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Evaluation of the Roles of Bioglass Nanoparticles and Er:YAG 2940 nm Laser in Occluding Human Dentinal Tubules (In Vitro Study)

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Aim: To evaluate the effect of 45S5 bioglass nanoparticles versus Er:YAG 2940 nm laser, either alone or in combination, on dentin and dentinal tubule occlusion.
Materials and Methods: Forty dentin discs were used for this study. All dentin discs were soaked in 1% citric acid and after that distributed equally into four groups. Group (1): received no treatment, only desensitization. Group (2): treated with bioglass nanoparticles alone. Group (3): treated with Er:YAG laser irradiation only. Group (4): treated with bioglass after Er:YAG laser irradiation. Dentin samples were morphologically assessed by scanning electron microscopy, and elemental analysis was carried out using energy dispersive X-ray analysis.
Results: Compared with other groups, the combined group demonstrated noticeably better dentinal tubule closure, as verified by SEM imaging. The EDX analysis, which revealed the greatest calcium and phosphorus mass percentages in the combined group, validated these results.
Conclusion: Dentinal tubule closure was deemed satisfactory with the use of bioglass and an Er:YAG laser. However, rather than employing each modality alone, it was discovered that combining bioglass and a laser produced better results and was more efficient.

Keywords: bioactive glass, dentin, dentine hypersensitivity, dental laser, 45S5 bioglass

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Introduction

Dentin hypersensitivity (DH) is a common oral manifestation in clinical dental practice. When exposed dentin is stimulated by thermal, tactile, osmotic, or chemical stimuli, it causes brief, intense pain that cannot be associated with another dental pathosis.[1–3]

The affected teeth in DH patients become sensitive to typically benign environmental stimuli. Light contact, minor cold or heat, chemicals (fruits, foods, beverages), and airflow stimulation may all create momentary, intense discomfort. This discomfort may hinder normal daily routines such as drinking, eating, brushing teeth, and even talking. Chronic dental pain that requires therapy, such as neuropathic pain, can be produced by more intense DH, which can last more than half a year and become a constant discomfort that causes emotional and psychological distraction.[2,3]

Dentin hypersensitivity can be induced by several physiological causes, including improper and aggressive tooth brushing techniques, occlusal pressure, attrition, erosion of the cementum and enamel, and abrasion. DH may also be influenced by iatrogenic factors in dental clinics, such as periodontics treatment, tooth bleaching, and tooth preparation.[2]

Dentin hydrolytic conduction, often termed as the hydrodynamic theory, is the most frequently believed clarification for dentinal sensitivity among the many theories that have been proposed. According to the hydrodynamic theory, sensitized dentin is caused by the displacement and motion of the tubular fluid, which sends a pain signal to the nerves.[3,4]

Different therapies have been suggested for DH; some concentrate on blocking dentin tubules, while others aim to reduce the conduction of unpleasant nerve stimuli. These therapies, which are frequently used in dental offices and at home, include toothpaste, gels containing strontium or potassium, and fluoride varnish.[2,3]

Laser therapy is another approach for treating DH. DH can be treated using a variety of laser types with adjustable energy levels and wavelengths. This sort of therapy has exhibited a desensitizing impact that can be either physical (the shrinkage or blockage of dentinal tubules) or connected to laser effect to the teeth innervation.[5]

The first laser was applied to treat dental necrosis in the middle of the 1960s with the introduction of medical laser appliances into several areas of dentistry. However, Matsumoto and his colleagues used laser therapy for the first time to treat DH in the middle of the 1980s.[6] Erbium laser therapy for non-carious cervical lesions that show hypersensitivity produces promising outcomes by lowering the DH and preserving pulp viability.[7]

Nanotechnology is a field of science that's concerned with nanoparticles (NPs). Nanomaterials are tiny solid particles with sizes between one and one hundred nanometers.[8] The utilization of nanotechnology in the treatment of dental disorders has attracted much interest. The range of applications for clinical dentistry, including the management of dentin hypersensitivity, tooth restoration, endodontic therapy, and surgical and orthopedic procedures, among others.[9]

Because of their characteristics, such as mechanical biocompatibility, the introduction of bioactive glasses (BAGs) represents a turning point in the evolution of biocompatible materials.[10] The phrase "Bioglass" alludes to Hench's original 45S5 composition.[11] BAG is used in dentistry for several purposes, including

coating implants, bone grafting, and restorative materials.[12]

45S5 bioactive glass nanoparticles were initially utilized for bone augmentation and as a covering for dental implants.[11] Their use for treating enamel and dentin defects was only recently introduced.[13]

The bioactive glass 45S5 nanoparticles cause an ionic reaction. Glass particles release calcium and phosphate ions at the oral cavity and after encountering the saliva, creating a calcium phosphate layer. The created layer could assist in remineralization of the enamel and dentin defects, exhibited good biocompatibility with pulp tissue, and showed resistance to brushing abrasion through the oral cavity.[13] Furthermore, these particles might abrade the tooth surface, forming a smear layer that clogs dentinal tubules (DTs).[14]

Therefore, the aim of the present study was to assess the effect of Er:YAG and Bioglass 45S5 nanoparticles on dentinal tubules occlusion.

The null hypothesis of the current investigation was that there would be no difference in the outcome in occlusion of the dentinal tubules among the control, 45S5 bioglass treatment, 2940-nm Er:YAG laser treatment, or combined treatment, unless the opposite is approved.

Materials and methods

Aim: The aim of the present study was to assess the effect of Er:YAG and Bioglass 45S5 nanoparticles on dentinal tubules occlusion.

A. Sample size:

The sample size was determined based on a 5% alpha error and 80% study power. According to Dilber et al., the mean (SD) Ca/P ratio was 2.08 (0.05) for the untreated group and 2.07 (0.04) for the Er:YAG laser group.[15] In accord with the outcome of Khan et al., the mean Ca/P ratio for bioactive glass was estimated to be 2. [16] The difference between the independent means was determined using the F test, and the pooled standard deviation (SD) was 0.045; there was a sample of 8 samples per group. This was elevated to 10 samples to compensate for procedure errors. Total sample = Number per group × Number of groups = 10 × 4 = 40 samples.

The sample size was established on Rosner's method and computed by G*Power 3.1.9.7.[17,18]

B. Sample selection:

40 Premolar teeth were gathered, cleaned and kept hydrated in distilled water containing 0.5% thymol solution (Khalil Pharmacy Laboratory, Alexandria, Egypt) to inhibit microbial growth at 4°C until use. Carious, fractured, and restored teeth were excluded.[19]

C. Sample preparation:

After the extracted teeth were placed in acrylic blocks, 40 dentin discs of about 1 mm thick were generated. The thicknesses were produced with an angle 90 degrees to the vertical axis of the tooth under the amelodentinal junction and above the pulp horn with a microtome machine (Micracut 150, Metkon Metallography, Bursa, Turkey).[19] The thickness of the specimens was verified with a calliper.[20]

D. Dentinal tubule opening protocol

The specimens were soaked in a 1 wt% citric acid solution (Khalil Pharmacy Laboratory, Alexandria, Egypt) for 20 seconds and then rinsed thoroughly with water to make a sensitive tooth model by opening the dentinal tubules.[21]

F. Sample grouping:

The samples were divided randomly into 4 gps, each containing 10 samples after dentinal tubule opening:

Group 1: Received no treatment (positive control group)

Group 2: Included those treated with 45S5 bioactive glass NPs only.

Group 3: Discs were exposed to Er:YAG 2940 nm laser radiation only.

Group 4: Discs were irradiated with Er:YAG 2940 nm laser and then treated with 45S5 bioactive glass NPs.

G. Characterization of the bioglass NPs 45S5

The Bioglass nanoparticles used in this study were prepared by a sol-gel method (Nanogate, Egypt).[22] Characterization was performed using transmission electron microscopy. Transmission electron microscopy (TEM) is the most widely used technology for analyzing nanoparticle size and form because it offers not only direct images of the material but also the most accurate estimate of nanoparticle homogeneity.[23,24] Fig. (1) shows that the average dimensions of the nanoparticles extended from 10.89 nm to 29.62 nm.

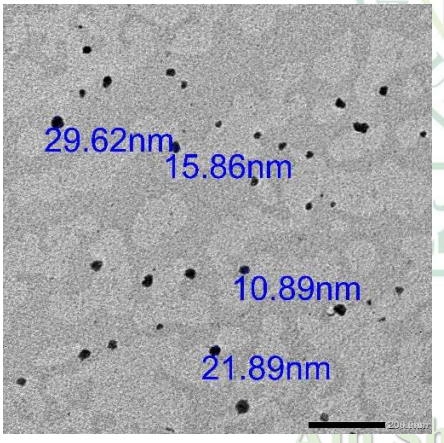

Figure (1): TEM image of Bioglass nanoparticles.

Er:YAG laser application:

Samples from groups 3 and 4 were irradiated with Er:YAG 2940 nm wavelength (Fotona lightwalker model 85979 CE ENG 34) according to the parameters described by Zhuang H. for effective occlusion of DTs and safety of dental pulp (0.5 W, 10 Hz, 50 mJ) for 30 seconds under water spray (level 9) and air spray (level 4) perpendicular to the dentin superficies (approximately 1 cm) with the tipless noncontact handpiece in a back-and-forth movement over the whole surface.[25]

The samples of group 4 after laser irradiation, a layer of bioglass NPs paste was added to the whole surface and retained by a layer of bond.

In a specially designated laser clinic run by the laser department of Alexandria University of Dentistry in Egypt, the laser was utilized in accordance with laser safety protocols, which included wearing gloves and eye goggles and avoiding direct exposure.

Application of Bioglass:

In groups 2 and 4 (after laser irradiation), 45S5 bioactive glass NPs were administrated to the surface of the specimens. The bioglass powder was combined with 50% phosphoric acid (Khalil Pharmacy Laboratory, Alexandria, Egypt) on a glass slab for 1 min to form a creamy paste and to use it as a liner over the whole dentin surface. This acidic gel (approximately 0.5 mm thick) was spread to the surface of the dentin discs with a microbrush.[26] The paste was then coated with a thin film of light curing bonding agent (all-bond universal, Bisco, USA), and it was subjected to a light cure for 20 seconds.[13,27]

The bioglass layer coated with the bond was retained for 24 hours and then removed with an excavator, and the samples were washed thoroughly with water.[13]

H. Sample storage:

All the samples were reserved in artificial saliva at 37°C for 24 hours and after that prepared for assessment.[21]

The artificial saliva used was 0.4 gm NaCl, 0.4 gm KC1, 0.795 gm $CaCl_2$, 0.78 gm NaH_2PO_4 (Mono basic), 1 gm urea, and 1000 ml Aqua (Khalil Pharmacy Laboratory, Alexandria, Egypt).[27]

F. Sample preparation for assessment:

The samples with a bioglass layer, following a 24-hour storage period, the bond-coated bioglass layer was removed using an excavator, and the samples were thoroughly cleaned with water and dehydrated.[13,27]

To prepare the samples, after assessment all the samples were removed from artificial saliva rinsed with water and dehydrated.[13,21,27]

G. Assessment:

I. Morphological analysis:[24]

After dehydration and drying of all the samples they were coated with a thin layer of gold by an ion sputter evaporator device. The dentin surface for all the samples was surveyed using a Scanning Electron Microscope JSM-IT200 (SEM) at the Faculty of Science, Alexandria University, to assess the changes in the dentin and dentinal tubules.[24]

II. Elemental analysis and chemical characterization:[24]

The dentin surfaces of all the samples were analysed via energy dispersive X-ray analysis by scanning electron microscopy (SEM-IT200; EDXA) at the faculty of Science Alexandria University for elemental analysis.[24]

III. Statistical analysis:

Q-Q plots and the Shapiro-Wilk test were used to verify the normality. The mass percentages of calcium, phosphorus, and oxygen were normally distributed, but the carbon mass was not. The data was presented using the mean, standard deviation, 95% confidence interval (CI), median, interquartile range (IQR), minimum and maximum values.

One-way ANOVA was utilized to compare groups for normally distributed variables, followed by Tukey's post hoc test with Bonferroni correction. For nonparametric variables, the Kruskal-Wallis test was employed. Every test was two tailed, and a p value of less than 0.05 was used as the significance threshold. IBM SPSS, version 23 for Windows (Armonk, NY, USA), was used to analyze the data. With the help of GraphPad Prism, version 10.0.0 for Windows (GraphPad Software, Boston, Massachusetts, USA), a graphical presentation was produced.

Results

Scanning electron microscopy

The dentin experimental discs were analysed by SEM for all groups. As shown in Fig. 2(a & b) & 3(a & b) for the control group, the examination revealed abundantly distributed dentinal tubules with wide openings and neither the formation of a smear layer nor smear plugs. The surface was almost completely clean except for some debris. The dentinal tubules were uniformly distributed, and the dentinal opening shows a defined circular or oval shape.

Fig. 2(c & d) for the bioglass NPs group, top surface examination showing a crystalline structure precipitated over the surface, obliterating the opening of the dentinal tubules. Fig (3c) Some openings were completely occluded with long calcified plugs, and others were partially occluded. Figure (3d) shows large numbers of densely packed openings.

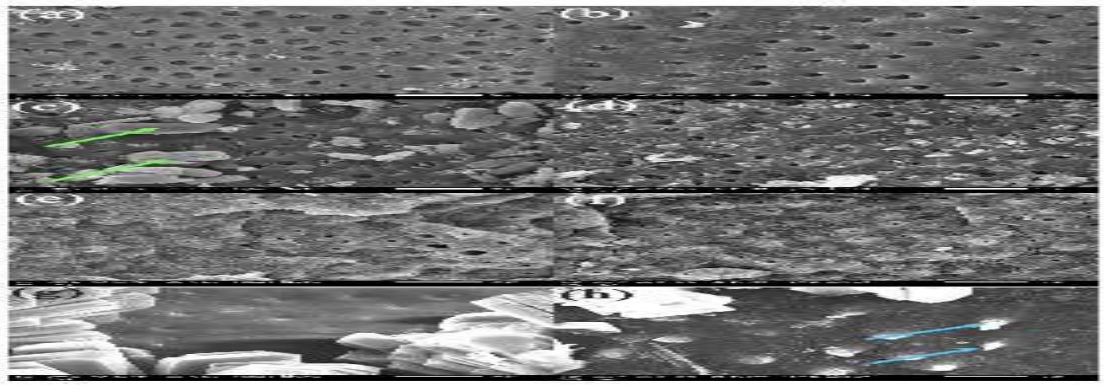

Figure (2): SEM images of the control gp (a & b), Bioglass NPs gp (c & d), Er:YAG laser gp (e & f) and combined gp (g & h) at magnifications of 2000×. Control gp observed in images (a & b) revealed abundantly distributed dentinal tubules with wide openings and neither the formation of a smear layer nor smear plugs. The bioglass gp in images (c & d) displayed a crystalline structure precipitated over the surface, obliterating the opening of the dentinal tubules. Green arrows (c) pointed at the crystalline structure over the surface treated with bioglass NPs. Er:YAG laser appeared in images (e & f), where the jagged surface showed a melting and solidification characteristic giving the unique scale-like appearance. Combined treatment gp images (g & h) displayed DTs openings that were completely closed. Note the smooth surface with a compact cluster of crystalline-like structures covering the dentine surface, plugging the dentinal tubules. Blue arrows (h) indicated the previous area of the DT opening after obstruction.

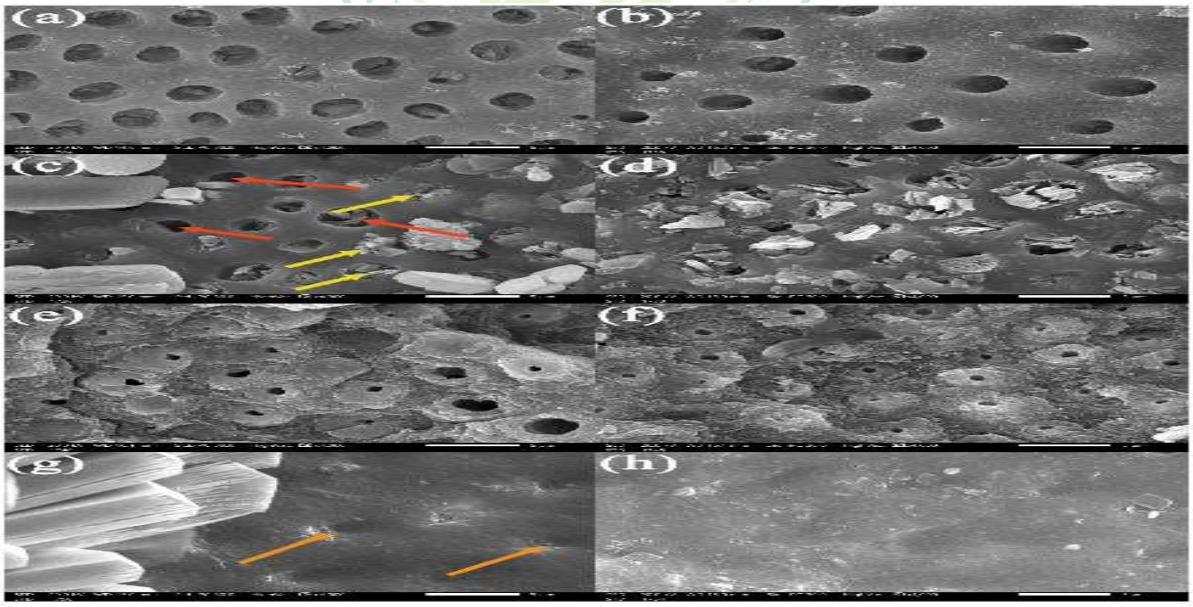

Figure (3): SEM images of the control gp (a & b), Bioglass NPs gp (c & d), Er:YAG laser gp (e & f) and combined gp (g & h) with 4,000x magnifications. Control gp images (a & b) demonstrated a wide DTs opening with uniform edges in circular or oval shapes. Bioglass gp images (c & d) revealed the obstruction of the DTs with bioglass. Red arrows (3c) pointed to partially closed DTs while yellow ones (3c) showed DTs stuffed with crystals. Er:YAG laser gp images (e & f) presented the irregular outline of DTs opening, with many others already totally sealed. Also showed the irregular surface with the molten appearance. Combined group images (g & h) revealed the smooth dentin surface surrounded by the crystal-like mineralized structure (3g). Orange arrows in (3g) pointed at the depression representing the site of DTs before obstruction

.

For the Er:YAG laser group observed in Fig. 2(e & f) and Fig. 3(e & f) the dentinal tubules were smaller with irregular shapes and irregular outlines in comparison with control group. Most of the openings were almost obliterated, with a few still widely opened Fig. 3(e & f). All the laser group images show a unique scale-like appearance. The jagged surface shows a melting and solidification appearance.

As shown in Fig. 2(g & h) and Fig. 3(g & h) for the combined group, the dentinal tubule openings were completely closed, and the surface was smoother than that of the other groups, with a compact cluster of crystalline-like structures covering the dentine surface plugging the dentinal tubules, which is more prominent than in laser group. The site of the openings only appears as a slight depression in Fig (3g) or a white colour representing the bioglass impeded in the opening in Fig (2h).

Energy dispersive X-ray analysis (EDXA)

EDXA exhibited a significantly greater Ca mass% in group 4 than in groups 3, 2, and 1, as we can see in Table (1).

Table 1: Comparison of calcium mass percentages among the groups

	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	Group 4 (n=10)
Mean ± SD	12.26 ± 2.22	18.87 ± 3.03	24.48 ± 1.97	29.34 ± 3.67
95% CI	10.67, 13.84	16.70, 21.04	23.07, 25.89	26.72, 31.96
Median (IQR)	13.12 (2.92)	17.99 (5.06)	24.60 (1.76)	29.94 (6.51)
Min – Max	7.88 – 14.71	15.21 – 24.47	19.60 – 26.93	24.04 – 35.58
F Test (*p* value)	68.94 (<0.0001*)			

*Statistically significant difference at *p* value≤0.05, CI: confidence interval, IQR: interquartile range, F test: one-way ANOVA

Nonetheless, group 4 had a considerably higher P mass percentage than the other groups (p <0.05). As indicated by Table 2, group 1 had the lowest P mass%, but there was no significant difference between groups 2 and 3 (p >0.05). The C mass percentage showed a different pattern. The largest C mass value was displayed in group 1, and the smallest was in group 3. Table 3 showed that there was no statistically significant variance between gp 1 and gp 2, or between gp 4 and either gp 2 or gp 3.

Table 2: Comparison of phosphorus mass percentages among the groups

	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	Group 4 (n=10)
Mean ± SD	7.97 ± 2.42	14.30 ± 2.71	14.62 ± 0.80	17.59 ± 3.07
95% CI	6.24, 9.71	12.36, 16.24	14.05, 15.19	15.39, 19.79
Median (IQR)	8.88 (3.30)	15.20 (3.57)	14.55 (1.46)	17.00 (4.10)
Min – Max	2.68 – 10.07	10.05 – 19.10	13.53 – 15.93	14.10 – 24.56
F Test (*p* value)	28.09 (<0.0001*)			

*Statistically significant difference at *p* value≤0.05, CI: confidence interval, IQR: interquartile range, F test: one-way ANOVA

Table 3: Comparison of carbon mass percentages among the groups

	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	Group 4 (n=10)
Mean ± SD	37.07 ± 7.27	27.61 ± 11.22	9.49 ± 2.01	17.69 ± 10.07
95% CI	31.86, 42.27	19.58, 35.64	8.05, 10.92	10.49, 24.89
Median (IQR)	37.36 (5.40)	24.86 (21.21)	10.12 (3.27)	15.71 (14.62)
Min – Max	21.83 – 51.08	13.61 – 43.30	6.52 – 12.57	6.06 – 39.45
H Test (*p* value)	24.76 (<0.0001*)			

*Statistically significant difference at *p* value≤0.05, CI: confidence interval, IQR: interquartile range, H test: Kruskal–Wallis test

Discussion

An elevation in the quantity and size of patent dentinal tubules on the tooth surface exposed to the oral environment is connected to dentin hypersensitivity.[2,3] Both the smear layer and the tubular plugs must be removed to sensitize the exposed dentin.[3] In this study, a sensitive tooth model was generated with 1% wt. citric acid to clear the smear layer and unblock the dentinal tubules, as shown in the

control group. As a result, the goal of dentin hypersensitivity treatment is to partially or permanently close the dentinal tubules.[2,3]

The use of nanomaterials for DT occlusion has the potential to improve the treatment of DH. Nanosized particles have increased reactivity and surface area because they can easily enter dentinal tubules.[28] When it comes to applications in dentistry, bone tissue engineering, and bone replacement, nanoscale bioactive glasses are more versatile than standard (micrometer-sized) bioactive glasses.[29]

Several varieties of bioactive glasses exist based on the components they contain. The primary commercial formulation, known as Bioglass 45S5, has FDA approval and comprises 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5.[30] the same formula was prepared for the current study. Bioglass 45S5 can be thought of as the "parent" glass of many compositions that were created by altering the original formulation.[31] When these glasses come upon tooth surfaces and saliva in acidic environments, they can dissolve, which releases phosphate and calcium ions while increasing the pH at the same time.[13] As we can see, blending bioactive glass with a phosphoric acid solution causes the release of calcium, phosphate, and sodium ions into an acidic environment. At the same time, the acidic solution has the ability to move phosphorus and calcium ions to the underlying tissue, where they can pass through the porosity of the tissue.[13]

The technique of coating the bioglass paste with a film of adhesive resin is used to protect the paste from being dissolved and keep it attached to the treated surface for the desired time to complete its bioactive stages of chemical reaction.[13] Er:YAG lasers with a wavelength of 2940 nm have provided a different approach to address DH due to their strong water absorption and are intended to reduce heat injury to pulp and dentin tissues.[2,25]

In consonance with the SEM results of the present experiment, the control group (sensitized sample) exhibited widely opened dentinal tubules with neither smear layer nor smear plug. These results are in agreement with those of Choi et al., who noted a dentine surface devoid of a smear layer and exposed DT after the same treatment with 1% citric acid.[32]

By using bioglass nanoparticles alone, some calcified crystal structures were found to partially occlude DTs with plugs; Curtis et al. supported these results by finding clusters of nanobioglass when observed immediately, while a day later, rod-like protrusions and agglomerations were found within and around DTs.[33] Solati et al. also reported the invasion of DTs by nanobioglasses.[34] In contrast, Huang et al. demonstrated a smooth dentin appearance with many opened DTs in the bioglass group, although in the longitudinal segments, a superficial sealing layer was observed, and tubules were incompletely blocked with finite penetration depth, possibly due to the difference in application technique and, mostly, the variation in particle size.[35]

The laser-only group of samples mostly exhibited closed DTs, and the opened tubules were narrow and ragged in shape and had an outline with a general melting bubble appearance on the dentine surface. Zhuang et al., with the same parameters used herein, reported a surface with a melted layer that almost blocks the openings of DTs.[25] Kurt et al. and Sahin et al., although with different parameters, supported constricted and plugged tubules.[36,37] While Srivastava et al. reported a heavily charred and dissolved surface that did not aid in DT obstruction

obviously due to using high power output.[38]

Compared with SEM image of other groups, the combined group had the smoothest surface, with almost complete closure of tubules and the aggregation of crystalline-like structures overlaying the surface. When compared with the laser only group which has an irregular surface this smooth surface emphasizes the effect of bioglass mineralization and alteration of dentin surface when combined with laser. Rashed et al. micrographs demonstrated many closed DTs with nanobioactive glass accumulations, while other openings were narrowed.[39]

Although Huang et al. reported that when laser and bioglass were combined, a small number of tubules were obstructed, while the majority had loose lumps that resembled blockages in the combined group, possibly because the difference in laser parameters and size of the bioglass particles resulted in this dissimilar outcome.[21]

According to the EDX analysis, although the nanobioglass group and laser group contained less calcium and phosphorus than did the combined group, they were still superior to the untreated control samples. In Souza Penha et al. 45S5 bioglass samples showed more calcium and phosphorus predomination than did untreated samples, while the carbon content was greater in the untreated group.[40] Regarding the EDX analysis of Sahin et al, the laser group using same wavelength, Ca and P content were almost maintained with that of the intact dentin which suggest a remineralised effect when compared with demineralized dentin.[37]

Calcium and phosphorus were most prominent in the group of Er:YAG + bioglass Nps with less carbon than in the sensitized group. Rashed et al. confirmed the same high percentage of Ca and P belongs to the combined gp of nanobioactive glass and laser.[39]

The limitations of the hereby study include the absence of assessments of dentinal tubule occlusion. The absence of follow-up after different periods and with acidic challenges. Another hindrance is the small quantity of samples.

Conclusion

The combination of nanobioglass and the Er:YAG laser had more impressive effects than either method independently on the blockage of dentinal tubules, with superior results.

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Data availability

All datasets and materials used and/or analysed during the current study are included in this published article.

Ethical approval

This in vitro study was approved by the Research Ethical Committee of National Institute of Laser Enhanced Sciences with approval reference: (NILES-EC-CU 24-10-18).

The teeth were collected from a dental clinic in Alexandria, Egypt. They were extracted for orthodontic treatment according to the treatment plan from the specialized orthodontist and accepted by the patients. Formal informed consent from orthodontic patients was needed to take part in this study.

Competing interest

The authors declare no competing interests.

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