Al-Azhar Journal of Dentistry

Manuscript 1613

Restorative Dentistry Issue (Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

The Effect of Eggshell Nanoparticles Combined with Titanium Dioxide Nanoparticles on Permeability of Dentinal Tubules After Exposure to Wear Challenges

Fadia A. Shosha

Wael E. Gamil

Fatma H. Mohamed

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Fadia A. Shosha*, Wael E. Gamil, Fatma Hussein

Department of Operative Dentistry, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

Abstract

Purpose: This study was conducted to evaluate the effect of monotherapy of eggshell (EB) nanoparticles or their combination with titanium dioxide (TiO_2) nanoparticles on the permeability of dentinal tubules after exposure to wear challenges. **Patients and methods:** The nanoparticles were prepared and then characterized using a transmission electron microscope. A total of 60 freshly extracted human premolars were sectioned longitudinally into two halves (buccal and lingual). Class v cavity was prepared on the cervical region of each half. After the simulation of hypersensitive dentin, cavities were divided into four groups (30 for each) according to the treatment materials: (A0) no treatment (negative control), (A1) monotherapy of EB. (A2) combination therapy of EB + TiO_2 nanoparticles, (A3) MI paste plus positive control. Each group was further subdivided into three subgroups (10 for each) according to the time of evaluation of the treatment outcome: (T1) immediately (T2) after 2 weeks, and (T3) after 4 weeks. Samples were evaluated for dentinal tubule occlusion using an Environmental Scanning Electron Microscope and for dentin permeability using a Stereomicroscope. Image analysis software was used for calculating the depth of dye penetration. **Results:** The lowest statistically significant mean value of dye penetration depth was recorded in the combination therapy of EB + TiO_2, then the monotherapy of EB followed by MI paste and negative control. **Conclusion:** EB monotherapy or its combination with TiO_2 can be considered a promising therapy for decreasing dentin permeability and treatment of dentin hypersensitivity.

Keywords: Dentin permeability, Eggshell nanoparticles, Titanium dioxide nanoparticles

1. Introduction

Dentin hypersensitivity (DH) is a commonly occurring dental problem characterized by short and sharp pain, which arises when the exposed dentin is exposed to different external stimuli, such as evaporative, thermal, chemical, tactile, or osmotic stimuli [1]. When the enamel is missing, hypersensitive dentin is mostly found. Regarding the mechanism of hypersensitive dentin, hydrodynamic theory represents the most accepted one. It proposes that the dentinal tubular fluid flow increases due to pain-provoking stimulus, stimulating the nerves and in turn leading to hypersensitive dentin [2].

The treatment of DH can be achieved either by dentinal tubule occlusion, or by inhibiting excitation of the intradental nerves [3,4]. Most dentin desensitizing agents have incomplete occlusion of the dentinal tubules, short-lived therapeutic effects, and low resistance to wear challenges such as tooth brushing and acidic beverages [5]. Due to these drawbacks, nanomaterials have been predicted to be promising materials for treating DH.

Amorphous calcium phosphate (ACP) is a soluble compound of calcium phosphate which releases calcium and phosphate ions, changing to apatite. When it comes in contact with saliva, it remineralizes the tooth structure. Previous studies have revealed that Casein PhosphoPeptide (CPP)-ACP-containing

Received 10 June 2023; revised 13 July 2023; accepted 30 August 2023.
Available online 25 September 2024

* Corresponding author. Yosef Abass, Nasr City, Cairo, 11651, Egypt.
E-mail address: fadiaali2020@yahoo.com (F.A. Shosha).

https://doi.org/10.58675/2974-4164.1613

products such as GC tooth mousse and MI paste plus were effective in dentinal tubule occlusion and decreasing their permeability [6].

In recent years, several studies investigated the eggshells (EBs) for their remineralization potential. EBs have rich bioavailable calcium content in the form of carbonates and oxides. It contains 94% calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4% organic matter. EBs can be considered a cost-effective, renewable, and sustainable source of nanohydroxyapatite. It was revealed by previous studies that EB powder is considered a promising agent for tooth remineralization, and a highly effective agent in dentinal tubule occlusion and decreasing DH [7].

Titanium dioxide (TiO_2) is one of the few bioactive materials, which induce hydroxyapatite formation [8]. A new EBs-TiO_2 composite will be an effective and promising agent for treating DH [9]. However, literature is sparse in evaluating the effects of combination therapy of EBs + TiO_2 in comparison to EB monotherapy, on the permeability of dentinal tubules under acidic and brushing challenges.

So, the purpose of this study was to evaluate the effect of monotherapy of EBs nanoparticles or their combination with TiO_2 nanoparticles on dentin permeability. The null hypothesis was that there was no difference in decreasing dentin permeability between the treatment agents.

2. Patients and methods

2.1. Sample size calculation

To study the effect of the three different treatment materials that were used in this study on the permeability of dentinal tubules, the analysis of variance (ANOVA) test was used for comparison of dye penetration. According to a previous study [10] a total sample size of 120 (30 in each group) was sufficient to detect; an effect size of 0.9, a power 1-β error of 0.8, using a two-sided hypothesis test, and a significant level (α error) of 0.05 for data. For compensate for the errors, eight samples were used in each subgroup. Two samples were added for each subgroup for scanning electron microscope evaluation.

2.2. Selection of teeth

The teeth selected for this study were 60 human premolars, which were freshly extracted for orthodontic treatment. The patients were informed about and consented to the use of their teeth following approval of the ethics committee of Faculty of Oral and Dental Medicine for Girls, Al-Azhar University (approval code; REC-CL-23-05). The attached soft tissues were removed from the teeth by a scalpel, while a low-speed handpiece with pumice paste was used to remove the remaining debris from them. Then they were stored in distilled water for a maximum period of 1 month till use [11].

2.3. Exclusion and inclusion criteria

All teeth enrolled in this study were selected according to the following criteria [12].

2.4. Inclusion criteria

Intact teeth extracted for orthodontic purposes, noncarious, and free of abrasion and erosion.

2.5. Exclusion criteria

Teeth with developmental defects, craze lines, fractures, wasting diseases, caries, or restorations.

2.6. Preparation of samples

Each tooth was sectioned longitudinally into buccal and lingual halves with a diamond disk mounted on a slow-speed water-cooled handpiece, using a double-faced diamond disk (BesQual Dia -Disc NY11373, USA size; S-22 mm) in a cutting machine (DEMCO, Dental Maintenance CO, Bonsall, Calf. USA, model E96). Cavities of 0.3 mm depth and 0.8 mm width were prepared on the cervical area of each half 1 mm coronal to the cement−enamel junction (Fig. 1), using a # 245 bur (tungsten carbide bur, made by Kerr, Canada, ISO No 245, head length 2.7 mm) in a low-speed handpiece (Coxo low-speed handpiece, push button,

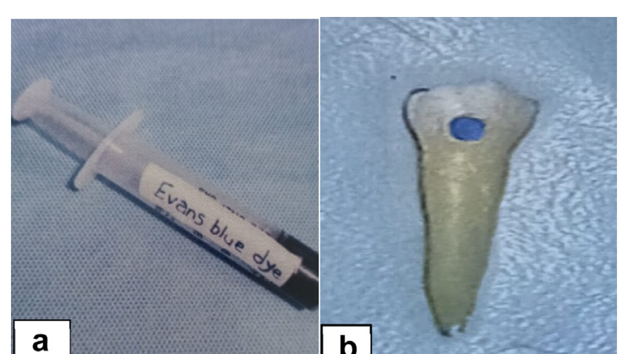

Fig. 1. (a) Evans blue dye, (b) cavities of 0.3 mm depth and 0.8 mm width were prepared on the cervical area of each half 1 mm coronal to the cement−enamel junction.

made in China, rotation speed MAX 40 000 rpm) with a copious coolant [13].

2.7. Sample grouping

After the simulation of hypersensitive dentin, the cavities were divided into four groups (30 each) according to the treatment material: (A) No treatment, (A0) monotherapy of EB nanoparticle, (A1) combination therapy of EB + TiO_2 nanoparticles (A2), and MI paste plus (A3). Each group has three subgroups (10 each) according to the time of evaluation of the treatment outcome (T), immediately (T1), after 2 weeks (T2), and after 4 weeks (T3). From each subgroup, eight cavities were evaluated for dentin permeability and two cavities for environmental scanning electron microscope (ESEM) evaluation.

2.8. Simulation of hypersensitive dentin

A 6% citric acid (pH; 1.5) solution was prepared at the Faculty of Science, Al-Azhar University, by dissolving 6 gm of citric acid in 100 ml distilled water. To simulate hypersensitive dentin, each sample was conditioned with immersion in the prepared acidic solution for 2 min, after that they were rinsed with distilled water [14].

2.9. Application of treatment materials

EB nanoparticles with and without titanium dioxide nanoparticles were used in the form of gel (prepared by Nanogate Company, Giza, Egypt). According to the selected therapy in the following groups: A1 and A2, micro brush (MicroBrush-Applicators Fine 1.5 mm Assorted Bonding and MicroBrushes Applic #400756-Mfg#MFA400) was used for the application of the prepared gel into the prepared cervical cavities. The gel was kept in the cavities for 5 min, after that, they were rinsed using distilled water and dried [10]. In the third group, the MI paste plus containing CPP-ACP was applied for 3 min [15]. No treatment agent was applied in the control group (A0). After treatment, artificial saliva (sodium chloride (NaCl 9.0), potassium chloride (KCl 0.4), calcium chloride hexahydrate ($CaCl_2$–$6H_2O$ 0.2), and sodium hydrogen carbonate ($NaHCO_3$), pH 7.0 was used as storage media till the evaluation time and replaced every day [16].

2.10. Wear challenges

The acidic challenge was performed by treating the cavities with 6 wt. % citric acid (pH, 1.5) for 1 min, and then the cavities were rinsed with distilled water. An electronic toothbrush (Oral-B DB4.010 Pro expert battery toothbrush powered by Braun) (48.800 movements per minute) was then used for the brushing challenge. The brush with medium hardness bristles and ~90° inclination was applied to the dentin surface for 2 min [17]. The cavities in all subgroups were subjected every day to acidic and then brushing challenges once per day. Postchallenge permeability was then measured immediately, after 2 weeks and after 4 weeks.

2.11. Measurement of dentin permeability

2.11.1. Application of the 5% Evans dye on dentin cavities

5% Evans blue dye solution was prepared and then infused into each dentin cavity using a plastic syringe. Then, it was left to penetrate the dentinal tubules for 20 min. After that, the dental unit triple syringe was used for rinsing and drying the cavities [18].

2.11.2. Dentin permeability analysis

The cavities were sectioned longitudinally in buccolingual direction through the center of the cavity. Then, a stereomicroscope (OlympusTM BX41, Olympus, Tokyo, Japan) was used to photograph the samples. Images of 5.1 megapixels were obtained. Three measurements in the analyzed area were taken for each image, indicating the depth of the dye infiltration. The dye penetration depth and its mean value were calculated for each specimen [12].

2.12. Environmental scanning electron microscope (ESEM) examination

Two cavities from each subgroup were used for examination using an environmental scanning electron microscope. Buccal and lingual surfaces of the prepared cavity were ground till the depth of the cavity, after that the crown was sectioned in the mesiodistal direction into buccal and lingual halves. Each half was then cut horizontally, and the cervical part was used for ESEM examination [19].

2.13. Statistical analysis

Statistical analysis was performed by applying one-way ANOVA to compare parametric data, followed by the post hoc test for multiple comparisons between different groups. *P* less than or equal to 0.05 was considered statistically significant (95% significance level). Quantitative parametric measures (dye penetration test) were expressed as mean ± SD. Statistical evaluation was performed using the SPSS statistical package (version 25, IBM Co. USA).

3. Results

3.1. The results of dentin permeability (dye penetration test) (Table 1):

3.1.1. Effect of time within each group on dye penetration (intragroup comparison)

For all groups; the mean of dye penetration was low after T1 (immediately), increased after T2 (2 weeks), and then decreased again after T3 (4 weeks).

For all groups, the overall *P* value from ANOVA was statistically significant (*P* value $\leq$ 0.05) between the three time intervals. T2 with the highest mean of dye penetration and T1 with the lowest mean of dye penetration.

According to the Tukey post hoc test, there was a significant difference between the three time intervals for groups A0, A1, and A3. For A2, there was no significant difference between T1 and T3, while there was a significant difference between T1 and T2 as well as T2 and T3.

3.1.2. Effect of material within each time interval on dye penetration

The overall *P* value from ANOVA was statistically significant, meaning there was a significant difference between groups, especially between A0 (the highest mean of dye penetration) and A2 (the lowest mean of dye penetration).

After T1: According to the Tukey post hoc test, there was a significant difference between group A0 and all other groups, while there was no significant difference between A1, A2, and A3 groups.

After T2: There was a significant difference between group A2 and A0 groups, while there was no significant difference between A0, A1, and A3 groups. Als, there was no significant difference between A1 and A2 groups.

After T3: There was a significant difference between A0 and all groups, while there was no significant difference between A1 and A3 groups, also there was no significant difference between A1 and A2 groups.

3.2. Results of environmental scanning electron microscope

SEM images for each subgroup, in each testing period at 4000× magnification are represented in Fig. 2. The SEM observation matched with dye penetration depth. Numerous closed and narrow dentinal tubules were presented in the combination therapy of EB + TiO_2 group at all time intervals. Eggshell monotherapy and MI paste showed less narrow, and more patent dentinal tubules. In the control group A0, the tubules were mostly patent and wider than in other groups.

(A) Negative control, (B) eggshell monotherapy, (C) eggshell + titanium dioxide combination, (D) MI paste. (1) immediate, (2) after 2 weeks, (3) after 4 weeks.

4. Discussion

DH treatment relies on partially closing or permanently blocking dentinal tubules. No material can be considered the gold standard for DH treatment [19].

Using EB waste material for the treatment of DH, it is an important way to recycle this material, strengthing the economic benefits, related to using a natural waste material, which is high on the global agenda for a greener environment [9,10].

Long-term durability is an important criterion for an ideal desensitizing agent, not only the immediate action after its application. Exposure to acidic challenges can decrease the durability of desensitizing effects [9]. Thus, this study compared the durability of monotherapy of EBs to their combination with TiO_2 after exposure to wear challenges.

Table 1. The mean ± SD of dye penetration depth (mm) within each group at the three time intervals and the effect of material within each time interval on dye penetration using one-way analysis of variance test.

	T1	T2	T3	
Material	Immediate	2 weeks	4 weeks	*P* value
(A0) No ttt	1.29 ± 0.36^{cA}	2.22 ± 0.91^{aA}	1.53 ± 0.14^{bA}	0.000*
(A1) Eggshell	0.2 ± 0.18^{cB}	1.83 ± 0.53^{aAB}	0.77 ± 0.63^{bBC}	0.000*
(A2) Eggshell + TiO_2	0.16 ± 0.11^{bB}	1.07 ± 0.71^{aB}	0.25 ± 0.17^{bC}	0.000*
(A3) MI paste	0.52 ± 0.36^{bB}	2.02 ± 0.49^{aA}	0.98 ± 0.63^{bB}	0.000*
P value	0.001*	0.012*	0.001*	

Means with different small letter superscripts within the same row are statistically significantly different.
Means with different capital letter superscripts within the same column are statistically significantly different.
* = statistically significant at P less than or equal to 0.05.

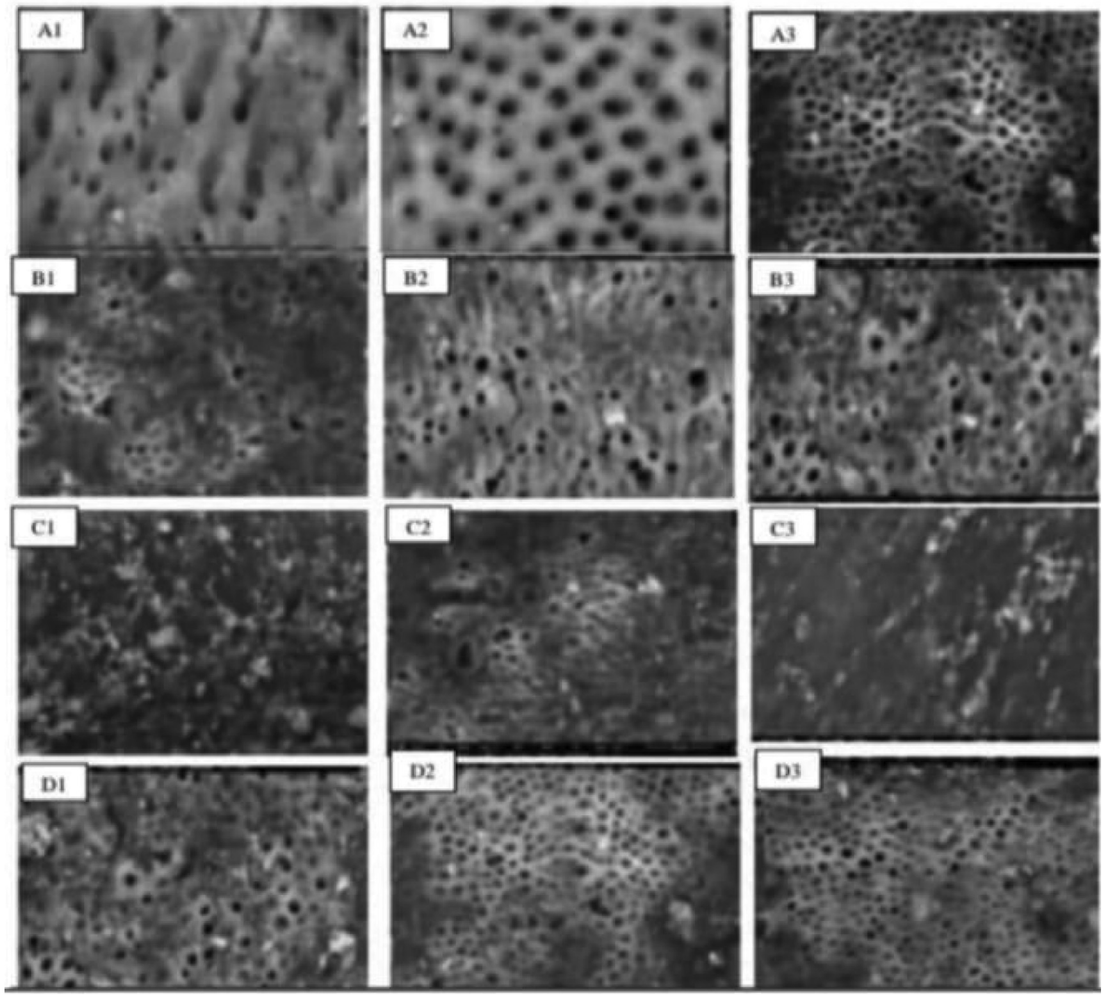

Fig. 2. Environmental scanning electron microscope photomicrographs showing representative samples of each subgroup in each testing period at 4000× magnification.

Previous studies have used Evans blue dye for measuring dentin permeability. Dye penetration into the tubules is a valuable and effective method for evaluating the efficacy of desensitizing products in dentin permeability reduction [13].

Regarding the effect of material on dye penetration, the tested materials showed a significant decrease in the mean value of dye penetration depth in comparison to the control. The lowest mean value of dye penetration was recorded in the combination therapy of EBs + TiO_2, and then the monotherapy of eggshell followed by MI paste, while the highest mean value of dye penetration was recorded in the negative control group. The results of dye penetration were confined to ESEM results as shown in Fig. 2.

Amorphous calcium phosphate (ACP) releases calcium and phosphate ions to change to apatite, which effectively occludes patent dentinal tubules, hence decreasing dentin permeability [6].

Eggshell nanoparticles achieved a significant reduction of dentin permeability, which can be attributed to the composition and the nano size of the prepared powder, which has rich $CaCO_3$ content effectively occluding the open tubules and hence decreases their permeability [20].

The results of our study were in agreement with a study that revealed that eggshell slurry occluded the open dentinal tubules effectively and reduced the permeability of dentin, after exposure to acidic challenges compared with the CPP-ACP desensitizing agent. This can be explained by the single application of the prepared eggshell gel being more effective than the single application of MI paste, and that was due to one application of MI paste not being able to form stable calcium precipitates within the DT [21].

Again, it was also in accordance with previous studies, which showed that EB nanoparticles have high remineralization potential and effectively occlude patent dentinal tubules [19,22,23].

The combination therapy of EB + TiO_2 showed more reduction in dentin permeability than EB monotherapy, and this may be attributed to the action of TiO_2, which is one of the few materials classified as bioactive, which spontaneously forms hydroxyapatite upon storage in simulated body fluid. Adsorption and dissociation of water by TiO_2 produces Ti−OH groups and a negative surface charge of TiO_2. Ca ions attracted to the surface forming a positively charged surface, forming hydroxyapatite by attraction of P ions to it [6]. It was also shown that TiO_2 can increase the resistance of calcium carbonates to acidic challenges and occlude the open dentinal tubules effectively [9].

This was in accordance with another study which reported that the combination of nano-sized eggshell, and titanium dioxide effectively occluded the patent dentinal tubules compared with other desensitizing agents [24,25].

Regarding the effect of time on dye penetration depth, the results showed a significant difference between the three time intervals, especially between T1 (the lowest mean of dye penetration) and T2 (the highest mean of dye penetration). The dye penetration increased at T2 and then decreased again after T3.

The higher mean values of dentin permeability at 2 weeks after the acidic exposure compared with that after application of the treatment agents, as revealed in the results and ESEM figures may be attributed to that the mineral precipitates formed by the treatment materials were weak and not able to withstand the acid challenge [21].

The low dentin permeability observed at 4 weeks compared with that observed at 2 weeks can be attributed to that the nucleation sites significantly increased over time, which resulted in additional apposition of crystals derived from the surrounding artificial saliva, which was highly supersaturated with calcium and phosphate ions [24].

The results matched with a previous study that compared hydraulic conductance, before and immediately after the application of the treatment agents and revealed that the hydraulic conductance decreased after their application. After tooth brushing for 1 and 2 weeks, there was an increase in dentin permeability, but a decrease after 6 weeks [26,27]; therefore, the null hypothesis of our study was rejected.

4.1. Conclusion

EB monotherapy or its combination with TiO_2 is considered a promising and effective therapy in decreasing dentin permeability and treatment of DH.

4.2. Recommendation

Other studies are needed for the evaluation of the clinical efficacy and durability of eggshell, and its TiO_2 combination in the treatment of DH.

Funding

The authors did not receive any specific funding for this study.

Conflict of interest

The authors do not have any financial or any other personal interest of any nature.

Acknowledgments

The author acknowledges Dr. Enas Mohamed Attia, Professor at the Faculty of Science, Al-Azhar University for Girls for her assistance in the preparation of artificial saliva and citric acid.

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