Long-Term In Vitro Effectiveness of A Bioglass Desensitizing Agent Investigated Using Electrochemical Impedance Spectroscopy, Atomic Force Microscopy and Scanning Electron Microscopy

Shuya Shi1, Q Wu2, YT Xu3 and Yaming Chen4
1Department of Stomatody, Affiliated Hospital of Jiangnan University, China
2Department of Polyclinic, Affiliated hospital of Stomatology, Nanjing Medical University, Nanjing, China
3Department of Endodontics, Suzhou Stomatological Hospital, China,
4Institute of Stomatology, Nanjing Medical University, Nanjing, China

Abstract

Objectives: To compare the long-term in vitro effectiveness of a newer desensitizing agent containing bioactive glass and two other commercial products employed for dentin hypersensitivity.

Methods: Fifty occlusal dentin disks from extracted sound human third molars were treated with 0.5M ethylenediaminetetraacetic acid for 2 min then randomly divided into five groups (n=10). Specimens were brushed for 2 min twice daily with Actimins Paste (Group1), Lesening Super Desensitizing Toothpaste (Group2), Colgate Sensitive Pro-Relief Desensitising Polishing Paste (Group3) and distilled water (Group4) at 9:00 am and at 5:00 pm. Specimens in Group5 had no brushings. All specimens were immersed sequentially for 5 min into coffee (pH=5.4) at 10:00 am and undiluted cola (pH=2.5) at 6:00 pm. Dentin permeability was measured weekly over one month using electrochemical impedance spectroscopy (EIS). Data were analyzed using two-way repeated measures ANOVA and post hoc LSD tests. Dentin microstructures were observed at one month by atomic force microscopy and scanning electron microscopy.

Results: The three desensitizing agents significantly reduced dentin permeability over one month. Post hoc LSD tests of the EIS values indicated that brushing with Actimins showed no significant difference from Colgate Sensitive (P=0.32), and that both agents had significantly less permeability than Lesening (P<0.05).

Conclusions: The three desensitizing agents were effective in occluding dentinal tubules and reducing permeability to varying extents over one month, with Actimins and Colgate Sensitive being the most effective. Actimins might be initially, a faster-working desensitizing agent.

Clinical significance: Actimins, a newer desensitizing agent containing bioactive glass, has the potential to relieve dentin hypersensitivity in the presence of long-term daily acidic beverage ingestion.

Keywords: Bioactive glass; Dentin hypersensitivity; Dentin permeability; Electrochemical impedance spectroscopy; Atomic force microscopy

Introduction

Dentin hypersensitivity (DH) has been characterized as a brief, sharp pain that occurs in response to thermal, evaporative, tactile, osmotic or chemical stimuli that may not be attributed to any form of dental defect or pathosis [1,2]. DH is closely bound up with the exposure of dentinal tubules as a result of enamel loss and/or gingival root surface exposure [1,2]. Many factors may contribute to the exposure of dentinal tubules, such as occlusal wear, abrasion, dietary erosion, parafunctional habits, gingival recession, aging, chronic periodontal disease, tooth abnormally positioned in the arch, periodontal surgery, incorrect tooth-brushing habits, root preparation and abfraction lesions [1-3].

The hydrodynamic theory was proposed as the basis for dentin hypersensitivity, which is caused by movement of fluid within open dentinal tubules [4]. Therefore, occlusion of the tubules should diminish fluid movements within them and the clinical symptoms of DH, accordingly [5].

Many topically applied materials and treatments can produce promising short-term dentin desensitization [6]. And, if daily brushing with a desensitizing agent blocks exposed open dentinal tubules, this method should be an effective way to treat the common painful symptoms of DH. However, if the occlusion of the tubule orifices is only superficial, then daily tooth-brushing abrasion or exposure to acidic beverages may re-open the tubules, resulting in only a short-term desensitizing effect [7,8]. Some in vitro studies have found that the risk for DH may increase with the presence of dietary acids [9]. Therefore, an ideal product for treating DH should not only reduce...
The application of a soluble bioactive glass with the potential to both occlude open dentinal tubules and to remineralize tooth structure offers a potential approach to the aforementioned problems. Bioactive glass was developed originally to stimulate the formation of new bone and was subsequently employed as a coating on orthopedic implants to promote adhesion between the implants and bone [11,12], and later to fill periodontal bone defects [13,14]. Recently, dentin desensitizing agents containing bioactive glass have been shown to have potential clinical efficacies for managing DH. A bioglass-containing toothpaste showed excellent in vitro dentinal tubule occlusive effects after brushing treatments and artificial saliva immersion [15], and the cytotoxicity testing of several bioactive glass pastes also indicated that they were highly biocompatible materials [16-18]. Although various desensitizing agents may be effective in reducing DH over a short period, little information is available on their long-term effectiveness.

The purpose of the present in vitro study is to compare the long-term effectiveness of a newer dentin desensitizing agent containing bioactive glass with two other commercial products used to treat dentin hypersensitivity. The null hypothesis proposed is that after one month of daily applications by brushing, the bioactive glass agent is no more effective for the occlusion of dentinal tubules than the other two commercial products.

### Materials and Methods

#### Dentin specimen preparation

Freshly extracted intact human third molars of Chinese adult patients, who were all living in the Nanjing non-fluoridated water supply region, were obtained from the affiliated Stomatological Hospital of Nanjing Medical University. The study was approved by the Ethics Committee of Nanjing Medical University, and the informed consent of the patients was obtained. All teeth were thoroughly cleaned, inspected carefully for any defects using a stereoscopic microscope at 10x magnification, and stored until required at 4°C in distilled water containing 0.5% thymol to inhibit microbial growth. Fifty selected teeth were sectioned perpendicular to their long axes 2.0-3.0 mm coronal to the buccal cemento-enamel junctions, to obtain single occlusal dentin disks with a thickness of approximately 1.0 mm, using a water-cooled diamond saw (Isomet Low Speed Saw, Buehler.
No coronal enamel or pulp horn exposures were present in the specimens.

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Active ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actimins Paste</td>
<td>Datsing Bio-Tech Co., Ltd, Beijing, China</td>
<td>Calcium phosphosilicate, silicon dioxide, plant glycerin</td>
</tr>
<tr>
<td>Lesening Super Desensitizing Toothpaste</td>
<td>Dencare Corp. Ltd, Chongqing, China</td>
<td>Potassium nitrate, strontium chloride hexahydrate</td>
</tr>
<tr>
<td>Colgate Sensitive Pro-Relief Desensitising Polishing Paste</td>
<td>Colgate-Palmolive Co., Guangzhou, China</td>
<td>Arginine, calcium carbonate, sodium monofluorophosphate</td>
</tr>
</tbody>
</table>

Table 1: Desensitization products employed, manufacturers and active ingredients.

Experimental procedures

The occlusal surfaces of all 50 dentin specimens were sanded with 600-grit silicon carbide paper for 30 s under continuous water irrigation before they were immersed into 0.5 M ethylenediaminetetraacetic acid (EDTA) solution (pH=7.4) for 2 min to remove the standardized smear layer produced, and then gently rinsed with copious amounts of distilled water. Specimens were allocated using a table of random numbers to five groups (n=10), and each brushed daily for 2 min at 9:00 am and at 5:00 pm with either Actimins Paste (Group 1), Lesening Super Desensitizing Toothpaste (Group 2), Colgate Sensitive Pro-Relief Desensitising Polishing Paste (Group 3) or distilled water (Group 4). Two electric tooth brushes with bristles (Colgate 360°, Colgate-Palmolive Co.) were employed, the heads being replaced after 15 days. No brushing was used for the control specimens (Group 5). The manufacturers and active ingredients of the desensitizing agents used are shown in Table 1. After the 9:00 am brushings the specimens were immersed for 5 min in coffee (pH=5.4) at 10:00 am and, following the 5:00 pm brushings, the specimens were immersed for 5 min in undiluted cola drink (pH=2.5) at 6:00 pm (Figure 1). All specimens were placed into distilled water in an ultrasonic cleaner after the brushing treatments and acidic beverage immersions. The treatment cycle, using two beverages to simulate a common daily acid challenge, was repeated every day over one month. Between the treatment intervals, to simulate the oral environment, the specimens were stored in artificial saliva at 36.0°C (S.D. 1.0). The composition of the artificial saliva was 1.5 mM/L CaCl₂, 50 mM/L KCl, 0.9 mM/L KH₂PO₄ and 20 mM/L Tris (pH=7.4).

Electrochemical impedance spectroscopy (EIS)

EIS values for the dentin specimens were first measured after 2 d and then every 7 d during treatments using an Electrochemical Workstation (IM6e, ZAHNER-Elektrik GmbH & Co. KG, Kronach, Germany). The specimens were placed in separate split U-shaped chamber devices containing a pair of rubber O-rings (6 mm diameter), which resembled the hydraulic conductance device described and perfected by Pashley and coworkers [19]. A glassy carbon electrode was used for the working electrode (CHI1104, CHI Instruments, Inc., Austin, TX, USA) and a platinum electrode was used for the counter electrode (CHI120, CHI Instruments, Inc.), each inserted into their respective half-cell. The occlusal surfaces of the specimens faced at the working electrode. A Ag/AgCl electrode was adopted for the reference electrode (CHI111, CHI Instruments, Inc.). Both half-cells were filled with 0.1 M KCl solution as the electrolyte. The dentin specimens were first soaked in the electrolyte for 2 d to expel any trapped air and to aid conductivity before the EIS measurements were made. The electrolyte was changed each EIS measurement. A sinusoidal voltage signal with an amplitude of 10 mV was applied. EIS of the dentin specimens were measured at frequencies ranging from 0.1 Hz to 60 kHz to obtain an impedance spectrum in the Nyquist (complex plane) plot. Factors affecting impedance (kΩ) are the electrical resistance of the electrolyte solution, the resistance of the dentin specimens, and the resistance of the electrodes. Therefore, the dentin disk impedance R (d) equals the total impedance R(t) minus the impedance of the electrolyte R(e). And, at the frequency where the Z-value (alternating current impedance vector) is the lowest, the Z-value is close to that of R(t). R (e) was acquired by measuring the impedance of the 0.1 M KCl electrolyte alone, which was quite stable over 2-3 h.

Atomic force microscopy (AFM)

After one month of daily treatments, the dentin specimens were individually embedded in acrylic resin, polished sequentially with alumina polishing powders (1.00 μm, 0.30 μm and 0.05 μm), and then placed into distilled water in an ultrasonic cleaner for 30 min to clean them before AFM examination. The scanning probe microscope (CSPM 5000, Ben Yuan Ltd, Beijing, China) employed was used with the tapping mode, in which the probe periodically touched the specimen surfaces to produce higher quality images with fewer artifacts [20,21]. An AFM probe (AC160TS-C2, Olympus Corp., Tokyo, Japan) was employed with a nominal resonant frequency of ~300 kHz, and a nominal spring force constant of ~42 N/m. Images were obtained using a slow scan rate of ~1.5 Hz, at a chosen resolution of 512 × 512 pixels. Images could be obtained with a maximum vertical resolution of 0.1 nm and a lateral resolution of 0.2 nm. Quantitative information regarding the dentinal tubule diameters was obtained using the AMF image data analysis software provided by the manufacturer.

Scanning electron microscopy (SEM)

After one month of daily treatments, the same specimens were first rinsed with copious distilled water, dried in a desiccator and then sputter-coated with gold in a vacuum to aid conductivity before the dentin surfaces were examined using SEM. Micrographs of the dentin surfaces were obtained using a scanning electron microscope (JSM6300, JEOL, Tokyo, Japan) at an accelerating beam voltage of 20 kV.

Statistical analysis

Statistical analysis of the ETS data was performed using the Statistical Package for the Social Sciences V17.0 software (SPSS Inc., Chicago, IL, USA). After confirming the equality of the variances using Levene's test, any significant statistical differences among the groups for dentin impedance were determined by two-way repeated measures ANOVA and Fisher's post hoc Least Significant Difference (LSD) tests. The probability level for statistical significance was set at α=0.05.
Results

EIS evaluation

The results for the dentin disk impedances (kΩ) after demineralization and treatments with desensitizing agents and acidic beverages are shown in Table 2 and illustrated graphically in Figure 2. Two-way repeated measures ANOVA revealed significant main effects for time (F=7.42; P<0.05) and group (F=12.07; P <0.05). Post hoc LSD testing indicated that brushing with Actimins significantly reduced dentin permeability compared to all other treatments (P<0.05) except Colgate Sensitive Pro-Relief (P=0.32). Brushing with Actimins produced the highest EIS values among all groups at all time periods. Brushing with distilled water showed lower EIS values at all time periods than for no brushing, but the differences were not significant (P 0.25). Although brushing with Lesening Super Desensitizing generally showed higher EIS values at all time periods than for no brushing, the differences again were not significant (P=0.29).

AFM evaluation

Figure 3 shows selected AFM micrographs of topographical representations from Groups 1, 2 and 3 after one month of different treatments. Brushing with Actimins (A,B) resulted in a fairly homogenous granular dentin surface having the appearance of an artificial smear layer and showing relatively few open dentinal tubules with significantly reduced diameters and the most pronounced tubule orifice occlusions. Brushing with Lesening Super Desensitizing (C,D) and with Colgate Sensitive Pro-Relief (E,F) created a reduced prominence of the dentinal tubules, with reduced tubule orifice diameters and small granular deposits on the dentin surfaces and partially occluding the orifices, which effect appeared to be more slightly more pronounced for Colgate Sensitive. Brushing with distilled water only (G,H) and the no-brushing controls (I,J) showed large open dentinal tubule orifices, with slightly more dentin debris present on the dentin surfaces and pushed into the tubule orifices in the distilled water specimens.
Figure 3: Tapping mode AFM micrographs of specimen surfaces in Group 1 (A, B), Group 2 (C, D) and Group 3 (E, F) after one month of desensitizing treatments. The mean diameters of the dentinal tubule orifices became progressively wider from Group 1 (Actimins), to Group 3 (Colgate Sensitive Pro-Relief), to Group 2 (Lesening Super Desensitizing). The two micrographs in each group were chosen from two individual samples. Note: arrows show some completely occluded dentinal tubule orifices in (B). (Original image sizes 30 µm × 30 µm).
Table 2: Dentin disk impedance (Mean, Standard Deviation) after EDTA demineralization and treatments with desensitizing agents and acidic beverages at 2, 9, 16, 23 and 30 days.

<table>
<thead>
<tr>
<th>Treatment (n=10grp)</th>
<th>EDTA</th>
<th>2 days</th>
<th>9 days</th>
<th>16 days</th>
<th>23 days</th>
<th>30 days</th>
</tr>
</thead>
</table>
| Group 1 | 1.55 (0.51)
| 2.14 (0.34)
| 2.33 (0.43)
| 2.20 (0.52)
| 2.09 (0.47)
| 1.93 (0.49)
| Actimins |
| Group 2 | 1.48 (0.68)
| 1.83 (0.75)
| 1.93 (0.61)
| 1.64 (0.44)
| 1.62 (0.85)
| 1.46 (0.54)
| Lesening |
| Group 3 | 1.48 (0.63)
| 1.87 (0.81)
| 2.24 (0.62)
| 2.14 (0.68)
| 2.02 (0.57)
| 1.86 (0.56)
| Colgate S. |
| Dist. water | 1.55 (0.63)
| 1.88 (0.53)
| 1.79 (0.36)
| 1.26 (0.36)
| 1.10 (0.46)
| 0.98 (0.41)
| No brush. |

Discussion

Treatments for DH may be classified into chemical and physical methods [22]. Chemical agents include stonzon chloride, sodium fluoride, formalin solution [23], ferric oxalate [24], calcium hydroxide, stannous hydroxide, calcium oxalate, ferric phosphate [25] and potassium nitrate [26]. Physical methods have included the use of a fluoride-releasing resin [27] and lasers [28-30]. All these treatments have produced promising yet relatively short-lived therapeutic effects. After daily tooth brushing and mastication, or following their immersion in artificial saliva and acidic beverages, these agents were gradually abraded or solubilized and eventually lost their dental tubule occlusive effect [6]. Therefore, when appraising any treatment method employed for treating DH, its long-time effectiveness must be evaluated. Based on the hydrodynamic theory for pulpal pain [4,31], attention has focused more recently on the tubular occlusive action and dentin remineralizing ability of bioactive glasses.

EIS is a non-destructive and sensitive technique for evaluating the patency of dentinal tubules. EIS has been used also to assess the effects of acids on dentin permeability [19,32], the effectiveness of resin-dentin bonds [33], and the microleakage of resin composite restorations [34] and temporary restorations after root canal therapy [35]. Measurements of dentin permeability using EIS require the penetration of an electrolyte into the dentin specimen to make them conductive. The conductive properties and, hence, the impedance spectra obtained are characterized by the microstructure of the specimen. In the present study, the electrochemical impedance of a dentin disk specimen, at various alternating current frequencies, depends on the differences in potentials between the two half-cells on each side of the dentin disk and on the electrical current through the dentinal tubules [19]. Occlusion of the dentinal tubules leads to a decrease in hydraulic conductance (less fluid shift) and an increase in resistance to electrical currents (less ionic movement). The value will be low when the tubules are patent and high when the tubules are occluded [19].

AFM provides not only topographical images but accurate quantitative information (dimensions, profile, roughness and periodicity) of the specimen surfaces [36]. For investigations of biological specimens, the tapping mode is preferred to avoid surface damage caused by probing friction [37]. Because the fine polishing powders used in the present study were of small diameters (1.00 μm, 0.30 μm and 0.05 μm), and the mean diameter of the dentinal tubules from human third molars in young adults was reported to be approximately 1.21 (0.08) μm [38], it was critical to place the specimens in an ultrasonic cleaner for 30 min before commencing the AFM observations. By comparison with SEM, there are reduced artefacts, and the physical or chemical fixations and coating of the specimen surfaces by sputtering for better contrast and conductivity are not necessary.

The results from EIS showed that Actimins Paste containing bioactive glass created the lowest dentin permeability among the groups tested (Table 2). As a biocompatible material, bioactive glass has a high surface reactivity in physiological systems that can deposit hydroxyapatite, a mineral chemically similar to natural tooth mineral. Such reactivity happens through a series of interdependent sequential reactions. The gradual dissolution of bioglass occurs by hydrolysis of the silicate network [11]. Free silicate ions spontaneously adsorb onto the dentin surface and undergo condensation to form surface functional silanol groups (Si-OH), which act as heterogenic nucleation sites. Positively charged calcium ions are
then followed by increases in dentin permeability (Table 2). A previous negatively charged erosive challenges by hypersensitive teeth. And, in vitro, strontium was associated with the groups.

dentin permeability than was no brushing (P=0.29).

In the present study, brushing with Lesening was generally no more effective in reducing dentin permeability than was no brushing (P=0.29).

Colgate Sensitive toothpaste containing 8.0% arginine and 1450 ppm fluoride has demonstrated remarkable clinical reductions in DH [42]. The Pro-Argin formula dentifrice was highly effective in occluding dentinal tubules, and the arginine-containing dentin plugs could resist an acid challenge [45]. In the present in vitro study, Actimins and Colgate Sensitive had low and similar dentin permeability results (P=0.32).

After the initial EDTA challenge, brushing with distilled water and even no brushing produced short-term reductions in dentin permeability following the artificial saliva immersions, which were then followed by increases in dentin permeability (Table 2). A previous study found that brushing with distilled water would leave some dentin smear debris in the tubules [15]. And, as in the present study, it was reported that brushing after undiluted cola drink immersion also resulted in some dentin smear debris in the tubules and that "no significant morphological differences were observed from samples without toothbrushing" [9]. Artificial saliva is able to solubilize some particles from dentinal tubules to make the dentin more permeable [10]. However, during remineralization in artificial saliva, mineral salts may create crystal precipitates on the dentin surface, leading to a reduction in permeability [7]. Apart from the brushing treatments, the resulting permeability of the dentin would also reflect the demineralization and remineralization cycle treatments.

The presence of pulpal pressure in vital teeth may be an additional influence on the long-term maintenance by desensitizing agents of dentinal tubule occlusion. Therefore, in situ and in vitro studies are necessary to confirm the findings of this in vitro study.

Conclusions

The three tested tooth desensitizing agents showed long-term decreased permeability of occlusal dentin specimens to varying degrees. Material deposited in the dentinal tubules during daily tooth brushings largely resisted concomitant acid challenges and artificial saliva immersions over one month. Actimins Paste reduced dentin permeability rapidly, and had a larger but not significantly greater long-term reduction than Colgate Sensitive (P=0.32). Both agents showed significantly more reductions in permeability than Lesening (P<0.05). Therefore, the null hypothesis was accepted only for Colgate Sensitive.

Acknowledgements

The authors would like to thank Professors Zhiping Bai and Jianwei Zhao from Nanjing University for assisting with the EIS and the AFM measurements, respectively. We also appreciated the financial support received from A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, Project No.: 2011-137, and Research into dentin hypersensitivity and bioactive glass, Project No.: 22011-001.

References


