INTRODUCTION

Dentinal hypersensitivity is characterized by typical short sharp pain on the exposed dentin which is aroused by the thermal, evaporative, tactile, osmotic or chemical stimuli. Clinical data indicated the manifestation of this symptom in various exposed dentin conditions, such as exposed dentin from traumatic brushing in abrasive and erosive lesions, as exposed dentin from traumatic brushing in abrasive lesion, denuded root surface due to loss of cementum following gingival recession, as well as loss of dental structure by chemical dissolution as in erosive lesions.

Theoretical etiology of the dentinal hypersensitivity has been attributed to the hydrodynamic theory proposed by Brännström. Ascribing to the movement of dentinal fluid inside the dentinal tubules from some stimuli; nerve fibers in pulp or predentin are stimulated resulting in fluid inside the dentinal tubules.

However, different types of hypersensitive dentin express different surface characteristics. For example, eroded dentin has a uniquely complex histological structure. Its organic fraction becomes increasingly exposed as a result of the continual action of acids. The outer zone of demineralized organic surface followed by a partly demineralized zone toward to the underneath zone of fully mineralized tissue was characterized in eroded dentin. The presence of exposed collagen fibrils leads to the question of whether the natural desensitization can occur spontaneously in eroded dentin. Moreover, dentin surface condition is of importance for the desensitizer deposition, penetration, as well as surface integration. However, there are few studies dealing with desensitizing effect on different types of dentin surface.

Several desensitizers have been introduced for hypersensitivity treatment with various unique mechanisms, mainly categorized as tubular occlusion and blockage of nerve activity. Currently, calcium-phosphate containing desensitizers have evoked considerable interest due to their biocompatible property, their outstanding characteristic in dentinal tubule occlusion and favorable reduction in dentin permeability in the oral environment. Teethmate Desensitizer (TMD; Kuraray Noritake Dental Inc., Tokyo, Japan) is a recently developed calcium-phosphate containing material; tetracalcium phosphate and dicalcium phosphate anhydrous (DCPA; CaHPO$_4$), whose combination could spontaneously transform to hydroxyapatite (HA; Ca$_5$(PO$_4$)$_3$(OH)). In the previous study, we demonstrated that TMD exhibited higher performance in permeability reduction after long-term artificial saliva (AS) immersion against the long-history-of-use oxalate containing desensitizer as the

Keywords: Calcium-phosphate, Desensitizer, Hypersensitivity, Dentin permeability, Surface integration
supersaturation of salivary fluid with respect to HA could promote the sustainability of calcium-phosphate containing material on treated dentin surface\(^1\). Furthermore, the interaction of this material to dentin surface is expected to be another benefit of calcium-phosphate containing desensitizer contributing to long-term sustainability. However, this aspect of retention has not been investigated so far.

Therefore, the aim of this study was to evaluate the effect of TMD in dentin permeability reduction and integration of desensitizer layer on dentin surface immediately after treatment and after immersion in AS, regarding two different surface characteristics of dentin, with and without exposed collagen fibrils.

**MATERIALS AND METHODS**

**Specimen preparation**

Sixty eight intact human molars were collected with ethical approval following the guideline of the Ethics Committee of Tokyo Medical and Dental University under protocol number 725. Mid-coronal dentin discs with a thickness of 0.5 mm were prepared by using low-speed water cooled diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) cut perpendicular to long axis of teeth away from the pulp chamber. Both occlusal and pulpal surfaces of each dentin disc were polished with 600-grit silicon carbide paper under running water for 30 s to create standardized smear layer. The occlusal dentin surface was partly covered by nail varnish leaving a round window exposed (D=5 mm) so as to enable a reproducible permeability measurement. Forty of the discs were divided into two groups according to dentin surface characteristics (N=20 per group) that were obtained by immersion in 0.5 M EDTA (pH 7.4) for 2 min representing collagen exposed dentin\(^1\), or immersion in 0.5 M EDTA (pH 7.4) for 30 s followed by 5% NaOCl for 2 min representing non-collagen exposed dentin\(^1\). After being copiously rinsed with water, the specimens in each group were randomly allocated to two subgroups (N=10 per subgroup) of no treatment (control) and TMD treatment according to manufacturer’s instruction as listed in Table 1.

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Application</th>
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</thead>
<tbody>
<tr>
<td>Teethmate Desensitizer (TMD)</td>
<td>Kuraray Noritake Dental Inc., Tokyo, Japan</td>
<td>Powder: TTCP, DCPA (Lot. 100616-6) Liquid: water (Lot. 100706-2)</td>
<td>Mix powders and water within 30 s, and rub on dry dentin surface for 30 s using a micro-sponge</td>
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</table>

Abbreviations: TTCP: tetracalcium phosphate \([\text{Ca}_4(\text{PO}_4)_2\text{O}]\); DCPA: dicalcium phosphate anhydrous \((\text{CaHPO}_4)_2\)

**Artificial saliva (AS) immersion**

Each specimen was then stored in AS with pH 6.5 at 37°C for 4 weeks. The composition of the AS was 1.0 mM CaCl\(_2\), 3.0 mM KH\(_2\)PO\(_4\), 0.02% NaN\(_3\), and it was replenished every 1 week\(^1\).

**Dentin permeability measurement**

By using a water-filled system and modified split-chamber unit\(^1\) at a simulated pulpal pressure of 10 psi\(^1\), fluid transudation through the dentin disc was measured in the steps after surface pretreatment (baseline), immediately after TMD application, and after AS immersion for 4 weeks.

Each dentin disc was positioned in between rubber O rings and split-chamber following the exposure window surrounded by the nail-varnish, leaving the 0.196 cm\(^2\) of available surface area for filtration of deionized water. The movement of an air bubble (mm) trapped within 1.0 mm-diameter micro-capillary tube which was horizontally connected to the split-chamber was recorded every 1 min for consecutive 3 min and the average data were then calculated into a hydraulic conductance \((Lp, \mu\text{Lmin}^{-1}\text{cm}^{-2}\text{cmH}_2\text{O}^{-1})\).

The permeability reduction percent (PR\%) after TMD treatment and after AS immersion was calculated for each specimen by using its Lp at the baseline as maximum permeability. The results were statistically analysed by \(t\)-test and paired \(t\)-test to determine significant differences in PR\% between and within surface pretreatment, respectively.

**SEM observation**

Two specimens from each 4-week AS-immersed subgroup were randomly selected and processed for SEM observation. Moreover, eight additional dentin discs were pretreated by EDTA or EDTA/NaOCl. Half of discs in each surface pretreatment were subjected to the desensitizer for observation of the specimens immediately after TMD application, whereas the other half served as untreated control. All of the discs were dehydrated in ascending grades of ethanol. They were dried and fixed by immersion in hexamethyldisilazane (HMDS) for 10 min, and then left on filter paper in a covered glass vial at room temperature for 24 h. The dentin discs were fractured by finger pressure revealing the cross-section of dentinal tubules without smear layer. After gold-sputter coating (SC-701AT, Elionix, Tokyo, Japan), the micromorphology was examined using a scanning electron microscope (JSM-5310LV, JEOL, Tokyo, Japan).
Integration test

The remaining 20 discs were prepared and treated by either EDTA or EDTA/NaOCl in the same manner as described. After that, the dentin discs in each group were allocated into 5 subgroups (N=2) as followed:
1: immersed in AS for 1 week
2: immersed in AS for 1 week and then ultrasonicated
3: treated with TMD and then ultrasonicated
4: treated with TMD and immersed in AS for 1 week
5: treated with TMD, immersed in AS for 1 week, and then ultrasonicated

The ultrasonication in distilled water for 10 min was done by using ultrasonic cleaning bath (SND Model US 102, Nagano, Japan) which had 0.010 W/cm² average power output and mean operating frequency of 38 KHz. SEM observation was done following the method described above.

The summary of sample distribution in each group is shown in Table 2.

RESULTS

Measurement of hydraulic conductance

The results of hydraulic conductance measurement expressed in PR% are summarized in Table 3. TMD application resulted in effective reduction of permeability. T-test showed no significant differences in PR% immediately after TMD application between two pretreatments (p>0.05), whereas, after AS immersion, EDTA/NaOCl pretreatment showed significant higher PR% than that of EDTA pretreatment (p<0.005). Paired t-test also confirmed that the AS immersion significantly improved PR% in EDTA/NaOCl pretreatment (p<0.005).

SEM observation

Both pretreatments resulted in removal of smear layer and smear plugs, where EDTA pretreatment left collagen exposed on dentin surface (Fig. 1a), but a plain surface was observed in EDTA/NaOCl pretreatment (Fig. 1b). After TMD application, regardless of pretreatment, dentin surface was covered by a thin layer of desensitizer

Table 2  Schematic diagram of sample distribution

<table>
<thead>
<tr>
<th>Mid-coronal dentin discs (N=68)</th>
<th>Dentin permeability measurement (N=40)</th>
<th>SEM observation (N=8)</th>
<th>Integration test (N=20)</th>
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<tr>
<td></td>
<td>Baseline permeability</td>
<td>Surface morphology of</td>
<td>Group</td>
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<td></td>
<td></td>
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<td>1</td>
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<td>5</td>
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</tbody>
</table>

*The number in [ ] represents the sample size in each surface pretreatment (EDTA or EDTA/NaOCl).

Abbreviation: TMD, Teethmate Desensitizer treatment; AS, artificial saliva immersion; US, ultrasonication

(-) indicates the treatment was not applied, (✓) indicates the treatment was applied.

Table 3  Mean and standard deviations of the PR% (%) (N=10)

<table>
<thead>
<tr>
<th>Surface pretreatment</th>
<th>Subgroup</th>
<th>After desensitizer treatment (immediate PR%)</th>
<th>After AS immersion (long-term PR%)</th>
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</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>No treatment</td>
<td>0</td>
<td>65.1(5.3)</td>
</tr>
<tr>
<td></td>
<td>TMD treatment</td>
<td>92.0(5.2)x&lt;sup&gt;LD&lt;/sup&gt;</td>
<td>92.2(5.0)x&lt;sup&gt;KE&lt;/sup&gt;</td>
</tr>
<tr>
<td>EDTA/NaOCl</td>
<td>No treatment</td>
<td>0</td>
<td>97.0(2.2)</td>
</tr>
<tr>
<td></td>
<td>TMD treatment</td>
<td>91.0(5.5)x&lt;sup&gt;LD&lt;/sup&gt;</td>
<td>98.3(2.0)x&lt;sup&gt;PF&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Significant differences in each row were represented by different lowercase superscript letters and those in each column by different uppercase superscript letters (p<0.05).
Fig. 1  SEM micrographs of dentin surface and dentinal tubules in EDTA pretreatment (a, c) and EDTA/NaOCl pretreatment (b, d) before immersion in AS. (a) EDTA pretreatment exposed the collagen fibrils (white arrow) on dentin surface. (b) EDTA/NaOCl pretreatment caused the patent dentinal tubules (white arrow). (c, d) After application of Teethmate Desensitizer, the layer of desensitizer (hand pointers) covered the dentin surface and occluded the dentinal tubules as well.

Fig. 2  SEM micrographs of dentin surface and dentinal tubules in EDTA pretreatment (a, c) and EDTA/NaOCl pretreatment (b, d) after 4-week immersion in AS. (a) EDTA pretreatment exhibited small amount of crystallites (white arrow) in dentinal tubules. (b) EDTA/NaOCl pretreatment showed the large amount of crystallites (white arrows) deposited on dentin surface and in dentinal tubules, which could be attributed to the AS penetration from both upper and lower sides of dentin disc, contributing to the dramatic increase in PR% upon the immersion. (c) TMD applied on EDTA-treated dentin promoted the deposition of crystallites (hand pointer) on the calcium-phosphate rich layer (blank arrow) upon AS immersion. (d) TMD application on EDTA/NaOCl-treated dentin resulted in the deposition of crystallites (hand pointer) over the calcium-phosphate rich layer (blank arrow) upon AS immersion, but only a deposition of few crystallites was found in the dentinal tubules (white arrow), indicating that the sustained or increased PR% in TMD treated groups was mainly attributed to the tubule occlusion by the TMD material itself.
which appeared to completely occlude dentinal tubules (Fig. 1c, d).

After 4-week AS immersion, control group of EDTA pretreatment exhibited limited formation of new crystallites deposited in some dentinal tubules (Fig. 2a), whereas EDTA/NaOCl pretreatment showed remarkable deposition of crystallites on dentin surface and in dentinal tubules (Fig. 2b). Both TMD treated groups revealed abundant deposition of newly formed crystallites on the desensitizer layer which remained on the dentin surface (Fig. 2c, d).

**Integration test**

After the TMD treated discs were vibrated in ultrasonicator for 10 min, the desensitizer layer was partially observed on dentin surface as well as within some dentinal tubules in both pretreatment groups (Fig. 3a, b). After 1-week AS immersion, the control (non-desensitizer treated) dentin showed the deposition of the crystallites in a similar manner as that of 4-week immersion (Fig. 2a, b), but in lesser extent (data not shown). However, after ultrasonication, these deposited crystallites were completely lost from the surface and dentinal tubules in the control group (data not shown). After 1-week of immersion in AS, in TMD treated groups without ultrasonication, the desensitizer layer and newly formed crystallites were observed regardless of dentin surface pretreatment (Fig. 3c, d). However, after ultrasonication, a dense layer was found on the dentin surface, sealing around the dentinal tubules with deposited islets (Fig. 3e, f).

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**Fig. 3**  SEM-micrographs of TMD treated dentin in EDTA pretreatment (a, c, e) and EDTA/NaOCl pretreatment (b, d, f). (a, b) Immediately after desensitizer application, ultrasonication resulted in partial removal of the calcium-phosphate rich layer. Some parts remained covered by the desensitizer (hand pointers) and dentinal tubules occasionally occluded (white arrows). (c, d) The 1-week AS immersion showed the deposition of crystallites (hand pointers) on the calcium-phosphate rich layer of the desensitizer (white arrows) which covered dentin surface. (e, f) The 1-week AS immersion followed by the ultrasonication revealed the integration of the calcium-phosphate rich layer of desensitizer on dentin surface as well as in dentinal tubules (white arrows), occasionally with islets remaining over dentinal tubules (blank arrows).
DISCUSSION

The dentin permeability measurement derived from the hydraulic conductance accompanying with the examination using SEM to visualize surface deposits and tubule occlusion are essential to evaluate the effectiveness of desensitizers\(^{17}\). Obtained data from both assessment methods revealed that the exposure of collagen fibrils on the dentin surface did not affect deposition or penetration of the desensitizer into dentinal tubules (Fig. 1 and Table 3). TMD showed an excellent immediate permeability reduction, regardless of surface characteristics.

The effectiveness of TMD in forming a layer on dentin regardless of pretreatment and maintaining tubule occlusion should be attributed to its chemical composition. TMD consists of TTCP and DCPA as the major starting components. The mixing of these two components provided a thick paste which could penetrate into the dentinal tubules (Fig. 1c, d) by mean of scrubbing on dry dentin surface. This occluding effect resulted in the immediate dentinal permeability reduction and, hence, clinical hypersensitivity reduction could be expected. Previous studies on combination of reduction and, hence, clinical hypersensitivity reduction effect resulted in the immediate dentinal permeability of scrubbing on dry dentin surface. This occluding penetrate into the dentinal tubules (Fig. 1c, d) by mean of two components provided a thick paste which could be formed due to the supersaturation of AS with respect to HA\(^{13}\), precipitation occurred solely along the outer surface of dentin. Moreover, it has been reported that the EDTA-extractable phosphoprotein which was found floating within the EDTA-treated dentinal matrices was analogous to free phosphoprotein in solution. This free phosphoprotein could act as an inhibitor for nucleation and crystal growth of HA on collagen\(^{26,27}\). Nevertheless, application of TMD on EDTA-pretreated dentin accompanied by physical agitation could transform the surface and result in deposition of a calcium-phosphate rich layer that induced crystallite growth upon immersion in AS, and maintained tubule sealing and PR\% of dentin.

The ultrasonication in distilled water was performed in this study to investigate the integration between the calcium-phosphate rich layer of TMD and the dentin surface\(^{28}\). The integration in this context was defined as the incorporation of the calcium-phosphate rich layer of TMD into the dentin surface and fusion between the two substrates. Ultrasonication, using the power and time parameters employed in the present study, could remove almost all of the smear layer and completely remove smear plugs as confirmed by SEM micrographs in the preliminary examination (data not shown). By taking into consideration that acoustic energy generating low- and high-pressure waves alternately in liquids would lead to the formation of strong hydrodynamic shear-forces\(^{29}\), partial remaining of the calcium-phosphate rich layer may imply its shear resistance immediately after application of TMD on dentin surface. Although the present study did not directly investigate the chemical interaction between the calcium-phosphate rich layer and dentin surface, the findings suggested that the calcium-phosphate rich layer of TMD had interacted closely with dentin surface. This finding was in line with the report showing that the calcium-phosphate based bioactive materials can effectively form a chemical bond with dentin tissue\(^{30}\). More interestingly, the significant resistance of the deposits against ultrasonication challenge after one week of AS immersion suggested maturation of the mineral substance by ions from the solutions and development of stronger bonds through time that ensured sealing of the dentin.

It should be noted that the occlusion in dentinal tubules of TMD was shown in the AS-immersed groups, even after being subjected to permeability test, where the high pressure of water was used pass through the dentinal tubules or after strong shear-forced ultrasonication. These results could be attributed to the effect of the potential chemical bond and the AS facilitating the maturation of TMD. The AS used in this study was simulated following the calcium and phosphate concentration similar to the human saliva\(^{31}\). Therefore, the results regarding the long-term maintained tubule sealing occurred in this in vitro study is expected to exist in the intra-oral condition under pulpal pressure. Nevertheless, clinical trials are necessary to confirm the assumption.

From present study, the permeability reduction and the stability properties of TMD in vitro were declared. However, there are other properties desired for a desensitizer to reach the best performance under
oral conditions including resistance against abrasion. Further studies are required to determine the clinical success of the newly introduced material.

CONCLUSIONS

Teethmate Desensitizer showed a great potential to reduce dentin permeability immediately after application, and developed an effective integration to the dentin surface after immersion in AS regardless of the dentin surface characteristic. It is expected to be a new generation of desensitizers forming a stable calcium-phosphate rich layer and enhancing the calcification under oral conditions.

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REFERENCES