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Digital image analysis and visualization of early caries changes in human teeth

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The primary aims were the visualization, digital image analysis and X-ray EDS microanalysis to evaluate caries lesions in human premolars, with and without resin sealants, and light and scanning electron microscopic observations of hard dental tissues before and after the use of experimental protective strontium-fluoride toothpaste. The material consisted of healthy premolars extracted for orthodontic reasons, sectioned into slices. Sections of tooth crowns were digitized with a light microscope. The area of enamel demineralization was then measured. The in situ model was developed by placing the samples on the buccal surfaces of the first molars in healthy subjects, and exposing them to the use of the experimental toothpaste for 3 or 6 months. X-ray EDS microanalysis was undertaken to examine the Ca and Sr content on the enamel surface and at 15, 30, 60 and 100 microns depth. The results showed that the toothpaste induces an increased deposition of Ca in sub-superficial layers of the damaged enamel. The role of digital imaging needs to be defined with the diagnostic problem of the patient to plan an effective prophylaxis and treatment of early caries.

Key words: *digital image analysis; EDS microanalysis; dental hard tissue restoration; enamel demineralization; fissure sealants.*

1. Introduction

Human tooth enamel is a hard tissue structure which can lose mineral substances due to unfavorable long term conditions involving low pH and the presence of carbo-

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hydrates and microorganisms which cause caries changes. To prevent demineralization of dental hard tissues, the enamel structure needs to be remineralized by supplementing fluoride, phosphorus, calcium, and other ions into the enamel apatites, for example strontium. It can be performed by application of toothpastes supplemented with cariostatic ions and/or placing fissure sealants or varnishes on the total enamel surface.

Pit and fissure sealing aims at providing a good protection against caries. To achieve the best benefit, sealants and varnishes should bond appropriately to the enamel surface. However, sealant retention depends on the sealant quality and procedures used for fissure preparation. Small fractures of sealant indicating failure or degradation of adhesion in pits, fissures and their environs were shown by scanning electron microscopy observations [4]. Penetration of sealant into fissures was reported to be significantly greater when a drying agent was used [1]. An assessment of microleakage around pit and fissure sealant with and without the use of pumice prophylaxis was recently reported [2]. Pumice cleaning prior to enamel etching removes plaque and debris from the enamel surface and improves sealant retention which reduces microleakage. These studies show applications of biomaterial science to induce remineralization of dental hard tissues through modelling and restoring enamel structures.

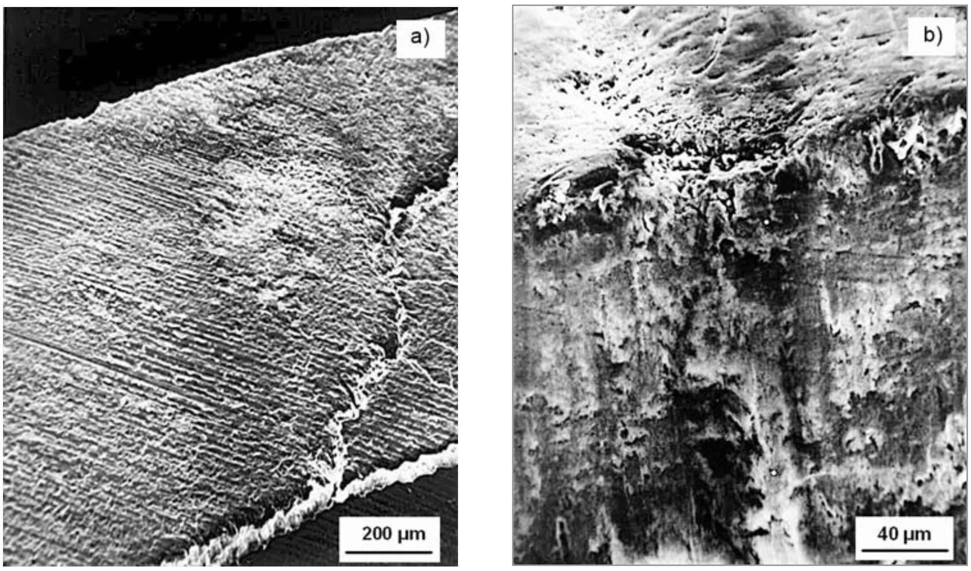

Fig. 1. SEM images of enamel lamellae, with demineralization, at 100 microns of depth (a) and at sub-superficial layer (b)

The authors' previous works were developed to quantitatively assess caries changes [5, 8, 9]. The aim of this in vitro study was the use of digital image analysis and X-ray EDS microanalysis for evaluation of quantitative differences between caries lesions in human premolars with and without resin sealants before and after the use of experimental strontium-fluoride toothpaste protecting hard dental tissues observed in light and scanning electron microscopy (Fig. 1).

2. Experimental

The material of the in vitro study consisted of seventy cavity free first premolars extracted for orthodontic reasons from twenty healthy young men and women of 16–19 years of age. Thirty teeth had fissures protected with sealant a year before extraction and formed the "fissure sealant premolar" group (Fig. 2). The remaining teeth established the "premolar" group (Fig. 3).

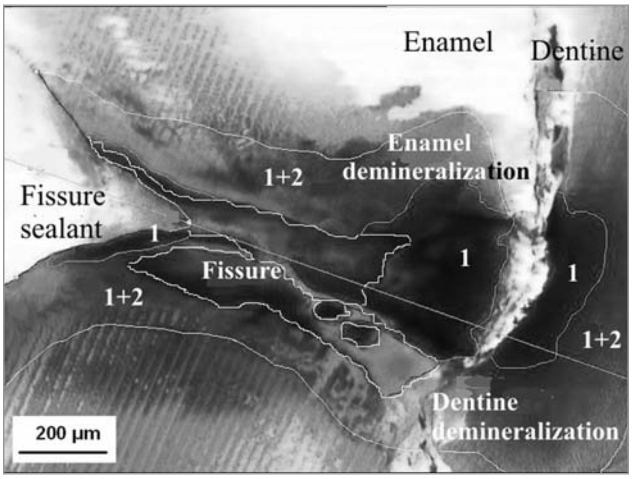

Fig. 2. Caries lesions in a fissure sealant premolar – a light micrograph

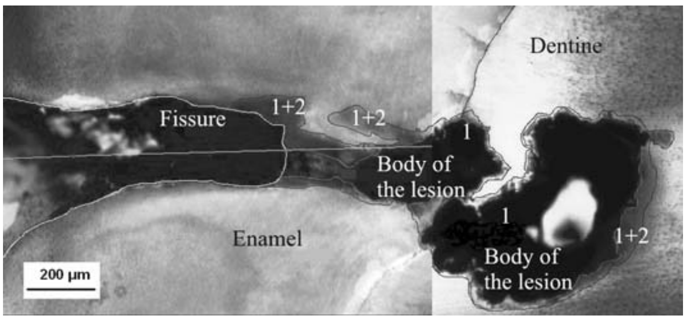

Fig. 3. Caries lesions in an unprotected premolar

Crowns of teeth were sectioned along the vertical axis of tooth using a low speed Buehler diamond disc, 0.1 mm thick, cooled with distilled water to obtain four sections each approximately 2 mm thick. Selected sections were smoothed out on both sides to remove the smeared layer. Next, the sections were Mallory stained and digitized with a computer-assisted Nikon Optiphot-2 light microscope, running with the MicroImage v 4.0 for Windows image analysis system. Dark areas representing the main demineralization of enamel were segmented by colour sampling and thresholding in HSB colour space. Brightness of pixels was introduced as the third dimension expanding 2D images into 3D space. The scenery behind objects of interest was reduced to a background by sets of threshold levels for hue, brightness and saturation of colours. The orthogonal projection of spatial objects of interest (Fig. 4) allowed us to determine their planar area [6].

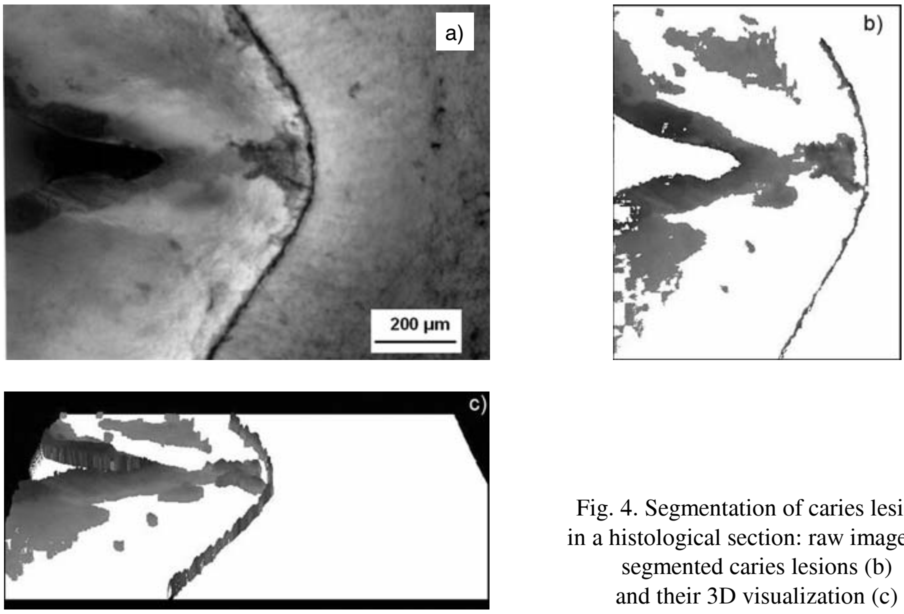

Fig. 4. Segmentation of caries lesions in a histological section: raw image (a), segmented caries lesions (b) and their 3D visualization (c)

Smooth smears surrounding the main demineralized areas of enamel and/or located around the fissure were also segmented in the same way. These areas, together with the main demineralized area, represented the area of total demineralization of enamel (Figs. 2, 3). The area of segmented main and total demineralization was then determined.

The in situ model was developed for twelve volunteers selected from the same group of young people. The remaining samples of 4 mm^2, prepared from selected sections were placed on the buccal surfaces of the first molars in the same subjects, and exposed for 3 or 6 months to use of the experimental strontium-fluoride (F^- and Sr^{2+}) toothpaste. The quantitative X-ray EDS microanalysis was performed to examine the Ca and Sr content on the enamel surface and layers at 15, 30, 60 and 100 microns deep (Figs. 5, 6).

The collected results were subjects of statistical analysis by using the Mann –Whitney test.

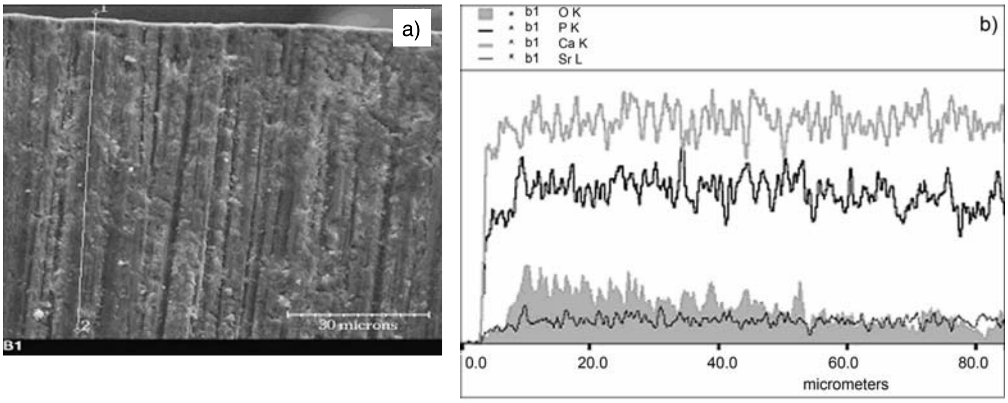

Fig. 5. A SEM image of enamel lateral wall after demineralization (a) and spectra of the examined elements on the enamel lateral wall (b)

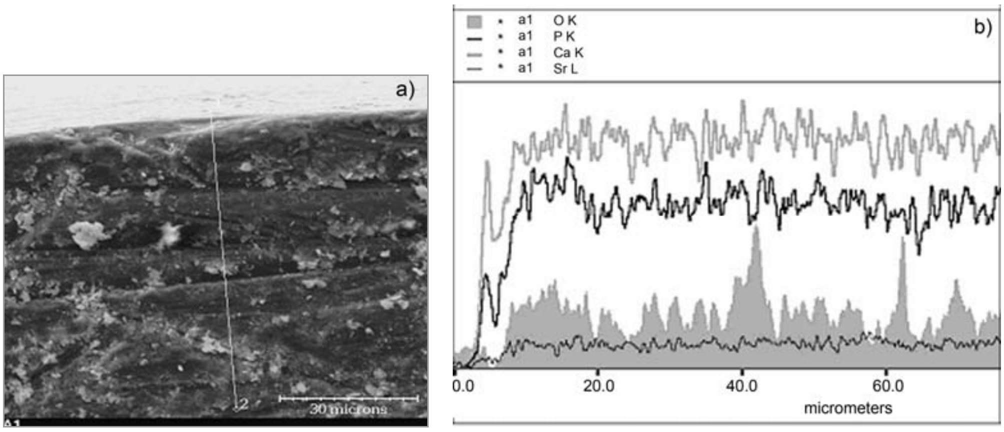

Fig. 6. A SEM image of enamel lateral wall after 3-months application of strontium-fluoride toothpaste (a) and spectra of the examined elements on enamel lateral wall (b)

3. Results

In the light microscopy images, the area of total demineralization was significantly greater in fissure sealant premolars than in unprotected premolars ($p < 0.05$), whereas the area of the main demineralization was not significantly different between the groups of teeth compared (Table 1).

The results of studies on the in situ model showed that the experimental strontium-fluoride toothpaste induces calcium deposition in sub-superficial layers of the demineralized enamel, thus inducing the recovery of its injury (Tables 2–4). The toothpaste significantly promotes the remineralization of deeper layers of damaged enamel.

Table 1. Evaluation of enamel demineralization in light microscopy observations

Quantitative assessment of enamel demineralization		Mean ± std. dev.	Significance level p
Area of the main demineralization (mm^2)	Premolars Fissure sealant premolars	0.09 ± 0.21 0.04 ± 0.02	non significant
Area of the total demineralization (mm^2)	Premolars Fissure sealant premolars	0.27 ± 0.35 0.29 ± 0.15	$p < 0.05$

Table 2. Enamel surface microanalysis of Ca and Sr at baseline and after 3 and 6 months of the experiment

Trace element (wet %)	At baseline	After 3 months	After 6 months
Ca	20.80 ± 3.84	21.36 ± 4.16	22.11 ± 2.05
Sr	0.71 ± 0.11	0.68 ± 0.08	0.72 ± 0.02

Table 3. Enamel lateral wall microanalysis of calcium at baseline, after 3 and 6 months of the experiment

Enamel depth (μm)	Ca (wet %) at baseline	After 3 months	After 6 months
15	15.70 ± 0.22	28.23 ± 0.38[3]	28.22 ± 2.49[1]
30	15.55 ± 0.25	28.54 ± 0.38[3]	28.50 ± 1.55[2]
60	15.76 ± 0.32	28.57 ± 0.48	29.04 ± 0.37
100	15.45 ± 0.31	28.68 ± 0.32[2]	29.65 ± 2.09[1]

[1] $p < 0.05$, [2] $p < 0.01$, [3] $p < 0.0001$ (the results after 3 and 6 months compared with the results at baseline).

Table 4. Enamel lateral wall microanalysis of strontium at baseline, after 3 and 6 months of the experiment

Enamel depth (μm)	Sr (wet %) at baseline	After 3 months	After 6 months
15	0.02 ± 0.01	0.44 ± 0.55	0.02
30	0.00	0.48 ± 0.19	0.16 ± 0.22
60	0.00	0.06 ± 0.05[1]	0.56 ± 0.26[1]
100	0.00	0.00	1.07 ± 0.21

[1] $p < 0.01$ (the results at 60 μm significantly different from the results at 15 and 30 μm).

4. Discussion and conclusions

Image analysis of histological sections of human premolars showed that caries changes can be present in teeth protected with fissure sealants [2, 5]. To prevent lesions progressing, teeth should be appropriately diagnosed and prepared before introducing the sealant. The quality of sealant and its placement can be assessed in in vitro studies by examining microcracks or a range of microleakage (if appeared) in light and/or scanning electron microscopy. The results presented in this paper, showed the usefulness of digital image analysis for this kind of data. SEM topographic analysis of the enamel surface revealed smoothing probably caused by mineral compound deposition and associated with caries incipience [9]. Early caries lesions are reversible and may heal. To induce enamel remineralization, sealants, varnishes, toothpastes and other agents of oral hygiene should be supplemented with the most efficient cariostatic ions. Fluoride has substantial benefits in the prevention of tooth decay, depending on the level and source of exposure. Fluorides also have adverse effects on human tissue [3]. The most common adverse effect of excess exposure to fluoride is dental fluorosis, a permanent hypomineralization of enamel, which shows unfavorable modelling of the structure. The role of trace elements with cariestatic potential, other than fluoride, in caries prophylaxis has long been investigated. Strontium has been one of the leading trace elements applied to induce remineralization of dental tubules in cases of dentine hypersensivity [7, 10]. Therefore, the idea arose to limit fluoride content by supplementing with strontium for remineralization of dental enamel. In the studies, after 6-months application of strontium-fluoride toothpaste, the level of calcium and strontium increased in the deeper layers of the enamel. It confirms a usefulness of Sr^{2+} for enamel remineralization and healing of early caries changes. Concluding, the increasing role of digital imaging needs to be defined with the diagnosis of further problems for the patient in mind in order to plan an effective prophylaxis and treatment of early caries changes.

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