To characterize the mineralization potential of CPP-ACP and a BIOMINF® glass using MAS- NMR at an acidic pH

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Abstract

Objectives: Casein Phosphopeptide and Amorphous Calcium Phosphate complex (CPP-ACP) is the main remineralizing additive in GC Tooth Mousse®. The objective of this study was to analyze the fluoride mineral phase formed on enamel after application of GC Tooth Mousse (GC), BIOMINF® (BF) and fluoride in an acidic environment.

Methods: Enamel blocks (n=1), measuring ~5×5mm with a maximum thickness of ~1mm, were cutfrom caries-freepermanenthumanmolars. Eachenamelblockwasimmersedin50mlofacidicsolution (0.1M acetic acid pH 4.0) at 37°C for 24hrs. Subsequently, the samples were subjected to remineralization by immersing them in a fresh acidic solution (0.1MaceticacidpH4.0) containing either 1g of BF, 1g GC, 1g GC +18ppm F – or 18ppm F. These samples were then stored for a further 96 hrs at 37°C. All five enamel blocks were accurately weighed (±0.0001g) before and after the demineralization and remineralization cycle to calculate the percent weight loss/gain. At the end of their mineralization cycle the enamel blocks were ground to a powder and analyzed for fluoride mineral phase using ^{19}FMAS-NMR. The remineralization solutions were tested for changes in pH using a calibrated pH electrode. Changes in F-concentration was monitored using ISE, whereas Ca and P concentrations were analyzed using ICP-OES.

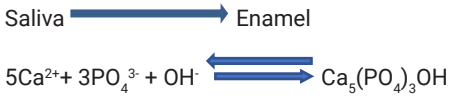
Results: BF showed highest weight increase during remineralization cycle. This was followed by GC + 18ppm F and GC Tooth Mousse. The18ppm F-samples showed a further weight loss. BF showed enhanced buffering capacity as compared to any of the materials tested. It also showed highest consumption of fluoride during remineralisation.^{19}FMAS-NMR spectra showed formation of fluorapatite and CaF_2 in varying proportions for all the samples. However, for 18ppm F- samples, the peak for CaF_2 was more prominent as compared to others.

Conclusion: Both BIOMINF® and GC tooth Mousse have enhanced remineralization properties and forms acid resistant appatite on the surface of enamel.

Keywords: ACP-CPP, remineralization, demineralization. Inductively coupled plasma-optical emission spectroscopy, Magic angle spin-nuclear magnetic resonance.

Introduction

Tooth structure is subjected to constant demineralization and remineralization processes in the oral cavity (1). Therefore, any disturbance in the pH of the oral cavity will destruct tooth structure. Saliva serves as a natural buffer of the mouth, providing free calcium and phosphates that aids in maintaining the integrity of the tooth (2). Therefore, any disturbance in the pH of the oral cavity will destruct tooth structure. At pH ≤ 5.5, the reaction between hydrogen ions, produced by bacterial metabolism, and the phosphate group of enamel crystals leads to enamel dissolution/demineralization (Robison et al., 1995). This process can be reversed at normal pH and in presence of calcium and phosphorus ions. Incipient enamel lesions can be remineralized, especially using treatments to promote remineralization (Lata et al., 2015). The equation for remineralization and demineralization in saliva is as such thatCa^{2+}, $PO4^{3-}$ and OH- ions in saliva are in dynamic equilibrium with apatite mineral in enamel.



$$5Ca^{2+} + 3PO_4^{3-} + OH^- \rightleftharpoons Ca_5(PO_4)_3OH$$

In case of an acidic pH, ionic substitutions take place in the apatite such that OH- can be substituted by F- to form fluorapatite that is a more acid resistant apatite. (Berkovitz, B 2010).Saliva serves as a natural buffer of the mouth, providing free calcium and phosphates that aids in maintaining the integrity of the tooth. (Edgar WM 1992). It buffers the pH back to neutral (7) during an acidic challenge, hence protecting the tooth (Anderson 2001). This is achieved by a rapid increase in the bicarbonates ions in saliva, the concentration of which increases 10-15 times during stimulated salivary flow. But if the tooth is continuously subjected to a lower pH for a prolonged time than saliva alone is insufficient to provide protection to the dental hard tissues and tooth is prone to demineralization. In such case it requires aid from an external source which would deliver adequate amount of calcium phosphate and fluoride to maintain the integrity of the dental hard tissue by forming fluorapatite which is more acid resistant than a carbonated hydroxyapatite. Topical application of fluoride has been proven to be more effective than systemic delivery of fluoride. (Ripa 1991).

In current approaches for caries prevention a paradigm shift is evolving in dentistry that is focused more towards a minimal invasive approach and preservation of tooth structure (Farooq et al 2013). In order to achieve this goal, Manufacturers have introduced multiple calcium and phosphate based remineralization systems which incorporate specific form calcium & phosphates to overcome the limited bioavailability of calcium and phosphate ions for remineralization process (3). Long bottom et al proposed in 2009 that an ideal caries preventive material should have the ability to release calcium and phosphate in the oral environment. Nowadays there are many novel dental materials that contain these fundamental ingredients of calcium phosphate and fluoride in the

form of ACP-CPP (GC), calcium sodium phophosilicates (Novamin) or fluoride containing bioactive glasses (4). But the mechanism of release of calcium, phosphate and fluoride are different which directly effects their bioavailability and efficacy to form a stable apatite on surface of enamel.

An overview of Bioactive glass

The emphasis of this study was on fluoride containing bioactive glasses and its effects regarding formation of fluorapatite. Therefore, structure and function of fluoride containing bioactive glasses needs to be understood.

The network connectivity is one of the key aspects in explaining the properties of glass. Network connectivity can be described as the number of bridging oxygen atoms per network forming elements (R. Hill 1996). Fluoride containing bioactive glasses is different than pure silicate glasses on the bases of network connectivity. The equation for network connectivity is given as,

$NC = (4\{SiO_2] - 2[M1/2\ O - MII\ \ O] + 6[P_2O_5])/SiO_2$

The network connectivity of pure silicate glasses is 4 while that of fluoride containing bioactive glasses is around 2 (Brauer et al., 2009). As mentioned above, the network connectivity provides vital information regarding the properties of the bioactive glass therefore by knowing the network connectivity we can easily predict how the glass would behave when it comes in contact with bodily fluids. We can assess the surface reactivity of the glass, solubility and bioactivity. The possibility of the glass undergoing glass into glass phase can also be predicted. (Hill 1996) (Elgayar et al., 2005). Therefore, a bioactive glass having Network connectivity of 4 is difficult to degrade and breakdown rendering it not suitable for use in dental applications as we would want the glass to degrade in a limited time frame to bring out the desired effect. Its beneficial if the network connectivity of a glass used for dental applications is low. With a network connectivity of 2, the glass network changes from cross linked to a linear chain of decreasing molar mass, it will result in decrease in the glass transition temperature and there would be an increase in the glass reactivity and solubility.

An overview of Fluoride containing Bioactive glasses:

Incorporation of fluoride in glass plays a vital role in the glass properties with respect to dental application.The composition of fluoride containing glasses is such that bioactive glass consists of SiO_2–P_2O5–CaO–Na_2O and CaF2 being amorphous in nature (Brauer et al.,2009). The fluoride is added to the glass in the form of CaF. While the network connectivity (NC) and the ratios of all other components are kept constant. This provides the network connectivity of 2.13 which is very suitable to be used for dental application. The addition of fluorine to the glass does not form Si-F bonds but instead forms multiple calcium sodium fluoride like species with in the glass. (Brauer et al., 2009).The phosphate is present in the form of orthophosphate which is similar to that present in human enamel. The phosphate that is present in the glass does not interfere with the actual glasses network backbone instead it is charge balanced by calcium and sodium cations present in the glass. (Brauer et al., 2009) with the help of Magic Angle Spin Nuclear Magnetic Resonance (MASNMR). It is a very useful tool to predict the form of fluoride, phosphate and silica present in the glass. It is a technique that is very sensitive to elements that are magnetic and respond to a magnetic field.

An overview of glass degradation in simulated body fluids and its effects on the pH:

Incorporation of fluoride in glass plays a vital role in the glass properties with respect to dental application. Fluoride has the ability to inhibit dental caries by preventing enamel and dentine demineralization hence promoting remineralization (Thuy et al., 2008).

The bioactive glasses without the incorporation of fluoride, when placed is simulated body fluid (SBF) causes a rise in the pH. This rise in pH is estimated to be around 7.95 to 8 according to a study conducted by R.G Hill et al 2010. Such a pH rise can cause damage to the oral tissues by disrupting the membranes therefore causing damage to the oral mucosa rendering it not suitable to be used in a tooth paste with such properties. On the other hand, when fluoride is incorporated into the glass a considerable decrease in the pH is observed. The pH dropped to 7.3 after 3 days post immersion into the SBF (Brauer, D 2010).When glass comes in contact with the physiological fluid e.g. (saliva or water) the H ions from the saliva hydrolyses the glass and the structure of the glass starts to break. As the glass degrades, there is a series of ion exchange on the surface of the glass. The cations from the glass primarily being calcium or sodium go into the solution in exchange for H+ ions. This process is added by dissociation of H+ and OH- ions of water if in the oral cavity which results in pH increase. In a similar manner the fluorine ions get substituted with the OH- and as a consequence of this process the OH ions are removed from the solution hence buffering the alkaline ion effect of the glass. (Brauer, D 2010).

The Structure and Function of ACP CPP:

The technology of ACP CPP is based on a milk protein called “casein” that forms nano clusters with calcium and phosphate and takes the shape of a micelle (Younus et al., 2016). Casein proteins and calcium phosphate form large colloidal particles called casein micelles (Rollema 1992). They belong to a family of secretory calcium binding protein phosphoproteins that precipitate from raw milk at pH 4.6 at 20 degree Celsius. (Whitney et al., 1976)the amorphous calcium phosphate is stabilized, by casein phosphopetide (Reynolds 2008).

The casein milk protein has this characteristic ability to stabilize calcium and phosphate ions in neonates. (Keith et al., 2005). The casein phosphoprotein being colloidal in nature consists of four different subunits, mainly–- αs1-casein, αs2-casein, b-casein and k-casein. These subunits form a compound with phosphorus, calcium, water, and enzymes to make a spherical shape called a micelle. The purpose of amicelle is to make large insoluble molecules soluble in water. As mentioned above the micelle is very much water loving (hydrophilic) and readily dissolves in water. The basic function of each micelle is to carry concentrated forms of calcium and phosphates on the outside of the sphere while the insoluble components are present on the inside. (Uversky and Lyubchenko, 2014).

Further to the structural properties of phosphopetide that are responsible for stabilization of large amounts of amorphous calcium phosphates are supposed to have a sequence of A-B-C. “A” is thought to be a phosphoamino acid as phosphoserine, “B” is any amino acid and “C” a glutamate. (Reynolds 2008). The amorphous calcium phosphate is bound to multi phosphoserine residues, therefore allowing formation of small ACP-CPP clusters. (Oshiro et al., 2007). The CPP forms complexes with calcium and phosphate ions and stabilize them within the structure and makes them bioavailable for remineralization of subsurface lesions. (Cross et al., 2005). The CPP-ACP has the ability to substantially increase the level of calcium and phosphates ions in supragingival plaque therefore promoting remineralization of enamel subsurface lesion. Reynolds, E. (2008). The ACP CPP is also thought increase the calcium and inorganic phosphate ions levels in supra gingival plaque. It sticks to salivary pellicle and to the surface of bacteria in the plaque biofilm to inhibit demineralization. Reynolds, E. (2008). The ACP-CPP also has antibacterial properties as it decreases the count of strept. Mutans as it integrates to the enamel pellicle. (Schupbach et al.,1996).

It also plays a vital role in buffering the plaque pH. It accomplishes this capacity to buffer the pH by delivering amino acids binding protein which acts as buffers; this in turn increases the calcium

and phosphates levels in the plaque. The ACP -CPP also has this characteristic ability to bind with hydroxyapatite of human tooth and increase the bioavailability of calcium and phosphate ions, thus maintaining a supersaturated state of these minerals in the oral environment. This aids in preventing demineralization and encouraging remineralization. (Rahiotis et al., 2008).

The CPP and ACP has a tendencyfor inhibition of caries (5), inhibition of enamel demineralization in vitro (6), promotion ofenamelsubsurface lesion remineralization (5). A combination of CPP-ACP with fluoride had asuperior remineralization effect over individual use of ACP-CPP or fluoride (3, 7). But this conclusion was in contradiction with study conducted by (Xiaojing Chenet al 2017) which showed that the GC Tooth Mousse plus was forming fluorapatite and calcium fluoride within the tube prior to its application on to the tooth. In this study18 ppm fluoride was added to ACP-CPP during the experiment to observe for formation of fluorapatite on the surface of enamel. The emphasis of this study was focusedon two materials, The (BIOMINF® Mousse) containing bioactive glassand (GC Tooth Mousse) containing amorphous calcium phosphate & casein phosphopetide.

There first objective of the study was to analyze the fluoride mineral phase formed onenamelafter application of GC Tooth Mousse (GC), BIOMINF® Mousse (BF) and fluoride in anacidicenvironment using ^{19}F MAS-NMR andISE. The second objective was to assess their bioactivity and ability to form a stable apatite on the surface ofenamel and to analyze the effect of ACP-CPP when fluoride was added to it separately during the study.

Methodology

A cross sectional in vitro study was done at Dental Institute of Queen Mary University of London.

Inclusion criteria:

Surgically extracted Caries free permanent molars and premolars.

Exclusion criteria:

Any tooth with restorations, fillings, caries, or any pathologies that may have affected the enamel would not be included in the study.

Preparation of Human Enamel blocks:

Caries free permanent Molars were used with approval from Queen Mary research Ethics committee QMREC2011/99) to cut five enamel blocks of approx. (5*5 mm) as shown in fig1with a maximum thickness of 1mm using a diamond blade (Struers Accutum 5 instrument).The location of each cut was recorded. The linear dimensions and weight of each specimen was also recorded. The specimens were stored in 0.1% thymol solution. (Figure1).

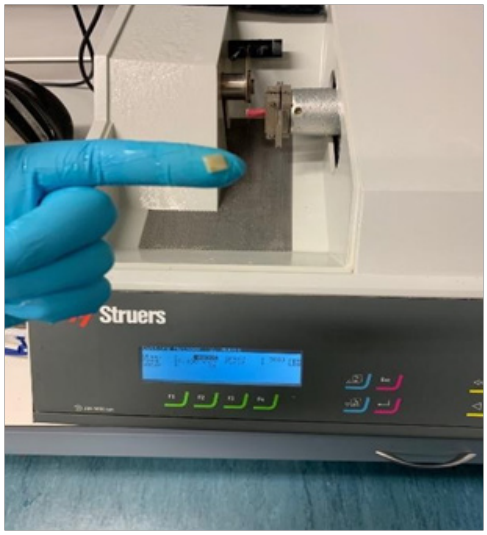


Figure1. Preparation of Enamel Block measuring ~5×5mm with a maximum thickness of ~1mm.

Demineralization of the samples:

A 1-liter batch of 0.1 molar acetic acid solution was prepared and buffered with potassium hydroxide to pH 4.0. Each enamel block was placed in a plastic container with 50 ml of de-mineralizing solution at 37°C in a shaking incubator (KS 4000i control IKA, UK) at 60 rpmfor24 hours. Before and after immersion each enamel block was blot dried and weighed using balance accurate to ± 0.0001 g (mettler HK, Switzerland) to record the baseline value of the sample. Each plastic container was labelled from 1 to5.

Remineralization of Specimens:

The acetic acid concentration and pH of the remineralization solution was kept similar to that of Demineralization solution. The samples were placed in the following order after application of the remineralizing agents.

Group 1: control sample immersed in 50ml acetic acid (control)

Group 2: 1g of BIOMINF® Mousse in 50ml acetic acid

Group 3: 1g of GC Mousse in 50ml acetic acid

Group 4: 1g of GC Mousse + 18ppm Fluoride in 50ml acetic acid

Group 5: 18ppm Fluoride in 50ml acetic acid

The above samples were stored for 96h in a shaking incubator at 37°C.

^{19}F MAS-NMR

After 96h the enamel blocks were taken out of their respective solutions, blot dried andweighed.The enamel blocks were ground to powder using mortar-pestle for solid state ^{19}FMAS-NMRusing 600MHz (14.1T) spectrometer (Bruker, Germany) and run at a Larmor frequency of564.5MHz under spinning conditions of 22 KHz in a 2.5mm rotor. It helped selectively probe the local environment for only fluorine atoms in the samples, permitting us direct identification of possible structural forms in which fluorine may existed with in enamel either fluorapatiteor calcium fluoride. The NMR spectra were obtained using a low fluorine background probe in a single pulse experiment with 30 s recycle duration. The samples were placed under MAS-NMR for 12-24 h and the acquired spectra were an accumulation of 600 and 1440 scans. The duration of the scans was dependent on the fluoride levels present in the sample. The chemical shift scale for fluorine was referenced to 120 ppm peak of 1 M NaF solution.

ICP-OES

The immersion solutions from the Remineralization cycle were analyzed for calciumandphosphorus concentration using ICP-OES. For quantitative analysis of calcium and phosphorous in immersion solution after reaction with enamel, the immersion solution was diluted to a factor of 1:20 to decrease background sodium levels. The solution was further acidified with 1% (0.1 ml of 69% nitric acid in 10 ml) and analyzed quantitatively by ICP_OES. (ICP;Varian Vista-PRO, Varian Ltd., oxford, UK). The measurements were taken repeatedly and the calibration solution used were similar to that of immersion solution (o.1 M acetic acid, pH 4) with similar ionic strength. The standards for calcium and phosphorus were used in concentration of 0.1-10 ppm.

ISE

Changes in fluoride ion concentration wereanalyzedusing a calibrated fluoride Ion Selective Electrode (ISE) (Orion 9609BN, 710Ameter, South Burlington, VT, USA) . The fluoride ion concentration in immersion solution was measured in ppm before and after the remineralizing cycle to get the baseline values for the total fluoride

present in each Group .ISE helped to assess the reduction in fluoride ion concentration in ppm after the remineralization cycle was complete. The changes in pH were also observed before and after the remineralizing cycle using a calibrated pH.electrode. As the pH of immersion solution was kept constant at pH "4" therefore any change from this pH for each group were monitored upon completion of remineralization cycle.

Results

The mineral weight loss/gain:

The mineral weight loss and gain was assessed by comparing the weights of the samples before and after the remineralization cycle. Figure 2 shows that for control sample (Group 1), in the absence of additional fluoride, calcium and phosphate the weight decreased by (16 %). (Group 2) containing BIOMINF® showed the highest weight increase by (3.17%) due to release of fluoride as it further reduced the demineralization of enamel. This was followed by (Group 3) containing GC tooth Mousse+18ppm fluoride that increased by (2.6%). (Group 4) containing GC tooth mousse without fluoride also showed a weight increase of (1.88%) due to release of calcium and phosphate which further inhibited the demineralization process of the enamel. (Group 4) containing fluoride alone showed a weight decrease of (3.25%).

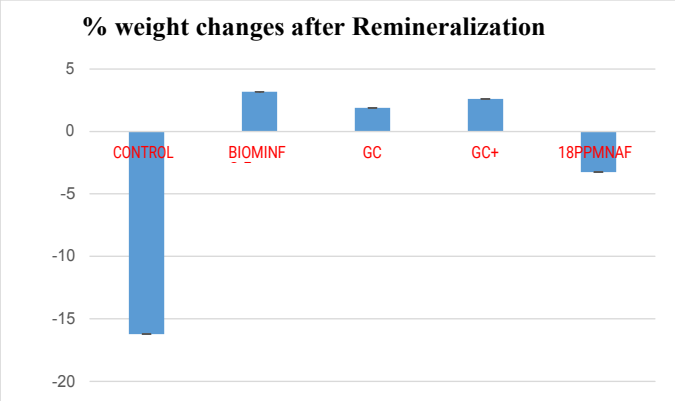


Figure 2. Percentage enamel weight loss and gain by each group after Remineralization.

pH changes:

The BIOMINF® showed highest increase in the pH (4.25) followed by GC that increased the pH to (4.20). GC +18 ppm NaF slightly increased the pH to (4.10). Sodium fluoride alone showed a decrease in pH to 3.85 as shown in figure 3.

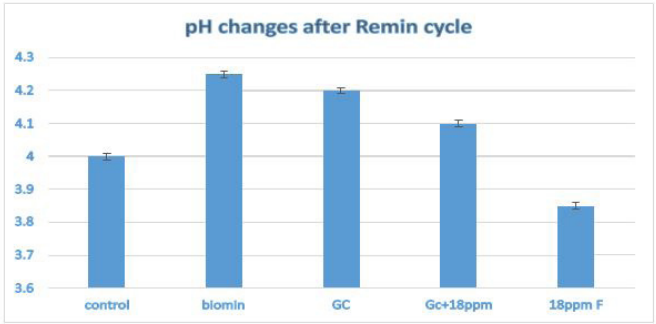


Figure 3.pH changes for each group after Remineralization cycle. The error Bar shows instrumentation error

ISE

As shown in figure 4, the consumption of fluoride during remineralization cycle from BIOMINF® was the highest. The fluoride ion concentration for BIOMINF® dropped from 10 ppm to 2 ppm. It was then followed by GC+ 18ppm NaF which showed almost 50% reduction in fluoride ion concentration. Sodium fluoride alone showed minimal reduction in fluoride ion concentration after remineralizing cycle. The reduction in fluoride ion concentration suggested formation of different fluoride compounds (calcium fluoride or fluorapatite). The results obtained by ISE were corroborated with 19F MAS-NMR to confirm for the presence of calcium fluoride or fluorapatite on surface of enamel.

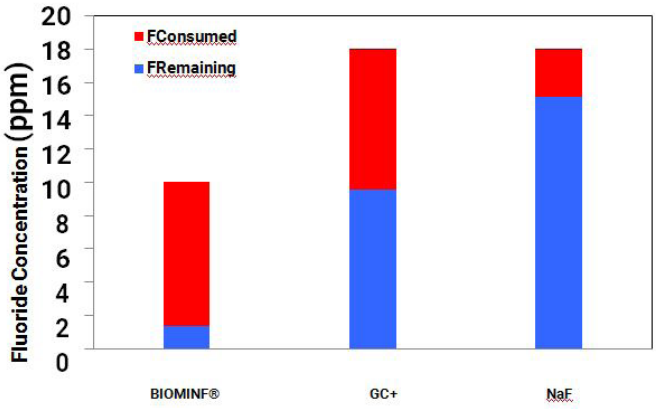


Figure 4.Fluoride Ion Concentration marked in Red and Blue showing fluoride remaining and fluoride consumed after remineralization.

^{19}F MAS-NMR

Figure 5 shows sequence of spectra obtained from 19F MAS-NMR after remineralization of enamel with Biomin, GC tooth mousse +18 ppm fluoride and 18 ppm fluoride alone. The reference spectrum for fluorapatite expressed a distinctive peak at -102 ppm which relates to the triangles F-Ca(3) of the apatite structure whereas the reference spectra for calcium fluoride showed a characteristic peak at 108 ppm corresponding to the F-Ca(4) site. The samples

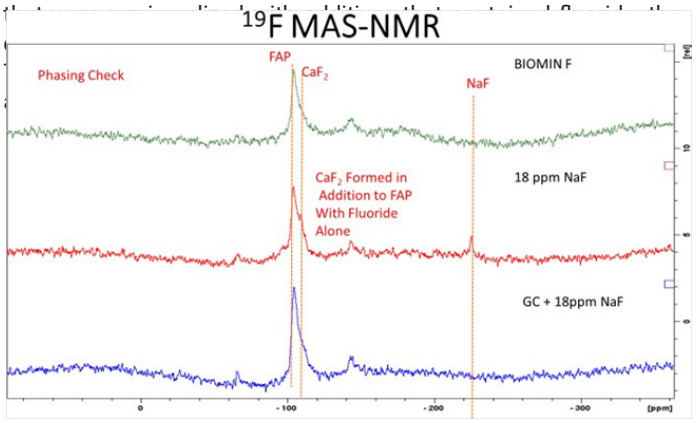


Figure 5.19 F MAS_NMR spectra of enamel blocks immersed in 1 g of BIOMINF®, GC + 18 ppm [F–] and 18 ppm NaF alone. Green spectra indicate for enamel block immersed in BIOMINF®. Red spectra indicate for enamel block immersed in 18ppm fluoride and blue spectra indicate for enamel block immersed in GC+18ppm [F–].

ICP-OES

Table 1 shows the Ca/P ratios that were calculated from ion release data taken from ICP-OES. The ICP-OES helped identify the release of calcium and phosphate into the reaction solution after demineralization and remineralization cycle. The ICP-OES analysis showed that there was controlled release of calcium and phosphate from GC and BIOMINF® in demineralization solution in the absence of enamel samples. After the enamel blocks were placed in the solution containing BIOMINF® and GC Tooth Mousse, a substantial decrease in calcium and phosphate was observed.

Table 1.Showing ICP- OES analysis of Calcium and Phosphorus detected in reaction solutions.

After Remineralization			
	[Ca] / ppm	[P] / ppm	Ca/P Ratio
Control	15.04	8.71	1.73
BIOMINF®	23.65	7.16	3.30
GC	32.17	26.11	1.23
GC+18ppm	33.71	28.00	1.20
18ppm NaF alone	11.17	5.79	1.93

Before Remineralization			
	[Ca] / ppm	[P] / ppm	Ca/P Ratio
BIOMINF®	22.28	6.91	3.23
GC	42.05	34.70	1.21

Discussion

The principal methodology used for this study was 19F MAS-NMR due to its ouststanding ability to identify for any structural form of fluorine present in a sample. This methodology has also been used previously by Yesinowsk and Mobley [1983] to distinguish between, (FAp), fluorohydroxyapatite and calcium fluoride (CaF 2). With the help of 19F MASNMR all F atoms in a sample can be detected [White et al., 1988]. The primary focus was to analyze for fluorapatite formed on the surface of enamel. The 19F MASNMR spectra (fig 5) provided a strong signal for fluorapatite at -102ppm and calcium fluoride at around -104ppm for powdered enamel samples that were immersed in BIOMINF®, GC + 18ppm fluoride and fluoride alone (group 5). The fluoride was predominantly present as fluorapatite and calcium fluoride for BIOMINF® and GC tooth Mousse. For 18ppm fluoride alone group, calcium fluoride was present in abundance as shown in figure 5. The 19F MAS-NMR results for BIOMINF® and GC + 18ppm were promising as they formed fluorapatite predominantly. The formation of Fluorapatite by these dentifrices is of great clinical importance as formation of fluorapatite is a key step in caries prevention and is more acid resistant than carbonated hydroxyapatite (Brauer et al., 2008).

The 19F MASNMR results were also corroborated by results obtained from ISE. The total ion concentration of fluorine was calculated for both BIOMINF® and GC PLUS in one gram of the paste. One gram of BIOMINF® consisted of 10 ppm fluoride and GC Plus contained 18 ppm fluoride. The fluoride ion concentration for BIOMINF® dropped to almost 2ppm and GC +18ppm dropped to 9 ppm which was suggestive of the fact that the fluoride is being taken up by enamel samples to form calcium fluoride or fluorapatite. The results were confirmed by 19F MASNMR which showed the presence of these compounds in powdered enamel samples.

In this study, fluoride was added separately to GC to assess its effectiveness to form fluorapatite. In a previous study conducted by (Xiaojing Chenet al 2017) showed a decrease in efficacy of GC PLUS that consisted of ACP-CPP with additional fluoride. ACP-CPP has been incorporated in a various material in the recent past as a fluoride replacement therapy to enhance remineralization of enamel (Chambers et al.,2013). GC also launched another Tooth Mousse which we mimicked in this study that was with addition of soluble fluoride along with ACP-CPP. The incorporation of fluoride was thought to enhance the remineralizing properties of the GC Tooth Mousse, but the effects were observed to be different. A recent characterization study conducted by (Chen et al., 2017) on ACP-CPP with fluoride suggested that there were varying degrees of conversion of the ACP to apatite prior to its application in the oral cavity. In addition to that, the GC Tooth Mousse containing fluoride was observed to be reacting with calcium and phosphate forming fluorapatite within the paste, hence compromising the remineralizing properties of the Mousse due to deficiency of ions that had to be Bioavailable for effective remineralization. (Chen et al., (2017). Addition of fluoride to GC during the experiment resulted in formation of fluorapatite on surface of enamel predominantly. It suggests that Incorporation of fluoride to GC in a different form may avoid its undesired conversion.

On the other hand, fluoride incorporated within the composition of glass exhibits 'smart properties' and enhanced remineralizing abilities in low pH environment (Braueret et al., 2010). A systematic review conducted by Taha et al 2017 suggested that based on in vitro finding in isolation, the bioactive glass may have enhanced enamel remineralizing properties compared to other topical materials including ACP-CPP and fluoride.

The ability of the glass to provide such enhanced remineralizing properties is due to the amorphous nature of the glass and the systematic degradation. When glass from the paste comes in contact with saliva, it readily reacts with the hydrogen cations (H3O+) that in turn causes the release of calcium and phosphate ions from the surface of the glass. This process of degradation of the glass also induces a rise in pH, which helps in increased precipitation of calcium and phosphate ions from the glass. This results in formation of calcium and phosphate layer. The reaction continues and the layer crystalizes into hydroxyapatites. (Hench 2006) and (Jones 2012). This buffering ability of the bioactive glass has also been confirmed in this study. Figure 3 show that the BIOMINF® buffered the pH from 4 to 4.5.

Similarly the GC asein phosphopeptide forms nanoclusters with amorphous calcium phosphate thus providing a pool of calcium and phosphate which can maintain the super saturation of saliva. Since CPP–ACP can stabilize calcium and phosphate in the solution, it can also help in the buffering of plaque pH and so calcium and phosphate level in plaque is increased asein phosphopeptide forms nanoclusters with amorphous calcium phosphate thus providing a pool of calcium and phosphate which can maintain the super saturation of saliva. Since CPP–ACP can stabilize calcium and phosphate in the solution, it can also help in the buffering of plaque pH and so calcium and phosphate level in plaque is increased Casein phosphopeptide forms nanoclusters with amorphous calcium phosphate thus providing a pool of calcium and phosphate which can maintain the super saturation of saliva. Since CPP–ACP can stabilize calcium and phosphate in the solution, it can also help in the buffering of plaque pH and so calcium and phosphate level in plaque is increased. Similarly GC tooth Mousse has characteristic ability to form nanoclusters with amorphous calcium phosphates and maintains a supersaturated state when it comes in contact with physiological body fluids. It also has the ability to stabilize calcium and phosphates. In solutions which aids in buffering the pH. (Farooq et al., 2013).

The results of this study confirmed as shown in figure 3 that GC buffered the pH from 4 to 4.20. The results obtained from ICP-OES showed a considerable decrease in calcium to phosphate ratios for GC after remineralization cycle was complete, which was suggestive of the fact that they became a part of the apatite.

Conclusion

In conclusion both BIOMINF® and GC had enhanced remineralization

properties. Both materials predominantly formed fluorapatite and calcium fluoride on the surface of enamel as confirmed by 19F MASNMR. Fluorapatite is more acid resistant than carbonated hydroxyapatite and is of great clinical significance. The controlled degradation of BIOMINF® increased its efficacy to form fluorapatite. It also helped to increase the bioavailability of calcium, phosphate and fluoride in demineralizing solution.

The study also suggests that GC plus can add fluoride in the form of sodium monofluorophosphate to avoid forming flourapatite and calcium fluoride within the tube prior to its application. This may increase its efficacy.

Conflict of Interest

There is no conflict of Interest.

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