ORIGINAL ARTICLE

Comparative Analysis of CPP–ACP, Tricalcium Phosphate, and Hydroxyapatite on Assessment of Dentinal Tubule Occlusion on Primary Enamel Using SEM: An *In Vitro* Study

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Abstract

Background: Over the last few decades, fluoride in various forms has been proved to reduce caries and dentinal hypersensitivity in both the primary and permanent dentitions. Recently, newer materials containing calcium and phosphate ions, tricalcium phosphate, and hydroxyapatite has received much attention.

Aim: The aim of the study was to compare CPP–ACP, tricalcium phosphate, and hydroxyapatite in relation to the assessment of dentine tubule occlusion on primary enamel using scanning electron microscope (SEM).

Materials and methods: Forty freshly extracted noncarious primary molars were randomly divided into 4 groups (I–IV) with 10 sections in each group—group I: negative control, group II: CPP–ACP, group III: tricalcium phosphate, group IV: hydroxyapatite. To assess tubule occlusion, twenty dentin sections of 2-mm thickness were obtained from the cervical third of sound primary molars. Each section was processed to simulate the hypersensitive dentin and the test agents were brushed over the sections with an electric toothbrush and observed under a SEM for calculation of the percentage of occluded tubules.

Results: Groups II and IV showed a greater percentage of tubule occlusion than group III. An intergroup comparison of tubule occlusion potential of groups II and IV was not significant.

Conclusion: Hydroxyapatite showed significantly higher dentinal tubule occlusion when compared to CPP–ACP and tricalcium phosphate.

Keywords: CPP–ACP, Dentinal hypersensitivity, Hydroxyapatite, Scanning electron microscope, Tricalcium phosphate.

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Introduction

Dentine hypersensitivity refers to the transient and severe pain arising from the stimulation of exposed dentine with cold, heat, and mechanical pressure. Many diseases (including physiological wear, dental erosion, enamel hypoplasia, wedge-shaped defects, bruxism, and deep carious lesions) can lead to exposed dentine.[1] This condition is described clinically as an exaggerated response to a non-noxious stimulus and is the result of dentin tubules' exposure due to the loss of enamel.[2] Its prevalence greatly varies between 3% and 98% depending on the population, study setting, and study design.[3,4]

Dental erosion and early childhood caries (ECC) are being recognized as a common condition in pediatric dentistry with complications of tooth sensitivity, altered aesthetics, and loss of occlusal vertical dimension. Recently the prevalence of erosion in children has been reported to range from 10 to over 80%,[5] whereas for ECC it has been reported to range from 5 to 85% in developed countries.[6] Despite the increase in prevalence of these conditions, limited literature is available in support with regard to primary dentition. Bearing this in mind, we have undertaken this study to find substantial evidence for treatment of the same.

Dentin tubules' occlusion is the most current therapeutic approach.[7] Recently, good clinical results were reported with products containing arginine/calcium carbonate, calcium sodium phosphosilicate (NovaMin), or strontium acetate.[8,9] Fluoride varnish was the first Food and Drug Administration (FDA)-approved agent for treatment of hypersensitivity, and it protects the dentin surface by forming a protective layer of calcium fluoride.[10]

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Recently, introduction of newer materials such as CPP–ACP (that is derived from bovine milk protein, casein, calcium, and phosphate),[11] tricalcium phosphates (calcium phosphate system, which is stable in aqueous environment and does not affect the fluoride activity when added into the dentifrices)[12] and Remin Pro (hydroxyapatite that fills eroded enamel, fluoride seals dentinal tubule and xylitol acts as an antibacterial agent) and has been recommended for the management of dentinal hypersensitivity.[13] Therefore, considering all the factors, the aim of the present study was to evaluate and compare CPP–ACP, tricalcium phosphate, and hydroxyapatite on assessment of dentine tubule occlusion on primary enamel using scanning electron microscope (SEM).

Materials and Methods

Forty noncarious primary molars extracted for therapeutic reasons were selected and were thoroughly cleaned free of debris and calculus using hand scalers and stored in 10% formalin till the time of the commencement of study.

Assessment of Dentine Tubule Occlusion

Forty primary molars were used to prepare dentin disks of 2-mm thickness from the coronal portion of the tooth just below the level of the cementoenamel junction using a double-sided diamond disk with a micromotor handpiece. These dentin disks were then polished with silicon carbide paper of 320, 600, and 800 grit, and were ultrasonicated in distilled water for 10 minutes to remove the residual smear layer and then etched by immersing the specimens in a tray containing 6% citric acid for 2 minutes to simulate dentin hypersensitivity.

The 40 dentine specimens were divided into the four above-mentioned groups ($n = 10$). The test agents were brushed over the specimens using an electric toothbrush (Colgate 360° sonic power®) at 20,000 strokes per minute for 2 minutes twice a day for 7 days. The specimens were rinsed in distilled water after each brushing session and stored in a closed container containing distilled water. After the last brushing session, specimens were washed with distilled water and coated (MED 010-Jeol, Japan) with a thin gold layer, following which the specimens were analyzed in a scanning electron microscope (DSM 840 A-Geol. Japan) operating at 10 kV in 2,000× magnification. The area in the center of each specimen was scanned so as to obtain tubules in a circular cross section. Photomicrographs were taken to analyze the following study parameters (Fig. 1).

- Number of tubules occluded per unit area.
- Number of tubules patent per unit area.

Quantitative analysis was performed by counting the numbers of dentinal tubules at 2,000× magnification; considering a total area of 1,600 μm^2, grids were applied on the photomicrograph using CorelDraw CS3 software.

Results

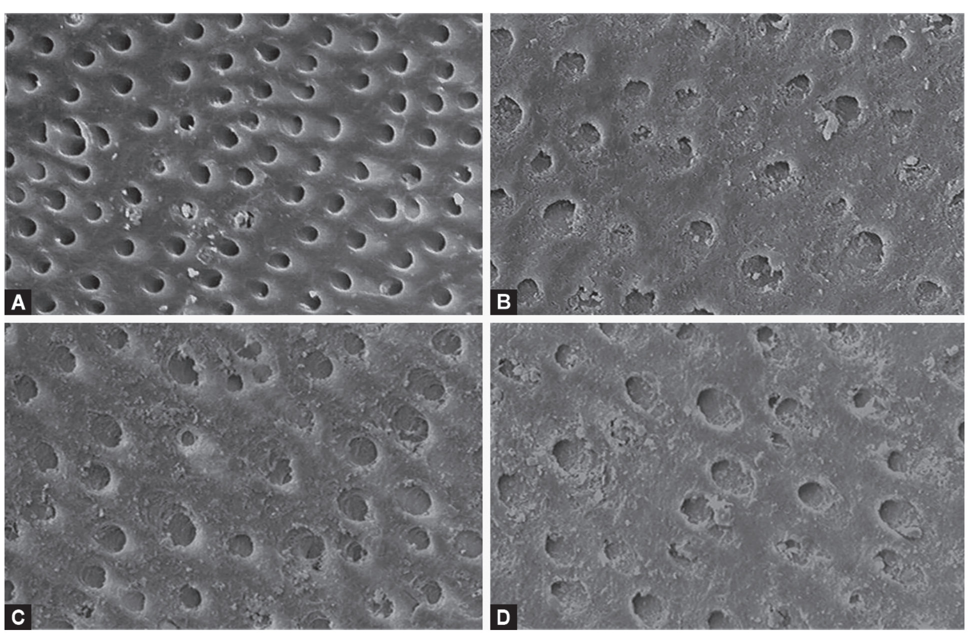
The results were subjected for appropriate statistical analysis; the Kruskall–Wallis test revealed that an intergroup comparison of the mean percentage of tubule occlusion was highly significant (p value < 0.001) (Table 1) and Mann–Whitney U test showed that the tubule occlusion potential was statistically significant among all groups except for Group II and IV, which was not significant (Table 2 and Fig. 1).

Discussion

Dentin hypersensitivity that can be a potential threat to the individual's oral health is closely related to the exposure and patency of dentinal tubules. Many factors may contribute to the exposure of dentinal tubules such as occlusal wear, deep carious lesion, abrasion due to brushing, dietary erosion, parafunctional habit, abnormal tooth positioning, and abfractive lesions. The condition has been treated by a number of agents, which have been claimed to reduce pain by occluding dentinal tubules.[14] Desensitizing dentifrice is the preferred treatment for hypersensitivities because tooth-brushing is one of the easiest methods in a home-care system.

Various *in vivo* studies have shown a considerable decrease in hypersensitivity when teeth were brushed with desensitizing dentifrices.[15] The *in vitro* dentine disc dentinal tubule blockage experiment has become the gold standard for assessment of dentine hypersensitivity.[16,17] Utilizing safe and effective biological materials through their physical or chemical properties to block exposed dentinal tubules, to reduce or inhibit the flow of tubular fluid, and to avoid stimulating pulp nerve endings is thus an effective means for controlling dentin sensitivities.[18]

Human cervical dentine has reported to have 19,000 tubules per mm^2 in superficial dentine. As the half way point between the superficial dentine and the pulp is reached, the number of tubules reached to 30,000 tubules per mm^2.[19] Dentinal tubule area was evaluated by SEM at 2,000 times magnification (total area of 1,600 μm^2). Quantitative analysis of dentinal tubule number could be a prejudice due to changes in direction of dentinal tubules and in the position of SEM samples. The presence of smear layer and



Figs 1A to D: Dentinal tubule occlusion seen in all four groups

Table 1: A comparison of difference in tubules closed

Study groups	*Mean (%)*	*SD*	*p value (Kruskall Wallis test)*
Group I	3.34	3.38	<0.001* HS
Group II	65.33	5.95	
Group III	48.18	7.33	
Group IV	71.81	5.51	

*$p < 0.001$; HS, highly significant

Table 2: Multiple comparisons of groups

Study groups	*Multiple comparisons (Mann–Whitney test)*
I and II	($p = 0.008$*) S
I and III	($p = 0.008$*) S
I and IV	($p = 0.009$*) S
II and III	($p = 0.008$*) S
II and IV	($p = 0.151$) NS
III and IV	($p = 0.009$*) S

*$p < 0.05$; S, significant
$p > 0.05$; NS, not significant

small analysed area could also introduce some bias. Hirayama et al.[20] reported that the tubules of primary dentin had smaller diameters because the peritubular dentin matrix was wider than that of permanent dentin.

Occluding dentinal tubule agents can create a barrier by precipitating proteins and calcium/phosphate ions on surface or within the tubule orifices. The mechanism of action of various chemical desensitizing agents is still not well understood.[21] Most of the previous measurements of dentin permeability have been carried out on the coronal[22,23] or radicular dentin of human permanent teeth. However, no studies have been performed on primary teeth. Permeability is defined as the ability of a membrane to permit solutes or solvents to pass through it.[24]

The agents used in the present study used are hydroxyapatite (Remin Pro), CPP–ACP (GC tooth mousse), and tricalcium phosphate (Clinpro tooth crème), which significantly shown occlusion of the dentine tubules by the above-mentioned topical desensitizing agents.

CPP–ACP is the acronym for a complex of casein phosphopeptides (CPPs) and amorphous calcium phosphate (ACP), which is used as a desinsitizing agent for the present study. Dentin surface treated with CPP–ACP showed substantial crystal-like deposits within the tubule lumen. Nevertheless, in few zones, the layer of amorphous calcium phosphate present on the dentin covered the orifices of dentinal tubules (Fig. 1). When the peptide complex binds to plaque or the tooth surface, it is said to deliver bio-available calcium and phosphate for remineralization, resulting in occlusion of dentin tubules.[25]

In the present study, CPP–ACP showed 65.33% of tubule occlusion when compared to TCP group with 48.18% tubule occlusion. Contradictory to our results, a study done by Prabhakar et al.,[14] stated that there was 29.51% tubule occlusion seen with GC MI paste plus as it had shown a weak ability to occlude dentinal tubule when compared to NaF and Clinpro tooth crème. GC MI paste had almost similar composition with that of GC tooth mousse, which was used for the present study.

Tricalcium phosphate (TCP) has been considered as one possible means for enhancing the levels of calcium in plaque and saliva. Combining calcium phosphate and fluoride ions in oral care products is problematic and can lead to the loss of bioavailable fluoride ion due to a reaction between the calcium phosphate phase and the fluoride ion. In an approach to overcome this incompatibility of calcium phosphates and fluoride ions, new technologies have been developed. This technology is functionalized tricalcium phosphate TCP, where tricalcium phosphate particles have been ball milled with sodium lauryl sulfate, and has been included in a tooth cream with sodium fluoride marketed as Clinpro tooth crème (3 M ESPE).[26]

In the present study, TCP showed 48.18% of tubule occlusion when compared to CPP–ACP (65.33%) and HA (71.81%). This SEM observation was supported by Prabhakar et al.[14] in which Clinpro tooth crèm showed 45.74 % of tubule occlusion and similar findings were noted in the previous study conducted by Mackey et al.[27] The presence of fluoride concentration of 950 ppm may contribute to the occlusion of tubules apart from calcium phosphate system.

Hydroxyapatite (HA) is one of the most biocompatible and bioactive materials and is widely applied to coat artificial joints and tooth roots. In the present study, hydroxyapatite (Remin Pro) was used as desensitizing agent, which is a water-based cream, containing hydroxyapatite, fluoride, and xylitol. Hydroxyapatite (which is the main constituent of Remin Pro) fills the superficial enamel lesions and the tiniest irregularities that arise from erosion. Fluoride, which is also one of the contents of Remin Pro, gets converted to fluorapatite when it comes in contact with saliva and thus strengthens the tooth and renders it more resistant to acid attacks and also it seals dentinal tubule. Xylitol reduces the harmful effects of bacteria and their metabolic product to lactic acid. Since HA has a crystal structure similar to human teeth, preliminary research exploring the effects of HA in easing dentine hypersensitivity, remineralization of early enamel lesion by adding HA to dentifrice have been reported in recent years.[28]

In 2012, a study by Yuan et al.[18] concluded that HA added to ordinary dentifrice showed a significantly increased effect of dentinal tubule occlusion, which was similar to commercially available anti-dentin sensitive dentrifices as reported by commercial research of such products. Similarly, in the present study, hydroxyapatite (Remin Pro) showed highly significant dentinal tubule occlusion when compared with CPP–ACP and tricalcium phosphate.

Dentine tubule occlusion in an *in vitro* may be quite different when compared with dynamic, complex biological system, which usually occurs in the oral cavity *in vivo*. Thus, direct extrapolations to clinical conditions must be exercised with caution because of the obvious limitations of *in vitro* studies. However, there is a need for the further long-term research under clinical conditions to prove the efficacy of these agents.

Conclusion

It was concluded that all the groups showed significant dentinal tubule occlusion; however, SEM observation revealed that dentinal tubule occlusion was seen significantly higher in hydroxyapatite when compared to CPP–ACP and TCP group.

References

1. Parolia A, Kundabala M, et al. Management of dentinal hypersensitivity: a review. J Calif Dent Assoc 2011;39:167–179.

2. Addy M, Hunter ML. Can tooth brushing damage your health? Effects on oral and dental tissues. Int Dent J 2003;53(3):177–186.
3. Splieth CH, Tachou A. Epidemiology of dentin hypersensitivity. Clin Oral Invest 2013;17(1):S3–S8. DOI: 10.1007/s00784-012-0889-8.
4. Cunha-Cruz J, Wataha JC, et al. Northwest Practice-based Research Collaborative in Evidence-based dentistry. The prevalence of dentin hypersensitivity in general dental practices in the northwest United States. J Am Dent Assoc 2013;144:288–296. DOI: 10.14219/jada.archive.2013.0116.
5. Taji S, Seow WK. A literature review of dental erosion in children. Australian Dental Journal 2010;55:358–367. DOI: 10.1111/j.1834-7819.2010.01255.x.
6. Çolak H, Dülgergil CT, et al. Early childhood caries update: a review of causes, diagnoses, and treatments. J Nat Sci Biol Med 2013 Jan–Jun;4(1):29–38. DOI: 10.4103/0976-9668.107257.
7. Schmidlin PR, Sahrmann P. Current management of dentin hypersensitivity. Clin Oral Invest 2013;17(1):S55–S59. DOI: 10.1007/s00784-012-0912-0.
8. Cummins D. Recent advances in dentin hypersensitivity: clinically proven treatments for instant and lasting sensitivity relief. Am J Dent 2010;23(Spec No A):3A–13A.
9. Gendreau L, Barlow AP, et al. Overview of the clinical evidence for the use of NovaMin in providing relief from the pain of dentin hypersensitivity. J Clin Dent 2011;22:90–95.
10. Ritter AV, de La Dias W, et al. Treating cervical dentin hypersensitivity with fluoride varnish: a randomized clinical study. J Am Dent Assoc 2006;137:1013–1020. DOI: 10.14219/jada.archive.2006.0324.
11. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76(9):1587–1595. DOI: 10.1177/00220345970760091101.
12. Balakrishnan A, Jonathan R, et al. Evaluation to determine the caries remineralization potential of three dentifrices: An *in vitro* study. J Cons Dent 2013;16(4):375–379. DOI: 10.4103/0972-0707.114347.
13. Benjamin S, Roshni, et al. Seal that heals. World J Dent 2012;3:243–246. DOI: 10.5005/jp-journals-10015-1164.
14. Prabhakar AR, Manojkumar AJ, et al. *In vitro* remineralization of enamel subsurface lesions and assessment of dentine tubule occlusion from NaF dentifrices with and without calcium. J Indian Soc Pedod Prev Dent 2013;31(1):29–35. DOI: 10.4103/0970-4388.112403.
15. Arrais GC, Micheloni CD, et al. Occluding effect of dentifrices on dentinal tubules. J Dent 2003;31:577–584. DOI: 10.1016/S0300-5712(03)00115-5.
16. Gillam DG, Mordan NJ, et al. The effects of oxalate-containing products on the exposed dentine surface: an SEM investigation. J Oral Rehabil 2001;28:1037–1044. DOI: 10.1046/j.1365-2842.2001.00775.x.
17. Kolker JL, Vargas MA, et al. Effect of desensitizing agents on dentin permeability and dentin tubule occlusion. J Adhes Dent 2002;4:211–221.
18. Yuan P, Shen X, et al. Effects of dentifrice containing hydroxyapatite on dentinal tubule occlusion and aqueous hexavalent chromium cations sorption: a preliminary study. PLoS One 2012;7(12):e45283. DOI: 10.1371/journal.pone.0045283.
19. Garberoglio R, Brannstrom M. Scanning electron microscope investigation of human dentinal tubules. Arch Oral Biol 1976;21:355–362. DOI: 10.1016/S0003-9969(76)80003-9.
20. Hirayama A, Yamada M, et al. Analytical electron microscopic studies on the dentinal tubules of human deciduous teeth. J Dent Res 1985;64:743.
21. West NX, Hughes JA, et al. The effect of pH on erosion of dentine and enamel by dietary acids *in vitro*. J oral Rehabil 2001;28:860–864. DOI: 10.1111/j.1365-2842.2001.00778.x.
22. Pashley EL, Talman R, et al. Permeability of normal versus carious dentin. Endod Dent Traumatol 1991;7:207–211. DOI: 10.1111/j.1600-9657.1991.tb00437.x.
23. Tagami J, Hosoda H, et al. Effect of aging and caries on dentin permeability. Proc Finn Dent Soc 1992;88(Suppl. 1):149–154.
24. Pashley DH. Dentin-predentin complex and its permeability: physiologic overview. J Dent Res 1985;64(Special Issue):613–620. DOI: 10.1177/002203458506400419.
25. Kowalczyk A, Botulinski B, et al. Evaluation of the product based on RecaldentTM technology in the treatment of dentin hypersensitivity. Adv Med Sci 2006;51:40–42.
26. Shen P, Manton DJ, et al. Effect of added calcium phosphate on enamel remineralization by fluoride in a randomized controlled in situ trial. J Dent 2011;39:518–525. DOI: 10.1016/j.jdent.2011.05.002.
27. Mackey AC. *In Vitro* Assessment of Dentin Tubule Occlusion by Hypersensitivity Dentifrices. 2009 April 1–4; available from: http://iadr.confex.com/iadr/2009miami/webprogram/Paper 120922.Html.
28. Kamath U, Sheth H, et al. The effect of Remin Pro on bleached enamel hardness: An *in vitro* study. Indian J Dent Res 2013;24(6):690–693. DOI: 10.4103/0970-9290.127612.