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Synchrotron radiation-based X-ray tomography reveals life history in primate cementum incrementation

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Cementum is a mineralized dental tissue common to mammals that grows throughout life, following a seasonally appositional rhythm. Each year, one thick translucent increment and one thin opaque increment is deposited, offering a near-complete record of an animal's life history. Male and female mammals exhibit significant differences in oral health, due to the contrasting effects of female versus male sex hormones. Oestrogen and progesterone have a range of negative effects on oral health that extends to the periodontium and cementum growth interface. Here, we use synchrotron radiation-based X-ray tomography to image the cementum of a sample of rhesus macaque (*Macaca mulatta*) teeth from individuals of known life history. We found that increased breeding history in females corresponds with increased increment tortuosity and less organized cementum structure, when compared to male and juvenile cementum. We quantified structural differences by measuring the greyscale 'texture' of cementum and comparing results using principal components analysis. Adult females and males occupy discrete regions of texture space with no overlap. Females with known pregnancy records also have significantly different cementum when compared with non-breeding and juvenile females. We conclude that several aspects of cementum structure and texture may reflect differences in sexual life history in primates.

1. Introduction

Cementum, the connective tissue that anchors tooth roots to the periodontal ligament (PDL), is unique among mammalian dental tissues, as its growth is continuous throughout life, recorded by a series of circum-annual growth increments [1]. Every year of life is recorded by one wide translucent increment and one narrow opaque increment, when viewed under transmitted light in thin-section histology [2]. Unfavourable seasons are thought to promote a reduction in the deposition rate of the cementum hydroxyapatite matrix, while the mineralization rate is unaffected, producing ultra-mineralization of narrow cementum increments during these seasons [3,4], though there is still uncertainty as to whether narrow or wide increments are more mineralized [2,5].

As the only mineralized dental tissue to grow throughout life without remodelling (apart from instances of severe gingival damage; figure 1a) [4,9], cementum is likely to be affected by several aspects of life history. As cementum receives collagenous 'Sharpey's fibres' from the PDL, its growth is also affected

Figure 1. Exploring the relationship between hormonal physiology and cementum increment tortuosity and cementum texture in propagation-based phase-contrast imaging (PPCI) CT data of *Macaca mulatta* cementum. (a) Flow chart describing potential negative effects of major female sexual hormones on oral health (green boxes) and their causes (orange boxes) [6–8]. (b, c), PPCI-CT slices of male (individual k39) (b) and female (individual I56) (c) tooth roots. Insets in b–c are straightened and isolated reconstructions of cementum following the cemento-dentine boundary (red bracketed lines), with individual increments highlighted (red arrows). (d–e) Heatmaps of greyscale values for straightened male (d) and female (e) cementum. (f–g) Outlines of outermost increment in straightened cementum following dashed red lines for male (f) and female (g). (h–i) Relationship between increment length (red line) and shortest possible length (dashed white line) in male (h) and female cementum (i).

by periodontal health and disease [9]. It is widely accepted in the clinical literature that females encounter higher rates of periodontal and dental disease than males, and studies on laboratory animal populations show that instances of dental disease increase proportionally with increasing levels of the female sex hormones oestrogen and progesterone (figure 1*a*) [6–8]. Conversely, increases in androgen levels (male sex hormones) have no significant effect on oral health [8]. The negative impacts of oestrogen on oral health have also been shown to fluctuate through the menstrual cycle [8] and accelerate during puberty and pregnancy. Damage to the gingiva related to oestrogen and progesterone extends to the PDL and its connective tissues, suppressing collagen production and breaking down collagen fibres [6] (figure 1*a*).

A major constraint of cementum studies is the restrictive nature of thin-section imaging, which provides a limited number of two-dimensional (2D) viewpoints of the tissue, but synchrotron radiation-based computed tomography (SRCT) applications have overcome this caveat [4,10]. A preliminary report [11] attempted to use SRCT to compare cementum between male and female archaeological humans, and, although a single increment of potentially abnormal shape/structure was identified in the cementum of one female, taphonomic alteration and low image quality meant the data were insufficient to confidently identify it as a stress marker linked to a pregnancy event. Several earlier, non-SRCT studies, have identified dimorphic characteristics of cementum between sexually mature male and female cementum in a range of mammals [12–14]. These studies, however, are unable to provide predictive means of identifying sex in individuals of unknown life history.

To address these challenges, we present the results of a preliminary experiment employing propagation-based phase-contrast imaging (PPCI) through SRCT (herein PPCI-CT) to characterize the cementum of 14 rhesus macaque (*Macaca mulatta*) individuals of known life history and sex. We subject PPCI-CT data to a novel image processing and analysis protocol to measure the shape of increments, and the texture of cementum for the first time, to compare these properties between individuals and test for significant differences between sexes.

2. Methods

2.1. *Macaca mulatta* sample

Lower right first molar teeth (m1) were extracted from a sample of deceased macaques with monitored life history (see electronic supplementary material, for further detail). The sample comprised nine sexually active females, three sexually inactive females, one sexually mature male and one juvenile female (table 1). The crown of each tooth was removed, and anterior/posterior roots were mechanically separated and glued to carbon fibre rods (2 mm diameter) for PPCI-CT imaging.

2.2. Image acquisition, processing and analysis

PPCI-CT data were collected from the TOMCAT beamline of the Swiss Light Source (Paul Scherrer Institute; Villigen, Switzerland) (see electronic supplementary material). The axial third of each root was imaged at an isotropic voxel size of 650 nm, with 20 keV X-ray energy of 20 keV, integration time of 150 ms for 1500 angular projections, at 14 mm sample-to-detector distance. X-ray projection phase was retrieved using the Paganin single-distance phase retrieval algorithm, implemented in-house at TOMCAT, using $\beta = 3.7 \times 10^{-8}$ for the imaginary part, and $\delta =$

1.7×10^{-10} for the decrement of the real part of the refractive index [15] (figure 1*b,c*). Phase-retrieved projections were reconstructed using an in-house implementation of the Gridrec algorithm [16] at TOMCAT.

We present here a novel protocol for standardized analysis and comparison of discrete properties of cementum that directly relate to its growth through life (see electronic supplementary material). For each individual, PPCI-CT cementum data were selected from 30 random CT slices. These slices were investigated for the predominance of cellular voids. If greater than 10 voids were found in a slice, they were deemed evidence of cellular cementum and the respective slice was replaced with another randomly selected PPCI-CT slice, so that only circum-annually deposited acellular cementum was compared [1]. Cementum in the selected data was straightened and isolated with the 'straighten' function in ImageJ/Fiji (Version 2.0) [17] (see electronic supplementary material), using the cemento-dentine junction as a reference for the straightening direction (figure 1*b–g*). Straightened data were then subjected to two custom-designed algorithms in Matlab (R2016a; The MathWorks, Inc., Natick, MA, USA) that assessed cementum texture (figure 1*d,e*) and increment shape (figure 1*f–i*), respectively.

Increment shape was defined as the tortuosity of an increment along its 2D path length along with straightened PPCI-CT slices. Tortuosity is here defined as the ratio between the path length followed by an increment and the shortest possible path length (figure 1*f,g*). The outermost five increments in each randomly selected PPCI-CT slice were isolated (see electronic supplementary material), and their path length was calculated as their *x*-axis distance (in pixels), plus the change in *y*-axis values between every concurrent pixel along the *x*-axis (figure 1*f–i*). Shortest path lengths were calculated as the hypotenuse between the *x/y*-axis coordinates at the endpoints of the increment (figure 1*h,i*). Non-parametric Kruskal–Wallis analysis was used to compare tortuosity values between individuals and subsamples.

Cementum 'texture' was defined as the spatial organization of greyscale values within the PPCI-CT cementum data. By measuring greyscale as a 'third dimension/z-axis', several metrics commonly used in biological surface profiling were applied to our data (figure 1*d,e* and table 2) [18]. These comprised height measures, spatial measures, hybrid measures and functional measures (see electronic supplementary material and references therein for definition different measure types). Twenty measures were derived from each PPCI-CT slice which had previously been used to estimate increment tortuosity, each measure was compared between two subsamples (male/adult female) using ANOVA (table 2), and measures with significant differences ($p < 0.05$) between subsamples were subjected to principal components analysis (PCA).

3. Results

Qualitative comparison between the cementum increments of male and female *Macaca mulatta* individuals suggests several discrete differences between their texture and shape. Male cementum increments follow relatively uniform (low-tortuosity) transects through the tissue and are of roughly equal thickness (figure 1*b*). Female increments are considerably more chaotic and follow conflicting, tortuous transects through the cementum, producing complex patterns and increment splitting/coalescence (figure 1*c*). These effects are compounded through the cementum, often resulting in increased tortuosity in the outermost increment. Increments in samples from female individuals also qualitatively show considerably lower image contrast than male increments

Table 2. Cementum texture measure descriptions and results of ANOVA comparisons and PCA correlations between measurements for the adult female subsample (breeding and non-breeding) and adult male individual. See Supplement for description and explanation of measures.

measure	category	description	F value	P value	PC1 correlation value (−1 to 1)	PC2 correlation value (−1 to 1)
1. absolute average height of surface	height	average greyscale value	10.7	<0.001	0.82	−0.29
2. relative average height of surface	height	average difference between greyscale values above average	15.6	<0.001	0.84	−0.28
3. root mean square height of surface	height	root mean square (RMS) greyscale value	9.6	<0.001	0.64	0.60
4. maximum peak height of surface	height	value of maximum greyscale peak above average	49.4	<0.001	0.65	0.23
5. average maximum peak height	height	average value of 10 highest greyscale peaks above average	53.5	<0.001	0.72	0.30
6. minimum valley depth	height	value of lowest greyscale trough found below the average greyscale value	12.9	<0.001	0.54	0.46
7. average minimum valley depth	height	average value of 10 lowest greyscale troughs found below the average greyscale value	51.6	<0.001	−0.64	0.56
8. average height	height	average height of greyscale peaks	0.3	0.57		
9. ten-point average height	height	ten-point moving average value of 10 greyscale peaks	0.3	0.57		
10. maximum skew	Hybrid	degree of symmetry of the surface heights about the mean plane	10.8	0.002	−0.78	0.62
11. summit density	hybrid	number of greyscale peaks per unit area	149.7	<0.001	0.16	0.73
12. maximum kurtosis	hybrid	prominence of inordinately high peaks and/or deep valleys	2.3	0.121		
13. root mean square gradient of surface	hybrid	root mean square (RMS) of greyscale slopes, evaluated over all directions	9.6	0.002	0.47	0.26
14. developed interfacial area ratio	hybrid	percentage of additional surface area contributed by the texture as compared to an ideal plane the size of the measurement's region	20.9	<0.001	0.90	−0.09
15. core roughness depth	functional	proportion of dataset occupied by the core	7.854	0.006	0.33	0.58
16. average peak height above core	functional	average difference in greyscale between peaks above the maximum core greyscale value versus the maximum core greyscale value	14.89	<0.001	0.93	−0.09
17. average valley depth below core	functional	average difference in greyscale between troughs below the minimum core greyscale value, and the minimum core greyscale value	7.842	0.009	0.39	−0.42

(Continued.)

Table 2. (Continued.)

measure	category	description	F value	P value	PC1 correlation value (−1 to 1)	PC2 correlation value (−1 to 1)
18. surface bearing area ratio	functional	proportion of the surface that consists of peaks above the core greyscale value	12.3	<0.001	−0.30	−0.47
19. autocorrelation length	spatial	horizontal distance of the autocorrelation function (ACF) that has the fastest decay to the value 0.2. Large value: surface dominated by low frequencies; low value: surface dominated by high frequencies	34.6	<0.001	0.18	0.02
20. texture aspect ratio	spatial	ratio between the distance with the fastest to the distance with the slowest decay of the autocorrelation function (ACF)	9.8	0.002	0.82	−0.06

and are more poorly organized with higher isotropy than male increments.

Following cementum isolation and straightening, quantitative analysis supports the qualitative inferences of higher increment tortuosity within samples from female individuals (figure 2a). Every female individual produced significantly higher values of increment tortuosity than the male individual (table 1). Samples from breeding adult females also had significantly higher tortuosity than from non-breeding adult females ($H = 438.4$; $p < 0.001$) and the latter in-turn produced significantly higher tortuosity than the juvenile female individual ($H = 18.2$; $p < 0.001$) (figure 2a). The female with the highest number of recorded pregnancies (female I59) also produced increments with the highest individual tortuosity, and the highest mean tortuosity was found for female I72, with four recorded pregnancies (figure 2a, table 1).

Cementum texture also showed significant differences between the adult male and female subsamples. Of the 20 assessed texture measures, 17 were significantly different when compared using ANOVA (table 2). When subjected to PCA, principal component 1 (41.2% of variance) reflected predominantly the image contrast between light/dark increments, with positive values reflecting higher image contrast. Principal component 2 (16.9% of variance) reflected principally the 'surface roughness' of cementum, with lower values corresponding to lower variability in greyscale values and less greyscale anisotropy (directional dependence) throughout the sampled PPCI-CT slice. Male cementum formed its own discrete 'texture space', with no overlap between values from male and adult female individuals (figure 2b). However, male texture space did overlap with juvenile female texture space, which in-turn overlapped with that of non-breeding females, but not breeding females. The texture space of non-breeding females was positioned between that of juvenile female and breeding female cementum. The cementum of I72, the individual with the highest mean increment tortuosity, also produced the most highly differentiating texture measure when compared to the male individual (figure 2b).

4. Discussion

We here demonstrate a significant relationship between sexual life history and patterns in cementum incrementation in a dataset of *Macaca mulatta* individuals. We interpret these differences in tortuosity and texture as reflecting sexual differences in the growth and maintenance of cementum due to distinct hormonal regimes, with an increasing influence of oestrogen and progesterone creating different conditions for cementum growth in females. Pregnancy is known to promote a significant increase in oestrogen and progesterone, as is the onset of sexual maturity. Hence, our hypothesis is further supported by the findings of significant differences between the tortuosity of increments found in breeding versus non-breeding females, and adult females versus the juvenile female. This pattern correlates with the occupation of texture space, with breeding females occupying the furthest texture space from male cementum, bridged by cementum signals from non-breeding and then juvenile cementum.

Our novel methods for image processing, analysis and validation have the potential to elucidate quantitative differences in cementum organization between/within populations and offer a new way to relate cementum growth to a range of life-history variables and events. If the sexual dimorphism we identify here

Figure 2. Cementum increment tortuosity and texture results. (a) Boxplots of tortuosity measurements for each *Macaca mulatta* individual. Red boxes: breeding adult females; orange boxes: non-breeding adult female; purple box: juvenile female; blue box: adult male. Thick black lines represent mean values, upper and lower box bounds represent upper and lower quartile ranges. (b) Results of principal components analysis of 17 texture measures for cementum of each *M. mulatta* individual. (c) Correlation plot of texture metrics along PC1. (d) Correlation plot of texture metrics along PC2.

is retained in larger samples of known life history, these methods, in combination with increment counting using non-destructive SRCT imaging, have the potential for estimating life-history data for individuals and populations of unknown provenance, including archaeological, palaeontological and forensic samples.

Data accessibility. Data supporting this study are openly available from the University of Southampton repository (<https://doi.org/10.5258/SOTON/D1467>).

Authors' contributions. E.N. secured and prepared all specimens and designed, validated and performed all automated analyses. All authors were involved in writing the proposal for synchrotron

beamtime and the synchrotron beamtime. All authors contributed to writing the manuscript.

Competing interests. The authors report no conflicts of interest.

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