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Strontium effects on root dentin tubule occlusion and nanomechanical properties

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ABSTRACT

Objectives. Dentin hypersensitivity often is treated by promotion of dentin tubule occlusion. In this in vitro study we evaluated nanomechanical properties and degree of tubule occlusion conferred to sound and demineralized human root dentin following treatment with a 10% (w/w) strontium acetate solution and its relation to the treatment duration and delivery method.

Methods. 24 human cervical root dentin disks (8 groups of 3) were polished through 0.25 μm . 12 disks were subjected to an acid challenge (1% citric acid, pH3.8) for 2 min. The specimens were incubated in artificial saliva, treated by soaking or brushing with deionized (DI) water or a solution of 10% strontium acetate for 2 min twice a day for 28 days. The occlusion percent and nanomechanical properties were determined at the baseline, 5, 14 and 28 days. Cross-sectioned specimens were prepared to evaluate the depth affected by strontium acetate / dentin interaction by SEM. Statistical analysis was performed using linear mixed effects models.

Results. A 10% strontium acetate treatment over 5–28 days significantly increased tubule occlusion for normal root dentin and to a lesser extent for demineralized dentin and increased the AFM based nanomechanical properties of demineralized dentin. Brushing was more effective than soaking in recovery of properties of demineralized dentin when treated with strontium. No difference in tubule occlusion was found between the two delivery methods.

Significance. Strontium acetate itself proved to have the ability to occlude dentin tubules and result in small changes in the mechanical properties of dentin.

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1. Introduction

Dentin hypersensitivity has become a significant oral health concern in developed countries, where an increased number

of people retain their teeth longer [1] and people live longer. In the 20th century life expectancy increased almost 30 years [2]. Increased tooth tissue wear and loss due to abrasion, attrition, abfraction and erosion has become a greater concern. It is reported that non-carious tooth tissue loss occurred in up

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to 80% of children and in up to 43% of adults [3–5]. The Centers for Disease Control and Prevention (CDC) first added tooth wear as a part of the National Health and Nutrition Examination Survey (NHANES) in 2003–2004 [6]. Physical structure loss of the enamel, cementum or surrounding soft tissue will lead to exposed dentin. Once dentin is exposed it is subject to various physical and chemical challenges in the oral cavity. In most cases, sound (not caries affected) exposed dentin can adapt to the environment, probably by tubules naturally being occluded and asymptomatic. In some cases exposed dentin leads to dentin hypersensitivity. It is characterized by sharp short pain triggered by thermal, tactile, or chemical stimuli or evaporation, without any form of dental defect or pathology. There is no reported evidence of pulpal inflammation such as irreversible or reversible pulpitis [7,8]. Common histological findings are enlarged dentinal tubules that occur in areas with larger numbers of tubules per area, when compared with dentin without sensitivity [9,10].

Symptom management ranges from the use of over the counter products (OTC) containing active ingredients that either desensitize the nerve tissue at the base of the dentin tubule or by encouraging the promotion of dentin tubule occlusion. Professional treatments may also include fluoride varnish application to exposed root surfaces, placing a restoration, a gingival graft to cover the exposed roots, or even pulpotomy in severe cases. Improvement of dentin hypersensitivity symptoms is thought to be related to tubule occlusion and/or desensitizing the nerve. KNO_3 is the most commonly used active ingredient in OTC toothpastes. Pashley suggested KNO_3 works by potassium salt reducing the excitability of intradental nerves by altering the extracellular potassium concentration [11]. Occlusion agents are less widely employed but can be favored over nerve depolarization primarily due to the rapid onset of pain reduction associated with the occlusion approach [12]. Several chemicals such as fluoride help to deliver Ca and P into tubules [13], and strontium salts, either strontium chloride or strontium acetate, are found to be very effective in reducing dentin hypersensitivity [14]. Strontium also was reported to have the ability to improve radiodensity and if given prenatally it improves caries resistance [15,16].

Strontium has an atomic radius slightly larger than calcium, and readily substitutes for calcium in minerals. Strontium is known to be able to replace calcium in bone [17]. In the oral environment, strontium and other divalent cations with a similar charge-to-size ratio to calcium can readily substitute in the lattice of hydroxyapatite. Dedhiya et al. confirmed a calcium strontium apatite $\text{Ca}_6\text{Sr}_4(\text{PO}_4)_6(\text{OH})_2$ is formed by the substitution of intracrystalline calcium in apatite by strontium [18]. Gedalia et al. reported a significant increase of radiodensity after immersing sound and etched dentin in a 25% (w/w) strontium chloride solution for 24 h. Increased radiodensity was due to formation of Ca–Sr apatite [15]. Strontium has shown high affinity to dentin and apatite similar to other metals used for treating hypersensitivity such as zinc and tin.

Epidemiologically, Curzon et al. reported that there was low caries prevalence where the drinking water contains fluoride and strontium [19]. This synergistic effect of strontium

and fluoride was examined by Featherstone et al. [20]. They showed synthesized carbonated apatites precipitated with strontium and fluoride had improved crystallinity and markedly reduced acid reactivity. For low-carbonated apatite, strontium and fluoride together improved the property but fluoride alone did not improve crystallinity. Thuy et al. showed co-presence of appropriate levels of fluoride and strontium showed synergistic effects in remineralizing artificial enamel lesions [21]. These findings are the foundation of using strontium salts in remineralizing tooth paste [22,23].

Generally two approaches have been taken to evaluate the efficacy of proposed treatments. One is evaluating clinical pain improvement *in vivo*, the other is evaluating morphological or structural change *in situ* or *in vitro*. Reports are lacking on the modification of mechanical properties of dentin and tubule occlusion treated with occlusion agents purported to operate by a mineralization mechanism. It is possible that occlusion by mineralization could alter the nano-structure of dentin. In this *in vitro* study we evaluated nanomechanical properties and degree of tubule occlusion conferred to sound and demineralized human root dentin following treatment with a 10% (w/w) strontium acetate solution and its relation to the treatment duration (up to 28 days) and delivery method (brushed/soaked).

2. Materials and methods

Sound, single-rooted teeth were collected from subjects requiring extractions following a protocol approved by the UCSF Committee on Human Research. Teeth were sterilized by gamma radiation and stored in Hank's balanced salt solution (HBSS) until used [24]. Hypersensitivity most commonly presents on buccal cervical surfaces of permanent teeth thus those areas are recommended for clinical studies [25]. We elected to use the root dentin to meet the same recommendation. Dentin blocks from the buccal surface of the root, just below the dentin-enamel junction were prepared by cutting with a diamond saw under water (Buehler, Lake Bluff, IL), then polished with diamond suspensions on polishing cloths (Buehler, IL) to 0.25 μm . Surfaces were prepared to be parallel to the root surface so the dentin tubules were approximately perpendicular to the surface (Fig. 1). Teeth with visible abrasion of the cervical area were not used. Specimens were prescreened for tubule occlusion with AFM and those with more than 25% occlusion, assessed visually at baseline, were excluded from the study.

Experiment 1 tested *in vitro* strontium acetate treatment of dentin blocks in artificial saliva up to 28 days. Variables evaluated were the delivery method (soaking or with brushing), length of treatment (5–28 days) and condition of the dentin (sound or etched). We evaluated changes in tubule occlusion and nanomechanical property change with AFM. Experiment 2 evaluated strontium acetate interaction with sound dentin, utilizing SEM/EDX. Cross-sectioned specimens were prepared to evaluate the depth affected by strontium since the evaluation in experiment 1 was detecting the accumulated changes at the treated surface.

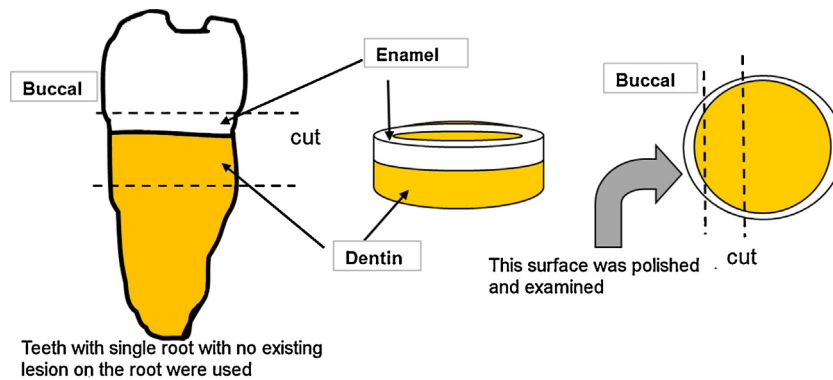


Fig. 1 – Dentin blocks obtained from the buccal surface of the root, just below the dentin-enamel junction were prepared by cutting with a diamond saw under water (Buehler, Lake Bluff, IL), then polished with diamond suspensions on polishing cloths (Buehler, IL) to 0.25 μm . Surfaces were prepared to be parallel to the root surface so the dentin tubules were approximately perpendicular to the surface.

2.1. Experiment 1: evaluation of *in vitro* strontium acetate treatment on tubule occlusion and nanomechanical properties

The root dentin of 24 teeth was treated as shown in Fig. 2 after half had been subjected to an acid etch to removed proteinaceous material covering the dentin surface, as outlined below. The specimens were treated by soaking or brushing with deionized (DI) water or a solution of strontium acetate.

2.1.1. Strontium treatment and acid challenge treatment

The 24 teeth were divided into 8 groups, 3 per group (see Fig. 2). 12 teeth were assigned for pretreatment with 1% citric acid (pH 3.8) for 2 min after the baseline occlusion percent and nanomechanical properties were determined. Half of each group (i.e. 6 teeth) was treated with 10% (w/w) strontium acetate solution (Strem Chemicals, Newburyport, MA, Lot#A5861018) for the strontium treatment and the other half ($n=6$) was treated with DI water. 10% was chosen as it falls between the two tested concentrations of 8%, clinically [26–28] and *in vitro* [29–31], and 12% clinically [32–34] for strontium acetate. For the tooth brushing procedure, an electric toothbrush (Walgreens' power whitening brush) was used. Each group had an assigned soft brush head and battery so that

there was the same amount of wear of brush and voltage change among test groups. Remaining battery voltage was monitored and it was changed as needed, at approximately 14 days for all groups when the voltage reached 1.2 V (fresh battery – 1.5 V) and there was detectable change in brushing noise. A force of 70 g was applied at the brush head during brushing. The strontium solution or water treatment was delivered by soaking the specimen in the treatment solution or by brushing for 2 min. Two treatments per day were provided for 28 days. AFM measurements were made at the baseline, 5, 14 and 28 days. Specimens were stored in artificial saliva (50 mM NaCl, 1.1 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 0.6 mM KH_2PO_4 , pH 7.0, with 0.05% sodium azide to prevent bacterial growth) at 37 °C in a double container with the outer container filled with argon gas to avoid pH changes of the artificial saliva (and resulting precipitation of calcium salts in the artificial saliva) due to the air in the inner container until the next treatment.

2.1.2. Atomic Force Microscopy (AFM) and nanoindentation

Conventional atomic force microscopy (AFM) (Nanoscope III, Bruker, Santa Barbara, CA) was used to obtain topographic

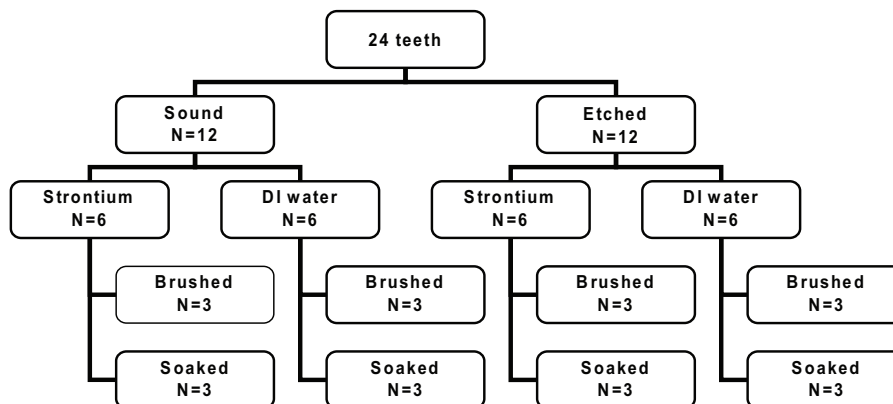


Fig. 2 – 24 dentin blocks were divided into 8 groups based on pretreatment with 1% citric acid (etched or sound), treatment solution (strontium acetate or water) and delivery method (soaked or brushed).

images of specimens, immediately after they were removed from artificial saliva and briefly rinsed with DI water and air dried with canned air. 50 $\mu\text{m} \times 50 \mu\text{m}$ images were obtained in the dry condition from 2 well-separated typical positions from each specimen for tubule occlusion assessment.

Mechanical properties of the specimens were measured after topographic imaging. AFM-based nanoindentations were made in a liquid cell containing distilled and filtered water (“wet” condition) with the standard head replaced by a Tribo-scope indenter system (Hysitron Inc., Minneapolis, MN), as described elsewhere [35,36]. A cube corner diamond indenter with a tip radius of about 20 nm was used for indentations and imaging. Fused silica was used for calibration of the machine compliance, the elastic modulus, and to define the tip area function for indentation depths over a range of 50–600 nm for the cube corner tip. Indentation loads of 400 μN on sound dentin resulted in indentation depths between 300 and 400 nm. A linear series of 6 indentations, spaced approximately 3 μm apart in 2 well-separated typical areas, were made in each specimen (12 indents in each specimen). AFM imaging ensured positioning the indenter on the intertubular dentin while avoiding tubules and any peritubular dentin.

The indentation load–displacement data were analyzed to determine the elastic modulus (E) according to the method of Oliver and Pharr [37,38].

$$E = \frac{\sqrt{\pi}}{2\sqrt{a}} S$$

$$H = \frac{F_{\max}}{a}$$

The hardness, H , was calculated on the basis of maximum force, F_{\max} , divided by the projected contact area at maximum load, a , while the elastic modulus, E , was calculated from the contact stiffness, S , defined as the slope of the linear portion of the force/displacement curve during unloading near the maximum load

2.1.3. Occlusion of tubules

Percent of tubule occlusion was determined from the AFM topographic images. Two examiners, who had more than 5 years of experience evaluating AFM images, were calibrated on post-editing AFM raw images and definitions of “open”(O), “partially filled” (P), “filled”(F) and “covered” (C) tubules as described elsewhere [39]. Each examiner scored the tubules independently. The percentage of occluded tubules was calculated by dividing the sum of “partial occlusion”, “complete occlusion” and “covered” tubules by the total number of tubules in an image. $((P)+(F)+(C))/ \text{total number} \times 100$.

2.2. Experiment 2: strontium acetate interaction with dentin

Further investigation with scanning electron microscopy (SEM) was carried out after we observed changes in nanomechanical properties and tubule occlusion. Cross-sectioned specimens were prepared to evaluate the depth affected by strontium acetate /dentin interaction.

The strontium treated specimens after nanoindentation measurements in a wet-cell did not have detectable strontium

acetate. To evaluate the interaction in more detail, three additional teeth were used for this section. After prescreening for tubule occlusion, they were treated with 10% strontium acetate, and brushed two times per day with the electric brush. They were stored in 100% humidity (sandwiched between artificial saliva saturated sponges, with the treated surface not touching the liquid) at 37 °C between treatments for 7 days. AFM topographic images were taken at day 3 and day 7. At day 7, accumulation of deposits was noted. This first layer was formed to establish dentin/strontium interaction. The second layer was built by applying a drop of strontium acetate solution to the layer every other day and specimens were kept covered by KimWipes™ so the strontium solution could evaporate at room temperature. This second layer was built to create a thicker layer to serve as a reference for SEM analysis.

2.2.1. SEM and energy-dispersive X-ray analysis (SEM/EDX)

SEM (Topcon ISI ABT SX-40A, Milpitas, CA) visualization of the samples was executed in backscattered mode without coating, using a charge-free anti-contamination system (CFAS) and EDX analysis for chemical information (Thermo-Noran Sigma2, Middleton, WI) with accelerating voltage 10.0 kV at magnification range from $\times 50$ to 5000. The strontium distribution was mapped (dwell time, 5600 μs ; 128×128 pixels).

2.3. Statistical analysis

Statistical analysis was performed using linear mixed effects models using the Kenward-Roger denominator degrees of freedom method; the best fitting (smallest corrected Akaike information criterion) of 8 correlation structures was used for a random tooth sample effect to account for repeated measures within samples (proc mixed Statistical Analysis Software (SAS) system ver9.3, Cary, NC). The 2- and 3-way interactions of the treatment (strontium/water), delivery method (soaked/brushed) and time (0 (baseline), 5, 14, and 28 days) were evaluated for each condition (sound or etched). Etched value was used as the reference value for statistical analysis. Bonferroni adjusted P -values were used to account for multiple comparisons.

3. Results

3.1. Nanomechanical property changes

We observed significant interactions among variables (treatment, delivery and time). Treatment differed in several groups, whereas delivery method only differed significantly in elastic modulus of sound dentin.

3.1.1. Elastic modulus

3.1.1.1. Etched condition. In analyzing elastic modulus with a mixed effects model no interactions with time were significant. The treatment (strontium/water) \times delivery (brushed/soaked) interaction was statistically significant (Table 1A). The “strontium brushed” group had significantly higher E values (estimated: 18.2 GPa) than the “water brushed” group (estimated: 15.9 GPa). However, the “strontium soaked”

Table 1 – Nanomechanical properties results after treatments. (Differences between Least Square Means with Bonferroni adjustment).

A: Strontium versus Water (Treatment × Delivery interaction)									
Etched condition: Elastic modulus					Etched condition: Hardness				
Delivery method	Estimated difference in modulus	Standard Error	t Value	Adjusted P	Delivery method	Estimated difference in hardness	Standard Error	t Value	Adjusted P
Brushed	2.344	0.787	2.98	0.020	Brushed	0.092	0.041	2.23	0.090
Soaked	-2.446	0.787	-3.11	0.020	Soaked	-0.087	0.041	-2.11	0.112
B: Strontium versus Water for each Treatment Time (Time × Treatment interaction)									
Sound condition: Elastic modulus					Etched condition: Hardness				
Treatment Time (days)	Estimated difference in modulus	Standard Error	t Value	Adjusted P	Treatment Time (days)	Estimated difference in hardness	Standard Error	t Value	Adjusted P
0	-0.111	0.606	-0.18	1.000	Etched	-0.073	0.046	-1.58	0.498
5	0.560	0.606	0.92	1.000	5	-0.103	0.046	-2.23	0.134
14	2.353	0.606	3.89	0.002	14	0.103	0.046	2.23	0.134
28	0.712	0.606	1.18	0.990	28	0.083	0.046	1.79	0.330
C: Pairwise Differences between Treatment Times									
Sound condition: Hardness					Etched condition: Hardness				
Treatment Time (days)	Estimated difference in hardness	Standard Error	t Value	Adjusted P	Treatment Time (days)	Estimated difference in hardness	Standard Error	t Value	Adjusted P
0 vs 5	0.118	0.034	3.48	0.009	Etched vs 5	-0.010	0.027	-0.37	1.000
0 vs 14	0.079	0.043	1.85	0.439	Etched vs 14	-0.068	0.031	-2.17	0.226
0 vs 28	0.120	0.044	2.75	0.059	Etched vs 28	-0.013	0.033	-0.41	1.000
5 vs 14	-0.039	0.040	-0.98	1.000	5 vs 14	-0.058	0.027	-2.17	0.228
5 vs 28	0.002	0.044	0.04	1.000	5 vs 28	-0.003	0.031	-0.11	1.000
14 vs 28	0.040	0.043	0.96	1.000	14 vs 28	0.055	0.027	2.05	0.298
D: Treatment × Delivery interaction in Hardness									
Sound condition									
Effect	Estimated difference in hardness			Standard Error	t Value		Pr		
Treatment:Strontium vs Water	0.081			0.034	2.34		0.032		
Delivery: Brushed vs Soaked	-0.033			0.034	-0.96		0.351		
Elastic modulus ((GPa), no significance interactions with time), Hardness ((GPa), with significant interactions with time included). Reported value of sound dentin elastic modulus: 18.7 (17.5–20), hardness: 0.86 (0.80–0.93) Mean (95%CI), Brauer et al. [40].									

(estimated 13.4 GPa) had lower and statistically significant values than the “water soaked” group (estimated 15.9). Although there was significant difference observed with the treatment-delivery interaction, the estimated difference is within the variation observed in sound dentin nanomechanical property (17.5–20.0 GPa, 95% CI) [40].

3.1.1.2. *Sound condition.* There was significant interaction of treatment and delivery method with time. The only significant differences occurred at 14 days in which the strontium treatment showed significantly higher E than water treatment group (Table 1B) ($p \leq 0.005$). Although there was statistical significance, the E values are still in the range of normal value for human sound dentin, but there could be a trend of strontium increasing elastic modulus more than water with longer treatment time.

3.1.2. *Hardness*

3.1.2.1. *Etched condition.* Treatment significantly interacted with time. No treatment changed the hardness of etched dentin (Table 1B and C).

3.1.2.2. *Sound condition.* Although there was no significant interaction with time, as there was in E, the strontium treatment yielded significantly higher hardness values than the water treatment (Table 1D) ($p=0.03$), but delivery method was not significant ($p=0.35$). There was a slight decrease in hardness at the beginning of the treatment, which was significant, but over the course of the treatment, it disappeared (Table 1C).

Detailed results and statistical analyses are shown in Table 1.

3.2. *Occlusion of tubules*

The total number of dentin tubules occluded using the classification: partial (P)+full (F)+covered (C) were evaluated for sound and etched dentin. In analyzing occlusion with a mixed effects model, the 3 way interaction of condition (etched/sound) × treatment (strontium/water) × delivery (brushed/soaked) was statistically significant with both sound and etched conditions. AFM topographic images suggested that brushing seemed to remove the demineralized collagen fibers from the surface with both strontium and water treatments.

3.2.1. *Etched condition*

There was a significant 3-way interaction between time, delivery and treatment ($p=0.02$). There was no significant difference in delivery method. With the 3-way interaction considered, strontium treatment with brushing occluded tubules significantly more at 28 days than other times. If soaked in strontium solution, 5 and 14-day treatments occluded the tubules significantly more than other times. Treatment with water did not occlude tubules at any evaluated time with either brushed or soaked method (Table 2).

3.2.2. *Sound condition*

There was a 3-way interaction between time, delivery and treatment ($p=0.01$). Strontium treatment resulted in higher tubule occlusion than water treatment ($p=0.001$). Delivery

Table 2 – Percentage of occluded tubules after treatments (Differences between Least Square Means with Bonferroni adjustment).

A: Strontium versus Water by Delivery and Treatment Time											
Sound condition: (including time interaction)					Etched condition: (3way interaction including time interaction)						
Delivery	Treatment Time (days)	Estimated difference in percent occlusion	Standard Error	t Value	Adjusted P	Delivery	Treatment Time (days)	Estimated difference in percent occlusion	Standard Error	t Value	Adjusted P
Brushed	0	1.70	7.899	0.22	1.000	Brushed	Etched	-0.633	5.147	-0.12	1.000
Brushed	5	13.42	7.899	1.70	0.843	Brushed	5	-1.817	5.147	-0.35	1.000
Brushed	14	21.01	7.899	2.66	0.122	Brushed	14	1.892	5.147	0.37	1.000
Brushed	28	31.55	7.899	3.99	0.006 ^a	Brushed	28	14.542	5.147	2.83	0.073
Soaked	0	10.03	7.899	1.27	1.000	Soaked	Etched	0.900	5.147	0.17	1.000
Soaked	5	45.68	7.899	5.78	0.001 ^a	Soaked	5	11.517	5.147	2.24	0.275
Soaked	14	27.20	7.899	3.44	0.021 ^a	Soaked	14	10.467	5.147	2.03	0.422
Soaked	28	26.18	7.899	3.31	0.029 ^a	Soaked	28	3.708	5.147	0.72	1.000

^a Significantly different.

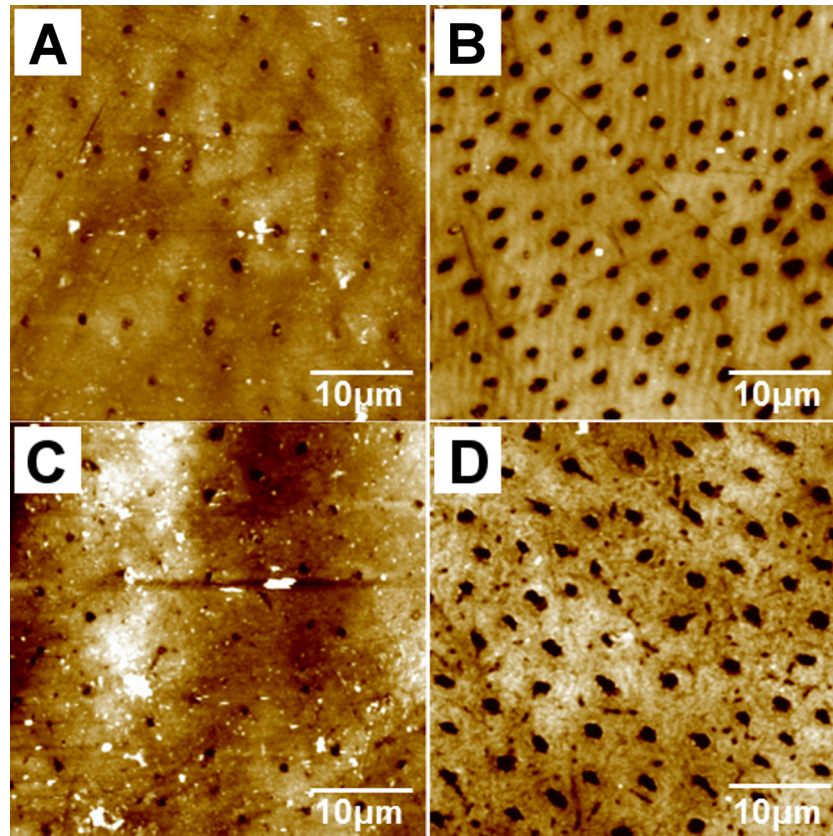


Fig. 3 – Representative AFM topographic images of root dentin, after 28 days of brushing treatment. A and B were sound dentin, A was treated with 10% strontium acetate, B was treated with DI water. C and D were pre-treated with 1% citric acid (pH3.8) for 2 min before starting strontium (C) or DI water (D) treatment. Note the enlarged dentin tubules when treated with DI (D). Specimens treated with strontium (A) and (C) showed occluded, smaller tubules and some precipitation on the surface. Images were taken in contact mode (dry).

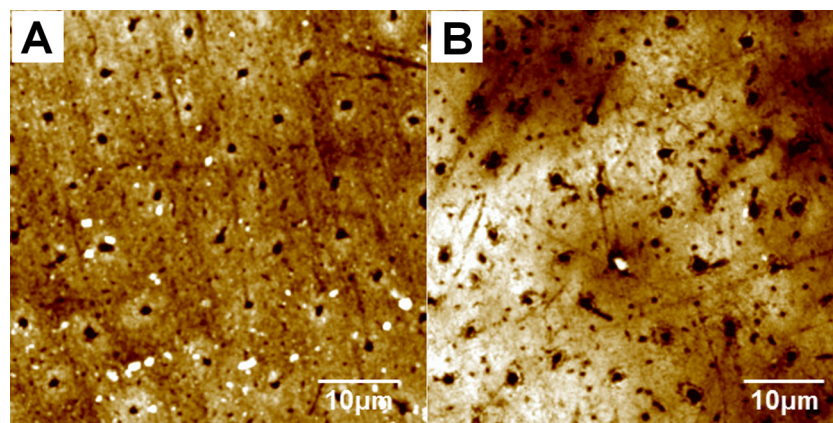


Fig. 4 – Comparison of delivery method on etched dentin. AFM topographic images of root dentin after 14 days of treatment. Both A and B were demineralized with 1% citric acid (pH3.8), before 10% strontium acetate treatment with different delivery methods. A was soaked in 10% strontium acetate solution, whereas B was brushed with 10% strontium solution. In B, the demineralized collagen layer appeared to be removed.

method did not differ. Soaking resulted in a significantly higher rate of tubule occlusion with time ($p < 0.001$). Soaking significantly increased occlusion at 5 days. Occlusion rate improved over time with brushing and reached significance at 28 days, (Table 2)

AFM topographic images after 28 days of brushing showed that the diameter of dentin tubules became smaller and they were mostly filled in the sound dentin group (Fig. 3A), whereas the sound group treated with water had enlarged tubules with no deposits (Fig. 3B). Cloudy deposits on the surface of intertubular dentin were also observed with the group treated with strontium (Fig. 3A and C). Etched dentin treated with water revealed the caniculi of dentin, similar to the dentin structure observed when treated with acid and subsequently treated with sodium hypochlorite [41]. The tubules remained enlarged, without deposits in the tubules (Fig. 3D). The soaking delivery method resulted in deposition of the strontium-containing material without removing the demineralized surface dentin (Fig. 4).

3.3. SEM and EDX analysis

Strontium-containing deposits did not form on the surface uniformly. On one specimen, areas with deposit (Fig. 5A) or no deposit (Fig. 5C) could be observed. EDX confirmed Sr peaks for strontium acetate powder (Fig. 5B), however there was no detectable strontium peak from the areas in either Fig. 5A or C. Fig. 6 shows SEM/EDX analysis of an additional set of specimens made by brushing with 10% strontium acetate. Instead of transferring them to artificial saliva solution immediately after treatment, specimens were kept in a chamber with a sponge saturated with artificial saliva. EDX analysis of those specimens showed a thick strontium containing layer deposited on the surface (Fig. 6A and B). The strontium peak was detectable at 20 μm from the surface (Fig. 6C). At higher magnification, we confirmed that strontium containing particles were deposited in dentin tubules (Fig. 6D).

4. Discussion

In this study, we selected tubule occlusion and mechanical properties as two objective measures, evaluated the effects on dentin-strontium interaction of delivery methods and condition of the dentin when strontium is used as an active ingredient over time up to 28 days. The mechanistic study design focused on elucidation of the interaction of strontium with dentin rather than the mechanism of action in fully formulated dentifrices.

Strontium can substitute for Ca in hydroxyapatite to form Sr-Ca-hydroxyapatite [15,17,18,20]. This substitution can occur naturally in our daily acidic food consumption with the presence of strontium [19,42] or be induced in dental calcified tissues [15,21] or synthesized [20,43,44] in various strontium/calcium ratios. Synthesized strontium apatite has been reported to demonstrate higher solubility than hydroxyapatite, and the solubility increases with greater strontium substitution (strontium ratio) [43,44]. However, it has also been reported that the dissolution rate of synthetic hydroxyapatite can be retarded in the presence of strontium. This

latter observation was thought to be due to strontium forming a very thin surface calcium-strontium apatite complex [18].

In terms of mechanical properties synthesized strontium-partially substituted hydroxyapatite (Ca-Sr-apatite) has been observed to result in an increasing tendency in Young's modulus compared with hydroxyapatite, whereas bending strength was decreased. For strontium apatite (where strontium replaced 100% of calcium), both modulus and bending strength were lower than hydroxyapatite. Interestingly, in this limited study when the hydroxyapatite was soaked in a strontium containing solution (1.0 mol/l $\text{Sr}(\text{NO}_3)_2$) for 24 h, higher modulus and bending strengths were observed compared to non-treated hydroxyapatite [45]. The authors suggested surface treatment with strontium resulted in strontium plugging into the Ca-deficient site of hydroxyapatite.

There is no report on the effect of strontium substitution on mechanical properties in dental tissues, thus we evaluated two strontium interactions scenarios, demineralized and sound, in our mechanical property study. For demineralized specimens, our results showed differences between treatments with strontium or water. Strontium-apatite can be formed at $\text{pH} > 7$. Pan suggested nucleation of strontium-substituted apatite is easier than biological hydroxyapatite in neutral pH and strontium apatite can work as a template for apatite formation [44]. A work on demineralized enamel showed strontium significantly improved remineralization with fluoride [21]. In our study, remineralization of the dentin matrix seemed to be accelerated by strontium, thus leading to the recovery of mechanical properties.

Secondarily, it is also possible that strontium led to the development of Ca-Sr apatite when the specimen was repeatedly exposed to strontium acetate, because our sound dentin specimens showed somewhat higher mechanical properties if treated with strontium, although the treatment did not increase the mechanical properties of sound dentin beyond normal variation [40]. Our finding is consistent with the result of synthesized apatite mentioned above [45].

The soaking method yielded better occlusion than brushing at the beginning of the treatment, whereas brushing increased tubule occlusion continuously through day 28. Soaking may saturate the surface of dentin quickly and inhibit further interaction between dentin and strontium acetate while brushing may allow strontium acetate to penetrate deeper and bind better to dentin. This finding is consistent with an *in situ* study that reported significantly higher strontium was detectable at deeper depth from the surface of demineralized enamel after brushing with strontium containing toothpaste for up to 6 months [46].

Brushing in water, used as a control group in the study, could still cause abrasion. Around 1 μm loss of dentin after 50,000 brushing strokes with 100 g weight was reported, although the dentin smear layer was still preserved [47]. In the present study, an electric toothbrush with around 3000 strokes per minute with 70 g weight was used. Brushing the demineralized surface may have removed the exposed collagen as deeper sound dentin was exposed and strontium rich precipitates leading to higher mechanical properties. This can explain the appearance of brushed specimens with

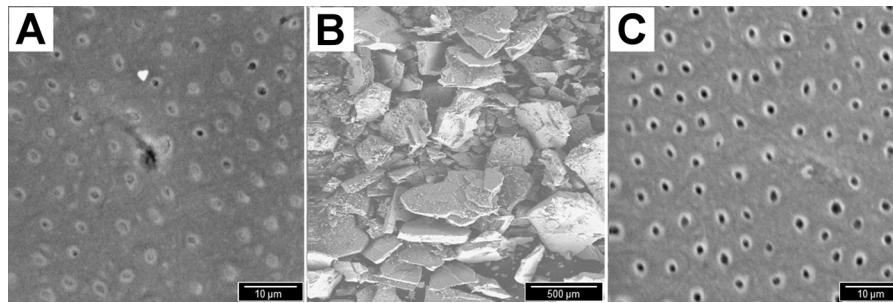


Fig. 5 – SEM images of dentin treated with strontium acetate solution. (A) Specimen brushed 28 days with 10% strontium acetate solution. Most of the tubules are occluded. (B) Crystals of strontium acetate. (C) Area not occluded from the same specimen as A. Tubule occlusion was not uniform, possibly because the specimen was soaked in DI water for mechanical property measurements before SEM analysis and the occluded materials were washed away. EDX confirmed peaks for strontium in B, however there was no detectable strontium peak from A or C.

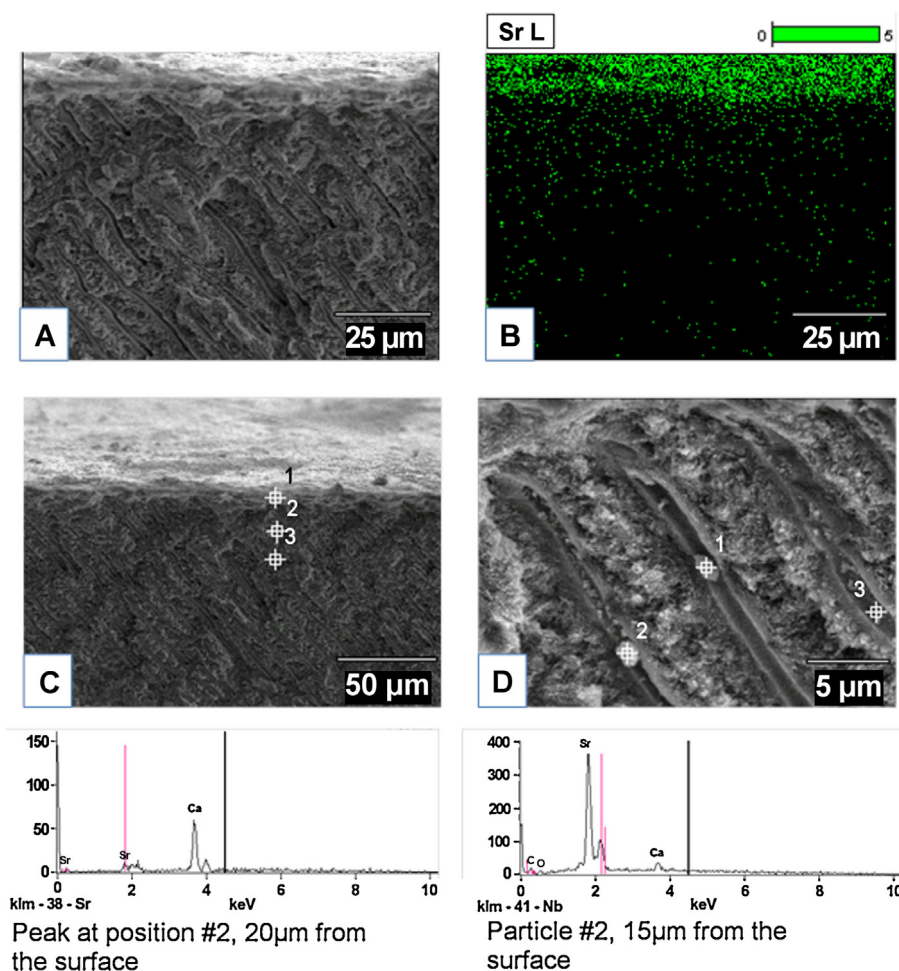


Fig. 6 – SEM images and EDX results from fractured specimen of strontium treated sound dentin. It was prepared by brushing for 7 days, stored moist. Then a thick strontium containing layer was fabricated by precipitating the strontium acetate solution on it, which is shown on the surface (A and B). EDX confirmed Sr peaks 20–30 μm from the surface and particles (#1–#3) were present in dentin tubules (C and D).

more exposed canaliculi, that was similar to demineralized dentin treated with NaOCl [41]. Although we did not directly measure surface loss, the reason delivery method differed in the elastic modulus of etched dentin can be explained by the removal of soft demineralized dentin by brushing,

whereas with the soaking method there was no mechanical removal.

Tubule occlusion can occur by deposition of an occluding layer on the top of dentin or occluding materials migration into dentin tubules. Strontium treatments created surface

crystals, deposits on demineralized dentin surface *in vitro*, but they were easily washed away with water [48]. *In vivo* and *in situ* studies with strontium acetate [26,27,30] show greater tubule occlusion compared to our *in vitro* study models. To explain this discrepancy, we should consider dynamics in the oral cavity in addition to the presence of fluoride and silica in the study design. We can speculate that the mechanism is similar to that of topical fluoride application and mechanism of fluoride retention in the oral cavity. Fluoride is retained in solution in saliva as well as being retained in the form of calcium fluoride. It can be retained on the tooth surface, in saliva, plaque, on soft tissues such as the tongue and lower posterior vestibule of the mouth [49]; thus these sites serve as reservoirs. Fluoride can penetrate through light biofilm layers, which can increase the retention of intra-oral fluoride availability [50]. But if rinsed with water immediately after using the fluoride containing dentifrice, bio-availability of fluoride was reduced significantly [51].

Because of the possibility of losing the strontium deposit from the surface, we carried out AFM contact mode tubule occlusion analysis dry prior to mechanical property testing in wet condition. Our SEM-EDX evaluation could not detect a strontium containing layer after the specimens were soaked in DI-water for mechanical property measurement in Experiment 1. The levels of strontium incorporated into the surface of dentin may be below the detection limits of EDX, or the strontium salts formed may be soluble under the analysis conditions in the present study as reported by Addy and Mostafa [48]. Conversely, specimens kept on moist sponges (saturated with artificial saliva, simulating the reservoir, as in Experiment 2, showed strontium acetate particles reaching 20 μm into tubules and general evidence of strontium presence through the dentin with SEM and EDX (Fig. 6).

In the present study specimens were placed in an artificial saliva solution immediately after the 2 min application of strontium acetate; this might not be sufficient for allowing strontium to optimally interact with dentin. The absence of reservoirs and immediate rinsing may explain the lower occlusion rate of various *in vitro* studies compared to *in vivo* studies. Comparative studies of fluoride and strontium on bio-availability are warranted to improve the delivery method.

Various components in modern dentifrice formulations can act to occlude dentin tubules in their own right, but such ingredients are generally inert and confer a short term level of occlusion. Despite the high uptake and deposit observed on the surfaces and in the tubules, in some cases tubule occlusion induced by dentifrice slurries can be washed away rather quickly by water [52]. Davies et al. suggested that the retention of the occluding particles and resistance to removal by acids are related to the specific combination of the active ingredient and abrasives [31]. In our study, there were no abrasives or chemicals in the study design except salts in artificial saliva, thus tubule occlusion can be attributed purely to strontium acetate, although the binding seemed to be loose and water soluble. This may be one of the reasons that the occlusion did not increase as much as *in situ* studies that have used strontium acetate tooth paste containing abrasives.

Dentin and bone share many characteristics, and substituting strontium for calcium has been reported to aid bone

formation [17,53,54] possibly by providing the template for rapid mineralization [44]. It is possible the natural demineralization/remineralization process occurring in the oral environment assists the uptake of strontium for incorporation into the dentin apatite [20] by the same mechanism. Further study will be needed to confirm if strontium can be taken into the structure of dentin, promote remineralization and alter the properties of dentin in the duration and delivery method we used.

5. Conclusions

A 10% strontium acetate treatment over 5–28 days significantly increased tubule occlusion for normal root dentin and to a lesser extent for demineralized dentin and increased the AFM-based nanomechanical properties of demineralized dentin. Brushing was more effective than soaking in recovery of mechanical properties of demineralized dentin when treated with strontium. No difference in tubule occlusion was found between the two delivery methods.

Strontium acetate itself proved to have the ability to occlude dentin tubules and result in small changes in the mechanical properties of dentin. However, strontium could not be detected in the samples when analyzed by EDX. Further research is required to understand the role strontium plays in the occlusion of dentin tubules. Strontium may be taken up by dentin and may form strontium-containing apatite, which could explain the changes in mechanical properties and tubule occlusion compared to the negative control. Strontium, due to its chemical similarity to calcium, and its ability to form complex strontium phosphate salts may have a direct chemical action on dentin.

Conflict of interest

The research was funded by grants from GlaxoSmithKline. Charles R. Parkinson is an employee of GSK, a manufacturer of a product containing strontium acetate. Others have no conflicts of interest to report.

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