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The Effect of Eggshell and Seashell Nanoparticles Alone and Combined With Nd: YAG Laser on Occlusion and Remineralization Potential of Patent Dentinal Tubules: An *In Vitro* Study

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Abstract

Introduction: There is an interest in developing materials with bioactive potential that could block exposed dentinal tubules. This study compared the effects of eggshell and seashell nanoparticles individually or combined with ND:YAG laser on dentinal tubules occlusion and remineralization.

Methods: Fifty radicular dentin discs were prepared from freshly extracted human premolars. The smear layer created by cutting was removed using 37% phosphoric acid gel for 15 sec. The discs were divided into five groups according to the applied treatment(A) (n = 10 each): (A1) control, (A2); Nano eggshells, (A3); Nano seashells, (A4); Nano eggshells + Nd: YAG Laser, and (A5); Nano sea shell + Nd: YAG Laser. Each specimen was evaluated for tubular patency and mineral contents before and after each therapy using ESEM-EDXA energy dispersive spectroscopy for the assessment of tubule occlusion and remineralization.

Results: ESEM results revealed a statistically significant decrease in the mean percent changes of the dentinal tubules number after the treatment of the experimental groups compared to the control. The greatest percent decrease was recorded in the seashell NPs + Nd: YAG laser, followed by the eggshell NPs + Nd: YAG laser, then Eggshell NPs only and then Seashell NPs only, while the lowest percentage decrease was recorded in the control group. EDXA revealed that the greatest percentage increase in Ca wt% was recorded in the Eggshell + Nd:YAG laser group, followed by Eggshell only, then Seashell only and then Seashell NPs + Nd: YAG laser, while the lowest percent increase was recorded in the control group. The post hoc test revealed no significant difference between the experimental groups.

Conclusions: Both eggshell and seashell nanoparticles are effective in the occlusion and remineralization of dentinal tubules. The combined treatments with Nd: YAG laser had no benefits when compared to the effect of treatments alone.

Keywords: Eggshell; Seashell; Neodymium-doped yttrium aluminum garnet laser; Combined therapy; Dentinal tubules occlusion.

Introduction

Dentin hypersensitivity is considered one of the most painful and noxious conditions in dentistry. It is characterized by short, intense pain of exposed dentin of vital teeth in response to thermal, evaporative, tactile, osmotic, or chemical stimuli. Dentin exposure is primarily caused by two processes, either by wear of tooth enamel or by exfoliation of the root surface due to the loss of cementum and periodontal tissues coating it.[1]

The most generally accepted theory for this mechanism is the hydrodynamic theory. It focuses on dentinal tubules, which are open on the dentin surface, allowing a direct channel to the pulp. In the presence of any trigger element, the interstitial fluid in the tubules moves inward or outward and the displacement of fluid in the tubules is interpreted as pain fibers at the pulp wall.[2,3] Therefore, the method of controlling the hydrodynamic mechanism has led to the development of two types of products, namely (1) agents that reduce fluid flow in dentinal tubules and (2) agents or products that interfere with nerve impulse transmission.[4]

Potassium oxalate, sodium fluoride, strontium salt, amorphous calcium phosphate containing casein phosphopeptide, and calcium glycerophosphate are

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occluding agents which are widely used as desensitizers because they can seal dentinal tubules. However, tubules blocked by some of these materials have been reported to be superficial with a limited depth of infiltration.[5] Recently, there has been an interest in developing materials with bioactive potential that can block exposed dentinal tubules and subsequently reduce fluid flow in the dentinal tubules.[6]

As the demand for environmentally friendly technologies increases, the synthesis of biomaterials from natural sources rich in calcium remains a viable and more economical option. One such biological waste is eggshell, which provides a cost-effective, renewable, and sustainable source for enriched nano-hydroxyapatite.[7]

Eggshells are abundant in calcium. 94% of their constituents are calcium carbonate ($CaCO_3$), calcium phosphate (1%), magnesium carbonate (1%), and organic matter (4%). Eggshells contain not only calcium but also other elements such as fluoride and strontium. They have a positive effect on bone and tooth metabolism.[8]

This idea provides an innovation to produce a new valuable product from other waste materials such as seashells.[9] Seashells contain a high calcium source which acts as a calcium precursor. Some researchers found that the content of $CaCO_3$ in seashells is about 98% and 99%.[10] The term seashell usually refers to the shells of a marine mollusk. The production of calcite nanoparticles using seashells as the source of $CaCO_3$ is of great significance for environmental protection and biomedical applications. Seashells with a natural ceramic structure are similar to human bone and tooth structures.[11]

Recently, treatments using desensitizing materials are supplemented by laser therapy; improvements in technology, scientific knowledge, and evolution of new lasers with wavelengths suitable for therapy, their success rate for treating hypersensitive dentin has increased.[12]

Neodymium-doped yttrium aluminum garnet (Nd:YAG) laser is used for dentin hypersensitivity treatment by melting and occluding the tubules. It has been considered quite successful in various studies.[13,14] Combination treatment with Nd:YAG laser has resulted in the sealing of dentinal tubules (DTs) and a reduction in the diameter and number of opened dentinal tubules compared to the treatment alone.[14]

Nanomaterials are one of the highest quality materials for dentinal tubules occlusion due to their excellent dispersion when entering dentinal tubules of about 2-3 mm diameters easily. The properties of nanoparticles are quite different from their equivalent bulk materials. The solubility and reactivity of nanoparticles can increase due to the fact that they have a large surface area, high surface energy, and easy deposition in irregular spaces.[15]

One of the most important advances of the last few years is the implantation of nanoparticles in the dentin hypersensitive treatment regimen. The conversion of large particles to nanoparticles results in a larger surface area that increases the reactivity of nanoparticles and hence its effectiveness. Nanoparticles are widely used and have extensively been studied in recent years due to their advantages and superior properties.[16]

Despite the great potential of eggshells and seashells and their calcium bioavailability, the literature is scarce in evaluating the efficacy of eggshells or seashells therapy alone or combined with Nd:YAG laser on the dentin surface. Therefore, this study aimed to evaluate the effects of nano eggshells and seashell nanoparticles alone or combined with Nd:YAG laser on dentinal tubules sealing and mineral deposition ability in vitro. The hypothesis of this study is as follows: the tested materials would significantly occlude and remineralize opened dentinal tubules. The study tested two null hypotheses: 1) there would be no difference in the occluding and remineralizing potential by the same desensitizing agents before and after the therapy, and 2) there would be no difference in occluding and remineralizing capabilities when different natural biowaste agents were applied. 3) there would be no difference in the occluding and remineralizing capabilities between the mono-therapy and in-combination one.

Materials and Methods

Preparation of Eggshell Nanoparticles and Characterization

Biological waste chicken eggshells were obtained through a calcination process according to the protocol given by the World Intellectual Organization (WO/2004/105912: Method of producing eggshell powder). Calcination is carried out to produce powder free of pathogens and to increase powder alkalinity.

Chicken eggs were collected from a local hatchery; their contents were removed, separated from the membranes, and cleaned in distilled water, and the dirty parts were brushed off. Eggshells were kept in a hot water bath at 100°C for 30 minutes. Eggshells were dried and then crushed into coarse powder using a kitchen grinder. Furthermore, 385 g of duck eggshells were milled at 100 rpm for 10 hours using the ball milling reactor[17] (planetary- ball-mills pm 400, Faculty of Nano technology, Cairo University, 6th October, Egypt).

The tiny, crushed particles obtained were then calcined in a furnace at 1200°C for 1 hour to make sure the resulting powder is a pathogen free.

A high resolution transmission electron microscope was used for the examination of the resulting powder. TEM (JEOL, JEM-2100) was at a voltage of 80.0 kV. TEM micrographs showed that the prepared white nanoparticles had a spherical shape as represented in Figure 1A-B). They were 17 ± 3 nm in size.

Preparation of Seashell Nanoparticles and Characterization

Cockleshells were collected from Red Sea beaches in

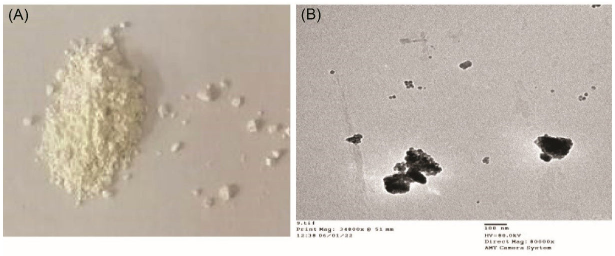

Figure 1. White Powder of Eggshell (A) TEM Photomicrograph Showing Spherical Shape Particles of Eggshells (B).

Egypt, and then they were washed to remove all dirt. 100 grams of seashells were boiled at 100°C for 30 minutes and dried for two days at 110°C in the oven, and then an agate mortar was used to grind the shells. A ball mill machine (planetary- ball-mills pm 400, Faculty of Nano technology, Cairo University, 6th October, Egypt) was used to mill the dried powder for 10 hours at 350 rpm at 3-minute intervals.[18]

The resulting gray powder as represented in Figure 2A was examined using a high resolution transmission electron microscope. TEM (JEOL, JEM-2100) was at a voltage of 80.0 kV. TEM micrographs showed that the prepared nanoparticles had a spherical shape as represented in Figure 2B. They were 21 ± 5 nm in size.

Artificial Saliva

Artificial saliva used in this study was prepared using 9 g NaCl + 0.24g $CaCl_2$ + 0.43 g KCl + 0.2 g $NaHCO_3$ all dissolved in 1 L of water according to a previous study.[19]

Sample Collection and Preparation

A total of fifty fresh intact human maxillary premolars were recruited in the current study. The teeth for orthodontic treatment were extracted from patients aged 18 to 24 years. Teeth with cracks, fractures, caries, restorations, or previous endodontic treatment were excluded. Teeth were scaled, cleaned, and then stored in distilled water with 0.2% thymol.[20]

Radicular dentin samples were obtained from the coronal third of the buccal surface of each root yielding 50 dentin discs of 4 mm long, 3 mm width and 1 mm thickness. Dentin specimens were embedded in rubber base impression materials using a rectangular hollow plastic mold to provide a means of immobilizing the specimen during the experiment (Figure 3).

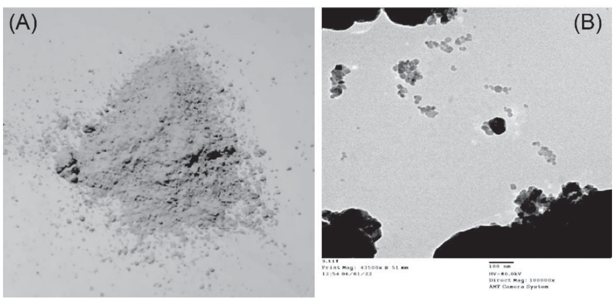

Figure 2. Gray Powder of the Seashell (A); TEM Photomicrograph Showing Spherical Shape Particles of Seashells

Treatment Modalities

Standardization of the Working Area

To standardize the working area in each sample, we made a facet at high speed in the center of each dentin disc using a small round diamond bur.

Simulation of Hypersensitive Dentin

37% phosphoric acid was used to remove the amorphous smear layer created by cutting. A mini-sponge was used to gently rub the acid on the surface of the dentin for 15 seconds, and the dentin was rinsed using distilled water for 60 seconds, and then it was dried gently with air for 60 seconds according to the manufacturer's instructions.[21]

Sample Grouping

The 50 dentin slabs were divided randomly into five groups (n = 10 each) according to the type of applied treatment (A): (A1) control, (A2) Nano eggshells, (A3) Nano seashells, (A4) Nano eggshells + Nd: YAG Laser, and (A5) Nano sea shell + Nd: YAG Laser. The specimens were digitally coded using a waterproof permanent marker on the pulpal side of each dentin disc for easy identification, and then they were stored in distilled water in individual small containers labelled according to the tested groups until they were used.

Treatment of Samples

The specimens of group (A) received no surface treatment and acted as the control. The specimens of groups (A2) and (A3) were treated using eggshells and seashell nanoparticles respectively. Slurry was mixed by adding 0.3 mL of distilled water into 1.8gm of the tested powder. It was applied on the surface of dentin discs using micro-brush, left for 7 minutes, washed using distilled water, and then gently air dried.[20,22]

The samples in groups (A4) and (A5) were treated with Nd: YAG laser (Surelite II laser device, Continuum, USA, National Institute of Enhanced Laser Science, Cairo

Figure 3. Dentin Slab Embedded in a Rubber Mold

University). It was applied after the application of the nano eggshell or seashell according to the divided groups as previously described.

Laser parameters used were 30 mJ power energy, 1W average power, 8 ns pulse width, and a 10 Hz repetitions rate for a 2-minute exposure time at a 1064-nm wavelength. The beam was directed to the sample surface using laser parameters as represented by Table 1. The beam was delivered perpendicular to the dentin surface as represented by Figure 4. The specimens of all groups were stored in artificial saliva in individual small containers labeled according to the tested groups until environmental scanning electron microscope/energy-dispersive x-ray spectroscopy (ESEM-EDX) evaluation.

Assessment of Tubular Occlusion Using the Environmental Scanning Electron Microscope

Dentinal surface characteristics and the patency of dentinal tubules were assessed for each specimen before and after the treatment using ESEM. No surface treatment or thin film deposition such as gold or carbon deposition was performed on the samples to facilitate the re-use of the same therapy. The investigations were performed at an accelerating voltage of 500 kV and 900X. The standardized working area (facet) was divided into quarters by drawing two radial lines within this facet (circle).

The photomicrographs were taken at 900X magnification at the intersection of the two radial lines at the center of the circle as represented by Figures 5A and 5B. The images were captured with good contrast and brightness settings which remained constant for all dentin specimens before and after the treatment.

Image Analysis

For quantitative evaluation, the number of opened DTs before and after the treatment was calculated for each sample using image analysis software (ImageJ software). Dentinal tubules located in the middle part of each ESEM photomicrograph were calculated. The program calculated the number of DTs at the standardized portion of each photomicrograph, which was subtracted to form an isolated image from the original one as represented by Figure 5C. This was repeated for each photomicrograph before and after the treatment

EDXA Analysis

All samples were analyzed with energy dispersive X-ray spectroscopy (EDXA) correlated with ESEM (FEG, Quanta 250, France). The calcium and phosphorus constituents of the dentin surface and the change in the percentage levels of these elements after each therapy were determined.

Results

ESEM Analysis (Dentinal Tubules Occlusion)

Table 1. Laser Parameters Used in This Study

Type of Laser	Nd: YAG
Wavelength (nm)	1064
Emission mode	Pulsed
Time of exposure	2 min
Delivery system	lens
Energy distribution	30 mJ
Peak power	3.75 MW
Average power	1 W
Spot diameter at the focus	432 µm
Spot diameter at the tissue	3.9 mm
Focus-to-tissue	-
Spot area at the tissue	0.12 cm^2
Peak power density at spot area	2.6×10^9 W/cm^2
Peak power density at the tissue	31 MW/cm^2
Average power density at spot area	682 W/cm^2
Average power density at the tissue	8.3 W/cm^2
Beam divergence	0.5 mrad
Water irrigation	-
Air and aspirating airflow	-
Pulse width ns	8-10 ns

Nd: YAG, neodymium-doped ytterbium aluminum garnet laser.

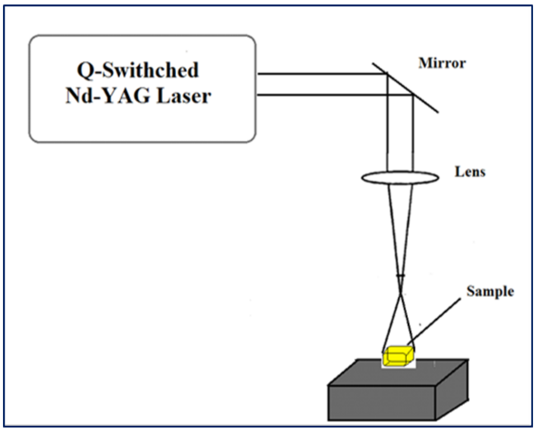

Figure 4. Schematic Diagram Representing the Experimental Setup of Dentin Sample Exposure

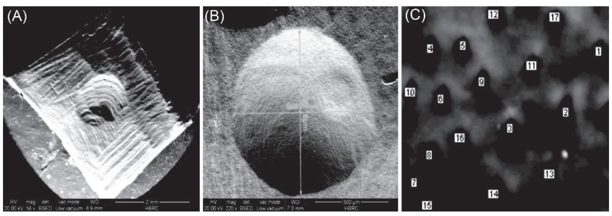

Figure 5. (A): ESEM photomicrograph showing the round facet in the center of the dentin slab, (B): ESEM photomicrograph showing the meeting point of two radial lines in the center of the circle, and (C): Subtracted image from original ESEM photomicrograph using ImageJ software

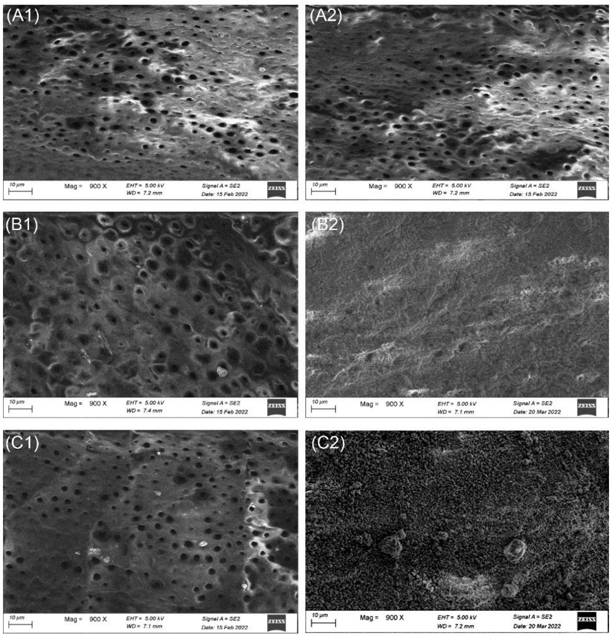

Figure 6. (A1,A2): ESEM photomicrographs show the dentin surface for the control group; (B1, B2): ESEM photomicrographs show the dentin surface in the eggshell group; at baseline they show patent numerous dentinal tubules; (B1), and (B2): after treatment they show closure of DTs and film deposit on the dentin surface; (C1, C2): ESEM photomicrographs of the dentin surface in the seashell group; at baseline they show patent numerous dentinal tubules (C1),and(C2): after the treatment they show closure of DTs and film deposit on the dentin surface

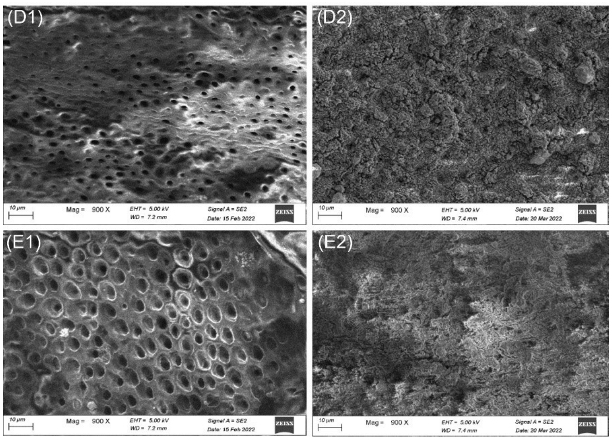

Figure 7. (D1, D2): ESEM photomicrographs show the dentin surface in the eggshell + Nd:YAG laser group; at baseline they show patent numerous dentinal tubules (D1) and (D2) after treatment, they show closure of DTs and film deposit on the dentin surface (E1, E2): ESEM photomicrographs show the dentin surface in the seashell + Nd:YAG laser group; at baseline (E1) they show patent numerous dentinal tubules and (E2) after treatment, they show closure of DTs with dentin surface morphology similar to an island like appearance

ESEM photomicrographs represented in Figures 6 and 7 show the morphological characteristics of the dentin surface in the tested groups before and after the treatment. ESEM photomicrographs of the control group represented by Figures 6A1 and A2 show frank opened dentinal tubules, and the surface was free from a smear layer coating before and after the experiment, which confirmed the sensitive tooth model.

In the eggshell and seashell groups, there was an obvious closure of the opened dentinal tubules after the therapy by mineral deposition, and film precipitation was observed as represented by Figures 6B2 and 6C2 respectively. For combined groups represented by Figure 7, the nanoparticles were melted, recrystallized, and trapped inside the tubules forming a plug inside their orifices. On the other hand, the dentin surface showed a more irregular surface having a characteristic shape similar to an island like appearance when treated with sea shell nano-powder combined with a laser (Figure 7E2).

Image Analysis Results

Effect of Therapy on the Number of Dentinal Tubules Within Each Group

Table 2 and Figure 8 compare the mean values of patented DTs before and after each treatment using a paired *t*-test. There was a statistically significant decrease in the mean values of opened DTs after each treatment in all groups.

Comparing Dentinal tubules Number and Percentage Changes Between the Groups

Table 3 and Figure 9 compare the mean values and mean percentage changes of patented DTs between the groups. Results of one-way ANOVA test showed that, there was no significant difference between the groups before the treatment.

On the other hand, there was a statistically significant difference in the mean values and mean percentage changes between the groups after the treatment. The greatest percentage decrease was recorded in the seashell NPs + Nd: YAG laser, followed by the eggshell NPs + Nd: YAG laser, then Eggshell NPs only and then Seashell NPs only, while the lowest percentage decrease was recorded in the control group. The post hoc test revealed no significant difference between the experimental groups.

EDXA Results (Dentin Remineralization)

Effect of Therapy on Calcium Weight % and Ca/P Ratio Within Each Group

The results of comparing the mean values before and after the treatment using a paired t-test showed that, there was a statistically significant increase in the mean values of Ca weight percent and Ca/P ratio within the experimental groups as represented by Tables 4 & 5 and Figures 10 & 11, respectively.

Effect of Materials on Percentage Changes of Calcium Weight% and Ca/P Ratio Between the Groups

The results of comparing the mean percentage changes of calcium weight % and Ca/P ratio between the groups using an analysis of variance (ANOVA) test are summarized in Tables 6 & 7 and Figures 12 & 13, respectively. There was no statistically significant difference between the

Table 2. Comparison of Mean Values Before and After the Treatment Within the Same Group Regarding the Dentinal Tubules number (paired t-test)

Group	Time	Mean	SD	Difference		*t*	*P*
				Mean	SD		
Control	Before	21.20	5.76	0	0	Not computed	
	After	21.20	5.76				
Eggshell NPs only	Before	21.80	2.59	19.60	2.51	17.46	0.000*
	After	2.20	0.84				
Seashell NPs only	Before	27.40	4.34	24.00	4.36	12.31	0.000*
	After	3.40	2.07				
Eggshell NPS+Nd:YAG laser	Before	22.20	2.95	20.60	3.36	13.70	0.000*
	After	1.60	1.52				
Seashell NPs+Nd:YAG laser	Before	25.20	2.77	23.40	2.88	18.16	0.000*
	After	1.80	1.92				

Significance level $P \leq 0.05$, *Significant.

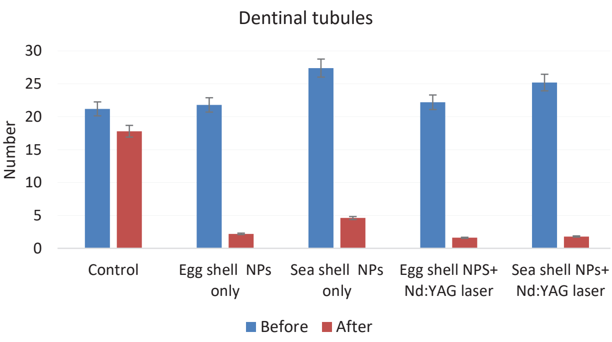

Figure 8. Bar Chart Illustrating the Mean Values of The Dentinal Tubules Number Before and After the Treatment in Different Groups

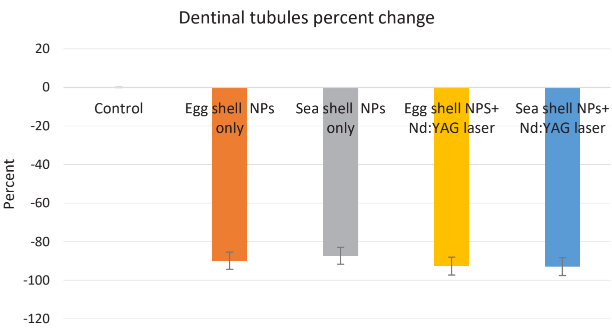

Figure 9. Bar Chart Comparing the Mean Percent Changes of the Dentinal Tubules Number Between Different Groups

groups before the therapy; on the other hand, there was a statistically significant difference between all groups after the treatment. The greatest percentage increase of the Ca wt% was recorded in the Eggshell NPS+Nd:YAG laser, followed by the Eggshell NPs only, then the Seashell NPs only and then the Seashell NPs+Nd:YAG laser, while the lowest percentage increase was recorded in the control group. The ANOVA test revealed that the control group was significantly lower than all other groups. The post hoc test revealed no statistically significant difference between the experimental groups.

Discussion

Dentin hypersensitivity is associated with an increase in the number and diameter of patent dentinal tubules on the tooth surface exposed to the oral environment. Tubule diameters are patent and more plentiful in numbers with hypersensitive dentin.[23,24] For this reason, the treatment of dentin hypersensitivity aims to partially close or permanently block dentinal tubules. Although there are different treatment options for DH, there is currently no treatment that can be accepted as the gold standard.[25]

Hydroxyapatite powder is characterized by bioactive properties and has chemical constituents similar to the hard tissue of the tooth, and it can be obtained from two different sources: synthetic and natural hydroxyapatite.[26] Hydroxyapatite can be produced from coral seashells, eggshells, and body fluids.[27]

Traditional methods of obtaining synthetic hydroxyapatite are performed using expensive chemical reagents; these synthetic routes are expensive and time-consuming.[28] Chicken eggshells were selected in the current study because they are rich in calcium with low cost. These eggshell wastes help reduce the cost of high-quality calcium sources while promoting the recycling of materials.[29]

Seashells were selected for this study owing to their high calcium content, abundance, and low cost. Cockle seashells used in this study belong to the species *Anadara granosa*, a sea molluscan. The cockle seashells are the products of the seafood industry after eating mussels. Cockleshell is one of the rich sources of $CaCO_3$, which acts as a calcium precursor.

The use of nanomaterials for dentinal tubules occlusion has the potential for revolutionizing the treatment of DH. The production of nano-sized particles enhanced the reactivity as well as surface area as nanoparticles can easily enter dentin tubules.[30] Several researchers have reported that Nd: YAG laser can be used as alternative or adjunctive therapy in the control and treatment of dentin hypersensitivity by reducing the patency of dentinal tubules.[13,14,31]

However, studies investigating the use of eggshells and seashells as desensitizers are rare in the literature. Furthermore, the use of Nd:YAG laser with this natural source is not known and has not been studied. Hence, the current study aimed to evaluate and compare these two nanoparticles in vitro to evaluate their effects as mono-therapy and combined with Nd:YAG laser on the occlusion of human dentinal tubules. 86 % of the total resistance to fluid movement is due to the smear layer coating. In this study, dentin slabs were etched using 37% phosphoric acid gel for 15 seconds for simulating DH. This ensures the efficient removal of dentin plugs and smear layers and the exposure of dentinal tubules.[21]

Table 3. Descriptive Statistics and Comparison of the Mean Values and Mean Percentage Changes of Dentinal Tubules Number Between the Groups

Dentinal Tubules Number		Mean	SD	Median	95% CI for Mean		Min	Max	*P* Value
					Lower Bound	Upper Bound			
Before	Control	21.20	5.76	20	14.05	28.35	15.00	29.00	0.092ns
	Eggshell NPs only	21.80	2.59	22	18.59	25.01	18.00	25.00	
	Seashell NPs only	27.40	4.34	26	22.02	32.78	23.00	32.00	
	Eggshell NPS+Nd:YAG laser	22.20	2.95	22	18.54	25.86	18.00	25.00	
	Seashell NPs+Nd:YAG laser	25.20	2.77	26	21.75	28.65	21.00	28.00	
After	Control	21.20^{a}	5.76	20	14.05	28.35	15.00	29.00	0.00*
	Eggshell NPs only	2.20^{b}	.84	2	1.16	3.24	1.00	3.00	
	Seashell NPs only	4.60^{b}	2.07	3	2.03	7.17	3.00	8.00	
	Eggshell NPS+Nd:YAG laser	1.60^{b}	1.52	1	-0.28	3.48	0.00	4.00	
	Seashell NPs+Nd:YAG laser	1.80^{b}	1.92	1	-0.59	4.19	0.00	5.00	
Percent change	Control	-0.001^{y}	0.00	0.00	0.00	0.00	0.00	0.00	0.007*
	Eggshell NPs only	-89.89^{x}	3.71	-89	-94.50	-85.28	-95.24	-86.36	
	Seashell NPs only	-87.34^{x}	2.94	-88	-90.98	-83.69	-90.63	-82.61	
	Eggshell NPS+Nd:YAG laser	-92.68^{x}	7.17	-94	-101.58	-83.77	-100.00	-80.95	
	Seashell NPs+Nd:YAG laser	-92.95^{x}	6.93	-96	-101.56	-84.34	-100.00	-82.14	

Significance level $P \leq 0.05$, ns=non-significant, *Significant.
Post hoc test: Within the same comparison, means sharing the same superscript letter are not significantly different.

Table 4. Comparison of Mean Values Before and After the Treatment Within the Same Group Regarding Calcium Weight Percent (paired t-test)

Group	Time	Mean	SD	Difference		*t*	*P*
				Mean	SD		
Control	Before	68.87	2.11	-0.07	.05	-3.13	.035*
	After	68.94	2.13				
Eggshell NPs only	Before	67.91	2.81	-7.70	4.52	-3.81	.019*
	After	75.61	2.66				
Seashell NPs only	Before	67.54	1.39	-7.41	.52	-31.80	0.000*
	After	74.95	1.17				
Eggshell NPS+Nd:YAG laser	Before	66.71	1.31	-9.50	4.60	-4.62	0.010*
	After	76.20	4.00				
Seashell NPs+Nd:YAG laser	Before	68.84	1.42	-7.18	0.96	-16.80	0.000*
	After	76.02	1.83				

Significance level $P \leq 0.05$, *Significant.

Table 5. Comparison of Mean Values Before and After the Treatment Within the Same Group Regarding the Calcium/Phosphorus Ratio (paired t-test)

Group	Time	Mean	SD	Difference		*t*	*P*
				Mean	SD		
Control	Before	2.22	0.20	-0.01	0.01	-3.06	0.38
	After	2.23	0.21				
Eggshell NPs only	Before	2.14	0.30	-1.00	0.64	-3.51	0.025*
	After	3.14	0.47				
Seashell NPs only	Before	2.09	0.13	-0.91	0.08	-24.65	0.000*
	After	3.00	0.19				
Eggshell NPS+Nd:YAG laser	Before	2.01	0.11	-1.29	0.75	-3.86	0.018*
	After	3.30	0.71				
Seashell NPs+Nd:YAG laser	Before	2.21	0.15	-0.97	0.20	-10.88	0.000*
	After	3.19	0.31				

Significance level $P \leq 0.05$, *Significant.

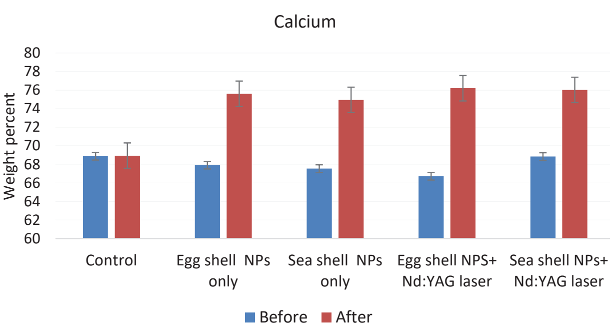

Figure 10. Bar Chart Illustrating the Mean Values of Calcium Weight % Before and After the Treatment in Different Groups

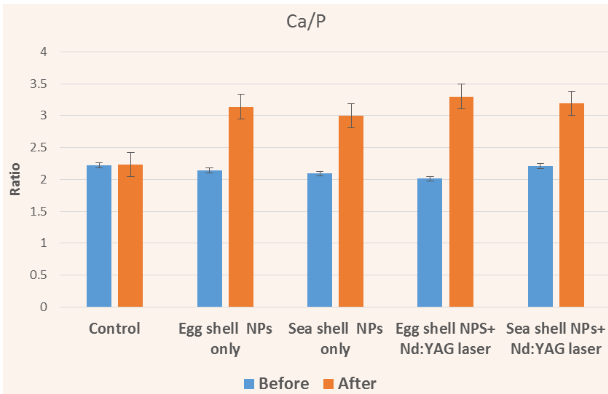

Figure 11. Bar Chart Illustrating the Mean Values of the Calcium/Phosphorus Ratio Before and After the Treatment in Different Groups

Table 6. Descriptive Statistics and Comparison of the Mean Values and Mean Percentage Changes of Calcium Weight % Between the Groups

Calcium		Mean	SD	Median	95% CI for Mean		Min	Max	*P* Value
					Lower Bound	Upper Bound			
before	Control	68.87	2.11	69.86	66.26	71.49	65.28	70.30	0.356 ns
	Eggshell NPs only	67.91	2.81	67.49	64.42	71.40	64.72	72.45	
	Seashell NPs only	67.54	1.39	67.41	65.82	69.26	65.78	69.44	
	Eggshell NPS + Nd:YAG laser	66.71	1.31	67.21	65.08	68.34	64.41	67.68	
	Seashell NPs + Nd:YAG laser	68.84	1.42	68.92	67.08	70.60	67.09	70.48	
After	Control	68.94^{b}	2.13	69.95	66.29	71.59	65.29	70.33	0.001*
	Eggshell NPs only	75.61^{a}	2.66	74.65	72.30	78.91	73.21	78.88	
	Seashell NPs only	74.95^{a}	1.17	74.89	73.50	76.40	73.51	76.19	
	Eggshell NPS + Nd:YAG laser	76.20^{a}	4.00	77.46	71.23	81.18	71.02	80.69	
	Seashell NPs + Nd:YAG laser	76.02^{a}	1.83	76.75	73.75	78.30	73.23	77.71	
Percent change	Control	0.09^{y}	.07	0.13	0.01	0.18	0.02	0.16	0.016*
	Eggshell NPs only	11.52^{x}	6.84	15.34	3.04	20.01	1.19	17.57	
	Seashell NPs only	10.98^{x}	.90	11.10	9.86	12.10	9.50	11.75	
	Eggshell NPS + Nd:YAG laser	14.30^{x}	7.04	17.10	5.56	23.05	5.51	20.56	
	Seashell NPs + Nd:YAG laser	10.43^{x}	1.37	10.87	8.73	12.12	8.90	12.10	

Significance level $P \leq 0.05$, ns = non-significant, *Significant. Post hoc test: Within the same comparison, means sharing the same superscript letter are not significantly different.

Table 7. Descriptive Statistics and Comparison of the Mean Values and Mean Percentage Changes of the Ca/P Ratio Between the Groups

Ca/P ratio		Mean	SD	Median	95% CI for Mean		Min	Max	*P* Value
					Lower Bound	Upper Bound			
Ratio before	Control	2.22	0.20	2.32	1.97	2.48	1.88	2.37	0.356 ns
	Eggshell NPs only	2.14	0.30	2.08	1.77	2.50	1.83	2.63	
	Seashell NPs only	2.09	0.13	2.07	1.92	2.25	1.92	2.27	
	Eggshell NPS + Nd:YAG laser	2.01	0.11	2.05	1.87	2.15	1.81	2.09	
	Seashell NPs + Nd:YAG laser	2.21	0.15	2.22	2.03	2.40	2.04	2.39	
Ratio after	Control	2.23^{b}	0.21	2.33	1.97	2.49	1.88	2.37	0.005*
	Eggshell NPs only	3.14^{a}	0.47	2.94	2.56	3.72	2.73	3.73	
	Seashell NPs only	3.00^{a}	0.19	2.98	2.77	3.23	2.78	3.20	
	Eggshell NPS + Nd:YAG laser	3.30^{a}	.71	3.44	2.42	4.17	2.45	4.18	
	Seashell NPs + Nd:YAG laser	3.19^{a}	0.31	3.30	2.81	3.57	2.74	3.49	
Ratio percent	Control	0.31^{y}	0.23	0.43	0.03	0.59	0.04	0.53	0.017*
	Eggshell NPs only	49.72^{x}	32.12	60.52	9.83	89.60	4.45	83.21	
	Seashell NPs only	43.85^{x}	3.45	44.19	39.56	48.14	39.67	49.00	
	Eggshell NPS + Nd:YAG laser	65.15^{x}	38.42	80.26	17.44	112.85	19.02	106.47	
	Seashell NPs + Nd:YAG laser	43.87^{x}	7.89	43.77	34.07	53.67	34.19	53.21	

Significance level $P \leq 0.05$, ns = non-significant, *Significant. Post hoc test: Within the same comparison, means sharing the same superscript letter are not significantly different.

Since premolars were the teeth most commonly affected by hypersensitivity (49%), followed by molars (34%) and canines (19%), this study was confined to upper first maxillary premolars.[1] The replication of the dentin substrate is not possible because of the natural variation in the number and diameter of dentinal tubules and the degree of mineralization as described by a previous study.[32] Nonetheless, attempts have been made to obtain dentin from the same area of root dentin and approximately the same thickness and dimensions. Therefore, each dentin disc served as its own control to standardize regional differences in the number of dentinal tubules.

The laser source used throughout the current work is the Q-switched oscillator Nd: YAG laser produced by Continuum (Surelite II Laser). It provides a laser beam with some parameters (1064 nm, 30 mJ, 10 HZ). It was

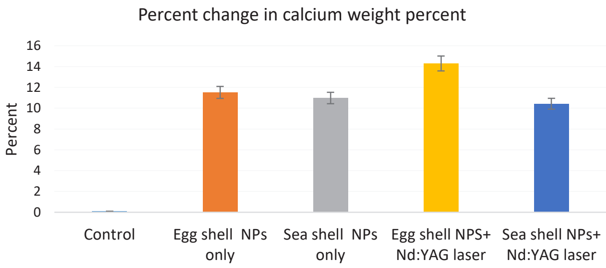

Figure 12. Bar Chart Illustrating the Mean Percentage Change of Calcium Weight % In Different Groups

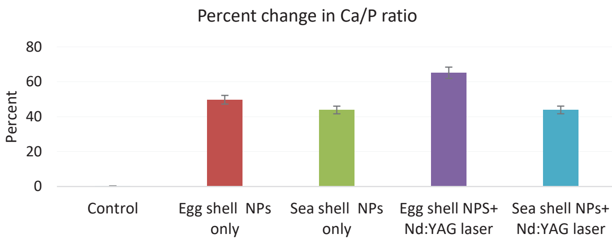

Figure 13. Bar Chart Illustrating the Mean Percent Change of the Calcium/Phosphorus Ratio in Different Groups

recommended by previous researches for treatment of dentin hypersensitivity.[33,34]

The ESEM and EDX were used to study the surface morphology, calcium, and phosphate composition of the specimens before and after the treatment. No metal sputter coating was required, so the same sample was continuously evaluated throughout the study. Also, several authors have warned that conventional SEM sample preparation could result in the dissolution of organic and inorganic chemical deposits that could be precipitated from the desensitizing agents. Third, the surface preparation and high vacuum drying of conventional SEM may introduce artifacts and cracks which may make the interpretation of the results difficult or even infeasible.[32]

To standardize the working area, we made a small facet in the center of each dentin disc using a round bur. For ESEM-EDX investigations, this area was divided into quarters by drawing two radial lines through the center of this facet (circle, then ESEM-photomicrograph was taken at the center of the circle), as shown in Figure 5B.

Regarding the effect of the tested materials on dentinal tubules occlusion, Ca wt%, and Ca/P ratio, ESEM-EDX analysis showed that the groups treated with eggshells or seashells nanoparticles alone or combined therapy had a significantly higher occlusion rate, Ca weight %, and Ca/P ratio after therapy compared to the control, thus the the first null hypothesis was rejected. This can be attributed to the blockage of open dentinal tubules by calcium phosphate deposits on the dentin surface which was confirmed by the EDX analysis of the Ca wt % and Ca/P ratio of the treated dentin. These results are consistent with the results of a previous study,[20] which showed the occlusion of DTs by nano hydroxyapatite synthesized from eggshells.

Likewise, the present findings are supported by a previous study, in which the toothpaste toothpaste containing $CaCO_3$ was able to form a new film on the dentin surface, thereby occluding the dentinal tubules. This fact strongly supports our ESEM results after treating dentin discs with nano seashell paste.[35]

Correspondingly, the current findings confirm the confirm the research results in which cockle shells are composed of approximately 96% $CaCO_3$, while other constituents include organic matter and oxides like SiO_2, MgO and SO_3.[36] Both Cockle shells and coral exoskeletons have similar minerals and physicochemical characteristics to bone and can be used as good alternative biomaterials for regeneration. Also, this fact was in the same line with another study, which recorded a significant increase in dentinal tubule occlusion using nano seashells.[37]

Contrasting effects of different treatments on the occlusion of DTs between the groups, with the greatest percentage reduction in the number of DTs, were recorded in the seashell NP_S + Nd: YAG laser, followed by the eggshell NPs + Nd: YAG laser. This contradicts the mean percent changes of Ca wt% and Ca/P ratio, and the greatest percentage increase was recorded for the eggshell NPS + Nd: YAG laser. This difference can be attributed to the blockage of the dentinal tubules on the dentin surface by mineral deposits other than calcium and phosphorus when treated with the seashells + Nd: YAG laser.

Furthermore, there are no significant differences between the eggshell and seashell groups either as a single or combined treatment with respect to the percent changes of the opened dentinal tubule number, Ca(wt)% and Ca/P ratio, thus the second null hypothesis was accepted. This could be related to two main reasons: first, both materials are based on similar chemical composition of $CaCO_3$; the second is the nano-scale particle size of the tested materials, which provides superior functional properties and high bioactivity due to its grain size, crystallinity, and surface area to the volume ratio in the oral environment compared to its micro-scale sized counterparts. Since the ratio of the surface area and surface atoms increases with decreasing the particle size, more ionic components penetrate into the dentinal tubules which may lead to better tubular occlusion.

The application of eggshell or seashell nanoparticles prior to Nd:YAG laser did not significantly reduce the number of open dentinal tubules compared to treatment alone. Similarly, there was no statistically significant increase in ratio, thus the third null hypothesis was accepted. These findings were confirmed by the EDX results. These outcomes may be related to the reduction in the absorption potential of calcium bicarbonate nanoparticles by the laser beam and thus the heating effect of Nd: YAG laser on the dentin surface by the nanoparticles.

This fact is in accordance with a previous study, which concluded that the diode laser and dentifrice containing both arginine and $CaCO_3$ were effective in reducing dentinal permeability, and the combination of the two treatments did not show better results than either one used alone.[38] This can be supported by a previous study, in which the combination therapy of the acidulated phosphate fluoride gel and Nd: YAG laser did not promote an additional effect.[39]

On the other hand, the literature contradicts a previous study that reported an effective treatment regimen for dentin hypersensitivity by applying nanohydroxyapatite combined with Nd:YAG laser as a desensitizing agent.[31] This inconsistency may be related to differences in material composition, form, and methodology which may affect the outcomes of the results.

Conclusions

Within the limitation of this study, both eggshell and seashell nanoparticles are affordable and effective materials in occluding dentinal tubules and remineralization. Under the condition of this study, Nd: YAG laser combined with the two materials provided no benefit compared to the effects of the treatments alone.

Conflict of Interests

No conflict of interest was declared by the authors.

Ethical Considerations

This is an in vitro study, not including work or Humans or Animals. The ethical approval of the local ethic committee of the Faculty of Dental Medicine, Al-Azhar University for Girls, in accordance with international guiding principles was obtained (Code: REC-OP-22-11).

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References

1. Addy M. Dentine hypersensitivity: new perspectives on an old problem. Int Dent J. 2002;52(5 Suppl 2):367-75. doi: 10.1002/j.1875-595X.2002.tb00936.x.
2. Brännström M, Garberoglio R. The dentinal tubules and the odontoblast processes. A scanning electron microscopic study. Acta Odontol Scand. 1972;30(3):291-311. doi: 10.3109/00016357209004598.
3. Berman LH. Dentinal sensation and hypersensitivity. A review of mechanisms and treatment alternatives. J Periodontol. 1985;56(4):216-22. doi: 10.1902/jop.1985.56.4.216.
4. Schiff T, Delgado E, Zhang YP, Cummins D, DeVizio W, Mateo LR. Clinical evaluation of the efficacy of an in-office desensitizing paste containing 8% arginine and calcium carbonate in providing instant and lasting relief of dentin hypersensitivity. Am J Dent. 2009;22 Spec No A:8A-15A.
5. Arnold WH, Prange M, Naumova EA. Effectiveness of various toothpastes on dentine tubule occlusion. J Dent. 2015;43(4):440-9. doi: 10.1016/j.jdent.2015.01.014.
6. Chiang YC, Chen HJ, Liu HC, Kang SH, Lee BS, Lin FH, et al. A novel mesoporous biomaterial for treating dentin hypersensitivity. J Dent Res. 2010;89(3):236-40. doi: 10.1177/0022034509357148.
7. Abdulrahman I, Tijani HI, Mohammed BA, Saidu H, Yusuf H, Jibrin MN, et al. From garbage to biomaterials: an overview on egg shell based hydroxyapatite. J Mater. 2014;2014:802467. doi: 10.1155/2014/802467.
8. Siva Rama Krishna D, Siddharthan A, Seshadri SK, Sampath Kumar TS. A novel route for synthesis of nanocrystalline hydroxyapatite from eggshell waste. J Mater Sci Mater Med. 2007;18(9):1735-43. doi: 10.1007/s10856-007-3069-7.
9. Santhosh S, Balasivanandha Prabu S. Thermal stability of nano hydroxyapatite synthesized from sea shells through wet chemical synthesis. Mater Lett. 2013;97:121-4. doi: 10.1016/j.matlet.2013.01.081.
10. Lee CH, Orloff ND, Birol T, Zhu Y, Goian V, Rocas E, et al. Exploiting dimensionality and defect mitigation to create tunable microwave dielectrics. Nature. 2013;502(7472):532-6. doi: 10.1038/nature12582.
11. Silva TH, Mesquita-Guimarães J, Henriques B, Silva FS, Fredel MC. The potential use of oyster shell waste in new value-added by-product. Resources. 2019;8(1):13. doi: 10.3390/resources8010013.
12. Bamise CT, Esan TA. Mechanisms and treatment approaches of dentine hypersensitivity: a literature review. Oral Health Prev Dent. 2011;9(4):353-67.
13. Al-Saud LM, Al-Nahedh HN. Occluding effect of Nd:YAG laser and different dentin desensitizing agents on human dentinal tubules in vitro: a scanning electron microscopy investigation. Oper Dent. 2012;37(4):340-55. doi: 10.2341/10-188-l.
14. Birang R, Poursamimi J, Gutknecht N, Lampert F, Mir M. Comparative evaluation of the effects of Nd:YAG and Er:YAG laser in dentin hypersensitivity treatment. Lasers Med Sci. 2007;22(1):21-4. doi: 10.1007/s10103-006-0412-z.
15. de Villiers MM, Aramwit P, Kwon GS. Nanotechnology in Drug Delivery. New York: Springer Science & Business Media; 2009.
16. Onwubu SC, Mdluli PS, Singh S. The effectiveness of nanomaterials in the management of dentine hypersensitivity-a review. J Clin Rev Case Rep. 2018;3(8):1-5.
17. Nuryantini AY, Sundari CD, Halimahtussa'diah, Nuryadin BW. Synthesis and characterization of calcium oxide nanoparticles from duck eggshells using ball milling methods. Jurnal Kimia Valensi. 2019;5(2):231-5. doi: 10.15408/jkv.v5i2.879.
18. Tram NX. Synthesis and characterization of calcite nano-particle derived from cockle shell for clinical application. ASEAN Eng J. 2020;10(1):49-54. doi: 10.11113/aej.v10.15538.
19. Hussein F, Hashem SN, Elsayed SR. The synergetic effect of silver diamine fluoride with potassium iodide and grape seed extract on dentin remineralization. Al-Azhar Dent J Girls. 2021;8(1):71-80. doi: 10.21608/adjg.2021.43369.1296.
20. Kunam D, Manimaran S, Sampath V, Sekar M. Evaluation of dentinal tubule occlusion and depth of penetration of nano-hydroxyapatite derived from chicken eggshell powder with and without addition of sodium fluoride: An in vitro study. J Conserv Dent. 2016;19(3):239-44. doi: 10.4103/0972-0707.181940.
21. Bastos-Bitencourt N, Velo M, Nascimento T, Scotti C, da Fonseca MG, Goulart L, et al. In vitro evaluation of desensitizing agents containing bioactive scaffolds of nanofibers on dentin remineralization. Materials (Basel). 2021;14(5):1056. doi: 10.3390/ma14051056.
22. Abdel Samie BM, Al-Yasaki MA, Gad NA. The effect of mesoporous silica nanoparticles desensitizing slurry alone or combined with calcium phosphate on dentin permeability (An in scanning electron microscope study). Al-Azhar Dent J Girls.

2021;8(1):19-26. doi: 10.21608/adjg.2021.14176.1183.

23. Absi EG, Addy M, Adams D. Dentine hypersensitivity. A study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentine. J Clin Periodontol. 1987;14(5):280-4. doi: 10.1111/j.1600-051x.1987.tb01533.x.
24. Tunar OL, Gürsoy H, Çakar G, Kuru B, Ipci SD, Yılmaz S. Evaluation of the effects of Er:YAG laser and desensitizing paste containing 8% arginine and calcium carbonate, and their combinations on human dentine tubules: a scanning electron microscopic analysis. Photomed Laser Surg. 2014;32(10):540-5. doi: 10.1089/pho.2014.3779.
25. Porto IC, Andrade AK, Montes MA. Diagnosis and treatment of dentinal hypersensitivity. J Oral Sci. 2009;51(3):323-32. doi: 10.2334/josnusd.51.323.
26. Mezahi FZ, Oudadesse H, Harabi A, Le GY, Cathelineau G. Sintering effects on physicochemical properties of bioactivity of natural and synthetic hydroxyapatite. J Aust Ceram Soc. 2011;47(1):23-7.
27. Ripamonti CI, Maniezzo M, Campa T, Fagnoni E, Brunelli C, Saibene G, et al. Decreased occurrence of osteonecrosis of the jaw after implementation of dental preventive measures in solid tumour patients with bone metastases treated with bisphosphonates. The experience of the National Cancer Institute of Milan. Ann Oncol. 2009;20(1):137-45. doi: 10.1093/annonc/mdn526.
28. Szcześ A, Hołysz L, Chibowski E. Synthesis of hydroxyapatite for biomedical applications. Adv Colloid Interface Sci. 2017;249:321-30. doi: 10.1016/j.cis.2017.04.007.
29. Sanosh KP, Chu MC, Balakrishnan A, Kim TN, Cho SJ. Utilization of biowaste eggshells to synthesize nanocrystalline hydroxyapatite powders. Mater Lett. 2009;63(24-25):2100-2. doi: 10.1016/j.matlet.2009.06.062.
30. Khan AS, Farooq I, Alakrawi KM, Khalid H, Saadi OW, Hakeem AS. Dentin tubule occlusion potential of novel dentifrices having fluoride containing bioactive glass and zinc oxide nanoparticles. Med Princ Pract. 2020;29(4):338-46. doi: 10.1159/000503706.
31. Sesen Uslu Y, Donmez N. The effects on dentin tubules of two desensitising agents in combination with Nd:YAG laser: an in vitro analysis (CLSM and SEM). Opt Laser Technol. 2020;129:106225. doi: 10.1016/j.optlastec.2020.106225.
32. Kolker JL, Vargas MA, Armstrong SR, Dawson DV. Effect of desensitizing agents on dentin permeability and dentin tubule occlusion. J Adhes Dent. 2002;4(3):211-21 .
33. Kumar NG, Mehta DS. Short-term assessment of the Nd:YAG laser with and without sodium fluoride varnish in the treatment of dentin hypersensitivity--a clinical and scanning electron microscopy study. J Periodontol. 2005;76(7):1140-7. doi: 10.1902/jop.2005.76.7.1140.
34. Birang R, Yaghini J, Shirani AM. Comparative study of dentin surface changes following Nd:Yag and Er:Yag lasers irradiation and implication for hypersensitivity. J Oral Laser Appl. 2008;8(1):25-31.
35. Wang Z, Sa Y, Sauro S, Chen H, Xing W, Ma X, et al. Effect of desensitising toothpastes on dentinal tubule occlusion: a dentine permeability measurement and SEM in vitro study. J Dent. 2010;38(5):400-10. doi: 10.1016/j.jdent.2010.01.007.
36. Awang-Hazmi AJ, Abu Bakar MZ, Noordin MM, Abu J, Norimah Y. Mineral composition of the cockle (*Anadara granosa*) shells of west coast of Peninsular Malaysia and it?s potential as biomaterial for use in bone repair. J Anim Vet Adv. 2007;6(5):591-4.
37. Ashraf R, Aidaros N. Evaluation the efficiency of nano seashell, sodium fluoride and a commercially available toothpaste on dentinal tubules occlusion after acid attack using scanning electron microscope: in vitro study. Egypt Dent J. 2021;67(3):2783-94. doi: 10.21608/edj.2021.73931.1609.
38. Rosa RR, Calazans FK, Nogueira RD, Lancellotti AC, de Sousa Gonçalves L, Geraldo-Martins VR. Effects of different desensitizing treatments on root dentin permeability. Braz Oral Res. 2016;30(1):e111. doi: 10.1590/1807-3107BOR-2016.vol30.0111.
39. Pereira DL, Freitas AZ, Bachmann L, Benetti C, Zezell DM, Ana PA. Variation on molecular structure, crystallinity, and optical properties of dentin due to Nd:YAG laser and fluoride aimed at tooth erosion prevention. Int J Mol Sci. 2018;19(2):433. doi: 10.3390/ijms19020433.