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 time scale. 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 enamel 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 Moorrees (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