Microhardness and SEM analysis of artificial white spot lesion treated with Magnesium gel and diode laser in combination: An in vitro study

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ABSTRACT

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Objectives. The present study was accomplished to study and compare the ability of Magnesium chloride gel at different concentration and diode laser in combination with commercially available 1.23%APF in improving microhardness of WSL of permanent teeth.

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Methods. 42 intact premolars were collected and immersed in demineralization solution for four days to create artificial WSL. Then the samples were then randomly assigned into six groups: Group A: Control, Group B: 0.5%MgCl2 gel, Group C: 1%MgCl2 gel, Group D: 0.5%MgCl2 gel & 36 Diode laser, Group E: 1%MgCl2 gel & Diode laser and 1 Group F: 1.23%APF. Assessment of surface microhardness properties using a Vickers tester. The surface topography of samples from each group was examined using a SEM. Collected data were investigated using one-way ANOVA followed by Duncan post hoc test at $p \leq 0.05$.

Result. Non-significant difference in microhardness was noticed among treatment groups. 1%MgCl2 & diode laser showed The highest VHN value, followed by 0.5%Mg Cl2 & diode laser, 1%MgCl2 , 0.5%MgCl2 and 1.23% APF gel.

Conclusion. In permanent teeth, magnesium gel alone had similar effects as 1.23% APF gel through increasing Surface microhardness. Diode laser could enhance remineralization of WSL.

Keywords: Magnesium, Diode laser, White spot lesion

Introduction

One of the main goals of orthodontic treatment is to enhance the patient's esthetics so the presence of white spots not only affect the consequence of the therapy but also makes the affected teeth more likely to dental caries [1]. Orthodontic braces, arch wires and bands have rough surface which affect the natural cleansing ability of saliva and facial muscles, leading to the buildup of debris on teeth surfaces and making it challenging in maintaining optimal oral hygiene [2]. These lesions commonly arise during orthodontic treatment. They are typically occurring at the gingival and buccal area of teeth; the canines and upper lateral incisors are more susceptible to developing these lesions [3]. White spot lesion is subsurface enamel demineralization with an intact outer surface and is typically regarded as the initial stage of dental caries. Under suitable circumstances, it can be reversed, and several methods have been proposed for the early treatment such as remineralization, microabrasion, bleaching, laser, and resin infiltration [4-6] Currently, the majority of treatments focus on remineralization process [7]. Demineralized tooth surface, causing the affected enamel to lose its natural color and optical properties, result a chalky, white appearance [8]. WSL start to appear within four weeks after the placement of brackets, while regular caries lesions generally take around six months to develop [9]. Clinically the Prevalence of white spot lesions (WSLs) ranging from 2% to 97% based on different epidemiological studies [10].

One of the main objectives of modern dentistry is rebuilding the demineralized tooth structures of enamel and dentin through "remineralization" instead of the traditional approach of "drilling and filling" dental caries therapy [11].

Different materials and methods were proposed for the enhancement of remineralization and the most used current method is fluoride topical application for the restriction of initial caries lesions [12]. But Fluoride utilization has raised certain concerns and far from complete treatment since the formation of disorganized remineralization layers that is notably different from the natural enamel structure. These disorganized structures can compromise the mechanical properties of the remineralized layer [13], and Fluoride can't penetrate deep layers of enamel and also prevent deep penetration of calcium and phosphate due to its macromolecule structures, so its effect is limited to the enamel surface and to overcome of drawbacks of fluoride a new advanced materials have been introduced which either assist in incorporation of Ca+ and ph+ ions or changing the solubility of the hydroxyapatite [13,14]. Enamel is the hardest and the most durable tissue in the body made up of 96% mineral and ~3% water, and ~1% residual organic materials and small amounts of trace element such as magnesium (0.2 to 0.5 wt % Mg2+), the amount of magnesium in enamel increases as you move deeper into the tooth towards DEJ, also it is an important element for numerous physiological functions and organizing of metabolic and biochemical processes [15-17].

The gel has several benefits over other forms such as simple in construction, non-oily in composition, exhibit excellent adhesion to the targeted area, resistant to stressful conditions and biocompatible [18].

In the 1960s, laser has introduced in dentistry by Miaman and offer a new method for reducing dental caries [11,19].

Among different types of lasers, laser diodes have become quite popular in clinical practice due to several advantages such as they are small in size, their usage in the oral cavity is comfortable and low cost12. Although previous studies have established the effect of Nd: YAG laser on increasing the microhardness of enamel [20,21], however, studies have obtained contradictory outcomes about the use of diode laser [22-24]. A previous studies used MgCl2 as solution [16,25-27]. So the aim of study was to quantify and compare the effect of magnesium chloride salt in form of gel at different concentration and in combination with diode laser to a commercially available1.23% APF gel on microhardness restoring of white spot lesion.

Method

Tooth Selection and study design

After receiving the ethical approval from the research ethics committee in University of XXXX/ College Of Dentistry (UOM.DENT.23/64).The study was planned according to the consolidated criteria of Reporting Trials (CONSORT) as shown in (fig. 1). Thirty six healthy premolar teeth were extracted for orthodontic treatment purpose [28]. The teeth samples were collected from the outpatient clinic, specialized dental centers and College of Dentistry in the city. The participants ranged in age from (13-26) years old. The samples were cleaned and rinsed with normal saline before being preserved in a 0.1% thymol solution and subsequently evaluated under a stereomicroscope at magnification 10X to ensure the absence of any enamel defects [29,30]. The samples were cleansed from soft tissue debris [31]. Then polished with a mixture of pumice and water without fluoride using white rubber prophylactic cup at low speed [32]. The crowns detached from the roots in by using a diamond disc bur at high speed hand piece cooled with water approximately 1 mm above the cement-enamel junction and mounted in cylinder-shaped plastic ring (16mm diameter×5mm depth) by using cold cure acrylic resin and the outer buccal enamel surface of the tooth sample exposed [33]. The surfaces were painted with a nail polish except for a [4×4] mm section on the outer side of the surface to facilitate handling and subsequently, they were immersed again in deionized water [34]. Total sample size calculation based in previous study using G.Power1.3.9.7, in which effect size (0.60), α error (0.05) and power (0.50) as 7 teeth for each group [35].

Test Grouping:

The specimens were randomly assigned to 6 groups (n=7/group):

Group A: Control (demineralization teeth received no treatments other than being submerged in artificial saliva for 24hr).

Group B: 0.5%MgCl2 gel (applied 4 min., once a week for 4 week).

Group C: 0.5%MgCl2 gel & Diode laser (laser irradiation applied once for 60 sec. after gel application).

Group D: 1%MgCl2 gel (applied 4 min., once a week for 4 weeks).

Group E: 1%MgCl2 gel and Diode laser (laser irradiation applied once for 60 sec. after gel application).

Group F: 1.23%APF (applied 4 min., once a week for 4 weeks).

Creation of white spot lesion

Before the application of treatment protocol, the formation of artificial demineralized lesions were occurred by immersing each tooth sample in a separate container holding a 10 ml of a demineralizing solution (2.20 mmol/L calcium chloride, 2.20 mmol/Monosodium_ phosphate, 1 mol/L potassium hydroxide and 0.05 mol/L acetic acid; pH 4.4) for four days. This process continued until a clear and noticeable alteration in the appearance of enamel observed in both wet and dry conditions, taking score (2) according to International Caries Detection Assessment System (ICDAS score 2) [30].

Gel preparation

Two measures (0.25gm and 0.5gm) of $MgCl_2$ salt was taken, based on daily recommended dose 36. Then dissolved in 50 ml of distilled water in beaker. Different concentration of Xanthan gum containing gel was formulated by modified cold mechanical process, finally (6gm) Xanthan gum was chosen and added in to above solution. Put the beaker aside to allow the Xanthan gum to swell for 90 minute and then stirring should be done by using magnetic stirrer for 25 min. Other component methyl paraben and propyl paraben were dissolved in suitable solvent (propylene glycol or ethanol), also added with continuous stirring to obtain the gel at required consistency, then placed in plastic container and PH of the gel was adjusted at (7) by adding (NaOH) solution [37].

Gel Application

The gels (1.23% Acidulated phosphate fluoride, 0.5% Magnesium chloride, 1% Magnesium chloride) were applied in the uncovered area of the samples with a disposable applicator38 and left it for four minutes undisturbed on demineralized tooth surface, the excess amount of the gel was cleaned with cotton rolls. Then the specimens were rinsed with de-ionized water and immersed back in artificial saliva between gel applications. The fluoride gel and magnesium gels were applied once a week for four weeks except for control group which received no treatment other than being stored in artificial saliva only [39].

Combined Laser-Gel Treatment

A Diode laser was utilized to irradiate the exposed surface of the samples of group E. and F. The laser's tip was applied in non-contact mode at a distance of (5) mm from the enamel surface. Standardization the distance of the laser tip and perpendicularity from tooth surface was maintaining by using a customized tip that was attached to the laser hand-piece. Laser application was carried out immediately after applying gel that was remained on the enamel surface for four minutes after laser irradiation. Laser was performed using a 400-lm fiber equipped with a pulse mode diode laser (Epic X, Biolase Inc, USA) at 940 nm wavelength with pulse comfort CP2 in which pulse duration (1 millisecond) and pulse interval (1 millisecond) . The laser was operated at average power of 2 watts while the peak power 4 watts for a duration of 60 seconds [40].

Vicker Microhardness test

A Vickers microhardness machines are quick and easy approaches to assess the mechanical characteristics and physical structure of the tooth samples. The exposed surfaces of teeth were polished to make the surface of each samples smooth and flat and free from any defects for static indentation test and to remove the debris [41,42]. Readings were taken at the sound enamel, after creation white spot lesion and after treatment. The surface of samples were subjected for 15 sec.to a fixed load of 500 G to all teeth samples. The load and period were uniform for all samples. The length of two diagonal of each indication was measured optically using a stereomicroscope / light microscope at a 70X magnification and the Vickers hardness number (VHN) is calculated from the following formula:

- VHN=0.0018544 x L/d2
- L-load in gram force (kilogram)
- d-mean of the two diagonals (in mm2) [43].

SEM Analysis

SEM analysis was performed on one specimen for each tested group at the (MIRA3 TESCAN). The samples were covered with gold–palladium (Au–Pd) and air-dried. The surfaces of the samples were observed and the images were taken from the testing area on each sample at magnifications of 5000 x.

Statistical analysis

SPSS version 26 was performed for analyzing the data, including descriptive statistics as mean and standard deviation beside ANOVA and Duncan post hoc to find out the significant differences between the tested groups; where $p \leq 0.05$.

Result:

Table 1: represents means, standard deviations and Duncun analysis of the surface microhardness (kg/mm2) of the considered groups. For surface microhardness, 1%MgCl2 gel + Diode laser irradiation showed the highest value(141.1±8.6), while control group showed lowest mean value(82.3±1.3) as shown in figure (2).

ANOVA revealed a significant difference among the studied groups ($p \leq 0.05$), However, no significant difference was detected between 1.23% APF, 0.5%MgCl2 gel, 1%MgCl2, and 0.5%MgCl2 gel & Diode, 1%MgCl2 gel + Diode. But there were a significant differences between control and among other treated groups as show in table (1).

Discussion

Remineralization is the process of reconstitution the mineral elements that were lost when the tooth tissue demineralized, which needs the accessibility of the same ions to restore the lost or damaged enamel rods. Demineralization refers to decline in the mineral content that in turn, reduces the hardness of the enamel and marks the necessity of remineralization, although there was a clear improvement in microhardness of teeth samples treated with 1.123% APF gel, but because it's effect is restricted to initially improve surface layer remineralization, making full treatment early caries lesion difficult to achieve, it's macromolecules prevent deep penetration and exchange of ions into lesion's body zone [44,45], while magnesium ions penetrate deeper into enamel, forming more regular and rigid structure [16].

The aim of Modern non-invasive management of early-stage dental caries lesion is to emphasize non-invasive method and substitute fluorides with non-fluoride enamel remineralization systems, avoiding some of the hazards associated with fluoride treatment, necessity for invasive restorative procedures and also support treatments of patients at high risk of dental caries[46,47].

The reasons behind choosing diode laser are it's small in size, comfortable and low cost [12], also the diode laser compared to high power laser considered safer regarding to their effects on enamel surface morphology, it had less hazard effect on the tooth structure, less cracks, less rough enamel Surface, making enamel less liable to dental plaque and less susceptible to pulp necrosis [48**,**49]. The diode laser's low absorption coefficient within enamel showed a great advantage, facilitating fast elevation of surface energy during exposure and rapid temperature reduction upon termination of lasing. Notably, there were no adverse effects observed on the dental pulp. The rise in pulp chamber temperature when employing a diode laser at 1 to 2 W remains below the critical threshold of 5.5°C, deemed necessary to avoid irreversible pulp damage. Therefore, it is imperative to ensure that this threshold is not surpassed during treatment [50].
Magnesium (Mg) has been recognized as one of the cationic substitutes for calcium in the hydroxyapatite lattice, typically, the inclusion of Mg in synthetic hydroxyapatite is limited (up to 0.4wt% of Mg)51**.**

The most possible method explaining how Mg impacts into tooth enamel is dissolution and re-precipitation, through recurring cycles of demineralization and remineralization, in which Mg ions inhibition of crystal growth resulting in the formation of smaller in size crystals on the enamel surface [16] as shown in (Fig. 3, G–I). previous investigations displayed that teeth with enamel nan-ocrystallites are firmer, whiter and more challenging to fracture [52,53].

This study was carried out on the permanent teeth and explored the qualitative modifications occurring in sound enamel, post application of magnesium chloride gel at different concentration and combined with diode laser and compared their remineralization action by means of hardness and morphological representation.

The second null hypothesis was accepted, because all the treatment protocols showed no significant difference, but gradual improve in microhardness in relation to the control.

In microhardness result, the mean value of microhardness was 342.2 ± 15.9 for sound enamel at baseline which matches previous researched [54], and decreased to 71.3± 30.9 after creating demineralized WSL. Values measured after demineralization which were similar to the finding described in previous studies [40].

In microhardness result, all treatment groups were significantly different from baseline. Combination diode laser with magnesium gels didn't improve remineralization significantly but provide further enhancement in enamel microhardness. This agreed with [55], who Concluded that diode laser irradiation does not increase the remineralization effect of fluoride varnish and CPP-ACP paste, and also agreed with [23], who found that the Nanoseal® and Zamzam water showed to be superior than a diode laser for the treatment of initial enamel carious lesions.

Ultimately agreed with [56,57], who compared the usage of laser followed by fluoride with the usage of fluoride followed by laser and found no significant difference in the results.

Also according to [22], who found that diode laser irradiation along with sodium fluoride or MI Paste Plus did not produce any additional effects in improving remineralization of WSLs, Although the combined diode laser with Remin Pro® was effective in enhancing hardness of initial caries lesion.

But the results of the present study did not agreed to the findings by21, who concluded that the Vickers hardness value obtained after Diode laser irradiation is significantly higher than the Vickers hardness value obtained from an untreated surface, despite the fact that diode laser used for soft tissue.

Magnesium gel significantly increased the microhardness of demineralized enamel and this agreed with [26,27,58], who showed that the crystals get smaller in size and more irregular as the magnesium level rises and has an impact on the Hydroxyapatite crystal's morphology, size, organization and thermal stability of it, therefor offered brilliant mechanical properties.

Morphological changes in enamel after application of different treatment strategies were evaluated using scanning electron microscopy (SEM).

A typical honeycomb organization is shown in figure (4, A). Enamel was subjected to demineralization solution, which lead to surface etching patterns, thus inter-prismatic enamel protrudes, indicating that demineralization has taken place, and the enamel is eroded as in [59].

The enamel surface after exposure to magnesium chloride gel as in figures (4,B) and figure (4,D) and Acidulated phosphate fluoride gel as in figure (4,f) show the rough surface of enamel and partially obliteration of rod ends with slight dissolution of the inter-prismatic area, while in diode laser irradiation followed by magnesium chloride gel topical application respectively, display the formation irregularities, cracks and showed enamel melting effects as in figure (4,C) and figure (4.E) and this agreed with the result of [60].

Finally, the discrepancy observed between the outcomes of this study and those of previous studies may be related to the different laser parameters including pulse energy or duration, repetition rate, energy density, and period of irradiation application, also thickness or consistency of magnesium gel may decrease or inhibit the effect of laser. This study is a relative study, so there is a need for more laser and magnesium gel comparative studies to compare their performance clinically.

Limitation

Although the result of this study are helpful but possible drawbacks could be that the evaluation was conducted in vitro, more in vivo clinical trials are required to obtain more important information, as the in vitro circumstances do not replicate the dynamic and complicated biological oral system and lesion depth of treatment groups was not measured after demineralization and compared with post-treatment outcome.

Conclusion

Within the limitations of present study, Magnesium is as effective as fluoride in producing remineralization of white spot lesion of the permanent teeth when used in the form of a gel. All tested groups exhibited varying degrees of remineralization following the four week of treatment procedure. The combination of diode laser-irradiation with topical magnesium gel application and high concentration provide further improvement than magnesium gel alone or low concentration. Surface changes following remineralization by magnesium exhibits uniform surface characteristics as shown in SEM images.

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Conflict of Interest

We certificate that we do not have any financial or personal relationships that might bias the content of this work.

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Figure 1. illustrated Study Design: Consort flow design

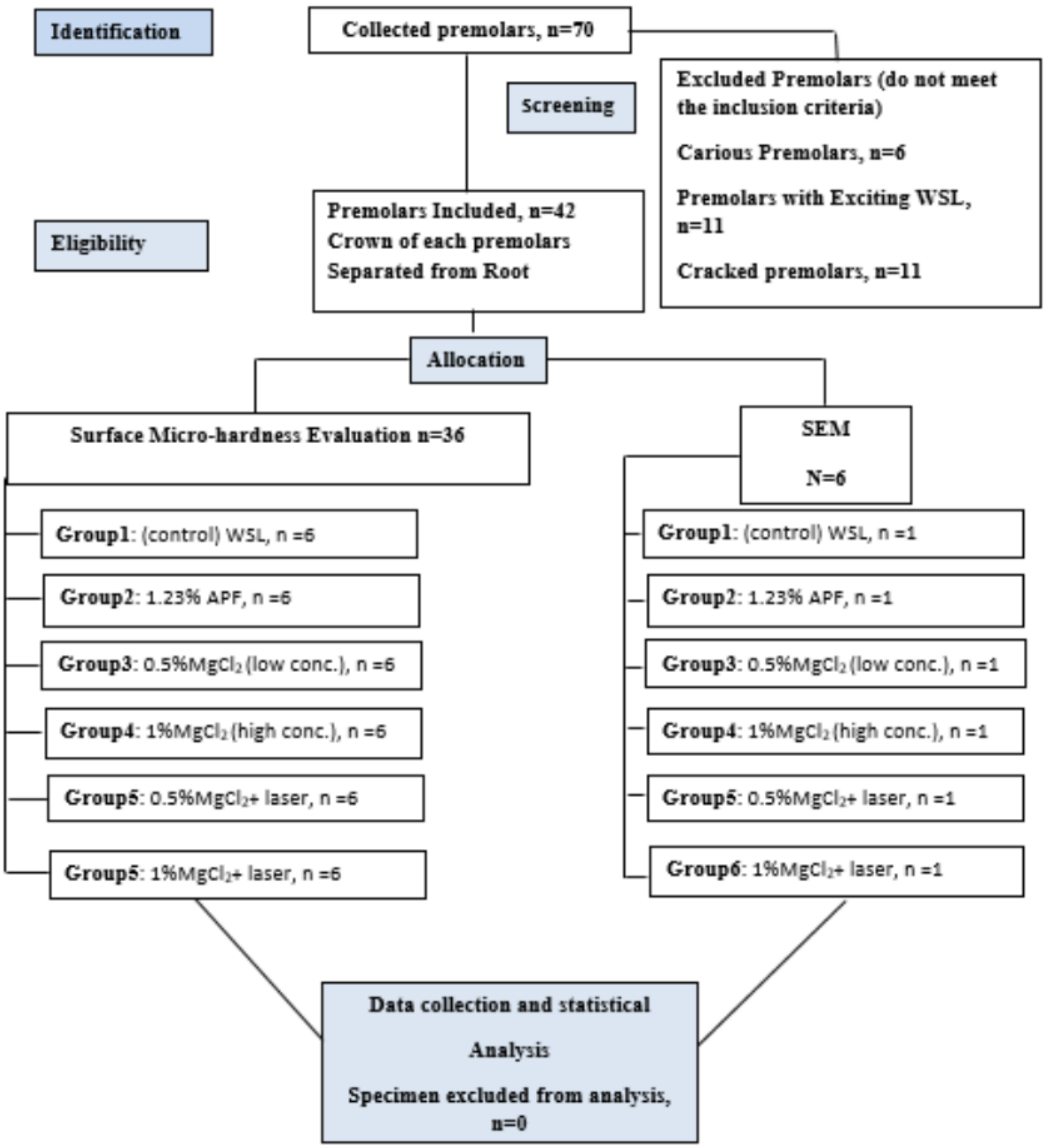

Table 1: descriptive statistics (Means, standard deviations) and Duncan post hoc of the surface Microhardness of all tested groups, $p \leq 0.05$

Groups	microhardness difference Mean ± SD	Duncan's test*	Anova
0.5%MgCl2 gel	127.5±32.3	B	0.043
0.5%MgCl2 gel + Diode laser	135±35.2	B	
1%MgCl2 gel	132.6±42.5	B	
1%MgCl2 + Diode laser	141.1±8.6	B	
1.23%APF	131.9±39.4	B	
Artificial saliva	82.3±1.3	A	

*same letter indicate a non-significant differences between groups.

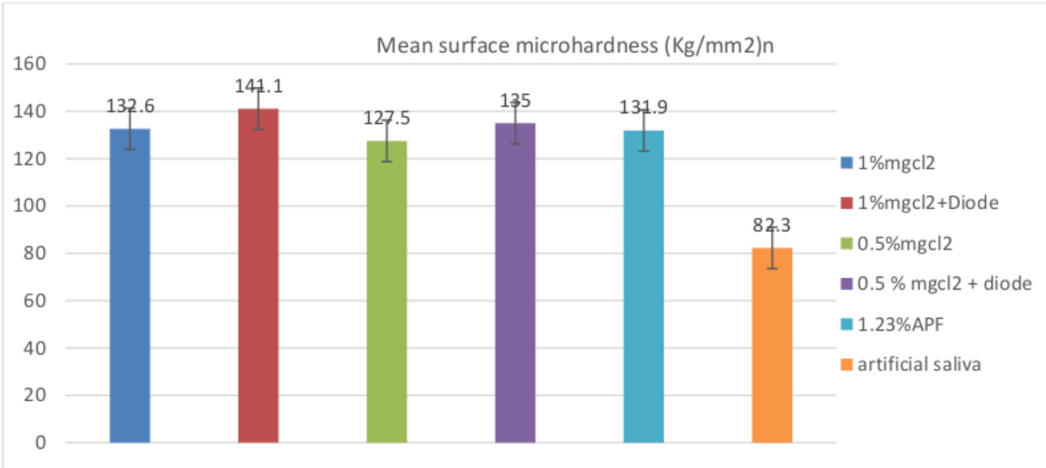

Figure 2: Surface microhardness results of the investigated groups

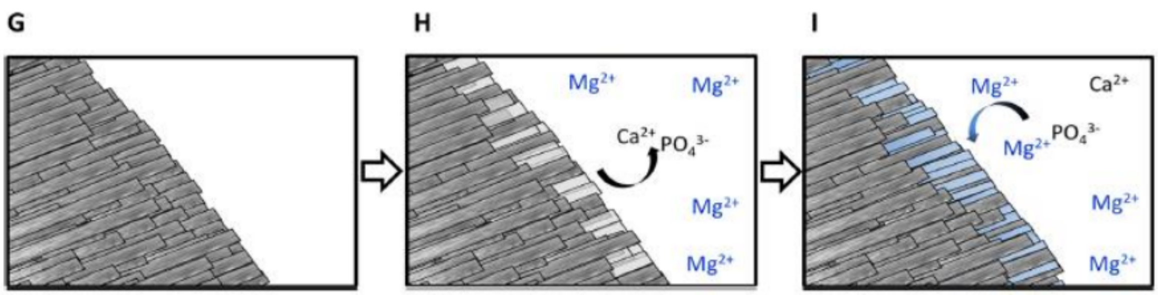

Fig. 3: Magnesium ion exchange. [26]

Figure (4), represent scanning electron micrographs illustrate the overall micro-morphology of the buccal surface in all groups at magnification (5000x).

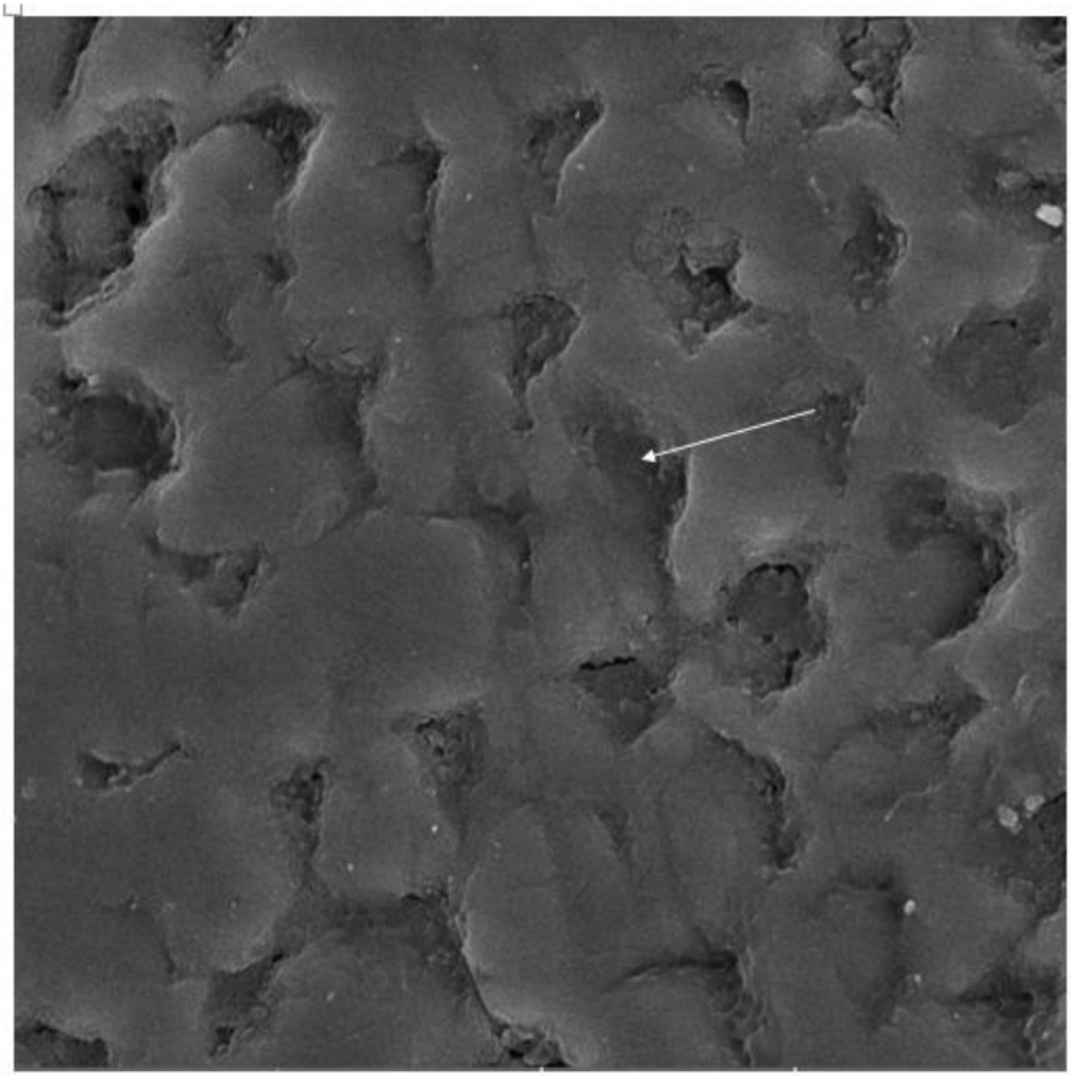

Fig. (4)(A), G1 (group treated with the demineralizing solution and then immersed in artificial saliva): an irregular surface with an early stage of destruction of enamel (white arrow) at 5000× magnification, with selective dissolution of the apatite crystals inside the prisms respectively.

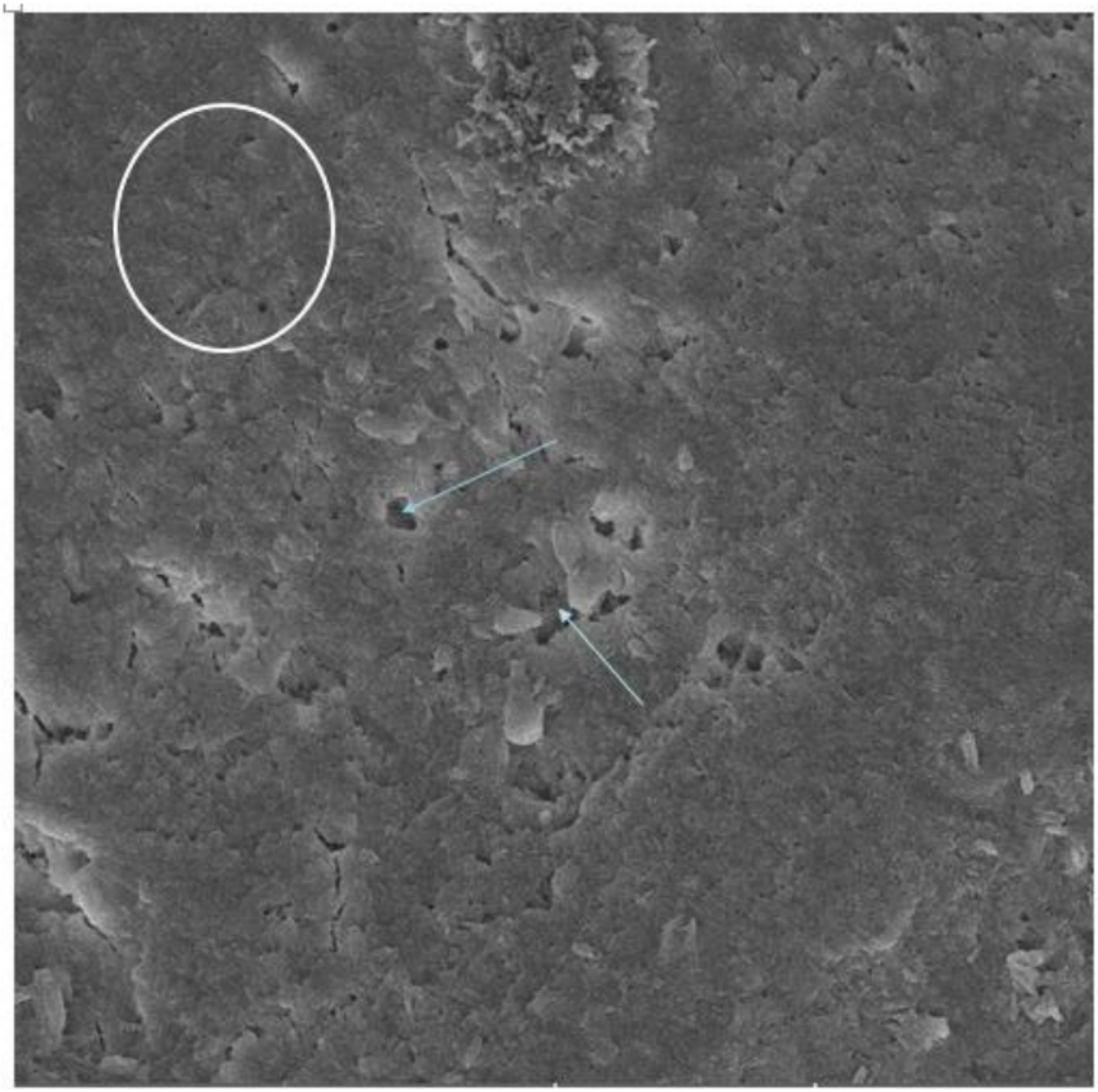

Fig. (4)(B): G2 (group treated 0.5%MgCl2 gel): An partial intact morphology of enamel surface (white circle) with the presence of a slight inter-prismatic dissolution (blue arrows) at 5000×.

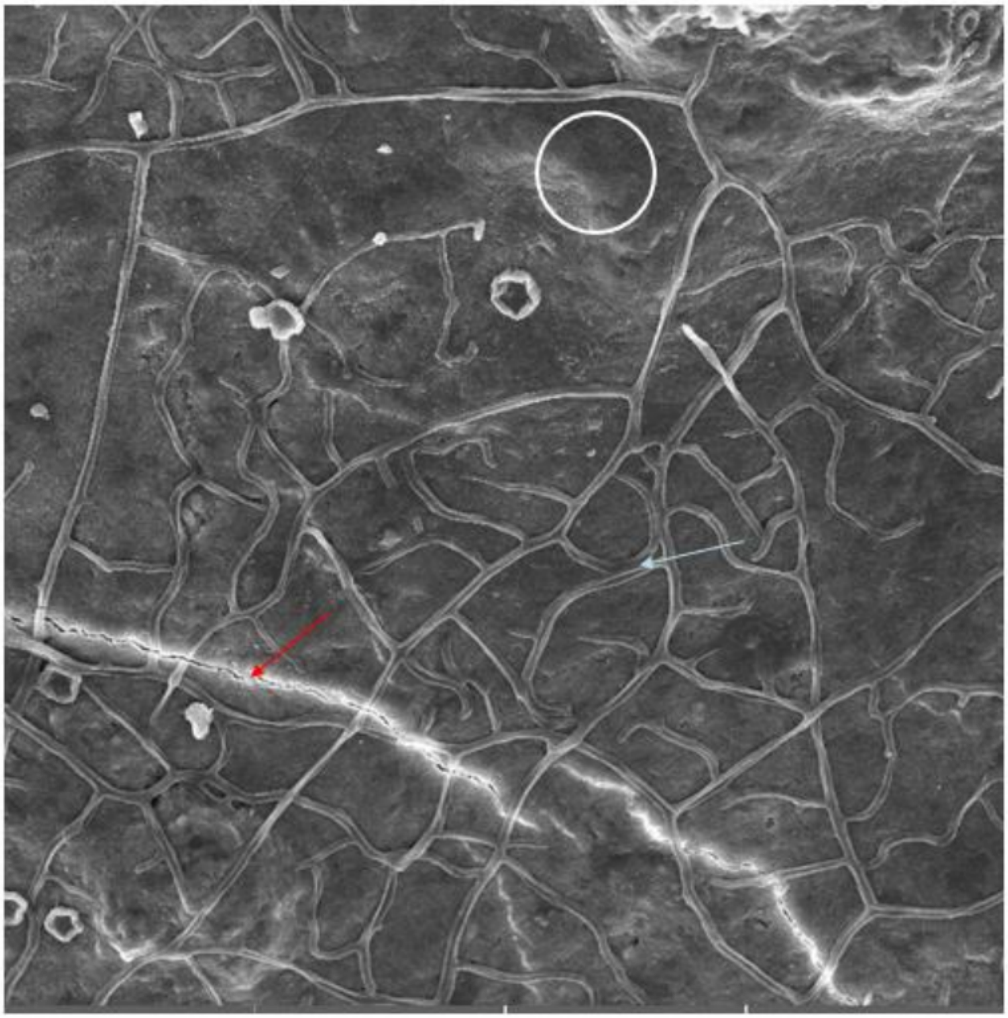

Fig. (4)(C): G3 (group treated 0.5%MgCl2 gel with diode laser): non-homogeneous surface with intact inter-prismatic areas (white circle) alternated by cracks (red arrows) and remnant of gel (blue arrow) in the enamel surface at 5000x.

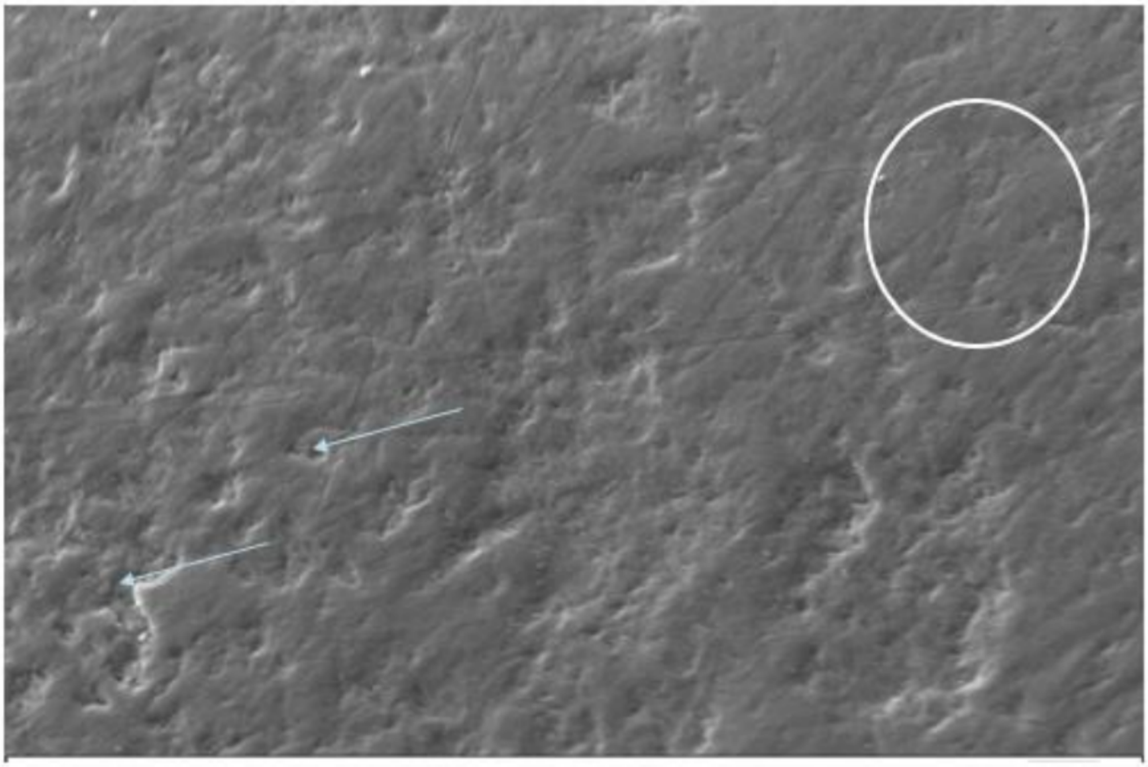

Fig. (4)(D): G4, G4 (group treated 1%$MgCl_2$ gel: an irregular surface with intact areas of enamel rod (white circle) at 5000× magnification and slight dissolution of the interprismatic area (blue arrows).

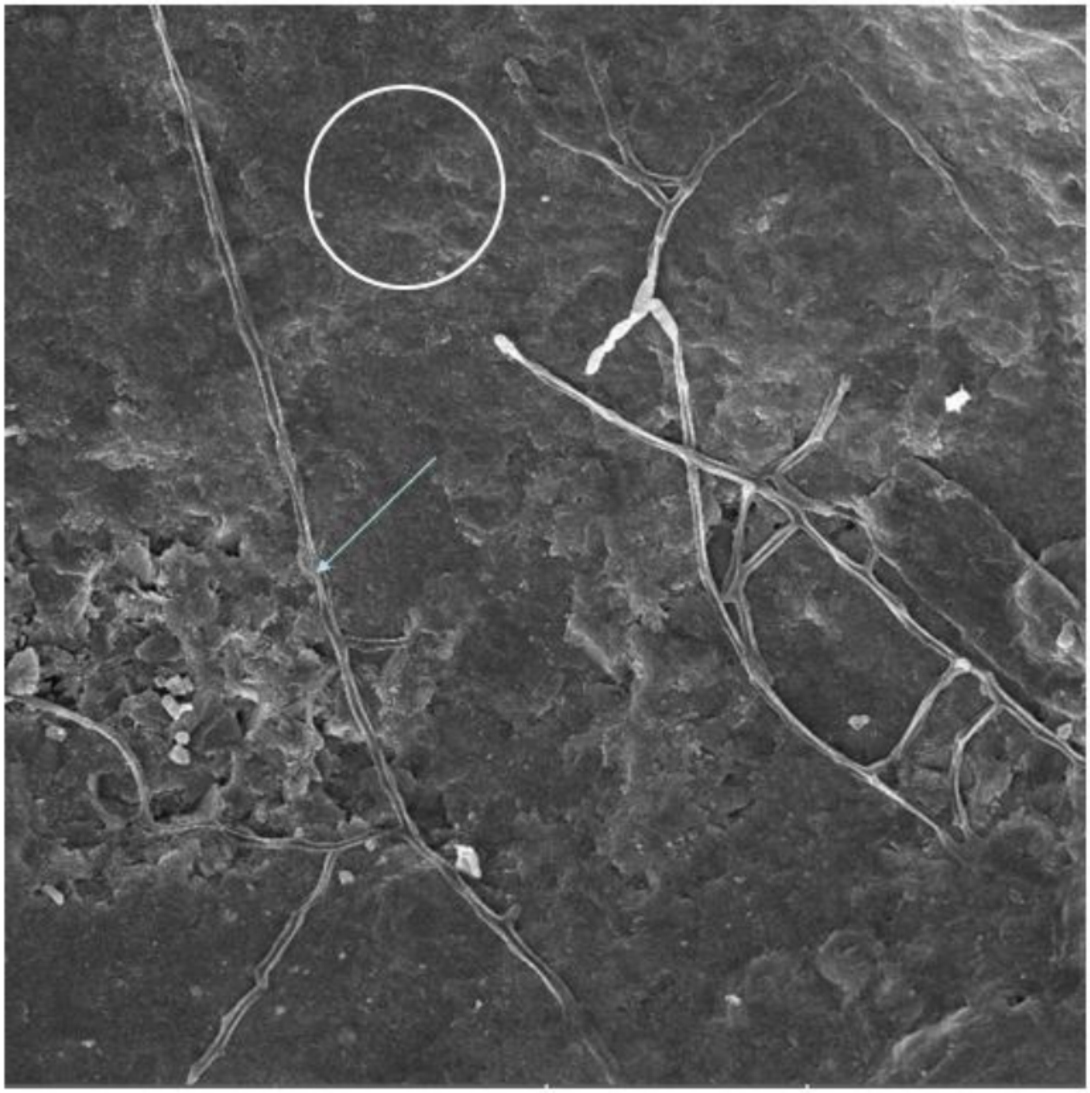

Fig. (4)(E): G5, (group treated 1%MgCl2 gel with diode laser): non-homogeneous surface with partially intact enamel rod areas (white circle) and remnant of gel on enamel surface(blue arrows) at 5000×, with slight dissolution of the inter-prismatic area

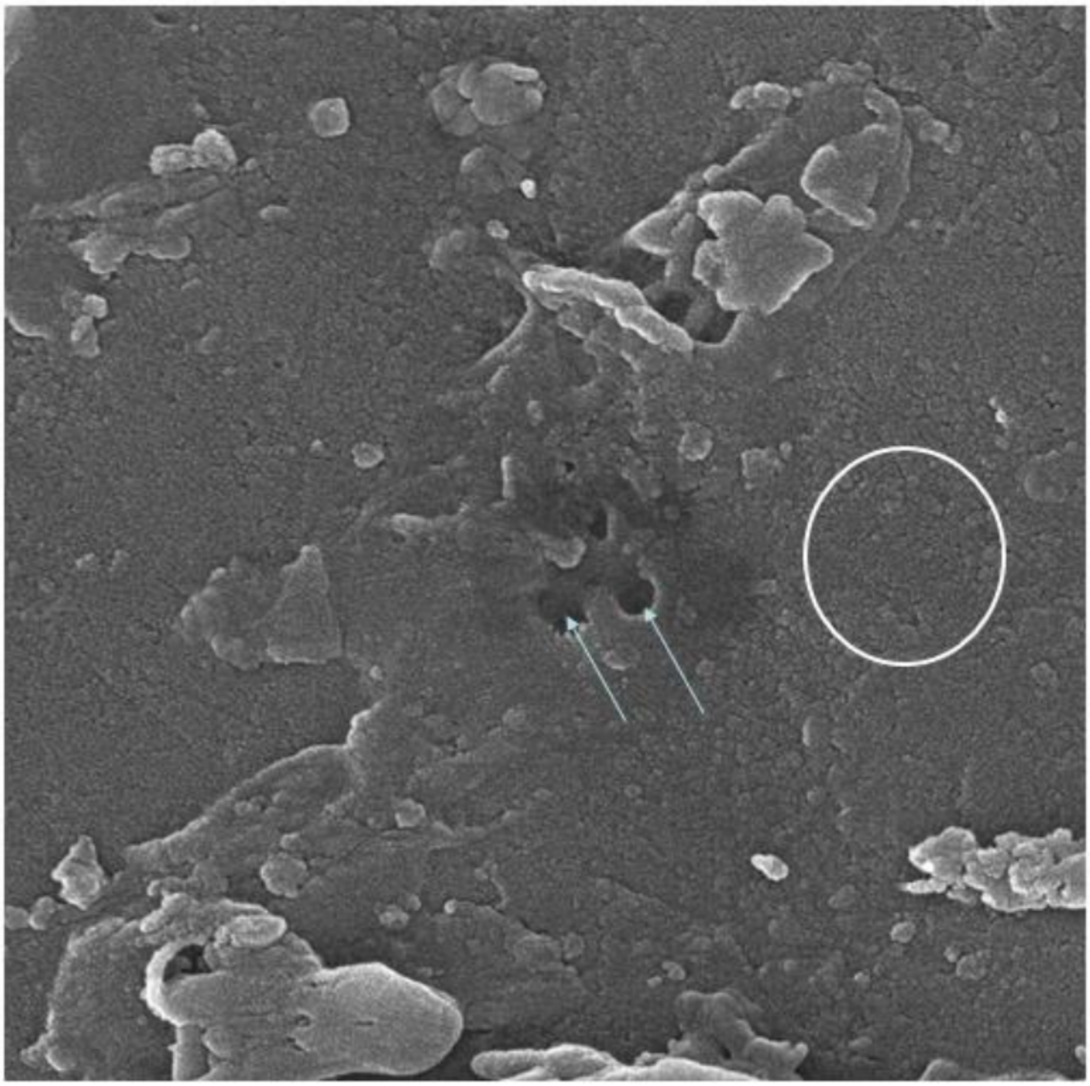

Fig. (4)(F): G6, (group treated 1.23%APF gel) an irregular surface with partially intact enamel rod (white circle) at 5000× with slight dissolution of the inter-prismatic area (blue arrows)