Effect of exposure of glass ionomer cements to acid environments

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ABSTRACT

This research focused on evaluating the effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) incorporated into a glass ionomer cement (GIC). Two conventional GIC materials were the main focus of the study, Fuji VII and Fuji VII EP (containing 3% w/w CPP-ACP), these materials were made into rectangular blocks and subjected to various acidic media (lactic, citric and hydrochloric acids), while daily observations of surface hardness, mass loss and ion release were carried out over a three day period. Acid storage solutions were specifically chosen to approximate conditions the materials would be exposed to in an oral environment; bacterial acid challenge, food acid challenge and stomach acid reflux. Later stages of the study also included topical CPP-ACFP (casein phosphopeptide-amorphous calcium fluoride phosphate) treatment of GIC surface, as well as a more detailed analysis of ion release of Fuji VII EP compared to other materials (Shofu Beautifil).

Blocks of GIC material were prepared and allowed to set in an incubator (37°C, 95%+ relative humidity) for 24 hours. Surface hardness was determined using micro-indentation using a microscope-aligned indenter. Acid storage media were changed every 24 hours and all solutions were kept for further analysis of ion release. Fluoride ion concentrations were measured using an ion selective electrode, phosphate ion concentrations were determined using a UV spectrophotometry assay and calcium ion concentrations were measured using atomic absorption spectroscopy (AAS). Mass loss was determined using an analytical microbalance. Later stages of the study also measured aluminium ion concentrations, which were acquired using AAS. Data from sample groups was found to follow the normal distribution, $\chi^2$ test was used to test normality. Single factor ANOVA was used to analyse the results using Bonferroni-Holm multiple comparison. Two-way ANOVA was used to determine the interaction...
between incorporation of CPP-ACP and exposure to different acids. Level of significance was set at $\alpha = 0.05$.

Over the course of three days of storage the surface hardness of both materials reduced significantly in all acid solutions as well as a control water solution. Results showed significant increases in calcium and phosphate ion release in Fuji VII EP groups compared to Fuji VII in the vast majority of acid solutions. Fluoride release remained stable for both materials with no significant differences measured between them. Further topical treatment provided additional increases in calcium and phosphate ion release for both materials. Exception to this were groups stored in citric acid, which exhibited far greater mass loss compared to other groups, very high phosphate ion release and an increase in surface hardness. Analysis of aluminium ion release to indicated that aluminium ions are possibly chelated from the GIC matrix, leading to additional release of phosphate ions and increased mass loss. Formation of a crust surface layer seems to be the cause of increased surface hardness in topically treated citric acid groups.

Comparison of fluoride, phosphate and aluminium ion release between Fuji VII EP and Shofu Beautifil showed that ion release profiles are significantly and fundamentally different between the two materials, which may be due to the level of maturation (age) of the glass and structure differences between the materials.
DECLARATION

This is to certify that this thesis is the original work of the author except where indicated; due acknowledgement has been made in the text. This thesis is less than 40,000 words in length exclusive of figures, tables and bibliography.

Ilya Zalizniak
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LIST OF ABBREVIATIONS

GIC : Glass ionomer cement
CPP-ACP : Casein phosphopeptide-amorphous calcium phosphate
CPP-ACFP : Casein phosphopeptide-amorphous calcium fluoride phosphate
F7 : Fuji VII
F7EP : Fuji VII EP
TM+ : Tooth Mousse Plus
SBF03 : Shofu Beautifil F03
AAS : Atomic absorption spectroscopy
EDXA : Electron dispersive x-ray analysis
SEM : Scanning electron Microscopy
MPa : Megapascal
VH : Vickers hardness
RH : Relative humidity
N : Newton
w : width
l : length
t : thickness
TISAB : Total ionic strength adjustable buffer
HCL : Hydrochloric acid
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Table 8.1. General composition of GICs
CHAPTER 1

Introduction

Originally introduced by Smith in 1968\(^1\), glass ionomer cements (GIC) underwent a period of rapid development in the late 1970s and early 1980s\(^2\)\(^-\)\(^21\) and have been the subject of continuing steady improvement ever since. Valued for their chemical adhesion to tooth structure and potential for long term fluoride release, GICs have established a unique role as a restorative material with anti-cariogenic properties able to promote remineralisation of caries-affected enamel and dentine\(^22\).

Over the years, numerous efforts to improve GICs have yielded mixed results. Incorporation of anti-bacterial agents, bio-active glass particles and silica fillers greatly reduced the workability of the material\(^23\)\(^-\)\(^27\). However, further developments of the GIC formula have provided fast setting times, enhanced radio-opacity and efforts to provide the release of calcium and phosphate ions in addition to fluoride ions to further enhance GIC’s biomimetic qualities\(^28\),\(^29\).

Ongoing research has increased our understanding of the chemical and physical properties of GICs and their broader applications within dentistry. Through this research the GICs have found wide clinical application as pit and fissure sealants, bonding agents and ‘low’ load-bearing restorative materials\(^30\). Lower mechanical
strength compared to other materials as well as lower retention rates and initial water sensitivity have limited the use of GICs to several specific applications\textsuperscript{30,31}.

To better understand more recent improvements to GIC materials a series of laboratory studies were undertaken and are presented in the following pages of this thesis. Physical and chemical properties of GIC materials were investigated in combination with a variety of environmental conditions and topical treatment protocols. Additional chemical analysis was then conducted based on obtained results to further understand the underlying structure of GIC materials.

A comparison of conventional GICs, a modified conventional GIC and a pre-reacted glass ionomer particle filled resin composite were investigated. The experimental work introduces a new approach to testing the GIC-based materials and how they might react under different environmental conditions. This work has shed light into their structure and finally suggests some possible ways the GIC formula can be further improved to ameliorate the effects the different environmental conditions.

The first part of the experimental work was centred on a series of pilot studies to develop an optimum specimen shape, the best methods to determine ion release and hardness of test materials. The following chapter (Chapter 5) investigated the changes in physical properties and ion release of a CPP-ACP-modified conventional GIC when it was subjected to an acidic environment. Chapter 6 describes the effects of the potential to recharge the GICs from the aspect of fluoride, calcium and phosphate ions. The cements were recharged with a CPP-ACP containing crème and then exposed to the acidic environment once more to determine changes in ion release. The outcomes showed some unexpected results that are believed to be related to the aluminium ions in the GICs. This formed the basis of Chapter 7. Finally, Chapter 8 draws together and discusses all of the aspects of this research project and briefly considers where GICs could be modified to overcome some of the changes that may occur when exposed to different acids.
1.1 References


2.1 Dental Caries

In today’s world, despite many advanced pharmaceuticals and our advanced understanding of medicine, dental caries remains a major health problem worldwide. In many countries it is the single most costly disease to treat. Many efforts have been made to better understand and treat the problem. It has long been commonly accepted that the prevalence of refined sugars in the diet are a major causative factor in dental caries. The effect of sugars on dental caries has been the subject of discussion for over 60 years despite the best efforts of the dental profession and others to curtail the problem. Raising public awareness has been at the forefront of efforts to combat dental caries.
2.1.1 Aetiology

In an attempt to describe the aetiology of caries lesion formation, several studies have attempted to correlate the onset of caries to trace elements found in caries-affected enamel. It was established that fluoride must possess anti-cariogenic properties due to the consistent observation of its presence in the surface layer found above the carious lesion and its absence in the de-mineralised enamel surrounding the lesion. For a long time there was a significant amount of empirical evidence linking caries to dietary habits, but the formation of carious lesions has been best described by Featherstone et al., in 1979. Their study investigated the chemical processes that occurred during the formation of carious lesions.

Beginning in the 1890s, studies focused on studying the bacteria present during the onset of dental caries and. Replicating the formation of lesions in the laboratory was the first step to understanding the relationship between bacteria and dental caries. It was found that certain strains of bacteria, *Streptococcus mutans*, which is present in healthy saliva, consumes glucose. Fermentation of glucose increases the growth of the bacterial colony and the metabolism of the bacteria which in turn produces lactic acid. The lower pH caused by increased concentrations of lactic acid results in tooth mineral dissolution and consequently dental caries. Other *Streptococcal* strains and *Lactobacilli*, both found in healthy dental plaque, were found to have similar characteristics as *S. mutans*. It is unclear what exactly causes some species of bacteria to increase in population in preference to others, but in almost all cases of dental caries a dominant glucose consuming strain emerges and as a result the pH of the oral environment drops as more lactic acid is produced. Thus the onset of dental caries depends on a number of factors; a mature biofilm containing the right bacterial strains, the supply of fermentable sugars and a substrate for bacterial colonies to grow on, namely a tooth surface (enamel and/or dentine). All these conditions need to be satisfied for caries to initiate and progress.

Fermentable sugars remaining after food consumption in the oral environment produce lactic acid over time when the bacterial biofilm is mature.
Lactic acid, in its un-ionized form, penetrates the enamel, dissociates and once a high enough concentration is reached begins attacking and removing calcium, phosphate and fluoride ions from the tooth. The largely intact surface layer is formed as subsurface ions diffuse to the surface where they are adsorbed back into the lattice of the enamel. The rates of lactic acid penetration, mineral loss and adsorption at the surface all depend on concentration gradients of lactic acid with respect to the intra-enamel fluid. As the lesion progresses further into sub-surface layers greater amounts of tooth mineral are displaced, which in turn leads to the drop in the concentration gradient at the surface creating an equilibrium between mineral loss and ion adsorption. From the outside this creates an intact surface layer while the lesion continues to progress further into the sub-surface. Hence, the presence of fluoride in the surface layer has been shown to be the result of carious lesion progression rather than hindering it. Despite this evidence, the cariostatic ability of fluoride has been promoted in favour of other agents (such as calcium and phosphate), that also have been found to slow the progression of dental caries.

2.1.2 Immunisation

Early efforts to characterize dental caries anti-bodies found that such antibodies either exist in very low concentrations in saliva or do not exist at all. More recent attempts at immunization against dental caries found some success in animal studies, however the efficacy of such vaccines on humans remains unknown as further research is needed. Although ultimately the onset of dental caries can be traced to various strains of bacteria, the underlying cause is driven by demineralisation of tooth structure caused by a falling pH in the oral environment. Clinical observations suggest that patients on broad-spectrum antibiotics exhibit reduced symptoms of dental caries; this highlights the problem surrounding immunisation. Any effective vaccine would need to be broad enough to immunise against all known caries causing bacteria.
2.1.3 Fluoride and Caries prevention

Since the introduction of fluoride into the oral environment, either through a fluoridated water supply, toothpaste or topical treatments, the prevalence of dental caries has fallen. The role of fluoride in caries prevention has long been established. Investigations have concluded that low concentrations of fluoride have a two-fold effect in inhibiting formation of caries-like lesions in human enamel. The interaction of fluoride with enamel mineral leads to an increase in re-mineralisation during periods of super-saturation with respect to hydroxyapatite and also resulted in reduced de-mineralisation during periods of under-saturation with respect to fluorapatite. The same study also determined that even low concentrations of fluoride led to reduced bacterial acid production resulting in smaller pH falls in dental plaque. It is now commonly accepted that fluoride is able to substitute for hydroxyl ions in hydroxyapatite found in enamel to form fluorapatite (Equation 1), which is less soluble thereby providing increased protection against acid attack.

(Equation 1) \[ \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 2\text{F}^- \rightarrow \text{Ca}_{10}(\text{PO}_4)_6\text{F}_2 + 2\text{OH}^- \]

Little research has been done to exactly determine the concentrations of fluoride required to achieve an optimal cariostatic effect. In contrast, numerous studies have investigated the effect of fluoride on the clinically observed levels of fluorosis, especially in children.

Furthermore, early evidence showed that topical fluoride applications prevent bacterial growth on the tooth surface. Reduced levels of \textit{S. mutans} have been reported under stannous fluoride (SnF$_2$) treatments in particular, more so than sodium fluoride (NaF$_2$). Later, studies investigating calcium fluoride have shown no change in \textit{S. mutans} levels under topical fluoride treatments. It has been proposed that in high enough concentrations of fluoride, topical treatments are able to substitute fluoroapatite into demineralised enamel thereby arresting the onset of carious lesions. By providing a physical barrier between tooth mineral and bacteria,
fluoride varnishes are able to create a favourable concentration gradient for inward diffusion of fluoride, thus promoting remineralisation\textsuperscript{23}. Sugars remaining after food consumption, which are metabolised into acid, are more likely to be found on the surface of teeth. Therefore, specifically protecting the surface of the tooth against acid attack further adds to the cariostatic ability of fluoride. Could deep caries-like artificial lesions also be re-mineralised using conventional calcium, phosphate and fluoride topical treatments? It was found that topical treatments could only re-mineralise the surface layer of enamel and that the ion penetration of the surface was not high enough to reach demineralised dentine\textsuperscript{24}. This is supported by previous theories about subsurface dissolution of minerals and subsequent adsorption on the surface layer of enamel. Further evidence in support of fluoride exposure is often reported\textsuperscript{25,26}, but other factors in the oral environment, such as dietary habits and the role of healthy saliva flow are gaining more significance and attention with regard to caries activity and risk\textsuperscript{27}. The composition of dental plaque, or biofilm as it is now called, has been an ongoing topic of interest. The anti-cariogenic potential of dental plaque has always been seen as an important factor in the development of caries, the biofilm is supersaturated (with calcium and phosphate ions) with respect to tooth mineral and consequently has the potential to re-mineralise carious lesions. However, fermentable carbohydrates will lower the pH of the biofilm and the degree of saturation will therefore decrease rapidly\textsuperscript{28}. A cycle of remineralisation and demineralisation at the tooth surface involving calcium and phosphate ions is currently the accepted mechanism of a carious lesion formation and repair (Figure 2.1)\textsuperscript{29}.
2.1.3a Minimal Intervention Dentistry

Born out of the idea of trying to re-mineralise apatite depleted dentine and enamel, minimal intervention dentistry is a concept that is now supported and promulgated by many individuals\textsuperscript{30-32}. The traditional approach of treating caries involves removing all ‘infected’ and ‘affected’ dentine from the tooth for the preparation of a defined cavity that will be filled with a material of some kind, either dental amalgam or a resin-based material. Caries-affected dentine is defined as de-mineralised dentine that is ‘free’ from bacterial contamination and still retains its collagen matrix structure \textsuperscript{33}. Caries-infected dentine contains a high concentration of bacteria and the caries has progressed to the point where the collagen fibre structure has broken down into an amorphous mass, consequently no re-mineralisation is
possible. Minimal intervention treatment is a concept where traditional restorative approaches of removing all caries-infected and -affected dentine is questioned and a method of removing only infected dentine and leaving affected dentine to be re-mineralised is advocated. The role of fluoride is a major one as fluoride incorporated into dental restorative materials may have the potential to re-mineralise surrounding tissue and tissue at the base of a cavity. Early studies reported on how fluoride release from dental materials would affect enamel; one of the conclusions made from these studies was that materials containing no fluoride reduce the fluoride content of enamel. This seems to indicate that fluoride saturation of the oral environment has a strong influence on fluoride content of enamel; lack of fluoride in plaque can lead to low fluoride levels in enamel. This ties in well with our understanding of carious lesion formation and the effect of fluoride saturation levels on arresting caries progression, low levels of fluoride ions can lead to an imbalance between plaque and enamel, which in turn can promote demineralisation.

2.1.4 Fluoride containing dental restorative materials

Historically, some dental materials have proven to be better fluoride releasing agents than others. One such type of material is a glass ionomer cement (GIC), first proposed in the late 1960s and actively developed from the early 1970s. GICs have established a niche amongst other dental restorative materials for not only their high fluoride release but reliable chemical adhesion to tooth structure as well as simple use.
2.2 Glass Ionomer Cements

2.2.1 Origin

In 1972 a glass ionomer cement (GIC) system was introduced by Wilson and Kent as a new type of dental material, described by the reaction between acrylic acid and fluoroaluminosilicate glass powder. The idea of adhesion and the formation of an ion exchange layer to tooth structure using a poly-alkenoic acid was first suggested by Smith a few years earlier. The bond is created when acidic carboxyl groups attack tooth structure liberating calcium and phosphate ions, in return aluminium, fluoride, silica and strontium ions are liberated from the glass powder and penetrate the tooth structure, resulting in a layer of exchanged ions between the GIC and tooth tissue. This type of chemical adhesion to tooth structure made GICs unique amongst dental restorative materials, many studies followed attempting to improve and understand the benefits of GICs.

2.2.2 Chemistry of the Setting Reaction

Research into possible alternatives to poly-alkenoic acids, which tend to thicken and gel at the required concentration of 50%, have led to tartaric acid being proposed as a possible accelerant to be used in GICs. It was also determined that certain copolymers of polyacrylic acid showed good mobility and low viscosity at a concentration of 50%. Of these, acrylic acid produced cements of higher tensile strength. In subsequent studies the relationships between the glass powder and acid liquid (P:L) ratio and its effect on setting time, compressive strength and hardness were explored. It was claimed that higher P:L ratio leads to increased compressive strength, setting rate, surface hardness and made the mix less sensitive to moisture. The optimal powder:liquid ratio, which will depend on the specific clinical application, should be tailored to accommodate working time and maximize strength and durability of the mix. Some studies found that the molecular weight of
the polyacid used in the reaction had a significant effect on the strength of the GIC\(^{46}\). Poly acids of higher molecular weight produced GICs with enhanced strength, but the working time of the mix was reduced limiting the GICs use. If a lower concentration of the polyacid, with the same P:L ratio, was used to increase the working time, the strength of the GIC was subsequently reduced\(^{46}\). Investigation of the effect of tartaric acid on the properties of GICs discovered that tartaric acid was able to accelerate the setting reaction, providing a “snap set,” as well as increase the strength of the cement. However, when used in excess it slowed the setting reaction and weakened the cement\(^{50}\). A chelating effect of tartaric acid on the setting properties of GICs was also observed, it was speculated that the tartaric acid extracted metal ions (e.g. aluminium) from the glass and was able to hold the ions in solution preventing early binding and thus extending the “working time.” The setting characteristics were further improved as this effect also increased the rate of hardening of the cement mix\(^{44}\).

A separate study focused on investigating the ability of polyacrylic acid to extract fluoride ions from the glass powder during the setting reaction\(^{57}\). It was concluded that the extraction of fluoride ions was aided by the formation of calcium and aluminium salt complexes. Understanding of the extraction mechanism is important in being able to control the reaction, if the fluoride is not extracted efficiently the pH of the setting cement rises too quickly producing an unworkable mix\(^{39}\). Further research into the effect of pH on fluoride release and mechanical properties concluded that while fluoride ion release was beneficial, more work is needed to be done to improve the mechanical properties of many materials if they are to be used for load bearing restorations\(^{58}\).

**2.2.2a Role of Aluminium**

Aluminium plays an important role in the acid-base setting reaction in GICs. It is predominantly present in the form of aluminium oxide. Although the initial setting reaction in the GIC involves calcium or strontium salts, a secondary setting reaction
relies heavily on aluminium salts. The presence of aluminium adds basicity and ensures that a complete acid-base reaction can take place, allowing the mix to fully set. Exposure to an acidic challenge may lead to aluminium being leached from the GIC restoration; the biological effects and structural significance are discussed in the paper by Nicholson et al.\textsuperscript{59}.

It is estimated that if a GIC restoration were to dissolve completely and be absorbed by the human body via the gastro-intestinal tract over a 5-year period it would only contribute 0.5% of the maximum allowable aluminium intake. Attempts to make a GIC containing high levels of fluoride proved successful, of particular interest was the relative ratio between aluminium oxide and silicon dioxide and its effect on the stability of the cement mix\textsuperscript{52}. It was also discovered that the Al\textsubscript{2}O\textsubscript{3}: SiO\textsubscript{2} ratio controlled the cements susceptibility to acid dissolution. The presence of Al\textsuperscript{3+} at network forming sites made the cement vulnerable to acid attack, but above a certain Al:Si ratio the inclusion of Al\textsuperscript{3+} ions made the GIC more resilient against an acid challenge\textsuperscript{51}. The attenuated total reflectance spectroscopy technique was used to investigate the various species present in the cement mix during the setting reaction of a GIC\textsuperscript{60}. It was discovered that both calcium and aluminium salts were present in the final mix in equal amounts. Calcium salts were formed first and were responsible for gelation and the initial set, aluminium salts were formed later which resulted in the hardening of the cement mix 40. In a follow-up study, it was discovered that calcium salts were fully formed within the first 3 hours of setting, while aluminium salts only started forming after an hour and the reaction continued for up to 48 hours after the initial setting. The initial stages of setting consisted of a precipitation reaction as fluoride and phosphate ions were extracted from the glass powder to form insoluble salts and complexes. The structure of the final matrix was characterized by chains of covalent bonds, which were further strengthened by ionic bridges cross-linking the chains, with glass particles embedded in the hard matrix\textsuperscript{38}. 
Investigation of wear and microhardness of GICs showed that hydration and exposure to lactic acid play a significant role in the hardness and wear properties of GICs. Characterization studies of GICs found that surface hardness predominantly develops during the first 24 hours after mixing, with slight increases being recorded up to one year following the initial set. The compressive strength of the investigated GICs continued to increase after a 24 hour period, and the authors speculated that the maximum compressive strength would be achieved after one year. A study of surface roughness and hardness of various types of GICs determined that the composition of the GIC has a significant effect on its roughness and hardness. GICs were found to have the highest surface roughness. A silver reinforced GIC was found to have the highest microhardness, however no correlation between roughness and hardness could be determined. Elsewhere it was reported that major chemical changes in some conventional GICs occurred when exposed to artificial saliva, which pointed to an increase in surface microhardness after a period of storage. In an attempt to find an appropriate laboratory test to predict the resistance to degradation of restorative materials three different methods were compared – mass loss, degradation of the area of thin cement films, and reduction in transverse strength. Degradation of the area of thin films made from cements showed the greatest correlation with in vivo results. Research into incorporation of hydroxyapatite (HA) into GICs found that the fracture toughness was improved with incorporation of HA, while at the same time leaving bonding and fluoride releasing benefits of the material unaffected.

It was shown that GIC had the largest initial water uptake in the first 24 hours compared to other water-based dental cements (dental silicate cements and zinc polycarboxylate cements), although erosion over a seven day observation period was similar. When exposed to lactic acid media, GICs demonstrated the least amount of erosion compared to other water-based cements. While GICs have several advantages over other materials, such as strong chemical adhesion to tooth structure,
they do have their shortcomings. Initial water sensitivity, setting time and other physical properties have been investigated and compared to many other restorative materials.

2.2.4 Fluoride Release from GICs

GIC’s ability to deliver a sustained release of fluoride ions provides unique benefits in combating caries. Studies of fluoride release and absorption from GICs at different pH levels show that fluoride release is dependent on surface degradation of the GIC, which is caused by varying the pH of the solution\textsuperscript{66}. However, it is important to note that Kim et al.\textsuperscript{67}, in their study of GIC’s ability to re-mineralise apatite depleted dentine, claim that mineral concentration alone is not a sufficient endpoint for assessing the success of contemporary remineralisation strategies\textsuperscript{67}. It is noted that GICs may be able to re-mineralise partially de-mineralised dentine, where remaining apatite crystals may serve as centres for nucleation and subsequent re-mineralisation. In a split-mouth study, proximal caries lesions treated with GICs were found to be more likely to remain in or regress to the outer half of enamel\textsuperscript{68}. A study of mineral loss on enamel adjacent to GIC restorations concluded that GICs can exert a protective effect only under a cariogenic challenge and not an erosive challenge\textsuperscript{69}, it is speculated that fluoride ion release may not be high enough to prevent a strong erosive challenge. However, this may also indicate GIC vulnerability against a citric acid attack. Fluoride ion release comparison between fluoride varnish (Duraphat, Colgate-Palmolive Pty Ltd, USA) and GIC, found that both products promote more re-mineralization of artificial lesions on proximal surfaces, with fluoride varnish (52%) providing higher reduction in carious surface area than GIC (33%)\textsuperscript{70}. Other studies also claim to have found a positive correlation between the dosage of fluoride and its ability to inhibit demineralization\textsuperscript{71}. De-mineralisation and re-mineralisation of enamel was found to have a strong dependence on the concentration of fluoride ions, it is claimed that fluoride has a significant effect on inhibiting demineralisation at the
tooth surface, and that fluoride contributes to sub-surface remineralisation potentially reversing the effect of caries\textsuperscript{72}.

A study by Williams et al\textsuperscript{73} investigated the relationship between fluoride release from GICs, volume of cement samples and their surface area. Several different sample shapes were tested (discs, cylinders and bars); a strong positive correlation was found between long-term fluoride release and surface area, as opposed to the sample volume\textsuperscript{73}. A short-term study proposed that fluoride releasing gels may prolong the fluoride release of GICs by replenishing fluoride lost from the GIC surface\textsuperscript{74}. However, it has been pointed out that the overwhelming evidence of superior fluoride release by GICs is not conclusive evidence of their cariostatic ability, which can only be ascertained through carefully designed clinical trials\textsuperscript{75}.

\textbf{2.2.5 Clinical Application and Comparison}

In a series of articles, McLean and Wilson discussed the clinical implications and uses of GICs, chemical adhesion to dentine and enamel was highlighted as one of the major benefits of the material, desirable aesthetic qualities were also mentioned. The authors suggested that the most promising use of GIC would be in preventive dentistry, sealing erosion lesions and fissures. In addition, GIC’s cariostatic properties could play an important role in inhibiting occlusal caries. Following a clinical trial of GICs as a restorative material for cervical lesions a number of recommendations were made and the limitations of GIC applicability were highlighted\textsuperscript{47-49}.

Since then a lot of progress has been made, both in developing GIC materials and tailoring them for specific applications. Further research has highlighted the effectiveness of GICs compared to resin-based fissure sealants, and concluded that no material had an advantage over the other and both were effective as fissure sealant materials\textsuperscript{76}. Additional evidence comparing the effect of resin-based infiltrants and sealants on the progression of caries lesions on proximal surfaces has shown significant benefit to using either approach. Using resin infiltrants and sealants promises to alleviate the issue of secondary caries, which non-fluoride releasing resin
restorations are susceptible to\textsuperscript{77}. In a review study of clinical trials pit and fissure
sealants were found to have a significant effect on prevention of dental caries, however when comparing various sealant types amongst themselves the results were conflicting \textsuperscript{78}. Studies showing resin-based sealants as being superior to GIC sealants
reported high retention rates, as opposed to very low retention rates for both sealant
types in studies that showed GICs to be superior. Long-term (7 years) studies, which
showed no difference between either sealant type reported high retention rates for
resin-based sealants and poor retention rates for GIC-based sealants \textsuperscript{79}. Fluoride
varnishes were compared with sealants in their ability to prevent dental caries and it
was found that sealants were superior in their ability to prevent occlusal caries \textsuperscript{79}. Some studies have claimed that resin-based sealants performed better in terms of
sealing ability than GICs \textsuperscript{80}. However, comparison of retention and caries prevention
of a resin composite sealant and a GIC has shown that although the retention of GIC
was lower, its caries preventative effect was higher than the resin-based material in
permanent first molars\textsuperscript{81}. In support of this evidence others have found that fissures
sealed with GIC continued to have a caries inhibiting benefit even after the
restoration was clinically deemed to have been totally lost. Only after careful
examination of tooth replicas was it revealed that some of the GIC still remained in
the fissure base\textsuperscript{82}. It would seem that complete retention is not necessary for GICs to
retain their caries inhibiting properties. This is further supported by Torppa-Saarinen
and Seppa \textsuperscript{83}, who suggested that as long as a small part of the GIC was still present in
the fissure it may still provide an anti-cariogenic effect. Examining secondary caries
around margins restored with amalgam, resins and GICs revealed that GICs were
superior in inhibiting secondary caries occurrence, although the margins themselves
were not as “tight” as the amalgam and resin materials tested\textsuperscript{84}. A five year clinical
evaluation of GICs used as luting cements on full cast restorations reported low rates
of secondary caries and excellent retention\textsuperscript{85}. A survey of GIC use by Australian
dentists found that GICs were mainly used for tunnel restorations, approximal
restorations in primary molars as well as “sandwich” restorations, where a GIC is used
for its chemical adhesion to dentine as an intermediate layer beneath a resin
Very few biological complications were reported, but the majority of surveyed dentists reported more complications with GICs than amalgam restorations, with fracture of the marginal ridge and wear being the two most cited complications with GICs. Only a few dentists reported occurrences of secondary caries and gingival inflammation when using a GIC compared with three quarters of surveyed dentists when using resin composites.

GICs are classified into several types depending on application, chemical formulation of each type are very similar and only vary in power:liquid ratio and glass particle size. A brief summary of these types and the specific differences between them is outlined below.

**Type I – luting cements**
Falling under a more general category of luting agents, luting cements fill the role of fluoride releasing adhesives used for dental prostheses, implants, crowns, bridges, veneers and orthodontic appliances. A strong chemical bond to tooth structure and to other restorative materials is the major advantage of using GICs as a luting agent.

**Type II – Aesthetic (II-1) and Reinforced (II-2) restorative cements**
GICs in this category are commonly used as cavity restorations. Depending on the cavity location and size, all types of GICs (conventional, resin-modified, fast setting, highly flowable) find use as restorative cements. The clinical evidence suggests that conventional GICs are only suitable in low stress restorations, such as anterior approximal and cervical restorations. High failure rates have been reported for conventional GICs used in posterior approximal restorations.

**Type III – liners and bases**
Commonly used in “closed sandwich” or laminate restorations, where a GIC is placed at the base of the cavity and covered by another restorative material. This
method provides a superior chemical bond between the dentine and restoration, while maintaining a durable, aesthetic restoration on the exposed surface\textsuperscript{55, 91}.

**Type IV – fissure and sealant**

Modern GICs have developed specific formulations for use as fissure sealants on the occlusal surface, the glass particles are finer than in other GIC formulations and the powder:liquid ratio is modified to be a more flowable mix\textsuperscript{92}.

### 2.2.6 Resin-Modified GICs

Resin-modified GICs (RM-GICs) were introduced relatively recently and have found applications as fluoride releasing restorative materials that could be used in load-bearing restorations, as well as broadening the range of fluoride delivery methods in general. Hydroxyethylmethacrylate (HEMA) is the resin included into the GIC, which introduces an additional polymerization reaction, either light activated or self-cured. Introduction of resin into the GIC reduces the initial water sensitivity that conventional GICs suffer from during the early stages of setting. Resin-modified GICs undergo an acid-base setting reaction like conventional GICs, with a separate resin polymerization reaction occurring. Some reports claim that like conventional GICs resin-modified GICs are able to bond via chemical adhesion to dentine and enamel, although studies on this topic are divided. Parallel efforts were also made to introduce fluoride releasing silicate glasses into resin composites. Poly-acid modified resin composites (PAMRC) as they are called, share some of the properties of resin composites and GICs, such as fluoride release. However, PAMRCs lack the chemical adhesion to tooth structure and fluoride recharge properties of GICs.

The study of fluoride release and recharge from different materials used as fissure sealants determined that GICs showed the highest initial and sustained fluoride ion release in comparison to RM-GICs and resin composites\textsuperscript{93}. An overview of the therapeutic effects of GICs surmised that GICs exhibit anti-cariogenic properties through their uptake and release of fluoride ions\textsuperscript{94}. Fluoride release from GICs and resin composites shows that composites release much lower levels of fluoride than
GICs in the first year, but following that, the reduced release from both materials is very similar\(^95\). The lower hydrophilicity of resin composites in comparison to GICs is a significant factor in the large difference in fluoride release between the two materials during the early stages of restoration. It has also been reported that GICs show better fluoride recharge, responding to NaF treatments better than comparable resins\(^96\). Some studies claim that RM-GICs have similar levels of fluoride ion release as conventional GICs, and that the compressive strength of RM-GICs did not change over time compared to a conventional GIC\(^97\). Further evidence of equivalent fluoride release from RM-GICs compared to conventional GICs has been reported\(^98\). In vitro comparison between RM-GICs and conventional GICs used to restore cervical cavities showed no significant difference between the two materials, however, RM-GIC proved to be more acid resistant\(^99\). A laboratory study of fluoride release from several GICs, RM-GICs and PAMRCs demonstrated that the amount of fluoride released is dependent on the storage medium perhaps more so than the type of material\(^100\). A coat of dentine adhesive over conventional GIC and RM-GIC restorations was found to significantly reduce fluoride release from these materials\(^101\). Under in situ conditions RM-GICs show significant remineralisation abilities compared to amalgam, which exhibited further demineralisation in the specimens\(^102\). A meta-analysis by Mickenautsch et al concluded that RM-GICs and composites containing fluoride are equally effective at reducing demineralisation in adjacent hard tooth tissue and that both are more effective than composites containing no fluoride\(^103\). A laboratory study of orthodontic brackets bonded with either RM-GIC or fluoride containing resin composites showed no difference in demineralisation between the two materials\(^104\). A four year clinical study comparing amalgam, resin composites and RM-GICs did not find any significant difference between the materials when used to restore overdenture abutments\(^105\).

It has been found that RM-GICs are weaker in diametral tensile strength than resin composites but stronger than conventional GICs. RM-GICs also showed less susceptibility to water loss or uptake over a 6 month storage period\(^106\). Load and pH have been found to have a significant effect on the wear rate of dentine and enamel,
the same study also demonstrated that resin composites are more resistant to wear than conventional and RM-GICs. Research into erosion of dental restorative materials compared with dental tissue at low pH showed that resin composites exhibited no change when exposed to citric acid (pH 3.0 to 7.0) for seven days, conventional GIC showed increasing signs of erosion with decreasing pH, although it was reported that the rate of erosion for the GIC was less than that of dentine and enamel. Another comparison of wear rates under acidic challenge between resin composite, RM-GIC and a conventional GIC reported that resin composite exhibited the lowest susceptibility to acid challenge, followed by a RM-GIC, with a conventional GIC having the lowest resistance to acid attack. A three-year clinical study by Ostlund et al. focused on retention of posterior approximal restorations using amalgam, resin composite and GIC materials. Restorations that used a GIC were found to have much higher failure rates than resins and amalgam. Surface properties of GICs were found to be greatly affected by acidic challenge (pH 4.3 to 6.2), which was not the case for resin composites. Additionally the pH of the demineralising solution was found to be strongly correlated with the release of fluoride ions. Further evidence of higher fluoride release for a RM-GIC compared to a resin composite has been reported, also of note is the resin’s slower polymerization rate due to interference by oxygen during the polymerization process.

2.2.6a Atraumatic Restorative Treatment

Atraumatic Restorative Treatment (ART) technique was first proposed by Frencken et al. as a means to provide oral healthcare to a wider population throughout the world, especially in those areas that are economically less developed. The need for such a treatment was first highlighted by Thorpe et al. The technique is an extension of Minimal Intervention Dentistry, and relies only on hand instruments and requires no electricity. Caries-infected tissue is removed and the cavity is restored using an adhesive filling material, usually a conventional GIC. A two year study using a GIC and a resin composite for occlusal pit and fissure sealing and
proximal restorations (class I and II respectively) using the ART approach determined that neither material was significantly better than the other\textsuperscript{115}. However, a comparison between high viscosity GICs and resin composites according to ART concluded that GICs were more effective under field conditions due to being less technique sensitive than resin composites\textsuperscript{116}. In a once-off application of composite and GIC using the ART approach, GIC was found to have a 3.1 to 4.5 times higher caries preventive effect\textsuperscript{117}. A review of GICs used as sealants for ART claimed that the treatments were effective and newer ART tailored GICs have shown an improvement over previous versions of GICs\textsuperscript{118}. Based around the ART approach, a study revealed that retention rates of occlusal and posterior approximal restorations using resin composite and GIC materials were more affected by the type of restoration rather than the type of material used, with occlusal restorations showing higher retention rates for both materials\textsuperscript{115}.

2.2.7 Proximal and Secondary Caries

An extensive survey showed that the main reason for restoration replacement was secondary caries\textsuperscript{119}. A study of proximal caries progression highlighted the need to develop better preventive measures for proximal surfaces in caries affected patients\textsuperscript{120}. The effect of restorative materials on secondary caries prevention indicates that conventional GICs exhibit a more pronounced inhibition zone around the restoration than a RM-GIC, and a fluoride releasing resin composite exhibited no inhibition zone at all\textsuperscript{121}. Consequently, a conventional GIC seems to provide more protection against secondary caries than a RM-GIC. Other studies report much higher numbers of \textit{Streptococcus mutans} when sampled from margins of resin composite restorations compared with the margins of GIC restorations\textsuperscript{122}. Furthermore, over a 5-year period retention rates in occlusal resin composite restorations were much higher than GIC restorations, but the resin composite group showed a higher rate of recurrence of caries\textsuperscript{123}. Additional clinical evidence of poor retention rates for RM-GIC compared to other sealants has been reported, studies also highlight significantly
lower *Streptococcus mutans* counts for RM-GIC restorations which is attributed to the presence of fluoride release\textsuperscript{124}. In cervical carious lesions a higher rate of recurrence of caries is claimed in resin composite restorations than GIC restorations\textsuperscript{125}. Consequently, several comparisons involving RM-GICs and fluoride containing resin composites showed that, although the inclusion of fluoride into the resin composite has a positive anti-cariogenic effect that prevents demineralisation in surrounding tooth tissue, RM-GICs provide a far greater benefit\textsuperscript{126,127}. A problem can arise when interpreting clinical trial results. It has been shown that fluoride releasing RM-GICs can provide an anti-cariogenic benefit in bovine enamel some distance from the restoration site, this can therefore make evaluating split mouth study designs very complicated, if not invalid\textsuperscript{128}. A two-year clinical trial found that retention rates for RM-GICs are much lower than for resin composites, consequently a higher caries recurrence rate was reported for the RM-GIC\textsuperscript{129}. A three-year clinical study evaluating a fluoride containing resin composite and a RM-GIC found that both materials were suitable for posterior approximal restorations, but the resin had a higher failure rate due to secondary caries than the RM-GIC\textsuperscript{89}. Wear rates of several RM-GICs revealed a cyclic behaviour in wear patterns of the materials tested over a two-year period\textsuperscript{130}. Although intermediate results at 6, 9 and 18 months showed great variability, long-term wear rates appeared to converge and the difference between the materials after two years was insignificant. This may explain some conflicting reports in comparison of resin composites and RM-GICs with regards to recurrence of caries.

### 2.3 Casein Phosphopeptide-Amorphous Calcium Phosphate

First demonstrated to have potential anticariogenic properties in the laboratory, animal and human *in situ* studies, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) has since been incorporated into several dietary products as well as dental materials\textsuperscript{131-134}. According to Reynolds et al. the proposed anticariogenic mechanism of CPP-ACP is that it incorporates nanocomplexes into
plaque and onto the tooth surface, the localized CPP-ACP nanocomplexes then act to buffer the free calcium and phosphate ion activities, thereby maintaining a state of supersaturation with respect to tooth enamel, preventing demineralisation and promoting remineralisation $^{135}$. The bond between CPP and ACP is dependent upon the pH of the surrounding environment, as the pH drops following an acid challenge, CPP-ACP dissociates releasing calcium and phosphate which counteracts the drop in pH and leads to an inhibition of demineralisation by maintaining a supersaturation of calcium and phosphate ions with respect to tooth enamel $^{136,137}$.

### 2.3.1 Chemistry and Role in Oral Environment

An *in vitro* study concluded that CPP-ACP complexes on dentine surface provoke lower demineralization and higher remineralization compared to the dentine surfaces without the agent $^{138}$. An *in situ* trial of dental products containing calcium, phosphate and fluoride, Tooth Mousse Plus (GC Corporation, Tokyo, Japan) and Clinpro Tooth Crème (3M ESPE, MN, USA) had significantly higher levels of enamel lesion remineralization than products containing only fluoride (1000ppm fluoride, 5000ppm fluoride)$^{139}$. Cochrane et al. have demonstrated subsurface lesion remineralization with CPP-ACP and CPP-ACFP with greatest effect recorded at pH 5.5$^{140}$.

Incorporation of CPP-ACP into a GIC has shown that addition of 3% w/w CPP-ACP has the potential to improve the GIC’s anti-cariogenic properties without adversely affecting its physical properties $^{141}$. Further studies looking at incorporation of CPP-ACP into GICs claim that incorporation of 1.56% w/w of CPP-ACP increased microtensile bond and compressive strengths, as well as increased the release of calcium and phosphate ions from the GIC. The authors also stated that the CPP-ACP containing GIC was more resistant to acid challenge *in vitro* $^{142}$.

Prior application of CPP-ACP containing paste (Tooth Mousse, GC Corporation, Tokyo, Japan) has been shown to reduce the fall of plaque pH following a sucrose challenge $^{143}$. Continuous lubrication with Tooth Mousse (TM) has also been shown to
reduce erosive dentine wear, while at the same time promoting remineralisation.

Further studies of enamel erosion in vitro concluded that ProNamel (GlaxoSmithKline plc., Brentford, Middlesex, UK) and Tooth Mousse may offer a degree of protection from erosion of permanent enamel. CPP-ACP’s ability to bind to plaque has also been investigated and a rise of free calcium in plaque biofilm as a result of application of CPP-ACP was demonstrated. This property of CPP-ACP may aid in remineralisation, additionally the ability to provide a large reservoir of calcium may also restrict mineral loss during a cariogenic episode.

Several attempts have been made to incorporate CPP-ACP into commercially available products. A trial of sugar-free chewing gum containing CPP-ACP presented evidence of subsurface lesion remineralisation following an acid attack, the study also claimed that the re-mineralised mineral is more resistant to further acid challenges. Further evidence outlining CPP-ACP’s ability to remineralise subsurface lesions when compared to conventional sources of calcium showed that mouth rinses and chewing gums containing CPP-ACP provided higher levels of remineralisation.

A significant correlation between tooth wear and a number of acidic dietary products and drinking habits has been reported in adults between 18-30 years of age. The addition of CPP-ACP into sports drinks in vitro demonstrated that concentrations of 0.063% w/w of CPP-ACP and higher prevented erosive lesions occurring in test specimens. However, surface irregularities were observed which may be attributed to re-deposited calcium and phosphate from CPP-ACP.

2.3.2 Interaction between CPP-ACP and Fluoride

Saturation of plaque with calcium and phosphate ions is an important aspect of preventing demineralisation and promoting remineralisation, however fluoride ions continue to play an important role in caries prevention. At the core of caries prevention is fluoride’s ability to substitute into hydroxyapatite to create fluorapatite, which is more resistant to acid attack. It has been shown that the presence of
amorphous calcium and phosphate ions can help localise fluoride ions in plaque thereby creating ideal conditions for fluorapatite formation\textsuperscript{153}. Several studies have investigated the mechanism by which calcium and phosphate ions help to localise fluoride ions on the tooth surface\textsuperscript{140, 154-156}, investigators conclude that amorphous calcium, phosphate and fluoride nano-complexes are formed in plaque in the presence of CPP creating a bioavailable stabilised reservoir of these ion.

\subsection*{2.4 Analytical Methods}

The overview of dental caries and GICs has identified several important parameters of interest such as concentrations of beneficial ions in the oral environment (calcium, phosphate and fluoride) and physical properties of restorative materials (compressive and tensile strength, hardness, and solubility).

A number of laboratory methods have been used to measure the concentration of ions released from dental materials. Atomic absorption spectroscopy has been used in a number of studies to detect concentration of calcium ions in solution released from GICs following a period of storage anywhere between 24 hours and a few weeks\textsuperscript{141, 142}. The same studies along with several others have used an ion selective electrode to measure the release of fluoride ions into storage solutions\textsuperscript{58, 66, 93, 141, 142, 157-159}. Phosphate ion release has been measured colorimetrically using a UV-visible spectrophotometer\textsuperscript{141, 142, 160}. Some studies have used ion chromatography to measure fluoride, calcium and phosphate ions at the same time\textsuperscript{139, 161}. One of the common factors in many studies is the use of lactic acid as an ‘acid challenge’ solution, the pH of the solution is often set to 5.0 as a critical level for dissolution of tooth mineral. These parameters are specifically chosen to be as close to clinical conditions. The levels of ion concentration measurements depend on the volume of the storage solution as well as the period of storage; sufficient accuracy is achieved by fine tuning these two parameters at the pilot stage of any experimental protocol.
Clinical evidence shows that GICs perform differently to other restorative materials when it comes to physical properties, especially at the early stages of polymerisation before a ‘complete set’ is achieved. Due to self-curing nature of conventional GICs these early stages are more critical to long-term performance of GICs compared to other restorative materials such as resin composites and RM-GICs. To better understand how GIC behaves many physical characteristics of these materials have been studied using a variety of techniques. Roughness of the surface can indicate a material’s ability to trap plaque and can have an undesired effect on the aesthetics of the material, which can lead to discoloration of the surface. In Dental Research, surface roughness has been measured using atomic force microscopy (AFM)¹⁶² and surface profilometry⁶². AFM is able to achieve a higher resolution so it is often used for measuring surface roughness of enamel. Restorative materials can undergo higher levels of degradation, especially when subjected to abrasive laboratory testing, in these instances surface profilometry has proven to be more suitable. In conjunction with surface roughness, surface hardness is also a property of interest, especially when looking at occlusal surfaces of restorations, where restorative materials are subjected to masticatory forces. Either Knoop or Vickers hardness tests are used to determine the surface hardness of materials, enamel and dentine. Knoop hardness is often chosen over Vickers when small sample size poses a restriction on the available surface area. Micro-indentation⁶² and nano-indentation¹⁶³,¹⁶⁴ are well established methods for measuring surface hardness.

GICs are very sensitive to the levels of moisture in the surrounding environment during the early stages of the setting reaction and can suffer from either water uptake or water loss. Analytical balances are often used to measure the mass of GIC samples¹⁶¹ as a way of monitoring the moisture level of the materials. When investigating ion release, mass measurements can also supplement the ion release data.
2.5 References


CHAPTER 3

Aims and Objectives

Review of published literature highlighted the lack of laboratory evidence exploring the effect of various acids on GICs. Anecdotal clinical evidence is abundant that points to acid exposure causing increased fluoride release from GICs but poor performance when it comes to the effects on physical properties. By restricting the number of variables, a laboratory study can help understand the specific factors responsible for influencing ion release and physical properties of GICs undergoing acid challenge.

The aims of this research were to:

a) Investigate the effects of various acids on selected physical properties of conventional GICs. Determine if surface hardness and mass loss vary depending on which acid the GIC is exposed to;

b) Investigate the effects of various acids on fluoride, phosphate and calcium ion release from GICs;
c) Evaluate the ion release and physical characteristics of GICs after topical applications of a calcium and phosphate releasing cream;

d) Investigate recharge potential of topically delivered CPP-ACFP when applied to the surface of conventional GICs with and without incorporated CPP-ACP.

e) To characterise the role of aluminium in the structure of GICs and the impact of chelating agents on the physical properties of GICs.
CHAPTER 4

Preliminary studies

4.1 Introduction

A series of pilot studies were developed to determine the most suitable methods for measuring the remineralisation and demineralisation properties of glass ionomer cements (GICs) and other GIC-like materials. Measurements of mass loss, surface hardness and ion concentrations were determined to be the most fundamental parameters related to remineralisation and demineralisation of tooth mineral. Several different materials were investigated, including conventional GICs and resin-modified GICs. In parallel, several acid solutions were evaluated as acidic challenge media to replicate various clinical scenarios.

Several measuring techniques were found to be incompatible with the selected materials and acid solutions. Difficulties with measuring fluoride ion concentration when using lactic acid required either the use of other methods to measure fluoride or using a different acid to provide an acid challenge for selected materials. Liquid chromatography, previously used to measure ion release in acidic
solutions was unable to measure fluoride ions in lactic acid. Results indicated that spectral peaks for fluoride and lactic acid overlapped to cause accuracy problems for determining fluoride release. At this stage a pilot study was run to investigate the use of other acids. Citric acid was chosen due to its presence in the diet and oral environment, with a specific acid concentration and pH value being chosen to retain relevance to clinical conditions. Although citric acid presented itself as a suitable replacement for lactic acid for the purpose of measuring fluoride ions, it was discovered that it posed a considerable threat of contamination to liquid chromatography equipment. Citric acid was noted to cause large mass loss in the GICs which was believed to be caused by chelation of aluminium ions, consequently a risk of aluminium contamination of the liquid chromatography column led to its abandonment as an analytical tool. Alternative methods were identified and validated for accuracy and consistency of measurement. The ion selective electrode was used to obtain fluoride ion concentrations in solution. Atomic absorption spectroscopy (AAS) was used to conduct calcium assays and colorimetric assays were used to measure phosphate ion concentrations. AAS was also later used to conduct aluminium assays.

Pilot studies with citric acid did show that the GICs responded to citric acid very differently to lactic acid, so exposure to citric acid became an additional focus of further study and following several pilots, citric acid was included in the study protocol. Results from pilot citric acid studies also highlighted the importance of investigating other low pH media that may be encountered in the oral environment, consequently hydrochloric acid was also included. The ionic concentration of each solution was carefully selected based on values found previously in plaque fluid. ¹

As several acid solutions were trialled during the early stages of the pilot studies the protocols for surface hardness and ion release had to be re-evaluated and a new set of parameters tested.

The aims of the pilot studies were to:
a) investigate suitable materials and methods to further the understanding of demineralization and remineralisation processes in dental restorative materials; and
b) establish protocols that provide consistent outcomes for measuring mass loss, surface hardness and ion release for dental restorative materials following an acid challenge.

4.2 Specimen shape

4.2.1 Materials and Methods

Various glass ionomer cement (GIC) restorative materials were used in pilot studies to determine suitable parameters for the main investigations undertaken in this research (Table 4.1). The GICs used were ChemFil Molar (CFM), ChemFil Rock (CFR) (Dentsply DeTrey GmbH, Germany), Riva Protect (SDI Ltd., Australia), which is a resin-modified GIC (RM-GIC), and the high fluoride releasing conventional GICs, Fuji VII (F7) (GC Corporation, Tokyo, Japan) and Fuji VII EP (F7EP) (GC Corporation, Tokyo, Japan). Materials were tested for mass loss, ion release and surface hardness.

<table>
<thead>
<tr>
<th>Material</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChemFil Molar</td>
<td></td>
</tr>
<tr>
<td>Chemfil Rock</td>
<td>Replaced ChemFil Molar</td>
</tr>
<tr>
<td>Riva Protect</td>
<td></td>
</tr>
<tr>
<td>Fuji VII</td>
<td>High fluoride release</td>
</tr>
<tr>
<td>Fuji VII EP</td>
<td>Fuji VII that contain 3% (w/w) CPP-ACP</td>
</tr>
</tbody>
</table>

Table 4.1. Materials used in pilot studies. CPP-ACP (casein phosphopeptide-amorphous calcium phosphate).
Polyvinyl siloxane impression material (eliteHD+ light body, Zhermack SpA, Badia Polesine, Italy) moulds were used to create standardised discs of the restorative materials that measured 1mm x 10mm (thickness x diameter). This was later changed to rectangular blocks measuring 3mm x 6mm x 6mm (thickness x width x length). Discs or blocks of each restorative material were prepared by placing the materials in the mould with the top and bottom surfaces covered by plastic strips, held between two glass slides. The glass slides were pressed together to extrude excess material. The specimens were allowed to set inside the moulds for 24 hours in an incubator (37°C, ~100% relative humidity). After cooling to room temperature the blocks were removed from the moulds, and lapped with 600-grit paper (Norton Tufbak, Saint-Gobain Abrasives Ltd., Auckland, NZ) under running distilled water.

4.2.2 Discussion

Specimen shape was changed to accommodate subsurface analysis of the restorative materials. The thickness of discs was insufficient for this purpose; additionally the disc shape lent itself to only two surfaces being suitable for subsurface analysis as opposed to six surfaces of a thicker block. Rectangular blocks instead of discs were proven to be more suitable for investigating depth profiles of tested materials following acid challenge. A pilot study was run to determine the feasibility of using blocks instead of discs. Later pilot studies, using blocks, were performed to investigate hardness as a function of depth as well as Energy Dispersive X-Ray Analysis (EDXA) assays of subsurface regions of dental materials, something which would not be possible using discs due to a thickness of only 1mm.
4.3 Ion release

4.3.1 Materials and Methods

Acid solutions, namely tartaric acid, citric acid, lactic acid and hydrochloric acid were trialled in conjunction with measuring equipment to be used to determine calcium, phosphate and fluoride ion concentrations to ensure compatibility.

Four different solutions were prepared to expose the blocks of GIC to a variety of acidic and neutral environments. The three acidic solutions were formulated to simulate a gastric erosive challenge (50mM NaCl adjusted to pH 2.0 with HCl), a dietary erosive challenge (50mM citric acid at pH 5.0) and a cariogenic acid challenge (50mM lactic acid at pH 5.0). A further pilot study was conducted using tartaric acid (pH 5.0, 50mM), which was used to investigate the effect of the disassociation strength of acids. Disassociation strength of acids varies depending on the molecular structure, specifically the number of H\(^+\) ions an acid can donate. Lactic and tartaric acid are able to donate a maximum of two H\(^+\) ions. Citric acid can donate three H\(^+\) ions; this means that citric acid is able to chelate aluminium ions (Al\(^{3+}\)), whereas lactic acid and tartaric acid cannot. Consequently, another important goal of the pilot work was to establish a protocol for measuring aluminium ion concentration in aqueous solution using AAS. In addition to mass loss and aluminium ion release, calcium and phosphate ion release were also measured. The control solution in all studies was distilled deionised water at pH 6.9 (MilliQ water, Millipore Corporation, Victoria, Australia).

Initial pilot studies used a 28 day duration for measurements that were carried out after 1, 2, 3, 7, 14, 21 and 28 days. The storage volume was initially 15mL (plastic containers), however this protocol was quickly replaced with a three-day duration setup in 5 mL of solution. Two to six blocks per group of each material were exposed to 5mL of one of the four solutions above. Solutions were changed every 24 hours and the samples were measured for change in mass and surface hardness. The solutions were analysed to determine calcium, phosphate and fluoride ion release.
Later experiments also focused on aluminium ion release, the protocol used for aluminium assays was similar to calcium assays as it used the same equipment, but was developed at a later stage.

The mass of each block was measured every 24 hours before surface hardness measurements were performed. Blocks were taken out of the solution, pat dried using Kimwipes (Kimtech Science, Kimberly-Clarke Professional, Australia) and then weighed using a microbalance (Precisa XT 120A, Dietikon, Switzerland). Readings were obtained to within ±0.0001 of a gram.

The ion release of calcium, aluminium, phosphate and fluoride after each 24-hour period of storage were determined using atomic absorption spectroscopy (for calcium and aluminium), colourimetry (for inorganic phosphate) and an ion-specific electrode (for fluoride). To determine the calcium concentration, sample solutions (0.5 mL) were diluted with water (0.5 mL, MilliQ), acidified with 1M HCl (0.5 mL) and diluted with 2% lanthanum chloride (0.5 mL) and analysed on a Varian AA240 atomic absorption spectroscope (AAS, Varian Australia Pty. Ltd.) against a set of seven standards ranging from 0 to 250 µM calcium. The Varian AA240 AAS was also used to determine the concentration of aluminium ions. Sample solutions (0.5 mL) were diluted with potassium chloride (0.5 mL, 8mg/L) and lanthanum chloride (0.5 mL, 2% w/w) and then acidified with 1M HCl (0.5 mL) and compared against a set of standards ranging from 0 to 125 µM aluminium. Inorganic phosphate ion concentrations were determined colourimetrically using a spectrophotometer (UV-visible spectrophotometer, Varian Australia, Pty. Ltd). The samples were prepared by taking 100 µL of solution, diluting it with 500 µL of 4.2% ammonium molybdate and adding 20 µL 1.5% of Tween ® 20. (Sigma-Aldrich, St. Louis, MO). The phosphate concentration was determined by comparing the spectrophotometer readings of the samples against a set of seven standards ranging in phosphate concentrations from 0 to 100 µM. The concentration of fluoride ions was determined using an ion-selective electrode (Radiometer analytical, ISE C301F, Lyon, France) connected to an ion analyser (Radiometer analytical, Ion Check 45, Lyon, France). Sample solutions (1mL) were diluted with 1mL total ionic strength adjustment buffer (Merck Pty Ltd, Kilsyth,
VIC, Australia) and measured against a set of eight fluoride standards ranging from 0 to 1000 µM. Liquid Chromatography (ICS-3000, Dionex Corporation, CA, USA) machine was used at the initial stage to measure the concentration of various ions (see table).

As the main body of experimental work progressed additional pilots were conducted. One of the parameters tested was the effect of topical treatment using CPP-ACFP containing cream (Tooth Mousse Plus, GC Corporation, Tokyo, Japan). The pilot established suitable parameters for dilution of the cream and standardised the duration of treatment duration. This additional step was integrated into already established protocol of testing surface hardness, mass loss and collecting solutions for later ion concentration assays.

Later stages of the main experimental work focused on aluminium ions and the effect on the structure of GICs under acid challenge. In order to rule out the buffering effect of the CPP-ACP containing cream on the initial setting reaction a pilot study was conducted. Some groups were allowed to set for 24 hours in an incubator (37C, 95% relative humidity), while other groups only set for one hour in an incubator (37C, 95% relative humidity). Blocks of F7 and F7EP were stored for 24 hours in either water or citric acid solution. F7 and F7EP groups were subjected to either Tooth Mousse Plus (TM+, GC Corporation, Tokyo, Japan) treatment or a placebo mousse treatment, while a control group had no topical treatment. After 24 hours, the mass loss of each block was measured, however no surface hardness or ion release measurements were made.

Data from sample groups were found to follow a normal distribution after use of χ² test. Single factor ANOVA was used to analyse the results using Bonferroni-Holm multiple comparison. Two-way ANOVA was used to determine the interaction between application of CPP-ACFP and exposure to different acids. The level of significance was set at α = 0.05.
4.3.2 Results

F7 results showed no significant differences between one-hour and one-day groups when stored in water (Table 4.2). When stored in citric acid, however, significant differences in mass loss were observed in both the placebo mousse and TM+ groups when one-day results were compared to one-hour results (p<0.05). However, when blocks were only allowed to set for one hour, the placebo group showed a decrease in mass loss compared to the one-day groups, whereas the TM+ group showed an increase in mass loss. F7EP results showed that allowing the blocks to only set for one hour before placing them in solution had a significant impact on all one-day/one-hour mass loss comparisons (Table 4.3). In both water and citric acid, the one-hour groups showed significantly (p<0.05) higher mass gain (water) or significantly (p<0.05) lower mass loss (citric acid).
Table 4.2. Comparison of mass loss between one hour setting time and 24 hour setting time followed by citric acid exposure of F7. Percentage change in **bold** numbers, all other numbers in grams. n=5.
Table 4.3. Comparison of mass loss between one hour setting time and 24 hour setting time followed by citric acid exposure of F7EP. Percentage change in **bold** numbers, all other numbers in grams. n=5.

The evaluation of ion release of the initial four restorative materials over a 28-day period in many cases showed very low concentrations for daily ion release, in some instances the low release levels were below the detection limit (phosphate ion release in water) of the instrumentation (Table 4.4). These results also highlighted the inability of liquid chromatography to measure fluoride ions in lactic acid, something of great interest to this study.
Table 4.4. Daily ion release of F7, F7EP, Riva Protect and ChemFil Molar using liquid chromatography. All concentration values are in mM.

In addition to the above four materials, ChemFil Rock (CFR) was investigated separately. Compared to F7 and F7EP, ChemFil Rock exhibited less mass loss over three days, this difference was found to be statistically significant (p<0.05) (Table 4.5). F7EP was also found to have lost more mass than F7 (p<0.05). Mass gains found in water storage were also found to be statistically significant among all groups (p<0.05). Precision and consistency of the mass measurements produced very small errors.
Table 4.5. Mass loss comparison between CFR, F7 and F7EP when stored in citric acid and water over a three-day period. All values are in grams.

F7EP showed a gain of mass when stored in tartaric acid, F7 showed a mass loss, the difference between the two materials was significant (p<0.05) (Figure 4.1). Citric acid showed a much higher aluminium ion release than either lactic or tartaric acids (p<0.05). There was no significant difference between F7 and F7EP in terms of aluminium ion release for all solutions (Figure 4.2 and 4.3). Significantly higher phosphate ion release (p<0.05) was measured in tartaric acid for both F7 and F7EP compared to phosphate ion release in lactic acid. Phosphate ion release was significantly lower for both materials in tartaric acid compared to citric acid (p<0.05).
Calcium ion release was significantly higher in tartaric acid compared to lactic acid in F7EP only (p<0.05).

**Figure 4.1.** Mass loss of F7 and F7EP in tartaric acid over a three-day period. n=5
Figure 4.2. Calcium, aluminium and phosphate ion release from F7 in three different acids. n=5. Al3+
Some of the acid parameters were investigated coincidently as part of other pilot studies, such as the tartaric acid and aluminium AAS study. The studies involving Riva Protect, ChemFil Molar and F7 and F7EP used storage volumes of 15mL for each block. This volume was deemed too large, as ion concentrations were too diluted and in many cases were falling below the detection limit of equipment. Surface hardness, which was measured up to 28 days, showed that past the seven-day point standard deviations became too large and therefore statistical comparisons became meaningless. The protocol was therefore changed to a three-day duration and a storage volume of 5mL for all further pilot work. The new protocol produced much greater precision and consistency when comparing ion release profiles between groups due to a higher concentration of ions, while still being able to detect
differences between materials and solutions in terms of mass loss and surface hardness.

For each material, two to six discs or blocks were divided into equal groups. Most groups were not subjected to topical treatments, however some later pilot work included an additional topical treatment step. Topical treatment pilot studies (Tooth Mousse Plus, placebo mousse) followed largely the same protocol as for the other ion release and mass/hardness change studies. The equipment used was the same as for the studies without topical treatment (mass loss, ion release and surface hardness). MilliQ water was used to rinse the blocks following topical treatment. Kimwipes were used to pat dry the blocks before they were placed in acid solution (or water control). Topical treatments were always carried out immediately before the blocks were placed back into an acid solution, after mass loss and surface hardness measurements.

4.4 Hardness

4.4.1 Materials and Methods

Vickers microhardness measurements were determined from indentations on the lapped GIC surface using a Microhardness tester (MHT-10, Anton Paar GmbH, Graz, Austria) attached to a microscope (Leica DMPL, Leica Microsystems Wetzlar GmbH, Germany). Indentations were made using a range of forces (0.5, 1.0, 1.5, 2.0 and 2.5N) on fresh and acid exposed blocks of F7 and F7EP. Dwell time was varied between 6 and 20 seconds at a fixed force of 1.0N. Rate of force application was fixed at 0.99 N/min for all measurements. The indentations were separated by a distance of at least three times the indentation size. Images of the indentations were acquired through a calibrated digital camera (Leica DFC320) mounted on the microscope and distance measurements made using Image Tool software (Version 3.0, UTHSC, San
Antonio, TX) which were then converted into Vickers hardness values. Blocks were then placed into fresh solution and returned to the incubator.

During investigations of surface hardness of F7 and F7EP high surface hardness was measured in some groups. This prompted a further pilot study to determine whether a crust layer was formed on the surface of the materials. In this pilot, surface hardness was measured in layers as a function of depth. The depth profile of surface hardness involved lapping the material surface in stages of 100 μm and measuring the hardness of the exposed surface at each depth, up to 500 μm (Figure 4.4). Microhardness was measured using the same equipment as described above (Vickers hardness). Blocks were lapped using wet 600-grit lapping paper and rinsed with MilliQ water.

![Figure 4.4.](image)

**Figure 4.4.** Depth profile comparison of TM+ treated F7EP following three-day exposure to four solutions. Initial surface VH shown as a black line.
To supplement the depth profile hardness study another pilot was conducted using electron dispersion X-ray analysis (EDXA). Samples for EDXA using scanning electron microscope (Fei Quanta Cryo SEM, Fei Corporation, ON, USA) were selected from groups already exposed to the three-day acid challenge. Blocks were fractured in two (using a sharp blade) in order to expose the central and subsurface regions. Samples were air dried and secured to stubs using two sided carbon tape. Ion concentrations were sampled and compared between the surface layer, subsurface region and ‘bulk’ material, only relative concentrations of ions could be measured, no absolute values were available (Figure 4.5).
4.4.2 Results

Surface hardness was measured over a 28-day period, statistically significant (p<0.05) differences between materials appeared by the third day (Table 4.6). In some cases statistically significant differences could only be detected by the seventh day, but by the 14th day the standard deviations became too large and no significant differences between materials could be measured.

ChemFil Molar was initially significantly harder (p<0.05) than the other three materials and Riva protect was significantly (p<0.05) softer than F7 and F7EP. Considerable softening was observed in the first 24 hours; at this stage no significant differences between any of the materials could be measured.

<table>
<thead>
<tr>
<th>Day</th>
<th>F7</th>
<th>F7EP</th>
<th>Riva</th>
<th>ChemFil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>58.2</td>
<td>47.0</td>
<td>65.6</td>
</tr>
<tr>
<td>1</td>
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<td>3</td>
<td>13.9</td>
<td>10.9</td>
<td>18.3</td>
<td>18.6</td>
</tr>
<tr>
<td>7</td>
<td>12.1</td>
<td>9.4</td>
<td>13.1</td>
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<td>7.1</td>
<td>3.4</td>
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</tr>
<tr>
<td>28</td>
<td>3.6</td>
<td>3.1</td>
<td>2.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Table 4.6. Surface VH of F7, F7EP, Riva Protect and ChemFil Molar in lactic acid over 28 days. Storage volume 15ml. n=5

In addition to the above four materials, ChemFil Rock (CFR) was investigated separately. Only mass loss and surface hardness were measured. ChemFil Rock was initially of very similar surface hardness to F7 and F7EP (p>0.05) (Table 4.7). At the 48-hour mark significant differences could be detected between CFR, F7 and F7EP. In fact, in the citric acid groups, following the softening observed in the first 24 hours, CFR did not record any further softening at the surface (p>0.05 between days 1,2 and 3). F7 and F7EP continued to gradually soften with each day when stored in citric acid. Blocks stored in water showed significant differences among all materials, with F7EP recording the least reduction in surface hardness, followed by CFR and F7.
Four groups were investigated for depth profile hardness, F7EP treated with TM+ exposed to citric, lactic and hydrochloric acids as well as a water control group. Results showed a statistically significant (p<0.05) decrease in subsurface VH (around 100 μm below the surface) in the citric acid group compared to ‘bulk’ VH (i.e. the hardness of the material in a region not directly exposed to the solutions). This decrease was also significant when compared to measurements taken at a depth of 100 μm in groups placed in the other solutions. No statistical significance could be detected when lactic acid, hydrochloric acid and water groups were compared amongst each other at the 100 μm depth (p>0.05).

EDXA of surface and subsurface regions of F7EP blocks showed there was a significant difference between blocks stored in water and those stored in citric acid. The concentration of ions in the ‘bulk’ of the material in both groups was very similar. However, in some cases the water storage groups showed lower ion concentrations on the surface and in subsurface regions compared to the citric acid groups and compared to the material bulk (aluminium and strontium ions) (Figure 4.6). There are also cases where citric acid exposed groups showed higher concentrations of ions (sodium and carbon) on the surface and in subsurface regions compared to ‘bulk’ of the material (sodium and carbon) (Figure 4.7).

Table 4.7. Surface VH of Chemfil Rock compared to F7 and F7EP stored in citric acid over a three day period. Standard error shown in secondary columns. All values in MPa VH1.0

<table>
<thead>
<tr>
<th></th>
<th>Chemfil Rock</th>
<th></th>
<th>F7</th>
<th></th>
<th>F7EP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>CA</td>
<td>Err</td>
<td>MQ</td>
<td>Err</td>
<td>CA</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>49.1</td>
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</tr>
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<td>1</td>
<td>36.9</td>
<td>1.0</td>
<td>46.8</td>
<td>1.0</td>
<td>34.6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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<td>1.6</td>
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<td>33.9</td>
</tr>
<tr>
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<td>3</td>
<td>37.6</td>
<td>2.3</td>
<td>42.8</td>
<td>1.2</td>
<td>29.5</td>
</tr>
<tr>
<td>% Loss</td>
<td></td>
<td>23.5</td>
<td></td>
<td>17.9</td>
<td></td>
<td>39.9</td>
</tr>
</tbody>
</table>
Figure 4.6. F7EP blocks treated with TM+ analysed using EDXA at various subsurface regions.
4.4.3 Discussion

Microhardness measurements showed an expected spread of results, higher load values produced larger indentations. When the surface of the material was softened following a three-day exposure to acid the indentations were even larger. Variations in dwell time produced no perceptible change in indentation size.

The microhardness pilot study was conducted to determine which range of indentation sizes would provide the most accurate results for all cases (soft and hard restorative material surfaces). The values obtained at this stage were not important, the goal was to establish a set of parameters (dwell time and applied force) that would work for all materials and conditions without changing the magnification of the microscope during post indentation image capture. In some cases very large
indentations were still observed and the magnification needed to be changed in order to obtain precise measurements. This was not ideal, however using lower applied force would have resulted in reduced precision for small indentations. A pragmatic set of parameters (1.0N, 6 sec dwell time) was chosen to minimise the impact of having to change magnifications during indentation measurements.

Inclusion of ChemFil Rock was only intended for pilot tests, CFR is a different type of GIC compared to F7/F7EP and Riva. Most GICs are based on aluminium, however some new materials include zinc in their formulation; CFR is one such material. The addition of zinc improves the flexural strength of material and potentially improves resistance to dissolution. The intended application of CFR is different to that of the other tested materials, nevertheless including CFR in pilot work forms a baseline measurement.

Changes with depth were marginal and there were many difficulties in determining the optimal depth interval. Ideally, it was not so compelling to find out what the hardness drop was but rather at which depth it occurred, if at all. Even using a very coarse resolution of 100 μm the significant drop and then rise in hardness for the citric acid group could still be measured. Additionally, EDXA surveys showed evidence of ion migration from the ‘bulk’ and sub-surface regions of the material to the surface layer.

4.5 Conclusion

The protocol for measuring surface hardness was established using the Anton Paar microhardness testing machine and a Leica microscope using parameters of force of 1.0N, dwell time of 6 seconds and rate of 0.99N/min. Protocols for measuring calcium and aluminium ions were established using atomic absorption spectroscopy (AAS). An Ion selective electrode was determined to be the only feasible way to measure fluoride ion concentrations for the tested combination of materials and acid storage media. Colourimetry was used to measure phosphate ion concentrations.
4.6 References


5.1 Abstract (249 words):

Recent commercialization of a new glass-ionomer cement (GIC) (Fuji VII™ EP) claims enhanced tooth protection due to the incorporation of 3% (w/w) casein phosphopeptide amorphous calcium phosphate (CPP-ACP). Objectives: Aims of this study were to assess this new GIC compared with a GIC without CPP-ACP (Fuji VII™) in regards to ion release, changes in surface hardness and in mass under a variety of acidic and neutral conditions. Methods: Eighty blocks of Fuji VII™ (F7) and Fuji VII™ EP
(F7EP) were subjected to three acidic solutions (lactic and citric acids pH 5.0, hydrochloric acid pH 2.0) and water (pH 6.9) over a three day period. Ion release, surface hardness and weight measurements were carried out every 24 hours. **Results:** Higher calcium ion release from F7EP was observed under all acidic conditions. Increased inorganic phosphate ion release was observed for F7EP in hydrochloric and citric acids. Fluoride ion release was similar between F7 and F7EP under all conditions but was significantly higher in acids compared to water. After three days there was no significant difference in surface hardness (p>0.05) between the two materials under all conditions except hydrochloric acid. Minimal change in mass was observed for F7 and F7EP in water, lactic and hydrochloric acids, however citric acid caused significantly more mass loss compared with water (p<0.001). **Conclusion:** Incorporation of 3% w/w CPP-ACP into F7 has enhanced calcium and phosphate ion release, with no significant change in fluoride ion release and no adverse effects on surface hardness or change in mass.

Keywords: GIC, CPP-ACP, hardness, ion release

### 5.2 Introduction

Glass ionomer cements (GICs) are widely used for a variety of purposes such as for intermediate restorations, caries stabilization, definitive restoration of microcavities or non-carious cervical lesions, adhering orthodontic brackets and bands, fissure sealing erupting molars and as a surface protecting material for high risk surfaces such as root surfaces. The main advantages of GICs are their strong ability to chemically bind to dentine and their ability to release fluoride ions. This fluoride ion release has been shown to slow the progression and aid the regression of early carious lesions. The tooth surface will only demineralise when the fluid bathing the tooth is undersaturated with respect to the tooth mineral which is composed of
hydroxyapatite hence the calcium and phosphate ion activity at the tooth surface will also be important.

A number of investigators have explored the modification of dental materials in attempt to have them release calcium, phosphate and fluoride ions. Skrtic et al. (2003) explored modifying dental composites with bioactive glasses. Mazzaoui et al. (2003) and Al Zraikat et al. (2011) assessed the addition of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) to GICs. The casein phosphopeptides stabilise calcium and phosphate ions in a bioavailable form that allow them to inhibit demineralisation and promote the remineralisation of early lesions. CPP-ACP is stable in the presence of fluoride and has been shown to work synergistically with fluoride. This makes CPP-ACP a promising additive to dental products and restorative materials. The study by Mazzaoui et al. (2003) was a proof of concept study that showed that CPP-ACP could be added to GIC. Al Zraikat et al. (2011) later explored the effect of CPP-ACP concentration on GIC mechanical properties. Additionally, both of these of studies showed that the addition of CPP-ACP improved calcium and phosphate ion release in lactic acid.

The tooth surface can be exposed to a variety of demineralization challenges including: acids formed from metabolic processes of oral bacteria (predominantly lactic acid); food acids such as citric and phosphoric acid that are commonly found soft drinks; and hydrochloric acid from the regurgitation of stomach acids. If these acids overwhelm saliva’s protective functions mineral may be lost from the teeth. Therefore, the overall aim of this study was to determine how GIC with CPP-ACP performed under a variety of acidic conditions compared with GIC without CPP-ACP in terms of ion release, change in hardness and change in mass. The research questions were: what effect would the addition of CPP-ACP have on; (1) surface hardness of F7EP compared to F7; (2) change in mass between F7EP and F7 under neutral or acidic environments and (3) change in calcium, phosphate or fluoride ion release between F7EP and F7 under neutral or different acidic environments.
5.3 Materials and Methods

F7 and F7EP containing 3% w/w CPP-ACP from the same batch were provided by GC Corporation (Japan) in capsule form. Polyvinyl siloxane impression material (eliteHD+ light body, Zhermack SpA, Badia Polesine, Italy) moulds were used to create standardised GIC blocks measuring 3mm x 6mm x 6mm (thickness x width x length). 40 blocks of each GIC were prepared by placing the materials in the mould with the top and bottom surfaces covered by plastic strips, which was held between two glass slides. The glass slides were gently pressed together to extrude any excess material. The specimens were allowed to set inside the moulds for 24 hours in an incubator (37°C, ~100% relative humidity). After cooling to room temperature the blocks were removed from the moulds, and the two major parallel surfaces of the blocks were lapped with 600 grit paper (Norton Tufbak, Saint-Gobain Abrasives Ltd., Auckland, NZ).

Four different solutions were prepared to expose the blocks to a variety of acidic and neutral environments. The three acidic solutions were formulated to simulate a gastric erosive challenge (50mM NaCl adjusted to pH 2.0 with HCl), a dietary erosive challenge (50mM citric acid at pH 5.0) and a cariogenic acid challenge (50mM lactic acid at pH 5.0) (this concentration was selected based on values found previously in plaque fluid \(^{11}\)). The neutral solution was distilled deionised water at pH 6.9 (Millipore Corporation, Victoria, Australia).

Ten blocks of each type of GIC were exposed to 5mL of one of the four solutions (stored in plastic containers). Solutions were changed every 24 hours and the samples were measured for change in mass, surface hardness and the solutions were analysed to determine ion release of calcium, phosphate and fluoride.

The mass of each block was measured every 24 hours before surface hardness measurements were performed. Blocks were taken out of solution, pat dried and then weighed using a microbalance (Precisa XT 120A, Dietikon, Switzerland).

Vickers microhardness measurements were determined from indentations on the lapped GIC surface using a Microhardness tester (MHT-10, Anton Paar GmbH,
Graz, Austria) attached to a microscope (Leica DMPL, Leica Microsystems Wetzlar GmbH, Germany). Two indentations were made on each block (Force, 1.0 N; Dwell, 6 s; Rate, 0.99 N/min). The indentations were separated by a distance of at least three times the indentation size. Images of the indentations were acquired through a calibrated digital camera (Leica DFC320) mounted on the microscope (Leica DMLP, Leica Microsystems Wetzlar GmbH, Germany) and distance measurements made using Image Tool software (Version 3.0, UTHSC, San Antonio, TX) which were then converted into Vickers hardness values. Blocks were then placed into fresh batch of solution and returned to the incubator.

The ion release of calcium, phosphate and fluoride after each 24 hour period of storage were determined using atomic absorption spectroscopy, colorimetry and an ion-specific electrode respectively. To determine the calcium concentration sample solutions (1 mL) were acidified with 1M HCl (0.5 mL) and diluted with 2% lanthanum chloride (0.5 mL) and analysed on a Varian AA240 atomic absorption spectroscope (Varian Australia Pty. Ltd.) against a set of seven standards ranging from 0 to 250 µM calcium. Inorganic phosphate ion concentrations were determined colorimetrically using a spectrophotometer (UV-visible spectrophotometer, Varian Australia, Pty. Ltd). The samples that were analysed were prepared by taking 100 µL of solution, diluting with 500 µL of 4.2% ammonium molybdate and adding 20 µL 1.5% of Tween® 20. (Sigma-Aldrich, St. Louis, MO). The phosphate concentration was determined by comparing the spectrophotometer readings of the samples against a set of seven standards ranging in phosphate concentrations from 0 and 100 µM. The concentration of fluoride ions was determined using an ion-selective electrode (Radiometer analytical, ISE C301F, France) connected to an ion analyzer (Radiometer analytical, Ion Check 45, France). Sample solutions (1mL) were diluted with 1mL total ionic strength adjustment buffer (Merck Pty Ltd, Kilsyth, VIC, Australia) and measured against a set of eight fluoride standards ranging from 0 to 1000 µM.

Data from sample groups was found to follow the normal distribution, χ² test was used to test normality. Single factor ANOVA was used to analyse the results using Bonferroni-Holm multiple comparison. Two-way ANOVA was used to determine the
interaction between incorporation of CPP-ACP and exposure to different acids. Level of significance was set at $\alpha = 0.05$.

5.4 Results

Table 5.1 shows surface hardness of F7EP and F7 in three different acidic solutions and distilled deionised water measured over three days. A significant decrease in surface hardness was measured from day to day in all solutions except in the following cases. F7EP in citric and hydrochloric acids between day two and three did not register a significant change in surface hardness. F7 in water did not register a significant change between days one and two, and two and three. The surface hardness of F7EP in water was not significantly different between its initial hardness and day one as well as between days two and three. The initial hardness of F7EP was significantly ($p<0.001$) lower than that of F7. However, after 24 hours of storage in lactic and citric acid F7EPP had a similar surface hardness to F7 and was no longer significantly weaker for citric, lactic acids and water ($p>0.05$), but it was still significantly ($p<0.01$) weaker in hydrochloric acid. After three days of storage no significant difference in surface hardness could be measured between F7EP and F7 ($p>0.05$) for citric, lactic acids and water, but F7EP still remained significantly ($p<0.05$) weaker in hydrochloric acid.
Table 5.1. Mean surface hardness of F7 and F7EP in acidic and neutral solutions measured over three days. Values marked with the same lower case subscript indicate significant difference between GIC materials within the same solution for the same time period.

<table>
<thead>
<tr>
<th>VH (MPa)</th>
<th>F7</th>
<th>F7EP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Lactic</td>
</tr>
<tr>
<td>Initial</td>
<td>53.8 (a)</td>
<td>51.8 (b)</td>
</tr>
<tr>
<td>Day 1</td>
<td>48.5 (e)</td>
<td>32.6 (f)</td>
</tr>
<tr>
<td>Day 2</td>
<td>44.4 (g)</td>
<td>28.5 (h)</td>
</tr>
<tr>
<td>Day 3</td>
<td>42.0 (i)</td>
<td>24.8 (j)</td>
</tr>
</tbody>
</table>

Table 5.2. Mean relative mass of F7 and F7EP when stored in the four different solutions over three days. Values marked with upper case superscript indicate significant difference between different solutions within the same material for the same time period. Each group is scaled according to its initial weight (100%).

<table>
<thead>
<tr>
<th>(%) relative to initial mass</th>
<th>F7</th>
<th>F7EP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Lactic</td>
</tr>
<tr>
<td>Day 1</td>
<td>100.3 (a)</td>
<td>99.8 (b)</td>
</tr>
<tr>
<td>Day 2</td>
<td>100.5 (b)</td>
<td>99.7 (b)</td>
</tr>
<tr>
<td>Day 3</td>
<td>100.6 (c)</td>
<td>99.6 (c)</td>
</tr>
</tbody>
</table>

Table 5.2 shows the mean relative mass change of F7 and F7EP blocks when stored in the four different solutions over three days. Each group is scaled according to its initial weight (100%). The greatest mass loss at all time points was from F7 and F7EP that were exposed to citric acid.

Figures 5.1a-c show the measured concentration of calcium, phosphate and fluoride ions released from F7 and F7EP when stored in the four different solutions over a three day period. The rate of calcium and phosphate ion release for F7EP was significantly greater than that of F7 for both citric and hydrochloric acids (Figures 5.1a and 5.1b), and there was also greater calcium ion release in lactic acid (Figure 5.1a). The fluoride ion release in all solutions was generally similar for both F7 and F7EP (Figure 5.1c).
Figure 1 (a)  
Calcium Ion Release from Fuji VII and Fuji VII EP

Days in Solution

- Water (F7)
- Lactic (F7)
- HCl (F7)
- Citric (F7)
- Water (F7EP)
- Lactic (F7EP)
- HCl (F7EP)
- Citric (F7EP)

Figure 1 (b)  
Phosphate Ion Release from Fuji VII and Fuji VII EP

Days in Solution

- Water (F7)
- Lactic (F7)
- HCl (F7)
- Citric (F7)
- Water (F7EP)
- Lactic (F7EP)
- HCl (F7EP)
- Citric (F7EP)
Figure 5.1. Measured calcium (a), phosphate (b) and fluoride (c) ion release from F7 and F7EP when stored in the four different solutions over a three day period. Significant increase (p<0.05) in calcium ions was measured for all groups (except water) between F7EP and F7. Significant increase (p<0.05) in phosphate ion release was measured for citric and hydrochloric acids compared to water. Significantly more phosphate was released from F7EP compared to F7 in citric and hydrochloric acids. Fluoride ion release was significantly higher (p<0.005) in all acids compared to water. Groups marked with same lowercase indicate no significant difference in fluoride ion release between F7 and F7EP when exposed to the same solution.

Where a change in mass was detected for an exposure to an acidic solution over the three days (Table 5.2) the calcium and phosphate ion release was found to be greater for F7EP (Figures 5.1a and 5.1b). The difference in calcium and phosphate ion release as a function of change in mass for F7 and F7EP is shown in Figures 5.2a and 5.2b for citric and hydrochloric acids respectively (lactic acid and water could not be plotted as there was no measurable mass loss). These figures show that per unit of mass loss there was a greater release of calcium and phosphate ions from the GIC containing CPP-ACP.
Figure 5.2. Difference in calcium and phosphate ion release as a function of change in mass between F7 and F7EP in citric acid (a) and hydrochloric acid (b).
5.5 Discussion

In all the tests conducted on F7 and F7EP, the materials behaved differently in water and acidic environments. The surface hardness of the GIC’s significantly decreased in the acidic environments although this did not correlate to a change of mass. The release of ions was greater in acidic conditions compared with water. The release of calcium and phosphate ions from the material only under acidic conditions is ideal as this is when they would be needed to protect the adjacent tooth structure from demineralization. The higher release of calcium and phosphate ions from F7EP under acidic conditions may saturate the fluid in the oral environment with appropriate ions thus acting as a "smart material" for the protection of at risk tooth surfaces.

Previous studies have investigated ion release and surface hardness of various GICs in acidic and neutral environments \(^4,^5,^{12}\). Past results have shown that fluoride release from F7 is decreased with incorporation of CPP-ACP when stored in neutral solution (water) or lactic acid \(^5\). It has also been reported that lower pH environments negatively affect surface hardness of GICs \(^13\). However, there have been no studies looking at ion release or surface hardness of GICs covering a wide variety of acids that can be encountered in the oral environment.

5.5.1 Surface hardness

The results indicate that the degradation of surface hardness is pH dependant rather than solution dependant. Lactic and citric acids (both pH 5) produced a similar reduction in microhardness over three days, while HCl (pH 2) showed the greatest and water (pH 6.9) the least. Even though initially F7EP was significantly weaker in surface hardness than F7, a converging pattern occurred across all solutions such that, except for HCl, the difference after three days was no longer significant. It is possible that F7EP has a greater buffering capacity than F7 alone as CPP-ACP has previously been shown to have buffering potential \(^14\).
5.5.2 Change in mass

As with surface hardness, there was an initial difference in the change in mass between these two GIC materials. This can be partly attributed to F7EP being slightly less dense than F7. Significant mass loss was observed for specimens placed in citric acid for both materials. While other solutions produced small changes from day to day the differences between them were not significant. The difference in the change in mass between F7EP and F7, in citric acid, was not significant when taking into account the difference in initial weights. Although surface hardness showed very similar trend in a decrease in hardness for all solutions this was not repeated for change in mass. For citric and lactic acids the mass loss shows a large difference between two acid solutions such that, in lactic acid a slight mass gain was observed for F7EP and a slight loss for F7. In citric acid the largest mass loss was observed for both materials, while a mass gain was observed in water. The chelating effect of the citric acid may contribute to the large mass loss. Citric acid is a triprotic acid, which can chelate transition metals, in this case aluminium. Aluminium is an initial component of the GIC matrix in the form of aluminium phosphate and the aluminium ion is involved in the acid-base setting reaction. The cross-linking between the polyalkenoic acid chains is formed by a slow final maturation reaction involving aluminium ions, which increases the strength of the GIC$. This hardening process may take days to complete. When exposed to citric acid $Al^{3+}$ ions are complexed by citrate removing them from the GIC matrix leading to the greater mass loss of the material. Further evidence to support this theory can be seen in the phosphate ion release data shown in Figure 5.1b. The GIC without CPP-ACP released high levels of phosphate although it contained no CPP-ACP supporting the chelation of aluminium and the dissolution of the aluminium phosphate present within all GICs.
5.5.3 Ion Release

When subjected to aggressive environments such as citric, lactic and hydrochloric acids a substantial release of ions occurred from both GIC materials. Calcium release in the three acids ranged from 0.07 to 0.20 μmol/mm² for F7 but was higher for F7EP, ranging from 0.36 to 1.34 μmol/mm², equating to an increase of 564%, 405%, 462% for citric, lactic and hydrochloric acids respectively. This can be explained by the CPP-ACP acting as a source of additional calcium ions. The CPP-ACP complexes may release calcium and phosphate ions by four proposed mechanisms with one being pH dependent release 16. An increase in calcium ions would work to maintain the degree of saturation with respect to tooth mineral and work to prevent demineralization. Phosphate ion release in lactic acid and water was not detectable for either GIC material. Daily phosphate ion release for citric acid ranged from 9.8 to 13.9 nmol/mm² for F7 and was higher for F7EP, ranging from 14.5 to 17.6 nmol/mm², an average increase of 39%. Daily phosphate ion release for hydrochloric acid ranged from 0.42 to 0.51 nmol/mm² for F7 and was higher for F7EP, ranging from 0.7 to 1.07 nmol/mm², an average increase of 85%. This implies that some phosphate is being supplied by CPP-ACP, but unlike the calcium ion release a lot of the phosphate is also being supplied by the GIC matrix most likely from dissolution of AlPO₄ at the surface of the GIC. Dissolution of the AlPO₄ by citric and hydrochloric acids releases PO₄³⁻ from the GIC matrix. Daily average fluoride release for lactic acid and water dropped with addition of CPP-ACP, from 63.79 to 54.38 μmol/mm² in lactic acid and from 11.47 to 6.02 μmol/mm² in water (Figure 5.1b). This effect has been observed in previous studies conducted on F7 with CPP-ACP 5. Daily average fluoride release remained steady for both GIC materials when exposed to citric (103.53 and 108.46 μmol/mm²) and hydrochloric acids (107.40 and 109.38 μmol/mm²) for F7 and F7EP respectively (Figure 5.1b).
5.6 Conclusion

It has been shown that, although initial surface hardness of F7EP is lower than F7, after three days of storage in three of the four types of solution there was no significant difference between the two GICs. Addition of CPP-ACP to F7 increased the release of calcium ions by an average of 477% when exposed to acidic solutions with very little or no change in fluoride ion release. However, phosphate ion release ranged greatly (below detection limit to 17.5 nmol/mm$^2$ daily) depending on the type of acid exposure to the GIC materials and whether CPP-ACP has been incorporated.
5.7 References


Recharge and ion release following topical Tooth Mousse treatment of CPP-ACP modified GIC in acid solutions

6.1 Abstract (235 words):

Recent commercialization of a glass-ionomer cement (GIC) (Fuji VII™ EP) claims enhanced tooth protection due to the incorporation of 3% (w/w) casein phosphopeptide amorphous calcium phosphate (CPP-ACP). **Objectives:** The aims of this study were to assess the ability for calcium and phosphate ion recharge of the modified GIC using a topical treatment containing CPP-ACP and comparing it with a GIC without CPP-ACP under a variety of acidic and neutral conditions. **Methods:** Eighty blocks of GIC Fuji VII™ and Fuji VII™ EP were subjected to three acidic solutions
(lactic and citric acids at pH 5.0, hydrochloric acid at pH 2.0) and water (pH 6.9) over a three-day period. Ion release, surface hardness and weight measurements were carried out every 24 hours. Every 12 hours half of the blocks were treated with Tooth Mousse Plus (TM+), the remainder received a placebo treatment. **Results:** Significantly higher calcium and phosphate ion release were recorded for both GIC materials treated with either placebo or TM+ when exposed to citric acid, accompanied by increased mass loss and increase in surface hardness. The majority of other cases with TM+ treatment resulted in lower mass loss and increased release of calcium and phosphate ions compared to groups treated with placebo mousse. **Conclusion:** Topical treatment affects the GIC in a way that is poorly understood. Topical Tooth Mousse Plus treatment increased surface hardness and ion release of both GICs in citric and hydrochloric acids.

Keywords: GIC, CPP-ACP, surface hardness, ion release, recharge, tooth mousse

6.2 Introduction

In restorative dentistry glass ionomer cements (GICs) are widely used for a variety of purposes such as restorations, caries stabilization as well as adhesion of orthodontic brackets. GICs bind chemically to dentine and enamel and release fluoride ions over time, which has been shown to slow the progression and aid the regression of early carious lesions. In an effort to further improve the caries inhibition and repair potential of GICs, a number of attempts have been made to modify them with the aim to allow release of calcium and phosphate ions in addition to fluoride. One such attempt has incorporated the compound, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) into the GIC. Previous studies assessed the potential of incorporating CPP-ACP into GICs and have shown very promising results. Higher calcium and phosphate ion release from the CPP-ACP modified GIC when exposed to lactic acid were demonstrated. CPP-ACP has
been shown to work synergistically with fluoride and the bioavailable phosphate and calcium ions from CPP-ACP, and demonstrated to inhibit demineralisation and promote the remineralisation of early carious lesions\textsuperscript{8,9}. Incorporation of fluoride into the ACP complex to form amorphous calcium fluoride phosphate (ACFP) has been characterised and demonstrated to be more effective than ACP at remineralising enamel subsurface lesions\textsuperscript{8,10}.

A variety of demineralization challenges including acids formed from metabolic processes, food acids and gastric acids can leave the surface of teeth exposed to loss of surface mineral\textsuperscript{11}. If these acids overwhelm the protective functions of saliva, tooth mineral will be lost\textsuperscript{12}. Loss of tooth mineral has been studied extensively, however loss of dental restorative materials has largely been examined from the clinical point of view only, and much of the mechanisms how the loss occurs are not well understood. Recently, an investigation of the effects of various acids on the physical properties of GICs revealed that the common food acid, citric acid, had a far more pronounced impact on GICs than lactic acid\textsuperscript{7}, which is the acid associated with the formation of carious lesions. A significant mass loss of GIC blocks exposed to citric acid, compared to lactic acid, was accompanied by an increase in the release of calcium and phosphate ions.

Early indications suggest that an increased release of phosphate ions from GIC in citric acid may be the result of chelation of aluminium ions. Aluminium is contained in four components of GICs - aluminium fluoride, cryolyte, aluminium oxide and aluminium phosphate. During the initial mixing between the glass powder and acid, aluminium ions are leached from the glass and replace hydrogen ions in carboxyl groups in the acid, forming salts\textsuperscript{13}. Many of the aluminium ions remain in the glass particles and do not initially participate in the setting reaction. The initial ratio of aluminium and silica controls the rate of the GIC setting reaction through the gelation and cross-linking phase, so presence of extra aluminium ions is an important consideration for the workability of the mix\textsuperscript{14}. This study will attempt to provide a further understanding of the mechanisms that produce such an unexpectedly large release of ions and mass loss in conventional GICs and compare these findings against
resin-modified GICs, which theoretically are not as susceptible to chelation by exposure to citric acid. Early indications from a previous study suggest that a much higher release of phosphate ions in citric acid may be the result of chelation of aluminium ions within the GIC\textsuperscript{7}. The aims of this study were to build on the data collected in the previous study\textsuperscript{7} and further investigate the effect of adding CPP-ACP in the form of a crème with respect to ion release and physical properties of Fuji VII (F7) and Fuji VII EP (F7EP) when subjected to an acidic challenge. The effect of CPP-ACFP will be investigated after a topical treatment using Tooth Mousse Plus (TM+), which contains 10% w/w CPP-ACFP compared to a placebo mousse without CPP-ACFP.

The null hypotheses to be tested are; 1) topical treatment using placebo mousse does not alter surface hardness, mass loss profile and provides no additional ion release, under acid challenge compared to “no treatment” groups; 2) topical treatment using Tooth Mousse Plus does not alter surface hardness, mass loss profile and provides no additional ion release under acid challenge compared to (a) “no treatment” groups and (b) groups treated with placebo mousse.

6.3 Materials and Methods

Encapsulated F7 and F7EP containing 3% w/w CPP-ACP from the same batch of glass powder were provided by GC Corporation (Tokyo, Japan). Polyvinyl siloxane impression material (eliteHD+ light body, Zhermack SpA, Badia Polesine, Italy) moulds were used to create standardised GIC blocks measuring 3mm x 6mm x 6mm (thickness x width x length). Eighty blocks of each GIC were prepared by placing the materials in the mould with the top and bottom surfaces covered by plastic strips, held between two glass slides. The glass slides were pressed together to extrude excess material. The specimens were allowed to set inside the moulds for 24 hours in an incubator at 37°C and 100% relative humidity. After cooling to room temperature the blocks were removed from the moulds, and lapped with wet 600-grit paper
(Norton Tufbak, Saint-Gobain Abrasives Ltd., Auckland, NZ) to provide a standardised surface finish.

For each material, the 80 blocks were divided into two groups of 40. One group received topical treatment of Tooth Mousse Plus (TM+, GC Corporation), the other received a placebo mousse treatment (mousse base with no active ingredient, provided by GC Corporation, Japan) (Table 6.1). TM+ (10% w/w CPP-ACFP) was diluted with water (MilliQ, Millipore Corporation, Victoria, Australia) to 2% w/w CPP-ACFP, placebo mousse was diluted using the same ratios. Each block was placed into 2 mL of diluted mousse solution, either TM+ or placebo, for 2 minutes, followed by a gentle rinse using distilled water after which the block was placed back into an acid or control solution (water). Topical treatments were delivered every 12 hours starting with a pre-treatment dose applied after the blocks were lapped but before being placed into the storage solution for the first time.

<table>
<thead>
<tr>
<th>Control solution</th>
<th>- Water (pH 6.7) control for Acid solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control treatment</td>
<td>- Placebo treatment control for TM+</td>
</tr>
<tr>
<td></td>
<td>- “No treatment” control for Placebo and TM+</td>
</tr>
<tr>
<td>Control material</td>
<td>- Fuji VII control for Fuji VII EP</td>
</tr>
</tbody>
</table>

Table 6.1. Treatments and control groups.

Four different solutions were prepared using three acidic and one neutral environment. The three acidic solutions were formulated to simulate a gastric erosive challenge (50mM NaCl adjusted to pH 2.0 with HCl), a dietary erosive challenge (50mM citric acid at pH 5.0) and a cariogenic acid challenge (50mM lactic acid at pH 5.0). The control solution was distilled deionised water at pH 6.9 (Millipore Corporation, Victoria, Australia). In the absence of saliva as a storage medium and taking into account the highly controlled nature of a laboratory study, the ionic concentrations of acidic solutions were selected based on values previously reported in plaque fluid\textsuperscript{15}. 

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Ten blocks per group of each GIC were exposed to 5mL of one of the four solutions (stored in plastic containers). Solutions were changed every 24 hours and the samples were measured for change in mass and surface hardness. The solutions were analysed to determine ion release of calcium, phosphate and fluoride. Topical treatments were always carried out immediately before the blocks were placed back into the solution, after mass loss and surface hardness measurements.

The mass of each block was measured every 24 hours before surface hardness measurements were performed. Blocks were taken out of solution, gently dried using Kimwipes Delicate Task Wipers (Kimtech Science, Kimberly-Clark Professional, NSW, Australia) and then weighed using a microbalance (Precisa XT 120A, Dietikon, Switzerland). Only single measurements were carried out to minimise dehydration of the GIC surface and maintain consistency amongst all samples.

Vickers microhardness measurements were determined from indentations on the lapped GIC surface using a Microhardness tester (MHT-10, Anton Paar GmbH, Graz, Austria) attached to a microscope (Leica DMPL, Leica Microsystems Wetzlar GmbH, Germany). Two indentations were made on each block (Force, 1.0 N; Dwell, 6 s; Rate, 0.99 N/min). The indentations were separated by a distance of at least three times the indentation size. Images of the indentations were acquired using a calibrated digital camera (Leica DFC320) mounted on the microscope (Leica DMLP, Leica Microsystems Wetzlar GmbH, Germany) and distance measurements were made using Image Tool software (Version 3.0, UTHSC, San Antonio, TX), which were converted into Vickers hardness values. Blocks were then placed into fresh solution and returned to the incubator. At no time were the blocks allowed to become dehydrated.

The ion release of calcium, aluminium, phosphate and fluoride after each 24-hour period of storage were determined using atomic absorption spectroscopy (for calcium and aluminium), colourimetry (for inorganic phosphate) and an ion-specific electrode (for fluoride). To determine the calcium concentration, sample solutions (0.5 mL) were diluted with water (0.5 mL, MilliQ), then acidified with 1M HCl (0.5 mL) and diluted with 2% lanthanum chloride (0.5 mL) and analysed on a Varian AA240
atomic absorption spectroscope (AAS, Varian Australia Pty. Ltd.) against a set of seven standards ranging from 0 to 250 µM calcium. The Varian AA240 AAS was also used to determine the concentration of aluminium ions. Sample solutions (0.5 mL) were diluted with potassium chloride (0.5 mL, 8mg/L) and lanthanum chloride (0.5 mL, 2% w/w) and then acidified with 1M HCl (0.5 mL) and compared against a set of standards ranging from 0 to 125 µM aluminium. Inorganic phosphate ion concentrations were determined colourimetrically using a spectrophotometer (UV-visible spectrophotometer, Varian Australia, Pty. Ltd). Samples were prepared by taking 100 µL of solution, diluting them with 500 µL of 4.2% ammonium molybdate and adding 20 µL 1.5% of Tween® 20. (Sigma-Aldrich, St. Louis, MO). The phosphate concentration was determined by comparing the spectrophotometer readings of the samples against a set of seven standards ranging in phosphate concentrations from 0 and 100 µM. The concentration of fluoride ions was determined using an ion-selective electrode (Radiometer analytical, ISE C301F, France) connected to an ion analyser (Radiometer analytical, Ion Check 45, France). Sample solutions (1mL) were diluted with 1mL total ionic strength adjustment buffer (Merck Pty Ltd, Kilsyth, VIC, Australia) and measured against a set of eight fluoride standards ranging from 0 to 1000 µM.

Control groups consisted of F7 and F7EP blocks that underwent acid challenge only (no topical treatments). Data from sample groups were found to follow a normal distribution, χ² test was used to test normality. Single factor ANOVA was used to analyse the results using Bonferroni-Holm multiple comparison (Microsoft Excel 2011). Two-way ANOVA was used to determine the interaction between incorporation of CPP-ACFP and exposure to different acids. Level of significance was set at α = 0.05.
6.4 Results

6.4.1 Surface Hardness

Topical treatment with TM+ of F7 surface in control solution (water) prevented nearly all of the softening compared to no treatment \((p<0.05)\), TM+ was also significantly better than the placebo treatment \((p<0.05)\) at reducing the softening of the surface (Table 6.2). Placebo treatment of the F7 surface showed no statistically significant improvement compared to the control \((p>0.28)\). F7EP showed no improvement when either of the topical treatments were applied \((p>0.42\) in both cases) compared to no treatment.

<table>
<thead>
<tr>
<th>Δ VH after 72 hours (%)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>F7</td>
<td></td>
</tr>
<tr>
<td>Citric</td>
<td>-51.4 ±4.8</td>
</tr>
<tr>
<td>Lactic</td>
<td>-52.1 ±2.1</td>
</tr>
<tr>
<td>HCl</td>
<td>-71.0 ±3.9</td>
</tr>
<tr>
<td>Water</td>
<td>-21.8 ±2.5</td>
</tr>
<tr>
<td>F7EP</td>
<td></td>
</tr>
<tr>
<td>Citric</td>
<td>-45.3 ±5.9</td>
</tr>
<tr>
<td>Lactic</td>
<td>-48.1 ±2.8</td>
</tr>
<tr>
<td>HCl</td>
<td>-71.5 ±3.9</td>
</tr>
<tr>
<td>Water</td>
<td>-11.8 ±1.7</td>
</tr>
</tbody>
</table>

Table 6.2. Surface Hardness (% change relative to fresh blocks).

\(n=10\). Standard error in brackets

GIC blocks exposed to citric and lactic acids with no topical treatment showed considerable softening on the surface, with an average a drop of 51% and 52% in Vickers hardness respectively (Table 6.2). When the topical treatment cycle occurred, blocks exposed to citric acid not only showed much less softening, but actually demonstrated an increase in surface hardness. This effect was more
pronounced in groups treated with TM+, however blocks treated with placebo mousse also showed an increase in surface hardness (Table 6.2). The increase was statistically significant for F7 and F7EP with the placebo and TM+ treatments compared to the control (p<0.001 in all cases); no statistical difference was measured between placebo and TM+ (p>0.05).

In lactic acid, only the placebo treatment of F7 showed any significant difference (p<0.05), where the surface hardness was lower than the control group, little or no change was measured for TM+ application for either F7 or F7EP. TM+ treatment showed a statistically significant reduction in softening of F7 treated groups in hydrochloric acid compared with the control and placebo treatments (p<0.05 both cases). Very similar results were also observed in blocks stored in water, where a significant improvement in surface hardness was measured in F7 treated with TM+ compared to the control and placebo (p<0.001 and p<0.01 respectively). No statistically significant differences in hardness change were measured for F7EP exposed to lactic and hydrochloric acids and water for both placebo and TM+ treatments.

Statistically significant differences between F7 and F7EP were observed in only two cases, placebo treatment in lactic acid (p<0.001) and TM+ treatment in water (p<0.05).

6.4.2 Mass Loss

Both materials that underwent topical treatments and were exposed to citric acid showed a significant increase in loss of mass compared to the controls (p<0.001 in all cases (Table 6.3). The loss in mass increased dramatically (an increase in the range of 4.8% to 8.7%) in all four groups. Placebo treatment showed significantly higher mass loss compared to TM+ treatment in citric acid for both F7 and F7EP (p<0.05). F7 and F7EP responded differently to placebo and TM+ treatments, F7EP was more resistant to mass loss under both placebo and TM+ treatments (p<0.05 and p<0.001 respectively). The largest daily loss of mass occurred on the last day of
measurement for all four groups (F7, F7EP in citric acid with TM+ and placebo treatments, Figures 6.1a-d).

<table>
<thead>
<tr>
<th></th>
<th>Δ mass after 72 hours (%)</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>Placebo</td>
<td>TM+</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>Citric</td>
<td>-12.2 (±1.4)</td>
<td>-20.9 (±1.3)</td>
<td>-19.2 (±1.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactic</td>
<td>-0.4 (±1.2)</td>
<td>-0.9 (±1.6)</td>
<td>-0.4 (±1.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCl</td>
<td>-2.3 (±1.5)</td>
<td>-2.1 (±1.5)</td>
<td>-1.8 (±1.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>+0.6 (±1.8)</td>
<td>+1.2 (±1.4)</td>
<td>+0.1 (±1.2)</td>
<td></td>
</tr>
<tr>
<td>F7EP</td>
<td>Citric</td>
<td>-12.4 (±1.4)</td>
<td>-19.2 (±2.1)</td>
<td>-17.2 (±1.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactic</td>
<td>+1.3 (±1.7)</td>
<td>+0.2 (±1.7)</td>
<td>+1.2 (±1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCl</td>
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<td>-1.3 (±1.3)</td>
<td>+0.5 (±1.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>+2.2 (±1.6)</td>
<td>+2.4 (±1.7)</td>
<td>+2.6 (±1.4)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.3. Mass Loss (% change relative to fresh blocks).

n=10. Standard error in brackets.

Figure 6.1. Daily mass loss of F7 blocks treated with placebo mousse and stored in four different solutions.
The largest measured difference occurred in the citric acid challenge, however statistically significant differences were also detected in other storage media. Placebo treatment in lactic acid showed a higher mass loss compared to the acid control and TM+ treatment for both F7 and F7EP groups (p<0.001 in all four cases), TM+ treatment was no different to the acid control (p>0.11 for both materials). F7EP also showed reduced mass loss compared with F7 for both placebo and TM+ treatments when stored in lactic acid (p<0.001 in both cases).

Figure 6.2. Daily mass loss of F7EP blocks treated with placebo mousse and stored in four different solutions.
Figure 6.3. Daily mass loss of F7 blocks treated with TM+ and stored in four different solutions.

In hydrochloric acid, the TM+ treated groups showed a statistically significant reduction in mass loss compared to the control and placebo groups for both F7 (p<0.01 and p<0.05 respectively) and F7EP (p<0.001 in both cases). As was the case for citric and lactic acids, F7EP showed less mass loss in hydrochloric acid compared with F7 for both placebo and TM+ (p<0.001 for both cases).

Topical treatments of the F7EP blocks stored in water for three days showed a significant difference in the measured mass compared to the control groups (no topical treatment). Both placebo and TM+ showed a significantly reduced mass loss compared with the control untreated blocks (p<0.05 both cases). No statistical difference between the two topical treatments was detected for F7EP. Only TM+ treatment showed a significant difference in mass loss when used to treat F7 stored in water compared to control and placebo treatment (p<0.05 in both cases).
6.4.3 Ion Release

Overall, F7EP showed consistently higher calcium ion release across all groups compared with F7 (p<0.001), with the exception of water where the detection limit was unable to measure the ion levels (Table 6.4). Calcium ion release for specimens that were exposed to lactic and hydrochloric acids showed an increased release of ions when treated with TM+ compared to the control and placebo treatment groups (p<0.05 in all cases), this was true for both F7 and F7EP. The increase was very similar in magnitude for both materials (around 130 µM for HCl and 20-50 µM for lactic acid). Placebo mousse treatment of F7 showed significantly less calcium ion release when stored in hydrochloric acid (p<0.05, 30.3 µM vs 16.6 µM), the difference in the F7EP group was similar in magnitude (173.0 µM vs 156.6 µM), but it was not statistically significant (Figures 6.5 and 6.6). F7EP blocks exposed to citric acid showed no change in calcium ion release when treated with TM+, but showed a large increase when treated with the placebo (p<0.01). F7 blocks treated with placebo also showed a
higher calcium ion release than those treated with TM+ (p<0.05). In most cases ion release during the first 24 hours was larger than on subsequent days. The exception to this was the TM+ treated groups that were exposed to hydrochloric acid; in both cases the daily release remained steady.

<table>
<thead>
<tr>
<th>Day</th>
<th>No t-ment</th>
<th>TM+</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80.9</td>
<td>107.5</td>
<td>69.2</td>
</tr>
<tr>
<td>2</td>
<td>47.9</td>
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<tr>
<td>3</td>
<td>44.3</td>
<td>103.2</td>
<td>42.1</td>
</tr>
<tr>
<td>Total</td>
<td>173.0 ± 3.25</td>
<td>304.2 ± 3.85</td>
<td>156.5 ± 0.89</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Citric acid (pH 5.0)</th>
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</thead>
<tbody>
<tr>
<td>Day</td>
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<td>-----</td>
</tr>
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<td>1</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>HCl acid (pH 2.0)</th>
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</thead>
<tbody>
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<td>Day</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water (pH 6.7)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
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<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lactic acid (pH 5.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

**Table 6.4.** Calcium ion release (all units in μM), n=10
F7EP consistently displayed a slightly higher release of phosphate ions than F7 (Table 6.5). Lactic acid groups treated with placebo mousse showed higher ion release compared to the respective control groups (p<0.05 for both materials), TM+ treated groups produced significantly higher ion release compared to the “no treatment” control (p<0.05 for both F7 and F7EP) and placebo treatment groups (p<0.05 for both F7 and F7EP).

### Table 6.5. Phosphate ion release (all units in μM), n=10

<table>
<thead>
<tr>
<th></th>
<th>HCl acid (pH 2.0)</th>
<th>Citric acid (pH 5.0)</th>
<th>Water (pH 6.7)</th>
<th>Lactic acid (pH 5.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>No t-ment</td>
<td>TM+</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>30.8</td>
<td>69.9</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.3</td>
<td>82.3</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>26.4</td>
<td>91.4</td>
<td>24.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>77.5 ± 6.67</td>
<td>243.6 ± 10.5</td>
<td>60.8 ± 1.06</td>
</tr>
</tbody>
</table>

Note: Values are mean ± standard deviation.
Figure 6.5. Cumulative three-day calcium ion release from F7 blocks across four solutions and three treatment groups.

Data for the control groups when blocks were exposed to citric acid showed very high phosphate ion release compared to other solutions (Figure 6.3a,b). F7 treated with placebo mousse showed a slightly higher amount of phosphate ion release compared with the control group (p<0.05), even though the reading for F7EP compared to the control was also higher, no significant difference was detected (p>0.25). However, F7 and F7EP treated with TM+ exposed to citric acid showed a significantly lower release of phosphate ions compared with the control groups (p<0.05), the TM+ treated groups also showed significantly lower phosphate ion release than placebo treated groups for both materials (p<0.05).
In hydrochloric acid exposed groups, the placebo treated blocks released a statistically lower amount of phosphate ions compared to “no treatment” control groups, the magnitude of difference was similar in both materials although only significant for F7 (p<0.05). Blocks treated with TM+ showed significantly higher phosphate ion release than control and placebo treated groups (p<0.05 in all cases). Water stored blocks displayed a similar pattern to the hydrochloric acid groups, many results fell below the detection limit, thus making statistical comparisons difficult. TM+ treated blocks released significantly more phosphate ions than both control and placebo groups (p<0.05 in all cases).
Figure 6.7. Cumulative three-day phosphate ion release from F7 blocks across four solutions and three treatment groups.

F7EP consistently produced higher phosphate ion release than F7 in all three acids; the only statistically non-significant result was for TM+ treated blocks in lactic acid (p<0.05 in all other cases), although the amounts were higher for F7EP they were also highly variable from day to day. In several groups, the largest daily release of phosphate was observed on the third day of measurement.
Topical treatment with TM+ had a similar effect in groups exposed to acid solutions, a modest increase in the amount of fluoride ions were released into the solution (Table 6.6). Blocks exposed to citric acid showed much higher release of ions than blocks stored in other solutions. Placebo mousse treatment produced an increase in fluoride ion release comparable to TM+ treatment, however this was only observed in citric acid exposed groups. TM+ treated group produced significantly higher fluoride ion release than the “no treatment” control in F7 (p<0.05), but was not significantly different from the placebo treated group; no significant differences were measured between control and both treatments for F7EP. In all other groups placebo treatment showed no significant difference in ion release compared to “no treatment” control groups.

Figure 6.8. Cumulative three-day phosphate ion release from F7EP blocks across four solutions and three treatment groups.
6.5 Discussion

Although no densitometry measurements were made during the experiment, there was no visible change in volume of the blocks in all groups. Measurements of volume change may shed more light on where the mass loss is coming from. It may be possible to correlate aluminium ion release with various sources of aluminium in GIC composition. Cross-linking aluminium ions in the matrix if chelated may cause a chain reaction leading to greater mass loss, whereas filler amorphous sources of aluminium ions may have a far less pronounced effect.

More calcium and phosphate ions are expected to be released from F7EP (compared to F7), where additional ions are provided by CPP-ACP incorporated into the GIC. Similarly, TM+ is a source of additional calcium, phosphate and fluoride ions due to the presence of CPP-ACFP in the mousse; these additional ions can also contribute to higher calcium, phosphate and fluoride ion release. Ion release from TM+ treatment of F7EP should be close to combined placebo treatment of F7EP and TM+ treatment of F7. This straightforward, additive ion release pattern is observed in lactic and hydrochloric acid groups for calcium and phosphate ion release.
6.5.1 Water control

<table>
<thead>
<tr>
<th>Water Treatment</th>
<th>F7</th>
<th>F7EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>---</td>
<td><em><strong>F</strong></em></td>
</tr>
<tr>
<td>Placebo</td>
<td><em><strong>P</strong></em></td>
<td>H<em><strong>P</strong></em></td>
</tr>
<tr>
<td>TM+</td>
<td>HMCP*</td>
<td>M***F</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F7</th>
<th>None</th>
<th>Placebo</th>
<th>TM+</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td><em><strong>P</strong></em></td>
<td>---</td>
<td>HMCP*</td>
</tr>
<tr>
<td>Placebo</td>
<td>HMCP*</td>
<td>---</td>
<td>M***F</td>
</tr>
<tr>
<td>TM+</td>
<td></td>
<td></td>
<td>H***F</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F7EP</th>
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<th>TM+</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>*M**F</td>
<td>---</td>
<td>*M**F</td>
</tr>
<tr>
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<td><em>M</em>PF</td>
<td>---</td>
<td><em>MCP</em></td>
</tr>
<tr>
<td>TM+</td>
<td>M*PF</td>
<td>*<em>CP</em></td>
<td>---</td>
</tr>
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Table 6.7. Statistical significance in water. (Hardness. Mass loss. Calcium, Phosphate and Fluoride ion release. * - no statistical significance)

The control solution, water provides a baseline for the rest of the results. There is a considerable softening of the surface of the GIC when stored in water for three days, the softening is more pronounced in F7 than in F7EP (Table 6.7). Topical treatment with TM+ does not seem to have any effect (positive or negative) on F7EP blocks, however TM+ treatment of F7 blocks makes a big difference and is the highest surface hardness value after three days of storage out of all water groups (albeit still 3.7% lower than fresh GIC blocks). Placebo mousse did not register any statistically significant results suggesting that it is in fact CPP-ACFP specifically that is responsible for improved surface hardness in F7 and F7EP blocks, as evidenced by higher surface hardness in F7EP and improved surface hardness in F7 only when treated with TM+.

All groups stored in water showed a slight mass increase, some comparisons were statistically significant even though the magnitude of the differences was small, this is possibly due to the accuracy of mass measurements leading to very tight error margins. The slight increase in mass could be due to natural variance but could also be due to the GIC’s water uptake, especially in the early stages of maturation. Since the samples were not desiccated prior to weight measurements it stands to reason that some moisture would remain in the GIC blocks. Detailed analysis of GIC water uptake is beyond the scope of this study, these numbers formed the basis as control groups for the rest of the study.
Calcium and Phosphate ion release in water groups was very low, often below the detection limits of our techniques, the exceptions were groups where TM+ treatments were used and the highest release of ions occurred in the first 24 hours, dropping off significantly in the following days.

6.5.2 Lactic acid

<table>
<thead>
<tr>
<th>Lactic Treatment</th>
<th>F7</th>
<th></th>
<th>F7EP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Placebo</td>
<td>TM+</td>
<td>None</td>
</tr>
<tr>
<td>F7</td>
<td>---</td>
<td><strong>HM*P</strong></td>
<td><strong>CPF</strong></td>
<td>---</td>
</tr>
<tr>
<td>Placebo</td>
<td><strong>CPF</strong></td>
<td>---</td>
<td><strong>MCP</strong></td>
<td>---</td>
</tr>
<tr>
<td>TM+</td>
<td><strong>CPF</strong></td>
<td><strong>MCP</strong></td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>F7EP</td>
<td>---</td>
<td><strong>HMCP</strong></td>
<td><strong>MCP</strong></td>
<td>---</td>
</tr>
<tr>
<td>Placebo</td>
<td>---</td>
<td><strong>M*P</strong></td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>TM+</td>
<td><strong>M*P</strong></td>
<td><strong>CP</strong></td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Table 6.8. Statistical significance in lactic acid. (Hardness. Mass loss. Calcium, Phosphate and Fluoride ion release. * - no statistical significance)

Hardness remained largely unchanged under the topical treatments, both placebo and TM+, the exception was placebo treatment for F7, which showed more softening of the surface, which was significant (Table 6.8). There is no clear pattern to explain this particular result, the difference is not great (although statistically significant) and may be due to natural variation.

Several groups showed statistical significance when it came to mass loss, however measurements fell between 0.5% and 1.1% of each other between groups, this suggests that the difference in mass loss is not substantial and the significance is almost entirely a result of high precision in absolute measurements. In many cases an increase in mass was observed, which indicates that GIC’s early stage water uptake is having a more pronounced effect than acid erosion. Competing factors of water uptake and acid erosion make it difficult to assign significance to these results, especially when the differences between groups are so small.
There was significantly more calcium and phosphate in TM+ groups for both F7 and F7EP compared to placebo and control treatments, presence of CPP-ACFP in TM+ is most likely responsible for this increase, extra ions were being released directly from topically applied TM+ as it is exposed to acid media and the peptide bond is broken. Fluoride levels were affected very little, this is not only true for topical treatments but also between different solutions tested. There was some variation between groups, but no firm pattern could be observed.

6.5.3 Hydrochloric acid

<table>
<thead>
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<th>HCL</th>
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<th>F7EP</th>
</tr>
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<td>Placebo</td>
<td>TM+</td>
</tr>
<tr>
<td>F7</td>
<td>None</td>
<td>**<em>CP</em></td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>---</td>
<td>HMCP*</td>
</tr>
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<tr>
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<td><em>MCP</em></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>**<em>F</em></td>
<td>****F</td>
</tr>
<tr>
<td></td>
<td>TM+</td>
<td>---</td>
<td>***F</td>
</tr>
</tbody>
</table>

Table 6.9. Statistical significance in hydrochloric acid. (Hardness. Mass loss. Calcium, Phosphate and Fluoride ion release. * - no statistical significance)

The only significant result in surface hardness was detected in F7 when treated with TM+, no significant difference was observed in F7EP, this may suggest that CPP-ACP/ACFP is helping to protect the surface and the already present CPP-ACP in F7EP is the reason no statistical significance was observed in the F7EP groups (Table 6.9).

As with the water and lactic acid groups the differences in mass loss between groups were minor, however there was a strong pattern in the HCl results that suggested TM+ treatment is having a protective effect. This result was even observed in the F7EP groups, where GIC already contains CPP-ACP.

More calcium and phosphate ions were being released when F7 and F7EP were treated with TM+. In the case of phosphate ion release from F7EP the daily
release was increasing each day suggesting that perhaps the peak release potential may not be reached until some time after three days. This effect was not observed in F7 groups treated with TM+. Fewer calcium and phosphate ions were released under the placebo treatment for both F7 and F7EP suggesting that TM+ was having an additive effect on ion release.

6.5.4 Citric acid

<table>
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<th>Treatment</th>
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<tr>
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<td>---</td>
</tr>
<tr>
<td>TM+</td>
<td><em>M</em>P*</td>
<td>---</td>
<td><em>MCP</em></td>
</tr>
</tbody>
</table>

Table 6.10. Statistical significance in citric acid. (Hardness. Mass loss. Calcium, Phosphate and Fluoride ion release. * - no statistical significance)

Surface hardness improved greatly under topical treatments for both F7 and F7EP. These results showed lot of variability and even though TM+ numbers were much higher than the placebo no significant difference between the two treatments could be measured (Table 6.10). The large standard error present in placebo and TM+ results may suggest they are unreliable, however the increase in surface hardness was so large that the effect was not in doubt. Since placebo groups followed the same pattern as TM+ it is clear that topically applied CPP-ACFP was not the main cause of the substantial increase in surface hardness, but something common to both topical treatments must be the reason for the increase.

Unlike the other solutions, which showed very minor variation in mass loss, the citric acid treated GIC blocks suffered from significant loss of mass. Under topical treatments this mass loss increased. Placebo and TM+ treatments were both significantly different from the control (untreated) groups and were very close to
each other. Statistical significance was also detected between the placebo and TM+ treatments.

More calcium and phosphate ions were released by the F7EP groups compared to F7, which can be attributed to CPP-ACP present in F7EP. However, TM+ treated groups released significantly less calcium and phosphate than the placebo treated groups for all groups (F7 and F7EP). In other acid groups TM+ application showed that CPP-ACP/ACFP has an additive effect on ion release, more calcium and phosphate ions were released from F7EP than F7 and this was further elevated under TM+ treatment. Furthermore, placebo treatment in the other acids did not demonstrate a consistent increase in ion release, this was completely the opposite of the citric acid measurements. It stands to reason that an ingredient, possibly glycerine, common to both the placebo and TM+ was interacting with the citric acid to produce high ion release, increased mass loss and hardening of the surface. Slightly lower mass loss and lower (although still increased relative to the control) ion release of TM+ compared to placebo may indicate that CPP-ACFP was inhibiting the interaction between the citric acid and glycerine.

6.5.5 Surface hardness

Exposure to any liquid storage medium has a softening effect on the GIC surface, as demonstrated by the reduction of Vickers hardness in water for all groups. The protocol followed was exactly the same as in that determined in Chapter 5. The results of repeated measurements were in good agreement with the previously published data\textsuperscript{7}.

Results from the control group indicated that reduction in surface hardness is strongly associated with the pH of the acid solution. However, the citric acid groups treated with TM+ and placebo mousse behaved very differently. Topical treatment with either TM+ or the placebo mousse not only showed reduction in the softening of the surface, but also an increase in hardness compared to initial measurements prior to topical treatment or acid challenge. The increase in mass loss for blocks treated
with TM+ and placebo suggests that more ions were displaced as a result of the topical treatments. Some of these displaced ions may have migrated to the surface of the specimen where they formed a zone of saturation and thus precipitated on the surface to form a crust. In addition, the topical treatment itself most likely left a film on the surface, which could have formed a nidus for other ions to precipitate onto. CPP-ACP has a demonstrated ability of localising calcium and phosphate ions on the tooth surface \(^8, ^9\), consequently formation of a surface crust-like layer seems quite possible and would explain the dramatic increase in surface hardness amongst the citric acid exposed groups.

Only in a few cases for the lactic and hydrochloric acid exposed groups were there any statistically significant changes in surface hardness. In one F7 group (lactic acid) the placebo treatment was associated with softening of the surface, also in lactic acid, but in the F7EP blocks treated with TM+ showed a slight reversal in softening.

### 6.5.6 Change in mass

Baseline measurements from the groups stored in water indicated that the GICs used in this experiment tended to swell and retain a portion of liquid storage media. This early stage water uptake was the most likely reason of the modest softening of the surface of many GICs and has been reported in the past \(^{13}\). Tooth mousse treated GIC blocks stored in lactic and hydrochloric acids were more resistant to dissolution and exhibited lower mass loss compared to the control and placebo treatments, F7EP also performed better than F7 in this regard. Although statistically significant, these differences were very small. Significant loss of mass in the citric acid exposed blocks with no topical treatment has already been reported\(^7\), and interestingly the mass loss was even higher for the topically treated groups. The presence of a hard surface layer suggests that any loss of ions due to erosion would be inhibited, it would seem that the mechanism for increased mass loss was slightly different in the topically treated
groups. F7 showed a slightly greater mass loss than F7EP, which may suggest that the CPP-ACP in the cement acts as a buffering agent. However, the greatest daily loss of mass almost always occurred on the third (last) day of measurement, indicating that the process seemed to be accelerating. This suggests that the topically applied CPP-ACP was not having the same buffering effect as the CPP-ACP already present within the GIC (F7EP).

6.5.7 Ion Release

In TM+ treated groups exposed to citric acid both F7 and F7EP showed a reduction in phosphate ion release, a steady calcium ion release and an increased fluoride ion release. At the same time an increased loss of mass was also observed. In the case of the placebo treated groups exposed to citric acid, increased phosphate, calcium and fluoride release was measured for both GICs, accompanied by the largest measured loss of mass amongst all groups. As demonstrated by the placebo mousse results, application of the topical treatment itself introduced an interaction between the acid storage media and the GIC. In some cases the ion release was greater than that which would be expected from the TM+ alone, which itself has additional ions in the form of CPP-ACFP. Understanding of additional mechanisms, such as formation of a crust surface layer and chelation of aluminium ions may help to explain the large differences observed between the control, placebo and TM+ groups. The presence of CPP-ACFP in TM+ seems to provide some mitigation against mass loss compared with the placebo treatment. Additional calcium, phosphate and fluoride ions provided by the TM+ itself were hard to distinguish from those released from the GIC as the additional buffering capacity produced by TM+ ions could potentially inhibit these same ions being released from the GIC, especially in the case of F7EP.
6.5.8 General Discussion

It would seem a lot of ionic movement is occurring as evidenced by the high mass loss and ion release, more so after the topical treatments, where more ions were not only being released into storage solutions but also being deposited on the GIC surface. This indicates that surface erosion of GIC may not be the main mechanism contributing to increased ion release. It is more likely that the inherent porosity of the GIC plays a key role in facilitating high ion release. Previous speculation suggested that chelation of aluminium ions in citric acid exposed groups may be responsible for the large phosphate ion release observed in the untreated control F7 and F7EP blocks. In many cases, the highest daily phosphate ion release and mass loss were observed on the last day of measurements, which not only suggests that the process may not have reached its peak, but also there may be a strong link between phosphate ion release and mass loss. A possible explanation for increased loss of mass and increased surface hardness could be that citric acid has an inherent ability to chelate aluminium from the GIC matrix producing an ion rich environment that when coupled with topical treatments of either TM+ or placebo mousse resulted in the formation of a crust-like surface layer. In turn, the presence of precipitation in the surface crust layer would produce a solubility concentration gradient that increases from the subsurface inwardly towards the bulk of the GIC, allowing citric acid to penetrate deeper into the GIC thus chelating additional aluminium as the acid continues to diffuse through the cement. Extra ions are believed to then increase the thickness and strength of the crust-like surface layer that in turn improves the resistance to dissolution, but results in deeper acid penetration into the GIC. The deeper ingress of the acid can then lead to increased loss of mass within the bulk of the cement but is associated with an increased surface hardness that exceeds the hardness of fresh GICs. This process is thought to be not unlike the process of an active carious lesion in teeth, where calcium, phosphate and fluoride ions are displaced due to solubility of fluorapatite in the presence of lactic acid creating a concentration gradient, which promotes the inward progression of
carious lesions\textsuperscript{17}, but is also associated with the initial maintenance of the surface integrity of the enamel. It would be interesting to perhaps extend the length of exposure of the GIC in acid to note whether the surface eventually dissolves to form a pit or cavity.

Clinical evidence suggests that conventional GIC restorations have poor retention rates and suffer from high wear rates when placed on load bearing surfaces\textsuperscript{18}. A lot of these drawbacks have been addressed with the introduction of resin-modified GICs, however sustained high fluoride release is something that resin-modified GICs are not currently able to achieve. High mass loss is certainly an undesirable property to have in a restorative material, but in this case it is also accompanied by increased calcium and phosphate ion release. These combinations of drawbacks and benefits have created a special role for GICs within the spectrum of restorative materials, particularly in the areas of caries prevention. However, it may be possible to reduce some of these limitations if interactions between citric acid, topical treatments and GIC materials are better understood. Greater control over ion release and potentially higher “on-demand” ion release may be possible through a better understanding of the mechanisms that cause loss of mass and accompanying ion release in the cements. This may provide great benefit in cases where high erosion of tooth mineral is present and may be counteracted by increased ion release from the GIC, which may be preferentially lost rather than tooth structure.

The null hypotheses tested, it was found that; 1) topical treatment using placebo mousse increased surface hardness, caused higher mass loss and produced increased calcium and phosphate ion release for F7 and F7EP blocks stored in citric acid compared to untreated blocks, no consistent significant difference was observed in other storage media; 2) topical treatment using TM+ increased surface hardness, caused higher mass loss and produced increased calcium and phosphate ion release for F7 and F7EP blocks stored in citric acid compared to (a) untreated blocks and (b) showed significant differences when compared to blocks also stored in citric acid but treated with placebo mousse. A significant increase in calcium and phosphate ion
release was also observed in TM+ treated groups in other solutions when compared to untreated groups.

6.6 Conclusion

Tooth Mousse Plus and placebo mousse topical treatments have shown that the topical treatment itself has a very pronounced effect on the GICs tested (F7, F7EP). In the case of placebo mousse, additional phosphate, calcium and fluoride ions were released in citric acid from the GIC at the expense of significant mass loss, no additional ions were released in the lactic and hydrochloric acid challenges however, no significant mass loss was observed either. Tooth Mousse Plus treated GIC blocks showed increased calcium, phosphate and fluoride ion release in lactic and hydrochloric acids, as well as increased calcium and phosphate ion release in water with no additional adverse effects. In citric acid, Tooth Mousse Plus showed similar results to the placebo mousse, with CPP-ACP providing some mitigation against mass loss and increased ion release compared to the “no treatment” control. Further research into the importance of aluminium compounds present in the GICs may explain the vulnerability of fluoroaluminosilicate glass materials to chelating agents such as citric acid.
6.7 References


CHAPTER 7

Aluminium ion release of conventional GICs and Giomer materials in acid solutions

7.1 Abstract (232 words):
Recent advances in the understanding of the interaction between GIC materials and acidic environments have shed new light on the clinically encountered problem of erosion of GIC restorations. Objectives: The aim of this study was to evaluate the importance of aluminium ions in the GIC matrix structure and its role in beneficial ion release from GICs and Giomer materials. Methods: Forty blocks of, Fuji VII™ (F7), Fuji VII™ EP (F7EP) and Shofu Beautifil (SBF03) were subjected to three acidic solutions (50mM lactic and citric acids at pH 5.0, 50mM hydrochloric acid at pH 2.0) and water (pH 6.9) over a three day period. Ion release, surface hardness and weight measurements were carried out every 24 hours. Results: A significantly higher
(p<0.05) mass loss and ion release were recorded for conventional GICs when exposed to citric acid compared to the Giomer material. SBF03 showed substantial fluoride ion release in the first 24 hours compared to following days (p<0.05). F7 and F7EP showed significantly higher daily fluoride ion release than SBF03 in all tested acids (p<0.05). **Conclusion:** Results show that pre-reacted glass additives in SBF03 produce substantial fluoride release in the first 24 hours only. When examining ion release profiles from SBF03, F7 and F7EP each material has a different source of fluoride ion release. Differences in ion release profiles are strongly linked to aluminium ions and aluminium compounds found in glass particles.

### 7.2 Introduction

Glass ionomer cements (GICs) are widely used for a variety of purposes such as for interim restorations, caries stabilization, definitive restoration of micro-cavities or non-caries cervical lesions, bonding orthodontic brackets and bands, fissure sealing erupting molars and as a surface protecting material for high risk surfaces such as root surfaces \(^1,2\). The main advantages of GICs are their strong ability to chemically bind to dentine and their ability to release fluoride ions. This fluoride ion release is believed to slow the progression and aid the regression of early carious lesions \(^3\). The tooth surface will only demineralise when the fluid bathing the tooth is under-saturated with respect to the tooth mineral (fluoride, calcium and phosphate ions), which is why ion activity at the tooth surface is an important topic to study.

A number of investigators have explored the modification of dental materials in attempt to have them release calcium, phosphate and fluoride ions \(^4-6\). Skrtic et al\(^6\) explored modifying dental composites with bioactive glasses. Mazzaoui et al\(^4\) and Al Zraikat et al\(^5\) assessed the addition of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) to GICs. The casein phosphopeptides stabilise calcium and phosphate ions in a bioavailable form that allow them to inhibit demineralisation and promote the remineralisation of early lesions \(^7\). CPP-ACP is stable in the presence of
fluoride and has been shown to work synergistically with fluoride. This makes CPP-ACP a promising additive to dental products and restorative materials. The study by Mazzaoui et al. (2003) was a proof of concept study that showed that CPP-ACP could be added to GIC. Al Zraikat et al. (2011) later explored the effect of CPP-ACP concentration on GIC mechanical properties. Additionally, both of these studies showed that the addition of CPP-ACP improved calcium and phosphate ion release in lactic acid.

The tooth surface can be exposed to a variety of demineralization challenges including: acids formed from metabolic processes of oral bacteria (predominantly lactic acid); food acids such as citric and phosphoric acid that are commonly found in soft drinks; and hydrochloric acid from the regurgitation of stomach acids. If these acids overwhelm the protective functions of saliva, mineral may be lost from tooth surfaces. Recently, a closer investigation of the effects of various acids on the physical properties of GICs has revealed that common food acids, such as citric acid, have a far more pronounced impact on GICs than lactic acid. A significant loss in mass of GIC blocks exposed to a citric acid challenge was found compared to lactic acid. This was also accompanied by an increased release of calcium and phosphate ions. Early indications suggest that an abnormally high release of phosphate ions in citric acid may be the result of chelation of aluminium ions.

Aluminium is contained in GICs in four compounds - aluminium fluoride, cryolyte (sodium aluminium fluoride), aluminium oxide, and aluminium phosphate (Table 7.1). During the initial mixing between the glass powder and acid some aluminium ions are leached from the glass and replace hydrogen ions in carboxyl groups in the acid, forming salts. A lot of the aluminium ions remain in the glass particles and do not participate in the setting reaction. The initial ratio of aluminium and silica controls the rate of the setting reaction through the gelation and cross-linking phase, so presence of extra aluminium ions is an important consideration for the workability of the mix. In this study it was aimed to better understand the mechanisms that produce such an unexpectedly large release of ions and loss of mass in conventional GICs and compare these findings against resin composite containing
pre-reacted glass ionomer fillers referred to as a Giomer, which theoretically is not as susceptible to chelation by exposure to citric acid.

<table>
<thead>
<tr>
<th>Powder</th>
<th>weight per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryolite (Na₃AlF₆)</td>
<td>5%</td>
</tr>
<tr>
<td>AlF₃</td>
<td>5%</td>
</tr>
<tr>
<td>SrF₂ or CaF₂</td>
<td>22-34%</td>
</tr>
<tr>
<td>AlPO₄</td>
<td>10%</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>16%</td>
</tr>
</tbody>
</table>

Table 7.1. General composition of GICs. SiO₂ makes up the rest up to 100%.

A comparison between a conventional GIC and a Giomer restorative material (Shofu Beautifil) is presented. Giomers are resin-based tooth coloured restorative materials that contain pre-reacted glass particles. The glass particles are included to facilitate fluoride ion release, enhancing the materials ‘therapeutic’ benefits. However, being a resin-based material the Giomer should be less susceptible to chelation caused by leaching of aluminium ions. The null hypothesis is; a) There is no difference in fluoride ion release between F7, F7EP (a conventional GIC containing CPP-ACP) and SBF03 (a resin-based material containing pre-reacted, fluoride containing, glass elements); and b) Aluminium ions are not related to fluoride ion release in either F7, F7EP or SBF03.
7.3 Materials and Methods

Two conventional glass ionomer cements, Fuji VII and Fuji VII EP, (GC Corporation Tokyo, Japan, F7 and F7EP) in capsule form, and a pre-reacted glass particle filled resin composite, Beautifil F03 (Shofu Inc., Kyoto, Japan, SBF03) were used in this study. Moulds made of a polyvinyl siloxane impression material (eliteHD+ light body, Zhermack SpA, Badia Polesine, Italy) were used to create standardised GIC and Giomer blocks measuring 3mm x 6mm x 6mm (thickness x width x length). Forty blocks of each material were prepared by placing the GIC or Giomer into the mould with the top and bottom surfaces covered by plastic strips, which was held between two glass slides. The glass slides were gently pressed together to extrude any excess material, SBF03 samples were light-cured for 10 sec on two major surfaces of the block using and LED light curing unit (bluephase C8, Ivoclar Vivadent AG, Liechtenstein). All specimens were allowed to set inside the moulds for 24 hours in an incubator (37°C, ~100% relative humidity). After cooling to room temperature the blocks were removed from the moulds, and lapped with wet 600-grit wet and dry silicon carbide paper (Norton Tufbak, Saint-Gobain Abrasives Ltd., Auckland, NZ) ensuring specimens did not become dehydrated.

Four different solutions were prepared to expose the blocks to a variety of acidic and neutral environments. The three acidic solutions were formulated to simulate a gastric erosive challenge (50mM NaCl adjusted to pH 2.0 with HCl), a dietary erosive challenge (50mM citric acid at pH 5.0) and a cariogenic acid challenge (50mM lactic acid at pH 5.0) (this concentration was selected based on values found previously in plaque fluid 13). The neutral solution was distilled deionised water at pH 6.9 (MilliQ, Millipore Corporation, Victoria, Australia).

Ten blocks of each each material, namely GIC, GIC containing CPP-ACP and the Giomer were exposed to 5mL of each of the four solutions (stored in plastic containers). The solutions were changed every 24 hours and the samples were measured for change in mass and surface hardness. The solutions were analysed to determine release of aluminium, calcium, phosphate and fluoride ions.
The mass of each block was measured every 24 hours before surface hardness measurements were performed. Blocks were taken out of the solution, pat dried and then weighed using an analytical microbalance (Precisa XT 120A, Dietikon, Switzerland). One measurement per sample was performed with an error of ±0.1 mg.

Vickers microhardness measurements were determined from indentations on the lapped GIC surface using a microhardness tester (MHT-10, Anton Paar GmbH, Graz, Austria) attached to a microscope (Leica DMPL, Leica Microsystems Wetzlar GmbH, Germany). Two indentations were made on each block (Force, 1.0 N; Dwell, 6 s; Rate, 0.99 N/min) at each time interval. A distance of least three times the indentation size separated the indentations and both of the major surfaces of the block were used for indentation. Images of the indentations were acquired through a calibrated digital camera (Leica DFC320) mounted on the microscope. Diagonals of the indent were measured using Image Tool software (Version 3.0, UTHSC, San Antonio, TX) that were converted into Vickers hardness values. Blocks were then placed into fresh batch of solution and returned to the incubator.

Ion release of calcium, aluminium, phosphate and fluoride after each 24-hour period of storage were determined using atomic absorption spectroscopy (for calcium and aluminium), colourimetry and an ion-specific electrode respectively. To determine the calcium concentration sample solutions (0.5 mL) were diluted with water (0.5 mL, MilliQ), were then acidified with 1M HCl (0.5 mL) and diluted with 2% lanthanum chloride (0.5 mL) and analysed on a Varian AA240 atomic absorption spectroscope (AAS, Varian Australia Pty. Ltd.) against a set of seven standards ranging from 0 to 250 µM calcium. The Varian AA240 AAS was also used to determine the concentration of aluminium ions. Sample solutions (0.5 mL) were diluted with potassium chloride (0.5 mL, 8mg/L) and lanthanum chloride (0.5 mL, 2% w/w) and were then acidified by 1M HCl (0.5 mL) and compared against a set of standards ranging from 0 to 125 µM aluminium. Inorganic phosphate ion concentrations were determined colourimetrically using a spectrophotometer (UV-visible spectrophotometer, Varian Australia, Pty. Ltd). The samples that were analysed were prepared by taking 100 µL of solution, diluting with 500 µL of 4.2% ammonium
molybdate and adding 20 µL 1.5% of Tween ® 20. (Sigma-Aldrich, St. Louis, MO). The phosphate ion concentration was determined by comparing the spectrophotometer readings of the samples against a set of seven standards ranging in phosphate concentrations from 0 and 100 µM. The concentration of fluoride ions was determined using an ion-selective electrode (Radiometer analytical, ISE C301F, France) connected to an ion analyser (Radiometer analytical, Ion Check 45, France). Sample solutions (1mL) were diluted with 1mL total ionic strength adjustment buffer (TISAB, Merck Pty Ltd, Kilsyth, VIC, Australia) and measured against a set of eight fluoride standards ranging from 0 to 1000 µM.

Data from sample groups were found to follow a normal distribution. The χ² test was used to test normality of the data. Single factor ANOVA was used to analyse the results using Bonferroni-Holm multiple comparison. Two-way ANOVA was used to determine the interaction between incorporation of CPP-ACP and exposure to different acids. The level of significance was set at α = 0.05.

7.4 Results

7.4.1 Surface Hardness

Surface hardness for SBF03 samples was initially higher than F7 and F7EP, however in citric acid, hydrochloric acid and water the percentage drop in hardness was higher compared to F7 and F7EP (Table 7.2). In citric acid the absolute hardness remained higher than F7 and F7EP, but in water the absolute hardness of SBF03 was lower than F7EP and higher than F7. The absolute hardness of SBF03 in HCl was lower than both F7 and F7EP following the three-day acid challenge. Lactic acid was the only group where SBF03 showed lower percentage drop and higher absolute hardness after three days compared to F7 and F7EP.
Table 7.2. Surface hardness comparison (VH1.0). Absolute values in **bold**, % drops in second row.

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Day Three</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Citric Acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F7EP</td>
<td>49.1</td>
<td>29.6</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>60.2</td>
</tr>
<tr>
<td>F7</td>
<td>47.2</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>62.5</td>
</tr>
<tr>
<td>SBF03</td>
<td>64.1</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>55.3</td>
</tr>
<tr>
<td><strong>Lactic Acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F7EP</td>
<td>46.3</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>48.3</td>
</tr>
<tr>
<td>F7</td>
<td>49.0</td>
<td>29.0</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>59.1</td>
</tr>
<tr>
<td>SBF03</td>
<td>61.5</td>
<td>38.6</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>62.7</td>
</tr>
<tr>
<td><strong>HCL</strong></td>
<td></td>
<td></td>
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<tr>
<td>F7EP</td>
<td>51.7</td>
<td>41.3</td>
</tr>
<tr>
<td></td>
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<td>80.0</td>
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<tr>
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<tr>
<td></td>
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<td>85.4</td>
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<tr>
<td>SBF03</td>
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<td>28.6</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>44.5</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
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<td>F7EP</td>
<td>51.1</td>
<td>48.8</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>95.7</td>
</tr>
<tr>
<td>F7</td>
<td>55.7</td>
<td>38.2</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>68.6</td>
</tr>
<tr>
<td>SBF03</td>
<td>64.3</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>66.9</td>
</tr>
</tbody>
</table>

7.4.2 Mass Loss

Shofu Beautifil F03 showed no significant mass loss in all solutions tested. After three days there was some change (increase and decrease depending on group), but no more than 0.5% in all groups (Table 7.3). Beautifil F03 blocks stored in water showed some variability in results, which may indicate some water absorption. F7 and F7EP experienced significant mass loss over three days; depending on the solution the loss of mass was substantial and significant as shown in Chapters 5 and 6.
### 7.4.3 Ion Release

Fluoride ion release comparison between F7 and F7EP has been described previously in Chapter 5, in many groups no statistical significance could be measured, for the purposes of further comparison only F7EP will be used. Fluoride ion release in F7EP was measured to be consistent over the 3 days of the study for each solution. The acidic solutions showed significantly higher \((p<0.05)\) fluoride ion release than the control (water). Fluoride ion release from SBF03 was significantly higher \((p<0.05)\) on the first day than the following days, but was significantly lower \((p<0.05)\) than F7EP for each day in all solutions (Figure 7.1).
A similar pattern was observed for the daily release profile of aluminium ions and fluoride ions in SBF03 (Figure 7.2). A similar pattern was observed for F7 and F7EP (Chapters 5 and 6), however for F7/F7EP it was aluminium and phosphate ions that shared similar daily release profiles (Figure 7.3).
Figure 7.2. Relationship between daily aluminium and fluoride ions released from SBF03.

Figure 7.3. Relationship between daily aluminium and phosphate ions released from F7EP.
7.5 Discussion

7.5.1 Surface Hardness

Initial surface hardness of F7 and F7EP was very similar, SBF03 on average had a 28.7% higher initial surface hardness than F7 and F7EP. The higher initial surface hardness of SBF03 did not always translate into higher surface hardness after the three-day acid challenge. Absolute values are ultimately more important, however percentage drops in hardness indicate how a material can potentially perform over a longer period of time if the acid challenge was extended beyond the three-day duration. SBF03 was especially vulnerable to hydrochloric acid, where the drop in hardness was largest out of all storage solutions, conversely F7 and F7EP performed best when exposed to hydrochloric acid compared to other acids tested. When looking at surface hardness, citric and lactic acids had a similar effect on all materials tested.

7.5.2 Change in mass

The results from Chapters 5 and 6 showed F7/F7EP underwent a significant mass loss, particularly when exposed to citric acid. A high phosphate ion release in the high mass loss groups suggested that aluminium chelation might be the cause for the high loss of mass; this hypothesis is further supported in that aluminium ion release has now been measured directly. It is worth noting that although the loss in mass was significant there was no obvious visible reduction in the size of the blocks. This needs to be investigated further. No significant mass loss was measured in Giomer groups. However, in the F7/F7EP groups the high mass loss was always accompanied by high ion release.
7.5.3 Ion Release

The Giomer material showed significantly lower fluoride ion release compared to F7EP in the first 24 hours, and fluoride ion release significantly reduced after 24 hours (p<0.05). Aluminium ion release in Shofu Beautifil closely followed fluoride ion release, which is different to the pattern observed in F7EP. The GIC materials demonstrated a steady fluoride ion release over 3 days, which was also higher than that of Beautifil, and aluminium ion release also closely followed phosphate ion release (Chapters 5 and 6). This may indicate that, while Beautifil contains glass particles, the source of fluoride ions may be different for each material. Table 7.1 shows the possible sources of aluminium, fluoride and phosphate ions contained in the GIC materials. In conventional GICs, cross-linking aluminium ions are the source of secondary setting reaction following the initial setting reaction involving calcium (or strontium) ions. The secondary setting reaction involving aluminium ions proceeds at a much slower rate over many days or even weeks (depending on material)\(^\text{12}\). As F7EP is subjected to acid challenge the setting reaction is still ongoing and aluminium ions are not as tightly bound as in the matrix of a completely matured material. This creates many potential sources of aluminium ions in F7EP, particularly under chelation of citric acid, which results in higher phosphate and fluoride ion release. Beautifil contains pre-reacted glass particles that contain mostly bound aluminium, phosphate and fluoride compounds. The most substantial release of fluoride and aluminium ions observed in Beautifil occurred in the first 24 hours. However, the greatest ion release may have been during a shorter period of time within the first 24-hour measurement period. There are two possible reasons for this pattern. The initial burst release of fluoride and aluminium ions is from the accessible surface regions of the material. Compared to GICs, resin-based materials are not as porous and permeable. GICs are able to sustain a higher fluoride release over time as additional ions are released from sub-surface regions; this compromises the structural integrity of the GIC materials as evidenced by the mass loss data and high ion release on the last day of measurement. Another possible explanation could be
that these aluminium and fluoride ions, contained in pre-reacted glass filler particles, are excess ions that have not taken part in the initial setting reaction of the glass. These ions would not be tightly bound and consequently could be washed out of the material much more easily than more tightly bound ions. These differences may be the reason behind the different fluoride ion release profiles between F7EP and Beautifil over the 3-day period.

The release of aluminium ions has emerged as an important piece in understanding how other beneficial ions are stored and released from GICs and GIC-based materials. When we consider acid challenge from not only lactic acid but also other acids encountered within the oral environment, such as citric acid the mechanisms involved in the ion release can be established. The evidence is building that points to the chelation of aluminium ions by citric acid as the main reason for high mass loss in F7 and F7EP (Chapters 5 and 6), as well as the formation of a ‘crust-like’ surface layer on F7 and F7EP samples following topical treatments. Aluminium ions facilitate better cross-linking of the GIC matrix and take part in the long-term maturing process of the GIC, however they are also present in many filler compounds within the GIC structure. Given the high mass loss and Al$^{3+}$ and PO$_4^{3-}$ ion release data the evidence suggests that it is not only the filler aluminium ions that are being chelated but also the cross-linking ions. This is of great importance, however the exact mechanisms that cause this are not clear, some parameters have been ruled out as potentially contributing factors. For example, low pH (HCl at pH of 2.0) was found to not be a major factor in high dissolution of GIC structure. Also, topical treatment of GIC surface did not always provide a benefit, and in some specific cases reduced the performance of the GIC material. These unknown interactions as well as the physical evidence presented in relation to aluminium ion release merits further study.
7.6 Conclusion

Fluoride ion release was found to be significantly higher in F7EP compared to Beautiful. Aluminium ions have a strong effect on ion release, especially when the materials are exposed to citric acid. Aluminium ion release holds a strong correlation with the release of other ions, fluoride for Beautiful and phosphate for F7EP. For each material the source of fluoride ions is different, which can be attributed to F7EP still being in a non-matured state and Beautiful containing elements from matured pre-reacted glass.
7.7 References


CHAPTER 8

Discussion

8.1 Pilot study outcomes

From the very start the intention was to only use lactic acid as it is the major acid associated with the formation of carious lesions\(^1\), however difficulties were encountered with the measuring equipment, so alternative options needed to be evaluated. Conflicts arose when using lactic acid and measurement of fluoride ion release. The pilot work focused on determining if a different acid could be used (citric acid instead of lactic acid), however it was found citric acid caused contamination of the equipment and hence was also deemed incompatible. The citric acid pilot study revealed some interesting findings (high phosphate ion release and higher mass loss compared to lactic acid). A different measuring setup needed to be used, but the citric acid pilot results warranted further investigation due to the different outcomes, this led to citric acid being included in the final testing protocol. It became apparent that when immersed in different acids, even at the same pH and ionic strength, the GICs seemed to react in very different ways. Consequently, hydrochloric acid was also
added to the protocol, even though there were no initial data to suggest that it would produce different results. After acid challenge solutions were established the remainder of the pilot studies were able to proceed.

Microhardness pilot work focused on determining the correct combination of test parameters such that upper and lower hardness values could be measured without adjusting the equipment. The upper values were expected at the start when GIC blocks were fresh, shortly after the initial set and not yet exposed to acid solution. After three days of storage however, it was expected that the surface of the material would deteriorate and soften leading to lower surface hardness values. The pilot tests focused on the two extreme hardness parameters.

Ion release assays were initially carried out on samples stored in 15mL of acid solution, this volume was found to be too large as some readings fell below the detection limits of the analytical apparatus used. The pilot was re-run using 5mL of solution, which produced an improvement in accuracy for the low reading groups, however, the high reading groups required dilution in some instances. This was established at the pilot stage and carried throughout the remainder of the experiments. The microhardness pilot was also re-run with 5mL storage volumes to determine if a smaller volume of acid was adequate for consistent measurements. Only in a few extreme cases did the magnification of the microscope need to be altered (and those readings were re-calibrated appropriately).

8.2 Acid challenge outcomes

The main focus of these studies began with testing the response of F7 and F7EP GICs to a 3-day acid challenge. The results showed that substantially increased calcium, phosphate and fluoride ion release was observed in F7EP compared to F7. The increase was attributed to the presence of CPP-ACP in F7EP. Statistical analysis showed that changes to physical properties (surface hardness, mass loss) were either not significant or showed an improvement for F7EP in comparison to F7. Mass loss in
citric acid was unexpectedly high and was observed for both F7 and F7EP. Statistical analysis could not attribute this effect to CPP-ACP, consequently no further investigation was conducted at the time as the focus of the study was on the effect of CPP-ACP. However, the citric acid groups also showed a much higher phosphate ion release than the other acid solutions.

It became evident that, at least, conventional GICs respond differently to the different acids that are readily found in the oral environment. Prior to this study the main focus of research has always been on lactic acid as the chief causative agent in cariogenic activity\(^1\). Lactic acid has proven to be an appropriate solution to use when studying the effect of caries initiation in teeth under laboratory conditions. However, when investigating its effect on restorative materials it may only provide part of the picture. Citric acid is present in many processed foods and its effect on GICs is therefore of great interest.

### 8.3 Topical treatment outcomes

Building on the knowledge of the benefits of CPP-ACP a further study investigating whether topically applied CPP-ACP (using TM+) could impart its benefits onto conventional GICs by enhancing calcium, phosphate and fluoride ion storage and release, in a manner similar to work done in the past to ‘recharge’ the fluoride content of GIC by topical fluoride applications. The study was designed with several control groups; essentially, each variable had its own control material, solution or treatment. Placebo mousse was used as the control treatment, water was used as the control solution and F7 was the control material for F7EP. In addition to placebo mousse served as a control treatment, the previous study’s results (no treatment) were also used as a further control group.

The presence of CPP-ACP in TM+ showed an increase in ion release, reduction in mass loss and increase in surface hardness when used to treat F7 blocks compared to placebo mousse treatment. However, data also showed, with a high level of
statistical significance, that mass loss compared to the ‘no-treatment’ control groups was much higher and surface hardness increased beyond its initial values. Phosphate ion release also increased from its already elevated levels observed in the ‘no-treatment’ groups of the previous study. The hypothesis that aluminium chelation was the main cause of the observations had gained a greater significance. The results were not only unusual for exposure to citric acid compared to other acids, but also carried important implications of how GICs potentially react to various acid challenges in clinical situations.

The first stage was to investigate the increased surface hardness. A pilot study was conducted to measure hardness throughout specimens and not just on the surface. The surface was progressively lapped (in 100 μm increments) and the hardness of the newly exposed surface measured. The results revealed a crust-like layer on the surface of the GIC blocks in citric acid with the highest values being measured at the initial exposed surface, while the lowest values were recorded just below the surface layer (100 μm below the surface). Values of the lowest hardness and the specific depth below the surface at which it occurs are of great importance, however due to the very coarse resolution of the pilot work only the initial evidence of this effect has been observed. This aspect of the effect of the acids needs a more comprehensive study. The presence of the crust-like layer indicates that there is a migration of ions from the material bulk and sub-surface regions of the GIC blocks to the surface. A more direct investigation of aluminium ions and their link to surface hardness, mass loss and general ion release was the focus of the follow-up study.

8.4 Importance and role of aluminium ions

The early studies (Chapter 5) found an unexpected correlation between high phosphate release and mass loss for F7 and F7EP stored in citric acid. At that stage no evidence was found that both of these measurements were caused by the same mechanism, however aluminium chelation by citric acid was a plausible reason for
both results. This outcome was interesting, but the focus of further investigations remained with GICs and CPP-ACP. In the follow-up study (Chapter 6), topical CPP-ACFP treatment in the form of Tooth Mousse Plus on the same set of GIC materials, F7 and F7EP, again showed the same effect. Under topical treatment, phosphate ion release and mass loss were higher compared to no treatment. This was true even for the control groups, which used a placebo mousse, where no additional phosphate was available. Whatever was the cause of the high phosphate ion release and high mass loss it was being further exacerbated by the topical treatment. An additional unexpected result was the increased surface hardness beyond that of fresh GIC blocks (before any treatment or acid challenge). Further investigation of this mechanism was warranted.

It was determined that the most simple and direct way to validate our hypothesis that aluminium was somehow involved was to measure the release of aluminium ions. A new protocol was developed to employ Atomic Absorption Spectroscopy assays to measure aluminium ions released into solution. Results showed that aluminium ion release correlated closely with phosphate ion release and mass loss. Even day-to-day and sample-to-sample variations between phosphate ion release and aluminium ion release followed each other closely.

Examining the composition of GICs (Table 8.1) revealed that aluminium is present in a number of compounds, of which aluminium phosphate is one, but there are many others. Chelation of aluminium phosphate would account for the high ion release and consequently high mass loss, however the relationship of other aluminium-based compounds and citric acid remains unclear.

Investigation of a resin composite containing so-called pre-reacted glass ionomer fillers presented an opportunity to investigate the role of aluminium in GICs from a different perspective. The material selected was Shofu Beautifil F03 (SBF03). SBF03 is a ‘giomer restorative’ material. It is resin-based with the inclusion of pre-reacted glass particles that are claimed to allow fluoride ion release. The results showed that SBF03 released a substantial amount of fluoride, however the daily release fell sharply after the first 24 hours. Examination of aluminium ion release
showed that, unlike in F7 and F7EP, SBF03 shared a close correlation with fluoride ion release but not phosphate. This major difference may be explained by the different levels of maturation of the glass present in each material. In order to understand this more clearly a closer examination of the polymerisation process of GICs is needed.

<table>
<thead>
<tr>
<th>Powder</th>
<th>weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryolite (Na₃AlF₆)</td>
<td>5%</td>
</tr>
<tr>
<td>AlF₃</td>
<td>5%</td>
</tr>
<tr>
<td>SrF₂ or CaF₂</td>
<td>22-34%</td>
</tr>
<tr>
<td>AlPO₄</td>
<td>10%</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>16%</td>
</tr>
</tbody>
</table>

**Table 8.1.** General composition of GICs

The initial setting reaction during the polymerisation process involves either calcium or strontium salts and was developed to give a fast setting property to GICs. Historically, tartaric acid was used to provide this ‘snap-set’. Aluminium salts participate in the polymerisation process at a much slower rate and the reaction continues for many weeks. This means that the relative composition of compounds present in a ‘fresh’ GIC would be very different to those present in a ‘mature’ GIC, aluminium ions would be bound to different compounds and with different binding strengths. This may explain the differences observed between F7/F7EP and SBF03 when it comes to fluoride, phosphate and aluminium ion release.

The studies examined two types of material, a conventional GIC that was mixed fresh and immediately subjected to acid challenge and a giomer material,
which contains ‘matured’ glass particles. The results indicated that glass particles might react differently to acid challenge depending on their stage of maturation. These results may carry implications for many conventional GICs. If the function of aluminium ions can be more fully understood and their interaction with external acids better traced over time the changes observed in the current work can be better explained. This could then possibly allow modifications of the cements to be introduced to improve their properties.
8.5 References


Conclusions and Areas for Further Research

Conclusions from the research undertaken are summarised below:

- CPP-ACP, incorporated into Fuji VII EP, significantly increased the release of calcium and phosphate ions compared to Fuji VII; no significant difference in fluoride ion release could be detected between Fuji VII EP and Fuji VII.

- GIC materials (Fuji VII EP and Fuji VII) responded to various acids in different ways. The type of acid was found to be a more significant factor than pH or ionic strength of the acid.

- Topical CPP-ACFP treatment (Tooth Mousse Plus) of GIC surface (Fuji VII EP and Fuji VII) was found to be more beneficial than placebo control treatment (no active ingredient) and “no treatment” when considering calcium and fluoride ion release. Topical treatment (Tooth Mousse Plus and placebo
mousse control) greatly increased phosphate ion release and mass loss of GIC blocks compared to “no treatment”. Tooth Mousse Plus was found to provide greater protection to mass loss compared to placebo treatment.

- Aluminium chelation was identified as a key factor in phosphate ion release from Fuji VII EP and Fuji VII when both GIC materials were exposed to citric acid. Increased phosphate and aluminium ion release combined with increased surface hardness suggests a formation of a “crust-like” layer on the surfaces of Fuji VII EP and Fuji VII.

- A comparison between Fuji VII EP and a glass-containing giomer material (Shofu Beautifil F03) showed different ion release profiles depending on the maturation of the glass in each material. Aluminium chelation was present in both cases, however aluminium ion release was closely related to fluoride release in Beautifil and phosphate release in Fuji VII EP.

Following the investigations covered in this thesis several areas of further research have been identified:

- Further study into how dental restorative materials (not just GICs) respond to acid challenge, especially relating to ion release. Laboratory studies have shown GICs respond differently to acid challenge depending on the acid used. Clinically the environment is more complex as different acids will exist concurrently as well as protein adsorption on the material surface. This more complex system may cause different interactions compared with the outcomes from individual acids used in the current research.

- A further investigation of recharge potential of CPP-ACFP (Tooth Mousse Plus) on GICs and other materials is required. The results presented in this thesis
showed a significant improvement in ion release potential of conventional GICs when topically treated with Tooth Mousse Plus. This may be important for use in patients who have a dry mouth and increased caries risk, as well as the appropriate selection of GIC products depending on environmental acid exposure.

- Topical treatment of GICs stored in citric acid showed the formation of what appeared to be a ‘crust-like’ surface layer with an ion-depleted sub-surface region. Mass loss, ion displacement and migration to the surface layer is not unlike the formation of an early enamel carious lesion in teeth, where a fluoride-rich surface layer is located above a sub-surface region of demineralised tooth structure. The effect of aluminium chelation in GICs needs further investigation.

- A more detailed examination of aluminium ions within GICs and how the loss of aluminium ions can be addressed to improve the longevity and its relation to the clinical application of GICs. Substitution of aluminium ions with another ion can potentially help mitigate the chelation caused by citric acid in GICs (and possibly other triprotic acids encountered in the oral environment). The substitution can occur either via a topical treatment or ideally during the manufacturing process. The manufacturing process of the glass of GICs to substitute aluminium could be considered.
APPENDIX
Ion release and physical properties of CPP–ACP modified GIC in acid solutions

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ABSTRACT

A new glass-ionomer cement (GIC) (Fuji VII™ EP) includes 3% (w/w) casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) to enhance ion release.

Objectives: To assess this new GIC compared with a GIC without CPP–ACP (Fuji VII™) with respect to ion release, changes in surface hardness and in mass under a variety of acidic and neutral conditions.

Methods: Eighty blocks of Fuji VII™ (F7) and Fuji VII™ EP (F7EP) were subjected to three acidic solutions (lactic and citric acids pH 5.0, hydrochloric acid pH 2.0) and water (pH 6.9) over a three-day period. Ion release, surface hardness and weight measurements were carried out every 24 h.

Results: Higher calcium ion release from F7EP was observed under all acidic conditions. Increased inorganic phosphate ion release was observed for F7EP in hydrochloric and citric acids. Fluoride ion release was similar between F7 and F7EP under all conditions but was significantly higher in acids compared with water. After three days there was no significant difference in surface hardness (p > 0.05) between the two materials under all conditions except hydrochloric acid. Minimal change in mass was observed for F7 and F7EP in water, lactic and hydrochloric acids, however citric acid caused significantly more mass loss compared with water (p < 0.001).

Conclusion: Incorporation of 3% (w/w) CPP–ACP into F7 enhanced calcium and phosphate ion release, with no significant change in fluoride ion release and no adverse effects on surface hardness or change in mass.

Clinical significance statement: GICs have the potential to release fluoride ions particularly under acidic conditions associated with dental caries and erosion. A new GIC containing CPP–ACP and fluoride releases not only fluoride ions but also calcium and phosphate ions under acidic conditions which should help to inhibit demineralisation associated with caries and erosion.

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1. Introduction

Glass ionomer cements (GICs) are widely used for a variety of purposes such as intermediate restorations, caries stabilisation, definitive restoration of micro-cavities or non-caries cervical lesions, adhering orthodontic brackets and bands, fissure sealing erupting molars and as a surface protecting material for high risk surfaces such as root surfaces.1,2 The main advantages of GICs are their strong...
ability to chemically bind to dentine and their ability to release fluoride ions. This fluoride ion release has been shown to slow the progression and aid the regression of early carious lesions.

Tooth enamel will only demineralise when the fluid bathing the enamel crystals is undersaturated with respect to the enamel mineral (carbonated hydroxyapatite). Hence, calcium and phosphate ion activity is an important component influencing the level of enamel mineral saturation. A number of investigators have explored the modification of dental materials in attempt to have them release calcium, phosphate and fluoride ions. Skrtic et al.6 explored modifying dental composites with bioactive glasses. Mazzaoui et al.7 and Al Zraikat et al.8 assessed the addition of casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) to GICs. The casein phosphopeptides stabilise calcium and phosphate ions in a bioavailable form that allow them to inhibit demineralisation and promote the remineralisation of early lesions. CPP–ACP is stable in the presence of fluoride and has been shown to work synergistically with fluoride. This makes CPP–ACP a promising additive to dental products and restorative materials. The study by Mazzaoui et al.9 was a proof of concept study that showed that CPP–ACP could be added to GIC. Al Zraikat et al.5 later explored the effect of CPP–ACP concentration on GIC mechanical properties. Additionally, both of these studies showed that the addition of CPP–ACP improved calcium and phosphate ion release in lactic acid.

The tooth surface can be exposed to a variety of demineralisation challenges including: acids formed from metabolic processes of oral bacteria (predominantly lactic acid); food acids such as citric and phosphoric acid that are commonly found in soft drinks; and hydrochloric acid from the regurgitation of stomach contents. If these acids overwhelm saliva’s protective functions mineral may be lost from the teeth. Therefore, the overall aim of this study was to determine how GIC with CPP–ACP performed under a variety of acidic conditions compared with GIC without CPP–ACP in terms of ion release, change in hardness and change in mass. Fuji VII (F7) is a low viscosity strontium glass ionomer cement and is commonly used as a surface protectant, providing effective wetting and intimate adhesion to tooth surfaces, as well as enhanced remineralisation capabilities. It has higher fluoride release than most other GICs which makes it a good candidate to establish if there is an additional benefit through the incorporation of CPP–ACP as a source of calcium and phosphate ions together with the fluoride ions. This study will establish whether F7 plus CPP–ACP has the potential to further protect surrounding hard tissue and enhance the remineralisation of demineralised tissue by additional ion release.

The research questions were: what effect on the GIC would the addition of CPP–ACP have on; (1) surface hardness; (2) change in mass under neutral or acidic environments and (3) calcium, phosphate and fluoride ion release under neutral or different acidic environments.

2. Materials and methods

Fuji VII GIC (F7) and F7 with added 3% (w/w) CPP–ACP (F7EP) from the same batch were provided by GC Corporation (Japan) in capsule form. Polyvinyl siloxane impression material (eliteHD + light body, Zhermack SpA, Badia Polesine, Italy) moulds were used to create standardised GIC blocks measuring 3 mm x 6 mm x 6 mm (thickness x width x length). 40 blocks of each GIC were prepared by placing the materials in the mould with the top and bottom surfaces covered by plastic strips, which was held between two glass slides. The glass slides were gently pressed together to extrude any excess material. The specimens were allowed to set inside the moulds for 24 h in an incubator (37 °C, ~100% relative humidity). After cooling to room temperature the blocks were removed from the moulds, and the two major parallel surfaces of the blocks were lapped with 600 grit paper (Norton Tufbak, Saint-Gobain Abrasives Ltd., Auckland, NZ).

Four different solutions were prepared to expose the blocks to a variety of acidic and neutral environments. The three acidic solutions were formulated to simulate a gastric erosive challenge (50 mM NaCl adjusted to pH 2.0 with HCl), a dietary erosive challenge (50 mM citric acid at pH 5.0) and a cariogenic acid challenge (50 mM lactic acid at pH 5.0) (this concentration was selected based on values found previously in plaque fluid). Ionic strength of all the acidic solutions was made up to 50 mM including the HCl solution which was modified with NaCl to approximate that found in stomach acid.

The neutral solution was distilled deionised water at pH 6.9 (Millipore Corporation, Victoria, Australia).

Ten blocks of each type of GIC were exposed to 5 mL of one of the four solutions. Solutions were changed every 24 h and the samples were measured for change in mass, surface hardness and the solutions were analysed to determine ion release of calcium, phosphate and fluoride every 24 h over three days.

The mass of each block was measured every 24 h before surface hardness measurements were performed. Blocks were taken out of solution, and then weighed using a microbalance (Precisa XT 120A, Dietikon, Switzerland). Mass loss measurement was performed under the same conditions for each sample. Blocks were gently pat dried in the same manner and weighed immediately to ensure consistent treatment before testing. All mass loss measurements were obtained under ambient conditions of 60 ± 5% relative humidity and 23 ± 2 °C. The percentage change in mass was a combination of water uptake (absorption) and dissolution (solubility) of the GIC.

Vickers microhardness measurements were determined from indentations on the lapped GIC surface using a Microhardness tester (MHT-10, Anton Paar GmbH, Graz, Austria) attached to a microscope (Leica DMPL, Leica Microsystems Wetzlar GmbH, Germany). Two indentations were made on each block (force, 1.0 N; dwelling, 6 s; rate, 0.99 N/min). The indentations were separated by a distance of at least three times the indentation size. Images of the indentations were acquired through a calibrated digital camera (Leica DFC320) mounted on the microscope (Leica DMLP, Leica Microsystems Wetzlar GmbH, Germany) and distance measurements made using Image Tool software (Version 3.0, UTHSC, San Antonio, TX) which were then converted into Vickers hardness values. Blocks were then placed into fresh batch of solution and returned to the incubator.

The ion release of calcium, phosphate and fluoride after each 24 h period of storage was determined using atomic
absorption spectroscopy, colorimetry and an ion-specific electrode respectively. To determine the calcium concentration sample solutions (1 mL) were acidified with 1 M HCl (0.5 mL) and diluted with 2% lanthanum chloride (0.5 mL) and analysed on a Varian AA240 atomic absorption spectroscopy (Varian Australia Pty. Ltd.) against a set of seven standards ranging from 0 to 250 μM calcium. Inorganic phosphate ion concentrations were determined colorimetrically using a spectrophotometer (UV-visible spectrophotometer, Varian Australia, Pty. Ltd.). The samples that were analysed were prepared by taking 100 μL of solution, diluting with 500 μL of 4.2% ammonium molybdate and adding 20 μL 1.5% of Tween® 20 (Sigma–Aldrich, St. Louis, MO). The phosphate concentration was determined by comparing the spectrophotometer readings of the samples against a set of seven standards ranging in phosphate concentrations from 0 to 100 μM. The concentration of fluoride ions was determined using an ion-selective electrode (Radiometer analytical, ISE C301F, France) connected to an ion analyser (Radiometer analytical, Ion Check 45, France). Sample solutions (1 mL) were diluted with 1 mL total ionic strength adjustment buffer (Merck Pty. Ltd., Kilsyth, VIC, Australia) and measured against a set of eight fluoride standards ranging from 0 to 1000 μM.

The χ² test was used to test normality and data were found to be normally distributed. Single factor ANOVA was used to analyse the results using Bonferroni–Holm multiple comparison. Two-way ANOVA was used to determine the interaction between incorporation of CPP–ACP and exposure to different acids. Level of significance was set at α = 0.05.

3. Results

Table 1 shows surface hardness of F7EP and F7 in three different acidic solutions and distilled deionised water measured over three days. A significant decrease in surface hardness was measured from day to day in all solutions except in the following cases. F7EP in citric and hydrochloric acids between day two and three did not register a significant change in surface hardness. F7 in water did not register a significant change between days one and two, and two and three. The surface hardness of F7EP in water was not significantly different between its initial hardness and day one as well as between days two and three. The initial hardness of F7EP was significantly (p < 0.001) lower than that of F7. However, after 24 h of storage in lactic and citric acid F7EP had a similar surface hardness to F7 and was no longer significantly weaker for citric, lactic acids and water (p > 0.05), but it was still significantly (p < 0.01) weaker in hydrochloric acid. After three days of storage no significant difference in surface hardness could be measured between F7EP and F7 (p > 0.05) for citric, lactic acids and water, but F7EP still remained significantly (p < 0.05) weaker in hydrochloric acid.

Table 2 shows the mean relative mass change of F7 and F7EP blocks when stored in the four different solutions over three days. Each group is scaled according to its initial weight (100%). The greatest mass loss at all time points was from F7 and F7EP that were exposed to citric acid.

Fig. 1a–c shows the measured concentration of calcium, phosphate and fluoride ions released from F7 and F7EP when stored in the four different solutions over a three-day period. The rate of calcium and phosphate ion release for F7EP was significantly greater than that of F7 for both citric and hydrochloric acids (Fig. 1a and b), and there was also greater calcium ion release in lactic acid (Fig. 1a). The fluoride ion release in all solutions was similar for both F7 and F7EP (Fig. 1c).

Where a change in mass was detected for an exposure to an acidic solution over the three days (Table 2) the calcium and phosphate ion release was found to be greater for F7EP (Fig. 1a and b). The difference in calcium and phosphate ion release as a function of change in mass for F7 and F7EP is shown in Fig. 2a and b for citric and hydrochloric acids respectively (there was no measurable mass loss in lactic acid and water). These figures show that per unit of mass loss there was a greater release of calcium and phosphate ions from the GIC containing CPP–ACP.

4. Discussion

In all the tests conducted on F7 and F7EP, the materials behaved differently in water and acidic environments. The surface hardness of the GIC’s significantly decreased in the acidic environments although this did not correlate to a change of mass. The release of ions was greater in acidic conditions compared with water. The release of calcium and phosphate ions from the material only under acidic conditions is ideal as this is when they would be needed to protect the adjacent tooth structure from demineralisation. The higher release of calcium and phosphate ions from F7EP under acidic conditions may saturate the fluid in the oral environment with

| Table 1 - Mean surface hardness of F7 and F7EP in acidic and neutral solutions measured over three days. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| HV (kg/mm²) n = 10 | F7 (Std. Dev.) | F7EP (Std. Dev.) | |
| | Water | Lactic acid | Hydro-chloric acid | Citric acid | Water | Lactic acid | Hydro-chloric acid | Citric acid |
| Initial | | | | | | | | |
| 53.8³ (6.3) | 51.8³ (5.5) | 53.3³ (7.6) | 53.2³ (7.1) | 47.1³ (6.0) | 46.2³ (6.2) | 46.6³ (6.4) | 46.7³ (7.0) |
| Day 1 | 48.5³ (6.8) | 32.6³ (5.1) | 28.1³ (4.5) | 39.5³ (7.4) | 45.6³ (4.3) | 34.0³ (5.9) | 22.6³ (4.0) | 38.1³ (9.0) |
| Day 2 | 44.4³ (4.2) | 28.5³ (3.1) | 22.0³ (3.9) | 32.9³ (5.8) | 42.2³ (3.3) | 28.3³ (4.8) | 15.5³ (3.5) | 28.5³ (6.3) |
| Day 3 | 42.0³ (4.7) | 24.8³ (2.3) | 15.4³ (2.7) | 25.8³ (5.6) | 41.5³ (3.2) | 24.0³ (3.0) | 13.3³ (2.3) | 25.6³ (6.8) |

Values marked with the same letter (a–h) indicate a significant difference between GIC materials within the same solution for the same time period.
appropriate ions thus acting as a “smart material” for the protection of at risk tooth surfaces. The three-day continuous exposure in vitro would have been equivalent to a much longer term periodic acid challenge in vivo. However, the in vitro performance of these GIC materials should be confirmed clinically.

Previous studies have investigated ion release and surface hardness of various GICs in acidic and neutral environments.4,5,13 Past results have shown ion release from F7 with incorporation of CPP–ACP when stored in neutral solution (water) or lactic acid.4 It has also been reported that lower pH environments negatively affect surface hardness of GICs.14 However, there have been no studies looking at ion release or surface hardness of GICs covering a wide variety of acids that can be encountered in the oral environment.

The results indicate that the degradation of surface hardness was pH dependent rather than solution dependent. Lactic and citric acids (both pH 5) produced a similar reduction

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### Table 2 – Mean relative mass of F7 and F7EP when stored in the four different solutions over three days.

<table>
<thead>
<tr>
<th>(%) relative to initial mass (100%) n = 10</th>
<th>F7 (Std. Dev.)</th>
<th>F7EP (Std. Dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Lactic acid</td>
</tr>
<tr>
<td>Day 1</td>
<td>100.3± (5.7)</td>
<td>99.8 (3.8)</td>
</tr>
<tr>
<td>Day 2</td>
<td>100.5± (5.7)</td>
<td>99.7 (3.8)</td>
</tr>
<tr>
<td>Day 3</td>
<td>100.6± (5.6)</td>
<td>99.6 (3.8)</td>
</tr>
</tbody>
</table>

Values marked with the same letter (a–f) indicate significant difference between different solutions within the same material for the same time period. Each group is scaled according to its initial weight (100%).

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### Fig. 1 – Measured calcium (a), phosphate (b) and fluoride (c) ion release from F7 and F7EP when stored in the four different solutions over a three-day period. Significant increase \( (p < 0.05) \) in calcium ions was measured for all groups (except water) between F7EP and F7. Significant increase \( (p < 0.05) \) in phosphate ion release was measured for citric and hydrochloric acids compared to water. Significantly more phosphate was released from F7EP compared to F7 in citric and hydrochloric acids. Fluoride ion release was significantly higher \( (p < 0.005) \) in all acids compared to water. Groups marked with same letter indicate no significant difference in fluoride ion release between F7 and F7EP when exposed to the same solution.
in microhardness over three days, while HCl (pH 2) showed the greatest and water (pH 6.9) the least. Even though initially F7EP was slightly softer in surface hardness than F7, a converging pattern occurred across all solutions such that, except for HCl, the difference after three days was no longer significant. It is possible that F7EP has a greater buffering capacity than F7 alone as CPP–ACP has previously been shown to have buffering potential.15,16

As with surface hardness, there was an initial difference in the change in mass between these two GIC materials. This can be partly attributed to F7EP being slightly less dense than F7. Significant mass loss was observed for specimens placed in citric acid for both materials. While other solutions produced small changes from day to day the differences between them were not significant. The difference in the change in mass between F7EP and F7, in citric acid, was not significant when taking into account the difference in initial weights. Although surface hardness showed very similar trend in a decrease in hardness for all solutions this was not repeated for change in mass. For citric and lactic acids the mass loss showed a large difference between the two acid solutions such that, in lactic acid a slight mass gain was observed for F7EP and a slight loss for F7. In citric acid a large mass loss was observed for both materials. A mass gain was observed in water. The chelating effect of the citric acid is likely to contribute to the large mass loss. Citric acid is a triprotic acid, which can chelate transition metals, in this case aluminium. Aluminium is an initial component of the GIC matrix in the form of aluminium phosphate and the aluminium ion is involved in the acid–base setting reaction. The cross-linking between the polyalkenoic acid chains is formed by a slow final maturation reaction involving aluminium ions, which increases the strength of the GIC.17 This hardening process may take days to complete. When exposed to citric acid Al3+ ions are complexed by citrate removing them from the GIC matrix leading to the greater mass loss of the material. Further evidence to support this theory can be seen in the phosphate ion release data shown in Fig. 1b. The GIC without CPP–ACP released high levels of phosphate although it contained no CPP–ACP supporting the chelation of aluminium and the dissolution of the aluminium phosphate present within all GICs.

When subjected to aggressive environments such as citric, lactic and hydrochloric acids a substantial release of ions occurred from both GIC materials. Calcium release in the three acids ranged from 0.07 to 0.20 μmol/mm² for F7 but was higher for F7EP, ranging from 0.36 to 1.34 μmol/mm², equating to an increase of 564%, 405% and 462% for citric, lactic and hydrochloric acids respectively. This can be explained by the CPP–ACP acting as a source of bioavailable calcium ions. The CPP–ACP complexes will release calcium and phosphate ions in a pH dependent manner.16,18 An increase in calcium ions would help to maintain the degree of saturation with respect to tooth mineral which would prevent demineralisation. Phosphate ion release in lactic acid and water was not detectable for either GIC material. Daily phosphate ion release for citric acid ranged from 9.8 to 13.9 nmol/mm² for F7 and was higher for F7EP, ranging from 14.5 to 17.6 nmol/mm², an average increase of 39%. Daily phosphate ion release for hydrochloric acid ranged from 0.42 to 0.51 nmol/mm² for F7 and was higher for F7EP, ranging from 0.7 to 1.07 nmol/mm², an average increase of 85%. This implies that some phosphate is being supplied by CPP–ACP, but unlike the calcium ion release a lot of the phosphate is also being supplied by the GIC matrix most likely from dissolution of AlPO₄ at the surface of the GIC. Dissolution of the AlPO₄ by citric and hydrochloric acids releases phosphate from the GIC matrix. Fluoride release from both materials was similar and highest in citric (103.53 and 108.46 μmol/mm³) and hydrochloric acids (107.40 and 109.38 μmol/mm³) for F7 and F7EP respectively (Fig. 1b).

5. Conclusion

Although initial surface hardness of F7EP was slightly lower than F7, after three days of storage in three of the four types of solution there was no significant difference between the surface hardness values of the two GICs. Addition of CPP–ACP to F7 increased the release of calcium ions by an average of 477% when exposed to acidic solutions with no change in fluoride ion release. However, phosphate ion release ranged greatly depending on the type of acid exposure and whether CPP–ACP had been incorporated.
REFERENCES


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