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Evaluation of diode laser along with 1.23% acidulated phosphate fluoride gel on dentinal tubule occlusion: An *in vitro* study

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Abstract

Context:

Several studies have recommended the use of lasers in treatment of dentinal hypersensitivity. These materials have been used alone or in combination with an active desensitizing agent.

Aims:

The present study aimed to evaluate the use of 1.23% acidulated phosphate fluoride (APF) gel and 810 nm diode laser when used alone and when used together on exposed dentinal tubules.

Settings and Design:

Sixty-one extracted teeth were sectioned with a diamond saw and dentin slices were prepared. They were then treated with 37% orthophosphoric acid to remove the smear layer.

Materials and Methods:

One of the 61 sections was left as an untreated control, whereas the remaining sixty sections were divided into three groups of twenty sections each. The first set of sections (Group 1) was treated with laser alone, whereas the second set (Group 2) was treated with APF alone. A third set of sections (Group 3) was treated with a combination of laser and APF. The sectioned teeth were then evaluated using field emission scanning electron microscopy, and the percentage of dentinal tubule occlusion was observed.

Statistical Analysis:

The Shapiro–Wilk normality test was performed to check for the normal distribution of data, whereas the Kruskal–Wallis test was used to compare the mean percentage of dentinal tubule occlusion between the three groups. The Chi-square test was used to compare the number of images with complete tubular occlusion between the three groups.

Results:

A total of 96 images were processed from the sixty sectioned samples. It was seen that the sections in Group 3 showed the highest percentage of dentinal tubule occlusion. Group 3 also showed the highest number of samples with complete dentinal tubule occlusion. Significantly lesser levels of dentinal tubule occlusion were seen in Groups 1 and 2.

Conclusions:

Diode lasers were effective in occluding dentinal tubules. The addition of 1.23% APF significantly increased the efficacy and thoroughness of dentinal tubule occlusion. Significantly lesser levels of dentinal tubule occlusion were seen in the sections treated with laser alone and APF alone.

Keywords: Acidulated phosphate fluoride, dentinal hypersensitivity, lasers

INTRODUCTION

With increased life expectancy and increasing rate of tooth retention into old age, the problem of dentinal hypersensitivity is more than ever before. The prevalence of dentinal hypersensitivity ranges from 4% to 74% depending on the population assessed.[1,2,3,4] Several therapies including home- and office-applied agents have been prescribed for the treatment of dentinal hypersensitivity, with varying degrees of success.[5,6,7,8,9]

Over the past decade, the use of lasers in dentistry has become commonplace.[10,11,12] They have been used alone or in combination with an active desensitizing agent in the treatment of dentinal hypersensitivity.

Fluoride gels such as those containing sodium fluoride have also been used in the treatment of dentinal hypersensitivity with a reasonable amount of success.[13] Even though acidulated phosphate fluoride (APF) has been recommended in the treatment of dentinal hypersensitivity,[14] till date it has not been used systematically in its treatment. This agent has been successfully used in preventive dentistry for a number of years for the prevention of dental caries and dental erosion.[15,16]

Dentinal hypersensitivity has been directly related to exposure of dentinal tubules and results from fluid movement through these tubules.[17,18] Occlusion of these tubules results in reduction in flow of fluid, consequently alleviating dentinal hypersensitivity. Several studies have used dentinal tubule occlusion as anin vitro model to study various desensitizing treatments.[10,11,12,16,19]

The present study aimed to evaluate the effect of 1.23% APF gel and 810 nm diode laser when used alone and when used together on exposed dentinal tubules in human extracted teeth.

Go to:

MATERIALS AND METHODS

Sixty one extracted teeth were collected after obtaining informed consent from patients aged 20-40 years from the Department of Oral and Maxillofacial Surgery, The Oxford Dental College, Bengaluru, Karnataka, India. Approval from the Institutional Ethics Committee of The Oxford Dental College was obtained for the same. The study was carried out between May 2018 and October 2018.

Only intact extracted human single-rooted teeth were taken up in the study. Fractured teeth, eroded teeth, or cracked teeth, carious teeth or restored teeth, hypoplastic teeth or teeth exhibiting signs of fluorosis, and teeth with developmental anomalies were excluded from the study.

The teeth were first cleansed of gross debris and stored in 10% formalin solution till processing. Following this, all teeth were placed in deionized water for 24 h before commencement of the experiment. Dentin slices, approximately 800 µ thick, were cut from the crown section of human single-rooted teeth in a parallel manner, slightly below the enamel-dentin junction, using a double-sided diamond disk operated on micromotor and a straight handpiece. The sectioned specimens were then polished with a 1000 grit sandpaper.

The polished sections were then placed in a jar of deionized water and sonicated (Confident Ultrasonic Cleaner C-80-M, Confident Dental, India) for 10 min to remove the polishing abrasive. After sonication, the sections were rinsed with deionized water. The tubules were opened by etching the dentin sections in a petri dish with a 37% orthophosphoric acid solution, using mild agitation for 20 s. After etching, the sections were rinsed and then placed in a jar of deionized water and finally sonicated once again for 10 min. The etched and sonicated sections were stored in phosphate-buffered saline.

One sectioned tooth sample was not treated with any of the active agents. It was only demineralized with 37% orthophosphoric acid. This sample was used as a control. The remaining sections were equally divided into three groups with each group comprising 20 samples each.

One of the sets (Group 1) was treated with diode laser (810 nm Fotona™) for a period of 60 s at each site in a continuous wave, “noncontact” mode with a spot diameter of 320 µm and a power setting of 0.25W.

A second set of sections (Group 2) was treated with Pascal™ 1.23% APF gel. The agent was uniformly applied for a period of 60 s. Following this, the samples were allowed to air dry.

A third set of sections (Group 3) was treated with a combination of Pascal™ 1.23% APF gel and 810 nm diode laser. The fluoride gel was uniformly applied for a period of 60 s. Following this, the sections were allowed to air dry. The sectioned tooth specimens were then treated with diode laser for a period of 60 s at each site with a spot diameter of 320 µm and a power setting of 0.25W in a continuous wave, “noncontact” mode.

The samples were then stored in artificial saliva for a period of 1 week which was changed every 6 h. This methodology was similar to that described by Suge *et al*.[19]

Before the evaluation with scanning electron microscopy (SEM), the samples were removed from the artificial saliva and allowed to air-dry for a period of 12 h. The samples were then taken to the Department of Nano Manufacturing Technology Centre, located at Central Manufacturing Technology Institute, Bengaluru. Here, the sections were first treated with a layer of gold–palladium sputter coating up to a depth of 4 nm. Then, these sections were mounted on SEM stubs and evaluated for dentinal tubule occlusion. The sections were then evaluated using a scanning electron microscope (Carl Zeiss, Germany; Model: Neon 40) [Figure 1].

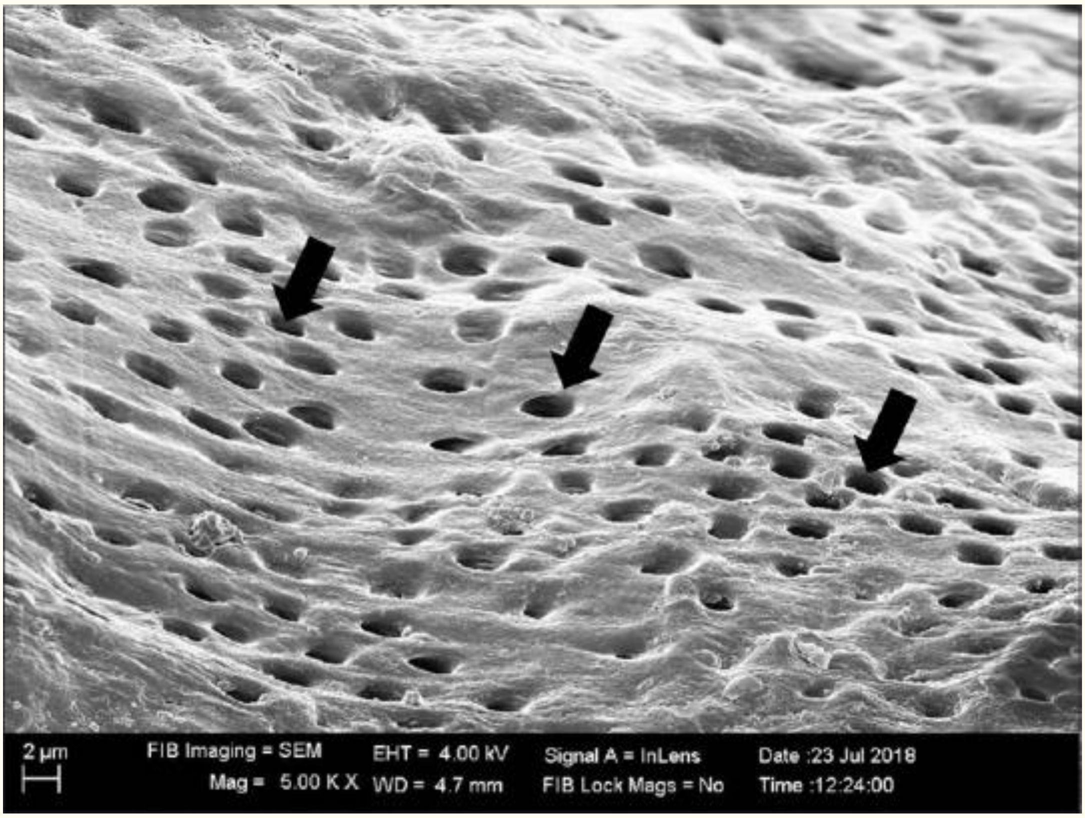

Figure 1

Scanning electron microscopy image of the untreated control showing open dentinal tubules (arrow) when viewed at ×5000

From the 61 samples that were obtained after tooth sectioning, a total of 97 images were processed. This amounted to 32 images in each of the three treatment groups, with another image being obtained from the untreated control. All samples were viewed with a magnification of ×5000.

Evaluation of tubule occlusion was done by calculating the percentage of dentinal tubule occlusion. The image from the untreated control was used to show complete dentinal tubule exposure before treatment. The images of the sectioned tooth samples from the three groups were compared to those of the untreated control. The percentage of dentinal tubule occlusion was calculated using the following formula:

$$\text{Percentage of dentinal tubule occlusion} = \frac{\text{Number of occluded tubules in treated dentine surface} \times 100}{\text{Maximum numbers of open tubules in the untreated control specimen}}$$

Statistical analysis

The Statistical Package for the Social Sciences for Windows, version 22.0. Released in 2013. IBM Corp., Armonk, NY, USA was used to perform statistical analyses.

Descriptive analysis was done for expression of percentage of dentinal tubule occlusion in terms of mean and standard deviation.

For the inferential statistics, the Shapiro–Wilk normality test was performed to check for the normal distribution of data. The test revealed that data were not following normal distribution. Hence, all relevant inferential analyses were performed using nonparametric tests. The Kruskal–Wallis test was used to compare the mean percentage of dentinal tubule occlusion between the three groups.

The Chi-square test was used to compare the number of images with complete tubular occlusion (100%) between the three groups.

The level of significance (P value) was set at $P < 0.05$.

Go to:

RESULTS

A total of 96 images were obtained from the sixty samples examined in the groups. This constituted to 32 images from 20 samples in each of the groups.

Among the 96 images examined through SEM, it was seen that the samples treated with a combination of laser and fluoride (Group 3) showed appreciable dentinal tubule occlusion (90.28 ± 17.07). Significantly lesser levels of occlusion were seen in the samples treated with fluoride alone (Group 2) (73.09 ± 30.37) and those treated with laser alone (Group 1) (71.38 ± 23.75). This difference was statistically significant ($P = 0.004$). The details of these figures are shown in Table 1 and Figure 2. Figures Figures11 and and33--55 show the SEM sections of all the groups.

Table 1

Comparison of mean percentage of dentinal tubule occlusion between the three groups using Kruskal-Wallis test

Groups	***n***	**Mean±SD**	**Min**	**Max**	***P***
Group 1	32	71.38±23.75	8	100	0.004*

Groups	*n*	Mean±SD	Min	Max	*P*
Group 2	32	73.09±30.37	7	100	
Group 3	32	90.28±17.07	44	100	

*Statistically significant, where *P*<0.05 was considered significant. SD – Standard deviation; Min – Minimum occlusion of dentinal tubules; Max – Maximum occlusion of dentinal tubules; *P* – *P* Value; *n* – Number

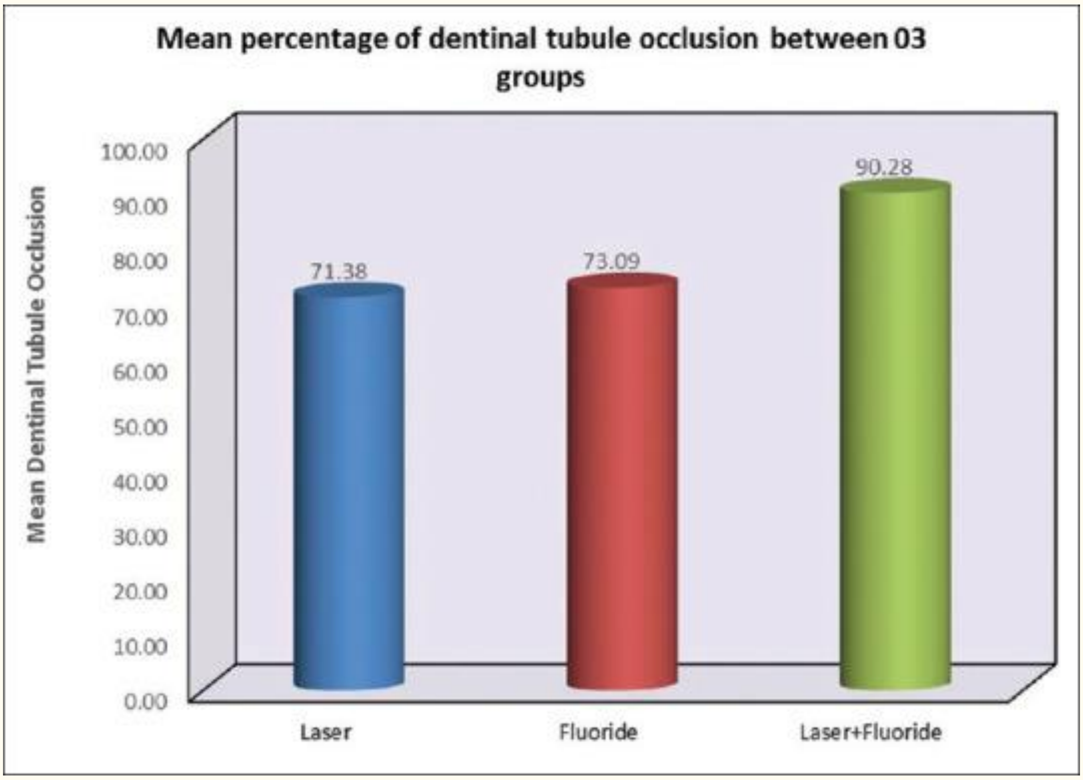

Figure 2

The mean percentage of dentinal tubule occlusion in each of the three groups

Figure 3

Scanning electron microscopy image of the sections treated with 810 nm diode laser alone (Group 1) showing linear deposition to cover the dentinal tubules (arrow) when viewed at ×5000

Figure 5

Scanning electron microscopy image of the sections treated with a combination of Pascal™ 1.23% acidulated phosphate fluoride gel and 810 nm diode laser (Group 3) showing even globular formations (arrow) when viewed at ×5000

Table 2 and Figure 6 show a comparison of the presence of complete dentinal tubule occlusion (100% occlusion) between the three study groups.

Table 2

Comparison of presence of complete occlusion (100%) of dentinal tubules between the three groups using Chi-square test

Complete occlusion	Group 1, *n* (%)	Group 2, *n* (%)	Group 3, *n* (%)	χ^2	*P*
Present	5 (15.6)	4 (12.5)	15 (46.9)	12.333	0.002*
Absent	27 (84.4)	28 (87.5)	17 (53.1)		

*Statistically significant, where $P<0.05$ was considered significant. *n* – Number of samples with complete tubular occlusion; % – Percentage of samples with complete tubular occlusion; χ^2=Chi-square; *P* – *P* Value

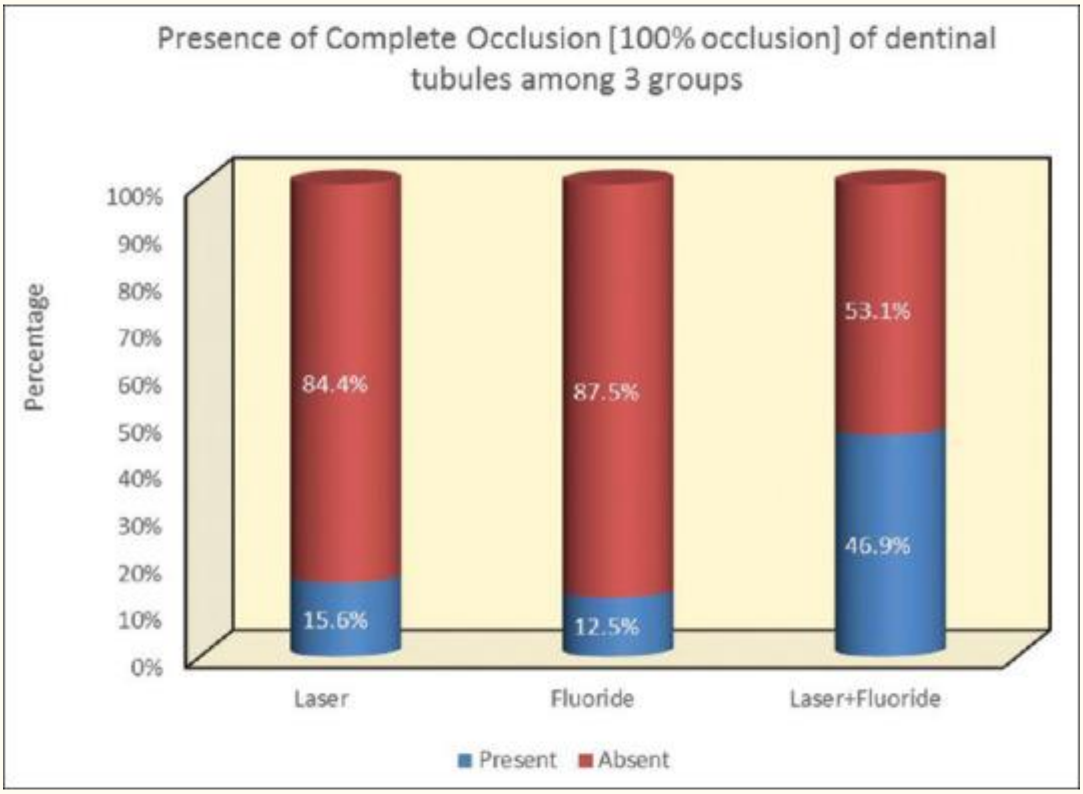

Figure 6

The prevalence of complete dentinal tubule occlusion in all the three groups

The test results showed that there was a significant difference in the percentage of complete occlusion of dentinal tubules between the three groups, wherein Group 3 showed the highest percentage (46.9%) of complete occlusion, followed by Group 1 (15.6%). The group with the least number of samples with complete dentinal tubule occlusion was Group 2 (12.5%) (P = 0.002).

The multiple comparisons between the three groups revealed that the percentage of complete occlusion of dentinal tubules in Group 3 showed a significant difference with both Group 1 (P = 0.003) and Group 2 (P = 0.007). However, the difference percentage of complete tubular occlusion between Group 1 and Group 2 was not statistically significant (P = 0.72). Table 3 displays a complete analysis of these values. The SEM images of the various groups after treatment are shown in Figures Figures44--66.

[Figure 4](#)

Scanning electron microscopy image of the sections treated with Pascal™ 1.23% acidulated phosphate fluoride gel (Group 2) showing uneven globular formations (arrow) when viewed at ×5000

Table 3

Multiple comparisons between the three groups for complete occlusion using Chi-square test

Groups	**Group 1 versus Group 2**	**Group 1 versus Group 3**	**Group 2 versus Group 3**
P	0.72	0.007*	0.003*

*Statistically significant, where P<0.05 was considered significant. P – P Value

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DISCUSSION

Application of fluorides has been used in the prevention of demineralization of teeth as well as in the treatment of dentinal hypersensitivity.[20,21] Diode lasers have been used alone as well as in combination with these agents in the management of sensitive teeth.[11,12,13] The current study tried to examine the effect of 810 nm diode laser and 1.23% APF each when used alone and in combination on dentinal tubule occlusion.

Group 1 comprised tooth sections treated with laser alone used for 60 s at power setting of 0.25 W. The mean percentage of occlusion of dentinal tubules was 71.38%. The current study used a diode laser with a wavelength of 810 nm to treat exposed dentinal surfaces. Previous studies have used diode lasers with a wavelength of 810 nm and found that they enhanced dentinal tubule occlusion and successfully treated dentinal hypersensitivity.[12,22,23,24] Our study used a continuous wave mode to uniformly apply the laser on all exposed surfaces. Similar continuous wave modes were used in earlier studies too.[12] Due to the poor absorption of diode lasers in water, the energy produced by the laser has been postulated to provoke a sufficient increase in temperature to melt and occlude dentinal tubules.[25]

Our study found that a power setting of 0.25 W was sufficient to significant dentinal tubule occlusion when an 810 nm diode laser was used. Femiano *et al*. found that 810 nm diode lasers used with similar power settings produced a reduction in dentin hypersensitivity.[23] However, Sicilia *et al*. found a reduction in dentinal hypersensitivity when 810 nm diode lasers were used at far lesser power settings.[24]

1.23% APF was chosen in this study, as it has the highest concentration of fluoride ions (12,300 ppm) among all the fluoride gels used in dentistry. It has been postulated that fluorides used in higher concentrations were more effective in treating dentinal hypersensitivity.[26] In our study, we found that the dentinal tubule occlusion in the tooth sections treated with a 60-s application of 1.23% APF alone (Group 2) significantly occluded dentinal tubules (73.09%). Earlier studies using other fluoride gels have been shown to significantly reduce dentinal hypersensitivity and improve dentinal tubule occlusion.[13,27]

It was seen that the samples treated with a combination of 1.23% APF and diode laser (Group 3) showed the maximum occlusion of dentinal tubules in all the samples treated (90.28%). This was significantly higher than those in Group 2 (73.09%) and in Group 1 (71.38%). This figure was statistically significant ($P = 0.004$). Sgolastra, in a recent systematic review, concluded that lasers were effective in reducing dentinal hypersensitivity.[28] Earlier studies have found that the addition of fluorides or other desensitizing agents reduced the incidence of dentinal hypersensitivity when compared to laser alone.[12,13,23,24]

The percentage of sites with complete occlusion of the dentinal tubules (100% tubular occlusion) was also seen to be higher in Group 3 when compared to Groups 1 and 2 ($P = 0.002$). It may be safe to assume that the addition of a “wetting agent” such as a fluoride gel may increase the thoroughness of dentinal tubule occlusion. Studies such as those done by Lan *et al*.,[10] Moritz *et al*.,[29] and Reddy *et al*.[30] found that the addition of additional chemical agents to lasers increased the effectiveness of dentinal tubule occlusion by up to 20%.

In our study, we found that the images of sections in the group treated with a combination of 1.23% APF and diode laser showed a “globular crystal formation” on the dentin surface. Similar findings were seen in a study by Serdar-Eymirli *et al*. in sites treated with a combination of fluoride and laser.[31] The sections in the other two groups showed a rather amorphous grainy appearance. Fluoride gels when used along with lasers have been shown to increase acid resistance.[26] Whether these globular formations are responsible for increased acid resistance and consequently reduced dentinal hypersensitivity remains to be seen.

One of the limitations of our study was that we could not assess the depth of penetration of any of the agents into the dentinal tubules. Increased penetration of agents into the tubules may lead to more lasting relief from dentinal hypersensitivity. A study of the mechanism of action laser in occluding dentinal tubules was also not done in our study. Our study only performed a surface examination of dentinal tubules following diode laser application. However, earlier studies by Umana *et al*.[12] and Liu *et al*.[25] suggested a possible melting effect on the dentin with laser, leading to dentinal tubule occlusion. We also did not assess the resistance of these occluded tubules to acid challenge. We did, however, partially simulate oral conditions by storing the sections in artificial saliva which was changed every 6 h.[19] Our study showed a significant dentinal tubule occlusion in all the three groups despite the sections being stored in saliva for 1 week.

Our study did not observe any damage to the dentin structure due to the application of any of the agents used in the study. 1.23% APF along with 810 diode lasers at a power setting of 0.25W can thus be used safely to treat dentinal hypersensitivity *in vivo*. The power settings mentioned in the study led to significant dentinal tubule occlusion.

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CONCLUSIONS

The present study shows that all treatments led to significant dentinal tubule occlusion. The use of 1.23% APF along with 810 diode lasers at a power setting of 0.25W led to significant dentinal tubule occlusion. 1.23% APF and 810 nm diode laser can also be used alone in the treatment of dentinal hypersensitivity. However, the degree of dentinal tubule occlusion of these treatments used alone was significantly less thorough when compared to when a combination of 810 nm diode laser and 1.23% APF was used.

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Conflicts of interest

There are no conflicts of interest.

Go to:

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