Abstract

• Objective: The purpose of this study was to compare the reactivity of three dentifrice formulations on smear layer-covered root dentin surfaces, and the effects of the formulation treatments on resistance to acid softening and dentinal tubuli disclosure.

• Methodology: Commercial dentifrices, including Crest® Cavity Protection Regular, Colgate® Total®, and a new dentifrice comprised of stannous fluoride/sodium hexametaphosphate (SnF2/HMP: Crest® Pro-Health™), were cycled through a pre-treatment period on smear layer-covered dentin surfaces, including intermittent soaking in dentifrice slurries and whole human saliva immersion. Following pre-treatments, the cycling treatments were modified to include dietary acid exposure, including soaks in an acidic soft drink. Vickers surface microhardness, variable pressure scanning electron microscopy (VP-SEM), and confocal laser scanning microscopy in reflection mode (CLSM) were used to characterize dentin reactivity and smear layer protection.

• Results: CLSM and SEM analyses showed that specimens treated with SnF2/HMP appeared to resist acid solubilization, evidenced by the absence of disclosed dentinal tubuli. The histo-tomographic observations in this study were in agreement with the hardness measurements. The superior surface protection of dentin with SnF2/HMP would suggest potential benefits in ameliorating dentinal hypersensitivity in the clinical situation.

• Conclusion: A stannous fluoride/sodium hexametaphosphate dentifrice prevents dietary acid softening and tubule exposure of smear layer dentin surfaces.

Introduction

Tooth (dentin or dentinal) sensitivity is a common condition.1 Epidemiological studies suggest that the percentage of the population which experiences sensitive teeth ranges somewhere between 8 and 30%, and the peak incidence has been reported to occur in 20 to 40-year-olds.2,3 Dentin sensitivity is characterized by patient experience of tooth pain (described as short, sharp, aching) arising from environmental stimuli, including thermal, tactile, or osmotic changes on the tooth surface.4 Although tooth (dental) sensitivity can develop as a result of numerous intraoral conditions, including chipped or cracked teeth, dental caries, marginal leakage of restorations, cracked cusps, or palatal/gingival grooves, the majority of patients who report tooth sensitivity present without structural damage to the tooth. Instead, these patients typically show signs of exposed root dentin as their most likely source of the condition.

Dentin hypersensitivity is unique in that it can affect people with both poor and good oral hygiene. Patients with poor oral hygiene may develop exposed dentin due to attachment loss of the gingiva associated with periodontal disease. Patients with enthusiastic hygiene may also develop exposed dentin due to brushing-induced gingival recession. While general hypersensitivity is not necessarily harmful to the dentition, the pain associated with the condition can be debilitating and the condition may have broader consequences, affecting nutrition and future oral hygiene activity.

Regardless of the source of the exposed root, the mechanism of dentinal sensitivity is believed to follow the so-called Bränström hydrodynamic model,5,6 which is supported by considerable empirical and fundamental evidence.1 In this model, the exposed root surfaces may contain open (exposed/disclosed) dentinal tubuli. These exposed channels provide a conduit for fluid movement in the dentin associated with pressure gradients induced on the tooth surface. Common environmental stimuli which may act to produce these gradients include abrupt temperature changes (hot or cold foods), tactile activity (rubbing on the dentin surface), or high osmotic pressures (foods/liquids with high salt or sugar content). The fluid movement serves as a stimulant that excites nerve terminals at the inner ends of the dentinal tubules or in the outer layers of the pulp. In this manner, external stimuli produce hypersensitivity sensory response.

With improved understanding of the cause and etiology of dentinal hypersensitivity, various technological approaches have been developed toward providing amelioration of symptoms, including strategies/ingredients applied in conventional oral products (toothpastes and mouthrinses). One important class of ingredients with proven efficacy toward controlling tooth hypersensitivity are those targeted at sealing dentinal surface tubuli. Successful physical blockage of tubuli and excellent clinical responses have been associated with dentist-applied dental restoratives and dental sealants.7,8 With respect to at-home treatments, incorporation of tubule-blocking ingredients in toothpastes has
also had a measure of success, with clinical efficacy reported for various formulations, including those containing stannous fluoride, strontium chloride salts, and a variety of oxalate preparations.9-12

Stannous fluoride formulations are attractive options for hypersensitivity control, with combined anticaries and antimicrobial benefits also reported for this fluoride source when applied in highly bioavailable formulations.13-15 Recently, stannous fluoride formulations have been developed containing highly bioavailable stannous fluoride in combination with sodium hexametaphosphate (HMP). HMP is a condensed polyphosphate molecule in the class of ingredients known as “calcium phosphate surface active builders,” whose inclusion in dentifrices has been shown to provide efficacy in the safe removal of dental stains and prevention of stain and calculus buildup between dental prophylaxes.16-18 Research has established clinical efficacy for SnF2/HMP formulations in the prevention of caries, calculus, gingivitis, and plaque formation.19-22

The purpose of this study was to examine a combination multi-benefit stannous fluoride/sodium hexametaphosphate dentifrice for its effects on root dentin smear layers in vitro, including the protection of root surfaces against dietary acid demineralization and smear layer solubilization, as a means to assess the potential of this technology for relief of dentinal hypersensitivity. The effects of this novel combination dentifrice have been compared with marketed control dentifrices, including a standard NaF dentifrice (Crest® Cavity Protection, Procter & Gamble Co., Cincinnati, OH, USA), with Vickers indents applied at 200 gram loading with five indentations made for each tooth specimen. Teeth were stored under refrigeration in saturated thymol solutions until preparation for testing. In these experiments, root sections from sound canine teeth were selected containing no evidence of restorations or dentinal caries. Rectangular sections, approximately 2 × 4 mm, were prepared under a water-cooled saw and mounted in methacrylate polymer. The natural root surfaces were lightly flattened with coarse grit, and a final smear layer was prepared by hand polishing (wet) with 300 grit aluminium oxide abrasive paper.

Materials and Methods

Tooth Preparation

Human teeth were collected by dentists and periodontists in the course of their typical practice in the Cincinnati region. This effort is part of a long-standing program of tooth preservation for P&G laboratory requirements. Teeth were stored under refrigeration in saturated thymol solutions until preparation for testing. In these experiments, root sections from sound canine teeth were selected containing no evidence of restorations or dentinal caries. Rectangular sections, approximately 2 × 4 mm, were prepared under a water-cooled saw and mounted in methacrylate polymer. The natural root surfaces were lightly flattened with coarse grit, and a final smear layer was prepared by hand polishing (wet) with 300 grit aluminium oxide abrasive paper.

Pre-Test Measures—Stratification

Root specimens were pre-measured for surface microhardness using a Buehler hardness tester (Buehler Ltd., Lake Bluff, IL, USA), with Vickers indent applied at 200 gram loading with five indentations made for each tooth specimen. Specimens were stratified 12/group based upon initial surface microhardness averages.

Phase A: Pre-Treatment Equilibration—Smear Layer Curing with Dentifrice Treatments

Following stratification, root dentin specimens were assigned to one of three dentifrice treatments:

- Sodium fluoride control dentifrice—Crest Cavity Protection
- Sodium fluoride test “all in one” dentifrice—Colgate Total: 0.243% NaF, silica abrasive, 0.3 % triclosan (antimicrobial), 2.0 % Gantrez (copolymer of methyl vinyl ether and maleic anhydride, anticalculus ingredient, delivery coating for triclosan retention; negative control);
- SnF2/Na Hexametaphosphate (SnF2/HMP) Test Dentifrice—Crest Pro-Health: 0.454% stannous fluoride, silica abrasive, sodium hexametaphosphate (anticalculus ingredient, stain control ingredient; Procter & Gamble Co., Cincinnati, OH, USA).

Specimen equilibrations and treatments were carried out in multi-well cell culture plates. Prior to dentifrice treatments, root dentin specimens were equilibrated with pooled human saliva for 24 hours at 37°C, with gentle shaking (4 ml/specimen) to provide pellicle conditioning to root surfaces. Following this, specimens were subjected to cycling regimen Phase A dentifrice treatment for eight days.

Phase A Regimen

9:00 a.m.—Fresh stimulated whole human saliva (WHS)
10:00 a.m.—Dentifrice treatment (Specimens soak 60 seconds in dentifrice slurry of 25% wt/vol dentifrice + 75% wt/vol distilled water followed by a 2× wash with water)—followed by saliva
12:00 p.m.—Dentifrice treatment—followed by saliva
2:00 p.m.—Dentifrice treatment—followed by saliva to morning

Specimens were stored in WHS between treatments, overnight and on weekends. Following eight days of Phase A cycling, specimens were removed from saliva, air dried, and subjected to a second surface microhardness analysis. Two specimens were set aside to serve as controls for assessment of surface effects by microscopy.

Phase B Experiment: Smear Layer Resistance to Acid Softening and Tubule Exposure

Following hardness testing, specimens were re-equilibrated with pooled human saliva for 24 hours at 37°C, with gentle shaking to again provide pellicle conditioning to root surfaces. Phase A-treated specimens were subjected to cycling regimen Phase B dentifrice treatment for three days.

Phase B Regimen

9:00 a.m.—Fresh saliva
10:00 a.m.—Dentifrice treatment—followed by acid treatment (specimens soaked in 5 ml cola, gentle shaking for three minutes, followed by a 2× wash with water)
12:00 p.m.—Dentifrice treatment—followed by acid treatment
2:00 p.m.—Dentifrice treatment—followed by acid treatment

Specimens were stored in WHS between treatments overnight and on weekends. Following three days of Phase B acid modi-
fied/dentifrice treatment cycling, specimens were equilibrated overnight in WHS and then removed for analysis.

**Post-Phase B Analysis**

Specimens were dried and measured a final time for surface microhardness. Following hardness analysis, two specimens, representing the average hardness values, were rehydrated “as is” for later microscopic analyses. Tooth specimens were examined for surface histology under a Confocal Laser Scanning Microscope (CLSM). CLSM measures were carried out with air immersion objectives (50×; NA = 0.8) reflection mode on surfaces of specimens using a Leica TCS SP 2 CLSM (Leica, Mannheim, Germany), with illumination provided by a focused He/Ne laser at a wavelength of 632 nm. Surface morphology changes were also assessed by Variable Pressure Scanning Electron Microscopy (VP-SEM) on a LEO 435VP (LEO Elektronenmikroskopie GmbH, Oberkochen, Germany). In the variable pressure mode of the VP-SEM, specimens were examined without gold coating under a pressure of some 20 Pa. Thus, charging artifacts of the insulating specimens are avoided. The back-scattered electrons (BSE) were detected via a quadrant backscatter detector (QBSD) at a high voltage of 20kV and 250 pA probe current. In our experience, the micrograph from VP-SEM gives a better impression of the surface topography than the SEMs which are collected from the gold-coated samples (standard SEM).

**Statistical Analysis**

Microhardness measurements on various treatments were compared using Student’s t-test.

**Results**

Microhardness values obtained following stratification, Phase A and Phase B (acid resistance) treatments are shown in Table I. Immediately after stratification there were no significant differences in surface microhardness between specimens in each treatment group. Average hardness values were consistent with literature observations on sound root dentin. Following Phase A dentifrice/saliva cycling, microhardness values did not significantly change, though a slight trend for hardening was observed in Crest Cavity Protection and SnF<sub>2</sub>/HMP treatments. Following acid cycling/treatment regimen in Phase B, all treatment groups showed a significant loss in surface microhardness. The SnF<sub>2</sub>/HMP-treated specimens maintained the highest hardness values, significantly greater than specimens treated with either Crest Cavity Protection or Colgate Total dentifrice.

Figure 1 shows low magnification VP-SEM images of dentifrice-treated specimens before (Phase A) and after dietary acid cycling (Phase B), as well as same scale CLSM images after an acid challenge (Phase B). Independent from the dentifrice regimen, smear layers remained intact for all treatments during Phase A, and, in fact, surface deposits were developed on treated dentin as shown in the top row of the figure. In this magnification, Colgate Total and SnF<sub>2</sub>/HMP appear to retain a fairly uniform surface smear layer, whereas Crest Cavity Protection appears to add deposits to the smear layer to generate a rough, spotted surface with some debris on it. Following a dietary acid challenge (bottom two rows in figure) for Phase B, the Crest Cavity Protection and Colgate Total smear layers appear significantly solubilized. Within the Crest Cavity Protection-treated specimens there is faint evidence for remaining spot-like surface coatings. The VP-SEM image after SnF<sub>2</sub>/HMP treatments in Phase B cycling shows some faint hints at tubuli, but these have less contrast and make a diffuse appearance. We reasoned that the extended visualization depth (about 20 μm) of VP-SEM under the present conditions could be recording a modestly thin surface layer, as well the underlying dentine tubuli at that low magnification. To test this, we recorded images in CLSM reflection mode of identical specimens. This analysis, shown in the bottom row, illustrates that Crest Cavity Protection and Colgate Total dietary acid-cycled specimens show tubuli patterns practically identical with the VP-SEM images. The SnF<sub>2</sub>/HMP specimens in CLSM, on the other hand, did not show any evidence for open tubuli. These results supported the retention of a surface smear layer resistant to an acid challenge, as compared to the other groups.

**Table I**

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<tr>
<td>Crest Cavity Protection</td>
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<tr>
<td>Regular</td>
<td>56.2 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.7 ± 5.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.4 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Colgate Total</td>
<td>56.9 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.3 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.4 ± 8.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SnF&lt;sub&gt;2&lt;/sub&gt;/HMP</td>
<td>57.0 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.1 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.7 ± 8.9&lt;sup&gt;b&lt;/sup&gt;</td>
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Between period comparisons within dentifrice treatments: nsd = not statistically significantly different; sd = statistically significantly different p < 0.05 Students paired t; comparisons between treatments (within period): a<sup>i</sup> b<sup>j</sup>; p < 0.05 Students t.
of VP-SEM, the SnF$_2$/HMP-treated specimen appears totally different, as in Figure 1. There is clear evidence for a surface coating with only faint hints for fragments of dentinal tubuli. Again, same scale CLSM images complemented the VP-SEM visualization. Most interestingly, the high resolution CLSM reflection mode images of Regular Crest and Colgate Total dietary acid cycled specimens are almost identical with the same scale VP-SEM images. In the case of SnF$_2$/HMP, there is still a slight difference between the VP-SEM and the CLSM visualization. In CLSM, the surface (identical with low magnification) appears totally deposit-covered. The laser light reflected from the surface obviously can not “see” the tubuli as long as the deposit totally covers the surface area.

**Discussion**

The clinical observation of tooth hypersensitivity in patients is typically associated with exposed root surfaces containing open (e.g., exposed or disclosed) dentinal tubuli. These channels provide an easy conduit for fluid movement in the dentin associated with pressure gradients induced by thermal, tactile, or osmotic stimuli. The fluid movement serves as a stimulant to nerve fibers. In this manner, external stimuli produce the familiar, painful hypersensitivity sensory response. One important class of technologies directed toward controlling tooth hypersensitivity includes ingredients targeted to seal surface tubuli. Indeed, physical blockage of tubuli associated with stannous fluoride, oxalate salts, dental restoratives, and dental sealants have proven effective in providing clinical relief of tooth sensitivity in patients.

From a mechanism point of view, the effective blocking of dentinal tubules may occur on surfaces with exposed tubuli and/or on surfaces with smear layers of dentin. The former reactivity may ameliorate existing sensitivity response, while the latter reactivity may provide a means to prevent reoccurrence of sensitivity. Deposits produced in exposed tubuli and/or in smear layers are ideally of low solubility in low pH environments, thereby providing a resistance of these layers to secondary disclosure through dietary acid challenges, which in combination with gingival recession, are known to be the environmental factors encouraging the development of sensitive teeth.

The provision of chemotherapeutic hypersensitivity relief in toothpaste formulations is complicated by the strong cleansing actions of these formulations, which can act in opposition to the formation and retention of “smear layer plugs.” The experiments described here sought to determine the relative efficacy of three dentifrice treatments toward producing smear layers resistant to acid solubilization. In Phase A of the experiment, smear layer-covered dentin was treated with topical slurries of dentifrices to permit mineral conditioning through chemical actions. Results show that all three pastes were effective in treating smear layer-coated dentinal tubuli without removing the smear layer through the dispersive actions of product surfactants or chelants. Colgate Total and SnF$_2$/HMP treatments appeared to be more effective in keeping dentin surfaces cleaner, consistent with the antibacterial and antitartar benefits of these products.

Phase B of the experiment was directed toward exploring whether dentifrice slurry treatments of smear layers would confer protective benefits against acid solubilization and tubule disclosure from dietary acid challenges. Following dietary acid treatment (represented generically by cola exposure), a clear differentiation of treatment efficacy was observed. Specimens treated with SnF$_2$/HMP appeared to resist acid solubilization, evidenced by the maintenance of greater surface microhardness and by the absence of disclosed dentinal tubuli (VP-SEM/CLSM). The superior surface protection of root dentin with SnF$_2$/HMP would suggest potential benefits in ameliorating dentinal hypersensitivity in the clinical situation. In fact, SnF$_2$/HMP dentifrice has been clinically shown to be effective in reducing tooth hypersensitivity in comparison to Crest Regular NaF dentifrice, as described by Schiff and co-workers.

SnF$_2$ dentifrices and gels may be expected to provide unique acid protection benefits to dentin surfaces, owing to the provision of insoluble layers of stannous salts on topical applications. The maintenance of this reactivity in a “high cleaning” multi-benefit dentifrice containing a whitening/tartar control ingredient such as hexametaphosphate is a unique discovery. The provision of hypersensitivity benefits through unique dentin chemistries is an additional benefit of topical applications of SnF$_2$/HMP dentifrice, complementing efficacy in reducing caries, gingivitis, plaque, and oral malodor.

These studies were also instructional from an analytical point of view. Here, the action of surface treatments on smear layer-
covered dentin was investigated by techniques of visualization using solely wet (non-fixed) samples. Our own experience and numerous literature citations caution against the dangers of specimen fixation and the use of dry samples when experiments focus on the structure and behavior of dentin and dental smear layers, respectively. Regardless of fixing techniques and/or drying procedures for high vacuum SEM, studies may bear the risk of artifacts due to the collapse of their structure by evaporation. Sample (e.g., gold) coating for avoiding charging effects in a high vacuum may additionally affect the structure of biological samples. Therefore, we concentrated on wet, non-fixed “native” samples in VP-SEM and CLSM examinations. Variable pressure electron microscopy generally provides high lateral resolution images of the outermost surface structures of tooth surfaces. It must be considered, however, that the in-depth information of SEM, depending on the instrument settings (in particular high voltage) and the material to be studied, may extend down to as much as 10 micrometers. That effect might be neglected as long as the materials to be investigated are homogenous. As soon as the surfaces are built up inhomogeneously (e.g., deposits), the VP-SEM information comprises varying in-depth structures or varying in-depth compositions. In consequence, the images obtained are average information and may be considered as overlays between different materials or layers. For these experiments, the combined analyses proved that deposits by dentifrice treatments might be thin enough, in particular when partially removed by dietary acid cycling, that SEM might “see” the underlying tubular dentin structure as well, leading to results that might be interpreted inconsistently. In this context, the combination of CLSM and VP-SEM analysis techniques were invaluable. Focused laser light used for CLSM illumination is reflected from non-translucent surfaces, and therefore might not penetrate by dietary acid cycling, that SEM might “see” the underlying tubular dentin structure as well, leading to results that might be interpreted inconsistently. In this context, the combination of CLSM and VP-SEM analysis techniques were invaluable. Focused laser light used for CLSM illumination is reflected from non-translucent surfaces, and therefore might not penetrate through dentifrice-generated surface deposits. Only supplementary reflection mode CLSM could prove the existence of a surface covering deposit with no tubuli left open for the thin smear layers, as contrasted with lower magnification VP-SEM. Clearly, the combined applications of VP-SEM and CLSM are attractive in studying surface structures of dentin and smear layers.

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References