J Clin Periodontol 2004; 31: 325–335 doi: 10.1111/j.1600-051X.2004.00488.x
Printed in Denmark. All rights reserved



Journal of Clinical Periodontology

# Dentine hypersensitivity: development and evaluation of a model in situ to study tubule patency

**N. Banfield and M. Addy**
Division of Restorative Dentistry, Dental School, Bristol, UK

*Banfield N, Addy M: Dentine hypersensitivity: development and evaluation of a model in situ to study tubule patency. J Clin Periodontol 2004; 31: 325–335. doi: 10.1111/j.1600-051X.2004.00488.x.* © Blackwell Munksgaard, 2004.

***Abstract***

**Background and aims:** Lesions of dentine hypersensitivity have numerous tubules open at the dentine surface as opposed to non-sensitive dentine where tubules are mostly covered by a smear layer. The present two studies were designed to model both states in situ and evaluate the effects of agents on the model.

**Method:** Etched (sensitive) and smeared (non-sensitive) dentine specimens prepared from human third molar teeth were retained in lower buccal acrylic appliances. The results of Study 1 led to the development of a method to ensure tubules were sectioned at right angles. Study 1 was a 5-day period, seven treatment regimens randomised, part blind cross over design involving five subjects. Treatments were 2 × day application of a desensitising (SA) or non-desensitising toothpastes (F) or chlorhexidine (CHX) mouthwash with or without drinking orange juice (OJ)(1 l/day). A no treatment group (P) allowed plaque to accumulate. The evaluation of effects was observational by scanning electron microscopy (SEM). Study 2 involved 1 subject, 4 treatments applied once and studied after 0, 6, and 12 h by SEM with image analysis. The 12 h groups were studied with and without imbibing water or OJ. Treatments were two desensitising (SA & SC) and one non-desensitising (F) toothpastes and an in office product (DS).

**Results:** Study 1: Treatment SA resulted in occlusion of tubules an outcome little changed by OJ. Treatment P produced a bio-film, which covered the dentine surface. CHX produced some tubule occlusion in three of the five subjects. F ± OJ and CHX+OJ had little effect and tubules remained open. For smeared specimens toothpastes and OJ removed the smear layer but SA ± OJ blocked tubules. P and CHX had no effect on the smear layer. Study 2: At 0 h, tubule occlusion was in order of magnitude DS ⩾ SA > SC > F. After 6 and 12 h with SA, SC and F some loss of occlusion occurred but not DS. Water and OJ by 12 h decreased occlusion for SA, SC and particularly F. Water and OJ removed virtually all of DS.

**Conclusions:** The aims of Study 1 were achieved and effect of treatments was not inconsistent with data in vitro. The need for more standardisation of specimens was appreciated and applied in Study 2 to allow image analysis to quantitatively record data. Further use of the model in randomised controlled clinical trials is envisaged.

Key words: densensitising agents; dentinal tubules; dentine hypersensitivity; method in situ; scanning electron microscope; toothpaste

Accepted for publication 17 June 2003

The hydrodynamic mechanism to explain dentine sensitivity was first proposed in 1900 (Gysi 1900), with evidence to support the hypothesis appearing much later (Brannstrom 1963). Consistent with the hydrodynamic theory teeth exhibiting dentine hypersensitivity, which may indeed be more accurately termed dentine sensitivity (for reviews, see Addy 1990, 2002, Pashley 1990, Dababneh et al. 1999), exhibit many more and wider dentinal tubules open at the dentine surface and patent to the pulp (Ishikawa 1969, Absi et al. 1987, 1989). Two

decades ago dentine hypersensitivity was described as an enigma (Johnson et al. 1982) a statement, which was reconsidered in more recent reviews (Dababneh et al. 1999, Addy 2002). These reviews presented the significant amount of information concerning dentine hypersensitivity that had accrued over 20 years and likened the condition to a tooth wear phenomenon. Also highlighted, nevertheless, was the lack of direct evidence concerning, the aetiology of the condition, the effect of treatment agents applied to dentine and the validity of proposed management strategies. Thus, much of the research associated with dentine hypersensitivity has been conducted in vitro, although associations have been drawn from epidemiological studies, lesions have been studied using replica techniques, biopsies or extracted teeth and treatments have been compared in classical randomised controlled clinical trials (for reviews see Jackson 2000, Pashley 2000, Addy 2002). Studies in vivo of the natural history of dentine hypersensitivity, the impact of potential aetiological agents on dentine and the changes produced by therapeutic formulations on open tubules are difficult, if not impossible, to perform at present. Faced with similar problems in many areas of dental science, researchers have resorted to models in situ to study dental phenomena or conditions. Many such models in situ are extensions of methods in vitro, indeed many models in situ involve techniques in vitro or ex vivo.

The aim of Study 1 was to develop a method in situ to investigate the impact of agents on dentine specimens, which had been prepared to simulate sensitive and non-sensitive dentine. Effects on dentine were evaluated subjectively by observing qualitative changes compared to untreated controls in the scanning electron microscope (SEM). Both quantitative and qualitative assessments of treatment effects were planned. In the event, Study 1 indicated the need for more standardisation of specimen preparation. This standardisation was applied to Study 2.

## Method and Materials

### Study 1

The study was an in situ single part blind, randomised 4-treatment-group, 7-treatments, split mouth crossover design with some treatments applied ex vivo. The United Bristol Healthcare Trust Ethics Committee approved the study and subjects were provided with verbal and written information on the study and gave signed consent to participate. Five healthy dentate volunteers participated; two males and three females aged between 26 and 54 years. All subjects had no relevant medical or pharmacotherapy histories, which might have influenced the conduct of the study. No subject wore removable prostheses or fixed or removable orthodontic appliances and all had sufficient teeth to retain two buccally positioned removable cold cured acrylic appliances. The appliances were constructed on plaster models of the lower arch prepared from alginate impressions. The acrylic body of the appliance spanned the arch from the disto-buccal surface of the lower canine to the disto-buccal surface of the second molar on the left and right segments. Retention of the appliances was by a ball-ended wire clasp between the embrasure space of the canine and first premolar and an Adams crib on the second molar. Clasping was into lingual undercuts at these specified sites. The buccal outer surface of the acrylic body of the appliances had a countersunk zone, approximately 20 mm × 6 mm × 2 mm, to hold four dentine specimens retained by sticky wax.

Dentine specimens were prepared from recently extracted, caries free, human third molar teeth. The crowns of the teeth were removed by transverse sectioning using a low speed diamond edge-coated microslice with water irrigation. The root portion was then sectioned longitudinally at approximately 1.5 mm widths. From these sections, specimens of root dentine measuring approximately 6 mm × 5 mm were fashioned using a lapping and polishing machine with 320-grit silicon carbide paper. One face, the test surface, was chosen and the opposite face marked with indelible ink. The test face was then polished to 1000 grit, giving a final dentine thickness of approximately 1 mm. All specimens were disinfected in sodium dichloro-1,3,5,triazinetrione (20,000 ppm) for 24 h, washed and then stored in distilled water at 4°C until used (usually no more than for 24 h). Immediately before placement, into the appliances, specimens were hand polished on 1000 grit paper with distilled water for 30 s each to recreate the smear layer partially removed by the disinfection technique. Four specimens were selected for placement in each appliance. Two specimens from each group had the smear layer removed by ultrasonication in 6% citric acid for 2 min and then washed in distilled water. All ''smeared'' and ''etched'' specimens were sectioned in half with dental wire cutters. Each half of each specimen was appropriately marked on the non-test face to allow re-identification of pairs. The left half (test face viewed) of each specimen was placed in the appliance and the right half served as the untreated control and stored in sealed tubes in distilled water. Appliances were worn from 8 a.m. to 10 p.m. Monday to Thursday and 8 a.m. to 5 p.m. on Friday. The seven treatments were as follows:

(1) Brush *ex vivo* twice daily for one minute with a strontium acetate desensitising toothpaste (SA) (MacLeans Sensitive, GlaxoSmithKline, Weybridge, UK).
(2) No treatment to allow plaque accumulation (P).
(3) Soak ex vivo twice daily for one minute in a proprietary 0.2% chlorhexidine mouthwash (CHX)(Corsodyl, GlaxoSmithKline, Weybridge, UK).
(4) Brush ex vivo twice daily for one minute with a conventional fluoride toothpaste (F) (Colgate, Colgate Palmolive, London, UK).
(5) Treatment 1 and drinking 250 ml of orange juice (OJ)(Sainsbury's Ltd., London, UK) 4 times daily.
(6) Treatment 4 and drinking 250 ml OJ, 4 times daily.
(7) Treatment 3 and drinking 250 ml OJ, 4 times daily.

Each subject wore two appliances when 6 treatments were combined as pairs, namely treatments 1 and 2, 3 and 4 and 5 and 6 using a split mouth approach. Treatment 7 was applied alone with one appliance in place. For the brushing treatments (1, 4, 5 and 6) applications to specimens in the respective appliance were ex vivo and performed by the individual subject using a standard flat trim multi-tufted toothbrush (Oral B 35, Oral B, London, UK).

Volunteers were asked to brush at approximately 9 a.m. and 6 p.m. OJ drinking was performed with the appliance in situ with the 250 ml consumed by sipping over a period of 10 min. Drinking times were at 3 h intervals commencing 10 a.m. of each day except Friday when drinking was at 2 h intervals

commencing 10 a.m. Overnight appliances were maintained at room temperature in sealed pots containing wet cotton wool. The exception was Treatment 2 (plaque accumulation) when appliances were placed in the same pot but containing 50 ml of phosphate buffered saline at 4°C in a household refrigerator. The orders of the four treatment groups was randomised by one investigator, to part blind the second investigator, who was to perform the SEM examinations. The study was part blind because specimens from Treatment 2 had to be treated differently for SEM preparation and therefore were identified to the second investigator who prepared and viewed specimens. Thus, specimens from all other treatments were removed from the appliances, matched with their respective control halves, both mounted on stubs and prepared for SEM by conventional vacuum gold sputter coating. For Treatment 2, one each of the smeared and etched specimens was gently wiped with cotton wool soaked in distilled water to remove any bio-film. The remaining two specimens from Treatment 2 were freeze dried before matching with control halves and sputter coating for SEM examination. A minimum of 2 days was allowed between study periods.

The SEM examination comprised capturing three images each near the fractured edge of test and control specimens. SEM photomicrographs were obtained as near as possible under the same conditions, HT = 20 KW, Working Distance 10.0 and magnification = 3060. The photomicrographs were assessed observationally for tubule patency by comparison of test and control images for each specimen for each treatment and for each subject. A proportion of test specimens from each treatment group were later fractured, re-sputter coated and viewed at the fracture surface.

#### Study 2

The study involved a single volunteer, wearing two removable lower buccal acrylic appliances containing etched dentine specimens. In this study to ensure tubules were sectioned at right angles to their long axis, the control part of each specimen was studied in the SEM. If tubule outlines were not judged to be circular the control and test parts were discarded. For each treatment and treatment condition, four etched specimens were placed in each appliance. The treatments applied to the 4 specimens were:

(1) A strontium acetate desensitising toothpaste (SA)(MacLeans Sensitive, GlaxoSmithKline, Weybridge, UK).
(2) A strontium chloride desensitising toothpaste (SC) (Sensodyne Original, GlaxoSmithKline, Weybridge, UK).
(3) An in-office desensitising product (DS)(D/Sense 2, Centrix Incorporated, Bridgeport, CT, USA) in the form of 2 liquids: one containing potassium phosphate and potassium carbonate and the other calcium chloride and strontium chloride, applied consecutively.
(4) A conventional fluoride toothpaste (F)(Colgate Regular, Colgate-Palmolive, London, UK).

The toothpastes were applied over the specimens ex vivo by tooth brushing with a standard toothbrush (Oral B 35, Oral B, London, UK) for 60 s. The in-office agent was applied according to the manufacturer's instructions. Prior to application of the treatments, the appliances, with contained specimens, were worn in situ for 60 min. After application of the respective treatment the appliances were replaced in situ and a 10 s mouth rinse with distilled water performed. For each treatment, five conditions in situ were performed, requiring groups of four etched specimens for each treatment condition, namely:

Group 1: 0 h in situ after treatment.
Group 2: 6 h in situ.
Group 3: 12 h in situ.
Group 4: 12 h in situ with 1 l of water imbibed.
Group 5: 12 h in situ with 1 l of orange juice imbibed.

The water and OJ were imbibed as 250 ml sipped over 10 min every 3 h.

Test specimens were recovered from the appliances, matched with their respective control specimens and fixed to SEM pin stubs with the fractured surface of control and test halves aligned as closely as possible. Specimens were then sputter coated with gold for examination in the SEM. For the control halves, four images, all in close proximity to one another, were captured from an area juxtaposed to the fracture edge. The exact adjacent corresponding area of test halves was viewed and six images captured. The working characteristics of the instrument were as used in Study 1. The SEM images from test and control specimens were qualitatively appraised and then quantitatively analysed using image analysis software (Scion Corporation, Frederick, MD, USA). The image analysis firstly calculated the area of tubule openings for each test and control image measured in $\mu m^2$. For the control specimens the tubule area was averaged across the four images to represent the mean permeability of the untreated specimen. For the test specimens, the highest and lowest areas were discarded and the mean permeability calculated by averaging across the remaining four images. The control mean permeability was then used to represent 100% permeability and the test mean permeability divided by the control mean permeability and then multiplied by 100 to calculate the percent reduction in permeability for each of the four specimens for each treatment and treatment condition. The image analysis was secondly employed to count the number of tubules on each test and control image. Again, the highest and lowest test image scores were discarded and the mean number of tubules averaged across the four remaining images. The percent reductions in tubules were then calculated for each treatment and treatment condition. The percent reductions in permeability and tubules for each treatment and treatment condition were expressed as means and standard errors and comparisons between treatments made using unpaired *t*-tests.

## Results

#### Study 1

All of the subjects completed the four treatment periods and no specimen losses occurred whilst appliances were worn. A small number of specimens (6) were damaged or lost in the laboratory.

A majority (70%) of the etched untreated control specimens revealed tubules cut at right angles so that orifices were circular in outline (Fig. 1). The remainder showed tubule orifices of varying ovoid outlines.

### Etched Specimens

#### Treatment 1: SA

All of the specimens revealed a heavy deposit covering most of the dentine surface and occluding the majority of tubules (Fig. 2a). Tubules not occluded

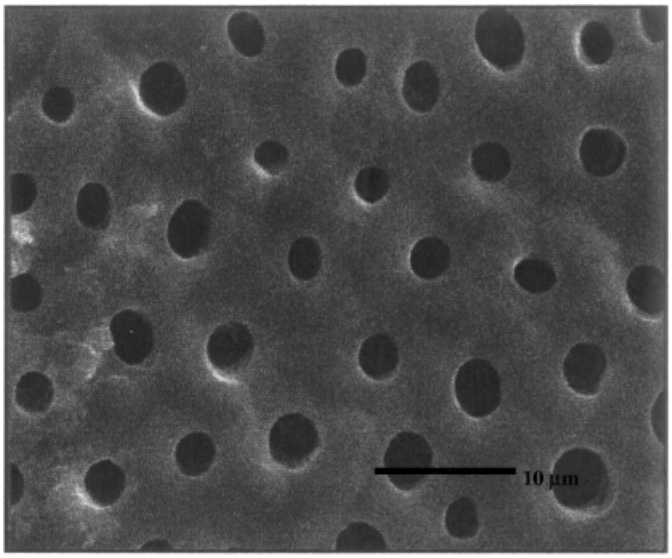


*Fig. 1.* Untreated etched, control dentine specimen with all tubules open and sectioned at right angles to the tubule long axis.

completely had reduced diameters. Some of these specimens were subsequently fractured and examination of the fracture edge revealed penetration of the deposit from 5 to >20 $\mu$m (Fig. 2b).

#### Treatment 2: P

In all cases etched specimens exhibited a plaque bio-film, which completely covered the dentine surface and no tubule orifices were seen (Fig. 3). A subsequent fracture of some specimens revealed a microbial film, which lay over the dentine surface but with little evidence of penetration into the tubules. Specimens where the bio-film had been wiped away appeared identical to control specimens with all tubules open.

#### Treatment 3: CHX

This treatment produced the greatest variation in morphological change in specimens and this appeared subject related. Three subjects showed either complete occlusion of some tubules or partial occlusion in others and in both etched specimens (Fig. 4). Two subjects showed no convincing changes in tubule outline compared to their respective controls and again in both samples.

#### Treatment 4: F

Virtually no deposited material was seen on the inter-tubular dentine surface in any specimen. However, all specimens studied showed a few tubules occluded and a large number reduced in diameter by comparison with respective controls (Fig. 5). Subsequent fracture of specimens failed to reveal material within the tubules.

#### Treatment 5: SA+OJ

There were only modest differences in the surface changes seen by this treatment when compared to Treatment 1. Observationally, less surface deposit was seen, otherwise many tubules appeared occluded or reduced in diameter.

#### Treatment 6: F+OJ

No surface deposits were seen on any specimens and the majority of tubules were clearly patent. However, direct comparison with the etched control indicated that the tubule diameters were narrower in this treatment group (Fig. 6).

#### Treatment 7 CHX+OJ

All of the specimens studied could not be distinguished from their respective controls and tubules remained patent.

### Smeared Specimens

All of the untreated control specimens exhibited a smear layer covering the dentine surface with no tubules visible (Fig. 7). At the magnification shown the smear layer was irregular with scratches criss-crossing the surface.

#### Treatment 1: SA

The dentine surface was covered by a granular deposit almost identical to that seen for the etched specimens in Treatment 1. A few dentinal tubules were partially open or their outline could be appreciated but occluded by a similar granular deposit.

#### Treatment 2: P

Plaque bio-films were seen on all specimens in this treatment group – with surface details similar to that noted with etched specimens. Some individual variations in surface morphology were apparent but all bio-films obliterated the underlying dentine surface. Removal of the plaque bio-films revealed the intact underlying smear layer seen in controls.

#### Treatment 3: CHX

Specimens in the group showed no discernable differences from control specimens and the smear layer remained to cover the dentine surface and obliterate the tubule orifices.

#### Treatment 4: F

All specimens brushed with the fluoride toothpaste showed variable, albeit extensive, loss of the smear layer and numerous tubules opened. Some tubules were partially occluded with what could be toothpaste residues or smear plugs. Tubule diameters appeared narrower than untreated etched specimens (Fig. 8).

#### Treatment 5: SA+OJ

The appearance was almost identical to that seen with Treatments 1 and 5 for etched specimens and Treatment 1 for smeared specimens. At most, a few more tubule orifices were visible than in both Treatment 1 specimens but all were partly occluded.

#### Treatment 6: F+OJ

Specimens showed no surface deposits, the smear layer appeared completely removed and tubules were opened.

#### Treatment 7: CHX+OJ

There were some subject variations but all specimens showed complete removal of the smear layer and opening of most or all of the tubules.

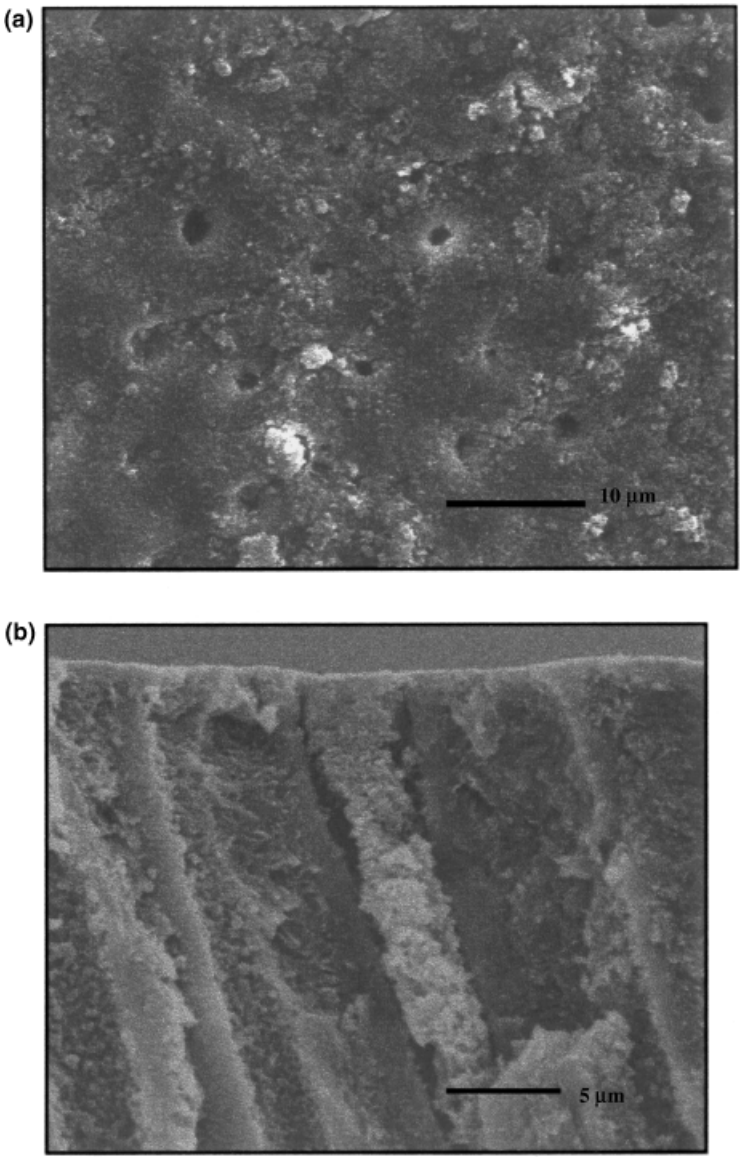


*Fig. 2.* (a) Etched dentine specimen brushed twice daily with Treatment 1, SA, after 5 days in situ. Most tubules are blocked partially or completely by a granular material, which also covers most of the inter-tubular dentine. (b) Fractured, etched, dentine specimen brushed twice daily with Treatment 1, SA, after 5 days in situ. A similar granular deposit noted in Fig. 2a penetrates the tubules to at least 20 μm.

#### Study 2

Data for percent change in permeability and tubule numbers for each treatment and treatment condition are shown collectively in Figs. 9 and 10. Reference will be made to these data to appraise both intra- and inter-treatment changes.

Fig. 1 (from Study 1) represents the typical appearance of the untreated control portion by which to make treatment comparisons. Small qualitative changes by the different treatment conditions will be described without photomicrographic illustration and some references will be made to photomicrographs in Study 1 where changes were essentially similar.

#### Treatment 1: SA

Immediately after application and single water rinse the entire dentine surface was covered with a granular material with many tubules occluded or reduced in diameter. The appearance was similar to Study 1 (Fig. 2a) except large particulate material was visible on the dentine surface. Percent reduction in permeability and tubule numbers was in excess of 90% (Figs. 9 and 10). Maintaining specimens in situ for 6 and 12 h or 12 h with water drinking decreased the granular layer and revealed more open tubules, however, reduction in permeability remained around 80% and reduction in tubule numbers $>50\%$ for all three conditions. Imbibing OJ over 12 h reduced further surface and intra-tubule deposition and permeability and tubule numbers increased further (Fig. 11 and Figs. 9 and 10). Analysis of changes revealed significant differences in percent reduction in permeability and tubule numbers for 0 h compared to 6, 12 and 12 h+water ($p<0.01$) but no significant differences between the three post 0 h conditions ($p>0.05$). OJ produced a further significant increase permeability and tubule numbers ($p<0.01$).

#### Treatment 2: SC

Immediate treatment effects were very similar to the effects of the SA product with the dentine surface and tubule orifices respectively covered or blocked partially or completely (Fig. 12). Indeed, the subsequent four conditions in situ produced similar changes to permeability and tubule numbers seen with Treatment 1. Percent reduction in permeability and tubule numbers was approximately $>70\%$ after initial application. At 6 h, 12 h and 12 h+water in situ the percent reduction in permeability was slightly less than time zero and only significant for the 12 h in situ treatment ($p<0.05$). Similarly, although the percent reduction in tubules was less under these three conditions compared to time zero, differences only reached significance for 12 h in situ ($p<0.05$). Twelve hours in situ with OJ resulted in a further increase in permeability and tubule numbers but differences only reached significance for percent permeability reduction compared to 12 h in situ with water conditions ($p<0.05$).

#### Treatment 3: DS

Observationally at 0 h the application of the product resulted in the deposition of a crystalline-like layer, which completely obliterated the dentine surface and dentinal tubules (Fig. 13). Retaining specimens in situ for 6 and 12 h had little visual effect on the deposit. However, retaining specimens in situ for 12 h and imbibing water or OJ removed virtually all trace of the

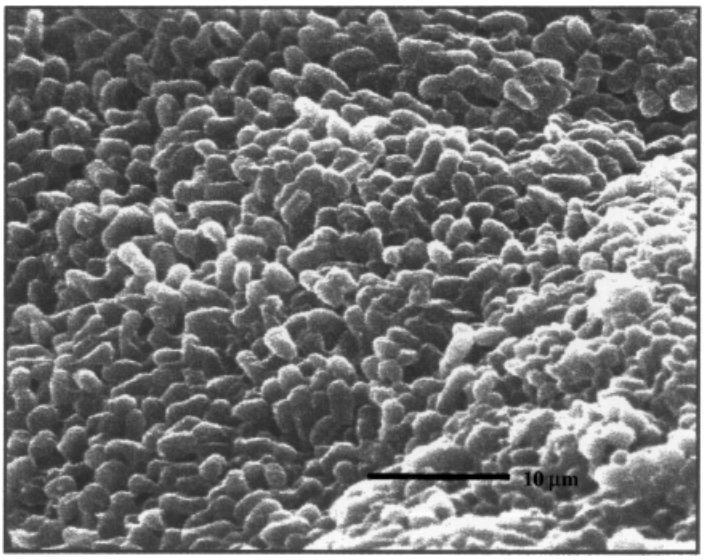


*Fig. 3.* Etched dentine specimen from Treatment 2, P, 5 days in situ. A bacterial bio-film completely covers the dentine surface.

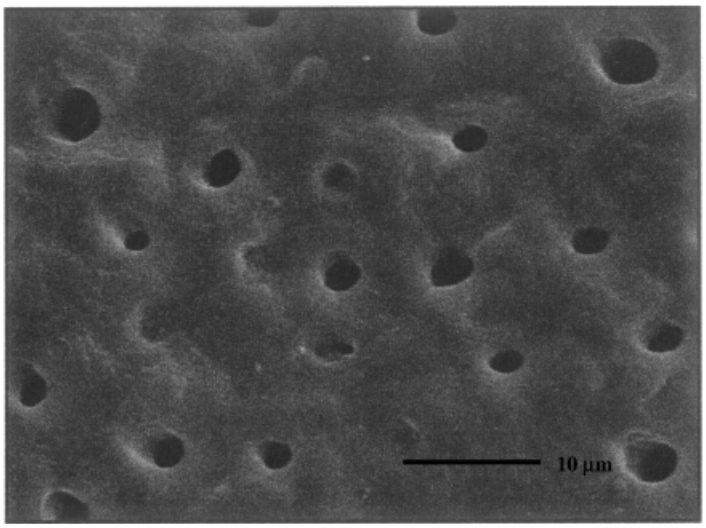


*Fig. 4.* Etched dentine specimen exposed twice daily to treatment 3, CHX, 5 days in situ. Tubules are either occluded or reduced in diameter: a characteristic seen in both specimens of three out of five subjects.

deposit and specimens had a similar appearance to controls. Image analysis overall tended to confirm the visual appraisal. Thus by comparison with respective controls the percent reductions in permeability and tubule numbers at 0, 6 and 12 h in situ were all in the order of 95%, with no significant differences between the 3 treatment conditions ($p > 0.05$). Samples left in situ for 12 h and exposed to water or OJ showed a significant increase in permeability and number of open tubules compared to the 12 h in situ alone ($p < 0.001$). Nevertheless, by comparison with respective controls for water and OJ, the percent reductions in permeability were approximately 30% and 35% and the percent reductions in tubule numbers approximately 17% and 4% respectively.

#### Treatment 4: F

Visual appraisal of 0 h specimens revealed a scant particulate deposit on the inter-tubular dentine and many dentinal tubules either occluded or of reduced diameter compared to control halves. Essentially specimens had the appearance of the same treatment in Study 1 as seen in Fig. 5. After 6 and 12 h in situ more open tubules were seen but many were narrower than control tubules. After 12 h in situ and imbibing water and more particularly OJ many more tubules were open and specimens were little different in appearance from controls. Image analysis of 0 h specimens recorded an approximate 70% reduction in permeability and 50% reduction in tubules. In mean terms, permeability and open tubules increased after 6 h in situ but the differences from 0 h were not significant ($p > 0.05$). After 12 h in situ percent reduction in permeability and open tubules from controls was around 30% and 18%, respectively, and significantly different from 0 h. Imbibing water or OJ when specimens were in situ for 12 h significantly increased permeability and the number of open tubules compared to 12 h in situ alone ($p$ ranged from $< 0.05$ to $< 0.01$). Thus with water reductions in permeability and closed tubules were 15% and 10% respectively and for OJ 6% and 5% respectively.

#### Inter-treatment comparisons

For permeability, SA and DS produced significantly greater percent reductions at 0, 6 and 12 h than SC and F ($p$ ranged from $< 0.001$ to $< 0.05$). The former two products were not significantly different for permeability at 0 h but SA was significantly less effective at 6 and 12 h compared to DS ($p$ ranged from $< 0.01$ to $< 0.05$). The SC and F products were similar for permeability at 0 and 6 h but F was significantly less effective at 12 h compared to SC ($p < 0.01$). Exposure to water had little effect on SA and SC deposits in terms of mean permeability and SA remained significantly the more effective product ($p < 0.05$). Water exposure markedly and similarly reduced ($p > 0.05$) the efficacy of both DS and F to levels significantly less than SC ($p < 0.05$). OJ consumption increased permeability for all treatments compared with water consumption. The percent reduction in permeability from highest to lowest was SA, SC, DS and F with differences between pairs of treatments all significant ($p$ ranged from $< 0.05$ to $< 0.001$).

For tubule numbers the pattern was essentially similar to permeability. At 0, 6 and 12 h the order from highest to least percent reduction was DS, SA, SC and F. In the majority of cases significant differences existed between pairs of products on the downward ranking ($p$ ranged from $< 0.05$ to $< 0.001$). Exceptions were SA and DS

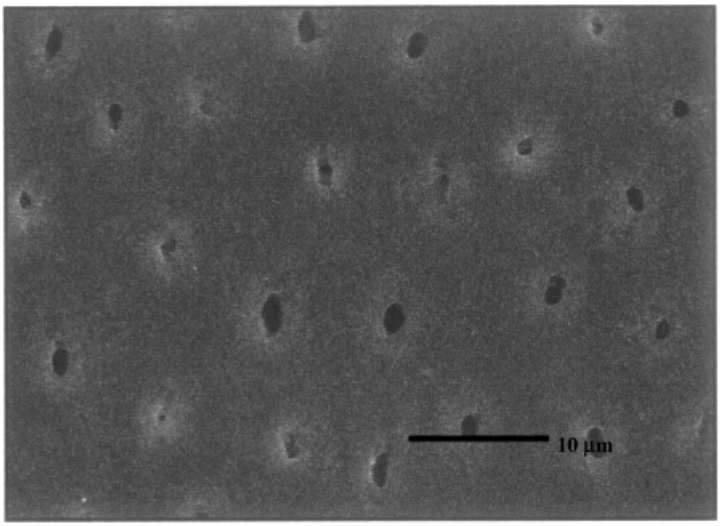


*Fig. 5.* Etched dentine specimen exposed to Treatment 4, twice daily brushing with F after 5 days in situ. Tubules are mostly reduced in diameter with a small proportion obturated.

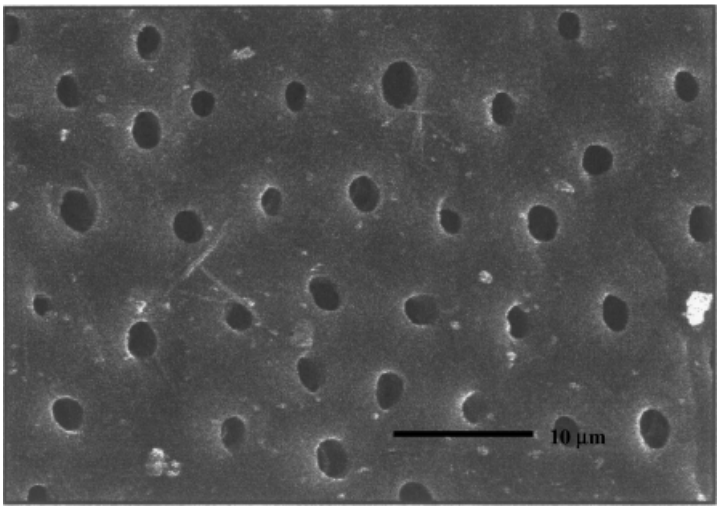


*Fig. 6.* Etched dentine specimen exposed to Treatment 6, F and OJ after 5 days in situ. Most tubules are open although diameters appear reduced compared to untreated controls (Fig. 1).

at 0 h ($p > 0.05$), SA and SC at 6 h and SC and F at 6 h. Exposure to water and OJ changed the ranking to SA, SC, DS and F (latter 2 products reverse order with OJ). In many comparisons significant differences existed between pairs on the downward ranking ($p$ ranged from $< 0.05$ to $< 0.01$) the exceptions being DS and F at 12 h with water or OJ ($p > 0.05$) and the two strontium pastes at 12 h with OJ ($p > 0.05$).

## Discussion

### Study 1

This study in situ was conceived first and foremost to evaluate the feasibility of a model to simulate lesions of dentine hypersensitivity and non-sensitive dentine surfaces in the mouth based on studies in vivo (Absi et al. 1987, 1989). The second aim was to employ a randomised controlled clinical trial methodology to study the effect of agents applied to the two dentine surfaces which may, from laboratory studies, abrade or erode the dentine surface or occlude tubules. The deficiency in any model in situ for dentine hypersensitivity is the lack of fluid flow outwards from the pulp, which could modulate the effects of materials which impact on the surface. However, models in vivo can, at present, only be evaluated by extraction, replication or dentine biopsy, all of which have their own problems and limitations.

The preparation of specimens was performed in a standardised manner in the hope that the orientation of tubules and thereby their shape and dimensions would be similar at baseline. This would have permitted the use of image analysis software to measure tubule numbers and dimensions. In the event, a significant proportion, approximately one third, of specimens did not have tubules sectioned at the expected right angle to the tubule direction. Although controls for each specimen were available, it was felt that image analysis data would be skewed by determination of changes across different tubule shapes. Study 2 was thus planned to address this problem.

The placement and retention of specimens was wholly successful with appliances well tolerated and with no loss of specimens in situ. The site of appliances and contained specimens was designed to coincide with reported areas of predilection for dentine hypersensitivity namely buccal cervical regions of canine and premolar teeth (Graf & Galasse 1977, Flynn et al. 1985, Addy et al. 1987, Fischer et al. 1992). For the reasons stated previously, the clinical trial part of the investigation had to be based on observation of treatment changes to the dentine surfaces under SEM. Photomicrographs were obtained from similar sites for each test and control specimen. With the exception of the plaque accumulations, this could be carried out blind with the specimens and photomicrographs coded by number into treatment groups. Despite the use of a subjective assessment method, with one exception, the changes produced by each treatment were similar in all specimens and in all subjects: the exception was CHX.

For the etched specimens, prepared to simulate sensitive dentine, most treatments had little effect on tubule patency. Thus, with OJ and F alone, F with OJ and plaque accumulation, many tubules were seen to be still patent and specimens remained very similar to their controls. At most, F left some debris on the inter-tubular dentine surface and some tubule diameters narrowed. The narrowing may have resulted from the deposition of material from the paste around the orifice but, as there was no evidence of penetration, the effect was probably dentine smearing due to an abrasive action of the

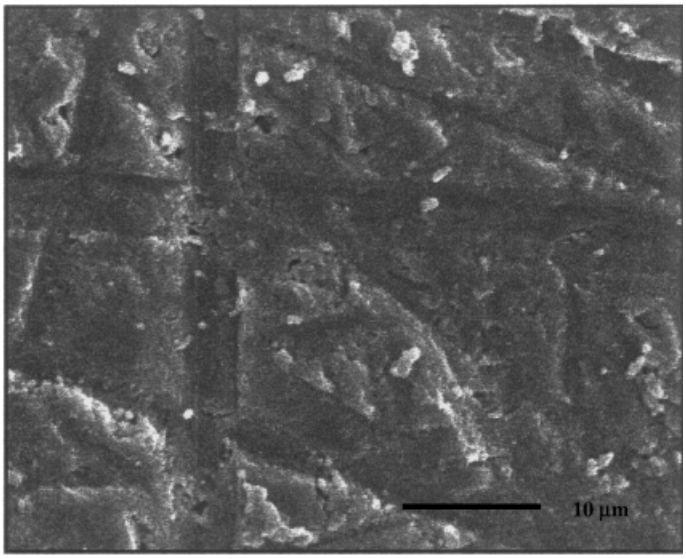


*Fig. 7.* Untreated smeared dentine specimen with a smear layer obliterating the tubule orifices.

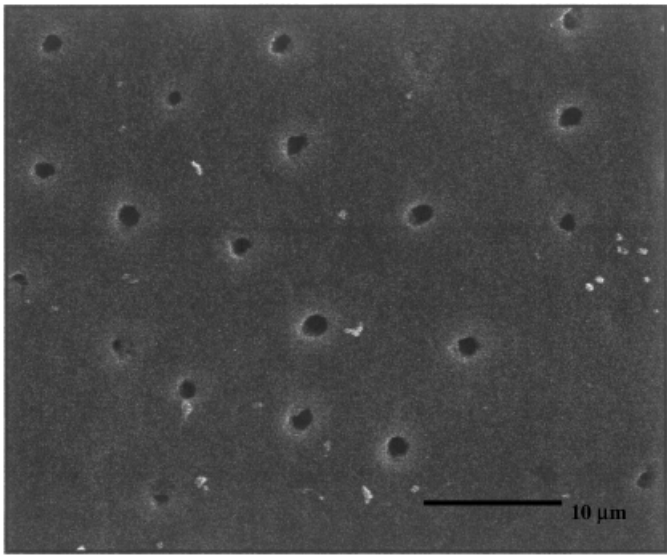


*Fig. 8.* Smeared dentine specimen exposed to treatment 4, brushed twice daily with F after 5 days in situ. Smear layer has largely disappeared and tubules openings are apparent albeit of reduced diameter to etched controls. Some tubules still remain obturated.

brush and paste (Absi et al. 1992, 1995). OJ, which is readily known to erode dentine both in vitro and in situ (Absi et al. 1992, 1995, Hunter et al. 2000a, b), not surprisingly maintained tubule patency both alone and combined with F. Plaque accumulation also had no effect on underlying dentine. If anything, these young bio-films might have been acidogenic and therefore tubule patency should not be affected. However, no food or drink intake occurred whilst specimens were in situ and therefore a more neutral plaque would have been present. This was borne out by the smeared specimens where the smear layer remained intact. Future studies involving longer periods of plaque accumulation and/or sucrose rinsing would be of interest. Bacterial penetration of tubules was also not seen within this time frame but evidence suggests that this could occur perhaps over longer study times (for a review, see Love & Jenkins 2002). The outcome for F alone and combined with OJ and OJ alone on smeared specimens was essentially the same as seen for etched specimens. This indicates that the toothpaste and OJ readily remove the smear layer by abrasion and erosion respectively. This, again, could have been predicted by studies in vitro (Addy et al. 1987, Absi et al. 1992, 1995).

CHX had no effect on smeared dentine and, as this rinse was at neutral pH, this was not unexpected. Studies with acidic rinses would be of interest, particularly in view of data for effects on dentine and enamel in vitro and in situ respectively (Addy et al. 1991, Pontefract et al. 2001). CHX did, however, produce some tubule closure in some subjects. This may have resulted from deposition of calcium phosphate into the tubules since chlorhexidine is known to increase supragingival calculus formation (Loe et al. 1976, Lang et al. 1982, Yates et al. 1993). The most significant and consistent tendency to occlude tubules was seen with the desensitising product (SA). This occurred with both etched and smeared specimens, which in the case of the latter, suggested the paste, probably through abrasion, removed the smear layer and then deposited material onto the dentine surface and into the tubules. The appearance of the material and the results from studies in vitro strongly suggest the material was the artificial silica abrasive (Addy & Mostafa 1989). Interestingly and again supported by studies in vitro, artificial silica, when available in a non-ionic detergent base as in this product, has a strong affinity for dentine and resists removal by acidic solutions such as OJ (Absi et al. 1995).

In summary, these studies suggest that dentinal tubule patency can be maintained or created by the erosive effects of acidic fluids imbibed and the abrasive effects of some conventional toothpaste products alone or combined. The cationic antiseptic, CHX, if anything, has a slow propensity to occlude tubules and, given its anti-plaque activity could be considered as a treatment for dentine hypersensitivity when used as the only oral hygiene measure: such a possibility clearly needs further investigation. Some desensitising toothpastes offer the potential of occluding tubules by virtue of contained ingredients, which may not necessarily be the supposedly contained active.

#### Study 2

As with Study 1, the present investigation in situ was foremost methodologi-

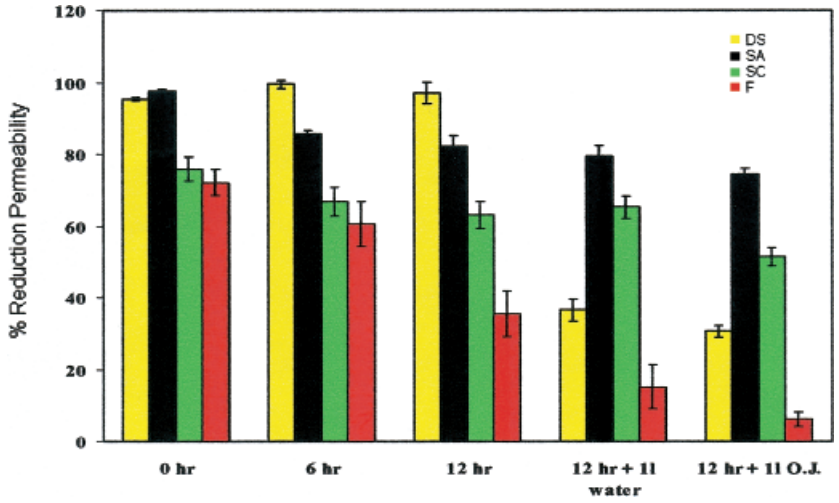


*Fig. 9.* Bar chart of the mean and standard errors for the percent reduction in permeability (tubule area) for the 4 singly applied, treatments at 0, 6, and 12 h and 12 h drinking water or OJ in situ.

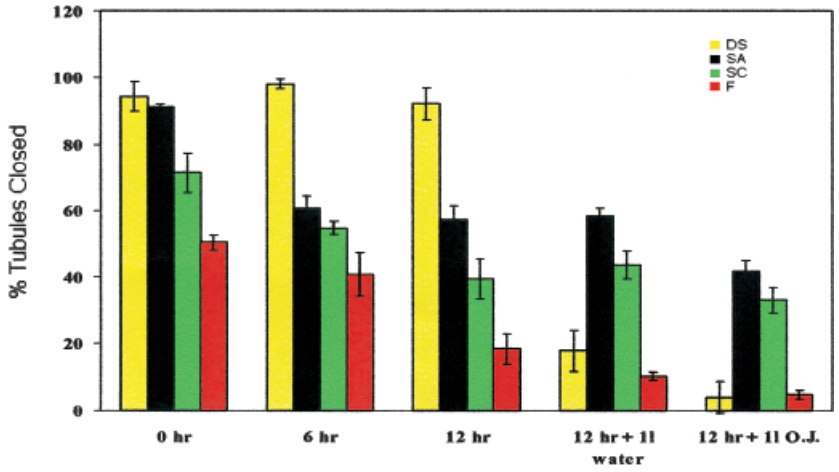


*Fig. 10.* Bar chart of the mean and standard errors for the percent tubules closed for the 4 singly applied, treatments at 0, 6, and 12 h and 12 h drinking water or OJ in situ.

cal and attempted to build on the experiences with and the findings of the first model. The refinements made to the first design in situ were standardisation of specimens and the use of image analysis to measure quantitatively the dentine surface in respect of tubule area and numbers. This of course could have been modelled totally in vitro. However, in an attempt to build on the findings of the first study and use image analysis in a more clinically relevant manner the short-term effects of treatments on dentine specimens maintained in situ were investigated. Study 1 took the form of a randomised controlled clinical trial, which if nothing else highlighted the labour intensity of the data collection. The findings however, revealed changes peculiar to each treatment, which for the majority were consistent for pairs of specimens in the same subject and across all subjects. As a result and for feasibility and logistic reasons the model was applied in a single subject, but with 4 control and test specimens used for each treatment and treatment condition, giving a total of 20 test specimens (plus respective 20 controls) per treatment. Although the data must be viewed as peculiar to the one subject, the consistency of the findings and their comparability to those from relevant parts of Study 1 allow cautious extrapolation.

Standardisation of specimens was guaranteed by the method employed but as noted in the previous study this required discarding a quarter to a third of the dentine sections originally cut. The treatments and treatment conditions chosen were to determine the immediate and lasting effects during the waking hours of some formulations, which may be applied to sensitive dentine. Tooth brushing with toothpaste is usually once or twice a day and if twice approximately 12 h apart: a time period which would seem reasonable to study the natural history of toothpaste on the dentine surface. In office desensitising treatments are usually applied once and reapplied over varying time periods. Immediate and short-term effects of such treatments would therefore also seem relevant.

Image analysis from standardised images was straightforward and produced data consistent with the qualitative assessments and permitted statistical analysis for intra- and inter-treatment effects. Multiple paired comparisons were made clearly increasing the chance of alpha errors. The importance of these is probably limited, since the overall pattern of the qualitative and quantitative data were consistent across specimens and treatments. Also many of the inter-treatment comparisons were highly significant.

The pattern for the inter-treatment effects and differences can easily be appraised from the bar charts (Figs. 1 and 2) and therefore the results will be discussed primarily by product. SA produced similar changes after one application and retention in situ for 12 h as seen and after multiple applications during 5 days in Study 1. The dentine surface was covered with a granular layer and many tubules partly or completely occluded such that the percent reductions in permeability and tubule numbers were high initially. Some loss of material occurred over the 12 h and numbers of exposed tubules increased but with much lesser effects on permeability. The occluding material was almost certainly the artificial silica abrasive (Addy & Mostafa 1989). Interestingly and again complimented by Study 1 and studies in vitro (Absi et al. 1995), the deposit was little influenced by drinking water or orange juice: this would attest to the apparent strong affinity of artificial silica for dentine, at least when in a non-ionic detergent vehicle. Cautious extrapolation from in situ to in vivo would support the efficacy of this product in the treatment of dentine hypersensitivity when used

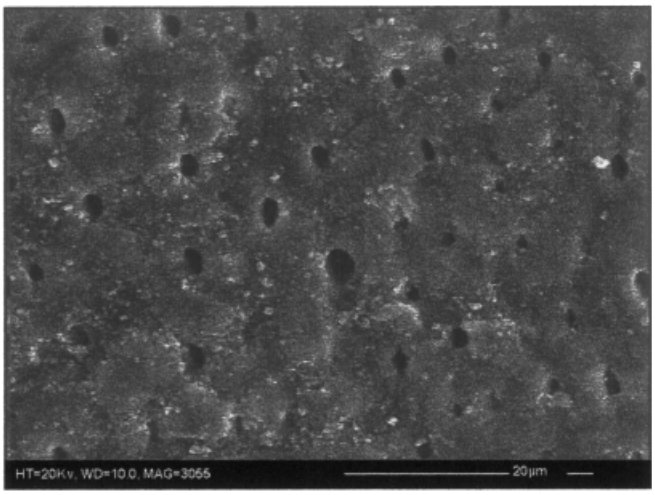


*Fig. 11.* Etched dentine specimen treated with SA after 12 h in situ with drinking OJ. Most surface aggregates have disappeared and proportionately more open tubules are apparent compared to Fig. 4, many tubules remain obturated or reduced diameter (cf Fig. 3).

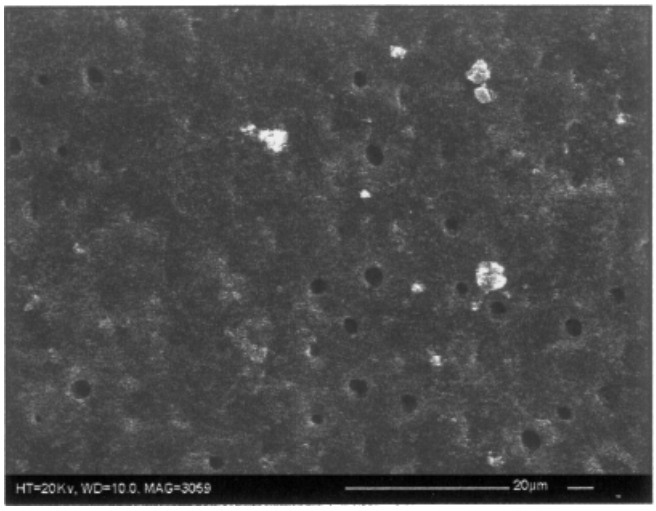


*Fig. 12.* Etched dentine specimen treated with SC after 0 h in situ. Appearance is similar to SA with many tubules occluded or of reduced diameter.

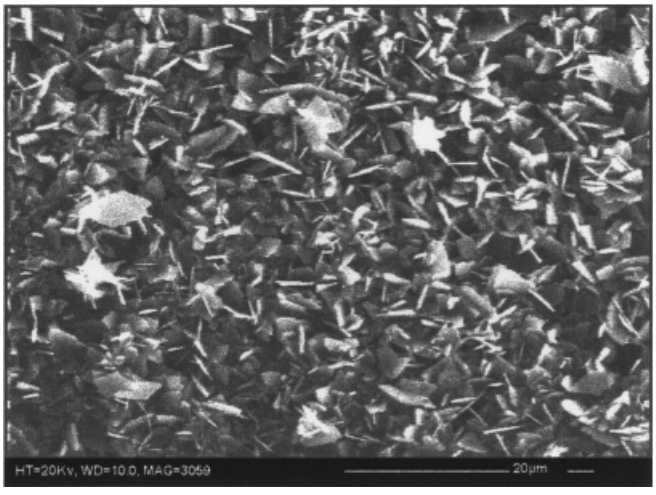


*Fig. 13.* Etched dentine specimen treated with DS after 0 h in situ. Crystalline-like deposit completely obliterates the dentine surface. This same appearance was apparent at 6 and 12 h in situ.

twice per day and through a reduction in dentine permeability. Certainly, this was reported from a clinical trial of a prototype formulation of the existing product (Addy et al. 1987). The pattern with SC were very similar to SA albeit in qualitative and quantitative terms less. The two pastes are now similar in their formulation; artificial silica in a non-ionic detergent base and the same conclusions must be drawn as to tubule occlusion mechanisms. Previously the SC used diatemaceous earth, which is a natural silica and from studies in vitro poorly retentive to the dentine surface (Addy & Mostafa 1989). Without further details of the two formulations, it is not possible to explain the differences in effects produced, some of which reached significance.

The in office product, DS, is formulated as two liquids, which when consecutively applied to the dentine surface react to form two insoluble calcium and strontium salts, namely the carbonates and phosphates. The appearance of specimens, so treated, suggest such precipitates were deposited on the dentine surface to completely occlude all morphological details. The changes if extrapolated to clinical effect would suggest that relief from dentine hypersensitivity would be immediate. Evidence in support of this comes from studies in vitro and in vivo using a similar two stage calcium phosphate precipitation system were tubule occlusion was noted with immediate clinical relief in the majority of patients (Imai & Akimoto 1990). As with all in office treatments, the persistence of tubule occlusion is the important parameter (for a review, see Pashley 2000). Indeed other authors have criticised the precipitation of insoluble salts onto dentine based on lack of tubule penetration, and therefore poor precipitate retention (Ishikawa et al. 1994). To achieve tubule penetration, acid calcium phosphate solutions have been recommended but conclusions are based only on data in vitro (Ishikawa et al. 1994, Suge et al. 1995). The poor retention of the crystalline deposit was shown in the present study. Thus if left relatively undisturbed, the deposit remained but was easily dislodged by drinking water or more particularly orange juice. Similar problems of retention in situ have been noted with calcium oxalates (Kerns et al. 1991). The poor retention of DS would be consistent with the failure to show superiority of a similar two-step

calcium phosphate precipitation formulation in the treatment of dentine hypersensitivity when water was used as the placebo control (Yates et al. 1998).

Finally, the results for the conventional fluoride toothpaste, F, which make no claim for the treatment of dentine hypersensitivity, were very similar to those from Study 1.

In conclusion, the proposed model in situ offers the possibility to study aspects of dentine hypersensitivity within the oral environment and over relatively short periods of time. Absolute standardisation of specimens is time consuming but allows both objective and subjective measures of changes. As always data even from studies in situ must be extrapolated with caution to effects in vivo particularly, as these primarily methodological studies, were based on 5 and 1 subject respectively. It would be hoped that the model could be applied to study the aetiology and management of dentine hypersensitivity using randomised controlled clinical trial designs.

## References


Absi, E. G., Addy, M. & Adams, D. (1987) Dentine hypersensitivity: a study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentine. *Journal of Clinical Periodontology* **14**, 280–284.

Absi, E. G., Addy, M. & Adams, D. (1989) Dentine hypersensitivity: the development and evaluation of a replica technique to study sensitive and nonsensitive cervical dentine. *Journal of Clinical Periodontology* **16**, 190–195.

Absi, E. G., Addy, M. & Adams, D. (1992) Dentine Hypersensitivity. The effects of tooth brushing and dietary compounds on dentine in vitro: a SEM study. *Journal of Oral Rehabilitation* **19**, 101–110.

Absi, E. G., Addy, M. & Adams, D. (1995) Dentine hypersensitivity: uptake of toothpastes onto dentine and effects of brushing, washing and dietary acid. *Journal of Oral Rehabilitation* **22**, 175–182.

Addy, M. (1990) Aetiology and clinical implications of dentine hypersensitivity. *Dental Clinics of North America* **34**, 503–514.

Addy, M. (2002) Dentine hypersensitivity: new perspectives on an old problem. *International Dental Journal* **52** (Suppl. 5/02), 367–375.

Addy, M., Absi, E. G. & Adams, D. (1987) Dentine hypersensitivity: effects in vitro of acids and dietary substances on root planed and burred Dentine. *Journal of Clinical Periodontology* **14**, 280–286.

Addy, M., Loyn, T. & Adams, D. (1991) Dentine hypersensitivity: effects of some proprietary mouthwashes on the dentine smear layer. An S.E.M. study. *Journal of Dentistry* **19**, 148–152.

Addy, M. & Mostafa, P. (1989) Dentine hypersensitivity. II. Effects produced by the uptake in vitro of toothpastes onto dentine. *Journal of Oral Rehabilitation* **16**, 35–41.

Addy, M., Mostafa, P. & Newcombe, R. (1987) Dentine hypersensitivity: the distribution of recession, sensitivity and plaque. *Journal of Dentistry* **15**, 242–248.

Brannstrom, M. (1963) A hydrodynamic mechanism in the transmission of pain-produced stimuli through the dentine. In *Sensory mechanisms in dentine*, ed, Anderson, D. J., pp. 73–79. Oxford: Pergamon Press.

Dababneh, R. H., Khouri, A. T. & Addy, M. (1999) Dentine hypersensitivity – an enigma? A review of terminology, epidemiology, mechanisms, aetiology and management. *British Dental Journal* **187**, 606–611.

Fischer, C., Fischer, R. G. & Wennberg, A. (1992) Prevalence and distribution of cervical dentine hypersensitivity in a population in Rio de Janeiro, Brazil. *Journal of Dentistry* **20**, 272–276.

Flynn, J., Galloway, R. & Orchardson, R. (1985) The incidence of hypersensitive teeth in the West of Scotland. *Journal of Dentistry* **13**, 230–234.

Graf, H. & Galasse, R. (1977) Morbidity, prevalence and intraoral distribution of hypersensitive teeth. *Journal of Dental Research* Special issue A **162**, 56, 2.

Gysi, A. (1900) An attempt to explain the sensitiveness of dentin. *British Journal of Dental Science* **43**, 865–868.

Hunter, M. L., West, N. X., Hughes, J. A., Newcombe, R. G. & Addy, M. (2000a) Erosion of deciduous and permanent dental hard tissues in the oral environment. *Journal of Dentistry* **28**, 257–264.

Hunter, M. L., West, N. X., Hughes, J. A., Newcombe, R. G. & Addy, M. (2000b) Relative susceptibility of deciduous and permanent dental hard tissues to erosion by a low pH fruit drink in vitro. *Journal of Dentistry* **28**, 265–270.

Imai, Y. & Akimoto, T. (1990) A new method for the treatment of dentine hypersensitivity by precipitation of calcium phosphate in situ. *Dental Materials Journal* **9**, 167–172.

Ishikawa, S. (1969) A clinico-histological study on the hypersensitivity of dentine. *Journal of Japanese Stomatology Society* **36**, 68–88.

Ishikawa, K., Suge, T., Yoshiyama, M., Kawasaki, A., Asaoka, K. & Ebisu, S. (1994) Occlusion of dentinal tubules with calcium phosphate using acidic calcium phosphate solution followed by neutralisation. *Journal of Dental Research* **73**, 1197–1204.

Jackson, R. J. (2000) Potential treatment modalities for dentine hypersensitivity: home use products. In *Tooth Wear and Sensitivity*, eds. Addy, M., Embery, G., Edgar, W. M. & Orchardson, R., pp. 327–338. London: Martin Dunitz.

Johnson, R. H., Zulgar-Nairn, B. J. & Koval, J. J. (1982) The effectiveness of an electroionising toothbrush in the control of dentinal hypersensitivity. *Journal of Periodontology* **53**, 353–359.

Kerns, G. D., Scheidt, J. M., Pashley, D. H., Horner, J. A., Strong, S. L. & Van Dyke, T. E. (1991) Dentinal tubule occlusion and root hypersensitivity. *Journal of Periodontology* **62**, 421–428.

Lang, N. P., Hotz, P., Graf, H., Geering, A. H., Saxer, U. P., Sturzenberger, O. P. & Meckel, A. H. (1982) Effects of supervised chlorhexidine mouthrinses in children. A longitudinal clinical trial. *Journal of Periodontal Research* **17**, 101–111.

Loe, H., Schiott, C. R., Glavind, L. & Karring, T. (1976) Two years oral use of chlorhexidine in man. I. General design and clinical effects. *Journal of Periodontal Research* **11**, 135–144.

Love, R. M. & Jenkinson, H. F. (2002) Invasion of dentinal tubules by oral bacteria. *Critical Reviews in Oral Biology and Medicine* **13**, 171–183.

Pashley, D. H. (1990) Mechanisms of dentine sensitivity. *Dental Clinics of North America* **34**, 449–474.

Pashley, D. (2000) Potential treatment modalities for dentine hypersensitivity: in-office products. In *Tooth Wear and Sensitivity*, eds. Addy, M., Embery, G., Edgar, W. M. & Orchardson, R., pp. 351–366. London: Martin Dunitz.

Pontefract, H., H, Hughes, J., Kemp, K., Yates, R., Newcombe, R. G. & Addy, M. (2001) Erosive effects of some mouthrinses on enamel. A study in situ. *Journal of Clinical Periodontology* **28**, 319–324.

Suge, T., Ishikawa, K., Kawasaki, A., Yoshiyama, M., Asaoka, K. & Ebisu, S. (1995) Duration of dentinal tubule occlusion formed by calcium phosphate precipitation method: in vitro evaluation using synthetic saliva. *Journal of Dental Research* **74**, 1709–1714.

Yates, R., Jenkins, S., Newcombe, R. G., Wade, W. G., Moran, J. & Addy, M. (1993) A 6-month home usage trial of a 1% chlorhexidine toothpaste. 1. Effects on plaque, gingivitis, calculus and tooth staining. *Journal of Clinical Periodontology* **20**, 130–138.



Address:
*Martin Addy*
*Division of Restorative Dentistry*
*Dental School*
*Lower Maudlin Street*
*Bristol BS1 2LY*
*UK*
E-mail: martin.addy@bristol.ac.uk