How the microstructure of dentine can contribute to reconstructing developing dentitions and the lives of hominoids and hominins

Comment la microstructure de la dentine peut contribuer à reconstruire le développement dentaire et la vie des hominoïdes et hominines

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ABSTRACT

Accounts of dentine microstructure are less well established in the primate life history literature than enamel microstructure. The aim of this paper is to draw some basic comparisons between enamel and dentine but at the same time to show how dentine microstructure can make a major but different contribution to reconstructing past lives than enamel can. Dentine has both an organic and an inorganic component. The organic component contains growth factors, stable isotopes and DNA that survive long after death. The mineral component contains trace elements and preserves an incremental record of tooth growth. These can be used to put a time scale to many past events when the chemistry or microstructure of dentine has become altered during tooth growth. Dentine microstructure allows us to reconstruct tooth root growth in the past and has contributed to a fuller understanding of the modular nature of developing dentitions among hominoids and hominins.

RÉSUMÉ

Au sein de la littérature sur l'histoire de vie des primates, il existe moins d'études portant sur la microstructure de la dentine que sur celle de l'émail. Le but de cet article est d'extraire des informations à partir de simples comparaisons entre émail et dentine, tout en montrant combien la microstructure de la dentine peut apporter une contribution majeure et différente de celle de l'émail pour reconstruire la vie passée. La dentine présente à la fois une composante organique et une composante non-organique. La composante organique contient des facteurs de croissance, des isotopes stables et de l'ADN qui survivent longtemps après la mort de l'individu. La composante minérale contient des éléments traces et enregistre de manière incrémentale les variations périodiques de la croissance dentaire. Toutes ces informations peuvent être utilisées pour retracer la chronologie de nombreux événements passés lorsque la chimie ou la microstructure de la dentine ont été altérées au cours de la croissance dentaire. La microstructure de la dentine nous permet de reconstruire la croissance de la racine des dents et contribue à mieux comprendre la nature modulaire du développement dentaire chez les hominoïdes et hominines.

1. Introduction

As a tissue, dentine predates the origin of teeth. The dermal placodes, or placode scales, of jawless fish evolved as reparative organs able to response to wear and wounding on the surface of the skin. In all likelihood their microstructure suggests they were able to detect changes in osmolarity and temperature giving them a protective role comparable to dentine in teeth today. The classic theory is that these structures in the skin at the margins of the mouth gave rise to teeth in the first jawed vertebrates, some 420 million years ago (Smith and Sansom, 2000; Smith et al., 2016). Subsequently, the evolution of an epithelial dental lamina along the jaws enabled what were scattered structures in the skin to become a precisely timed and serially ordered morphogenetic field of developing teeth in the mouth (Smith et al., 2016). The placode dentine of jawless fish, 380 million years ago, contained fine tubules the same size (~2 μm diameter) as human dentine tubules that formed at the periphery of a vascular pulp cavity seated in supporting bone beneath (Smith et al., 2016). The odontoblast cells, who's long cell processes created the tubules as they retreated towards the vascular space within the trabecular bone, also clearly laid down reparative (or secondary) dentine in response to wear and damage (Smith et al., 2016). In mammals, odontoblasts are among the longest-lived of all post-mitotic cells (Couve et al., 2013). For the whole life of a tooth (and an individual) they are capable of pre-dentine and dentine formation.

Dentine makes up the bulk of all teeth. Dentine surrounds the vascular pulp of a tooth and is covered with enamel over the crown and with cementum over the tooth root (Fig. 1). Only 72% by weight of dentine is made up of an inorganic component that is largely hydroxyapatite, compared with enamel where 96% by weight is mineral. Dentine contains 10% water while enamel contains only 2%. Various organic components make up 20% of dentine by weight compared with only 1 or 2% in enamel. The organic constituents in dentine are largely type I collagen, but there are also other non-collagenous proteins such as proteoglycans (important for collagen fibre assembly), dentine phosphoproteins and sequestered growth factors (Linde, 1984). Dentine phosphoproteins have a very high phosphate content and have calcium binding properties that are presumably involved in the mineralisation process (Berkovitz et al., 2002; Linde, 1984). These fundamental compositional differences give dentine and enamel completely different physical properties. While harder than

bone and cementum, dentine is less hard than enamel, but it resists crack propagation better than enamel and has greater compressive and tensile strength being both rigid and elastic (Berkovitz et al., 2002).

Dentine is characterised by tubules that pass from the pulp to the enamel dentine junction (EDJ) in the crown and to the cementum dentine junction (CDJ) in the root (Fig. 1). They follow a long sinuous curving path that reflects the changing rate of formation as dentine is laid down from the EDJ pulpwards (Fig. 1). The cell bodies of odontoblasts line the pulp cavity and their cell processes remain embedded within dentine tubules through life. Some accounts (Schroeder, 1991) claim that during life an odontoblast cell process can reach as far as the EDJ, while others suggest it may only extend just a third of the total tubule length (Shellis, 1981). Dentine tubules also contain tissue fluid and some unmyelinated nerve fibres that are largely distributed beneath the crown. This ensures that dentine remains hydrated, vital (alive) and also constantly sensitive and responsive to changes in temperature, osmotic pressure and other external stimuli. Enamel, on the other hand, is a non-vital tissue containing no cells and is completely insensitive to external stimuli and unable to repair itself. Pulp and bone are both richly innervated and vascularised tissues but enamel, dentine and cementum contain no blood vessels at all.

Enamel, dentine and cementum all form incrementally by apposition of one tissue layer secreted upon another and once formed are not replaced or turned over during life. The general incremental pattern of dentine (Fig. 1) is primarily determined by the rate of differentiation of the forming odontoblast cell sheet and was first described by Victor von Ebner (1902). Dentine formation, unlike enamel formation, can continue in various forms after the tooth has fully grown in response to abrasion, attrition, erosion, tooth fracture and caries so long as the pulp has a blood supply and remains vital. At the end of tooth formation, so-called primary dentine formation is complete, but dentine formation continues at an extremely slow rate in the form of regular secondary dentine (Fig. 1). The reduced outline of the pulp chamber, when examined radiographically, gives some indication of the history of how this has occurred, both locally and generally within a tooth. It has even been used to estimate the age of individuals and may proceed at different rates in males and females (Zilberman and Smith, 2001). Irregular secondary dentine and sclerosed dentine (Fig. 1) are protective and/or reactive forms of dentine and are described and discussed further below.

During tooth formation, ameloblasts secrete enamel matrix and odontoblasts secrete dentine matrix but here the similarities end. A layer of predentine between 10 and 40 μm thick remains unmineralised until the organic component of the predentine has become highly organised into a mesh or felt-work of collagen fibres arranged parallel with the forming dentine surface (Fig. 2). The predentine is essentially a working space within which individual collagen fibres are assembled and densely packed to run randomly between dentine tubules in the plane parallel to the odontoblast cell sheet (Fig. 2). Anders Retzius (1837) first demonstrated that the complex branching pattern of dentine tubules (Fig. 2) is in many cases taxon specific, but there are no studies of this in primate dentine. Towards the CDJ and close to the EDJ dentine tubules are very finely branched but more sparsely distributed. They become more crowded together as the dentine forming odontoblasts converge towards to the pulp chamber, and so there is less space for intertubular-dentine here. The felt-work of collagen fibres run strictly orthogonal to the tubules, but randomly between them, and are well illustrated in the drawings of von Ebner (1902) and in the micrograph reproduced here (Fig. 2) from Bradford (1958). Only very close to the CDJ and the EDJ do collagen fibres in dentine ever run in the long axis of the dentine tubules or the odontoblast and its cell process.

Collagen fibres are composed of smaller tropocollagen strands that are in turn made up of three polypeptide chains that coil around each other in a long helix. This arrangement of the collagen fibre components acts as a template for hydroxyapatite crystallites to lay down in the same orientation both within the intermolecular spaces of the tropocollagen strands and along their surface (Bradford, 1967). The crystallites in dentine are 60-70 nm long, 20-35 nm wide and 3-4 nm thick (Berkovitz et al. 2002; Schroeder, 1991) and much smaller than those in enamel (>100 μm long, 70 nm wide and 25 nm thick). The extraordinary elasticity of dentine and superior crack resisting and fracture toughness properties of dentine result in part from these small hydroxyapatite crystallites being pre-compressed within the collagen framework as water is lost and the fibres contract during the biomineralisation process (Forien et al., 2015). Because the long axis of the crystallites (the c-axis) in the majority of dentine aligns with the collagen fibres in this way even when collagen is lost from fossil teeth, the birefringence pattern observed in polarised light that is determined by the hydroxyapatite crystallite orientation still reflects the original packing pattern and orientation of collagen fibres established during tooth formation (Smith et al., 2000).

During dentine formation the odontoblast process functions simultaneously to transport mineral to the mineralising surface farthest away from the pulp periphery and organic components to the unmineralised predentine zone that lies closest to the pulp (Linde, 1984). Experiments have demonstrated that labelled proline (^{3}H-proline), an amino acid component of collagen, appears in predentine within 3-4 hours and then subsequently in mineralised dentine 20-30 hours later, whereas labelled calcium ions appear in the odontoblast cell process just 1 hour after entering the cell and appear only 2 hours later in mineralised dentine (Schroeder, 1991). Enamel mineralisation is quite different and occurs initially within 24 hours of matrix secretion (Boyde, 1989). While there is clearly some slow continuous mineralisation of enamel (Rosser et al., 1967; Smith, 1998), it is not until an intense period of enamel maturation, after the whole thickness of enamel has been formed, that the enamel mineralisation process is completed. How much enamel mineral is laid down soon after matrix secretion has become an important question in studies of trace elements preserved in enamel and dentine (Humphrey et al., 2008), which will be discussed later. Even when erupted into the oral environment, enamel continues to mineralise as calcium and phosphate ions from saliva are incorporated into the outer surface layer.

2. Dentine and the record of life preserved in teeth

Because of the differences in structure and formation between enamel and dentine, briefly outlined above, dentine preserves a different record of events that reflect the past life of an individual than enamel does. Dentine is a living tissue with dynamic response capabilities so long as a tooth remains vital. Because of this, it is possible to read aspects of the past history of a tooth from its chemistry, histology and radiographic appearance long after death. The organic component of dentine also provides a unique record of its cell biology in a way that enamel can never do.

2.1. The organic phase

Dentine and pulp are really both part of an inseparable functional unit, the pulpodentinal complex. The periphery of the pulp chamber is lined with odontoblasts. The most proximal part of the odontoblast cell processes that run through predentine contain organelles including mitochondria. Afferent nerve axons, especially in the

coronal dentine tubules where 40% may contain nerve fibres, also contain mitochondria (Berkovitz et al., 2002). These, together with cell debris in the pulp chamber, are presumably the source of mtDNA in teeth when it has been successfully extracted by drilling out the circumpulpal regions of the dentine in modern, archaeological and fossil teeth. Rapid evaporation of fluid from exposed dentine tubules at the tooth surface (during life or post-mortem), as well as some clinical tooth cutting processes, can cause odontoblasts and their contents to be aspirated into the proximal parts of dentine tubules. It follows that nuclear DNA may in this way also come to be preserved within the circumpulpal dentine (Brännström, 1963).

The dietary intake of an individual, as well as the environment in which they live, influences the chemical composition of bone and tooth tissues forming at any one time. The organic component of dentine, particularly the collagen component, incorporates carbon and nitrogen stable isotopes that have been used to provide information about diet and to estimate the duration of breast feeding and the time of weaning. Nitrogen isotopes, ^{15}N, reflect the composition of dietary proteins. A shift from higher to lower values in dentine collagen reflects the decline in protein intake from breast milk to protein obtained from non-breast milk sources after weaning (Humphrey, 2014). Nutritional deprivation and reduced protein intake can also be detected in this way (Beaumont et al., 2013). Stable carbon isotopes, ^{13}C, derived from dietary proteins are also incorporated into collagen. Certain foods are enriched in ^{13}C, such as maize, and tooth collagen chemistry also reflects this (Beaumont et al., 2015; Dupras and Tocheri, 2007). The interplay between diet, the physiological response to nutritional stress and tooth chemistry is complicated, but Beaumont et al. (2015) have been able to use dentine in an archaeological sample to identify a ^{13}C and ^{15}N signal from collagen that is consistent with maize used as a famine relief food during times of growth stress during tooth formation.

Interestingly, and something that has not yet been exploited in comparative cell biology, the dental pulp, which throughout life maintains dentine as a vital tissue, is also a rich source of stem cells. Exfoliated deciduous teeth have now become a resource for stem-cell therapies including autologous stem-cell transplantation and even tissue engineering (Miura et al., 2003). As it mineralises, circulating growth factors such as (IGF)-II, (BMP)-2 and (TGF)-ß become sequestered in dentine (Sloan et al., 2000; Smith, 2000; Zhao et al., 2000). These growth factors can also be retrieved post mortem from teeth. In life they can be released again during

demineralisation, for example as a result of caries. This triggers the differentiation of new odontoblast-like cells able to form a plug of so-called irregular secondary dentine (Fig. 1) that blocks off the proximal dentine tubules at the pulp periphery (Berkovitz et al., 2002). In the face of an extreme insult exposing the distal ends of the dentine tubules to the external environment, this effectively preserves the life of the tooth. It is a trade-off between some local loss of sensory feedback (but also often relief from persistent pain) and the continuing vitality of the pulp that is essential for maintaining the elastic fracture resistant properties of dentine that otherwise becomes brittle. The tubules, now blocked off from the pulp, become air-filled in histological sections of teeth and so appear dark in transmitted light microscopy. These so-called dead tracts within the dentine of archaeological and fossil teeth (Fig. 1) persist as a record of the local response of the tooth to its external environment during life.

An important record of certain stress events is laid down as dentine forms that can subsequently be retrieved from a tooth long after the event. Austin et al. (2016) have recently used immunohistochemistry and Raman spectroscopy to detect heat shock protein-70 (HSP70) in dentine. Adenosine triphosphate (ATP) is the source of intracellular chemical energy which, when it loses a phosphate group, liberates energy and becomes adenosine diphosphate (ADP). It seems that lower ATP (and so, excess ADP) presence triggers HSP70 to be released during dentine formation. This, in turn, captures an important component of the organic dentine matrix, a regulator of dentine mineralisation, dentine matrix protein-1 (DMP-1), that alters the nature of the predentine matrix. But exactly how the rate of secretion of predentine matrix and assembly of and density of collagen fibre packing, or even the non-collagenous component of the matrix and the mineralisation process, are each affected by this remains unclear. This approach has great potential for human biology and primatology, and potentially for archaeology and palaeontology, where stress events in the life of an individual can be identified if the organic components of the dentine remain intact. But not all stress events trigger this particular reactive mechanism, and the neonatal line that results from the stress of being born is apparently one such case (Austin et al., 2016).

2.2. The mineral phase

When dentine mineralises it does so completely all at one time, although within the dentine tubules slow deposition of peritubular dentine continues throughout life. Peritubular dentine is quite different to intertubular dentine in that it contains no collagen at all. It gradually fills in the tubule lumen, slowly encroaching upon the odontoblast cell process and reducing the tissue fluid content, eventually to the point of occluding it. Regions of sclerosed or transparent dentine often form adjacent to dead tracts (Fig. 1), where the reaction to caries, attrition or abrasion, for example, has been less severe than to cause irregular secondary dentine formation and a resulting dead tract. One method of age assessment makes use of this process (Miles, 1963) and is based on the more gradual increasing sclerosis, or transparency, of the root dentine that occurs with age. Through life, the apex of the root, and then the root margins along the CDJ towards the cervix, become increasingly transparent as more dentine tubules are filled in with peritubular dentine (Fig. 1).

A problem with both dentine and enamel is the loss of temporal resolution of mineral laid down during tooth formation with increasing age. In dentine, the problem arises from the continual deposition of peritubular dentine, even though initial mineralisation is fast and complete. However, this can be minimised by only using newly formed teeth from young individuals. There is also the greater likelihood of post mortem change and diagenesis in archaeological and fossil dentine, since empty tubules draw up fluid by capillary action potentially contaminating dentine with both organic and inorganic material from beyond the tooth. Enamel, on the other hand, continues to mineralise over a long period of time, blurring the distribution of mineral secreted by ameloblasts over a wider area. Fig. 3 illustrates this in a ground section of a tooth labelled with the antibiotic oxytetracycline during life. In incident ultraviolet light, the oxytetracycline fluoresces as bright sharp bands in the dentine, but as more diffuse bands of less intensity in the enamel. At higher resolution, when the same label lines in dentine are imaged with confocal microscopy (Fig. 5 bottom left), it becomes clear how specific this temporal resolution is in mineralising dentine. Clearly, some oxytetracycline binds with calcium ions (chelates) in enamel and remains localised at the time of initial mineralisation throughout the subsequent maturational processes, but not as fully as it does in dentine. As much as ~86% of the mineral content of mature enamel has been estimated to be deposited during the maturational stage (Humphrey et al., 2008; Smith, 1998).

3. Incremental growth of dentine

Several lines of evidence point to the circadian control by clock genes of both enamel and dentine matrix secretion and dentine mineralisation (Lacruz, 2016; Lacruz et al., 2012; Zheng et al., 2013, 2014). It also seems likely these occur maximally at different times of day in an alternating manner (Miani and Miani, 1971; Zheng et al., 2014). Incremental growth markings are clearly visible in predentine when tooth sections are stained appropriately to demonstrate this (Fig. 4), which suggests the circadian clocks that operate on odontoblast function modulate organic matrix secretion during the period that collagen fibres are being assembled and organised ahead of mineralisation.

The pattern of dentine mineralisation is often initially from minute spherical centres known as calcospherites within the predentine, which spread concentrically and then coalesce (Fig. 5). Adjacent to the CDJ, these minute calcospherites fail to coalesce completely and give a granular appearance to the dentine (the granular layer of Tomes; see Fig. 1). Small calcospherites can be seen mineralising freely in the predentine ahead of the main mineralising front (Fig. 4). As dentine formation proceeds, new and bigger calcospherites often become more arcade-shaped and flattened and fuse together to form a more continuous laminar mineralising front (Fig. 4). A proportion of the mineral crystallites within a calcospherite run out radially from a focal point and cut across those aligned along the collagen fibres. In polarised light, these calcospherites are, therefore, very prominent features of dentine. Their rate of initiation, together with the rate of dentine mineralisation, governs the predictable geometric selection of their size and distribution (Shellis, 1981; Swan and Kershaw, 1994; Ubukata, 1994).

When there is failure of calcospherites to coalesce completely within the body of the dentine, the unmineralised spaces remaining between them form what are known as interglobular spaces that often distribute broadly along the incremental lines (Fig. 1). Interestingly, dentine tubules run unhindered through regions of interglobular dentine, suggesting the rate of matrix secretion by odontoblasts is not affected. Vitamin D deficiency and rickets is one cause of hypomineralisation that may manifest as regions of interglobular dentine (D'Otenzio et al., 2016). The concentric pattern of calcospheritic incremental growth clearly reflects the mineralisation process, but the more laminar increments, especially when close to the CDJ, might

either be overlain on top of the increments visible in predentine, or be superimposed out of phase in some way on this predentine template. What seems clear from experiments is that one dark and one light dentine increment form over a 24 hour period (Kawasaki et al., 1980; Okada, 1943; Yilmaz et al., 1977).

A longer period growth marking also exists, at least in primate dentine (Dean, 1995). The simplistic assumption has been that these long-period markings are homologous with long-period Retzius lines in enamel (Fig. 1), if not causally at least in their periodicity. Long-period lines in dentine are best expressed in coronal and early root dentine (Figs. 4, 5), but are often not expressed at all in cuspal dentine and/or further apically into the root dentine. In other words, they are most often visible during the period perikymata are forming in the lateral enamel of the same tooth, and usually a bit beyond this time into early root formation. The visibility of long-period dentine lines is, however, strongly subject to section obliquity: they may be well expressed in a true plane of section through a tooth cusp, but not visible at all in a serial section taken only a few hundred micrometers lateral to this.

Whether these long-period growth markings are yet again superimposed onto the predentine daily markings, or onto the more laminar daily mineralisation lines, or are simply an enhanced increment of one or the other (or both), is again unclear. They certainly do not superimpose onto the daily calcospheritic mineralising lines close to the CDJ, which favours an underlying rhythmic change to organic matrix secretion as the visible expression of whatever their proximate cause might be.

The dentine layers adjacent to the CDJ are structurally complicated. They include the clear glass-like hyaline layer immediately beneath the cementum, that contains no dentine tubules and the darker granular layer of Tomes (GLT) just deep to this (Fig. 1). Matrix secreted from early cementum forming cells (that also contain some enamel proteins), as well as matrix from odontoblasts, may in fact mix together to form the hyaline layer. This layer is also special, in that mineralisation within it is delayed at the root surface until the first fibres of the cementum have formed and are firmly bound together with it (Berkovitz et al., 2002). The implication of this developmental and structural complexity at the root surface is that any regular (i.e., non-hypoplastic) long-period markings in root dentine are unlikely to run straight through to the cementum root surface in the same way long-period lines in enamel crop out at perikymata grooves (Risnes, 1985). Long-period dentine lines are strongly enhanced in ground sections viewed in transmitted polarised light, but are also visible

in demineralised sections where polarised light then has little effect on their expression (Fig. 5). This suggests a strong mineral crystallite size or orientation effect on their birefringence in ground sections of dentine, but also that the most probably primary expression of their periodicity is an underlying rhythmic shift in the density of the collagen, and/or non-collagenous component of the organic matrix (Dean, 1995).

In short, the best understood, and in that sense reliable, incremental markings in dentine are the daily mineralisation lines. In both enamel and dentine, it is the daily incremental markings that form the basis of reconstructing tooth formation times and of putting a timescale to stress markings in teeth. Distinguishing regular long-period lines in dentine, especially when few are well expressed, from closely spaced accentuated hypoplastic lines is often impossibly difficult (Fig. 5). As markers of the mineralising front, however, all these lines are extremely useful, but as the basis of counts, and so of estimates of dentine formation times long-period lines in dentine (especially root dentine), are of questionable reliability. The main problem that has hindered the collection of data about rates of dentine formation and root growth from modern teeth is the difficulty seeing daily incremental markings in dentine at all.

4. The record of trace elements preserved in dentine

The biological hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$) that makes up 96% of enamel and 72% of dentine by weight contains many trace element substitutions and inclusions. Apatite structure and chemistry has been reviewed by Elliott (1997) and a number of elements are known to substitute for calcium (Na, Mg, Mn, Zn, Sr, Ba). Carbonate ions (CO_3^{2-}) contained in apatites can substitute either for PO_4^{3-} ions or for two hydroxyl ions, while fluoride ions commonly substitute for one hydroxyl ion (OH^-). Other elements (i.e., lead) may exist as inclusions within the apatite lattice, but all elements also exist in an amorphous form within enamel and dentine. For this, and other reasons, and because substitutions within the apatite lattice occur regularly, the ratio of calcium to phosphorus (Ca/P), varies in enamel from 1.8 to 2.4 and in dentine from 2.1 to 2.2 (Hillson, 1996; Schroeder, 1991). However, the first-formed dentine crystallites, particularly the minute crystallites in peritubular dentine, seem not to be hydroxyapatite, but rather octacalcium phosphate crystallites ($Ca_8H_2(PO_4)_6.5H_2O$) (Bodier-Houllé et al., 1998; Shellis, 1981) with a different calcium to phosphorus

ratio. These variations in enamel and dentine chemistry are important to bear in mind, because calcium is often used to normalise the concentration of trace elements in enamel and dentine in laser ablation studies. The ratio of calcium to phosphorus differs between enamel and dentine, and may well also differ within dentine, for example, where large amounts of late-formed peritubular dentine exist in proportion to the early-formed intertubular dentine, particularly close to the pulp chamber in chronologically older teeth where dentine tubules are crowded together more.

The mineral phase of dentine also captures oxygen isotopes as it forms. Seasonal climate changes have been identified in mastodon dentine that reflect the $\delta^{18}O$ values derived from precipitation. Oxygen isotope analysis of the carbonate fraction of dentine apatite, within the thinner darker increments formed in the winter, contained substantially lower $\delta^{18}O$ values of the CO_3 fraction than those formed at other times of the year (Koch and Fisher, 1986; Koch et al., 1989).

Unlike enamel, dentine contains ~1% magnesium (Schroeder, 1991), that is associated with the very small crystallite size found within the dentine collagen fibrils (Bigi et al., 1992). So far, there is no clear evidence for any elemental variation at the daily or long-period incremental markings in enamel or dentine. However, a candidate for further investigation is zinc. Levels of zinc are higher where there is active dentine formation near the pulp, and zinc is also non-uniformly distributed on forming Haversian bone surfaces (Gomez et al., 1999; Schroeder, 1991).

Trace element distributions in enamel and dentine are a powerful way of reconstructing infant diet and the time of first supplementary food intake during breast feeding, as well as the incidence of stress events during the period of tooth formation (Humphrey, 2015). The drawback of using dentine rather than enamel in archaeology and palaeoanthropology, has been its permeability and propensity for diagenetic change over time, as well as the complicated chemical variation that accrues with peritubular dentine formation over a lifetime. Enamel also has its drawbacks, in particular the degree to which the temporal record of enamel chemistry is smothered during the extended maturation process (Sponheimer and Cerling, 2014). Humphrey et al. (2008) have provided some evidence that enamel preserves a temporal record of strontium and calcium in sufficient concentrations to track changes that reflect an individual's nursing history. The fact that there appears to be a sharp shift in enamel chemistry immediately after the neonatal line in deciduous teeth, in both enamel and dentine, is itself evidence that a temporal record of enamel chemistry, like that of

dentine, persists and is not entirely overwhelmed during the enamel maturation process (Humphrey et al., 2008). Good experimental evidence exists that tracks the temporal resolution of dietary nitrogen and carbon isotopes in dentine, as well as carbon and oxygen isotopes through the enamel during tooth formation from cusp to cervix (Balasse, 2002; Balasse et al., 2001). Nonetheless, as these authors note, each sampling point of a time series cuts across many oblique time layers within enamel and/or dentine, and so cannot yet precisely enough demonstrate the degree of difference in the temporal isotopic resolution between enamel and dentine.

Austin et al. (2013, 2016) have now extended studies of trace element distributions in enamel to dentine and have used recent well-preserved primate teeth, where they found that barium normalised to calcium gives the most reliable dietary signal during early dentinogenesis. Interpreting the distribution and pattern of individual trace elements normalised to calcium in both enamel and dentine depends on a knowledge of how various systems and organs of the body discriminate or favour the transport of each element, and whether they are actively transported (as calcium is) or simply diffuse passively (as hypothesized for strontium) through ameloblasts and odontoblasts. Moreover, the normal background gradients of trace elements need to be understood to quantify the over-riding effects of dietary trace element intake laid down in enamel and dentine (Humphrey, 2015). So far, shifts in Ba/Ca and Sr/Ca ratios look the most likely candidates for tracking the record of nursing history from birth to the end of the weaning period, both of which can be retrieved from the mineral phase of dentine.

5. The record of accentuated stress lines in dentine

Hypoplasias (regions of reduced tissue secretion and/or mineralisation) are important indicators of reduced enamel and dentine formation during tooth growth. They are also potentially useful temporal markers across teeth from the same individual. Linear hypoplasias in enamel and dentine have become synonymous with stress events during tooth formation (Hillson, 2014). Many things are known to disrupt enamel and dentine formation, including malnutrition, dysentery, respiratory infections, exanthematous childhood illnesses, and other high fevers (such as that resulting from malaria) can cause disruption to either the secretion of enamel and predentine matrix, or to the mineralisation of the enamel and dentine matrix, or to

both of these processes (Molnar and Ward, 1975; Pindborg, 1970, 1982; Schuman and Sognnaes, 1956; Suckling and Pearce, 1984; Vitzthum and Wikander, 1988).

Odontoblast differentiation and dentine matrix secretion occur ahead of enamel formation, and predentine secreted at the EDJ is always ahead of the enamel front at the EDJ. When hypoplasias affect only matrix secretion, the distance of the mismatch as accentuated hypoplastic lines converge at the EDJ is greater than when hypoplasias affect only mineralisation. The apparent spatial delay in dentine mineralisation means a hypoplastic line might potentially be 10-40 μm behind the predentine front, and so will often converge closer to the same hypoplastic event in enamel at the EDJ (see the chlortetracycline labels in enamel and dentine in Fig. 3, that almost coincide at the EDJ). In practice, however, there can be few things that don't affect both matrix secretion and mineralisation, but cell function is very sensitive to temperature, and the mineralisation process very sensitive to shifts in pH. Hypoplastic stress lines in dentine, including the neonatal line, are often harder to define than their corresponding lines in enamel, which may be partly because the effects of a short sharp stress event are buffered by changes taking place over a longer period of time within the predentine zone ahead of mineralisation.

Parturition lines in root dentine are a special kind of stress record in the teeth of mothers (Dean and Elamin, 2014). It has been hypothesised by Okada (1943) that the metabolic acidosis that occurs during labour has an initial effect on mineralising dentine, a slightly lowered pH being associated with a zone of less well mineralised dentine. Following birth, there is then swift recovery and a period of more intense mineral deposition. Accompanying these apparent changes to the degree of mineralisation are changes in collagen fibre packing pattern and/or orientation, and likely also other changes to the non-collagenous organic matrix (Okada, 1943). The overall picture is one of a characteristic dark then light zone of dentine when viewed in transmitted polarised light, indicating disruption to both the organic and inorganic components of the dentine. While polarising light microscopy clearly suggests a change in crystallite orientation or density at a parturition line and/or a neonatal line in dentine, there remains no clear evidence for a zone of hypomineralisation, as suggested by Okada (1943), persisting in mature teeth.

6. Reconstructing dentine formation times in fossil teeth

A remarkable thing about tooth tissues is their durability and preservation at the microstructural level long after death. Fossil dentine and bone belonging to a specimen of *Paranthropus boisei* 1.6 million years old (KNM-ER 1817) from Koobi Fora (Furseth Kinge et al., 2005, 2009) still contained a significant proportion of biological hydroxyapatite with less replacement of hydroxyl groups by fluoride ions than might be expected. At a microstructural level when gently demineralised with EDTA (ethylenediaminetetracetic acid, an agent able to bind and remove calcium ions), fragments of alveolar bone prepared for transmission electron microscopy (TEM) contained areas of fibrous material with the characteristic 64 nm banding pattern typical of collagen fibres. Similar electron dense material was seen in EDTA demineralised dentine with TEM, suggesting that not all the organic matrix, and even collagen originally present in dentine and bone, is necessarily lost during fossilization. This finding is encouraging for future research that might seek to explore the chemistry of this residual organic matrix in fossil hominin bone and dentine.

The microanatomy of the CDJ is often well preserved in fossil tooth root material, and daily incremental markings in dentine are often clear (Fig. 5). Paradoxically, daily incremental markings in fossil dentine appear to be more consistently visible than those in modern primate material. The reason for this is obscure but it may be that in inorganic fossil dentine the mineral density alternates in step with the daily incremental pattern and may in fossil dentine have calcite formed within the more porous layers. While speculative, it may be that alternating calcite-rich and apatite-rich increments within dentine, with different refractive indexes, enhance the distinction between the alternating light and dark bands visible in transmitted light microscopy.

Where it has been possible to measure daily rates of dentine formation in different tooth types of the same individual, or at different positions along the forming root from cervix to apex (for example, more extensively than any other specimen in *Paranthropus boisei,* KNM-ER 1817), the gradient from CDJ to deeper formed dentine remains much the same over a 200 μm thickness of first-formed root dentine (Dean, 2012). Beyond this thickness, rates of dentine formation begin to differ greatly between tooth types and taxa, and are after all the basis of differing tooth size and shape.

Fig. 6 represents some of the data for the spacing between daily dentine increments that it has been possible to gather from teeth close to the root surface, just

deep to the granular layer of Tomes (GLT). There appear to be small differences between taxa, but more teeth of different tooth types are needed to confirm this. *Oreopithecus, Hispanopithecus* and *Pan* have slightly slower rates than *P. boisei, H. erectus* and *H. sapiens* in the teeth represented here. *Gorilla* stands out as having markedly faster rates within the 100-200 μm zone. Even so, the average differences within a given 100 μm zone deep to the GLT of most taxa are less than 1 micrometer (Fig. 6). This kind of consistency in formation rates at the CEJ contrasts with what we know of enamel at the EDJ, where rates vary considerably more across taxa (Zanolli et al., 2016: table 7). Data for living taxa are hard to come by and more are needed, as Fig. 6 demonstrates. Nonetheless, direct counts of daily increments across a 200 μm zone of dentine just deep to the GLT average 80 to 90 days in most taxa studied so far (Dean and Cole, 2013).

Direct observation of daily incremental markings, together with clear accentuated (regular or irregular) markings in root dentine that indicate the former position of the dentine mineralising front, provide a basis for reconstructing the rates of root extension and the timing of root formation in fossil hominoid and hominid teeth (Dean and Cole, 2013).

Fig. 7 shows how the general pattern of incremental markings in dentine changes from very fast-forming teeth, where many secretory odontoblasts are active at one time, to more slow-growing teeth, where proportionately fewer odontoblasts are active along the mineralising front. The angle the mineralising front made with the root surface during tooth formation is still reflected by the orientation of incremental markings that are visible in fully formed teeth. Unfortunately, daily dentine increments are not visible in all teeth studied any more than enamel increments are, and estimates are usually used for each taxon from what data there are. Using average daily rates of dentine formation estimated across the first formed 200 μm of root dentine and then tracking an accentuated marking representing the position of the former mineralising front at this point back to the CDJ gives the length of root formed in a given time (usually, between 80-90 days in hominoids). Fig. 8 illustrates this in a portion of *Oreopithecus* M2 root. Thick lines lie parallel with the dentine tubules and extend 200 μm from the CDJ. Thin arrows then pass from the end of the thick lines back to the CDJ along the direction of oblique accentuated markings that represent the former mineralising front. By repeating this procedure over the length of a tooth root, and cumulating the time taken to form each 200 μm length of root dentine and each

length of root formed, it has been possible to reconstruct what is essentially a longitudinal record of root growth along the CDJ in several Miocene hominoid teeth and several Plio-Pleistocene hominin teeth (Dean and Cole, 2013).

7. Evidence for developmental dissociation within developing dentitions

From the preserved microanatomy of dentine to being able to reconstruct tooth root growth in the past, a bigger picture emerges about the evolution of dentitions and the processes involved. Individual hominoid and hominin teeth may take broadly similar times to grow from cusp initiation to root apex closure (Dean, 2010; Dean and Cole, 2013), but they are each individual components of a developing dentition that, as a whole, must complete in whatever time is available before adulthood is reached. Delaying the time each individual tooth begins to form (particularly molars), draws out the time a whole dentition takes to complete. But this mechanism for prolonging dental maturation may have its limits. Root growth rates in most hominoid teeth studied rise to a peak, or spurt, as a tooth erupts into functional occlusion (Dean and Cole, 2013, 2014) and the age at peak velocity can be used as a kind of marker event (Fig. 9). In modern humans, eruption of the molar teeth into functional occlusion has been drawn out beyond the time that the root spurt occurs, suggesting a degree of developmental dissociation between the process of root formation and the process of tooth eruption. Indeed, enamel formation times may also on the one hand be constrained by the time available to grow them in the bone before tooth eruption occurs but, on the other hand, they are clearly under strong selection pressure to grow enamel thick or thin, or crowns short or tall, as required to best last a lifetime of chewing and food processing. Enamel crown formation times seem to vary greatly among hominoids, and appear then also to vary independently of the total time taken to grow the dentine core of a particular tooth type, or the total time taken to complete a dentition (Dean, 2010; Dean and Cole, 2013; Smith, 2016). Making use of the incremental growth markings in dentine and enamel to put a time scale to events that contribute to the development of whole dentitions in the past will ultimately allow us to better understand the modular nature of dental development and the ways in which evolutionary processes have occurred during human evolution (Dean, 2016).

8. Summary

Odontoblasts secrete an organic dentine matrix that subsequently mineralises. Both the organic component and the mineral component of dentine contain constituents that can be recovered after death. When the record of stable isotopes, growth factors and trace elements has been altered by either seasonal change, stress or diet during tooth formation evidence for this persists in dentine and can be used to reconstruct past events in the life of an individual. Long odontoblast cell processes trail behind the forming tissue front as dentinogenesis proceeds and create tubules within the dentine. During life, fluid-filled dentine tubules function to nourish dentine. They also form part of the sensory feedback system that protects teeth from damage, and that can trigger a reparative response to tooth wear or fracture within the tooth pulp. Empty dentine tubules in archaeological or fossil teeth may draw organic and inorganic material into a tooth from the external environment by capillary action, making dentine a less satisfactory tissue than enamel for retrieving an unaltered chemical signal post mortem. On the other hand, dentine mineralises quickly during development, capturing a temporal record of stable isotope and trace element distribution with greater resolution than enamel. The incremental record of dentine formation, like enamel, does not turn over during life. Dentine, therefore, contains an incremental record of tooth growth that can be used to reconstruct the rates and formation times of individual teeth long after death. Irregular accentuated markings resulting from hypoplasias or stress events leave marks in all teeth forming at the same time, such that a time-line can be cross-matched across the different individual stages of tooth development within a dentition. Information of this kind from many teeth growing at the same time in an individual can then be used to reconstruct the time taken to grow whole dentitions and to estimate the age of key dental growth events, such as tooth eruption ages and root completion ages. When associated partial skeletons and dentitions are preserved in the fossil record, enamel and dentine, when studied together, can sometimes be used to put a chronological time scale to skeletal growth events and to body mass and stature attainment in the past. In this way, a bigger picture of some of the evolutionary events that have taken place during primate and human evolution can be pieced together.

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Fig. 1. Three longitudinal sections of anterior teeth illustrating the microanatomy of dentine. Left and middle after figs. 116 and 126a of von Ebner (1902); right after fig. 7.19, Aiello and Dean (1990). SR, striae of Retzius in enamel; HSb, Hunter-Schreger bands in enamel; P, pulp cavity; Igd, interglobular dentine; C, cementum; GLT, granular layer of Tomes; D, dentine incremental lines; E, enamel; DT, dentine tubules; Rsd, regular secondary dentine; Ca, caries; Sc, sclerosed dentine tubules; Isd, irregular secondary dentine; Pd, primary dentine; Trd, translucent dentine.

Fig. 1. Trois sections longitudinales de dents antérieures montrant la micro-anatomie de la dentine. Les images à gauche et au milieu sont tirées des figs. 116 et 126a de von Ebner (1902); celle de droite est tirée de la fig. 7.19 de Aiello et Dean (1990). SR, stries de Retzius dans l'émail; HSb, bandes de Hunter-Schreger dans l'émail; P, cavité pulpaire; Igd, dentine interglobulaire; C, cément; GLT, couches granulaires de Tomes; D, lignes incrémentales de la dentine; E; émail; DT, tubules de dentine; Rsd, dentine secondaire normale; Ca, caries; Sc, tubules de dentine sclérosés; Isd, dentine secondaire irrégulière; Pd, dentine primaire; Trd, dentine translucide.

Fig. 2. Dentine tubules showing the fine branching pattern in a human incisor root after plate A2, fig. 1b from Retzius (1837). Lower left: tubules in cross section showing collagen fibres (CF), peritubular dentine (Ptd) and odontoblast processes (Op) within the tubules (after fig. 127 in von Ebner, 1902); lower middle: dentine tubules (Dt) sectioned transversely running left to right and collagen fibres (CF) running at right angles to them (after fig. 127 in von Ebner, 1902); lower right: light micrograph of predentine with dentine tubules cut end-on showing collagen fibres running randomly between tubules (fig. 22 from Bradford, 1958). Dentine tubules are ~2 μm in diameter.

Fig. 2. Tubules de dentine montrant un fin niveau d'interconnexions dans une racine d'incisive humaine selon la planche A2, fig. 1b de Retzius (1837). En bas, à gauche: tubules en coupe transversale montrant les fibres de collagène (CF), la dentine péri-tubulaire (Ptd) et l'empreinte des odontoblastes (Op) dans les tubules (d'après la fig. 127 dans von Ebner, 1902); en bas, au centre: tubules de dentine (Dt) sectionnés transversalement, allant de gauche à droite et fibres de collagène (CF) arrivant à angle droit par rapport à ces derniers (d'après la fig. 127 dans von Ebner, 1902); en bas, à droite: photographie en microscopie optique de prédentine avec les tubules de dentine

coupés de face, montrant les fibres de collagène circulant aléatoirement entre les tubules (fig. 22 de Bradford, 1958). Les tubules de dentine font ~2 µm de diamètre.

Fig. 3. Modern human M2 longitudinal ground section viewed in incident UV light. Label lines of oxytetracycline antibiotic fluoresce in the dentine (to the right) and the enamel (to the left). Those in the enamel are more diffuse and less intense than their matching labels in the dentine.
Fig. 3. Section histologique longitudinale d'une M2 humaine modern vue en lumière UV incidente. Les lignes fluorescentes de l'antibiotique à oxytétracycline sont visibles dans la dentine (à droite) et dans l'émail (à gauche). Celles de l'émail sont plus diffuses et moins intenses que leurs équivalents dans la dentine.

Fig. 4. Top left: demineralised section (silver block-stained by Beilschovsky technique) showing dentine formation. Odontoblasts form a layer at the bottom of the image. A darkly stained line defines the boundary between predentine and what was mineralised dentine. The ~30 µm zone of predentine contains a few free calcospherites to the left. Both predentine and dentine show daily incremental markings. Odontoblast cell processes show as stained extensions of the cells passing into tubules that run vertically through the dentine. Top right: demineralised and silver block-stained section showing dentine formation; the same features can be seen as in the previous image but daily increments in the predentine are more marked. Bottom left: ground longitudinal section of human crown dentine in transmitted polarised light showing long-period lines ~30 µm apart. Bottom right: demineralised sliver block-stained section of human coronal dentine showing long period incremental markings remaining after all mineral has been removed.
Fig. 4. En haut, à gauche: section déminéralisée (teintée en bloc à l'argent selon la technique Beilschovsky) montrant la formation de la dentine. Les odontoblastes forment une couche au bas de l'image. Une ligne teintée plus sombre marque la limite entre la prédentine et la zone qui correspondait à la dentine minéralisée. La zone de ~30 µm de prédentine contient quelques calcosphérites isolées sur la gauche. La prédentine et la dentine montrent des lignes incrémentales de croissance journalière. Les empreintes des odontoblastes sont visibles comme des extensions teintées des cellules passant dans les tubules qui sont orientées verticalement le long de la dentine. En haut, à droite: section déminéralisée et teintée en bloc à l'argent montrant la

formation de la dentine ; les mêmes structures que dans l'image précédente peuvent être observées ici, mais les lignes incrémentales de la dentine sont plus marquées. En bas, à gauche : section histologique longitudinale de dentine coronale humaine en lumière transmise polarisée montrant les marqueurs incrémentaux de longue période séparés de ~30 µm. En bas, à droite: section déminéralisée et teintée en bloc à l'argent de dentine coronale humaine montrant les marqueurs incrémentaux de longue période restant après la suppression de la phase minérale.

Fig. 5. Top left: scanning electron micrograph of mineralising calcospherites on the forming dentine front close to the root surface. Top right: daily incremental markings in *Victoriapithecus macinnesi* M2 crown dentine (longitudinal section, transmitted polarised light). Bottom left: laser confocal image of human M2 root dentine showing fluorescent labels of oxytetracycline antibiotic outlining the calcospheritic pattern of mineralisation; some peritubular dentine is labelled in the dentine tubules that run obliquely from top left to bottom right. Bottom right: mineralising calcospheritic pattern of daily lines close to the root surface in a ground section of a fossil hominin molar seen in non-polarised transmitted light.

Fig. 5. En haut, à gauche: Image par microscopie électronique à balayage de calcosphérites en cours de minéralisation sur le front de formation de la dentine, proche de la surface de la racine. En haut, à droite: marqueurs incrémentaux quotidiens dans la dentine d'une M2 de *Victoriapithecus macinnesi* (section longitudinale, en lumière transmise polarisée). En bas, à gauche: image par microscopie confocale à balayage laser de dentine radiculaire d'une M2 humaine montrant les marques fluorescentes de l'antibiotique à oxytétracycline entourant le patron de minéralisation calcosphéritique; de la dentine péritubulaire est marquée dans les tubules de dentine qui sont orientés de manière oblique d'en haut, à gauche, à en bas, à droite. En bas, à droite: patron de minéralisation calcosphéritique des lignes de croissance quotidiennes proches de la surface de la racine sur la coupe histologique d'une molaire d'un hominine fossile, vu en lumière transmise non-polarisée.

Fig. 6. Measurements of daily dentine increments at 0-100 µm and at 100-200 µm from the granular layer of Tomes (GLT). The average taken across 5 days was possible N times. Or = *Oreopithecus*; Hi = *Hispanopithecus*; Pa = *Pan*; Go = *Gorilla*; P = *Paranthropus boisei*; He = *Homo erectus*; Hs = modern *Homo sapiens*. There is a

gradient of increasing rate in all taxa over 200 µm of dentine formation. Average rates are slower in *Oreopithecus, Hispanopithecus* and *Pan* but slightly faster in *P. boisei, H. erectus* and *H. sapiens*; *Gorilla* stands out as having markedly faster rates within the 100-200 µm zone.

Fig. 6. Mesures des incréments quotidiens de la dentine à 0-100 µm et à 100-200 µm de la couche granulaire de Tomes (GLT). La moyenne estimée sur 5 jours était possible N fois. Or = *Oreopithecus*; Hi = *Hispanopithecus*; Pa = *Pan*; Go = *Gorilla*; P = *Paranthropus boisei*; He = *Homo erectus*; Hs = *Homo sapiens* actuel. Il y a un gradient de croissance chez tous les taxons au-delà de 200 µm de formation de dentine. Les taux moyens sont plus lents chez *Oreopithecus*, *Hispanopithecus* et *Pan* mais légèrement plus rapides chez *P. boisei*, *H. erectus* et *H. sapiens*; *Gorilla* se distingue par des taux bien plus rapides dans la zone des 100-200 µm.

Fig. 7. Incremental markings in enamel and dentine showing the transition in orientation from fast growing deciduous teeth (left) and slower growing permanent teeth (right). The teeth are truncated as if root growth was incomplete. The angle subtended by the incremental marking to the root surface reflects the orientation of the former mineralising front during root formation (after fig. 7.24, Aiello and Dean, 1990).

Fig. 7. Marqueurs incrémentaux de l'émail et de la dentine montrant la transition dans l'orientation entre les dents déciduales qui ont une croissance rapide (à gauche) et les dents permanentes qui ont une croissance plus lente (à droite). Les dents sont tronquées de la même manière que si la croissance de la racine était incomplète. L'angle entre chaque marqueur incrémental et la surface de la racine reflète l'orientation des anciens fronts de minéralisation durant la croissance de la racine (d'après la fig. 7.24, Aiello and Dean, 1990).

Fig. 8. Longitudinal ground section of a fossil *Oreopithecus* M2 root in transmitted polarised light (specimen IGF4883V from Baccinello, Tuscany, Italy). A bright thin layer of cementum covers the root surface on the left. The prominent small arcade-like structures with their domed aspect directed towards the root surface are calcospherites. Dentine tubules sweep from left to right towards the pulp. Incremental markings that define previous positions of the forming dentine surface run obliquely from top right to bottom left. Thick blue lines are 200 µm long and run parallel with

dentine tubules. Thin blue arrows pass back to the cement dentine junction along incremental markings.

Fig. 8. Section histologique longitudinale d'une racine fossile de M2 d'*Oreopithecus* en lumière transmise polarisée (spécimen IGF4883V de Baccinello, Toscane, Italie). Une fine couche de cément brillant recouvre la surface de la racine sur la gauche. Les petites structures marquées en formes d'arcades, avec leur aspect bombé dirigé vers la surface de la racine sont des calcosphérites. Les tubules de dentine sont orientés de gauche à droite vers la pulpe. Les marqueurs incrémentaux qui soulignent la position précédente de la surface de dentine en formation sont orientés de manière oblique, d'en haut, à droite vers le bas, à gauche.

Fig. 9. Distance curves (solid lines) of fossil primate tooth root growth overlain with velocity plots (first derivative of the distance curves) shown as broken dashed lines. Rates of root formation rise to a peak and then decline. The age at peak velocity (avp) is shown above each plot and indicated with a vertical dotted line. Velocity (in μm / year) is shown on the right Y axis and length along the CDJ of the root (in μm) on the left Y axis (from Dean and Cole, 2013).

Fig. 9. Courbes de distance (lignes continues) de la croissance de la racine dentaire de primates fossiles superposées avec les courbes de vélocité (première dérivée des courbes de distance) illustrées par des lignes pointillées. Les taux de formation de la racine croissent pour former un pic puis déclinent. L'âge au pic de vélocité (avp) est montré au-dessus de chaque graphique et indiqué avec une ligne verticale en pointillés. La vélocité (en μm / année) est montrée sur l'axe Y de droite et la longueur le long de la CDJ de la racine (en μm) est indiquée sur l'axe Y de gauche (d'après Dean et Cole, 2013).