

Clinical Evaluation of Theobromine-containing Toothpaste for Dentin Tubule Occlusion

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Abstract

Objective: This double-blind, randomized, parallel group clinical study compared dentin tubules occlusion by theobromine-containing dentifrices with (Theodent-classic-F[®]) and without (Theodent-classic[®]) fluoride with commercially available standard fluoride (Colgate[®] Regular) and Novamin[®]-containing (Sensodyne[®]-5000-Nupro) dentifrices. **Design:** Each of 80 participants 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 corresponding control (untreated) blocks were examined with Scanning Electron Microscope (SEM). The efficacies of the dentifrices were compared statistically (ANOVA/Tukey's test; $\alpha=0.05$) based on the % surface area covered by deposited smear layer (%DSL) and the 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 show increased %COT and %DSL with increasing usage of each product. After 1 and 2 days, %COT was significantly ($p<0.05$) higher with Theodent-classic[®] (TC) and Theodent-classic[®]-F (TCF) compared with Sensodyne and Colgate. Following 3 and 7 days, %COT was not different among TC, TCF and Sensodyne but remained significantly ($p<0.05$) lower in Colgate compared to these three. No difference in %COT between TC and TCF at all measurement points. Within each dentifrice, %COT increased significantly ($p<0.05$) with increasing usage except in Colgate. At any measurement points, %DSL were significantly ($p<0.05$) higher in TC, TCF and Sensodyne compared with Colgate. Within each dentifrice, %DSL increased with increasing usage but the differences at various

measurement points were only significant ($p < 0.05$) in Sensodyne and Colgate. **Conclusions:** Regarding dentin tubule occlusion and smear layer deposition, theobromine-containing toothpastes with and without fluoride were equally more effective in a shorter time period than novamin[®]-containing toothpaste; however, the three were equally efficacious after one week but not the standard fluoride toothpaste.

Introduction

Dentin hypersensitivity (DHS) 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 4 to 57% in the general population studied and 60 to 98 percent in patients with periodontitis (3, 4), with more than 90% of hypersensitive tooth surfaces located at the cervical margin on the facial aspects of the teeth (3). DHS still is an underestimated problem in daily clinical practice, possibly because most patients develop coping strategies.

Present approaches to treat DHS employed agents that either chemically suppress or modify the nerve impulse by direct neurological interaction or mechanically occludes the dentin tubules to decrease dentin permeability and prevent fluid movement, 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 (5, 6). Dentin tubules occlusion is the most current therapeutic approach. Some pastes or aqueous solutions containing potassium oxalate, ferric oxalate, and glutaraldehyde achieved this by precipitative intratubular occlusion (7-15). However, available evidence does not currently support the recommendation of dentin hypersensitivity treatment with oxalates (15) while the potential biocompatibility hazards associated with glutaraldehyde cannot be ignored (16). Recently, good clinical results from dentin tubule occlusion were reported with products containing arginine/calcium carbonate, calcium sodium phosphosilicate (NovaMin®) or strontium acetate (17-19).

Fluoride varnish was the first 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 iontophoresis (23) showed some cumulative efficacy. Adhesive bonding techniques have also been used to seal the tubules but due 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 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 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, 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 measure of their efficacy to treat dentin hypersensitivity. The toothpaste was compare with commercially available standard 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 causes dentin tubule occlusion and deposition of smear layer, of percentage that is significantly greater than zero. The second hypothesis is that the four dentifrices differ with respect to percentage of dentin tubule occlusion and deposited smear layer. Of

special interest is whether the non-fluoride theobromine-containing dentifrices promote greater dentin tubule occlusion and deposition of smear layer relative to the novamin-containing dentifrice.

Materials and Methods

Study Design: This was a double-blind, randomized, parallel group, single center, controlled clinical trial to test the ability of theobromine-containing toothpaste with and without fluoride to physically occlude dentin tubules, comparing them with those of a novamin-containing toothpaste (Sensodyne®-5000 Nupro) and a standard fluoride dentifrice (Colgate regular™). The primary outcome is precipitative occlusion of dentin tubules and deposition of smear 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 smear layer. In this trial, each of 80 participants wore four intraoral appliances 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 smear 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 all participants provided written informed consent. The majority of participants were recruited from among patients receiving treatment in the dental schools' clinics.

Specimen Preparation: Using Water cooled diamond wire saw (Well Diamond Wire Saws, Inc. Norcross GA, USA), rectangular dentin blocks measuring approximately 4 mm length x 1.5mm

width x 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, Inc. CA, USA) initially with 30 μ m diamond grit and finishing with 1 μ m grit. Each block was then sectioned into two halves to produce a pair of blocks, each measuring 2 mm length x 1.5mm width x 0.75mm 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, USA) each pair of blocks (control and test) simultaneously in 200ml beaker with 6% citric acid (pH 2.0) for 2 minutes (power setting 1). This was followed by another 2 minutes 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 hours in laminar hood.

Following drying, the control dentin blocks were examined with SEM 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 Inc.; Model: JSM-6610LV; JEOL Company, 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 1500X (pre-treatment 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 intra-oral appliance, a customized orthodontic bracket, described in our previous publication (29). 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.

Participants 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 intra-oral 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 ml/min and ≥ 0.7 ml/min, respectively, measured according to Sreebny and Valdini (1987) procedure (30), 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 participant or appointment to receive dental treatment which may affect their participation.

Study Treatment: Participants were randomized to one of four commercially available toothpastes (20 participants/product); theobromine-containing toothpaste without (TC) and with (TCF) fluoride (Theodent classic®; Theocorp Holding Company, Metairie, LA, USA), Novamin-containing toothpaste (Sensodyne 5000 Nupro®; DENTSPLY Professional; York, PA, USA) and Standard fluoride toothpaste (Colgate regular™; Colgate Pharmaceuticals, New York, NY, USA). All Participants 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. On each occasion, subjects brushed for one minute using at least a one-inch strip of their respective toothpaste and then wait for another one minute before rinsing with 10 ml of water for 10 seconds. 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 minutes after brushing. A diary was provided to each subject to keep a record of the number of times brushed each day. All subjects were asked to maintain their normal dietary habits. The use of any other oral hygiene product, such as mouthwashes, prescription products, etc, was prohibited.

After the 7 days washout period, the *in situ* appliances, bearing the dentin blocks, were assigned and fitted to each participant. Each subject wore four dentin blocks to permit efficacy assessments after 1 day (2 times product usage), 2 days (4x product usage), 3 days (6x product usage) and 7 days (14x 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 seconds, in accordance with current principles of dental practice, washed and dried for a further 30 seconds, 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 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 2x 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, 4x usage), day-4 (for 3 days, 6x usage), and day-8 (for 7 days, 14x usage) when the remaining dentin-bearing appliances were 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, and were then visualized and scanned with SEM 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 1500X (post-treatment 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 Pre- and post-treatment SEM image was assessed, by two calibrated blinded examiners, for the extent of tubule occlusion based the numbers of fully-open, partially and completely occluded dentin tubules as well as the extent of dentin surface covered by smear layer on each of 1500X 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. The free-margin Kappa 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 smear layer (%DSL) 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 (31,32,33) 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 % 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* multi-step comparisons using Tukey's HSD test, the efficacies of the four toothpastes in occluding dentin tubules were compared based on the %FOT, %POT, %COT and %DSL. The efficacies of the four toothpastes were compared at 1 day (2 product usage), 2 days (4 product usage), 3 days (6 product usage) and 7 days (14 product usage) time points. Intra-product comparison of efficacies after 2, 4, 6 and 14 product usage was performed to determine longitudinal effect.

RESULTS

The 80 subjects recruited for this trial completed the study without any dropout. There was no incidence of adverse event reported. As shown in figures 1, %COT was comparable in TC and

TCF at all measurement time points, but significantly ($p < 0.05$) higher in TC and TCF when compared with Colgate at all time points and when compared with Sensodyne after 2 and 4 product usage. However, after 6 and 14 product usage, %COT was comparable in Sensodyne, TC and TCF. After 2 and 4 product usage, %COT was comparable in Sensodyne and Colgate. Within each dentifrice, %COT increased significantly ($p < 0.05$) with increasing number of usage. Figure 2 shows that %FOT were significantly ($p < 0.05$) different among the four dentifrices at every measurement time point except between TC and TCF. Within each dentifrice, %FOT decreased with increasing times of use; however, this was only significant ($p < 0.05$) after 6 product usage in all dentifrices except in Colgate where the decrease was not significant. In figure 3, %POT was significantly ($p < 0.05$) higher with TC at every time point when compared with Sensodyne and Colgate, but only after 4 and 6 product usage when compared with TCF. Further, with regards to %POT, Sensodyne and Colgate were comparable at every measurement time points and the two were comparable with TCF after 4 and 6 product usage. Within each dentifrice, %POT decreased significantly ($p < 0.05$) with increasing number of usage. Figure 4 shows that at all measurement time points, %DSL were not different in TC and TCF but were significantly ($p < 0.05$) higher in these two theobromine-containing dentifrices when compared with either Sensodyne or Colgate. Smear layer deposition was more than 90% with one day usage of the two theobromine-containing dentifrice, and 100% deposition was achieved with 4 and 6 product usage of TCF and TC respectively. Sensodyne deposited significantly ($p < 0.05$) more smear layer than Colgate. Within each dentifrice, %DSL increased with increasing number of usage but the differences at various measurement time points were only significant ($p < 0.05$) in Sensodyne and Colgate.

Figures 5, 6, 7 and 8 show the typical SEM images of the surface of the dentin blocks with tubules before (bf) and after the usage of the toothpastes for 2 (1 day), 4 (2 days), 6 (3 days), and 14 (7 days) times. The images demonstrate the increasing occlusion of the dentin tubules and deposition of smear layer with increased usage of TC, TCF and Sensodyne but not Colgate. Colgate Regular toothpaste deposited a negligible amount of smear layer even after 14 usage of the product.

Discussion

In this clinical study the efficacy of theobromine-containing toothpaste (Theodent classic™) to physically occlude dentin tubules as a surrogate measure of its ability to relief dentin hypersensitivity was investigated and measured based on percentage of completely-occluded, partially-occluded, and fully-open tubules as well as the percentage of dentin surface covered by deposited smear layer. These variables were monitored after 2, 4, 6 and 14 usage of the product to determine the number of applications required to achieve efficacy. The present study demonstrated a steady increase in percentage of both the completely occluded tubules and deposited smear layer, and concomitant decrease in partially-occluded and fully-open tubules, with increased usage of the dentifrice (Figures 1-4). Addition of 1500 ppm fluoride in this dentifrice did not produce a significant difference to its tubule occlusion and smear layer deposition efficacies although there was a non-significant increase in both %COT and %DSL (Figures 1-4). This ability of the theobromine-containing dentifrice, as demonstrated in the present study, to bring about the precipitative occlusion of dentin tubules and deposition of smear 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 microns were observed when grown in the presence of 1.1 mmol/L of theobromine, while a crystallite measuring only 0.5 micron was obtained in the absence of theobromine (27, 28). The promotion of remineralization of early caries lesion by theobromine present in artificial saliva has also been demonstrated (26).

The above efficacy of Theodent Classic™ was compared with those of a standard 1500 ppm fluoride dentifrice (Colgate® Regular) and a novamin®-containing dentifrice with 5000 ppm fluoride (Sensodyne® 5000 Nupro) in the present study. While Theodent Classic™ rapidly produced a relatively high amount of completely-occluded tubules (27%) with just two times (1 day) usage of this product, it took 6 to 14 times (3-7 days) usage for the amount of completely-occluded tubules produced by Sensodyne® to be comparable but still lower, to that of Theodent Classic™ (Figure 1). The amount of smear layer (90%) deposited by two times (1 day) usage of Theodent Classic™ doubled that produced by Sensodyne® (44%) with the same number of usage and remained significantly higher than that of Sensodyne® at all measurement points (Figure 4). At any time point the percentage of fully-open tubules was greater and partially-occluded tubules less with Sensodyne® than with Theodent Classic™ (Figures 2 and 3). The standard toothpaste, Colgate®, produced a relatively negligible amount of both completely-occluded tubules and deposited smear layer (Figure 1 and 4) within this study period (one week usage). Thus this study demonstrated that while Theodent Classic™ has a quick reaction in occluding dentin tubule and as such very efficacious in a shorter period of time (within first day of use), novamin®-containing dentifrice required relatively more usage time (about one week usage) before its efficacy can manifest (Figures 1-4). 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 (34, 35). Thus 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 examination and images (Figures 5-8) confirmed the data depicted in Figures 1 through 4. Almost the entire dentin surface was covered by smear layer deposition with only two times usage of either Theodent Classic® or Theodent Classic®-with-fluoride, thus confirming the rapid action of the two products. Smear layer and occluded tubules were hardly noticeable with the use of Colgate® at any measurement time point (Figures 1-4).

In conclusion, the result of this clinical study demonstrated that Theodent Classic®, Theodent Classic®-with-fluoride, and Sensodyne-Nupro-5000® toothpastes are efficacious in occluding dentin tubules as well as depositing smear layer on the dentin surface in one week but Colgate toothpaste was not. Theodent Toothpaste with or without fluoride was more effective in a shorter period of time than Sensodyne Nupro 5000® when measuring complete and partial tubule occlusion, smear layer deposition and the concomitant reduction of fully open tubules. Based on these variables, Theodent Classic® and Theodent Classic® with Fluoride were equally more efficacious than the Novamin-containing Sensodyne® 5000 Nupro.

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Figure Legends

Figure 1: The % of completely occluded tubules after twice daily use of the four dentifrices for 1, 2, 3 and 7 days. T1=Theodent-Classic[®], T2 = Sensodyne 5000[®] Nupro, T3 = Colgate regular[™] and T4 = Theodent-Classic[®] with Fluoride. Letters compared the efficacy of the four toothpastes at each usage time point (1, 2, 3 & 7 days). Different letters (a, b, c, d) denote significantly different ($p < 0.05$) % of completely occluded tubules, while similar letters means not significantly different. Symbols (*, **, γ , β) compared the efficacy of the same toothpaste after different lengths (1, 2, 3 & 7 days) of usage. c & d compares T2 and T4 after 3 days use of product. Different symbols denote significantly different ($p < 0.05$) % of completely occluded tubules, while similar symbols means not significantly different.

Figure 2: The % of fully-open tubules after twice daily use of the four dentifrices for 1, 2, 3 and 7 days. T1=Theodent-Classic[®], T2 = Sensodyne 5000[®] Nupro, T3 = Colgate regular[™] and T4 = Theodent-Classic[®] with Fluoride. Letters compared the efficacy of the four toothpastes at each usage time point (1, 2, 3 & 7 days). Different letters (a, b, c) denote significantly different ($p < 0.05$) % of fully-open tubules, while similar letters means not significantly different. Symbols (*, **, β) compared the efficacy of the same toothpaste after different lengths (1, 2, 3 & 7 days) of usage. Different symbols denote significantly different ($p < 0.05$) % of fully-open tubules, while similar symbols means not significantly different.

Figure 3: The % of partially-occluded tubules after twice daily use of the four dentifrices for 1, 2, 3 and 7 days. T1=Theodent-Classic[®], T2 = Sensodyne 5000[®] Nupro, T3 = Colgate regular[™] and T4 = Theodent-Classic[®] with Fluoride. Letters compared the efficacy of the four toothpastes at each usage time point (1, 2, 3 & 7 days). Different letters (a, b, c, d) denote significantly different ($p < 0.05$) % of partially-occluded tubules, while similar letters means not significantly different. Symbols (*, **, β) compared the efficacy of the same toothpaste after different lengths (1, 2, 3 & 7 days) of usage. Different symbols denote significantly different ($p < 0.05$) % of partially-occluded tubules, while similar symbols means not significantly different.

Figure 4: The % of surface area covered by deposited smear layer after twice daily use of the four dentifrices for 1, 2, 3 and 7 days. T1=Theodent-Classic[®], T2 = Sensodyne 5000[®] Nupro, T3 = Colgate regular[™] and T4 = Theodent-Classic[®] with Fluoride. Letters compared the efficacy of

the four toothpastes at each usage time point (1, 2, 3 & 7 days). Different letters (a, b, c, d) denote significantly different ($p < 0.05$) % of completely occluded tubules, while similar letters means not significantly different. Symbols (*, **, β) compared the efficacy of the same toothpaste after different lengths (1, 2, 3 & 7 days) of usage. Different symbols denote significantly different ($p < 0.05$) % of completely occluded tubules, while similar symbols means not significantly different.

Figure 5: Before treatment (BF); After 2 uses (1 day) of toothpastes T1=Theodent-Classic®, T2 = Sensodyne 5000® Nupro, T3 = Colgate regular™ and T4 = Theodent-Classic® with Fluoride.

Figure 6: Before treatment (BF); After 4 uses (2 days) of toothpastes T1=Theodent-Classic®, T2 = Sensodyne 5000® Nupro, T3 = Colgate regular™ and T4 = Theodent-Classic® with Fluoride.

Figure 7: Before treatment (BF); After 6 uses (3 days) of toothpastes T1=Theodent-Classic®, T2 = Sensodyne 5000® Nupro, T3 = Colgate regular™ and T4 = Theodent-Classic® with Fluoride..

Figure 8: Before treatment (BF); After 14 uses (7 days) of toothpastes T1=Theodent-Classic®, T2 = Sensodyne 5000® Nupro, T3 = Colgate regular™ and T4 = Theodent-Classic® with Fluoride.

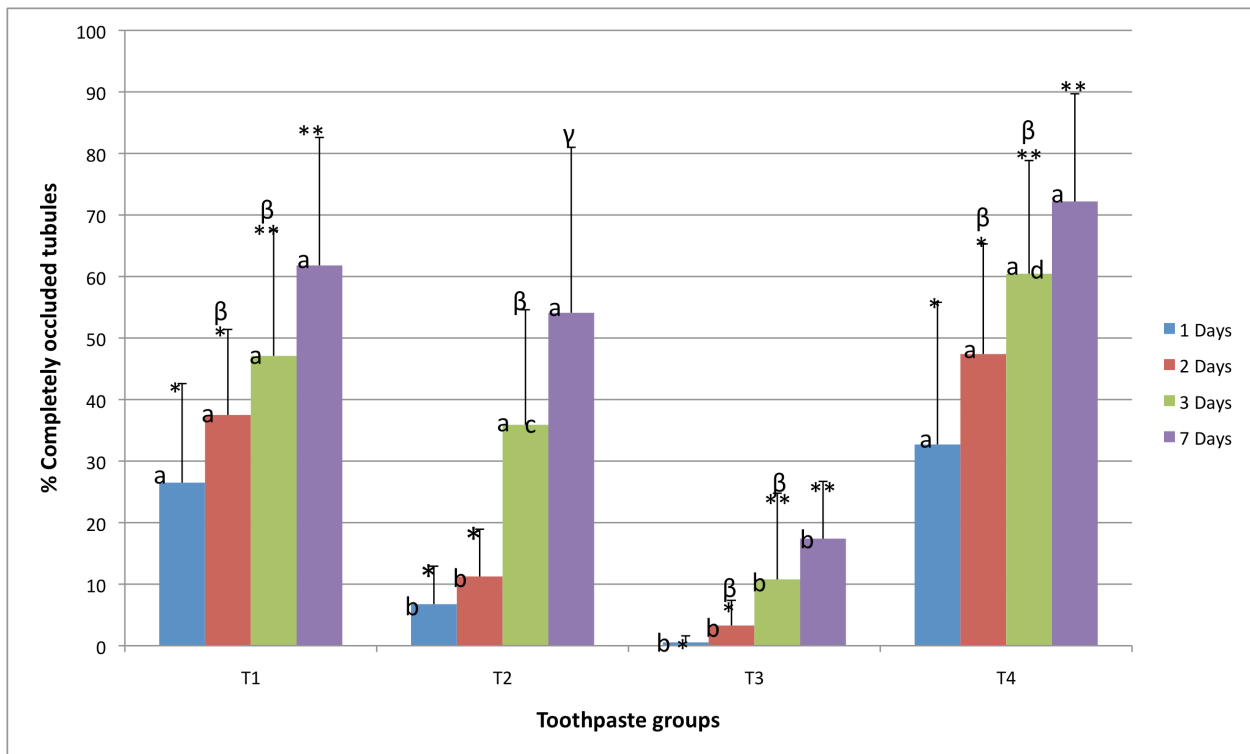


Figure 1

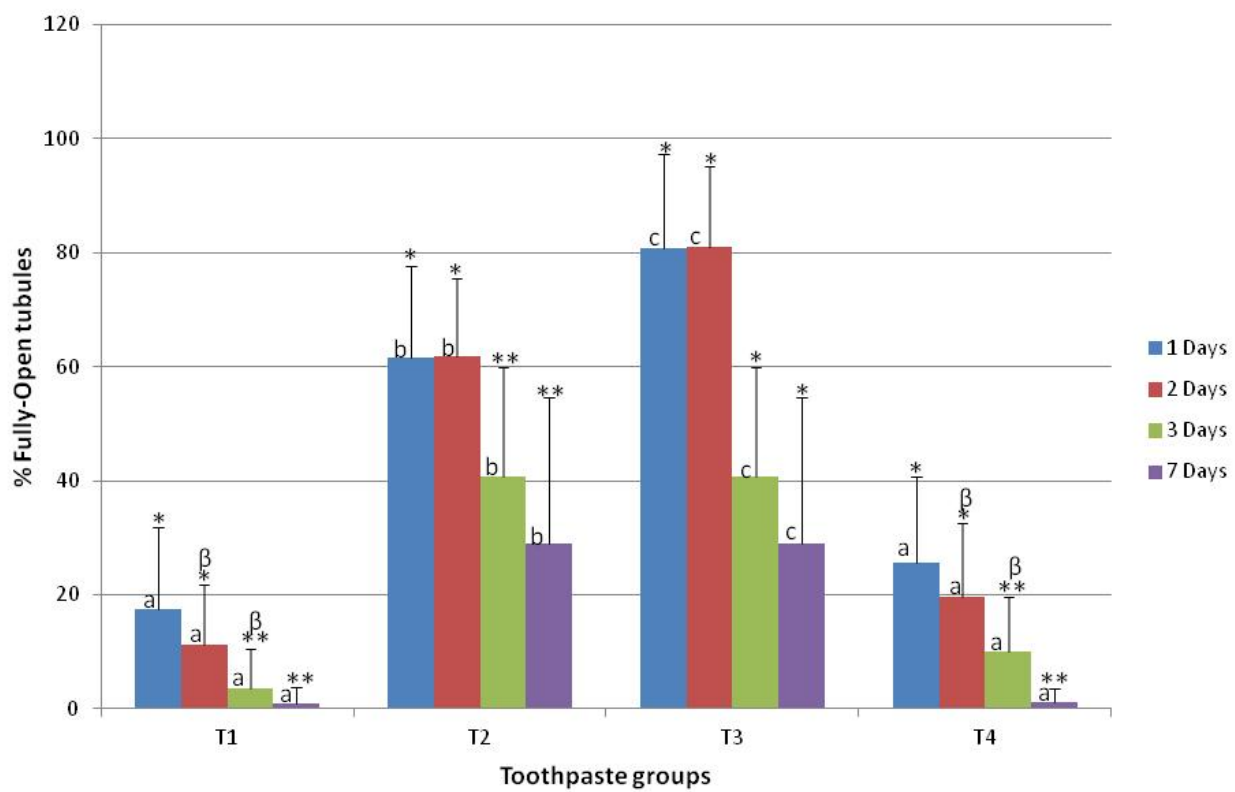


Figure 2

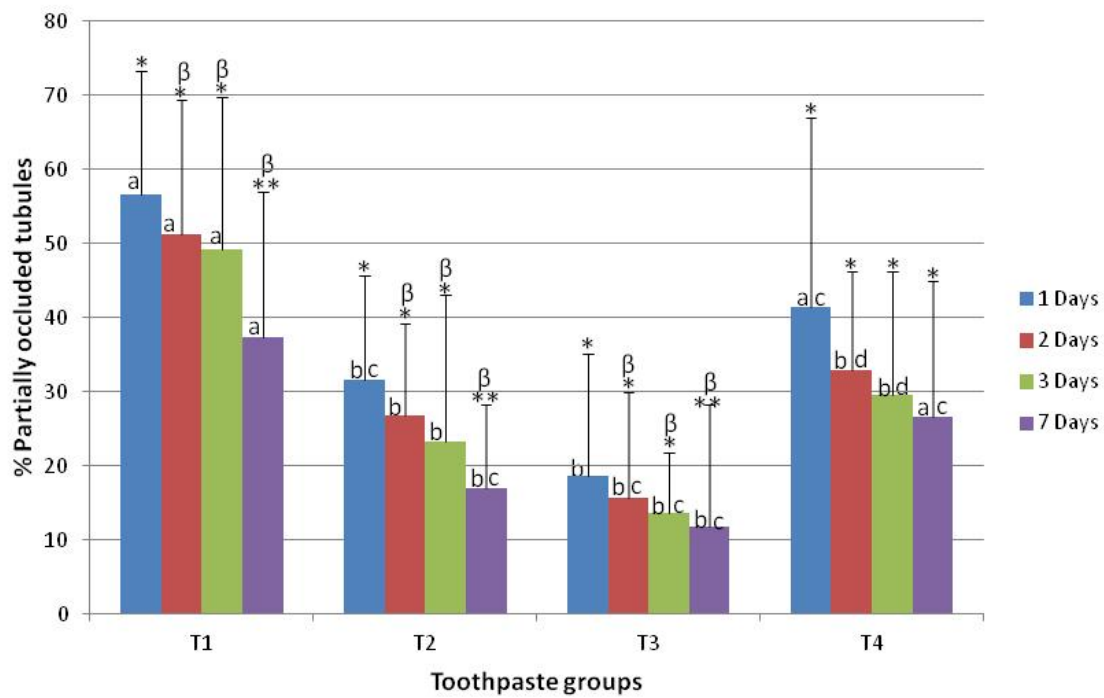


Figure 3

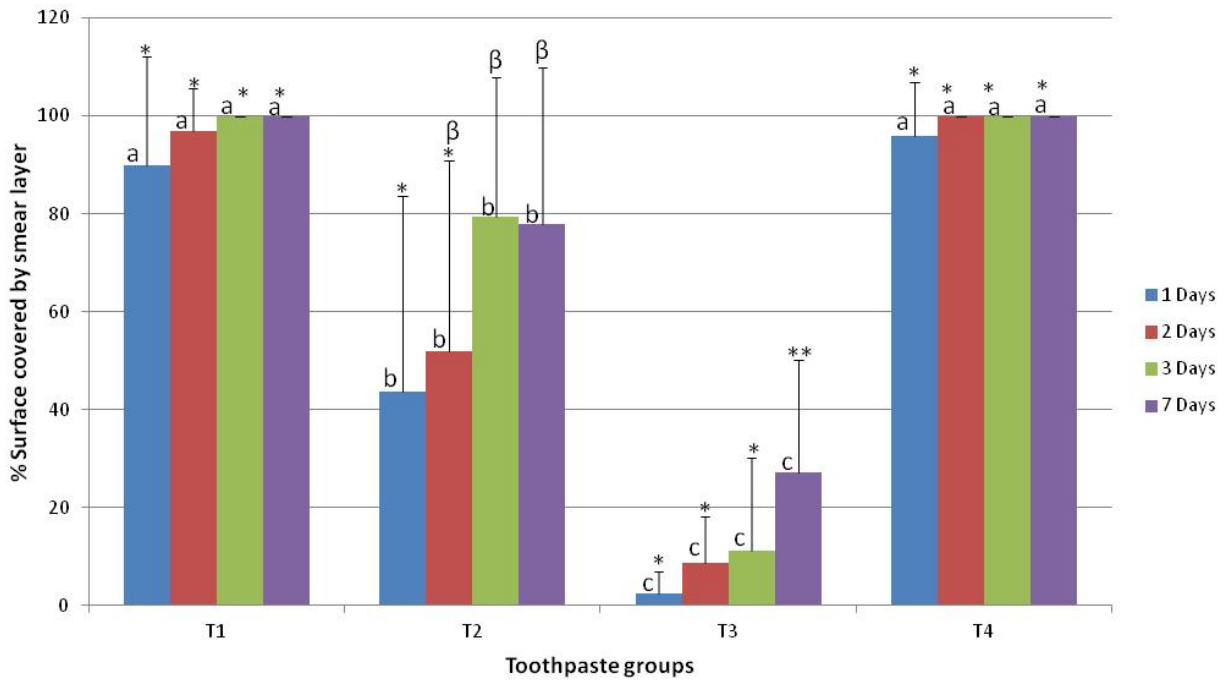


Figure 4

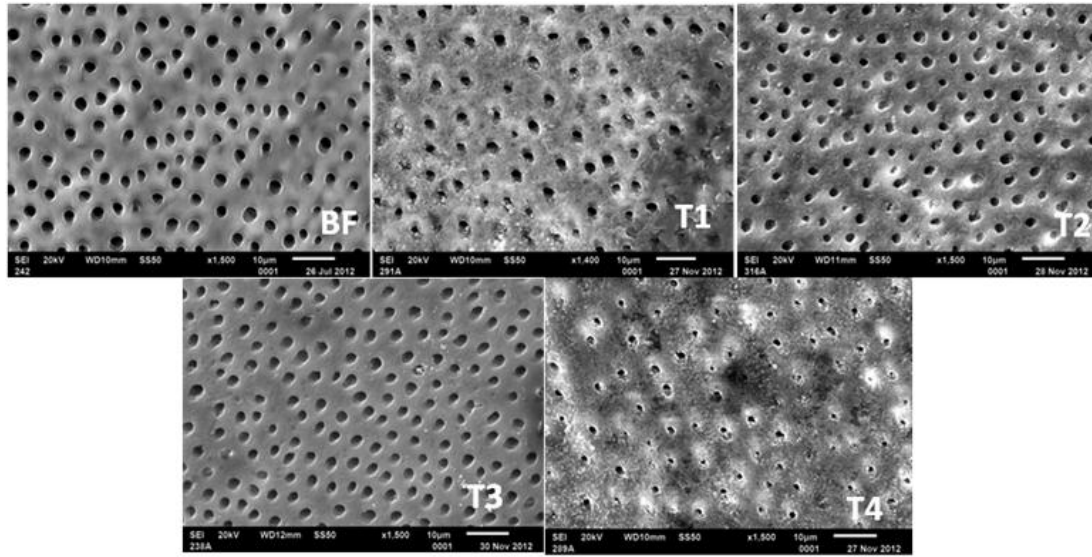


Figure 5

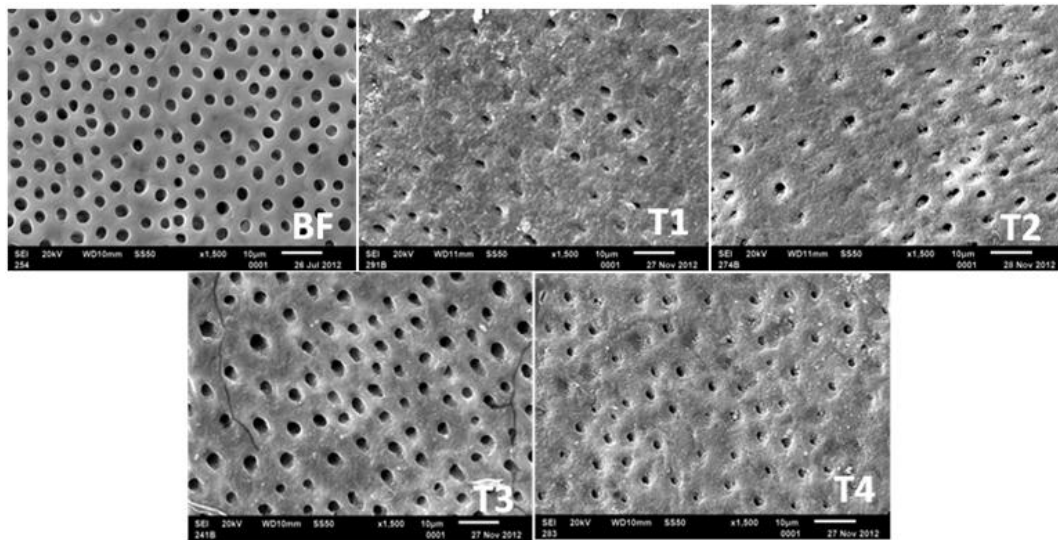


Figure 6

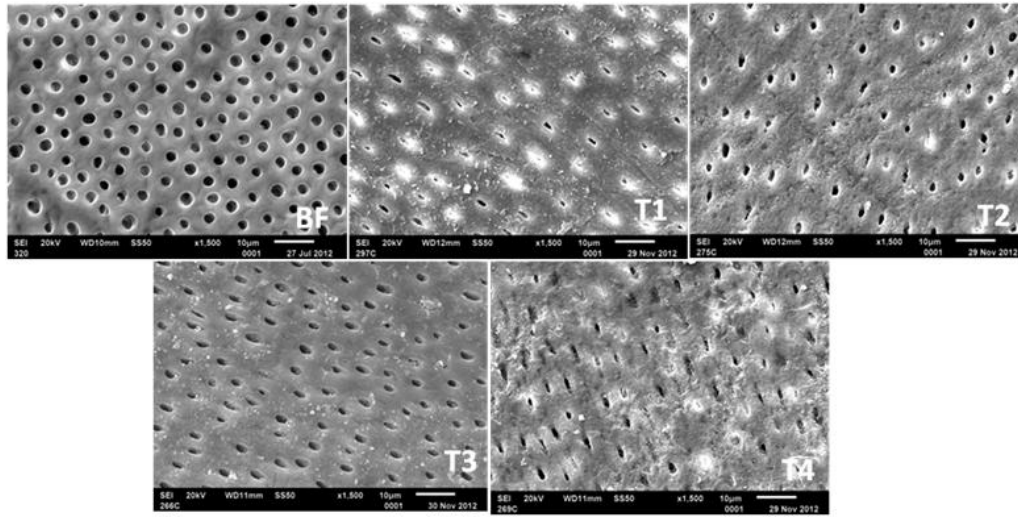


Figure 7

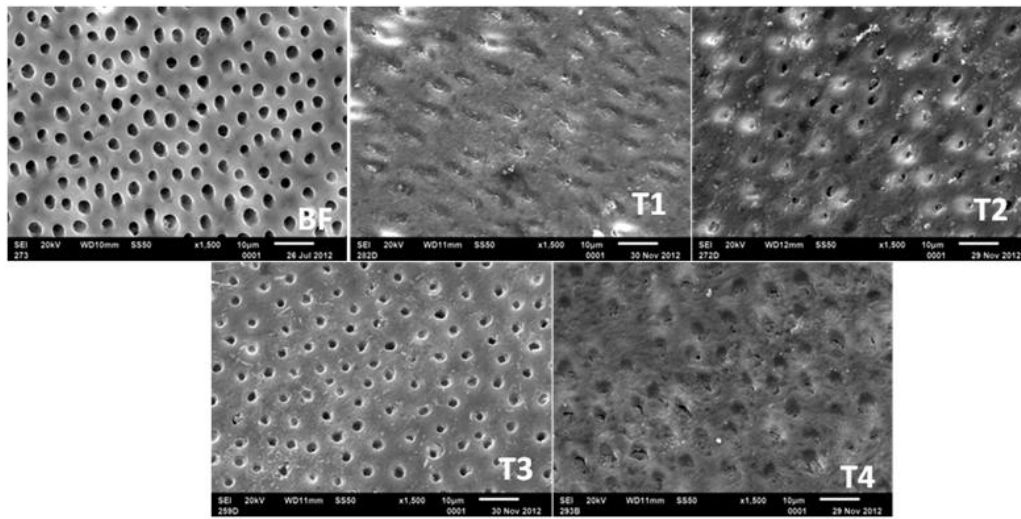


Figure 8