Remineralization Effect of Fluoride on Incipient Caries: An in vitro Evaluation with "Digital Subtraction Radiography" Technique

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Abstract

Objectives: Caries prevention is the first priority in the oral healthcare system. Fluoride use is also one of the ways to assure tooth health. The purpose of this study was to quantitatively evaluate the remineralization effect of fluoride on induced enamel lesions, using "digital subtraction radiography" (DSR) technique.

Materials & Methods: This was an analytical laboratory study. Sixty caries-free premolars were selected. Thirty teeth were exposed to the remineralization solution and the rest were placed in the deionized water in incubator for 28 days. Radiographic X-rays were taken of the study samples before the induction of caries lesion, at the 14th and the 28th day of immersing. The images were subtracted from the baseline and the mean value of the gray levels was calculated. Paired t-test and student's t-test were used for statistical analysis.

Results: The mean value of the gray level for control group was 124.7 ± 2.1 (day 14th) and 124.4 ± 1.2 (day 28th). (P<0.24) (*t*-test=1.177) However, this value for experimental group was 128.5 ± 2.4 (day 14th) and 130.6 ± 2.6 (day 28th). (P<0.001) (*t*-test=7.89) The values were statistically significant different at both stages of study when the experimental group was compared to control. (P<0.001) The gray scale's mean was statistically significant within the experimental group at the 14th and 28th day (p<0.001).

Conclusion: Remineralization effect of fluoride is detectable and increases along the time. The mineral deposition of fluoride is also quantifiable by means of the DSR technique.

Key Words: Enamel demineralization, Digital subtraction radiography, Enamel remineralization, Fluoride, Primary tooth, Tooth caries

Introduction

Prevention of tooth caries is a major goal in the oral healthcare system in all communities [1]. Fluoride material is a reservoir and enhances chemical structure of enamel by replacing hydroxyl ion [2]. This replacement forms a stable and a caries-resistant chemical compound of fluoro-apatite. Besides, this compound has the ability to remineralize incipient caries [3]. There are a compiling clinical and laboratory evidence that prove the positive quantitative effect of fluoride on incipient dental caries [4].

Quantifying the fluoride intake by enamel would be a valuable measurement for defining the level of fluoride penetrated in tooth enamel. There are ways like: microradiography, polarized light microscopy to measure the level of fluoride penetration in enamel, but a few of them can be used clinically. Among clinically acceptable techniques, Quantitative Laser/Light-induced Fluorescence (QLF) is recommended for evaluation of remineralization level of enamel [5,6]. According to clinical studies, QLF method is capable of early diagnosis of incipient caries lesion. It, furthermore, can diagnose the regression of such lesion. However, the limitation of this technique is its incapability of assessing interproximal caries [7].

Another technique is "digital Subtraction Radiography" (DSR) which is introduced by Ziedess des Plants in 1934 [8]. The principle of DSR technique is subtraction of the density of 2 pictures from a single object or structure at 2 different occasions. The DSR technique is capable of detecting even up to 1-5% of mineral loss [9].

The DSR method was first used in medicine. Later on, it was introduced to dentistry [8]. In periodontics [10,11], implantology [12,13] endodontics [14] and orthodontics [15,16]. DSR technique are frequently used for evaluation of the level of osteogenesis, root resorption and etc. There has been so much enthusiasm in the field of caries research with DSR technique. Applying this technique, Halse et al. [17], Maggio et al. [18], Nummikoski et al. [19], and lately Haiter-Neto et al. [20] evaluated the demineralization changes of enamel. Some studies also introduced different advanced software for analysis of radiographic images. These programs would improve technical shortcomings of current imaging methods and the geometric dimension of images, as well [21-24]. These attempts were for to introduce an imaging method that can prepare an image with the highest precision and the least error. According to Takeshita, DSR is the best method of radiography for such purposes due to its high technical sensitivity [25].

The other advantage of digital subtraction radiography is detecting the areas of caries regression that occur between periods of radiographic examinations [26]. It is an image processing algorithms which can relate the magnitude of the changes on the subtraction image to the reference wedge, thereby allowing calculation of the extent of the remineralization area. According to this technique, practically, a radiograph of a decayed tooth is compared with of the tooth after it was exposed to fluoride. Finally, similarities of superimposed images are omitted and the extent of remaining shade of the affected structure would be evaluated.

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In spite of the important role of fluoride in promoting oral health, the remineralization phenomenon of incipient caries by fluoride has little been evidence-based in the literature [18,27]. This study has been conducted to quantitatively evaluate the remineralization effect of fluoride on the induced enamel lesion.

Materials and Methods

This study was designed as an in vitro type. For this purpose, sixty extracted maxillary and mandibular premolar teeth were collected from the patients referred for orthodontic treatment. The inclusion criteria were the teeth of no restorations, no hypoplastic defects, and no caries. All samples were disinfected with a 0.1% concentration sodium hypochlorite solution for 10 minutes. All surfaces of teeth were covered with a layer of varnish except a rectangular area with the dimension of 3x1.5 mm^2 on mesial or distal surface. Teeth were stabled vertically by placing roots in a cube of self-cure acryl. An "L" shaped orthodontic round wire (0.9 mm diameter) was also embedded inside acryl, for accurate conformity of images *(Figure 1)*. For repeatability of radiographic images, the distance between X-ray source and CCD image receiver was fixed at 24cm when taking radiographs. A plexiglas plate with 18mm thickness was also placed in between to simulate the soft tissue texture *(Figure 2)*. A four-wedged aluminum block was designed in 1, 2, 3, 4, and 5mm thickness to simulate the gray scale of images, additionally *(Figure 3)*.

All teeth were immersed in a 20cc volume of demineralization solution (buffered acetic acetate acid with pH=4.8 and concentration of 2.282 gr/100cc of CH3COONa) in room temperature. When demineralization was visible macroscopically on tooth surface (after 21 days) with naked eye, samples were removed from the solution and rinsed with deionized water. Base-line digital radiographs were taken of the 60 specimens by the Planmeca panoramic machine (t=0.1", mA=8, KVP=68) (Planmeca Co., Finland).

Thirty specimens were submerged in remineralization solution, according to Iijima [28] study. They all were retained in an incubator (Shimaz Co., Iran) under 37°C temperature for the duration of 28 days. The solution formula was 20mM HEPES*, 0.9mM KH_2PO_4, 1.5mM $CaCl_2$ (Sigma-Aldrich, Co., UK), and 1ppm fluoride (NaF) with a pH level of 7 [14]. The solution was also renewed every 48 hours.

The remaining specimens (control group) were also immersed in 20cc deionized water held in an incubator at 37°C temperature for 28 days. After the length of time of 14 and 28 days, radiographs were taken as previously mentioned. At the end of experiment, one case and 3 control samples were dismissed from the study, because of the poor geometric adaptation, remaining 29 experimental and 27 control samples.

(*HEPES buffer (C_8 H_{18} N_2 O_4 S) directly bought from Sigma – Aldrich Co.)

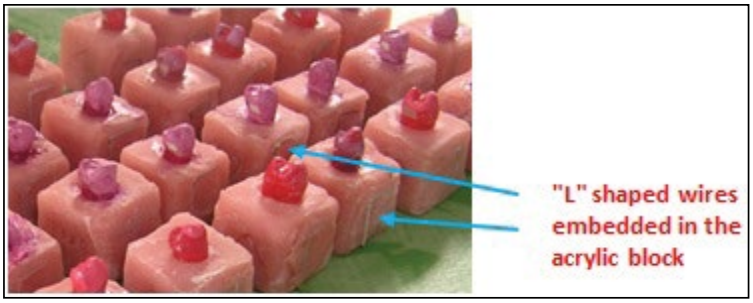

Figure 1. *The teeth are stabled in acrylic blocks. They are coated with varnish but a 3x1.5 mm^2 square on mesial or distal surface, as an exposed area for fluoride penetration.*

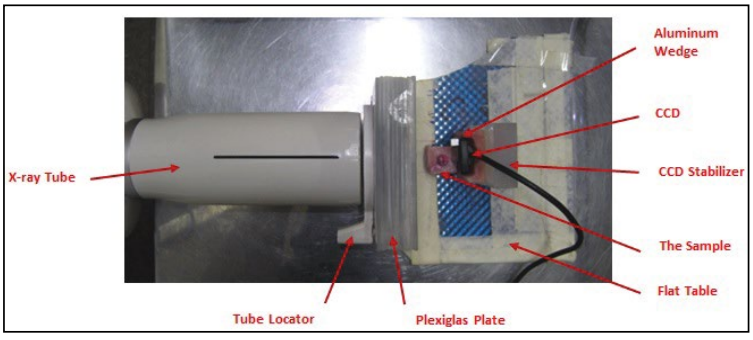

Figure 2. *The DSR imaging setup used in the study: Source of X-ray (tube & locator), plexiglas plate, the sample tooth embedded in acryl, CCD, and stabilizer for CCD.*

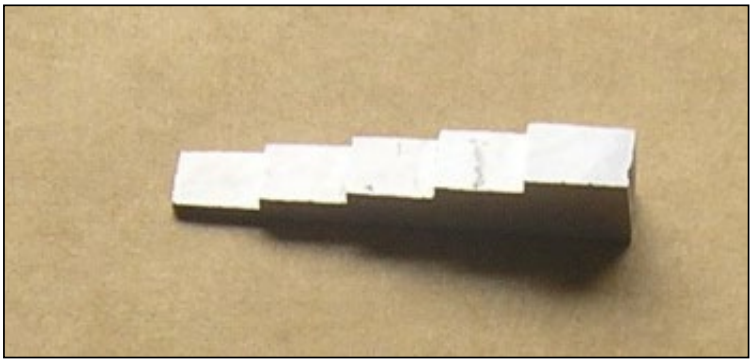

Figure 3. *An aluminum wedge in 1, 2, 3, 4, and 5mm thickness was used to simulate the gray scale of images.*

(https://www. Sigmaaldrich.Com/catalog/search/product. detail/SIGMA/H3375).

X-ray Analysis

Radiographs of the 3 stages of experiment (day zero, 14th, and 28th) of both groups and were compared using Dimaxis software (Planmeca Co., version 2.4.1). The images were saved in the "jpg" format. The Adobe Photoshop software was also used for analysis of radiographs. X-ray images of the day 14th and 28th of all samples were calibrated for "gray level" compared to the day first, for subtraction. The calibration was done using an aluminum wedge *(Figure 3)*. Then, subtraction of images was performed by superimposing the reference L-form wire on each couple (before and after exposure) of images *(Figure 4)*. For quantitative evaluation, as indicated, the defined unique rectangular area in the demineralized surface of all samples was the criteria for evaluation. They all had identical sizes and covered almost whole experimental area [22,27,29,30]. The mean gray scale was calculated in the interproximal surface. The software provided a histogram and the mean of gray level of the rectangular area *(Figure 5)*. The flowchart of the procedures of this study is also presented briefly in *Figure 6***.**

The collected data was analyzed using "paired t-test" for both groups and "student's t-test" for independent evaluation of samples using the SPSS software (α=0.05 and $p<0.05$ is assumed significant). The P-value of changes is also calculated for "inter-group" and "intra-group" analysis.

Results

At the day 28th, the 27 teeth in control group and 29 teeth in experimental group were evaluated. At the end of this period, the average gray scale of images in experimental group was 130.6 ± 2.6 (median 130.1, at the range of 127.0 to 135.9) and in control group was 124.4 ± 1.2 (median 124.2, at the range of 122.3 to 126.7). The *t*-test analysis for the mean values was 11.032 and the P-value was 0.001 for this period of time.

The average gray scale of images related to experimental samples was 128.5 ± 2.4 and related to control samples was 124.7 ± 2.1, at the day 14th. For the same period, the *t*-test result for the mean values analysis was 6.316 and the P-value was 0.001 *(Table 1)*.

There was a statistically significant difference between the gray scale results of the day 14th with the 28th day "within" the experimental group. ($t=7.89$, $P \leq 0.001$) However, this difference was not significant "within" the control group. ($P=0.24$, $t=1.177$) The mean paired difference in the gray scale of the experimental group in the time duration of 14 to 28 days was 2.2 ± 0.3. According to the range of gray scale (0-256), the value for all experimental samples was above 127 and for control samples was below 127. Thus, the number 127 was determined as the cut-off point for the level of remineralization.

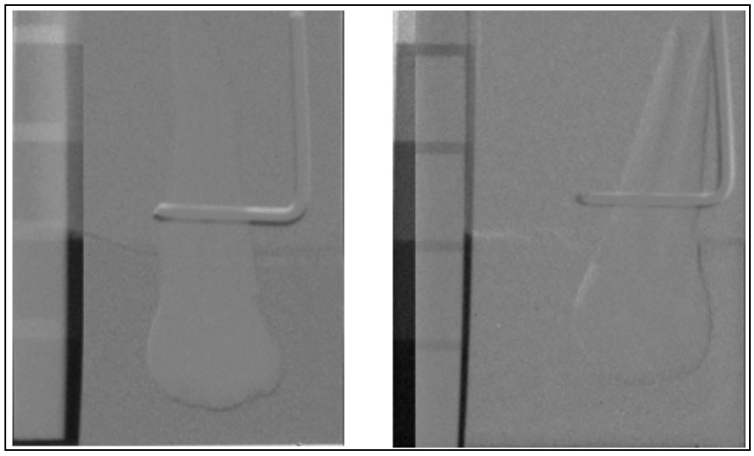

Figure 4. *Subtraction image: Sample of deionized water after 28 days/ sample of remineralization solution after 28 days (left to right).*

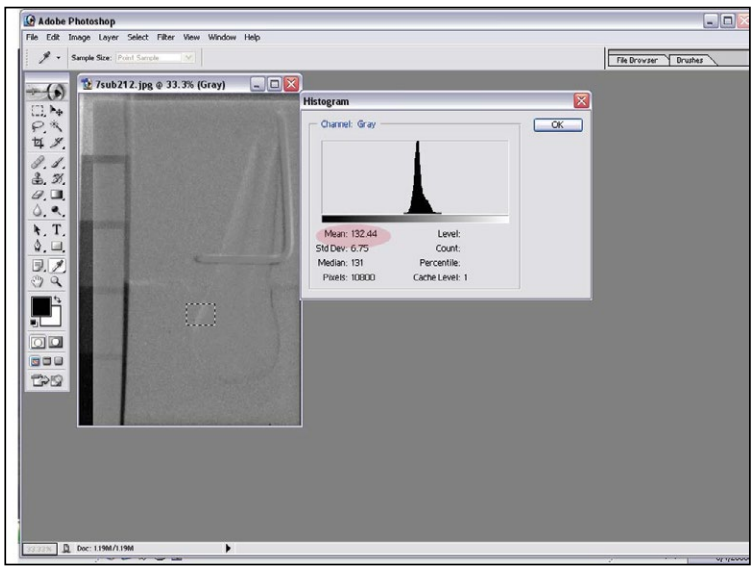

Figure 5. *Histogram and mean gray shades of selected interproximal areas.*

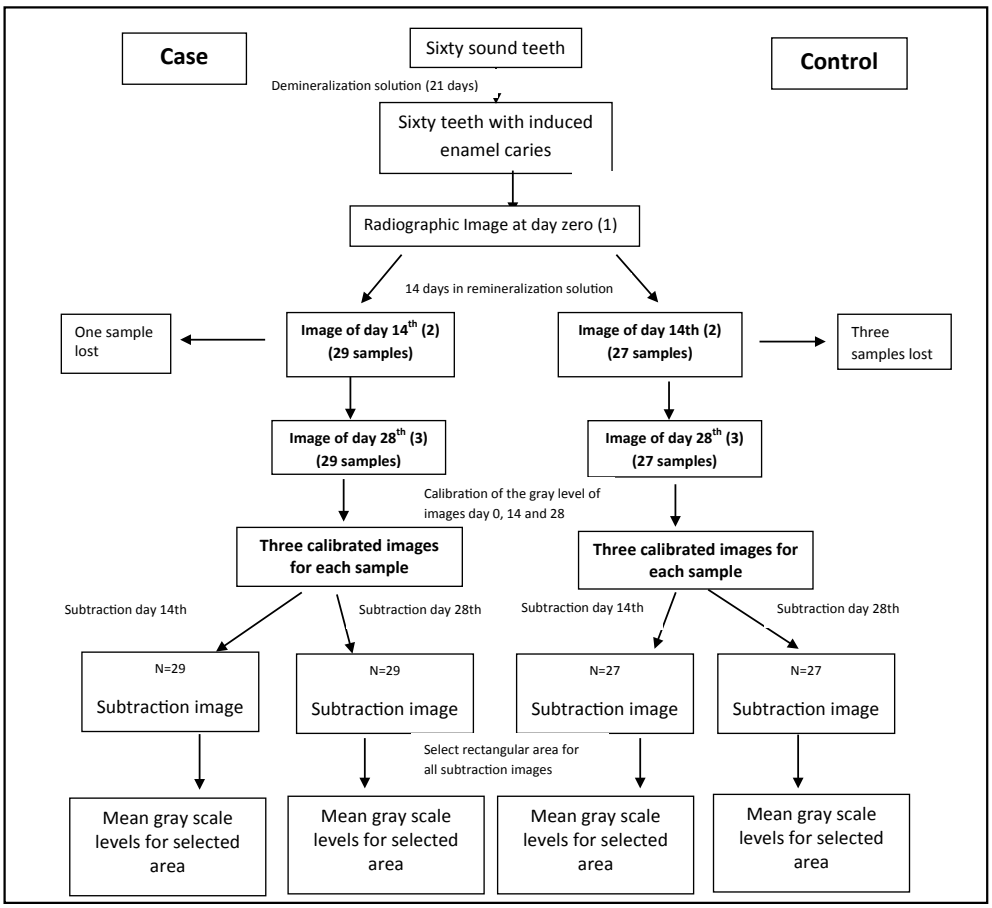

Figure 6. *The diagram of all stages of the study for qualitative evaluation of remineralization effect of fluoride using the DSR technique.*

Table 1*. The mean gray scale for the experimental and control groups at two periods of 14 and 28 days. The statistical analysis was based upon "intra-group" and "inter-group" evaluations.*

Mean gray scale groups	Sample Size	Day 14th (Mean ± SD)	Day 28th (Mean ± SD)	P-Value (intra-group)	*t*-test (intra-group)
Control	27	124.7 ± 2.1	124.4 ± 1.2	0.24	1.177
Experimental	29	128.5 ± 2.4	130.6 ± 2.6	0.001	7.89
P-Value (inter-group)		0.0001	0.001		
t-test (inter-group)		6.316	11.032		

Discussion

DSR technique has proved to be an efficient method for evaluation of fluoride effect on early cries formation. This radiographic method has additionally the capability of scaling the remineralization level of tooth surface. Compiling evidence has emphasized concerning the precision of this approach as a diagnostic tool for quantitative assessment enamel changes. Plus, this is a noninvasive method for mineralization changes in the interproximal surface along the time.

Conventional radiographic methods have little validity to prove fluoride penetration into interproximal surfaces of teeth, when remineralization happens. Capability of digital subtraction radiography technique to detect interproximal caries has been indicated by many researchers in recent years [18-20]. According to the result of this study, there was a statistically significant difference between the gray scale of both experiment and control groups at the day 28th. In addition, the difference between the gray scale at the day 14thand 28th was also statistically significant within the experiment group. This implies that the regular use of fluoride has an incremental effect in the remineralization process of enamel. This was in agreement with the result of a study by Carneiro [27] after a 2-month period of observation. Maggio [18] also concluded with a similar finding when the artificial saliva was used as the media for the remineralization process.

Another study by Eberhard et al. [29] extended the remineralization period to 42 days; and the cases were evaluated on the weekly basis. However, they noticed that remineralization changes of the affected tooth surfaces, adjacent to the cavity filled with glass ionomer cement (case), failed to reach the statistically significant level in comparison with the unfilled cavity surfaces (control).

The most important feature of DSR technique is its comparable geometric images before and after experiment. Additionally, the present study found the "aluminum wedge" a dependable tool for accurate evaluation.

In earlier studies, DSR technique was used to analyze images, visually [29-31]. This procedure is nowadays performed by "gray scale" method and is measured quantitatively, free of any visual error. In some other studies, the affected areas were selected for assessment based upon the operator choice [20-27]. For better quantitative evaluation, an identical confined rectangular area was proposed on proximal surfaces of samples [20]. Then, the mean value of changes was evaluated using the Photo-Shop software.

Different investigations have proposed different cut-off

point for re- and de-mineralization phenomena, due to using a variety of gray scale analysis software. Okano et al. [30] found 128 as the border of density changes for alveolar bone following periodontal treatment. This study observed 127 for tooth mineralization changes.

Conclusions

Within the limitation of the present study, the phenomenon of remineralization changes of dental enamel is accurately detectable with DSR technique. This can be monitored and quantified even after few days of application of fluoride. This technique is also useful for longitudinal evaluation of enamel remineralization. For definite, fluoride can change the chemical structure of enamel to a caries-resistant material.

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