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The Histological Paradox: Methodology and Efficacy of Dental Sectioning

Christopher Aris

Abstract: Within the last two decades, the fields of dental anthropology and bioarchaeology have seen a drastic increase in the number of studies investigating the internal structures of human enamel in archaeological populations. Due to its relatively low cost and preparation time, combined with a high degree of accuracy, destructive histological analysis has become a common methodology in enamel research. However, despite its accuracy and presence within academic literature, institutions often reject applications to perform histological analysis as standard procedure. Most frequently this is justified because destructive analysis negatively impacts future research. As a result, many studies are forced to utilise published data or attempt to access the small number of dental histological slides already in existence. This paper details the processes and procedures followed during histological sampling, with the aim to provide an easily accessible reference for curators allowing them to make more informed decisions regarding requests to conduct histology on samples within their care. Moreover, this paper highlights the preservative methods available to researchers which, when employed, both limit the negative impact to future research and expand the type of material which institutions can provide access to. Access to these new materials provides curators with alternative responses to applications rather than rejecting proposals entirely. Methods include high quality resin casting, which allows for future metric and micro-wear analysis, and digital stitching methods for producing dental cross section databases which institutions can offer access to instead of further destructive sampling.

Keywords: dental sectioning, histology, human variation, sampling permission

Introduction

Dental anthropology is a rapidly expanding field with research spanning the breadth of primate and hominin evolution, variation, and taxonomy (e.g. Beynon et al. 1991; Schwartz 2000; Skinner et al. 2008), hominoid dietary variation, (e.g. Martin et al. 2003; Vogel et al. 2008; Lucas et al. 2013; Pampush et al. 2013; Le Luyer et al. 2014; Le Luyer and Bayle 2017), and the impact of human health on dentition (e.g. Lukacs 1991, 1992, & 1999; Birch and Dean 2014; Primeau et al. 2015). This research relies on analysis of both external and internal dental features with the study of internal structures becoming progressively more frequent. Histology is the most commonly

used method to study internal tooth structures, as a plethora of information can be gathered through the use of microscopy on histological samples, including: linear, relative, and average enamel thickness (e.g Suwa and Kono 2005; Smith et al. 2006a; Reid and Dean 2006; Olejniczak et al. 2008; Mahoney 2010), regional secretion and growth rates of enamel (e.g. Lacruz and Bromage 2006; Smith et al. 2006b; Mahoney 2008), and enamel periodicity (e.g. Dean et al. 1993; Fitzgerald 1998; Smith et al. 2007; Mahoney 2008). Histology can therefore be seen to provide access to ample data to justify its use. However, due to the destructive nature of histology, the method can be unattractive to institutions curating dental material (e.g. museums and universities) and can lead to the rejection of applications associated with histology-based research projects. This paper will discuss this issue and how, thanks to preservative and novel digital techniques, institutions would benefit from rethinking their policies regarding histological analyses.

While this paper will encourage institutions to consider histological research proposals, their concerns remain valid. This paper will address institution concerns by providing alternatives and new methodologies that can be considered when responding to histological applications. The ethical standards associated with histological research on archaeological material will also be detailed. These will focus on guardianship of produced and leftover material, the longevity of sectioned specimens, and the benefits of histological analysis over non-destructive methods.

Background

The history of histological analysis within anthropology is long and multifaceted, including the study of extant and extinct hominoids. Since the turn of the century, many projects have focussed on the analysis of human remains. In the past, morphology and growth rates of human enamel was thought to be relatively consistent between populations. However, more recent research has begun to identify intraspecific variation in enamel thickness measures (e.g. Reid and Dean 2006; Smith et al. 2006a; Mahoney 2008; Le Luyer and Bayle 2017). These discoveries have raised questions regarding the extent of intraspecific variation in human enamel. Such questions can only be answered by conducting more expansive histological analyses within archaeological and bioarchaeological research projects.

It is therefore pertinent to review the process of conducting a histological study of human dentition, detail the modern methodologies associated with such analysis, and discuss what this means for curators receiving associated applications.

Research into the internal structures of human dentition date back as far as 1873, with the work of Retzius (1873), and other pioneer studies including Asper (1916) and Gysi (1931). These early researchers took an ontogenetic approach to dental analysis, working to outline the growth mechanisms which determine the composition and structure of human enamel. Where the existence of these mechanisms correlated with visible internal enamel structures, more recent research has worked to compare them at an intraspecific level by conducting histological analyses on archaeological human remains. Reid and Dean (2006) published the most expansive study of this kind, sampling 326 molars and 352 anterior teeth (canines and incisors) from five collections, including four archaeological assemblages. Subsequent analyses revealed a wide range of enamel growth patterns and enamel thicknesses between the populations (Reid and Dean 2006). Smith et al. (2006a) identified the first significant intraspecific differences, between similar interior enamel thickness features and different archaeological populations. Using data from four of the five populations sampled by Reid and Dean (2006), Smith et al. (2006a) identified significant differences in the third molar bi-cervical diameters between South African, Northern American, and Northern English populations (Smith et al. 2006a). Most recently Le Luyer and Bayle (2017) compared enamel thickness features of 40 human upper second molars from the Palaeolithic, Mesolithic, and Neolithic periods. Significant differences were reported in relative enamel thickness and functional cusp expression between the Early Mesolithic and Early Neolithic samples, which was suggested to be due to dietary shifts and the transition to agriculture (Le Luyer and Bayle 2017).

Despite the breadth and volume of the dental histological studies cited above, results are, for the most part, derived from a relatively small number of histological collections of human dentition. These consist of a number of small archaeological samples from a 10th century Slavic cemetery, Spitalfields Crypt (London), St Gregory's Priory (Canterbury) and Medieval Denmark and England, and larger

collections from Northern Europe and Southern Africa (Macho and Berner 1993; Liversidge 1995; Dean and Scandrett 1996; Schwartz et al. 2001; Reid and Dean 2006; Smith et al. 2007; Mahoney 2008; 2012). The relatively low number of populations analysed, and their limited geographic variability, means our understanding of how they reflect human dental variation is limited. Many internal dental features which may significantly vary between human populations, particularly those relating to enamel, can only be accessed through histological methods. Therefore, future research will invariably require a wider application of histological methods to archaeological populations. It is therefore important that curators fully understand the methods and processes associated with histological sampling, and appreciate its value both to their institution and to ongoing research.

Aims

This paper aims to: (1) Provide an easily comprehendible outline for the process of histological analysis of human dentition; (2) present an exhaustive list of the data made available from histological analysis of dental enamel; (3) discuss the better and lesser-known preservative aspects of histology; (4) and the advantages of institutions more routinely permitting such analyses on their collections.

Dental histology methodology

Preservative methods

Before any destructive sampling takes places, high resolution pictures are taken for all aspects and angles of each tooth. Many histological analyses will also include the production of a 1:1 resin cast using the same methods and materials used in dentistry (e.g. Mahoney 2008). Casts are produced by creating a dental mould using silicone-based light body putty (Coltene®). The mould is subsequently filled using a 4:1 hardener and epoxy resin solution (Buehler®), which dries over a 24 hour period to produce the final cast. Finished casts can be measured against the original tooth by taking select diameter measures of the crown and root. Where these are not identical, the casting process is repeated until the required accuracy is achieved. Casts produced using this method are more durable and easier to curate than the original tooth (Schmidt 2001).

Destructive stages

Once pictures and casts are produced, teeth are embedded in the same solution of 4:1 hardener and epoxy resin (Buehler®). Next, the embedded samples are cut at a low speed, most commonly by a diamond-edged wafering blade and a precision cutter (Buehler® IsoMet), through the required plane (typically longitudinally through a dental cusp). Cut samples are mounted on glass microscope slides. The mounted section is cut again so that the material adhered to the slide is around 2-3 mm thick before being lapped using increasingly fine grinding pads, until between 100-120 μm thick. At this thickness, interior enamel features of enamel formation can be observed using light microscopy. Accounting for the most common thickness of wafering blades, and the volume of material destroyed through grinding, sectioned material will lose between 2-4 mm of material permanently, alongside the remaining dental material being sectioned into three pieces. Ground sections are then polished using 0.3 μm aluminium oxide powder, which acts to remove any evidence of lapping which obscures enamel features. Once polished, samples are placed within an ultrasonic bath to remove any material debris, and subsequently dehydrated using progressively higher concentrations of ethanol solutions. Finally the dental samples are cleared (typically using Histoclear®) and mounted with a glass cover slip. Cover slips are typically adhered using a mounting medium (DPX®) or an identical 4:1 epoxy resin solution. The use of a cover slip protects dental samples from outside contaminants and preserves them indefinitely.

Digital methods

Once complete, a histological sample can be observed and analysed under light or polarised light microscopy. Recently developed software (Olympus cellSens) allows for stitching methods to be conducted, where microscopic images are tracked and recorded in live action while a microscope lens is in motion. This produces a composite image of the whole dental cross-section, with specific save files recording set scale parameters, allowing the slide to later be accessed and used for data collection without the need of the slide itself. Enamel features used in anthropological and bioarchaeological research can be observed under 20x magnification, and current stitching techniques are accurate to magnifications above 40x. This means that stitching of human enamel sections is adequately reliable for academic research.

Discussion

Data made available by histology

The above methodologies provide access to the internal structures of dentine and enamel. These structures allow researchers to measure specific thicknesses of enamel and their growth rates which cannot be observed from exterior analyses. Thickness measures have particular value in analysing dietary variation between populations, as enamel is known to thicken in response to hard or highly wearing diets in hominoids (e.g. Dumont 1995; Martin et al. 2003; Pampush et al. 2013). However, thickness measures can also be taken using non-destructive micro computed tomography (CT). Conversely, growth rates of dentine (Kawasaki et al. 1979) and growth rates and cross striations of enamel (Boyde 1963; Berkovitz et al. 2002), can only be accurately measured through histological analyses. These growth lines are highly regular in their formation and can thus be used to map variation in enamel growth across dental crowns (e.g. Beynon et al. 1991; Lacruz and Bromage 2006), examine the influence of external stimuli on enamel growth (e.g. Mahoney 2015), aid in ageing remains (e.g. Boyde 1963; Antoine et al. 2009), examine the variation in growth patterns between species (e.g. Schwartz et al. 2001; Smith et al. 2006b), and allow enamel growth to be calculated at a daily rate (e.g. Beynon et al. 1991; Reid et al. 1998; Lacruz and Bromage 2006). Given the expansion of dental anthropological research discussed previously, and the wealth of data made available only by

histological analysis, institutions should strongly consider the importance of histological analysis when reviewing associated applications to sample material in their care.

The wide use of histological methods in dental anthropology and bioarchaeology speaks to its high applicability. However, experience in applying to conduct histological analysis on human teeth shows that these requests can be rejected due to the availability of non-destructive alternatives. The most commonly available alternatives include radiography and micro-CT. In regards to radiography, research has found it to be significantly less accurate when analysing incremental features and thickness of teeth. In particular, radiographic analysis has been found to overestimate the age of enamel mineralisation, and underestimate the time for crown completion (Beynon et al. 1998). Micro-CT however can analyse teeth to a comparable accuracy as histology (both to μm) when analysing enamel thickness (e.g. Le Luyer and Bayle 2017). However, such analyses are exponentially more expensive and are not widely accessible to many researchers, in particular PhD students and early career researchers. Moreover, as stated above, micro-CT scans cannot provide access to all the same data histological methods can. Applications to conduct histological analyses should therefore still be strongly considered, despite the existence of non-destructive methods, as these do not always offer accurate or viable alternatives.

Preservative methods

Despite the preservative measures now taken in modern histological analyses of human material, it is still undeniably destructive, and curators should take care when reviewing applications to conduct such methods on valuable biological material. However, what is evident to those whose research relies on histology is that many institutions are not informed on the number of preservative methods that are implemented alongside destructive histological sampling. Moreover, it should be understood that standard guidelines for histological sampling of dentition permits only one tooth per individual per project to be sectioned (Mitchell and Brickley 2004). This means no histological project poses a serious destructive risk to any single set of individual remains.

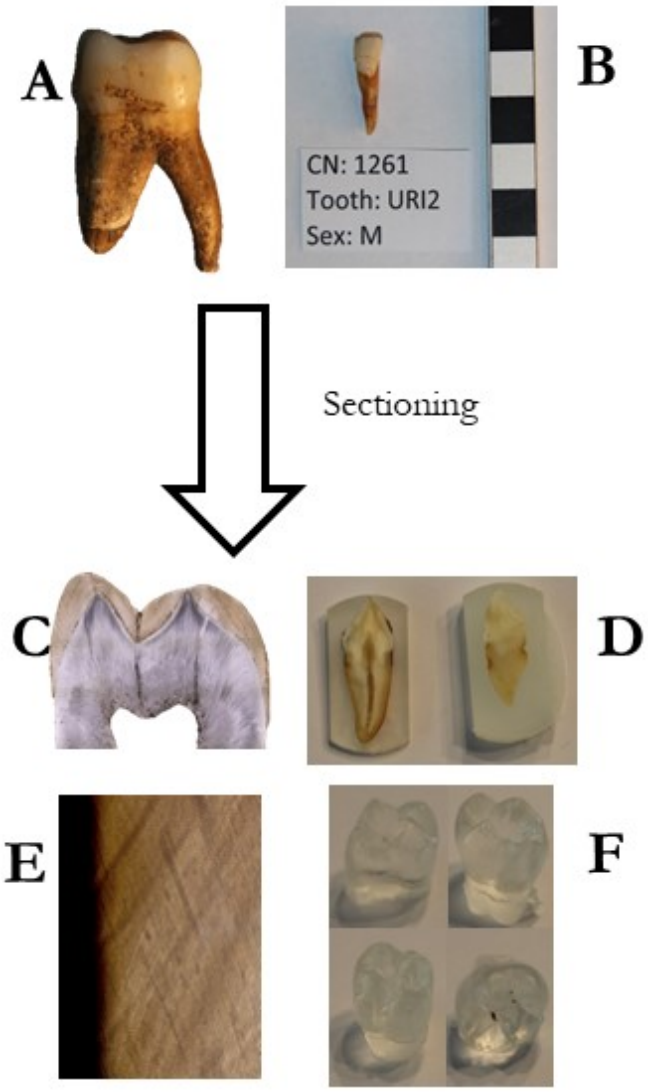

Figure 1: Images depicting the before and after products of histological analysis. **A.** Photograph of a Roman maxillary first molar. **B**. High resolution photograph, with identification card and scale, of a Medieval first incisor. **C**. Cross section of the dental crown of the same Roman molar (**A**) produced using stitching software at 20x magnification. **D.** The remaining embedded material of a Roman canine after histological sectioning. **E.** Microscopy image taken from an Anglo-Saxon canine at 20x magnification displaying features of internal enamel. **F.** Images of a 1:1 resin cast produced from the dental crown of a Medieval molar before sectioning.

One preservative method available is the production of a micro-CT scan of each tooth before sampling. However, the equipment necessary for this is not widely available, can be expensive and requires extensive training. As a result, early career

researchers and PhD students in particular may not have access to micro-CT scanning. The method of producing a 1:1 scale dental cast, detailed previously, provides a more readily available and less expensive preservative method, requiring less training while retaining precision. While precision is necessary, accurate casts can be produced with relative ease provided the tooth is thoroughly cleaned and care is taken to produce a mould which is tight to the sample, encompassing every aspect of the tooth. If care is taken, the detail of the cast allows future research to accurately analyse micro-wear patterns, allowing for associated dietary and health studies (e.g. Schimdt 2001; Mahoney 2007), and the study of external morphology and morphometrics (e.g. Ferrario et al. 1993; Boaz and Gupta 2009). Alongside producing additional material which institutions can add to their available collections, the non-mounted remains of histological sampling can be utilised in future research. As detailed in figure 1, the mesial and distal aspects of the tooth not mounted for microscopic analysis remain embedded in resin. The dentine in particular is viable for subsequent isotopic analysis once cleaned. The use of embedded dentine material in isotopic analyses can be observed in the literature (e.g. Beaumont et al., 2013; 2014). The accuracy of the isotopic data produced can vary according to the thickness of material available, and the reliability can vary according to the material used for embedding. However, when care is taken to retain and document embedded material, curating institutions can allow isotopic research and provide viable material without further destructive analysis on untarnished teeth. All these methods provide additional material, or uses for returned material, which institutions can provide access to for future research in addition to the cross-section slide produced.

The value of digital material must also be emphasised when discussing the products of histological analysis. Thanks to recent advances in microscopy software, digital stitching techniques provide a further preservative method which can be utilised when conducting histological sampling. This increases the accuracy of dental analysis and furthers the preservation of additional valuable dental material. As discussed previously, the produced composite images allow researchers to observe the dental cross section in its entirety. This image can thus be used to take accurate measurements of multiple dental features using the same program, thereby avoiding

incurring further error by having to manually create a collage of individually captured images. When saved in a specific format, these images retain accurate scale measurements of the sectioned material. This allows future researchers to access the image and collect more metric and non-metric data without the need of the slide itself, or most importantly, without needing to section another tooth. When these images are curated alongside the main collection, this would allow institutions to offer access to the digital files, thereby avoiding further destructive measures.

Conclusions

While destructive, when histological analysis is implemented alongside digital techniques it provides affordable access to a wealth of valuable data and produces both physical and digital resources that can be curated by institutions for future research. It is also important to note that histological slides remain a part of their original collection. Any additional materials produced (casts, digital images, etc.) are also considered a part of the collection and are presented to the curating institution at the culmination of research. Therefore, the resources made available to institutions through histological analyses should be considered when reviewing associated sampling applications, alongside the value of the resulting data. Museums, universities, and other institutions curating human remains are thereby encouraged to address their policies regarding histological-based projects. Moreover, it is clear that both researchers and relevant institutions would benefit from application systems more willing to approve histological analysis of curated material. A more open discussion between histologists and curators based on both the potential volume of novel data, and on the minimal impact their work has on future research, would greatly benefit both parties and bioarchaeology as a field.

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