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ORIGINAL ARTICLE

Evaluation of the effects of Er:YAG laser, Nd:YAG laser, and two different desensitizers on dentin permeability: in vitro study

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Abstract

The purpose of this in vitro study was to evaluate and compare the efficacy of erbium-doped yttrium aluminum garnet (Er:YAG) laser, neodymium-doped yttrium aluminum garnet (Nd:YAG) laser, PrevDent nano-hydroxyapatite toothpaste plus Repairing Serum Kit (PNH), and NUPRO Sensodyne Prophylaxis Paste with NovaMin (NPP) on dentin permeability reduction. Forty dentin discs obtained from bovine incisors were divided into four study groups: Er:YAG laser-treated (2940 nm; 0.2 W, 80 mJ/pulse, 3 Hz); Nd:YAG laser-treated (1064 nm; 1 W, 10 Hz); PNH-treated; and NPP-treated groups. The quantitative changes in permeability of each dentin disc were measured using a computerized fluid filtration method (CFFM) before and after desensitizer treatments. The data were analyzed using the Wilcoxon, paired-samples *t*, Kruskal-Wallis, and Mann-Whitney *U* tests. The dentin surfaces and tubules were also morphologically detected by scanning electron microscopy (SEM). In all groups, dentin permeability was significantly reduced after the desensitizer and laser treatments ($p < 0.05$). Among the groups, we detected a significant difference in only when comparing the Er:YAG laser- and NPP-treated groups ($p = 0.034$). SEM analysis revealed physical changes in the dentin surface in all groups. This in vitro study shows that all tested desensitizers and laser treatments reduced dentin permeability. Also, surface changes, such as complete or partial occlusion or shrinkage of dentin tubules, were observed in all groups. Although the laser groups performed best, the PNH protocol can be considered as an alternative therapeutic product. In addition, clinical and laboratory studies should be performed for this product, and their efficacy should be assessed by combined therapy with lasers.

Keywords Dentin sensitivity · Dentin permeability · Computerized fluid filtration device · Laser

Introduction

Dentin hypersensitivity (DH) is a common condition that can be defined as a sharp, short pain arising from exposed dentin surfaces [1, 2]. Thermal, mechanical, and osmotic changes in dentinal tubules cause DH; the movement of fluids within the tubules stimulates a nerve receptor sensitive to pressure that leads to transmission of the pain stimuli [2]. This theory, which is the most accepted explanation for the sensitivity-response mechanism, proposes that pain arises via a hydrodynamic mechanism [3–5].

DH is more frequently encountered in patients with periodontitis. DH also occasionally appears following dental procedures, such as periodontal therapy, tooth whitening, and restorative procedures [3, 6]. Various desensitizing procedures and agents can be used in the treatment of DH [7]; many investigators have also successfully used various types of lasers [6]. Studies have detected reduced dentin permeability following all of these treatment procedures, which is a change that can be explained by hydrodynamic theory [8, 9]. Erbium-doped yttrium aluminum garnet (Er:YAG), neodymium-doped yttrium aluminum garnet (Nd:YAG), carbon dioxide (CO_2), and surgical diode lasers are frequently used to treat

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DH [6, 7, 10]. Nd:YAG laser treatment provides occlusion or narrowing of the dentin tubules and nerve analgesia; Er:YAG lasers dissolve and evaporate the surface layer, causing insoluble salts to accumulate in the dentin tubules [6, 11, 12]. In short, lasers seal tubules by recrystallization or melting of the dentin [6, 11].

Nano-hydroxyapatite (n-Hap) agents, generally considered among the most biocompatible and bioactive materials available in dentistry, are commonly used for this [13, 14]. n-Hap also has the potential to repair dental enamel, and recent studies have found that n-Hap is effective in occluding dentinal tubules [13, 14]. Several products are available, including one tested in this study, the PrevDent n-Hap Toothpaste and Repairing Serum Kit procedure (PNH).

Calcium sodium phosphosilicate, known by the trade name NovaMin, contains calcium, sodium, phosphate, and silica and is initially used for bone regeneration [15, 16]. NovaMin has also been used as a desensitizing agent for occluding dentin tubules [16]. Recent studies have reported promising clinical findings for NovaMin. In the literature, several NovaMin-containing agents, including NUPRO Sensodyne Prophylaxis Paste with NovaMin (with and without fluoride (NPP)), which is used to reduce DH following periodontal dental treatment, such as scaling-root planing [16].

There are studies in the literature that compare different treatment procedures for DH, but several issues remain to be clarified. Studies on n-Hap products are limited, and treatment protocols containing a combination of serum and restorative toothpaste have not yet been evaluated. To our knowledge, the efficacies of laser and n-Hap therapy for DH have not been compared. This study aimed to evaluate and compare the effects of using the different chemical desensitizing agents and laser irradiation on dentin permeability and surface morphology.

Materials and method

This in vitro study used a computerized fluid filtration device modified from Orucoglu et al., as well as dentin discs obtained from bovine central incisors [17]. Post-/pre-treatment measurements, treatments, and SEM imaging were performed by different researchers, who were unaware of which study group the samples belonged.

Preparation of dentin discs and the use of computerized fluid filtration method

The bovine central incisors were obtained from newly cut animals of less than 2 years old (from slaughterhouse materials). All organic and inorganic additives in the teeth were removed under running water using a periodontal curette. The teeth were kept in 10% formalin solution at 4 °C until the experimental study and were used within 4 weeks.

For the preparation of dentin disc, crown sections were separated from the teeth, and the roots were then fixed to the precision cutting device (Buehler, IL, USA; Isomet 1000) perpendicularly to the long axis of the teeth. The cementum part was removed from the root at 200 rpm with abundant water cooling. Afterward, 1-mm thick sections were obtained from the root dentin portion of the teeth. Following this phase, dentin discs were examined under a stereomicroscope (Leica Microsystems, Heerbrugg, Switzerland; Leica EZ4) at × 20 magnification, and the discs containing pulp residue or enamel residue in the area to be measured (diameter, 3.4 mm) were excluded from the study.

Two surfaces of 40 dentin discs without cracks, enamel or pulp residue were sanded with 600 grit silicon carbide abrasive paper (Struers, Tokyo, Japan) for 30 s to form a standard smear layer under running water. The discs were then stored in distilled water at 4 °C until the measurement process.

In our study, the rubber-based O-rings (inner diameter, 3.4 mm; outer diameter, 8.16 mm) and dentin discs were connected to the computerized fluid filtration device for measurements [18, 19]. The standardization of the area to be measured was provided by the empty part in the middle of the rubber-based rings, while the impermeability standard was provided by the rubber and brass parts of the device. The rubber-based rings were glued to the pulpal surfaces of the dentin discs with fast-drying acrylamide glue (Pattex; Henkel, Dusseldorf, Germany). After, the dentine discs were made to be the size of the rubber rings which were placed inside the device, using aerators and fissure burs under water cooling (Fig. 1).

When using the computerized fluid filtration method (CFFM) to measure the permeability of the dentin discs, distilled water was used as a perfusion fluid. The discs were also checked for leaks. A pressure vessel was filled with distilled water and the hydraulic pressure set at 1.8 bar (or 1835.49 cm H_2O). Air bubbles are created by the microsyringe in the system, and measurements are then performed. All of these procedures were performed, and a computer program described by Orucoğlu et al. was used for the measurements [17].

The smear layer on each disc was removed by placing the discs in a 6% citric acid solution for 2 min before the first measurements were conducted [20]. After washing with water, maximum permeability was measured; six measurements were taken for each sample, and the averages of these measurements were then obtained.

Before performing the treatment procedure, the samples were divided into four groups, as follows: group 1, ($n = 10$) Er:YAG laser-treated group; group 2, ($n = 10$) Nd:YAG laser-treated group; group 3, ($n = 10$) PNH-treated group; and group 4, ($n = 10$) NPP-treated group.

The measurements of maximum permeability were different because each dentine disc was obtained from a unique

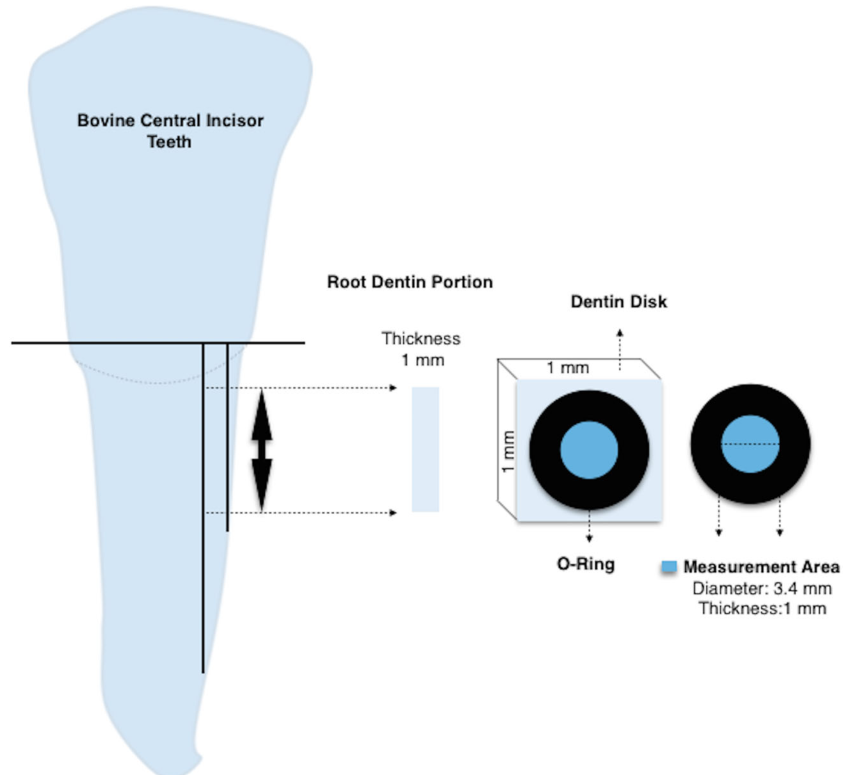

Fig. 1 The preparation of dentin discs and measurement area

animal. To minimize deviation in intra-group measurements, discs within similar measurement ranges were assigned to a single group. Therefore, this is a non-randomized study, and the sample distribution was performed after the initial analyses of permeability.

Treatment procedures applied to groups

The Er:YAG laser-treated group—The Er:YAG laser was applied using the Fotona laser system (Fotona AT, Fidelis III, Ljubljana, Slovenia). The laser application was conducted according to the parameters recommended for Fotona laser devices and studies in the literature [21]. The power indicator was set to 0.2 W, energy per pulse to 80 mJ/pulse, frequency to 3 Hz, and the device was set to short pulse (pulse duration, 300 μs) mode. An R02-handpiece (Fotona AT, Fidelis III, Ljubljana, Slovenia) was used for laser application. Also, during this time, water irrigation was applied to the surface of rubber-based rings (this application was 25 ml/min, to protect from thermal changes), but not the laser application area. The application was slowly and regularly applied to the surface (surface diameter, 8.16 mm). The distance between tissue and tip was 6 mm (i.e., the application was unfocused). For each sample, the entire procedure took ~2 min to complete. Ten dentin discs were washed with distilled water for 10 s and stored in sterile distilled water [19].

The Nd:YAG laser-treated group—The Nd:YAG laser was also applied using the Fotona laser system (Fotona AT, Fidelis III, Ljubljana, Slovenia). The application was made according to the parameters recommended for the Fotona laser device and previous studies [22]. The power indicator was set to 1 W, the frequency to 10 Hz, and the device was set to short pulse mode (pulse duration, 180 μs). A 300-μm Nd:YAG fiber was used; this application was slowly and regularly applied to the surface. The distance between tissue and tip was 2 mm. This procedure was done three times for each sample. Ten dentin discs were washed with distilled water for 10 s and stored in sterile distilled water [19]. For each sample, the entire procedure took ~2 min to complete.

The PNH-treated group—The protocol for PrevDent-DeSensiDent Home (PrevDent, Baroniestraat, Amsterdam, The Netherlands) was applied to the surface of the dentine discs. This protocol consists of repair tubes and repair serum. Each day, a repair tube was applied to the surface of the dentine discs for 2.5 min using its sponge application head, followed by a 20-min wash with distilled water. This treatment lasted 4 days, during which the treatment was supported by toothbrush brushing with the repair serum. The brushing experiments were conducted for 2 min twice per day (at 8 am and 8 pm) by a single toothbrush and roll technique; the repair tubes were applied only once per day (1 pm). In short, four repair tubes plus repair serum were simultaneously applied to the surface of the dentin discs, as suggested by the manufacturer's protocol. During and after application, the dentin discs were stored in distilled water. In this product, nano-hydroxyapatite crystals are identical to natural hydroxyapatite minerals, which make up 97% of tooth enamel and 64% of dentin. This product's ingredients are water, hydrated silica, sorbitol, glycerin, xylitol, potassium nitrate, nano-hydroxyapatite, magnesium aluminum silicate, mentha piperita oil, sodium lauroyl sarcosinate, xanthan gum, phenoxyethanol, potassium chloride, sodium sulfate, sodium saccharin, linalool, limonene, and titanium dioxide (Ci 77891).

The NPP-treated group—NPP (NUPRO Sensodyne Prophylaxis Paste with NovaMin (Dentsply, London, UK) was applied using a rubber cup on a slow-speed handpiece for 1 min on the dentine surface; 10 dentin discs were washed with distilled water for 10 s and stored in sterile distilled water. The NPP contained hydrated silica, glycerin, water, bicarbonate flavoring, cellulose, sodium saccharin, and NovaMin. Our application was performed according to the recommendations of the manufacturer.

The applications were applied to each group of 10 dentine discs, and the measurements were repeated by CFFM; six measurements were taken for each sample, and the averages were calculated.

In short, two measurements were taken for the dentine discs in each group. These measurements were for (1) maximum permeability (Lp1) after standing in 6% citric acid solution for 2 min and washing with water; and (2) after the application of the relevant sensitizer to the dentine discs (Lp2).

Scanning electron microscopy analysis

The dentine discs for each group were prepared and treated with the respective treatment procedure for that group. To display the morphological changes that the desensitization treatments caused in the dentine tubules, the dentine discs were broken following the treatment phase. Also, a dentin disc was prepared by only etching with citric acid. The discs were placed in glass Petri dishes (taking care not to touch the top surface) and desiccated; they were then kept under a vacuum in the desiccator for 12 h. The specimens were coated with gold/palladium (Au/Pd) and were examined using an scanning electron microscopy (SEM) device (JOEL JSM-7001F, Japan) at 10.0 kV and various magnification ratios ranging from × 500 to × 15,000.

Statistical analysis

The data were analyzed using the SPSS software program (SPSS Inc., version 19.0, Chicago, IL, USA). The normality of distributions was tested using the Shapiro-Wilk test. A Wilcoxon test or paired-samples *t* test was used to determine whether any significant differences existed between the pre- and post-treatment values for each group ($p < 0.05$). The Kruskal-Wallis test ($p < 0.05$) and the Mann-Whitney test were applied using Bonferroni's correction for the comparison of the differences between the groups.

Results

The outcomes of the Shapiro-Wilk test are shown in Table 1. The fluid-conductance measurements are summarized in Table 2. When the fluid conductance measurements were compared by the Wilcoxon test or paired-samples *t*, all treatment procedures for DH were observed to have reduced fluid conductance ($p < 0.05$). When differences between the groups were evaluated by the Mann-Whitney test, statistically significant differences were observed between group 1 (Er:YAG) and group 4 (NPP) ($p = 0.034$) (Table 2). The median permeability changes were 82.01, 75.34, 73.07, and 48.40% for the Er:YAG laser-treated, Nd:YAG laser-treated, PNH-treated, and NPP-treated groups, respectively. Among the treatments, the Er:YAG laser-treated group performed best, but this was significantly better than only the NPP-treated group (Table 2).

Figure 2 shows an untreated dentin disc after etching with citric acid. Images of the dentin surfaces and tubules in each treatment group are shown in Figs. 3 and 4 at various magnifications. Also, the closures and deeper closures with crystallization are shown in cross-sectional images (Figs. 3d, h and 4d, h). When SEM images were examined in all groups, different morphologic surface coverings were observed, which support the decrease in dentin permeability. When the SEM image of the untreated dentin disc after etching with citric acid was evaluated, it was seen that the dentin tubules are open, and the disc has a clean surface appearance (Fig. 2). In the laser-treated groups, the tubules were narrowed or clogged; there are also various surface irregularities and some cracks, which, in the Er:YAG laser-treated group, are thought to originate from ablation. In the PNH-treated and NPP-treated groups, the presence of accumulation, constriction, or closure in the dentin tubule mouths were observed (Figs. 3 and 4).

Discussion

Various clinical and laboratory investigations have been conducted to evaluate the efficacy of treatment procedures used for dentin hypersensitivity [4, 23, 24]. In clinical trials, the lack of objective assessments of pain, placebo effects, and other uncontrollable factors (often patient-related) can lead

Table 1 The outcomes of the Shapiro-Wilk test

Treatment groups	Shapiro-Wilk Sig.	
	Pre-treatment	Post-treatment
Er:YAG	0.049	0.014
Nd:YAG	0.139	0.196
PrevDent	0.525	0.376
NovaMin	0.040	0.002

Table 2 Dentin permeability before and after treatment for DH. Percentages of treatment success and differences between groups

	N	Mean-SD			Median (min-max)			*p*
		Before	After	Change %	Before	After	Change %	
Er:YAG	10	3.50 ± 0.44	0.90 ± 0.63	73.89 ± 18.81	3.44 (2.90–4.58)	0.64 (0.37–2.08)	82.01 (36.52–89.68)	$p < 0.05$[a]
Nd:YAG	10	2.55 ± 0.39	0.73 ± 0.38	70.08 ± 18.18	2.43 (2.14–3.23)	0.68 (0.28–1.60)	75.34 (28.14–87.82)	$p < 0.05$
PrevDent	10	1.47 ± 0.22	0.40 ± 0.24	72.70 ± 16.13	1.41 (1.20–1.91)	0.44 (0.10–0.79)	73.07 (50.52–92.78)	$p < 0.05$
NovaMin	10	1.42 ± 0.66	0.75 ± 0.55	47.01 ± 21.71	1.28 (0.82–2.66)	0.65 (0.32–2.13)	48.40 (19.89–77.63)	$p < 0.05$[a]

Values are means ± standard deviation and median (min-max). Permeability expressed in $\mu L\ cm^{-2}\ min^{-1}\ cm\ H_2O^{-1} \times 10^3$

[a] Statistically significant differences exist between the groups ($p = 0.034$)

to inconsistencies. Such inconsistencies are avoided in quantitative laboratory studies [25, 26].

DH is linked to the hydrodynamic theory, and there is a consensus that the sensitivity problem can be solved by reducing dentin permeability. Studies often examine hydraulic permeability changes to determine the success of tenderness treatments [8]. Numerous laboratory studies in the literature have been conducted on hydraulic permeability changes to evaluate the efficacy of various tenderness treatments [8, 19, 27, 28]. This method has several advantages, including the provision of objective and comparable numerical data and the method's ability to be repeatedly tested [19, 29]. Our study was conducted using a liquid filtration device based on hydraulic permeability changes; the post-treatment success of four different treatment procedures was evaluated using this device, as previously reported [8, 19].

Our study used bovine incisor teeth, which have standardizable permeability characteristics and are easily obtained [30]. For standardization, all discs were obtained (of the same thickness) from the dentin of bovine incisor teeth. O-rings were used to standardize the measurement areas and to determine the same area during re-measurements [18, 19]. So that the maximum permeability values of all discs could be obtained, the smear layer was removed with 6% citric acid solution before the measurements were applied [20]. Several studies have examined the various treatment procedures used, which have achieved favorable outcomes in treatment. These practices can be used in clinical routines [7, 11, 15, 16, 31, 32]. For the four different treatment procedures, we observed that the permeability decreased significantly after each treatment application ($p < 0.05$).

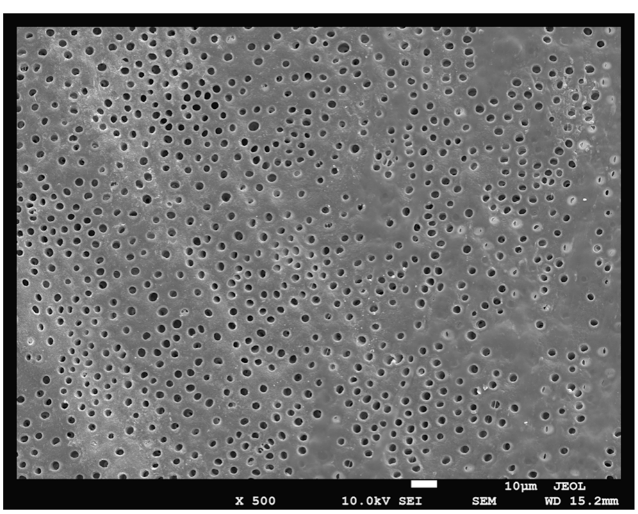

Fig. 2 SEM micrograph of untreated dentin disk after etching with citric acid (× 500)

Several studies have successfully demonstrated the use of lasers for DH treatment [21, 32–36]. Er:YAG and Nd:YAG lasers are thought to be able to treat dentin hypersensitivity by occlusion or narrowing of the dentinal tubules [37]. The Nd:YAG laser helps to obtain a non-porous structure by melting the surface; Nd:YAG laser treatment also has an additional analgesic effect by blocking nerve conduction [38, 39]. Various clinical and laboratory studies have proven the superiority of various laser types; studies have shown that Er:YAG and Nd:YAG lasers provide similar results [34] and that two lasers can be used successfully in the treatment of DH [35, 39]. Similarly, our study shows that the percentages of permeability reduction using Er:YAG and Nd:YAG lasers are not statistically different. Among the tested treatments, the greatest permeability reductions were seen in the Er:YAG laser-treated group. This suggests that the Er-YAG laser is more effective than the other procedures that we did not apply to narrowing or closing the tubules, and that treatment can be more tolerated in terms of permeability. Orchardson et al. reported that the efficacy of the Nd:YAG laser in DH treatment increased via neurotransmission blockage [38]. However, because we were unable to examine this neurotransmission blockage and analgesic effect under laboratory conditions, here, we only examined the mechanical permeability changes. The effectiveness of the lasers used in the treatment of DH also depends on the wavelength and power settings [40]; therefore, when choosing our parameters, we took into account the current literature [21, 22, 41] and the Fotona laser-application recommendations.

Several studies in the literature have examined the effectiveness of lasers, as well as the combined use of lasers

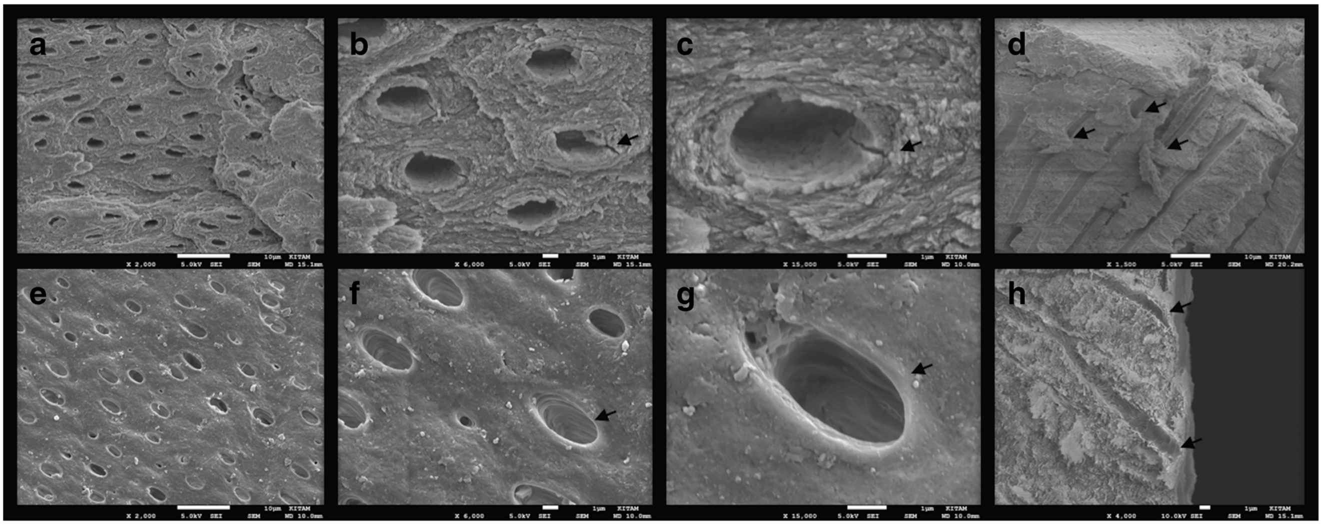

Fig. 3 SEM micrographs of the Er:YAG laser group: **a** (× 2000), **b** (× 6000), **c** (× 15,000), **d** Cross-sectional image (× 1500). SEM micrographs of the Nd:YAG laser group: **e** (× 2000), **f** (× 6000), **g** (× 15,000), **h** Cross-sectional image (× 4000)

and desensitizing agents [33, 34]. Studies have reported that lasers provide more meaningful and better results than desensitizing agents in stand-alone applications [21, 42]. Similarly, the results of our study show that the Er:YAG laser is more successful than NPP and that the permeability percentages of the Nd:YAG laser were considerably higher than with NPP.

The results of n-Hap treatment were similar results to the laser groups. To our knowledge, this is the first study to compare this product against laser applications. Costs and possible pulp damage must be taken into account when treating DH [42]. The PNH procedure does not cause pulp damage (but it has been reported that laser treatments can cause heat-induced pulp damage) and, therefore, might be preferable because of its easy applicability in clinics; also, in the future, PNH might be combined with laser treatments. Previous studies have compared n-Hap paste application products against other desensitizing agents [31, 43] and NovaMin [44, 45]. Kulal et al. demonstrated that the n-Hap paste outperformed NovaMin and that n-Hap paste was the best available material for DH treatment [44]. Gopinath et al. reported that both treatments showed similar results [45]. In our study, serum cursors were applied together with n-Hap-containing paste, resulting in better results (although the difference was not statistically significant).

When the SEM results were examined, the tubules appeared to be small or blocked when using the Nd:YAG laser. When using the Er:YAG laser, the surface of the tubules had an open and tubular appearance, although a deeper closure with crystallization was found in the cross-sectional images.

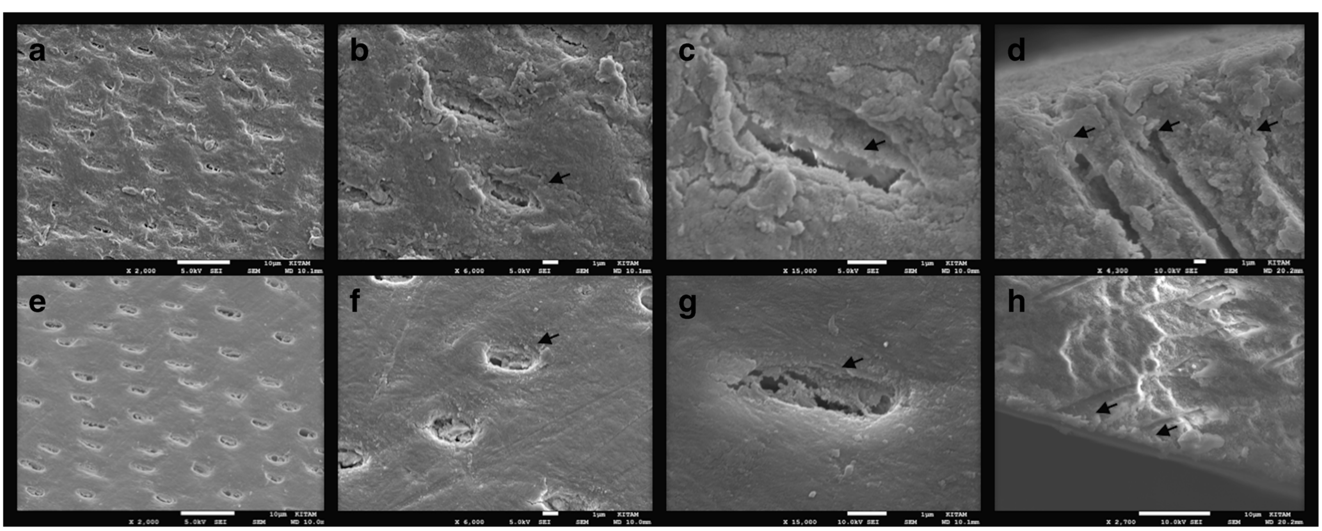

Fig. 4 SEM micrographs of the group PNH-treated group: **a** (× 2000), **b** (× 6000), **c** (× 15,000), **d** Cross-sectional image (× 4300). SEM micrographs of the NPP-treated group: **e** (× 2000), **f** (× 6000), **g** (× 15,000), **h** Cross-sectional image (× 2700)

One study has reported that Er:YAG laser light is absorbed by the water molecules in the hydroxyapatite and that dentin ablation results in morphological changes on the tooth surface, thus forming rough surfaces, cracks, and craters [34, 46]. The main reason for this activity is the micropatterns that form in the small particles of hard tissue, which originate from the sudden evaporation of water. The goal is not to destroy or melt the surface but to change its structure or chemical composition [34, 46]. Our SEM images were consistent with the literature since the various craters and cracks formed by the Er:YAG laser application were visible. Our SEM scans showed that tubules blocked or shrank after treatment in each group. For example, in the NPP-treated group, where the percentage of permeability was lower, the surface appeared to be covered with a crystalline layer. Because of its working protocol, some pressure is applied to the discs when measuring with a liquid filtration device. This must be taken into account when interpreting SEM images because there is no pressure in SEM analyses (in fact, in SEM analyses, closed dentin tubules even if observed, the current closures may be affected by external influences such as this pressure). If this pressure could have been closer to the pulpal pressure in teeth, then it would have been possible to obtain more meaningful Lp values and to make more clinically appropriate interpretations [19].

To conclude, four hypersensitivity treatment methods were evaluated in our study, each of which was able to reduce dentin permeability. Although the success of laser treatments has been demonstrated in many studies, our study also includes a non-laser treatment, PNH. These results are promising for the future of sensitivity treatment and suggest that a combination of lasers of different wavelengths and PNH therapy would increase treatment effectiveness. More extensive work and clinical studies are now needed to address this possibility.

Compliance with ethical standards

Conflicts of interest The authors report no conflicts of interest related to this study.

Ethical approval This article does not contain any studies with human participants or animals. The study is an in vitro study, and the bovine teeth obtained from slaughterhouse materials were used.

Informed consent Formal consent is not required for this study.

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