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Dentinal Tubule Occluding Efficacy Of Er: YAG Laser And Universal Adhesive Loaded With Nano-Carbonated Apatite

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ER:YAG laser and experimental resin-based dental adhesive loaded with functionalized carbonated apatite filler were used in this study to evaluate the dentin interaction in terms of penetration and occlusion of the dentinal tubules aiding in the control of dentin hypersensitivity (DH). Spheroidal Carbonated apatite nanoparticles (N-CAP), with an average size of 20±5 nm diameter, were synthesized, characterized, and incorporated in a universal adhesive "All Bond Universal, Bisco, USA", in (2% weight) concentration. Er:YAG laser "Lightwalker, FOTONA, EU" was adjusted to an energy output of 40mJ/ pulse and pulse repetition of 10 Hz for 10 seconds. Dentin specimens were prepared from the buccal surface of 75 extracted sound human molars. The specimens were randomly divided into five groups (n=15) according to the surface treatment: Group (L): Laser only; Group (LB): Laser in combination with adhesive; Group (LBN): Laser in combination with adhesive loaded with N-CAP; Group (B): adhesive only; and Group (BN): adhesive loaded with N-CAP. Depth of penetration and occlusion of the dentinal tubules were assessed using Environmental Scanning Electron Microscope Examination (ESEM). One-way ANOVA was used to compare groups, followed by a pairwise test for multiple comparisons (α=0.05). Groups (LB), and (LBN) showed the highest mean of dentinal tubules' penetration, with a non-significant difference between them. In contrast, the specimens treated with laser only (L) showed the most minor penetration. The employment of ER-YAG laser irradiation with the adhesive loaded with N-CAP was evaluated to be effective in penetrating and occluding the opened dentinal tubules.

Key Words: ER-YAG Laser, Bioactive carbonated apatite nanoparticles; Universal adhesive; dentinal-tubules occlusion; Dentin hypersensitivity.

Introduction

Dentin is a calcified tissue that forms the majority of the tooth structure which is formed of microscopic channels called dentinal tubules and an intra-tubular matrix. Dentin hypersensitivity (DH) results from the exposure of these tubules due to erosion, abrasion, attrition, abfraction, and dental caries, also due to loss of the enamel or cementum by parafunctional habits or poor dental repair procedures (1). The leading additional causes of dentin exposure are gingival recession, periodontal disease, or poor toothbrushing techniques. Dentin hypersensitivity is a common complaint of patients in dental practices that affects 10–30% of the population. DH is characterized by short and sharp pain caused by stimuli to the exposed dentin. Although "Hydrodynamic Theory " is considered the most accepted theory illustrating the mechanism of DH, however, other theories as direct innervation of dentinal tubules, neuroplasticity and sensitization of nociceptors, odontoblasts serving as sensory receptors, and algoneurons should be considered to suggest different treatment modalities to DH (2).

The conventional acceptable treatment protocols for tooth hypersensitivity are mainly based on two mechanisms: reducing the excitability of nerve fibers within the pulp and occluding the open dentinal tubules in different ways. The occlusion of the exposed dentinal tubules orifices is recognized as a temporary and relatively short-lived treatment modality as the used agents could be gradually worn away by the oral salivary clearance, daily tooth brushing, or maybe acid labile (3).

Different dental lasers such as He-Ne, CO_2, Nd: YAG, Er: YAG, or laser diodes were found to have a good impact in managing DH through denaturation and occlusion of the exposed dentinal tubules, which provides long-term desensitizing effects. In particular, the Er: YAG laser has a high absorption on the tooth surface; therefore, it has been applied on dental hard tissue as a treatment method, and its impact on controlling DH has already been reported (4).

Bonding agents are used for a variety of dental applications, one of which is restorative dentistry. Another use for dentin bonding agents could be the treatment of DH. Dental adhesives could be considered a long-lasting solution to control DH by penetrating the dentinal tubules and blocking their orifices. Self-etch bonding systems typically contain acidic ingredients that condition the dentin, as well as monomers that combine to dentin, forming a hybrid layer. This layer provides a coating over the dentin and significantly reduces hypersensitivity over four weeks (5).

Furthermore, adding an active ingredient, especially bioactive materials in a nanoscale, can help in penetrating the exposed dentinal tubules, leading to their partial blockage or occlusion of the orifices of the opened dentinal tubules. Carbonated apatite, one of the calcium phosphates, which mimics the natural biologic apatite found in dental and osseous tissues was supposed to be used as a treatment for DH. Nano-sized carbonate apatite (N-CAP) was found to have a significant impact on occluding the opened dentinal tubules (6) .

Finding an aiding method for more long-lasting desensitization was always proposed for better patient satisfaction. It was suggested that the penetration and mineral deposition inside the dentinal tubules may be an efficient and permanent strategy for diminishing dentin permeability and minimizing dentinal fluid movement, which in turn will aid in relieving pain (7). This study aimed to assess the performance of ER-YAG Laser and experimental resin-based dental adhesive loaded with functionalized N-CAP filler on the dentin interaction regarding penetration and occlusion of the dentinal tubules. The study hypothesized that combining Er: YAG Laser with universal adhesive loaded with N-CAP could enhance the occluding efficacy and the penetration power of the adhesive in the dentinal tubules.

Materials and methods:

Ethical Approval:

The study protocol was approved by the Ethics Committee at the National Institution of Laser Applications, Cairo University, under approval number 139 on 23/5/2020 and is in accordance with the declaration of Helsinki and its later modification.

Study Design:

Experimental, in vitro study, between five parallel groups.

Sample Size Calculation:

The sample size was calculated based on previously published research work by Lee S-Y et al (8). For testing the dentin penetration, a total of 50 samples were included. The effect size (f) was 0.674 resulting in 95% power and the $\alpha=0.05$. For each group, the minimum sample was to be 10 to achieve that power. However, the number of samples was increased to 15 in each group for statistical analysis reliability.

Laser device used in the study.

The laser equipment used was a pulsed (Er. YAG) 2940 nm laser system, which is considered a solid-state type of laser, with an active medium represented as (erbium-doped yttrium aluminum garnet laser, erbium YAG laser) wavelength in the infrared range, (Lightwalker, FOTONA, EU).

Synthesis and characterization of Carbonated apatite nanoparticles (N-CAP)

Nanoparticles were precipitated by a batch heating method according to the procedure developed by *López-Macipe et al*. (9) using two solutions: 100 ml of 0.1 M $CaCl_2$ + 0.4 M trisodium citrate and100 ml of 0.12 M Na_2HPO_4 + 100 mM Na_2CO_3 were mixed at 4 ^{0}C. The pH was adjusted to 8.5 with hydrochloric acid. Citrate anions were used as a calcium complexing agent to prepare homogeneous metastable solutions to avoid the instantaneous calcium carbonate or calcium phosphate precipitation. The mixture was then introduced in a 250 ml round-bottom flask, sealed with a glass stopper, and immersed in a water bath at 80 ^{0}C. The experiment was performed by collecting the powder after immersing the metastable and solution in the flask at 80 ^{0}C. After precipitation, the particles were repeatedly washed with distilled water and centrifugation (M1324, RWD, USA) . The resultant precipitated particles were dried at 37 ^{0}C for 48 hours.

N-CAP was characterized using a transmission electron microscope (TEM) (JEOL JEM-2100, Tokyo, Japan.) and X-ray diffraction (XRD) (XPERT-PRO., U.S.A). With the aid of specialized software. Data was gathered, and an absorption rate curve was drawn. The TEM was used to record size, shape, and particle distribution. An XRD pattern was performed using a powder diffractometer system, with 2Θ between (20^0 - 80^0) at a wavelength (Kα) = 1.54614^0, to identify the structure of the cellular units (d-spacing) used for the confirmation of a successful CAPNs synthesis. Minerals analysis was done by Energy Dispersive Analytical X-ray "EDAX" (Quanta 250, FEG, Netherland) and used for elemental mapping of the nano-carbonated apatite nanoparticles (N-CAP). Environmental Scanning Electron Microscope "ESEM" (Quanta 250, FEG, Netherland) was used for detecting the morphology and structure of N-CAP Also, dispersion and distribution of the nanoparticles throughout the adhesive were performed.

Adhesive system

Box 1. Adhesive used in the study (Specifications, Components, Manufacturer, Lot no):

Adhesive	Specifications	Components	Manufacturer	LOT no.
All Bond Universal	Universal dental adhesive	MDP[1], Bis-GMA[2], HEMA[3], ethanol, water, and initiators	BISCO Schaumburg, USA	2100008511

Methacryloyloxydecyl dihydrogen phosphate, 2 Bis-GMA: Bisphenol A di glycidyl methacrylate, 3 2-hydroxyethyl methacrylate

Incorporation of N-CAP into the adhesive system:

A concentration of 2% by weight was added to the universal adhesive (6.1ml) according to *Leitune VCB et al.* (10). 5% silane was added to the acetone solvent 95% and then added to the N-CAP for silanization and left for 24 hours to ensure complete solvent evaporation. 0.122 gm of silanized N-CAP was added to the adhesive to form the desired 2% concentration of N-CAP according to the following equation:

$$\frac{\text{The volume of the adhesive X concentration of N-CAP}}{100}$$

$$\frac{6.1 \times 2}{100} = 0.122gm$$

The procedure was performed entirely in a dark environment. To ensure complete homogeny and distribution of the N-CAP in the adhesive solution sonication was done in an ultrasonic device at 20 kHz (Anonkia, Shenzhen Guan Yijia Technology Co., China) for 1-hour. Sample preparation:

75 sound freshly extracted human molars were collected, mechanically debrided using an ultra-sonic scaler (NSK., Varios 570), and examined under a surgical operating microscope (G6, Global Surgical Corp; USA) for caries, fractures, or cracks. IsoMet cutting machine (IsoMet 4000 micro-saw, Buehler, Illinois, USA), was used to prepare dentin blocks from the buccal surface 1mm above the cemento-enamel junction (4 width × 4 length× 2 height mm). With the aid of Teflon mold with polymethyl-methacrylate resin the specimens were mounted and stored in deionized water. The dentin specimens were etched using 6% citric acid for one minute to remove the smear layer and open the dentinal tubules to simulate the oral condition of opened dentinal tubules followed by washing with distilled water for one minute (11) .

Samples were randomly distributed into five groups (n=15) according to the type of treatment received. (Box 2)

Box 2. Different treatment modalities of dentin specimens.

Group	Type of dentin treatment
L	Er:YAG laser was applied directly on the surface of the dentin.
B	The universal adhesive was applied directly on the exposed surface of the dentin.
LB	Er:YAG Laser was applied for 10 seconds after the application of universal adhesive on dentin.
BN	Adhesive containing N-CAP was applied on the surface of the dentin.
LBN	Er:YAG Laser was applied for 10 seconds after the application of universal adhesive containing N-CAP on dentin.

Laser irradiation

Er:YAG laser was adjusted to an energy output of 40mJ/ pulse and pulse repetition of 10 HZ for “10 secs”, using an angled handpiece (0.9) mm spot size. ER: YAG laser beam was applied in a non-contact mode without water coolant. The ER: YAG laser beam was controlled by the autofluorescence signal from the tooth surface induced by a blue-infrared diagnostic laser, emitting energy at 655nm. To keep the same diameter of the laser beam, the handpiece was fixed at a 25mm distance from the exposed surface using a transparent plastic block. The laser beam was applied with zigzag movement for 10 seconds to ensure uniform application of laser energy (11).

Application of the adhesive system

Regarding group (B), the adhesive was applied according to the manufacturer’s instructions. 1-2 drops of All Bond Universal “ABU” were dispensed and well-scrubbed on the exposed dentin surface using a micro brush for 20 seconds in two successive coats. Excess solvent was evaporated with the aid of oil-free air flow for 5 seconds until the dentin surface was glossy and a uniform adhesive thickness was obtained. The bonded surface was exposed to a fully charged LED light curing unit (radii plus SDI, Australia) for 20 seconds, with a light intensity of 1200 w/cm^2.light intensity was tested before application using the integrated radiometer. Similarly, in the groups where the experimental adhesive containing the N-CAP was used (BN, LBN), the adhesive was applied with the same protocol as the original adhesive. For groups (LB and LBN) the specimens were exposed to Er:YAG Laser beam before light curing the adhesive layer containing the N-CAP.

Environmental Scanning Electron Microscope Examination (ESEM):

ESEM was used to clarify the effect of different methods of treatment on the surface of dentin specimens. First dentin specimens were examined after citric acid etching (dentin demineralization) before any treatment. After treatment of each group, Image analysis was used to assess the opened, occluded, and partially occluded dentinal tubules. The specimens of all the study groups were sectioned longitudinally into two halves using the Iso Met cutting machine (Iso Met 4000 micro saw, Buehler, USA) to assess the depth of penetration of the testing materials inside the dentinal tubules. Images taken by the ESEM were transferred to Image Tool 3.0 (UTHSCSA, San Antonio). All images were presented on a micrometer scale, which was essential for the calibration of software measurement tools. The software polygon tool was used to identify dentinal tubules based on gray pixel intensity differences between dentinal tubules and the outer area. The length of the precipitate formed along the dentinal tubules from the dentinal surface to the bottom of the precipitate was measured in at least 20 dentinal tubules for each sample.

Statistical analysis

Data was checked for normality using Kolmogorov–Smirnov test. One-way ANOVA was used to compare between all groups. Followed by a pairwise test for multiple comparisons. A significant level was set at $\alpha=0.05$ (SPSS IBM, version 23, Armonk, NY, USA).

Results

Characterization of Nanoparticles

The prepared carbonated apatite nanoparticles were in powder form of white color. The resultant TEM images recorded and analyzed with a histogram to obtain the average size of nanoparticles showed that the prepared nanoparticles were of spheroidal shape with an average size of 20±5 nm diameter. (Figure 1)

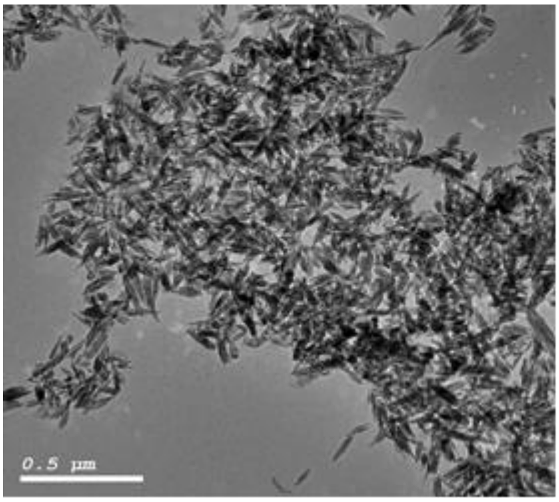

Figure 1. TEM images of the prepared N-CAP

By X-ray diffraction Analysis (XRD), the most intense peaks appeared at 25.88 (related to the 0.02 planes) and about 32 (broadband due to the triplet 211, 112, and 3.00) revealing the resultant nanoparticles were carbonated apatite (Figure 2).

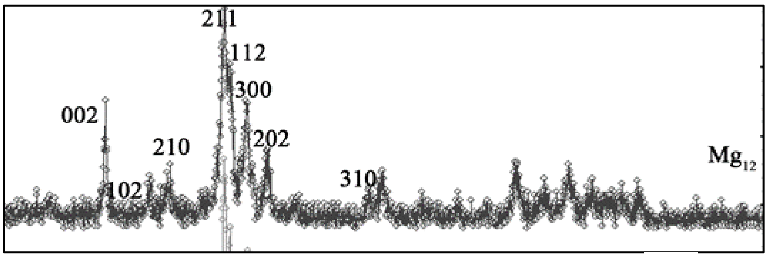

Figure 2. Absorption rate curve of N-CAP

Energy Dispersive Analytical X-ray (EDAX) of carbonated apatite nanoparticles N-CAP, showing evidence of oxygen, calcium, and phosphorus in the carbonated apatite samples (Figure 3).

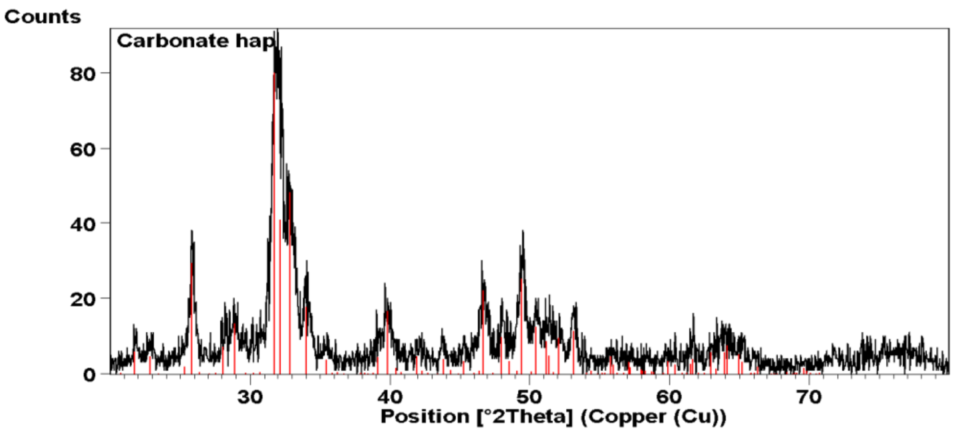

Figure 3. EDAX pattern of prepared N-CAP

ESEM (Environmental scanning electron microscope) images of dentinal tubules in dentin samples

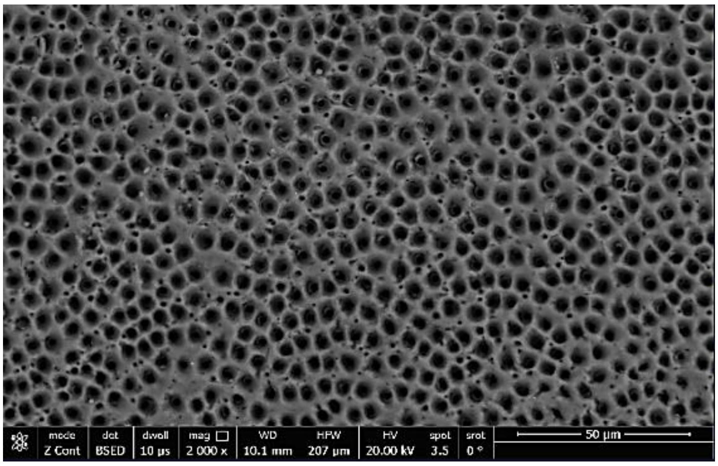

Figure 4. ESEM Image Of Orifices of the opened dentinal tubules before any treatment method (Mag X2000)

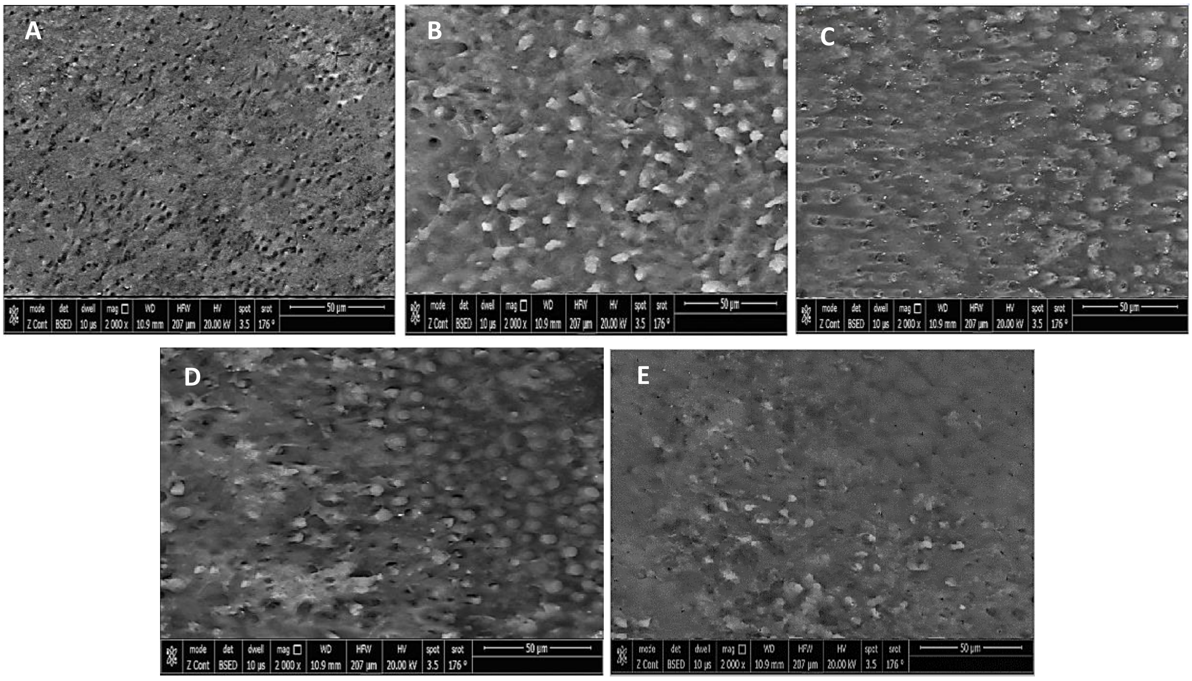

Figure 5. Effect of different methods of treatment on the surface of dentin: **a:** Er:YAG laser (L); **b:** Universal adhesive(B); **c:** Adhesive with Er:YAG Laser (LB); **d:** Adhesive containing the N-CAP; **e:** Adhesive containing N-CAP with Er:YAG Laser.

Effect of different treatment modalities:

From a top view, all five groups showed narrowing or occlusion of the surface dentin with different degrees when compared to the original untreated dentin (Figure 4), while the combination of the adhesive containing N-CAP with Er:YAG Laser showed the maximum occlusion where N-CAP appeared on the dentinal tubules' orifice.

Image analysis of ESEM on dentin samples with different treatment methods on the depth of penetration (longitudinal section):

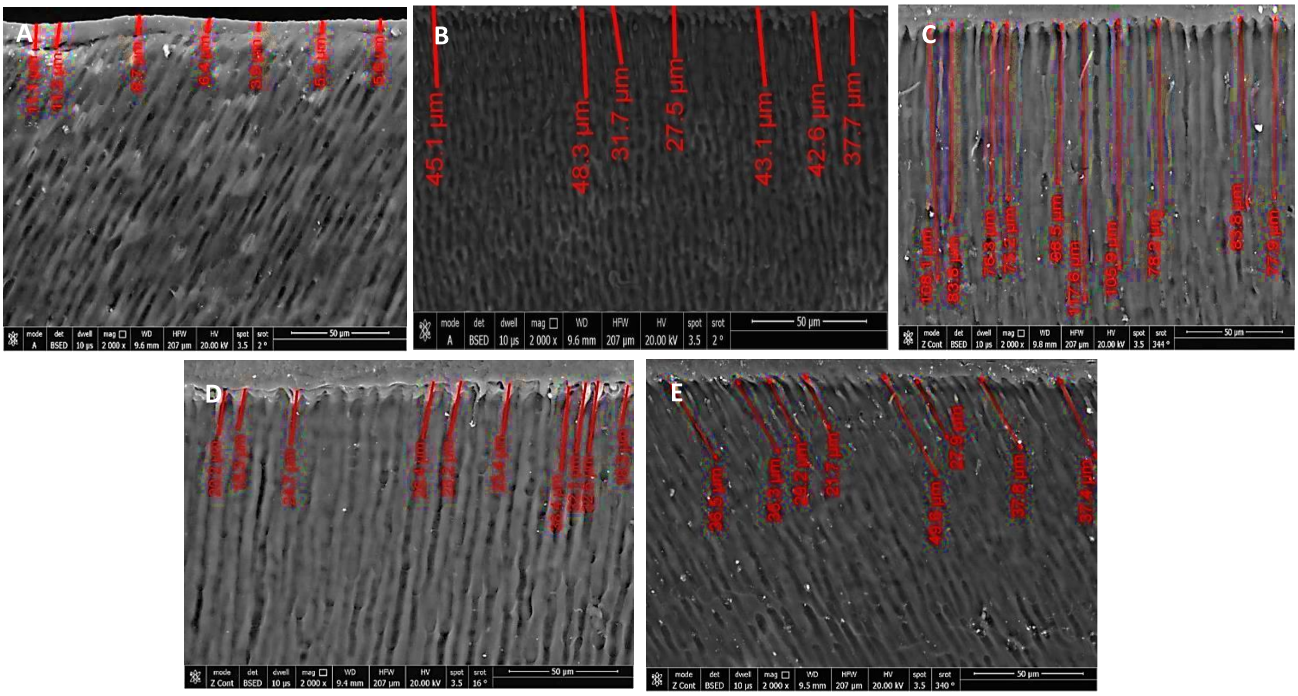

Figure 6. Image analysis of ESEM images of a longitudinal section of dentin samples with (x2000) magnification showing the depth of penetration and occlusion of dentinal tubules. **A:** Er:YAG Laser (L); **B:** Universal adhesive (B); **C:** Adhesive with Er:YAG Laser (LB); **D:** Adhesive containing the N-CAP (BN); **E:** Adhesive containing N-CAP with Er:YAG Laser (LBN).

Table 1. Comparison between different dentin treatment.

	Type of treatment	Mean	SD
Penetration (µm)	Laser beam alone (L)	8.6 [a]	2.5
	Adhesive only (B)	16.0 [a]	12.5
	Adhesive/N-CAP (BN)	15.7 [a]	6.5
	Laser+ Adhesive only (LB)	61.1 [b]	16.6
	Laser+ Adhesive/N-CAP (LBN)	48.5 [b]	23.4

Table (1) and Figure (6) show the effect of different testing techniques on the mean penetration depth of the tested materials (µm). The significant difference between all tested groups at $p<0.001$. Group LB where the laser was applied with adhesive showed the highest significant mean for penetration values within the dentinal tubules (61.1±16.6) followed by group LBN (48.5± 23.4) where the laser was applied to the adhesive that was loaded by nanoparticles treated group with the non-significant difference between them. Treating the dentin with laser only (8.6±2.5) adhesive only (16.0 ±12.5) or adhesive containing CAP Ns (15.7±6.5) showed the least penetration values with insignificant difference between them.

Discussion:

DH is one of the major challenges in dental practice. A usual approach to control the pain arising from exposed dentin, causing DH, would thus depend on the blockage or the reduction of the diameter of the dentinal tubules. For this reason, the current study was performed to assess the depth of penetration of a universal adhesive system only or when loaded with N-CAP with and without the aid of Er:YAG laser irradiation on dentin, which was treated to simulate the clinical condition. All the tested groups showed a barrier layer made through either occlusion or penetration of different treatment modalities to the exposed dentinal tubules. (Figures 2 and 3). This finding could be considered with high value, giving dental professionals different options to deal with DH.

Group (LB) where the laser was applied with adhesive showed the highest significant mean value for penetration within the dentinal tubules with a non-significant difference to the group (LBN) where the laser was applied to the adhesive loaded with N-CAP. Treating the dentin with laser only or adhesive only or adhesive containing N-CAP showed the least penetration values with insignificant differences between them.

The use of laser technology in dentistry has been widely studied because of its potential use in several dental applications. Recent studies suggest that lasers could be used as an alternative or complementary method for the modification of dental surfaces (12). Applying the Er:YAG laser only (L) on the dentin surface was not effective in penetrating dentinal tubules and recorded the most inferior penetration value (8.6 ± 2.5). This result is credited to the mode of action of ER:YAG laser which depends on the obliteration of the dentinal tubules by melting and solidification of the irradiated dentin (13). The irradiation of the dentin surface with Er:YAG laser decreases dentin permeability and increases acid resistance of dentin, however, the effect of laser desensitization is depleted over time because of mastication and friction from external objects such as the dental brush, leading to the removal of the barrier layer formed, which suggest the use of a more sophisticated alternative treatment options, gathering the effect of laser and other desensitizing agents (3,14).

The penetration and occlusion of the opened dentinal tubules were significantly achieved using Er:YAG laser irradiation following adhesive application (LB) and with adhesive loaded with N-CAP (LBN). In these two groups, the laser beam was applied after adhesive application and before polymerization to give a chance for the adhesive to penetrate and flow inside the tubules as the use of the laser before the adhesive procedure is not recommended, based on the fact that this type of irradiation leads to depletion of the dentinal tubules with partial or total occlusion, hindering its infiltration (15). Laser irradiation after the application of adhesive had the superiority of elevating the temperature of the irradiated dentin surface and thus decreasing the viscosity of the adhesive, and in turn increasing its penetration power (16).

Er:YAG laser was selected over other types like Nd:YAG and Diode Lasers as it has a great affinity to interact with structures that are bonded to water especially dentin, which forms 20-25% of its structure. The dentin interaction with Er:YAG laser leads to a sudden expansion of the crystalline structure forming dentin, destructing dentinal tubules in an irregular pattern by the reaction occurring in the peritubular dentin (13). Although some studies showed the superiority of Nd:YAG laser to deal with occlusion of dentinal tubules especially when combined with desensitizing agents (17), however, in a study conducted by Aghayan, S. et al(18) no significant difference in the results that gained from comparing the Nd:YAG, Er:YAG, or Diode lasers in occluding the dentinal tubules. Moreover, pulsed Er:YAG, independent of the pulse duration, has high selectivity to water and lower thermal penetration to deeper dentin layers (19), which secures the superiority of Er:YAG laser for treating DH.

The power and exposure time of the pulsed (Er. YAG) 2940 nm laser system are two contributing parameters that affect the amount of energy emitted to the tooth. The maximum safe power of a laser beam for dentin with a thickness of 1 mm had been suggested to be 40mJ/ pulse. This value is generally applied in clinical studies where the dentin thickness is minimal in cervical regions(11). However other studies that used water coolant in conjunction with the Er:YAG laser found no harm when using the 60 mJ power (20). The 10-second exposure time used in the present study was reported by *Han SY, et. al.* (11) who found optimum results with Er:YAG laser irradiation of 40mJ/ pulse and pulse repetition of 10 Hz for 10 seconds. The Er:YAG laser was applied in this low power parameters, in a trial to decrease the heat generation, and any negative effect generated by the Er:YAG laser on the dentin surface, especially since the ER:YAG laser was used with no water coolant so as not to wash away the adhesive in groups combining laser with adhesive (LB and LBN) (11). Fortunately, the adhesive cannot absorb the Er:YAG laser wavelength, based on the fact that it is a transparent solution, and the dentin exposed to low energy densities will heat up below its melting point, consequently, decreasing the viscosity of adhesive and improving the adhesive's ability to penetrate deeper into the dentin (15).

Laser irradiation of the adhesive at low energy densities appears to lead to a better, more intimate interaction of the adhesive with the dentinal tubules (Table 1). However, this result was contradicted by *Kouros P. et al.* (21) where the Er:YAG laser displayed lower adhesive penetration values in almost every combination with the adhesive system.

Recently, different nanomaterials have been given considerable attention in dentistry because of their potential qualities, high surface area to volume ratio, high surface energy, and large proportion of surface atoms (22). The N-CAP, as one of the nanomaterials that could be applied in dentistry, offers superior biocompatibility to act as a source of calcium and phosphate, in addition to possessing a strong ability to bond with tooth proteins , as well as a source of free calcium ions, which are required for remineralization (6). Spheroidal-shaped N-CAPs were used as they aid in more penetration and infiltration through the dentinal tubules compared to other nanoparticles' shapes (23).

The universal self-etch adhesive system integrates with the smear layer rather than its removal aiding in reducing the danger of DH (24). Adding 2% N-CAP by weight to the adhesive did not affect the flowability and penetration power of adhesive to dentinal tubules, which appeared in the results gained when comparing either group B to group BN, or group LB to LBN. This result was accepted in literature where different concentrations of nanoparticles were added to the adhesive and found that 2% w. concentration of nanoparticles aided in the best adhesive quality (10). However, SEM images (Figure 6) demonstrated that the N-CAP not only covered the dentin surface but also infiltrated through the dentinal tubules. The integration of the N-CAP with adhesive (BN) as an aid to block the dentinal tubules' orifices could be the reason for this outcome (6). Although, Leitune et al (10) found better adhesive quality when adding 2% w. nanoparticles to adhesive, they reported less penetration mean values to dentin when loading adhesive with nanoparticles. This finding was justified by the fact that nanoparticles added to the adhesive system act as fillers which increase the viscosity of the adhesive and decrease its seeping ability. The exposure of the dentin surface to Er:YAG laser after applying N-CAP loaded universal adhesive (LBN) secured the blockage of movement of nanoparticles through the dentinal tubules and more penetration through dentinal tubules, giving this treatment modality a predicted permanent maneuver for the treatment of the DH (12). To our knowledge, loading dental adhesive systems with carbonated apatite crystals has not been evaluated before.

The limitation of the current study was that it did not assess the longevity of laser treatment combined with N-CAP as a treatment for DH, more studies concerning these issues are encouraged in the near future to guide dentists for a more predictable and permanent solution for this daily-based problem faced worldwide.

Combining different treatment modalities to manage DH can give better results. The penetration power of adhesive loaded with N-CAP could be enhanced by using the Er:YAG laser beam on dentin surface which can aid in a more promising way to treat DH.

Conflict of interests

The authors declare that they have no conflict of interest .

Resumo

O laser ER-YAG e o adesivo dentário experimental à base de resina carregado com carga de apatita carbonatada funcionalizada foram usados neste estudo para avaliar a interação com a dentina em termos de penetração e oclusão dos túbulos dentinários, auxiliando no controle da hipersensibilidade dentinária (HD). Nanopartículas de apatita carbonatada esferoidal (N-CAP), com tamanho médio de 20±5 nm de diâmetro, foram sintetizadas, caracterizadas e incorporadas em um adesivo universal "All Bond Universal, Bisco, EUA", na concentração de 2% em peso. O laser Er:YAG "Lightwalker, FOTONA, EU" foi ajustado para uma saída de energia de 40mJ/pulso e repetição de pulso de 10 Hz por 10 segundos. Os espécimes de dentina foram preparados a partir da superfície vestibular de 75 molares humanos sadios extraídos. Os espécimes foram divididos aleatoriamente em cinco grupos (n=15) de acordo com o tratamento da superfície: Grupo (L): Somente laser; Grupo (LB): Laser em combinação com adesivo; Grupo (LBN): Laser em combinação com adesivo carregado com N-CAP; Grupo (B): somente adesivo; e Grupo (BN): adesivo carregado com N-CAP. A

profundidade de penetração e a oclusão dos túbulos dentinários foram avaliadas por meio do Exame de Microscópio Eletrônico de Varredura Ambiental (ESEM). ANOVA de um fator foi usada para comparar os grupos, seguida pelo teste de pares para comparações múltiplas (α=0,05). Os grupos (LB) e (LBN) apresentaram a maior média de penetração dos túbulos dentinários, com uma diferença não significativa entre eles. Em contraste, os espécimes tratados apenas com laser (L) apresentaram menor penetração. O emprego da irradiação do laser ER-YAG com o adesivo carregado com N-CAP foi avaliado como eficaz na penetração e oclusão dos túbulos dentinários abertos.

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