Evolution of Human Enamel Growth Analyzed by Confocal Microscopy

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INTRODUCTION

Tooth formation is the end result of a long developmental process, which results from interactions between oral epithelium and cranial neural crest-derived ectomesenchyme (Kollar, 1983; Ruch, 1984; Thesleff and Hurmerinta, 1981). The timing, rate and duration of these events vary along the tooth row and are attributed to the activity of numerous genes encoding growth factors and structural molecules. These activities are associated with the pattern of tooth development taking place in a staggered fashion along the dental arch, during a limited period of time (MacKenzie et al., 1992; 1991; Smith and Hall, 1990; Snead et al., 1988; Wagner, 1989; Weiss, 1993). However, little is known about how the timing and rate of dental development relate to final tooth size and form (Butler, 1956; Jernvall et al., 1994; Slavkin, 1990).

A hierarchical change in the timing and expression of regulatory genes can be postulated to account for the observed differences in homologous teeth on an evolutionary timescale. Over the course of human evolution, from ancient to modern times, there has been a decrease in molar tooth size. These size changes have been associated with a change in cusp pattern (Dahlberg, 1961; Smith, 1978, 1982, 1988; Wood, 1981; Wood and Abbott, 1983; Wood et al., 1983). The main change observed is a relative decrease in size of the distal portion of the crown (Dahlberg, 1945, 1961).

Enamel has two important properties, durability and structural complexity. Because of its durability teeth are preferentially preserved in the fossil record and its structural complexity reflects its development. To examine the relationship between tooth formation and changes in final tooth size we have analyzed the microstructural organization of mature molar enamel and particularly its arrangement at the occlusal surfaces of distinct regions. Confocal laser scanning microscopy (CLSM) was the method of choice to study mature mineralized teeth because it allows a non-destructive analysis.

MATERIALS AND METHODS

CLSM images were taken in modern and fossil teeth of hominids from Israel. On each CLSM image, three parameters of dental structure were studied at different locations and these were 1) shape, 2) size, and 3) density of the enamel prisms.
Skeletal remains found in archaeological excavations in Israel that date from 6000-200 B.P. were used as representative of *Homo sapiens* samples and the remains of Qafzeh, dated to circa 90,000 B.P represented early *Homo*.

This study was carried out on 21 lower permanent molars, of which 18 were modern teeth and 3 were fossil teeth. Two modern teeth were excluded from this group because prisms were not visible in any cusp. None of the teeth had any observable wear facets, caries, cracks or other post-mortem damage. The developmental stage of each tooth was assessed both metrically and non-metrically (Smith et al., 1995). The mesiodistal (MD) and buccolingual (BL) crown widths as well as the mesiobuccal crown height were measured using a Digimatic needle-point sliding caliper (Mitutoyo) with a resolution of 0.01 mm and the product of the first two diameters was used as an index of crown size (Haydenblit, 1996). Measurements were taken according to the definitions described by Moorees (1957).

CLSM images were taken through cusp tips of the protocone (mesio-buccal cusp) and hypoconulid (distal cusp) of the first permanent lower molars. Confocal optical sections (images perpendicular to the surface) were acquired from successively deeper sections in steps of 1µm. The above parameters were determined for the deep, intermediate, and superficial enamel of the cusp tips. The recording of the deep enamel region was limited until the image lacked sufficient contrast to record it, the superficial enamel level was examined at an area at the outer margin of the cusp, and the intermediate depth was defined as an area midway between the superficial and deep optical sections. To insure uniform magnification among the confocal images all enlargement factors (i.e. magnifying lenses in the microscope and zoom in the computer) were held constant. All the images were recorded using a Bio Rad MRC1024 confocal laser microscope using the 488nm line of a Kr/Ar laser, an Eclipse E800 Nikon microscope and a 60X/NA=1.4 Nikon Plan Apo objective (W.D. 0.21 mm). All the confocal images were obtained by a reflection mode. The principle of the confocal microscope is based on reflected light microscopy that optically scans a specimen by sending and receiving light through pinholes of variable apertures (Pawley, 1995). Thus, it is possible to focus on a chosen plane in a thick specimen while rejecting the light that comes from out-of-focus regions above and below that plane. The CLSM images were obtained directly from archaeological teeth. Each tooth was placed on a slide with plasticine and the tip of the cusp was placed directly perpendicular under the lens with immersion oil and a coverslip. A second drop of oil was placed between the coverslip and the objective lens.

Measurements of prism spacing followed the methods developed by Fosse (Fosse, 1968a-e). Briefly stated, up to 10 of each of four linear and one area measurements were collected randomly from each image. Three linear measurements (x, y, and d) were used to calculate the average ameloblastic cross-sectional secretory area (ASA), and the estimated prism density (EPD). Prism diameter (PD) and prism area (PA) data were collected to provide an estimate of prism size. To obtain an estimate of the prismatic matrix (PM), prism area was compared with the average ameloblastic area for the same region (PA/ASA x 100) (see Grine et al., 1987) for a detailed description of the formulae for these calculations). All measurements were analyzed with Scion image (a modified version from NIH image) and Lasersharp Software. In addition, depending on the
alignment and packing of the ameloblasts, one of the three basic developmental prism pattern configurations (described by Boyde, 1965) was documented for each specific region.

Intra-observer error was assessed for both crown and enamel prism measurements. This was checked by repeated measures of 10 randomly selected teeth. The mean intra-observer measurement difference was 0.05 mm for crown measurements and 0.06 mm for enamel prism measurements. Statistical tests were performed using SPSS 8.0 for Windows 95.

RESULTS

For the recent archaeological samples the laser light was reflected from the outer surface of the tooth to about 100 to 180 µm (depending on each tooth) under the surface. Figure 1 illustrates prism shape for three different depths and shows the well-known prismatic structure of enamel. Distinction was made between prisms and interprismatic enamel. Pattern 3 prisms according to Boyde’s (1964) classification were observed in all of the enamel surveyed. No significant differences were found among superficial, intermediate and deep reflections in ASA, EPD, PA and PA/ASA x 100 in either cusp (data not shown).

Since we are interested in looking at the changes between final tooth size and enamel formation we examined:

a) Prism size and spacing on the mesio-buccal and distal cusps of fully formed teeth.

b) Whether these prism parameters differ according to tooth size.

Student’s t-test and non-parametric tests were used to examine the null hypothesis that there is no change in prism area or density between the mesio-buccal and distal cusps and that there is no correlation to tooth size.

Table 1 shows the mean values of prism parameters found in the mesio-buccal and distal cusps. Significant differences were found in ASA(P<0.01), EPD(P<0.02) and PA(P<0.02) between both cusps and no significant difference was found in PA/ASA. Mean ASA and PA on the mesio-buccal cusp were larger than those of the distal cusp. Figure 2a shows the scatter plot between ASA against the crown area on both cusps. ASA on the distal cusp increases together with an increase in crown area. Linear regression analysis shows a significant if low correlation (R²=0.41) between ASA and crown area in the distal cusp, but none for the mesio-buccal cusp where ASA does not increase. The value of EPD decreases as the crown area increases on both cusps (data not shown). This result was expected since ASA and EPD are intimately related. PA on both cusps increases as crown area increases (Fig. 2b). Linear regression analysis shows a significant if low correlation (R²=0.20) for the distal cusp but there is no correlation between PA and crown area for the mesio-buccal cusp. To summarize, there is an increase in ASA and PA as the crown area increases, and both prism parameters, ASA and PA appear to be larger for the MB cusp than the D cusp.
Fig. 1. Confocal images of enamel (parallel to the surface) from the mesio-buccal cusp of the lower first permanent molar of modern teeth illustrating prism packing arrangement. a: Superficial (depth 30 μm). b: Intermediate (depth 60 μm). c: Deep (depth 100 μm) enamel. The optical section of the enamel surface reveals the orientation of the keyhole structures. The CLSM image shows the intensity of light reflected or scattered from an in-focus plane. The dark appearing prismatic areas of the enamel structure allow the laser light to pass without reflection. Scale = 10 μm.

Table 1. Comparison between ASA (ameloblast secretory area), EPD (estimated prism density), PA (prism area) and PA/ASA x 100.

<table>
<thead>
<tr>
<th>Cusp</th>
<th>ASA (μm²)</th>
<th>EPD(# per μm²)</th>
<th>PA (μm²)</th>
<th>PA/ASA x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Mesio-buccal (n=18)</td>
<td>42.80</td>
<td>11.04</td>
<td>67.68-26.47</td>
<td>23805</td>
</tr>
<tr>
<td>Distal (n=18)</td>
<td>37.00</td>
<td>6.58</td>
<td>46.41-20.72</td>
<td>29331</td>
</tr>
</tbody>
</table>

See text for explanation on prism parameters. t-test, P<0.02. n=number of teeth. Means are based on the average of the superficial, intermediate and deep reflection images.

To further study if the prism parameters differ according to tooth size and/or cusp type, the relationship between tooth size and enamel prism spacing was examined. We have taken the value of 111 mm² (crown area, see Figure 2) as the cut-off point for dividing small and large teeth. There was a significant difference between small and large teeth on the distal cusp for ASA(P<0.03), EPD(P<0.01) and PA(P<0.01). On the mesio-buccal cusp PA(P<0.03) and PA/ASA X 100 (P<0.03) (Figure 3). Our results suggest that large teeth have larger PA and ASA than small teeth.

To study enamel microstructure in fossil homininid teeth from Israel, confocal images were obtained from the mesio-buccal and distal cusps of teeth from Qafzeh. Figure 4 illustrates the appearance and quality of the confocal images obtained from the specimens of Qafzeh. From the outer surface of the enamel tooth to about 100 μm under the surface,
dental enamel was recorded by the CLSM as a horse-shoe microstructure. The interior of the prisms appears dark and the surrounding interprismatic margins are brighter due to the reflection of the laser light on the mineralized tissue as seen in Figure 1.

Fig. 2. Plot of correlations between prism parameters and crown area for the mesio-buccal (MB) and distal (D) cusps. a: Ameloblast secretory area (ASA) and crown area. b: Prism area (PA) and crown area. Note tooth size: small teeth include all teeth in which crown area is below 111 mm² and large teeth are above this value. Means are based on the average of superficial, intermediate and deep reflection images.
Fig. 3. Histogram showing the differences in ASA and PA of the mesio-buccal and distal cusps for large and small teeth. Means are based on the average of superficial, intermediate and deep reflection images. Small teeth, n=8, large teeth, n=10.

Fig. 4. CLSM image of enamel (superficial depth) from the distal cusp of a mandibular first permanent molar from Qafzeh (depth 30μm). Scale=10 μm.

DISCUSSION

Mature enamel has a complex three-dimensional structure (Hillson, 1986; Osborn, 1981). The enamel prism packing patterns reflect the past history of the position and movements of ameloblasts providing information about growth patterns in tooth development (Boyde, 1990) and on the relationships of fossil species to one another and to living forms (Boyde, 1989). Thus, the development of teeth is permanently recorded in their microstructure. This research, used as an exploratory study to analyze growth patterns of enamel, illustrates the potential of the non-invasive technique of confocal laser scanning microscopy as a means of examining the microstructure of recent and fossil human teeth.

The present study sample comprises 18 modern teeth. Enamel prisms were not visible in two teeth and this fact probably depends on the state of preservation of the teeth
sampled. Furthermore, there is a big variation on the color of the crown of teeth sampled (from white to brown). Although no relation may exist between the external gross morphology and preservation of archaeological bone (Bell and Jones, 1991) there may be a correlation between external morphology, tooth microstructure and the ability of acquiring confocal images from teeth. Analysis on the relationship between the state of preservation of teeth and quality of images need to be further investigated.

Our working hypothesis is that the microstructure of enamel in modern teeth determines the macrostructure (size and form) of the mineralized mature teeth. We found that the mesio-buccal cusp contains prisms at a lower density and of larger size than the prisms on the distal cusp. Based on our results we propose that the decrease in tooth size may be the result of a reduction in ameloblast secretory area and prism area.

The novel CLSM technique for investigating the microstructure of teeth opens a wide field of three-dimensional, non-destructive analysis to study evolutionary growth and developmental processes in mineralized tissues of modern and fossil hominid teeth.

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LITERATURE CITED


