Novel Materials for Desensitizing and Remineralizing Dentifrices

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ABSTRACT

Titanium dioxide (TiO_2), calcium bentonite, and sodium alginate are three non-toxic materials have been explored as components of novel dentifrices capable of occlusion of dentin tubules. Chondroitin sulphate (ChS) has been shown to be a better surface modifier than caffeic acid (CA), for increasing affinity of TiO_2 to the dentin surface. Calcium bentonite, another constituent of existing toothpastes, has significantly higher affinity to dentin in the presence of external Ca^{2+} ions. The sequential treatment of dentin by sodium alginate and Ca^{2+} ions was proved to be another efficient method for the tubule occlusion, based on the cross-linking mechanism. The latter two materials are also promising vehicles for the calcium delivery to the tooth surface, essential for remineralization, including the biopolymere-templated bone restoration. Utilizing scanning electron microscopy (SEM), we qualitatively assessed both adhesion of the new compositions to dentin, and their ability to occlude dentin tubules. The deposited materials persisted on the dentin surface even after 30 s sonication, which makes them good candidates for desensitizing and antibacterial compositions, whose pharmaceutical effect will be linked to their ability to occlude dentin tubules.

Keywords: bentonite, hypersensitivity, occlusion, remineralization, cross-linking, alginate, titania

1 INTRODUCTION

Dentinal hypersensitivity arises from the exposure of dental tubules to saliva and the subsequent demineralization as a consequence of decaying enamel or receding gum line [1,2]. Its prevalence in recent years is due to the heightened longevity of oral biominerals (teeth) as a result of increased oral health [3,4,5]. For decades, the primary goal of marketed dentifrices have been to combat the demineralizing effects of acidogenic bacteria and a low pH diet [3]. Recently, the efforts for treating tooth hypersensitivity have focused on the occlusion of dentin tubules, connected to mechanoreptors, via solid materials adhesion that can be directly observed by SEM imaging [2]. This specific adhesion or bonding may involve delivery of components in the adherant to tooth surface [6]. This inspired us to focus on the tubule ovvlusive materials able to promote remineralization as well.

It was found that compositions of hydroxyapatite and silica nanoparticles are able to infiltrate demineralized dentin, but not occlude the tubules [7]. Apparently, adhesion of particles to dentin has to be the first necessary step of occlusion, which can then progress to several contributing mechanisms: 1) aggregation of particles; 2) triggering deposition of other insoluble materials in the tubules; 3) delivering their components to the tooth surface. Exploration of the effect of the particle types and their surface functionalization on the relative contributions of those mechanisms is critical for the design of novel desensitizing, antibacterial, and remineralizing materials. Our work focuses on activation of the existing dentifrice excipients (used for tooth polishing, composition texturing, etc) by their surface functionalizations, which allows to curcumvent many possible toxicity and sefety issues. Harnessing of the approved food chemicals further helps in pursuing this approach. We characterized interaction of both modified and unmodified toothpaste encipients with human dentin through examination of the morphological or structural changes of the dentin surface via SEM [8].

2 MATERIALS AND METHODS

Caffeic acid (CA) was purchased from Alfa Aesar, chondroitin sulfate (ChS) and sodium alginate (alginate) from Bioalternatives, $CaCl_2$ and titanium dioxide (rutile) from Fisher Scientific (Cat. No. 79586), and calcium bentonite (bentonite) was obtained from Aztec Secret Health & Beauty LTD. These experiments were conducted with a Sigma Zeiss Field Emission Scanning Electron Microscope (SEM) and a 28H Neytech ULTRAsonik Cleaner.

2.1 Preparation of Functionalized TiO_2 Nanoparticles

Nanoparticles of caffeic acid TiO_2 conjugate (TiO_2-CA) were synthesized as we previously reported [8]. In a similar manner, a stirred mixture of titanium dioxide (320 mg, 0.6 mmol) and chondroitin sulfate (ChS; 200 mg, ~ 0.4 mmol of repeating units) was refluxed in 5 mL of deionized water for 2 h, cooled to room temperature, and stirred overnight for 16 h. The suspension was centrifuged (3,000 rpm, 20 min). The supernatant was decanted, and the precipitate was resuspended in 2 mL H_2O. The prepared after another centrifugation (2,000 rpm, 5 min) precipitate was stored in 5 mL of fresh nanopure water preceeding paste formulation.

2.2 Formulation of Calcium-Loaded Bentonite Pastes

Calcium bentonite and $CaCl_2$ were ground and mixed in 1:2 and 1:4 weight ratios, respectively. To the mixture, nanopure water was added until the consistency of a thick slurry was obtained. The resultant formulations were heated to 37 °C prior application.

2.3 Preparation of Dentin Samples

Dentin segments were obtained from healthy adults with informed consent, through caries-free human molars extracted by Dr. Joshua Brower at *Smiles for Siouxland*. The teeth were rinsed and the outer enamel layer was removed using a dental bur. Samples were then wrapped in a paper towel and mechanically split with a hammer into segments about 2X2 mm. To remove smear layer and expose tubules, dentin specimens were sonicated in water for 20 s and drained, repeatedly, until water was no longer turbid. This protocol excludes inadvertent surface modification by common acid etchants that affect the dentin's affinity to nanoparticles. Samples were then dried at 100°C overnight and stored at 37°C until treatment.

2.4 Dentin Treatments Protocols

Upon selection, specimens were rinsed for 5 seconds with nanopure water. Paste-like materials were then applied, following adequate preparation, and gently spread on the dentin surfaces upon a glass slide for 2.0 min to simulate a brushing technique. Persistence of deposition and occlusion was tested by subsequent sonication for 30 s. Sodium alginate was applied by submersing a dentin section into a 1% alginate solution (pH 6) for 30 s followed by brushing for 10 s, followd by another submersion and 2 min of brushing. For cross-linking, the samples were next submersed in a $CaCl_2$ aqueous solution for 30 s while shaking (30 rmp). All specimens were then gently washed with nanopure water for 5 s, stored overnight, and dried in an oven for 3 h prior to SEM analysis. Each experiment was performed twice to ensure consistency of results.

2.5 SEM Analysis

Dentin tubule occlusion and material deposition was evaluated both before and after treatment by the tested material. Occlusion was evaluated in three separate locations on each batch of two specimens. The most characteristic SEM micrographs are presented in Fig. 1.

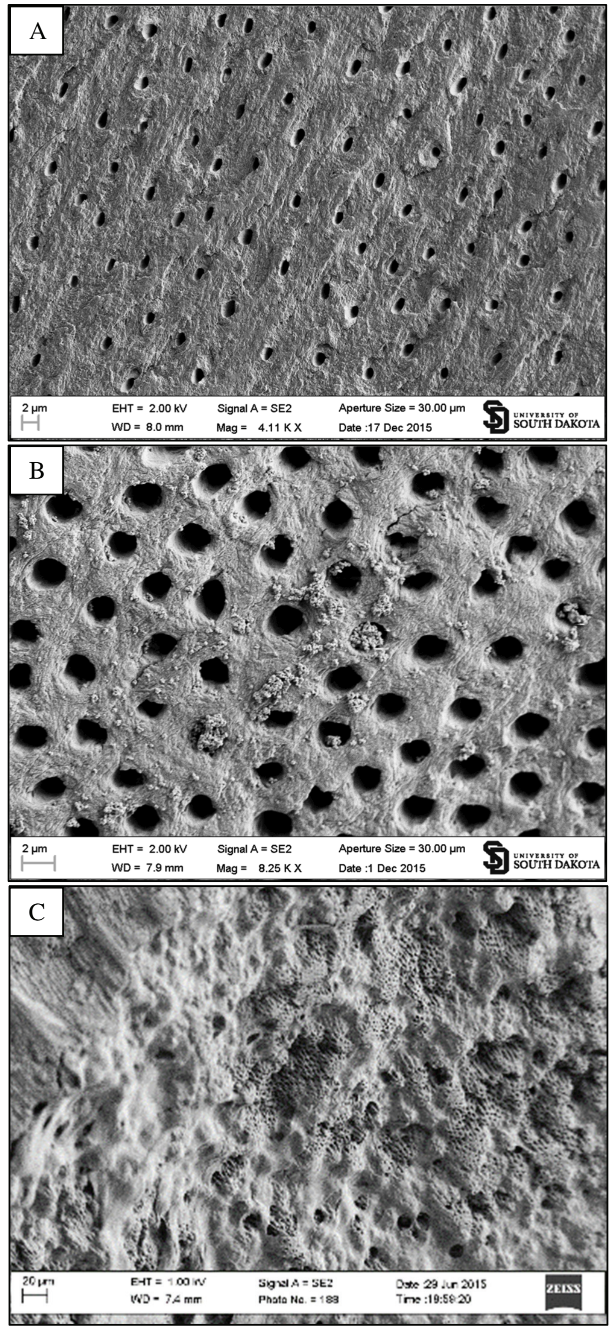

Fig. 1: SEM micrographs. (A) Dentin before treatment. (B) Dentin after treatment with TiO_2-ChS after sonication for 30 s. (C) Dentin after sequintial treatment with sodium alginate and calcium chloride.

3 RESULTS AND DISCUSSION

It is known that even slight occlusion of dental tubules mitigates dentinal hypersensitivity by consequential reduction in fluid flow pressure changes due to a variety of external stimuli [2, 9]. This begets hydroxyapatite (HA), the major component of human dentin and almost sole component of enamel, as the primary target of any classical homogeneous remineralization process. HA is known to bind with carboxyl groups of amino acids through directed interactions with salivary proteins [10,11, 12]. Furthermore, it is known that bacteria adhere to the tooth surface by conjugation with salivary phosphoproteins, after they are attached to hydroxyapatite [13]. These oral infections could possibly be prevented by interfering with adhesion of microorganisms to the tooth surface. Consequently, we have investigated the interactions of dentine samples with modified toothpaste excipients including functionalized TiO_2 nanoparticles, calcium-laden bentonite. The second approch was based on the sequential treatment of dentin by the food component sodium alginate, next cross-linked by $CaCl_2$ during the second application. These materials with higher affinity to the dentin substrate have the potential to occlude dentin tubules and compete with bacteria for adhesion to the tooth surface.

3.1 The Effect of TiO_2 Functionalization on Dentin Distribution and Adhesion

We have previously demonstrated that surface functionalization of TiO_2 with caffeic acid increased its adhesion to dentin due to the interaction of carboxy-groups with the hydroxyapatite component of dentin [8]. Here, we report an alternative linker, chondroitin sulfate (ChS), which belongs to the class of sulfonated glycosaminoglycans. This moiety is naturally occuring as part of proteoglycans related to the negatively charged salivary glycoproteins, inherent in the naturally-formed dental pellicle [14,15]. We have taken advantage of the natural affinity of ChS to the tooth pelicle to transfer this affinity to ChS-encased particles of TiO_2. This material may suppress adhesion of acidogenic bacteria to the tooth surface due to the possible repulsion between the receptors of acidogenic bacteria and the negatively charged ChS-encased-TiO_2. Importantly, the deposited material has demonstrated some resistence to sonication challenge modeling the in-vivo challenges (Fig. 1b).

The naturally occuring ChS is already approved as a food supplement, and TiO_2 has been a component of commercially available common toothpastes for years, which significantly decreases safety concurns with the new material.

3.2 Sequential deposition of Sodium Alginate and $CaCl_2$

Next, we explored the ability of alginate (a polysaccharide that can chelate calcium ions) to soidify by the dentin surface after the treatment by a cross-linking reagent - $CaCl_2$. The approach has paved the way to the utilization of the Layer-by-Layer (LbL) deposition technique common for the thin film production [16, 17], Alginate is known to increase adhesion of calcium carbonate to dentin [18], which demonstrates its potential as a component for the dentin tubule occlusion by sequintial treatments. Variation of the concentration of calcium ions offer a high degree of control over the surface adhesion. We found that this cross-linked material deposited by the sequential treatment, did produce a robust coating of dentin resistant to sonication (Fig. 1c).

3.3 Adhesion of Calcium Bentonite to Dentin in the Presence of External Ca^{+2}

Bentonite, a phosphoaluminum silicate clay comprised primarily of montmorillonite $(Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O)$, is used in most homemade toothpaste formulations as both as buffering agent for oral acidity and as an abrasive [19, 20]. Bentonite is an intercalated system composed of tetrahedral, octahedral layesrs, and exchangeable interlayer cations within the octehedral lattice [21]. It is known that bentonites are excellent absorbents and ion-exchangers due to the isomorphic substitution of ions similar to that of hydroxyapatite [3, 21]. This makes bentonite an excellent tool for delivery of calcium to the tooth surface.

By comparison of bentonite laden with calcium chloride in the weight ratios of 1:2 and 1:4 respectively, we have demonstrated that the increased availability of external Ca^{2+} made the bentonite's coating of dentin more resilient to sonication following its application (Fig. 2 & 3). Replacing highly soluble $CaCl_2$ with much less soluble calcium citrate further decreased the adhesion, which is consistent with the observed trend. Further, the geometrical fit between the bentonite particle size (0.75-1.0 µm) and the dental tubule opening (2-3 µm) makes the bentonite-calcium combinations very promising leads as active ingridients of desensitizing dentifrices.

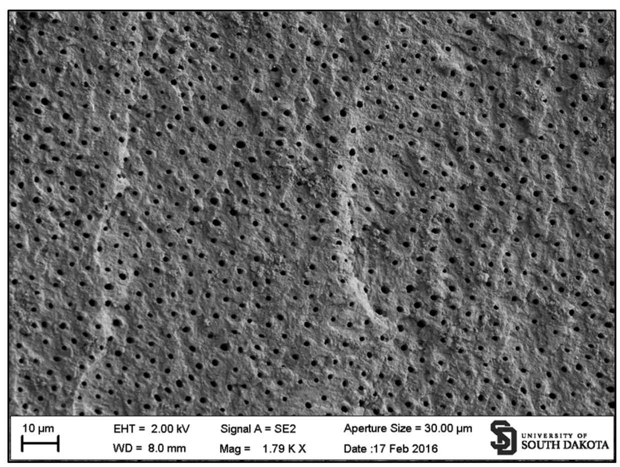

Fig. 2: SEM micrograph of calcium bentonite applied to the dentin substrate with no additional calcium.

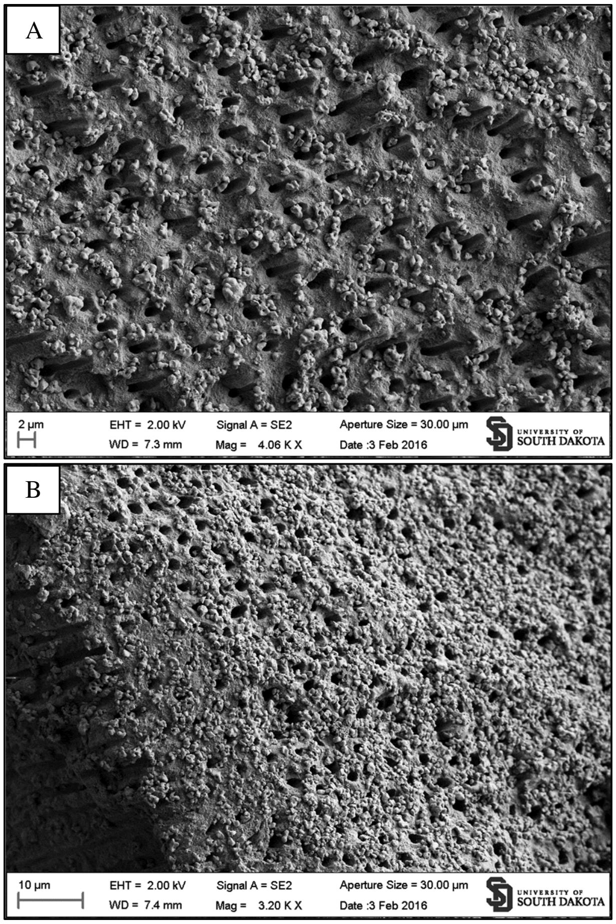

Fig. 3: SEM images comparing the effect of calcium to bentonite deposition. (A) Bentonite laden with x2 $CaCl_2$ (B) Bentonite loaded with x4 $CaCl_2$.

4 CONCLUSIONS

We have demonstrated that the excipients of existing toothpastes and food supplements can be harnessed for occlusion and possible remineralization of human dentin by their surface functionalization or cross-linking. The new compositions are promizing leads as components of desensitizing, anti-bacterial, and remineralizing dentifrices. Currently, we are expanding our research toward new biopolymers as components for the approach of sequential treatments, especially focusing on the LbL tequnique, shifting the scope of our search toward the natural components of food. Quality of the dental tubule occlusion will be evaluated by the X-ray computer tomography.

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