

IN VITRO COMPARATIVE SEM ANALYSIS OF NANO-HYDROXYAPATITE TOOTHPASTE AND DIODE LASER: EVALUATION OF EFFICACY OF DENTINAL TUBULAR OCCLUSION

Mohamed Shamel[1], Sara El Banna[2]

[1]Department of Oral Biology, The British University in Egypt, Cairo, Egypt
[2]Department of Oral Biology, October University for Modern Sciences and Arts, Cairo, Egypt

ABSTRACT

Introduction: Dentin hypersensitivity (DH) is a very common problem that faces many patients and maintaining treatments with a long term effectiveness remains a challenge. Based on hydrodynamic theory, the main approach of overcoming DH are tubular occlusion.

Objectives: The aim of this study was to discover the efficacy of diode laser alone and in combination with a desensitizing toothpaste containing nano-hydroxyapatite (nHap) to evaluate dentinal tubule occlusion after acid challenge under scanning electron microscope (SEM).

Material and methods: Forty dentin discs were divided into 4 groups: group 1, control; group 2, dentin disc treated with nHap toothpaste for one week; group 3, discs treated with diode laser in non-contact mode for 60 seconds; group 4, discs treated with nHap toothpaste for one week, followed by laser application. SEM analysis was performed after acid challenge to the discs.

Results: Tubular occlusion was evident in all groups after acid challenge; however, group 4 showed the most occluded tubules, which was the most resistant to acid challenge.

Conclusions: Combination of diode laser and toothpaste containing nHap has a greater effect on occluding dentinal tubules rather than each treatment alone.

Key words: dentin hypersensitivity, diode laser, hydroxyapatite.

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INTRODUCTION

Dentin hypersensitivity (DH) is described as short, sharp pain emerging from exposed dentinal tubules in response to various stimuli. This pain is not related to any underlying dental defect or pathology [1]. It is a very common problem among patients, especially those with periodontal and gingival diseases, and consequently, dentists face this problem in everyday practice due to its' high prevalence [2].

Many theories have been proposed to explain DH pathogenesis. However, Brannstrom and Astrom's hydrodynamic theory is the most extensively accepted concept, which states that both the inward and outward movement of fluids inside the dentinal tubules causes mechanical irritation of the nerve endings at the den-

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Address for correspondence: Dr. Mohamed Shamel, Department of Oral Biology, The British University in Egypt, El Sherouk City, Suez Desert Road, Cairo 11837 – P.O. Box 43, Egypt, e-mail: mohamed.shamel@bue.edu.eg

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tin pulp interface, which in turn would end in a painful sensation [3]. Fluid movement is caused by any external stimulus and the resulting mechanical irritation would be transmitted as a painful sensation, which is known as DH. Based on this theory, two main approaches of overcoming DH are tubular occlusion and blockage of nerve activity, or both [4].

Many treatment methods for DH depend on local application of different types of agents, either at home or professionally by dentists. These desensitizing agents work by occluding the dentinal tubules through forming precipitates on the exposed tubules [5]. However, superficial layers of these precipitates may wear out by brushing or by dietary acids, and DH-associated pain reoccurs once again and re-treatment is required [6]. Therefore, treatments with long-term effect have been guided towards the idea of intra-tubular deposition of minerals. Toothpastes containing bioactive glass ceramics or nanohydroxyapatite (nHap) have proven to occlude open dentinal tubules through mineral deposition [2].

Lasers have been introduced as another option for in-office treatment of DH. Low-power output lasers, such as diode laser, produces a continuous wave without overheating their substrate. Wavelengths between 780 and 980 nm have been shown to be clinically effective in the treatment of dentine hypersensitivity [7-9]. Diode lasers is thought to ameliorate DH through decreased nerve transmission and melting down dentin structure, which causes blockage of the dentinal tubules [10].

Some studies showed that laser application can augment the effect of desensitizing agents, which results in a greater efficacy and duration in managing DH rather than using the laser or desensitizing agent alone [11]. Hence, the goal was to discover the efficacy of diode laser alone and in combination with a desensitizing toothpaste containing nHap (Biorepair; Dr. Kurt Wolff GmbH, Bielefeld, Germany) to evaluate dentinal tubule occlusion after acid challenge, under scanning electron microscope (SEM).

OBJECTIVES

The aim of this study was to discover the efficacy of diode laser alone and in combination with a desensitizing toothpaste containing nHap to evaluate dentinal tubule occlusion after acid challenge under SEM.

MATERIAL AND METHODS

Forty non-carious human premolars, extracted for orthodontic treatment were obtained from dental hospital of the British University in Egypt, after informed consent obtained from the patient. Research design for the study was granted ethical approval from research ethics committee.

One dentin disc of 1 mm thick was sectioned from each tooth and then, the discs were immersed in citric acid (1%) for 20 seconds to expose the dentinal tubules and simulate the tooth with DH. Then, specimens were thoroughly rinsed with water and kept in distilled water until use [12].

Dentin discs were randomly and equally divided into four groups:

- group 1 – control group, where no treatment was applied to the dentin disk;
- group 2 – dentin discs treated with Biorepair, a desensitizing toothpaste with nHap (Dr. Kurt Wolff GmbH, Bielefeld, Germany) twice daily for 7 days [12];
- group 3 – dentin discs subjected to diode laser (980 nm; Doctor Smile, Italy) once in non-contact mode for 60 seconds;
- group 4 – dentin discs treated with Biorepair twice daily for 7 days followed by diode laser.

For the purpose of standardization of brushing procedure, a specially designed brushing apparatus was designed and fabricated (Figure 1) [13, 14]. Brushing apparatus was set at 120 strokes /min, with a load of 250 g. Then the discs were rinsed with water for 30 seconds to wash off any residual paste. Toothpaste treatment was applied for 2 minutes twice daily for 7 days and were kept in artificial saliva at 37°C after each application till the following day. At the end of experimental procedures, the discs were re-immersed in 1% citric acid for 20 seconds to detect acid resistance and then, they were washed and were stored in artificial saliva at 37°C. SEM examination of the dentin disc treated surfaces were observed and electro-micrographs were captured (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA) using Quanta FEG 250 (field emission gun) with accelerating voltage of 30 kV.

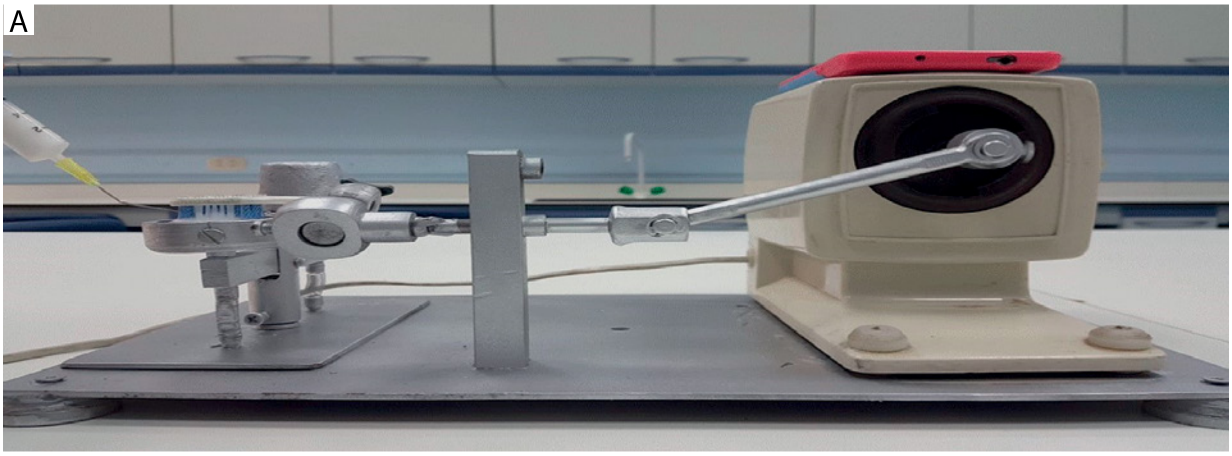
RESULTS

Surface morphology of dentine after nHap toothpaste and diode laser application are presented in Figure 2. After citric acid application, the control group specimens revealed no smear layer and frankly open dentinal tubules, thus simulating a tooth with DH (Figures 2A-B).

Samples from group 2, with dentin treated with Biorepair twice daily for a week followed by acid challenge, showed that the dentinal tubules were occluded, most likely due to deposition of minerals from the nHap paste used (Figures 2C-D).

Samples from group 3, lased dentin using diode laser followed by acid challenge, revealed that dentinal tubules were occluded and an irregular dentin surface was present, which indicated melting and re-solidification of the dentin (Figures 2E-F).

Samples from group 4 (dentin treated with Biorepair followed by diode laser and then acid challenge) showed that the tubules of irradiated dentin were not visible, in



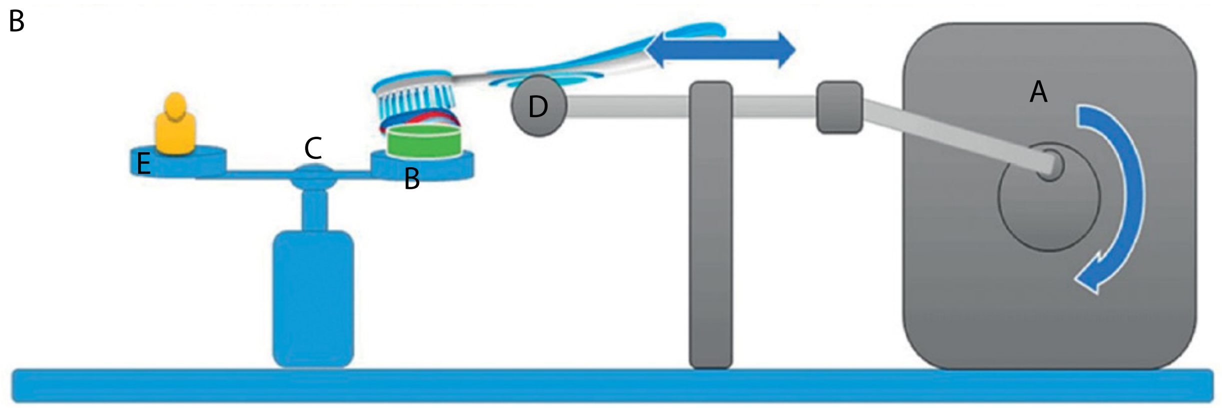


FIGURE 1. A) Custom-made brushing apparatus used for the experiment. **B)** Diagrammatic drawing showing the brushing apparatus. **A)** A gear box to reduce the speed of the motor to 2 cycle/second, with a crankshaft and connecting rod attached to a slider in order to change rotation movement into linear movement for standardized 5 mm horizontal movement. **B)** Samples holding pane. **C)** Double-pane balance. **D)** A brush holder. **E)** Weight holding pane

which some areas showed deposition of various minerals from the toothpaste, and other areas showed irregular dentin surface due to melting and re-solidification. Other samples from group 4, showed completely obliterated dentinal tubules as well as scarcely opened tubules were found (Figures 2G-H).

DISCUSSION

Patients complaining from DH usually prefer treatments with long-term relief of pain. Failure to obtain satisfying results has proven to cause DH to be one of the most encountered diseases in dental practice.

In the current study, after using nano-hydroxyapatite toothpaste (Biorepair) for a week, partial tubular occlusion in the dentin discs could be observed in SEM examination, with precipitates formed on the tubular surface. Similar precipitate layer formation on the dentin surface was reported in previous studies using nHap toothpastes to occlude dentinal tubules [15-17].

The efficacy of nano-hydroxyapatite to occlude the dentinal tubules is most probably related to the fact that these crystals are completely similar to the mineral that forms dental hard tissues. There nano-size are small enough to simulate the size of natural dentinal hydroxyapatite, which is approximately 20 nm [18].

The occluding effect of nHap found in this study might be attributed to the formation of a homogenous hydroxy apatite layer on the demineralized dentin surface. This can be explained by the facts that nHap has hydrophilic and wetting characteristics, which enable to produce a thin but tightly bound layer on the tooth surface, resulting in re-mineralization in addition to increasing surface hardness [19].

Another explanation for the occluding effect of nHap is that it also acts as a filler, because it repairs small holes and depressions on enamel as well as dentin surfaces, a property enhanced by nano-size of the particles [20].

The partial tubular occlusion found in this study is in accordance with several studies, which demonstrated that nHap toothpastes had a rapid action in treating DH but for a short period of time [12, 15, 18]. This might be explained by the fact that the acid challenge caused demineralization of the precipitates formed inside the tu-

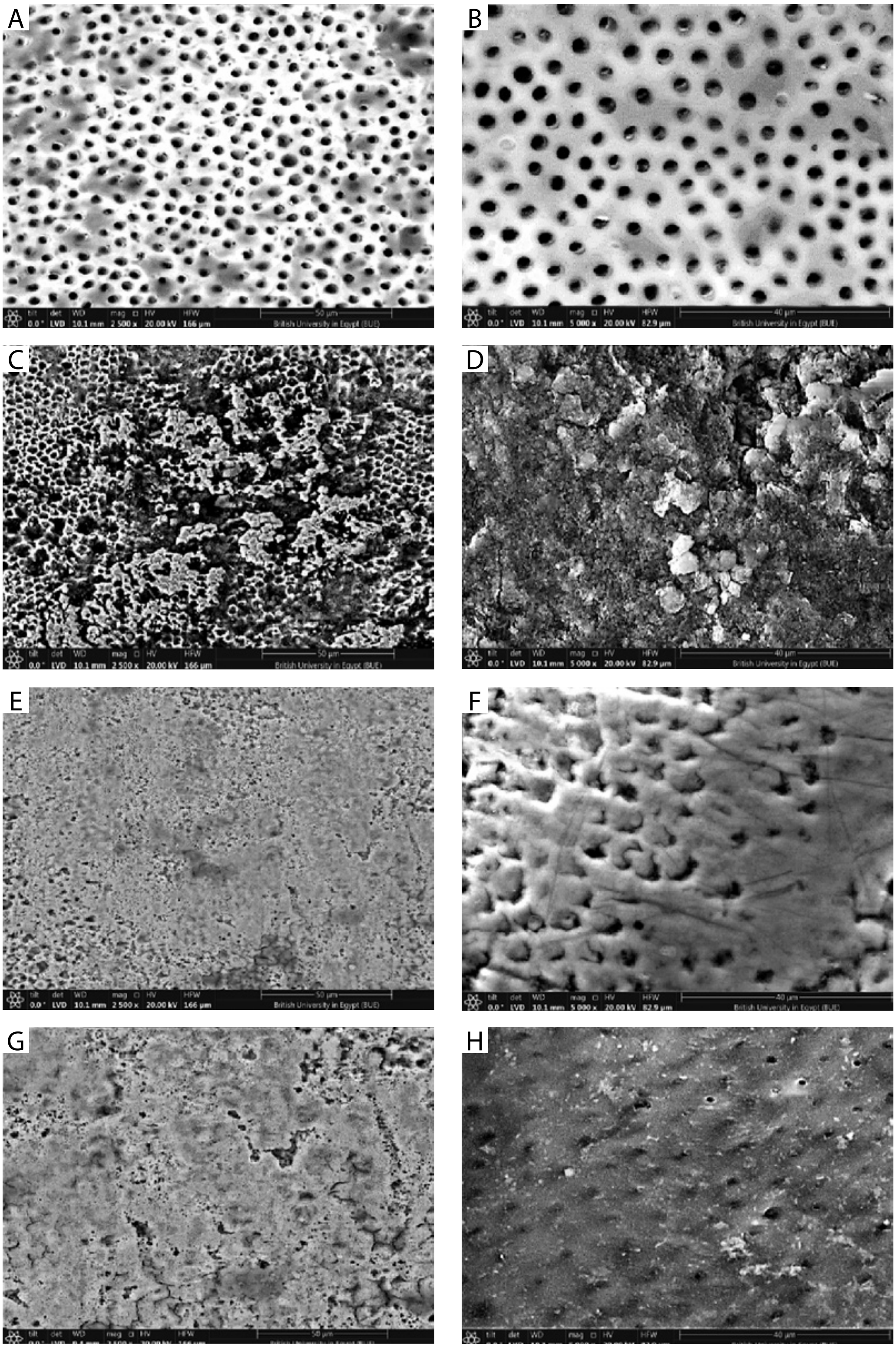


FIGURE 2. SEM images of dentin samples. **A-B)** Control group of untreated dentin sample showing widely opened dentinal tubules with no smear layer observed (×2,500 and ×5,000, respectively). **C-D)** Samples of group 2 dentinal tubules not visible due to deposition of minerals from the toothpaste (×2,500 and ×5,000, respectively). **E-F)** Group 3 irregular dentin surface with some areas melted and re-solidified, and other areas showing completely obliterated dentinal tubules after laser application, also very few opened dentinal tubules are seen in **F** (×2,500 and ×5,000, respectively). **G)** Tubules of the irradiated dentin are not visible and some areas show deposition of various minerals from the toothpaste, other areas showing irregular dentin surface due to melting and re-solidification (×2,500). **H)** Obvious total closure of most of the dentinal tubules as well as few partially occluded and scarcely opened dentinal tubules can be seen, with areas of irregular dentin present due to melting and re-solidification (×5,000)

bules, the same process that caused open dentinal tubules in the first place and subsequently, DH.

Diode laser was used in this study as many studies have showed its' efficiency in the treatment of DH. Moreover, it is also inexpensive, lightweight, and frequently available in dental clinics. It is a semi-conductor laser, whose different wavelengths have been used to treat DH [21, 22].

Parameter of the power used in our study was 2 W, and is in accordance with a study by Liu *et al.* [23]. Their study demonstrated that 2 W is a suitable parameter for 980nm diode laser that seals dentinal tubules without excessive melting of the dentine, thus achieving a good level of dentinal tubule occlusion, which is comparable with the results of the present study.

SEM examination revealed that the specimens irradiated with diode laser showed occlusion of the dentinal tubules but to a greater extent than the nHap group. These results are in accordance with several studies, which showed that the diode laser caused a mild, random, and irregular melting in peritubular and intertubular dentine throughout a relevant site, thus causing obliteration of dentinal tubules [24, 25].

This is explained by the fact that a laser wavelength of between 800 and 980 nm is poorly absorbed in water and hydroxyapatite. Accordingly, this poor absorption allows propagation, scattering, or diffused transmission of the laser radiation through the dentin [26]. Energy absorbed by the dentin surface provides a sufficient increase in temperature to attain a melting effect that causes occlusion of the dentinal tubules [10].

In the current study, SEM of the nHap and diode laser combination group showed that the dentinal tubules were almost completely occluded after the treatment and acid challenge. These results might be attributed to ultrastructural changes including crystal size growth and recrystallization of dentin as a result of high temperature rise in the surface caused by the diode laser. It is possible that these chemical and structural changes facilitated nanohydroxyapatite deposition [27], thus improved sealing ability of dentinal tubules and acid resistance.

CONCLUSIONS

The results of this study prove that the combination of diode laser and toothpaste containing nHap has a greater effect on occluding dentinal tubules rather than each treatment alone. Moreover, the acid resistance was more evident when using this combination, indicating a long-term relief from DH.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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