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An in Vitro Comparison of the Enamel Remineralisation Potential of Bioactive Glass, Hydroxyapatite and CPP-ACP

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The objective of this research was to investigate the comparative in vitro enamel remineralisation potential of commercial toothpastes containing bioactive glass (BG) particles, hydroxyapatite (HAP) particles or casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) nanocomplexes. Eighteen extracted permanent teeth were coated with varnish leaving a window on the buccal surface and placed in demineralising solution for 24 h to create artificial caries-like white spot lesions (WSLs). The teeth were randomly assigned to six groups and sectioned longitudinally through the WSLs. The roots were removed and the teeth were re-varnished, leaving the WSLs exposed. Groups A, B and C were subjected to an optimum remineralisation protocol in which the “control” half of each tooth was incubated in artificial saliva for 24 h at 37 °C and the “treatment” half of each corresponding tooth was cyclically exposed to artificial saliva and to 1:2 toothpaste solution containing either BG, HAP or CPP-ACP, respectively. Groups D, E and F were subjected to an acid-challenge remineralisation protocol which was similar to that of Groups A, B and C but which also incorporated cyclic exposure to demineralising solution. Scanning electron microscopy and energy dispersive X-ray analysis were used to compare the remineralisation of the surface and depth of the control and treatment WSLs. Under optimum conditions BG and CPP-ACP provided sub-surface repair by diffusion of calcium and phosphate ions into the WSLs. HAP did not influence remineralisation under neutral pH conditions. Conversely, under acid-challenge conditions, HAP was able to dissolve to release calcium and phosphate ions which diffused in to the WSLs and also protected the enamel surface from further erosion. BG and CPP-ACP both coated the enamel surface under acidic conditions, although their ability to remineralise the body of the lesion was compromised at low pH.

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1. Introduction

Dental caries is the destruction of tooth tissue under the action of organic acids produced by the fermentation of carbohydrates by cariogenic bacteria [1, 2]. Enamel comprises ~ 90% substituted hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), which is cyclically subjected to dissolution (demineralisation) when the pH of the mouth is reduced and recrystallisation (remineralisation) via the diffusion of calcium and phosphate ions into the vacancies created during the acid-mediated demineralisation episodes [1, 2]. Incipient white spot lesions (WSLs) are the first visual indication that the complex dynamic physico-chemical processes that maintain healthy enamel have shifted in favour of demineralisation.

It is possible to reverse the early stages of enamel caries [1, 2]. A combination of good oral hygiene, dietary control and fluoride therapy is a widely recommended strategy for the prevention and reversal of early caries. In most cases, the concentration and bioavailability of calcium ions are the limiting factors in the remineralisation process and accordingly a number of home-use and clinical products has been developed to

enhance the calcium and phosphate concentrations of saliva and plaque [2–5]. These include dentifrices and topical pastes which contain bioactive calcium-sodium-phosphosilicate glass, BG, (Sensodyne Repair & Protect, GalaxoSmithKline, UK), synthetic hydroxyapatite, HAP, (mirasensitive hap+[®], Hager Werken, Germany) and casein phosphopeptide-amorphous calcium phosphate, CPP-ACP, (GC Tooth Mousse, GC JAPAN, Japan).

Bioactive glasses are calcium sodium phosphosilicate glasses that dissolve under neutral conditions in aqueous media to release sodium ions, which elevate the pH, and calcium and phosphate ions which are reported to remineralise enamel [6]. Hydroxyapatite particles are incorporated into toothpastes to exploit their abrasive and remineralising properties [7]. In the latter case, nanoparticles appear to be more effective than micron-sized particles owing to their superior solubility and release of bioavailable calcium and phosphate ions.

CPP-ACP, a milk-derivative, comprises phosphorylated seryl- and glutamic acid-rich peptide residues bound to amorphous calcium phosphate nanoparticles [8]. The peptide residues stabilise the amorphous calcium phosphate phase and inhibit its premature crystallisation to HAP in the oral cavity, thus maintaining a supply of bioavailable calcium and phosphate ions for subsurface remineralisation of WSLs. These CPP-ACP

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nanoclusters are reported to adhere to enamel, to inhibit bacterial adhesion to the tooth, and to act as a pH buffer against acid assault [5, 8, 9].

The purpose of this study was to compare the *in vitro* enamel remineralisation potential of commercial toothpastes containing bioactive glass (BG) particles, hydroxyapatite (HAP) particles or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes under optimum and acid-challenge conditions.

2. Materials and methods

Eighteen sound, extracted molars and premolars were debrided, polished with pumice and paste, copiously rinsed under flowing water, air-dried and coated with acrylic nail varnish leaving a 2 mm×3 mm window on the buccal surface. The teeth were then individually immersed in 10 cm³ of demineralising solution (2.2 mM CaCl₂, 2.2 mM KH₂PO₄, 0.05 M acetic acid, adjusted to pH 4 with 1.0 M NaOH_(aq)) for 24 h to create artificial WSLs [10].

The demineralised teeth were then rinsed thoroughly, air-dried and sectioned longitudinally through the WSLs. Pulpal remnants were discarded and the roots were removed at the cemento-enamel junction. Varnish was re-applied to the sectioned teeth leaving the WSLs exposed.

7.0) approximates the supersaturation of hydroxyapatite found in human saliva [10]. Groups D, E and F were subjected to an acid-challenge remineralisation protocol which was similar to that of Groups A, B and C but which also incorporated cyclic exposure to the demineralising solution (Fig. 1).

The toothpastes used in this study were Sensodyne Repair & Protect (GalaxoSmithKline, UK) which contains BG; mirasensitive hap+[®] (Hager Werken, Germany) which contains micron-sized HAP and the GC Tooth Mousse (GC JAPAN, Japan) which contains CPP – ACP. The composition of these toothpastes can be found on the manufacturers' websites [11–13].

The surfaces of the original enamel, control WSLs and treatment WSLs for each of the groups were observed by scanning electron microscopy (SEM) using secondary electrons (SE). The cross-sections of the lesions were analysed by SEM using back scattered electrons (BSE) and by line scanning energy dispersive X-ray analysis (EDX), as detailed elsewhere [7].

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Erciyes University, Turkey (Approval Number 2016/316).

3. Results and discussion

3.1. *In vitro* remineralisation under optimum conditions

Secondary electron SEM images of the surfaces of the original enamel, the control WSL and the treated WSL of a random tooth from Group A are shown in Fig. 2a, b and c, respectively. The control WSL presents a textured eroded surface with regions of broken hydroxyapatite

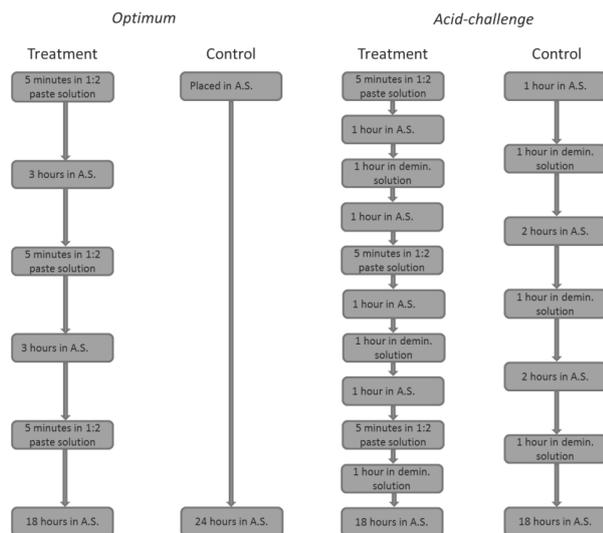


Fig. 1. Treatment and control regimes under optimum and acid-challenge conditions.

The teeth were randomly assigned to six groups. Groups A, B and C were subjected to an optimum remineralisation protocol in which the “control” half of each tooth was incubated in 5 cm³ of artificial saliva for 24 h at 37 °C and the “treatment” half of each corresponding tooth was cyclically exposed to artificial saliva and to 5 cm³ of 50% (v/v) toothpaste solution containing either BG, HAP or CPP-CAP, respectively (as shown in Fig. 1). The remineralising artificial saliva solution (1.5 mM CaCl₂, 0.9 mM NaH₂PO₄, 0.15 M KCl at pH

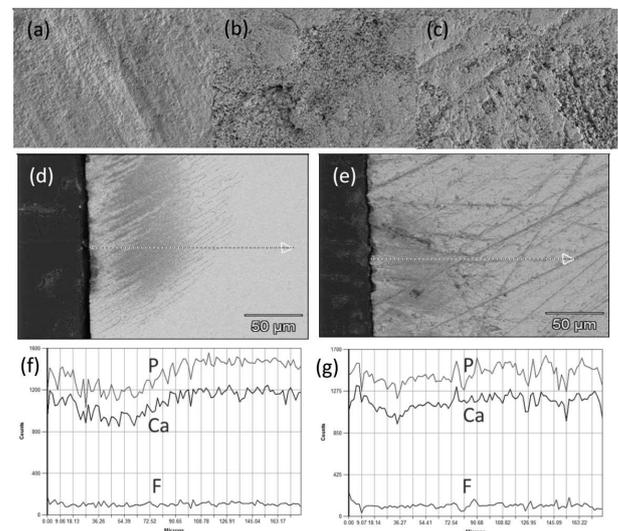


Fig. 2. Group A: SEM images ($\times 10\,000$) of (a) original enamel, (b) control enamel and (c) BG-treated enamel surfaces (under optimum conditions). BSE images of the sectioned artificial WSLs of the (d) control and (e) BG-treated enamel, and the corresponding EDX line-scans for P, Ca and F through the (f) control and (g) BG-treated WSLs.

rods (Fig. 2b), which is in contrast with the relatively smooth surface of the original enamel (Fig. 2a). Treatment with BG indicates incomplete remineralisation of the surface of the WSL with some areas of exposed rods remaining (Fig. 2c).

Back scattered SEM images of the cross-sections of the control and treated WSL of the same Group A tooth are presented in Fig. 2d and e, respectively. The extent of sub-surface demineralisation is indicated by darker regions in these micrographs. Comparison of the corresponding EDX line-scans demonstrates that exposure to BG afforded some sub-surface repair of the WSL by diffusion of calcium and phosphate ions into the body of the lesion (Fig. 2f and g).

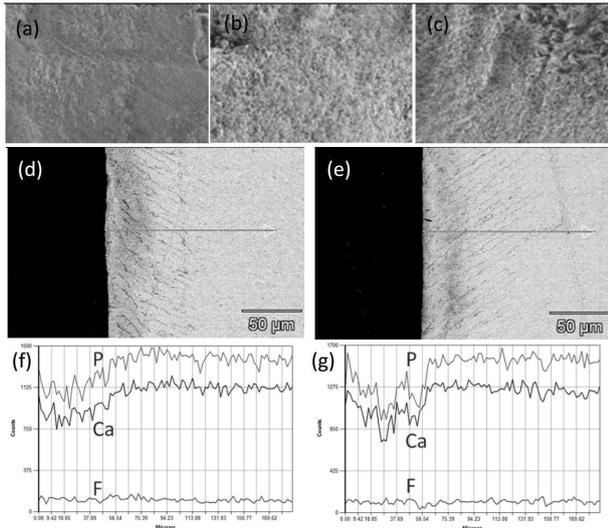


Fig. 3. Group B: SEM images ($\times 10\,000$) of (a) original enamel, (b) control enamel and (c) HAP-treated enamel surfaces (under optimum conditions). BSE images of the sectioned artificial WSLs of the (d) control and (e) HAP-treated enamel, and the corresponding EDX line-scans for P, Ca and F through the (f) control and (g) HAP-treated WSLs.

Figure 3a, b and c shows SE SEM images of the original enamel, control WSL and treated WSL surfaces of a random tooth from Group B. The extents of enamel erosion of the control and treated WSLs are similar and indicate that exposure to HAP provided no apparent surface remineralisation. Corresponding EDX line-scans also indicate that HAP failed to effect any restoration of the calcium and phosphate levels within the body of the WSL (Fig. 3f and g).

SEM images and EDX line-scan data for a random tooth from Group C are presented in Fig. 4. The surface of the control WSL is typically characterised by eroded enamel (Fig. 4b), whereas, the WSL exposed to CPP-ACP exhibits some surface remineralisation and also features a mixture of organic and inorganic deposits (Fig. 4c). The BSE images (Fig. 4d and e) and EDX line-scans (Fig. 4f and g) for this tooth indicate that the calcium and phosphate components of CPP-ACP have substantially remineralised the body of the WSL.

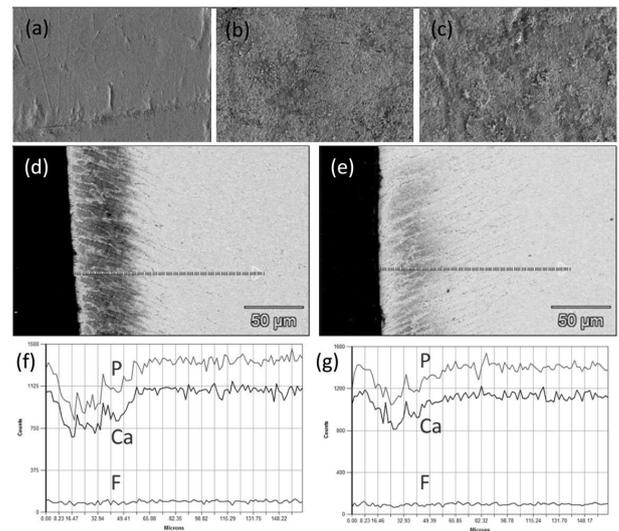


Fig. 4. Group C: SEM images ($\times 10\,000$) of (a) original enamel, (b) control enamel and (c) CPP-ACP-treated enamel surfaces (under optimum conditions). BSE images of the sectioned artificial WSLs of the (d) control and (e) CPP-ACP-treated enamel, and the corresponding EDX line-scans for P, Ca and F through the (f) control and (g) CPP-ACP-treated WSLs.

3.2. In vitro remineralisation under acid-challenge conditions

Secondary electron SEM images of the original enamel, control WSL and treated WSL of a random tooth from Group D are shown in Fig. 5a, b and c, respectively. The corresponding BSE images of the cross-sections of the control and treated WSL are shown in Fig. 5d and e,

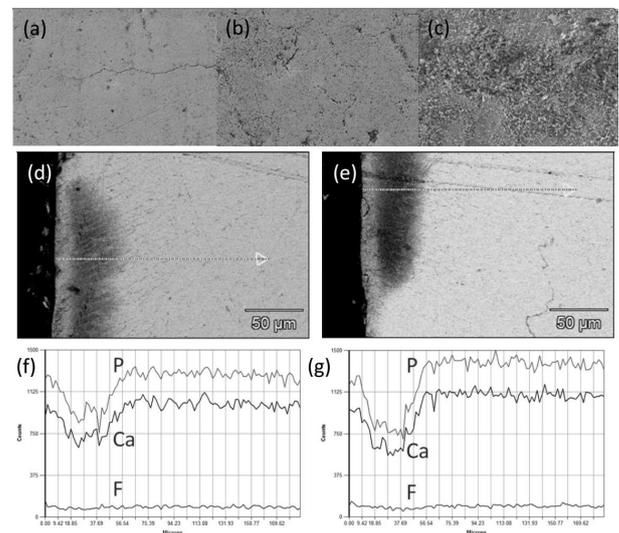


Fig. 5. Group D: SEM images ($\times 10\,000$) of (a) original enamel, (b) control enamel and (c) BG-treated enamel surfaces (under acid-challenge conditions). BSE images of the sectioned artificial WSLs of the (d) control and (e) BG-treated enamel, and the corresponding EDX line-scans for P, Ca and F through the (f) control and (g) BG-treated WSLs.

respectively; and the EDX line-scans are given in Fig. 5f and g. These data indicate that under acid-challenge conditions the components of BG coated the surface of the WSL but did not provide any sub-surface repair within the body of the lesion.

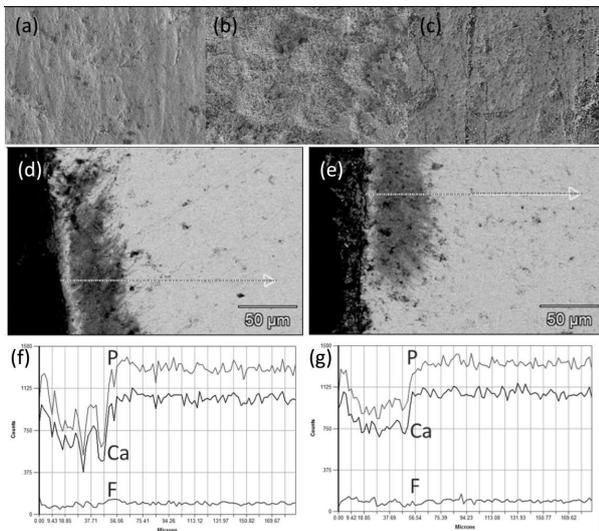


Fig. 6. Group E: SEM images ($\times 10\,000$) of (a) original enamel, (b) control enamel and (c) HAP-treated enamel surfaces (under acid-challenge conditions). BSE images of the sectioned artificial WSLs of the (d) control and (e) HAP-treated enamel, and the corresponding EDX line-scans for P, Ca and F through the (f) control and (g) HAP-treated WSLs.

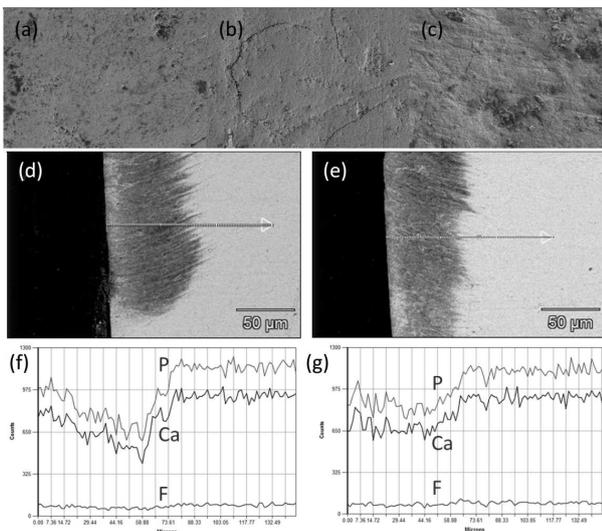


Fig. 7. Group F: SEM images ($\times 10\,000$) of (a) original enamel, (b) control enamel and (c) CPP-ACP-treated enamel surfaces (under acid-challenge conditions). BSE images of the sectioned artificial WSLs of the (d) control and (e) CPP-ACP-treated enamel, and the corresponding EDX line-scans for P, Ca and F through the (f) control and (g) CPP-ACP-treated WSLs.

Conversely, the SEM and EDX data for Group E (Fig. 6) indicate that, under acid-challenge, the exposure to HAP had significantly protected and restored the

surface, and also provided some sub-surface repair of lesion. The original enamel surface of the Group E tooth (Fig. 6a) is significantly eroded during acid-challenge (Fig. 6b); and exposure to HAP is seen to mitigate the extent of this damage (Fig. 6c). Modest sub-surface repair afforded by exposure to HAP is also evident from the BSE images of the cross-sections of the control and treated WSL (Fig. 6d and e), and the corresponding EDX line-scans (Fig. 6f and g).

Figure 7a, b and c shows SE SEM images of the original enamel, control WSL and treated WSL surfaces of a random tooth from Group F. Again, a mixture of organic and inorganic deposits is seen to have populated the surface of the lesion (Fig. 7c); although, under acid-challenge conditions the extent of sub-surface restoration of calcium and phosphate levels within the WSL (Fig. 7d and e) is lower than those observed under optimum conditions (Fig. 4f and g).

3.3. Remineralisation mechanisms

The findings of this study indicate that, under optimum conditions, the calcium and phosphate components of both BG and CPP-ACP are released in bioavailable forms which readily diffuse into the WSL at near neutral pH. This is in contrast with HAP which is essentially insoluble under neutral conditions and does not discharge bioavailable calcium and phosphate ions. However, under the selected acid-challenge conditions (\sim pH 4), HAP is soluble and its constituent ions are able to diffuse into the lesion and also to protect the surface from further erosion. Both BG and CPP-ACP have coated the enamel surface under acidic conditions, although their ability to remineralise the body of the lesion was compromised at low pH.

A number of other studies also report that proprietary toothpastes containing either CPP-ACP or BG possess the potential to remineralise enamel [3, 6, 10]. Our findings confirm these observations and also reveal that under acid-challenge conditions, the effectiveness of these remineralising agents is significantly compromised. Toothpastes containing micron-sized HAP are reported to inhibit dentinal erosion, to remineralise bleached enamel and to inhibit caries [4, 7]. In addition, our study demonstrates that, unlike CPP-ACP and BG, HAP affords superior remineralising capacity and greater erosion-resistance under acid-challenge conditions. This phenomenon is related to its dissolution and release of calcium, phosphate and hydroxide ions at low pH and its comparatively poor solubility under neutral conditions.

4. Conclusions

The objective of this research was to investigate the comparative in vitro enamel remineralisation potential of commercial toothpastes containing bioactive glass (BG) particles, hydroxyapatite (HAP) particles or casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) nanocomplexes. Under optimum conditions BG and

CPP-ACP provided sub-surface repair by diffusion of calcium and phosphate ions into the WSLs. Conversely, HAP did not influence remineralisation under neutral pH conditions (at which it is essentially insoluble). Conversely, under acidic conditions, HAP was able to dissolve to release calcium and phosphate ions which diffused in to the WSLs and also protected the enamel surface from further erosion. BG and CPP-ACP both coated the enamel surface under acidic conditions, although their ability to remineralise the body of the lesion was compromised at low pH.

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