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The chromatograph evaluation of white spot lesion treated with magnesium gel and diode laser: an in vitro study

Rasha M. Salim, Sarmad S. Salih. Al Qassar, Aisha A. Qasim

Department of Pedo Ortho Prevention, College of Dentistry, University of Mosul, Iraq

Sarmad S. Salih. Al Qassar **ORCID ID:** 0000-0002-2758-761X

ABSTRACT

Objective. White spot lesions are one of the most common concerns of 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.

Methods. 42 extracted sound upper premolar teeth were recruited for this study. WSL was artificially induced, until it appears in wet and dry condition on buccal surface of each tooth. Different approaches have been utilized for treatment WSL. 6 experimental groups were involved: Group A: Control, Group B: 0.5%MgCl2 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. In present study, topical application of 0.5% $MgCl_2$ gel ensure color reinstating compared to other groups.

Keywords: color, diode laser, magnesium, white spot lesion

INTRODUCTION

The primary goal of orthodontic procedures is to achieve both functional occlusion and pleasing aesthetics. The aesthetic appearance of teeth plays a crucial role in evaluating the outcome of orthodontic treatment. 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 (RI)of 1.62, while in white spot lesions, the enamel has many small holes occupied with either water (RI 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

Corresponding author:
Sarmad S. Salih. Al Qassar
E-mail: sarmadsobhi@uomosul.edu.iq

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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 Ca10(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 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. Additionally, Mg ions exert influence on the synthesis of apatite by competing with calcium (Ca) ions at sites of crystal growth, so Magnesium has important effect on crystal structure and mechanical characteristics, despite being united into apatite at relatively low levels [11]. Previous investigation utilized MgCl2 as solution and its effect on physical properties [12-15]. As Fluoride 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] and due to the diode laser is small in size, comfortable and low cost [17,18]. So the aim of study was to quantify and compare the effect of magnesium chloride salt in a gel form at different concentration and in combination with diode laser to a commercially available1.23% APF gel on esthetic restoring of white spot lesion.

METHOD

Tooth Selection and preparation

The ethical approval from the research ethics committee in University of XXXX / College Of Dentistry was obtained and issued (UOM.DENT.23/64), at 14/11/2023.

42 human premolar teeth were recruited for this study. The teeth have been extracted for orthodontic reasons [19], and cleaned with normal saline 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 [22], and then polished with a mixture of pumice and water without fluoride using white rubber prophylactic cup at low speed [23]. Enamel specimens were created by separating the roots from the crown 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 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%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 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 Central laboratory in the University, following the general safety rules of the laboratory, chemical materials (3.9 mmol/L Na_3PO_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 H_2SO_4 and 3.27 mmol/L $NaHCO_3$) were weighting by accurate digital balance, then mixing it in one liter of distal water until it totally dissolved. pH meter (pen type) was utilized to know the pH of the prepared solutions [21].

Gel preparation

Two doses (low conc. 0.5% and high conc. 1%) of $MgCl_2$ 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 to 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 measured in terms of the coordinate value L*, value a* and value b* established by the Commission International de l'Eclariage (CIE) which positions the color of an object in 3D color space. The CIE L*a*b* system is made of three components: L* denotes 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 information for the present investigation was gathered, organized in tables, and subsequently analyzed statistically employing SPSS software to know the significant difference between groups. Statistical analysis was made using T-test and ANOVA for comparison between Mean and SD of six groups. All data were examined for normality using the Shapiro-Wilk test. The significance level was set at $p < 0.05$.

RESULT

Table 1 represents 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 represents means, standard deviations, and Duncan analysis of the lightness mean value difference among the studied 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 showed a significant difference among the different groups ($p \leq 0.05$), as shown in Figure 2.

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

DISCUSSION

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. Remineralization,

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	p-value
0.5%MgCl2 gel	9.4 ±1.6	A	0.006
0.5%MgCl2 gel + Diode laser	10.2±1.1	A	
1%MgCl2 gel	12.9±2.3	B	
1%MgCl2 + Diode laser	13.2±1.3	B	
1.23%APF	10.4±2.6	A	
Artificial Saliva (control)	11.6±1.3	AB	

Means followed by same letter in the column do not differ statistically according to the Duncan's.

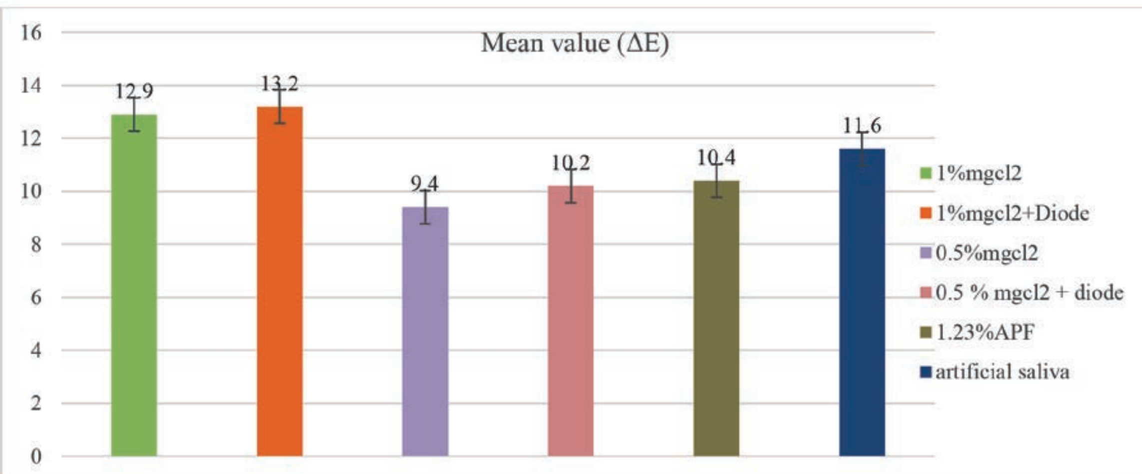

FIGURE 1. Color changes (ΔE) of the investigated groups

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	p-value
0.5%MgCl2 gel	-3.76±1.7	B	0.028
0.5%MgCl2 gel + Diode laser	-5.87±2.3	AB	
1%MgCl2 gel	-7.97±3.2	A	
1%MgCl2 + Diode laser	-7.84±2.1	A	
1.23%APF	-4.33±3.7	B	
Control	-5.83±0.6	AB	

Means followed by same letter in the column do not differ statistically according to the Duncan's.

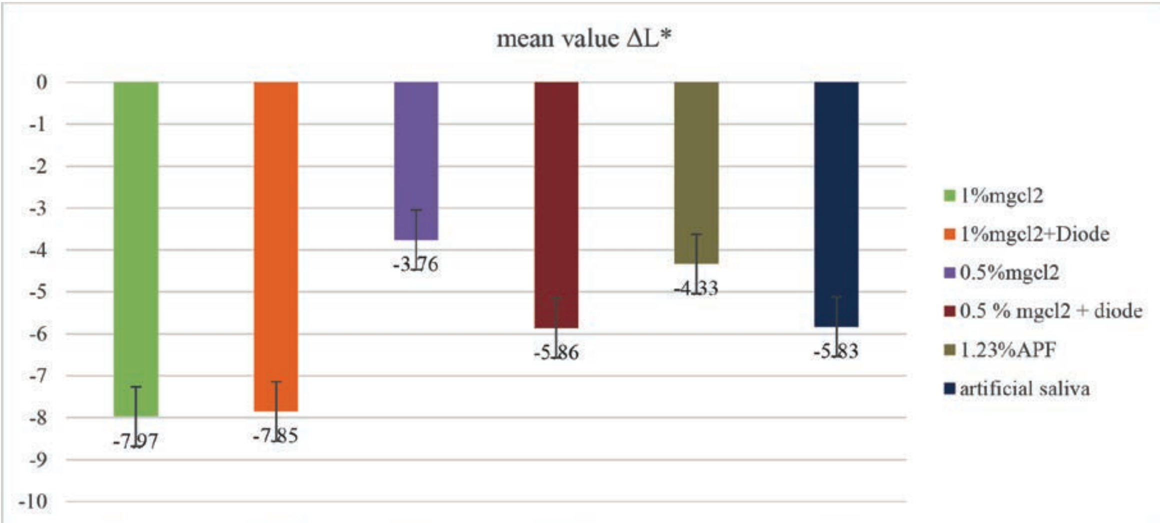

FIGURE 2. Color changes (ΔL*) of the investigated groups

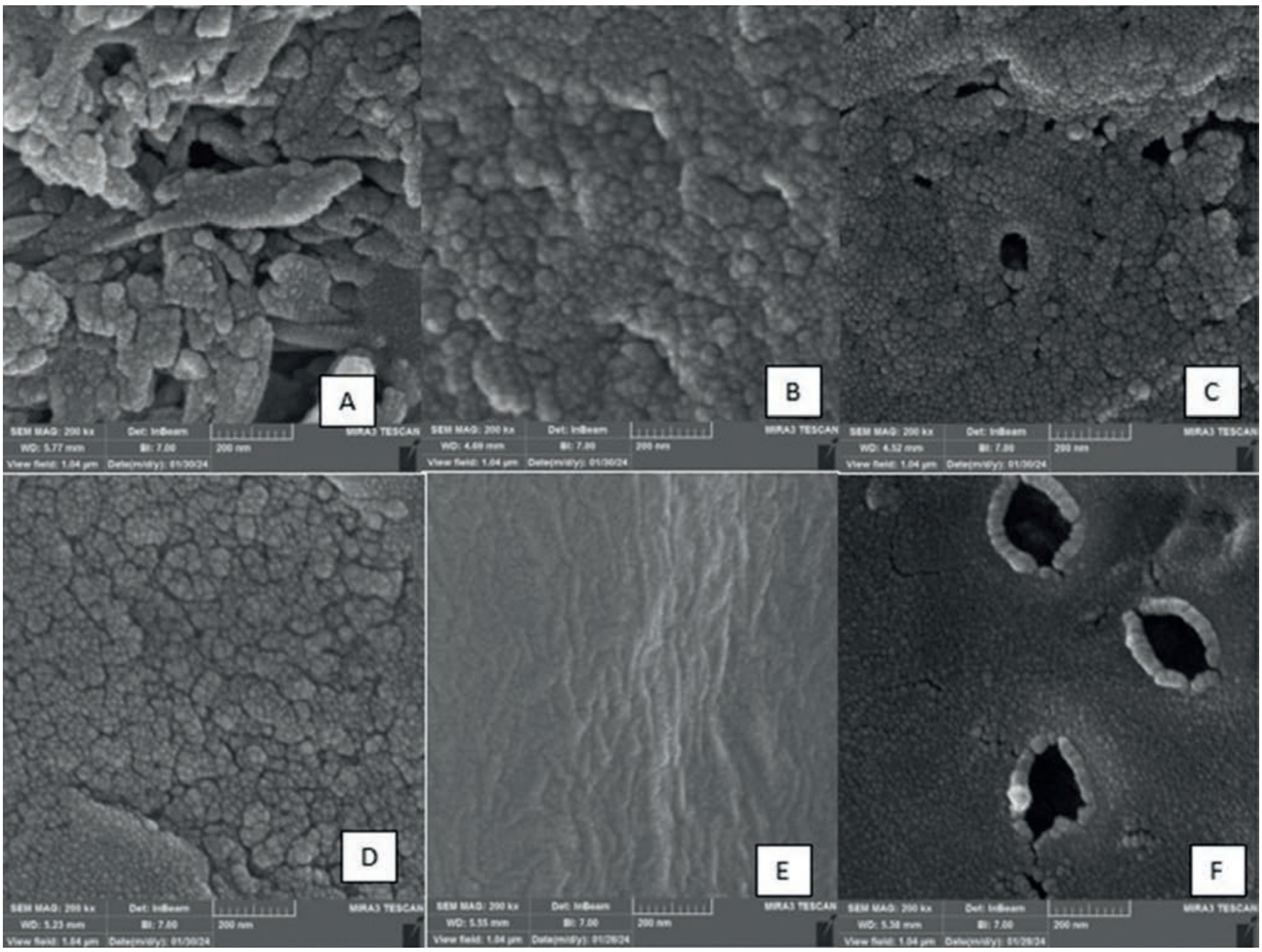

FIGURE 3. (A) Artificial saliva group (control), (B) 0.5% $MgCl_2$ gel group, (C) 0.5% $MgCl_2$ gel and diode laser, (D) 1% $MgCl_2$ gel group, (E) 1% $MgCl_2$ gel group and diode laser, (F) 1.23% APF gel

being a natural process, facilitates the partial reverse of initial caries lesions [35].

Willmote, 2004 [36], Investigated the impact of fluoride and saliva on the management of white spot lesions subsequent to the removal of fixed orthodontic appliances. He observed that the sizes of the post-orthodontic demineralized white spot lesions were reduced within six months following the treatment to approximately half the original size; however, the lesions were not completely removed.

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 to determine the color difference (ΔE) due to its accuracy, sensitivity, repeatability, and objectivity [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.

These lesions were 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 the fixed orthodontic therapy, 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 fibers and cost effectiveness 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 (970 nm) 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 remineralization [12].

Mg2+ ions slow 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, Mg^{2+} 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 Mg^{2+} 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 restoring was established by a significant decrease in ΔE values. The best result was observed in the 0.5%$MgCl_2$ group at (9.4 ±1.6). This result indicates that the lesion was restored closer to the initial enamel color. 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 smooth remineralization layer, which appeared to mask the surface appearance, consisted of round-shaped globules. Figure 3 F showed covered with a new remineralization layer around the porous surface.

Although this outcome, treated groups did not entirely take the color 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, such as pulse energy, duration, repetition rate, energy density, and irradiation period. Additionally, 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 involving lasers and magnesium gels are needed to clinically assess their performance and ascertain which 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

Vivo clinical trials are required to obtain more important information. The in vitro circumstances do not replicate the dynamic and complicated biological oral system, also lesion depth of treatment groups was not measured after demineralization and compared with post-treatment outcome.

CONCLUSION

Topical application of 0.5% $MgCl_2$ provide better color restoring than 1.23% APF gel, while 1% $MgCl_2$ gel make WSL more whiter. Diode laser application after magnesium chloride gel application provide further color enhancement. Scan electron microscope image represent homogenous surface topography.

Authors' contribution:
RMS, conception and design, acquisition of data, analysis and interpretation of data, software, writing – original draft, final approval of the version to be published.
SSSA, conception and design, analysis of data, software, reviewing & editing, final approval of the version to be published.
AAQ, Conception, interpretation of data, final approval of the version to be published.

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