

# Dentine Tubule Occlusion by Novel Bioactive Glass Based Toothpastes

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## Abstract

There are numerous over-the-counter (OTC) and Professionally-applied (In-office) products and techniques currently available for the treatment of Dentine Hypersensitivity but more recently the use of bioactive glasses in toothpaste formulations has been advocated as a possible solution to managing DH. *Aim:* The aim of the present study, therefore was to compare several bioactive glass formulations to investigate their effectiveness in an established *in vitro* model. *Material & Methods:* A 45S5 glass was synthesized in the laboratory together with several other glass formulations 1) a mix glass (fluoride and chloride), 2) BioMinF, 3) a chloride glass and 4) an amorphous chloride glass. The glass powders were formulated into five different toothpaste formulations. Dentine discs were sectioned from extracted human teeth and prepared for the investigation by removing the cutting debris (smear layer) following the sectioning using a 6% citric acid solution for 2 minutes. Each disc was halved to provide a test and control half for comparison following the brushing of the five toothpaste formulations onto the test halves for each toothpaste group. Following the toothpaste application, the test discs were immersed in either artificial saliva or exposed to an acid challenge. *Results:* The dentine samples were analysed using scanning electron microscopy (SEM) and observation of the SEM images indicated that there was good surface coverage following the artificial saliva immersion. Furthermore, although the acid challenge removed the hydroxyapatite layer on the dentine surface for most of the samples, except for the amorphous chloride glass, there was evidence of tubular occlusion in the dentine tubules. *Conclusions:* The conclusions from the study would suggest that

the inclusion of bioactive glass into a toothpaste formulation may be an effective approach to treat DH.

## **1. Introduction**

### **1.1 Overview**

Dentine hypersensitivity (DH) affects approximately 10-30% of the adult population and may have a direct impact on the individual's quality of life. There are numerous over-the-counter (OTC) and Professionally-applied (In office) products and techniques available for the treatment of Dentine DH. Recently the use of bioactive glasses in toothpaste formulations has been advocated as a possible long-term solution for managing DH. Currently, most of the research activity focusses on the Hydrodynamic theory as the basis for the therapeutic treatment of DH. The rationale being that by blocking the dentinal tubules (tubular occlusion) there will a corresponding reduction of the fluid flow through dentine (dentine permeability) and a subsequent relief of pain. The mechanisms underpinning the Hydrodynamic Theory are generally investigated in several recognised models, e.g. *in vitro*, *in situ*, *in vivo* human studies as well as animal studies (for nerve desensitizing mechanisms). The aim of the present *in vitro* study, therefore was to investigate effectiveness of experimental bioactive glasses designed for toothpaste formulations.

An ideal desensitising agent should have a rapid action with long-term effects, be non-irritant to pulp, painless, easy to apply and should not stain the tooth. Toothpastes are considered the most economic method for using desensitising in-home treatments and generally are classified by the Regulatory authorities on the ingredients within the formulation (e.g., cosmetic, medicine/drug) (5, 19). This are a plethora of products that are being developed for this condition but currently there does not appear to be one ideal product that can completely resolve the problem.

Bioactive materials have been considered for both medical and dental use particularly in bone defects. For example, in 1969, bioactive glasses were discovered as a second-generation alternative for the bonding of bone to an implant within the host's tissue through a chemical reaction. One of the advantages of these materials was that they have the capacity of producing hydroxyapatite and induce osteogenesis in physiological systems (4, 6). Since 1985, 45S5 Bioglass has been used clinically, as a third generation of biomaterials, for tissue reparation using gene activation proprieties. The first Bioglass containing material established in the market was initially used to treat conductive hearing loss and also in several head and neck surgery (4). In dentistry, the application of bioactive glasses was related to bone replacement implants in edentulous patients, to provide more stable ridge for denture construction. It was also used for periodontal diseases (Perioglas®) and bone defects as a method of reconstruction of bone. Dentine and bone have similarities in terms of tissue composition (e.g., hydroxyapatite); hence biocompatible glasses may be an efficient material for incorporating into toothpaste formulations as a tubular occludent (4, 6, 20). The composition of bioactive glasses is silicon, sodium, calcium and phosphorus oxides with specific percentages. In addition, fluoride can be incorporated into the glass and both fluoride and calcium ions are released in the presence of saliva (20, 21). When in contact with a biological fluid such as saliva, bioglass particles react and three processes occur 1) Leaching and formation of silanols, 2) dissolution of the glass network and 3) precipitation. Precipitation is an important process for occluding dentine tubules. The formation of a layer composed of calcium and phosphate induced by the release of these ions from the glass can mechanically occlude dentinal tubules and lower fluid flow

within the dentine. This layer is crystallized into hydroxyapatite and the presence of silica can accelerate the maturation of hydroxyapatite. In bone, bioactive glass triggers an osteoblast cell cycle leading to rapid cell proliferations and differentiation (4, 17, 20, 22).

Toothpaste formulations containing potassium designed to treat sensitive teeth can also have an analgesic effect. For example, potassium saline is responsible for maintaining high levels of potassium ion extracellularly, preventing re-polarization of the nerve cell membrane and inhibiting the transmission of impulses. In brief, potassium nitrate has been postulated to act by blocking neural transmission to reduce DH symptoms. However, there are limited clinical data in humans to support the mode of action of potassium ions reducing DH. Several studies have reported that toothpastes containing calcium sodium phosphosilicate or NovaMin® (GSK) can occlude the dentinal tubules more effectively than potassium salts as evidenced in clinical studies as well as *in vitro* studies immersing the products in artificial saliva (17, 19, 22). Usually toothpaste formulations are based on fluoride (to protect against caries), an abrasive component (that provides the cleaning ability), substances that inhibit bacterial growth, and other ingredients (3, 19, 22). Fluoride is a compound that may aid remineralization in enamel although evidence regarding its effect on DH is limited. It has been demonstrated, however that fluoride in toothpastes can create a precipitation onto the dentine surface and block the dentine tubules as well as increasing resistance against an acidic challenge (19). The precipitation however may also contain silica particles that can occlude the dentinal tubules rather than the fluoride ion *per se*. The suggested actions of a bioactive glass toothpaste were through its chemical ability to occlude dentine tubules by the

formation of calcium-phosphorous precipitates, calcium fluoride and fluorapatite. Fluoride in toothpaste formulations, however is important for its role in caries prevention by reducing the rate of demineralization, promoting remineralization of damaged tissue and decreasing acid production by interfering with oral bacterial in the tooth biofilm, functioning as a biocide against *S mutans*. Nevertheless, fluoride in combination with other ions may also enhance the effectiveness of desensitising effects (10, 21). Toothpaste formulations may also induce the formation of calcium, phosphate and fluoride and contribute to intratubular mineralization (3, 19, 22). In addition, the use of calcium phosphate products has been considered promising for the treatment of DH. Calcium is also important for the remineralization in tooth restorations as it was the primary component of hydroxyapatite with apatite as a form of calcium phosphate which can be used in dental materials (e.g., hydroxyapatite and fluorapatite).

Precipitation of hydroxyapatite on to exposed dentine surface can occlude dentine tubules and it has been shown that calcium phosphate may occlude dentinal tubules without inhibiting the spontaneous remineralization of tooth surface (16, 21). Moreover, toothpaste formulations can also contain strontium, stannous and calcium phosphate which can form physical barriers that may occlude the dentin tubules. These mechanisms occur by precipitating insoluble metal compounds on the dentine surface. Stannous chloride has also been reported to be effective in occluding dentine tubules although, NovaMin® has been reported to be more effective than a strontium chloride and placebo toothpaste, particularly when exposed to citric acid and artificial saliva. (9) Stannous fluoride may also block the dentine tubules by forming  $\text{SnF}_2$  and  $\text{CaF}_2$ . Additionally, strontium chloride has been reported to block the dentine tubules

(19). Tubule occlusion may however, occur naturally through the normal remineralization processes by saliva and by dentine sclerosis through secondary dentine formation. Saliva also has a protective function against tooth wear. The biofilm layer has been reported to promote remineralization and reduce any mineral loss (10). Therefore, using some of these dental products may help protect the dentine order to enhance its resistant to both mechanical and chemical attack. One method of increasing the dentine surface resistance to wear by acid erosion and abrasion is to increase its mineral density, alternatively occluding the dentine tubules with a mineral substance, such as calcium and phosphate toothpaste, would increase acid resistance of dentine (3, 9).

### **1.3.3 Aim of the project**

The aim of the project, therefore was to compare different bioactive glass formulations to investigate their effectiveness in the *in vitro* environment.

## **1.4 Materials and Methods**

A 45S5 glass was synthesized in the laboratory together with several other glass formulations and subsequently formulated into five different toothpaste formulations (see section 1.4.4).

### **1.4.1 Preparation of samples**

Caries free extracted mandibular and maxillary molars were collected from tooth bank with approval from Queen Mary Research Ethics Committee OMREC 2011/99.

The teeth were cleaned with deionised water and stored in 70% ethanol. Each tooth was sliced into mid coronal sections by a diamond cut off wheel machine.



The teeth were embedded in an impression material to make blocks to stabilize them. The sections were required to be less than 1mm thick, therefore the machine was set to approximately 0,600mm thick. Subsequently, the dentine discs were polished using a carbide abrasive paper of P800, P2500 and P4000 consequently. A micro meter was used to measure the thickness after polishing, and it was established to be approximately 0,3mm.

#### **1.4.2 Glass manufacture**

The 45S5 Bioactive glass was manufactured in the laboratory with the University Department. The reagents including SiO<sub>2</sub> (45%), CaO (24,5%), P<sub>2</sub>O<sub>5</sub> (6,0%) and Na<sub>2</sub>O (24,5%). were mixed and placed in a crucible, melted at 1390°C for 1 hour in an electric furnace. The mixture was then quenched into cold water to prevent crystallization and ground in a Gyro Mill for two sets of 7 minutes. Finally, the particles were separated into two groups, 1) ≥38 microns and 2) ≤38 microns using a 38um sieve. The glass with particle size ≤ 38 microns were used for further characterization.

#### **1.4.3 Artificial saliva and toothpaste application**

The artificial saliva was formulated using potassium chloride (2.236g/L), potassium dihydrogen phosphate (1.361 g/L), sodium chloride (0,759 g/L), calcium chloride dihydrate (0,441 g/L), Mucin (2.200 g) and sodium azide (0,2g). These reagents were weighed in an electronic balance and dissolved in 8000mL of deionised water, the pH was adjusted to 6.5 with KOH.

The paste was manufactured following the protocol by Mahmood *et al.* (22). The components included glycerol, Carbopol (polyacrylic acid), PEG400 (polyethylene glycol) and Syloid 63, synthetic amorphous silica, K acesulfame, titanium dioxide and Na laurylS (sodium dodecyl sulfates) were also used. The

ingredients were weighed and mixed thoroughly in a plastic container. The pastes were divided into five portions with approximately 17g each. Five batches of bioactive glass containing toothpastes (laboratory manufactured 45S5, mixed glass, commercial BioMin F, CDL Chloride glass and amorphous chloride glass) were formulated by adding 1g of bioactive glass into the paste and mixed thoroughly.

#### 1.4.4 Experimental design

Five different types of bioactive glasses were investigated. The laboratory manufactured 45S5, mixed glass containing fluoride and chloride, commercial BioMinF (Biomin Technologies Ltd, London, UK), Chloride glass and Amorphous chloride glass (Table 1.1).

Table 1 Five different types of bioactive glass formulations used for the study

1)	Laboratory manufactured 45S5
2)	Mix glass containing fluoride and chloride
3)	Commercially available BioMinF
4)	Chloride glass
5)	Amorphous chloride glass

#### 1.4.5 Scanning electron microscopy study

The discs were immersed into 6% citric acid for 30 seconds to remove the smear layer. The etched discs were then rinsed with deionised water and dried prior to the commencement of the experiment. For each glass-based toothpaste, one disc was cut into four halves. One half was the control without brushing, and the other ones were brushed with a fixed amount of 50mg toothpaste for 30

seconds. The second half was rinsed with deionised water, dried and stored for SEM analysis. The third half was subsequently to brushing, immersed in artificial saliva for 1 hour and then dried and stored for SEM. The fourth half was immersed into 6% citric acid for 2 minutes, rinsed with deionised water, dried and stored for SEM (Table 2).

#### **1.4.6 Quantification of Tubule Occlusion based on SEM observation**

The number of tubules was assumed to be constant between the control section of etched dentine and the treated dentine section. This assumption was made because in many cases the individual tubules are no longer visible post treatment. The number of tubules in each section of control etched dentine varied between 56 and 72. However for each sample the control etched section was taken from the same mid coronal dentine slice and in close proximity to the etched dentine. Tubules were classified as fully open, partially occluded where particles were observed within the tubule or where materials was observed to have reduced the diameter significantly compared to the control section or fully occluded. Since fully occluded tubules in many cases could not be observed the number of fully occluded tubules was assumed to be the number of tubules in the control section minus the number of open tubules and the partially occluded tubules. The results were converted to a percentage. Typically about 100 tubules were examined for each treatment representing a random statistical error of about 10%.

Table 2 Test and control discs with the specific method of application of the toothpaste formulations

Section 1:	Control
Section 2:	Brushed with toothpaste

Section 3:	Brushed with toothpaste + Artificial saliva
Section 4:	Brushed with toothpaste + Acid challenge

The dentine discs were mounted onto stubs with conducting carbon cement. Each part of the disc was mounted flat with the upper side exposed for brushing. The samples were subsequently sputter coated with gold/palladium for SEM. The images were obtained from different fields of the disc at alternative magnifications such as 5000x and 10000x.

## 1.5 Results

1.5.1 It was evident from previous research in the Department that bioactive glasses have the capacity of occluding dentine tubules and as such may be an effective treatment for DH. In the present investigation, we used laboratory made artificial saliva to mimic the *in vivo* environment following brushing of the discs. The results showed very good surface deposition with characteristic plate-like crystals or needle crystals like formations in the various toothpaste samples. In the 45S5 sample, there was evidence that the Bioglass did not form apatite directly, but may have formed a phase called octacalcium phosphate (OCP) which displays a plate-like morphology which then may convert to a hydroxycarbonated apatite. The use of artificial saliva immersion following the toothpaste application was a useful addition to the methodology normally employed in *in vitro* studies in that it provided a similar environment to the oral cavity. For example, in the SEM images, there was clear evidence for hydroxyapatite formation with several hexagonal crystals observed following artificial saliva immersion. Apatite often has a hexagonal structure that can be recognized in the SEM images. Moreover, hydroxyapatite is a more stable crystal

compared to other calcium phosphates, under physiological conditions. However, when exposed to an acid challenge most of the hydroxyapatite layer formed with the artificial saliva dissolved, although there was still a degree of tubule occlusion present.

SEM analysis of the test and control discs can be observed in the following figures (Figs 1-5) for example, an SEM image of a control etched dentine surface with visible open tubules can be observed in Fig 1), please note a residual smear layer deposit was present due to the samples not being cleaned ultrasonically before the commencement of the study in order to mimic real life conditions.

The SEM images for the toothpaste containing 45S5 can be observed in Fig. 1A. In image B it may be noticed that after brushing some of the tubules were occluded by the particles. However, in image C, when immersed in artificial saliva for 1 hour, all the tubules were occluded, as indicated by the clear formation of a layer covering the treated surface. This formation would lend some support to the fact that the effects of the product were improved when immersed to artificial saliva compared to when the discs were only brushed without immersion in saliva. After the acid challenge, in image D, a significant part of that layer covering the tubules was removed, although it can be noted that some of the tubules were still occluded.

In Fig. 2, image B showed an improved coverage of the mix glass product compared to 45S5. Nevertheless, the layer formed with the artificial saliva immersion was similar but with different formations of particles. Following the acid challenge, as image C revealed, the layer was again rinsed away although some of the tubules remained occluded.

Fig.3 showed good coverage of the dentine surface with BioMinF. However, when immersed into artificial saliva, the layer formed was different than that formed with 45S5 and the mix glass. Particles of various sizes can be observed on the dentine surface and within the tubule lumen. Following the acid challenge, the results showed improvement compared to the other glasses. BioMinF was not totally washed away, and image C would suggest that almost the same amount of the dentine tubules maintained their occlusion (see image B).

In Fig. 4, the Chloride glass sample showed basically the same coverage as with the other images. This observation would suggest that although the layer covering the tubules was not formed with artificial saliva, the particles were not dissolved away with the acid challenge. Nevertheless, some of the tubules still remained open.

Finally, Fig. 5 showed an excellent surface coverage with the amorphous chloride glass based toothpaste. Particles of various sizes occluded most of the tubules. In image C, with artificial saliva immersion, the dentine surface appeared covered by the layer and there were different formations covering the tubules. After an acid challenge, the results were also encouraging as many of the dentine tubules were still occluded, and although the surface layer was dissolved away, there was evidence of tubular occlusion within the tubules.

Figure 6 shows the percentages of open tubules, partially occluded tubules and occluded tubules estimated from the SEM micrographs. It can be seen that all the toothpastes exhibit significant increases in occlusion post brushing with over >90% of all treatments resulting in complete and partially occluded tubules post brushing. Furthermore the tubule occlusion generally increases upon immersion

in AS but generally declines after the acid challenge in citric acid. The exception here is the BioMinF which shows almost 100% tubule occlusion after the acid challenge.

When comparing the control images with those images following brushing with the bioactive glass based toothpaste formulations it may be concluded that all samples provided a degree of tubule occlusion following brushing with the respective toothpastes (Figs. 1-5).

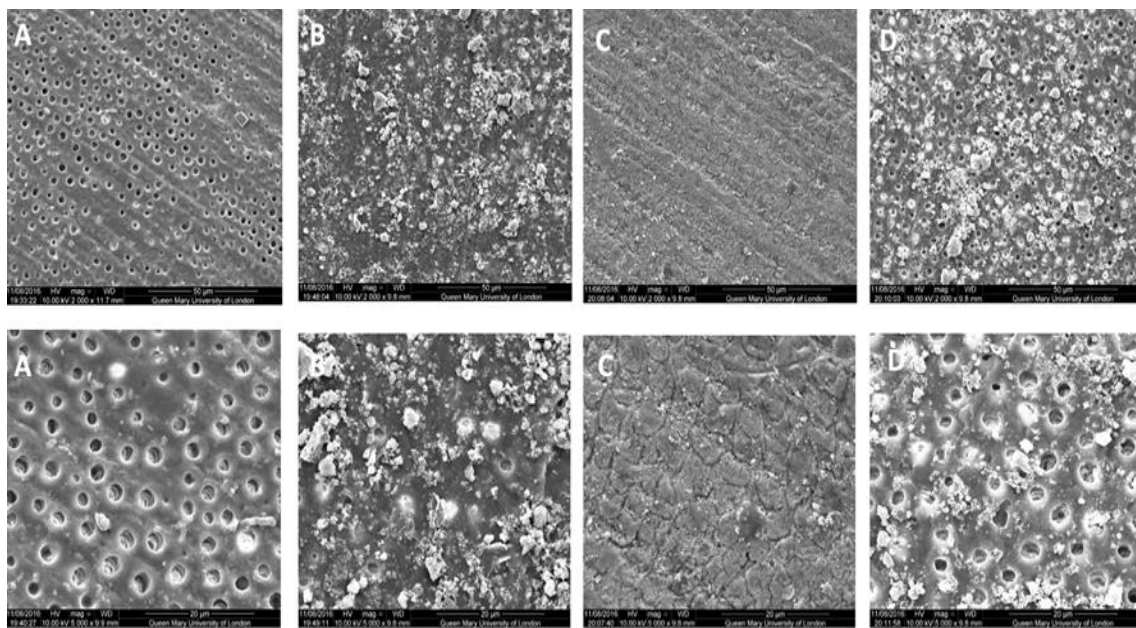


Fig. 1 SEM image of the dentine surface morphology in disc 1 treated with a 45S5 glass based toothpaste at 5000x (top) and 10000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

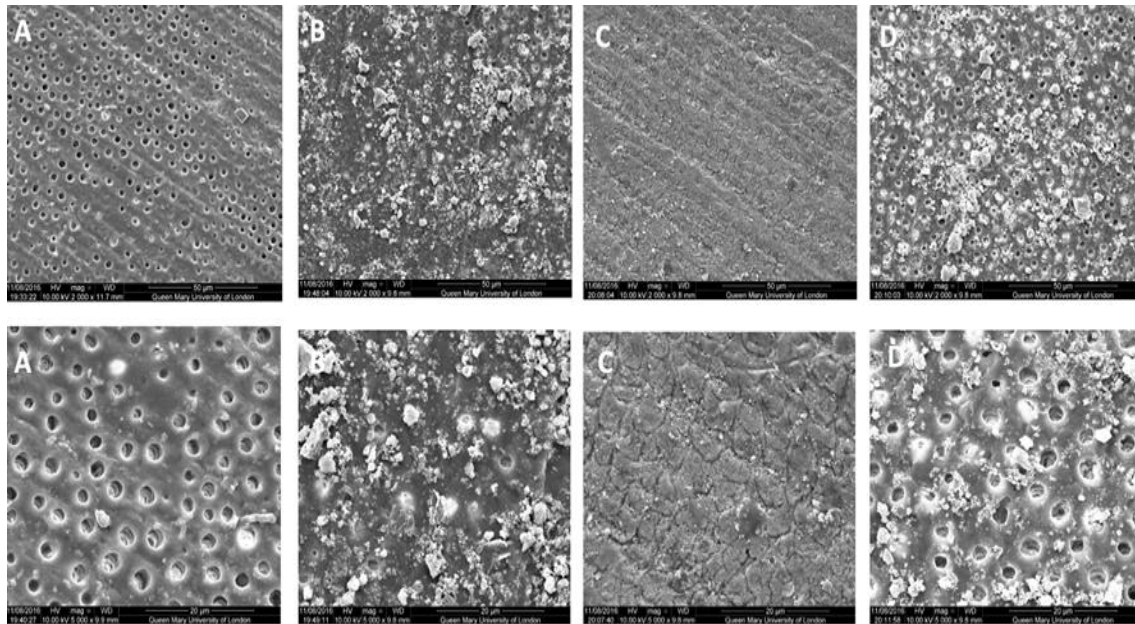


Fig. 2 SEM image of the dentine surface morphology in disc 2 treated with a mix glass based toothpaste at 2000x (top) and 5000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

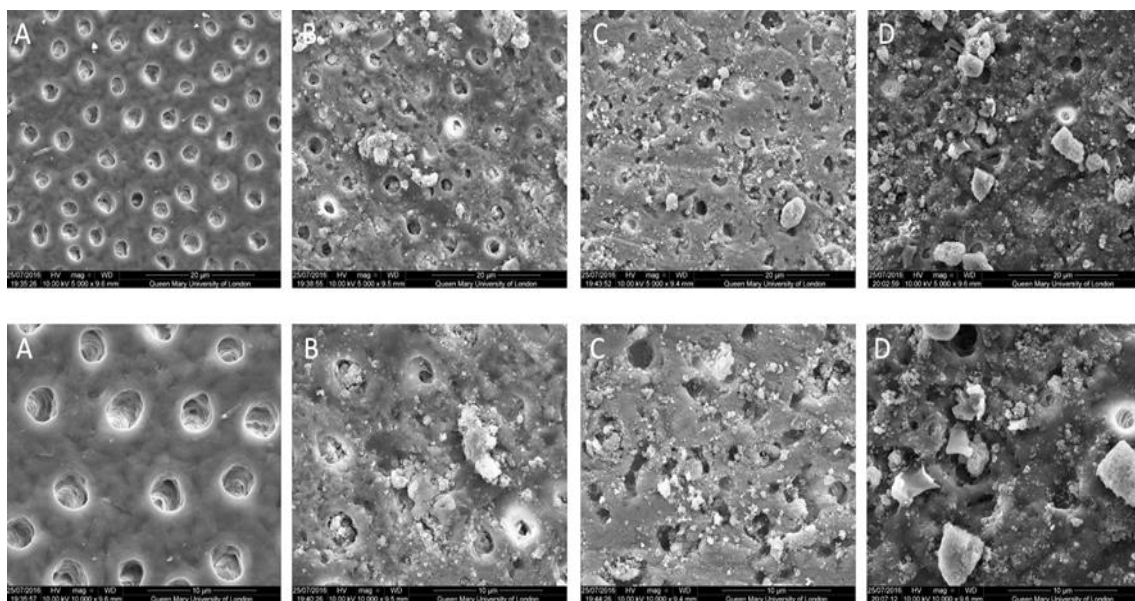


Fig. 3 SEM image of the dentine surface morphology in disc 3 treated with a BioMinF glass based toothpaste at 5000x (top) and 10000x (bottom)



magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

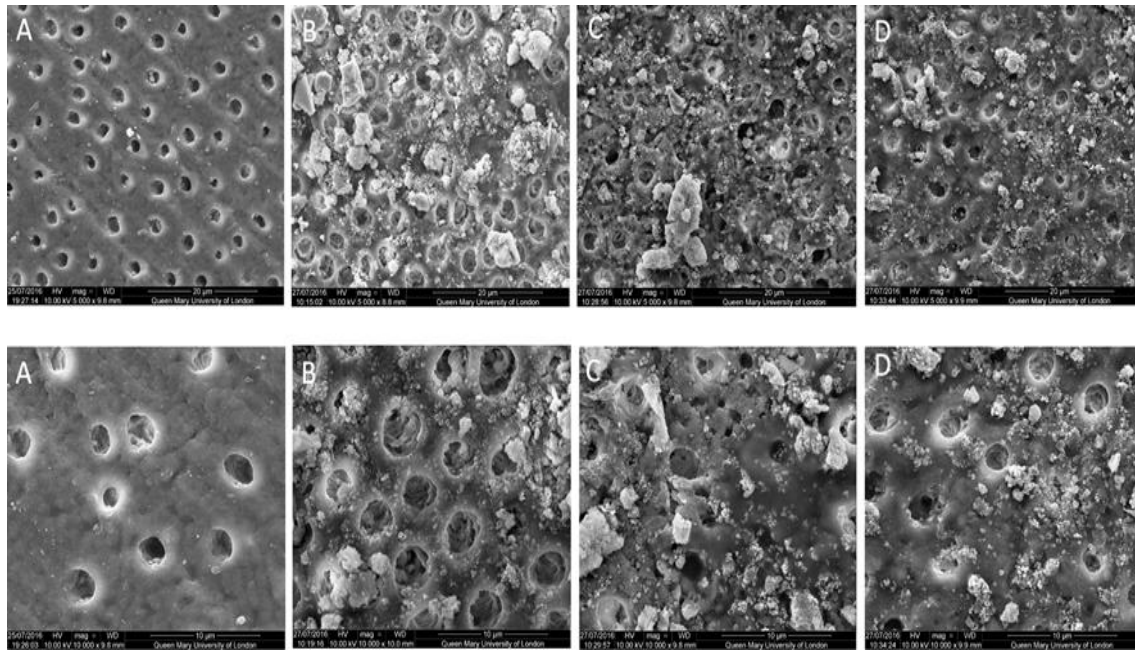


Fig. 4 SEM image of the dentine surface morphology in disc 4 treated with a chloride glass based toothpaste at 5000x (top) and 10000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

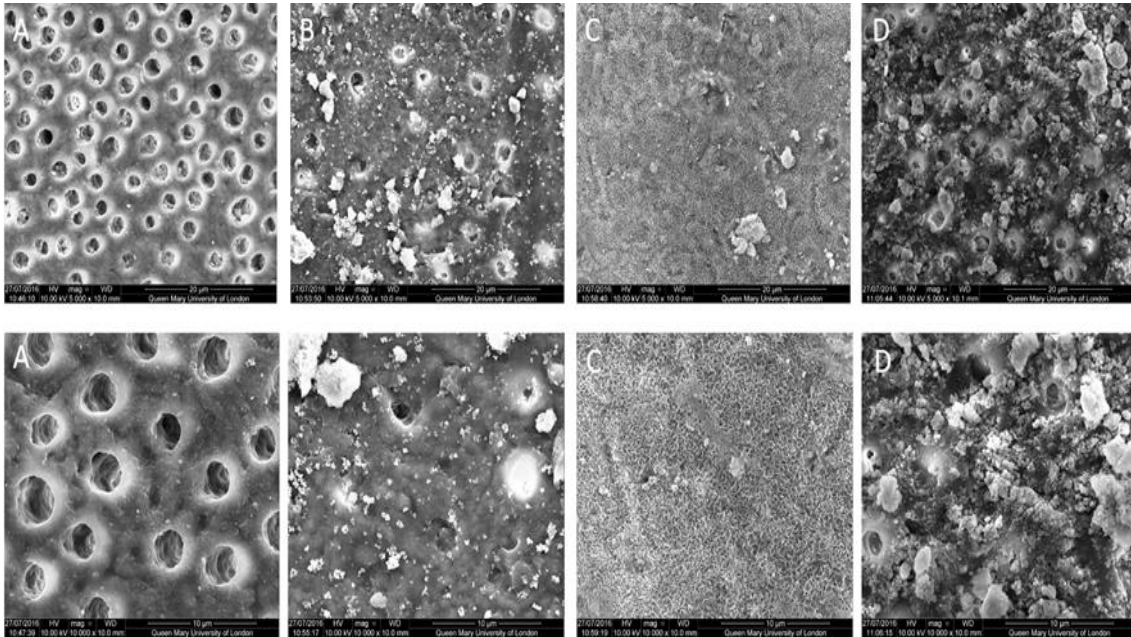
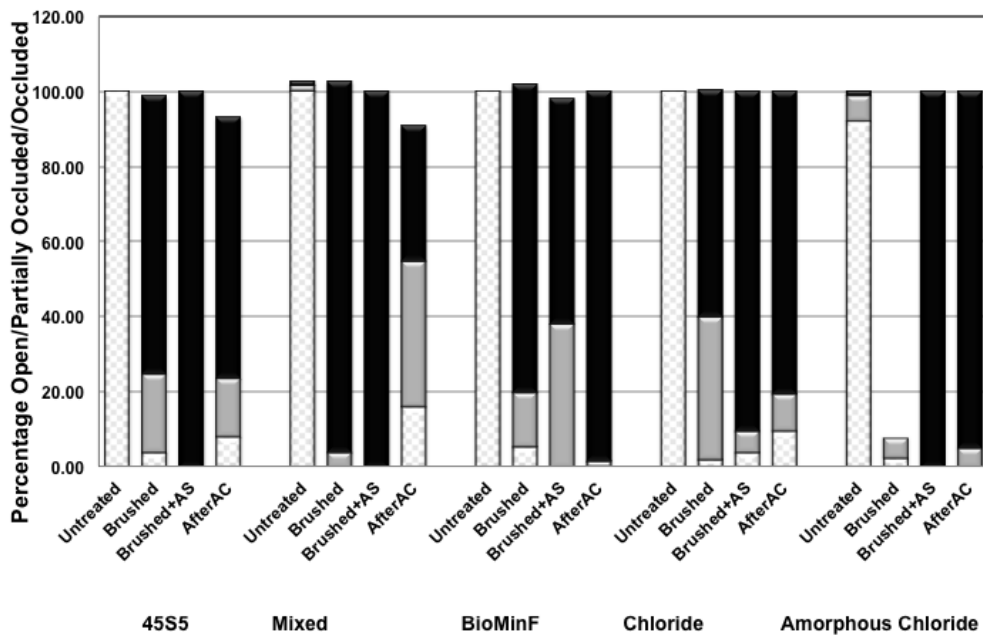


Fig. 5 SEM image of the dentine surface morphology in disc 5 treated with an amorphous chloride glass based toothpaste at 5000x (top) and 10000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

Fig 6 Percentage of open tubules (□) partially occluded (■) and occluded tubules (■) present after different treatments.



## 1.6 Discussion

It was evident from previous *in vitro* studies that bioactive glasses have the capacity of occluding dentine tubules and as such incorporating these glasses into toothpaste formulations may be beneficial for those individuals who suffer from DH. In the present *in vitro* study, artificial saliva was formulated to simulate the *in vivo* environment following brushing the teeth. The results indicated that there was a good surface deposit with characteristic plate-like crystals and needle crystals formations in the different samples.

A study by Wang *et al.* (23) compared different types of bioactive glass based toothpastes. Although these formulations were designed to deliver potassium ions to treat DH, the silica and calcium particles clearly occluded the tubules together with the smear layer produced by the application of the toothpaste. The SEM images of the toothpastes indicated that the toothpastes showed no resistance to an acid challenge, and the citric acid removed the particulate-coating layer from the tubules orifices. Furthermore, the precipitates

formed were more resistant to an acid challenge when inside the dentine tubules. Another observation from this study which was of interest was that when a tooth was immersed in artificial saliva there was an increase in fluid flow as measured in a hydraulic conductance model. This observation is somewhat contradictory as one would have expected a decrease in fluid flow following immersion.

In the 45S5 sample investigated in the present study, there was an indication that the Bioglass formulation does not form any apatite directly and may form an octacalcium phosphate (OCP) phase, which has a plate-like morphology. OCP is recognised to be a precursor for hydroxyapatite formation when the pH is  $\leq 9$  in the absence of fluoride. Moreover, the fluoride ions can either aid the in the conversion of OCP to apatite or result in direct apatite formation. Therefore, OCP has an attractive potential for remineralisation since OCP can incorporate a source of fluoride for catalysing the transformation of OCP to apatite and for the formation of a more acid-durable fluoridated apatite (1).

In a novel laboratory study (16), the Investigators synthesized a varnish containing potassium chloride (KCl) and fluoridated hydroxyapatite (FHA). The SEM images obtained from the study indicated that the dentine tubules were occluded in the varnish FHA group. KCl-FHA varnish could release potassium ions and reduce hydraulic conductance of the dentine discs and may therefore be a suitable option for the treatment of DH. FHA had the ability to occlude dentine tubules over time, however the samples were not subjected to an acidic challenge or any other food and beverages that would have an impact in the clinical environment.

In the present study, the SEM images demonstrated evidence of hydroxyapatite formation as several hexagonal crystals were observed within the

images following artificial saliva immersion. Apatite has a hexagonal structure that can be easily recognized. Moreover, hydroxyapatite is relatively stable when compared to many other calcium phosphates, under physiological conditions though it is not as stable as fluorapatite. When exposed to an acid challenge most of the layer formed with the artificial saliva, which is thought to be hydroxyapatite was dissolved, although there was still evidence of some tubule occlusion. The BioMinF treatment resulted in a layer consisting of fine highly elongated needle like crystals in contrast to the more plate like crystals or short stubby crystals formed with the fluoride free toothpastes. Fluoride is known to promote the formation of fluorapatite, which generally forms as needle like crystals and is much more acid durable. The BioMinF treatment resulted in the greatest tubule occlusion following the acid treatment, which probably reflects the formation of more acid resistant fluorapatite.

The needle-like formations similar to that observed in the images, were also observed in both the chloride glass and amorphous chloride glass images. A study by Masahiro *et al.*, elucidated that the formation of needle-like structures after immersion in artificial saliva were enriched with both Ca and P, and as such the bioactive glass-coated alumina produced a crystal which may be calcium phosphate (24, 25).

Recent studies have made considerable progress in elucidating the effects of Bioglass particles on the tooth structure through the *in vitro* evaluation of different chemical composition(s) of Bioglass containing toothpastes (20). For example, several studies have reported different outcomes when evaluating chloride containing toothpastes. Several *in vitro* studies have demonstrated that a small crystalline deposit is precipitated on to the dentine surface which can

easily be rinsed away, however other studies have reported positive effects of the chloride containing products on the relief of DH (16, 26).

When comparing chlorine and fluoride toothpaste formulations there were conflicting results reported in the published literature with several clinical studies indicated that there were no differences in efficacy between the two products and other studies indicating there were differences in favour of chloride containing toothpastes. This observation was also true when comparing *in vitro* studies alone as well as in comparative studies using cross -sections of dentine where no tubular occlusion was observed following treatment with a SnF<sub>2</sub>-containing toothpaste (18).

A study published in 2013 (27) aimed to compare the effectiveness of a one-minute application of a polishing prophylaxis paste containing 15% calcium sodium phosphosilicate with and without fluoride compared to a on fluoride polishing control in reducing post therapy DH following a dental scaling and root planing procedure. DH was assessed by both tactile and air blast stimuli at baseline, immediately following polishing and 28 days after the single application (Subjects were provided with a non-desensitizing toothpaste for the duration of the study). The results showed a significant reduction of sensitivity after 28 days of treatment for both groups with or without fluoride which would suggest that any improvement in DH was independent of the presence of fluoride (Note; There a many confounding factors in running DH studies which could affect the results in this type of study) (28).

A further *in vitro* study (6) reported that toothpaste formulations with different proportions of Bioglass replacing the silica compounds (2.5% and 7.5%) provided greater surface coverage than that of the original Bioglass product. However, the

study did not assess the composition of the particles deposited on the dentine surface or within the dentine tubules. Furthermore there was no reported determination on whether the deposit was an abrasive component e.g., silica or whether the deposit consisted of Bioglass particles or a precipitation of calcium phosphate following ion exchange on the surface of Bioglass.

Although the *in vitro* effectiveness of potassium containing toothpastes was not the focus of the present study, a brief comparison of the effectiveness of products that occlude the dentine tubules (tubular occlusion) compared to the effect of potassium containing toothpaste claimed to work by blocking a pulp nerve response is worthy of some comment. For example, Acharya *et al.* compared the efficacy of toothpastes containing calcium sodium phosphosilicate and potassium nitrate and reported that calcium sodium phosphosilicate had a greater reduction in DH than potassium nitrate (17). Moreover, this systematic review presented an overview on clinical trials of calcium sodium phosphosilicate (CSPS) to treat DH. CSPS was reported to provide superior results in reducing DH compared to potassium nitrate containing toothpastes. Investigators have also suggested that when in contact with body fluids CSPS reacts forming a layer of hydroxyapatite that can occlude dentine tubules. The results with CSPS were also reported to be more effective than negative controls (14).

It is recognised, however that the quantification of the number of occluded tubules is somewhat subjective and perhaps the limitations in the study was the lack of fractures specimens to view the depth of penetration into the tubules. Nevertheless the semi-quantification method used in the study does support the observations from the SEMs that there was a degree of tubular occlusion and that this varied between the experimental toothpastes. Furthermore, evidence

from an unpublished report that included this study indicated that the hydraulic conductance values of the BioMinF toothpaste supported the SEM observations indicating that the dentinal tubules were occluded (29).

One final review that may be relevant in this discussion on the effectiveness of CSPS is a systematic review by Talioti *et al.* (30). These Investigators compared the evidence of OTC desensitising products (e.g., calcium sodium phosphosilicate, amorphous calcium phosphate, nanohydroxyapatite and tooth mousse toothpaste/gels) in reducing DH. One of the problems reported by these Investigators that there was a lack of published studies directly comparing these four products. Furthermore, although there was evidence for the effectiveness of CSPS in occluding the dentine tubules from the *in vitro* studies, no conclusion could be made regarding the clinical efficacy of the various desensitizing toothpastes compared in the review. This was due in part to different study designs and methodologies used in the various studies and the fact that there were relatively few randomized controlled trials (RCT) available for analysis (28). A systematic review and meta-analysis by Zhu *et al.* (31) also recognised the limitations of the published studies evaluating CSPS formulations in both toothpaste for DH and in prophylaxis polishing pastes for post-periodontal therapy hypersensitivity. These investigators recommended that further non-industry Independent) supported clinical studies should be conducted prior to making any definitive recommendations regarding the efficacy of these formulations

## **1.7 Conclusions**

Of the various compositions of bioactive glasses assessed in the present *in vitro* study, all glass compositions demonstrated surface coverage after



brushing with the formulated toothpaste. The formation of a hydroxyapatite layer occluding the dentine tubules following artificial saliva immersion, may be considered an important stepping stone for further evaluation of these bioactive glass compositions. One of the innovative features of the study was the incorporation of an acid challenge to mimic the oral environment. Although the glasses, in particular the BioMinF composition were resistant to an acid challenge, there was no doubt that further research was required to identify a different formulation or component that was not removed when immersed in a citric acid solution. In conclusion the results from the present *in vitro* study would appear to support the growing evidence in the published literature that toothpaste formulations containing bioactive glasses occlude dentine tubules and therefore may be an effective approach treating DH.

#### *Conflict of Interest*

Robert Hill, Xiaojing Chen and David Gillam have one or more patents on the bioactive glass formulations as described in the study. Robert Hill and David Gillam are co-founders and Directors of Biomin Technology Ltd.

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An earlier version of this work was presented as a poster at IADR/AADR/CADR General Session & Exhibition. San Francisco, Calif., USA - March 22-25, 2017.

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## Legend for Tables and Figures

### Tables

Table 1 Five different types of bioactive glass formulations used for the study

Table 2 Test and control discs with the method of application of the toothpaste formulations

### Figures

Fig. 1 SEM image of the dentine surface morphology in disc 1 treated with a 45S5 glass based toothpaste at 5000x (top) and 10000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

Fig. 2 SEM image of the dentine surface morphology in disc 2 treated with a mix glass based toothpaste at 2000x (top) and 5000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

Fig. 3 SEM image of the dentine surface morphology in disc 3 treated with a BioMinF glass based toothpaste at 5000x (top) and 10000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

Fig. 4 SEM image of the dentine surface morphology in disc 4 treated with a chloride glass based toothpaste at 5000x (top) and 10000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

Fig. 5 SEM image of the dentine surface morphology in disc 5 treated with an amorphous chloride glass based toothpaste at 5000x (top) and 10000x (bottom) magnifications. A: Control; B: After brushing with a bioactive glass based toothpaste; C: After brushing with a bioactive glass based toothpaste + artificial saliva immersion for 1 hour; D: After brushing with a bioactive glass based toothpaste + acid challenge with 6% citric acid.

Fig. 6 Percentage of open tubules (□) partially occluded (□) and occluded tubules (□) present after different treatments.