Enamel Paste in the Treatment of Dentin Hypersensitivity

by

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DEDICATION

To my parents, sisters and brothers.. I could not have accomplished this without your constant support and guidance. My love and respect to you is beyond imagination…

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I would like to thank the following people for their invaluable contribution to this research;

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Background and Significance:

Dentin hypersensitivity (DH) is a common problem for about half of adults in their thirties and forties (1). For many people, this condition is severe. First line therapy for this condition typically involves the use of desensitizing toothpaste, which most commonly includes potassium nitrate or other potassium salts as the key ingredient desensitizing agent. Sensodyne® form GSK is the leading toothpaste for sensitive teeth on the market. In spite of the huge market success for Sensodyne and commercial acceptance for sensitive teeth toothpaste products, the efficacy of potassium nitrate and other potassium salts for the treatment of DH is not supported strongly by the literature. A Cochrane review of toothpastes containing various potassium salts in 2006 failed to show a significant effect on DH after 6 to 8 weeks of use (2).

A second line therapy for the treatment of DH involves in-office dentist application of topical desensitizing agents. A recent study in the Journal of Contemporary Dental Practice (3) comparing four commonly used dental applications for the treatment of DH, such as fluoride varnish and sealants, showed that only two had statistically significant effect on DH mean pain scores (the metric and endpoint in the study) at seven days after application. The successful therapies at 7 days were a sealant and fluoride iontophoresis. None of these applications involved an easy to apply material that can be applied over wet surfaces.

Aims and objectives:

This project focused on the application of synthetic FA crystals in the treatment of DH. FA crystals were produced and dispersed in a viscoelastic gel to form a paste, which adheres to wet surfaces, and can be applied by patients or dentists to:

- Provide immediate relief for DH, a condition also referred to as "sensitive teeth" and characterized by discomfort or pain when brushing teeth or when eating or drinking cold, hot or acidic food. The FA/viscoelastic gel paste is designed to occlude patent dentinal tubules which are the cause of the sensitivity.

- Provide long term relief from DH. Over longer periods of time the FA/viscoelastic gel paste is designed to release ions on dentin surfaces at neutral pH and when the intra oral pH drops (occurs usually during and after eating). The ions deposition on dentin surfaces over time will further reduce tooth sensitivity by forming a mineralized barrier.

The proposed paste has the advantages of being: biocompatible; allowing for the diffusion of ions; reacting positively to pH change in the oral environment i.e. releasing Ca, P and F at neutral pH and acidic pH; being easily applied and adherent to tooth structure in the wet environment of the mouth. The crystals were mixed with three different viscoelastic gels (Alginic acid, P(HEMA) and P(DOPA)), and tested for the desired properties mentioned above.

Specific aims:
1. Demonstrate the physical occlusion of the patent tubules in dentin by the FA crystals.
2. Demonstrate the release of Ca, P and F from the FA crystals at neutral and acidic pHs.

Hypothesis:

<u>Primary</u>

H_o1: The paste will not cause physical obstruction of the patent dentinal tubules.

$Ha1$: The paste will cause physical obstruction of the patent dentinal tubules.

<u>Secondary</u>

H_o2: The paste will not release Ca, P and F at neutral and acidic pHs.

$Ha2$: The paste will release Ca, P and F at neutral and acidic pHs.

Literature Review:

Definition of DH

The Canadian advisory board on DH (4) defined DH as "a short, sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which can not be ascribed to any other form of dental defect or disease".

A similar definition was suggested by Pashley (5); "a sharp, transient, well-localized pain in response to tactile, thermal, evaporative, or osmotic stimuli, which does not occur spontaneously and does not persist after removal of the stimuli." Generally, this definition has been applicable to exposed cervical dentin, but Pashley (5) recommended it to include any sensitive dentin. He also illustrated the difference between dentin sensitivity and hypersensitivity. He stated that the term hypersensitive dentin is a widely used term but poorly understood. Patients perceive exposed dentin as hypersensitive because of lack of sensation before the exposure. Dentin is an innervated tissue, and usually covered by enamel or cementum. Once these protective non-innervated layers are lost, patients will start feeling sensitivity. Nevertheless, in this investigation the term 'Dentin Hypersensitivity' (DH) will be used, because most of the literature refers to the condition using this terminology. Clinically, DH is usually diagnosed after other possible conditions have been eliminated (6).

Prevalence of DH

Contrary to what is generally believed, the literature on the prevalence of DH is not abundant. However, DH seems to be a common condition among adult population with prevalence values ranging form 1.34 to 98% (Table 1). The diversity in the reported prevalence values is primarily attributed to the difference in the methods used to diagnose DH. Questionnaires and clinical examination were the methods most commonly used. Studies that relied on questionnaires only to diagnose DH yielded higher values of prevalence than those combined with clinical examination (Table 1). Fifty percent prevalence was reported relying on a questionnaire administered to adult patients attending for a routine dental appointment (7). Gillam et al. reported 52% prevalence for a UK population and 55.4 for a Korean population (8). There was no statistical difference between the two populations. Questionnaires used in these studies involved questions evaluating three aspects of DH; questions related to pain or discomfort experienced by the patient, oral hygiene habits, periodontal treatment and discomfort following treatment. A question to evaluate possible erosive factors was added by some investigators (7,8). The use of questionnaires that require self-reporting of DH may well overestimate the magnitude of the problem, because of sensitivity caused by other pathologies, such as caries (9).

Reviewing the literature, the primary methods used clinically to diagnose DH were: sensitivity to a blast of air, tactile sensitivity and sensitivity to a cold water mouth rinse (CWMR). Bamise et al. studied the prevalence of DH among adult patients attending a Nigerian teaching hospital (10). Patients were interviewed and the relevant history was recorded. All standing teeth were examined for tooth surface loss such as attrition, erosion, abrasion, and any evidence of DH was confirmed with the use of an air blast of an air-water jet and probing the suspected surfaces with a dental explorer. A total of 2165 patients were examined, from which only 29 patients gave a positive response to intraoral testing of DH. There was no significant difference in prevalence between males and females. The majority of patients with DH were between 31-40 years of age. Approximately 86% of the patients described the pain of DH as sharp, whereas 13.8% of patients claimed dull pain. Thermal stimuli were the commonest initiating factors and sour/sweet were the least. DH interfered with eating 31% of the time, 41.4% with brushing, and 72.4% of the patients had pain on drinking water. Only one patient (3.4%) had sought professional treatment for DH. Nearly

13% of the patients had gingival recession; 10.9% had attrition; 7.4% had abrasion; 2.7% had abfraction-like lesions and 3.5% had erosion. Interestingly, DH was more frequently elicited on the occlusal surface in 56% of the cases, and 28% in the cervical area.

Flynn et al. (11) determined the prevalence of DH among patients attending clinics in the Glasgow dental hospital. They compared the subjective questionnaire reporting of DH with intraoral tests using CWMR and examination with a sharp dental explorer. A total of 369 patients were questioned and examined. CWMR test was carried out by asking the patients to swill 15 ml of cold water (7°C) around their teeth for a few seconds. Patients were asked to report any source of discomfort and to indicate, as precisely as possible, the location of the discomfort. Patients reporting sensitivity to CWMR were given a more detailed examination with a sharp dental explorer, in which sensitivity was evoked by gentle probing of the tooth. Twenty eight percent of the patients reported sensitivity in the questionnaire. However, only 18% of the patients were sensitive to CWMR, and that number dropped to 8.7% when combined with probing. The highest proportion of subjects with sensitive teeth (24%) was in the group aged 30-39 years. The commonest location of sensitive dentin was on the vestibular surfaces at the cervical margin (85%). Premolars were the most frequently affected teeth. The questionnaire revealed that only one quarter of the subjects who thought they had sensitive teeth had received desensitizing treatment. Desensitizing toothpaste was the most commonly used treatment. The authors raised a concern about the 75% of patients who did not receive any treatment. They attributed this to either the patients did not consider the condition severe enough to warrant attention, or the condition had not been diagnosed. Furthermore, the efficacy of the used desensitizing dentifrices appeared questionable, as over 60% of the users had demonstrable sensitivity; however, information about the compliance and method of applying the desensitizing dentifrice treatment was not investigated casting doubt on the accuracy of such a conclusion.

Rees and Addy carried out a cross sectional study of the buccal cervical sensitivity in a UK general practice population, and reviewed studies on DH prevalence (9). They suggested reasons for the variability of prevalence values recorded in the literature, including: the different study designs used to assess the condition; variation in patients' oral hygiene habits; consumption of erosive foods and drinks; and the type of setting where the

study was carried out. Authors claimed that questionnaire studies tend to overestimate the prevalence as mentioned previously. Furthermore, studies carried out at hospitals or specialty practices tend to report higher prevalence values, presumably because of the greater risk of root exposure as a result of periodontal attachment loss and gingival recession following periodontal treatment. However, this was not the case in the study by Bamise et al. (10) where only 1.34% prevalence value was reported despite the fact that the study was conducted in a teaching hospital setting. For their study, Rees and Addy recruited 18 dental practitioners to participate in the study. All patients attending the dentists' practice during a period of one month were screened for DH. The diagnosis was confirmed using air blast and by ruling out other causes of sensitivity. Periodontal disease was assessed and recorded. Buccal recession associated with the sensitive teeth was recorded. One thousand fifty four teeth were diagnosed as having DH in 152 patients, with an overall prevalence figure of 2.8%. The average age for patients was 42.9 years with a range of 15-80 years. The highest percentage of DH was within the 4th decade. The male: female ratio was 1:1.5. The upper 1st molar teeth were most commonly affected, followed by the 1st premolars, canines, and then 2nd molars. Cold drinks were the mostly associated initiating factor. Ninety three percent of the sensitive teeth were associated with buccal gingival recession in the range of 1-3 mm.

In a study done by Rees et al. (12), the same methodology was used to determine the prevalence of DH in a hospital clinic population in Hong Kong. Of the 226 patients examined 153 patients (67.7%) were diagnosed with DH. The commonest teeth affected were the lower incisors and the commonest initiating factor was cold drinks. In a similar study, Fischer et al. (13) had 25% of 635 patients subjectively reporting DH, while only 17% were confirmed to have cervical DH by intraoral tests.

Many studies reported a higher prevalence of DH among patients referred to periodontology specialty clinics than in general practice. Chabanski et al. (14) studied the prevalence of DH (cervical dentin sensitivity) in an adult population referred to a specialist periodontology department. Fifty one patients were clinically evaluated for DH using a Yeaple® explorer, cold air blast. Teeth were evaluated for plaque accumulation and gingival recession. The results demonstrated a prevalence value ranging between 72.5 and 98% which

is higher than prevalence values reported elsewhere. A value of 52% was reported for the prevalence of DH among a general practice population (1).

In conclusion, cold was the most frequently reported stimulus to DH (7,8,13). DH was not regarded as a severe complaint by the majority of patients, with pain being reported as low grade and occasional. Commercially available desensitizing toothpastes were the most commonly used treatment. Only a minority of patients sought professional treatment (7,8,13). The effectiveness of desensitizing dentifrices appeared questionable as over 60% of the users had demonstrable sensitivity (13).The condition was mostly prevalent among the young population in the 3rd and 4th decades (Table 1). The prevalence may shift in the future to a younger age group because of the increase in acidic food/drink intake and the influence of greater oral hygiene awareness and measures (7,14). There was a significant association between DH and patients who receive periodontal treatment (13), and in patients who have gingival recession. Furthermore, higher prevalence values were reported among patients referred to periodontology clinics than the general practice clinics (14). This would suggest a significant role of periodontal disease in the etiology of DH (12). In general, a slightly higher prevalence of DH was reported in females than in males, which may reflect their better oral hygiene awareness (15). That difference was not statistically significant in the majority of the studies.

Table 1. Summary of prevalence studies on DH

Study	Country	n	Study type	Method of clinical assessment	Setting	Prevalence (%)	Peak of age	M: F ratio	Commonly affected teeth	% with GR[a]
Bamise et al. (19)	Nigeria	2165	Q[b] + CE[c]	AB[d] / Probing	University	1.34%	4th decade	1.4:1	Molars	12.8%
Rees and Addy (18)	UK	5477	Q + CE	AB/ PDA[e]	GDP[f]	2.8%	4th decade	1:1.5	Max[g] 1st molars	93%
Rees et al. (21)	Hong Kong	226	Q + CE	AB/ PDA	PSC[h]	67.7%	5th decade	1:1.5	Mand[i] incisors	76.8%
Clayton et al. (16)	UK	250	Q	NA[j]	GDP	50%	3rd decade	1:1	Mand right sextant	NA
Tanni and Awartani (25)	Saudi Arabia	259	Q + CE	AB/ PDA	GDP + PSC	GDP 42.4% PSC 60.3%	4th decade	GDP 1:4 PSC 1:2	Max molars and mand anteriors	5%
Gillam et al. (17)	UK and Korea	557	Q	NA	GDP	52- 55.4%	3rd and 4th decades	NA	NA	NA
Fischer et al. (22)	Brazil	635	Q + CE	AB/ Probing	Marine dental clinic	17%	M: 6th, F: 3rd decade	1:1	Incisors and premolars	NA
Flynn et al. (20)	UK	369	Q + CE	CWMR[k]/ Probing	University	8.7%	4th decade	1:1	Premolars	NA
Gillam et al. (1)	UK	277	Q	NA	GDP	52%	3rd decade	1:1.4	NA	NA
Chabanski et al. (23)	UK	51	Q + CE	AB/ Probing	PSC	72.5-98%	5th decade	1:1	Molars	NA
Liu et al. (26)	Taiwan	780	Q + CE	AB/ Probing	University	32%	NA	1:1	Premolars & molars	23%

[a] gingival recession, [b] questionnaire, [c] clinical examination, [d] sensitivity to air blast, [e] periodontal disease assessment, [f] general dental practice, [g] maxillary, [h] periodontal specialty clinic, [i] mandibular, [j] not applicable, [k] cold water mouth rinse

Mechanism of DH

Three main theories have been proposed to explain the mechanism of DH, these are: the odontoblast-receptor theory; the direct nerve stimulation theory and the hydrodynamic theory (16). The latter theory is the most accepted (4).

The hydrodynamic theory has been tested as a possible mechanism for dentin sensitivity in the original work reported by Brannstrom (17). So far, this theory has been the prevailing theory explaining why dentin can be sensitive to different stimuli. It presupposes that dentin is filled with fluid that is clear, thin and devoid of cells. Different stimuli can cause a movement of the dentinal fluid and that in turn stimulates the nerve cells in the tooth pulp (A-δ fibers surrounding the odontoblasts). This putative mechanism requires individual tubules be open at the external dentinal and pulpal surfaces. A series of experiments have been conducted to examine the manner in which the removal of dentinal fluid can give rise to pain. The first experiment aimed to find whether on application of a reduced pressure to a cavity, pain was elicited and odontoblast nuclei were drawn into the tubules. A cavity was prepared on the buccal aspects of teeth which were to be extracted for orthodontic reasons, and a vacuum pump was connected to it. As soon as the pump was started, individuals felt pain that lasted as long as the pressure was reduced-up to 2 to 3 minutes. The teeth were then extracted, and histological examination disclosed numerous odontoblast nuclei in the tubules radiating from the cavity. It was concluded that the pain and aspiration of odontoblast nuclei on application of a reduced pressure to the cavity were due not so much to the pressure difference between the pulp and the cavity, but to the intense evaporation of the fluids on the dentin surface. The second experiment used an air stream. Clinical experience indicated that teeth can be extremely sensitive to an air blast. Therefore, in this experiment, the application of an air stream over a fairly long period resulted in numerous nuclei penetrating the tubules; a reaction that was not found in corresponding control cavities. Pain was elicited and lasted as long as the air blast was applied-up to 2 minutes. An air blast lasting one second can remove fluid from patent dentinal tubules. Although it may be a few microns into the dentin, there will be a corresponding outward flow until equilibrium is attained. When the air stream was discontinued after a second, the pain ceased. The rapid outward displacement of dentinal fluid produces a

corresponding movement in the pulp and would be expected to exert a mechanical effect directly or indirectly on the free nerve endings, thus eliciting an action potential resulting in pain. The effect of heat was tested in the third experiment. Previously, it has been reported that aspiration of odontoblast nuclei had been produced by the action of heat in cavity preparation, however, Brannstorm hypothesized that it was due to dentinal fluid evaporation resulting from the generation of frictional heat. In a series of tests, moist heat provided by circulating steam was applied to a cavity in one of a pair of teeth. The cavity in the contra-lateral tooth was subjected for the same period (45 seconds) to dry heat at 100° C. Aspiration was found to have occurred beneath the cavity exposed to dry heat but usually not beneath that exposed to wet heat. This lead to the conclusion that, as in the case of pressure reduction and application of an air stream, the pain and aspiration caused by dry preparation might be due to evaporation and removal of the dentinal tubule contents at the apertures of the exposed tubule. This resulted in a simultaneous rapid outward displacement of the contents of the tubules; the outward movement was probably the cause of pain rather than the aspiration of odontoblasts into the tubules. To investigate the validity of the theories that proposed that dentin is sensitive because of the presence of nerves in the tubules Brannstrom (17) initiated a series of experiments using an isotonic solution of potassium chloride which stimulates pain when applied to tissues containing pain fibers. Pain was produced in every case in which this salt solution was applied to an exposed pulp, but not at all when applied to dentin. That result provided an indirect support for the hydrodynamic theory. Another set of experiments was initiated to prove that the removal of some of the dentinal fluid using an absorbent paper would elicit pain. Brannstrom concluded that extremely small displacements can have a great effect because of the simultaneous involvement of large number of tubules. Although his series of studies constituted good support for the hydrodynamic theory, they did lack basic scientific elements: details of how the experiments were performed; study design; sample size; statistical analysis; etc.

To determine the magnitude and direction of fluid shift across dentin and hence their hydrodynamic-related pain stimulation Pashley (18) performed an *in vitro* study to evaluate dentin sensitivity to clinically relevant stimuli and transform them to

equivalency values. For this purpose, eight extracted unerupted human third molars were used in the study. Teeth were cut to remove occlusal enamel and the root 1mm below the CEJ. An apparatus was assembled to measure linear displacement of fluids using an electric flow detector (Fig. 1). Each specimen received the hydrodynamic stimuli in the order of an air blast, hot, cold, tactile and osmotic. The stimuli were repeated at three dentin levels (superficial, middle and deep) in eight crown segments. Results showed that hot water and tactile stimuli produced an inward fluid movement from the dentin surface toward the pulp chamber. Cold water, air blast and osmotic stimuli produced an outward fluid movement from the pulp chamber toward the dentin surface. The magnitude of the response varied with the location of the dentin surface. In the superficial dentin, which simulates hypersensitive dentin, hot water produced the largest fluid shift followed by cold water, air blast, osmotic, and tactile stimulus produced the least shift.
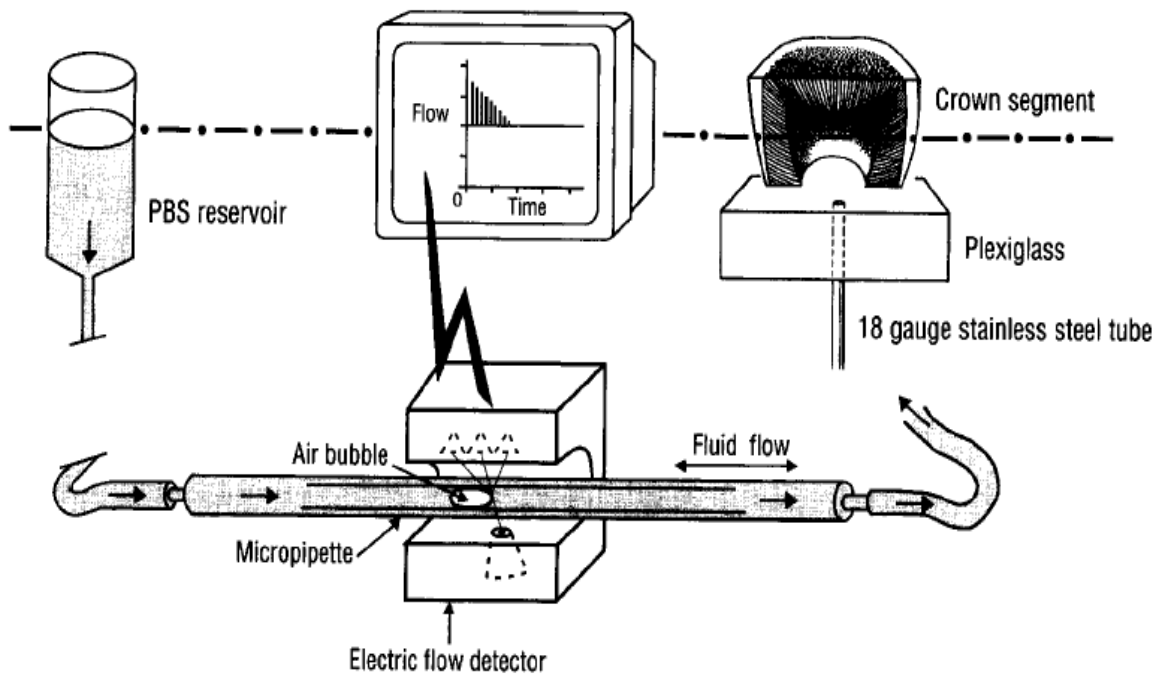
Fig. 1. Diagram showing permeability testing of crown segments (18)

The in vivo relationship of tubule patency and DH was established (19) in a study where twenty adult patients, with teeth requiring extraction, participated. The included teeth exhibited chronically exposed cervical dentin. A stream of air was used to provoke

sensitivity. The patients were asked to grade the stimuli on an individually determined numeric scale which ranged from zero "non-sensitive" to four "very sensitive". The exposed dentin was then treated with 0.5 M EDTA for 4 minutes to remove any smear layer and the air test repeated. After that, the dentin surfaces were treated for 2 minutes with either a 3% monopotassium-monohydrogen oxalate solution or 3% sodium chloride solution. The pH of both solutions was 2.4. The air test was repeated, and the patients' response recorded. The experimental teeth were then extracted, sectioned and examined under Scanning electron microscopy (SEM). The degree of tubule occlusion was determined by using a computer digitalized scanner. A mean value of 1.54 on the numeric pain scale was recorded as baseline for patients with sensitive teeth. This value increased to a mean of 2.5 after the treatment with EDTA. This increase was assumed to be the result of the removal of a superficial layer of debris or smear layer residing over the exposed dentin. The sodium chloride solution was more effective in reducing dentin hypersensitivity than the potassium oxalate solution. This conclusion was supported by the lower mean score value of the air test for the sodium chloride group along with a reduction of the dentinal tubules size on SEM (1.72 square µm mean dentin tubule aperture size after EDTA treatment alone, 0.564 square µm following potassium oxalate treatment and 0.386 square µm following sodium chloride treatment). The authors claimed they proved an *in vivo* relationship between tubules patency and DH, despite the absence of controls for the dentin sections illustrating the mean aperture size of the tubules before any treatment. The validity of the reduction in tubule aperture size calculation is thus questionable. Clinically, dentinal tubules are often exposed after periodontal surgery involving root planning. This sensitivity develops in 3-5 days, and disappears spontaneously over the next 2-4 weeks (5).

A comparison between sensitive and non-sensitive cervical dentin areas using SEM was conducted (20). Seventy-one vital teeth planned for extraction were examined for exposed cervical dentin. A cold, air blast and probing with a dental explorer were used to test the sensitivity of the teeth. Thirty four teeth were assessed clinically as hypersensitive and 37 as non-sensitive. All the teeth were then extracted, sectioned through the area of exposed dentin and examined with SEM. Counts and diameters of

open dentinal tubules from two randomly selected photomicrographs of each tooth were recorded. Half of the teeth from each group were placed into 2% methylene blue dye, and the penetration of the dye was scored. The number of open tubules in the sensitive teeth was eight times more than the non-sensitive teeth (P<0.001). The dentinal tubule diameter was two times wider in the sensitive group (P<0.001). Furthermore, the dye showed varying penetration into the dentinal tubules in the sensitive group with 7 of the teeth showing complete penetration to the pulp. However, the non-sensitive group only 5 teeth showed dye penetration to the outer third of the dentin, and only one through to the pulp. The difference in dye penetration between the two groups was significant. According to the hydrodynamic theory of dentin sensitivity, the amount of fluid flow in hypersensitive teeth should be greater than the flow in the non-sensitive teeth in response to stimuli. This study demonstrated a wider diameter of dentinal tubules in sensitive teeth compared to the non-sensitive ones. Flow in a capillary obeys Poiseuille's law which states that the rate of movement is dependent on the radius of the capillary to the power four. Thus, doubling the dentinal tubule radius would increase the flow 16 times. From here came the idea of decreasing the flow in the sensitive teeth's dentin via reducing the diameter of the tubules.

Banfield and Addy (21) did a study to develop an in situ method to investigate the impact of different agents on dentin specimens prepared to simulate sensitive and non-sensitive dentin. Two separate studies were performed; the second one being more standardized than the first. In the 1st experiment, five healthy dentate volunteers participated. Acrylic appliances, made with a place to retain sections of recently extracted teeth in the buccal sulcus, were fabricated for the subjects. Two specimens of each group were treated with 6% citric acid for 2 min to remove the smear layer. All "smeared" and "etched" specimens were sectioned in half; one half was placed in the appliance and the other served as the non-treated control. Appliances were worn for 5 days. Treatments were 2-day application of a desensitizing toothpaste (strontium acetate-SA), non-desensitizing toothpastes (F), and chlorhexidine (CHX) mouthwash with or without drinking orange juice (OJ). A no treatment group (P) allowed plaque to accumulate. The effects were observed by SEM. The second study involved a single volunteer wearing

two removable lower buccal acrylic appliances containing etched dentin specimens. In this study, to ensure tubules were sectioned at right angles to their long axis, the control part of each specimen was studied with SEM. If tubule outlines were not judged to be circular the test and its counterpart control were discarded. Four etched specimens were placed in each appliance. The treatments applied to the 4 specimens were: SA toothpaste, strontium chloride desensitizing toothpaste (SC), in-office desensitizing product (DS), and a conventional fluoride toothpaste (F). The toothpastes were applied to the specimens by tooth brushing with a standard toothbrush for 60 sec. Test specimens were recovered from the appliances, matched with their respective control specimens and examined with SEM. Results were analyzed using unpaired t-test. The results showed that the treatment with SA toothpaste occluded the tubules and was little affected by OJ, contrary to the treatment with F and CHX. For the smeared specimens toothpastes and OJ removed the smear layer but SA±OJ blocked the tubules. At 0 hr, tubule occlusion was in order of magnitude DS>SA>SC>F. After 6 and 12 hr with SA, SC and F some loss of occlusion occurred but not DS.

Addy et al. (22) performed an in vitro study to observe the effects of commonly used mouthwashes and toothpastes on the removal of dentinal smear layer, and the hypothetical initiation of DH. Sections of freshly extracted teeth (1 mm thickness) were cut. This procedure produced a smear layer on both sides of the sections. Pairs of dentin sections were selected and placed individually into 10 ml volumes of the experimental mouthwashes for varying time periods (3, 10 60, 120, 180 and 300 minutes). Sections were then examined with SEM. The experiment was repeated twice. In the first experiment, the sections were brushed with water for 2 minutes after soaking them in mouthwashes. In the second experiment, the sections were brushed with a fluoride containing toothpaste and SEM photomicrographs were then taken. The micrographs were scored using a subjective scale. The results of the study showed that most of the mouthwashes tested produced no consistent visible changes to the dentin surface at any time, with or without post-treatment brushing with water or toothpaste. The smear layer remained intact and the tubules were not visible. However, three mouthwashes (Double Amplex, Oraldene, and Listerine) did produce visible changes compared to the controls.

The use of these mouthwashes caused a slight removal of the smear layer, and their effect was enhanced by post-treatment brushing. Despite the subjective scale used to evaluate the smear layer removal, this study indicated the need to determine whether the intermittent use of some mouth rinses would produce cumulative effects on dentin.

Etiology and predisposing factors

The etiological and predisposing factors related to DH were investigated in a study by Bamise et al. (23). Twenty nine patients suffering from DH were recruited to participate in the study. A detailed relevant history was taken, which included dietary consumption and oral prophylaxis habits. Intraorally, all teeth were examined for position, mobility, and occlusal relations. Any evidence of gingival recession, caries, attrition, erosion, abrasion, abfraction, fractures and chipping were recorded. The suspected DH sites on the teeth were stimulated by an air blast and by probing the exposed area with a dental explorer until the patient reported discomfort. Frequencies and percentages were calculated. A Chi square test was used to examine the association between the discreet variables with a $P<0.05$ level of significance. Erosive lesions were reported in 90.6% of patients consuming orange juice, 50% with carbonated beverages consumption, and 40.6% with chewable vitamin C consumption. Patients who used a hard bristle toothbrush had 38.5% of the teeth with gingival recession and 40.3% of the teeth with abrasion. Patients who used a soft bristle toothbrush had six teeth (5.1%) with gingival recession and two teeth (3%) with abrasion. Sixty-five teeth (97%) showing abrasion were found in patients who cleaned their teeth with a toothbrush and toothpaste. DH was correlated to gingival recession (36.8%); 41.4% with attrition; 59.7% with abrasion and 64% with abfraction. The authors concluded that gingival recession followed by attrition were the most common etiological factors in this study, while erosive lesions showed the most frequent association with dentin hypersensitivity. Bamise et al. study suggested the possible etiological and predisposing factors to DH, however, the study lacked defined criteria in which attrition, abrasion, erosion, and abfraction were diagnosed. The complexity of diagnosing some of those conditions in addition to the overlap of these conditions in many clinical cases cast doubt on the conclusions drawn.

Two phases have been described for the progression of DH: lesion localization and lesion initiation. The former occurs by exposure of dentin, either by loss of enamel or gingival recession. However, not all exposed dentin is sensitive. The localized DH lesion has to be initiated. This occurs when the smear layer or tubular plugs are removed opening the outer ends of the dentinal tubules. Abrasion and erosion may be implicated in this process. Gingival recession seems to be the predominant localization factor and erosion is the predominant initiating factor. DH is more frequently encountered in patients with periodontitis and transient hypersensitivity may occur after periodontal procedures such as deep scaling, root planning or gingival surgery. Hypersensitivity also may occur after tooth whitening and restorative procedures (6).

Treatment of DH

Management of a patient suffering from DH should be based on a correct diagnosis of the condition by the dentist, who should be aware of other clinical conditions which are similar in their presenting features. Conditions that can produce symptoms mimicking those of DH are: cracked tooth syndrome, fractured restorations, chipped teeth, dental caries, post-restorative sensitivity and teeth in acute hyperfunction. Patients generally complain that pain arising from DH is usually rapid in onset, sharp in character and short in duration. More rapid response to stimuli or the persistence of pain after removal of the stimuli has been ascribed to inflammatory changes in the pulp. In such cases conventional approaches to the treatment of DH are unlikely to be successful and recourse to endodontics even exodontia may be necessary (15).

Numerous treatment modalities have been used to manage DH. The mechanism of action of the therapies used varies and could be divided into four main categories: dentinal tubules blocking agents, nerve desensitizers and placebo treatment. Many authors classified the treatments used for DH according to their mode of delivery to: self administered by the patient at home, or professionally applied in the dental office (15,24). At-home methods tend to be simple and inexpensive and can treat simultaneously generalized DH affecting many teeth. In-office treatments are more complex and generally target DH localized to one or few teeth.

Dentinal tubule occluding agents

Toothpastes containing strontium chloride have been widely used to treat DH. Minkoff and Axelrod (25) did a 12-week double blind clinical study to determine the efficacy of 10% strontium chloride hexahydrate used in a commercial dentifrice (Sensodyne®) in alleviating the symptoms of DH. Sixty one healthy adult patients with clinically confirmed DH accompanied by cervical erosion, gingival recession or both participated in the study. Subjects were randomly assigned to one of two groups (active product or placebo). Instructions were given to the subjects to brush their teeth with the assigned product for at least 30 seconds twice daily. The level of DH that the patients had was recorded at baseline and at weeks 2, 4, 8, and 12. Three methods were used to evaluate DH: tactile stimulus, thermal stimulus and a patient administered questionnaire. The analysis of variance t test was used to test the differences in changes from baseline, and Spearman's Rank Correlation Coefficient was used to perform a correlation analysis of the three methods used to quantify DH. Three subjects dropped out the study due to minor side effects which occurred upon the use of the active product. Analysis by subject showed no statistically significant differences between the two groups in response to tactile stimulus at all the recalls, however, it was significantly reduced (P=0.02) in the active toothpaste group. The same was observed for the thermal stimulus, where the active toothpaste group reported a significant reduction in DH. Fifty five percent of the 29 subjects who used the active toothpaste reported a total relief of the symptoms (questionnaire evaluation), compared to 14% only in the placebo group. The authors concluded that the regular use of a 10% strontium chloride hexahydrate containing dentifrice provided an effective treatment for patients with DH. Details about the placebo treatment and the double blinding process were not clarified in this study. The tactile assessment method that the authors used indicated that the change in pressure applied by the explorer is the prime stimulus for pain; however, Brannstrom (17) attributed the pain to the movement of the explorer rather than the pressure.

Products containing oxalates have been used to treat DH. These products reduced dentin permeability and occluded tubules more consistently in laboratory studies than they did in clinical trials (6). Sauro et al. (26) carried out a study to evaluate the changes

in dentinal permeability after application of several phytocomplexes containing oxalates. Eighteen different treatments were tested: experimental pastes, gels and solutions of phytocomplexes, an experimental potassium-oxalate-based paste, Elmex and Sensodyne toothpastes were used as controls. Each treatment was applied undiluted to dentin sections of extracted human teeth for 3 min using a soft brush. Permeability was calculated for each of the teeth sections: before treatment, after etching with 0.5M EDTA, after application of the treatment and after H_3PO_4 acid attack for 5 min. SEM was used to analyze the samples. Differences between the different treatment groups were tested using ANOVA. Fisher's least significant difference (LSD) test and Bonferroni's test were used to isolate and compare the significant differences ($P < 0.01$) between the groups. The most effective treatments were 25% spinach extract solution, 25% rhubarb + 25% spinach extract paste and 25% rhubarb + 25% spinach extract solution (oxalate-containing natural substances). In contrast, Elmex and 25% mint extract solution were the least effective treatments. The authors attributed the reduction in permeability to the formation of calcium oxalate crystals that would obstruct the patent dentinal tubules. However, the reduction in permeability ranged from 42.3% to 47.3% which does not give the oxalates any greater advantage in the treatment of DH.

Pereira et al. (27) compared the reduction of hydraulic conductance of three formulations of potassium oxalate (PO) and an acidified sodium fluorophosphate (APF) gel. Two hundred dentin discs were divided into 20 groups (10 discs each). Five different treatments were performed namely: etching with 0.5 M EDTA, washing and air-drying; washing and blot-drying; washing and left wet; no washing and air-drying; no washing and blot-drying. The materials used were; 3% PO (pH: 4.1), 6% PO (pH:4), 3% PO (pH:2.5), 1.23% APF (pH:3.6-3.9). The hydraulic conductance was measured after the application of the materials to the dentin discs. Specimens were then treated with 6% citric acid for one minute and the conductance was measured again. General MANOVA and post-hoc Duncan tests were performed. Results showed that the 3% potassium oxalate gel (pH 2.5) produced the greatest reduction in dentinal conductance (p<0.05), even after citric acid challenge, regardless of surface pre-treatment. On the other hand,

the APF gel produced the smallest reduction in hydraulic conductance when compared with the other materials.

Toothpastes containing various formulations of fluoride have been used to treat DH. However, the mechanism of its action is not clear. Fluoride decreased the permeability of dentin in vitro by precipitation of the insoluble calcium fluoride within the tubules is perhaps one explanation (6).

Calcium phosphates have been also used to treat DH. They occluded dentinal tubules and decreased dentin permeability in vitro (6). Kaufman et al. (28) carried out a double-blind, 8 week clinical study to compare the desensitizing efficacy of a conventional NaF-containing dentifrice (control), Enamleon® (which contained NaF, Ca and P salts) and a toothpaste containing MFP with Ca and P salts at a higher pH than the commercial Enamelon formula. One hundred and five healthy subjects with sensitive teeth participated in the study. Questionnaire evaluations and examinations were made at baseline and after 3 and 8 weeks of product use. Student-t test, Kruskal-Wallis and Friedman's 2-way analysis of variance were used to analyze the data collected. The number of sensitive teeth that became non-sensitive over time was greater at 3 and 8 weeks for Enamelon and the MFP test product than for the over the counter (OTC) control, which still showed a net decrease in sensitivity at week 8. Enamelon appeared to offer the greatest benefit ($P<0.001$). The decrease in the number of sensitive teeth at 8 weeks was 18% for Enamelon, 10% for MFP dentifrice and 5% for the OTC control. This was compared to a 28% decrease for potassium citrate and 19% decrease for potassium nitrate after 8 weeks application. Therefore, Enamelon was not a good alternative compared to already used sensitive teeth dentifrices. However, Enamelon had been claimed to have a unique delivery system where calcium and phosphate remain separated by a plastic divider and only get mixed when applied to the teeth. This allows high levels of remineralizing ions to be delivered to the tooth surface while still in the soluble state.

Stannous ions have been also used in desensitizing toothpaste formulas. Those ions along with the Ca and PO_4 from the saliva may precipitate on the dentinal surfaces thus occluding the dentinal tubules. Although these occluding technologies were

sometimes successful, none of them currently are used in OTC toothpastes which have received the ADA Seal of Acceptance for DH (29).

All the previously mentioned toothpaste formulations were intended to treat DH through occluding the patent dentinal tubules. However, these toothpastes contain abrasive agents that would help not only clean the tooth surface, but probably abrade the tooth structure and re-open the obstructed tubules. Kodaka et al. (30) investigated the effect of brushing with abrasive toothpaste used to treat DH on the surfaces of sound dentin that is exposed to the oral cavity. Thirty six dentin sections from 18 caries free teeth, extracted for orthodontic reasons, were used for the study. Three pairs of samples were divided and arranged in both sides of an acrylic dental plate. One third of the sample's surface was covered with acrylic resin as a control. The dentifrice used contained diatomaceous earth and silica abrasives, and strontium chloride hexahydrate as the active ingredient. Five adult subjects participated in the study. The resin plates were exposed to the oral cavity for 8 weeks, except for mealtimes, and subjected to experimental and usual tooth brushing. In each subject, the three pairs of samples were brushed with and without dentifrice every day for one minute, respectively, after their removal from the oral cavity. Sections were examined using SEM and scanning laser microscopy every 2, 4 and 8 weeks. The average number of occluded tubules was calculated for the samples. Brushing with the dentifrice gradually decreased the mean average of occluded tubules from about 91 to 77% during 2 to 8 weeks, although there was no significant difference among the individual values. The mean abrasive loss of the dentin surfaces brushed with the dentifrice significantly increased from about 52 to 143 μm. The brushed surfaces of the dentin showed a rough topography with numerous toothbrush scratches but no organic pellicle was found. On the other hand, brushing without dentifrice caused about 99% of the dentinal tubules to occlude in 2 and 4 weeks and 100% in 8 weeks. The brushed dentin surfaces at 8 weeks were entirely covered with organic pellicle containing fine mineral granules derived from saliva, and the abrasive loss was about 1.4 um.

The tremendous variety of sensitive teeth toothpastes offers an affordable easily accessible treatment modality. Nonetheless, these desensitizing dentifrices often take 1 to

3 months for the required results to be realized. Moreover, if the acid challenge persists, the balance is easily reversed and the sensitivity reappears (31).

The use of Ozone has been one of the proposed treatments for DH. The mechanism of which ozone might reduce DH has not been fully understood. One of the theories is; ozone is an oxidizing agent which leads to the formation of calcium oxalate when applied to calcium containing surfaces, hence blocking the dentinal tubules. Another theory proposes damage to the nerve endings of the intradental A-type nerve fibers through oxidation caused by ozone (16). The later supports the direct nerve ending theory in the etiology of DH, which has not been as widely accepted as the hydrodynamic theory.

Dahnhardt et al. (16) carried out a 54 weeks clinical trial to demonstrate the effectiveness of treating hypersensitive teeth with ozone for 60 sec. Thirty one patients with at least two hypersensitive teeth were recruited, and a split mouth design was chosen for the study. The baseline pain score to an air blast was recoded using the Visual Analogue Scale (VAS). Two weeks after, the same stimulation was performed and VAS was recorded. After which, the test tooth was treated with ozone for 60 sec, and pain level recorded. Ozone application and the subsequent testing were repeated at weeks 6 and 14. At week 22 the control tooth cross-over took place and the control tooth received the ozone treatment at weeks 30, 34, 38, 46 and 54, and pain level recorded. ANOVA and Wilcoxon rank sum test were used to analyze the data (P< 0.05). The results showed a temporal trend in pain level reduction with an average of 55% ± 5.5% before and after each treatment episode. There was a significant reduction of pain level in the control teeth as well. Comparing test and control teeth, over time, there was no statistically significant differences in pain reduction (P=0.58). The study was not blinded for the patient, the dentist, or the treatment rendered. The authors suggested some reasons for the absence of a significant difference between the test and the control. Those reasons were: the pain reduction on one side caused the reduction in the other side; a natural reduction of pain overtime; and the Hawthorne phenomenon, which is the response to non-intervention procedures such as frequent examination. The other explanations, which were not proposed by the authors, might be the placebo effect or the reduction in

sensitivity due to the use of desensitizing toothpastes which 17 subjects (55%) used and did not discontinue using during the study. Overall, if ozone was to be effective in treating DH, its efficacy is relatively low and requires a long duration.

Similar results have been reported by Azarpazhooh et al. (32). They performed a triple-blinded, randomized controlled clinical trial over 8-week (3 visit) on 44 subjects. All subjects reported a clinically significant reduction of pain at each follow-up relative to baseline; however, the difference between the study groups was not statistically significant. The authors attributed that to the placebo effect.

Various adhesives and resins have been used claiming a more permanent solution to DH. Because many topical desensitizing agents do not adhere to tooth surfaces, their effects are temporary. Current DH treatment includes the use of varnishes, bonding agents and restorative materials. The studies on the use of adhesives and resins to treat DH were mainly single-blinded. The clinical trials on those materials, however, tend to be pragmatic (6).

Lambrechts et al. (31) associated an increased resistance to acid and caries attack if the exposed dentin or root surface was impregnated with monomers from a dentin bonding system. However, this technique does not offer recontouring for the eroded area, but creates a durable wear resistant coat, combined with filled resin tags of several hundreds of μm length. While successful penetration has been achieved with respect to enamel surfaces, adhesion to cementum and dentin substrates has been more challenging.

The sealing ability of dentin adhesive/desensitizers was studied by Fu et al. (33). Standardized class V cavities were prepared on the buccal and lingual surfaces of 55 freshly extracted teeth. The teeth were longitudinally cut into two sections, and randomly assigned to 11 groups of 10 specimens each. One group was left untreated and the other groups received different treatments with adhesive agents. SEM examination was performed on two randomly selected specimens from each group. The sealing ability of the adhesives used was also assessed by measuring the penetration of 0.5% methylene blue dye slowly injected into the prepared cavities 0.5 mm below the cavosurface margin. The dye penetration scores (0–4) were recorded using a stereomicroscope at a 10-fold magnification. The maximum dye penetration score in each specimen was recorded

instead of the mean score. All dentin desensitizers significantly reduced dentinal permeability ($P<0.05$). The sealing ability of the different dentin adhesives/desensitizer was significantly different ($P<0.001$). Nevertheless, none of the dentin adhesives/desensitizer could completely block fluid percolation through the dentinal tubules. Fu et al. studied the sealing ability of some desensitizers without any prior treatment to the dentin sections. A smear layer would usually cover the cut dentin sections, in addition to the intra-tubular minerals that usually obstruct the lumen of non-sensitive dentin. That would necessitate etching the studied sections before further treatments. Failure to do so may overestimate the effect of the adhesives used.

The hydraulic conductance of five dentin desensitizing agents was evaluated by Kolker et al. (34). The agents tested were: Seal & Protect; Gluma Desensitizer; HurriSeal; D/Sense 2; and Super Seal. Thirty extracted human molars were sectioned into 1-mm dentin disks. Treatments were applied to the occlusal surfaces of dentin according to the manufacturer's instructions. Dentin permeability was measured at baseline and after treatment using bovine serum and phosphate-buffered saline at 10 psi. Two disks from each group were randomly selected for SEM analysis. Results showed that Super Seal may be the most effective adhesive when treating DH with a mean percent reduction of permeability of 97.5 ± 4.0, compared to HurriSeal (54.2 ± 35.3, D/Sense 2 (46.6 ± 20.4), Gluma Desensitizer (39.6±26.7), and Seal & Protect (33.8 ± 19.4). The difference in the effectiveness of the adhesives used was statistically significant ($P<0.01$).

A similar study was reported by Camps et al. (35). The study compared the effectiveness of three desensitizing agents; Protect, Gluma Desensitizer and MS coat. The reduction in permeability was compared at baseline, immediately after treatment and a month after storage in deionized water. No statistical difference was found between the immediate hydraulic conductances of the three groups after treatment. After 1-month storage, the control group showed a statistically higher hydraulic conductance than the three treated groups. There was no statistical difference between the three dentin desensitizing agents evaluated. Overall, the effectiveness of the agents used in reducing dentin permeability was around 60-85%.

Many studies have investigated the efficacy of Gluma desensitizer in the treatment of DH. Gluma reduced dentin permeability by only 39.6% in a Kolker et al. study (34), which is relatively low for an agent that penetrated the dentinal tubules to a depth of approximately 200 μm, as reported by Schupbach et al. (36). In their study dentin sections were tested for the desensitizer's penetration into the dentinal tubules after treatment with Gluma and compared to untreated control sections. The penetration was assessed with confocal laser scanning microscopy (CLSM), SEM and TEM. Gluma is an aqueous solution containing 5% glutaraldehyde (GA) and 35% hydroxyethyl mathacrylate (HEMA). It was concluded that GA is the compound responsible for the occlusion of the tubules due to its effect on serum proteins in the dentinal fluid. HEMA in this solution may promote deep penetration of the GA component into the tubules due to its water solubility. The penetration of Gluma to a depth of 200 μm compared to only 50 μm penetration when 5% GA was applied alone to the teeth sections was what lead the authors to such a conclusion.

In order for desensitizing agents to replace OTC dentifrices, they have to provide long-term relief for DH. The in vitro resistance of two dentin-bonding agents (One coat Bond and Optibond FL) and a dentin desensitizer (Gluma desensitizer) to acid erosion was investigated by Brunton et al. (37). Each of the three agents was applied to 20 dentin sections. Ten samples of which were immersed in distilled water and the other 10 were immersed in *Coca Cola*® (pH: 3.15) for 14 days. Profilometric tracings were recorded for the samples at 0 and 14 days. Thus, the mean reduction in desensitizer thickness and or tooth loss was determined. Results showed the mean change in vertical profile of the sites treated with Gluma, One Coat Bond, and Optibond FL groups after exposure to Coca Cola® were 38.3 μm±9.8 μm, 14.0 μm±13.0 μm, and 7.9 μm±5.7 μm respectively. Teeth treated with Gluma showed complete loss of the coating with additional loss of tooth tissue. While sections treated with dentin bonding agents retained a substantial thickness, therefore, no tooth tissue was lost. The average loss in film thickness for One Coat Bond was 52%±15.2%, while Optibond FL showed a loss of only 20%±4.7%. The authors concluded that the dentin bonding agents were far more acid resistant than the

dentin desensitizer used. The long term effects of the adhesives used, however, is questionable.

Restorative treatment has been widely discussed in the literature, especially the treatment of Non-Carious Cervical Lesions (NCCL). However, it is beyond the scope of this review to discuss the treatment options of NCCL, and the focus will be the treatment of the symptom that might be associated with NCCL; which is DH.

Iontophoresis has been described as a method of facilitating the transfer of ions by means of an electrical potential into soft or hard tissues of the body for therapeutic purposes (38). Iontophoresis of fluoride has been used for the treatment of DH; however, its real benefit is controversial. Many studies have found Iontophoresis a safe and effective method for treating cervical DH although some investigators consider it time consuming and technique-demanding. Many mechanisms have been proposed for its action: it may desensitize hypersensitive dentin by formation of a secondary dentin as a result of the electrical current employed; production of paresthesia by altering the sensory mechanism of pain conduction; lastly, iontophoresis may increase the concentration and depth of penetration of fluoride ions into dentinal tubules (precipitate calcium fluoride) thereby occluding the tubules and reducing the conduction of stimuli (38).

The effectiveness of sodium fluoride (NaF) treatment for teeth with hypersensitivity with and without Iontophoresis was investigated by Kern et al. (39). Sixteen patients with at least two teeth with DH were included in the study. A fresh solution of 2% NaF was applied to the teeth. A COE4 (tube) electrode from an Iontophoresis unit was used to apply 0.5mA current for 2 minutes to the test tooth and a 0mA to the control tooth. Patients and operators were blinded for the treatment delivered. Teeth were evaluated for sensitivity subjectively by patient's grading the sensitivity; tactile response and air blast. Evaluations were performed before and immediately after the treatment, and at 1, 3 and 6 months. Results have shown that the Iontophoresis group demonstrated a significant reduction in DH immediately after treatment ($P<0.0001$), whereas NaF alone had no effect. However, post treatment effects of Iontophoresis returned to baseline levels at 3 and 6 months. There was a small placebo effect with the

NaF-alone group, but it was not statistically significant. The results in this study contradicted the results reported by Brough et al. (40) where there was no significant difference in the application of NaF with and without Iontophoresis for the treatment of DH (one month duration). Nevertheless, Brough et al. reported an interesting finding. They found that the use of distilled water without iontophoresis was the only treatment that had a significant effect in reducing sensitivity to cold stimulus at 1 week and continued to be significant throughout the study.

Many of the studies evaluating the effect of iontophoresis in the treatment of DH have lacked some elements of randomized controlled trials (RCTs) including; suitable controls; standardization; randomization and blinding. Thereby, it is difficult to differentiate the topical effects of the active agent from any additional iontophoretic effect, as well as possible associated placebo and non-placebo effects (38).

Kimura et al. (41) reviewed the use of lasers in the treatment of DH. Since ruby laser was developed in 1960; researchers have investigated laser's use in dentistry. The first laser used for the treatment of dentin hypersensitivity was Nd:YAG laser. The lasers used for the treatment of dentin hypersensitivity are divided into two groups: low output power (low-level) lasers [helium-neon (He-Ne) and gallium/aluminum/arsenide (GaAlAs) (diode) lasers], and middle output power lasers (Nd:YAG and CO2 lasers). Low level lasers have been used to support wound healing, and they were an effective anti-inflammatory tool. In addition, low level lasers did stimulate nerve cells in the clinical environment. The effectiveness of He-Ne laser in the treatment of DH ranged from 5.2% to 100%. He-Ne laser irradiation at 6 mW does not affect the enamel or dentin surface morphologically, but a small fraction of the laser energy is transmitted through enamel or dentin to reach the pulp tissue. With low output power lasers, there is no danger of causing skin burns, or damaging cells. However, the mechanism of action of this type of laser is unknown. Three wavelengths (780, 830, and 900 nm) of GaAlAs have been used for the treatment of dentin hypersensitivity. It is postulated that this type of low output power lasers mediates an analgesic effect related to depressed nerve transmission. According to physiological experiments using the GaAlAs laser at 830 nm, this effect is caused by blocking the depolarization of C-fiber afferents; as with the He-Ne laser, a

small fraction of the laser energy is transmitted through enamel or dentin to reach the pulp tissue. The treatment effectiveness ranged from 30 to 100% depending on the wavelength used. With the use of Nd-YAG laser the treatment effectiveness ranged from 5.2 to 100%. When using this type of laser, the use of black ink as an absorption enhancer is recommended, to prevent deep penetration of the Nd:YAG laser beam through the enamel and dentin and excessive effects in the pulp. The mechanism of Nd:YAG laser effects on DH is thought to be laser-induced occlusion or narrowing of dentinal tubules, as well as direct nerve analgesia. The sealing depth achieved by Nd:YAG laser irradiation at 30 mJ/pulse and 10 pps on dentinal tubules is usually measured to be less than 4 um, but it is dependent on the irradiation parameters. The treatment effectiveness of CO_2 laser ranged from 59.8 to 100%. Its effect in treating DH is due to the occlusion or narrowing of the dentinal tubules. There have been no reports of nerve analgesia by CO_2 laser irradiation. The sealing depth achieved by CO2 laser irradiation at 0.3 W for 0.1 s on dentinal tubules is usually measured to be 2–8 um. Two main concerns have been raised in the literature for the use of lasers in the treatment of DH. The first concern was its thermal effect on the pulp. Nevertheless, previous studies have demonstrated that healthy pulp tissue is not injured thermally if the laser parameters are correct so that any temperature rise within the pulp remains below 5℃. Furthermore, pulpal disruption did occur in laser-treated specimens with remaining dentin thickness of less than 1 mm, while it did not happen when the dentin thickness exceeded 1 mm. The second concern was the recurrence of DH with time. The recurrence rate varied from 34 to 75% with the different lasers used. The mechanism of recurrence is unknown.

Nerve desensitization

Potassium salts containing toothpastes are the most widely used at-home treatment for DH (24). Potassium ions are thought to diffuse along dentinal tubules and decrease the excitability of the intradental nerves by altering their membrane potential; which has been proven in animal models. The efficacy of potassium nitrate to reduce dentin permeability in vitro and subsequently DH, however, has not been strongly supported in the literature (6). A systematic review (2) aimed to assess the effectiveness

of potassium containing toothpastes in reducing DH. Studies that were included in the review were randomized control trials (RCTs) comparing potassium to non-potassium containing toothpastes. Subjects participated in the studies were healthy human adults (18 years or more), for whom daily use of potassium containing toothpaste versus control toothpaste was prescribed. Sensitivity was assessed at baseline and 6-8 weeks after the treatment. Four methods were used to assess DH; tactile, thermal, air-blast, and patient's subjective assessment of pain. The following databases were searched: Cochrane Oral Health Group Trials Register (searched until August 2005); CENTRAL (until August 2005); EMBASE/MEDLINE, PubMed, Web of Science (until September 2005). The review did not exclude non-English studies, therefore, those were translated. Six studies only fulfilled the inclusion criteria for this review. In all the studies the experimental toothpaste contained 5% potassium nitrate. Two of the studies used toothpastes containing sodium monofluorophosphate, two used sodium fluoride toothpastes, while two studies used no fluoride as the control. The mean difference between the groups at 6-8 weeks for the one study where tactile sensitivity was measured by an ordinal scale was -0.65 indicating that the test toothpaste significantly reduced sensitivity. The mean difference for the 5 studies that measured tactile sensitivity as a continuous variable was 1.19. Chi^2 for heterogeneity indicated a significant reduction in sensitivity (P=0.02). Six studies were included in the meta-analysis for air-blast. The mean difference was -1.25 (P=0.008), which also indicated a significant reduction in sensitivity. Only one study measured thermal stimulation for DH with a mean difference of 1.1, indicating a significant difference compared to the control toothpaste. On the other hand, three studies were included for the subjective measurement for DH. The meta-analysis led to a mean difference of -0.67 (P=0.002), failing to detect a significant difference between the mean subjective assessments. Although the results of this review show a significant reduction of DH with the use of potassium containing toothpaste as assessed with tactile, thermal, and air-blast, it did not cause the same reduction as assessed subjectively. However, these findings were based on fairly small number of patients (390), and those studies evaluating the subjective assessment involved only 108 patients.

Placebo effect

As any treatment or medication, the efficacy of DH treatment modalities has been complicated by the placebo effect. The exact mechanism of placebo is yet to be known.

West et al. (42) performed a study to compare three commercially available toothpastes for the alleviation of DH. The three pastes were: SAF-UK Macleans Sensitive (strontium acetate+ sodium fluoride), PN-US Aquafresh Sensitive (potassium nitrate+ sodium fluoride) and SMFP-UK Aquafresh (sodium monofluorophosphate). The latest was used in the wash-in phase, where participants were asked to brush twice daily with the paste for 4 weeks, and it was used during the testing phase as a control. Afterwards, the participants were assigned one of the three toothpastes to use at home for 6 weeks. One hundred and thirty one volunteers with a history of DH, subjectively evaluated in addition to a clinical examination, were recruited. At each of the 3 clinical evaluation visits (treatment baseline, 2 weeks and 6 weeks), volunteers were asked to report their overall assessment of pain on a scale of 0 to 10, where 0=no pain, 10=excruciating pain. Further, tactile and cold air stimuli were undertaken on all teeth and a score using the same scale was recorded. Means and standard deviations were calculated and comparisons made using Wilcoxon's sum of ranks test. Results showed that the three treatment groups resulted in reduction of DH with time for all variables. However, there was no statistical difference between the three toothpastes. Interestingly, there was no significant change in sensitivity between wash-in baseline and treatment baseline for the cold air stimulus with the fluoride-only-based paste. However, for the group using the same fluoride toothpaste, there was a significant improvement between (wash-in baseline-week 6) and (treatment baseline-week 6) for this stimulus. The authors suggested a substantial placebo effect have occurred.

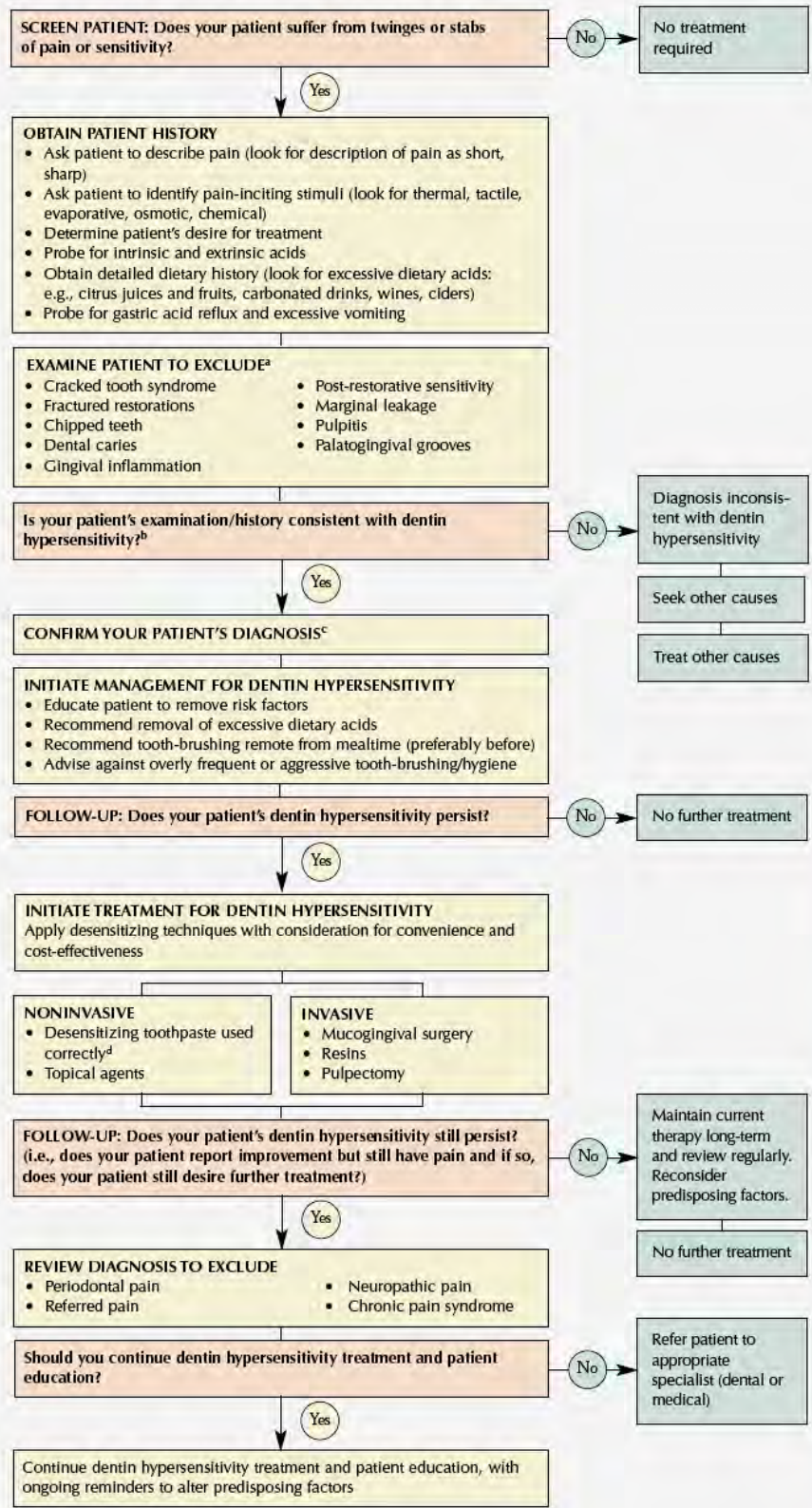The same authors, Addy et al. (43), performed a study aiming to determine whether the application of a periodontal dressing as a treatment regimen would help stop or reduce the pain of DH. For this purpose, patients were screened for DH. Patients included in the study (n=22) were; more than 18 years of age; have a minimum of two sensitive teeth (in different quadrants); showing buccal recession and exposed dentin; and

displaying a response of ≥30 mm on a 100-mm VAS to a one second evaporative stimuli. The two identified teeth in different quadrants were assigned, based on severity of pain, to either the test or control treatment group using stratification of the VAS score results for the evaporative stimulus. The baseline sensitivity was recorded. The dressing (Coe-Pak®) was then applied to cover the buccal surfaces and gingival margins of all the sensitive teeth in the test quadrant and one tooth either side of those with sensitivity. On the control side, Coe-Pak was placed either side of the sensitive control tooth, to cover all other sensitive teeth. Both the control and the test sides were then tested with the air and thermal stimuli and sensitivity score was recorded. Results showed that dressing application produced significantly greater reduction in pain compared with no periodontal dressing. However, there has been some pain reduction in the control side (no numerical value has been reported in the study), which the authors attributed to the placebo response. This study had a small sample size. Further studies with a larger sample size are needed to determine the effect of placebo.

The treatment of DH could simply rely on the elimination of its predisposing factors. For example, proper oral prophylaxis techniques could help prevent abrasion that could aggravate DH. Excessive force, hard toothbrushes and highly abrasive toothpastes should be avoided. Tooth brushing should be avoided after consuming acidic foods and drinks since tooth brushing, in combination with acid decalcification of superficial dentin, is capable of accelerating the loss of tooth structure and opening dentinal tubules. Dietary counseling is an important factor for the management of the condition. A written diet history should be obtained from patients with DH in order to identify etiological agents and form a basis from which to provide advice (15).

The Canadian advisory board on DH summarized the steps to the clinical diagnosis and management of DH in the following flowchart (Fig. 2).

SCREEN PATIENT: Does your patient suffer from twinges or stabs of pain or sensitivity? — No → No treatment required

Yes ↓

OBTAIN PATIENT HISTORY
- Ask patient to describe pain (look for description of pain as short, sharp)
- Ask patient to identify pain-inciting stimuli (look for thermal, tactile, evaporative, osmotic, chemical)
- Determine patient's desire for treatment
- Probe for intrinsic and extrinsic acids
- Obtain detailed dietary history (look for excessive dietary acids: e.g., citrus juices and fruits, carbonated drinks, wines, ciders)
- Probe for gastric acid reflux and excessive vomiting

EXAMINE PATIENT TO EXCLUDE[a]
- Cracked tooth syndrome
- Fractured restorations
- Chipped teeth
- Dental caries
- Gingival inflammation
- Post-restorative sensitivity
- Marginal leakage
- Pulpitis
- Palatogingival grooves

Is your patient's examination/history consistent with dentin hypersensitivity?[b] — No → Diagnosis inconsistent with dentin hypersensitivity / Seek other causes / Treat other causes

Yes ↓

CONFIRM YOUR PATIENT'S DIAGNOSIS[c]

INITIATE MANAGEMENT FOR DENTIN HYPERSENSITIVITY
- Educate patient to remove risk factors
- Recommend removal of excessive dietary acids
- Recommend tooth-brushing remote from mealtime (preferably before)
- Advise against overly frequent or aggressive tooth-brushing/hygiene

FOLLOW-UP: Does your patient's dentin hypersensitivity persist? — No → No further treatment

Yes ↓

INITIATE TREATMENT FOR DENTIN HYPERSENSITIVITY
Apply desensitizing techniques with consideration for convenience and cost-effectiveness

NONINVASIVE
- Desensitizing toothpaste used correctly[d]
- Topical agents

INVASIVE
- Mucogingival surgery
- Resins
- Pulpectomy

FOLLOW-UP: Does your patient's dentin hypersensitivity still persist? (i.e., does your patient report improvement but still have pain and if so, does your patient still desire further treatment?) — No → Maintain current therapy long-term and review regularly. Reconsider predisposing factors. / No further treatment

Yes ↓

REVIEW DIAGNOSIS TO EXCLUDE
- Periodontal pain
- Referred pain
- Neuropathic pain
- Chronic pain syndrome

Should you continue dentin hypersensitivity treatment and patient education? — No → Refer patient to appropriate specialist (dental or medical)

Yes ↓

Continue dentin hypersensitivity treatment and patient education, with ongoing reminders to alter predisposing factors

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a. Potentially useful diagnostic tools
- Air jet
- Cold water jet
- Other thermal tests
- Dental explorer
- Periodontal probe
- Radiographs (if needed)
- Percussion testing
- Assessment of occlusion
- Bite stress tests

b. Definition of dentin hypersensitivity
Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or disease.[1]

c. Other potential diagnostic aids
- Selective anesthesia
- Transillumination

d. The best results are achieved if desensitizing toothpaste is applied via twice-daily brushing, performed on an ongoing basis according to a regular schedule (not applied topically, as in "dabbing").

Fig 2. Algorithm for the diagnosis and management of DH (4).

References

(1) Gillam DG, Seo HS, Bulman JS, Newman HN. Perceptions of dentine hypersensitivity in a general practice population. J.Oral Rehabil. 1999 Sep;26(9):710-714.

(2) Poulsen S, Errboe M, Lescay Mevil Y, Glenny AM. Potassium containing toothpastes for dentine hypersensitivity. Cochrane Database Syst.Rev. 2006 Jul 19;3:CD001476.

(3) Olusile AO, Bamise CT, Oginni AO, Dosumu OO. Short-term clinical evaluation of four desensitizing agents. J.Contemp.Dent.Pract. 2008 Jan 1;9(1):22-29.

(4) Canadian Advisory Board on Dentin,Hypersensitivity. Consensus-based recommendations for the diagnosis and management of dentin hypersensitivity. Journal (Canadian Dental Association) 2003 Apr;69(4):221-6.

(5) Pashley DH. Theory of dentin sensitivity. J.Clin.Dent. 1994;5 Spec No:65-67.

(6) Orchardson R, Gillam DG, Orchardson R, Gillam DG. Managing dentin hypersensitivity. Journal of the American Dental Association 2006 Jul;137(7):990-8; quiz 1028-9.

(7) Clayton DR, McCarthy D, Gillam DG. A study of the prevalence and distribution of dentine sensitivity in a population of 17-58-year-old serving personnel on an RAF base in the Midlands. Journal of Oral Rehabilitation 2002 Jan;29(1):14-23.

(8) Gillam DG, Seo HS, Newman HN, Bulman JS. Comparison of dentine hypersensitivity in selected occidental and oriental populations. Journal of Oral Rehabilitation 2001 Jan;28(1):20-5.

(9) Rees JS, Addy M. A cross-sectional study of buccal cervical sensitivity in UK general dental practice and a summary review of prevalence studies. International Journal of Dental Hygiene 2004 May;2(2):64-9.

(10) Bamise CT, Olusile AO, Oginni AO, Dosumu OO, Bamise CT, Olusile AO, et al. The prevalence of dentine hypersensitivity among adult patients attending a Nigerian teaching hospital. Oral Health & Preventive Dentistry 2007;5(1):49-53.

(11) Flynn J, Galloway R, Orchardson R. The incidence of 'hypersensitive' teeth in the West of Scotland. Journal of Dentistry 1985 Sep;13(3):230-6.

(12) Rees JS, Jin LJ, Lam S, Kudanowska I, Vowles R. The prevalence of dentine hypersensitivity in a hospital clinic population in Hong Kong. Journal of Dentistry 2003 Sep;31(7):453-61.

(13) Fischer C, Fischer RG, Wennberg A. Prevalence and distribution of cervical dentine hypersensitivity in a population in Rio de Janeiro, Brazil. Journal of Dentistry 1992 Oct;20(5):272-6.

(14) Chabanski MB, Gillam DG, Bulman JS, Newman HN. Clinical evaluation of cervical dentine sensitivity in a population of patients referred to a specialist periodontology department: a pilot study. J.Oral Rehabil. 1997 Sep;24(9):666-672.

(15) Dababneh RH, Khouri AT, Addy M. Dentine hypersensitivity - an enigma? A review of terminology, mechanisms, aetiology and management. British Dental Journal 1999 Dec 11;187(11):606-11; discussion 603.

(16) Dahnhardt JE, Gygax M, Martignoni B, Suter P, Lussi A. Treating sensitive cervical areas with ozone. A prospective controlled clinical trial. Am.J.Dent. 2008 Apr;21(2):74-76.

(17) Brannstrom M. Sensitivity of dentine. Oral Surg.Oral Med.Oral Pathol. 1966 Apr;21(4):517-526.

(18) Pashley DH, Matthews WG, Zhang Y, Johnson M. Fluid shifts across human dentine in vitro in response to hydrodynamic stimuli. Arch.Oral Biol. 1996 Nov;41(11):1065-1072.

(19) Cuenin MF, Scheidt MJ, O'Neal RB, Strong SL, Pashley DH, Horner JA, et al. An in vivo study of dentin sensitivity: the relation of dentin sensitivity and the patency of dentin tubules. Journal of Periodontology 1991 Nov;62(11):668-73.

(20) Absi EG, Addy M, Adams D. Dentine hypersensitivity. A study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentine. J.Clin.Periodontol. 1987 May;14(5):280-284.

(21) Banfield N, Addy M. Dentine hypersensitivity: development and evaluation ofamodel in situ to study tubulepatency. Journal of Clinical Periodontology 2004 May;31(5):325-35.

(22) Addy M, Loyn T, Adams D. Dentine hypersensitivity--effects of some proprietary mouthwashes on the dentine smear layer: a SEM study. Journal of Dentistry 1991 Jun;19(3):148-52.

(23) Bamise CT, Olusile AO, Oginni AO, Bamise CT, Olusile AO, Oginni AO. An analysis of the etiological and predisposing factors related to dentin hypersensitivity. Journal of Contemporary Dental Practice [Electronic Resource] 2008;9(5):52-9.

(24) Orchardson R, Collins WJ. Thresholds of hypersensitive teeth to 2 forms of controlled stimulation. J.Clin.Periodontol. 1987 Feb;14(2):68-73.

(25) Minkoff S, Axelrod S. Efficacy of strontium chloride in dental hypersensitivity. J.Periodontol. 1987 Jul;58(7):470-474.

(26) Sauro S, Gandolfi MG, Prati C, Mongiorgi R. Oxalate-containing phytocomplexes as dentine desensitisers: an in vitro study. Arch.Oral Biol. 2006 Aug;51(8):655-664.

(27) Pereira JC, Segala AD, Gillam DG. Effect of desensitizing agents on the hydraulic conductance of human dentin subjected to different surface pre-treatments--an in vitro study. Dent.Mater. 2005 Feb;21(2):129-138.

(28) Kaufman HW, Wolff MS, Winston AE, Triol CW. Clinical evaluation of the effect of a remineralizing toothpaste on dentinal sensitivity. J.Clin.Dent. 1999;10(1 Spec No):50-54.

(29) Jacobsen PL, Bruce G. Clinical dentin hypersensitivity: understanding the causes and prescribing a treatment. Journal of Contemporary Dental Practice [Electronic Resource] 2001 Feb 15;2(1):1-12.

(30) Kodaka T, Kuroiwa M, Kuroiwa M, Okumura J, Mori R, Hirasawa S, et al. Effects of brushing with a dentifrice for sensitive teeth on tubule occlusion and abrasion of dentin. J.Electron.Microsc.(Tokyo) 2001;50(1):57-64.

(31) Lambrechts P, Van Meerbeek B, Perdigao J, Gladys S, Braem M, Vanherle G. Restorative therapy for erosive lesions. Eur.J.Oral Sci. 1996 Apr;104(2 (Pt 2)):229-240.

(32) Azarpazhooh A, Limeback H, Lawrence HP, Fillery ED. Evaluating the effect of an ozone delivery system on the reversal of dentin hypersensitivity: a randomized, double-blinded clinical trial. J.Endod. 2009 Jan;35(1):1-9.

(33) Fu B, Shen Y, Wang H, Hannig M. Sealing ability of dentin adhesives/desensitizer. Oper.Dent. 2007 Sep-Oct;32(5):496-503.

(34) Kolker JL, Vargas MA, Armstrong SR, Dawson DV. Effect of desensitizing agents on dentin permeability and dentin tubule occlusion. J.Adhes.Dent. 2002 Fall;4(3):211-221.

(35) Camps J, Pizant S, Dejou J, Franquin JC. Effects of desensitizing agents on human dentin permeability. Am.J.Dent. 1998 Dec;11(6):286-290.

(36) Schupbach P, Lutz F, Finger WJ. Closing of dentinal tubules by Gluma desensitizer. Eur.J.Oral Sci. 1997 Oct;105(5 Pt 1):414-421.

(37) Brunton PA, Kalsi KS, Watts DC, Wilson NH. Resistance of two dentin-bonding agents and a dentin densensitizer to acid erosion in vitro. Dent.Mater. 2000 Sep;16(5):351-355.

(38) Gillam DG, Newman HN. Iontophoresis in the treatment of cervical dentinal sensitivity--a review. J.West.Soc.Periodontol.Periodontal.Abstr. 1990;38(4):129-133.

(39) Kern DA, McQuade MJ, Scheidt MJ, Hanson B, Van Dyke TE. Effectiveness of sodium fluoride on tooth hypersensitivity with and without iontophoresis. J.Periodontol. 1989 Jul;60(7):386-389.

(40) Brough KM, Anderson DM, Love J, Overman PR. The effectiveness of iontophoresis in reducing dentin hypersensitivity. J.Am.Dent.Assoc. 1985 Nov;111(5):761-765.

(41) Kimura Y, Wilder-Smith P, Yonaga K, Matsumoto K. Treatment of dentine hypersensitivity by lasers: a review. J.Clin.Periodontol. 2000 Oct;27(10):715-721.

(42) West NX, Addy M, Jackson RJ, Ridge DB. Dentine hypersensitivity and the placebo response. A comparison of the effect of strontium acetate, potassium nitrate and fluoride toothpastes. J.Clin.Periodontol. 1997 Apr;24(4):209-215.

(43) Addy M, West NX, Barlow A, Smith S. Dentine hypersensitivity: is there both stimulus and placebo responses in clinical trials? Int.J.Dent.Hyg. 2007 Feb;5(1):53-59.

Chapter II

Abstract

OBJECTIVES: Dentin hypersensitivity (DH) is a prevalent problem with many treatment modalities that have not been successful in completely eliminating the condition. This study aimed to formulate a paste using previously synthesized fluorapatite (FA) enamel-crystals under ambient conditions to treat DH. FA-crystals were dispersed in 3 different carriers. The pastes were tested for mechanical obstruction and adherence to wet dentin surfaces (the short term relief of DH), as well as its ability to release fluoride (F), calcium (Ca) and phosphate (PO_4) at neutral and acidic pHs (the long term relief of DH). The ion release experiment at acidic pH aimed to mimic the daily fluctuation of intraoral pH (daytime application), and the second experiment at neutral pH aimed to mimic the overnight application of the paste.

METHODS: FA-crystals were synthesized, examined with TEM/EDX, then were mixed with Alginate (20%w/w), P(HEMA) (40%w/v) and P(DOPA) (40%w/v). The pastes were applied to etched dentin samples and examined with SEM to determine the degree of dentinal tubule occlusion. The pastes were either left on the dentin surface or were cleaned and rinsed off the surface to determine the degree of dentinal tubules coverage in the SEM examination. In the first ion release experiment design, mimicking the daily fluctuation of intraoral pH (daytime application), the pastes (FA+carrier) were subjected to lactic acid (LA) at pH 4.5 for 30 min, and then the pH were brought back to neutrality. This procedure was repeated 3 times in 24 hours. Fluoride, Ca and PO_4 concentration were measured at baseline (neutral pH) and after each LA episode. In the second ion release experiment (overnight application), the released ions concentration was calculated at the baseline and after 8 hours of submersion in distilled water without any acid challenge. The cytotoxicity of the synthesized P(DOPA) gel with or without FA fillers was tested. Kruskall-Wallis test for nonparametric data will be

used to test the differences in F, Ca, and PO$_4$$^-$ release between the groups. Statistical analysis will be carried to the 0.05 level of significance.

RESULTS: when the pastes were cleaned and rinsed off the dentin surface, FA/P(DOPA) paste obstructed more than 50-80% of the dentinal tubules. FA/P(HEMA) paste covered 50-70% of the open tubules, while FA/Alginic acid covered less than 50% of the tubules. All the pastes tested covered more than 90% of the dentin surface when applied and left on the surface without being removed. The F release in the daytime application design in descending order was; FA/P(HEMA) (7.2 ± 0.7 ppm); FA/P(DOPA) (7 ± 0.6 ppm) and FA/Alg (6.6 ± 0.7 ppm). Calcium concentrations were; FA/P(HEMA) (139.8 ± 32.5 ppm); FA/Alg (135.8 ± 38.2 ppm) and FA/P(DOPA) (117.3 ± 41.7 ppm). The average concentration of PO$_4$ was; FA/Alg (46.2 ± 16.4 ppm); FA/P(HEMA) (45.3 ± 13.4 ppm) and FA/P(DOPA) (44.3 ± 13.4 ppm). In the overnight application experiment, the [F] measured in descending order was; FA/Alg (5.2 ± 0.1 ppm) and 3.5 ± 0.2 ppm for both FA/P(HEMA) and FA/P(DOPA). The [Ca] measured for FA/Alg and FA/P(HEMA) was around 40 ppm, and 24 ppm for FA/P(DOPA). The [PO$_4$] was; FA/Alg (9.8 ± 0.1 ppm); FA/P(HEMA) (8.6 ± 0.5 ppm) and FA/P(DOPA) (10.4 ± 0.5 ppm). The Kruskall-Wallis analysis did not reveal any significant differences in F, Ca and PO$_4$ release between the groups in both the daytime and overnight application designs.

CONCLUSIONS: P(DOPA) and P(HEMA) pastes may offer immediate short term relief because of their ability to obstruct the tubules and adhere to wet dentin surfaces. The significant F, Ca and PO$_4$ release from the pastes in the daytime and overnight application designs would encourage mineral formation in the patent tubules and give long term relief of DH.

Introduction

In previous work, hydroxyapatite crystals of varying composition (that is containing varying amounts of fluoride and carbonate) and length (short and long- the long are similar to those seen in human enamel) were synthesized (1-4). This was achieved without the aid of cells (ameloblasts), enamel proteins or polymers. The enamel paste developed in this study simply used the synthetic fluorapatite (FA) crystals produced under ambient conditions. Crystals were incorporated as fillers into three different viscoelastic gels; alginate (Alginic acid), a hydrogel P(HEMA) and a mucus-like substance P(DOPA).

Alginic acid, also called alginate, is a viscous gum that is abundant in the cell walls of brown algae. It ranges from white to yellowish-brown, and takes filamentous, granular and powdered forms. It absorbs water quickly; it is capable of absorbing 200-300 times its own weight in water. Alginate is used in various pharmaceutical preparations, and is used extensively as an impression-making material in dentistry, prosthetics and lifecasting. It is also used in the food industry, for thickening soups and jellies. Calcium alginate is used in different types of medical products, including burn dressings that promote healing and can be removed with less pain than conventional dressings (5).

Poly (2-hydroxyethyl methacrylate) P(HEMA) is a polymer that forms a hydrogel in water. It was invented by Drahoslav Lim for use in soft contact lenses (6). Copolymers of P(HEMA) are still widely used today.

Many marine invertebrates form strong, temporary attachments to rocks in water environments. Limpets are renowned for their adhesiveness and use mucus as their glue (7,8). Limpets' mucus is 95% water, and shear attachment strength of 200-500 kPa have been recorded (9). To mimic these gels and to be biologically acceptable, a glycolic acid derivative of glycerin was used to make the precursors and then calcium oxide was used to make them gel. This method produced a P(DOPA) gel (polymer of 3,4-dihydroxyphenylalanine) in a matter of minutes with a "shelf life" of 10-14 days in conditions similar to those found in the oral environment.
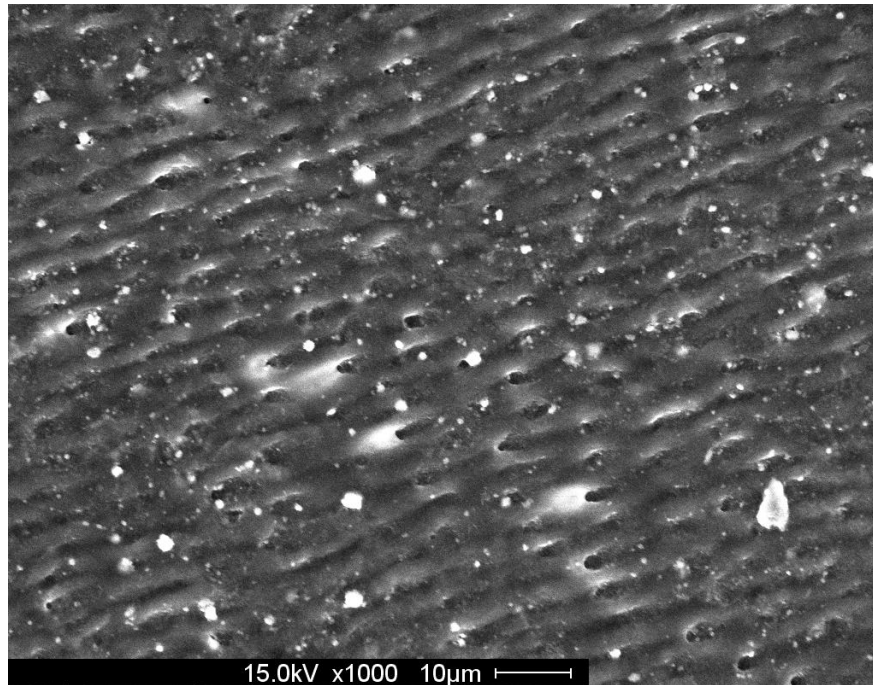
A paste which can be easily applied by patients at home to provide an effective means of relieving the pain associated with DH, presents a compelling commercial opportunity for healthcare consumer goods companies involved with over the counter dental products. The viscoelastic gels plus FA crystal filler described above have the potential to provide the key ingredients for a product that meets these criteria.

This present study aimed to formulate a paste using synthetic FA crystals to treat DH. The paste should provide short term relief of DH by blocking patent dentinal tubules and long term relief by releasing F, Ca and PO_4 which will help forming a mineralized barrier.

Preliminary studies

A series of experiments was performed to optimize the FA crystal to gel ratio. The best ratio was considered to be the one which obstructed most of the patent dentinal tubules based on SEM analysis. Different filler ratios were used for the FA/P(DOPA) paste; 10%; 20%; 40% and 50% weight/volume (w/v). The higher the filler particles in the gel, the better the obstruction achieved (Fig. 3-6). Forty percent and 50% ratios gave the best result; however, the 50% paste was overfilled and difficult to handle due to the solvent's volatility. Therefore, 40% FA/P(DOPA) was considered the optimum ratio giving good obstruction of the dentinal tubules and still being easy to mix and apply. Based on these results, 40% FA/P(HEMA) paste was also used, however, this ratio could not be achieved with FA/Alginate due the high viscosity of the alginate. Therefore, a 20% weight/weight (w/w) FA/Alginate was used for the study. Different methods were used to determine the best technique for applying the paste to the tooth sections. Applying it directly to a tooth section with a spatula was determined to be the most effective method of application. To test the efficacy of the paste's coverage to wet dentin surfaces, which is a desirable property for over the counter products, the paste was applied on both dry and wet dentin samples. SEM evaluation did not reveal any differences between applying the paste to the wet or dry tooth sections (Fig. 7, 8). Therefore, all the samples were stored in distilled water and blotted to remove excess water before the application of the paste. The effect of applying pressure on
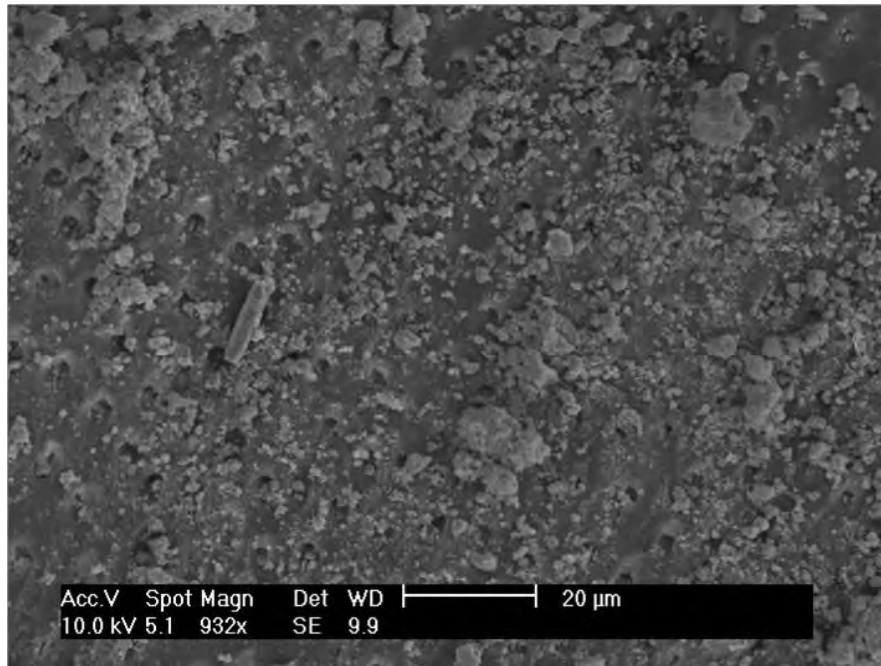
the penetration of the pastes into the tubules was also investigated. On one sample, the paste was applied onto the dentin surface using a metal spatula then rubbed gently using finger pressure to disperse the paste and force the crystals into the dentinal tubules. On another sample the paste was applied to the dentin surface without rubbing. SEM comparison of the two techniques did not reveal any difference (Fig. 9, 10). Therefore, the non-rubbing non-pressure technique was adopted and used on all the dentin specimens. In another set of preliminary experiments the paste was applied to the teeth, and allowed to dry for 3 min, 30 min and 60 min. No difference in tubule occlusion among the three different drying times was seen. Therefore, in later experiments, the paste was allowed to dry for 3 minutes before the samples were examined with SEM.
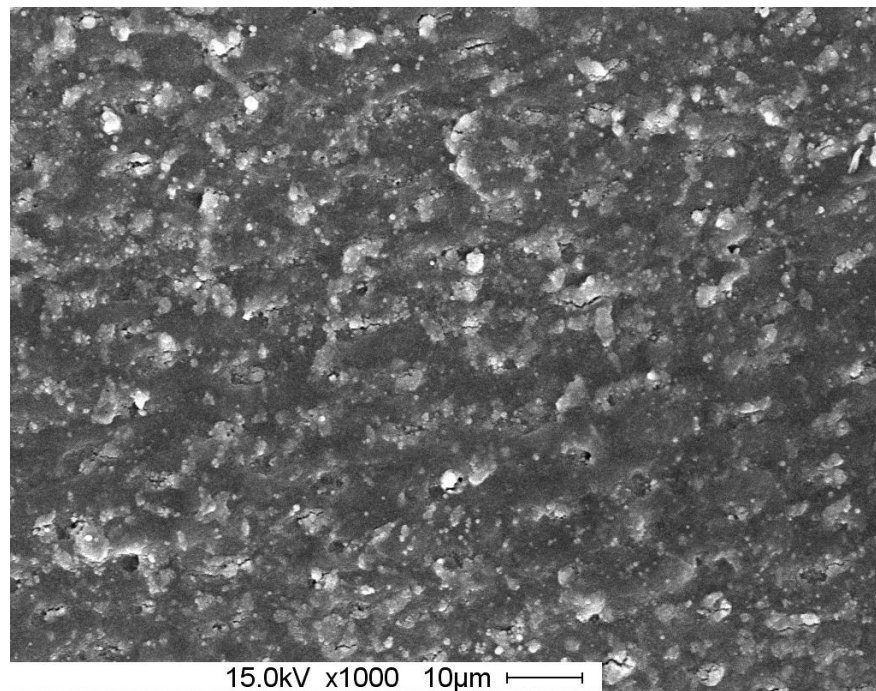
Fig 3. SEM image 10% FA/P(DOPA) paste applied to a dentin section
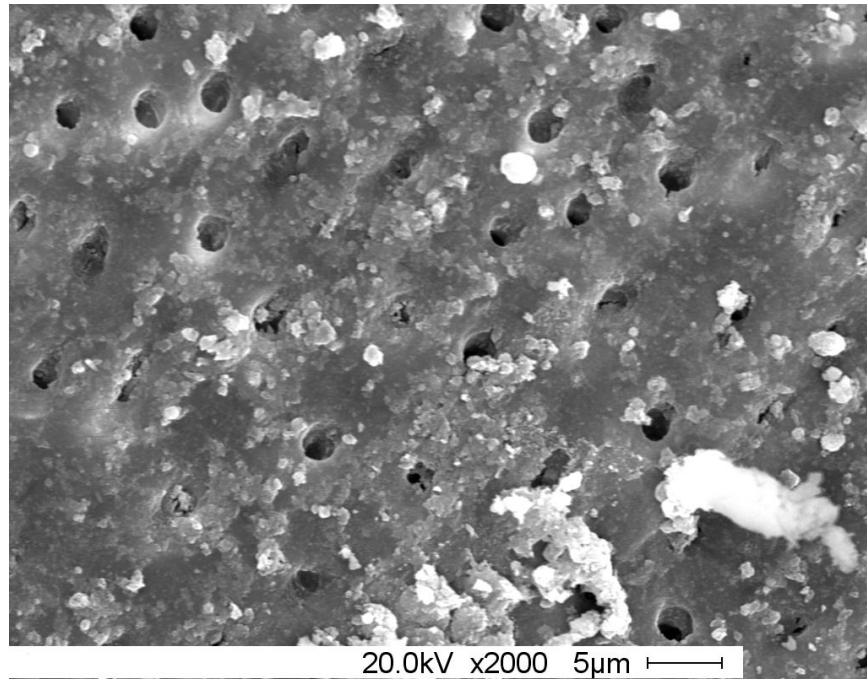

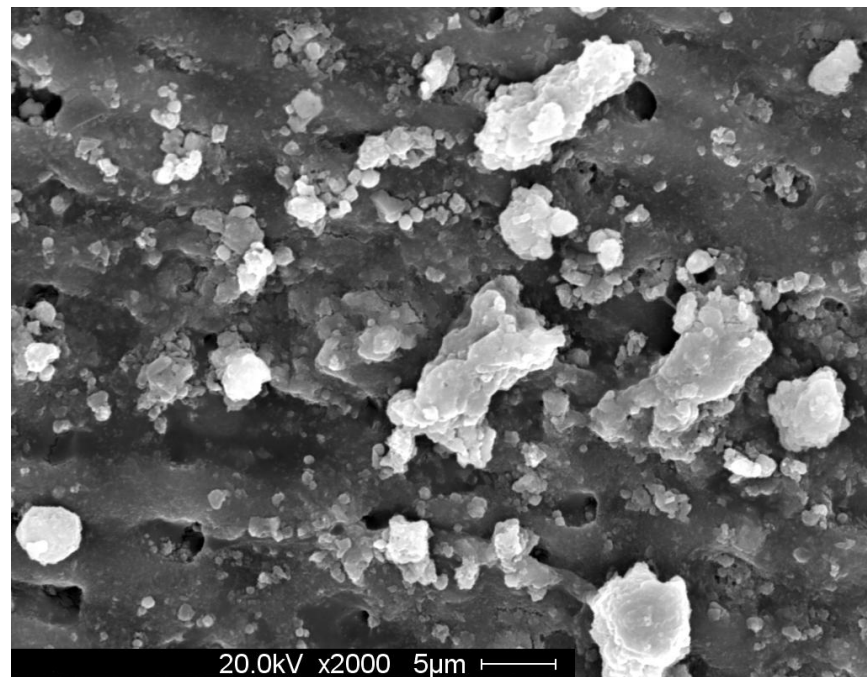**Fig 4. SEM image 20% FA/P(DOPA) paste applied to a dentin section**

Fig 5. SEM image 40% FA/P(DOPA) paste applied to a dentin section

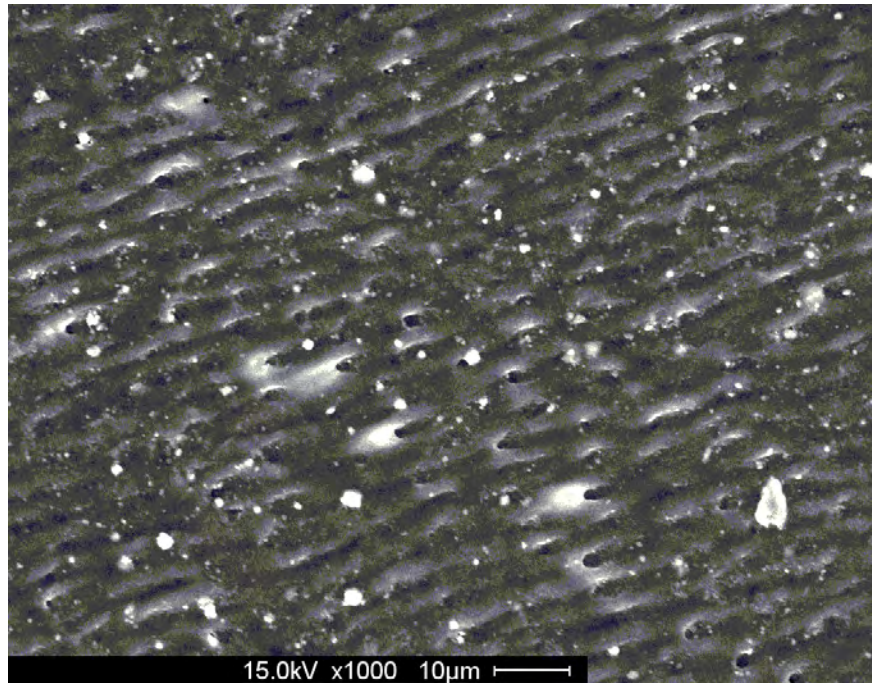**Fig 6. SEM image 50% FA/P(DOPA) paste applied to a dentin section**

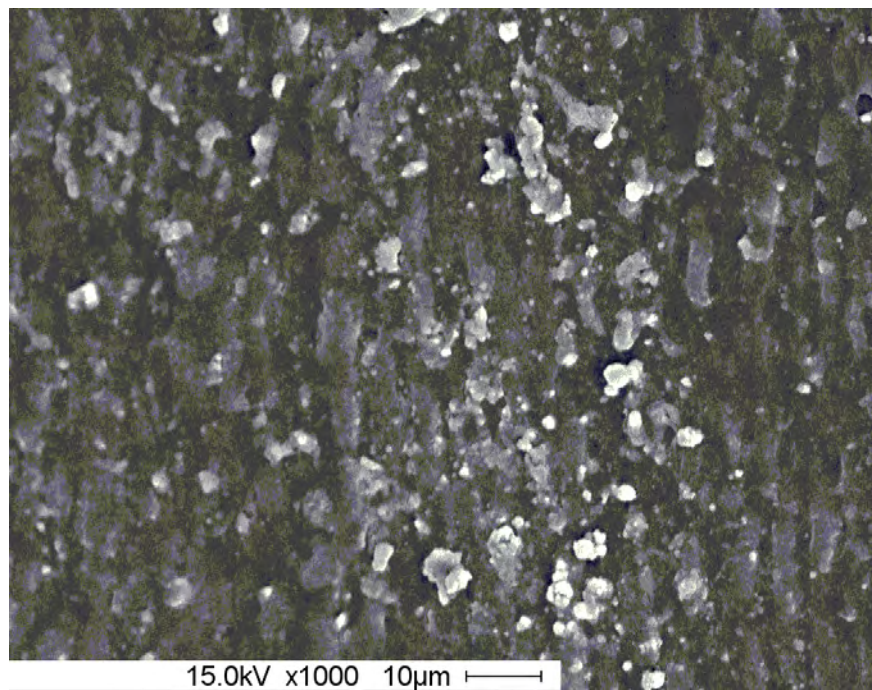**Fig 7. SEM image 40% FA/P(DOPA); dry tooth section**

Fig 8. SEM image 40% FA/P(DOPA); wet tooth section

Fig 9. SEM image 40% FA/P(DOPA); application without rubbing

Fig 10. SEM image 40% FA/P(DOPA); application with rubbing

Research Design and Methods

Fluorapatite Crystal Synthesis

Synthesis of FA crystals under ambient conditions has been described by Chen et al.(3). Hydroxyapatite powder (104.6 mg) and 8.4 mg of sodium fluoride were mixed with 100 ml distilled water. The suspension was stirred continuously, and HNO_3 was added until the powder dissolved, after which the pH was adjusted to 2.4. Ammonium hydroxide was then added drop wise to the solution with continuous stirring until pH 6 was reached. The suspension was sealed in a plastic tube and kept in a water bath at 70°C for 2 days, which did not affect the crystalline and compositional properties of the collected crystals (Chen et al. (3) incubated for 5 days). Transmission electron microscopy (TEM) technique including bright field (BF) high resolution electron microscopy (HREM) imaging, selected area electron diffraction (SAED) and X-ray energy dispersive spectroscopy (EDS) were used for studying morphology, crystallinity and composition of the materials, and to compare the 2 day and 5 day incubation periods.

A JEOL 3011 High-Resolution Electron Microscope (JEOL USA, Peabody, MA, USA) operating at 300 kV was used for normal TEM imaging, high-resolution electron microscope (HREM) and EDS analysis. The crystals were mixed with methanol and pipetted onto a holey-carbon film coated copper grids and left to dry before TEM examination.
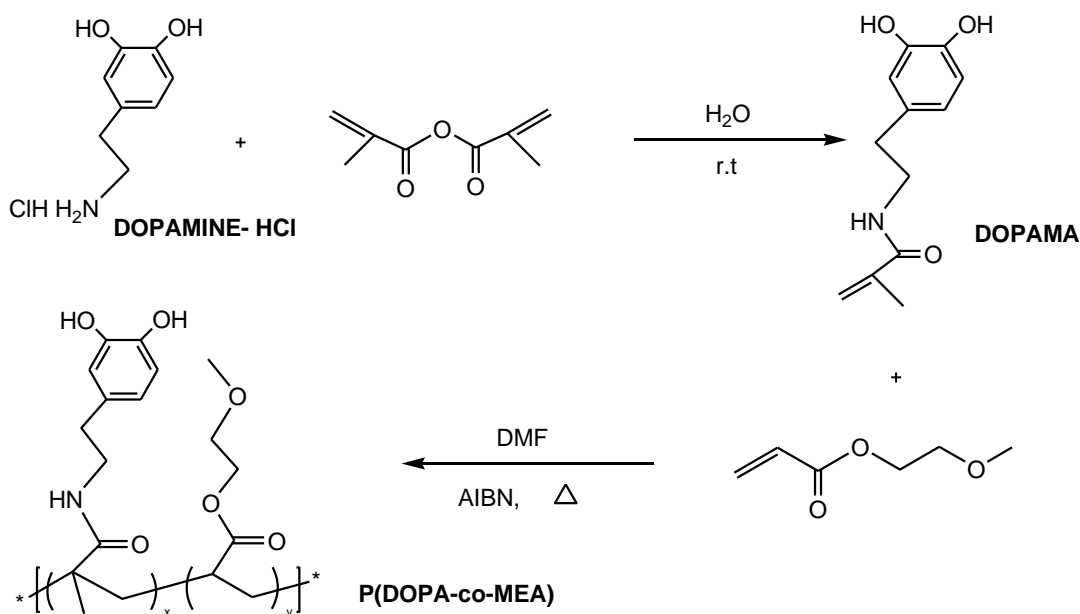
Gels Synthesis

- **Synthesis of monomer dopamine methacrylamide (DMA)**

The monomer dopamine methacrylamide (DMA) was prepared following the procedure described by Lee et al. (10). 1.6g of $NaHCO_3$ and 4g of $Na_2B_4O_7$ were dissolved in 50 ml of H_2O under N_2. Dopamine-HCl (2g) was added into the aqueous solution, and then 1.9 ml of methacrylate anhydride in THF (10 ml) was added drop-wise. NaOH aq (1M) was added so that the pH was kept above 8, and the mixture was stirred overnight with N_2

bubbling. The pH of the aqueous solution was adjusted to 2 and extracted with ethyl acetate (100 ml) three times. The combined organic layer was dried over MgSO$_4$. After removing the solvent under reduced pressure, the solution was added into hexane (100 ml) and then stored at 4 °C overnight. The grey precipitate was filtered and washed with hexane. (1.6 g, 68.9% yield).

- **Synthesis of copolymer poly (DOPA-co-MEA)**

The copolymer poly (DOPA-co-MEA) was prepared from DMA and methoxyethyl acrylate (MEA). The mole % of DMA was fixed at 15% in the feed monomer compositions. DMA (66.5 mg, 0.3 mmol) and MEA (234.5 mg, 1.7 mmol) were dissolved in DMF (5 mL) and then 2, 2'-azobisisobutyronitrile (AIBN) (3.3 mg, 0.02 mmol) was added. The solution was bubbled with N$_2$ for 5 minutes and heated at 60 °C overnight. The resultant polymers were precipitated in diethyl ether (200 ml). The polymer was dissolved in a small amount of methanol and precipitated again in diethyl ether. This procedure was repeated three times (195 mg, 65% yield). The chemical reaction in the preparation of the P(DOPA) gel is illustrated in fig. 11.
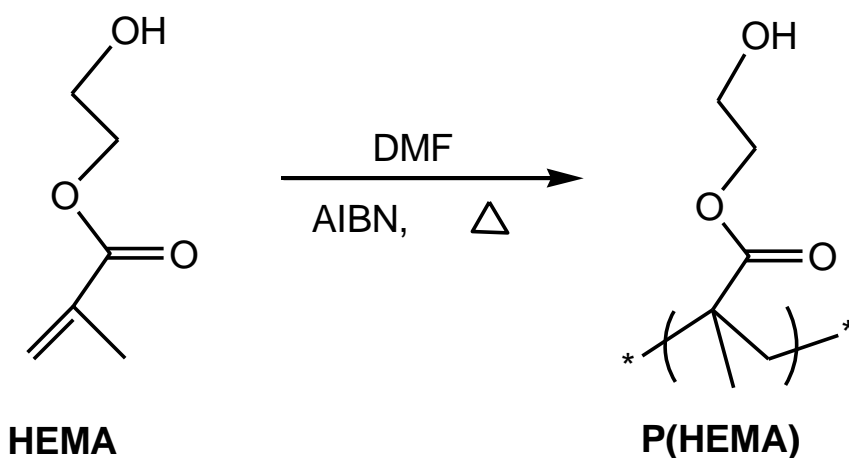

Fig 11. The synthesis of P(DOPA)
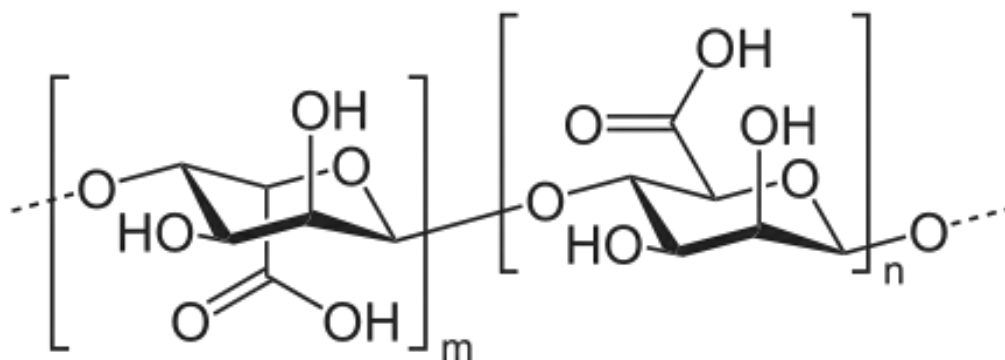
- **Synthesis of polymer poly(HEMA)**

HEMA (260 mg, 2 mmol) were dissolved in DMF (3 ml) and then 2, 2'-azobisisobutyronitrile (AIBN) (3.3 mg, 0.02 mmol) was added. The solution was bubbled with N_2 for 5 minutes and heated at 60 °C overnight. The resultant polymers were precipitated in diethyl ether (200 ml). The polymer was dissolved in a small amount of methanol and precipitated again in diethyl ether. This procedure was repeated three times (210 mg, 81% yield). The synthesis of the P(HEMA) is illustrated in fig.12.

Fig 12. The synthesis of P(HEMA)

- **Preparation of polymer solutions**

The P(DOPA) gel was prepared by dissolving 25 mg of P(DOPA-co-MEA) in 1 ml of acetone. The P(HEMA) gel was prepared by dissolving 80 mg of P(HEMA) in 1 ml of ethanol. Finally, the alginate gel was prepared by dissolving 10 mg of alginic acid sodium salt $(C_6H_7O_6Na)_n$ in 1ml of ethanol. The chemical structure of alginic acid is illustrated in fig.13.

Fig 13. The chemical structure of Alginic acid (Alginate)

Cytotoxicity testing

The cytotoxicity of the polymer P(DOPA) was tested to insure its safety for intraoral application. The following steps were followed to execute the test:

➢ 20mg P(DOPA-co-MEA) and 20mg P(DOPA-co-MEA) with 20mg FA crystal were dissolved in 2 ml EtOH, respectively.

➢ 25uL and 50uL of dissolved polymers with or without crystals were added into a 96-well plate (PS: Costar 3370); 50uL of EtOH was added into solvent control wells. Then, EtOH was evaporated from the open plate in the flow hood after 1.5 h.

➢ 150uL of medium MEM with 10% fetal bovine serum, 1% pyruvate and 1% nonessential amino acids was added into wells with dry polymers, into solvent control wells, and into empty wells (medium control wells). The plate was incubated 24h at 37°C/5% CO_2.

➢ HEp-2 cells (HeLa derivate) were seeded at the concentration of 10^4 cells per well in medium MEM with 10% fetal bovine serum, 1% pyruvate and 1% nonessential amino acids into a 96-well plate (tissue-culture PS: Falcon 3072) after 24h (cell confluency around 80%), cultivating medium from the plate with cells was replaced with the medium

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from the plate with polymers (100uL per well) and the plate was incubated for 24h at 37°C/5% CO2.

➢ The cytotoxicity was measured using XTT assay. The exposure medium was removed and cells were incubated with XTT for 4h, then absorbance at 450/750nm was measured.

Sample Collection

Extracted human permanent teeth were collected anonymously from the oral surgery department/School of Dentistry at the University of Michigan. Teeth were immersed in 70% ethyl alcohol for 24 hours, and then sectioned vertically at the center of the tooth in the mesial-distal plane. Any remnants of the pulp tissue were removed with the use of a dental excavator. Superficial layers of the cervical facial dentin were exposed using a diamond saw while preserving the specimen in the wet state. Teeth were then stored in 0.9% saline solution. Teeth with extensive fillings or decay; signs of dentinogenesis imperfecta; obliterated or narrow pulp spaces (signs of aging) were excluded from the study.
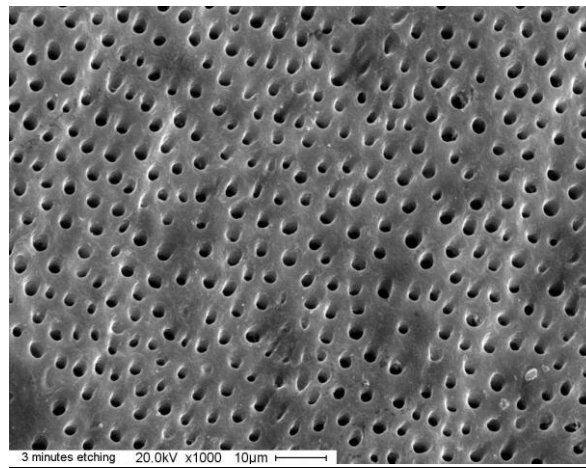
Sample Size

Thirty teeth were collected from which 20 specimens were tested for physical obstruction of dentin tubules by the FA paste; 10 specimens were used for ion release measurements.

Preparation of samples for scanning electron microscope (SEM)

Dentin sections were etched using 17% EDTA (Pulpdent, Watertown, MA, USA) for 3 minutes (11) to ensure the patency of the dentinal tubules and remove the smear layer (Fig.14). Specimens were then rinsed with distilled water for 1 minute. Each section was cut into two halves using a wire cutter (to prevent formation of a smear layer). The two sections

were labeled, one as an experimental and the other section served as a control. The FA paste was applied to the wet dentin sections. The pastes used were; 20% FA/Alginate (w/w); 40% FA/P(HEMA) (w/v) and 40% FA/P(DOPA) (w/v), The pastes were allowed to dry for 3 minutes before the excess visible paste was removed from the dentin surfaces using a flat metal spatula. The sections were then rinsed thoroughly with distilled water for 1 minute and left to dry. Afterwards, the test sections and their control counterparts were coated with gold for 60 sec (200 Å thick) preparing them for SEM analysis. The surface of the dentin was examined with SEM to demonstrate physical obstruction of the patent dentinal tubules by the paste.
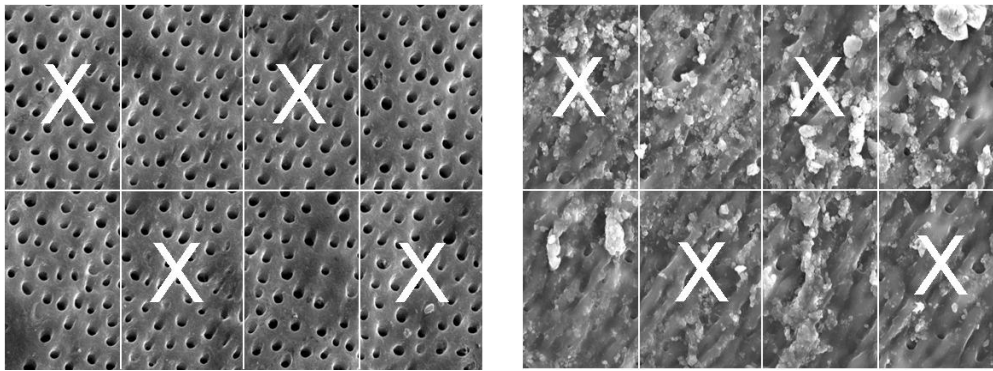

Fig 14. SEM image for a dentin sample etched for 3 minutes with 17% EDTA

Clinically, the paste would adhere to the dentin surface until it is removed by saliva or by oral hygiene practices. Therefore, another experiment was performed where the three pastes were applied to etched dentin samples without being removed or rinsed and then examined with SEM.

SEM analysis was conducted using a Hitachi S3200N Scanning Electron Microscope operated at 20 kV (resolution: 2.0 nm at 30 kV; 5.0 nm at 1 kV). Three representative images for each of the pastes, having the same magnification, were analyzed using a grid divided

into 8 equal 3x4 inches rectangles. Four rectangles of each image were chosen for counting the number of completely open dentinal tubules (fig. 15). The numbers were averaged for each test image, and the same was done for its control counterpart. Thereby, the number of totally or partially obstructed tubules was calculated (subtracting the average number of open tubules from the average number of tubules on the control section). From that, a percentage for dentinal tubules coverage was calculated.

Fig 15. The grid used for analyzing SEM images where 4 rectangles (3x4") were averaged for the control sections (left side) as well as the test sections (right side)

A further set of experiments were carried out to compare the degree of obstruction of the dentinal tubules by the crystals alone and gels alone (Table 2).

Crystals only control:

1. Dry FA crystals were applied to a dentin surface, gold coated, and examined with SEM.

Gels only control:

2. Each of the gels used was applied to an etched dentin section, left to dry for 3-4 minutes, the excess gel was removed from the surface, rinsed, and gold coated, then examined with SEM.

3. Each of the gels was applied to a dentin section, left to dry without scrapping or rinsing, gold coated and examined with SEM.

4. FA crystals were mixed with water (40% w/v), left to dry for 3 minutes, scrapped with a flat metal spatula, rinsed with distilled water for 1 minute, gold coated, then examined with SEM.

Table 2. Control specimens used

	No FA	FA
No carrier		Control # 1
Water carrier		Control # 4
Gel carrier	Control #2 and #3	

Ion release measurement

The ability of the synthetic FA crystals to release fluoride (F), calcium (Ca) and phosphate (PO_4^-) was investigated.
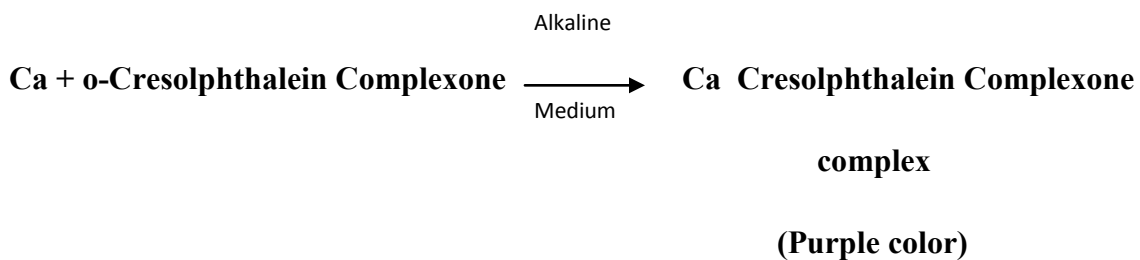
Fluoride concentration was measured with the use of an Orion combination fluoride electrode (Thermo Fisher Scientific Inc. USA). The fluoride electrode used was calibrated using 10 samples each containing 10 mg FA crystals in 10 ml deionized water. The fluoride concentration was measured for all the samples starting 5 min after preparation of the samples. Measurements were compared. Means and standard deviations were calculated.

The F release experiment was designed to mimic the daily fluctuations of pH in the oral cavity after eating 3 meals per day. For that purpose; a control sample was prepared with 10 mg of FA crystals placed independently in Scintilation vials with 20 ml of deionized water (continuously stirred). Three times per 24 hrs (2-3 hours intervals) the pH of the water

was reduced to pH 4.5 for 30 minutes using lactic acid and then returned back to neutrality. Fluoride concentration was measured at 25°C by withdrawing 10 ml of the solution and mixing it with 10 ml TISAB II (Orion Research Incorporated, Boston, MA). Deionized water was then added back to the vials to keep the specimen's water volume at 20 ml. The same experiment was performed for 3 test and 3 control samples. The test samples were: 20% FA/Alginate; 40% FA/P(HEMA) paste and 40% FA/P(DOPA) paste. The control samples were: 50 mg Alginate; 25 µl P(HEMA) and 25 µl P(DOPA),.

The second experiment was designed to mimic the overnight application of the enamel paste. Square dentin sections of known surface area (3x3x1 mm) were cut and etched. Twenty percent FA/Alginate, 40% FA/P(HEMA) and 40% FA/P(DOPA) were applied to the dentin surfaces (5mg FA added to each corresponding gel amount). Two control samples were used for this experiment; FA crystals only with no tooth section and a tooth section with no pastes added. Each of the specimens was submerged in 10 ml of deionized water. The solution was continuously stirred. F concentration was measured at baseline and after 8 hours.

Ca release was measured using a spectrophotometric color reaction (Pointe Scientific Inc. Canton, MI, USA). The principle of this reaction is demonstrated in the following equation:

$$\textbf{Ca + o-Cresolphthalein Complexone} \xrightarrow[\text{Medium}]{\text{Alkaline}} \textbf{Ca Cresolphthalein Complexone}$$

$$\textbf{complex}$$

$$\textbf{(Purple color)}$$

Calcium reacts with CPC in an alkaline medium to form a purple-color that absorbs at 570 nm (550-580 nm). The intensity of the color is proportional to the calcium concentration.

Phosphorous (P) release was also measured spectophotometricly. A color is formed by the reduction of a phosphomolybdate complex. This color is absorbed at 820 nm. This method was developed by Chen et al. (12) to utilize the greater sensitivity of the previously

used ascorbic acid method for the determination of phosphorus in smaller quantities. The concentration and weight of released phosphate (PO_4) were then mathmetically calculated from P concentration.

The same experimental design for F release (daily fluctuation in pH and overnight application) was followed for Ca and PO_4 analysis. All experiments were performed in duplicate for statistical analysis.

### *Data Analysis*

Linear regression analysis was used to calculate the unknown ions concentration form a standard curve of known solutions concentration. Kruskall-Wallis test for nonparametric data was used to test the differences in F, Ca, and PO_4^- release between the groups. Statistical analysis was carried to the 0.05 level of significance.

Results

The method used in this study produced short FA crystals, which ranged between 20 and 100 nm in length and approximately 10 to 20 nm in cross section based on TEM images (Fig 16 and 17). The corresponding selected area electron diffraction (SAED) patterns indicate that these synthetic crystals have a typical apatite crystalline structure (Fig 18). The EDS data (Fig 19) show that the Ca/PO_4 ratio is around 1.6, which approached the theoretical ratio of FA (Ca/ PO_4= 1.67).
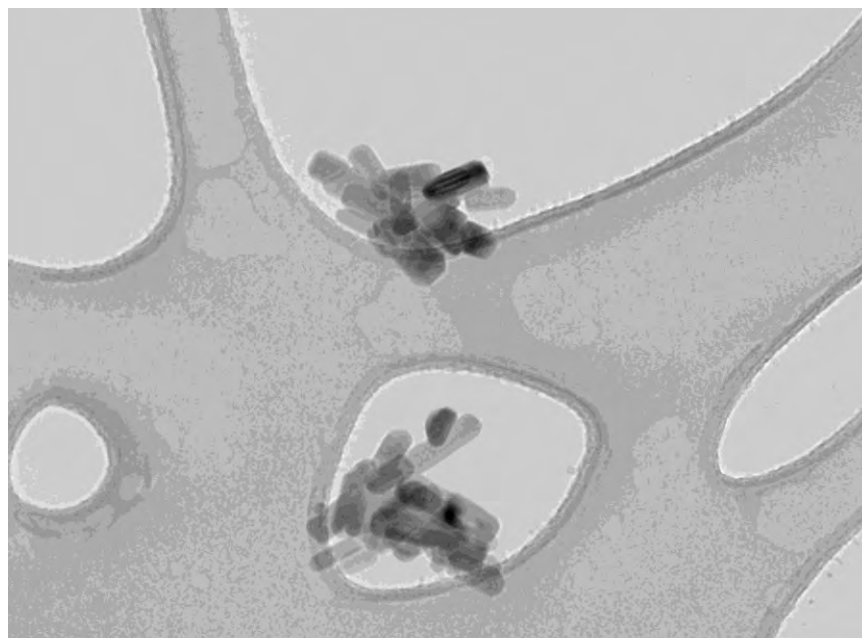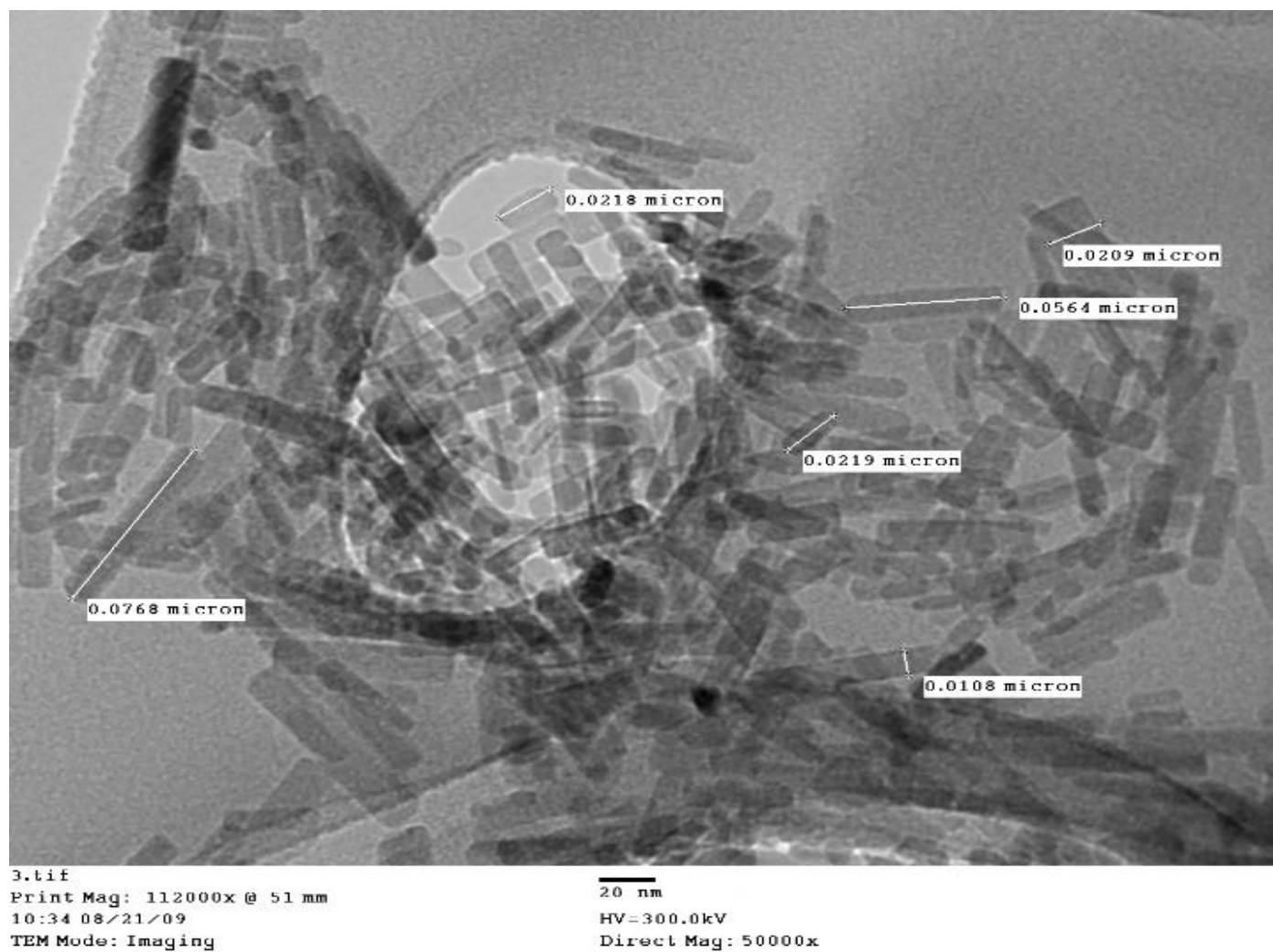
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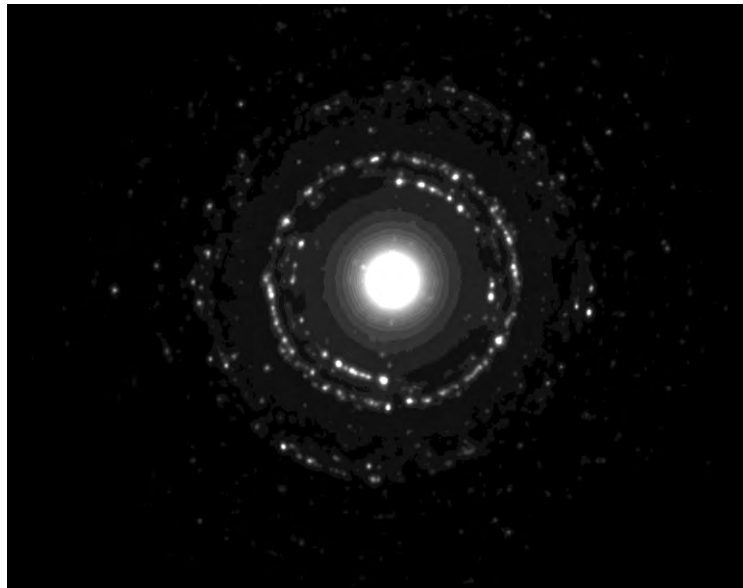HV=300.0kV
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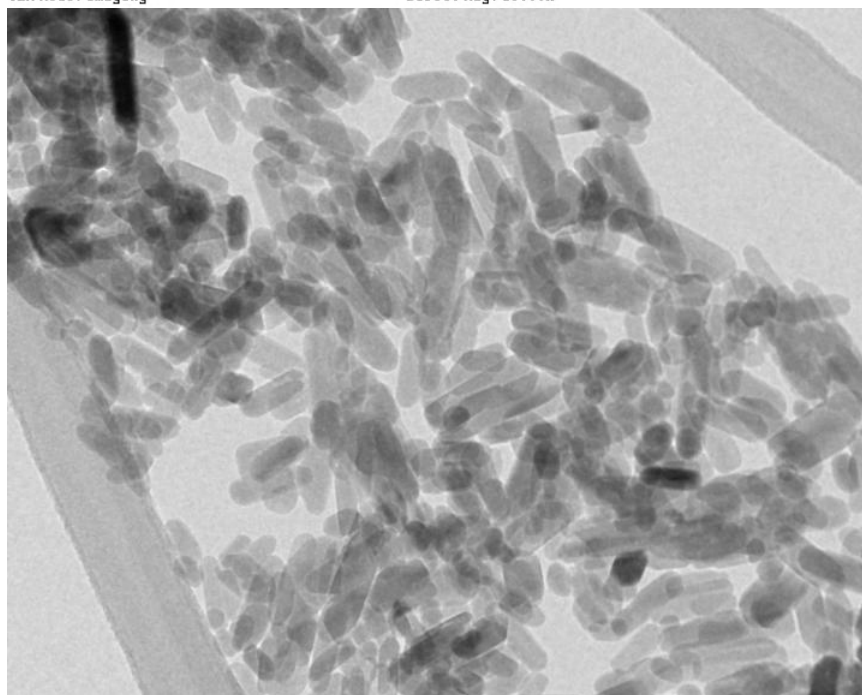
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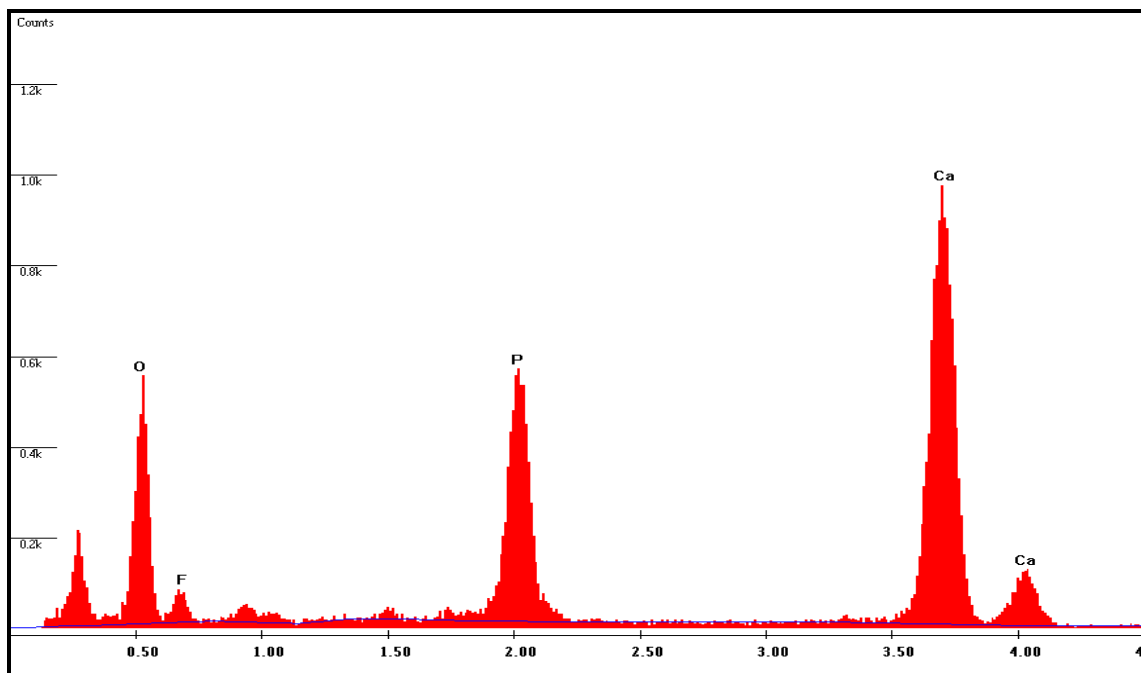HV=300.0kV
Direct Mag: 30000x

Fig 16. TEM BF images of FA crystals produced in ambient conditions

Fig 17. TEM BF image showing random measurements for FA crystal length and width

Fig 18. SAED pattern taken from a group of FA crystals indicating the crystals have a hexagonal crystal structure

Fig 19. EDS spectrum collected from FA crystals. It gives the Ca/P ratio of 1.6

Cytotoxicity Testing

The toxicity testing results of the gel P(DOPA) are displayed in table (3). The first step of testing for cytotoxicity indicated that the polymers P(DOPA-co-MEA) or a mixture of polymers P(DOPA-co-MEA) with FA crystals were free or have an insufficient quantity of harmful leachable compounds to cause cell death under the extreme conditions to which the cells were subjected.

Table 3. Average reduction in cell viability compared to the solvent control (EtOH) from hexaplicate (%)

| Polymers	Amount of dissolved polymer or EtOH (uL) per well	Cell viability (%)	SD
P(DOPA-co-MEA)	25	98	9
P(DOPA-co-MEA)	50	97	7
P(DOPA-co-MEA)/Crystal	25	97	11
P(DOPA-co-MEA)/Crystal	50	100	3
Ethanol	50	100	10
Medium	0	98	6

SEM Analysis

The SEM examination showed variable degrees of dentinal tubules coverage for the pastes used. SEM images for the control samples are shown in figures (20-27). The application of FA crystals without any carrier was able to partially obstruct (<50%) the open dentinal tubules (Fig. 20). Nevertheless, the addition of a carrier resulted in more obstruction of the patent tubules (Fig. 27-30). The addition of the different carriers resulted in the crystals clustering together even when the carrier was water (Fig. 27). P(DOPA) polymer left more remnants on the dentin surfaces after it has been washed away (Fig. 23) than P(HEMA) and Alginic acid. P(HEMA) and Alginic acid left almost no remnants after being

removed and rinsed away from the surface (Fig. 21, 22). P(DOPA) and P(HEMA) gels covered more than 90% of the open dentinal tubules when they were left to dry without being removed from the surface (Fig. 25, 26). On the other hand, most of the tubules were not covered by the alginic acid (less than 20% of coverage) (Fig. 24).

The experimental pastes coverage of the tubules is illustrated in Figures (28-33). The best coverage was achieved by the FA/P(DOPA) paste (Fig. 30). FA/P(DOPA) paste's remnants obstructed more than 50-80% of the tubules. Some of that coverage could be attributed to the FA crystals (Fig. 30 f, h), while some could be attributed to the P(DOPA) gel itself (Fig. 30 e, g). FA/P(HEMA) paste covered about 50-70% of the open tubules (Fig. 29). The P(HEMA) gel itself contributed to the obstruction achieved (Fig. 29 d). FA/Alginic acid covered less than 50% of the tubules (Fig. 28). All the pastes tested covered more than 90% of the dentin surface when applied and left on the surface without being removed (Fig. 31-33).
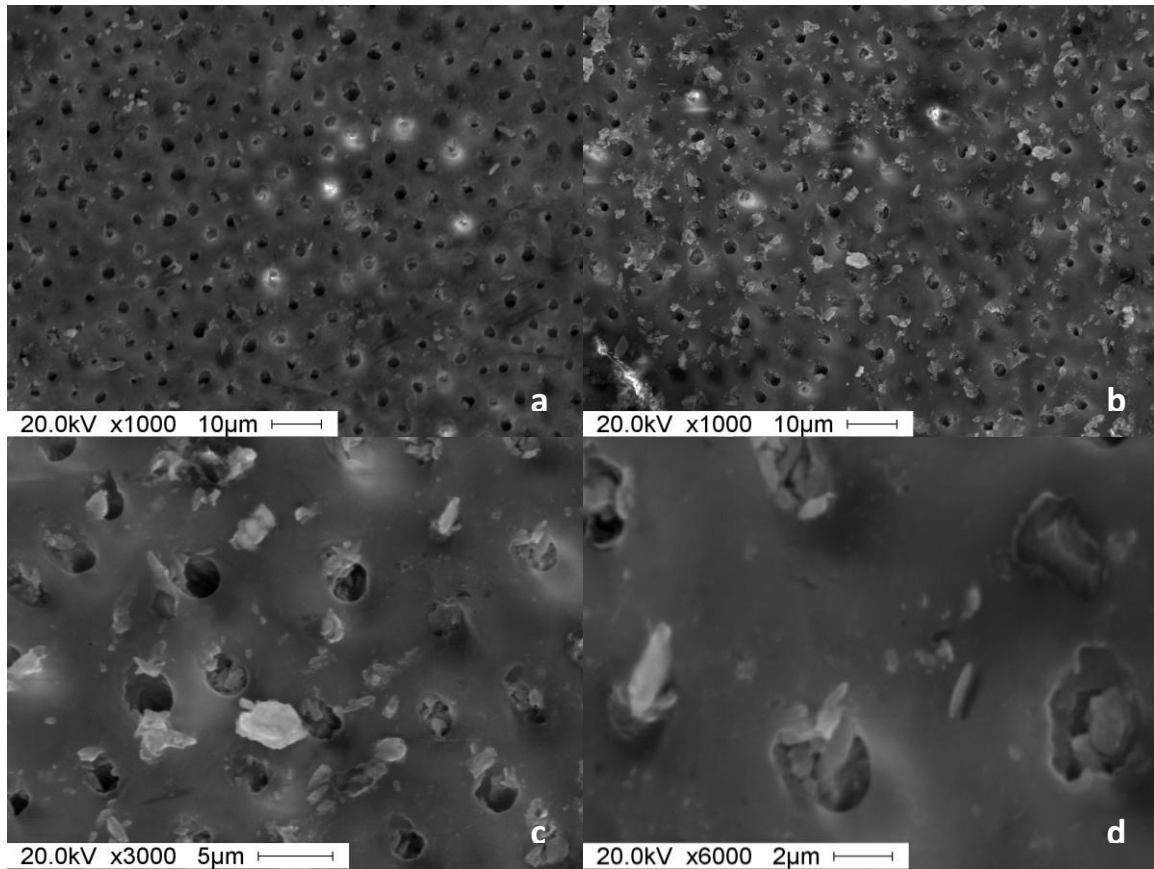
Fig 20. SEM images of dry FA crystals application on a dentin section (a) Control side (b-d) Test side
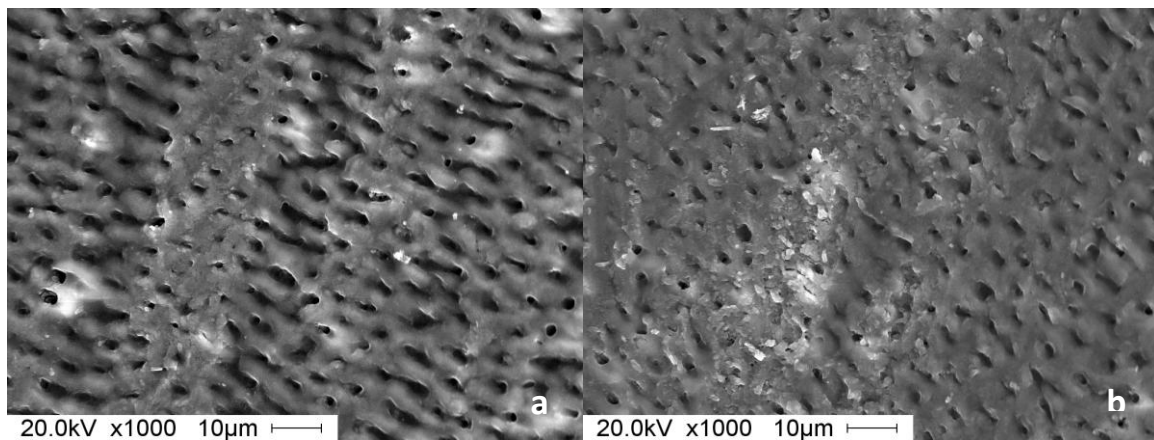
Fig 21. SEM images of remnants of Alginic acid gel after its application on a dentin section (a) Control side (b) Test side
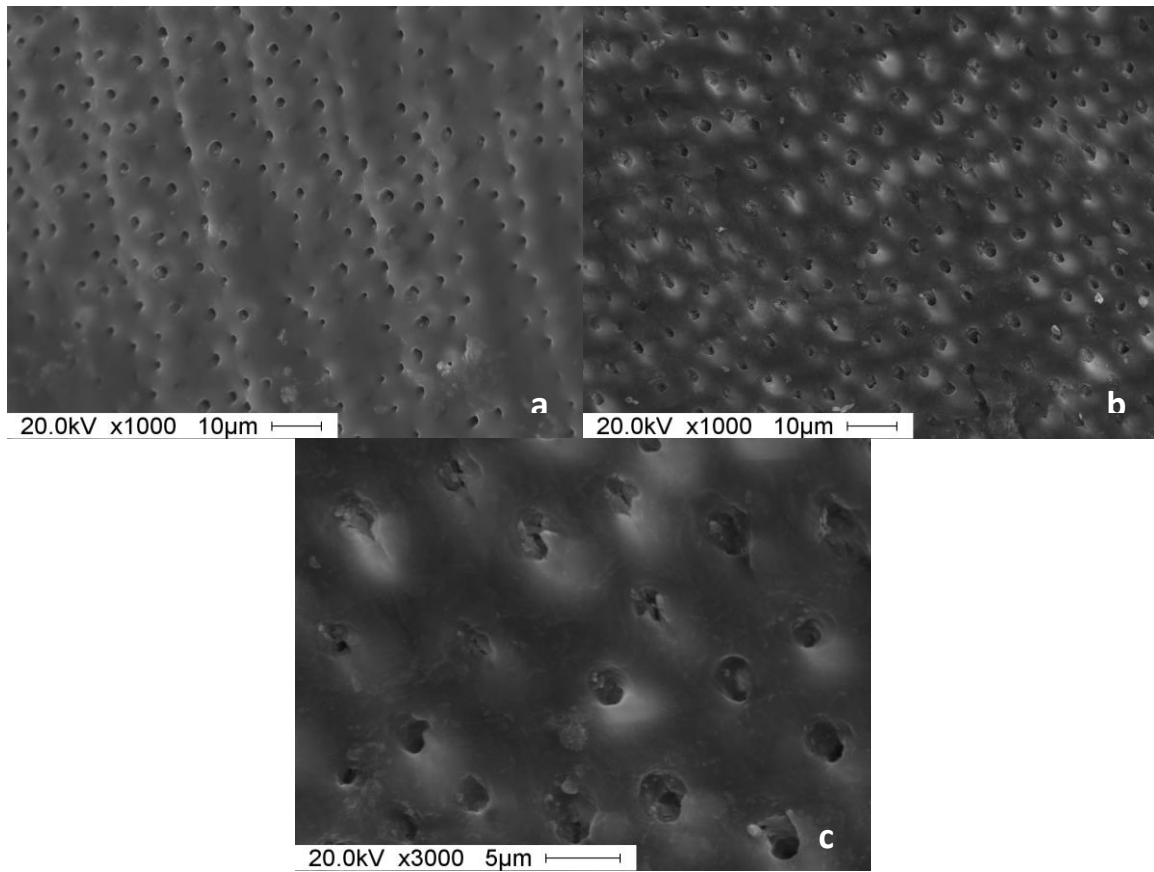
Fig 22. SEM images of remnants of P(HEMA) gel after its application on a dentin section (a) Control side (b & c) Test side
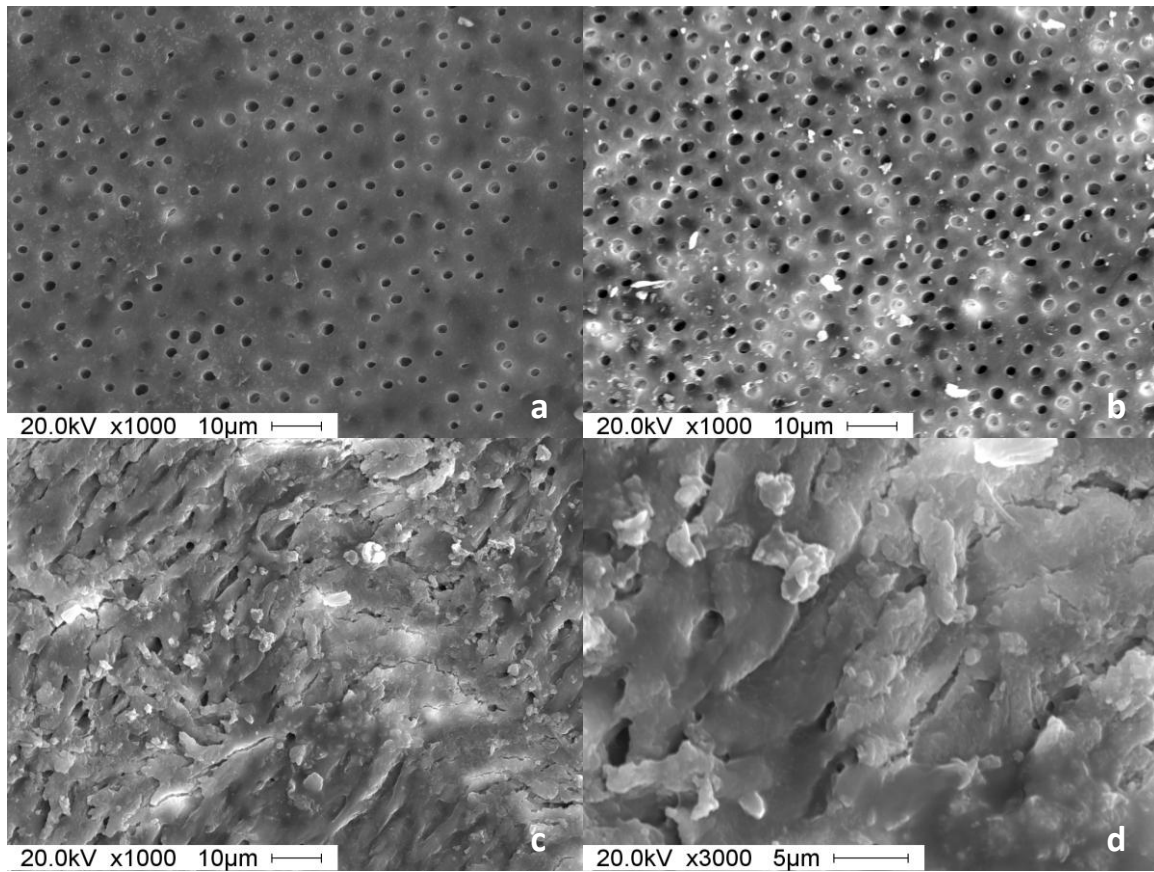
Fig 23. SEM images of remnants of P(DOPA) gel after its application on a dentin section (a) Control side (b-d) Test side
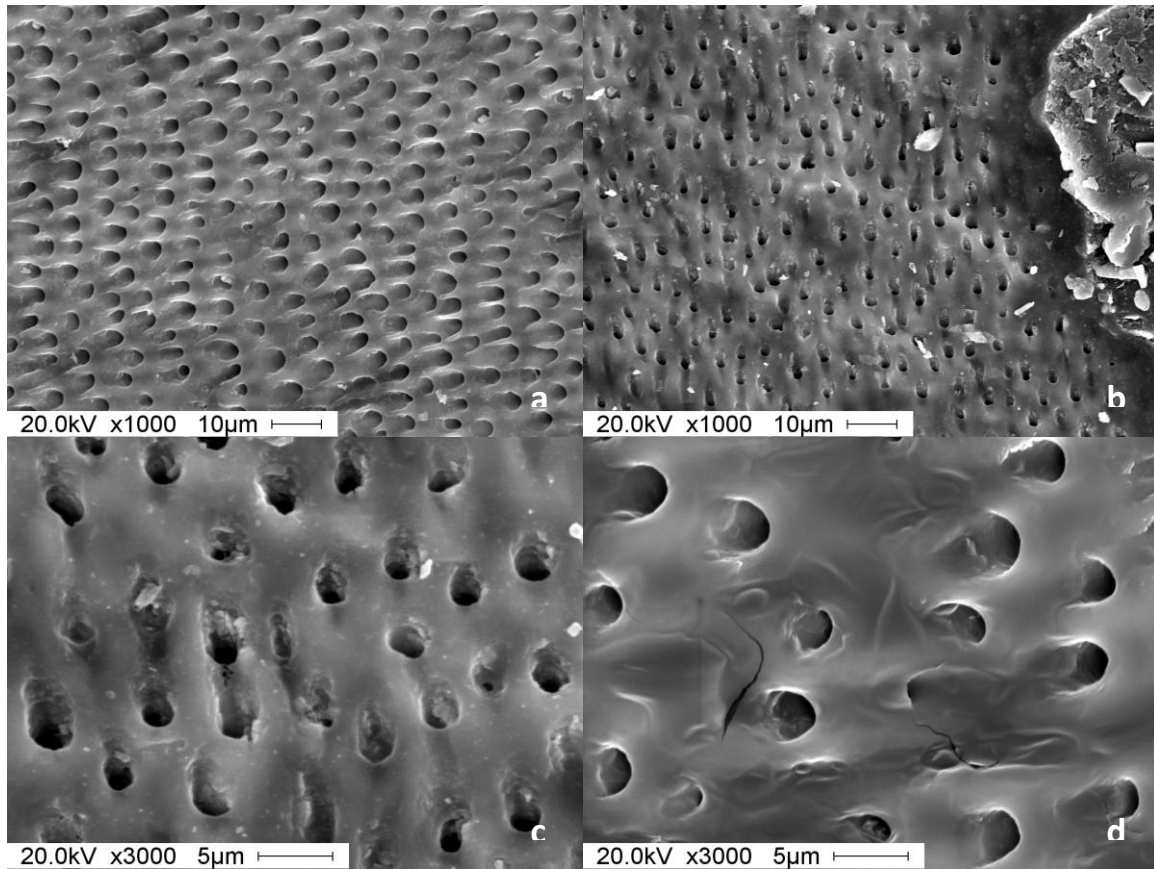
Fig 24. SEM images of Alginic acid gel after its application on a dentin section (without being removed) (a) Control side (b-d) Test side
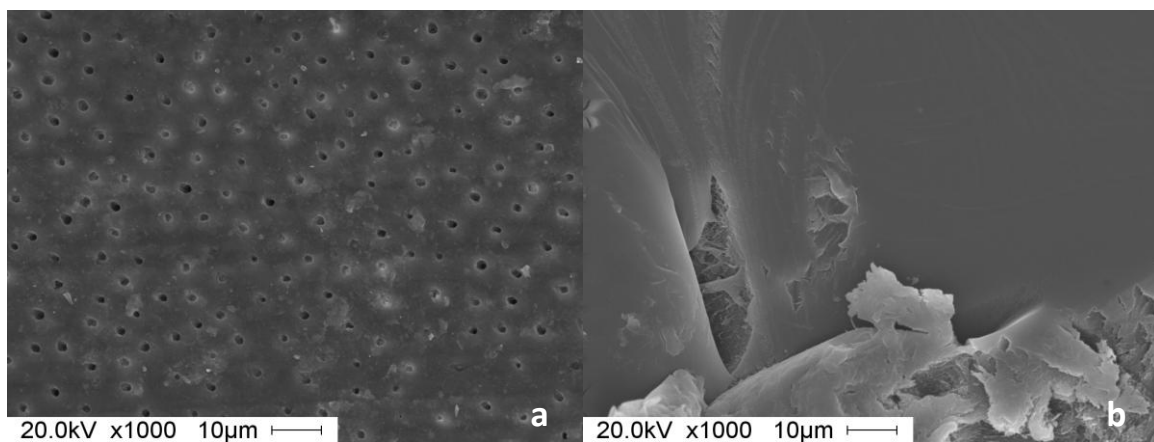
Fig 25. SEM images of P(HEMA) gel after its application on a dentin section (without being removed) (a) Control side (b) Test side

Fig 26. SEM images of P(DOPA) gel after its application on a dentin section (without being removed) (a) Control side (b) Test side
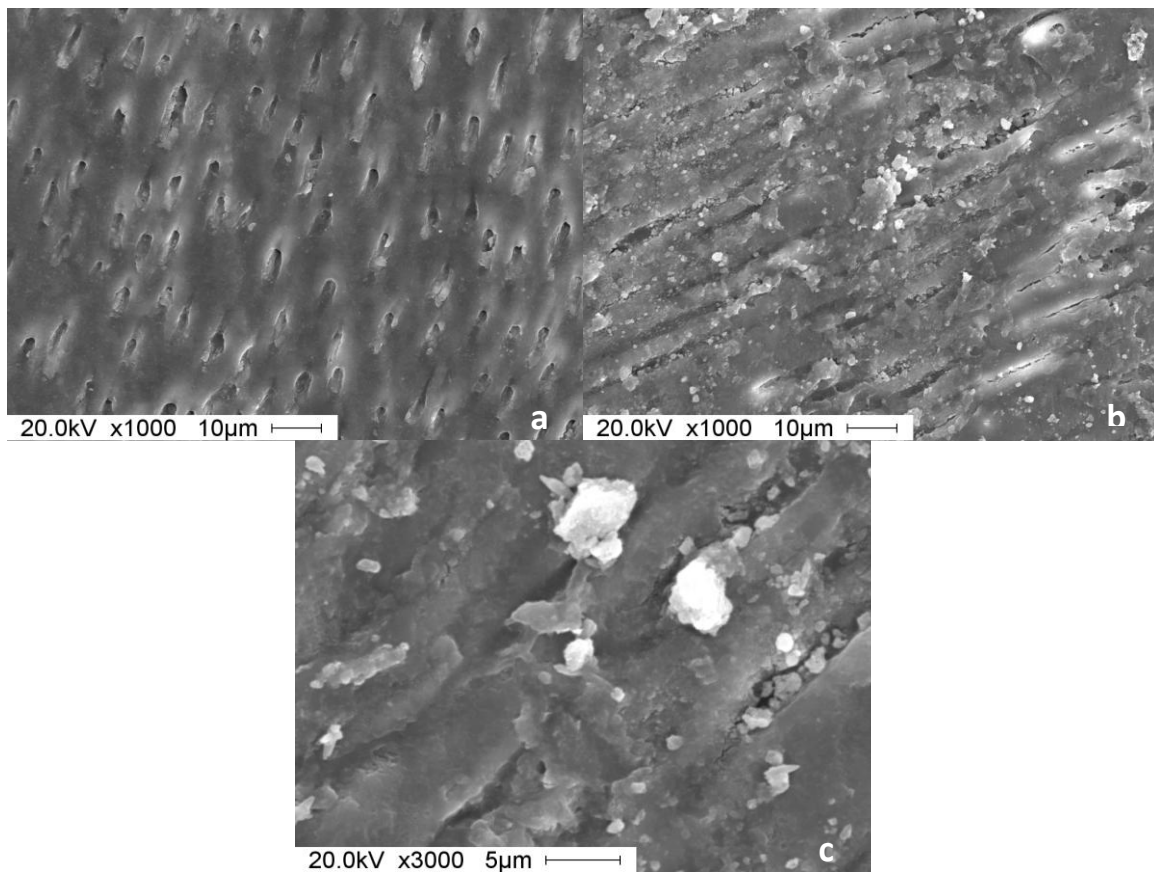
Fig 27. SEM images of 40% FA crystals/Water mixture applied on a dentin section (a) Control side (b, c) Test side

Fig 28. SEM images for remnants of 20% FA/Alginic acid paste applied on dentin sections (after being removed from the surface) (a) Control side (b-d) Test side
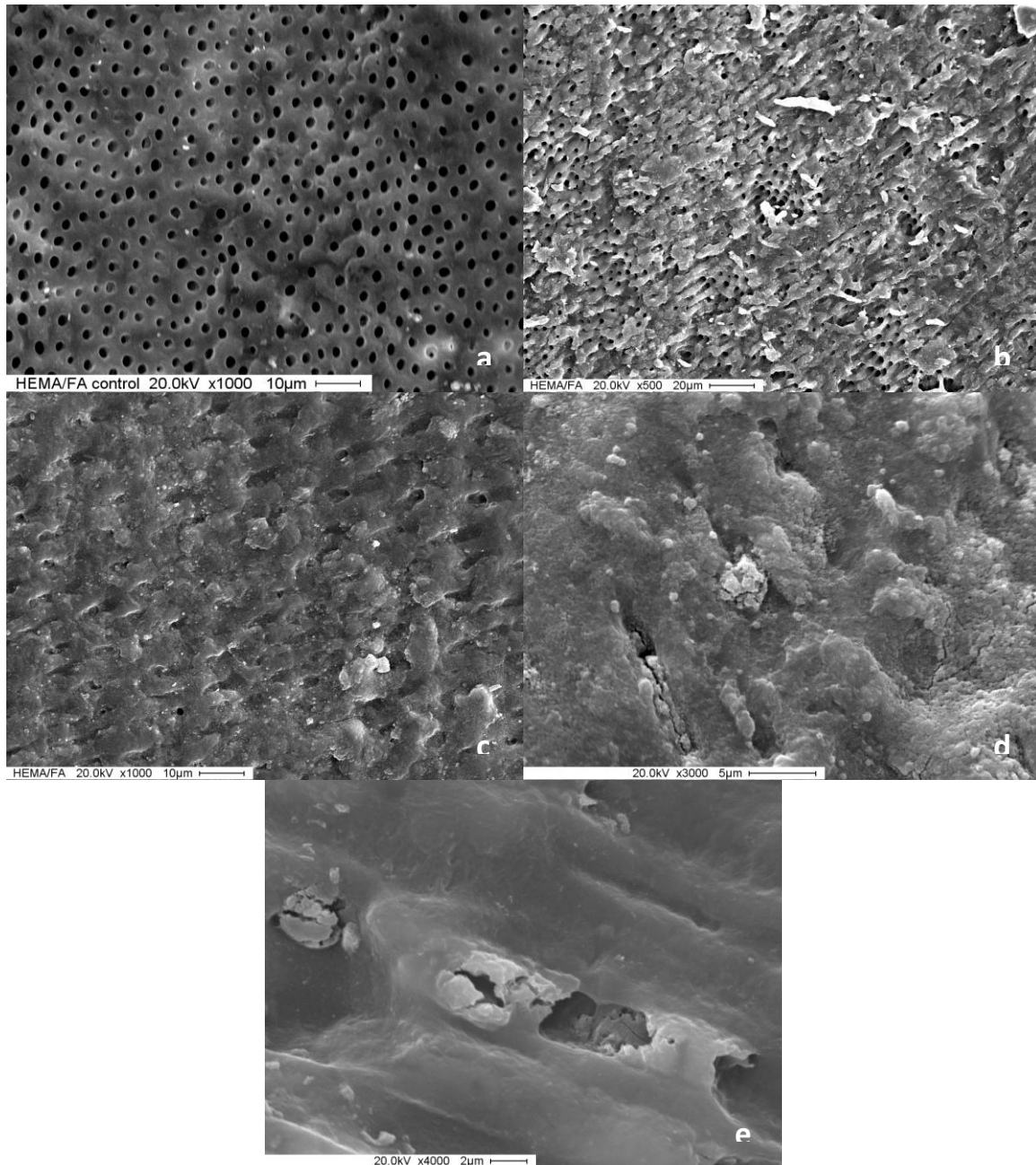
Fig 29. SEM images for remnants of 40% FA/P(HEMA) paste applied on dentin sections (after being removed from the surface) (a) Control side, (b-e) Test side
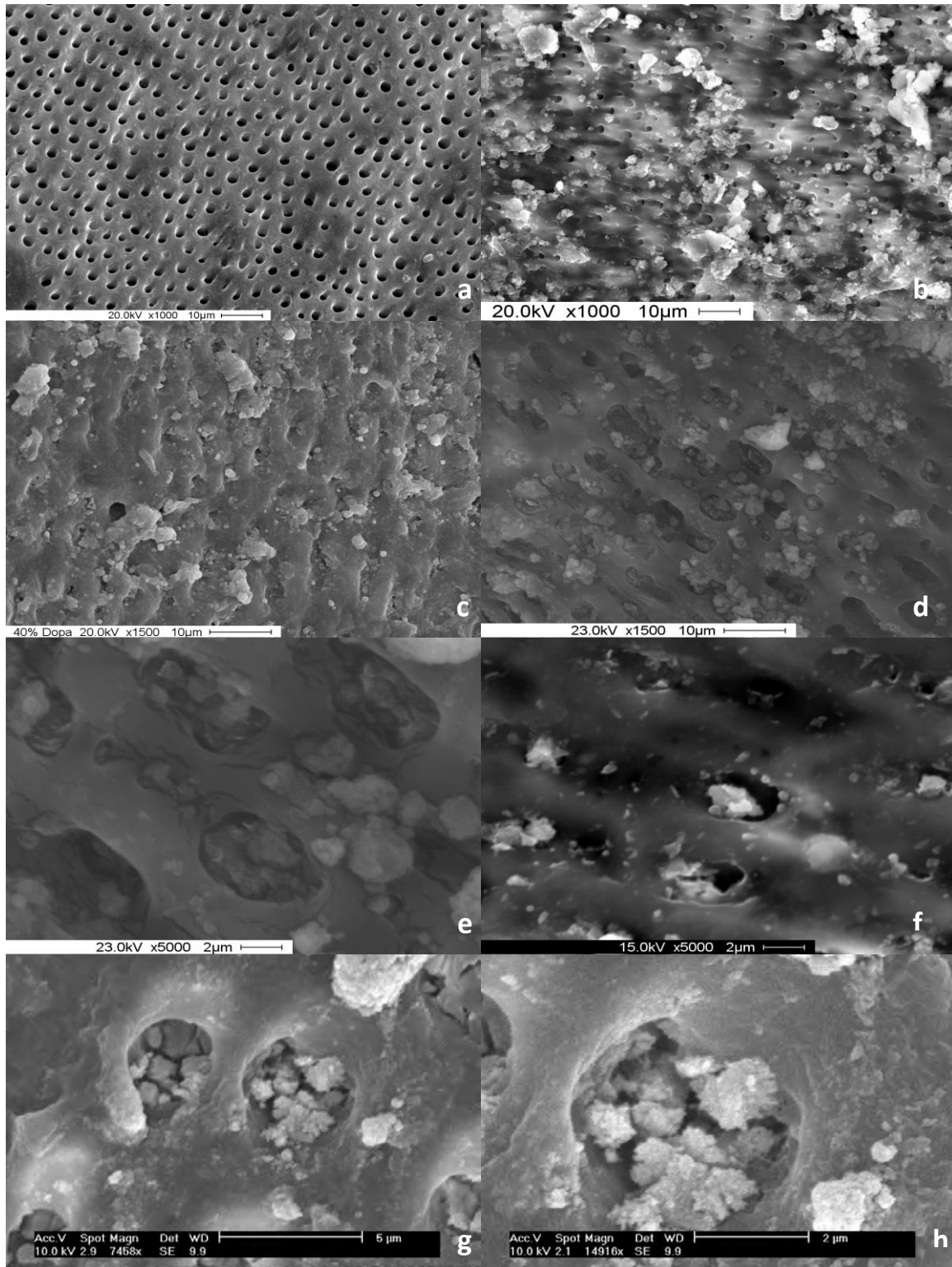
Fig 30. SEM images for remnants of 40% FA/P(DOPA) paste applied on dentin sections (after being removed from the surface) (a) Control side (b-h) Test side
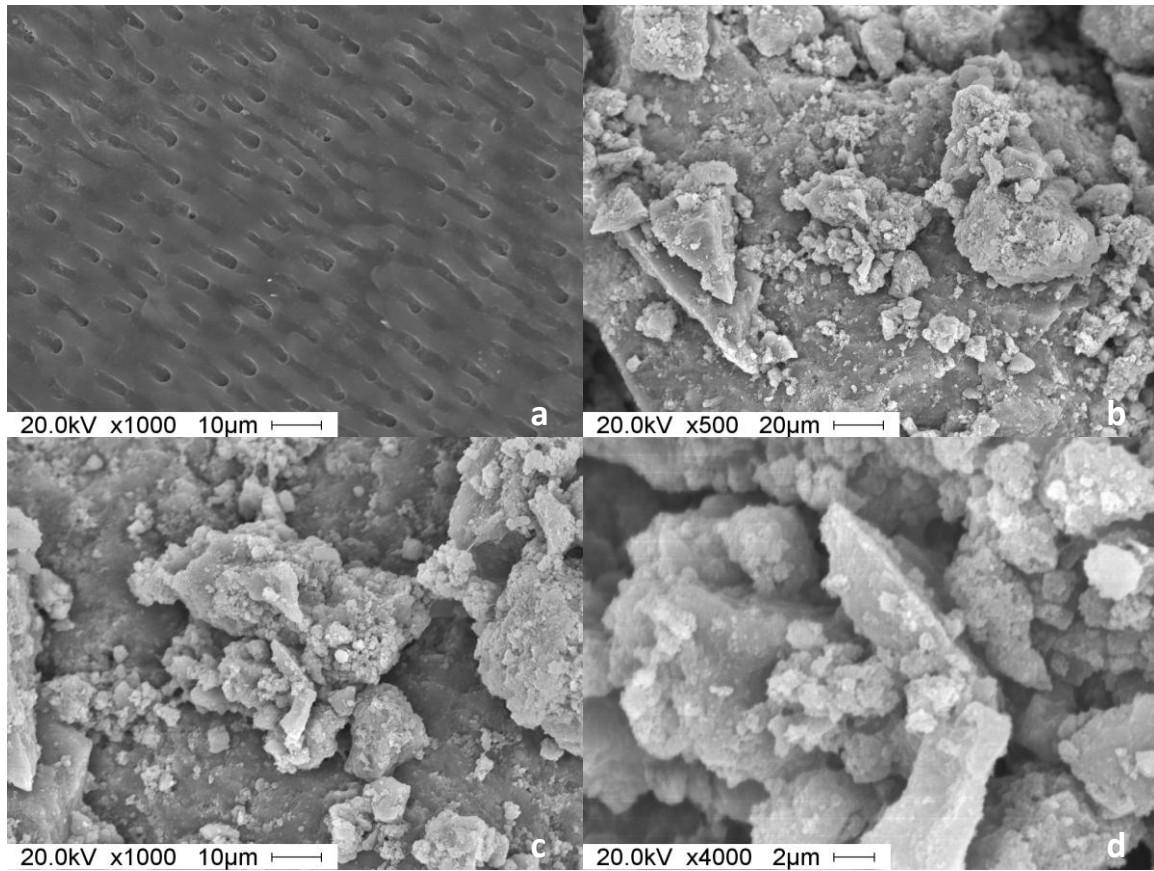
Fig 31. SEM images of 20% FA/Alginic acid paste applied on dentin sections (without being removed) (a) Control side (b-d) Test side
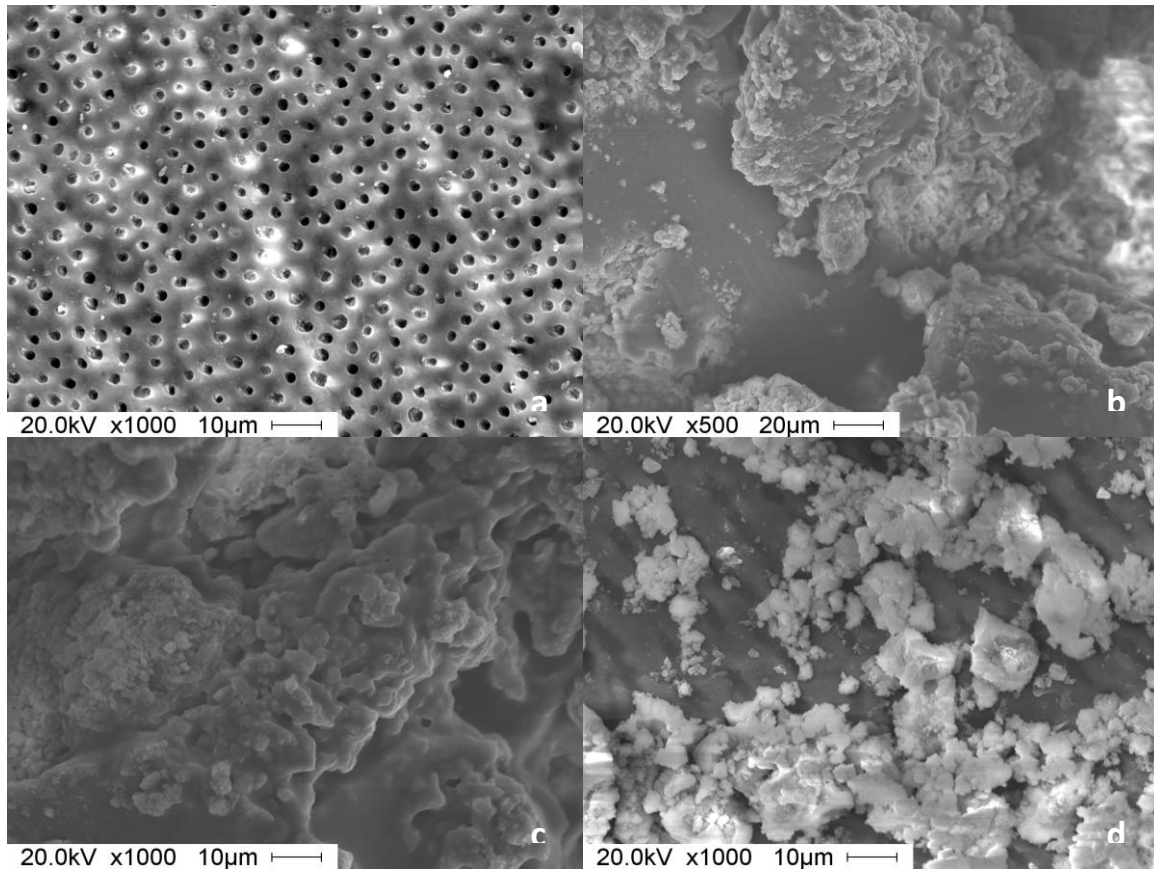
Fig 32. SEM images of 40% FA/P(HEMA) paste applied on dentin sections (without being removed) (a) Control side (b-d) Test side
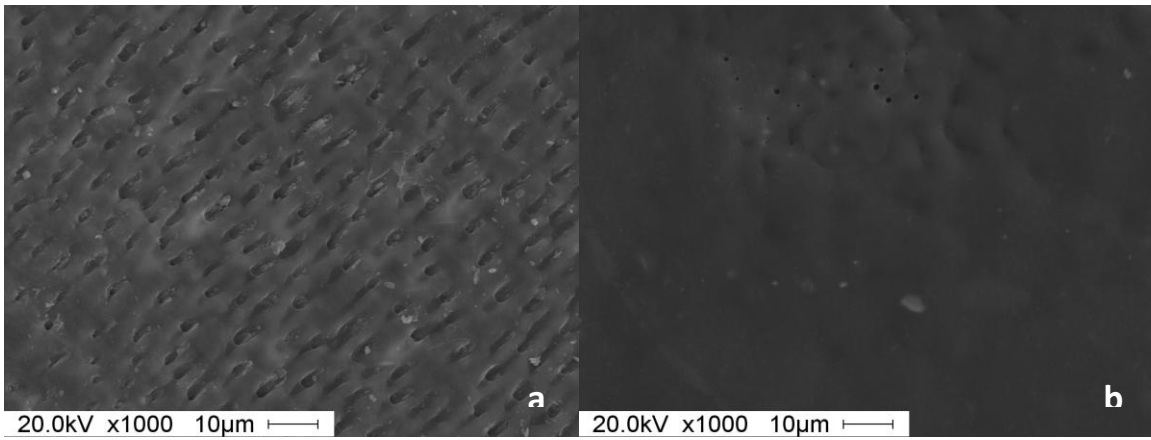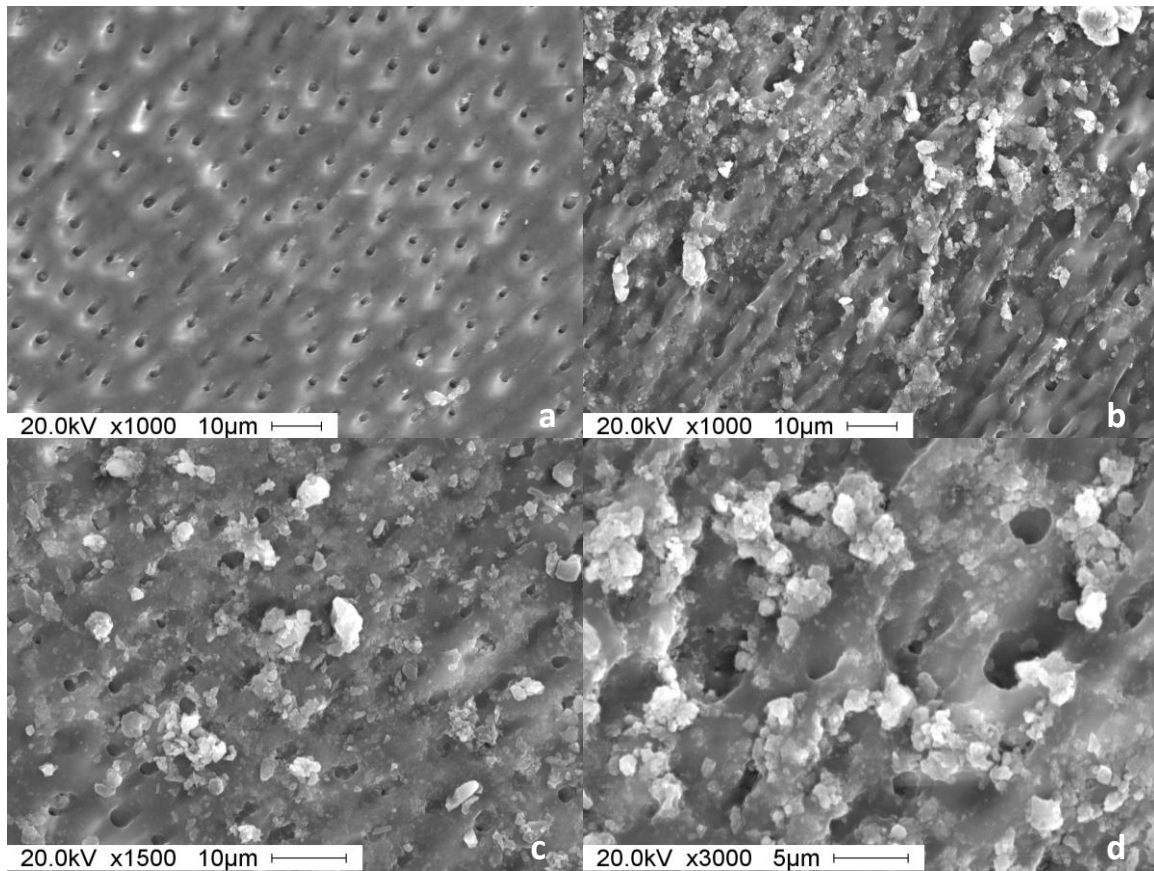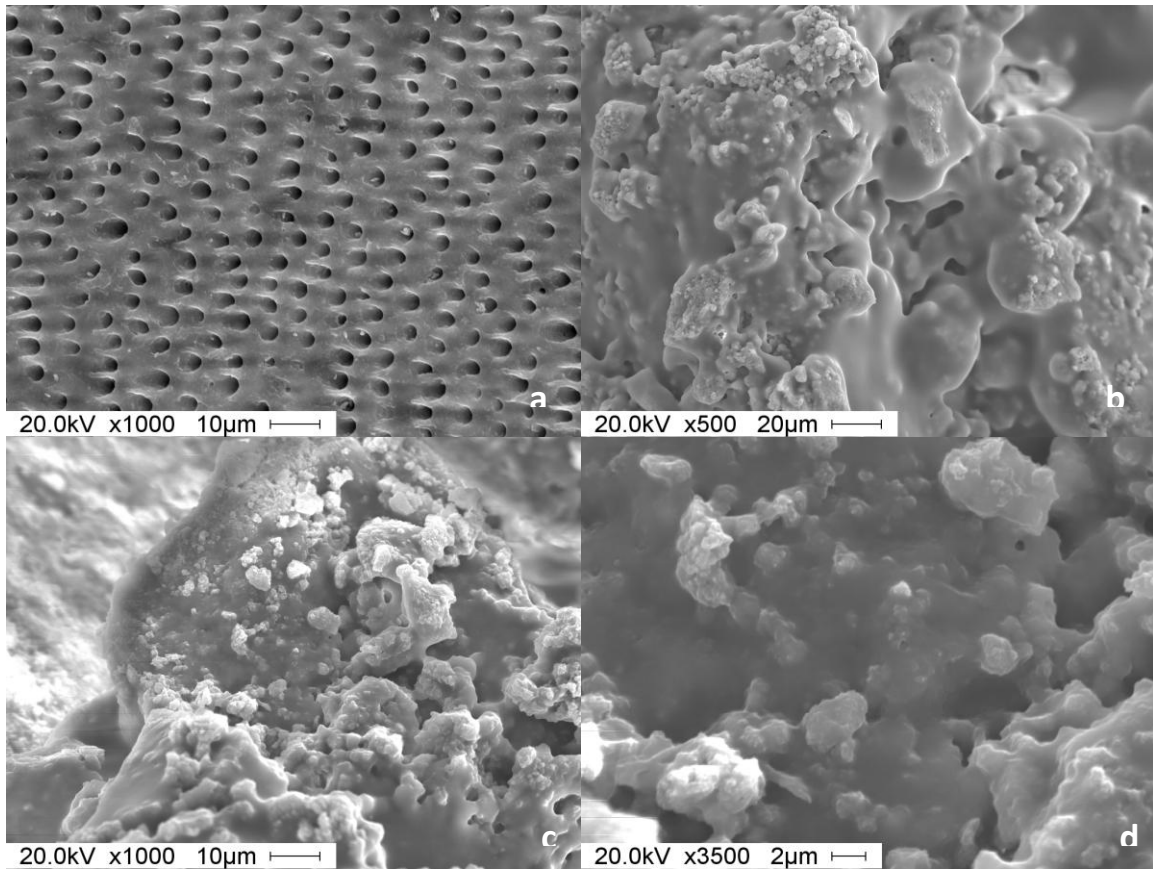
Fig 33. SEM images of 40% FA/P(DOPA) paste applied on dentin sections (without being removed) (a) Control side (b-d) Test side

Ion release

The calibration measurements for the F release performed on 10 samples of FA crystals submerged in distilled water gave an average of 4.3 ppm with a standard deviation of 1.54 (Table 4). The Kruskall-Wallis test revealed no statistically significant difference between the measurements ($P> 0.05$).

Table 4. Fluoride electrode calibration measurements for baseline F release of 10 mg FA crystals in 10 ml of distilled water (ppm)

N	Average [F]	SD	Chi-Square	df	Asymp. Sig
10	4.3	1.54	9.0	9	0.437

In the first experiment of F release (mimicking daily oral fluctuation in pH) (Table 5), FA crystals released an average of 4.8 ± 0.02 ppm fluoride ions in distilled water. The F release was less for the FA/Alg paste (3.1 ± 0.3 ppm); 1.9 ± 0.1 ppm for FA/P(HEMA) and 1.9 ± 0.3 ppm for FA/P(DOPA) paste. The amount of F release increased after the addition of lactic acid. The average cumulative concentration of F after the 3 episodes of LA was in descending order: FA only (7.8 ± 1.0 ppm); FA/P(HEMA) (7.2 ± 0.7 ppm); FA/P(DOPA) (7 ± 0.6 ppm) and FA/Alg (6.6 ± 0.7 ppm). However, the Kruskall-Wallis test demonstrated no statistically significant differences between the groups at baseline and after each LA episodes ($P>0.05$) (Table 5 and Figure 34). There was no significant amount of F ions released from the gel-only controls (Alg only, P(HEMA) only and P(DOPA) only) at baseline and after each LA episodes (Table 5). The calculated total subtractive weight of F ions released in 20 ml of distilled water solution is illustrated in Table 6.

N.B. The negative values calculated for [F] indicate less than 1 ppm concentration of F. The relationship between the electrode reading in milli Volt and the F concentration is a straight line when [F] is between 1-100 ppm.

Table 5. Average cumulative fluoride release at baseline and after each lactic acid episodes (ppm)

	Baseline [F]	[F] 1st LA	[F] 2nd LA	[F] 3rd LA	Average [F] after LA episodes	SD baseline	SD After LA
FA only	4.8	8.1	8.0	7.4	7.8	0.02	1.0
FA/Alg	3.1	7.2	6.5	6.2	6.6	0.3	0.7
FA/P(HEMA)	1.9	7.9	7.1	6.4	7.2	0.1	0.7
FA/P(DOPA)	1.9	7.6	6.9	6.5	7.0	0.3	0.6
Alg only	-1.4	0.8	-1.0	-2.2	-0.8	1.4	2.3
P(HEMA) only	-3.6	1.9	0.1	-1.1	0.3	0.7	1.5
P(DOPA) only	-4.1	0.1	-0.8	-2.1	-0.9	1.2	1.2
Chi-Square	12.4	12.11	11.79	10.51			
Df	6	6	6	6			
Asymp. Sig.	.054	.059	.067	.105			

Fig 34. Fluoride release at baseline and after LA episodes (ppm)
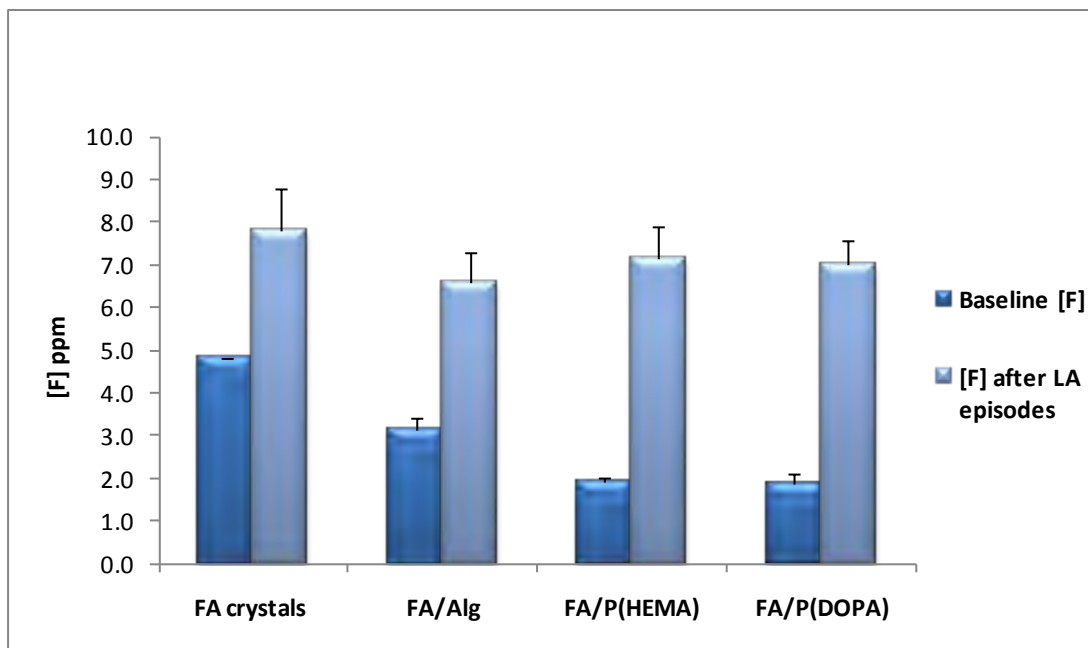
Table 6. Total subtractive[1] F weight released in 20 ml of solution at baseline and after each lactic acid episodes (µg)

	Baseline F wt	*F wt 1st LA*	*F wt 2nd LA*	*F wt 3rd LA*	*Total wt*
FA only	96.2	114.6	102.5	95.8	409.1
FA/Alg	62.5	112.9	72.5	87.0	335
FA/P(HEMA)	38.3	139.0	73.2	92.3	342.7
FA/P(DOPA)	37.5	134.1	70.4	95.2	337.1

Fluorapatite crystals released an average of 32.5 ± 17 ppm calcium ions in distilled water before the addition of lactic acid (Table 7). Calcium release was less for the FA/Alg paste $(12.8 \pm 5.6$ ppm); 8.1 ± 2.8 ppm for FA/P(HEMA), 16.9 ± 2.5 ppm for the FA/P(DOPA) paste, Alg-only $(8.7 \pm 9.4$ ppm), P(HEMA)-only $(2.7 \pm 2.1$ ppm) and 13 ± 2.1 ppm for

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[1] Subtractive weight is the calculated weight of the released ions in each episode independently, and the baseline ions weight was subtracted from the subsequent lactic acid episodes.

P(DOPA)-only. The amount of Ca release increased significantly after the addition of lactic acid. The average cumulative concentration of Ca after the 3 episodes of LA was in descending order: FA only (176.7 ± 119.1 ppm); FA/P(HEMA) (139.8 ± 32.5 ppm); FA/Alg (135.8 ± 38.2 ppm) and FA/P(DOPA) (117.3 ± 41.7 ppm). No significant Ca release was recorded for the gel-only controls after LA episodes. Kruskall-Wallis demonstrated no statistically significant differences between the groups at the baseline and after each LA episodes ($P>0.05$) (Table 7 and Figure 35). The calculated total subtractive weight of Ca ions released in 20 ml of distilled water solution is illustrated in table (8).

Table 7. Average cumulative calcium release at baseline and after each lactic acid episodes (ppm)

	Baseline [Ca]	[Ca] 1st LA	[Ca] 2nd LA	[Ca] 3rd LA	Average LA episodes	SD baseline	SD after LA
FA only	32.5	97.1	186.5	246.6	176.7	17.0	119.1
FA/Alg	12.8	105.3	150.6	151.4	135.8	5.6	38.2
FA/P(HEMA)	8.1	121.0	139.1	159.3	139.8	2.8	32.5
FA/P(DOPA)	16.9	72.1	130.8	148.9	117.3	2.5	41.7
Alg only	8.7	5.6	-3.0	-22.1	-6.5	9.4	13.8
P(HEMA) only	2.7	6.1	-1.8	-24.4	-6.7	2.1	16.5
P(DOPA) only	13.0	4.1	-9.7	-25.3	-10.3	2.1	15.1
Chi-Square	9.6	10.5	10.3	10.3			
df	6	6	6	6			
Asymp. Sig.	.143	.103	.111	.112			

Fig 35. Calcium release at baseline and after LA episodes (ppm)
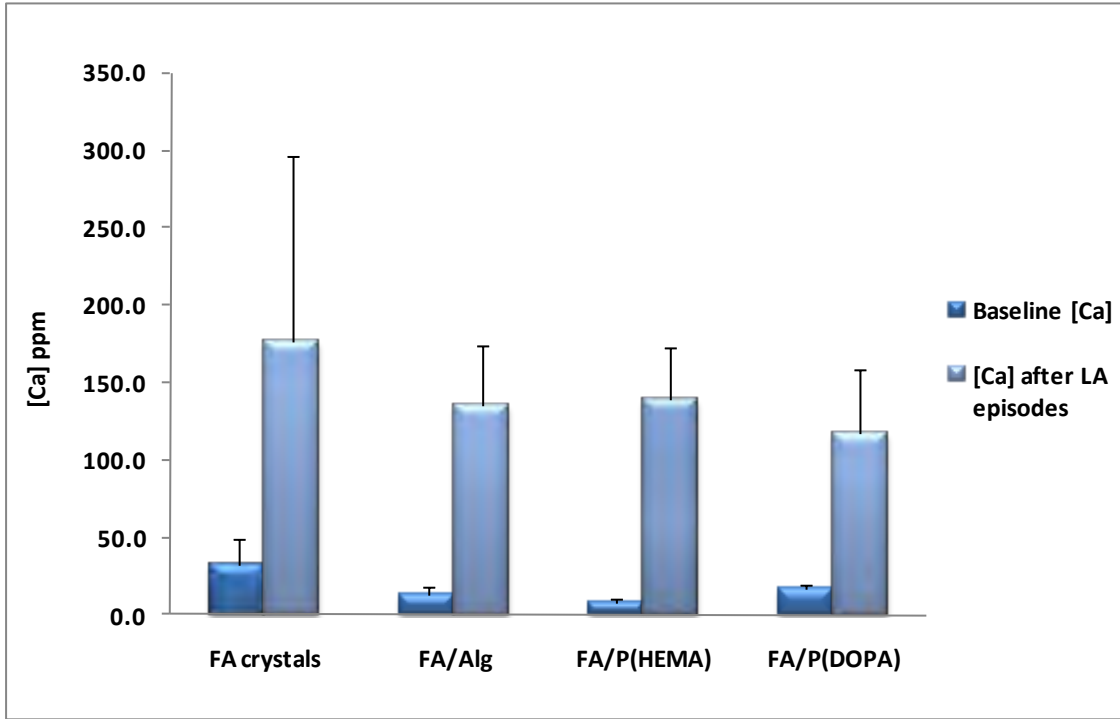
Table 8. Total subtractive Ca weight released in 20 ml of solution at baseline and after each lactic acid episodes (µg)

	Baseline Ca wt	Ca wt 1st LA	Ca wt 2nd LA	Ca wt 3rd LA	Total wt
FA only	339	1772	2843	3511	8465
FA/Alg	111	2051	1986	2035	6183
FA/P(HEMA)	56	2392	1586	2392	6426
FA/P(DOPA)	49	1416	1908	2024	5398

Fluorapatite crystals released an average of 12.9 ± 6.1 ppm of phosphate ions in distilled water before the addition of lactic acid (Table 9). Phosphate release was 13.4 ± 7 ppm for FA/Alg paste; 11.1 ± 6.3 ppm for FA/P(HEMA) and 12.7 ± 5.9 ppm for the

FA/P(DOPA) paste. No significant release of phosphate was recorded at the baseline for the gel-only controls. The amount of PO_4 release increased significantly after the addition of lactic acid. The average cumulative concentration of PO_4 after the 3 episodes of LA was in descending order: FA/Alg (46.2 ± 16.4 ppm); FA/P(HEMA) (45.3 ± 13.4 ppm); FA only (44.6 ± 14.7 ppm) and FA/P(DOPA) (44.3 ± 13.4 ppm). No significant PO_4 release was recorded for the gel-only controls after LA episodes. The Kruskall-Wallis test demonstrated no statistically significant differences between the groups at baseline and after each LA episodes ($P>0.05$) (Table 9 and Figure 36). The calculated total subtractive weight of PO_4 ions released in 10 ml of distilled water solution is illustrated in Table 10.

Table 9. Average cumulative phosphate release at baseline and after each lactic acid episodes (ppm)

	Baseline [PO₄]	[PO₄] 1st LA	[PO₄] 2nd LA	[PO₄] 3rd LA	Average [PO₄] after LA episodes	SD baseline	SD After LA
FA only	12.9	42.0	29.6	62.0	44.6	6.1	14.7
FA/Alg	13.4	45.2	28.7	64.6	46.2	7.0	16.4
FA/P(HEMA)	11.1	41.6	32.6	61.8	45.3	6.3	13.4
FA/P(DOPA)	12.7	41.9	30.7	60.3	44.3	5.9	13.4
Alg only	0.1	5.3	4.3	3.7	4.4	0.1	1.4
P(HEMA) only	-0.2	6.3	4.2	3.4	4.7	0.0	1.7
P(DOPA) only	0.5	4.7	4.6	23.3	10.9	0.2	10.1
Chi-Square	1.1	1.8	2.2	4.6			
Df	3	3	3	3			
Asymp. Sig.	0.79	0.6	0.54	0.20			

Fig 36. Phosphate release at baseline and after LA episodes (ppm)
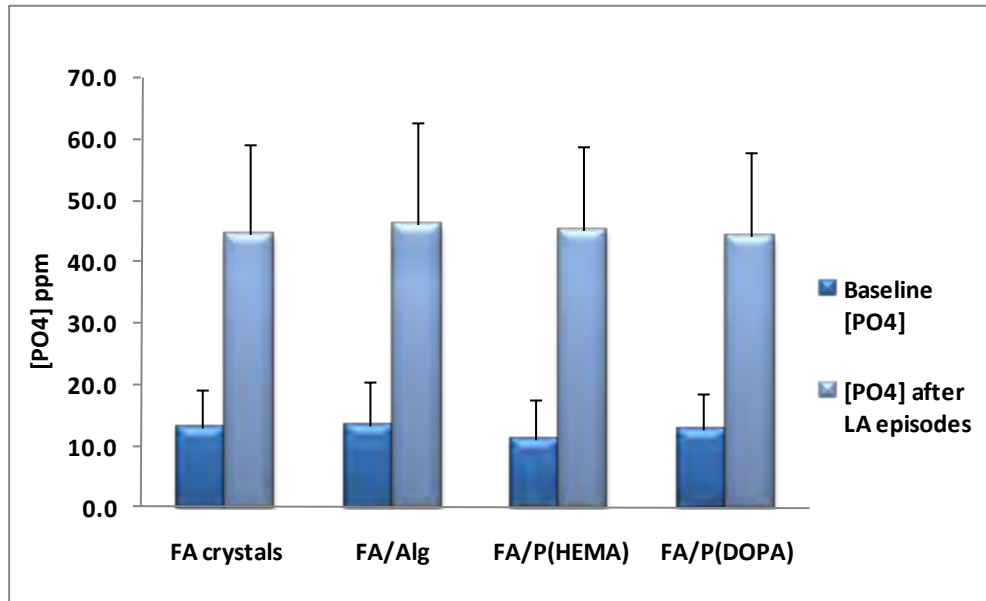
Table 10. Total subtractive phosphate weight released in 20 ml of solution at baseline and after each lactic acid episodes (µg)

	Baseline PO_4 wt	PO_4 wt 1st LA	PO_4 wt 2nd LA	PO_4 wt 3rd LA	Total wt
FA only	257.8	711.2	236.8	772.6	257.8
FA/Alg	267.9	769.6	189.0	828.6	267.9
FA/P(HEMA)	222.2	721.3	290.4	761.1	222.2
FA/P(DOPA)	254.6	710.3	258.7	756.6	254.6

In the second experiment of ion release, F concentration was measured at baseline and 8 hours after the samples submersion in distilled water without any acid challenge (at neutral pH). The average F concentration at baseline was the highest for the FA crystals-only without any gel (2.6 ± 0.2 ppm), followed by; FA/Alg (1.3 ± 0.2 ppm); FA/P(HEMA) (1.2 ± 0.2 ppm). There was no significant F release at baseline for the FA/P(DOPA) paste and the

tooth-only section with no pastes added (Table 11 and Fig. 37). The amount of F release increased after 8 hours. The cumulative [F] measured in descending order was: FA/Alg (5.2 ± 0.1 ppm); FA crystals-only (4.5 ± 0.3 ppm) and 3.5 ± 0.2 ppm for both FA/P(HEMA) and FA/P(DOPA). Insignificant release of F was recorded for the tooth-only sample. The Kruskall-Wallis analysis did not reveal any significant differences in F release between the groups at baseline and after 8 hours. The calculated total subtractive weight of released F ions at baseline and after 8 hours at neutral pH is illustrated in table 12.

Table 11. Average cumulative F release at baseline and after 8 hours at neutral pH (ppm)

	Average [F]0	*Baseline SD*	*Average [F]8*	*SD after 8 hrs*
FA only	2.6	0.2	4.5	0.3
FA/Alg	1.3	0.2	5.2	0.1
FA/P(HEMA)	1.2	0.2	3.5	0.2
FA/P(DOPA)	-0.2	0.3	3.5	0.2
Tooth only	-4.9	0.4	-1.4	0.9
Chi-Square	8.4		8.39	
Df	4		4	
Asymp. Sig.	.078		.078	

Fig 37. Average F release at baseline and after 8 hours at neutral pH (ppm)
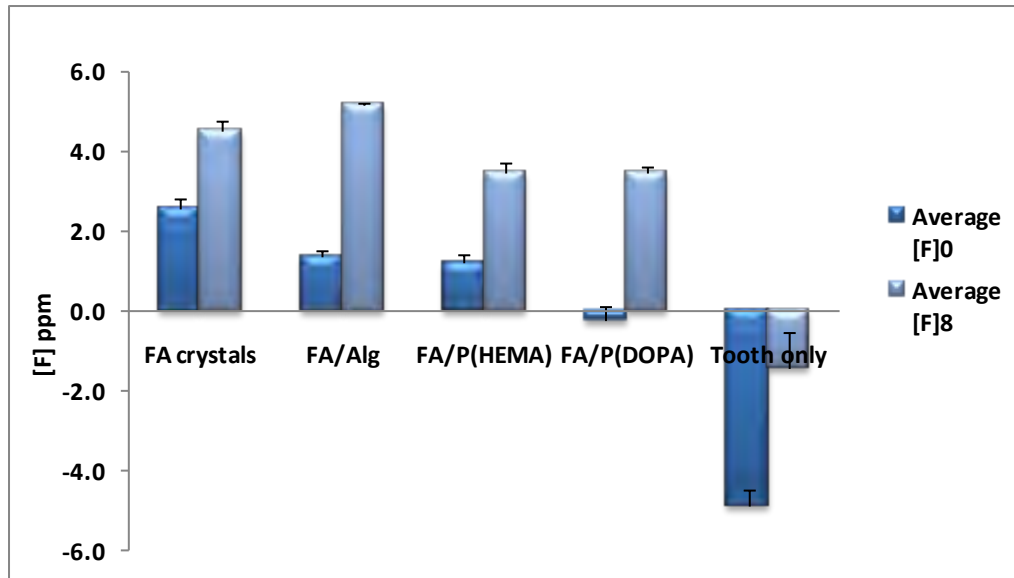
Table 12. Total subtractive F weight released in 10 ml of solution at baseline and after 8 hours at neutral pH (µg)

	Baseline F wt	*F wt after 8 hours*	*Total wt*
FA only	25.6	32.3	57.9
FA/Alg	13.5	44.9	58.3
FA/P(HEMA)	11.9	28.7	40.7
FA/P(DOPA)	---	35.7	33.4
Tooth section	---	10.3	10.3

The average Ca concentration at baseline was the highest for the tooth-only sample (18.1 ± 0.7 ppm), followed by FA/Alg (17.5 ± 5.4 ppm), FA crystals-only (10.1 ± 6.8 ppm), FA/P(DOPA) (9.6 ± 8.4 ppm) and FA/P(HEMA) (6.3 ± 3.6 ppm) (Table 13 and Fig. 38). The amount of Ca release increased after 8 hours. The [Ca] measured was very similar for FA

crystals-only, FA/Alg and FA/P(HEMA) with about 40 ppm. FA/P(DOPA) and the tooth-only control yielded less Ca release of about 24 ppm. The Kruskall-Wallis analysis did not reveal any significant differences in Ca release between the groups at baseline and after 8 hours ($P>0.05$). The calculated total subtractive weight of released Ca ions at baseline and after 8 hours at neutral pH is illustrated in table 14.

Table 13. Average cumulative Ca release at baseline and after 8 hours at neutral pH (ppm)

	Average [Ca]0	Baseline SD	Average [Ca]8	SD after 8 hrs
FA only	10.1	6.8	40.1	20.7
FA/Alg	17.5	5.4	42.4	12.6
FA/P(HEMA)	6.3	3.6	42.4	30.8
FA/P(DOPA)	9.6	8.4	24.3	2.9
Tooth only	18.1	0.7	24.4	6.4
Chi-Square	4.91		2.51	
Df	4		4	
Asymp. Sig.	0.297		.643	

Fig 38. Average Ca release at baseline and after 8 hours at neutral pH (ppm)
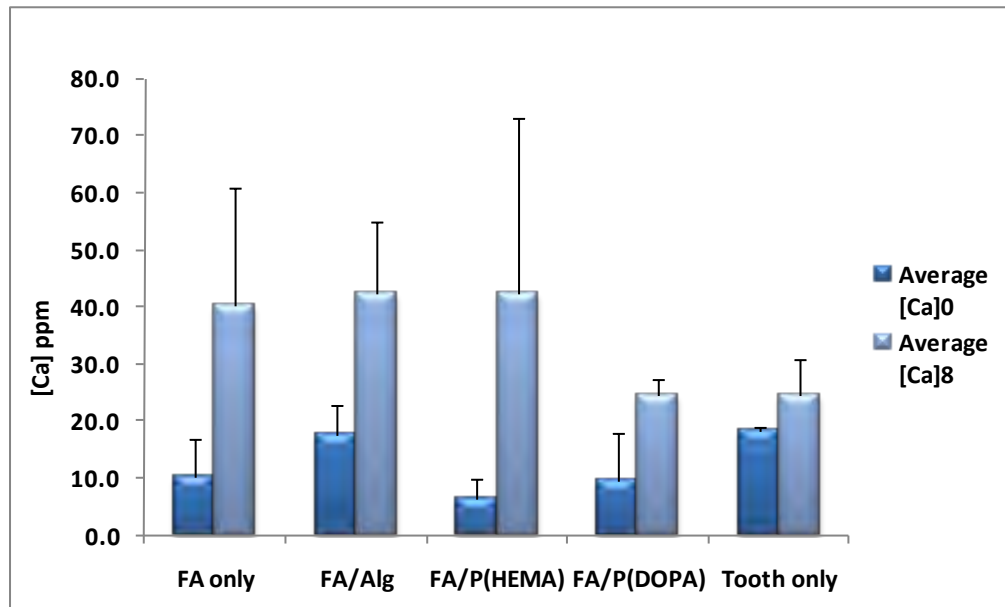
Table 14. Total subtractive Ca weight released in 10 ml of solution at baseline and after 8 hours at neutral pH (µg)

	Baseline Ca weight	*Ca weight after 8 hours*	*Total wt*
FA only	101.2	350.8	452.1
FA/Alg	174.6	336.4	511.0
FA/P(HEMA)	63.3	392.5	455.8
FA/P(DOPA)	96.2	195.4	291.6
Tooth section	181.4	153.5	334.9

The average PO_4 concentration at baseline was the highest for the FA crystals-only (21.5 ± 5.8 ppm), followed by FA/Alg (17.7 ± 0.1 ppm); FA/P(HEMA) (8.5 ± 0.8 ppm);

FA/P(DOPA) (7.7 ± 0.6 ppm) and the tooth-only sample (6.1 ± 0.9 ppm) (Table 15 and Fig. 39). The amount of PO_4 release decreased after 8 hours for FA crystals-only (11.5 ± 2.4 ppm); FA/Alg (9.8 ± 0.1 ppm). Phosphate release did not change for FA/P(HEMA) (8.6 ± 0.5 ppm) and the tooth-only sample (6.5 ± 0.4 ppm), however, it increased for FA/P(DOPA) paste (10.4 ± 0.5 ppm) (Table 15 and Figure 39). The Kruskall-Wallis analysis did not reveal any significant differences in PO_4 release between the groups at baseline and after 8 hours. The calculated total subtractive weight of released PO_4 ions at baseline and after 8 hours at neutral pH is illustrated in table 16.

Table 15. Average cumulative PO_4 release at baseline and after 8 hours at neutral pH (ppm)

	Average $[PO_4]0$	*Baseline SD*	*Average $[PO_4]8$*	*SD after 8 hrs*
FA only	21.5	5.8	11.5	2.4
FA/Alg	17.7	0.1	9.8	0.1
FA/P(HEMA)	8.5	0.8	8.6	0.5
FA/P(DOPA)	7.7	0.6	10.4	0.5
Tooth only	6.1	0.9	6.5	0.4
Chi-Square	7.96		7.6	
Df	4		4	
Asymp. Sig.	.093		.107	

Fig 39. Average PO$_4$ release at baseline and after 8 hours at neutral pH (ppm)
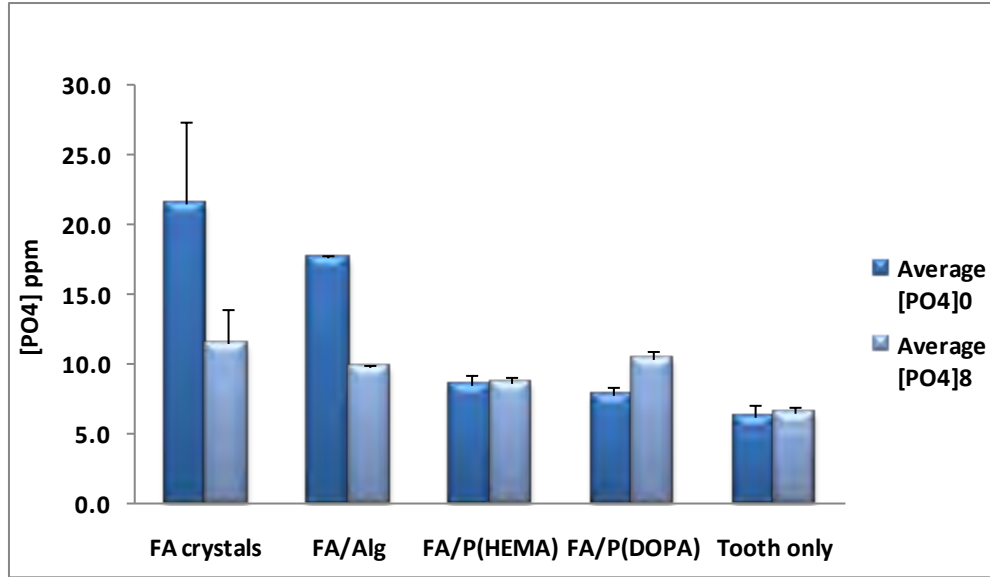
Table 16. Total subtractive PO$_4$ weight released in 10 ml of solution at baseline and after 8 hours at neutral pH (µg)

	Baseline PO$_4$ *wt*	PO$_4$ *wt after 8 hours*	*Total wt*
FA only	215.4	115.4	330.8
FA/Alg	176.6	98.1	274.7
FA/P(HEMA)	84.7	85.8	170.5
FA/P(DOPA)	77.4	104.0	181.4
Tooth only	61.5	64.7	126.2

Discussion

The method used in the current study for FA crystals synthesis was compared to Chen's method (3) where the crystals were incubated for 5 days. The change in the incubation time introduced in this study did not have a negative effect on the shape or crystalline structure of the FA crystals produced. However, the crystals in the current study were slightly longer and more variable in length. Chen's crystals had an average length of approximately 20–50 nm and a cross section of approximately 10 nm (fig. 40a). While in this study the crystals ranged between 20 and 100 nm in length and approximately 10 to 20 nm in cross section (fig 16 and 17). The EDS data (fig. 40b) showed that the Ca/P ratio is between 1.6–1.7, which is the same as the ratio obtained in this study (fig 19). Chen incubated the crystals in two different pHs (pH 6, and pH 9). However, all the prepared nanorods retained the same apatite crystalline structure at the experimental pH range 6-11. The crystals were synthesized at pH 6 in the current study.
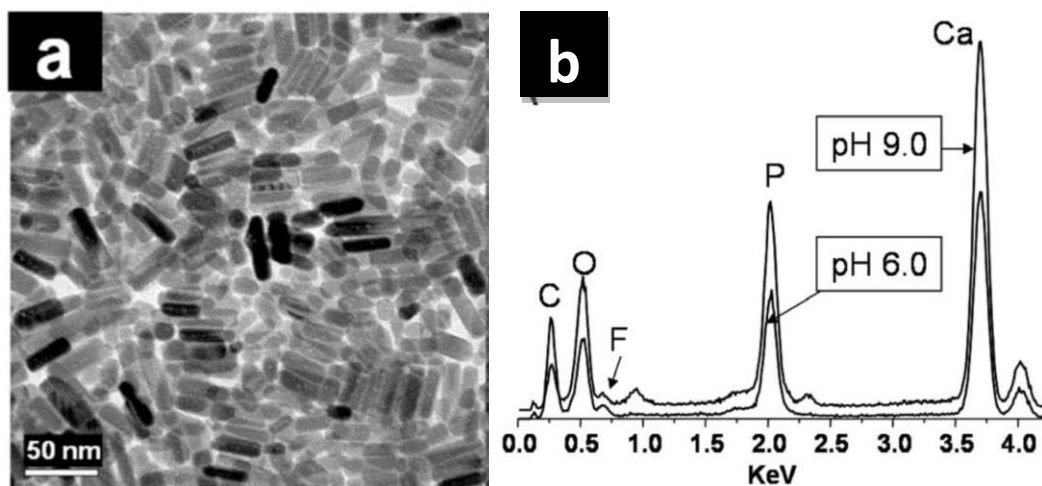
Fig 40. (a) TEM image of the synthetic FA crystals prepared at pH 6, (b) EDS of FA nanorods (3)

The cytotoxicity of the polymer P(DOPA) non filled or filled with FA crystals was tested. The test was performed in collaboration with the biologic and materials sciences

department at the University of Michigan. A standard XTT assay using a MEM medium plus 10% fetal bovine serum and HEp-2 cells were used to test the cytotoxicity of FA/P(DOPA) and P(DOPA) alone. The test showed that the polymer or a mixture of the polymer and FA crystals were free or have an insufficient quantity to cause harmful extractable effects under the extreme conditions used in this assay. Prasitslip et al. (13) studied the toxicity of homopolymers and copolymers of 2-hydroxyethyl methacrylate P(HEMA) on human dermal fibroblasts. It was found that a concentration of 10-25 % by weight was non-cytotoxic. The concentration of the P(HEMA) gel used in this study was 10% by weight. Alginic acid has been widely used in dental and medical applications with no reported effects in low concentrations. The cytotoxicity of alginate has been tested on cats by Chenoweth (14).

SEM Analysis

Two main methods have been used for the *in vitro* evaluation of potential dentin desensitizing agents; SEM analysis of dentin sections and permeability studies. The former method only was employed in this study.

Scanning electron microscope images of dentin sections have been evaluated for the obstruction of the dentinal tubules. This method uses a readily available and reproducible test substrate; however, careful attention to the details is mandatory to avoid potential errors. Several previous studies have used dentin discs for the SEM testing. However, a modification of the proposed model has been used in this study where sections of the facial cervical dentin were obtained instead of dentin discs cut in a coronal direction in the dentin disc model. Mordan et al.(15) examined the applicability of the dentin disc as a model for the *in vitro* testing of DH. They found that dentinal tubules from different dentin discs or even in the same disc have variable morphology. The authors attributed that to two factors: the changes in diameter, orientation and density of the dentinal tubules throughout the tooth; and the relation of the area of the cut to the smear layer morphology. Controls have not always been used in DH studies. However, some studies used control SEM images from the same tooth as the test disc or from a different tooth. Alternatively, many trials covered the control side on the same test disc with barriers like tapes and varnishes. Nevertheless, these methods have not always been successful. Mordan suggested that the dentin disc be halved after

etching the sample and preparing it for the treatment. Afterwards, the halves should be mounted as close as possible to each other for SEM analysis. This way, similar areas of dentin can be examined from both the test and control areas in an attempt to overcome the morphological variation. The study also suggested the dentinal tubules in the center of the disc be examined, as they were more circular than dentinal tubules at the periphery (dentin tubules at the peripheries are obliquely cut due to their orientation). Jain et al. (16) in a qualitative study, devised a methodology to overcome tubule variation, whereby a dentine disc was prepared for SEM, imaged, treated and once again prepared for SEM. However, the desiccation and gold coating of the disc before treatment and the difficulty in precisely relocating the same area may make this a less realistic model for quantitative studies. The use of an environmental SEM would negate the need to dry and coat the samples, thus the ability to use the same sample as a control and a test is applicable. However, this type of instrument is not readily available and the samples cannot be examined at a later date, because the sections will be altered with the treatments rendered.

The interpretation of the resulting SEM images and drawing relevant conclusions has been challenging. Many hypersensitivity studies (17-20) relied on subjective evaluation of the images, where the examiners compared the resultant images before and after treatment and ranked the treatments in terms of the "best to worst" coverage of the tubules. No specific criteria were quoted for either what is considered a good coverage, or the degree of dentinal tubule obstruction. This method entails a huge examiner bias and casts doubt on the accuracy of the evaluation. Some attempts have been made to quantify the coverage achieved with the desensitizing agents. Ahmed et al. (21) developed an analysis method for SEM images using automated digital image analysis software. Four micrographs of the control and four corresponding test images were taken at a constant magnification of 1000X. The images were then analyzed with the ImageProPlus software. The grayscale used ranged from 0=black to 255=white, and a level was chosen for the threshold such that only the tubules were highlighted. This threshold could be determined automatically or manually. However, automatic determination was more accurate and reproducible. Minimum and maximum diameters of the dentinal tubules were calculated using the software. The authors claimed the methodology used in this study was reliable and reproducible with no significant differences

within or between the examiners. They emphasized the importance of appropriate statistical analysis as it was shown that neglecting the complexity of clustered data, as often found in studies of this type, could lead to erroneous results and conclusions. It is probable that such measurements were accurate, but this method may be prone to bias and error, as the determination of the areas of interest for conducting the digital analysis is examiner dependent.

The SEM images obtained in this study were evaluated by one examiner for the occlusion of the dentinal tubules. No image analysis softwares were used in the current study. Alternatively, grids were created on 1000X magnification images to quantify occlusion of the tubules and calculate the average of obstructed tubules. This method has been previously used by Absi et al (22). Totally and partially obstructed tubules were counted obstructed in the current study, because a small reduction in tubule diameter would result in profound changes in fluid flow according to Poiseuille's law. For example, a reduction of the radius of the dentinal tubule by half would decrease the flow in the tubule 16 times.

In the current study, many control samples were prepared for the SEM analysis. Application of the crystals only, gels only, and water plus crystals were the controls chosen. The application of dry FA crystals resulted in partial obstruction of the open dentinal tubules. This could be attributed to the nano-size of the crystals, where a few crystals were able to penetrate the lumen of the dentinal tubules (fig. 20). As the TEM images showed, the synthesized FA crystals were 20 to 100 nm in length and approximately 10 to 20 nm in cross section (fig. 16 and 17). The size of the dentinal tubules ranges from 1-2 μm in areas of dentin the closer to the pulp. The dentin sections prepared in this study were taken from the cervical dentin approximately halfway to the pulp on the facial of the extracted teeth to mimic the parts of the tooth prone to sensitivity. Although the surface of the dentin samples was cleaned and rinsed for 2 minutes after the application of the dry FA crystals, many particles remained on the surface.

The three gels (Alginate, P(HEMA) and P(DOPA)) were applied to the dentin sections and left to dry, then the gel was removed and rinsed for 2 minutes. This procedure demonstrated the ability of remnants of the gels to obstruct the open dentinal tubules. The

P(DOPA) gel left more remnants on the surface than P(HEMA) and alginate. This could be attributed to the less hydrophilic nature of the P(DOPA) gel as opposed to the water-soluble nature of both P(HEMA) and alginate. Mechanically cleaning and rinsing the sections with distilled water for 2 minutes removed most of the P(HEMA) and alginate gels from the surface, but only partially removed the P(DOPA) gel. This adhesion of the P(DOPA) gel to the wet dentin surface is a desirable property for treating DH.

When the 3 gels were applied to the wet dentin surface and were not removed, P(HEMA) and P(DOPA) covered more than 90% of the patent dentinal tubules. This was not the case with the alginate gel which covered less than 20% of the tubules. This may be because of the hydrophilic nature of the alginate gel allowing its infiltration into the dentinal tubules.

The optimum manner of the synthesized pastes' application to the hypersensitive dentin surfaces was investigated; mimicking daytime or nighttime application. If the patients were to apply it in the daytime it is expected that faster removal of the paste will occur because of the salivary flow and by brushing and eating. Therefore, to mimic this daytime activity, the examined dentin sections were mechanically cleaned and rinsed. The daytime application will also be challenged by fluctuations in intra-oral pH, which inspired the experiment design for ion release. The overnight application would entail minimal physical removal of the paste on the dentin surface and would be subjected to minimal variation in intra-oral pH. For this reason the paste was left on the dentin surface for SEM examination and was not subjected to an acidic challenge during ion release testing.

The best dentinal tubule coverage achieved in this study was for FA/P(DOPA) paste (50-80%), FA/P(HEMA) (50-70%) and FA/Alginate (<50%) respectively. This was achieved after the paste's removal from the dentin surface. This could be attributed to the more tenacious adhesion of P(DOPA) and P(HEMA) to the dentin surface than Alginate. However, more studies are needed to quantify the adherence of the pastes to the dentinal tubules. Extrapolating the results of this SEM analysis to the application of the paste clinically (daytime) leads to the conclusion that about 50-80% of the FA/P(DOPA) and FA/P(HEMA) may remain in the dentinal tubules after its mechanical removal. The amount of the paste will

steadily accumulate in the dentinal tubules after repeated applications and continue releasing F, Ca and PO_4. For the overnight application, when the pastes were left on the surface and were not removed, all the pastes covered more than 90% of the tubules.

The crystals, because of the electrostatic nature, had a tendency to cluster with the addition of any of the carriers, which is a natural phenomenon of powders wetted by gels or liquids. This is especially so with the nano size crystals.

The two components of the pastes (crystals+carrier) contributed to the obstruction of the patent dentinal tubules. Nevertheless, the differentiation of the two components on SEM images is not easy. For example, in fig. 29e it was proposed that the tubule was obstructed with the P(HEMA) gel, as the shape of the particles inside that tubule are lacking an organized form. Conversely, it is proposed that the substance in the dentinal tubule in fig. 30h consist mainly of FA crystals. This is based on the organized shape of the particles in that tubule. Density of the particles inside the tubules helped distinguishing the obstruction caused by crystals vs. gel. Denser images were attributed to the FA crystals. Furthermore, the gel-only controls provided an idea for the shape of the gel's remnants in the tubules, which was compared to the paste experimental sections.

Scanning electron microscopy evaluation is a two dimensional analysis. The pastes constituents seemed to be obstructing and penetrating into the dentinal tubules. However, the use of atomic force microscopy (AFM) would provide a more objective evaluation of the paste's penetration into the tubules since it provides a true three-dimensional surface profile. Therefore, the depth of paste's penetration can be calculated.

Future experiments on this desensitizing paste should evaluate the duration of the pastes retention overtime and adhesion to the dentinal tubules. It would be of a great value to test this paste's ability to occlude dentinal tubules within an oral simulated environment preferably with artificial saliva that is continuously replaced. SEM images should be taken hourly for the experimental samples until no paste remains in the dentinal tubules. Suge et al. (19) designed a study to evaluate the duration of dentinal tubules occlusion after treatment with SiF (ammonium hexafluorosilicate) in a simulated oral environment. Etched dentin

disks were treated with 0.476 mol/L SiF for 3 minutes. The disks were then immersed in synthetic saliva, which was regularly replenished to maintain its ionic concentration, for up to 7 days. The occluding ability of the dentin tubules was evaluated using SEM, and the hydraulic conductance was measured at regular intervals. SEM images demonstrated that dentin tubules were occluded homogeneously and completely with the precipitate at 7 days. The authors claimed a newly formed calcium phosphate precipitate was present at the dentin surface.

Ion release analysis

An Orion combination fluoride electrode was used for F release measurements. This electrode offers fairly accurate measurements for fluoride concentrations above 0.02 ppm, and ±2% reproducibility. The temperature of the measured solution affects the electrode measurement, thus all the measurements were performed at 25°C. A calibration test of the electrode's reliability was performed by measuring 10 independent samples consisting of 10 mg FA+ 10 ml distilled water. The mean F concentration measured at neutral pH was 4.3±1.54 ppm (Table 4). The relatively large standard deviation could be explained by the difficulty in weighing 10mg of powder precisely; in addition to this is the aforementioned electrostatic force that causes the loss of some crystals during the transfer of the measured weight to the test tubes. Scaling up the production of the FA crystals and using larger weights of FA crystals would help resolve this problem.

The F release at neutral pH suggests an amorphous/crystalline mixture produced during the FA crystal synthesis because pure FA crystals would not release F under neutral pH. However, this F release is a desirable property for the synthesized crystals, insuring ions release at neutral and acidic pHs, thus making the ions available under a wide range of oral conditions. Interestingly, there was a difference in the baseline ion concentration in the 1st and 2nd ion release experiments despite the similarity in the amount of FA crystals used and the solution constituents (Tables 5-16). This might be indicative of variability in the concentration of the Ca, PO_4 and F ions in the crystals prepared at different time points; therefore resulting in the variable release of Ca, PO_4 and F ions.

In the ion release experiment designed to mimic the daily fluctuation of intra-oral pH, four controls were used; the FA crystals only; Alg gel only; P(HEMA) gel only and P(DOPA) gel only. Three experimental samples containing 20% FA/Alg; 40% FA/P(HEMA) and 40% FA/P(DOPA) were tested. The use of 'FA crystal only' control aimed to test the effect of mixing the crystals with the different carriers on the ion release. It was proposed that the addition of carriers would decrease the amount of ions released.

Analyzing the F, Ca, and PO_4 release results revealed some general tendencies. For example, FA crystals without any carrier released more ions than when it was combined with the carriers (tables 5-16); insignificant amounts of ions were released from the non-filled gel controls. More ions were released with the addition of lactic acid compared to the experiments where no acid was added. The total subtractive weight of F released in the 1st ion release experiment (mimicking daily fluctuation in intra-oral pH) was 6-10 times more than the total F weight released in the 2nd ion release experiment (mimicking overnight application) (tables 6 and 12). The total subtractive weight of Ca released at in the 1st ion release experiment was 12-19 times more than the total Ca weight released in the 2nd ion release experiment (tables 8 and 14). The total subtractive weight of PO_4 released in the 1st ion release experiment was 2.5-4.5 times more than the total PO_4 weight released in the 2nd ion release experiment (tables 10 and 16).

Kruskall-Wallis demonstrated no significant differences in F, Ca and PO_4 release at the baseline and after each of the lactic acid episodes. This test is used to examine the differences between groups of non-parametric data, and the small sample size employed in this study mandated its use. The value of statistical analysis of this type of data has been questioned in the literature because statistical significance does not always relate to clinical significance. It is of importance within the scope of this study to demonstrate the release of ions at baseline and after the acid challenge regardless of the small variations in the measured concentrations. More than 1.9 ppm of F, 8 ppm of Ca and 11 ppm of PO_4 were released from all of the pastes at the baseline. Moreover, there was more than 6.2 ppm of F, 72 ppm of Ca and 29 ppm of PO_4 released at any single lactic acid episode from all of the pastes tested (Tables 5, 7 and 9). These ion release values would suggest they would be clinically relevant

during the lowering of the oral pH. Not only would this concentration of ions result in mineral deposition to help occlude the dentinal tubules, but it would be available as a remineralizing therapy to combat demineralization of the dentin. However, in the oral environment, the pastes would be subjected to many mechanical and chemical challenges which may cause fluctuation in the amount of ions released.

Most of the fluoride release occurred after the 1st lactic acid episode, and then it stayed constant through the 2nd and 3rd episodes of lactic acid addition (Table 5). In contrast, Ca and PO$_4$ release continued after the 1st lactic acid episodes (Tables 7, 9). This could be attributed to the surface location of the fluoride ions on the FA crystals, thus allowing early release of the F ion. In contrast, the Ca and PO$_4$ ions are released overtime as the crystal dissolves, because of its presence throughout the crystal. As the surface of the crystal gets dissolved more Ca and PO$_4$ and less F are released.

The standard deviation of the mean F concentration during the experiments is small contrary to those seen for Ca and PO$_4$. The methods used for analysis of Ca and PO$_4$ relied on spectrophotometric analysis of a color producing reaction. These methods have been used mainly for detecting small concentrations of these ions in plasma and urine rapidly, conveniently, simply and inexpensively. Optimum detection levels using these methods range for Ca from 85-105 ppm and PO$_4$ 0.01-100 ppm respectively. In many instances, the measured Ca and PO$_4$ concentrations in this study were higher than the expected range of the methods used. Other accurate methods of measuring a wider range of ions concentration are atomic absorption or inductively coupled plasma-mass spectrometry (ICP-MS).

In the second experiment of ion release, mimicking the overnight application of the proposed paste, three experimental samples were used: 20% FA/Alg; 40% FA/P(HEMA) and 40% FA/P(DOPA) along with two controls; FA crystals only and tooth section only. The negative control 'tooth section only' did not release any significant amount of F. Nevertheless, it did release Ca and PO$_4$ at an average of 18.1 ppm and 6.1 ppm at baseline respectively, and after 8 hours 24.4 ppm of Ca and 6.5 ppm of PO$_4$. However, in the oral environment, such ion release values would not occur. Saliva is usually supersaturated with Ca and PO$_4$. Thus, tooth mineral will not dissolve in saliva or plaque fluid (which is even

more supersaturated than saliva during fasting), unless the saliva or plaque pH drops below 5.5 (23). The exaggerated hypotonic environment used in this study would not exist in the oral cavity. Testing the ion release in artificial saliva instead of distilled water would help providing a more realistic environment to test ion release from the pastes.

The F and Ca released at 8 hours were significantly more than the release at the baseline (Fig 37, 38 and 39). The availability of these ions in addition to the relatively constant but significant PO_4 release would be of great benefit by providing ions for mineral deposition into the tubules. The overnight application in a clinical situation would have decreased mechanical and acidic challenges compared to a daytime application, thus resulting in a longer retention time and a constant ion release. The longer the topical fluoride is in contact with the tooth surface the higher fluoride accumulation will be in enamel and dentin (24). Both the daytime and overnight application procedures showed promising results for tubule obstruction and ion release.

Conclusions

The filler for the desensitizing paste formulated and tested in this study was laboratory synthesized FA crystals. These synthetic FA crystals mimic the FA crystals in mature enamel in composition and their ability to release F, Ca and PO_4. They differ, however, in their size and the presence of an amorphous phase. The modification of these crystals from mature enamel FA crystals was advantageous in allowing ion release under fluctuating pH more easily than would have occurred from mature enamel. The crystals incorporation into viscoelastic gels facilitated its adherence to the wet dentin surfaces. The greatest dentinal tubules obstruction was achieved in descending order using 40% FA/P(DOPA), 40% FA/P(HEMA) and 20% FA/Alginate. P(DOPA) and P(HEMA) gels appeared to adhere more tenaciously to the wet dentin surfaces. Ion release was successful in both the daytime and nighttime ion release experiment designs, with higher concentrations achieved in the daytime design. All the pastes used were relatively similar in their ion release capacity.

References

(1) Cao M, Wang Y, Guo C, Qi Y, Hu C. Preparation of ultrahigh-aspect-ratio hydroxyapatite nanofibers in reverse micelles under hydrothermal conditions. Langmuir 2004 May 25;20(11):4784-4786.

(2) Chen H, Clarkson BH, Sun K, Mansfield JF. Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure. J.Colloid Interface Sci. 2005 Aug 1;288(1):97-103.

(3) Chen HF, Sun K, Tang ZY, Law RV, Mansfield JF, Czajka-Jakubowska A, et al. Synthesis of fluorapatite nanorods and nanowires by direct precipitation from solution. Crystal Growth & Design 2006 Jun;6(6):1504-1508.

(4) Chen HF, Tang ZY, Liu J, Sun K, Chang SR, Peters MC, et al. Acellular synthesis of a human enamel-like microstructure. Advanced Materials 2006 Jul;18(14):1846-+.

(5) Wikipedia.org. Alginic Acid. Available at: http://en.wikipedia.org/wiki/Alginic_acid.

(6) Wichterle O, Lím D. Hydrophilic Gels for Biological Use. Nature 1960;185(4706):117-118.

(7) Smith AM. The Role of Suction in the Adhesion of Limpets. Journal of Experimental Biology 1991 Nov;161:151-169.

(8) Smith AM. The structure and function of adhesive gels from invertebrates. Integrative and Comparative Biology 2002 Dec;42(6):1164-1171.

(9) Branch GM, Marsh AC. Tenacity and Shell Shape in 6 Patella Species - Adaptive Features. Journal of Experimental Marine Biology and Ecology 1978;34(2):111-130.

(10) Lee H, Lee BP, Messersmith PB. A reversible wet/dry adhesive inspired by mussels and geckos. Nature 2007 Jul 19;448(7151):338-341.

(11) Gandolfi MG, Silvia F, H PD, Gasparotto G, Carlo P. Calcium silicate coating derived from Portland cement as treatment for hypersensitive dentine. J.Dent. 2008 Aug;36(8):565-578.

(12) Chen Jr. PS, Toribara TY, Warner H. Microdetermination of phosphorus. Anal.Chem. 1956;28(11):1756-1758.

(13) Prasitsilp M, Siriwittayakorn T, Molloy R, Suebsanit N, Siriwittayakorn P, Veeranondha S. Cytotoxicity study of homopolymers and copolymers of 2-hydroxyethyl methacrylate and

some alkyl acrylates for potential use as temporary skin substitutes. J.Mater.Sci.Mater.Med. 2003 Jul;14(7):595-600.

(14) Chenoweth MB. The Toxicity of Sodium Alginate in Cats. Ann.Surg. 1948 Jun;127(6):1173-1181.

(15) Mordan NJ, Barber PM, Gillam DG. The dentine disc. A review of its applicability as a model for the in vitro testing of dentine hypersensitivity. J.Oral Rehabil. 1997 Feb;24(2):148-156.

(16) Jain P, Vargas MA, Denehy GE, Boyer DB. Dentin desensitizing agents: SEM and X-ray microanalysis assessment. Am.J.Dent. 1997 Feb;10(1):21-26.

(17) Gillam DG, Khan N, Mordan NJ, Barber PM. Scanning electron microscopy (SEM) investigation of selected desensitizing agents in the dentine disc model. Endod.Dent.Traumatol. 1999 Oct;15(5):198-204.

(18) Sauro S, Gandolfi MG, Prati C, Mongiorgi R. Oxalate-containing phytocomplexes as dentine desensitisers: an in vitro study. Arch.Oral Biol. 2006 Aug;51(8):655-664.

(19) Suge T, Kawasaki A, Ishikawa K, Matsuo T, Ebisu S. Ammonium hexafluorosilicate elicits calcium phosphate precipitation and shows continuous dentin tubule occlusion. Dent.Mater. 2008 Feb;24(2):192-198.

(20) Dijkman GE, Jongebloed WL, de Vries J, Ogaard B, Arends J. Closing of dentinal tubules by glutardialdehyde treatment, a scanning electron microscopy study. Scand.J.Dent.Res. 1994 Jun;102(3):144-150.

(21) Ahmed TR, Mordan NJ, Gilthorpe MS, Gillam DG. In vitro quantification of changes in human dentine tubule parameters using SEM and digital analysis. J.Oral Rehabil. 2005 Aug;32(8):589-597.

(22) Absi EG, Addy M, Adams D. Dentine hypersensitivity. A study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentine. J.Clin.Periodontol. 1987 May;14(5):280-284.

(23) Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. J.Am.Dent.Assoc. 2008 May;139 Suppl:18S-24S.

(24) Holler BE, Friedl KH, Jung H, Hiller KA, Schmalz G. Fluoride uptake and distribution in enamel and dentin after application of different fluoride solutions. Clin.Oral Investig. 2002 Sep;6(3):137-144.