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DEGREE OF DENTINAL TUBULES OCCLUSION USING DIFFERENT OCCLUDING AND SEALING TECHNOLOGIES

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ABSTRACT

Aim: the purpose of this study is to evaluate the effect of using nanohydroxyapatite, self assembly peptides, resin modified glass ionomer (RMGI) technologies on the degree of dentinal tubules occlusion in comparison to conventional applied sodium fluoride varnish.

Methods: Forty flat dentin specimens were prepared from sound human premolars. The specimens were immersed in 6% citric acid solution for 3min. Each dentin specimen was divided into two equal halves. One half acted as a control and the other received the treatment. The specimens were then divided into four equal groups according to desensitizing agent applied (n=10): GI: The nanohydroxyapatite based (Desensibilize NanoP, Brazil), GII: The self assemply peptide based (Curodont D'SenZ, Schweiz), GIII: RMGI based (Vanish™XT, USA), GIV: Sodium fluoride based (Durasheild, USA). The extent of dentinal tubules occlusion was assessed using environmental field emission scanning electron microscopy (EFESEM). EFESEM photomicrographs were captured from each half (Control and treated) after; 24 hours, 7 days and exposure to acidic challenge. EFESEM photomicrographs were imported into Image analysis software (NIH, USA) and percentage of dentinal tubules occluded areas was calculated. Data was analyzed using ANOVA test.

Results: The two ways ANOVA revealed that both desensitizing agents and time intervals and their interaction have a statistical significant effect on the degree of dentinal tubules occlusion. The groups after both 24 hours were ranked: GIII>GI=GII>GIV, after 7 days: GIII = GII > GI> GIV, and after the exposure to acidic challenge: GIII>GI>GII>GIV

Conclusion: The RMGI sealing technology showed a more promising effect on the degree of dentinal tubules occlusion compared to the innovative occluding technologies, based on the self assembly peptides, or conventional sodium fluoride varnish

Clinical relevance: The use of RMGI sealing technology can show immediate relief of dentin hypersensitivity owing to its immediate and lasting effect. Meanwhile the innovative occluding technologies based on the nanohydroxyapatite and self assembly peptides, need sometime to show a more profound effect. Nevertheless they are not recommended to patients with high citrus acid products consumption.

KEYWORDS: Dentin hypersensitivity, dentinal tubules occlusion, nanohydroxylapatite, self assembly peptides, resin modified glass ionomer

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INTRODUCTION

Dentin hypersensitivity is a common dental problem.[1] Although its prevalence varies among populations, it shows a clear up trend throughout the whole world.[2,3] Some research has placed the incidence as high as 74%, but in most populations, it appears to range between 10 and 30%, depending on the population studied, study setting as well as design.[4]

Dentin hypersensitivity is described clinically as an exaggerated response to a non-noxious sensory stimulus, such as osmotic changes, thermal changes, or mechanical stimuli. It is viewed as originating from the underlying exposed dentin after the enamel or cementum at the root surface has been eroded away.[5,6] Although different theories have been put forward to explain the mechanism behind the dentin hypersensitivity, still the most widely accepted theory is the hydrodynamic. The theory was first reported by Gysi in 1900, studied heavily and corroborated in the 1950s and 1960s by Bränström.[7,8] The theory stated that sensitive dentin is based on the stimulus-induced fluid flow in the dentinal tubules and consequent nociceptors activation in the pulp/dentin border area.[8]

Accordingly, there are two major approaches for treatment of dentin hypersensitivity; nerve desensitization and/or tubular occlusion.[1,9,10] Nerve desensitization process can be done using chemical agents as potassium nitrate that causes reduction in sensitivity due to prevention of pain impulses via depolarization of the nerve synapse. Meanwhile, occlusion of the patent dentinal tubules alleviates pain by preventing intratubular fluid shifts. This could be accomplished either physically using lasers[2,11] or chemically using either occluding or sealing agents. [7,12]

Occlusion of the dentinal tubules can be done using fluorides, oxalates or different calcium phosphate based remineralizing agents presented in its either amorphous form as the casein phophospeptites calcium phosphates or crystalline forms as the bioactive glass. Recently novel remineralizing technology has been developed via the introduction of nanostructures having good biocompatibility and bioactivity forming both organic and inorganic analogue of the human dentin. Since HA is principal inorganic component, synthetic HA is considered a logical mineral compound to substitute the natural mineral constituent of dentin.[13] Nanohydroxylaptite toothpastes have been recently investigated in exploring its effect in casing dentin hypersensitivity and their findings encouraged its use as effective desensitizing agents, taking into consideration that those over the counter products were used on daily bases,[14-17] however professionally applied products were not investigated.

Meanwhile the basic organic part of human dentin is type I collagen. Hence, synthetic Oligopetide104, self assemply peptide, is considered as a logical analogue to the natural organic part of dentin (P11-4). This raised the concept of biomimetic remineralization, which has been recently adopted in enamel remineralization.[18,19] The Self-assembly peptide is named Oligopeptide 104 by the International Nomenclature Committee to describe a cosmetic ingredient, INCI (P11-4). It consists of the natural occurring amino acids Glutamine, Glutamic acid, Phenylalanine, Tryptophan and Arginine. The peptides spontaneously assemble in response to environmental triggers to form 3D biomimetic scaffolds capable of nucleating hydroxyapatite de novo.[18,20] The Swiss company Credentis has licensed the peptide technology that has been marketed under the registered trademark curolox® technology. It was claimed that this technology could be used in enamel repair, protection and treatment of dentin hypersensitivity.

Sealing could be done using dentin adhesives or glass ionomer varnishes. To date, however, the clinical use of glass ionomers for desensitization has been limited, perhaps because the prevalent powder-liquid format is less convenient than alternative

liquid materials. The recent availability of RMGI in paste-liquid or paste–paste formats might encourage the wider use of these materials; however, little information is available in the literature regarding their performance.

However; no research work is available till now to compare between the contemporary professionally applied desensitizing agents either based on the occluding ability using both the nanohydroxyapaptite or self assembly peptide analogues and on the sealing capability using the recently launched RMGI based varnish and. Thus the aim of the study was to compare the degree of dentinal tubules occlusion using, nanohydroxyapaptite as professionally applied, self assembly peptide, RMGI and sodium fluoride based products. The hypotheses tested; First; there is no difference in the degree of tubular occlusion between the nanohydroxyapaptite, self-assembly peptide, RMGI and sodium fluoride based products. Second; the time and exposure to acidic challenge will not affect the performance of the tested desensitizing agents.

MATERIALS AND METHODS

Materials

Four professionally applied dentin desensitizing agents were used in the present study; (a) Nano P (FGM, Dentscare LTDA, Brazil) based on the use of nanohydroxyapatite technology. (b) Curodont D'SenZ (Credentis ag, MS Dental, Schweiz) as desensitizing agent based on the use of self assembly peptide technology (c) Vanish™ XT (3M ESPE, USA) as extended contact varnish based on resin modified glass ionomer technology (RMGI), (d) Durasheild (Sultan Health Care, USA) as sodium fluoride varnish. Materials composition, manufacturer and lot number are presented in table (1).

Specimen preparation:

Forty dentin specimens were prepared from sound human premolars extracted for orthodontic reasons. The extracted premolars were stored in a .1% thymol solution at 4^0C for a maximum period of one month till usage.[21]

TABLE (1) Materials used in the study

Material	Composition	Manufacturer and lot number
Desensibilize NanoP	Nanometer-sized calcium phosphates (in form of hydroxyapatite), calcium fluoride, potassium nitrate, distilled water, surfactant, thickener, flavoring, sweetener and preservatives	FGM, Dentscare LTDA, Brazil, 231013
Curodont D'SenZ	Hydrogenated starch hydrolysate, Aqua, Hydrated Silics, PEG-8, cellulose Gum, Sodium Monofluorophosphate, Aroma, Sodium Saccharin, citric acid, sodium hydroxide, Dicalcium phosphate, Oligopeptide-104, calcium Glyerophosphate, sodium choride, sodium sulfate, limonene, cinnamal,	Credentis ag, MS Dental, Schweiz, 0231
Vanish™XT	The liquid component consists of methacrylate- modified polyalkenoic, 2-hydroxyethylmethacrylate (HEMA), water, initiators (including camphorquinone), and calcium glycerophosphate. The paste is a combination of HEMA, 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl] propane, water, initiators and fluoroaluminosilicate glass.	3M ESPE, USA, 2405
Durasheild	5% Sodium Fluoride Varnish	Sultan Health Care, USA, 140115

Each tooth was sectioned using a diamond disc (Komet, Rock Hill, USA) in low speed under water cooling in mesiodistal direction to divide it into buccal and lingual halves. Standardized slabs of 4.4 mm width, 3 mm length and 3mm thickness were obtained from the cervical third of the buccal halves of teeth.[22] The slabs were embedded in acrylic resin molds (Acrostone Dental Factor, England) with the buccal surfaces facing upwards. Superficial dentin was exposed by wet grinding the buccal enamel using a 180-grit silicon carbide paper. The dentin specimens were then wet polished using a 600-grit silicon carbide paper for 30 seconds to create an even and uniform surface.[23] The polished specimens were then placed in a jar of distilled water and sonicated for 10 minutes to remove the polishing abrasive.[24] The flat dentin surfaces were etched by immersion in 6% citric acid solution (pH 2) for 3min to remove the smear layer and produce fully opened tubules and then rinsed with distilled water.[25] Each dentin specimen was divided into two equal halves by making a trench using a diamonad disc of 0.25mm thickness (Oker, Barazil). One half was labeled with a permanent marker to act as a control and the other half received the treatment. The specimens were divided into four equal groups according to desensitizing agent applied (n=10):

Group I: The nanohydroxyapatite based desensitizing agent (Desensibilize NanoP, FGM, Dentscare LTDA, Brazil) was applied according to the manufacturer's instruction, in which the paste was scrubed on the dentin surface for 10 seconds using a diamonad flex disc mounted on low speed contra. It was to be left on the surface for 5 minutes. The paste was the removed using a slightly moist cotton roll.

Group II: The self assembly peptide based desensitizing agent (Curodont D'SenZ, MS Dental, Schweiz) was applied according to the manufacturer's instruction, in which the gel was scrubed on the dentin surface for 2 minutes using a polishing cup mounted on low speed contra. Then, the gel was to be removed using a slightly moist cotton roll.

Group III: resin-modified glass ionomer based desensitizing agent (Vanish™XT, 3M ESPE, USA) was applied according to the manufacturer's instruction. Specimens were dried for 10s, then material coating was applied to cover specimen surface and light cured for 20 s (EliparTM S10 LED Curing Light, 1200 mW/cm^2)

Group IV: Fluoride based desensitizing agent (Durasheild, Sultan Health Care, USA) was applied according to the manufacturer's instruction, in which the varnish was applied to cover the specimens.

The treated specimens were placed in a closed polyethylene vials containing artificial saliva (20 ml per specimen).[26] Artificial saliva consisted of 22.1mM hydrogen carbonate, 16.1mM potassium, 14.5mM sodium, 2.6mM hydrogen phosphate, 0.8mM boric acid, 0.7mM calcium, 0.2mM thiocyanate, and 0.2mM magnesium.[27] The pH was adjusted to 7.2 using KOH. [26] Artificial saliva was refreshed every 24 hours. The specimens were examined after 24 hours, after 7 days, and after exposure to acidic challenge which comprised of exposing the same specimens to 6% citric acid (pH 2) for one minute, followed by rinsing in saline for two minutes.[25]

Evaluation the degree of dentinal tubules occlusion

The extent of dentinal tubule occlusion was assessed using environmental field emission scanning electron microscopy (Quanta Field Emission Gun 250, Holland 115/230 V, Inspect SFEI, Phillips, Holland) under low vacuum without gold sputtering. In such a way, the same specimen was examined several times (after 24 hours, 7 days and after exposure to acidic challenge). In each specimen, three EFESEM photomicrographs were captured from each half (Control and treated) at each testing period at 5000x magnification.

EFESEM photomicrographs were imported into Image analysis software (NIH, USA) and converted into binary images (black and white). The black (open dentine tubules) pixels area (Fig. 1) was measured using the particle analysis tool.[28] In each half of the specimens, the three readings obtained from the three EFESEM photomicrographs were averaged to obtain one reading from each specimen at each time interval. The percentage of tubular occlusion was calculated from following equation:

$$\frac{\textit{Pixels area in the control half} - \textit{pixels area in the treated half}}{\textit{pixels area in the control half}} \times 100$$

Statistical analysis

The calculated degree of tubular occlusion percentage of tested desensitizing agents was analyzed using two way Analysis of variance (ANOVA) to determine the effect of both; the desensitizing agents and the time interval with exposure to acidic challenge. One way ANOVA was used, to analyze the effect of each desensitizing agent at each time, interval (24 hours and 7 days), and after the exposure to acidic challenge and to compare between the different desensitizing agents at each time, interval (24 hours and 7 days), as well as after the exposure to acidic challenge. This was followed by multiple comparisons using Tukey's post hoc test. The significance level was set at 0.05. All data were processed by the SPSS (version 16.0) software package (SPSS Inc., Chicago IL, USA).

RESULTS

Two way ANOVA results revealed that both the desensitizing agents and time interval with exposure to acidic challenge and their interaction had a statistical significant effect on the degree of dentinal tubules occlusion as presented in table 2.

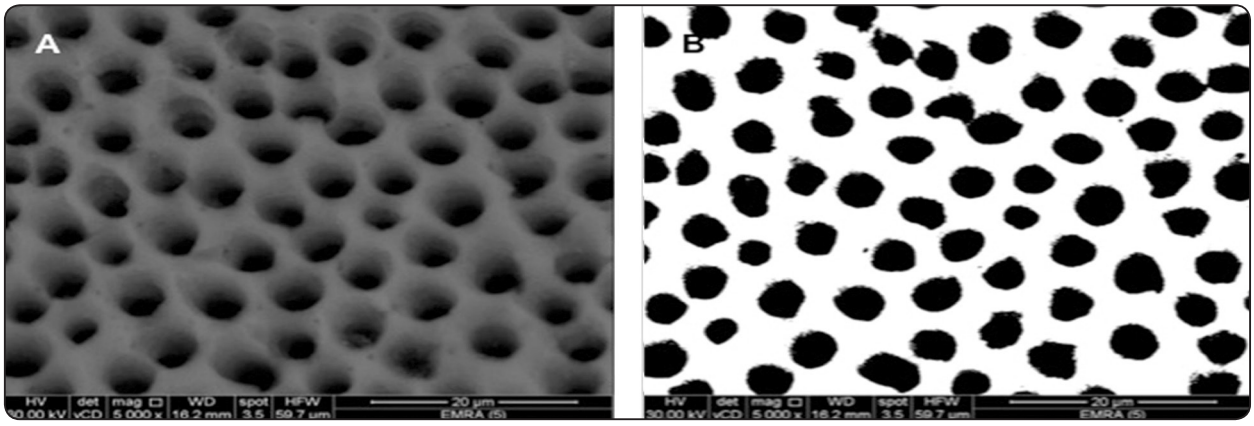

Fig (1) Preparation of EFESEM photomicrographs for the quantitative determination of closed dentine tubules. (a) EFESEM image and (b) converted binary black and white image.

TABLE (2) Two way ANOVA results:

Source of variation	Sum of Squares	df	Mean Square	*f*-value	*P*-value
The desensitizing agent	16420	2	8212	217.8	P<0.0001
Time interval and exposure to acidic challenge	109700	3	36550	969.5	P<0.0001
The desensitizing agent* Time interval and exposure to acidic challenge factor	14410	6	2402	63.71	P<0.0001

ANOVA: Analysis of variance; df,: degrees of freedom

The mean percentage of the dentinal tubules occluded areas (± SD) of all tested groups is presented in table 3. It was evident that the highest mean percentage of dentinal tubules occlusion either after 24 hours, 7 days or exposure to acidic challenge was found in group III which represent the, Vanish™XT, RMGI extended contact varnish treated group. The lowest percentage of dentinal tubule occlusion was presented in group IV, which represent the, Durasheild, sodium fluoride varnish treated group. Similar to the Vanish™XT, this result was found after 24 hours, 7 days or exposure to acidic challenge. There was no statistical significant difference between groups; I and II which represent the Nanohydroxyapaptite paste (Desensibilize NanoP), and Self assembly peptides

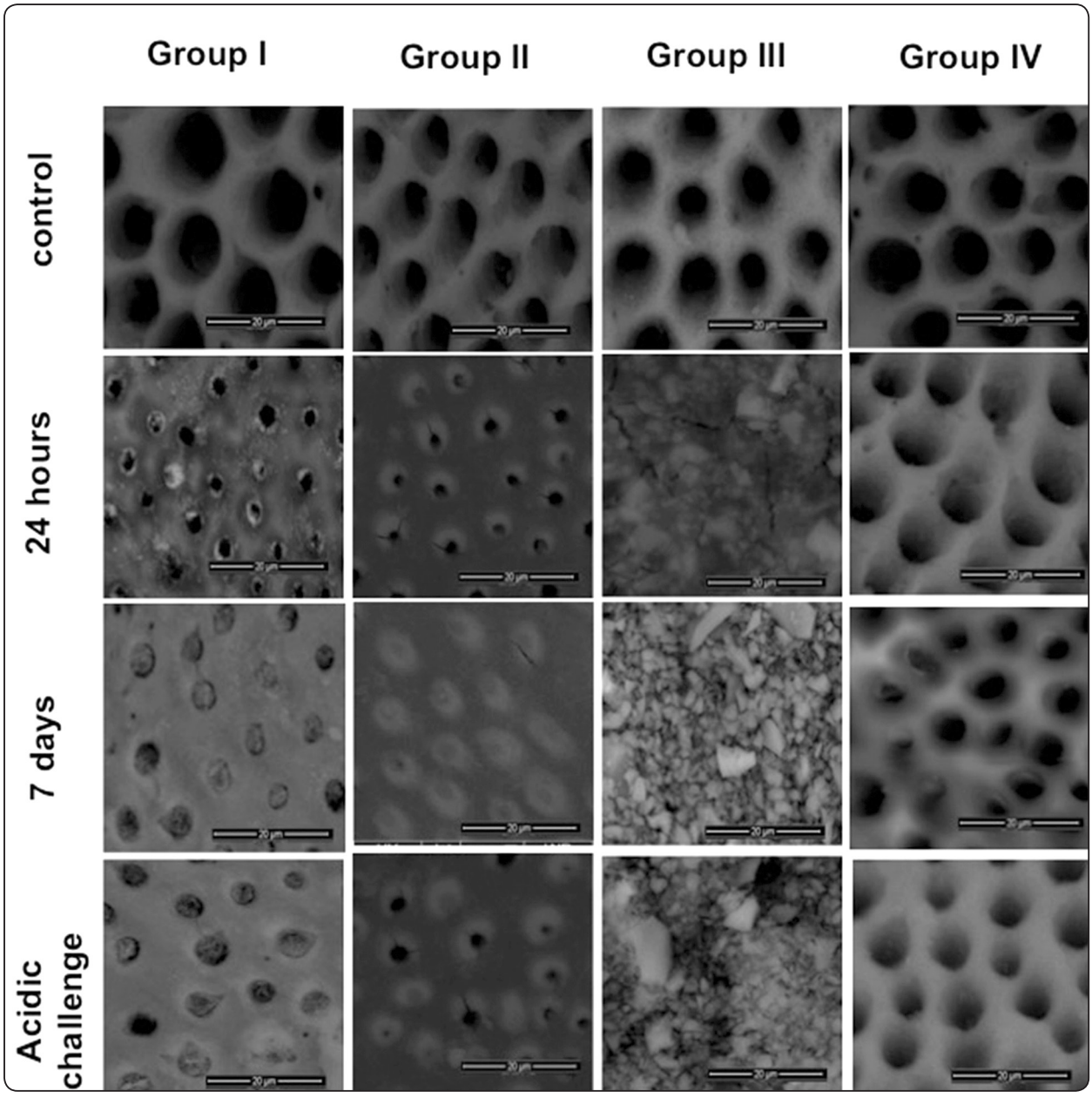

Fig (2) EFESEM photomicrographs of all tested groups at different time intervals (after 24 hours, 7 days and after the exposure to acidic challenge); Group I: treated with Nanohydroxyapatite paste, Group II: treated with the self assembly peptide gel, Group III: treated with resin modified glass ionomer, Group IV: treated with sodium fluoride varnish

TABLE (3) Mean percentage (± SD) of the dentinal tubules occluded areas of all tested groups.

	Group I (Desensibilize NanoP)	**Group II** (Curodont D'SenZ)	**Group III** (Vanish^{TM}XT)	**Group IV** (Durasheild)	**P value**
24 hours	24.55 ± 4.7^{Bc}	26.99 ± 6.^{Bc}	91.65 ± 5.2 ^{Aa}	6.29 ± 1.3 ^{Cb}	P<0.0001
7 days	67.33 ± 9.7 ^{Ba}	90.35 ± 4.4^{Aa}	95.62 ± 1.4 ^{Aa}	10.8 ± 2.7 ^{Ca}	P<0.0001
Acidic challenge	51.46 ± 12^{Cb}	68.34 ± 8.8 ^{Bb}	85.33 ± 3.1 ^{Ab}	3.77 ± 1.4 ^{Dc}	P<0.0001
P value	P<0.0001	P<0.0001	P<0.0001	P<0.0001	

Superscripts with capital letters are used for comparison between the groups within the same row (between different desensitizing agents at each time interval). Superscripts with small letters are used for comparison between the groups within the same column (between different time intervals for each desensitizing agent).

(Curodont D'SenZ) respectively, after 24 hours. Meanwhile, after 7 days and exposure to acidic challenge, Group II; the Self assembly peptides (Curodont D'SenZ) showed significantly higher percentage in the degree of dentinal tubules occlusion compared to Group I; Nanohydroxyapaptite paste (Desensibilize NanoP). Representative EFESEM photomicrographs of all tested group was presented in Fig 2.

DISCUSSION

In vitro examination of desensitizing products using a reproducible model, such as dentin slabs, was proven useful in the initial screening and evaluation of available desensitizing agents.[26,29] Since the buccal cervical third of premolar teeth, is considered the common site for dentin hypersensitivity due to the presence of a thin layer of enamel in this area the dentin slabs used in the present study were prepared from this area.[30]

The SEM photomicrographs captured in this study was via the Enviromental field emission scanning electron microscope in which no gold sputtering is required. Thus the same specimen can be assessed several times at different intervals. Analyzing the captured images protocol adopted in this study to evaluate the professionally applied desensitizing agents was in accordance to both Lee et al.,[21] and Arnold et al.,[28] in which an image analyzer was used to assess the dentinal tubules occluded area rather the number which gives a precise quantitative assessment of the dentinal tubule occluding effects.

Results of the present study revealed that there was a statistical significant difference in the degree of dentinal tubules occlusion between the tested desensitizing agents. Hence, the first null hypothesis is rejected and extended to reject the second null hypothesis as the time and/or exposure to acidic challenge affected the degree of dentinal tubules occlusion of all tested desensitizing agents.

The highest percentage of the occluded dentinal tubules area was found in the group treated with the RMGI vanish^{TM} XT. In addition this group showed the highest resistance upon exposure to acidic challenge. This could be attributed to its sealing and healing potentials. Its sealing ability arises from its chemical bonding ability as its composition is based on the same RMGI chemistry as the VitrebondTM and VitremerTM families of products, which include a methacrylate-modified copolyalkenoic acid molecule that participates in both the ionomeric reaction and the visible light-activated methacrylate curing.[7] Meanwhile, its remineralizing potential arises from sustained fluoride, calcium and phosphate release. The fluoride resides in fluoroaluminosilicate glass particles: reaction at the surface provides the immediate release, while the bulk provides a reservoir of fluoride for sustained release. The calcium glycerophosphate in

Vanish™XT provides continual release of calcium and phosphate over the life of the coating.[27] Similar findings were found in Rusin et al.,[7] a study who found that the use of Vanish™XT, showed significant reduction in the dentin permeability.

The occluding technologies based on the biomimetic remineralization using either inorganic analogue, nanohydroxyapaptite particles, or the organic analogue, self assembly peptides, showed equal degrees of dentinal tubules occlusion after 24 hours. Meanwhile, after 7 days and after the exposure to acidic challenge the self assembly peptides treated group showed superior results. Remineralization of dentin can occur either by simple precipitation of calcium phosphates into the loose demineralized dentin matrix between collagen fibrils (net remineralization), or by the chemical tight association of mineral to the dentin matrix structure (functional remineralization).[19]

The self-assembly peptide based desensitizing agent (Curodont D'SenZ) used in the present study contains the self-assembly peptide, which is named Oligopeptide 104 by INCI (P11-4). Self-assemsbling peptides P11-4 undergoes well characterized hierarchical self-assembly into three-dimensional fibrillar scaffolds. The formed scaffold-like structures with negative charge domains would attract Ca++ ions, inducing de novo precipitation of hydroxyapatite via the functional remineralization approach. [31,32] This product also contains other ingredients that maintain a stage of super saturation of calcium, phosphate and fluoride ions that foster remineralization of dentin. That is to say, dicalcium phosphate which is considered as one of the more soluble crystalline calcium phosphate phases[33] which in turn, increases the levels of free calcium ions that fosters improved remineralization promoting the net remineraliztion approach. [34]

Nanohydroxyapaptite products are to gain wider acceptance because of its similar composition to tooth and bone and its very high level of biocompatibility and bioactivity. Since the material is presented at a nano-scale level, it is speculated that its chemical reactivity allows it to be electrostatically bonded to the negative charged terminals of the demineralized intra tubular collagen fibrils.[35] This was depicted in the FESEM Fig. 2; in which remineralization process started as circumferential intra tubular peripheral deposits after 24 hours. Those nanoparticles then acted as bioactive templates that attracted a large amount of Ca2+ and PO3–4 from the remineralization solution promoting dentinal tubules occlusion. [35–37] The study finding, is in line with Bulkina et al,[38] and Hill et al.,[39] who found that the novel nanohydroxyapaptite based desensitizing agent oral rinse demonstrated an ability to occlude the dentine tubules to a certain degree.

Although the use of fluoride varnish is considered as one of the commercially known products for treatment of hypersensitivity among dental profession, the results revealed that it showed the lowest percentage of dentinal tubule occlusion. The results of the present study are in agreement with Lochaiwatana et al. Although the mechanism of action of fluoride varnish is not yet clear, a study has suggested that the dentinal tubule occlusion results from CaF_2 crystal formation by the chemical reaction between fluoride ions from fluoride varnish and calcium ions from dentin. CaF_2 crystals are generally very small in size, approximately 0.05 μm, hence a single varnish application may not completely occlude the tubules. Moreover, CaF_2 crystals are soluble, thus, they can dissolve in saliva.[40]

In the desensitizing agents based on the remineralzing approach, the percentage of dentinal tubules occlusion increased with time. As the percentage of dentinal tubules occlusion was significantly higher in nanohydroxyapatite, self assembly peptides and sodium fluorides treated groups after 7 days compared to 24 hours. Similar scenario was previously addressed in remineralizing the incipient carious lesion owing this to that increase the time is associated with increase in the dose of calcium and phoshate ions delivered to the demineralized substrate.[27]

After exposure to acidic challenge surface-precipitated phases were removed with different degrees and the morphology of the treated dentine surfaces showed partial reopening in the previously occluded dentinal tubules (Fig 2). This observation was significantly higher in the desensitizing agents based on the occlusion technology compared to those based on sealing technology which resisted the acidic challenge to a greater extent. Eliades et al., attributed this to the relative weak retention of the precipitated phases within dentine.[41] However, further studies should be carried to clarify the clinical performance of the tested desensitizing agents.

CONCLUSION

Under the parameters of the present study, the following conclusions can be drawn:

1- The RMGI sealing technology showed a more promising effect on the degree of dentinal tubules occlusion compared to the innovative occluding remineralyzing technologies, based on either the use of nanohydroxyapatite or self assembly peptides analogue, or conventional sodium fluoride varnish.

2- The biomimetic remineralyzing technology based on the use of self assembly peptides analogue showed superior performance compared to those based on the use of nanohydroxyapatite analogue.

3- The desensitizing agents based on occluding remineralyzing technologies using either nanohydroxyapatite or self assembly peptides analogues, or conventional sodium fluoride varnish is a time dependent process.

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