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COMPARATIVE ANALYSIS OF REMINERALIZATION IN PATIENTS UNDERGOING ORTHODONTIC TREATMENT SUBJECTED TO VARIABLE CONCENTRATIONS OF FLUORIDE SUPPLEMENT.

RESEARCH STUDY

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ABSTRACT

Aim: Evaluation of the linear depth of remineralization after subjecting the Enamel to fluoride supplements, under polarized light microscope. **Method:** Fifteen patients undergoing orthodontic treatment were divided into 3 groups of 5 each and they were asked to brush their teeth using 3 different dentrifice for 3 months. Group A were remineralized using non-fluoridated dentifrice (control), those in group B and group C using 500ppm and 1000ppm of fluoride containing dentifrice, respectively. Teeth were sectioned into 100 µm thick sections and images were captured using polarized light microscope [PLM]. **Results:** The values were tabulated and statistically analyzed by Anova. The highest values of linear depth for demineralization under PLM were seen in the group A which was found to be 184.68±6.43 µm and the highest values of linear depth for remineralization was seen in group C and was found to be 156.07±4.76 µm which was significantly higher than that for the group A. **Conclusion:** Study concluded that 1000 ppm fluoridated dentifrice showed a greater degree of remineralization than other groups and polarized light microscope gives promising results in the detecting the depth of demineralization and remineralization over the conventional methods.

KEYWORDS :

Demineralisation, Remineralization, fluoridated dentifrice, polarized light microscope

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Introduction :

Tooth minerals are lost and regained constantly in the human oral environment. Enamel demineralization is an undesirable but common complication of orthodontic fixed appliance therapy.[1] The health of the tooth is hence dependent upon equilibrium of this mineral exchange. A break in the equilibrium causes the tooth to either demineralize or remineralize depending upon the concentration of the mineral saturation in the oral cavity (fig 1).[2]

Over the last few decades, Fluoride in various forms has been proven to reduce demineralization in both the primary and permanent dentitions. It acts as a catalyst and influences reaction rates of dissolution and transformation of hydroxyapatite to fluorapatite that resists the

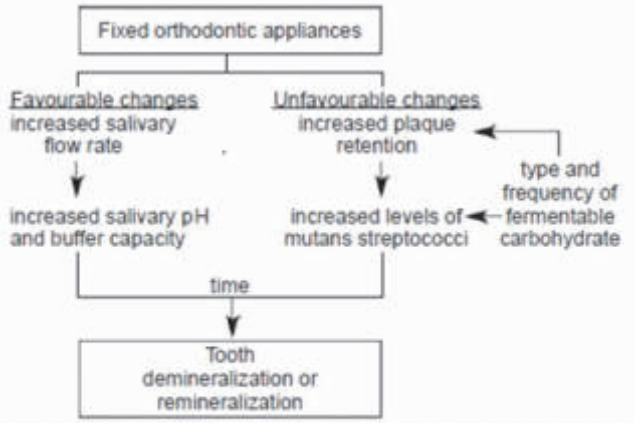


Fig. 1.–Hypothesized sequence of events in enamel demineralization during fixed orthodontic treatment. The distribution and severity of demineralization is influenced by the interaction between the various factors and the balance of mineral loss and repair.

demineralization of the tooth. Various topical agents like fluoridated solutions, gels, mouth rinses and dentifrices have been used to promote the remineralization. Fluoridated dentifrice in various concentrations have been used to bring about remineralization since it is a most common and easily available vehicle that is used to cleanse the teeth worldwide and can deliver fluoride topically to the oral cavity.[3]

Remineralization by the action of fluoride supplements have been analyzed by various qualitative and

quantitative techniques of measurement of tooth mineral changes that include Polarized Light Microscopy (PLM), light scattering, Polarization-Sensitive Optical Coherence Tomography, Transverse Microradiography, Energy Dispersive X-ray analysis, cross-sectional microhardness determination and Confocal Laser Scanning Microscopy.[4]

Under polarized light microscopy, the qualitative evaluation of the zones of demineralization and remineralization are assessed with respect to the area occupied, width and depth. The optical property of this technique helps in the detection of the depth of demineralisation and remineralization.[5]

Therefore, the aim of the present study is to evaluate the remineralization of orthodontically treated teeth through polarized light microscopy after subjecting it to 3 different concentrations of fluoridated dentifrice.

Materials and methods:

Method of collection of teeth and sample preparation:

Fifteen patients who were planned for orthodontic treatment with extraction of all first premolars were selected for this study. Maxillary and mandibular teeth were etched with 37 % phosphoric acid for 15 seconds. The teeth were then air dried for 15 seconds. Transbond XT Primer (3M Unitek) was applied and teeth were bonded with Gemini Series metal brackets (3M unitek) using Transbond XT light cure adhesive.

These fifteen patients were then divided into 3 groups of 5 each. They were asked to brush their teeth using different dentifrice as mentioned below.

Group A : Remineralization using non-fluoridated dentifrice - Meswak tooth paste from Dabur

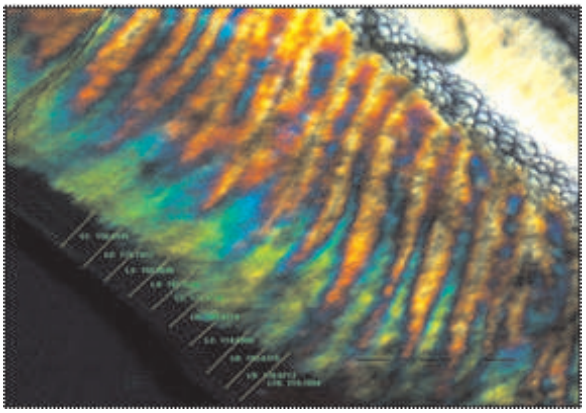
Group B : Remineralization using 500 ppm of fluoride containing dentifrice - Colgate Bubble Fruit tooth paste

Group C : Remineralization using 1000 ppm of fluoride containing dentifrice- Colgate Total tooth paste

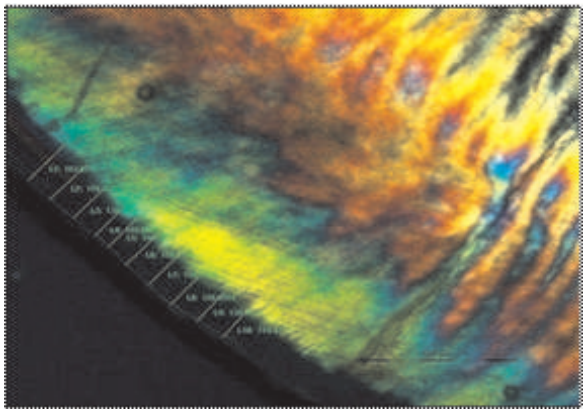
After 3 months of levelling and aligning, maxillary and mandibular first premolar brackets were debonded from the teeth and extracted. These teeth were cleaned thoroughly with normal saline and sectioned 1 mm below the cemento-enamel junction with a slow speed diamond disc and the crowns were used for the study.

The specimens were mounted in self-cure acrylic resin, sectioned with a hard tissue microtome (Leica SP 1600) to obtain specimens of 100 microns thickness, polished with an abrasive stone, stained with freshly prepared 0.1mM Rhodamine B solution for 1hr and washed thoroughly with phosphate buffer solution and were mounted on frosted glass slides with 80% glycerol mountant for further analysis through the Polarized Light Microscope (PLM), Olympus BX51 model with a 1 CCD c-mount adapter.

The imaging with PLM was done under 4X magnification and two images were captured from the buccal surface, (one each from either side of the midpoint measured from the occluso-cervical length of the tooth) and calibrated for linear depth of the lesion from the enamel surface using Image Pro Express software (Fig 2).



(a)



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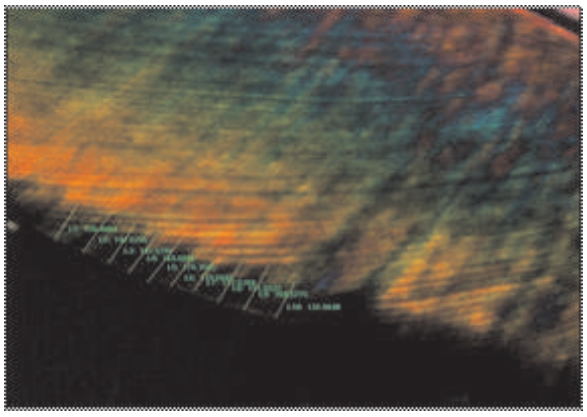
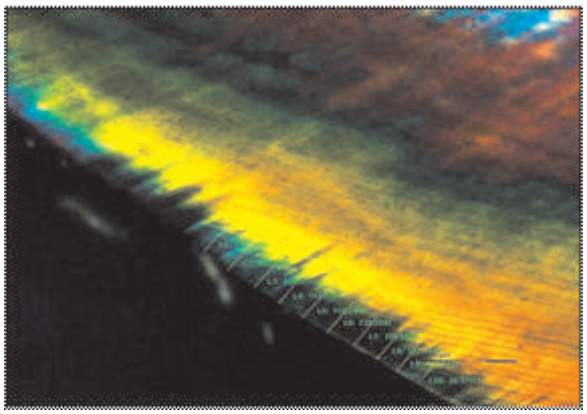
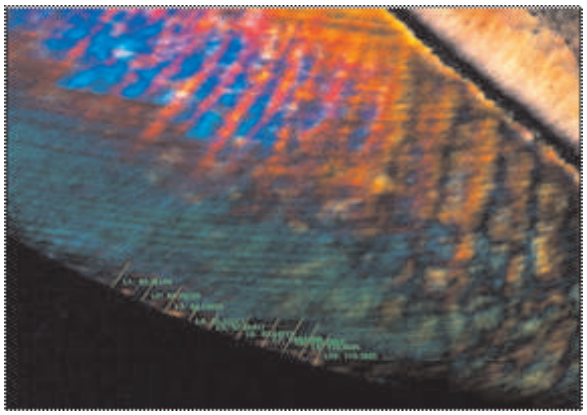


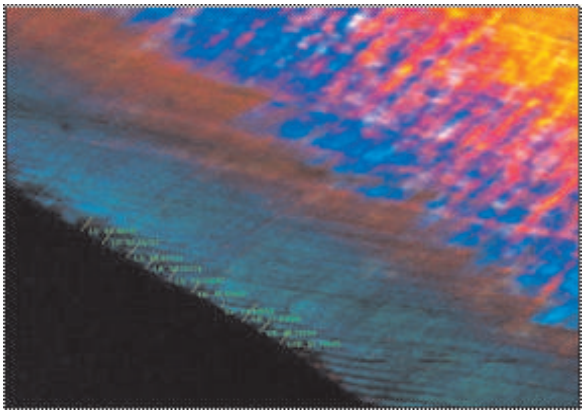
(c)



(d)



(e)



(f)

Fig.2: PLM images of Demineralized [(a), (c) and (e)] and remineralized zones [(b), (d) and (f)] of Group A, Group B and Group C specimens under 4x magnification.

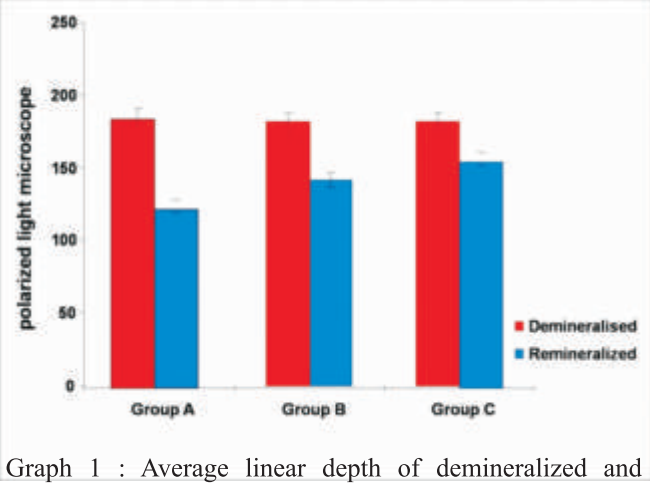
Results :

A Comparative evaluation study was undertaken to study the linear depth of demineralization and remineralization in µm as seen through polarized light microscope. The averages of ten measurements in each image were tabulated and statistically analyzed (Table 1, Graph 1).

Zone	Group A	Group B	Group C	P value
Demineralised	181.68±6.43	181.51±6.38	181.54±5.84	0.288
Remineralized	122.07±4.61	141.57±4.98	156.07±4.76	<0.001**
Difference	59.61±8.83	39.94±7.14	25.47±8.45	-
P value	<0.001**	<0.001**	<0.001**	-

+ Suggestive significance (P value: 0.05<P<0.10)
* Moderately significant (P value: 0.01<P 0.05)
** Strongly significant (P value: P0.01)

Table 1 : Evaluation of linear depth of demineralized and remineralized areas in µm as seen through polarized light microscope.



Graph 1 : Average linear depth of demineralized and remineralized areas in µm as seen through polarized light microscope

Results on continuous measurements were presented on Mean SD (Min-Max) and results on categorical measurements are presented in number (%). Significance was assessed at 5 % level of significance. Analysis of variance (ANOVA) was used to find the significance of study parameters, Student t test (two tailed, independent) was used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) and within each group on metric parameters.

Discussion:

Factors that affect the phase of mineralization include the oral pH, the contents and concentration of saliva, the oral bacteria present, frequency of sucrose ingestion, presence of fluoride or other chemicals and the duration of time all of these factors are present. Both of the demineralization and remineralization phases can be occurring at the same time in different parts of the mouth.[6]

Frequent, use of low fluoride concentration products which promote low and constant salivary fluoride levels have been accepted as an efficient way to prevent dental caries. [7] Fluoride affects the deminerlization process by enabling the formation of high quality fluorapatite that aids remineralization and inhibits glycolysis of plaque microorganisms.[8] Several methods of fluoride administration have been investigated including professionally applied gels and varnishes, home rinses and fluoride containing etchants, bonding agents, cementing media and elastic modules.[9]

Flouride is known to react with calcium to form calcium fluoride. There are three principle reactions with fluoride ion for remineralization: 1) Iso-ionic exchange of F^- for OH^- in apatite. 2) Crystal growth of fluorapatite from a supersaturated solution. 3) Apatite dissolution with CaF2 formation.[10] In the present study a non-fluoridated dentifrice has been used as a control along with two higher concentrations of fluoride, i.e 500 ppm and 1000 ppm for comparison.

Conventional microscopy suffers from the problem as light scattering, namely multiple scattering from objects that are out of focus within the illuminated region prevents imaging deep within a sample. Further, if care is not taken, optical microscopy can lead to the observation of certain artifacts which in turn leads to incorrect physical interpretation of the system in question. Most of the above disadvantages can be avoided by using fibre optic visible light spectroscopy, polarized light microscopy.[11]

The images viewed in the polarized light microscope will be a reduction, cancellation or pseudo-isotropy, or a reversal of the intrinsic birefringence of the enamel. The percentage of volume of spaces can be calculated from the observed birefringence using the known intrinsic birefringence of the enamel. Changes in enamel can be determined using various staining media with differing molecular sizes and refractive indices. [12] For this purpose 0.1mM of Rhodamine B dye has been used in this study.

Fontana performed a study to correlate area of demineralization, average fluorescence and total fluorescence obtained by confocal microscopy to lesion depth and mineral loss obtained from microradiography and polarized light microscopy. The findings show that when a 0.1 mM solution of rhodamine B dye was used, the deminaralized area correlated well with the mineral loss obtained from microradiography. However, the average lesion fluorescence best represented mineral loss, based on their hypothesis that rhodamine B penetrates the voids and pores created during enamel demineralization.[13]

In the present study, PLM images showed the linear depth of demineralization in the group A [non-fluoridated dentifrice (control)] was found to be 181.68±6.43 µm and remineralization was 122.07±4.61 µm and the difference 59.61±8.83 µm. In the group B it was found to be 181.51±6.38 µm and remineralization was 141.57±4.98 µm, and the difference 39.94±7.14 µm. In the group C it was found to be 181.54±5.84 µm and remineralization was 156.07±4.76 µm, and the difference 25.47±8.45 µm.

These findings suggest that the remineralization was more promising with the 1000 ppm fluoridated dentifrice, these results were similar to the values obtained by Celso Silva Queiroz.[14] who used 500ppm and 1100 ppm dentifrices on bovine teeth who used 1100 ppm dentifrice and casein phosphopeptide-amorphous calcium phosphate along with a placebo as control. [15] This was attributed to the capacity of the fluoride to improve the crystalline tooth structure, generation of fluorapatite and accelerate remineralisation. All these findings were also on par with the earlier studies showing that PLM is an advanced tool to diagnose and measure early enamel lesions.[16]

Conclusion :

Fluoride supplements show promising remineralization of the demineralised area of the tooth during orthodontic treatment and polarized light microscopy is one of the valuable tools for its diagnosis.

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