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Limiting the impact of destructive analytical techniques through sequential microspatial sampling of the enamel from single teeth☆

Alexis E. Dolphin [a,*], Mathew A. Teeter [a], Christine D. White [a], Fred J. Longstaffe [b]

[a] Department of Anthropology, The University of Western Ontario, London, Ontario N6A 5B7, Canada
[b] Department of Earth Sciences, The University of Western Ontario, London, Ontario N6A 5B7, Canada

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ABSTRACT

A fundamental research concern within contemporary bioarchaeology is the sensitive balance between the preservation of human remains and the use of destructive techniques to collect information. Here we describe one example of how multiple microspatial destructive/semi-destructive techniques may be carried out in sequence using only the enamel of a single tooth. With careful planning of both sample preparation strategies and sequencing of sampling methods, it is possible to produce multiple datasets, and yet to retain material for future analyses. In this case, enamel from the teeth of 27 individuals who lived during the early medieval period (AD 1170-1198) in Bergen, Norway, was subjected to histological, trace element (LA-ICP-MS), diagenetic (FTIR), and isotopic analyses ($\delta^{18}O$ and $\delta^{13}C$, via micromill/multiprep/IRMS).

1. Introduction

A fundamental research concern within contemporary bioarchaeology is the sensitive balance between the preservation of human skeletal and dental remains and the use of destructive analyses to collect data. Archeological human remains not only provide unique opportunities to gain understandings of an individual and/or group's past, but they also hold significant meaning and power for the descendant communities to which they are tied. And so, research involving human remains merits the development of sampling methodologies that will best satisfy the preservation ethic, even as the potential for data collection and interpretive possibilities are maximized. The sampling strategy and sequence of microspatial sampling presented here represents but one way by which we may work toward such an achievement.

In recent years the development of various microspatial sampling techniques has made it possible to minimize destruction of irreplaceable materials, and also to access discrete regions of interest within those materials. This has especially been the case for studies involving the microstructure and chemical composition of dental tissues (enamel, dentine, cementum) — although there has also been growing exploration of the meaning of microspatial chemical variations in bone (Scharlotta et al., 2013). A significant effort to develop microspatial sampling techniques for teeth (human and non-human) comes from within fields such as anthropology and archeology (Dolphin et al., 2005; Cucina et al., 2007; Copeland et al., 2008; Humphrey et al., 2008; Richards et al., 2008; Aubert et al., 2012; Metcalfe and Longstaffe, 2012; Farell et al., 2013; Pfeiffer et al., 2013), and health and environmental sciences (Lochner et al., 1999; Arora and Austin, 2013; Becker, 2013), among others. Such research stems from a desire to capture information that is only accessible through assessment of the incremental formation (at a known rate) of dental tissues, and examination of their incorporation of elements from the environment during development. The ability to understand temporal variation in the chemical signatures preserved in teeth relies on the availability of data derived from histological analyses of dental microstructures such as accentuated striae of Retzius (Wilson bands) and cross-striations. It also relies on a careful consideration of the impact that variability in mineralization rates will have on the ability to interpret such data (Montgomery et al., 2010; Scharlotta and Weber, 2014).

Histological analysis of dental microstructures in archeological teeth can be problematic as it requires the permanent embedding of samples in a resin block, thus making their later removal for bulk dissolution techniques difficult to impossible. Similarly problematic are the traditional bulk dissolution methods themselves, which homogenize the variable chemical composition data recorded in each tooth (Copeland et al., 2008). In terms of stable isotope and trace element research, some researchers have moved away from the traditional bulk sampling approaches that see entire teeth and/or tooth crowns destroyed, and toward other techniques that are less destructive. Some of these

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* Corresponding author at: Department of Anthropology, The University of Western Ontario, 1151 Richmond Street, London, Ontario N6A 5C2, Canada.
E-mail address: adolphin@uwo.ca (A.E. Dolphin).

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techniques may involve mechanical grinding and drilling of small areas within a sample (Hufthammer et al., 2010), or acid dissolution of successive layers of tissue. While chemical analyses such as electron probe X-ray microanalysis (EPXMA), secondary ion mass spectrometry (SIMS), proton-induced X-ray emission spectroscopy (PIXE), or synchrotron X-ray fluorescence (sXRF), have all been applied to the analysis of the chemical composition of dental tissues, laser ablation-inductively coupled plasma–mass spectrometry (LA-ICP-MS) is the most commonly utilized technique for conducting microspatial analyses of dental hard tissues (Lochner et al., 1999; Goodman et al., 2003; Kang et al., 2004; Grün et al., 2008; Humphrey et al., 2008; Dolphin and Goodman, 2009; Hare et al., 2011; Vašinová Galiová et al., 2012; Austin et al., 2013; Farell et al., 2013). Laser ablation-ICP-MS allows for rapid multi-elemental analysis, with samples extracted from ablated spots with diameters as small as 5 μm. Together with its high sensitivity and low detection limits, LA-ICP-MS makes possible the mapping of chemical variation with minimal sample destruction.

While histological and microspatial chemical techniques take advantage of the incremental growth structure of teeth, and their variable responses to physiological stress, diet/nutrition, or exposure to environmental pollutants, they are still inescapably semi-destructive. In keeping with the preservation ethic of bioarcheology, then, the work presented here offers an example of how multiple microspatial techniques may be combined *in sequence* so as to maximize the kinds of data that may be retrieved from a single sample — in fact, from a *single tooth tissue*. These data include estimates of the frequency and timing of childhood stress episodes via histological analyses, pollutant exposure data gathered using trace element analyses (LA-ICP-MS), assessment of tissue preservation using Fourier transform infrared spectroscopy (FTIR), and information regarding origins/migration and paleodiet from $\delta^{18}O$ and $\delta^{13}C$, respectively.

2. Materials and methods

One permanent tooth from each of 27 individuals excavated from St. Mary's (Mariakirken) churchyard at Bryggen, representing burials occurring between 1170 and 1198 AD in Bergen, Norway, was subjected to multiple, semi-destructive, microspatial analytical techniques. Nitrogen and carbon isotope ratios derived from bone samples were also assessed, but are not discussed here as they used more traditional dissolution, rather than microspatial, methods. The human skeletal and dental remains from Bryggen are fragmentary and incomplete for most individuals excavated, and also exhibit considerable dental wear. Thus, teeth were chosen for this study if macroscopic evaluation indicated that that they were likely well-preserved, with at least one intact cusp (no wear, cracks, caries, etc.) and no visible signs of alteration. The tooth sample ultimately included a combination of intact permanent canines (N = 6), premolars (N = 3), first molars (N = 9), second molars (N = 3) or third molars (N = 6). A schematic showing sectioning and sampling regions of the teeth is provided in Fig. 1.

In the interests of focusing on the sequence of sampling techniques, and the preservation of sampled material, and because only standard microspatial methods were applied and have been cited in all cases, details of the operating conditions of the various instruments used, and of subsequent data analysis, are not addressed here.

2.1. Step 1: histology

Once embedded in a resin block, longitudinal thin sections (~250 μm) were cut from the center of the best preserved cusp of each tooth using a Buehler Isomet 1000 slow speed saw. The remaining portions of each embedded tooth were retained for microspatial trace element and isotope analyses. Each thin-section was double-polished using a series of aluminum oxide slurries.

Sections were examined and photographed at 40× magnification under polarized light and images were knit together using the photomontage feature in Adobe Photoshop CS. To be counted as accentuated striae of Retzius, bands of dense enamel had to be visible from the enamel surface to the dentine–enamel junction (see Goodman and Rose, 1990; FitzGerald and Saunders, 2005). The frequency and timing of periods of physiological disruption were documented for each individual in the sample.

2.2. Step 2: laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)

From one of the remaining tooth halves, a cut was made parallel to the tooth surface that was exposed when the thin section was taken. This second cut produced a longitudinal thick section (~250 μm), referred to here as "Block A". A Thermo Fisher Scientific Element 2 ICP-MS and New Wave UP-213 laser was used to assess the presence of the following elements/isotopes in the enamel of each tooth: ^{23}Na, ^{24}Mg, ^{25}Mg, ^{31}P, ^{43}Ca, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{68}Zn, ^{85}Rb, ^{88}Sr, ^{119}Sn, ^{120}Sn, ^{137}Ba, ^{138}Ba, and ^{208}Pb. Spot ablations (80 μm diameter) provided samples from the earliest to the latest layers of enamel. Thus, samples (of 15 to 22 spots, depending on the size of the crown) were taken from enamel at the dentin–enamel junction (DEJ), moving up toward the surface of the cusp, and then down its side toward the root (Fig. 2). Outer and/or worn enamel was not sampled as it has been shown to be consistently enriched in, or depleted of, a number of trace elements examined here (see Budd et al., 1998; Lee et al., 1999; Reitznerová et al., 2000; Dolphin et al., 2005).

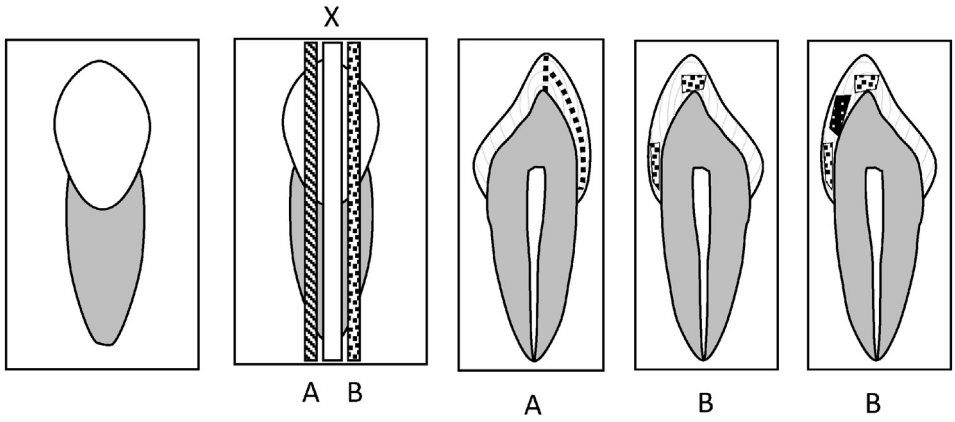

Fig. 1. Schematic illustrating the sequence of sample preparation and microsampling procedures. X = thin section for histological analysis; A = longitudinal thick section for LA-ICP-MS; B = longitudinal thick section for micromilling. Laser ablation spots are represented by the square points located in the tooth enamel shown for section A. The white speckled trapezoidal boxes located within the tooth enamel of thin section B were micromilled first for the purposes of conducting isotope analyses. The black speckled trapezoidal box also located in section B indicates the removal of additional enamel for diagenetic testing.