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Title:

Enamel thickness and growth rates in modern human permanent first molars over a 2000 year period in Britain

Short Running Title:

Molar enamel thickness and daily secretion rates in Britain

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Key Words:

Modern humans, molars, enamel thickness, daily secretion rates, enamel development

ABSTRACT

Objectives

This study explores variation and trends in first molar enamel thickness and daily enamel secretion rates over a 2000 year period in Britain.

Methods

Permanent first molars (n=89) from the Roman, Anglo-Saxon, and Medieval periods, as well as modern day Britain, were analysed using standard histological methods. Relative enamel thickness (RET) and linear measurements of cuspal and lateral thickness were calculated for mesial cusps. Daily secretion rates (DSRs) were calculated for inner, mid, and outer enamel regions in both cuspal and lateral enamel. Significant differences and trends were identified between samples using non-parametric statistical tests.

Results

Enamel thickness differed between some populations, but no temporal trends were identified. Early Anglo-Saxon molars had significantly thinner RET than both Late Anglo-Saxon (p<0.00) and Medieval (p<0.00) molars. Lateral enamel from the Roman molars was significantly thinner than the modern day sample (p=0.04). In contrast, DSRs slowed significantly from the more ancient to the modern day samples in every comparison except the mid lateral enamel region.

Discussion

This study presents the first evidence for a gradual slowing in the daily rate that enamel is secreted in molars over the past 2000 years in Britain. However, this trend was not matched by a change in enamel thickness, which remained fairly consistent over this time period. These findings suggest that modern human molars of similar enamel thickness, from different modern and ancient populations, formed at different rates.

1 | INTRODUCTION

This intraspecific study of modern human permanent molars investigates enamel thickness (e.g. Macho and Berner, 1993; Reid and Dean, 2006; Smith, Olejniczak, Reid, Ferrell, and Hublin, 2006a; Suwa and Kono, 2005) and daily enamel secretion rates (DSRs) (e.g. Beynon, Dean and Reid, 1991b; Lacruz and Bromage, 2006; Mahoney, 2008) in modern and ancient British populations. Enamel thickness and DSRs will be compared across a 2000 year period, between populations from St James' Place and Bath Gate (70-400AD), Ozengell Grange (500-600AD), Black Gate (800-1200AD), St Gregory's Priory (1100-1500AD), Fishergate House (1000-1600AD), and a modern collection of clinical dental extractions. The objective here is to identify variation in these two components of dental development when compared between these populations over this period of time.

Some components of enamel thickness, including average enamel thickness and the area of the enamel cap, can vary amongst modern humans when compared between populations, the sexes, and tooth types (e.g., Smith, Olejniczak, Ferrel, and Hublin, 2006a). Variation in enamel thickness has also been identified amongst modern human populations from different time periods (e.g. Le Luyer and Bayle, 2017; Le Luyer, Rottier, and Bayle, 2014). Thicker enamel has been observed in modern human molar crown regions where surface wear was expected to be greatest (Grine, 2005; Le Luyer et al., 2014; Le Luyer and Bayle, 2017). Subsequent research suggests that a shift in the distribution of enamel thickness upon a molar, when compared between Mesolithic and Neolithic groups from France, may represent a functional adaptation related to the transition to agriculture (Le Luyer and Bayle, 2017). One aim of this study is to further explore this finding in British samples, to determine if molar enamel thickness changed in Britain over the last 2000 years. During this time period there was substantial population movement into and out of Britain, as well as cultural,

dietary, and social developments (e.g. Scull, 1993; Privat, O'Connell, and Richards, 2002; Lightfoot et al., 2009). Given the potential association between these factors and enamel thickness, we could expect significant changes between British populations.

Enamel thickness of primate teeth is the product of several growth mechanisms including the daily rate at which ameloblasts secrete enamel (Grine and Martin, 1988; Macho, 1995; Mahoney et al., 2016). Daily secretion rates (DSRs) are typically highly variable when compared within or between primates (e.g. Boyde, 1989; Schwartz et al., 2005; Shellis, 1998; Smith et al., 2007a, 2007b, 2007c), and are typically slower in modern humans relative to earlier fossil hominins (Dean et al., 2001). Several studies have reported mean DSRs for modern humans (e.g. Beynon et al., 1991b; Lacruz and Bromage, 2006; Mahoney, 2008; Smith et al., 2007b). However, while a previous study calculated DSRs for both archaeological and modern day human sample (e.g. Smith et al., 2007b), the aim in that study was not focused on variation in DSRs through time. One aim in the present study is to determine if there is a temporal trend in DSRs amongst modern humans from Britain.

1.1 | Amelogenesis and enamel secretion rates

Amelogenesis is the process by which enamel cells (ameloblasts) secrete and mineralize protein matrix (Boyde, 1989; Nanci and Smith, 1992; Smith and Nanci, 1995). During the secretory stage of amelogenesis, ameloblast secretion is periodically altered generating short and long-period markers that are retained in enamel along prisms (e.g., Asper, 1916; Dean, Beynon, Reid, and Whittaker, 1993; Gysi, 1931; Kajiyama, 1965; Massler and Schour, 1946; Okada, 1943; Smith and Nanci, 2003). The short period markers are cross striations which correspond to a daily (24-hour) circadian rhythm of enamel secretion in primates (e.g. Antoine, 2000; Antoine, Hillson, and Dean, 2009; Boyde, 1963; 1990; Bromage, 1991; Dean,

1995; Fitzgerald, 1995; 1998; Kajiyama, 1965). Cross striations alter the refractive index of enamel prisms which makes these markers observable in thin sections under transmitted light (e.g. Berkovitz, Holland, and Moxham, 2002; Zheng et al., 2013).

Daily secretion rates (DSRs) can be calculated from cross striations. Typically, these rates change from inner enamel regions near the enamel dentine junction (EDJ) towards the outer enamel surface of primate deciduous and permanent teeth (e.g. Beynon, Clayton, Ramirez-Rozzi and Reid, 1998; Lacruz and Bromage, 2006; Mahoney, 2008; Reid, Beynon, and Ramirez-Rozzi, 1998; Beynon et al., 1991b). Daily secretion rates are also slower near the cervix compared to those near the dentine horn (Beynon et al., 1991b). As DSRs can vary within a tooth, comparisons of these rates between tooth types, or between populations, are usually undertaken for specific regions within a crown (e.g., Dean, 1998) where the molar crown is divided into cuspal, lateral, and cervical enamel, and then further subdivided into inner, mid and outer regions. However, DSRs are broadly similar when equivalent regions are compared between cusps within a molar (Mahoney, 2008).

The majority of research into modern human DSRs generally utilises single human populations (Beynon et al., 1991b; Lacruz and Bromage, 2006; Mahoney, 2008). When multiple human populations are examined it is often part of a larger, interspecific study of a grouped single representative sample (e.g. Smith et al., 2007b). Recently, Nava and colleagues (2017) reported slower DSRs in deciduous incisors from the present day compared to equivalent samples from imperial Rome. While their analysis was not focused on a temporal transect, their results do suggest a temporal trend in the DSRs of these populations. No study has specifically compared DSRs from equivalent enamel region of permanent teeth, between modern human populations from different time periods. Thus, our understanding of how DSRs can vary between populations from different time periods is limited.

1.2 | Enamel thickness

The thickness of enamel is established at the end of the secretory stage of amelogenesis (Nanci and Smith, 1992; Smith and Nanci, 1995). A number of measures associated with enamel thickness can be calculated from 2D dental thin sections and have featured heavily in anthropological research, often in studies of diet in non-human primates (e.g. Molnar and Gantt, 1977; Martin, 1983; Dumont, 1995), extinct hominins (e.g. Kay, 1981; Olejniczak et al., 2008; Skinner et al., 2015; Zanolli et al., 2016) and humans (e.g. Feeney et al., 2010; Grine, 2005; Reid and Dean, 2006; Le Luyer and Bayle, 2017; Le Luyer et al., 2014).

Hlusko and colleagues (2004) reported enamel thickness to be highly heritable in baboons over relatively short periods, in an evolutionary perspective. Moreover, examination of the human genome and the genetic components involved in enamelin activity (and thereby enamel formation: enamelin is one of the three major enamel matrix proteins expressed by ameloblasts), have revealed differences between humans and non-human primates consistent with the selective pressures of natural selection (Daubert et al., 2016; Horvath et al., 2014; Kelley and Swanson, 2008). These interspecific variations in enamelin appear to correlate with similar variations in diet between species, strongly supporting the evolutionary link between dietary changes and enamel thickness (Kelley and Swanson, 2008; Horvath et al., 2014).

Similar variations have been observed in humans, with significant differences in the frequencies of single-nucleotide polymorphism associated with enamel thickness identified between individuals of African and non-African ancestry (Daubert et al., 2016). Human enamel thickness might therefore, under some circumstance, be considered heritable, and associated with the selective pressures of diet. This current study focuses on linear measures

of lateral (e.g. Macho and Berner, 1993; Mahoney, 2010; Suwa and Kono, 2005) and cuspal molar thickness (e.g. Reid and Dean, 2006; Schwartz, 2000a; Suwa and Kono, 2005), and relative enamel thickness (Smith et al., 2006a; Olejniczak et al., 2008; Martin, 1983) of the molar enamel cap (Figure 1). The aim is to determine if enamel thickness, and its linear distribution over a molar crown, changes over a 2000 year time period when compared between British populations.

INSERT FIGURE 1 HERE

1.2.1 Inter-specific studies of permanent enamel thickness and diet

Inter-specific studies of primates suggest thicker enamel may relate to the increased consumption of abrasive foods. Thicker enamel may allow teeth to retain their ability to chew and process foods throughout an individual's lifetime, delaying exposure of dentine and protecting the pulp chamber while the enamel continues to wear (e.g. Lucas, et al., 1985; Molnar and Gantt, 1977; Martin et al., 2003; Pampush et al., 2013). Within hominins for example, quartz dust in or on foods has been suggested to serve as an abrasive additive that may select for thicker enamel (Lucas et al., 2013).

Others have proposed a second positive model explaining how thicker enamel may relate to food hardness in some mammalian species (e.g., Kay, 1991). Dumont (1995) observed significantly greater molar relative enamel thickness (RET) values in species consuming a hard diet compared to a closely related non-durophagous species. Martin, Olejniczak, and Maas (2003) observed a similar relationship in extant primates, noting that primates with thickly enamelled molars regularly consumed hard diets. The relationship between the mechanical properties of food and enamel thickness has been more specifically compared in *Pan troglodytes* and *Pongo pygmaeus* (Vogel et al., 2008). While both species

display preferences for ripe fruit, *Pongo* molar enamel is relatively thicker and possesses occlusal crenulations that may facilitate increased fracture resistance in the face of consumption of a harder diet during fallback episodes. It has been theorised that these traits are derived from ancestral hominin species, and possibly retained through the selective pressure of regular seed consumption (Vogel et al., 2008). Moreover, genetic evidence indicates that enamelin, one of the genes associated with enamel production and thickness, has undergone evolutionary selection since our divergence from chimpanzees, potentially due to shifts in diet (Horvath et al., 2014; Kelley and Swanson, 2008).

Differences in molar enamel thickness have also been attributed to variation in dietary hardness and composition between hominoid species. Kay (1981) found Ramapithecine species to have thicker enamel that may have related to the consumption of hard-rind nuts or seeds. Moreover, studies regarding Neanderthal populations have suggested that thin enamel in the post-canine teeth of European Neanderthal populations, as compared similarly aged human samples, allude to variation in diet between the two groups (Olejniczak et al., 2008) with particular reference to softer meat-based diets in Neanderthals (Smith et al., 2012). This alternative model suggests that consuming softer foods relaxes selective pressures leading to thinner enamel between species. Skinner and colleagues (2015) analysed a wide range of hominins, finding enamel thickness to follow a trend towards thickening throughout the Pliocene, culminating in the thick enamelled robust *Australopithecus*, followed by a thinning trend throughout the genus *Homo* (Skinner et al., 2015). Evans and colleagues (2016) have further suggested the variation in enamel thickness between hominin species to be result of developmental inhibitory cascade mechanism, wherein decreases in mesenchymal activation and inhibition maintenance within dentition resulted in progressively thinning teeth in *Homo*. While this developmental feature could potentially

further explain inter-species hominin variation, it cannot necessarily be used to predict patterns within a species over a period as short as 2000 years. Moreover, as enamel thickness has undoubtedly decreased over time within the *Homo* genus, we could expect a similar thinning of molar enamel to continue in modern human populations.

1.2.2 Studies of enamel thickness within human permanent molars

Enamel thickness can vary between modern human populations. Cuspal enamel thickness varies between South African, North American, and European populations samples (Reid and Dean, 2006). Mean average enamel thickness (AET) of molars differed between Southern African, Northern English, Northern American, and medieval Danish samples (Smith et al., 2006a). More recently, Le Luyer and Bayle (2017) reported AET and RET for 40 maxillary second molars from individuals in France representing a 7000 year period between the Upper Paleolithic, Mesolithic, and Neolithic periods. They found a significant difference between the RET of the Early Mesolithic and Early Neolithic molars, with Mesolithic dentition possessing the thinnest enamel. Enamel thickness can also vary over the cusps of a tooth crown (e.g., Kono, 2004; Kono et al., 2002; Kono and Suwa, 2000; Mahoney, 2010).

Molar enamel thickness can also differ between males and females within populations. Macho and Berner (1993) found linear measures of maxillary third molars from females to have marginally thicker enamel than their male counterparts. Schwartz and Dean (2005) reported that female mandibular third molars had slightly thicker enamel (RET) on average compared to males. Others (Saunders, Chan, Kluge, and FitzGerald, 2007; Smith, Olejniczak, Reid, Ferrell, and Hublin, 2006; Sorenti, Martinón-Torres, Martín-Francés, and Perea-Pérez, 2019) have similarly found thicker RET in female teeth and greater dentine volumes in male molars. Several studies have related increases in enamel thickness along the

molar row to increased bite forces along the molar row during chewing, and a change in the proportions of enamel or dentine within a molar (e.g. Grine, 2005; Macho and Berner, 1993; Schwartz, 2000; Mahoney, 2013).

All these findings highlight the actual and potential variability in human molar enamel thickness. They thereby justify conducting wider inter-population studies of modern human enamel thickness. In this particular study, it is therefore reasonable to expect to see significant variation in enamel thickness measures between the populations spanning the last 2000 years in Britain.

1.3 | Relationship between enamel thickness and underlying growth mechanisms

The relationship between DSRs and enamel thickness within humans is poorly understood. Past research has alluded to links between DSRs and enamel thickness of equivalent teeth in modern humans. When compared along the deciduous tooth row, incisors with thinner enamel had faster mean DSRs compared to molars with thicker enamel that had slower DSRs (Mahoney, 2015). However, permanent first molar cusps with thicker enamel do not necessarily present faster DSRs than first molar cusps with thinner enamel (Mahoney, 2008). The possible association between thicker enamel and faster or slower secretion rates requires further work in humans. Smith and colleagues (2006a) also presented molar DSRs alongside thickness data. However, it was not an aim of their study to explore relationships between this measure of enamel growth and thickness. Moreover, whether DSRs vary alongside molar enamel thickness when compared between human populations from different time periods has not been examined previously.

2 | MATERIALS AND METHODS

2.1 | Dental sample

Permanent first molars (n = 89) were selected from British populations representing five time periods. These populations date from the Roman period (n = 11), Early Anglo Saxon (n = 12), Late Anglo-Saxon (n = 19), Medieval (n = 32) and Modern day (n = 15). Only non-pathological teeth and unworn molars were selected. Right first molars were selected unless it was unavailable or the left was better preserved. Sex was not known for the majority of these samples as many of the molars were separate from the rest of the skeleton.

Figure 2 illustrates the location of the samples in Britain. The Roman population (70-400AD) is represented by individuals excavated from, St James' Place and Bath Gate, in Cirencester, Gloucestershire (McWhirr, Alan, Viner, and Well, 1982). The Early-Anglo Saxon samples (500-600AD) came from a population excavated from Ozengell Grange, Ramsgate, Kent (Millard, Jarman, and Hawkes, 1969). The Late Anglo-Saxon samples (800-1200AD) came from a population excavated from the Black Gate Cemetery, Newcastle-Upon-Tyne, Northumberland (Swales, 2012). The Medieval samples are represented by St Gregory's Priory, Canterbury, Kent (1100-1500AD) (Hicks and Hicks, 2001), and Fishergate House, York, North Yorkshire (1000-1600AD) (Holst, 2005).

The modern day sample was collected by dental surgeries in northern England and Glasgow (Southern Scotland) between 1964 and 1973. These samples are held in the Skeletal Biology Research Centre, University of Kent, as a part of the UCL/Kent Collection. Ethical approval for histology research on this sample of teeth was obtained from the UK National Health Service research ethics committee (REC reference: 16/SC/0166; project ID: 203541).

For details pertaining to the specific breakdown of each population sample by tooth position, the curated state of samples and the information regarding sex, see Supplementary Table 1.

<mark>INSERT FIGURE 2 HERE</mark>

2.2 | Sample preparation

Images and a resin cast were produced for each molar before destructive analysis. These were produced using standard methods (Aris, 2019). The casts reproduced the surface morphology of the tooth crown which will allow future study of microwear, crown morphology, and enamel surface features including perikymata and linear enamel hypoplasia, after destructive sampling.

Thin sections were produced using standard histological procedures (e.g. Mahoney, 2008; Schwartz et al., 2005). The molars were embedded in a hardener and epoxy resin solution (Buehler®) to minimise the chance of the teeth fracturing during sectioning. The embedded samples were cut at a low speed using a diamond-edged wafering saw blade (Buehler® IsoMet 1000 Precision Cutter) at a longitudinal angle through the apex of the mesial cusps: the paracone and protocone cusps for the maxillary molars, and the metaconid and protoconid of the mandibular molars. The samples were then mounted on glass microscope slides and lapped using progressively finer grinding pads (Buehler®) until between 100-120µm in thickness. Ground samples were polished using 0.3µm aluminium oxide powder until all evidence of lapping was removed from the mounted dental specimen. Polished samples were placed within an ultrasonic bath for two minutes to remove any remaining debris before being dehydrated using 90% and 100% ethanol-based solutions (Fisher scientific®). The dehydrated thin sections were finally cleared using Histoclear® and mounted with a glass cover slip using

a mounting medium (DPX®). All sections were examined using polarised light microscopy (Olympus BX53 Upright Microscope). Analyses and image capture was conducted using micro imaging software (cellSens) (see below for detail).

2.3 | Enamel thickness

For each molar, relative enamel thickness (RET), cuspal thickness (CT), and lateral thickness (LT) were measured. Each was measured and calculated on a composite image produced by stitching together 20x magnified images using cellSens digital software. Figure 1 illustrates the enamel thickness measurements.

RET is a scale free derivative of AET (Martin, 1983). Relative enamel thickness was chosen for analysis instead of AET as it removes the effect of tooth size on the quantified thickness of enamel (Martin, 1983). This was considered important because human tooth size can change, even over relatively short periods of time (Brace et al., 1987; Brace and Nagai 1982). Average enamel thickness (AET) is calculated by dividing the area of the entire enamel cap by the length of the EDJ in millimetres (Martin, 1983). RET is subsequently calculated by dividing AET by the square root of the dentine area (the area of dentine under the EDJ, enclosed by the bi-cervical diameter) and multiplying by 100 (e.g. Smith et al., 2006a; Olenjniczak et al., 2008). In this project, RET was calculated using the mesial cusps of M1 teeth. These cusps are the paracone and protocone cusps for the maxillary molars, and the metaconid and protoconid of the mandibular molars.

Two linear measures of enamel thickness (CT and LT) were measured on the paracone of maxillary molars, and the metaconid of mandibular molars. These cusps experience reduced masticatory loads compared to the protocone and protoconid (Schwartz, 2000a). While cusps experiencing higher masticatory loads and abrasion levels have been found to be

more responsive to external selective pressures, particularly in the in the maxillary molars (e.g. hypocone; Kono, Suwa, and Tanijiri, 2002), the variable wear levels and preservative states (particularly cracks, chips, and chalk damage) of the archaeological population highly influenced the sample sizes for these cusps. The paracone and metaconid were less affected by wear, meaning the sample sizes for each population were more consistent. As a result, the paracone and metaconid was selected to explore temporal variation in enamel thickness.

Cuspal thickness (CT in mm) was calculated by measuring the distance between the apex of the dentine horn and the outermost enamel surface of the cusp tip (e.g. e.g. Beynon and Wood, 1986; Grine 2005; Grine and Martin 1988; Le Luyer et al., 2014; Mahoney, 2010; Schwartz 2000; Suwa and Kono 2005) (Figure 1). Lateral thickness (LT in mm) was calculated by measuring, perpendicular to the EDJ, the maximum length between the EDJ and the enamel surface within the area of the tooth between the dental cervix and the first Retzius line to emerge on the outer enamel surface (e.g. Beynon and Wood, 1986; Grine, 2005; Grine and Martin, 1988; Le Luyer et al., 2014; Mahoney, 2010; Schwartz, 2000a) (Figure 1). This was typically around 1mm from the dentine horn.

2.4 | Daily secretion rates of enamel

Daily secretion rates were calculated for the inner, mid, and outer regions of the lateral and cuspal enamel areas of each tooth using standard methods (e.g. Beynon, Reid, and Dean, 1991a; Mahoney, 2008; Schwartz, Reid, and Dean, 2001). The regions were determined by dividing the length of the associated enamel area into three equal parts along the length of the enamel prisms. Cuspal enamel regions were determined within the appositional enamel of each tooth. Lateral enamel regions were determined within the area of imbricational enamel equidistant between the dentine horn and the dental cervix. For each region, a

measurement was made along the long axis of an enamel prism corresponding to the length of five consecutive cross striations. The measurement was then divided by five to give a mean daily rate of secretion in µm/day. This procedure was repeated five times in each region analysed to give a grand mean and standard deviation.

All cross striations were measured at magnfications between 20x and 40x magnification for the paracone and metaconid. Secretion rates appear to be consistent between the same regions of different cusps within individual molar teeth (e.g., Mahoney, 2008). Figure 3 illustrates how cuspal and lateral regions were split and how cross striations were counted along enamel prisms.

INSERT FGURE 3 HERE

2.5 | Statistical analyses

Differences in dentin area were sought between populations using a Kruskal-Wallis test. Dentine area is used here as a proxy for tooth size. A subsequent Kruskal-Wallis test was then conducted to test for differences in dentine area between maxillary and mandibular teeth. Next, Mann Whitney-U tests were conducted to test for differences in enamel thickness (RET, LT, and CT) and DSRs (from each region), between the maxillary and mandibular M1s within each sample population. Following this, a Kruskal-Wallis test combined with Dunn-Bonferroni pairwise comparison was used to compare enamel thicknesses and DSRs, between archaeological time periods. Jonckheere-Terpestra tests were conducted to identify any significant trends in enamel feature changes through time between populations. All statistical analyses were performed using SPSS 24.0.

3 | RESULTS

3.1 | Tooth Size and Maxillary and Mandibular molars

No significant differences were identified in the dentine areas between any two populations for either maxillary or mandibular teeth (Table S2). Thus, no scaling of the CT and LT measures was required in subsequent analyses. Dentine area varied significantly between maxillary and mandibular molars ($p < 0.00$; Table S3). This meant RET was analysed rather than AET for subsequent analyses so that the maxillary and mandibular molars could be combined when comparing populations. There were no significant differences between RET or linear measures of enamel thickness, or the DSRs, when compared between the maxillary and mandibular molars of any population (Table S4 and S5 respectively). Therefore, the data for maxillary and mandibular molars of each population were combined to form time-period representative samples for subsequent statistical tests.

3.2 | Enamel thickness measures

Table 1 reports the mean RET, LT, and CT of each population along with the standard deviations and results of the Kruskul-Wallis tests. The Early Anglo-Saxon RET was significantly larger than both the Late Anglo-Saxon ($p < 0.00$) and Medieval ($p < 0.00$) populations (Fig. 4). Cuspal enamel thickness (CT) did not differ significantly between any of the populations (Fig. 5), but the lateral enamel thickness (LT) of the modern day population was significantly thicker than the Roman population ($p = 0.04$; Fig. 6). No significant trends were found in RET ($p < 0.24$), CT ($p < 0.21$) ($p < 0.27$), or LT measurements across the 2000 years represented in this sample (Table 1).

INSERT FIGURE 4 HERE

INSERT FIGURE 5 HERE

INSERT FIGURE 6 HERE

INSERT TABLE 1 HERE

3.3 | Daily secretion rates

3.3.1 Cuspal DSRs

Table 2 reports the mean inner, mid, and outer cuspal DSRs of each population along with the standard deviations and results of the Kruskul-Wallis, post-hoc Dunn-Bonferroni pairwise comparisons, and Jonckheere-Terpstra tests.

Inner cuspal DSRs show a significant slowing trend of ameloblast daily secretion rates across time ($p < 0.00$). The modern day mean is 0.52μm/day slower than the Roman mean rate ($p < 0.00$; Fig. 7). The mean DSRs from the modern day sample were also significantly slower than both the Roman ($p < 0.00$) and Early Anglo-Saxon ($p < 0.00$) secretion rates. The Late Anglo-Saxon DSRs were significantly slower than the Roman ($p = 0.04$) and Early Anglo-Saxon ($p = 0.04$) secretion rates. Differences between the DSRs of inner enamel of the more ancient populations were less marked. Rates only increased slightly from the inner enamel of the Roman period to the Early Anglo-Saxon period (0.03 μm/day mean difference), and only a 0.02μm/day difference between the Late Anglo-Saxon and Medieval population.

Mid cuspal DSR's show a similar significant trend of slowing ($p < 0.00$); the modern day mean is 0.69μm/day slower than the Roman mean (Fig. 7). DSRs from this enamel region in the Roman sample were significantly faster compared to the Late Anglo-Saxon ($p = 0.04$), Medieval ($p < 0.00$), and the modern day ($p < 0.00$) populations. Rates did not differ significantly between the Late Anglo-Saxon and Medieval populations.

Outer cuspal DSRs also showed a significant slowing trend ($p < 0.00$); the modern day mean is 0.81μm/day slower than the Roman mean, which represents the most significant decrease in cuspal DSR across this 2000 year period (Fig. 7). Pairwise comparisons revealed significant differences between five pairs of populations. The mean DSRs were significantly

faster in the Roman population compared to the Late Anglo-Saxon (p = 0.02), Medieval ($p <$ 0.00), and modern day ($p <$ 0.00). The Early Anglo-Saxon population also presented significantly faster secretion rates than the Medieval ($p < 0.01$) and the modern day ($p < 0.00$) populations. Again, the Roman and Early Anglo-Saxon, and Late Anglo-Saxon and Medieval population pairs showed notably less reduction in secretion rates over time – 0.16μm/day and 0.09μm/day respectively.

INSERT FIGURE 7 HERE

INSERT TABLE 2 HERE

3.3.1 Lateral DSRs

Table 3 reports the mean inner, mid, and outer lateral DSRs of each population along with the standard deviations and results of the Kruskul-Wallis, post-hoc Dunn-Bonferroni pairwise comparisons, and Jonckheere-Terpstra tests.

The inner lateral DSR data show DSRs slow over time, with a significant negative trend from the more ancient to the modern period ($p < 0.01$; Fig. 8). The modern day mean DSR is 0.52µm/day slower than the Roman mean. The Late Anglo-Saxon and Medieval populations again presented similar secretion rates with no significant differences. Dunn-Bonferroni revealed a single difference where the Roman population presented significantly faster secretion rates compared to the modern day population ($p < 0.00$).

Secretion rates slowed slightly between the more ancient to the modern period in the mid lateral DSR data, but the trend was insignificant (Fig. 8.). The mean secretion rate of the modern day population was 0.77µm/day slower than the Roman population. However, the Roman population presented significantly faster DSRs than the Late Anglo-Saxon ($p < 0.00$), Medieval ($p < 0.00$), and the modern day ($p < 0.00$) populations. The Early Anglo-Saxon population also presented significantly faster secretion rates than the modern day population ($p < 0.00$). The Medieval and Late Anglo-Saxon populations again presented similar secretion rates (only 0.02µm/day mean difference).

Outer lateral DSRs show a significant negative, slowing trend over time ($p < 0.00$; Fig. 8.). The modern day mean rate was 0.86 µm/day slower than the Roman mean, which like the cuspal outer DSR, represents the most significant slowing. The mean DSRs were significantly faster in the Roman population compared to the Late Anglo-Saxon ($p < 0.00$), Medieval ($p < 0.00$), and modern day ($p < 0.00$). The Early Anglo-Saxon population also presented significantly faster secretion rates than the Medieval ($p = 0.03$) and the modern

day ($p < 0.00$) populations. Again, the Late Anglo-Saxon and Medieval population pairs were nearly identical (0.01 μm/day mean difference).

4 | DISCUSSION

With the exception of the Early Anglo Saxon sample, all three measures of molar enamel thickness (RET, CT, and LT) remained generally similar when compared between the British populations that spanned a 2000-year period of time. In contrast, there was a significant trend towards slower DSRs in samples from the more recent periods when equivalent regions of enamel were compared between populations, with the exception of the mid lateral region.

4.1| Relative enamel thickness

All measures of RET, CT, and LT lie within previously published ranges for human permanent first molars (Macho and Berner, 1993; Mahoney, 2010; Olejniczak et al., 2008; Reid and Dean, 2006; Schwartz, 2000a; Smith et al., 2006a; Suwa and Kono, 2005; Table 4). Compared to the other modern human groups that have been studied, all the British populations (except the Early Anglo-Saxons) have fairly thin first molar RET. Smith and colleagues (2006a) published mean RETs ranging from 20.16 (maxillary Medieval Danish M1s) to 22.62 (mandibular modern South African M1s). A study by Olejniczak and colleagues (2008) presented mandibular modern human mesial cusp RET ranges more similar to those presented here of 15.87 to 17.05. Here, the Early Anglo-Saxons had a mean RET of 21.52, and while the other populations

ranged from 16.21 to 19.24 (Table 1). The Early Anglo-Saxon values are still well within the range of published modern human RETs.

Despite falling within previously reported ranges, the RET value for the Early Anglo-Saxon sample was significantly greater than Late Anglo-Saxon ($p < 0.00$) and Medieval ($p < 0.00$) populations (Fig. 4; Table 1). It is possible that this thicker enamel in the Early Anglo-Saxon population compared to the late Anglo-Saxon and Medieval periods might reflect a shift to a diet containing a high prevalence of harder or abrasive foodstuffs (e.g. Dumont, 1995; Grine, 2005; Hlusko et al., 2004; Pampush, et al., 2013). Alternatively, these slight but significant variations between populations could relate to differences in the linear dimensions of the tooth crown. This seems less likely though, as dimensions of first permanent molars for Anglo-Saxon and Medieval populations do not seem to vary greatly between these time periods (Aris, Nystrom, Craig-Atkins, 2018).

Comparative analyses of non-human primate (e.g. Grine, 1981; Lucas, et al., 1985, 2008, 2013; Martin et al., 2003; Molnar and Gantt, 1977; Pampush et al., 2013) and human populations (Le Luyer et al., 2014) have found that those groups consuming foodstuffs that incur heavy wear tend to have thicker molar enamel and greater RET. Given the coastal location of the Early Anglo-Saxon population, it is possible this population had a greater dependence on marine foods compared to the later Anglo-Saxon populations, resulting in greater attrition. High levels wear have also been linked to increased consumption of grit from sand in marine diets (Littleton and Frohlich, 1993). Isotopic analysis by Mays and Beavan (2012) has identified increased levels of marine food dependency in Early Anglo-Saxon samples. It is therefore possible that the greater RET of the Early Anglo Saxons could relate to a more abrasive diet.

Migration and gene flow might have contributed to the difference in RET between the Early Anglo-Saxon and Late Anglo-Saxon and Medieval samples. Rapid increases in migration into Britain followed the end of Roman rule, with particular influx of Germanic people corresponding with political and cultural upheavals (Scull, 1993; Privat, O'Connell, and Riachrds, 2002; Lightfoot et al., 2009). The high level of immigration from mainland Europe at the beginning of the Anglo-Saxon period following the end of Roman rule (Scull, 1993; Privat, O'Connell, and Riachrds, 2002; Lightfoot et al., 2009) may have also factored into the high RET observed in the Early Anglo-Saxon sample, if the immigrating groups had relatively thicker RET than those of the indigenous populations. Shifts towards more abrasive marine diets in the Anglo-Saxon period, and the migration and subsequent gene flow following the end of Roman rule in Britain provide two explanatory options for the high RET of the Early Anglo-Saxon population. However, further research into this period is required to support of refute these hypotheses.

4.2 | Linear measures of enamel thickness

There were few differences in cuspal and lateral thickness between populations and no significant directional trends over time (Table 1). The only significant difference ($p = 0.04$) was thicker lateral enamel in the modern sample (1.68 ± 0.57 mm) compared to the Roman sample (1.34 ± 0.12 mm) (Table 1). Otherwise, both LT and CT remained relatively consistent across all populations with means ranging from 1.34mm to 1.68mm and 1.16mm to 1.46mm respectively (Table 1). Given the similarity of RET values for these populations, it is unsurprising that their linear enamel thickness measures are similarly indistinguishable. This is especially true considering that the maxillary paracone and mandibular metaconid

experience less pressure during mastication compared to other mesial cusps of M1 teeth (protocone and protoconid) and are therefore less likely to undergo selective pressure (Grine, 2005; Kelley and Swanson, 2008; Macho and Berner, 1993; 1994; Shellis, et al., 1998).

4.2.1 Cuspal enamel thickness

Despite slight variation between populations, all CT means fell within the known ranges for modern human cuspal thickness (Table 4), including those of previously analysed modern day archaeological British (Mahoney, 2008) and Slavic (Schwartz, 2000a) populations and geographically diverse modern populations including southern African (Reid and Dean, 2006), North America, Japan, and Germany (Suwa and Kono, 2005).

The CT data shows no significant directional trends over the time periods considered here (Table 1; Fig. 5). While the Roman mean CT was the thickest at 1.46mm, and the remaining archaeological populations were thinner, ranging from 1.16mm to 1.18mm, there were no discernible temporal trends. Although the Early Anglo-Saxon had high RET values compared to the other populations, it seems that they possessed a reduced volume of dentine encapsulated by the enamel cap (Table S2) resulting in a larger RET, rather than a noticeable increase in enamel at any crown region.

4.2.2 Lateral thickness between populations

The lateral thicknesses of the M1 cusps analysed here are comparable to those in the published literature (Table 4: Macho and Berner, 1993; Mahoney, 2010; Suwa and Kono, 2005). The Roman population presents significantly thinner LTs than the modern day sample ($p = 0.04$, Table 1). The biological and evolutionary significance of this difference is difficult to

identify. However, lateral enamel undergoes increased stress from increasing occlusal loads, resulting in higher levels of hoop stress that can cause margin fractures (Lawn and Lee, 2009). Interspecific analyses of lateral molar thickness have suggested that the thickening of lateral aspects of molar cusps could be related to dietary differences between modern humans and extinct members of the *Homo* genus (Pan et al., 2016). The significant thickening of enamel between the Roman and modern day LTs could relate to a shift in mechanical demands of food. However, there was no significant trend to the changes in LT across the 2000 year periods, so this possibility should be considered with caution.

4.3 | Daily secretion rates

Daily secretion rates fall within the known ranges of published modern human enamel DSRs (Beynon et al., 1991a; Dean 1998; Lacruz and Bromage, 2006; Lacruz, Ramirez-Rozzi, and Bromage, 2006; Mahoney, 2008; Table 5).

INSERT TABLE 5 HERE

4.3.1 Daily secretion rates compared between enamel regions

The daily enamel growth rate of each population followed the expected pattern of ameloblast activity, with increasing DSRs from inner to outer enamel regions (e.g. Mahoney, 2008; Schwartz et al., 2001). Lacruz and Bromage (2006) reported an increase in the mean secretion rates of 10 modern human molars, when compared between the inner to mid, and mid to outer cuspal enamel region (mean increase in the mean secretion rate of 1.70 and 0.70µm/day, respectively). Similar increases were observed by Mahoney (2008) in 13 British Bronze Age mandibular M1s, where metaconid secretion rates increased by 1.25µm/day

between the inner and mid regions and 0.35μm/day between the mid and outer cuspal areas.

While this pattern was present in the modern clinical sample, the rate of increase was not

nearly as steep as reported elsewhere (mean increase of 0.32 and 0.13μm/day between inner

to mid and mid to outer, respectively, Table 2). This suggests that such rapid increases in DSRs

through the inner to outer regions are not always present in modern human molars.

4.3.2 | Cuspal daily secretion rates compared between populations

Cuspal enamel secretion rates decreased over time ($p < 0.00$; Table 2). A significant decrease

in cuspal DSRs over such a short period of time has not been documented previously in

modern human populations for permanent teeth. There was however a notable similarity

between the mean cuspal DSRs of the Roman and Early Anglo-Saxon populations

(respectively: inner +0.03μm/day, mid +0.23μm/day, outer +0.13μm/day), and between the

Late Anglo-Saxon and Medieval populations (respectively: inner +0.02μm/day, mid

0.00μm/day, outer +0.09μm/day). Considering these population pairs are dated to similar

periods (Roman/Early Anglo-Saxon − 70-400AD/500-600AD; Late Anglo-Saxon/Medieval −

800-1200AD/1100-1600AD) this is unsurprising.

4.3.3 Lateral daily secretion rates compared between populations

Like cuspal DSRs, lateral DSRs slowed over the last 2000 years. However, this trend was only

significant in the inner and outer cuspal regions (Table 3). Similar to the cuspal DSRs, there

was minimal difference between the mean DSRs of the Late Anglo-Saxon and Medieval

populations across all regions of lateral enamel (inner 0.00μm/day; mid 0.02μm/day; outer

0.01μm/day). This is unsurprising, considering these two populations are dated to

overlapping periods (800-1200AD and 1100-1600AD respectively). Moreover, analysis of the

lateral thicknesses of the M1 cusps identified minimal variation between the British populations presented here and those within published literature (Table 4: Macho and Berner, 1993; Mahoney, 2010; Suwa and Kono, 2005). Given the large number of significant variations observed in DSRs that are linked to enamel of the same relative thickness (both cuspal and lateral), it appears that underlying growth mechanisms of enamel can change even though the final enamel thickness does not, when compared between human populations.

The volume of published modern human lateral enamel thickness data is considerably smaller than that of cuspal regions (Table 5). The lateral DSRs for the British populations included here generally fall a bit above the published inner means, below the middle means, and below the outer means (Table 5). However, the limited available data regarding M1 lateral enamel DSRs (Beynon et al., 1991b; Lacruz and Bromage, 2006) is too small to be considered representative of modern humans as a species. Thus, deviation from the published ranges is not surprising when analysing five new human populations.

4.4 | Relationship between DSRs and linear thicknesses

Previous research has considered potential relationships between DSRs and enamel thickness within human first molars (Mahoney, 2008). Additional papers have considered other periodic biorhythms associated with enamel growth, in particular Retzius periodicity (a circaseptum enamel growth pattern) has been associated with first molar enamel thickness. In permanent first molar slower periodicities have been found to predict thicker enamel (Mahoney et al., 2018). The opposite has been observed in deciduous molars (Mahoney, Miszkiewicz, Pitfield, Schlecht, Deter, and Guatelli-Steinberg, 2018). However, the results presented here suggest that there is not a strong connection between daily enamel secretion rates and thickness when compared between samples of molars from different periods. While lateral and cuspal

DSRs slowed significantly over this 2000 year time period in Britain, there was no consistent change in RET, LT, and CT over the same period of time (Tables 1, 2, and 3).

Lacruz and Bromage (2006) found that thicker enamel in fossil hominins is associated with faster secretion rates. However, the results presented here suggest that, while first molar enamel thickness can remain generally similar across time between human populations, DSRs may fluctuate independently. This is most evident in the lateral molar enamel, where the modern day sample was found to have significantly thicker lateral enamel than Romans (Table 1) yet have significantly slower DSRs than Romans in every single enamel region (Tables 2 and 3), which is opposite of what would be expected given the results of Lacruz and Bromage (2006). Similarly, the Roman and Medieval populations show no significant RET, LT, or CT differences (Table 1), but the Romans had significantly faster DSRs in the cuspal and lateral middle and outer enamel regions (Tables 2 and 3).

Given the numerous significant differences observed in DSRs and the lack of significant differences in enamel thickness measures from the same populations, it appears that inter-population variations in DSRs and enamel thickness are not tightly correlated. Future research could consider if other developmental variables such as ameloblast lifespan, crown formation time, periodicity, and/or enamel extension rate, are more strongly linked to enamel thickness when compared between different populations.

4.5 | Conclusions

Modern human enamel thickness has remained relatively stable across British populations throughout the last 2000 years. Conversely, the underlying enamel secretion rates of both the cuspal and lateral enamel regions present a significant slowing trend from the Romano-British period to present. Thus, it seems secretion rates can vary significantly over relatively

short periods of time in modern human populations. Moreover, the differences in DSRs were independent of enamel thickness, suggesting that factors influencing the rate of enamel growth do not necessarily influence the final thickness of enamel, when compared between populations.

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Figure legends

Fig. 1. *Cross-sectional diagram of 2D enamel thickness measures taken. **C**. the enamel cap area and **B**. the dentine encompassed by the enamel and bi-cervical diameter (double-headed arrow). The area of **C**. was divided by the length of the EDJ (marked by arrows) to give the average enamel thickness (AET) in mm. The AET is divided by the square root of the area of **B** and multiplied by 100 to give the relative enamel thickness (RET) (e.g. Martin, 1983), which is a dimensionless index. The first Retzius line that emerges on the outer enamel surface (solid red line) marks the border between cuspal and lateral enamel. The dashed line (CT) illustrates the cuspal enamel thickness measurement (e.g. Beynon and Wood, 1986). The dotted line (LT) illustrates the lateral enamel thickness measurement (e.g. Grine and Martin, 1988).*

Fig. 2. *Map of the United-Kingdom and Northern Ireland displaying the geographic location where the archaeological samples were excavated/modern day samples were extracted. Shapes denote the samples geographic origin, colour the time period they associated with: Roman = Orange, Early Anglo-Saxon = Purple, Late Anglo-Saxon = Blue, Medieval = Green, Modern Day = Red.*

Fig. 3. *Digital image of a paracone from one individual dating to the Roman period. White squares show the areas of the cuspal and lateral enamel subdivided into inner, mid, and outer regions. These regions were used for DSR calculations. The black square shows a 40x magnified superimposition of the mid lateral enamel. White arrows indicate individual cross striations.*

Fig. 4. *Plot of RET data distribution of each population. The central line displays the median thickness for the associated population.*

Fig. 5. *Plot of CT data distribution of each population. The central line displays the median thickness for the associated population.*

Fig. 6. *Plot of LT data distribution of each population. The central line displays the median thickness for the associated population.*

Fig. 7. *Circles depict the mean cuspal DSRs of each enamel region for each population. The figure illustrates the general decrease in secretion rates over time. The error bars display one standard deviation either side of the mean.*

Fig. 8. *Circles depict the mean lateral DSRs of each region and population, displaying their general decrease over time. The error bars display one standard deviation around the mean.*