



Correlation between Two Evaluation Methods for the Effect of Two Desensitizing Materials: An *In Vitro* Study

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Abstract

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AIM: This study was conducted to assess the efficiency of enamel matrix derivative (EMD) on dentin remineralization at two different application times.

MATERIALS AND METHODS: Thirty-two human dentin specimens of 1 mm thickness were prepared and randomly divided into two groups according to the treatment material (M) used: fluoride varnish with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (M₁) and Enamel matrix derivative (EMD) (M₂). Following treatment, each group was subdivided into two subgroups (n = 8) based on the storage time in artificial saliva into: (S); subgroup (S₁); storage for 15 days and subgroup (S₂); storage for 30 days. Dentin permeability was measured for all specimens at baseline and after applying the treatment protocol. All specimens were photomicrographed using ESEM before and after the treatment protocol.

RESULTS: The highest permeability reduction percent mean value was recorded for M₁S₂ specimens (90% ± 20), with a statistically significant difference with other subgroups, followed by subgroup M₂S₂ (56% ± 8) and subgroup M₁S₁ (51% ± 11), respectively. Meanwhile, the subgroup M₂S₁ had the lowest mean value (36% ± 9). In addition, the dentin permeability test was confirmed by the ESEM results.

CONCLUSION: EMD could be used as a promising remineralizing agent for dentin remineralization.

Introduction

Dentin hypersensitivity (DH) is defined as “a short, sharp, localized pain emanating from exposed dentin in response to thermal, evaporative, tactile, osmotic or chemical stimuli. It is not attributable to any other shape of dental deformity or disease” [1]. Brännström’s hydrodynamic hypothesis proposes two basic approaches for treating dentin hypersensitivity; dentin blocking agents that occlude patent tubules or nerve desensitization that reduce nerve excitability, or both simultaneously [2].

Dentin blocking strategy is one of many treatment approaches involving the use of ions and salts (oxalates, calcium phosphate, fluoride, and hydroxyapatite), proteins that promote remineralization, the application of restorative materials (dentin sealers), and the use of lasers [3]. Unfortunately, due to the heterogeneous structure, lack of residual seed mineral crystals in dentine lesions, and high organic content, dentine remineralization is less effective than enamel remineralization. Preliminary research of dentine remineralization significantly depends on the conventional crystallization route; it starts with crystal nucleation and progresses to crystal growth

via ion-by-ion attachment. However, the classical ion-based crystallization is not very effective due to factors related to dentin nature, as previously stated [4].

Recently, multiple review articles have reported a wide range of biomimetic remineralization strategies. These techniques have been demonstrated to be effective at regenerating dentin tissue microstructures. It represents a different approach to this issue by imitating the natural mineralization process using liquid-like amorphous calcium phosphate nano-precursor particles to backfill dentine collagen. This technique for bottom-up remineralization is not dependent on seed crystallites and may be considered a feasible method for remineralization and blockage of patent dentinal tubules [5]. (Emdogain®), a contemporary commercially available product comprising enamel matrix proteins (EMPs) has received significant attention in the past few years. EMPs’ biomineralization activity has been utilized to support the synthesis of artificial hydroxyapatite *in vitro* [6].

It is noteworthy that, while the findings in the research of dentin tissue regeneration are promising as a treatment for dentin hypersensitivity, there are still difficulties in implementing and evaluating biomimetic methods in dentistry. Therefore, this study was conducted to assess the efficiency of enamel matrix

derivative (EMD) at two different times of application on dentin remineralization using two methods of evaluation.

Materials and Methods

Materials' description, composition, manufacturers, and batch numbers are presented in Table 1.

Specimens preparation

After sample size calculation, 32 human impacted third molars freshly extracted from patients aged 20–30 years old were collected, cleaned, and stored in distilled water containing 0.2% thymol antiseptic solution for no more than 1 month at 4°C until testing [7]. The utilization of extracted human teeth was approved by the Research Ethics Committee of Faculty of Dentistry, Suez Canal University, Egypt (Ethical approval NO 93/2018). The teeth were carefully examined under a stereomicroscope to rule out the presence of cracks and/or defects. A cylindrical Teflon mold (15 mm diameter and 40 mm height) was used to mount chosen teeth on acrylic resin blocks. A periodontal probe (UNC-15, Paterson Dental) was used to adjust the level of the acrylic resin 2 mm below each tooth's cemento-enamel junction (CEJ).

Each selected tooth was prepared by removing the occlusal enamel in a direction parallel to the occlusal surface with a slow-speed diamond-impregnated saw (Isomet 5000, Buehler Ltd., Lake Bluff, USA) under copious water cooling. Subsequently, teeth were sectioned parallel to the occlusal surface, 2.5–3 mm above the cemento-enamel junction, and occlusal.

Thirty-two dentin specimens in the form of discs of 6 mm in diameter and 1 mm thickness were prepared, and their dimensions were confirmed by a digital caliper (Mitutoyo, Japan). Following sectioning, each specimen was examined thoroughly under a stereomicroscope to confirm it was devoid of coronal enamel or pulpal tissue [8]. The occlusal surface of each specimen was marked with a permanent marker to guarantee proper materials application and ensure that the specimens were adequately mounted in the filtration apparatus every time. All prepared specimens

were kept in deionized water until the performance of the treatment procedure [9].

Permeability test

All specimens were treated for one minute with phosphoric acid etching gel (Meta, South Korea) [10], then thoroughly rinsed. For hydraulic conductance measurements, a fluid filtration system with a split chamber device as described by Pashley and Galloway in 1985 was constructed. The fluid transport apparatus featured a Teflon split chamber device, which is made up of female and male pieces that are screwed together. Two pairs of identical rubber "O" rings with inner space diameter (0.6 cm) were used to adapt the chamber to the specimens intimately and to control the surface area of dentin to be studied. Hydrostatic pressure was applied to one side (male part) of the chamber to induce filtration through the dentin specimens. To imitate dentinal fluid, the entire device was immersed in distilled water [11].

Measurement of the fluid filtration rate at the baseline

Each specimen was placed in the split chamber device between the two rubbers "O" rings, with the pulpal side facing the pump side and the occlusal side facing the pipette side of the chamber (Figures 1 and 2).

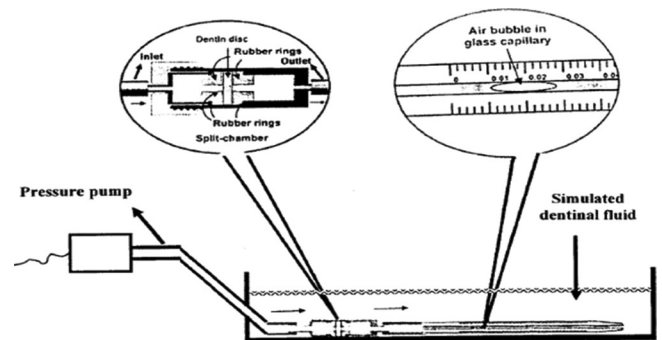


Figure 1: Diagram of fluid filtration apparatus assembly

The position of the air bubbles was then identified and documented for each specimen. For 10 min, the pressure pump was turned on, and the location of the air bubble was re-determined and recorded. Any specimens that showed signs of dentinal blockage or leakage were excluded immediately from the experiment. The linear displacement of the air bubble in the glass pipette was then determined and fluid flow (Q) was derived from

Table 1: Materials' description, composition, manufacturers

Materials	Description	Composition	Manufacturers	Batch numbers
Meta® Etchant	Etchant gel	37% phosphoric acid, blue color for visual control	Meta, South Korea	MET 1902111
MI Varnish™	Fluoride varnish with CPP-ACP	30%–50% polyvinyl acetate, 10%–30% hydrogenated rosin, 20%–30% ethanol, 1%–8% sodium fluoride, 1%–5% CPP-ACP, 1%–5% silicon dioxide	GC, Tokyo, Japan	1805011
Emdogain® gel	Enamel matrix derivative gel	Protein (porcine amelogenin) Propylene Glycol Alginate Acetic acid/acetate Salts	Straumann, Biora, Sweden	Table 2
Artificial saliva		Calcium, albumin, methylcellulose, sodiumcarboxy methyl cellulose, hydroxypropylmethyl cellulose, potassium chloride, di-potassium hydrogen phosphate, sodium fluoride, magnesium chloride, glucose, methyl paraben	Faculty of Pharmacy, Cairo university	MX 842

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate.

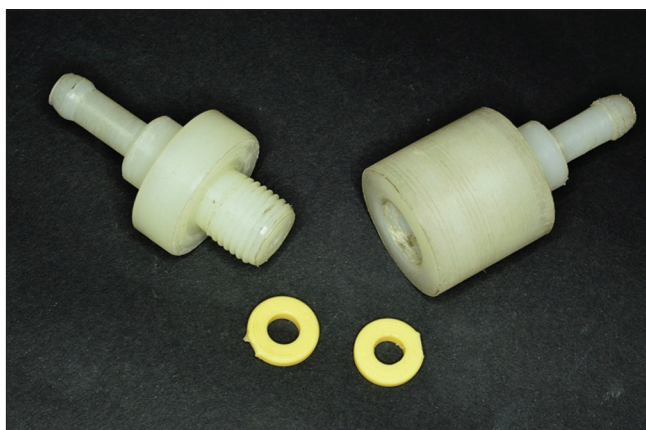


Figure 2: Split chamber device with pairs of identical rubber O rings

this linear displacement. Permeability was expressed in terms of fluid filtration (J_v) [8] where: $J_v = Q/AT$

J_v = fluid filtration rate in $\mu\text{l cm}^{-2} \text{min}^{-1}$

Q = displacement χ cross-sectional area of the pipette (fluid flow in μl).

A = dentinal (or O ring central hole) surface area in cm^2 .

T = time in minutes.

Treatment fluid filtration rate measurement

After determining the baseline fluid filtration rate, the specimens were demounted from the split chambers and dried with compressed air. They were treated according to their assigned groups, with each tested material applied on the occlusal surface of the specimen and in accordance with the manufacturer's instructions. Fluoride varnish with casein phosphopeptide-amorphous calcium phosphate CPP-ACP (MI varnish™, GC, Tokyo, Japan) was stirred then applied using an applicator brush as a thin uniform layer on the surface of (M_1) group and left undisturbed. Meanwhile, specimens of group (M_2) were treated with enamel matrix derivative gel (Emdogain®, Straumann, Biora, Sweden). The gel was applied and left for five min. on the occlusal surface of each specimen. Then, the gel excess was carefully removed using a towel. Each group was subdivided into two subgroups according to storage time in artificial saliva (S_1) stored for 15 days and (S_2) stored for 30 days. Each specimen was separately stored, and the storage media was changed every day. After completion of the storage period, each specimen was remounted in the split chamber, fluid filtration was remeasured as described in baseline measurements, and results were tabulated. The dentin permeability reduction percent of each specimen after applying different treatments protocols was obtained using the following equation, with each specimen serving as its own control:

= (Difference between fluid filtration rate at the baseline and after treatment protocol)/(Baseline fluid filtration rate) $\times 100$ [12].

Environmental scanning electron microscope

All tested specimens were examined by Environmental Scanning Electron Microscopy (ESEM model Quanta 250 FEG Field Emission Gun, Philips, Netherlands) at baseline and after application of treatment protocol of each subgroup to reveal the micromorphological changes that occurred in the dentinal tubules. Images were taken at a magnification of $\times 4000$. The percentage of obliteration of dentinal tubules for each specimen was detected using image analysis software (Image J-Fiji program).

Statistical analysis

Two-way ANOVA analysis was used to test the effects of different variables on the permeability and occlusion of patented dental tubules, as well as the interactions between different variables. One-way ANOVA followed by *post hoc* most minor square difference (LSD) was used to compare more than two sub-groups in non-related samples. Paired student t-test was utilized to compare between two groups in non-related samples. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 25 for Windows.

Results

Dentin permeability results

Data revealed that the effect of storage time (T) in artificial saliva was significantly more significant than the effect of treatment materials (M) (Table 2). Regarding the effect of the treatment materials (M) irrespective of storage times (S), the results showed that the highest permeability reduction percent mean value was recorded for MI varnish™ group (M_1) ($70.24\% \pm 25.29$), followed by Emdogain® gel group (M_2) ($45.96\% \pm 12.80$) with a statistically significant difference between them at $p \leq 0.05$. At the same time, the effect of storage times (S) irrespective of treatment materials (M), the highest permeability reduction percent mean value was recorded for (S_2) subgroup ($72.63\% \pm 22.76$), followed by (S_1) subgroup ($43.58\% \pm 12.22$) with statistically significant difference between groups at $p \leq 0.05$.

Table 2: Results of two-way ANOVA for the effect of different variables on dentin permeability reduction

Source of variation	F	P	Partial eta squared
Material	29.004	< 0.001*	0.509
Storage time	41.494	< 0.001*	0.597
Material \times Storage time	4.573	0.041*	0.140

*Significant at $P \leq 0.05$.

Considering the effect of both treatment materials (M) and different storage times (S) on permeability reduction percent, the results demonstrated

that the M_1S_2 subgroup had the highest permeability reduction percent mean value ($90\% \pm 20$) (Table 3).

Table 3: Mean and standard deviation values of permeability reduction of different subgroups

Subgroups	Permeability reduction percent, mean \pm SD	p
M_1S_1 subgroup	51 ± 11^b	$p1 < 0.001^*$
M_1S_2 subgroup	90 ± 20^a	$p2 0.029^*$
M_2S_1 subgroup	36 ± 8.5^c	$p3 0.461$ (NS)
M_2S_2 subgroup	56 ± 8^b	$p4 < 0.001^*$
		$p5 < 0.001^*$
		$p6 0.005^*$

*Significant as $P < 0.05$. NS: $P > 0.05$, means with different letters indicates significant difference. NS: Nonsignificant, SD: Standard deviation.

Environmental scanning electron microscope results

As depicted in Table 4, data showed that the effect of storage time in artificial saliva was significantly higher than the effect of treatment materials.

Table 4: Results of two-way ANOVA for the effect of different variables on dentinal tubules occlusion

Source of variation	F	p	Partial eta squared
Material	18.814	$< 0.001^*$	0.402
Storage time	51.883	$< 0.001^*$	0.649
Material \times Storage time	3.930	0.057 (NS)	0.123

*Significant at $P \leq 0.05$. NS: $P > 0.05$. NS: Nonsignificant.

As demonstrated in Figure 3a, the results of etched specimens represent the maximum patent dentinal tubules percent of each specimen, where dentinal tubules were widely opened with different shapes demonstrating removal of the smear layer. Regarding

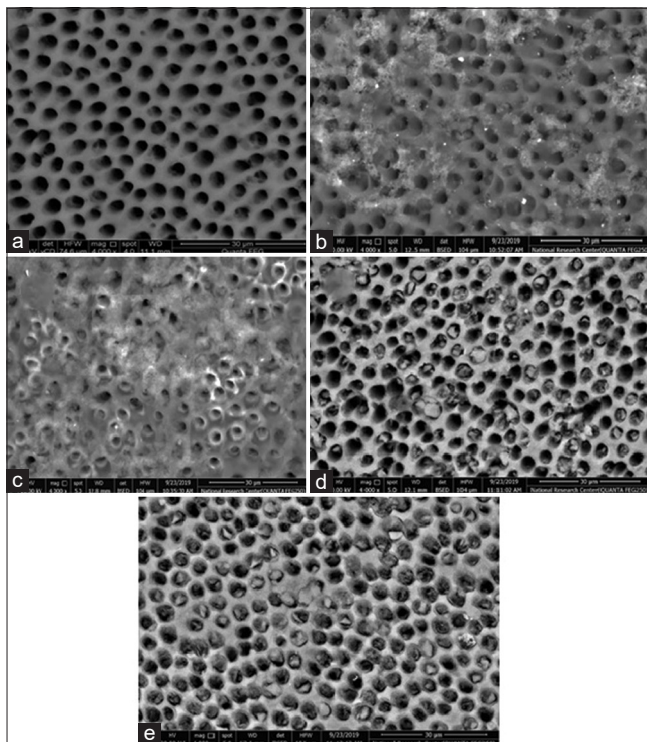


Figure 3: (a-e) Environmental scanning electron microscope photomicrographs

the effect of the treatment materials (M) irrespective of storage times (S), the results revealed that the highest dentinal tubules occlusion percent mean value was recorded for MI varnish™ group (M_1) ($54.27\% \pm 20.87$), followed by Emdogain® gel group (M_2) ($37.65\% \pm 12.09$) with statistically significant difference between them at $p \leq 0.05$. In terms of the effect of storage times (S) regardless of treatment materials (M), the results showed that the highest percent of dentinal tubules occlusion mean value was recorded for storage time in artificial saliva for 30 days subgroup (S_2) ($59.32\% \pm 17.27$). It was followed by 15 days group (S_1) ($32.6\% \pm 7.10$) with a statistically significant difference between groups at $p \leq 0.05$.

Table 5 reveals the effects of both treatment materials (M) and different storage times (S) on the percent of dentinal tubules occlusion. ESEM photomicrographs expressed these results where M_1S_2 subgroup images showed that almost all dentinal tubules orifices were obliterated by deposits (Figure 3c). Dentin surfaces ESEM photomicrographs of M_1S_1 subgroup specimens demonstrated few occluded dentinal tubules orifices, as depicted in (Figure 3b). For dentin specimens treated with Emdogain gel, fewer occluded dentinal tubules orifices were represented in M_2S_1 than the M_2S_2 subgroup (Figure 3d and e).

Table 5: Mean and standard deviation values of dentinal tubules occlusion of different subgroups

Subgroups	Percent of dentinal tubules occlusion%, mean \pm SD	p value
M_1S_1 subgroup	37 ± 6^{bc}	$p1 < 0.001^*$
M_1S_2 subgroup	69 ± 15^a	$p2 0.107$ (NS)
M_2S_1 subgroup	28 ± 5^c	$p3 0.052$ (NS)
M_2S_2 subgroup	47 ± 9^b	$p4 < 0.001^*$
		$p5 < 0.001^*$
		$p6 0.001^*$

*Significant as $P < 0.05$. NS: $P > 0.05$, means with different letters indicates significant difference. NS: Nonsignificant, SD: Standard deviation.

Discussion

Dentin remineralization through biomimetic remineralization is thought to be promising for treating dentin hypersensitivity. This non-classical particle-based crystallization pathway involves a multistage process that depends on a coordinated series of highly complex macromolecular, cellular, and matrix-mineral interactions. One of the contemporary commercially available organic analogs is Emdogain® gel, which consists of enamel matrix proteins (EMPs). It is made of 90% amelogenin and 10% pig enamel matrix protein derivatives [6]. Amelogenin in enamel and non-collagenous matrix proteins (NCPs) in dentin influences the organization and growth of hydroxyapatite (HAP) crystals during the biomineralization processes. It has been demonstrated that amelogenin peptides include the apatite-binding and self-assembly regions. These promote the regrowth of multilayered HAP nanostructures on the demineralized enamel surface,

integrating them with the underlying tissue and enhancing mechanical function and uniquely suited for dual enamel-dentin remineralization and repair [13].

In general, MI varnish™ is based on fluoride and CPP-ACP. In addition to the remineralizing effect of NaF, the dental remineralizing mechanism of MI varnish™ includes the precipitation of Ca²⁺ and PO₄³⁻ ions from ACP through the phosphorylated fibrils of the exposed intertubular dentin collagen, promoting the formation of apatite [14]. Therefore, based on its documented dental remineralizing mechanism, it was selected as a positive control group in the current study. Dentin permeability measurement was used to track changes in dentin hydraulic conductance, which was used to evaluate the effectiveness of the tested remineralizing agents [15]. Furthermore, ESEM was used as a confirmatory method to the dentin permeability test as it was proven to be essential for studying the ultrastructural changes associated with dentin reactivity with remineralizing materials [16].

The current study results showed that significant dentin permeability reduction percent was recorded after applying each tested remineralizing material irrespective of storage times. Moreover, the highest permeability reduction percent mean value was recorded for the MI varnish™ group with a significant difference with the Emdogain® gel group, confirmed by ESEM photomicrograph and image analysis findings. This result can be attributed to the synergistic effect of fluoride and CPP-ACP in MI varnish™, where CPP stabilizes nanoclusters of ACP and increases fluoride incorporation in subsurface dental tissue. This maintains the calcium and phosphate ions bioavailable for effective remineralization of demineralized crystals [17], [18]. In addition, it was revealed that the rapid and high ion release from MI Varnish™ is related to the high water solubility of the CPP-ACP complexes [19], [20]. This result was in accordance with Bächli *et al.*, 2019, and Zhou *et al.*, 2020, [21], [22], while came in disagreement with Poggio *et al.* 2013, who found that even when strong fluoride boosters of calcium phosphate-based compounds such as CPP-ACP complex are used, they lead to the remineralization of demineralized enamel to a limited depth and to incomplete occlusion of the exposed dentinal tubules of demineralized dentin [23].

It is worth mentioning that there is a different mechanism of action related to both tested materials where MI Varnish™ remineralizing effect occurs through occlusion of dentinal tubules by precipitation of calcified deposits. In contrast, Emdogain® gel depends on inter and intrafibrillar collagen remineralization [24]. During specimens preparation and treatment, specimens' surfaces had to be dried, the exposed collagen fibers became very fragile and may have been damaged during this process. Furthermore, we assume that the collagen fibers partially collapsed, which may be related to the inferior results related to Emdogain® gel compared to MI Varnish™ [21].

Regarding the effect of different storage times on the percentage of dentin permeability reduction, regardless of the processing material, the results showed that the specimen with the highest permeability reducing value was stored for 30 days demonstrated better results, as confirmed by the results of ESEM photomicrograph and image analysis. This effect can be attributed to a significant increase in nucleation sites over time, resulting in additional apposition of crystals derived from the surrounding artificial saliva, which was highly supersaturated with calcium and phosphate ions [25].

Conclusion

Based on the current study's limitations, the following conclusions could be drawn:

1. Using fluoride varnish with CPP-ACP decreases dentin permeability significantly compared to enamel matrix derivatives at 30 days storage period in artificial saliva.
2. Enamel matrix derivatives showed demonstrated dentin permeability reduction percent to the fluoride varnish with CPP-ACP when stored for a more extended period in artificial saliva.
3. A significant positive correlation was detected between dentin permeability reduction percent and environmental scanning electron microscope results when used to evaluate the tested desensitizing materials.

References

1. Canadian Advisory Board on Dentin Hypersensitivity. Consensus-based recommendations for the diagnosis and management of dentin hypersensitivity. *J Can Dent Assoc.* 2003;69(4):221-6. PMID:12662460
2. van Loveren C, Schmidlin PR, Martens LC, Amaechi BT. Dentin hypersensitivity management. *Clin Dent Rev.* 2018;2:1-6. <https://doi.org/10.1007/s41894-017-0019-8>
3. Öncü E, Karabekiroğlu S, Ünlü N. Effects of different desensitizers and lasers on dentine tubules: An *in-vitro* analysis. *Microsc Res Tech.* 2017;80(7):737-44. <https://doi.org/10.1002/jemt.22859> PMID:28251725
4. He L, Hao Y, Zhen L, Liu H, Shao M, Xu X, *et al.* Biom mineralization of dentin. *J Struct Biol.* 2019;207(2):115-22. <https://doi.org/10.1016/j.jsb.2019.05.010> PMID:31153927
5. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: A review of literature. *Int J Clin Pediatr Dent.* 2019;12(2):139-44. <https://doi.org/10.5005/>

- jp-journals-10005-1603
PMid:31571787
6. Li D, Lv X, Tu H, Zhou X, Yu H, Zhang L. Remineralization of initial enamel caries *in vitro* using a novel peptide based on amelogenin. *Front Mater Sci*. 2015;9:293-302. <https://doi.org/10.1007/s11706-015-0298-4>
 7. Elgalaid TO, Youngson CC, McHugh S, Hall AF, Creanor SL, Foye RH. *In vitro* dentine permeability: The relative effect of a dentine bonding agent on crown preparations. *J Dent*. 2004;32(5):413-21. <https://doi.org/10.1016/j.jdent.2004.01.013>
PMid:15193791
 8. Farghal NS, Abdalla AI, El-Shabrawy SM, Showaib EA. The effect of combined application of new dentin desensitizing agent and deproteinization on dentin permeability of different adhesive systems. *Tanta Dent J*. 2013;10:138-44. <https://doi.org/10.1016/j.tdj.2013.11.006>
 9. Yiu CK, Hiraishi N, Chersoni S, Breschi L, Ferrari M, Prati C, et al. Single-bottle adhesives behave as permeable membranes after polymerisation. II. Differential permeability reduction with an oxalate desensitiser. *J Dent*. 2006;34(2):106-16. <https://doi.org/10.1016/j.jdent.2005.05.001>
PMid:15979228
 10. Cao Y, Mei ML, Xu J, Lo EC, Li Q, Chu CH. Biomimetic mineralisation of phosphorylated dentine by CPP-ACP. *J Dent*. 2013;41(9):818-25. <https://doi.org/10.1016/j.jdent.2013.06.008>
PMid:23810733
 11. Pashley DH, Stewart FP, Galloway SE. Effects of air-drying *in vitro* on human dentine permeability. *Arch Oral Biol*. 1984;29(5):379-83. [https://doi.org/10.1016/0003-9969\(84\)90164-x](https://doi.org/10.1016/0003-9969(84)90164-x)
PMid:6588935
 12. Carvalho AO, de Oliveira MT, Nikaido T, Tagami J, Giannini M. Effect of adhesive system and application strategy on reduction of dentin permeability. *Braz Oral Res*. 2012;26(5):397-403. <https://doi.org/10.1590/s1806-83242012005000020>
PMid:22892877
 13. Mukherjee K, Visakan G, Phark JH, Moradian-Oldak J. Enhancing collagen mineralization with amelogenin peptide: Toward the restoration of dentin. *ACS Biomater Sci Eng*. 2020;6(4):2251-62. <https://doi.org/10.1021/acsbomaterials.9b01774>
PMid:33313393
 14. Madrid-Troconis CC, Perez-Puello S del C. Casein phosphopeptide-amorphous calcium phosphate nanocomplex (CPP-ACP) in dentistry: State of the art. *Rev Fac Odontol Univ Antioq*. 2019;30:248-62. <https://doi.org/10.17533/udea.rfo.v30n2a10>
 15. Kolker JL, Vargas A, Armstrong SR, Dawson DV. Effect of desensitizing agents on dentin permeability and dentin tubule occlusion. *J Adhes Dent*. 2002;4(3):211-21. PMid:12666757
 16. Schlueter N, Hara A, Shellis RP, Ganss C. Methods for the measurement and characterization of erosion in enamel and dentine. *Caries Res*. 2011;45 Suppl.1:13-23. <https://doi.org/10.1159/000326819>
PMid:21625129
 17. Salman NR, El-Tekeya MM, Bakry NS, Soliman S. Remineralization effect of fluoride varnish containing casein phosphopeptide amorphous calcium phosphate on caries-like lesions in primary teeth (*in vitro* study). *Alexandria Dent J*. 2019;44:13-6. <https://doi.org/10.21608/adjalexu.2019.57568>
 18. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. *J Dent Res*. 2008;87(4):344-8. <https://doi.org/10.1177/154405910808700420>
PMid:18362316
 19. Thakkar P, Badakar C, Hugar S, Hallikerimath S, Patel P, Shah P. An *in vitro* comparison of casein phosphopeptide-amorphous calcium phosphate paste, casein phosphopeptide-amorphous calcium phosphate paste with fluoride and casein phosphopeptide-amorphous calcium phosphate varnish on the inhibition of demineraliza. *J Indian Soc Pedod Prev Dent*. 2017;35(4):312-8. https://doi.org/10.4103/jisppd.jisppd_308_16
PMid:28914243
 20. Savas S, Kavrik F, Kucukyilmaz E. Evaluation of the remineralization capacity of CPP-ACP containing fluoride varnish by different quantitative methods. *J Appl Oral Sci*. 2016;24(3):198-203. <https://doi.org/10.1590/1678-775720150583>
PMid:27383699
 21. Bächli K, Schmidlin PR, Wegehaupt F, Paqué F, Ramenzoni L, Botter S. Remineralization of artificial dentin caries using dentin and enamel matrix proteins. *Materials*. 2019;12(13):2116. <https://doi.org/10.3390/ma12132116>
PMid:31266157
 22. Zhou Z, Ge X, Bian M, Xu T, Li N, Lu J, et al. Remineralization of dentin slices using casein phosphopeptide-amorphous calcium phosphate combined with sodium tripolyphosphate. *Biomed Eng Online*. 2020;19(1):18. <https://doi.org/10.1186/s12938-020-0756-9>
PMid:32245476
 23. 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(6):366-74. <https://doi.org/10.1002/sca.21077>
PMid:23427062
 24. Cao Y. Biomimetic Remineralisation of Hydroxyapatite on Human Enamel and Dentine. *HKU Theses Online*; 2015. Available form: https://doi.org/10.5353/th_b5570774 [Last accessed on 2016 Aug 19].
 25. Kanehira M, Ishihata H, Araki Y, Takahashi H, Sasaki K, Finger WJ. Effect of artificial saliva on permeability of dentin treated with phosphate containing desensitizer measured by digital flow meter. *Dent Mater J*. 2019;38(6):963-9. <https://doi.org/10.4012/dmj.2018-134>
PMid:31434831