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Original Article

Effects of Magnesium Gel and Diode Laser in Combination on the Color Masking of Artificial White Spot Lesions: An in Vitro Study

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ABSTRACT

Objective: White spot lesions are one of the most common problem during and after orthodontic therapy. The present study was accomplished to evaluate the ability of Magnesium gel at different concentration and diode laser in combination compared with commercially available 1.23%APF in color restoring of WSL on permanent teeth. **Materials and Methods**: In this in vitro study, 42 human caries-free premolar teeth were randomely divided into 6 experimental groups: Group A: Control (artificial saliva), Group B: 0.5%$MgCl_2$ gel, Group C: 1%$MgCl_2$ gel Group D: 0.5%$MgCl_2$ gel and Diode laser, Group E: 1%$MgCl_2$ gel and Diode laser and Group F: 1.23%APF. The difference between the displayed color and the original color standard (ΔE) was assessed by colorimeter. The surface topography of teeth samples from each tested group was examined by a SEM. Collected data were investigated using one-way ANOVA followed by Duncan post hoc test at $p \leq 0.05$. **Result:** A significant difference in (ΔE) was noticed among groups (p= 0.006). Group (B) showed the lowest ΔE value (9.4±1.6), followed by group (D) (10.2±1.1), group (F) (10.4±2.6), group (A) (11.6±1.3), group (C) (12.9 ±2.3) and group (E) (13.2±1.3). **Conclusion**: 0.5% magnesium gel is as 1.23% APF gel in color restoring of white spot lesion. No synergistic effect between materials and diode laser.

Keywords: Magnesium, color, Diode laser, White spot lesion.

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INTRODUCTION

Orthodontic procedures aim not only to enhance the functional alignment of teeth but also to significantly improve the aesthetic appeal of a patient's smile, which is crucial for both health and self-confidence.

During orthodontic treatment, Plaque deposit creates a problem and different parts of the orthodontic appliance make oral hygiene procedure more difficult. Bacteria in the dental biofilm make acids, which remove the mineral (particularly calcium) from tooth enamel, resulting in white chalky appearance of tooth surface [1,2], in which the enamel crystals and the media inside the porosities have different refractive index causing light to scatter, giving the lesions a white or opaque look.

The healthy enamel has a refractive index of 1.62, while in white spot lesions, the enamel has many small holes filled with either water (refractive index of 1.33) or air (refractive index of 1.00) [3], and it can quickly advance to a cavitated carious lesion, if the patient is not continuing excellent oral hygiene practice. Although of many approaches have been proposed for control of WSLs, no specific material or approach has been selected as a perfect solution for this common concern. It is essential to identify the suitable treatment methods and the effectiveness of the re-mineralizing agents [1,2].

The management of white spot lesion typically involves non-invasive methods, like, re-mineralization as the primary treatment option, followed by minimally intrusive techniques like resin infiltration, or if necessary, more aggressive interventions such as micro abrasion, composite restorations, and in advanced cases, the options like veneers or crowns may be considered [4].

The color of teeth, which is influenced by a lot of factors such as light and the condition of the enamel surface, can be evaluated through two main methods: visual assessment and instrumental analysis. Numerous studies have concluded that the using of instrument for assessing tooth color yields a more precise and dependable measurement in humans compared to the visual approach [5].

In the early 20th century, Prof. Albert D. Munsell introduced a color roll that included the dimensions of value, Chroma, and hue. Hue is the characteristic that distinguishes one color from another and refers to the name of a color, such as red, yellow or orange. Chroma, on the other hand, refers to the strength, or saturation of a hue. Lastly, value refers to the relative lightness or darkness of a color [6].

Enamel is the outermost layer of the dental crown, and is the most mineralized and strongest tissue in the human body [7], and is made up of millions of enamel rods, surrounded by rod sheaths, and an inter-rod substance that helps bind them together. These enamel rods, which are the main structural elements [8].

It is composed of 96% inorganic materials, including hydroxyapatite crystals, which are long and thin. The remaining 4% is made up of organic matter and water. Enamel crystals are primarily made up of calcium and phosphorus, in the form of hydroxyapatite (HAp), which has the chemical formula $Ca_{10}(PO4)_6(OH)_2$. [9], with several trace elements such as sodium, carbonate, chlorine, magnesium, potassium, and fluoride [10].

In mature human teeth, the concentration of magnesium changes from 0.1% near the outer

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enamel surface to about 0.4% near the DEJ. The concentration of magnesium (Mg) present in the enamel fluid during the formation of apatite crystallites plays a significant role in regulating both tooth enamel development and mineralization [11].

Since the ability of magnesium in the reminerlization of teeth with its effect on physical properties [12, 13, 14, 15], and Fluoride's effect is limited to surface layer due to its macromolecule and can't penetrate into deep layers of lesion, additionally preventing deep penetration of calcium and phosphate [16], also the **diode laser is small in size,** comfortable and low cost [17, 18].

So, the aim of study was to study and compare the effect of magnesium chloride salt in a gel form at different concentration and in combination with diode laser with commercially available1.23% APF gel on color restoring of white spot lesion.

METHOD

Tooth Selection and preparation

The ethical approval was obtained and approved from the research ethics committee in University of Mosul / College Of Dentistry (UOM. DENT.23/64).

42 human premolar teeth were recruited for this study. Teeth with visible White spot lesion, cavities, cracks, discoloration on the teeth and erosion were excluded from the study. The teeth have been extracted for orthodontic reasons [19]. Cleaned with normal saline to remove blood and for initial cleaning before being stored in a 0.1% thymol solution, then subsequently valued under a stereomicroscope to ensure the absence of any enamel defects or dental restorations [20,21]. The soft tissue debris were cleaned from teeth samples using spoon excavator and tooth brush [22], and then polished with a mixture of pumice and water without fluoride for more realistic result using white rubber prophylactic cup at low speed [23]. Enamel specimens were created by separating the roots from the crown by diamond bur at approximately 1 mm above the Cemento-Enamel Junction (CEJ) and embedding in plastic ring (16mm diameter×5mm depth) by using cold cure acrylic resin where with the buccal surface facing upward [24]. The enamel surfaces were painted with a nail varnish excluding a [4 × 4] mm window. The samples were submersed again in deionized water at room temperature in glass containers for two days until testing procedure conduction [1]. Total sample size calculation based in previous study using G. Power 1.3.9.7, in which one tail option was selected and the effect size was (0.60), α error was (0.05) and power was (0.50). the final sample per group was 7 teeth for each group [25].

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%$MgCl_2$ gel (applied 4 min., once a week for 4 week).

Group C: 0.5%$MgCl_2$ gel & Diode laser (laser irradiation applied once for 60 sec. after gel application).

Group D: 1%$MgCl_2$ gel (applied 4 min., once a week for 4 weeks).

Group E: 1%$MgCl_2$ 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 procedure, the specimens were kept individually in 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 procedure continued until a clear and noticeable change in the appearance of enamel observed in both wet and dry conditions [21].

Artificial saliva preparation

Chemical materials were obtained from the Main laboratory in the University after getting the formal transaction to deal and barrow the chemical materials. According to the laboratory general safety rules, chemical materials (3.9 mmol/L Na_3 PO_4, 4.29 mmol/L NaCl, 17.98 mmol/L KCl, 1.1 mmol/L $CaCl_2$, 0.08 mmol/L $MgCl_2$, 0.5 mmol/L H2 SO_4 and 3.27 mmol/L $NaHCO_3$) were handled after weighting it by accurate digital balance, the digital balance should be calibrated at first, then mixing it in one liter of distal water until it completely dissolved. pH meter (pen type) was used to check the pH of the prepared solutions [21].

Gel preparation

Two concentrations (low conc. 0.5% and high conc. 1%) of MgCl2 salt were measured in accordance to the daily recommended intake [26]. Subsequently, they were then dissolved in 50 milliliters of distilled water in a beaker. Various amounts of Xanthan gum-containing gel were prepared using a modified cold mechanical process and finally (6) grams of the gum powder were selected and added to the aforementioned solution. The beaker was set aside to allow the Xanthan gum powder to swell for 90 minutes, following which stirring was performed using a magnetic stirrer for 25 minutes. Methyl paraben and propyl paraben, dissolved in a suitable solvent (propylene glycol), then were added with continuous stirring to above mentioned to achieve the desired consistency of the gel. The mixture was then transferred to a plastic container, and the pH of the gel was adjusted to 7 by adding NaOH solution [27].

Gel Application

The three types of gels (1.23% Acidulated phosphate fluoride, 0.5% Magnesium chloride, 1% Magnesium chloride) were administered in the exposed areas of the teeth samples with a one-use applicator [28] and allowed it to sit undisturbed for (4) minutes on demineralized tooth surface, Any gel excess material was removed using cotton rolls. Subsequently, the specimens were rinsed with de-ionized water and returned back into artificial saliva between gel treatments. The fluoride and magnesium gels were applied weekly for 4 weeks except for control group which received no treatment other than being submersed in artificial saliva [29].

Combined Laser-Gel Treatment

A Diode laser was employed to irradiate the exposed area of the samples in groups C. and E. The laser's tip was positioned at a distance of (5) mm from the enamel surface. To ensure consistent distance and perpendicularity from the tooth surface, a customized tip attached to the laser handpiece was used for standardization. Laser application was conducted immediately after applying gel that was stayed on the enamel surface for 4 minutes post-irradiation. Laser utilized a 400-lm fiber with a pulse mode diode laser (Epic X, Biolase Inc, USA) emitting at a wavelength of 940. The laser operated at a power of 2 watts of duration of 60 seconds [30].

Color Measurement

The baseline and after treatment color measurements for all specimens was assessed by using a colorimeter (The measuring head has an 4-mm-diameter opening) [31,32]. Three independent measurements were taken with the colorimeter's head positioned at the center of the buccal surface of each sample. The instrument then automatically calculated the average of these readings, which were subsequently employed in the overall data analysis [33]. The colorimeter device directed perpendicular the tooth sample, and contact with tooth surface to prevents the effect of external illumination on the measurement surface also to prevent positioning errors [32]. Color measurements were quantified in terms of the coordinate value L*,value a* and value b* established by the Commission International de l'Eclariage (CIE) which locates the color of an object in 3D color space. The CIE L*a*b* system is composed of three components: L* represents the lightness while a* and b* represent red–green and yellow–blue color axes, respectively [39, 40]. The color difference was calculated using the following equation: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½}$ [34].

ΔE after remineralization = $[(L\ s\text{-}L\ T)^2+(a\ s\text{-}a\ T)^2+(b\ s\text{-}b\ T)^2]^{1/2}$

SEM analysis: SEM analysis was performed on one specimen for each studied group at the (MIRA3 TESCAN). The samples were covered with gold–palladium (Au–Pd) and air-dried. The surfaces of the teeth samples were examined at magnifications of (200 kx).

Statistical analysis

The data of the present study were collected, tabulated, and then subjected to statistical analysis using SPSS to interpret the significant difference between groups. Statistical analysis was performed using T-test and ANOVA for comparison between Mean and SD of six groups. All data were tested for normality using the Shapiro-Wilk test. For data following a normal distribution, a paired-sample t-test was used to test for changes within the same sample, and the two-sample t-test was used to compare between different groups. The significance level was set at $p < 0.05$.

RESULT

Table 1 denotes means, standard deviations, and Duncan analysis of the color difference among the investigated groups. Demineralized enamel treated with 1%$MgCl_2$ gel with diode laser irradiation showed highest mean value (13.2±1.3), while demineralized enamel treated with 0.5% $MgCl_2$ gel showed lowest mean value (9.4 ±1.6). ANOVA stated a significant difference among the different groups ($p \leq 0.05$). There were a significant differences between 1.23% APF, 0.5%$MgCl_2$ gel, 1%$MgCl_2$, and 0.5%$MgCl_2$ gel & Diode, 1%$MgCl_2$ gel + Diode. But no significant differences between control and among other groups as show in figure 1.

Table 2 denotes means, standard deviations, and Duncan analysis of the lightness mean value difference among the investigated groups. Demineralized enamel treated with 1%$MgCl_2$ gel showed highest mean value (-7.97±3.2), while demineralized enamel treated with 0.5% $MgCl_2$ gel showed lowest mean value (-3.76±1.7). ANOVA stated a significant difference among the different groups ($p \leq 0.05$), as shown in figure 2.

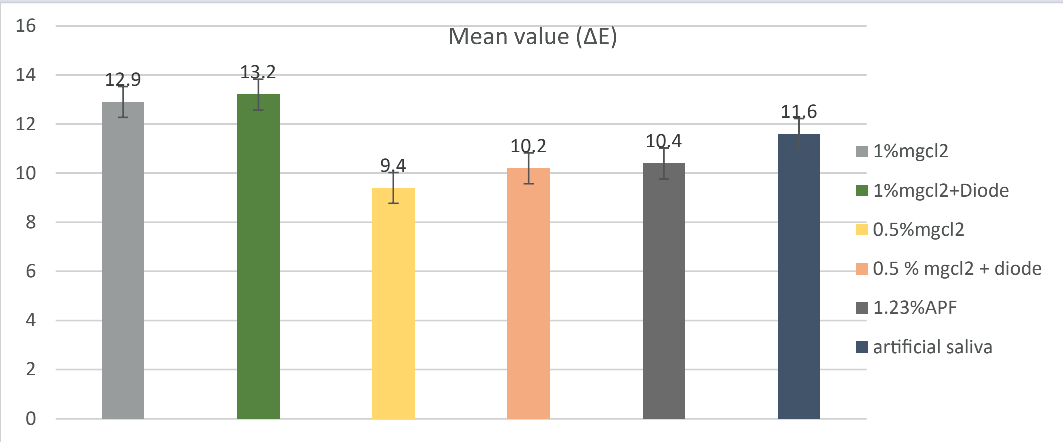

Figure 1: Showing Color changes (ΔE) of the investigated groups.

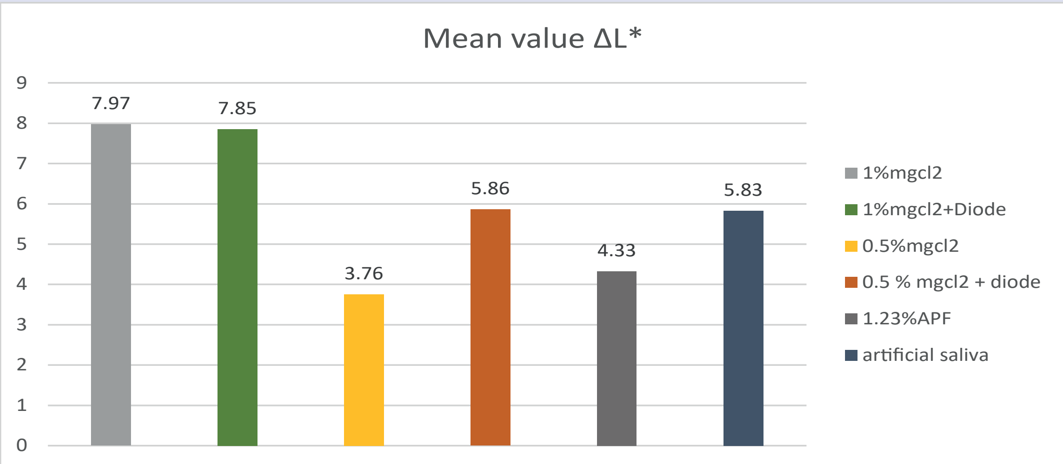

Figure 2: Showing Color changes (ΔL*) of the investigated groups.

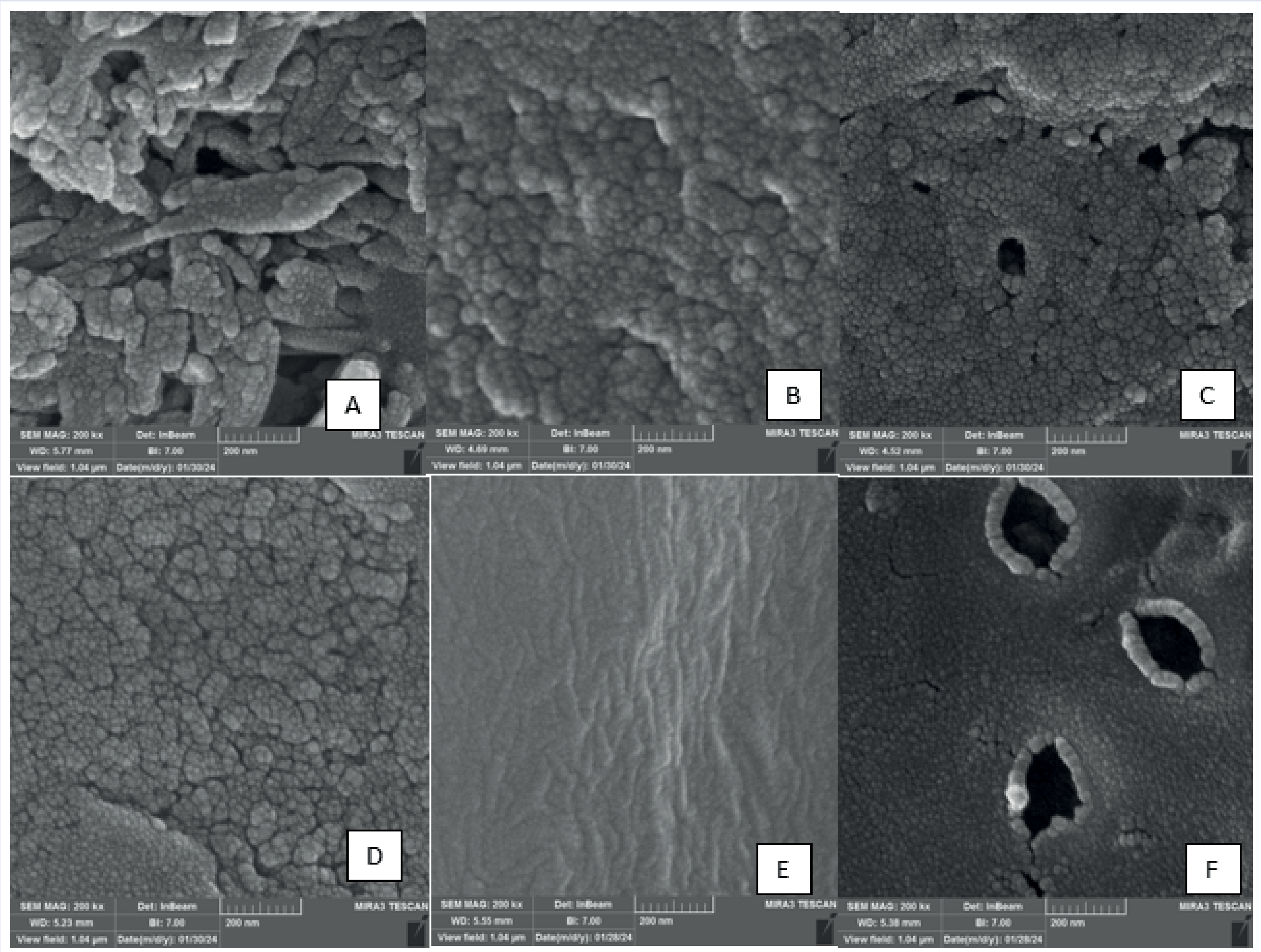

Figure 3: (A) Artificial saliva group (control), (B) 0.5% $MgCl_2$ gel group, (C) 0.5% $MgCl_2$ gel and diode laser, (D) 1% $MgCl_2$ gel group, (E) 1% $MgCl_2$ gel group and diode laser , (F) 1.23% APF gel.

Table 1: Means, standard deviations and Duncan's of the Color changes (ΔE) of the studied groups (p < 0.000).

Groups	Color difference (ΔE) Mean ± SD	Duncan's test	Anova
0.5%$MgCl_{2\ gel}$	9.4 ±1.6	A	
0.5%$MgCl_{2\ gel\ +}$ Diode laser	10.2±1.1	A	
1%$MgCl_{2\ gel}$	12.9±2.3	B	0.006
1%$MgCl_{2\ +}$ Diode laser	13.2±1.3	B	
1.23%APF	10.4±2.6	A	
Artificial Saliva (control)	11.6±1.3	AB	

Table 2: Means, standard deviations and Duncan's of the Color changes (ΔL*)of the studied groups (p < 0.000) .

Groups	Color difference (ΔE) Mean ± SD	Duncan's test	Sig.
0.5%$MgCl_{2\ gel}$	3.76±1.7	B	
0.5%$MgCl_{2\ gel\ +}$ Diode laser	5.87±2.3	AB	
1%$MgCl_{2\ gel}$	7.97±3.2	A	0.028
1%$MgCl_{2\ +}$ Diode laser	7.84±2.1	A	
1.23%APF	4.33±3.7	B	
Control	5.83±0.6	AB	

Figure 3 represent scanning electron micrographs illustrate the overall micro-morphology of the buccal surface in all groups at magnification (200 kx).

DISCUSSION

The chalky appearance or change in color to opaque is as a result of the process of demineralization, which increases the porosity at subsurface level and affects the way light is absorbed on that part and the remineralization is the process of reconstitution the mineral elements that were lost when the tooth tissue demineralized to restore the lost or damaged enamel rods and respectively restore color of the teeth. Numerous strategies have been suggested to enhance the aesthetics of white spot lesions. Among these, remineralization emerges as the primary approach to address these lesions as it being a natural process, facilitates the partial reverse of initial caries lesions [35].

Willmote, 2004 [36], studied how fluoride and saliva affected white spots (WSL) on teeth after braces were removed. He found that the white spots got smaller over six months after treatment and reduced to approximately half the original size, however, but they were not completely cured.

Ogaard (1988) [37], argues that the direct application of high fluoride concentrations for treating white spot lesions may yield unsatisfactory aesthetic outcomes, Also would arrest the lesion and prevent complete repair. The outermost layer of enamel is likely to undergo remineralization and even hypermineralization, hindering the movement of Fluoride ions towards body zone of lesion. Consequently, this interference affects the reflection of light on the enamel's hard surface. The aesthetics of teeth are important for assessing the visual outcome of a finished orthodontic procedure [5].

Colorimeters can measure the slight changing in color more accurately than the naked eye. Color measurement employs CIELAB color system which is very common, it provides a suitable, standardized method for analysis of (ΔE) values specifically and was chosen due to it is accurate, reliable, dependable and more precise outcomes in shade change [38].

Healthy premolar teeth were carefully selected as a samples of this study, which were collected from orthodontic clinics and dental centers. The aim was to investigate the potential effects of magnesium gel at different concentration with diode laser and compare the result with commercially available 1.23%APF. On the color of the teeth.

The WSL was formed in the center of the buccal surface to reduce the difficulty of measurement on the convex surface of premolars teeth [39].

The middle third area of the buccal surface was chosen to collect the data of color, due to it Comprises a moderate percentage of the features between cuspal and cervical thirds. This decision was informed by a research conducted by[40], who examined the difference between the basic composition of the cuspal and cervical area of human teeth enamel and their finding indicated lower calcification level (Ca and P wt %) at cervical compared to the cuspal areas. Additionally, the study noted the prevalence of WSL associated with fixed orthodontic appliance treatment, particularly at the bracket sites where food impaction occurred, ranging from 2% to 96% [41], and also The color of central third of tooth surface, usually indicates the color of the overall tooth structure, according to a number of prior studies. The cervical area is affected by the dispersed light from the nearby gingival tissues, but the incisal area is translucent to the light and consequently influenced by its own background [42].

Irradiation dental enamel with laser induces specific morphological and structural alterations, leading to enhanced acid resistance of the treated enamel and modifications in its resistance to acid and permeability [43].

Depending on the temperature attained through laser irradiation, varied effects can be obtained, particularly concerning the solubility of enamel. The mildest level of enamel acid dissolution occurs following heating to 300–350°C, with suggestions that this effect is produced from the denaturation and swelling of the organic matrix, thereby blocking the diffusion routes within the enamel. Beyond 200°C, there's a depletion of carbonate, which may enhance acid resistance. Microscopic voids formed due to the loss of water or carbonate, along with organic substances, could impede demineralization by filling with dissolved ions. Elevating pyrophosphates due to heating to 200–400°C significantly diminishes the dissolution rate of hydroxyapatite and the utilization of diode laser was chosen in this study, due to it has numerous unique features such as, small in size (footprint), comfortable, because of the optic fibres and cost effectivness in comparison to high power lasers, yet yielding similar effects in soft tissue comparable to the Nd: YAG laser, Notably, the wavelength of diode laser (970nm) is in proximity to that of Nd: YAG laser (1064 nm) [50], also the diode laser compared to high power laser considered safer regarding to their effects on enamel surface morphology, had less hazard effect on the tooth structure, less cracks, less rougher enamel Surface, making enamel less liable to dental plaque and acid and less susceptible to pulp necrosis [44,43].

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. There is quick elevation of surface energy during diode laser exposure and rapid reduction of temperature at end of lasing, due to low diode laser's absorption coefficient within the enamel [45].

Magnesium (Mg) has been acknowledged as one of the cationic alternatives to calcium within the hydroxyapatite lattice [46]. It has ability to incorporate with fully mature permanent [12] and primary teeth as in prior studies [13] and the most possible method explaining how Mg impacts tooth enamel is dissolution and re-precipitation, through recurring cycles of demineralization and reminerlization [12].

Mg2+ ions slows the growth of the crystal by competing with calcium ions at the growth site during mineralization, thus impacting in the physical and chemical stability of crystals. Consequently, Mg2+ acts as a competitive inhibitor, guiding the formation of narrower crystal columns and facilitating a highly organized arrangement, ultimately enhancing the hardness of mineralized tissues. Notably, the increase in the concentration of Mg2+ on the enamel surface leads to a significant rise in enamel nanohardness [11].

Previous investigations displayed that teeth with enamel nano-crystallites are firmer, whiter and more challenging to fracture [47, 48] Therefore, effect of Mg on structural changes in sound permanent premolars and diode laser in combination are discussed as follow.

Color resotring was obtained by a significant decrease in ΔE values. The best white spot color improvement was observed in the 0.5%$MgCL_2$ group at (9.4 ±1.6). This result indicates that the lesion was improved closer to the initial enamel color which represent clinically acceptable appearance and brings about patient satification. This is likely since 0.5%$MgCl_2$ is able to fill the subsurface enamel pores [49].

This study shown that the difference in the white spot color improvement between the fluoride gel application group and 0.5%$MgCl_2$ gel topical application group was not statistically significant but significantly different from 1% $MgCl_2$ gel and show increase in ΔE mean value (12.9±2.3).

The alteration in the L* value of the CIELAB system seems to be the most important parameter to perceive the color restoring of (WSL). As L* value decrease and b* increase indicate decreasing in lightness of WSL and restoring color of tooth [52].

As in 1%$MgCl_2$ gel application, the L* value increase and the mean value (7.97±3.2) as shown in table (3), and this agreed with previous study [12], who concluded that the treatment of erupted tooth enamel with hypersaline solution with $MgCl_2$ salt leading to increase enamel shade and appear whiter , while in 0.5% $MgCL_2$ and 1.23% APF the L* value decrease and the mean value of 0.5% $MgCL_2$ (3.76±1.7) and 1.23%APF (4.33±3.7) as shown in table (3), which agreed with previous study [50].

SEM Examining teeth sample without causing harm, generating 3D images, and producing a sensitive and comprehensive analyses [51].

SEM images of the test samples showed varying degree of remineralization, figure (3.A) demineralized enamel placed in artificial saliva without applying any remineralization agents showed negligible amount of remineralization, resulted from destruction of enamel surface when placed in demineralizing solution, while groups treated with 0.5%$MgCl_2$ gel, 0.5%$MgCl_2$ gel and diode, 1%$MgCl_2$ gel and 1%$MgCl_2$ gel and diode as shown in figure (3, B, C, D, E) showed a new regular smooth remineralization layer indicating color resorting. Figure (3, F) showed covered with a new remineralization layer around the porous surface.

Although this outcome, treated groups did not entirely take the colour elements return back to the baseline reading. Clinically, the color changes have considerably improved which has a big effect on esthetic.

Finally, the observed difference between the results of this study's laser application and those of prior studies might be associated to the differences in laser parameters, variations in magnesium gel thickness or consistency, gel manufacturing techniques, and the utilization of water- and air-cooling during treatment could contribute to these differences. Further comparative studies are needed to improve their performance and provide system offers optimal efficacy within shorter time frames and low costs [49].

The first hypothesis was accepted, as there was a significance difference between treatment groups.

LIMITATION

The current study has some limitations that need to be known. The use of extracted teeth which was difficult to completely reproduce the complex oral environment of the oral cavity, so more in vivo study are required, the limited sample size, and the lesion depth of treatment groups was not measured after demineralization and compared with post-treatment outcome. Future studies are recommended to study the effect Diode and other types of lasers with magnesium chloride gel with different periods of time and powers on artificially created white spot lesion.

CONCLUSION

The remineralizing substance was certainly efficient in handling white spot lesion. Topical application of 0.5% $MgCl_2$ provide color improvement to the baseline color as 1.23%APF, while 1% $MgCl_2$ gel change the color of WSL and make it more whiter. Diode laser application after magnesium chloride gel topical application provide further improvement but did not have a significant effect on enamel color enhancement as no synergistic effect between materials and diode laser. Scan electron microscope image represent smooth regular appearance of enamel rod. Magnesium gel could be used clinically in the treatment of white spot lesion.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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