Dental Research Journal

Original Article

In vitro evaluation of dentin tubule occlusion by three bioactive materials: A scanning electron microscopic study

Maryam Ghafournia[1], Maryam Hajnorouzali Tehrani[1], Afsaneh Nekouei[2], Reyhaneh Faghihian[1], Mehrnaz Mohammadpour[2], Atiyeh Feiz[3]

[1]Dental Research Center, Department of Pediatric Dentistry, School of Dentistry, Isfahan University of Medical Sciences, [2]Department of Pediatric Dentistry, Dental School, Shahrekord University of Medical Sciences, Shahrekord, [3]Dental Materials Research Center, Department of Restorative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Background: Dentin hypersensitivity (DH) is a common problem characterized by a short, sharp pain. Reduction of dentin permeability by occluding dentin tubules is considered as a method for treating DH. Many substances are available to decrease hypersensitivity. The aim of this study is to evaluate the ability of three different remineralizing agents for occluding dentinal tubules in comparison with positive and negative controls.

Materials and Methods: In this *in vitro* scanning electron microscopic (SEM) study, 75 extracted premolars were cut into crown and root fragments with a bur and divided into five groups: group 1: Sodium fluoride 5% varnish was applied (positive control), Group 2: No treatment (negative control), Group 3: Treated with Remin Pro (contains hydroxyapatite and fluoride), Group 4: Treated with MI paste (contains casein phosphopeptide-amorphous calcium phosphate [CPP-ACP]) and Group 5: Treated with GC tooth mousse (contains CPP-ACP). SEM images were obtained and mean tubular diameter was measured in each group. One-way ANOVA and Duncan's tests were used for statistical analysis. A significant level of $\alpha = 0.05$ was set for comparison between the groups.

Results: Statistically significant difference was observed between Group 2 (negative control) and other four groups ($P < 0.05$). There was no significant difference between Groups 1, 3, 4, and 5 ($P > 0.05$).

Conclusion: Under the limitations of the present *in vitro* study, it can be concluded that the application of a CPP-ACP paste as well as a paste which contains fluoride is effective on reduction of dentin permeability.

Key Words: Bioactive, dentin permeability, scanning electron microscope

Received: January 2018
Accepted: July 2018

Address for correspondence:
Dr. Afsaneh Nekouei, Department of Pediatric Dentistry, Dental School, Shahrekord University of Medical Sciences, Shahrekord, Iran.
E-mail: reyhane.fgh@gmail.com

INTRODUCTION

Dentin hypersensitivity (DH) is a common problem which has 10%–30% prevalence in the population.

This condition is generally characterized by a short, sharp pain. However, sometimes, the initial pain is severe and followed by a dull ache.[1]

Each tooth contains many thousands of dentinal tubules which are microscopic tubular structures that radiate outward from the pulp. Dentinal tubule diameters are 0.2–0.5 μm and are connected to pulp

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Ghafournia M, Tehrani MH, Nekouei A, Faghihian R, Mohammadpour M, Feiz A. *In vitro* evaluation of dentin tubule occlusion by three bioactive materials: A scanning electron microscopic study. Dent Res J 2019;16:166-71.

by plasma-like fluid. The most probable theory for occurrence of DH is hydrodynamic theory. Pain intensity depends on the condition of dentinal tubule openings; thus, the aim of many treatment agents for hypersensitivity is closing dentinal tubules.[2]

The pain of hypersensitivity is caused by pressure on the nerves, resulting from changes in fluid flow within the exposed dentinal tubules, according to hydrodynamic theory which is the most probable theory justifying DH. Two different methods have been suggested for desensitizing dentin:

1. Reduction of intradental nerves response to fluid shift
2. Reduction of dentin permeability.[3]

Many substances decrease dentin permeability and have been shown to reduce tooth hypersensitivity.[4]

Desensitizing toothpaste is considered by many as the first option in relieving DH. These compounds use both approaches described above to reduce hypersensitivity.[2]

Laser therapy and application of different materials such as fluoride, hydroxyapatite, strontium and zinc chloride, potassium chloride as well as dental adhesive and glass ionomer cement were also considered for treating DH.[5]

Oxalate, Bioglass, Portland cement, and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) have been also used as desensitizing agents.[5,6]

ACPs have the potential to remineralize the structure of tooth. ACP is a soluble calcium phosphate compound that discharges calcium and phosphate ions to change to apatite and remineralize the tooth structure when it comes in contact with saliva. Forming on the enamel and within the dental tubules, ACP provides a reservoir of phosphate and calcium ions in the saliva. CPP is a milk-derived protein that joins to the tooth's biofilm and is applied to stabilize ACP. In recent years, remineralization products are used CPP as a vehicle to deliver and preserve a supersaturation state of ACP near the surface of tooth.[7] Two different materials containing CPP-ACP are commercially available: GC tooth mousse and MI paste.

GC tooth mousse contains CPP-ACP. By virtue of its remineralizing capacity, it has been proposed by the manufacturers that can help in the prevention and treatment of DH.[6]

CPP-ACP and fluoride are active ingredients in MI paste which is approved by the Food and Drug Administration for treating hypersensitivity.[6,8]

Remin Pro combines three components for effective protection against demineralization: hydroxyapatite, fluoride, and xylitol. It has been shown that these agents are effective for tubular occlusion. Thus, this material may be beneficial for the treatment of DH.[9,10]

Since desensitization by tubule occlusion using chemical compounds is a viable treatment modality for hypersensitivity, the best commercially available material for reducing the condition by tubular occlusion should be recognized.

Thus, the aim of this study is to compare the ability of three different commercially available remineralizing agents for occluding dentinal tubules. To our knowledge, there is no study to compare the ability of evaluated products.

Two of these agents contain CPP-ACP, and the other contains hydroxyapatite and fluoride.

MATERIALS AND METHODS

This *in vitro* study was approved by the Ethical Committee of Isfahan University of Medical Sciences with the code number 394330.

Seventy-five extracted human sound premolar teeth extracted for orthodontic reasons were collected for this study. Teeth were cleaned thoroughly; they were disinfected in 5% sodium hypochlorite solution for 1 h and stored in artificial saliva. The composition of artificial saliva is included in Table 1.[6]

Then, the teeth were split into crown and root segments using fissure bur. The crown sections were mounted in acrylic resin blocks. Seventy-five buccal surface dentin discs with a thickness of 1.0 ± 0.1 mm were cut perpendicular to the long axis of the tooth by means of a low-speed water cooled diamond saw (CNC Cutting Section Machine, Iran).

Table 1: Composition of the artificial saliva

Composition	Amount
Natrium chloride	0.400 g/L
Calcium chloride	0.795 g/L
Natrium sulfate	0.005 g/L
Potassium sulfate	0.400 g/L
Sodium di hydrogen sulfate/water	0.690 g/L
Distilled water	1000 mL

For smear layer removal, all of the specimens were ultrasonicated in distilled water and dipped in EDTA 0.5 M (pH = 7.4) for 2 min. An exposed window with 2 mm × 3 mm dimension was left on each disc, and other parts of the disc were covered with a protective tape. The specimens were divided into five groups of 15 discs.

- Group 1 (positive control): Sodium fluoride (NaF) 5% varnish was applied
- Group 2 (negative control): No treatment material was applied
- Group 3: Specimens were treated with Remin Pro (VOCO, Cuxhaven, Germany)
- Group 4: Specimens were treated with MI paste (GC, Melbourne, Australia)
- Group 5: Specimens were treated with GC tooth mousse. (GC, Melbourne, Australia).

Each treatment agent was applied twice a day (with 12-h interval for 3 min) for a week. Specimens were kept in the artificial saliva after each time of treatment.

Compositions of agents are summarized in Table 2. Specimens were stored in the artificial saliva (Department of Pharmacology, Esfahan University of Medical Sciences) at 37°C and pH = 7.4 to resemble mouth environment. The composition of artificial saliva is summarized in Table 1.

In the next step, the specimens were prepared for scanning electron microscopic (SEM) analysis as follows: samples were dried in desiccators and coated with gold in a vacuum evaporator.

Dentinal tubule changes were observed with ×1500 magnification and SEM micrographs were obtained [Figures 1-5]. The largest tubular diameter in each group was measured. Data were analyzed using one-way ANOVA and Duncan's test.

RESULTS

The SEM images were examined to determine the level of tubular occlusion. The maximum, minimum, and mean tubular diameters for specimens of each group are shown in Table 3.

Statistically significant difference was determined between five groups, with regard to the largest tubular diameter.

Duncan's test was also applied, and the results demonstrated that the mean tubular diameter in negative control group differed from other four groups significantly. However, no statistically significant difference was observed between other four groups, including GC tooth mousse, Remin Pro, MI paste, and positive control.

DISCUSSION

DH is a clinical situation characterized by a short, sharp pain initiated by many types of stimulation such as tactile, thermal, osmotic, or chemical. It is one of the most common and uncomfortable conditions affecting oral comfort. There is a need to develop new treatment or products which permit the relief of symptoms.[11]

To be hypersensitive, dentin must be exposed and the exposed tubules must be open and patent to both the oral cavity and the pulp. Most DH therapy works by

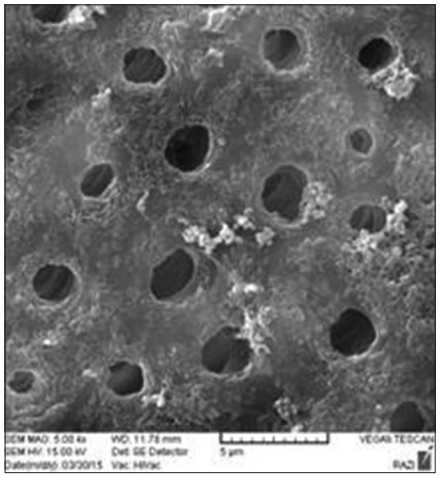

Figure 1: Scanning electron microscopic image of a sample treated with GC tooth mousse.

Table 2: Composition of treatment agents

Treatment agents	Composition	Country/company
Remin Pro	Sodium fluoride (1450 ppm), hydroxy apatite, xylitol	VOCO, Cuxhaven, Germany
GC tooth mousse	CPP-ACP	GC, Melbourne, Australia
MI paste	CPP-ACP, sodium fluoride 0.2% (900 ppm)	GC, Melbourne, Australia
Sodium fluoride varnish	Sodium fluoride 5%	VOCO, Cuxhaven, Germany

CPP: Casein phosphopeptide; ACP: Amorphous calcium phosphate

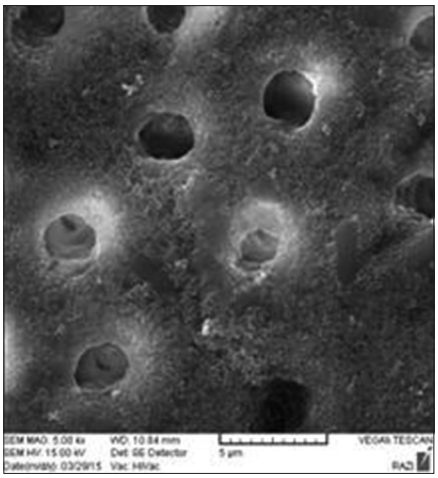

Figure 2: Scanning electron microscopic image of a sample treated with MI paste.

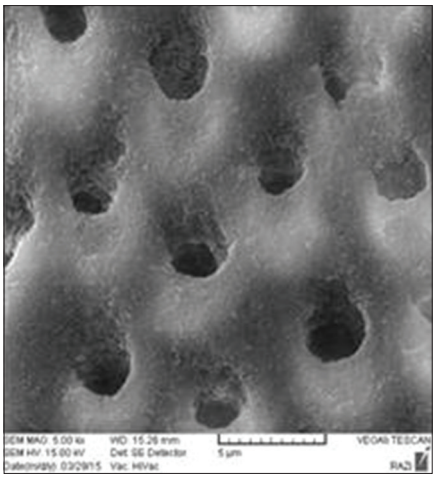

Figure 4: Scanning electron microscopic image of a sample treated with flouride varnish as positive control.

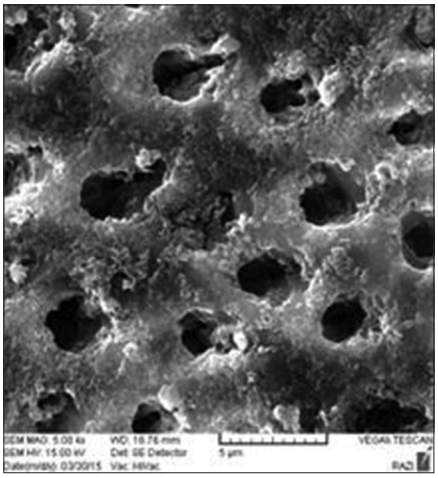

Figure 3: Scanning electron microscopic image of a sample treated with Remin Pro.

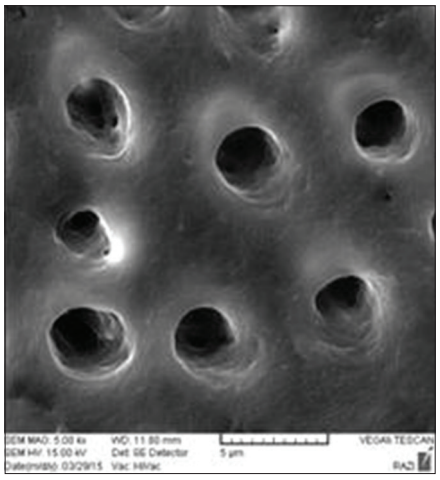

Figure 5: Scanning electron microscopic image of a sample with no treatment as negative control.

Table 3: Mean and standard deviation of tubular diameter after treatment (μm)

Material	*n*	Mean	SD	SE	95% CI for mean	
					Lower bound	Upper bound
Positive control	15	3.13	0.55	0.14	2.82	3.44
GC tooth mousse	15	2.85	0.52	0.13	2.56	3.14
MI paste	15	3.19	0.73	0.18	2.78	3.60
Remin Pro	15	2.94	0.40	0.10	2.72	3.17
Negative control	15	3.70	0.46	0.11	3.44	3.95
Total	75	3.16	0.60	0.07	3.02	3.30

SD: Standard deviation; SE: Standard error; CI: Confidence interval

either nerve desensitization or occlusion of exposed dental tubules.

Mineralization-promoting cements are cements containing calcium and phosphates. They effectively occlude dentinal tubules when applied.[12] GC tooth mousse, Remin Pro, and MI paste are the examples of these agents that their ability for occluding dentinal tubules was compared in this study.

Intergroup comparisons of three intervention methods revealed no significant difference in mean change of tubular diameter. The difference between intervention and positive control group was also not significant.

To our knowledge, this study is the first to compare three different materials including GC tooth mousse, Remin Pro, and MI paste for the treatment of DH. No significant difference was observed between mean tubular diameters of specimens in four treatment groups, including GC tooth mousse, Remin Pro, MI paste, and positive control (NaF varnish). This issue indicates the similar ability of these materials in occluding dentin tubules; however, further studies for clinical evaluation and comparison of these materials should be conducted. Mean tubular diameter

measurements in the negative control group in which no treatment was applied differed significantly with intervention groups, indicating that application of one of the remineralizing agents tested in this study can reduce dentin permeability significantly by occluding dentin tubules.

Kanaparthy and Aruna in their clinical study have used GC tooth mousse for the treatment of tooth hypersensitivity and compared it with placebo. The outcomes revealed significant reduction of pain in GC tooth mousse group.[11] Similar results were also found in a study by Saraf *et al.*[12]

Another *in vitro* investigation by Hongal *et al.* compared GC tooth mousse with Indian Propolis for the treatment of tooth hypersensitivity, which revealed that GC tooth mousse is more effective than Indian Propolis.[13]

In a study by Kowalczyk *et al.*, it was concluded that variables such as patients' oral hygiene, frequency of material application, and sample size affect the results of clinical studies.[14] However, the results of this study and other similar studies are valuable for developing clinical investigations.

In the present study, we found that two commercially available materials which contain CPP-ACP can reduce dentinal tubules diameter significantly in comparison with negative control. As a result, these agents may be helpful for the treatment of tooth hypersensitivity.

Remin Pro and MI paste were also evaluated in previous studies as the treatment materials for tooth hypersensitivity. These studies have shown both materials occlude dentine tubules and consequently reduce tooth hypersensitivity. To our knowledge, there is not any study about the comparison of GC tooth mousse, Remin Pro, and MI paste for the treatment of tooth hypersensitivity.

Mean tubular diameter can be a reliable index for SEM analysis of specimen in *in vitro* studies as used in our study.

Wang *et al*. evaluated desensitizing toothpaste effect for tooth hypersensitivity treatment by means of dentine tubule occlusion. They found that these agents can be useful and the mean tubular diameter measurement was applied for evaluation similar to our study.[3] In another study by the same author, it was reported that CPP-ACP paste occluded most of the dentin tubules in SEM observation compared to the control group.[15]

CONCLUSION

Application of GC tooth mousse, Remin Pro, and MI paste can reduce dentin permeability by occluding dentin tubules. It can be hypothesized that these materials can be effective for treatment of DH in a similar way as described in this study.

Financial support and sponsorship
Nil.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

1. Torwane NA, Hongal S, Goel P, B R C, Jain M, Saxena E, *et al.* Effect of two desensitizing agents in reducing dentin hypersensitivity: An *in vivo* comparative clinical trial. J Clin Diagn Res 2013;7:2042-6.
2. Chu CH, Lam A, Lo EC. Dentin hypersensitivity and its management. Gen Dent 2011;59:115-22.
3. 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;38:400-10.
4. Sauro S, Mannocci F, Watson TF, Piemontese M, Sherriff M, Mongiorgi R. The influence of soft acidic drinks in exposing dentinal tubules after non-surgical periodontal treatment: A SEM investigation on the protective effects of oxalate-containing phytocomplex. Med Oral Patol Oral Cir Bucal 2007;12:E542-8.
5. Miglani S, Aggarwal V, Ahuja B. Dentin hypersensitivity: Recent trends in management. J Conserv Dent 2010;13:218-24.
6. Poggio C, Lombardini M, Vigorelli P, Ceci M. Analysis of dentin/enamel remineralization by a CPP-ACP paste: AFM and SEM study. Scanning 2013;35:366-74.
7. Heymann H, Swift E, Ritter V. Art and Science of Operative Dentistry. 6th ed. Melbourne: Elsevier/Mosby; 2013. p. 118-42.
8. Sarah EC. Remineralization Effectiveness of MI Paste Plus – A Clinical Pilot Study. Iowa: Graduate Colledge of the University of Iowa; 2011.
9. Shiny B, Roshni B, Shamina P, Mohan N. Seal that heals. World J Dent 2012;3:243-6.
10. Kamath U, Sheth H, Mullur D, Soubhagya M. The effect of remin pro® on bleached enamel hardness: An *in vitro* study. Indian J Dent Res 2013;24:690-3.
11. Kanaparthy R, Aruna K. Clinical efficacy of amorphous calcium phosphate, GC Tooth Mousse and Gluma Desensitizer in treating dentin hypersensitivity. Int J Dent Clin 2011;3:65-9.
12. Saraf DJ, Amirisetty DR, Zade DR, Mangalekar DS, Zade DV, Ansari DS. Evaluation of effectiveness of CPP-ACPcombination in treating dentinal hypersensitivity following non surgical periodontal therapy – Arandomized clinical trial. Chhattisgarh J Health Sci 2013;1:32-4.

13. Hongal S, Torwane NA, Goel P, Chandrashekar B. The effect of 30% ethanolic extract of indian propolis on replica of human dentin compared against commercially available desensitizing agent: A methodological SEM study *in vitro*. Pharmacognosy Res 2014;6:113-9.
14. Kowalczyk A, Botuliński B, Jaworska M, Kierklo A, Pawińska M, Dabrowska E. Evaluation of the product based on recaldent technology in the treatment of dentin hypersensitivity. Adv Med Sci 2006;51 Suppl 1:40-2.
15. Ma X, Jiang T, Wang Y, Feng Y, Li R, Wang Z. The dentin tubule occlusion effects of desensitizing agents and the stability against acids and brushing challenges. Am J Dent 2015;28:128-32.