible post-mortem alteration of trace element concentrations [30]. Previous researchers found that lead and strontium profiles in tooth enamel show similar distributions in archaeological and modern teeth, suggesting that they are not affected by diagenesis [9,34]. The exception to this was seen in lead when the remains were in contact with very high levels of lead such as a lead coffin or very high concentrations of lead in the soil [47,6]. Although iron has not been extensively studied in relation to diagenesis, some studies on archaeological teeth have suggested that iron may accumulate in teeth post-mortem [42,11,32,12]. However, only Mansilla et al. [32] and Rodriguez-Fernandez et al. [42] recorded iron levels above those reported in the literature on modern teeth and in the latter case higher levels were only recorded in associated with discolouration of the tooth enamel. It has also been shown experimentally that iron can be taken up by tooth enamel [4] and that in vitro demineralisation of enamel results in significant changes in iron concentrations [38].

In this paper iron levels in a large sample of archaeological and a smaller sample of modern teeth were measured using ion beam surface analysis techniques. Additionally, microprobe mapping techniques were applied to a smaller number of teeth to investigate the distribution of iron within the enamel in order to strengthen the evidence of possible post-mortem alteration.

2. Materials and methods

The archaeological teeth investigated in this study came from children who died between 1863 and 1891 at the Randwick Destitute Children's Asylum, Sydney, Australia. The Asylum cared for children aged 3–12 years whose parents were unable to look after them. The children came from a background of poverty and sometimes of neglect and ill health [31]. Historical records and anthropological analysis of the remains indicate they were short for their age and suffered, at some stage, from chronic severe illnesses and/or malnutrition [31].

During 1995–96 the remains of 65 children were excavated from the unmarked cemetery. The soil at the cemetery was sandy, the pH varied from 3.03 to 5.62 and the exchangeable cation content was extremely low [31]. Iron levels in the grave soil ranged from 140 to 4400 $\mu g\, g^{-1}$ with an average iron level of 700 $\mu g\, g^{-1}$ [50].

The teeth from the asylum children (permanent molars $n = 56$, premolars $n = 34$) were stained light brown and were frequently in a fragile condition; permanent teeth were selected over deciduous teeth as they were in a much better state of preservation. Caries free teeth were selected. The teeth had been stabilised at the time of excavation by coating them with paraloid, a co-polymer of methyl acrylate and ethyl methacrylate and initial tooth preparation consisted of removal of the paraloid with acetone $(CH_3)_2CO$. Further cleaning was undertaken using distilled water and a soft brush; extensive cleaning of the teeth was not possible due to their fragile condition.

The modern teeth ($n = 22$) used for comparison were permanent teeth removed from children living in Sydney, Australia, who were undergoing orthodontic treatment. Teeth were caries free and in good condition.

Proton Induced X-ray Emission (PIXE) analysis of the buccal enamel was performed using the 3 MV Van Der Graff Accelerator at The Australian Nuclear Science and Technology Organisation, Sydney, Australia. PIXE is a surface analysis technique providing elemental concentrations averaged to a depth of 50–60 μm. The buccal surfaces of both the modern and archaeological teeth were analysed using 2.5 MeV protons and a 3 mm beam spot size, a target current of 20–50 nA and total charge between 16–40 μC. Analysis of the results was undertaken using the ANSTO in house PIXE analysis program PIXAN and were calibrated against ANSTO in-house standards. Following the surface analysis, the buccal enamel of the archaeological teeth was removed at the dentine-enamel junction and the subsurface enamel was analysed using the same technique as for the surface enamel. Separation of the enamel from the dentine of archaeological teeth was possible due to the deterioration of the teeth in the post-mortem environment, whereas in modern teeth the enamel and dentine were closely adherent. This made it impossible to remove the enamel of the modern teeth in the same manner used for the archaeological teeth, therefore in subsurface analysis of the modern teeth was only undertaken using the microprobe.

Previous investigation of the distribution patterns of elements in the teeth indicated a non-normal distribution [50] therefore non-parametric analysis was performed using SPSS version 20 for PC. Variation between the surface and subsurface iron measurements was tested using the Wilcoxon signed ranks test and the Mann Whitney *U* test. Soil samples from the graves were also analysed using PIXE. The Spearman Correlation coefficient was used to examine any relationship between the grave soil iron levels, grave soil pH and iron tooth levels.

Microprobe analysis has the advantage that elemental concentrations can be mapped and depth profiles can be measured. This can provide strong indications for post-mortem for the diffusion of elements into the teeth. Microprobe analysis was undertaken for four archaeological teeth and three modern teeth. Microprobe analysis only allows mapping of elemental concentrations across a flat surface of the specimen, therefore the teeth were set in resin, cut in the mid sagittal plane with a diamond saw, polished and carbon coated. The cut surfaces were then analysed using the heavy ion microprobe at ANSTO, which runs off the 10 MV Tandem accelerator. PIXE data across the whole sagittal section of tooth enamel were taken in 2×2 mm sections. Elemental maps as well as selected line profiles from external through to subsurface enamel were extracted from the data using the program GeoPIXE. Results were calibrated against ANSTO in-house standards.

3. Results

The results of the surface and subsurface analysis of the teeth are presented in Table 1. For the microprobe analysis, the results for the modern teeth were consistent for all teeth analysed; Fig. 1 shows the iron distribution for one modern tooth but is representative of the findings from all the teeth. Fig. 2 shows the iron distribution for an archeological molar tooth and Fig. 3 shows the iron distribution for an archeological premolar tooth, these results are representative of the findings for all four teeth. The scan area of the microprobe is limited to a $2 \times 2\ mm^2$ square. Therefore, in order to cover the enamel across the whole surface of the tooth a number of adjacent scans were taken. The images in Figs. 1–3 are composed of a number of individual maps. Line profiles were taken on the buccal, lingual and occlusal surfaces of both the modern and archaeological teeth. Each modern tooth had between 2 and 9 profiles produced and each archaeological tooth had