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Synchrotron radiation analysis of possible correlations between metal status in human cementum and periodontal disease

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Periodontitis is a serious disease that affects up to 50% of an adult population. It is a chronic condition involving inflammation of the periodontal ligament and associated tissues leading to eventual tooth loss. Some evidence suggests that trace metals, especially zinc and copper, may be involved in the onset and severity of periodontitis. Thus we have used synchrotron X-ray fluorescence imaging on cross sections of diseased and healthy teeth using a microbeam to explore the distribution of trace metals in cementum and adhering plaque. The comparison between diseased and healthy teeth indicates that there are elevated levels of zinc, copper and nickel in diseased teeth as opposed to healthy teeth. This preliminary correlation between elevated levels of trace metals in the cementum and plaque of diseased teeth suggests that metals may play a role in the progress of periodontitis.

Keywords: periodontitis; metals; cementum; synchrotron radiation analysis.

1. Introduction

Periodontitis is a chronic inflammatory condition of tissues supporting the teeth affecting humans worldwide leading to loss of periodontal support and attachment with eventual tooth loss. It can affect up to 50% of an adult population, 10% of whom are likely to experience severe symptoms (Albandar, 2002). The progress of the disease is episodic, affecting some 15–20% of the adult population severely at any one time.

Human teeth are secured in the jaw by a system of collagen fibers attached to the tooth root. These fibers are visible under polarized light as a series of concentric rings similar to the annual growth rings in trees; in fact, it is well established that the individual rings are deposited annually (Kagerer & Grupe, 2001). The collagen fibers become mineralized over time by the deposition of a calcium- and phosphorous-rich mineral phase, bio-apatite. As a result, the width of the mineralized region increases with individual apposition rates of up to 20 μm per year. The complete structure of collagen and mineral matter represents the cementum system.

Trace metals, present in the individual's diet or environment, may be incorporated into the mineral phase during apposition. There is some evidence that metal status is involved in the onset and severity of periodontitis (Freeland *et al.*, 1976; Polenik, 1993; Barrea *et al.*, 2001; Meisel *et al.*, 2005), raising the question of the role of metals in periodontal diseases. Metal uptake during the formation and mineralization of cementum could have both positive and negative effects; since the cementum once formed is relatively stable, unlike bone which is continually remodeled (Bilgin *et al.*, 2004), any negative influence could affect the stability of the cementum system. As a consequence, not only could the cementum system be altered in the young, but also metal uptake could be an influence throughout life thereby affecting periodontal tooth loss. Also, since the system is not remodeled, the trace metal concentration and distribution have the potential to provide a chronological record of exposure through an individual's lifetime (Martin *et al.*, 2004, 2007).

While the etiology of periodontitis is likely multi-factorial, it is unlikely that events leading to inflammatory disease would occur without dental bacterial plaque present. However, the characteristics of certain periodontal diseases (*e.g.* juvenile periodontitis) suggest that not all instances of periodontal tissue loss result solely from the presence and influence of bacterial plaque; other mechanisms in these abacterial systems may compromise the integrity of the cementum system (Gottlieb & Orban, 1938) leading to destruction of the periodontal ligament.

Synchrotron X-ray fluorescence analysis (SXFA) provides a technique capable of probing very small volumes of material and thereby providing detailed analysis in individual cementum rings (Martin *et al.*, 2004, 2007). In SXFA, an intense X-ray beam may be focused to a spot a fraction of a micrometer on edge. The resulting X-ray fluorescence may be used to identify the elements present in a sample as well as their concentrations and spatial distribution. In addition, detailed analysis of the X-ray absorption spectrum can provide the chemical environment of each species. Not surprisingly, synchrotron radiation analysis has been widely used to investigate the distribution of metals in human teeth (Carvalho *et al.*, 1998, 2000; Pinheiro *et al.*, 1999; Anjos *et al.*, 2004; Marques *et al.*, 2004; Pérez *et al.*, 2004; Abraham *et al.*, 2007; Arora *et al.*, 2007; Harris *et al.*, 2008*a*), including the crown, dentine and plaque formations and has even been used *in vivo* (Zaichick & Ovchjarenko, 1998) and to examine hominoid dental microstructure (Tafforeau & Smith, 2008)

2. Materials and methods

The teeth used in this study were supplied by the College of Dentistry, University of Saskatchewan: a healthy unerupted third molar from a 20 year old female and two teeth with periodontal disease, the first a molar with moderate periodontitis (Fig. 1) from a 40 year old male, the second an incisor with advanced periodontitis.

The unerupted molar was stored for about six weeks in H_2O_2 before being stored in 10% buffered formalin at the University of Saskatchewan. The other teeth were stored in buffered formalin only while at Saskatchewan. At the University of Western Ontario all the teeth were soaked overnight in 15% H_2O_2 and rinsed with distilled water, adhering tissue was removed using a hard bristle toothbrush and then the tooth was air dried. Some mobilization of metals is possible during this pre-treatment; however, only adhering soft tissue (removed during cleaning) is likely to be severely affected. While the treatment with H_2O_2 at the University of Saskatchewan may have affected metal distributions, it is reasonable to assume that the overnight exposure to 15% H_2O_2 used on all teeth at the University of Western Ontario would mask any differences owing to the pre-treatment. This is equivalent to assuming that any reactions involving metals and H_2O_2 had achieved equilibrium. There is no evidence that the subsequent cutting and polishing of the material introduced any metal contamination. The teeth were embedded in Struers EpoFix resin, which exhibits low shrinkage and little heat release during setting (Beasley *et al.*, 1992), prior to vertical sectioning using a low-speed diamond saw. The resulting thin sections were polished to a thickness of approximately 300 µm using 2.25 µm diamond polishing powder followed by 0.05 µm diamond powder with 2-propanol as lubricant; no substrate was used to support the sections during analysis.

Micro-X-ray fluorescence maps were collected at the PNC/XOR (Pacific Northwest Consortium Collaborative Access Team) facility, beamline 20-ID-B, Advanced Photon Source, Argonne National Laboratory, with incident X-ray energy of 16000 eV selected using a Si (111) monochromator. The beam was focused to a 2 × 1 µm spot using a pair of Kirkpatrick–Baez mirrors; the sample was scanned by moving it in 5 µm steps in the x and y directions with a counting time of 0.3 s per point.

The incident beam was at 45° relative to the plane of the sample, and X-ray fluorescence was monitored using a seven-element Ge detector placed at 90° relative to the incident beam to minimize interference from scattered X-rays. Ca, Zn, Cu and Ni $K\alpha$ and Hg $L\alpha$ lines were monitored; for Pb, both $L\alpha$ and $L\beta$ were monitored and the resulting signal was identified with Pb only if these two lines resulted in identical distributions to avoid confusion with any signal from As. Note the As $K\alpha$ interferes with the Pb $L\alpha$ line.

In the absence of suitable standards, no effort has been made to obtain quantitative results; however, the composition of dentine and cementum are sufficiently similar (Ten Cate, 1998) that the large differences in fluorescence intensity between these two materials may be confidently assigned to differences in concentration.

3. Results

Fig. 2 shows the distributions of Ca and Zn in the unerupted healthy molar (tooth 1). The Ca distribution shows enrichment in the tooth crown, as expected; the crown/dentine boundary is clearly visible. Cracking, the result of drying the tooth, is

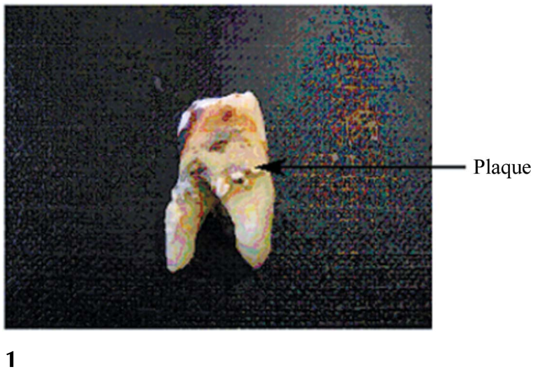

Figure 1
Photograph of the moderately diseased molar (tooth 2).

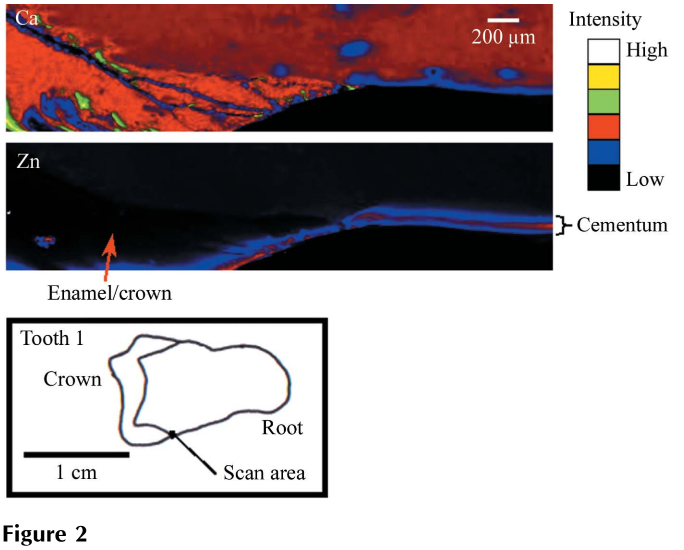

Figure 2
X-ray fluorescence images of the Ca and Zn distributions in the healthy tooth (tooth 1). The schematic shows the approximate location of the scan area.

evident; any other Ca irregularities are either the result of polishing defects or fissures in the dentine (Galil & Gwinnett, 1975). Zn enrichment on the crown surface and in the cementum is clearly evident. The Zn enrichment at the edge of the enamel (crown surface) is probably a result of proximity to the cemento–enamel junction. This Zn enrichment is not surprising since this region is reported to be frequently covered with a particular type of cementum (Bosshardt & Selvig, 1997).

Previous work (Martin *et al.*, 2004, 2007) on the trace metal distribution in teeth has shown the cementum to be enriched in Zn by at least a factor of five over the underlying dentine. This increased level of Zn may be used to identify the cementum region in X-ray fluorescence maps. No other metals were detected in this tooth at levels much above the estimated detection limits.

Fig. 3 shows the distributions for Ca, Zn, Cu, Pb, Ni and Hg in the molar exhibiting moderate periodontal disease (tooth 2). The Ca is confined to the tooth and may be used to define the tooth margin. In the healthy tooth this represents the boundary between the tooth and the embedding resin; however, in this tooth adhering plaque, also rich in Ca, extends beyond the tooth edge. Zn defines the cementum region, but there is a significant increase in fluorescence intensity from the plaque, especially where it is in contact with the cementum.

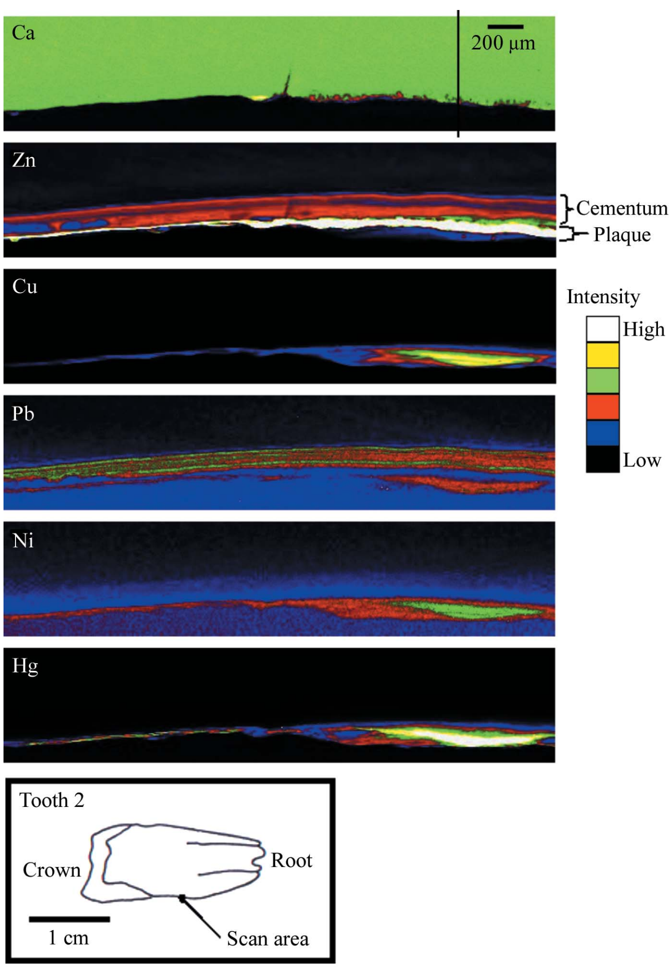

Figure 3
X-ray fluorescence images of the Ca, Zn, Cu, Pb, Ni and Hg distributions in the moderately diseased tooth (tooth 2). The schematic shows the approximate location of the scan area. The vertical line in the Ca image indicates the transect plotted in Fig. 5.

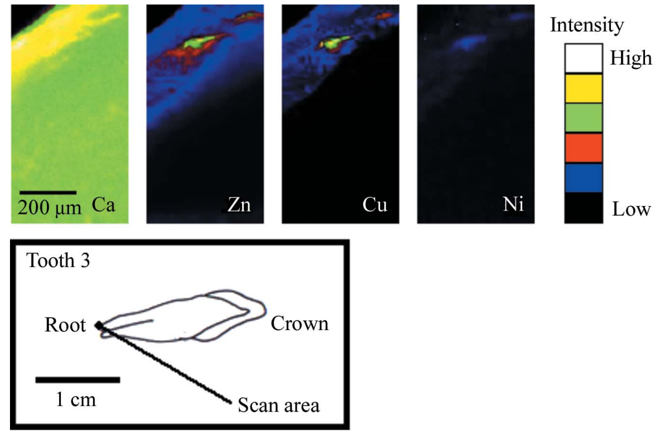

Figure 4
X-ray fluorescence images of the Ca, Zn, Cu and Ni distributions in the severely diseased tooth (tooth 3). The schematic shows the approximate location of the scan area.

The Cu image shows enrichment in the plaque in immediate contact with the cementum. Pb shows a banded structure in the cementum region, consistent with repeated lifetime Pb exposure (Martin *et al.*, 2004, 2007) with some Pb in the plaque material. It is possible that lead may induce structural changes in the hydroxyapatite component of the cementum (Bigi *et al.*, 1991).

Ni is enriched in the plaque with some penetration into the cementum. Some Ni fluorescence is detectable in the resin but at very low concentrations relative to the plaque and the cementum edge. Ni in the dentine is present below, or just at, detection limits. Finally, Hg from a tooth filling can be seen on the tooth surface and in the plaque. Hg has been studied using synchrotron radiation analysis as a result of the widespread use of dental amalgams (Carvalho *et al.*, 1998; Harris *et al.*, 2008*a*).

Fig. 4 was obtained close to the root tip of the third tooth which displayed severe periodontitis. The results for Ca, Zn, Cu and Ni are similar to those obtained in tooth 2, except that Pb and Hg are absent.

Fig. 5 shows a comparison of the X-ray fluorescence intensity for Zn and Cu obtained in a single scan from the resin through the cementum region, into the dentine of the moderately diseased tooth. Fig. 5 also effectively highlights an area of interest in the images of Fig. 3. At the outer edge of the plaque there is an area with no Ca, little Zn, but high in Cu, Ni and Hg (to the left in Fig. 5, bottom of images in Fig. 3); this is likely to be an area of uncalcified biofilm (recently formed plaque) or adhering soft tissue.

4. Discussion

The images from the two diseased teeth clearly indicate the increased concentrations of Zn in the cementum and Cu

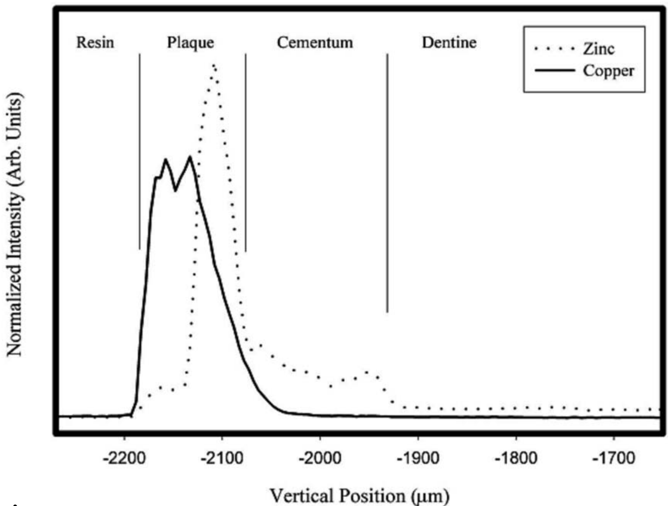

Figure 5
Vertical transect through the image of tooth 2 showing the relationship between Zn and Cu intensities across the plaque, cementum and dentine. The *y*-axis plots normalized X-ray fluorescence intensity for each element and thus does not correspond to absolute concentrations.

localized in the plaque in immediate contact with the cementum. Abraham *et al.* (2007) have noted a strong correlation between Zn and Cu in plaque deposits; interestingly they report no significant Cu concentration within the associated teeth tissues in their sample. Clearly Fig. 3 shows a similar distribution of Cu in the moderately diseased molar, namely, elevated in the plaque with little in the underlying cementum.

Fig. 5 provides a summary of the observations of the spatial relationship between zinc and copper. The zinc is clearly enriched in the cementum, relative to dentine, while it is further enriched in the plaque; copper is present in relatively high concentrations in the plaque material.

In the diseased teeth the increased metal concentrations within the plaque and cementum suggest that, individually or collectively, metals may play a role in the progress and severity of periodontal disease. The most probable synergy appears to be between Zn and Cu. The distributions we observe are consistent with observations of a correlation of the concentration of serum copper with the severity of periodontal disease (Freeland *et al.*, 1976) and the suggestion that inflammation of the gingival tissue causes hepatic cells to increase serum Cu (Poleník, 1993). Cu also appears to have a role in the formation of dental caries (Harris *et al.*, 2008*b*). In general, the incorporation of Cu and Zn in biological tissues has been identified as potential indicators of periodontal disease (Freeland *et al.*, 1976; Poleník, 1993). The presence of Ni, in both diseased teeth, which has been shown to damage oral epithelial cells, is consistent with a role for this metal in the progression of the disease (Trombetta *et al.*, 2005).

5. Conclusions

The results are based on only one set of teeth and so must be taken primarily as indicative of directions for future research; nevertheless, they show the efficacy of synchrotron micro-X-ray fluorescence in the study of trace metals in the cementum region of human teeth and a possible role for trace metals in the progression of periodontal disease. Most striking is the apparent reduction in cementum zinc with enrichment of zinc and copper in the adhering plaque, suggestive of a synergistic interaction between these metals, or at least a common involvement in periodontal disease. Detection of local nickel concentrations and evidence in the literature that this metal damages oral tissue may implicate this metal as well. Lead and mercury, likely from exogenous sources and/or dental amalgams, are also present in one diseased tooth. Overall the results are suggestive of a role for metals in the progression of periodontal disease.

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