



## The Effect of Minimally Invasive Treatments on Enamel Microhardness and Resistance to Further Demineralization

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### Research Article

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### ABSTRACT

**Objectives:** This study compared the microhardness of inactive proximal lesions after different remineralizing treatments, and investigated the resistance of treated lesions to a further demineralization challenge.

**Materials and Methods:** In this in-vitro study, 30 human molars with inactive proximal lesions were selected and randomly divided into three groups of 10. In group 1, the lesions were treated with a resin infiltrant (Icon). In group 2, the surface was conditioned by an Er:YAG laser before resin infiltration. The specimens in group 3 were remineralized by the bioactive glass. The specimens were kept in artificial saliva for 1 week and then immersed in a demineralization solution for 8 weeks. Microhardness was measured at baseline (T0), after remineralization (T1), and after exposure to the demineralization (T2) solution. Hardness values were compared between the treatment intervals in each group. The alterations in microhardness after the treatment and the demineralization challenge ( $\Delta$ VHN) were calculated and compared among the groups.

**Results:** In all groups, microhardness after demineralization was significantly lower than other intervals, but no significant difference was found between the T0 and T1 values ( $P > 0.05$ ). The alterations in microhardness between T1 and T0 ( $\Delta$ VHN<sub>T1-T0</sub>) and between T2 and T1 ( $\Delta$ VHN<sub>T2-T1</sub>) were not significantly different among the groups.

**Conclusions:** Pretreatment by Er:YAG laser before resin infiltration was more effective than other treatments in enhancing microhardness and protecting the tooth against the acidic challenge. However, the difference between groups did not reach statistical significance, implying the need for further studies to achieve more conclusive results.

**Keywords:** Bioactive glass, proximal caries, Er:YAG laser, remineralization, resin infiltration.

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### Introduction

Restorative therapy for an interproximal lesion usually involves the removal of the marginal ridge, which inevitably compromises the strength of the residual tooth structure.<sup>1</sup> Therefore, special attention should be paid to conservative treatment of early proximal lesions to preserve tooth integrity. Even easily detectable proximal caries in radiography may be associated with an intact enamel surface<sup>2</sup>, making non-invasive or minimally-invasive treatments the ideal treatment options. The non-invasive therapy has been defined as reducing caries activity and remineralizing the enamel lesion through oral hygiene instructions, diet education, and the application of remineralization agents such as fluoride or casein phosphopeptide amorphous calcium phosphate.<sup>3,4</sup> On the other hand, minimally invasive therapy involves mechanical blocking or sealing of the lesion via the placement of resin-based sealants or infiltrates.<sup>5</sup> It is believed that the application of minimally invasive therapy for early proximal caries is more sensible in patients with high caries risk, due to the greater progression speed of infection in these cases, as compared to the normal population.

Resin infiltration is a minimally invasive treatment for initial dental caries.<sup>6</sup> This technique is particularly recommended to postpone the need for restoration when caries have been extended into the inner half of enamel or the outer one-third of dentin.<sup>7</sup> It is believed that the pores within the enamel and dentin caries could be filled by the resin infiltrant, thus enhancing tooth strength and preventing lesion progression. Several systematic reviews have shown that resin infiltration or surface sealing has a better therapeutic effect on arresting lesion progression compared to non-invasive treatments.<sup>7-11</sup>

Lasers can be employed for numerous procedures in dentistry including caries prevention and treatment. The erbium family lasers including erbium:yttrium-aluminum-garnet (Er:YAG) and erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) are mainly absorbed by water and hydroxyapatite, making them applicable for hard tissue treatment.<sup>12,13,14</sup> Erbium lasers have been employed for the surface conditioning of enamel and dentin before restorative treatments<sup>15,16</sup> or cleaning and sterilizing enamel fissures before sealant therapy.<sup>17</sup> It is

also possible to use erbium lasers for the pretreatment of initial caries lesions before resin infiltration therapy to create a rough and micro-fractured pattern on the surface, which possibly enhances resin penetration.

Bioactive glass (BAG) is a relatively new and biocompatible agent that has been applied in many healthcare fields.<sup>5</sup> The first bioactive glass introduced in 1969 was a sodium, calcium, and phosphorus silicate glass.<sup>5</sup> Currently, there are different types of bioactive glasses, such as silicate-based glass and phosphate-based glass.<sup>5</sup> It has been demonstrated that the bioactive glass could be effective in preventing and arresting dental caries through different mechanisms including the antibacterial effect on cariogenic bacteria, inhibition of tooth demineralization, and promotion of remineralization.<sup>5</sup> It is assumed that bioactive glasses are dissolved after being implanted in the human body and the accumulation of dissolved particles not only alters the chemical composition and pH of the environment but also creates a layer of hydroxyapatite or hydroxycarbonate apatite (HCA) on the surface, which enhances remineralization.<sup>18</sup>

There are few studies concerning the comparison of resin infiltration and bioactive glass for the treatment of arrested proximal lesions.<sup>19</sup> In addition, no study evaluated the effect of pretreatment by Er:YAG laser on the results of resin infiltration. Therefore, the present study was conducted to compare the microhardness of inactive proximal lesions treated by resin infiltration, Er:YAG laser conditioning + resin infiltration, and bioactive glass, and investigate the resistance of treated lesions to a further demineralization challenge. The null hypothesis of this study was that there is no difference in microhardness and resistance to demineralization of proximal lesions treated by different remineralization methods.

## Materials and Methods

### Specimen preparation

This in-vitro study was conducted with the permission of the Ethics Committee of Mashhad University of Medical Sciences. Thirty human maxillary or mandibular molars with inactive proximal caries lesions were selected. The sample size was estimated as  $n=9$ , according to the data extracted from Torres *et al.*<sup>20</sup>, using an alpha significance level of 0.05 and a beta of 0.9. The sample size was then increased to 10 teeth per group.

The Nyvad Criteria (visual-tactile clinical examination)<sup>21</sup>, was used to detect inactive white spot lesions. Accordingly, lesions with matt and rough enamel were classified as active, whereas those with a glossy and smooth appearance were classified as inactive lesions, and selected for experimentation. The teeth were cleaned of any residual tissue with a water slurry of pumice and brush and then were kept in a 0.1% thymol solution at room temperature.

The teeth were cut in the buccolingual direction by a low-speed water-cooled diamond saw parallel to the proximal surface of the tooth. In this way, thirty disks containing carious proximal surfaces were created. The disks were then mounted in epoxy resin with the WSL

surface parallel to the ground. The proximal surface was then polished with 800- and 1200-grit silicon carbide sandpapers (AsiaSayesh, Iran) to create a "window" containing WSL for further treatments.

### Treatment procedures

The specimens were randomly assigned into three groups of 10 each; then underwent the following treatments:

**Group 1 (resin infiltration):** In this group, a resin infiltrant (Icon, DMG, Hamburg, Germany) was applied to proximal lesions according to the manufacturer's instructions. The dental proximal surface was etched with hydrochloric acid 15% for 120 seconds, then washed and dried for 30 seconds. After that, ethanol 95% (Icon-Dry) was applied for 30 seconds to dehydrate the surface and air dried. The lesion was coated by the Icon-infiltrant twice, the first one for 180 seconds, and the second for 60 seconds.<sup>1</sup> After each application, the infiltrant was light-cured for 40 seconds from different directions. Finally, the specimens were polished for 20 seconds using 4000- grit, aluminum oxide abrasive papers.

**Group 2 (Er:YAG laser conditioning + resin infiltration):** In this group, Er:YAG laser (KaVo Key Laser 3, KaVo Co., Biberach, Germany) was applied for surface conditioning before resin infiltration. The laser emitted photons at a wavelength of 2.94  $\mu\text{m}$  and was set at the energy of 50 mJ, and frequency of 10 HZ, using air/water spray.<sup>13</sup> The tip was positioned at a distance of 1 mm from the enamel surface (focused mode) and the window was irradiated through scanning movements for 10 seconds. The resin infiltration was then performed similarly to that explained in group 1.

**Group 3 (bioactive glass):** In this group, the bioactive glass was applied on the surface of the samples. The glass was prepared by mixing a tenth of a gram of Bioglass 45S5 (Nikceram, Iran; containing 24.5 wt% Na<sub>2</sub>O, 24.4 wt% CaO, 6 wt% P<sub>2</sub>O, and 5.45 wt% SiO<sub>2</sub>) with 0.2 ml of phosphoric acid 50%, for one minute, creating a substance with the consistency of the dough. The phosphoric acid-bioactive glass gel was applied on the samples by a micro-brush; then covered by a bonding layer (Clearfil SE Bond, Kuraray Medical, Tokyo, Japan), and cured for 20 seconds.

The treated specimens in all groups were stored in artificial saliva for one week. The artificial saliva used in this experiment contained 4.3 g xylitol, 1 g sodium carboxymethylcellulose, 0.1 g potassium chloride, 40 mg potassium phosphate, 1 mg potassium thiocyanate, 5 mg calcium chloride, and 100 g distilled deionized water.<sup>22</sup>

After 1-week storage in artificial saliva, the treated samples were immersed in a demineralization solution for 8 weeks. This solution contained 2.2 mM CaCl<sub>2</sub>, 2.2 mM NaH<sub>2</sub>PO<sub>4</sub>, and 50 mM acetic acid, with PH adjusted at 4.8. Each sample was individually placed in the demineralization solution and the solution was changed weekly.

### Microhardness assessment

A Vickers microhardness tester (model MH3, Koopa Pazhoohesh, Iran) was employed to measure the microhardness of the specimens at baseline (T<sub>0</sub>), after the

treatment and 1-week storage in artificial saliva (T1) and after exposure to the demineralization solution (T2). Two indentations were made, 100  $\mu\text{m}$  apart, at the center of the treatment window using a load of 100 g applied for 10 seconds, and the mean value was recorded as the Vickers hardness number (VHN) for that specimen. Hardness values were compared between the treatment intervals in each group. Furthermore, the alterations in microhardness after the remineralizing treatment ( $\Delta\text{VHN}_{\text{T1-T0}}$ ) and the demineralization challenge ( $\Delta\text{VHN}_{\text{T2-T1}}$ ) were calculated and compared among the groups.

### Statistical analysis

The normality of the data was evaluated using the Shapiro-Wilk test, which revealed that only  $\Delta\text{VHN}$  values followed the normal distribution ( $P > 0.05$ ). The intragroup comparisons of hardness values were made by the Friedman test, followed by Dunn's test for pairwise comparisons. One-way analysis of variance (ANOVA) was run to detect any significant difference in  $\Delta\text{VHN}$  values among the three groups. The statistical analysis was performed through SPSS software (version 16.0; SPSS Inc., Chicago, IL), and the significance level was set at  $P < 0.05$ .

### Results

Table 1 presents the mean and standard deviation (SD) of microhardness values at baseline (T0), after various surface treatments (T1), and after exposure to the demineralization solution (T2) in the study groups. Microhardness increased after treatments and decreased following exposure to the acidic challenge. Friedman test displayed a significant alteration in VHN values throughout the experiment in all the study groups ( $P < 0.005$ ). Further analysis by the Dunn test revealed that microhardness after demineralization (T2) was significantly lower than the T0 and T1 values, but no significant difference was found in VHN between the T0 and T1 time points in any of the treatment groups.

Table 2 presents the alterations in the Vickers hardness number after the remineralizing treatment ( $\Delta\text{VHN}_{\text{T1-T0}}$ ) and the demineralization challenge ( $\Delta\text{VHN}_{\text{T2-T1}}$ ). The greatest enhancement in microhardness after the remineralization treatment belonged to the Er:YAG laser conditioning + resin infiltration group and the lowest to the bioactive glass group. Following exposure to the demineralization solution, the greatest loss in microhardness was observed in the resin infiltration group and the lowest was found in the specimens treated by Er:YAG laser + resin infiltration. The statistical analysis failed to reveal a significant difference either in  $\Delta\text{VHN}_{\text{T1-T0}}$  or in  $\Delta\text{VHN}_{\text{T2-T1}}$  among the study groups ( $P > 0.05$ ; Table 2)

### Discussion

The present study compared the effects of resin infiltration, Er:YAG laser conditioning + resin infiltration, and bioactive glass for remineralization of arrested caries lesions. Hardness testing is a simple and accurate method

to detect changes in the mineral content of teeth and has been widely used in the literature.<sup>23,24</sup> Based on the findings of this study, pretreatment of enamel caries by Er:YAG laser followed by resin infiltration caused the highest increase in microhardness and created the greatest resistance against the demineralization attack. However, the difference between groups was not statistically significant, possibly due to the small sample size and great variations in the hardness values. So the null hypothesis of this study was accepted, implying that there is no difference in microhardness and resistance to demineralization of proximal lesions treated by resin infiltration, Er:YAG laser conditioning + resin infiltration, or bioactive glass.

During Resin infiltration, a low-viscosity resin fills the pores within the demineralized enamel to hamper further caries progression.<sup>6</sup> Resin infiltration also strengthens the enamel structure and thus prevents cavitation.<sup>21</sup> Icon is a methacrylate-based resin containing TEG-DMA, Bis-GMA, initiators, and solvents. The presence of TEG-DMA leads to the high elasticity of the resin<sup>25</sup>, whereas Bis-GMA reduces polymerization shrinkage and increases lesion hardness due to its greater molecular weight. Adding ethanol as the solvent improves the permeability of the resin by increasing the penetration coefficient.<sup>26</sup> In the present study, the infiltrant was applied twice; because repeated resin application is assumed to enhance lesion microhardness and provide beneficial effects on demineralization resistance.<sup>25</sup> It should be noted that proximal infiltrated lesions do not withstand chewing forces.<sup>25</sup> Therefore, an excessively high microhardness is not required at the proximal surface, but resin infiltration should provide sufficient microhardness to restore the proximal contact, provide resistance against proximal attrition and abrasion, and prevent lesion cavitation.<sup>26</sup> In the present study, resin infiltration did not show a significant superiority compared to the other techniques for enhancing remineralization and improving the resistance of treated lesions to acidic attack. Paris *et al.*<sup>27</sup> also reported that some demineralization can still occur after exposure to a new acidic challenge in lesions infiltrated with Icon. They attributed this finding to the incomplete inclusion of some resin minerals in the lesion or the occurrence of resin shrinkage during light curing, which leads to leakage and thus reduction in acid resistance of the substrate.<sup>21</sup>

The caries prevention effects of lasers have been demonstrated in previous studies and explained through different mechanisms. The laser absorption in tooth tissue can cause physical and chemical changes through the oxidation of organic components, conversion of acid phosphate to pyrophosphates, and reduction of carbonate content.<sup>13,29</sup> The caries-preventive effects of erbium lasers may be related to the induction of physical and structural changes in enamel and dentin and also to the creation of a rough and etched surface, which could increase the absorption and penetration of mineral agents.<sup>30</sup> Laser etching combined with conventional etching has also revealed satisfactory results in enhancing

bond strength to the tooth structure.<sup>31-34</sup> In the present study, pretreatment with Er:YAG laser before resin infiltration lead to higher microhardness and greater resistance to demineralization than resin infiltration alone, although the difference between groups failed to achieve statistical significance. It can be assumed that the

formation of micro-cracks by Er:YAG laser can increase the penetration of infiltrant within the pores of demineralized enamel, or enhance the bond strength of infiltrant to mineral tissues. However, further studies with larger sample sizes are warranted to accept or reject this hypothesis.

**Table 1.** Comparison of microhardness values in the study groups at T0 (baseline), T1 (after treatment application), and T2 (after demineralization)

Group	T0		T1		T2		P-value
	Mean	SD	Mean	SD	Mean	SD	
Resin infiltration	205.56 <sup>Aa</sup>	87.01	223.93 <sup>Aa</sup>	110.44	82.57 <sup>Ba</sup>	48.03	P<0.001
Er:YAG laser + resin infiltration	169.06 <sup>Aa</sup>	117.76	222.86 <sup>Aa</sup>	135.66	92.74 <sup>Ba</sup>	50.98	P=0.001
Bioactive glass	197.03 <sup>Aa</sup>	129.81	200.85 <sup>Aa</sup>	125.32	92.5 <sup>Ba</sup>	49.41	P=0.003

\*The different uppercase superscript letters in the rows indicate statistically significant differences at P<0.05.

**Table 2.** Mean and standard deviation (SD) of alterations in surface microhardness ( $\Delta$ VHN) between the treatment stages

Group	$\Delta$ VHN <sub>T1-T0</sub>		$\Delta$ VHN <sub>T2-T1</sub>	
	Mean	SD	Mean	SD
Resin infiltration	18.37 <sup>a</sup>	111.26	-122.99 <sup>a</sup>	83.53
Er:YAG laser + resin infiltration	53.8 <sup>a</sup>	82.44	-76.32 <sup>a</sup>	76.23
Bioactive glass	3.82 <sup>a</sup>	94.46	-104.53 <sup>a</sup>	101.50
P value	P=0.503		P=0.497	

\*The different uppercase superscript letters in the rows indicate statistically significant differences at P<0.05.

Bioactive glasses have been used in the structure of different products such as bone grafts, scaffolds, coatings of dental implants, and dental desensitizers.<sup>35</sup> One of the most important properties of bioactive glass is its antibacterial activity, which is due to the existence of antibacterial components such as silver, copper, or zinc within the glass structure, and the release of alkaline ions (Na<sup>+</sup>, Ca<sup>2+</sup>) that raise the pH of the environment.<sup>35-37</sup> The bioactive glass also could be a therapeutic choice for caries management through inhibiting demineralization and promoting remineralization of caries lesions. After placing bioactive glass in the salivary environment, it takes at least 2 hours to complete the bioactive cycle of the substance, resulting in the release of calcium and phosphorus ions from the silicate network.<sup>28</sup> In this study, a layer of bonding agent was applied over the surface of the bioactive glass, and cured. It has been demonstrated that the temporary coating of the glass with a thin layer of bonding agent for 24 h protects calcium and phosphorous ions against being washed out by saliva.<sup>38</sup> The bioactive glass also bonds tightly to materials and tissues and facilitates remineralization through the formation of stable hydroxyapatite crystals on the lesion surface. In the present study, however, the bioactive glass did not show any superiority over other treatments for the remineralization of caries lesions and for preventing further demineralization.

In the present study, neither of the remineralization agents caused a significant increase in the microhardness of inactive proximal lesions, nor increased the enamel resistance to a further acidic attack. These findings are in contrast to most of the previous investigations, which revealed significant enhancement in the mineral content of teeth exposed to a variety of remineralizing products.<sup>39-44</sup> This controversy may be attributed to the small sample size in the study groups. The use of arrested caries lesions which are usually high in mineral content may also

contribute to achieving insignificant results in this study. Furthermore, the study design did not include an untreated control group, and this may be considered a limitation of the present investigation. Further studies with larger sample sizes are warranted to assess the efficacy of resin infiltration (with or without laser pretreatment) and bioactive glass on remineralization of carious lesions in clinical conditions.

## Conclusions

- 1- The greatest enhancement in microhardness after the remineralization treatment belonged to the Er:YAG laser conditioning + resin infiltration group and the lowest to the Bioactive glass group.
- 2- Following exposure to the demineralization solution, the greatest loss in microhardness was observed in the resin infiltration group and the lowest was found in the specimens treated by Er:YAG laser + resin infiltration.
- 3- Although pretreatment by Er:YAG laser before resin infiltration was more effective than other treatments in enhancing microhardness and preventing the loss of mineral content, the difference between groups did not reach statistical significance; implying the need for further studies to achieve more conclusive results.

## Conflict of Interest

The authors declare no conflict of interest.

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