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

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Abstract

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: (S1); 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 M1 specimens (90% ± 20), with a statistically significant difference with other subgroups, followed by subgroup M2S1 (56% ± 8) and subgroup M2S2 (36% ± 5). 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 disea” [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’ biomimetic 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
Background: The aim of this study was to evaluate the scientific literature on the effect of two desensitizing materials on dentin remineralization. The two materials were identified as Meta® Etchant and MI Varnish™. The study was designed to compare their effectiveness in preventing and reversing dentin hypersensitivity.

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 cementoenamel 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 cementoenamel 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 pulp side facing the pump side and the occlusal side facing the pipette side of the chamber (Figures 1 and 2).

Table 1: Materials’ description, composition, manufacturers

<table>
<thead>
<tr>
<th>Materials</th>
<th>Description</th>
<th>Composition</th>
<th>Manufacturers</th>
<th>Batch numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meta® Etchant</td>
<td>Etchant gel</td>
<td>37% phosphoric acid, blue color for visual control</td>
<td>Meta, South Korea</td>
<td>MET190211</td>
</tr>
<tr>
<td>MI Varnish™</td>
<td>Fluoride varnish with CPP-ACP</td>
<td>30%–50% polyvinyl acetate, 10%–30% hydrogenated rosin, 20%–30% ethanol, 1%–8% sodium fluoride, 1%–5% CPP-ACP, 1%–5% silicon dioxide</td>
<td>GC, Tokyo, Japan</td>
<td>1805011</td>
</tr>
<tr>
<td>Emdogain® gel</td>
<td>Enamel matrix derivative gel</td>
<td>Protein (porcine amelogenin) Propylene Glycol Alginate Acetic acid/acetate Salts</td>
<td>Straumann, Biora, Sweden</td>
<td>MX 842</td>
</tr>
<tr>
<td>Artificial saliva</td>
<td></td>
<td>Calcium, albumin, methylcellulose, sodiumcarboxy methyl cellulose, hydroxypropylmethyl cellulose, potassium chloride, di-potassium hydrogen phosphate, sodium fluoride, magnesium chloride, glucose, methyl paraben</td>
<td>Faculty of Pharmacy, Cairo university</td>
<td></td>
</tr>
</tbody>
</table>

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate.
this linear displacement. Permeability was expressed in terms of fluid filtration \( J_v \) \[8\] where:

\[
J_v = \frac{Q}{AT}
\]

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

\( Q \) = displacement \( \chi \) cross-sectional area of the pipette (fluid flow in \( \mu l \)).

\( A \) = 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:

\[
\text{Dentin permeability results} = \left( \frac{\text{Difference between fluid filtration rate at the baseline and after treatment protocol}}{\text{Baseline fluid filtration rate}} \right) \times 100 \ [12].
\]

**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% ± 25.29), followed by Emdogain® gel group \((M_2)\) (45.96% ± 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% ± 22.76), followed by \((S_1)\) subgroup (43.58% ± 12.22) with statistically significant difference between groups at \( p \leq 0.05 \).
that the M₃S₂ subgroup had the highest permeability reduction percent mean value (90% ± 20) (Table 3).

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

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Permeability reduction percent, mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₁S₁ subgroup</td>
<td>51 ± 11*</td>
<td>p1 &lt; 0.001*</td>
</tr>
<tr>
<td>M₁S₂ subgroup</td>
<td>90 ± 20*</td>
<td>p2 0.029*</td>
</tr>
<tr>
<td>M₂S₁ subgroup</td>
<td>36 ± 8.5*</td>
<td>p3 0.461 (NS)</td>
</tr>
<tr>
<td>M₂S₂ subgroup</td>
<td>56 ± 8*</td>
<td>p4 &lt; 0.001*</td>
</tr>
</tbody>
</table>

*Significant as P < 0.05. 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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>F</th>
<th>p</th>
<th>Partial eta squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>18.814</td>
<td>&lt; 0.001*</td>
<td>0.402</td>
</tr>
<tr>
<td>Storage time</td>
<td>51.883</td>
<td>&lt; 0.001*</td>
<td>0.649</td>
</tr>
<tr>
<td>Material × Storage time</td>
<td>3.930</td>
<td>0.057 (NS)</td>
<td>0.123</td>
</tr>
</tbody>
</table>

*Significant at P ≤ 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 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 (M₁) (54.27% ± 20.87), followed by Emdogain® gel group (M₂) (37.65% ± 12.09) with statistically significant difference between them at p ≤ 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 (S₂) (59.32% ± 17.27). It was followed by 15 days group (S₁) (32.6% ± 7.10) with a statistically significant difference between groups at p ≤ 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 M₁S₂ subgroup images showed that almost all dentinal tubules orifices were obliterated by deposits (Figure 3c). Dentin surfaces ESEM photomicrographs of M₁S₁ 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 M₂S₁ than the M₂S₂ subgroup (Figure 3d and e).

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

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Percent of dentinal tubules occlusion, mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₁S₁ subgroup</td>
<td>56 ± 8*</td>
<td>p1 &lt; 0.001*</td>
</tr>
<tr>
<td>M₁S₂ subgroup</td>
<td>28 ± 5*</td>
<td>p2 0.107 (NS)</td>
</tr>
<tr>
<td>M₂S₁ subgroup</td>
<td>37 ± 6*</td>
<td>p3 0.052 (NS)</td>
</tr>
<tr>
<td>M₂S₂ subgroup</td>
<td>47 ± 9*</td>
<td>p4 &lt; 0.001*</td>
</tr>
<tr>
<td>M₃S₁ subgroup</td>
<td>69 ± 15*</td>
<td>p5 &lt; 0.001*</td>
</tr>
<tr>
<td>M₃S₂ subgroup</td>
<td>47 ± 9*</td>
<td>p6 0.001*</td>
</tr>
</tbody>
</table>

*Significant as P < 0.05. NS: P > 0.05. 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,
incorporating 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 Ca+2 and PO4-3 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
