Morphological characteristics of primary enamel surfaces versus permanent enamel surfaces: SEM digital analysis

ABSTRACT

**Aim** The morphology of permanent and primary enamel surface merits further analysis. The objective of this study was to illustrate a method of SEM digital image processing able to quantify and discriminate between the morphological characteristics of primary and permanent tooth enamel.

**Methods** Sixteen extracted teeth, 8 primary teeth and 8 permanent teeth, kept in saline solution, were analysed. The teeth were observed under SEM. The SEM images were analysed by means of digitally processed algorithms. The two algorithms used were: Local standard deviation to measure surface roughness with the roughness index (RI); Hough’s theorem to identify linear structures with the linear structure index (LSI).

**Results** The SEM images of primary teeth enamel show smooth enamel with little areas of irregularity. No linear structures are apparent. The SEM images of permanent enamel show a not perfectly smooth surface; there are furrows and irregularities of variable depth and width.

**Conclusion** In the clinical practice a number of different situations require the removal of a thin layer of enamel. Only a good morphological knowledge of both permanent and primary tooth enamel gives the opportunity to identify and exploit the effects of rotary tools on enamel, thus allowing for a correct finishing technique.

**Keywords**: Primary and permanent enamel; SEM analysis; Digital image processing.

Introduction

A great deal of clinical evidence and data reported in the literature suggest that erupted teeth, permanent and primary, responding to the criteria of normality, constantly show superficial defects. In all the enamel surface grooves, roughness and scratches with different size and depth are present [Ballard, 1944; Mortimer, 1970; Tuverston, 1980]. The enamel surface of an erupted and functional tooth may be irregular for the structural variations due to the occlusal function or the oral environmental condition. Furthermore, the deciduous enamel seems more porous and less mineralised [Clergeau-Guerithault, 1988] with more irregular crystals due to their bigger surface and a post-eruptive growth, which also have a less defined spatial layout with slightly inferior prismatic diameters. All of the above-mentioned characteristics make the colour of the deciduous enamel more matt, and thus whiter than in permanent teeth [Clergeau-Guerithault, 1988]. The enamel surface of healthy teeth shows the typical aspect of the shape of the enamel prisms’ extremities due to the natural etching of the tooth surface. In 50% of premolars and with different percentages in the other teeth it is possible to observe numerous and little dome relieves of 50 µm diameter and 10 µm height, sometimes defined by slender furrows [Stroud et al., 1998]. SEM (Scanning Electron Microscope) digital analysis shows that these are formed by aggregated radius-oriented crystallites regularly ordered [Ballard, 1944; Peck and Peck, 1972; Shillinburg and Grace, 1973].

The present study was intended to illustrate a method of SEM digital image processing able to quantify and discriminate between the morphological characteristics of primary enamel surfaces, when compared with permanent enamel surfaces.

Methods

The present study is based on an assessment of primary (N=8, group 1) and permanent teeth (N=8, group 2), extracted from nonbruxer healthy young patients, aged 6 to 16 years, for orthodontic and surgical reasons. Teeth extractions were carefully performed in order to avoid enamel scratches. The teeth were kept in saline solution T=37° for a maximum of seven days. Tooth selection criteria excluded teeth with white spots, cavities or morphological and structural changes of the enamel.

Analysis of samples

The morphological characteristics of primary and permanent enamel were assessed in two ways: the teeth were observed under scanning electron microscope (SEM) in order to assess quality; the SEM images were digitally analysed for an objective view of the morphological characteristics of the enamel in order to assess quantity.

SEM analysis

The primary and permanent enamel samples were washed with deionized water and blow-dried. Each sample was then mounted on metal supports, dehydrated and gold-coated, (thickness: 30 nm; time: 2 min; current: 25 mA), for SEM observation (Cambridge). SEM images were evaluated using a double-blind method, in other words, the evaluator was unaware of which group an image belonged to.
Digital analysis
The observation of the images related to primary and permanent enamel shows two main morphological characteristics: various degrees of roughness; presence of furrows.

SEM images were analysed by digitally processed algorithms for objective assessment of primary and permanent enamel morphologies. The two morphological characteristics which were assessed separately were the following: roughness; presence of furrows.

The two algorithms used for this assessment were: local standard deviation to measure surface roughness with the roughness index (RI); Hough’s theorem to identify linear structures with the linear structure index (LSI).

Data were subsequently located in the plane defined by RI and LSI axes, thus identifying the surface domain specific to each type of sample. The two parameters were calculated for each sample analysed and each sample was plotted on the plane defined by RI and LSI axes.

Roughness evaluation
Roughness was evaluated by measuring the brightness variability of pixels in the image. The method consisted in measuring the average value and variance of brightness of a defined area (21x21 pixels = 2.5 micron). This approach was repeated in 20 different areas of the images. The average value of variances was used as a measure of image’s roughness (Roughness index=RI).

Furrows evaluation
A sub-image (512x512 pixels, i.e. 62 micron side), equal to one fourth of the original image, big enough to include complex and wide structures, was selected for this purpose. However, the focus of the image was not consistent across the whole image, producing artificial alterations of the structures, hence the reason for selecting a sub-image. The selection of such a sub-image allowed also speeding up calculations. To proceed with the evaluation we selected the sub-image with the highest value of standard deviation and applied Roberts’ filter. This filter highlights edge structures and provides an image where only highly contrasted pixels are shown. On the resulting image Hough’s theorem was applied in order to highlight linear structures, obtaining an angular distribution of incidence of highly contrasted pixels. Peaks of these distributions are at the angle of furrows in respect of vertical axe. The value of the peak is a measure of the importance of the furrows oriented towards the corresponding angle in increasing the roughness of the image. This value represents the identifier of linear structures if the image.

For each sample two parameters were calculated and plotted on the plane defined by the two axes RI and LSI. In this way it was possible to identify the surface domain of each type of sample.

Results

Group 1 (primary enamel)
The SEM images of the primary enamel (Group 1) show smooth enamel with little areas of irregularity (Fig. 1). Roberts’ Filter is applied to a sub-image of Figure 1 (Fig. 2). The light area indicates the high-contrast pixels. No linear structures are apparent. The angular diagram of Hough’s theorem shows no peaks and LSI values are very similar for all angles (Fig. 3).

Group 2 (permanent enamel)
The SEM images of permanent enamel (Group 2) show a not perfectly smooth surface; it is possible to observe furrows and unevenness of variable depth and width with smoothed edges along with areas of smoother enamel (Fig. 4). Roberts’ Filter is applied to a sub-image of figure 4 (Fig. 5). The angular diagram of Hough’s theorem shows some peaks with different LSI values (Fig. 6).

The two parameters, roughness index (RI) and linear
structure index (LSI), were calculated for each sample (primary enamel N=8; permanent enamel N=8).

The mean for each group were plotted on the plane defined by axes RI and LSI (Fig. 7). Group 2, permanent enamel surface, shows LSI value really similar to Group 1; on the contrary, Group 2 shows RI values higher than Group 1.

Discussion

In the clinical practice, in the fields of paedodontics, restorative dentistry, periododontics, orthodontics and gnathology, a number of different situations require the removal of a thin layer of enamel, using rotary devices and abrasive strips, both in primary and permanent dentition [Mason, 2000; McDonald, 1994; Sheridan, 1987].

FIG. 3 - Angular diagram of Hough Transform applied to figure 1 (Group 1) shows no peaks and LSI values are very similar for all angles.

FIG. 4 - SEM image (x 1000) from Group 2 showing a not totally smooth surface with furrows and irregularities with variable depth and width and rounded contour, joined to smoother areas.

FIG. 5 - Roberts' Filters applied to sub-image from Figure 4 (Group 2). The light area indicates the high contrast pixel. No linear structures are apparent.

FIG. 6 - Angular Diagram of Hough Transform applied to Figure 4 (Group 2) shows no peaks and LSI values are very similar and low for all angles.

FIG. 7 - Distribution of roughness index (RI) and Linear structure index (LSI) values of the analysed sample. The diagram shows the mean value of the two parameters for primary enamel (white round) and permanent enamel (black round).
Sequential slicing of deciduous teeth is a procedure introduced by Hotz and revived in 1990 by Van der Linden [Hotz, 1970; Van der Linden 1990]. To permit the natural alignment of permanent incisors it is possible to reduce the interproximal enamel of primary canines; to facilitate a more distal eruption of primary canines or first permanent premolars it is possible to reduce the interproximal enamel of first primary molars or second primary molars [Arman et al., 2006; Graves et al., 2004; Jost-Brinkmann et al., 1991; Kalicanin and Nikolic, 2008].

In the mixed dentition sequential stripping allows to correct a deviated midline and to solve permanent incisor crowding, thus also preventing periodontal disease or cavities. Serial extractions, that reduce the arch length and deepen the bite, can so be avoided [Sheridan, 1985; Stroud et al., 1998]. A number of other situations better respond to treatment with reduction of the enamel: improvement of a Bolton index alteration and of the intermaxillary occlusion as well; prevention and treatment of interdental gingival recession in association with periodontal treatment in adults; control of relapse after orthodontic treatment; redesign of dental morphology for aesthetic purposes (enamel cracking); interproximal reduction of mandibular arch when maxillary canines replace agenesic lateral incisors; interproximal reduction of maxillary teeth in the case of agenesis or extraction of a lower incisor [Arman et al., 2006; de Harfin, 2000; Graves et al., 2004; Jost-Brinkmann et al., 1991; Kalicanin and Nikolic, 2008; Lucchese et al. 2001; Radiansky, 1988; Reller et al. 1989; Sheridan 1987; Strang et al., 1987; Zachrisson, 1986; Zhong et al., 1999].

Previously, research studied the morphological characteristics of nontreated or treated enamel (both primary and permanent) by SEM: primary enamel morphology was evaluated after etching for different time periods and using different etching systems; morphological changes in eroded enamel tissue; diagnosis of early enamel cracks, and morphology of enamel treated with different rotary devices [Boy et al., 2004; Clark et al., 2003; Jost-Brinkmann et al., 1991; McDonald, 1994; Radiansky, 1988; Reller et al., 1989]. The SEM study showed the presence, in the deciduous enamel, of the “aprismatic layer”: according to most of the authors, this layer is originally present, while for other authors it is a structure which is acquired through post-eruptive growth. The apismatic layer does not cover the entire crown, but it is found mainly at the interproximal surfaces, and only partially on the buccal aspect of the teeth and in the occlusal sulci [Clergeau Guerthault, 1988; Haikel and Frank, 1982; Ripa et al., 1966].

The morphological characteristics of the deciduous and permanent enamel untreated and treated with rotating tools, have already been highlighted by other authors [Clark et al., 2003; Radiansky, 1988].

We too have decided to investigate the morphological characteristics of primary and permanent enamel by means of scanning electron microscopic evaluation in order to assess its quality, and by means of SEM digital image processing in order to assess its quantity. Observation of the SEM images showed the presence of two main morphological characteristics, presence of roughness and furrows; automatic algorithmic digital image analysis provide exact, objective data on enamel morphology. Our SEM digital image processing of deciduous and permanent teeth, has given us a more precise method compared to a purely subjective and morphologic analysis of the surface of the enamel. Our study differs from the previous ones because, through the method of SEM algorithm digital image processing, we can provide a quantitative measurement of roughness and streaks. In particular, the surface roughness, measured using IR, was higher in permanent enamel, while the linear structures, measured using the index of linear structures (LSI) have shown quantitatively similar results in both groups.

Conclusion

The SEM analysis and digitally-processed algorithms used in this study permit to define primary and permanent enamel morphology and gives the opportunity to identify and ascribe the effects of rotating devices and abrasive strips on the enamel, thus permitting to refine the therapeutic technique and select the most appropriate materials in the treatment of primary and permanent teeth.

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