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PATENCY OF OPENED DENTINAL TUBULES AFTER TREATMENT WITH BIOMIMETIC MATERIALS

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ABSTRACT

Objectives: To estimate the dentinal tubules occlusion potential of biomimetic materials as nano-hydroxyapatite and self-assembling peptides and to compare their results with a traditionally used sodium fluoride material.

Materials and methods: Standardized forty dentin disks were obtained from extracted molars. They were treated with citric acid 6% for 2 min to simulate the hypersensitive dentin. Disks were classified into four equal groups (n=10); Control group without treatment; Sodium fluoride **(Bifluorid 10**, VOCO, Germany); self-assembling peptide (**Curodont™ Protect**, Credentis, Switzerland) or nanohydroxyapatite nHAp containing material (**Remin Pro**, VOCO, Germany). Fluoride varnish was applied twice during the test period (7 days) and the biomimetic desensitizing agents were left for 5 minutes/ once daily. Specimens were stored in artificial saliva during the seven days. Specimens were analyzed using scanning electron microscope (500X). Quantitative analysis of the surface area (μm^2) of the patent dentinal tubules in the scanned images was done by using digimizer image analysis software (version 3.7.0.0). Statistical analysis was done using One way-ANOVA followed by Tuckey's post hoc test for comparison ($P \leq 0.05$).

Results: In the control group, the dentinal tubules were widely opened after being demineralized with the highest dentinal tubules mean surface area recorded (592.5±68.0 μm^2). Sodium fluoride group revealed partially obliterated dentinal tubules with intratubular deposits leaving a mean surface area of 260.0**±** 53.3 μm^2. Opened areas in the fluoride group are statistically equal to those in the self-assembling peptide group (203.5±99.3). In the nanohydroxyapatite group, almost complete sealing of the dentinal tubules was evident with the least mean surface area of the opened parts(35.0±5.3). The difference between the study groups was statistically significant.

Conclusions: The desensitizing agents studied showed different abilities to change the dentin surface micromorphology, with partial or total occlusion of dentinal tubules. Nanohydroxyapatite containing agent is a promising biomimetic material for management of dentin hypersensitivity.

KEYWORDS: Biomimetic, Dentin, Dentinal tubules, Desensitizing, Occlusion, Patency**.**

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INTRODUCTION

Exposed dentin with widely opened dentinal tubules is a common problem that affects dental patients causing sever sharp pain.[1,2] This situation occurs after cavity preparation, the gingival recession with subsequent cemental loss or loss of protective enamel layer. Hydrodynamic theory is the most accepted theory that explains dentin hypersensitivity and it stated that any external stimulus affects the exposed dentinal tubules could induce movement of the dentinal fluids inside the dentinal tubules. This movement will stimulate the free nerve endings in the dentinal tubules and the pulp side and the pain will be induced. [3]

Sealing of such patent dentinal tubules is from the treatment options that solve the problem of hypersensitive dentin. It can be conducted either by using mechanical plugging of such opened dentinal tubules or physically by using LASER beam. [4,5] Diversity of chemical agents are available in the dental markets or even bought on the shelf for mechanical occlusion. [6] However, all of them occlude the tubules by their crystals deposition and so leaving some opened areas in the tubules. Together with providing only transient action and require many applications to perform its effect. Sodium fluoride is commonly used as a desensitizing agent especially the varnish form which enables it to have long-lasting desensitization. [7]

PeptideP11-4 self-assembles into a matrix triggering biomimetic mineralization and repair mechanism. It demonstrated promising results in the enamel remineralization, however no sold clue was obtained to demonstrate its effect on the occlusion of the patent dentinal tubules. [8-10] Nano Hydroxyapatite (HAP) is another biomimetic material which has a tendency to cause occlusion of the dentinal tubules and it is claimed to be stable. [11]

HAP agents containing sodium fluoride induces mineral gaining, forming a biomimetic apatite covering the tooth surfaces either enamel or dentin, which rapidly occurs due to the innovative nano-structured HAP particles.[12]

Calculation of the diameter, number of dentinal tubules, and percentage of dentinal tubules occluded have been done which showed a higher percentage of tubular occlusion with decreased diameter and number of dentinal tubules, and increased closed tubular area of the dentin. [13,14] However, calculation of dentinal tubules opened areas seems to give more reliable information about the actual occlusion of the dentinal tubules. Thus, the present study was undertaken to assess the effect of sodium fluoride varnish, two biomimetic materials; self-assembling peptide and nano-hydroxyapatite containing agents on the patent dentinal tubule surface areas. The null hypothesis stated that the used treatments will not show any effect on the occlusion of patent dentinal tubules.

MATERIALS AND METHODS

Forty sound recently extracted human molars were collected and stored in distilled water containing 0.2% thymol to be used in this study at 4˚C. [15] They were cleaned from any soft or hard deposits using hand scalers (Primadent, Germany) and examined using magnifying lens 6x (CarsonR Magnilook) to exclude any defective teeth. Teeth were mounted inside an acrylic blocks to facilitate the cutting procedures of them.

Preparation of dentin discs

Enamel surfaces were cut across the buccolingual and mesiodistal planes using a diamond disk (MTI Corporation, Richmond, CA, USA) in a low-speed micro-slicing machine (Isomet, Buehler, Lake Bluff, IL, USA) under water-cooling. This procedure allowed proper identification of enamel from the dentin and then enamel was removed from the occlusal surfaces of the specimens using the same slicing machine. Another cut was made 1mm apical to the exposed dentin surface to obtain one centralized dentin disc of 1 mm thickness from each molar relatively in the same depth. Further trimming of the dentin specimens was done to standardize the dimension of the discs regarding width, depth, and thickness (5 X 5X1 mm). Specimens were put in the ultrasonic cleaner for cleaning of any surface deposits. Simulation of hypersensitive dentin with widely opened dentinal tubules was achieved by immersion of the specimens in Citric acid 6% for 2 min.

Grouping of the specimens

The dentin discs thus obtained were randomly divided into 4 groups (10 each) according to the treatment used, as follows:

Group 1: Hypersensitive simulated (Control), no surface treatment was done.

Group 2: Sodium fluoride varnish group (Bifluorid 10)

Group 3: Self-assembling peptide (Curodont)

Group 4: nHAp (Remin Pro)

Materials, compositions, and manufacturers are summarized in **Table 1**.

Treatment of the specimens

Single-dose units were used in case of sodium fluoride varnish to standardize the amount of material applied to the specimen and it was applied twice during the test period which was 7 days. On the other hand, a standardized amount of the biomimetic desensitizing agents either Curodont or Remin Pro (1ml) was uniformly distributed over the demineralized surface using a disposable brush for each application and left for 5 minutes/ once daily for the 7 days. A graduated plastic syringe was used to carry the predetermined amount of the biomimetic desensitizing agents. The applied agent was thoroughly rinsed with an air-water jet for 15 s. Specimens were stored in daily changed artificial saliva at 37˚C in an incubator. Saliva was composed of [Na3PO4 (3.90mM), NaCl (4.29mM), KCl (17.98mM), CaCl2 (1.10mM), MgCl2 (0.08mM), H2SO4 (0.50mM), NaHCO3 (3.27mM) and distilled water with pH adjusted to 7.2]. [16]

TABLE (1) Specifications of the materials used in this study

Material	Description	Composition	Manufacturer
Remin Pro	Nano-hydroxyapatite containing desensitizing agent	20nm Hydroxyapatite, Sodium Fluoride (1,450 ppm fluoride), Xylitol, water	VOCO, Germany
Bifluoride 10	Sodium Fluoride Varnish	5% Sodium Fluoride (22.600 ppm Fluoride) and 5% Calcium Fluoride, ethyl acetate, Cellulose nitrate with alcohol, isopentyl propionate, Clove oil (≤2.5%)	
Curodont Protect	Self-assembling peptides	900 ppm fluoride as sodium monofluorophosphate, 0.1% di-calcium-phoshate , 0.028% calcium-glycerophosphate , self-assembling peptide P11-4	Credentis, Switzerland

Analysis of the treated specimens qualitatively and quantitatively

All specimens were put again in the ultrasonic cleaner for cleaning of any surface deposits**.** Specimens were then mounted on aluminum stubs and further dried in vacuum, and then sputter coated with gold (Ladd sputter coater, USA). Coated specimens were inspected under scanning electron microscope (JEOL_JSM_5500LV) at magnifications (500X). Scanned images (Three for each specimen) were analyzed using digimizer image analysis software (version 3.7.0.0). It was used to measure the surface areas of the opened parts in the dentinal tubules using the magic contour tool after being calibrated with the SEM images scale bar (10 μm). Automatic tracing of the opened areas with different contrast was done and surface area of each tubule was obtained. Finally, all obtained areas from the same image were summited and the total opened surface area was calculated.(Figure 1)

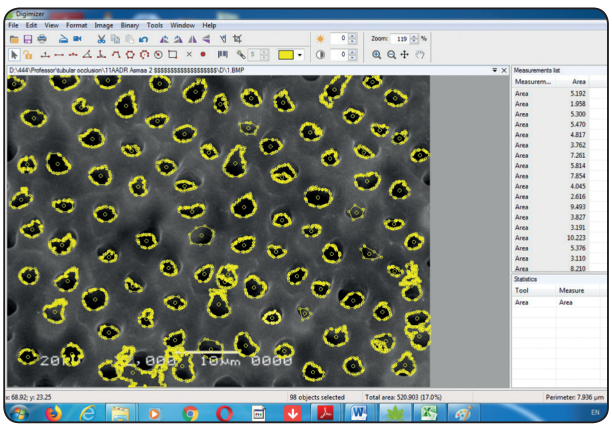

Fig. (1) Screen shoot for Digimizer software

Statistical analysis

Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. They showed normal distribution and so One Way-ANOVA test was used to study the effect of the different treatments used on the mean surface area of the dentinal tubules (μm^2) values. Tukey's post hoc test was used for pairwise comparison when ANOVA was significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 22 for Windows.

RESULTS

Qualitative analysis results of the dentinal tubules patency:

The photomicrographs of the control group and groups treated with desensitizing agents are shown in figures 2(a) to 5(a). The hypersensitive simulated control group showed widely opened numerous dentinal tubules without any evidence of smear layer and smear plugs (Figures 2a). Sodium fluoride group revealed partially obliterated dentinal tubules with irregular intratubular deposits. (Figures 3a) Biomimetic groups including the self-assembling peptides and nano-hydroxyapatite showed variable results. In the first biomimetic group, areas of complete sealing with narrowing of others dentinal tubules were seen. (Figures 4a) The second biomimetic group demonstrated almost complete sealing of the dentinal tubules with minor opened parts. (Figures 5a)

Quantitative analysis results of the patent dentinal tubules surface areas:

The analyzed photomicrographs of the different study groups are shown in figures 2(b) to 5(b). The results of the descriptive statistics are shown in table 2. In the control group, the mean surface area recorded for the opened dentinal tubules in μm^2 was the highest value recorded (592.5±68.0). However, in the Sodium fluoride group, the mean surface area of 260.0**±** 53.3 μm^2 was obtained and it was statistically similar to that obtained from the Self-assembling peptide group (203.5±99.3). On the other side, the nano-hydroxyapatite group had the least mean surface area of the opened parts(35.0±5.3). The difference between the study groups was statistically significant at $P < 0.0001$.

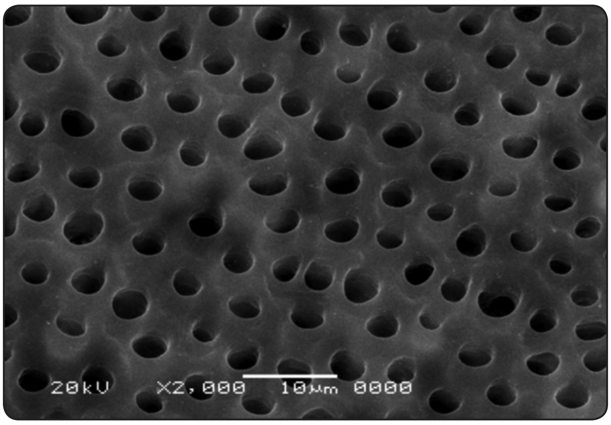

Fig. (2a) Scanning electron photomicrograph for the demineralized dentin showing numerous patent dentinal tubules.

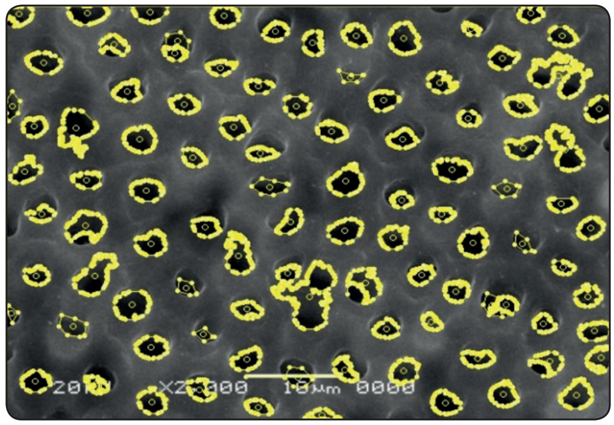

Fig. (2b) Analyzed scanning electron photomicrograph for the demineralized dentin showing traced patent dentinal tubules.

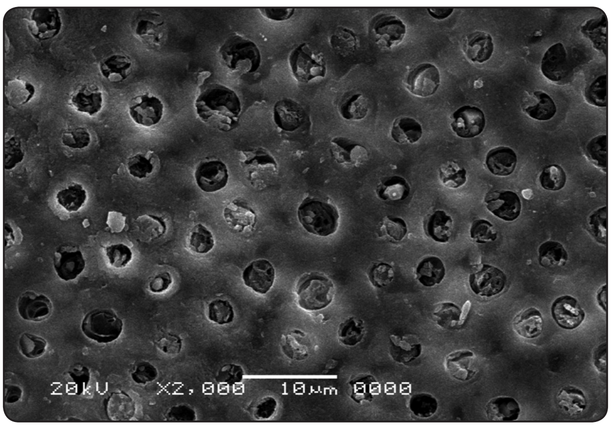

Fig. (3a) Scanning electron photomicrograph for the demineralized dentin treated with sodium fluoride varnish showing intratubular deposits blocking the dentinal tubules.

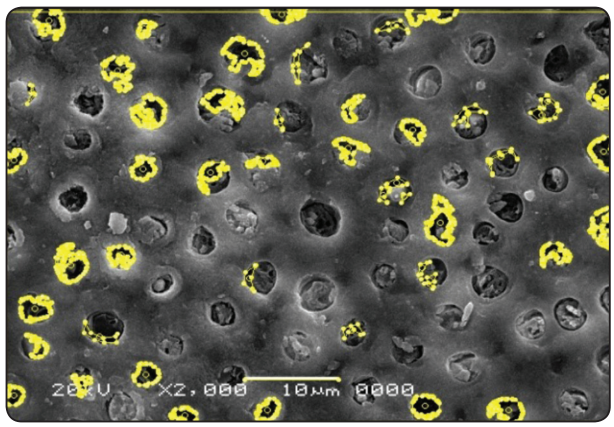

Fig. (3b) Analyzed scanning electron photomicrograph for the demineralized dentin treated with sodium fluoride varnish showing traced patent dentinal tubules.

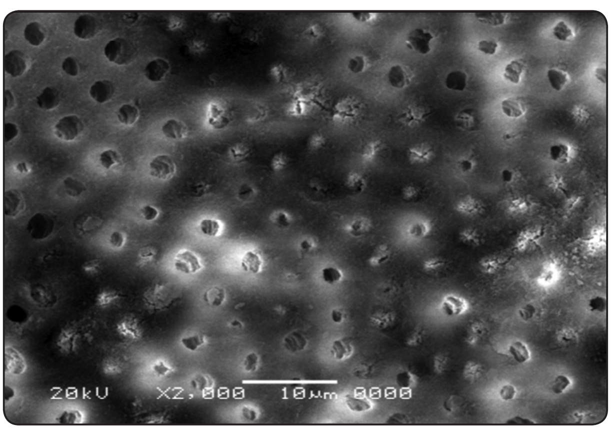

Fig. (4a) Scanning electron photomicrograph for the demineralized dentin treated with self-assembling peptides (Curodont) showing narrow sealed dentinal tubules.

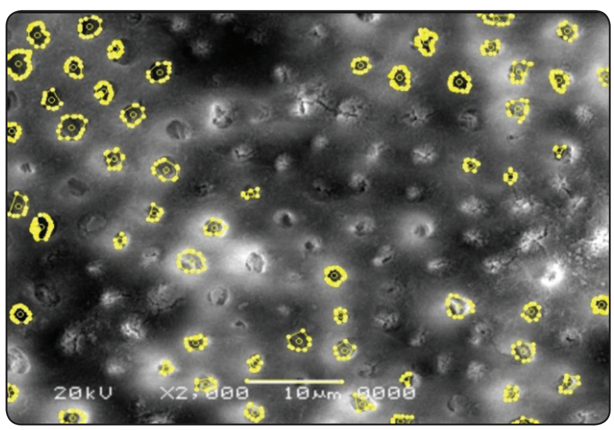

Fig. (4b) Analyzed scanning electron photomicrograph for the demineralized dentin treated with self-assembling peptides (Curodont) showing traced patent dentinal tubules.

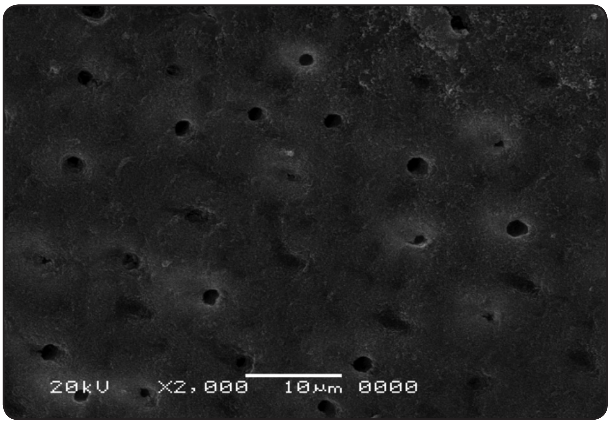

Fig. (5a) Scanning electron photo-micrograph for the demineralized dentin treated with nanohydroxyapatite (Remin Pro) showing few narrow sealed dentinal tubules.

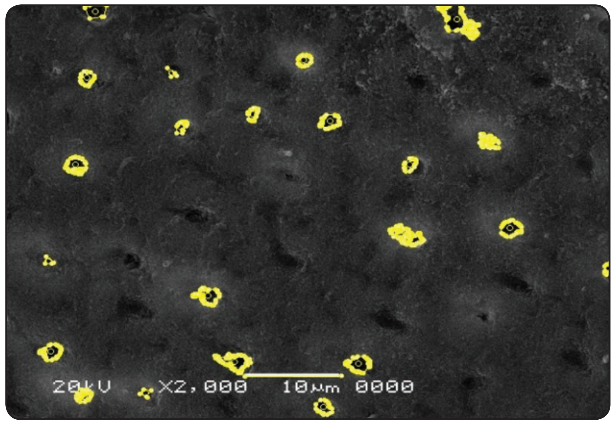

Fig. (5b) Analyzed scanning electron photomicrograph for the demineralized dentin treated with nanohydroxyapatite (Remin Pro) showing traced patent dentinal tubules.

TABLE (2) Mean, standard deviation (SD) and descriptive statistics for the patent dentinal tubules surface area (μm2) of the study groups

	Mean	SD	SE	Mini	Max	Lower 95% Confidence	Upper 95% Confidence
Demineralized	592.5^a	68.0	24.1	500	703	535.6	649.4
Fluoride	260.0^b	53.2	18.8	179	315	215.5	304.5
Curodont	203.5^b	99.3	35.1	97	350	120.5	286.5
Remin pro	35.0^c	9.3	3.3	20	45	27.21	42.8
P value	< 0.0001*						

Same letters in the column indicate no significant (ns) differences (p > 0.05).

DISCUSSION

In the present study, dentin discs were obtained from the coronal portion of the teeth because dentin standardization can be controlled in the coronal portion compared to the cervical parts.(17) Dentine disc model is a feasible and non-invasive approach to evaluate the efficacy of desensitizing agents and SEM micrographs were able to demonstrate the morphological modifications in dentinal tubules. Simulation of hypersensitive dentin was done by a commonly used technique which is the immersion in citric acid 6% for 2 minutes. Also, the occluding materials used were selected on the basis of being widely used as Sodium fluoride varnish, recent biomimetic as Curodont and nHA. Evaluation of dentinal tubules patency was done qualitatively and quantitatively using scanning electron microscope and image analysis software.

Sodium fluoride varnish (Bifluorid 10) is known as a unique material with natural ingredients. It contains 5% sodium fluoride and 5% Calcium fluoride, this unique combination allows fast fluoride release from the sodium fluoride and long-lasting effect from the insoluble calcium fluoride.(18) Regarding being varnish, a film over the dentin provides a barrier against further demineralization.

Nevertheless, this film may easily be removed or displaced by washing the surface, leading to partial occlusion of the tubules and formation of an irregular layer, which may offer a treatment without any long-lasting effect. [19] This group showed narrowing of the dentinal tubular lumen but failed to produce complete tubular occlusion **(Table 2 and Figure 3a and b)**. It reacts with the calcium of dentin resulting in the formation of calcium fluoride crystals, which are deposited onto the opening of the dentinal tubules. [20] However, these crystals are small in size and hence not effective in occluding the tubules totally. This result was in agreement with Rafaat et al in 2011. [21]

Self-assembling peptides (P11-4, Curodont) undergo self-assembly into three-dimensional (3D) fibrillar scaffolds in response to specific environmental conditions. The P11-4 side attracts calcium ions, activating precipitation of new hydroxyapatite, thereby promoting mineral deposition in situ. [8,9] In the current study, the self-assembling peptide P11-4 group showed similar quantitative results as the sodium fluoride group.**(Figure 4a and b)** This could be attributed to the ability of the peptide to induce biomimetic mineralization by nucleating hydroxyapatite crystals. [22]

The other biomimetic group (nHAp, Remin Pro) showed a higher level of dentinal tubule occlusion with a sporadic few opened tubules when compared to control group and the other tested materials. .**(Figure 5a and b)** Similar results were obtained by Ohta et al. [23] and Wang et al. [12] who showed that nHAp sealed the dentinal tubules. The occluding potential of nHAp could be attributed to its nanoscale particle size (20 nm) (Table 1). This size provides the HA with great bioactivity and osteoconductivity. .[24,25] Great penetration of ion resulted in better tubular occlusion and the relatively low pH together with sodium fluoride contents of the used nHA agent also could play a role in its bioactivity. [26] This finding was in disagreement with Dhillon et al in 2014 and the discrepancies between the results of the present study and other studies may be related to the processing differences in dentin specimen preparation and mode of application of the desensitizing agent.[27] Based on the abovementioned results the null hypothesis was rejected as all the treatment used had an effect on the occlusion of the patent dentinal tubules in comparison with the control group.

Further studies including the role hydraulic conductance of dentin in the desensitization process will be of great value.

CONCLUSIONS

Under the conditions of the present study the following conclusions are evident;

1) Sodium fluoride varnish and self-assembling peptides have similar tubular occlusion potential.
2) Nano-HAP is the most effective agent for occluding the dentinal tubules, and it will be a promising treatment for the hypersensitivity.

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