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RESEARCH

In vivo study of pixel grey-measurement in digital subtraction radiography for monitoring caries remineralization

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Objectives: The aim of this study was to evaluate the performance of a quantitative method, based on pixel grey value measurements, for monitoring caries remineralization.
Methods: Proximal radiographs of 11 patients (61 enamel caries lesions) were taken both before and after a 2 month remineralization protocol. Radiographs were digitized and for each area a follow-up image was subtracted from a baseline image. A quantitative analysis was undertaken using ImageTool software (University of Texas Health Science Center, San Antonio, TX). For each caries lesion diagnosed, a mean pixel value was obtained in digital subtraction radiographs (SR) using a scale ranging from 0–255 grey values. To identify caries status after the remineralization protocol, a subjective analysis of SR was undertaken by 6 radiologists who graded the lesions as demineralized ($n = 10$), unchanged ($n = 34$) or remineralized ($n = 17$).
Results: There was a statistically significant difference between the mean pixel values for demineralized (112.1 ± 14.4), unchanged (127.3 ± 12.3) and remineralized (137.5 ± 13.8) lesions.
Conclusions: It was concluded that the status of proximal caries lesions after remineralization therapy can be assessed by pixel grey measurements in SR and may constitute a suitable complementary method for monitoring the results of remineralization protocol in clinical practice.
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Introduction

Advances in knowledge about caries have allowed for more conservative treatments with the development of non-operative/preventive care for the initial stages of caries lesions.[1] However, diagnosis must be performed early at the non-cavitated stage, when the dynamic nature of lesion progression still makes the arrest of further mineral loss possible.[2] At this stage, remineralization therapy can be applied in order to reverse incipient lesions.[3] This leads to changes in mineral quality and quantity.[4]

Clinicians need resources of high diagnostic accuracy for both detecting incipient caries and monitoring remineralization of lesions. Wenzel[5] reported that conventional radiography has limited ability to detect lesions confined to enamel, and there is little information available about the use of digital systems in clinical practice. Digital imaging has the potential to improve diagnostic accuracy and make quantitative diagnoses.[6,7] In recent decades, software for radiographic analysis has been investigated and developed for the detection of lesions and the quantitative assessment of the depth of a caries lesion.[8,9] Digital subtraction radiography (SR) is a useful tool for the clinical assessment of caries lesion behaviour[10] and for the detection of small lesions.[11] SR is highly sensitive in the diagnosis of changes in alveolar defects, detecting amounts of bone loss ranging from 1–5%.[12] However, reproducible exposure geometry for serial radiographs and identical contrast and density of both radiographs that are to be subtracted are essential prerequisites for a successful SR evaluation.[13–15]

In non-operative care, early detection of lesions is as important as monitoring remineralization therapy. However, methods used for the clinical detection of

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proximal surface caries are still inadequate for differentiating the different stages of the disease process,[16] and there is still no generally accepted method of measuring mineral changes during remineralization procedures.[17] In addition, Wenzel[5] stated that most studies on diagnostic performance of new digital imaging systems are restricted to laboratory settings. Many *in vitro* studies detected mineral changes using SR,[14,18] computer-assisted densitometric image analysis (CADIA),[19] electrical resistance measurements[17] and microradiography.[20] However, further studies are still necessary to determine the clinical applicability of these methods. Hence the purpose of this study was to evaluate a quantitative method, based on pixel grey values in SR images, used for *in vivo* monitoring of dental caries remineralization.

Materials and methods

Sample preparation

60 subjects were invited to participate in the study. They were made aware of the research protocol and the need for thorough collaboration throughout the clinical experiment. Inclusion criteria were age between 16 years and 30 years, complete natural dentition, sound posterior teeth or with minor occlusal restoration and healthy gingival tissues. Subjects who refused to participate in the study and subjects with teeth loss, large restorations, dentin caries lesions or teeth malformations, systemic diseases and previous remineralization therapy were excluded from the sample.

44 subjects agreed to participate and underwent interproximal radiographs for caries examination using an X-ray unit (Heliodent 70, 70 kVp, 7 mA; Siemens, Bensheim, Germany) at 30 cm focus-receptor distance and an exposure time of 0.32 s for molars and 0.25 s for premolars. Bite-wing radiographic film holders (Indusbello; Londrina, Paraná, Brazil) and vinyl polysiloxane-based material (Re'Cord; Bosworth, Skokie, IL) were used for bite recording. Vertical angulation of the X-ray beam was also recorded for each subject. The radiographic films (IP-21, F speed; Eastman Kodak Co., Rochester, NY) were developed by an X-ray film processor (Peri-Pro III; Air Techniques, Hicksville, NY) containing fresh developer and fixer solutions (Kodak Brasileira Com. Ind. Ltda., Campinas, São Paulo, Brazil). Film holders were disinfected and identified before storage.

Radiographs were examined by three independent radiologists for detection of proximal caries lesions. Divergences were solved by consensus. 5 patients were free of caries (Group 1), 16 patients had at least 1 dentin or enamel lesion involving the dentin–enamel junction (Group 2) and 23 patients had only proximal enamel caries before the dentin–enamel junction (Group 3). To confirm radiographic diagnosis, orthodontic elastics were placed in proximal contacts over 24 h to separate contiguous teeth and allow direct visualization. After dental prophylaxis using a Robinson brush, water and pumice, teeth were dried and isolated with cotton rolls. A trained examiner carefully inspected all caries-suspected proximal surfaces. After clinical confirmation of enamel lesions, 11 patients were diagnosed as true positive cases (experimental group) and in 12 patients enamel caries lesions were not confirmed.

Caries-free subjects received oral hygiene orientation and those with dentin caries lesions were referred for restorative treatment. All of these were excluded from the experimental phase of the study. The experimental group ranged in age from 19 years to 27 years (mean 21.8 years; standard deviation (SD) 2.5 years), two were male and nine were female. 61 proximal lesions situated below points of contact were identified in these 11 subjects. There were 19 superior premolar lesions, 17 inferior premolar lesions, 11 superior molar lesions and 14 inferior molar lesions. The number of caries lesions ranged from 2 to 18 per patient (mean 5.5 lesions; SD 4.8 lesions), which were distributed over 20 interproximal radiographs.

Remineralization therapy protocol

Dental hygiene and diet instructions were given to the 11 patients at the beginning of the experiment and reinforced throughout the treatment period. A protocol for remineralization of proximal incipient caries lesions was performed weekly for 8 weeks. In each session, the following procedures were performed: (1) dental prophylaxis using a Robinson bristle brush, water and pumice; (2) isolation of the operative area with cotton rolls; (3) air-drying; (4) topical application of 37% phosphoric acid gel (Magic Acid; Vigodent, Rio de Janeiro, Brazil) for 30 s only in the first session; (5) washing with water and air-drying; (6) application of 0.05 M aluminium nitrate (Botica ao Veado D'ouro, São Paulo, Brazil) with a brush and drying with absorbent paper; (7) topical application of 1.23% acidulous phosphate fluoride gel (Frutti Flúor; Biodinâmica, Ibiporá, Paraná, Brazil) with a brush and dental floss; (8) instructions were given not to consume solid foods or liquids for 30 min. McCann[21] reported that the use of 0.05 M aluminium nitrate increased fluoride uptake in human enamel in both *in vitro* and *in vivo* remineralization protocols. The research protocol had been previously approved by the Institution's ethical committee and informed consent was obtained from all patients. Patients were referred for restorative treatment if remineralization therapy was unsuccessful after 8 weeks.

Final radiographic examination

1 week after the finalization of the remineralization protocol, another radiographic examination was undertaken. Posterior proximal radiographs of the 61 lesions were taken using the same X-ray unit, film holders for bite recording and vertical angulations for standardizing the geometric projection of the X-ray beam. The

radiographic films were developed using the same standardized procedures.

Digitization of radiographs and digital subtraction radiography
DSR system (Electro Medical Systems, Nyon, Switzerland) was used for the digitization of all the radiographs (20 baseline and 20 follow-up radiographs) as well as for digital radiographic subtraction. A 400 dots per inch (dpi) resolution scanner transparency system included in the DSR system was used for digitization. For each area, follow-up image was subtracted from baseline. For alignment of pairs of radiographs, four marked points were recorded at reproducible dental structures. Before subtraction, gamma correction was applied. 20 SR images were obtained and saved as Tagged Image File Format (TIFF).

Radiographic analysis

Quantitative analysis: SR images were analysed using ImageTool software (University of Texas Health Science Center, San Antonio, TX).[22] Each caries lesion was considered to be the region of interest (ROI). Differences in brightness intensity in SR images were used as a parameter to outline the caries lesions. In remineralized lesions, the ROI was characterized by greater whiteness compared with the homogeneous grey background and in demineralized lesions the ROI was defined by greater darkness compared with the background. In unchanged lesions, in which the grey level is similar to the background, the baseline image was used to guide the outlining of the ROI. The histogram method for pixel grey value measurement was performed in the selected area and the software automatically displayed the mean pixel grey value and the standard deviation of the mean (Figure 1). All lesions were outlined by the same operator, who had been previously trained and calibrated to reproduce consistent measurements.

In order to assure that quantitative evaluation detected the actual mineral change of the lesions, identical measurements were performed in areas without signs of demineralization (sound enamel), which were considered as internal control. These additional measures were taken using the same DSR images, focusing on sound enamel underneath the caries lesions.

Subjective analysis: A visual analysis of SR images was undertaken by six previously trained and calibrated radiologists. Examiners were blinded as to the quantitative analysis. The 61 lesions were classified as demineralized (closer to black), remineralized (closer to white) or unchanged (next to homogeneous background). Considering interexaminer agreement, the final status of the lesions was declared using the following criteria:

(1) Demineralized lesion: if at least four examiners agreed that the lesion had demineralized
(2) Remineralized lesion: if at least four examiners agreed that the lesion had remineralized
(3) Unchanged lesion: if less than four examiners agreed about the diagnosis

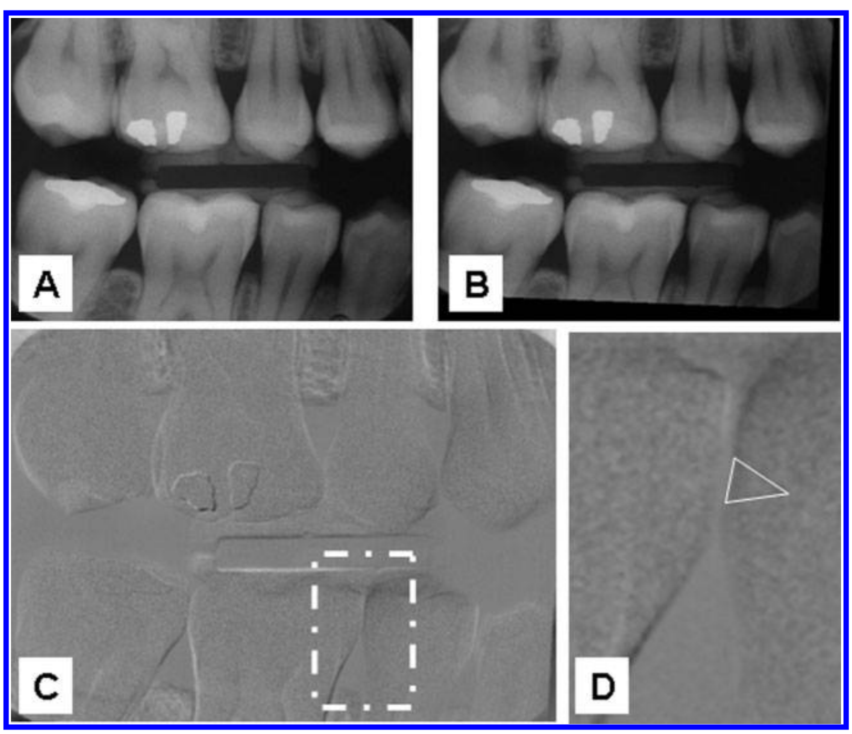

Figure 1 (a) Baseline image and (b) follow-up image. Subtraction image with (c) selected ROI area of the lesion and (d) delimitation of caries lesion

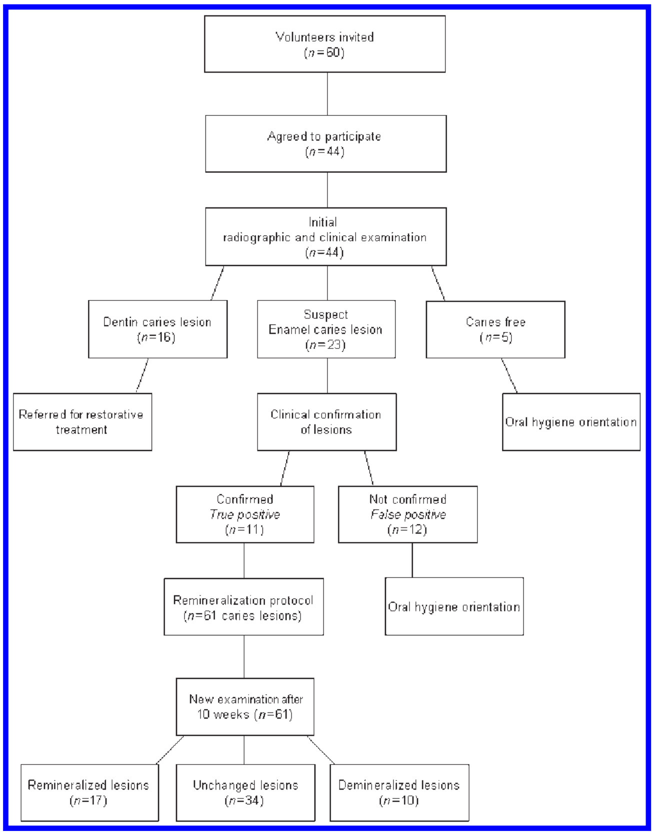

Figure 2 Research protocol and sample distribution

Data analysis

A descriptive analysis was undertaken for each lesion group (demineralized, remineralized and unchanged) and an unpaired *t*-test was used for comparative analysis. Differences were considered significant at a 5% level ($P < 0.05$). SPSS v10.0 software (SPSS Inc., Chicago, IL) was used for data analysis.

Results

Research protocol and sample distribution of patients and lesions are detailed in Figure 2.

According to the subjective analysis, 10 lesions were demineralized, while 17 were remineralized and 34 remained unchanged. The 95% confidence intervals and differences between means of pixels grey values (Tables 1 and 2) reveal significantly different groups after quantitative analysis (demineralized, unchanged and remineralized).

Table 1 Descriptive analysis of pixel grey values of lesions groups

Lesion groups	n *(%)*	*Min–Max*	*Mean (±SD)*	*95% CI*
Demineralized	10 (16.4)	81–127	112.10 (±14.35)	101.84–122.36
Unchanged	34 (55.7)	109–158	127.29 (±12.29)	123.01–131.58
Remineralized	17 (27.9)	105–157	137.47 (±13.83)	130.36–144.58

CI, confidence interval; Max, maximum; Min, minimum; SD, standard deviation.

Table 2 Comparison of pixel grey values for lesions groups

Lesion groups	n	*Mean*	*Mean difference*	t	P
Demineralized	10	112.10	15.19	3.311	0.002*
Unchanged	34	127.29			
Demineralized	10	112.10	25.37	4.540	0.000*
Remineralized	17	137.47			
Unchanged	34	127.29	10.18	2.674	0.010*
Remineralized	17	137.47			

*Statistically significant difference ($P < 0.05$)

Discussion

The International Consensus Workshop on Caries Clinical Trials, 2002, recommended the use of software tools for the quantitative assessment of caries lesion progression in clinical trials.[23] However, few studies have focused on the quantitative radiographic analysis of mineral changes, especially in *in vivo* experiments.[5,14,24] This is probably due to the difficulties involved in standardizing methods and controlling clinical variables in human settings.

In the present study, experimental procedures were standardized by using beam-aiming devices, a vinyl polysiloxane-based material for bite registration, recorded orientation of the X-ray source, and by controlling the conditions of film development and the digitization of radiographs. This research protocol simulates controlled clinical procedures which may vary in routine clinical practice. The effectiveness of such protocol is dependent on standardized procedures, including image acquisition, film developing and image digitization, that are essential for monitorization of caries progression or remineralization.

Digitized radiographs were used since no differences between digital (direct method) and digitized radiographs (indirect method) have been reported for assessing enamel subsurface demineralization.[18,25] In a clinical validity study, Nummikoski et al[26] found that digitized radiographs and subsequent subtraction presented greater accuracy in the detection of alveolar crestal bone loss when compared with conventional film viewing. The same procedures were also successfully used by Eberhard et al[14] for monitoring *in vitro* dental demineralization and by Ortman et al[12] for detecting changes in alveolar bone defects with bone loss in the range of 1–5%.

It is known that digital radiographic imaging has the advantage of reducing work time, but some practical disadvantages, such as the difficulty in positioning the rigid sensor intraorally, reduces image reproducibility and commonly results in undesired retakes. It is not known how many exposures are needed for sensors to cover a conventional bitewing.[5] PSPs were recently introduced to minimize positioning problems, but high cost and availability restrict clinical use.[25]

In some cases, the delimitation of lesion limits was difficult because caries lesions did not have well-defined radiolucency,[27] especially in overlapping sites. This cannot be completely avoided in clinical routine,[8] but in experimental research designs it is important to assure precision in operator's measurements to avoid outlining ROIs of larger or smaller selected areas than actual lesions. Another limitation of *in vivo* caries study is that the ground truth is not known because it is not possible to know *a priori* the actual status of the teeth. To minimize this problem, false positive teeth were excluded after a careful clinical examination including separation of teeth with elastics and a standardized clinical examination protocol.

For diagnosis of teeth status after remineralization, since there is no "gold standard" method for identifying the effectiveness of the remineralization processes in human settings, a criterion based on interexaminer agreement was used for subjective analysis. Final lesion status (demineralized, remineralized or unchanged) was based on the agreement of two out of three of the examiners (at least four). This was considered the most probable diagnosis for the caries lesion status after the remineralization protocol.

According to the subjective analysis, remineralization therapy was unsuccessful in only 10 (16.4%) caries lesions and 6 of those were in a single patient. The remaining lesions were stable (55.7%) or remineralized (27.9%). These results are consonant with Pitts,[1] Wenzel,[4] and Ferreira and Mendes,[3] who endorsed remineralization therapy for the control or even regression of incipient enamel lesions. It must be emphasised however, that remineralization therapy success is strictly dependent on following a rigorous protocol by both patient and clinician. Therefore, the success rates in controlled experimental conditions may differ from clinical daily routine.

The quantitative analysis showed that mean pixel grey values can be used for monitoring caries remineralization, revealing lesion status. Quantitative values correlated with lesion status in subjective analysis. Greater values were observed for remineralized lesions, lower values for demineralized while intermediary values (near 128 grey value) were consistent with stabilized lesions. 95% confidence intervals values of groups were independent and statistically significant differences between groups confirmed that quantitative analysis in SR images matched categories of lesion progression in subjective analysis. These findings corroborate the hypothesis of the usefulness of subtraction method for monitoring caries behaviour in the clinic, as suggested by Wenzel et al[10] and Wenzel[4] in *in vitro* settings using only qualitative assessment methods. However, it must be stated that pixel grey values may be combined with clinical and radiographic parameters, together with awareness of the patient's individual caries risk, diet control and hygiene habits.

Considering that *in vivo* remineralization is a slower process than demineralization, since mineral uptake occurs in very small amounts,[28] the proposed quantitative method demonstrated promising results for detecting mineral changes. This high sensitivity to detect such alterations is explained by the eliminatin of all anatomical structures other than those of immediate interest.[13] The quantitative assessment of caries lesions is not observer dependent because the operator's task is restricted to the delimitation of the ROI and the software automatically displays pixel grey values.

In conclusion, pixel grey value measurements in SR images may constitute a suitable complementary method for monitoring outcomes of remineralization protocol. Future studies are needed to guarantee the cost benefits and effectiveness of quantitative assessment methods in clinical practice.

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