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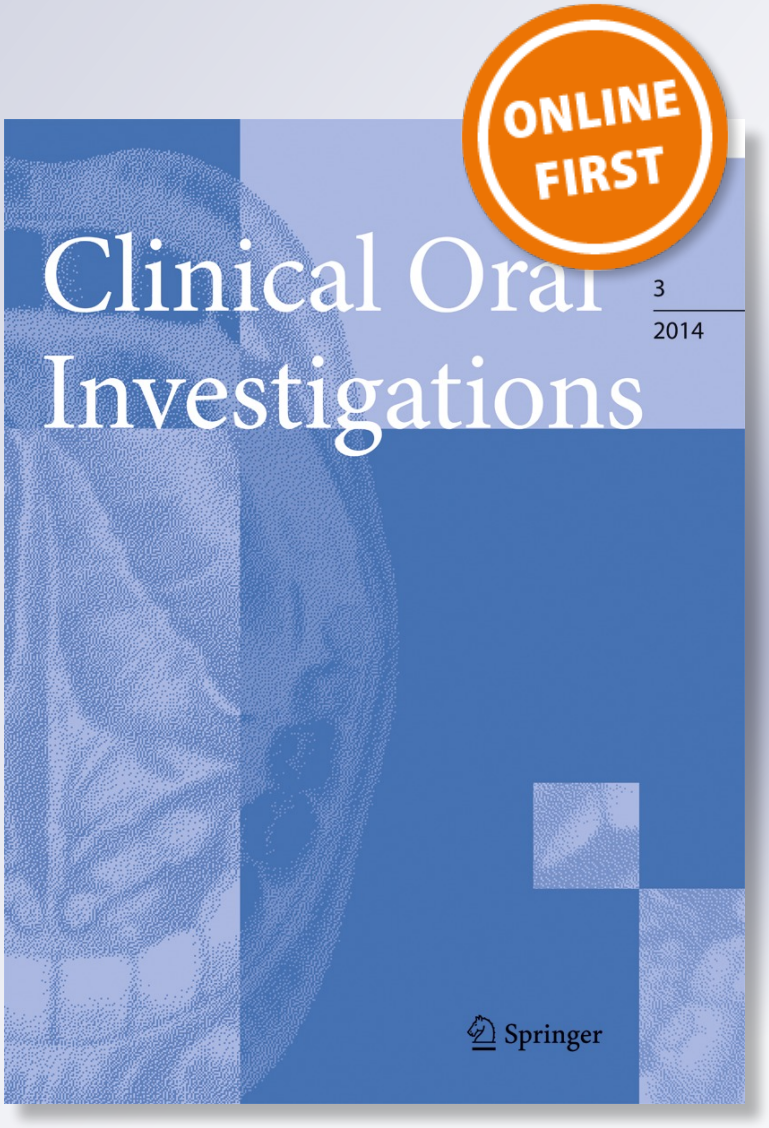

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ORIGINAL ARTICLE

Effect of theobromine-containing toothpaste on dentin tubule occlusion in situ

Bennett T. Amaechi · Sapna M. Mathews · Poornima K. Mensinkai

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Abstract
Objectives Dentin hypersensitivity (DH) is treated by either occlusion of dentin tubules or nerve desensitization. This in situ study compared dentin tubules occlusion by theobromine-containing dentifrices with (Theodent-classic-F®, TCF) and without (Theodent-classic®, TC) fluoride with 1,500 ppm fluoride toothpaste, Colgate®-Regular (Fluoride) and Novamin®-containing toothpaste, Sensodyne®-5000-Nupro (Novamin®).
Methods Each subject wore four intraoral appliances bearing dentin blocks while using one of four test dentifrices (n=20/dentifrice) twice daily for 7 days. The four appliances were removed successively after 1, 2, 3, and 7 days. Treated blocks and their control (untreated) blocks were examined with scanning electron microscopy (SEM). Effects were compared statistically (ANOVA/Tukey's) based on percentage of surface area covered by deposited precipitate layer (%DPL) and percentage of fully open (%FOT), partially occluded (%POT), and completely occluded (%COT) tubules in each block calculated relative to the number of tubules in their control blocks.
Results SEM observation indicated an increased %COT and %DPL over time. After 1 and 2 days, %COT was comparable with TC and TCF, and significantly (p<0.05) higher compared with Novamin® and Fluoride. Following 3 and 7 days, %COT was comparable among TC, TCF, and Novamin®, but remained significantly lower in Fluoride. At any time, %DPL was significantly (p<0.05) higher in TC, TCF, and Novamin® compared with Fluoride.
Conclusions Theobromine-containing toothpastes with and without fluoride have equal potential in occluding dentin tubules within a shorter time period than Novamin®-containing toothpaste; however, the three demonstrated equal potential after 1 week, but not the fluoride toothpaste.
Clinical relevance Theobromine-containing toothpaste promoted dentin tubule occlusion thus shows potential to relief DH.

Keywords Dentin hypersensitivity · Theobromine · Dentifrice · Theodent · Novamin® · Tubule occlusion

Introduction

Dentin hypersensitivity (DH) is characterized by distinctive short, sharp pain arising from exposed cervical dentin in response to various external stimuli that are typically thermal, evaporative, tactile, electrical, osmotic, or chemical, which cannot be ascribed to any other form of dental pathology, defect, or disease [1]. This condition is described clinically as an exaggerated response to a non-noxious stimulus and is the result of dentin tubules exposure due to either gingival recession or loss of enamel [2]. Its prevalence greatly varies between 3 and 98 %, depending on the population, study setting, and study design [3, 4].

Present approaches to treat DH employed agents that either chemically suppress or modify the nerve impulse by direct neurological interaction or mechanically occlude the dentin tubules to decrease dentin permeability and prevent fluid movement [5], thus reducing hypersensitivity discomfort/pain. Although its effectiveness is debatable, potassium ions, present in toothpastes containing 5 % potassium nitrate, can decrease the excitability of A fibers, which surround the odontoblasts, thus resulting in reduction in tooth sensitivity [6, 7]. Dentin tubules occlusion is the most current therapeutic approach [5]. Some pastes or aqueous solutions containing potassium oxalate, ferric oxalate, and glutaraldehyde achieved

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this by precipitative intratubular occlusion [6, 8–15]. However, available evidence does not currently support the recommendation of dentin hypersensitivity treatment with oxalates [15], while the potential biocompatibility hazards associated with gluteraldehyde cannot be ignored [16]. Recently, good clinical results were reported with products containing arginine/calcium carbonate, calcium sodium phosphosilicate (NovaMin) or strontium acetate [17–19]. Fluoride varnish was the first Food and Drug Administration (FDA)-approved agents for treatment of hypersensitivity, and it protects the dentin surface by forming a protective layer of calcium fluoride [20, 21]. Fluoride gels combined with either laser [22] or iontopheresis [23] have been demonstrated to show some cumulative clinical efficacy over time. Adhesive bonding techniques have also been used to seal the tubules, but due to the absence of fillers in these bonding agents, they tend to wear easily [24, 25].

Theobromine (3, 7 dimethylxanthine), a white crystalline powder, is an alkaloid readily available in cocoa (240 mg/cup) and chocolate (1.89 %). A recent study reported that theobromine in an apatite-forming medium can enhanced the potential of the medium to remineralize a demineralized tooth tissue [26]. In this study, it was demonstrated that theobromine, at a molar level 71 times less than that of fluoride, has an enamel lesion remineralization effect comparable to that of fluoride. The authors attributed this effect to previous observations that crystallite size was increased and crystallinity of teeth improved by growing hydroxyapatite in an apatite-forming-system containing an effective amount of theobromine [27, 28]. Based on these studies, a commercially available non-fluoride theobromine-containing toothpaste (Theodent classic®) was developed for prevention and treatment of dental caries. It is envisioned that, due to the ability of theobromine to stimulate crystallite growth [27, 28], the use of this toothpaste may cause occlusion of dentin tubules by crystallites precipitation. The objective of the present study was to determine the ability of this theobromine-containing toothpaste with and without fluoride to physically occlude dentinal tubules of human root specimens as a surrogate measure of their potential to treat dentin hypersensitivity. The toothpaste was compared with commercially available standard 1,500 ppm fluoride dentifrice (Colgate regular™) and Novamin®-containing toothpaste (Sensodyne®-5000 Nupro). This study sought to test two hypotheses. The first hypothesis is that each of the four dentifrices can promote the occlusion of dentin tubule and deposition of precipitate greater than zero (%). The second hypothesis is that the four dentifrices differ with respect to percentage of dentin tubule occlusion and deposited precipitate layer. Of special interest is whether the non-fluoride theobromine-containing dentifrices promote greater dentin tubule occlusion and deposition of precipitate layer relative to the novamin®-containing dentifrice.

Methods

Study design

This was a double-blind, randomized, parallel group, single center, in situ study to test the ability of theobromine-containing toothpaste with (TCF) and without (TC) fluoride to physically occlude dentin tubules, comparing them with those of a Novamin®-containing toothpaste (Novamin®) and a standard fluoride dentifrice (Fluoride). The primary outcome is precipitative occlusion of dentin tubules and deposition of precipitate layer. The efficacies of the four products were compared after 2, 4, 6, and 14 product usage based on four variables, the percentage of (a) completely occluded tubules (b) partially occluded tubules, (c) fully open tubules, and (d) surface area covered by precipitate layer. In this trial, each of 80 subjects wore four intraoral appliances [29, 30] bearing dentin blocks while using one of four test products twice daily for 7 days. The four appliances were removed successively after 1, 2, 3 and 7 days for scanning electron microscope (SEM) examination for calculation of the level of tubule occlusion and amount of deposited precipitate layer. The study was conducted at the Clinical Research Facility (CRF) of the dental school of University of Texas Health Science Center at San Antonio (UTHSCSA). The Institutional Review Board (IRB) of UTHSCSA approved the study (approval #: HSC20120238H), and the study have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all subjects prior to their participation in the study. The majority of subjects were recruited from among patients receiving treatment in the dental schools' clinics.

Specimen preparation

Using water-cooled diamond wire saw (Well Diamond Wire Saws, Norcross GA), rectangular dentin blocks measuring approximately 4 mm length × 1.5 mm width × 0.75 mm height were cut from the cervical region of the roots of freshly extracted human teeth (avoiding bifurcation and cement–enamel junction) stored in thymol disinfectant prior to use. A smooth working surface was obtained by polishing the samples using diamond lapping films in a MultiPrep precision polishing machine (Allied High Tech Products, Rancho Dominguez, CA) initially with 30 μm diamond grit and finishing with 1 μm grit. Each block was then sectioned into two halves to produce a pair of blocks, each measuring 2 mm length × 1.5 mm width × 0.75 mm height, one serving as the test sample and the other as control. The smear layer and debris generated during cutting and polishing was removed to obtain patent (fully open) dentin tubules by sonicating (Branson Sonifier 450, Danbury, CT) each pair of blocks

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(control and test) simultaneously in 200 ml beaker with 6 % citric acid (pH 2.0) for 2 min (power setting 1). This was followed by another 2 min sonication (power setting 2) in distilled water (pH 7.0). Blocks were allowed to dry in clean Petri dish, covered with sparsely perforated parafilm, for 16 h in laminar hood.

Following drying, the control dentin blocks were examined with SEM as described by Claydon et al. [31] to establish patency of dentin tubules and complete removal of smear layer generated during polishing. The blocks were sputter-coated with gold palladium, and then visualized with SEM (Joel Scanning Electron Microscope; Make: JEOL USA; Model: JSM-6610LV; JEOL, Tokyo, Japan) at a beam voltage at 15 kV. The center of the surface of each dentin block was scanned and the image acquired at a magnification of 1,500× (pretreatment image). Samples with fully open (patent) dentin tubules and without surface artifacts were selected and save for future analysis. The "test" blocks corresponding to the selected "control" blocks were chosen for study.

Each test dentin block was mounted within an intraoral appliance, a customized orthodontic bracket, which is a modification of our in situ caries model described in our previous publications [29, 30]. Briefly, the appliance consisted of an orthodontic molar pad with retentive mesh backing, which had a stainless steel band welded to it so that the band closely enclosed each test dentin block. The block was retained within the bracket using fluoride-free intermediate restorative material, exposing only the working surface of the block to the oral cavity. The specimens were mounted slightly recessed below the edges of the band to prevent contact of the dentin surface with oral mucosa surface. The appliances were sterilized with ethylene oxide gas [32, 33].

Subjects recruitment

Eighty healthy adults (27 males, 53 females) with mean (SD) age of 38.8 (13.9) from different ethnic origins and socioeconomic status participated in this study. The subjects were identified with code numbers generated by the data management team, and this number was used for the patient randomization. After providing informed written consent, subjects underwent a complete intraoral examination and completed medical and dental history questionnaires. The inclusion criteria were: age≥18 years in good general and oral health without known allergy to any commercial dental products or cosmetics; having at least 18 healthy teeth exposed to the oral environment; the ability to read and understand English; and having both left and right mandibular first and second molars with sound, unrestored buccal surfaces. Other inclusion criteria were normal salivary function with unstimulated and stimulated salivary flow rates≥0.2 and ≥0.7 ml⁄min, respectively, measured according to Sreebny et al. [34] procedure, and no evidence of significant oral soft tissue pathology. Exclusion criteria were history of adverse effects with the use of any oral hygiene product, periodontal disease requiring aggressive treatment, residing in the same household with another subject or appointment to receive dental treatment which may affect their participation.

Study treatment

Subjects were randomized to one of four commercially available toothpastes (20 subjects/product); theobromine-containing toothpaste without (TC) and with (TCF) 1,500 ppm fluoride (Theodent classic®; Theocorp Holding Company, Metairie, LA), Novamin®-containing toothpaste (Novamin®) with 5,000 ppm fluoride (Sensodyne 5000 Nupro®; DENTSPLY Professional, York, PA) and standard fluoride toothpaste (Fluoride) with 1,500 ppm fluoride (Colgate regular™; Colgate Pharmaceuticals, New York, NY). All subjects received a soft bristled manual toothbrush and their respective toothpaste for use throughout the duration of the study. They started 7 days washout period (without the intraoral appliance) and were instructed to brush two times daily, morning and last thing before bed, in their usual manner of toothbrushing. On each occasion, subjects brushed for 1 min using at least a 1-in. strip of their respective toothpaste and then wait for another 1 min before rinsing with 10 ml of water for 10 s. The first brushing occasion occurred at the Clinical Research Facility and was supervised by the Study Coordinator. Subjects were asked not to take any drink for at least 30 min after brushing. A diary was provided to each subject to keep a record of the number of times brushed each day. In addition to the diary-keeping as a monitor of compliance, the toothpastes were weighed before dispensing and on each study visit. All subjects were asked to maintain their normal dietary habits. The use of any other oral hygiene product, such as mouthwashes, prescription products, chewing gum, etc., was prohibited.

After the 7 days washout period, the in situ appliances, bearing the dentin blocks, were assigned and fitted to each subject. Each subject wore four dentin blocks to permit effectiveness assessments after 1 day (two times product usage), 2 days (4× product usage), 3 days (6× product usage), and 7 days (14× product usage). The appliances were fitted by a qualified dentist, who was different from the Laboratory Assistant that processed and analyzed the samples to produce the final data. The buccal surfaces of the subject's mandibular first and second permanent molar teeth chosen to carry the appliances were carefully acid-etched for 30 s, in accordance with current principles of dental practice, washed and dried for a further 30 s, and isolated using cotton rolls. The bottom of the appliance was loaded with the adhesive composite resin and the appliance was carefully positioned on tooth surface to avoid causing occlusal interference and soft tissue irritation. Subjects were advised to continue using their respective

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toothpaste as directed during the washout period. However, immediately after attachment of the appliance (on study day 1) subjects used their product supervised by the Study Coordinator. Then on day 2 (after 2× product usage on the previous day), subjects arrived at the clinic without using the product that morning, and had one of the four appliances detached and send to the laboratory for analysis. Immediately after the detachment of one appliance, the subject used the product for that morning at the clinic before going home. This process was repeated on day 3 (for 2 days, 4× usage), day 4 (for 3 days, 6× usage), and day 8 (for 7 days, 14× usage) when the remaining dentin-bearing appliance was detached. Any bonding agent left on the tooth surface was carefully and completely removed with composite-removing burs.

Following intraoral exposure, the dentin blocks were processed for SEM examination [31]. An experienced SEM technician, who was blinded with respect to group allocation of the samples, used the SEM to visualize and scan the dentin blocks as described for the control blocks. An image taken from the center of the surface of each dentin block was acquired at a magnification of 1,500× (posttreatment image).

At each visit, the clinical examiner inquired about adherence, assess adverse effects, and screen for possible serious adverse events (SAEs) or continuing symptoms since the previous visit.

Study outcomes and statistical power

Each acquired pretreatment and posttreatment SEM image was assessed by two calibrated examiners, who were blinded with respect to group allocation of the samples. The images were assessed for the extent of tubule occlusion based on the numbers of fully open, partially and completely occluded dentin tubules as well as the extent of dentin surface covered by precipitate layer on each of 1,500× image. The examiners were calibrated against a standard set of 20 images of mixed samples of fully open, partially and completely occluded dentin tubules from a previous study. Agreement to the set standard was quantified by Kappa analysis [35]. The free-margin Kappa [35] scores were 0.81 and 0.87 (any score> 0.70 was considered to be acceptable as adequate agreement). The average of the two assessments was calculated for each specimen. The numbers of fully open, partially and completely closed tubules in each block were counted and expressed as a percentage of the number of tubules on the corresponding control block. The mean of the percentages of fully open (%FOT), partially occluded (%POT), and completely occluded (%COT) tubules were calculated for the individual products. Also the mean of the percentage of the surface area covered by deposited precipitate layer (%DPL) was calculated for each product.

Our power analysis and sample size calculation were performed using nQuery Advisor software (Statistical Solutions, Cork, Ireland) and was based on the results of previous studies on dentin occluding agents [36–38] and on a hypothesized dentin tubule occlusion significantly greater than zero. For our null hypothesis that each of the four dentifrices promotes tubule occlusion that is significantly greater than zero, the proposed sample size of $n=20$ per product will have power greater than 0.95 with a 0.05 one-sided significance level to detect a difference between a null hypothesis mean of zero and a sample mean percentage of tubule occlusion equal to or greater than 10 %.

Statistical methods

Statistical analysis of the data was conducted using statistical software (PASW Statistics 18.0, IBM), with $\alpha=0.05$ set as the level of significance. With one-way repeated ANOVA, followed by post hoc multistep comparisons using Tukey's HSD test, the effects of the four toothpastes in occluding dentin tubules were compared based on the %FOT, %POT, %COT, and %DPL. The effectiveness of the four toothpastes were compared at 1 day (two product usage), 2 days (four product usage), 3 days (six product usage), and 7 days (14 product usage) time points. Intraproduct comparison of effectiveness after 2, 4, 6, and 14 product usage was performed to determine longitudinal effect overtime.

Results

The 80 subjects recruited for this trial completed the study without any dropout. There were no adverse events reported by subjects during the trial. As shown in Table 1, %COT was comparable and significantly ($p<0.05$) higher in TC and TCF than in Fluoride at all measurement time points and in Novamin® after two and four product usage. However, after 6 and 14 product usage, %COT was comparable in Novamin®, TC, and TCF. %POT was significantly ($p<0.05$) higher with TC at every time point when compared with Novamin® and Fluoride, but only after four and six product usage when compared with TCF (Table 1). %POT in Novamin® and Fluoride were comparable at every measurement time points and the two were comparable with TCF after four and six product usage (Table 1). Within each dentifrice, over time, the %COT increased significantly ($p<0.05$) and %POT decreased significantly ($p<0.05$); however, the concomitant decrease in %FOT was only significant ($p<0.05$) after six product usage in all dentifrices except Fluoride (table). Fig. 1 shows that %DPL was comparable and significantly ($p<0.05$) higher in TC and TCF at all time points when compared with either Novamin® or Fluoride. However, Novamin® deposited significantly ($p<0.05$) more precipitate layer than Fluoride. The precipitate layer deposition was more than 90 % with two

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Table 1 The %COT, %FOT, and %POT after 2, 4, 6, and 14 times use of the four dentifrices

Dentifrice	# of usage times	%COT Mean(SD)	%FOT Mean(SD)	%POT Mean(SD)
TC	2	26.5 (16.1)^{a,*}	17.6 (14.3)^{a,*}	56.6 (16.6)^{a,*}
	4	37.5 (13.9)$^{a,*,\beta}$	11.2 (10.7)$^{a,*,\beta}$	51.2 (18.1)$^{a,*,\beta}$
	6	47.1 (20.4)$^{a,**,\beta}$	3.7 (6.9)$^{a,**,\beta}$	49.2 (20.5)$^{a,*,\beta}$
	14	61.8 (20.8)^{a,**}	0.9 (3.0)^{a,**}	37.3 (19.7)$^{a,**,\beta}$
Novamin®	2	6.8 (6.2)^{b,*}	61.6 (16.0)^{b,*}	31.6 (14.1)^{b,c,*}
	4	11.3 (7.7)^{b,*}	61.9 (13.6)^{b,*}	26.8 (12.4)$^{b,*,\beta}$
	6	35.9 (18.7)$^{a,c,\beta}$	40.7 (19.2)^{b,**}	23.3 (19.8)$^{b,*,\beta}$
	14	54.1 (26.9)$^{a,\gamma}$	28.9 (25.7)^{b,**}	16.9 (11.4)$^{b,c,**,\beta}$
Fluoride	2	0.54 (1.1)^{b,*}	80.7 (16.6)^{c,*}	18.7 (16.4)^{b,*}
	4	3.3 (4.1)$^{b,*,\beta}$	81 (14.2)^{c,*}	15.7 (14.2)$^{b,c,*,\beta}$
	6	10.8 (14.0)$^{b,**,\beta}$	40.7 (19.2)^{c,*}	13.6 (8.3)$^{b,c,*,\beta}$
	14	17.4 (9.3)^{b,**}	28.9 (25.7)^{c,*}	11.7 (16.6)$^{b,c,**,\beta}$
TCF	2	32.7 (23.1)^{a,*}	25.7 (15.1)^{a,*}	41.5 (25.6)^{a,c,*}
	4	47.4 (17.9)$^{a,*,\beta}$	19.6 (13.0)$^{a,*,\beta}$	32.9 (13.3)^{b,d,*}
	6	60.5 (18.4)$^{a,d,**,\beta}$	10.00 (9.6)$^{a,**,\beta}$	29.6 (16.6)^{b,d,*}
	14	72.2 (17.5)^{a,**}	1.1 (2.5)^{a,**}	26.7 (18.2)^{a,c,*}

TC Theodent-Classic®, *Novamin*® Sensodyne 5000® Nupro, *Fluoride* Colgate regular™, *TCF* Theodent-Classic® with Fluoride. Letters compared the effect of the four toothpastes at each usage time point (2, 4, 6, and14 times). Different letters (a, b, c, and d) denote significantly different ($p<0.05$) data, while similar letters means not significantly different. For %COT, c and d compare Novamin® and TCF after 6 times usage of the products. Symbols (*, **, γ, and β) compared the effect of the same toothpaste after different lengths (2, 4, 6, and 14 times) of usage. Different symbols denote significantly different ($p<0.05$) data, while similar symbols means not significantly different

times use of either theobromine-containing dentifrice, with 100 % deposition achieved with four (TCF) and six (TC) times usage (Fig. 1). Fluoride toothpaste deposited a negligible amount of precipitate layer even with 14 times usage of the product (Figs. 1 and 2). Within each dentifrice, %DPL increased overtime but the differences between usage periods were only significant ($p<0.05$) with Novamin® and Fluoride.

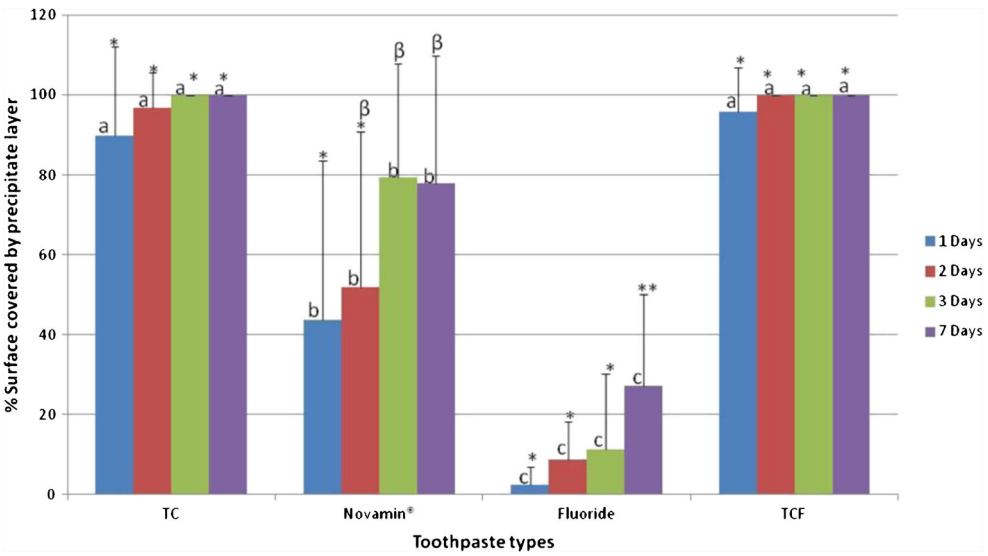

Fig. 1 Mean (SD) %DPL after twice daily use of the four dentifrices. *TC* Theodent-Classic®, *Novamin*® Sensodyne 5000® Nupro, *Fluoride* Colgate regular™, *TCF* Theodent Classic® with Fluoride, for 1, 2, 3, and 7 days. Letters compared the efficacy of the four toothpastes at each usage time point (1, 2, 3, and 7 days). Different letters (a, b, c, and d) denote significantly different ($p<0.05$) percentage of deposited precipitate layer, while similar letters means not significantly different. Symbols (*, **, and β) compared the efficacy of the same toothpaste after different lengths (1, 2, 3, and 7 days) of usage. Different symbols denote significantly different ($p<0.05$) percentage of deposited precipitate layer, while similar symbols means not significantly different

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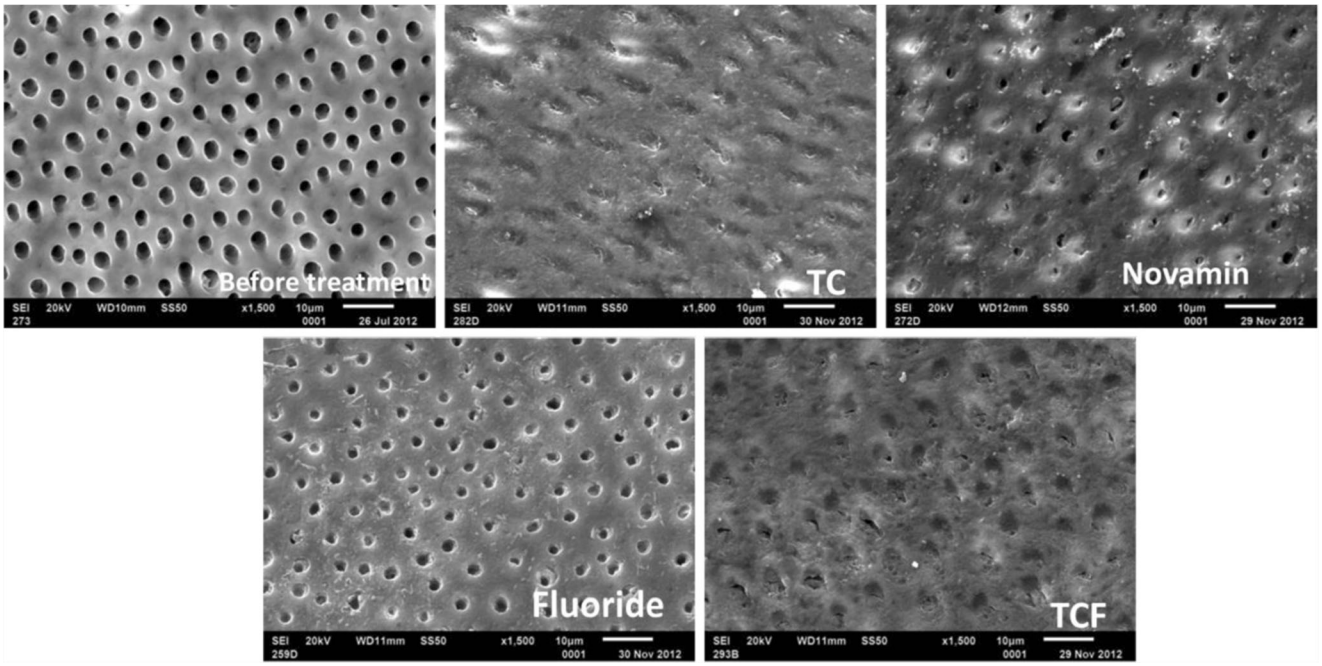

Fig. 2 Representative SEM images of dentin blocks; before treatment and following 14 times (7 days) use of the toothpastes. *TC* Theodent Classic®, *Novamin*® Sensodyne 5000® Nupro, *Fluoride* Colgate regular™, *TCF* Theodent Classic® with Fluoride

Discussion

In this in situ study, the ability of theobromine-containing toothpaste (Theodent classic™) to physically occlude dentin tubules as a surrogate measure of its potential to relief dentin hypersensitivity was investigated and measured based on %COT, %POT, %FOT, and %DPL. These variables were monitored after 2, 4, 6, and 14 times usage of the product to determine the number of applications required to achieve adequate tubule occlusion. The present study demonstrated a steady increase in %COT and %DPL, and concomitant decrease in %POT and %FOT overtime (Table 1 and Fig. 1). Addition of 1,500 ppm fluoride in this dentifrice did not produce a significant difference to its tubule occlusion and precipitate layer deposition potential, although there was a nonsignificant increase in both %COT and %DPL (Table 1 and Fig. 1). This ability of TC, as demonstrated in the present study, to bring about the precipitative occlusion of dentin tubules and deposition of precipitate layer on dentin surface can be attributed to the reports of previous studies [27, 28], in which the presence of theobromine in an apatite-forming system caused increase in crystallite size. A crystallite or clusters of crystallites measuring over 2 μm were observed when grown in the presence of 1.1 mmol/l of theobromine, while a crystallite measuring only 0.5 μm was obtained in the absence of theobromine [27, 28].

The above potential of TC was compared with those of a standard 1,500 ppm fluoride dentifrice and a Novamin®-containing dentifrice with 5,000 ppm fluoride in the present study. While TC rapidly produced a relatively high %COT (27 %) with just two times (1 day) usage of this product, it took 6 to 14 times (3–7 days) usage for %COT produced by Novamin® to be comparable but still lower to that of TC (Table 1). The amount of precipitate layer (90 %) deposited by two times (1 day) usage of TC doubled that produced by Novamin® (44 %) with the same number of usage and remained significantly higher than that of Novamin® at all measurement points (Fig. 1). At any time point, %FOT was greater and %POT less with Novamin® than with TC (Table 1). The standard toothpaste produced a relatively negligible amount of both %COT and %DPL (Table 1 and Figs. 1–2) within this study period (1 week usage). Thus this study demonstrated that while TC has a rapid reaction in occluding dentin tubule and as such exerts its effect within a shorter period of time (within first day of use), Novamin® required relatively more usage time (about 1 week usage) before its tubule occluding potential can manifest (Table 1 and Figs. 1–2). This may be attributed to the modes of action of the two agents. While the result of this study suggests that theobromine promotes rapid formation of crystallites, the glass particles protecting the calcium and phosphate ions in Novamin® need to be trapped for the calcium and phosphate to be localized for formation of apatite layer. When Novamin® is introduced into the oral environment, calcium and phosphate ions are released, which then interact with the oral fluids to form crystalline hydroxycarbonate apatite layer [36, 39]. Thus

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the mode of action of Novamin®, which is based on the chemical reactivity with aqueous solution, might have delayed the deposition of apatite layer relative to theobromine.

The SEM images (Fig. 2) further confirmed the data depicted in Table 1 and Fig. 1. Almost the entire dentin surface was covered by precipitate layer deposition with only two times usage of either TC or TCF, thus confirming the rapid action of the two products. Precipitate layer and occluded tubules were hardly noticeable with the use of Fluoride at any measurement time point (Figs. 1–2). Thus the fact that the two theobromine-containing dentifrices have equal ability, and in a shorter period of time (Table 1 and Figs. 1–2), on all four measurement criteria more than Novamin® and Fluoride, despite the high content of fluoride in Novamin® (5,000 ppm) and equal amount of fluoride (1,500 ppm) in TCF and Fluoride (1,500 ppm), suggests that the presence of fluoride did not play any significant role to the tubule occlusion effect of these products as measured in the present study.

Of the in situ model used in the present study, it is important to note the use of cervical dentin, rather than coronal dentin as some investigators do. We considered the use of cervical dentin more clinically relevant since most dentin hypersensitivity cases are consequences of gingival recession exposing cervical dentin tubules to the oral cavity, and thus their nerve and fluid contents to external stimuli. Furthermore, one may argue that the absence of dentin liquor (tissue fluid from the pulp tissue filling out the hollows of the dentin) in dentin tubules employed in the present study might have influenced the results. It is conceivable that during intraoral exposure of the dentin blocks saliva perfused the dentin tubule by capillary action, thereby acting like dentin liquor. However, whether this has happened or not, one must admit the fact that the presence of dentin liquor in vivo would influence the rate of tubule occlusion. Thus the potential of a dentifrice to occlude dentin tubule in vivo may not be absolutely extrapolated from the result of an in vitro study in which the dentin tubules were not perfused with dentin liquor.

In conclusion, the result of this in situ study demonstrated that theobromine-containing (with and without fluoride) and Novamin®-containing toothpastes were able to occlude dentin tubules as well as deposit precipitate layer on the dentin surface in 1 week, but standard fluoride toothpaste was not. Theobromine-containing toothpaste with or without fluoride exert these effects in a shorter period of time than novamin®-containing toothpaste when measuring complete and partial tubule occlusion, precipitate layer deposition and the concomitant reduction of fully open tubules. Based on these variables, the ability of the theobromine-containing toothpaste with or without fluoride to occlude dentin tubules was equal and more than that of the Novamin®-containing toothpaste.

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