Effects of Replacing Calcium Phosphate with Strontium Phosphate in Remineralising and Antibacterial – Releasing Dental composites

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Submitted by

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Dedication

I would like to dedicate this work to my parents, Fuad Alqadi and Samia Alqadi, for their endless love, support and encouragement throughout my life.

Declaration

I, Soha Fuad Alqadi confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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In the Name of Allah, the Most Merciful, the Most Compassionate all praise be to Allah, the Lord of the worlds; and prayers and peace be upon Mohamed His servant and messenger.

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Abstract

Introduction

The main cause of dental composite restoration failure is recurrent caries. This occurs due to micro-gap formation upon polymerisation shrinkage allowing bacterial penetration and continuing demineralization of dental tissue beneath the restoration. The aim of this study was to compare the effect of replacing calcium phosphate with strontium phosphate on water sorption induced expansion, surface hydroxyapatite layer formation and release of two different antimicrobial agents from new composite materials being developed to address these issues.

Methods

Composite pastes were prepared by mixing dimethacrylate monomers with dental glass filler at 3:1 ratio. The glass contained 10 wt% chlorhexidine diacetate (CHX), 10 wt% mono-calcium phosphate monohydrate, 10 wt% tri-calcium or tri-strontium phosphate and PLS (2 or 10 wt %).

Monomer conversion of the experimental composite was assessed using Fourier transform Infra-red Spectroscopy. The change in mass and volume of light cured composite discs were determined in water and simulated body fluid (SBF) at different time points. Surface microstructure and chemistry were assessed using Scanning Electron (SEM), Raman Microscopy and EDX. CHX and PLS release was measured through UV spectroscopy. The biaxial flexural strength of the experimental composites (BFS) and modulus of elasticity were assessed up to one month of water storage.

Results

Monomer conversion of all the dental formulations was between 70.6% and 76.2% and higher than 61.1% for Z250. Higher monomer conversions were obtained with higher PLS content. In all formulations MCPM played an essential role in promoting water sorption and subsequent diffusion of the remineralising ions and antimicrobial agents from the set materials. Effects of replacing TCP by TSrP on water sorption were minimal. Addition of PLS at

higher percentages, however, encouraged greater water sorption and more release of CHX. A calcium phosphate layer precipitated on the composite surfaces that were soaked in SBF. The Ca/P ratio was close to that of mineral in dentine. Whilst high levels of antibacterial and remineralising agents may be beneficial for the prevention of recurrent caries the levels must be optimised to ensure mechanical properties are not severely compromised.

Conclusion

To sum up, this work proved that replacing TCP by TsrP had limited effect on the properties of dental composites investigated in this thesis. Conversely, addition of MCPM and different percentages of PLS played a more important role in generating a remineralising and antibacterial releasing dental composite; however high percentages of antimicrobial drugs decreased the strength of the material dramatically.

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List of Abbreviations

ACP	Amorphous calcium phosphate
APS	3-acryloxypropyl trimethoxysilane
BAC	Benzalkonium chloride
BFS	Biaxial Flexural Strength
Bis-GMA	bisglycidyl ether dimethacrylate
CaP	Calcium and phosphate
CHX	Chlorhexidine
CQ	Camphorquinone
DCPA	Dicalcium phosphate anhydrate
DCPD	Dicalcium phosphate dehydrate
DMFT	Decayed, missing, filled teeth
DMPT	N, N-dimethyl-p-toluidine
ECC	Early childhood caries
EDX	Energy-Dispersive X-ray Spectroscopy
FTIR	Fourier Transform Infrared
GIC	Glass ionomer cement
HA	Hydroxyappatite
HEMA	2-hydroxyethyl methacrylate
MC	Monomer Conversion
MCPM	Mono calcium phosphate monohydrate
4-META	4-Methacryloxyethyl trimellitic anhydride
MMP	Matrix Metalloproteinase
MPS	3-methacryloxypropyl trimethoxysilane
Nm	Nanometre
OA	Osteoarthritis
OCP	Octacalcium phosphate
PPD	1-phenyl-1,2- propanedione
PG	Proteoglycan
PLR	Powder to liquid ratio
PLS	Polylysine
PPGDMA	Poly(propylene glycol) (425) dimethacrylate
RMGIC	Resin modified glass ionomer cement

SD	Standard deviation
SBF	Simulated body fluid
SE	Standard error
SEM	Scanning Electron Microscopy
SQRT	Square root of time
TB	Trypan blue
TCP	Tri calcium phosphate
TEGDMA	Tri (ethylene glycol) dimethacrylate
T_g	Glass transition temperature
TSrP	Tri strontium phosphate
UDMA	Urethane dimethacrylate
UV	Ultraviolet-visible spectroscopy
ZnO	Zinc Oxide

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Chapter One

Introduction and Literature Review

1 Introduction and Literature Review

1.1 Teeth

Teeth form around 20% of the surface area of the oral cavity. They have several functions, which include mastication, speech and aesthetics. Dental tissue is composed of four distinct structures; enamel, dentine, pulp and cementum.

1.1.1 Enamel

Enamel, the outer layer that covers the clinical crown, is the most highly mineralised tissue in the body. It is made up of 96% inorganic material and 4% organic material and water. The inorganic content of enamel consists of a crystalline calcium phosphate (hydroxyapatite) substituted with carbonate ions that are also present in bone, calcified cartilage, dentine and cementum. Several other ions such as strontium, magnesium, lead and fluoride might be incorporated within the crystals during enamel formation. Enamel is formed by ameloblasts which are then lost as the tooth erupts into the oral cavity. Since these cells disappear during tooth eruption, enamel is non-replaceable tissue. Regeneration of enamel is impossible if it is damaged by any cause eg caries or wear. To overcome this intrinsic limitation, enamel is a very complex and organised tissue and possess very high levels of mineralisation. These particular structures and compositions enable enamel to resist the high masticatory forces and frequent stimuli from acidic intake and bacterial invasion (Miller, 2012).

The hardness property of enamel makes it very brittle; hence supporting such a tissue by the underlying layer of more resilient dentine to preserve it is essential. The apatite crystals inside enamel are organised in a very packed manner and they create enamel rods which are separated by an inter-rod substance. These substances are also composed of apatite crystallites, which are organised in a different direction from those found in the rods. Although the enamel is a non-vital and insensitive tissue, it is still permeable. Ion exchange takes place in the oral cavity between the enamel and saliva. In addition, application of fluoride can possibly change the structure and make

it more resistant to acids by replacing the hydroxyl group by fluoride ions (Miller, 2012).

Enamel is translucent and shows differences in the colour and thickness. The shade can be varied from light yellow to grey-white, while the thickness is about 2.5 mm over the working surfaces and decreases dramatically to a feather-edge at the cervical portion of the crown. These differences have an effect on the enamel colour as the underlining dentine shows through the thinner areas (Miller, 2012).

1.1.2 Dentine

Dentine is defined as " hard, elastic, yellow-white, a vascular tissue enclosing the central pulp chamber" (Miller, 2012). It is composed of 70% inorganic material, 20% organic components and 10% water by weight while 45%, 33% and 22% by volume, respectively. The inorganic material represents itself as hydroxyapatite, while the organic component is approximately 30% collagen with some lipids and non-collagenous matrix proteins. Odontoblasts are the cells that form dentine in which their cell bodies are aligned along the inner edge of the dentine, where they create the peripheral border of the dental pulp. Dentine, differs from enamel, as it is a vital tissue and able to repair, due to the ability of odontoblasts or mesenchymal cells in the pulp to produce more dentinal tissue when they are affected by any stimulus. Radiographically, it looks more radiolucent than enamel but more radiopaque than pulp (Miller, 2012).

1.1.3 Pulp

Dental pulp consists of two main portions; the coronal part or pulp chamber and the radicular part or the root canal. The pulp chamber always follows the anatomy of the clinical crown. The root canal ceases at the apical foramen where the pulp and periodontal ligament can assemble and the main blood vessels as well as nerves enter and leave the dental tissue. Histologically, it can be divided into four different zones. The first zone represents the

odontoblastic zone and it is located at the pulp periphery. The second one is called the cell free zone under the odontoblasts, which is prominent in the coronal portion of the pulp. Cell rich zone is the third layer which characterised by a high cell density. Finally, the pulp core which consists of high percentage of blood vessels and nerves (Nanci, 2007).

1.1.4 Cementum

Cementum is one of the main structures of dental tissue and it covers the root of the tooth. It appears as a light yellow colour, lighter than dentine and easily can be differentiated from enamel due to lack of shine. In addition, it is a very thin structure compared to enamel and dentine but it is harder than bone. It is composed of 50% organic and 50% inorganic material. Several types of fibres are attached to it to anchor the tooth in the bone.

There are two types of cementum; primary and secondary. The primary cementum (also known as acellular cementum), covers the whole root surface and does not have the capability of additional growth. Secondary cementum is however known as cellular cementum and develops after the tooth has reached its functional occlusion. It persists through the life of the tooth at the apical third of the tooth. This continuation in growth takes place due to the specialised cells that are called cementoblasts. These cells are able to produce more cementum to keep the tooth in functional occlusion (Bosshardt and Selvig, 1997).

1.2 Caries

1.2.1 Definitions

Dental caries is the most common disease that affects the oral cavity. In 2003, Fejerskov *et al* defined it as "localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates". It can also be described as an extension of a process of a disease that rises in severity and destruction of tooth structure which starts from ultra-structural, subclinical alterations to more advanced and obvious

dentinal changes which can be seen clinically, either leaving the surface intact or showing noticeable cavitations (Kidd and Fejerskov, 2004, Pitts, 2004). 'Cavity in a tooth' is a general term that most dentists, health care providers and patients use to describe dental caries in their daily practice.

1.2.2 Epidemiology

It is known that dental caries is the most common disease that affects oral cavity. It has been stated that around 60%-90% of school children experience dental decay while 100% of adults suffer this lesion which then lead to pain and discomfort (Who.int, 2015).

According to the Child Dental Health Survey 2013, (England, Wales and Northern Ireland), it has been stated that an obvious decline in the severity and extension of dental caries in the permanent teeth of 12 and 15 years old between 2003 and 2013. The recent data in 2013 indicates that around 45% of 15 year olds and 34% of 12 year old children suffer decay in their permanent teeth compared to 56% and 43% in 2003, respectively.

Furthermore, the data in 2013 illustrates dental caries in the primary teeth among children aged 5 years old and 8 years old. The percentages were 31% and 46% respectively. However, the percentage of untreated dentinal caries was 28% and 39% in 5 and 8 years old, respectively. (http://www.hscic.gov.uk/catalogue/PUB17137).

Necrotic pulp or extractions are the expected outcomes in those who have untreated carious teeth. Around 66% of children admitted into hospital for general anaesthesia was due to dental treatment between 1997 and 2006 and this is due to the difficulty in managing them (Moles and Ashley, 2009). Moreover, it has been stated that the quality of life can be disturbed in those who suffer oral diseases. The idea of oral health related quality of life relies on how much these oral diseases affect the lives of people. For instance, early childhood caries (ECC) leads to eating difficulties, loss of appetite, sleeping disturbances, weight loss and low school performance (Abanto et al., 2011).

With regards to the financial aspect, treating oral disease is very costly and considered the fourth most expensive disease to treat. In industrialized countries, the oral lesion burden has been managed through establishing advanced oral health systems that provide therapeutic services to patients. In Scandinavia and UK, oral health care is offered to children and populations in need. Expenditure on oral health consumes about 5-10% of the total public health budget which makes an extra load on the overall economy of the country (Widström and Eaton, 2004).

1.2.3 Pathogenesis

Dental caries is a dynamic process that takes place as a result of interactions over a period of time between the microorganisms which are responsible for generating acids, a substrate that accumulates on the teeth surfaces in which bacteria can metabolize, dietary carbohydrates and other host factors such as saliva. Metabolism of fermentable carbohydrates and production of acids as a by-product of this process are due to endogenous bacteria mainly streptococci (Streptococcus mutans and Streptococcus sobrinus) and Lactobacillus spp (Caufield and Griffen 2000). This acid brings about significant decline of the pH value to a level that leads to demineralization of dental tissue (Caufield and Griffen, 2000, Featherstone, 2000, Featherstone, 2004). Obvious dental cavity can be diagnosed if persistent diffusion of some minerals such as calcium, phosphate and carbonate out of the tooth (Featherstone 2004).

Fortunately, this process can be inverted in the early phases of demineralization via the reuptake of lost minerals; calcium, phosphate and fluoride. Fluoride is considered as a significant mineral which helps the infusion of calcium and phosphate into the tooth and eventually remineralisation of the decayed crystalline structures. These restored crystalline surfaces demonstrate a greater resistance to the bacterial acidic damage than the original ones due to fluoridated hydroxyapatite and fluorapatite (Axelsson, 2000) . The tooth is in a place where a constant demineralization and remineralisation take place through the day and the

outcome of the lesion depends on the balance of this process (Featherstone 2004).

Furthermore, the buffering capacity of saliva and its ability to raise the pH level of the plaque make the remineralization process continuous during the day. The greater level of fluoride and the less microporous enamel can be noticed in the remineralised areas of the tooth structure compared to the original tissue as a result of gaining of calcium and phosphate from saliva. Maturation of dental plaque and its accumulation on the teeth surfaces for long period of time, can lead to the development of dental caries. If the cavity has already formed, this will offer a suitable place for the bio-film microbes to remain and get used to a declined pH (Fejerskov, 2004). These cavitations provide protective sites for the plaque, and if the patients don't pay attention to their oral hygiene, the progression of carious lesion persists (Kidd and Fejerskov, 2004).

Pre-school children can demonstrate the most destructive presentation of dental caries known as early childhood caries (ECC). This striking form of the lesion affects mainly the surfaces of upper primary anterior teeth; however the posterior primary molars can be involved too. It starts at the gingival third of the teeth as a white chalky spots then progress to destruct the whole structure of the dental tissue. The moderate stage of the lesion shows obvious cavitation and involvement of upper primary molars contrary to the severe form which destroys the upper molars as well as the lower ones (De Grauwe et al., 2004, Curzon and Preston, 2004).

1.2.4 Caries Management

Preserving the dental tissue can be carried out by careful removal of the biofilm, application of fluoride and fissure sealants (Selwitz et al., 2007). If restorative intervention is necessary, several methods, such as new adhesive materials, can be used to prevent excessive tooth destruction.

1.3 Current Restorative Materials

In Dentistry, multiple restorative materials can be used to restore defective teeth. These include amalgam, composite, glass ionomer and others such as gold and porcelain. Several factors should be taken in consideration when selecting the filling material; design of the cavity, caries risk, patient's desire, economical status and dentist's judgement.

1.3.1 Dental Amalgam

Dental amalgam is an alloy composed mainly of mercury (50%), silver (22%-32%) and other metals in smaller percentages such as tin and copper (Ferracane, 2001). This restorative material has been utilized in restoring cavities for more than hundred years. Several advantages make amalgam the primary choice of fillings; the good strength, durability, simple application and low cost. The main reasons behind the filling failure are recurrent caries, poor adaptation at the margin and fracture of the bulk of the filling (Shenoy, 2008). Recently, replacement of dental amalgam has become progressively more of concern because of the mercury content and inferior esthetics. The Minimata Convention on Mercury set limits and called for ongoing restriction of the use of mercury. One of the consequences of this is that dental amalgam is likely to be phased out (Mercuryconvention.org, 2015)

1.3.2 Glass Ionomer Cement and Resin Modified Glass Ionomer

One of the essential aims of restorative materials is their sealing of the tooth structure. Glass ionomer filling materials can resemble the tooth structure, have similar characteristics and adheres to the enamel and dentine to enable an effective seal. The glass ionomer cement powder phase is composed of calcium, strontium aluminosilicate glass (base) while the liquid is a water-soluble polymer (acid). GIC has several functions; it can be applied as a luting agent to cement the crown and bridges, filling material as well as base in deep cavities. The different types of GIC depend on the powder- liquid ratio and the size of the powder particles. There are many advantages of GIC

which makes it a favourable restorative material. These are 1) good adhesion to the dental tissue, 2) fluoride release and thus it acts as anti-cariogenic agent, 3) biocompatible and 4) shows similar thermal expansion to tooth structure. On the other hand, GIC has some drawbacks and they include the short working time, long setting time, either sensitivity to the water or dehydration in the primary phase of reaction and low wear resistance (Nagaraja Upadhya and Kishore, 2005).

Therefore, to overcome the moisture sensitivity and to enhance the mechanical properties of GIC, resin modified glass ionomer were developed. The advantage of this modified material is the combination between the basic acid-base reaction of conventional GIC and good physical properties of composite. So, RMGIC are defined as hybrid materials that keep the essential acid-base reaction during the curing process. It is made of leachable glass powder and a water-soluble polymeric acid such as poly acrylic acid. In addition, small amounts of resin, such as 2-hydroxyethyl methacrylate (HEMA) associated with initiators were added (McLean et al., 1994). This initiator is known to be sensitive to the light and thus make the RMGIC light cured (Nicholson and Czarnecka, 2008). Consequently, favourable working time, setting upon curing, good adhesion to dental tissue, fluoride release enhancement as well as acceptable appearance are considered advantages of this RMGIC (Nagaraja Upadhya and Kishore, 2005).

On the other hand, the limitations of RMGIC include low surface hardness and wear resistance (Peutzfeldt, 1997, Xie et al., 2000) . The reason may be as a result of low level of cross linking that is associated with high levels of mono-methacrylates. In addition, it has been shown that the leaching of HEMA from such a material can lead to further toxicity. Designing fast polymerisation kinetics to overcome HEMA toxicity may lead to more, but shorter and quicker wearing polymer molecules. Moreover, the silane bond between the matrix and filler can be weakened due to the reaction of glass particles. A further limitation with RMGIC is water sorption because of hydrophilicity of poly (HEMA) which might increase the pressure on the tooth and possibly crack it (Smith, 1998).

1.3.3 Compomer

Polyacid-modified composite resins, known as compomers, are aesthetic dental materials that can be used to restore cavitated teeth (McLean et al., 1994). Compomers were developed to find a material that combines between the therapeutic effect of conventional glass ionomer and aesthetic properties of composite (Burke et al., 2006).

The components of compomers are similar to composite. It is mainly composed of macro-monomers such as bisglycidyl ether dimethacrylate (BisGMA) or urethane dimethacrylate (UDMA) and co monomers to control the viscosity such as triethylene glycol dimethacrylate (TEGDMA). This monomer is mixed with strontium or barium aluminosilicate glass particles (Eliades et al., 1998). Moreover, additional monomer that has acidic functional groups is added. TCP, di-ester of 2-hydroxyethyl methacrylate with butane tetra carboxylic acid, is commonly used. Water sorption enables this monomer to react with the glass to release fluoride providing an anticariogenic agent.

Several advantages of compomers have been stated, which include easy manipulation, good polishing, excellent aesthetics, radiopaque and less likely to get dehydrated. On the other hand, the main drawbacks of this material include requirement of a bonding agent to adhere to dental tissue, less wear resistance and more marginal leakage compared to composite. In addition, the mechanical properties of compomer exhibited low flexural strength and moduli after 24 hours in comparison with composite (Chung et al., 2004a, Janda et al., 2006, Piwowarczyk et al., 2002). While the mechanical properties of compomer meet the ISO 4049 criteria, they still have lower flexural strength than those of composites (Rodrigues Filho et al., 2006). This is a result of high water sorption and fluoride release. In addition, it has been stated that the compressive strength of the compomer (120-260 MPa) is also less than the composites (Gömeç et al., 2005, Xu and Burgess, 2003). So, it is more practical to use composite than compomer in the dental clinic.

1.3.4 Composite

1.3.4.1 Introduction

The word "composite" means mixture (Ferracane, 1995). In the science of biomaterials, a composite is a combination of two or more different types of materials such as metals, ceramics as well as polymers. In dentistry, dental composite is a tooth-coloured restorative material which is made of organic resin matrix, inorganic reinforcing fillers and silane coupling agent. Each one of these constituents plays an important role in the success of the final filling.
It is believed that dental composite is the most appropriate restorative material for anterior teeth. The several advantages of this white filing material make it more desirable than dental amalgam. These advantages include high aesthetic, as well as no toxicity due to mercury. Thus, replacement of amalgam with composite is highly recommended. In addition, composite can be utilized for several clinical purposes which include cavity liners, pit and fissure sealants, cores and build up, inlays, onlays, crowns, provisional restorations, orthodontic devices, cements for single or several prosthesis, endodontic sealers and root canal posts (Ferracane, 1995).

Recently, several attempts have been carried out to improve the formulations of dental composite and make them applicable for back teeth. The use of radiopaque glass fillers that can be cut down to very small particles sizes is one of the modifications that could generate favourable filling with enhanced polishability and wear resistance. The longevity of such composite has been improved in the posterior teeth and has been proved from long-term clinical applications (el-Mowafy et al., 1994, Taylor et al., 1994).

However, this dental filling material is still has some disadvantages that limit its frequent uses. They include polymerisation shrinkage, recurrent caries, technique sensitivity and cost. Thus, broad understanding of the composition and characteristics of each component as well as the different ways of changing them helps to improve the final outcome of such material. The

essential parts of dental composite and their roles in the final restoration will be discussed in the following review.

1.3.4.2 Composition of Dental Composite

The typical dental composite includes the resin matrix, usually dimethacrylates, reinforcing fillers that are made from glass and the silane coupling agent that is considered as the bridge which facilitates the joining of reinforcing fillers to the matrix (Ferracane 1995).

1.3.4.2.1 Resin Monomer

Chemical reaction between dimethacrylate resin monomers generates a stiff cross-linked polymer network surrounding the filler particles. The level of this reaction is known as the degree of cure. It is very crucial in determining the physical and mechanical properties of the dental composite. It has been stated that many factors affect the degree of cure. These include the polymerisation promoter and inhibitor levels (Yoshida and Greener, 1994), the chemical composition of the monomers (Ferracane and Greener, 1986) and the filler composition / shape and size (Kawaguchi et al., 1994).

The involvement of monomer in dental composites started around 25 years ago. The most common monomer applied in commercial dental products is Bis-GMA. This material is very viscous and needs to be mixed with further dimethacrylates, for example TEGDMA, UDMA or any other monomers (Peutzfeldt, 1997) (Figure 1.1). Urethane Dimethacrylates (UDMA) is used as a base monomer in many present dental composites and it has two main advantages in comparison with Bis-GMA; the lower viscosity and the higher flexibility due to urethane link lead to enhanced toughness (Glenn, 1982). Nevertheless, UDMA viscosity is still high and addition of a diluent monomer such as (TEGDMA) is beneficial (Fig 1.1).

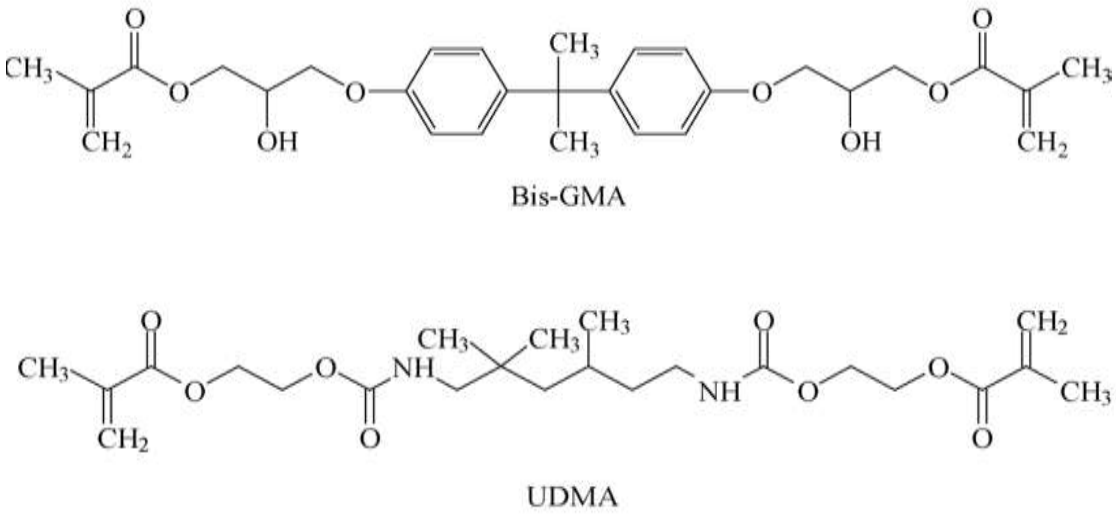

Figure 1.1: Chemical structure of Bis-GMA and UDMA

1.3.4.2.2 Diluent Monomer

1.3.4.2.2.1 Triethylene glycol dimethacrylate (TEGDMA)

Triethylene glycol dimethacrylate (TEGDMA) is a fluid resin that has lower viscosity and is commonly used as a diluent comonomer. The advantage of adding it in the mixture of the resin matrix for UDMA or Bis-GMA is to improve the handling property and allow more filler loading and better crosslinking between polymer chains (Ferracane, 1995). The molecular mass of TEGDMA is 286 Da and it is frequently used as a diluent monomer in different commercial composite (Figure 1.2).

1.3.4.2.2.2 Poly (propylene glycol) (425) dimethacrylate (PPGDMA)

Poly(propylene glycol) (425) dimethacrylate (PPGDMA) is another diluent monomer that has 660 Da molecular mass. This high molecular mass gives PPGDMA an advantage of lower shrinkage and less heat generation upon curing. Moreover, the concentration of double bonds in PPGDMA is one third that of TEGDMA. Because there is a proportional relationship between the concentration of double bonds and polymerisation shrinkage, lower shrinkage has been observed in a composite containing three parts UDMA to one part

PPGDMA in comparison with the same composite that has the same ratio of UDMA and TEGDMA. The small size of TEGDMA raises a concern regarding the capability of TEGDMA to leech out and penetrate the cells and disrupts their functions. In addition, several exposures to methacrylates, and small molecules such as TEGDMA that also penetrates gloves can lead to allergic reaction. Since the molecular mass of PPGDMA is higher than TEGDMA, it shows better biocompatibility. The complete conversion of PPGDMA to polymer decreases it leeching from the material and increases its longevity (Zimmerli et al., 2009, Leung et al., 2005, Moszner and Salz, 2007, Van Landuyt et al., 2007, Furuse et al., 2011).

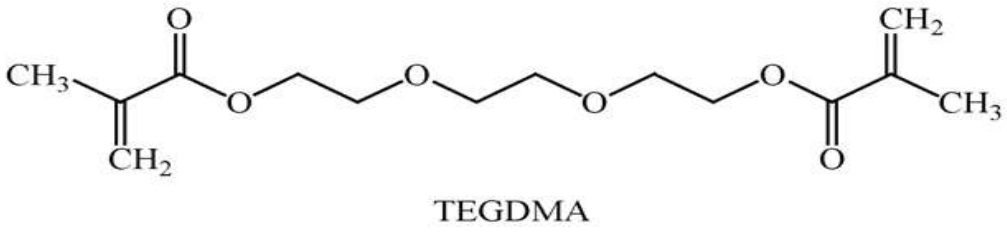

Fig1.2: Chemical Structure of TEGDMA

1.3.4.2.3 Photo Initiator System

Camphorquinone (CQ) is frequently used as a photoinitiator which is stabilized by a tertiary amine such as N, N-dimethyl-p-toluidine DMPT used as co-initiator (Stansbury, 2000) (Figure 1.3 and 1.4). It has been stated that free radicals are generated when CQ is exposed to 450-500 nm radiation. This process is accelerated by the reaction with tertiary amines (Cook, 1992). CQ, however, is yellowish in colour and might compromise the esthetics of restorative material. For this reason, multiple photoinitiators have been developed to improve this issue and provide more natural colour. These materials include PPD (1-phenyl-1,2- propanedione) (Park et al., 1999) Lucirin TPO (monoacylphosphine oxide) and Irgacure 819 (bisacylphosphine oxide) (Fig 1.3 and 1.4).

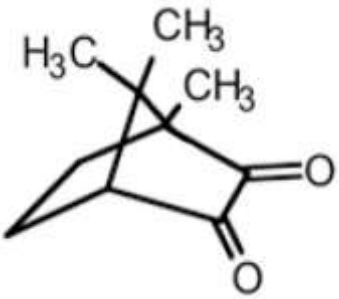

Figure1.3: Chemical Structure of CQ ($C_{10}H_{14}O_2$)

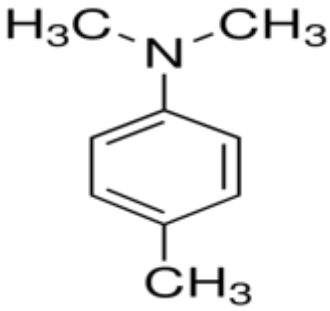

Figure1.4: Chemical Structure of DMPT ($C_9H_{13}N$)

1.3.4.2.4 Fillers

The significant roles of fillers include providing strength (Ferracane et al., 1987, Chung and Greener, 1990), enhancing stiffness (Braem et al., 1989, Kim et al., 1994), decreasing the polymerisation shrinkage, providing radiopacity (Cramer et al., 2011) and improving aesthetics (Ferracane, 1995). In general, the physical and mechanical properties of the dental composite are proportional to the amount of fillers added. The filler size or paste consistencies provide different composite classifications.

Several factors should be taken in the consideration when selecting the fillers. One of the most significant issues is the optical characteristic of the resultant composite. The refractive index of the monomer used is around 1.55. Utilizing fillers with highly different refractive index yields a very opaque composite that reduces the aesthetics. To optimize the aesthetics of composite material and maximise depth of cure, the difference between the refractive index of monomer and fillers should be minimized.

Originally, dental composites contained fused or crystalline quartz and borosilicate or lithium aluminosilicate glasses as fillers. These glasses or quartzes were crushed into different sizes ranging between 0.1 µm to 100µm (Rw, 1991). After that, they were added to the monomer at 70-80 wt% (55-65

vol %) to produce a good paste that has better stiffness and strength than the unfilled resins. In addition, quartz demonstrates favourable optical match to the polymer resin. One of the disadvantages of quartz and glass is the large size of the particles in comparison with the resin. This characteristic makes them raised when the polymer wears down and thus affects the surface of the restoration and brings about roughness which compromises the polishability and appearance (Ferracane, 2011).

Development of micro filled composites that contain amorphous silica helped to overcome this problem. The diameter of these silicon dioxide particles is ~ 0.04 µm. The larger surface area, however, that occurs with small particles increases the amount of monomer required to get good surface wetting and favourable viscosity. The lower filler content reduces both the strength and stiffness of the material. To overcome this problem the particles were combined with polymer and ground to produce a new product known as "small particle hybrid" composite. These composites are also named as "midifills". The size of filler particles is somewhat larger than 1 µm and in the same time has a part of the "microfillers" that measure 40-nm. These particles undergo more amelioration throughout milling and grinding procedures yielding composites with particles which measure about 0.4-1.0 µm that primarly named "minifills" (Bayne et al., 1994) and finally identified as "microhybrids". These products are used worldwide as a result of their compatibility for the anterior and posterior teeth as well as the favourable strength and polish-ability.

Recently, a new composite has been created and involving nanoscale particles which referred to as "nanofill". Most of the formulations of "microhybrids" have been altered and included additional nanoparticles, pre-polymerized resin fillers comparable to those of microfill composites, and ultimately termed "nanohybrids". Overall, differentiation between nanohybrids and microhybrids is not easy since they share some properties such as flexure strength and modulus, though the group of nanohybrids is in the inferior range of the microhybrids, however both are superior than microfills (Ilie and Hickel, 2009, Sarrett, 2010). Some evidence illustrated that the

involvement of pre-polymerized resin fillers could be the reason behind the slightly inferior characteristics of some nanohybrid composites.(Blackham et al., 2009).

With regards to the consistency of dental composites, filler distribution, amount of filler and type of monomer can have an effect on the ability of the material to flow. (Ferracane, 2011). These kinds of fillings can be applied either with a syringe or instrument, for instance flowable composite is produced to be dispensed through a very thin syringe to approach the tiny places for better adaptation, while the packable composite is fabricated to provide considerable resistance to the amalgam condenser as well as to give better mesial and distal contacts. In the flowable composite, the low viscosity is obtained by decreasing the amount of fillers or by involving some agents, such as surfactants, that has a role in the consistency of the material and to also evade the low quantity of the fillers which affect the mechanical properties and thus raise shrinkage (Bayne et al., 1998). However, the thick consistency of packable composite is not due to adding more filler but as a result of altering their size spreading or involvement of some particles such as fibers (Choi et al., 2000).

1.3.4.2.5 Silane Coupling Agent

When the composite was primarily produced, it was revealed that the reason behind the superior characteristics of filling material is the formation of a good bond between the inorganic fillers and the organic polymer matrix (Bowen, 1963b). These two materials can be joined together by coating the filler with an agent that has the ability to connect to both filler and matrix. This agent has silanol groups (Si-OH) that can make covalent bonds to silica fillers and methacrylate groups that bond to the resin matrix. Many studies have proven the significance of this agent in enhancing the mechanical properties of the restorative material (Nishiyama et al., 1991, Mohsen and Craig, 1995)a,b.

3-methacryloxypropyl trimethoxysilane (MPS) is frequently used in modern composites. Many trials were carried out with other coupling agents e.g alternative silanes, 4-META, titanates and zirconates) but they did not show as favourable results as MPS. For example, Mohsen and Craig in 1995 proved the ability of MPS to create more durable composites with quartz or zirconia silicate in comparison with 3-acryloxypropyl trimethoxysilane (APS).

1.4 Dental Adhesives

The adhesion between the tooth structure and dental composite is encouraged by dental adhesives. Several approaches can be used to apply these adhesives trying to create a strong and durable bond between the composites and both enamel and dentine. The essential mechanism of bonding to dental tissue is an exchange process that includes replacement of dental tissue minerals by resin monomers. This procedure includes two steps; firstly, calcium and phosphates are dissolved resulting in exposure of micro porosities in enamel and Dentine. Secondly, the resin penetrates into these created porosities (Van Meerbeek et al., 2003). Upon setting, this resin monomer is interlocked micromechanically in the created porosities (De Munck et al., 2005). Nakabayashi et al. in 1982 explained this interlock firstly and named it 'hybridization' or formation of a 'hybrid layer'. Adhesives can be categorized according to the underlying adhesion strategy in “etch & rinse,” “self-etch” and “(resin-modified) glass-ionomer adhesives.

1.4.1 Etch and Rinse adhesives

This technique is still considered the most successful way to obtain good and favourable bond to the enamel. Firstly, application of an acid (commonly 30-40% phosphoric acid) is carried out then rinsed off. This step is followed by a priming phase and then application of adhesive resin leading to a three-step application procedure. To reduce and simplify the clinical procedure, two steps are combined into one application; primer and adhesive (De Munck et al., 2005). Dissolution of hydroxyapatite crystals is accomplished through etching followed by in situ polymerisation of the bonding agent which is

absorbed by capillary attraction in the created etch pits, thus, enveloping individually exposed hydroxyapatite crystals (Van Meerbeek B, 2003).

1.4.2 Self-Etch Adhesive

Self-Etch Adhesive is another method of bonding in which no applications of separate acid etch is needed, however utilizing of non-rinse acidic monomer that condition and prime dentine at once. This technique has a useful clinical application by reducing the time and sensitivity of procedure and thus the risk of errors. Based on the pH, self-etch adhesives has been divided into two types; mild and strong (Van Meerbeek et al., 2001). The strong type of self-etch adhesives show pH less than 1 and this helps to obtain a favourable bond in dentine similar to the one that is formed by etch and rinse adhesives, whereas the mild self-etch adhesive has pH around 2 which leads to incomplete dissolving of dentine and therefore considerable amount of hydroxyapatite crystals stay in the hybrid layer. Generally, one-step self-etch adhesive composite shows the lowest bonding effectiveness compared to two-step and three-step adhesives (Van Landuyt et al., 2006). In addition, the sudden de-bonding stress can be prevented through micromechanical component while the hydrolytic breakdown is resisted via chemical interaction; hence, better marginal seal is obtained for long time (De Munck et al., 2005).

1.4.3 Glass Ionomer Adhesives

Glass Ionomer adhesive is the only restorative material that has the property of self-adherence to the tooth surface (Yoshida et al., 2000). In this restorative material, the tooth surface is cleaned by a short poly alkenoic acid followed by removing the smear layer that leads to exposure of collagen fibrils up to about 0.5-1 µm deep (Inoue et al., 2001); there, glass ionomer infiltrate and create a micromechanical bond following the idea of hybridization (Van Meerbeek B, 2003). Moreover, establishing chemical bond takes place through ionic interaction between the carboxyl groups of the polyalkenoic acid and calcium ions of hydroxyapatite which attach to the collagen fibrils (Yoshida et al.,

2000). These two bonding mechanisms are advantageous to increase the resistance to hydrolytic degradation.

1.5 Dental adhesives with antibacterial properties

The main limitation of current dental adhesives is the insufficient infiltration into the demineralised collagen network within the hybrid layer (Yuan et al., 2007) that will enable nanoleakage (Suppa et al., 2005). Consequently, passage of fluid and bacterial byproducts takes place and which leads to degradation of the resin adhesive (Wang and Spencer, 2003) and collagen fibrils (Pashley et al., 2004). Eventually, the bond strength will be weakened (Koshiro et al., 2004), enhance the microleakage and recurrent caries (Banerjee et al., 2001).

A second limitation of the adhesive systems is the inclusion of a smear layer that is a good shelter for bacteria. To solve this problem, a trial has been carried out to produce dentine bonding agent with antimicrobial property. By incorporation of particular materials, such as glutaraldehyde and acidic co-monomers, a dentine bonding agent with antibacterial property has been generated.

1.5.1 Glutaraldehyde

Gluteraldehyde (figure 1.5) is a fixative material that is commonly used in the medical and dental fields to fix tissues and to disinfect equipment (McDonnell and Russell, 1999).

The primary advantage of using Glutaraldehyde in the dental adhesive systems was to preserve the integrity of collagen fibrils within the hybrid layer. This was anticipated to enhance bond strength (Cilli et al., 2009) and decrease the risk of postoperative pain (Chermont et al., 2010). The highest amount of gluteraldehyde added to the bonding agent is 5 %.

Fig 1.5: Chemical structure of Glutaraldehyde (C5H8O2).

Elimination of bacteria from dentinal tubules has been observed in dental adhesives containing glutaraldehyde in one *in vivo* study (Yu et al., 2010). Moreover, a strong inhibitory effect on a several bacteria involving the cariogenic species has been proved when using cured dental adhesives that contained glutaraldehyde. These bacteria include *S. mutans*, *Streptoccocus sorbinus, L.casei* and *A. viscosus* (Cilli *et al*. 2009; Yu et al. 2010). The agar diffusion assay illustrated that the anti-bacterial effect took place as a result of releasing this glutaraldehyde. Although the evidence has shown the beneficial effects of using such a material, it has some toxic and mutagenic consequences which have given an important concern with regards to its application in the clinical field (Takigawa and Endo, 2006).

1.5.2 Acidic co-monomers

The primers of self-etching adhesive systems which contain phosphoric and carboxylic acid groups has been developed, mostly to aid in demineralization of dentine and assist the infiltration of resin with no an extra etching step (Tay and Pashley, 2001). Some evidence has revealed that the antibacterial activity was proven with un-cured self-etching primers (Elsaka and Elnaghy, 2012). Growth suppression of *S. mutans* and *A. viscosus* has been observed by self-etching primers when using agar diffusion assay (Cehreli et al., 2002, Kitasako et al., 2004).

It has been stated that 30 seconds contact with self-etching primers eliminated all the *S. mutans*. Furthermore, inhibition of *S. mutans* growth was also observed in both model cavity test and agar diffusion assay when applying self-etching primer solution. The reason behind this inhibitory effect was attributed to low pH values of un-cured self-etching primers (Kuramoto et al., 2005, Ye et al., 2007).

With regards to instant antimicrobial characteristics of un-cured self-etching adhesives, this could be due to eradication of remaining bacteria in the cavity. On the other hand, this property can be diminished significantly after light curing or buffering by dentinal fluid (Ye et al. 2007).

1.5.3 Antibiotics

Antibiotics have been introduced into dentine bonding agent. Addition of Vancomycine or metronidazole to the dentine adhesive system based on 4-META/MMA-TBB (4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane) has been tried (Kudou et al., 2000).

The suppression of six types of bacterial strains as well as the tensile bonding strength to dentine has been examined by adding vancomycine at 1, 2 and 5wt% and metronidazole at 1 wt%. It was reported that 1-5% of vancomycine demonstrated its ability to inhibit the growth of all tested streptococci and actinomyces in an agar diffusion assay. In contrast, only three bacterial strains have been inhibited when 1 wt% metronidazole was incorporated. Neither vancomycine nor metronidazole exhibited any significant effect on the tensile bond strength of the bonding agent. Since bacterial resistance can happen with the low release of antibiotics, adding antibiotics to the dental adhesives are not ideal method to produce bonding agent with antibacterial property (Kudou et al., 2000).

1.5.4 Fluoride

Various dentine adhesives with fluoride releasing property are available for use in clinical application (Han et al., 2002, Kameyama et al., 2002, Okuyama et al., 2006). Fluoride is diffused from these adhesives to the margins of the filling and hybrid layer. It has been mentioned that using these kinds of adhesives may enhance the tooth resistance to demineralization and hence diminishing the risk of developing secondary caries (Dubroc Jr et al., 1994, Xu and Burgess, 2003). However, other studies have shown that inhibition of secondary caries (Marya et al., 2010, Bapna et al., 1988)and maintain the bond strength to dentine is not achieved by fluoride releasing adhesives.

1.5.5 Chlorhexidine

The unprotected demineralized collagen fibrils are more prone to be degraded by enzymatic activity. This leads to deterioration of the bond between adhesives and dentine. It has been illustrated that the endogenous enzyme which is responsible for this collagenolytic activity is matrix metalloproteinases (MMPs) (Gendron et al., 1999). The antimicrobial agent, chlorhexidine, acts as an inhibitor of MMPs (Van Strijp et al., 2003)and therefore the integrity of the hybrid layer is maintained (Carrilho et al., 2007, Hebling et al., 2005). Consequently, this inhibitory effect of chlorhexidine on MMPs has promoted several studies on the chlorhexidine-containing dental adhesives (Carrilho et al., 2007, Hebling et al., 2005). In one *in vivo* study, it has been reported that bond strength had been preserved for up to 14 months when the dentine surface was treated with 2% chlorhexidine solution (Hebling et al. 2005).

In another in vitro study, the beneficial effect of chlorhexidine has been observed in preserving composite bond strength following 6 months storage in artificial saliva (Carrilho *et al*. 2007). Recently, the application of dental adhesives which contain either 0.2 or 2 wt% of chlorhexidine digluconate, exhibited less decline in bond strength after 6 months storage using similar in vivo conditions (Carrilho *et al*. 2007;(Stanislawczuk et al., 2009, Zhou et al., 2009).

The advantage of chlorhexidine in preserving the marginal integrity of dental composites is considered as an important characteristic that should promote more studies to produce restorative materials with antimicrobial property.

1.6 Anti-microbial Dental restorations

1.6.1 Composite

Due to the high percentages of recurrent caries following teeth restorations (Mjör, 1996, Wilson et al., 1997), there is a strong need to produce dental restorative materials with therapeutic effects. Numerous advantages can be obtained upon adding antimicrobial compounds to dental restorative

materials. They involve reduction of cariogenic bacteria in the saliva and oral cavity, inhibition of plaque accumulation around the filled cavities and consequently diminishing the risk of secondary caries. Antibiotic, for example, is one of the options that can have a therapeutic effect; however it is not highly recommended as it increases the risk of bacterial resistance. Consequently, other agents are suggested to be involved in the formulations of dental composites. They include chlorhexidine, triclosan, benzalkonium chloride (BAC), zinc oxide, fluoride, silver and Polylysine.

1.6.1.1 Chlorhexidine

Incorporation of antimicrobial compounds in dental restorative materials would provide several advantages to patients. These advantages involve reduction of micro-organisms in the saliva and oral cavity, inhibition of plaque formation around the filled cavities and consequently decreasing the risk of secondary caries.

Chlorhexidine is one of the most common antimicrobial dental materials that is widely used in dental practice. This agent has been introduced into dental restorative materials such as composites, GIC and adhesives and titanium implant coatings to impede the formation of dental plaque (Verraedt et al., 2011, Hu et al., 2013). The drug molecule works through chemical reaction between the negatively charged groups on the cell surface and the positively charged CHX. Consequently, losing of bacterial cytoplasmic components, damaging the membrane and inhibiting enzymes take place (Shen et al., 2011). At low doses, the antimicrobial drug has the ability to retard the metabolism of dental plaque via interfering with sugar transport, production of acids and membrane functions of streptococci (Marsh, 2010).

Hence, the concept of producing chlorhexidine releasing dental composite has arisen. Researchers confirmed the inhibitory effect of chlorhexidine against bacterial growth by agar-diffusion tests (Imazato, 2003). Unfortunately, the mechanical properties of the filling material and limited action of the drug were the two main drawbacks (Mehdawi et al., 2009).

1.6.1.2 Triclosan

Triclosan (2,4,4-trichloro-2-hydroxidiphenilethere) (figure 1.6) is another wide spectrum anti-microbial agent (Kolenbrander, 2000) that showed its effectiveness against bacterial growth through interruption of the enzymes activities (Imazato et al., 1995). It is commonly used in dentifrices, detergents and cosmetic products.

Since triclosan has been used within toothpastes as an antimicrobial agent, it introduced into commercial dental composites and its action against lactobacilli was evaluated (Badet and Thebaud, 2008). When adding 1 wt% of triclosan in the formulation of dental composite, a direct contact test exhibited significant inhibition of bacterial growth. In contrast, dental composite containing 0.3% triclosan did not suppress the activity of *lactobacilli*. This observation has been confirmed with two different tests; direct test and agar diffusion assay (Sehgal et al., 2007). The reason of that might be due to the releasing level of triclosan is below its minimum inhibitory concentrations as a result of limited solubility in water.

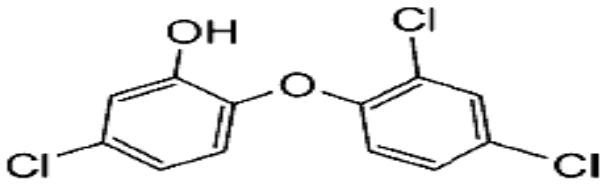

Figure (1.6) Triclosan chemical structure (C12H7Cl3O3)

1.6.1.3 Benzalkonium chloride

Benzalkonium chloride (BAC) is a wide spectrum quaternary ammonium antibacterial agent that has the ability to disrupt the bacterial cell membrane through attraction to the negatively charged membrane (Allmyr et al., 2006, Beyth et al., 2007) (Figure 1.7).

A trial was carried out to introduce this drug into the light cured dental composite. The effect of 0.25%, 0.75%, 1.25%, 1.75%, 2.5%, and 5% (wt) BAC was evaluated (Saito et al., 2007). It has been affirmed the effectiveness of BAC as anti-bacterial agent in suppressing the cariogenic bacteria; *S. mutans* and *S.sorbinus* , in an agar diffusion assay and direct contact test (Saito et al., 2007).

Moreover, it has been reported the role of quaternary ammonium-containing composite in providing anti-bacterial action. This action can be carried out through releasing cationic monomers that shows its effect against gtf gene, the gene that is responsible for increasing plaque accumulation. (Li et al., 2009). Therefore, the biofilm building enzymes are reduced, extracellular polysaccharide matrix (EPS) formation is diminished and bacterial attachment is inhibited. Interestingly, while the enhancement of anti microbial action is proportionally related to the percentage added, this material did not exhibit any effect on the mechanical properties (Othman, Wu et al. 2002).

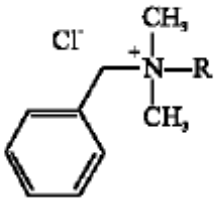

Figure 1.7: Chemical structure of benzalkonium chloride (BAC) ($C_{22}H_{40}ClN$)

1.6.1.4 Zinc Oxide

The beneficial effect of Zinc Oxide (ZnO) against microbes can be achieved by releasing both oxygen and zinc. The action of active oxygen is represented as H2O2 which is capable to inhibit the planktonic microbes (Aydin Sevinç and Hanley, 2010), while the Zn works through leaching into the growth medium and impeding the metabolism of sugars and disrupting enzyme systems of the plaque. Another function of Zn is it has the ability to decrease

the bacterial production of acids by inhibition the action of glycosyl transferase enzyme (He et al., 2002).

1.6.1.5 Fluoride - releasing dental composites

Flouride is a well-known anticariogenic element, which has the ability to remineralise the demineralized dental tissue. The mechanism of fluoride actions to impede bacterial metabolism involve inhibition of glycolytic enzyme enolase, bacterial colonization and competition. Additionally, it affects the intracellular or plaque associated enzymes such as acid phosphatase, pyrophosphatase, perox-idase and catalase (Hamilton, 1990).

Hence, development of different fluoride-releasing dental materials has been established to reduce the risk of dental caries. These materials involve glass ionomer cements, resin composites and toothpastes (Wang et al., 2014).

The amount of fluoride released from dental material plays an important role in determining the anti-cariogenic effect (Zimmerman et al., 1984, Donly and Gomez, 1994, Griffin et al., 1992). At the mean time, dental materials that have the ability to release fluoride are divided into four categories; glass ionomers, resin modified glass ionomers, compomers and composite resins (Xu et al., 2006). These categories illustrate differences in the fluoride releasing, mechanical and bonding properties. Xu and Burgess in 2003 have pointed out that low mechanical properties such as compressive strength are always associated with high fluoride release materials. On the other hand, materials which diffuse low amount of fluoride have high strength, good wear resistance and excellent aesthetics but low fluoride-recharge capabilities (Xu and Burgess, 2003).

Several trials have been carried out trying to produce composite filling materials that liberate fluoride either through passive leaching from selected fillers or through addition of fluoridated monomers (Burke et al., 2006). Three fluoridated resin composite materials have been analyzed through measuring the amount of Fluoride released into distilled water and the amount of fluoride which is uptaken by enamel and cementum. It was reported that the three

resins materials released fluoride in different percentages (Kawai et al., 1998). A sharp decrease in the amount liberated after 24 hours and then slowly reached a plateau. Both enamel and cementum absorbed the released fluoride but the amount was different among composite materials.

Another study compared the fluoride release from two resin composites (a commercial and experimental) with compomers, RMGIC and conventional GIC for more than one month. It was revealed that the least amount of F release was registered with composite resins and had ceased after two weeks (Yap et al., 1998). In addition, analysis of the fluoride release and uptake properties of a fluoride-releasing resin composite into distilled water over 33 days revealed that the discharged fluoride from this composite was less than conventional GIC by 54 and 14 times after 24 hours and one month, respectively. Therefore, no beneficial effect has been shown when adding fluoride into resin composite materials with regards to decreasing demineralization of carious lesions in root surfaces in comparison with GIC (Griffin et al. 1992).

Regarding the mechanical properties, addition of sodium fluoride (NaF) and strontium fluoride (SrF_2) to the resin phase of composite (Sales et al., 2003) resulted in porosities in the material and therefore reducing the mechanical characteristics (Rawls, 1986, Xu and Burgess, 2003, Arends and Van der Zee, 1990). In contrast, incorporation of, ytterbium trifluoride (YF_3) and fluoride - leaching glass fillers enhanced the mechanical properties regardless fluoride release (Boeckh et al., 2002, Itota et al., 2002). Incorporation of new fluoride releasing monomers such as dimethacrylate monomer and ternary zirconium fluoride chelate have been carried out, however it was very difficult to formulate dental composites which can combine sustained fluoride release and superior mechanical properties (Ling et al., 2009).

1.6.1.6 Silver - containing dental composites

Silver (Ag^{+})is one of the elements that show its effectiveness against bacteria (Ryu et al., 2011) and minimum toxicity to human cells (Park et al., 2009, Kang et al., 2009). The silver ions are capable of diffusing from dental materials into aqueous solution, which then adhere to the bacterial cell membrane and penetrate the biofilm. This penetration causes bacterial inactivation and retarding the microbes' replication through attachment to DNA and to the sulf hydryl group of the metabolic enzymes in the bacterial electron transport chain (Darouiche, 1999, Liao et al., 2010). In another way, it works through interfering with the enzymatic activities of strains (Jelinek et al., 2013, Liu et al., 2013). In the medical field, various usages of silver as antimicrobial agent have been mentioned. They include coating for surgical catheters, bone cements and wound dressings (Rupp et al., 2004, Alt et al., 2004).Therefore, attempts have been done to incorporate silver into the filler phase of dental composites trying to obtain this advantage against intraoral microbes. Moreover, the bacteriostatic action of dental composite containing silver was proved against three types of oral streptococci that are always separated from the surfaces of dental composites (Lansdown, 2002, Yamamoto et al., 1996).They involve Streptococcus oralis, Streptococcus sanguinis and Streptococcus mitio.

However, the main drawback of using silver as an antimicrobial agent in the resin materials is the discolouration. Silver zeolite, for instance, displayed instability in the colour following 24 hours in a storage solution contrary to 10% silver-apatite which exhibited much better result (Van der Burgt and Plasschaert, 1985). Silver-zirconium phosphate and siver silica gel are other compounds which have been introduced into dental composites. It has been proved the ability of 5 % silver-zirconium phosphate or 7 % silver-silica gel in suppressing the growth of S.mutans (Yamamoto et al., 1996).

1.6.1.7 Antibacterial prepolymerized resin fillers

The antimicrobial characteristic of a filler system that is composed of pre-polymerised resin fillers with immobilized methacryloyloxydodecylpyridinium bromide (MDPB) (PPRF) have been investigated (Imazato et al., 2003). This filler system is made of milled pre-polymerised methacrylate and antibacterial MDPB monomers (at 15.8 wt %) with glass silica particles. An inhibitory effect has been noticed on the growth of S.mutans after 18 hours contact with this filler. The investigators have stated that some of un-polymerised MDPB (~1 µg/ml) was eluted after 24 hours immersion in water however because this is approximately 16 times less than the MIC of MDPB against *S. mutans* , direct contact between particles and bacteria was the most likely mode of antibacterial action.

Furthermore, several studies formulated experimental composites with 17.9wt % of PPRF. These formulations suppressed the accumulation of S.mutans in vitro (Ebi et al., 2001). The reason behind this was interference with bacterial adhesion, glucan synthesis and bacterial growth. Fortunately, no elution of un-polymerised MDPB was reported. Additionally, this kind of filler did not reveal any roughness in the surface or hydrophobicity, which is known as causative factor that affects the bacterial adhesion.

1.6.1.8 Polylysine

1.6.1.8.1 Introduction

ε-Polylysine is a pale yellow powder that has a relatively bitter taste. . Epsilon (ε) stands for the linkage of lysine molecules. ε-Polylysine is a member of the cationic polymer group and the systemic name of it is (S)-poly (amino (2-amino-1-oxo-1, 6-hexanediyl). It is considered as basic polyamide which composed of 25-30 residues of L-lysine, one of essential amino acids. The peptide bond formed with α-carboxyl of L-lysine and ε-azyl from another L-lysine links this ε-Polylysine. . It is highly hygroscopic and has the ability to dissolve in water and hydrochloric acid but not in organic solvents such as alcohol and ether. The chemical formula of polylysine is shown in Figure 1.8.

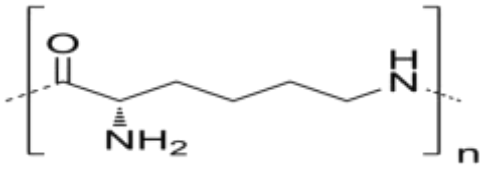

Figure 1.8: The chemical structure of polylysine

It has been stated that Streptomyces albulus ssp generates the ε-polylysine under aerobic conditions. This Gram-positive bacterium was isolated primarily from Japanese soil (Shima et al., 1984). A production of higher amount (four times) of polylysine was observed from a mutant of strains 346 which was later isolated (Kahar et al., 2001, Hamano et al., 2007).

Furthermore, it has been mentioned that the ε-polylysine solution has not been decomposed even when it was boiled at 100 °C for half an hour or autoclaved at 120 °C for 2 min (Kawai et al., 2003). This material has several properties and they include water solubility, odourless and have no effect on food flavour. The molecules of ε-polylysine are cationic and have an active surface which has a role in inhibition of the microorganism proliferation.

1.6.1.8.2 Applications of ε-polylysine

Polylysine has many properties such as water soluble, biodegradable, biocompatible toward humans and environmentally safe. Consequently, it has multiple uses in the industrial world. They include food preservatives, emulsifying agent, dietary agent, biodegradable fibers, highly water absorbable hydrogels, drug carriers, anticancer agent enhancer, and biochip coatings (Shih et al., 2006).

1.6.1.8.2.1 Polylysine as preservative

The use of ε-polylysine as an antimicrobial preservative in food is evident. It has been involved in several food types in Japan. They include sushi, boiled rice, soup stocks, sukiyaki, noodles and cooked vegetables. Around 10ppm to 5000ppm of ε-polylysine level is used in the food. It has been stated that the application of high amount of ε-polylysine, such as 20,000 ppm and 50,000

ppm, in the diet of rats did not cause any toxic effects in the animals. Consequently, ε-polylysine was safe and biocompatible (Fukutome et al., 1995).

Likewise, studies of the absorption, distribution as well as excretion of the ε-polylysine have pointed out the low absorption of this agent in gastrointestinal tract. Therefore, high percentage of ε-polylysine (approximately 94%) passes unabsorbed through the digestive system and excretes in the faeces.

It has been stated that when autography was used, the precipitation of ε-polylysine in the body organs was poor (Hiraki et al., 2003, Fukutome, M et al. 1995). The poor absorption of ε-polylysine in the body systems as well as the lack of harmful moiety in its polymer makes this material safe and biocompatible (Hamano, 2011).

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1.6.1.8.2.2 Polylysine as antimicrobial agent

Polylysine is stable in a broad range of pH and also can inhibit the growth of wide spectrum of micro-organisms such as yeast, fungi, Gram-positive and Gram-negative bacteria (Kito et al., 2002, Yoshida and Nagasawa, 2003). The mechanism of antimicrobial activity arises in part due to its ability to be adsorbed onto the cellular membrane electrostatically and therefore cutting the membrane off and disturbing the distribution of the cytoplasm (Shima et al., 1984).

In addition, ε-polylysine is effective against streptococcus mutans and aerobic oral microflora. In one study a proportional relationship was found between the amount of ε-polylysine utilized and the reduction rate of microbial counts (Najjar et al., 2009). Furthermore, immobilization of ε-polylysine into polyethylene terephthalate fabrics had an advantageous effect against both Gram positive and Gram negative bacteria (Lin et al., 2011).

1.6.1.8.2.3 Polylysine as lipase inhibitor

ε-Polylysine and taurocholate produce a surface-active complex that has the capability to bind to emulsion particles that can inhibit lipase adsorption and triacylglycerol hydrolysis in both *in vivo* and *in vitro*. This inhibition enhances with the degree of ε-Polylysine polymerisation. Hence, the action of ε-Polylysine is represented as anti-obesity agent (Tsujita et al., 2006, Tsujita et al., 2007).

1.6.2 Fissure Sealant

A number of materials have been investigated for the sealing of pits and fissures involving amalgam, GIC, however the current materials are most commonly based on the dental composite. The setting of these materials takes place through polymerisation and they are activated either chemically or by using light. Other components might have several materials for different purposes. They include diluent monomers to control the viscosity of bulk monomer, opaquers such as TiO_3 to allow monitoring of the sealant after its placement and finally fluoride-releasing agents such as YbF2 (Burke et al., 2006).

The amount of fluoride release from fluoride releasing pit and fissure sealants have been evaluated for thirty days in five commercial materials. The first day showed the most fluoride release in all types followed by the second day when the release decreased sharply. After that, a slow decrease in the diffusion has been recorded for the remainder of the trial period (Garcia-Godoy et al., 1996).

Another study compared the amount of fluoride release from light cure composite resin-based pit and fissure sealant and GIC based fissure sealant. The authors concluded that the first material diffused twice the quantity of fluoride for more than nine days and illustrated a small continual release over six months (Rock et al., 1996).

A significant release of fluoride for over six months has been exhibited from composite sealant compared to light cured resin composite adhesive agents. The primary fluoride release was approximately 10ppm per week into deionised water and this diffusion continued for up to six months although the progressively declining levels (Burke et al., 2006).

1.7 Current problems with dental composites

Dental composite materials still display some disadvantages. They are wear resistance, surface roughness, proximal contact adhesion, marginal adaptation, polymerisation shrinkage (Inoue et al., 2003, Kidd and Beighton, 1996, Peutzfeldt and Asmussen, 1996) and that they lack the ability to remineralise the tooth structure and to release the antibacterial agent and hence killing cariogenic bacteria. The excessive wear that leads to the loss of composite filling can be determined beneath enamel margins or can be found at proximal contact in class II restorations which cause open proximal contacts (Piwowarczyk et al., 2002, da Rosa Rodolpho et al., 2006). This can result from several factors which involve polymer or filler composite, filler size, and filler-polymer matrix binding quality, especially in early resin composite systems (Kusy and Leinfelder, 1977).

Polymerisation shrinkage (Bowen, 1963b) is an intrinsic characteristic of resin restorative materials . During polymerisation, denser structure is formed due to the formation of a polymer network which produces alterations in the volume contraction. This process leads to shrinkage and then enhancement of bacterial by-products to penetrate the dental tissue. So, micro-leakage is defined as "the passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it " (Kidd, 1976).

Development of several laboratory methods has been initiated trying to understand and analyze the marginal leakage at the tooth restoration interface. These techniques involve the use of dyes, radioactive isotopes, air pressure, bacteria and neutron activation analysis as well as artificial caries methods. The observations of these researches concluded that the margins

of fillings are not stable and have no impassable borders, but 'dynamic micro-crevices' that contain lots of ions and molecules.

When the covalent bond is formed during monomer reaction, a reduction in the distance between the two groups of atoms takes place and causes decreasing in free volume, which leads to volumetric shrinkage. The filler volume fraction and the composition as well as the degree of conversion of the resin matrix of composite influence the amount of this shrinkage. The shrinkage values for both BisGMA and TEGDMA have been estimated and they were 5.2% and 12.5%, respectively whereas 2-3% was registered with typical composite. The reason behind this difference is that around 60% of the hybrid composite is occupied by filler particles. Microfilled composite has the same shrinkage as hybrid one because of the presence of pre-polymerized composite particles. Furthermore, all the commercial composites lack the antibacterial and remineralising properties that can help in reducing secondary caries.

1.8 Remineralising composites

Dental caries occurs when there is imbalance between the remineralisation and demineralization with general increase in the latter. Therefore, finding other sources to produce calcium and phosphate in the oral cavity might play a role in enhancing the process of tooth remineralisation.

1.8.1 Calcium phosphates

Calcium phosphates involve different salts of tribasic phosphoric acid (H_3PO_4). Formation of ions such as $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-} can be performed through removal of H^+ ions from this acid. Both skeletal and dental tissues can form these ions naturally which make them of special interest to clinicians and biomedical scientists. The advantages of such compounds include biocompatibility and osteoconductive materials (Goto et al., 2006)and have been applied widely as bone substitutes and as carriers for drug delivery (Ginebra et al., 2006).

Neutral or basic solutions have the ability to dissolve various calcium phosphate compounds which then precipitate as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$. This hydroxyapatite is known to have similar characteristics to apatite present in bone and teeth. One of the crucial properties that should be taken into consideration is the solubility of calcium phosphate phases into the aqueous solution. This property is mainly associated with the calcium (Ca)/phosphorous (P) ratio. As a general rule, the solubility decreases with high Ca/P ratio. At physiological pH, the solubility of calcium phosphate compounds for instance diminishes in the order MCPM > DCPD = DCPA > OCP > β-TCP > HA. (Table 1.1).

Name	Abbreviation	Formulae	Ca/P ratio
Monocalcium phosphate monohydrate	MCPM	$Ca(H_2PO_4)_2.H_2O$	0.5
Dicalcium phosphate anhydrate (monetite)	DCPA	$CaHPO_4$	1.0
Dicalcium phosphate dihydrate (brushite)	DCPD	$CaHPO_4.2H_2O$	1.0
Octacalcium phosphate	OCP	$Ca_8H_2(PO_4)_6.5H_2O$	1.33
β-Tricalcium phosphate	β-TCP	$Ca_3(PO_4)_2$	1.5
Amorphous calcium phosphate	ACP	$Ca_3(PO_4)_2.nH_2O$	1.5
α-Tricalcium phosphate	α-TCP	α-$Ca_3(PO_4)_2$	1.5
Hydroxyapatite	HA	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Tetracalcium phosphate	TetCP	$Ca_4(PO_4)_2O$	2.0

Table 1.1: The main Calcium phosphates arranged according to Calcium (Ca) and phosphorus (P) ratio (Bohner et al., 2006, Mehdawi and Young, 2014)

1.8.2 Amorphous calcium phosphate

Utilizing of amorphous calcium phosphate has been documented in many biomaterials (Combes and Rey, 2010) such as coatings or ceramics and in cements and composites. The calcium and phosphate ions can be released

from the ACP composites, especially in the low pH environment (Llena et al., 2009). In vitro studies of dental composites proved the release of these ions and their role in promoting remineralization of dental tissue. However, incorporation of ACP affected the biaxial flexural strength and reduces it in comparison with conventional dental composite (Lee et al., 2007).

1.8.3 MCPM and TCP filled composite

Involvement of Mono calcium phosphate monohydrate (MCPM) and tri calcium phosphate (TCP) was achieved in different dental composites (Mehdawi et al., 2009). When blended with water, reaction through hydrogen ion exchange takes place and this leads to precipitation of brushite (di calcium phosphate di hydrate, CaHPO4·2H2O) or the anhydrous form, monetite (Hofmann et al., 2006). In the experiment of Mehdawi et al, they confirmed the release of chlorhexidine and calcium phosphate ions. This observation was due to addition of water-soluble MCPM fillers which played a significant role in encouraging the water sorption into the composite. It has been stated that this absorbed water facilitate the chemical reaction between MCPM and β-TCP and consequently brushite formed into the polymerized methacrylate. However, this study also proved the low strength of that composite.

1.8.4 Strontium (Sr^{+2})

Strontium was primarily identified by Humphry Davy using electrolysis in 1809 and was called Strontiate, a town in Scotland. It is a soft and silvery metal found naturally as nonreactive element. Strontium oxide is immediately formed upon strontium cutting lead to changing its colour to yellow. It has the ability to react with water producing strontium hydroxide and hydrogen gas. Strontium in its elemental form found naturally in rocks, soil, water and air and food such as corn (0.4 ppm), orange (0.5 ppm) and cabbage (45 ppm). In the periodic table, Strontium is element number 38 and has two positive charges in its ionic form (similar characteristics to Ca). The physical and chemical properties of Sr^{+2} are comparable to Calcium and barium. As a result of the

similarity between Sr and Ca, it is believed that Sr can replace the Ca in hydroxyapatite (Thuy et al., 2008) and has some effects on the human bone.

1.8.4.1 Application

In United States and Europe, strontium has been applied as a medical substance and therapeutic agents for decades. Some of its applications are summarized in the following;

1.8.4.1.1 Strontium as remineralising agent

For more than twenty years, researches have been focused on some elements, other than fluoride, that can prevent dental caries. Sr is one of the elements showed a relation in lowering caries process in both humans and animals' studies, which then attracted the researchers to investigate. In United States, Navy Dental Service conducted a 10- year study among 270,000 naval recruits. All of them had their teeth examined and the result revealed that caries free teeth were found in 360 individuals. Further examination clarified that 10% of those had lived in a small geographic region around Rossburg, Ohio where the water supply contain high concentrations of strontium (Curzon and Spector, 1981). Another studies supported the role of Sr in remineralisation and reducing dental caries (Lippert, 2012, Koletsi-Kounari et al., 2012).

In addition, both fluoride and strontium encourage the remineralisation process of dental tissue though the function of fluoride does not rely on the presence of strontium and vice versa. It has been reported the benefits of combination fluoride and strontium in improving the apatite crystallinity and reducing the acid reactivity of synthetic carbonated apatites (Featherstone et al., 1983). Presence of fluoride with calcium and phosphate helps the calcium ions to be adsorbed to the tooth surface and thus remineralise it (Featherstone, 1999). So, this study aims to formulate new dental composites replacing the calcium phosphate with strontium phosphate and confirm if it has an effect on the remineralisation process.

1.8.4.1.2 Osteoporosis

Osteoporosis is a medical term used to describe fragile and brittle bones which are more prone to fracture. This medical problem is a worldwide concern in the aging society. This happens due to imbalance between bone deposition and bone resorption during bone remodelling process. To treat such a condition, researchers have found that supplementation of some minerals such as strontium, calcium, magnesium and zinc showed favourable improvement in bone condition (Grynpas and Marie, 1990, Matsunaga and Murata, 2009, Buehler et al., 2001, Marie et al., 2001, Dahl et al., 2001).

The effect of strontium in reducing bone resorption and enhancing bone formation is dose dependant. Low doses of strontium stimulate osteoblast to form bone, while high doses cause improper remineralisation. Consequently, the physical and chemical characteristics of apatite minerals are affected too (Matsunaga and Murata 2009).

Furthermore, it has been stated that the growth and dissolution of hydroxyapatite (HA) in vitro are influenced by absorption of Sr^{+2} (Christoffersen et al., 1997); so it is essential to investigate the distribution of this element in the apatite minerals at the atomic level.

The mechanism of involving Sr^{+2} into apatite minerals occurs through ion replacement with Ca^{+2} in hydroxyapatite [$Ca_{10}(PO4)_6(OH)_2$ (HAp)]. This HA is the major inorganic constituents of bones. On the other hand, it has been demonstrated that the solubility of Sr^{+2} into HA is very little and considered less than one Ca^{2+} in 10 Ca^{2+} ions of the HA unit cell is replaced by Sr^{+2} (Boivin et al., 1996). Moreover, by assessing the surface of HA crystals, it was observed that the adsorption of Sr^{+2} occupied around 25% of Ca^{+2} sites (Christoffersen et al., 1997).

Strontium ranelate is one of the strontium compounds that reveal its efficiency in treating postmenopausal osteoporosis. One of the studies involved 353 women who suffered osteoporosis history of vertebral fracture as well as low scores of bone density in the lumber area. Strontium with different doses

(170, 340 or 680 mg/day) was administered into one group for two years while other group did not receive anything (placebo). The results were promising in which a significant alteration in bone metabolism and reduction in vertebral fractures were reported among the group who received 680 mg/day of strontium for two years (Meunier et al., 2002).

This result was a point of interest to many researchers to conduct a larger trial to prove the effect of Strontium ranelate in the enhancement of bone degenerations. In 2009, 1,649 postmenopausal osteoporotic women were randomized to either strontium ranelate or a placebo for 4 years. The results supported the previous study and proved the beneficial effect of using Strontium ranelate in treating osteoporotic postmenopausal women (Meunier et al., 2009).

1.8.4.1.3 Arthritis

It has been reported that strontium may improve cartilage formation. One trial that proved this observation was conducted in the University Hospital Bone and Cartilage Metabolism Research Unit, Liege in Belgium. Researchers tried to prove the hypothesis that says strontium may enhance the metabolism of cartilage in patients who are diagnosed with osteoarthritis (OA). In this study, cartilaginous cells were added to strontium and cultured for two to three days. They found that strontium stimulated the production of proteoglycan (PG) which has a role in promoting cartilage growth (Henrotin et al., 2001).

1.9 Statement of the Problem

Although dental composites have several advantages, a major drawback of this filling material is higher failure rates due to recurrent caries. This occurs due to the polymerisation shrinkage which leads to micro-gaps which with lack of material antibacterial action enables bacterial micro-leakage. The problem is to combat shrinkage, and enhance the antibacterial properties of the dental composite without affecting mechanical properties. Multiple attempts have been carried out to develop antibacterial and remineralising formulae. Examples include composites with chlorhexidine and reactive calcium phosphates but usually with the latter in particular there has been an associated large decrease in strength. This study addresses if antibacterial

and remineralising Polylysine and strontium phosphates can be added instead without as much loss of strength.

1.10 Aims and objectives

1.10.1 Aim

To compare the effect of replacing calcium phosphate with strontium phosphate on the formation of hydroxyapatite layer on the surface of composites and to evaluate the release of two different antimicrobial agents
The following objectives are required to accomplish the aim;

- Generation of composite with high glass filler to provide good strength.
- Assessment of the monomer conversion in all experimental formulations and compare them to a commercial composite. This will be achieved by FTIR.
- Involvement of MCPM and TCP or TSrP to promote the water sorption in set material and consequently to release calcium and phosphate ions for remineralisation purposes. This experiment will be assessed by using density kit.
- Addition of Chlorhexidine diacetate and Polylysine to act as antimicrobial agents to prevent recurrent caries by inhibition of bacterial micro-leakage. UV Spectroscopy will be utilized to assess the release of these two drugs.
- Precipitation of apatite on the composite surfaces will be assessed by Raman Spectroscopy, SEM and EDX.
- Replacement of TCP with TSrP and comparison of the remineralising effect of it with the calcium.
- Assessment of the strength of different formulations, to and detect if the added fillers influence this property. Instron machine will be utilized to evaluate this property.

Chapter Two

Material and Method

2 Materials and Methods

In this research, four different composites that contained antimicrobial and remineralising agents were prepared and tested against a commercial composite. Each formula was made up of monomer phase and filler phase.

2.1 Commercial Material (Z250)

Name	Supplier	Product Code	Filler content (wt %)	Filler Size (µm)	Shade	Components		Description
						Monomers/Additives	Filler	
Z250	3M	202458	78	0.01-3.5	B3	Bis-GMA UDMA Bis-EMA TEGDMA	Zirconia Silica	Pre-mixed syringe- light cure

Table 2.1: Details of commercial material used

2.2 Experimental Composites

2.2.1 Experimental control Composite without Reactive Fillers (Glass)

Resin Components		Filler Component
UDMA	**68%**	100 % Glass Particles
TEGDMA	**25%**	
HEMA	**5%**	
CQ	**1%**	
DMPT	**1%**	

Table 2.2: Components of the experimental control composite

2.2.2 Experimental Composite with Reactive Fillers

2.2.2.1 The Resin Phase

The resin matrix content was similar in all formulae .It was composed of 68% UDMA, 25% TEGDMA, 5% HEMA, 1% CQ, 1% DMPT as shown in table 2.3.All these materials have been discussed in the introduction chapter. The source of these materials is listed in table 2.4.

Contents of Resin Phase	
Composition	**Percentages**
UDMA	68 %
TEGDMA	25 %
HEMA	5 %
CQ	1 %
DMPT	1 %

Table 2.3: the components of resin phase in all dental formulations.

Name	Abbreviation	Chemical Formulae	Supplier and Product Code	Function
Urethane dimethacrylate	UDMA	$C_{23}H_{38}N_2O_8$	DMG, Germany 84118	Helps in the chemical reaction which generate a stiff cross-linked polymer network surrounding the particles of fillers
Tri ethylene glycol dimethacrylate	TEGDMA	$C_{14}H_{22}O_6$	DMG,Germany 80665	diluent monomer used to control the viscosity of UDMA and then improve handling
2-Hydroxyethyl methacrylate	HEMA	$CH_2=C(CH_3)COOCH_2CH_2OH$	DMG,Germany 768867	Monomethacrylate improves conversion. Low viscosity improves handling
N, N-Dimethyl-p-toluidine	DMPT	$C_{16}H_{20}NO_5Na$	Sigma Aldrich, UK15205BH	Polymerisation (Amine) accelerator
Camphorquinone	CQ	$C_{10}H_{14}O_2$	Alfa Aasar 10120023	Photo- initiator

Table 2.4: Summary of liquid content of the experimental composite and their sources.

2.2.2.2 The Filler Phase

The filler phase mainly consisted of glass in addition to antimicrobial agents, and remineralising materials. The percentages of each component were different in each formulae but the powder to liquid ratio was fixed at 3:1. The source of these materials is listed in table 2.5. Table (2.6) and (2.7) show the contents of powder phase and formulae used.

Name	Abbreviation	Supplier	Particle size	Function
DMG Glass Norm.Sil	DMG 7µm	DMG,Germany	D_{50}= ~ 7µm	Increase the strength and decrease the shrinkage
Monocalcium phosphate monohydrate	MCPM	Himed	fine	Remineralising agents
β-tricalcium phosphate	β-TCP	Plasma Biotal	5.78$^{\mu}$	
Chlorhexidine Diacetate	CHX Diacetate	Sigma-Aldrich, UK		Antibacterial agent
Polylysine		Handary S.A		

Table2.5: Summary of the sources for the material used in the filler phase.

Composition		Formula 1 (F1)	Formula 2 (F2)
Filler Phase	Glass	60 %	68 %
	MCPM	10 %	10 %
	TCP	10 %	10 %
	CHX	10 %	10 %
	PLS	10 %	2 %

Table 2.6: The content of the powder for formula 1 (F1) and formula 2 (F2).

Composition		Formula 3 (F3)	Formula 4 (F4)
Filler Phase	Glass	60 %	68 %
	MCPM	10 %	10 %
	TSrP	10 %	10 %
	CHX	10 %	10 %
	PLS	10 %	2 %

Table 2.7: The content of the powder for formula 3 (F3) and formula 4 (F4).

2.3 Sample preparation:

All components were weighed using a four-figure analytical balance in accordance with percentages shown above. With regards to the liquid (monomer phase), the constituents were put in a brown glass bottle and mixed using a magnetic stirrer bar and left stirring at room temperature till they became homogenous and no CQ particles were seen. Subsequently, the monomer was kept in the fridge for up to 1 month. The experimental specimens were made by dispersing the fillers of each formula into the matrix resin using an automatic mixer (Speed Mixer, Manufacturer; Hauschild Engineering) at 3600 rpm for 1 minute. The obtained homogenous composite pastes were loaded into split brass rings and covered with acetate sheets to get smooth surfaces. Then, all the samples were exposed to light cure (460-480 nm) for 40 sec from each side and the excess material was removed. Forty circular samples were obtained (10 for each formula). Tests included mass change, volume change, CHX release and PLS release.

2.4 Simulated Body Fluid Preparation (SBF)

Simulated Body Fluid was prepared following a concise version of British standard ISO (2012). This solution can be prepared by adding the following components (Table 2.8) to 700 ml water. The addition of these chemicals must be in order.

Components	Chemical formulae	Quantity
sodium chloride	NaCl	8.035 g
sodium hydrogen carbonate	$NaHCO_3$	0.355 g
potassium chloride	KCl	0.225 g
di-potassium hydrogen phosphate trihydrate	$K_2HPO_4 \cdot 3H_2O$	0.231 g
magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	0.311 g
1 M hydrochloric acid solution	HCl	39 mL
calcium chloride dehydrate	$CaCl_2 \cdot 2H_2O$	0.371 g
sodium sulfate	Na_2SO_4	0.072 g
tris-hydroxymethyl aminomethane (TRIS)	$NH_2C(CH_2OH)_3$	6.118 g

Table 2.8: components of the SBF solution.

The pH of the solution was maintained between 7.42 and 7.45 at 36.5ºC ± 0.2. This solution was then kept in the fridge.

2.1 Fourier Transform Infrared (FTIR) spectroscopy

2.1.1 Introduction

Spectroscopy means “the study of interaction of electromagnetic radiation with matter” (Bumbrah and Sharma, 2015). This spectroscopic method can be relied on the light that is emitted, scattered or emitted. There are several spectroscopic methods that can be applied to characterize a variety of samples. The qualitative technique is carried out to determine the identity of sample whereas the concentration of analyte can be estimated though quantitative analysis. Ultraviolet spectroscopy is an example of qualitative technique. Infrared Spectroscopy provides confirmatory information about the samples (Settle, 1997).

FTIR has been chosen to measure the amount of monomer conversion of the materials. IR spectroscopy is one of the most reliable techniques that have the ability to recognize the fingerprinting of the substances. There are several substances that can be identified and quantified by this machine. They include liquids, gases and solids. In addition, the functional groups of a variety of materials can be detected by the FTIR such as C=O, C-H and N-H (Azom.com, 2015).

2.1.2 Principle of Attenuated Total Reflection (ATR)

ATR is capable to measure the changes in a totally reflected infrared beam when the beam hits the specimen (Anon, 2015). The IR radiation can target the crystal with high refractive index at a specific angle. As a result of this internal reflectance, an evanescent wave is created. This wave protrudes only few microns (0.5 µ - 5 µ) beyond the crystal into the specimen. Thereafter, a good contact between the sample and the crystal is essential. When the sample absorbs the energy, the evanescent wave will be altered. The altered energy from every wave is passed back to the IR radiation which thereafter

exits through the other end of the crystal to the detector. The system then produces an infra-red spectrum (Anon, 2015).

2.2 Test Method

Setting reaction and kinetics of the composites was assessed using a Perkin Elmer Series 2000 Fourier Transform Infra-Red (FTIR) spectrometer (Beaconsfield, UK). The background of the instrument was scanned first using an Attenuated total reflectance (ATR) diamond attachment without any sample. This was done to ensure that the ratio of light intensity through the instrument either with or without the sample could be calculated by the computer.

The temperature was set at 37ºC to simulate normal human body temperature. The top surface of the specimen was covered with acetate sheet to prevent oxygen inhibition of the polymerisation. Spectra of the sample in contact with the diamond were obtained with resolution set at 4 cm^{-1} and wave number range between 600 and 2000 cm^{-1}. Number of scans was fixed at 8 and the total run time was 15 minutes for all formulations. After 1 min from start of spectral collection, samples were light cured for 40 sec using a L.E. Demetron I curing unit (Kerr, USA). The degree of monomer conversion was quantified through change in the height of the absorbance of the monomer peak at 1320 cm^{-1}. The degree of monomer conversion was calculated using the following equation

$$\% \text{ degree of conversion } = \frac{100[A_0 - A_t]}{A_o}$$

Where A_0 and A_t are the peak height of the C–O bond stretch peak at 1320 cm^{-1} before and after polymerisation respectively.

2.3 Mass Change and Volume Change

In this research, 10 samples for each formula were examined to investigate the mass changes as a result of the precipitation of hydroxyapatite on the surface of the samples or due to water sorption or mass loss. Five samples were immersed in 10 ml de-ionized water in sterilin tubes while the other five were soaked in simulated body fluid (SBF). The sterilin tubes were kept in an incubator at 37˚C for 2, 4, 6, 24, 48, 72 hours, 1, 2, 3, 4, 6 weeks and three months. At each time interval, the samples were removed, dried and weighed in air and water using a density kit and then the samples were put in a new tube with fresh storage media. The density, volume and mass change of each sample were calculated using the following equations;

$$\rho = \frac{A}{A-B}(\rho_o - \rho_l) + \rho_l \qquad \text{Equation 1}$$

$$V = \alpha \frac{A-B}{\rho_o - \rho_l} \qquad \text{Equation 2}$$

$$\Delta M = \frac{A_1 - A_o}{A_o} \times 100 \qquad \text{Equation 3}$$

Where

ρ= density of the sample

A= weight of the sample in air

B= weight of the sample in water

V= volume of the sample

ρ_o= density of water

ρ_l = density of air (0.0012 g/cm^3)

α = weight correction factor (0.99985).

A_1= new mass at each time point

A_0= original mass

2.4 Ultraviolet-visible spectroscopy (UV):

2.4.1 CHX Release Test

In order to calculate the percentage of CHX release, the storage medium (H2O) from the mass change experiments was examined using a Unicam Ultraviolet-visible (UV) 500 spectrometer (Thermo Spectrotonic, UK) at all-time intervals mentioned above. To obtain a calibration curve, CHX solutions of different ppms (20, 10, 5, 2.5 and 1.25) were made. 1 cm length quartz cells were used with distilled water as reference. Spectra were recorded between 200-400 nm. Peak absorbance of this antimicrobial drug was noticed at 231 and 255 nm. Following this a calibration curve for CHX was obtained by plotting CHX concentrations against the absorbance.

Five samples from each formulation were prepared and soaked in deionized water (10 ml at 37°C). Then each composite disc was removed and placed in a fresh medium at 2, 4, 6, 24, 47, 72 hours and 1, 2, 3, 4, 6 and 12 weeks. Samples were stored at 37°C before CHX quantification. A maximum absorption for CHX was registered at 255 nm, and then curves for CHX were drawn by plotting the percentages of release vs. square root (SQRT) of time.

The amount of CHX released (D, in grams) from the specimen was calculated using equation 4. Cumulative mass of CHX release in gram (C_D) at time “t” was calculated using Equation . The percentage release (P (%)) at time “t” was calculated using Equation .

$$D = \left(\frac{A}{k}\right) \times V_s$$

Equation 4.

$$C_D = \sum_{0}^{t} D_t$$

Equation 5

$$\mathrm{P} = \frac{C_D}{C_M} \times 100\%$$

Equation 6

Where

A = absorbance due to CHX at 255 nm,

k = gradient of the calibration curve for CHX.

V_S = storage solution volume (ml).

C_M = original CHX mass in specimen.

2.4.2 ε-Polylysine Release Test Method

For ε-polylysine release studies, the absorbance of storage solution (H2O) at different time points from mass experiment after reaction with Trypan Blue was obtained using the Unicam Ultraviolet-visible (UV) 500 spectrometer (Thermo Spectrotonic, UK) for a period of up to 3 months. Different techniques were previously used to determine the release of this material (Grotzky et al., 2010). In the current experiment, Trypan blue assay (TB assay) method was carried out.

The following steps were followed to produce the TB solution (also known as working reagent solution). First of all, 8000 ppm of TB solution was prepared by dissolving 0.080 g of TB in 10 ml distilled water. The solution then was diluted further by mixing 1 ml of 8000 ppm solution with 99 ml of distilled water to produce 80 ppm of TB solution which can be used up for 6 months.

To generate a calibration curve, standard PLS solutions were prepared in distilled water. ε-polylysine solutions of 2, 4, 8, 10, 14, 18 and 20 ppm were made using the following dilutions;

i) 10,000 ppm solution: 0.1000 g of PLS mixed with 10 ml distilled water

ii) 100 ppm solution: 1 ml of 10,000 ppm mixed with 99 ml distilled water

iii) 20 ppm solution: 10 ml of 100 ppm mixed with 40 ml of distilled water

iv) 18 ppm solution: 18 ml of 20 ppm mixed with 2 ml of distilled water

v) 14 ppm solution: 14 ml of 20 ppm mixed with 6 ml of distilled water

vi) 10 ppm solution: 1 ml of 100 ppm mixed with 9 ml of distilled water

vii) 8 ppm solution: 8 ml of 10 ppm mixed with 2 ml of distilled water

viii) 4 ppm solution: 4 ml of 10 ppm mixed with 6 ml of distilled water

ix) 2 ppm solution: 2 ml of 10 ppm mixed with 8 ml of distilled water

The same amount of prepared TB solution and the above PLS solutions were mixed to make PLS standard solutions of 1, 2, 4, 5, 7, 9 and 10 ppm. The mixture was kept in the incubator for 1 hour at 37^{o}C and then left to cool at room temperature for about 4 hours. All the mixture solutions were then centrifuged at 13,000 rpm for 20 minutes to remove any TB dye that had reacted with PLS. The supernatant solution was taken carefully and dispensed in 1.5 ml disposable plastic cuvettes. The absorption spectrum was recorded against distilled water as reference between 200 nm – 800 nm with the maximum absorbance of the TB dye being observed at 580 nm. A standard graph was then generated by plotting the different concentrations of PLS against the absorbance (Grotzky, Manaka et al, 2010).

In order to determine the amount of antimicrobial release, the solutions from the mass and volume study (n=5) at different time points were kept in the fridge at 4°C until analysis. For analysis, equal amount of TB solution and storage solution were mixed, reacted and tested as mentioned above. Finally, the amount of release was calculated using the following equation:

$$\% \text{ PLS} = \frac{Cumulative\ PLS\ released from\ disc\ X\ 100}{Mass\ of\ PLS\ in\ Original\ Disc}$$

Equation 7

2.5 Microstructure Study (Scanning Electron Microscopy)

Scanning electron microscopy (SEM) was used to study the surfaces of composite formulations and to evaluate effect of replacing TCP with TSrP on the microstructure of these surfaces. A magnified image can be produced by using this machine and the concept of it based on using the electrons instead of light. These electrons should be focused beam of high energy to generate a variety of signals at the surface of composite. The electron gun that is located at the top of microscope is able to produce a beam of electrons which then travels through electromagnetic fields and lenses. These beams are then focused down toward the specimen.

The electrons that are reflected off the specimen are collected and converted into a signal directed at a screen, similar to a television screen. This produces the final image. The signals that derive from electron-sample interactions reveal information about the sample which includes external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. All non-metals samples need to be made conductive by covering the sample with a thin layer of conductive

material. All water also must be removed from the samples because the water would vaporize in the vacuum.

2.5.1 Test Method for Microstructure

Each formulation of experimental dental composite was tested to assess the morphology of the surface and thus the precipitation of calcium phosphate and strontium phosphate layers. Two specimens from each formula were soaked in SBF and distilled water. After 2 weeks, samples were dried for 24 hours and then coated with gold-palladium in a sputter coating machine (Polaron E5000, East Sussex, UK) for 1 minute and 30 seconds at 20 mA. Surface scanning was obtained using a scanning electron microscope (Phillip XL-30, Eindhoven, The Netherland) instrument operating with primary beam energy of 5 kV and a current of approximately 200 pA. High resolution images of the composite were taken at different magnifications.

2.6 Raman Spectroscopy

2.6.1 Principle of the technique

Raman Spectroscopy is known as a vibrational spectroscopic method based on an inelastic scattering of monochromatic light that is usually a laser source. Inelastic scattering suggests that the frequency of photons in monochromatic light alters after interaction with a specimen (Bumbrah and Sharma, 2015).

The nature of molecular transitions can determine the difference between the two methods. In Raman technique, the molecule should change the polarization during vibration. This means that a positional change in the electron cloud must happen. In contrast, in case of IR, a dipole moment change to the molecule must happen during the vibration. Another difference between the two techniques is the relative frequencies when sample scatters radiation is determined in Raman spectroscopy, whereas absolute frequencies when sample absorbs radiation is assessed by IR spectroscopy (Wilson et al., 2012).

In Raman Spectroscopy, a laser source is essential to produce a monochromatic light that interacts with the sample and generates a scattered light. This scattered light is having different frequencies that differ from the incident light (inelastic scattering). Raman spectra results from inelastic collision among the incident radiation and molecules of the specimen. When the incident light hits the specimen, it scatters in all direction. Most of the scattered radiations have similar frequencies and this is called Rayleigh scattering. However, a small amount of this scattered radiation has different frequencies from the incident radiation and constitutes stoke and anti-stoke lines. In another way, Stoke lines takes place when the frequency of incident radiation is higher than the frequency of scattered one, while anti-stoke line occurs when the frequency of the incident radiation is lower than the frequency of the scattered one (Smith and Dent, 2013).

Stoke bands are more intense because the transition take place from lower to higher energy vibrational levels and therefore a conventional raman spectroscopy is used to measure these bands. On the other hand, anti-stoke bands are quantified with fluorescing samples because fluorescence interferes with stokes bands (Smith and Dent, 2013).

2.6.2 Test Method for Raman Spectroscopy

In this study, Raman Spectroscopy has been used to analyze the chemical components of the experimental composite and thus to assess the formation of a phosphate bond. Raman spectrum was run for every single component of the powder phase, dry sample from each formula and a representative sample of the four different formulations which were stored at 37ºC in sterlin tubes containing 10 ml of deionized water or SBF for two weeks. After that, A LabRam spectrometer (Horiba Jobin Yvon, Stanmore, UK), equipped with a 633 nm laser, grating set at 1800, x50 objective and wave number range of 700 to 1650 cm^{-1} was utilized. Run time and number were chosen to ensure adequate peak intensity above background and noise. Peak assignments were obtained by comparison with spectra of glass (1400 cm-1) and hydroxyapatite (960 cm^{-1}) using LabRam software (Horiba Jobin Yvon).

2.7 Energy-Dispersive X-ray Spectroscopy (EDX)

2.7.1 Principle of the Technique

Energy-Dispersive X-ray Spectroscopy is an analytical method that can be used with Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Scanning Transmission Electron Microscopy (STEM).

When it is linked to these imaging tools, EDX can provide elemental analysis on areas as small as nano-meters in diameter. This technique can be applied to determine the elemental composition of individual points of a sample which has a unique atomic structure giving unique set of peaks on its X-ray emission spectrum (Shindo and Oikawa, 2013).

2.7.2 Test Method

In this study, composite specimens were prepared from each formulae and soaked in SBF for 2 weeks. The sample surfaces were then analysed to detect the level of Ca and P and then the ratio of Ca to P or Sr to P. After 2 weeks, samples were dried and coated with gold-palladium in a sputter coating machine (Polaron E5000, East Sussex, UK) for 1 minute and 30 seconds at 20 mA. Surface scanning was carried out using a scanning electron microscope (Phillip XL-30 FEGSEM, Eindhoven, The Netherland) instrument operating with primary beam energy of 20 kV and a current of approximately 200 mA. Each dental composite disc was divided into nine areas and each area scanned separately to obtain 9 spectra from which the ratio of Ca/P, Sr/P and Ca/Si was calculated. Furthermore, SEM images of each area were obtained to evaluate the morphology of the surface

2.8 Biaxial Flexural Test

2.8.1 Biaxial Flexural Strength (BFS)

Flexural strength of a material is defined as " the maximum stress that it can resist before failure when subjected to bending load" (Chung et al., 2004a). The clinical application of a material determines the required flexural properties. For example, if the restoration is located in areas where high masticatory forces are applied, the flexural strength should be high. There are two common techniques applied to determine the flexural characteristics of dental materials. They are ISO 4049 three-point bending test and the biaxial test method. The biaxial test is considered more applicable for determining the flexural strength of brittle ceramic materials. For flexural strength testing of glass-ceramic restorative materials and glass ionomer cements piston-on-three-ball and ball-on-ring have been used (Frankel et al., 1998). Currently, the ball-on-ring is commonly used to determine the flexural strength of dental porcelain discs (Fleming et al., 1999, Fleming et al., 2000).

It has been reported that the lab results of both ball-on-ring and ball-on-three-ball loading methods have the highest accuracy in determining the flexural strength of brittle dental materials (Shetty et al., 1980). In the following study, the strength of experimental dental composites has been tested using ball-on-ring jig method. The jig is composed of a metal ring that holds the composite disc and supports it around all boundaries. The metal ball helps in spreading the load uniformly across the entire composite surface which decreases the variability in comparison with ball-on-three-balls flexural test.

2.8.2 Biaxial Flexural Modulus of Elasticity

Elasticity of the material is a mechanical property which indicates the stiffness or rigidity of that material (Monteiro and Montes, 2010). It reveals how much a material will expand when put under a given stress and resists elastic deformation.

Experimentally, this can be obtained from the slope of a stress-strain curve produced during tensile tests conducted on a sample of the material.
The Instron machine gives data in pound (lbs.) and inches, thus the data needed to be converted to Newton (N) and millimetres (mm). From the load - displacement data, a graph was plotted for each sample (Load against displacement) to determine the slope required for the calculation of modulus based on the following equation shown below:

$$E = \left(\frac{\Delta P}{\Delta W_c}\right) \times \left(\frac{\beta_c\, a^2}{t^3}\right)$$

Where:

$\Delta P/\Delta W$ change in force/change in displacement
gradient of the force displacement curve.

E elastic modulus of the disc (from BFS test).

β_c central deflection function. For a ball on ring geometry βc is 0.502 (Higgs et al., 2001)

2.8.3 Test Method for Biaxial Strength

The four different composite formulations, glass and commercial composite (Z250) were soaked in distilled water for 24 hours, 2 weeks and 1 month at 37˚C. 6 discs were produced from each formula. After incubation, biaxial flexural strength was obtained using an Instron Model 4505 Universal Testing Machine (Instron, High Wycombe, UK). The crosshead speed was set at 1mm/min. Before load application, the thickness t (mm) of each sample was recorded from three different points and the average was obtained. Every soaked sample was put on the knife edge ring support (radius equals 4 mm) and then loaded by a spherical tip in an Instron mechanical machine.

Maximum load (kN) at fracture and load versus central displacement gradient were obtained. The strength was then calculated using the maximum load and average of the thickness of the composite. The following formula was applied (Timoshenko);

$$\sigma = \frac{P}{t^2}\left[(1+\Omega)\left(0.485\ln\left(\frac{a}{t}\right)+0.52\right)+0.48\right]$$

Where

σ is biaxial flexural strength.

P is maximum load.

a is support radius.

t is average thickness of specimen.

Ω is poisson's ratio= 0.3(Chung et al., 2004b).

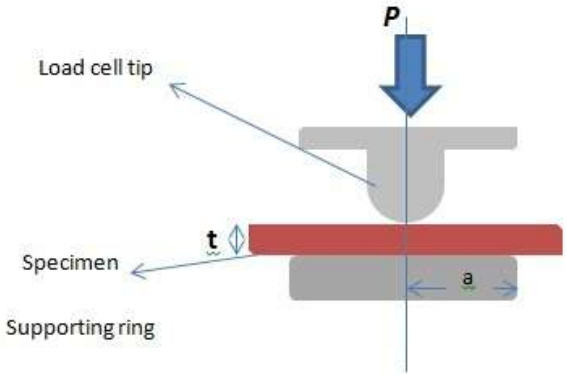


Figure 2-1 Schematic of biaxial flexural test

2.9 Data Analysis

2.9.1 Univariate Analysis of Variance (Two Way Anova)

The Two way ANOVA is one of the statistical methods that is used to calculate and compare the mean differences between the groups after dividing them into two independent variables. In addition, it helps to

understand the interaction between the two independent variables on the dependent variable.

In this study, six samples were made for each formula for testing the effect of replacing CaP by SrP as well as the influence of different percentages of PLS on the mechanical behaviour of the new dental composites. Another 5 specimens were prepared for every formula to study the impact of water sorption on the mass and volume after soaking them in water and SBF for different time points. In addition, this method helps to explore any evidence of differences between these formulations at the significance level 0.05.

2.9.2 Linest

Linest is one the excel functions that helps to work out a straight line that best matches the data, and then returns an array that describes the line. In this study, it has been used in the mass and volume changes and antimicrobial release. It gave the initial gradient of every experiment with R^2 and the standard error (SD).

Chapter Three

Results

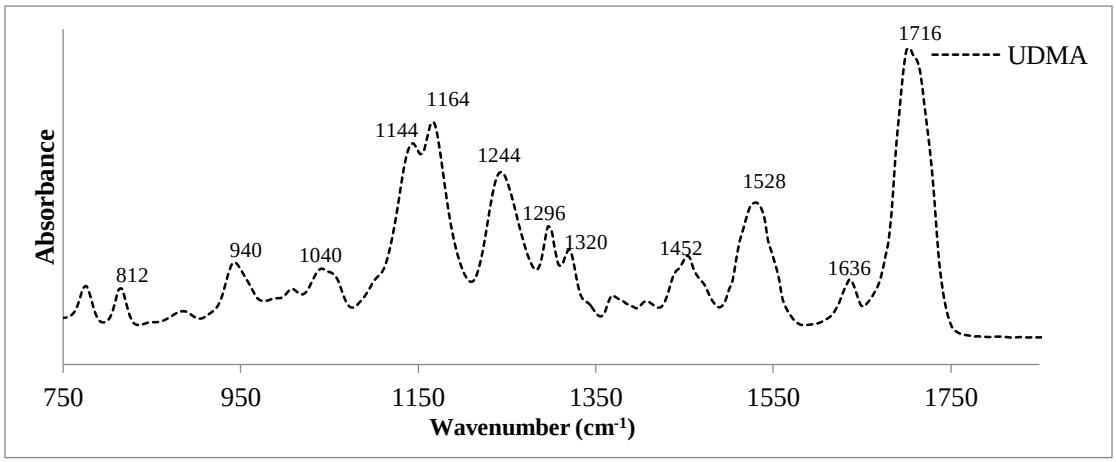
3 Results

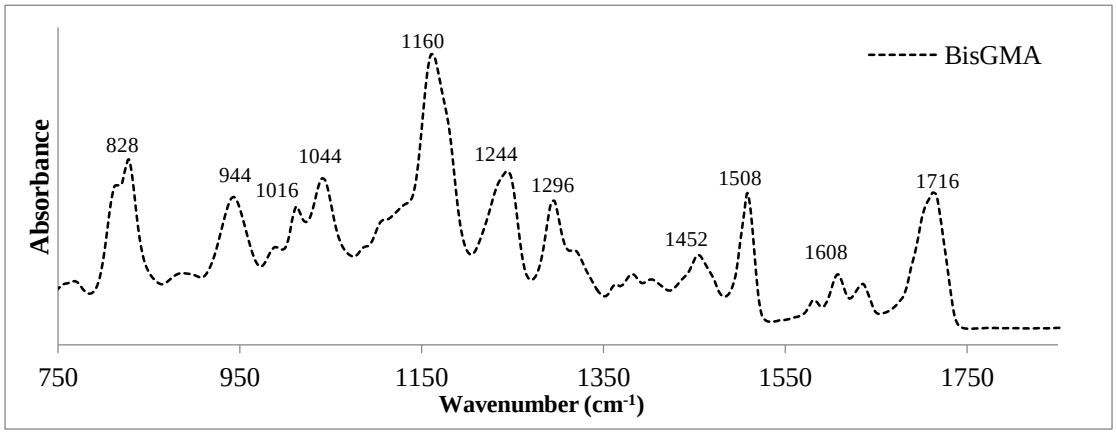
3.1 Degree of monomer conversion

The monomer conversions for each component as well as the glass filler were kindly provided by Saad Ligat, one of the PhD students, and they are illustrated in figure 3.1. There are several peaks that be recognized and each one represents a different functional group. The most important peak that can be identified is 1716 cm^{-1} which corresponds to C=O stretch. The area between 1608-1612 cm^{-1} represents C=C which exists in aromatic carbon ring in Bis-GMA. The peaks that were seen in all monomers were 1452 cm^{-1}, 1320 cm^{-1} and 1160 cm^{-1}. Theses peaks represent aliphatic C-H, C-O stretch and C-O-C stretch respectively. The very intense peak of glass spectrum was noticed mainly at ~ 988 cm^{-1}.

Figure 3.2 illustrates representative FTIR spectra for dental composites with reactive fillers prior and after light cure. All the changes noted due to light exposure were noted in the region of 1320 cm^{-1}. This region is characteristic of methacrylate monomer polymerisation. The 1320 cm^{-1} peak heights before and after cure were calculated to obtain conversion. This represents the stretching of C-O in the polymerizing methacrylate group.

The final level of polymerisation in experimental dental composites with reactive fillers (between 76.2% and 71.5%) was higher than with commercial composite (Z250) (Figure 3.3). The final monomer conversion of the commercial composite (Z250) was 61.1% whereas 70.6% was obtained for the composite containing only glass. Replacing CaP by SrP did not change the level of monomer conversion. Average result for samples with high PLS (10%) was 75.7% while with low PLS (2%) the result was 71.3% (Table 3.1).


UDMA
Absorbance
1716
1164
1144
1244
1528
1296
1320
940
1040
1452
812
1636
750
950
1150
1350
1550
1750
Wavenumber (cm-1)


BisGMA
Absorbance
1160
828
1244
944
1044
1296
1508
1716
1016
1452
1608
750
950
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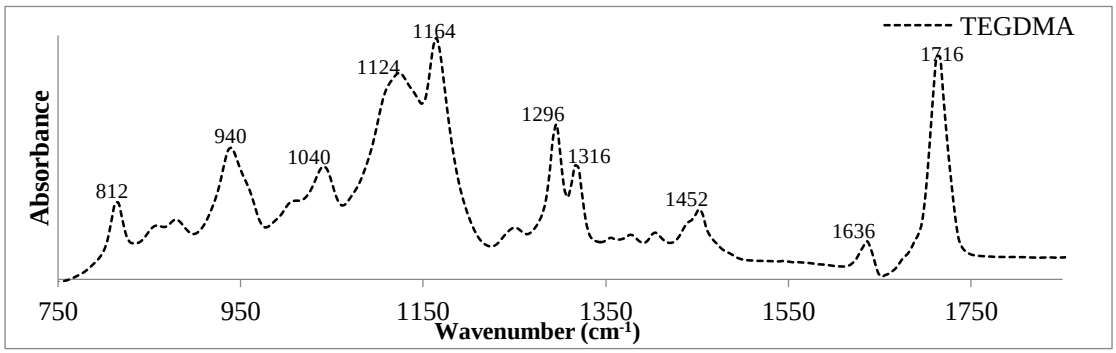
1750
Wavenumber (cm-1)


TEGDMA
Absorbance
1164
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1124
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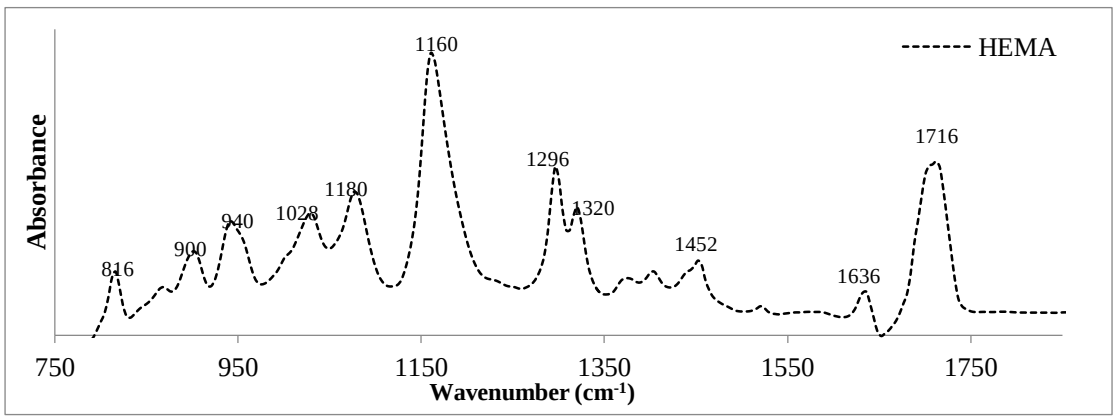
Wavenumber (cm-1)


HEMA
Absorbance
1160
1716
1296
1180
1320
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1028
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816
1636
750
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1750
Wavenumber (cm-1)

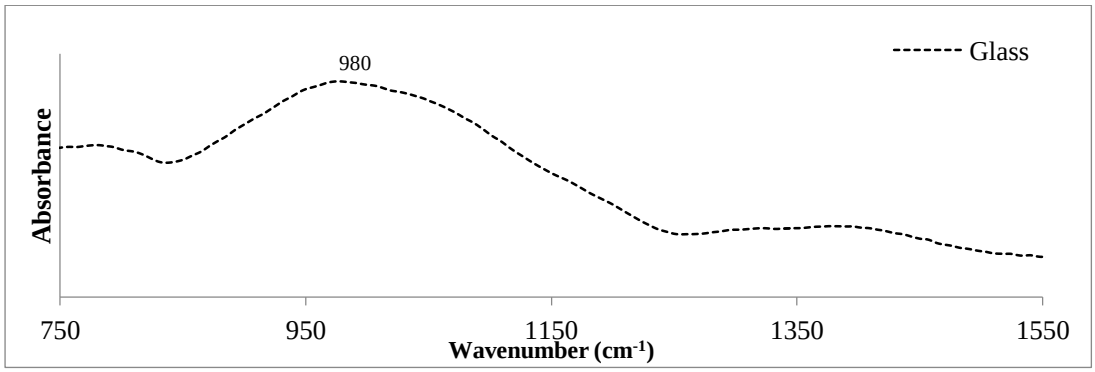


Figure (3.1): A representative FTIR spectra of each monomer used in the commercial and experimental composite. A separate example of the glass filler spectrum is given.

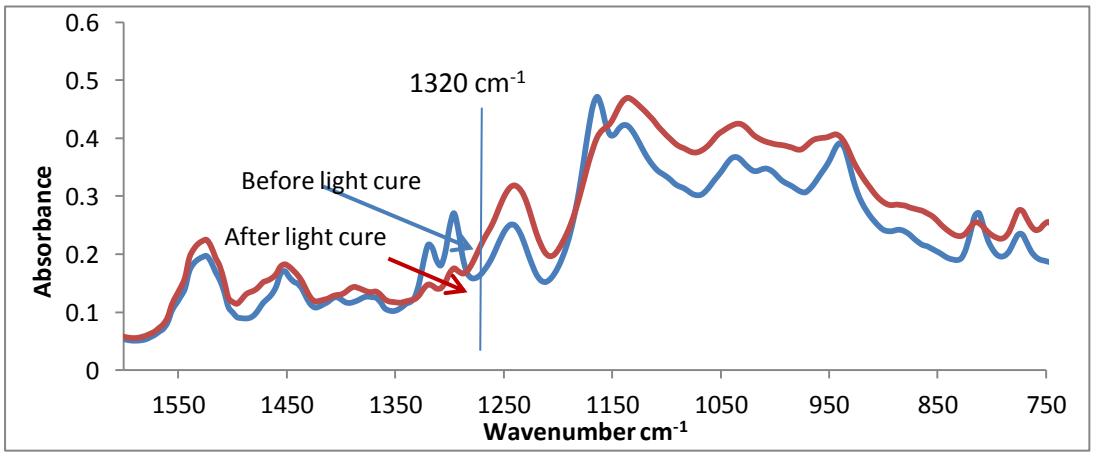


Figure 3.2: Representative sample of monomer conversion shows the difference in peaks before and after light cure. The peak of the monomer is 1320 cm $^{-1}$.

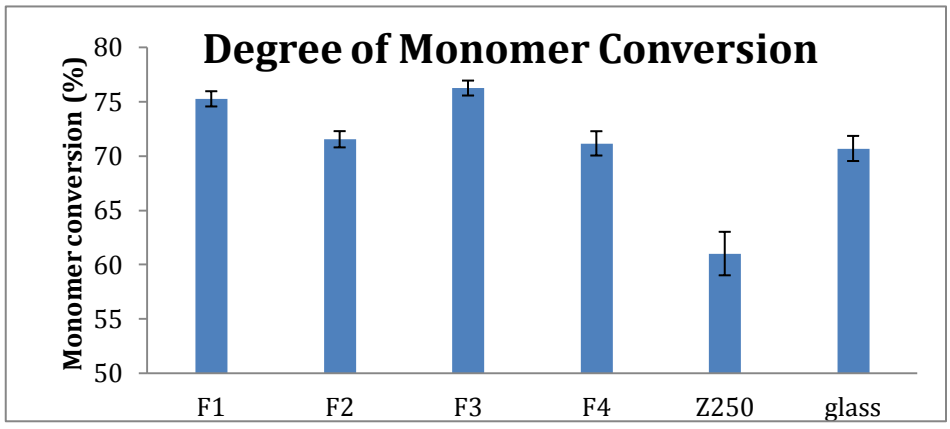


Figure 3.3; Degree of monomer conversion of four experimental composites, control (glass only) and the commercial composite (Z250).Errors bars shown are standard deviation, (n =3).

Formulation	Degree of Monomer Conversion (%)
F1	75.2
F2	71.5
F3	76.2
F4	71.1
Glass	70.6
Z250	61.1

Table 3.1: the final level of monomer conversion in all experimental dental composites, glass and Z250.

3.1.1 Multiple Comparisons

Post Hoc Tests were carried out to detect any statistically significant differences between the means of different groups and it is shown in Appendix 7.1. The results showed that there were significant differences in the means of monomer conversions between the groups.

The post-hoc multiple comparisons revealed a statistical significant difference between F1 and F2, F1 and F4, F1 and Z250, F1 and glass only composite with P<0.001. No significant difference was observed between F1 and F3, F2 and F4 with P>0. 99.

3.2 Mass and Volume Change

3.2.1 Mass Change in Water and SBF:

In the control formulations with no reactive fillers, water sorption was always less than 1 wt% in both water and SBF. In comparison the water sorption for the composites containing reactive fillers was much higher.

Statistically, Univariate Analysis of Variance revealed that different levels of PLS, replacing TCP by TSrP and type of medium (water or SBF) had a significant effect on the mass change after 2 weeks with $P < 0.05$ (Appendix 7.2). Moreover, the statistical analysis shows the dominant effect of PLS in

mass increase. Furthermore, it confirmed an interaction between PLS and medium with P<0.001. The type of medium revealed its effect when the percentage of PLS in the formulae was high (10%). 2% extra increase in mass was observed in SBF compared with water with both Ca and Sr when the percentage of PLS was 10% while the effect of medium was not noticed when the percentage of PLS was low (2%).

Additionally, increase in mass was significantly different between Ca and Sr formulae with P=0.02. There was also an interaction effect between antimicrobial and Ca vs Sr (P=0.006). Mass increase was greater in samples containing TSrP than the ones containing TCP. This was particularly high when the PLS was10%.

For all formulae, the mass increased linearly with the square root of time up to either the first or the third week before slowly declining or remaining steady over the following weeks. (Figure 3.4)

(CaP, 10% PLS)

The first formula with 20% CaP and 10% PLS had maximum mass increase in the first week and second week in water and SBF respectively. This was 7.25% in water and 8.42% in SBF. The mass of specimens immersed in water started to decrease after the first week and reached 4.46% in the third month. On the other hand, the mass of those that were soaked in SBF levelled off after 14 days and was around 7.34 % in the third month.

(CaP, 2%PLS)

The second formulae with 20% CaP and 2% PLS demonstrated its highest mass in water in the third week of 5.7%. The mass of the same samples in SBF, however, continued to rise until the third month and registered a maximum of 6.5%.

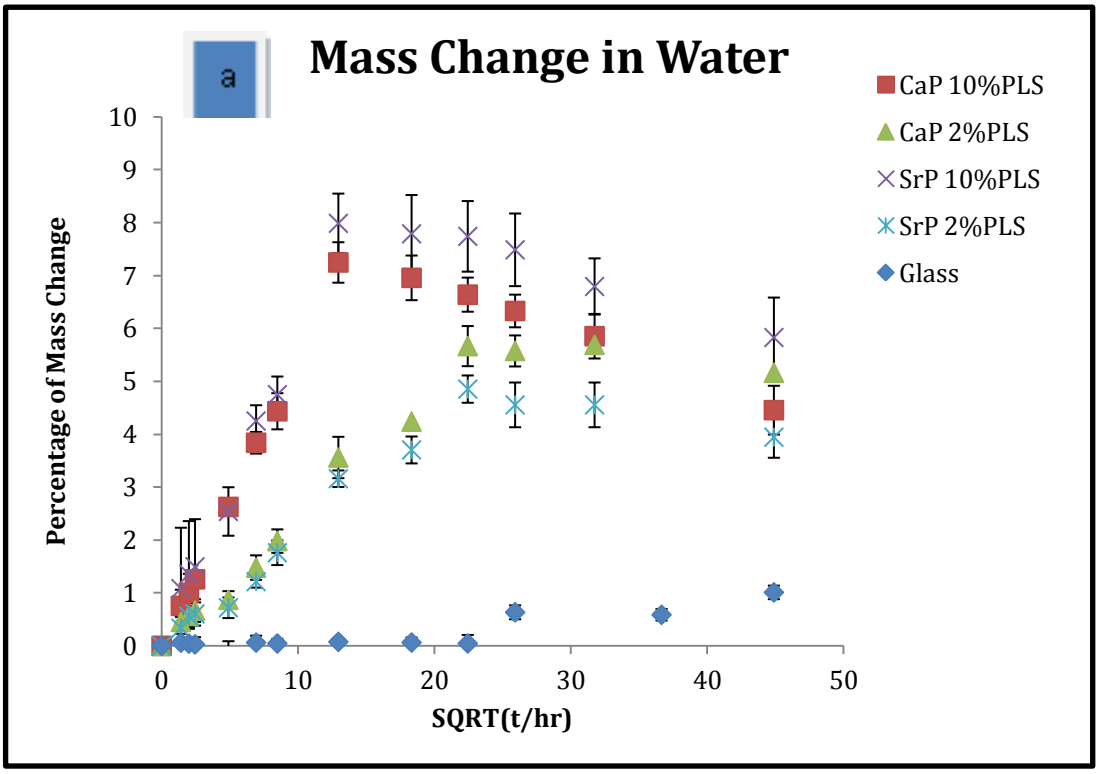
(SrP, 10%PLS)

The 10% PLS and 20% SrP formula, similar results in the first few hours to that with CaP and 20% SrP. The SrP containing 10% PLS composite had its

greatest mass in water after one week of 8%. This then remained in a steady state before it declined to 5.8% in the 12th week. In SBF, however, the maximum mass was observed in the third week (9.7%) then remained levelled off until the 12th week. By comparing the two media and their effect on the mass of F3, it has been noticed that the SBF had only a slight effect on the mass at early times but enhanced by 3.4% in the third month.

(SrP, 2%PLS)

In the 2%PLS and 20% SrP containing composite, the highest mass change in water was observed at the third week (4.8%) with only minor changes thereafter. In comparison the equivalent CaP formula, had slightly higher mass increase at this time and was 5.6%. In addition, the SBF had an influence on the mass of this formula and elevated it by 1.4% in the third month.



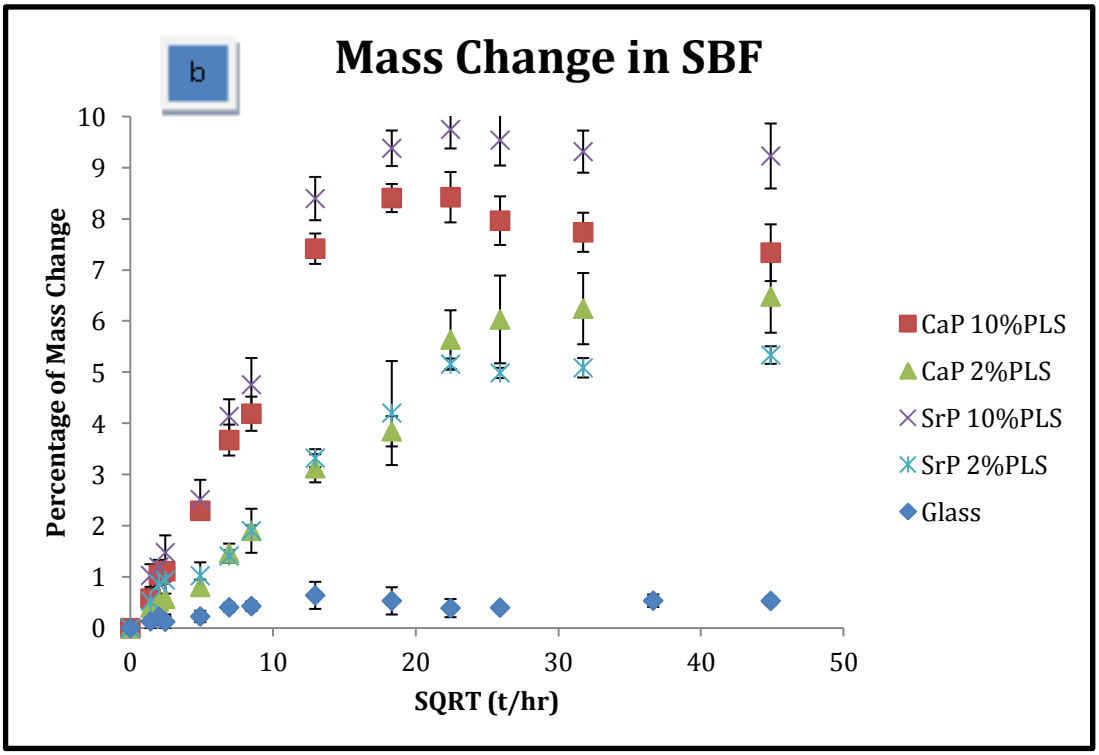


Fig 3.4 (a) and (b): Mass changes in deionized water and SBF as a function of square root of time for all formulations. PLR is 3:1 in both media. The error bars are standard deviation with n=5.

3.2.2 Volume Change in water and SBF

In the control formulation with no reactive fillers, volume change was always less than 1% in SBF while no increase in volume was observed in water. In comparison, the composites containing reactive fillers showed much higher volume increase.

Univariate Analysis of variance revealed a significant effect of PLS on volume changes after two weeks with P= 0.003. Replacing TCP by TSrP as well as the type of medium (water or SBF) did not have a significant effect on the volume changes and P values were 0.056 and 0.808, respectively (Appendix 8.2).

In water and SBF, volume versus square root of time up to the first week with the formulation containing CaP with 10% PLS curved upwards after the first few hours. Subsequently, it either slowly declined or remained relatively steady over the following weeks. By 3 months the volume change was on average 4.9%. (Figure 3.5 a and b).

3.2.2.1 Volume Change in water

All samples with low PLS as well as SrP with 10% PLS revealed their maximum volume change in the third week where the values were almost comparable. These values were on average 4.3%. By three month the average results between CaP and SrP containing 2%PLS was only 1%. (Figure 3.5, a).

3.2.2.2 Volume change in SBF

In SBF, all samples containing low PLS had their maximum values in the second week with average results 4%. In contrast, SrP with 10% is the only formulae which reached to its highest values after one month and it was 4.8%. By the third month all results of all samples were almost similar and showed an average equal to 5% (Figure 3.5, b).

Table 3.2 summarizes the most important values in all formulations including the glass.

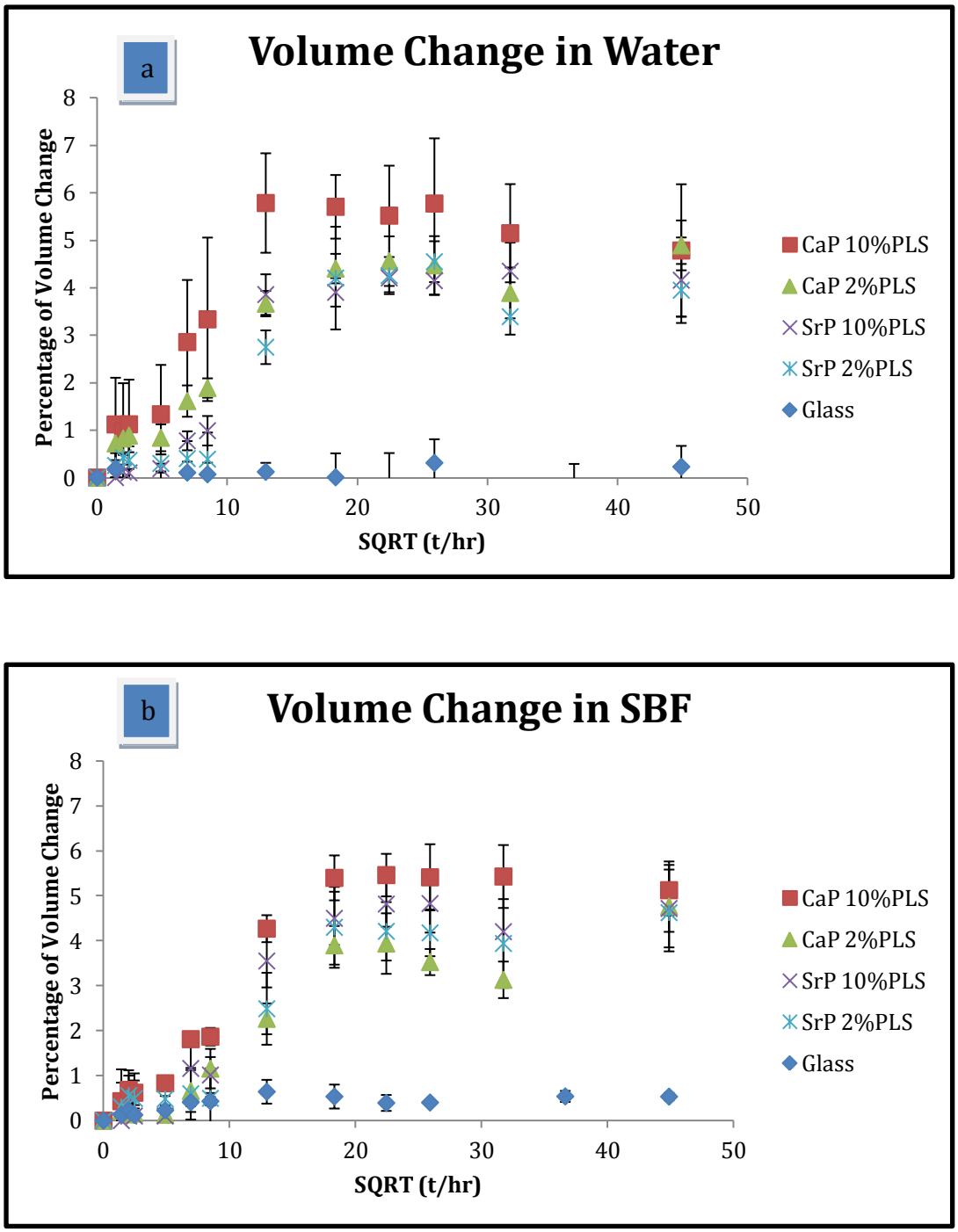


Fig 3.5 (a) and (b): Volume Changes in deionized water and SBF as a function of square root of time for all formulations. PLR is 3:1 in both media. The error bars are standard deviation with n=5.

Formulae	Storage Solution	Initial Gradient for the mass vs sqrt (time) with (± SE) %hr $^{-0.5}$	R^2	SD	Maximum Mass Increase (%)	Maximum Volume Increase (%)	Final value mass 3 months (%)	Final value volume 3 months (%)
F1	Water	0.55±0.01	0.99	0.01	7.3	5.8	4.5	4.8
F1	SBF	0.53 ± 0.01	0.99	0.04	8.4	5.5	7.3	5.1
F2	Water	0.25 ± 0.01	0.98	0.03	5.7	4.8	5.1	4.8
F2	SBF	0.22±0.01	0.99	0.0	6.4	4.7	6.4	4.7
F3	Water	0.59 ± 0.01	0.99	0.03	8.0	4.3	5.8	4.1
F3	SBF	0.61 ± 0.01	0.99	0.04	9.8	4.8	9.2	4.7
F4	Water	0.21 ± 0.01	0.97	0.03	4.8	4.5	3.9	3.9
F4	SBF	0.24 ± 0.01	0.97	0.03	5.1	4.6	5.3	4.6
Glass	SBF	0.017±0.002	0.74	0.005	0.64	0.77	0.53	0.42

Table 3.2: Initial mass gradient, maximum mass and volume increase for all formulations in both deionized water and SBF. Initial mass gradient was calculated using data up to 1 weak assuming a zero intercept whereas the final mass and volume change were obtained at 3 months. The errors are standard deviations with (n=5).

3.3 Chlorhexidine Release in Water

3.3.1 Calibration Curve of CHX

Table 3.3 shows the absorbance of CHX at 255 nm with different concentrations in water. Figure 3.6 provides the calibration curve of CHX.

Concentration of CHX (ppm)	Absorbance at 255 nm
1.25	0.044
2.5	0.114
5	0.185
10	0.465
20	0.939

Table 3.3 absorbance of CHX at 255 nm with different concentrations.

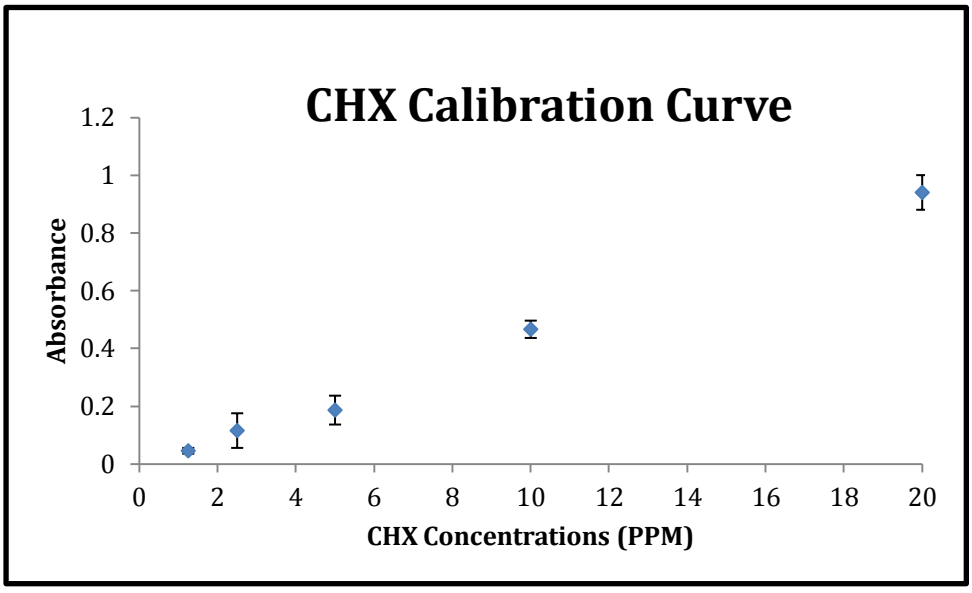


Figure 3.6: standard curve of CHX at 255 nm in water

3.3.2 CHX Release in Water

The level of chlorhexidine release was proportional to the square root of time for similar period to the mass and volume changes as evidenced from Figure 3.7. In this experiment, the release of this drug was assessed in four different formulae in which the amount of CHX added was fixed (10%) whereas the variables were Polylysine (10%) and (2%) and TCP vs TSrP.

It has been noticed that the release of CHX in all formulae was linear versus sqrt (time) at all time points. Samples containing higher percentage PLS encouraged more release of CHX compared to those with low PLS. For example, 23% and 19% CHX release was observed after three months with CaP and SrP, respectively whereas the release reduced to 14% and 11% when the percentage of PLS decreased to 2% for the same period.

Table 3.4 provides the gradient of chlorhexidine release up to three months and the percentage of chlorhexidine release for all formulations at three months.

Statistically, Univariate Analysis of variance revealed that replacing TCP by TSrP as well as changing the percentages of PLS in the formulations had a significant effect on the CHX release after three month with $P< 0.002$ (Appendix 7.3).

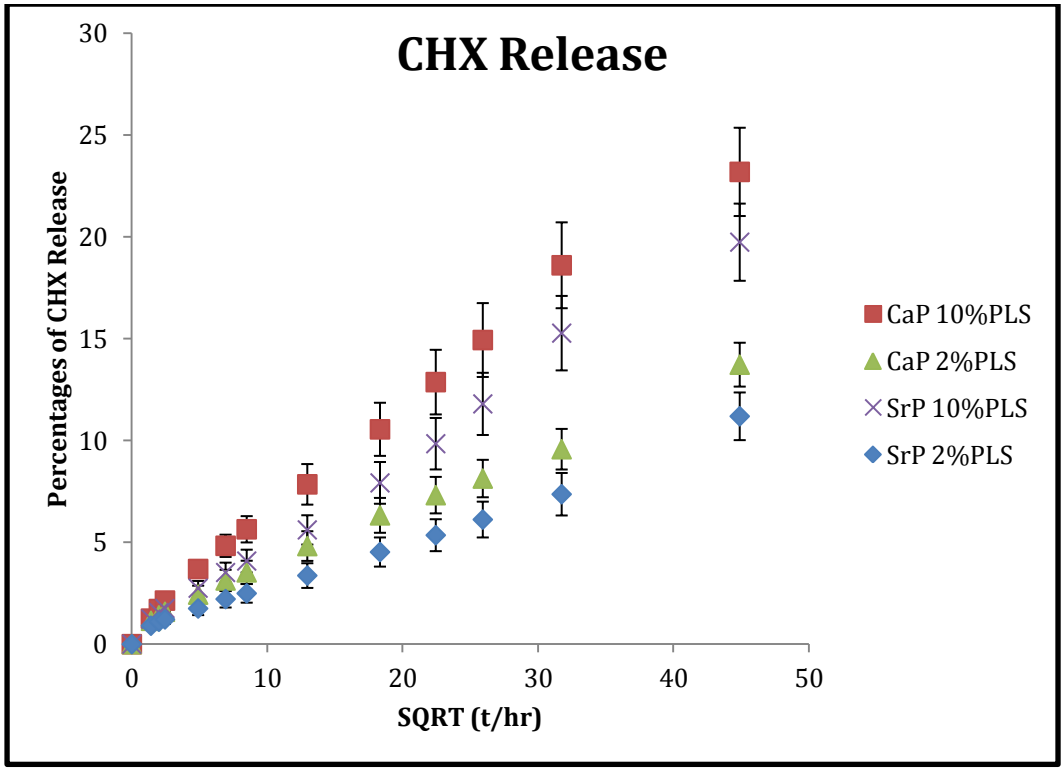


Figure 3.7: CHX release into 10 ml deionized water versus square root of time. Error bars are standard deviation with n=5.

Formulation	Storage Solution	Gradient of CHX release vs sqrt (time)with(± SE) (%hr $^{-0.5}$)	R^2	SD	Percentage of release in the 12th week (%)
F1	Water	0.66 ± 0.01	0.99	0.04	23.2
F2	Water	0.32 ± 0.01	0.99	0.03	13.7
F3	Water	0.45 ± 0.01	0.99	0.02	19.7
F4	Water	0.24 ± 0.01	0.99	0.02	11.1

Table 3.4: The gradient of CHX release from different experimental composites. Chlorhexidine gradient was measured from data up to 12th week assuming a Zero intercept. The errors represent standard deviations with n=5.

3.4 Polylysine Release

3.4.1 Calibration curve of ε-polylysine

Table 3.5 shows the average absorbance due to trypan blue dye after its reaction with increasing concentrations of ε-polylysine from 1 ppm to 10 ppm. This dye has peak absorbance at 580 nm and upon reaction with PLS forms a precipitate that was removed by centrifugation. The calibration curve was obtained by plotting the absorbance at 580 nm against the various level of ε-polylysine concentration in aqueous solutions (Figure 3.8).

Concentration of ε-polylysine (ppm)	Average absorbance at 580 nm
0	0.966
1	0.945
2	0.911
4	0.605
5	0.556
7	0.334
9	0.168
10	0.092

Table 3.5: The average absorbance of various concentrations of ε-polylysine at 580 nm.

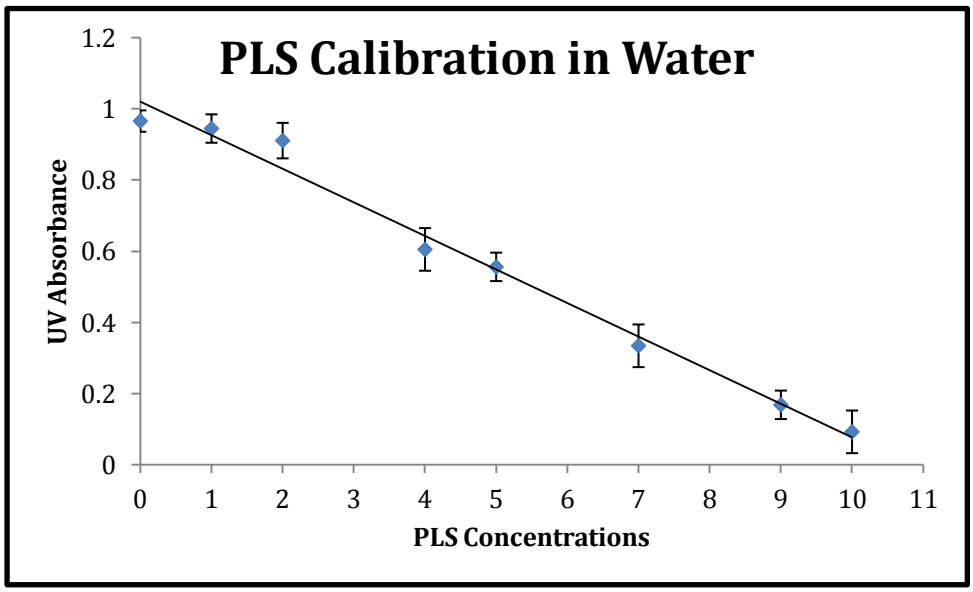


Figure 3.8: Calibration Curve of PLS. This gives the UV Absorbance due to TB dye at 580 nm after reaction with different concentrations of PLS in water

3.4.2 ε-polylysine Release in Water

Figure 3.9 reveals the cumulative percentage of PLS released in deionized water up to three months. This percentage was plotted against square root of time. The variables are the PLS with 10% and 2%, TCP and TSrP (10%). CHX is constant in all formulations (10%).

This release percentage has been calculated using the same equations as with CHX release.

It has been noticed that the burst release of PLS took place in the first 6 hours in all formulations followed by slow diffusion of the drug. Samples which contain high PLS revealed low release of the drug after 24 hours. The average release of PLS after 3 months was around 8.2%. However, samples containing lower percentage of PLS had their maximum release in the second week with average results 26.8%. Following this time point, the amount of release levelled off.

Statistically, univariate analysis of variance revealed that replacing TCP by TSrP did not have a significant effect with P=0.780 while changing the percentages of PLS in the formulations had a significant effect on the PLS release with P = 0.000 (Appendix 7.4).

Table 3.6 provides the gradient of PLS from 3rd day up to three months in F1 and F3 and from the first day up to the 1st week in F2 and F4

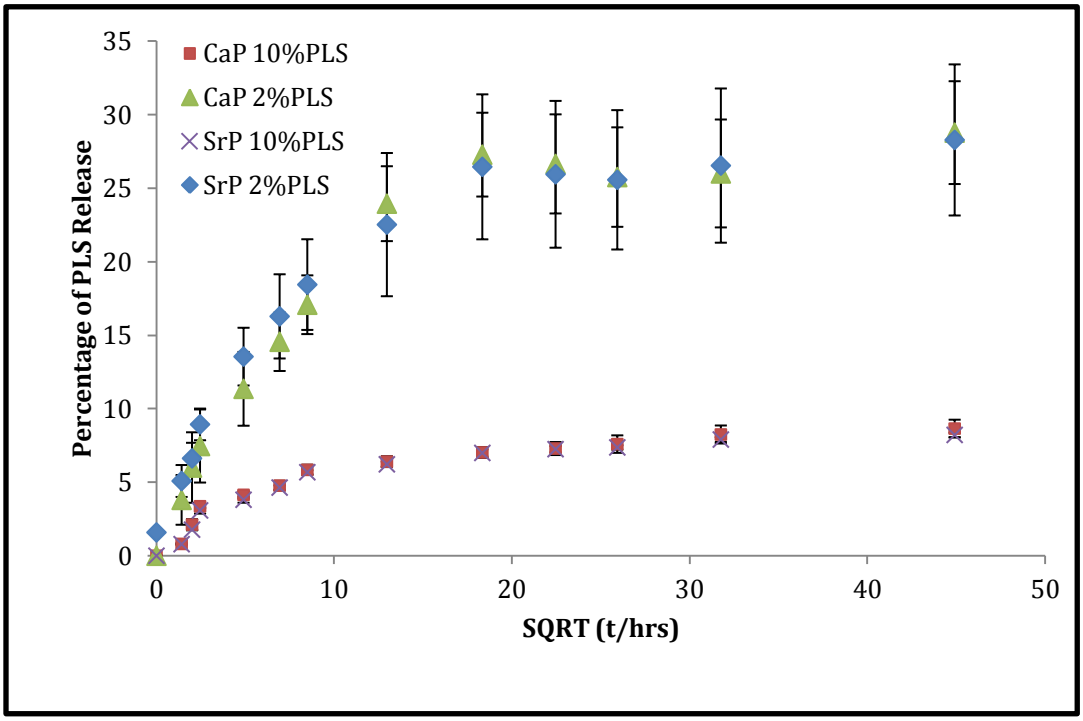


Figure 3.9: PLS release into 10ml deionized water versus square root of time (SQRT) with 3:1 PLR. The error bars are standard deviation with n=5.

Formulation	Storage Solution	Intercept (%)	Gradient of PLS vs sqrt (time) with (±SE) (%hr $^{-0.5}$)	R^2	SD	Percentage of release in the 12th week (%)
F1	Water	5.4 ± 0.2	0.07 ± 0.00	0.94	0.01	8.6
F2	Water	3.7 ± 0.1	1.5 ± 0.01	0.99	0.23	28.7
F3	Water	5.4 ± 0.2	0.06 ± 0.01	0.90	0.02	8.2
F4	Water	8.5 ± 0.7	1.1 ± 0.08	0.98	0.19	28.2

Table 3.6: The release gradient, total release of PLS from different experimental composite. PLS gradient was measured from 3rd day up to three months in F1 and F3 and from the first day up to the 1st week in F2 and F4 assuming a none Zero intercept. The errors represent standard deviations with n=5.

3.5 Microstructure study (Scanning Electron Microscopy)

Four different composite formulations were mixed, cured then soaked in water and SBF for two weeks. The cured composites were then dried, coated and observed under scanning electron microscopy (SEM) at different magnifications.

3.5.1 Water

All specimens in all different formulations which had been soaked in deionized water for two weeks demonstrated homogenous surface with small areas of porosity and voids and no signs to any precipitation of apatite on the composite surface. Figures (3.10 a, b, c) are representative examples of these composite surfaces.

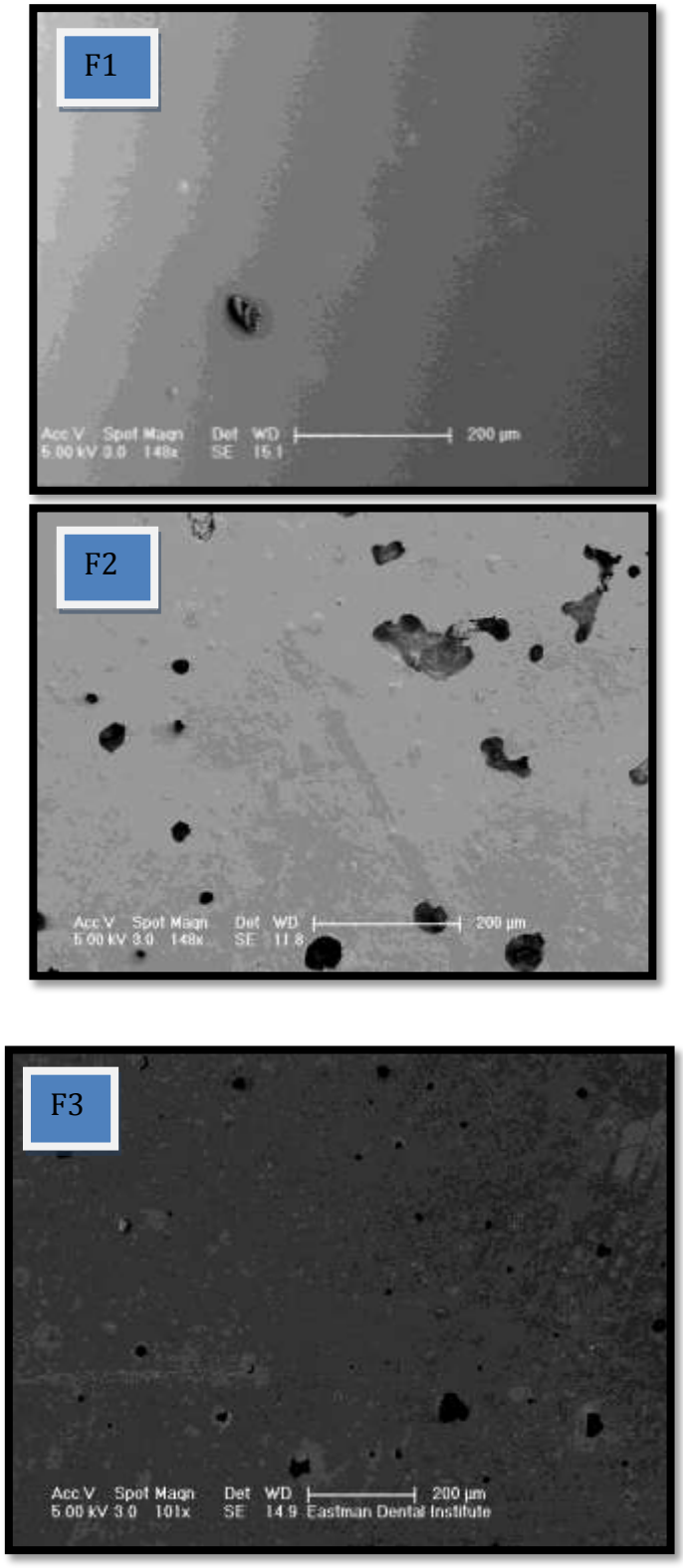


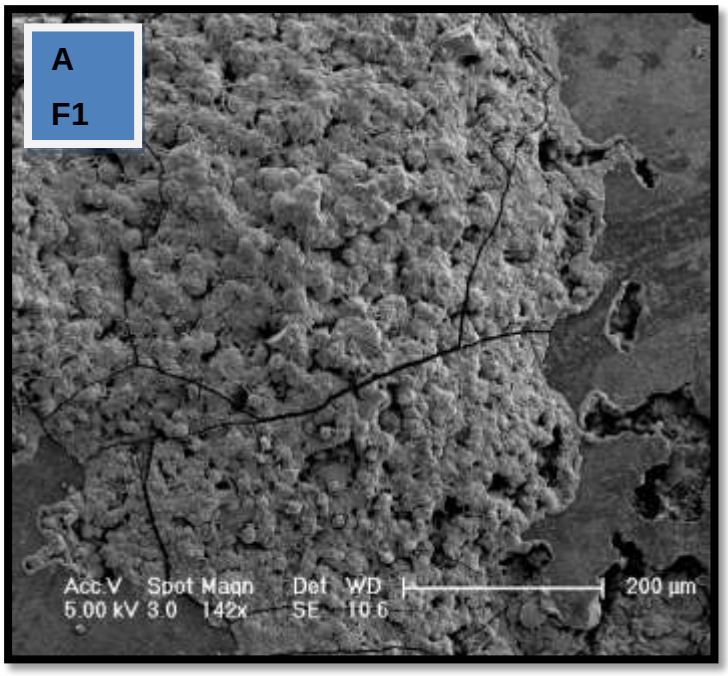
Figure 3.10: Representative images of composites surfaces which were soaked in deionized water for 2 weeks. No precipitation of any remineralising material is noticed.

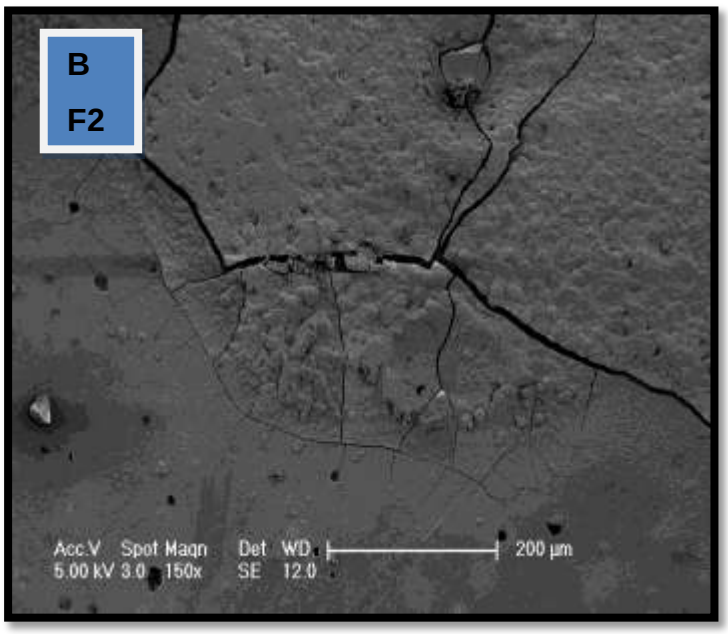
3.5.2 SBF

On the other hand, the SBF showed some effects on the composite. Different outcomes were noticed when the samples were kept in SBF for 14 days. Figures (3.11) and (3.12) show the texture of these composite surfaces and how they vary at different magnifications.

Low magnifications of SEM images display a layer of rough and porous texture that covers most of the sample. Some cracks in this layer can be seen as well. Figure (3.11)

At higher magnification, distinctive circular and lobular depositions with different sizes of crystals can be observed. In addition, the samples that contain SrP demonstrate denser and smother precipitation compared to CaP ones. Figure (3.12)


A
F1
Acc.V Spot Magn Det WD
5.00 kV 3.0 142x SE 10.6
200 µm


B
F2
Acc.V Spot Magn Det WD
5.00 kV 3.0 150x SE 12.0
200 µm

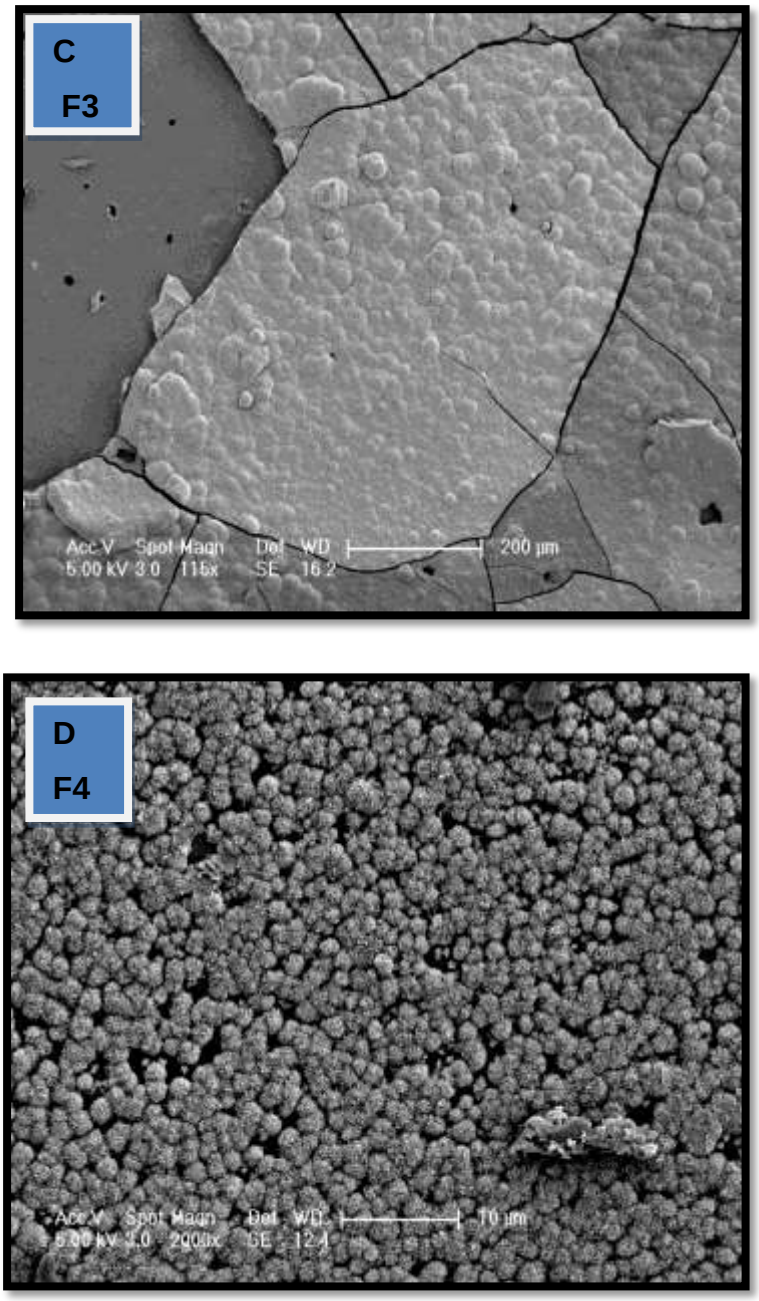
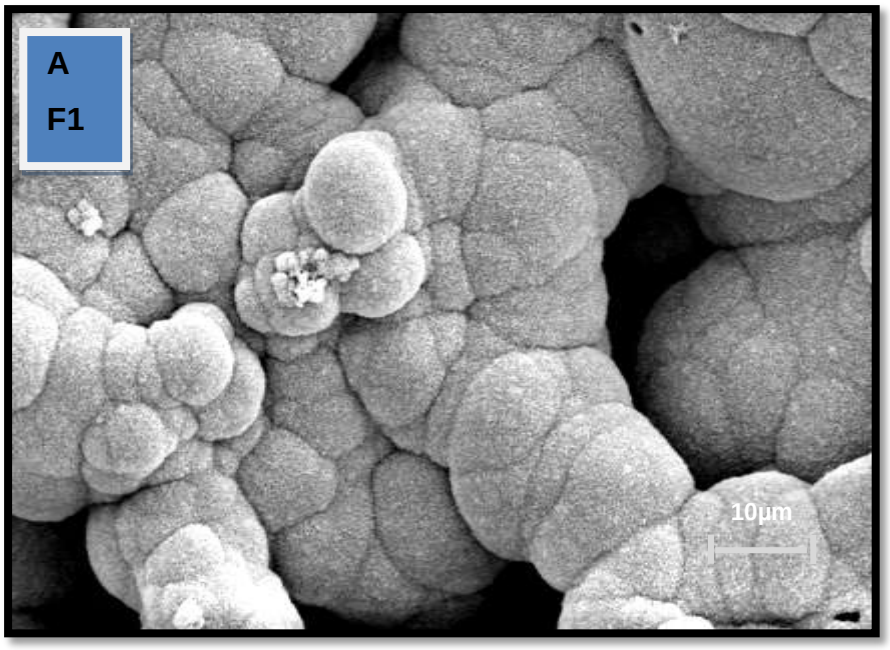
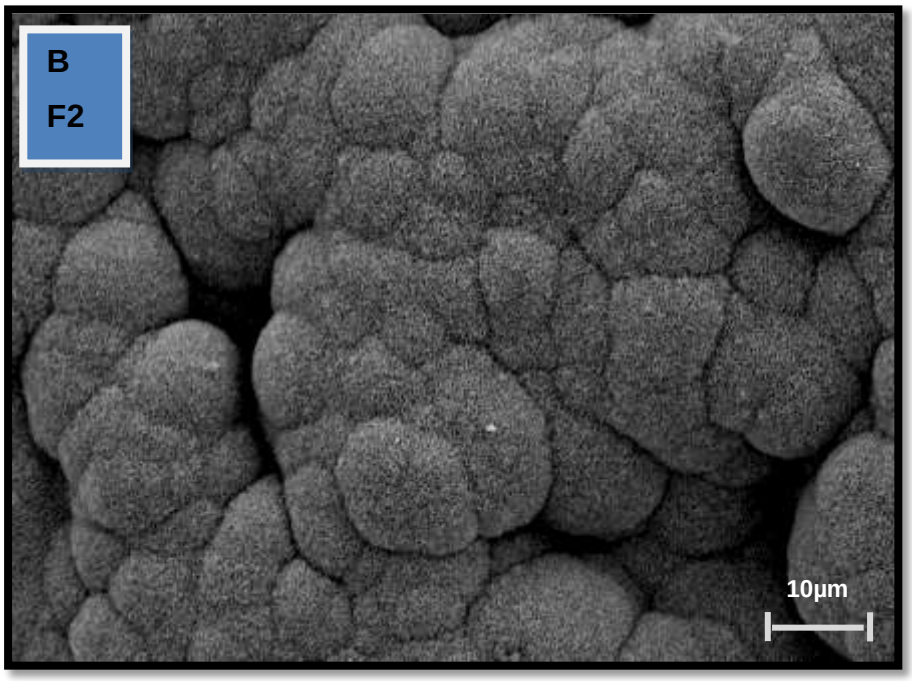
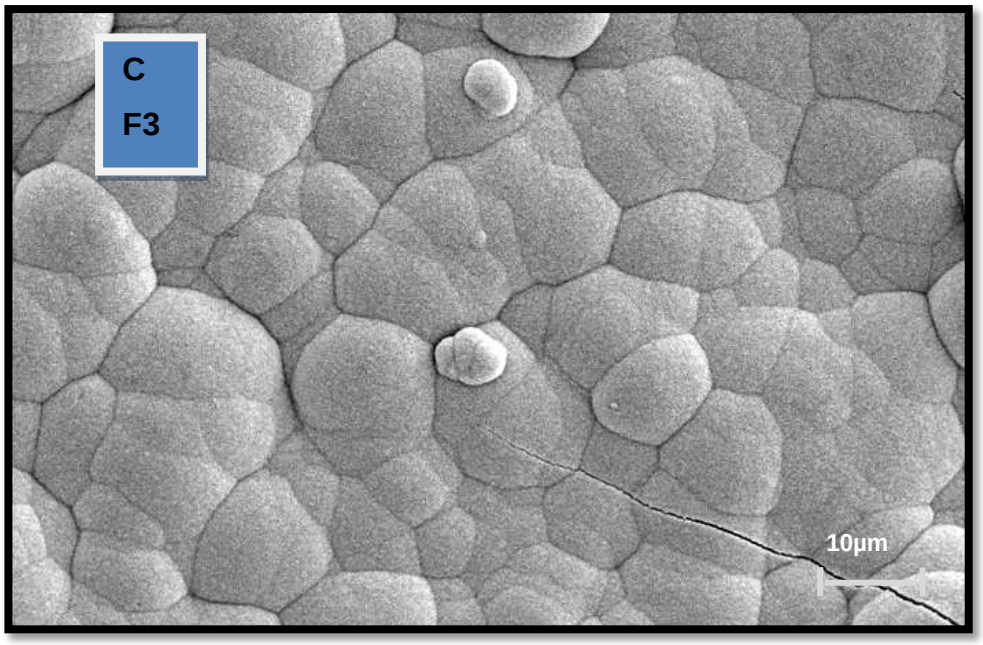


Figure 3.11: Representative images of composite samples in SBF under SEM at low magnifications show Porous layer as shown in F1 (a). More homogenous and dense texture with obvious cracks as shown in F2 (b) and F3(c). globular structures as in F4 (d).


A
F1
10µm


B
F2
10µm



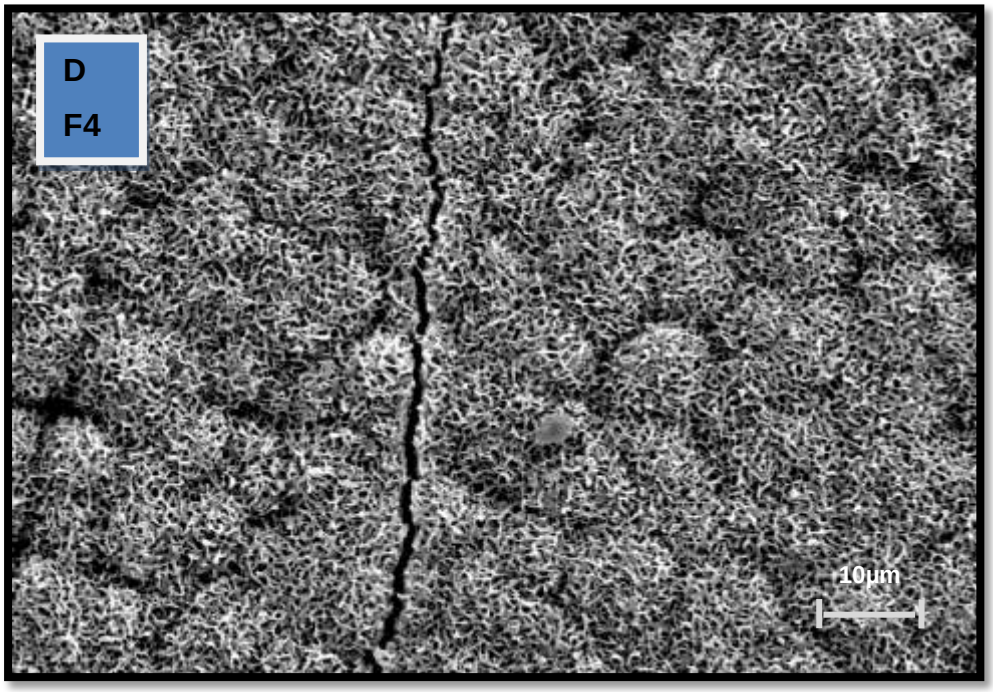


Figure 3.12: Representative images of composite samples in SBF under SEM at higher magnifications show globular structures with some voids as in F1 (a) and F2 (b) and crack lines interpreting the precipitated layer as in F3 (c) and F4 (d).

3.6 Raman Spectroscopy

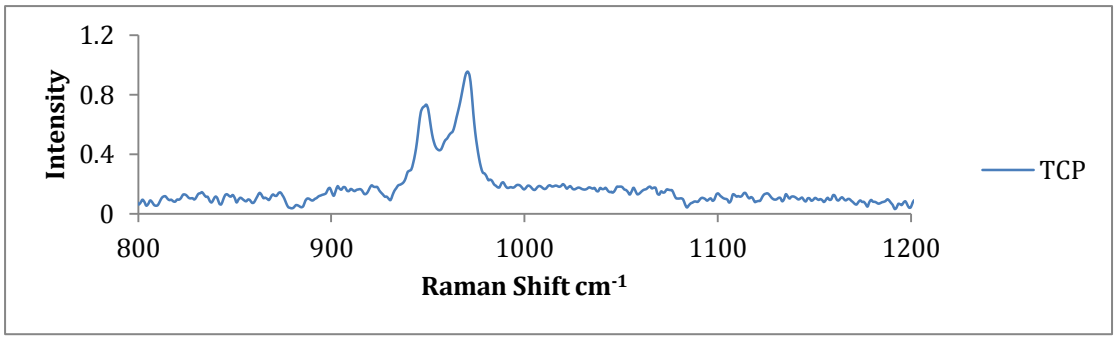
The following spectra show the Raman vibrational frequencies of different components that can be found in the composite samples. The β-TCP reveals two main bands at 949 and 969 cm^{-}, MCPM shows its main bands at 902, 913, 1113 cm^{-1} (Penel et al., 1999), TSrP band was detected at 947 cm^{-1}. The band of the main component of dental composite, glass, was seen at 1400 cm^{-1} , CHX has different and many bands and the dominant ones were at 1286 and 1598 cm^{-1}. The raman spectrum has a very characteristic strong bond that represents the phosphate group in HA at 960 cm^{-1} (Koutsopoulos, 2002). PLS demonstrates many bands and they represent themselves at 1440, 1577, 1673 cm^{-1} (Ma et al., 2007). (Figure 3.13)

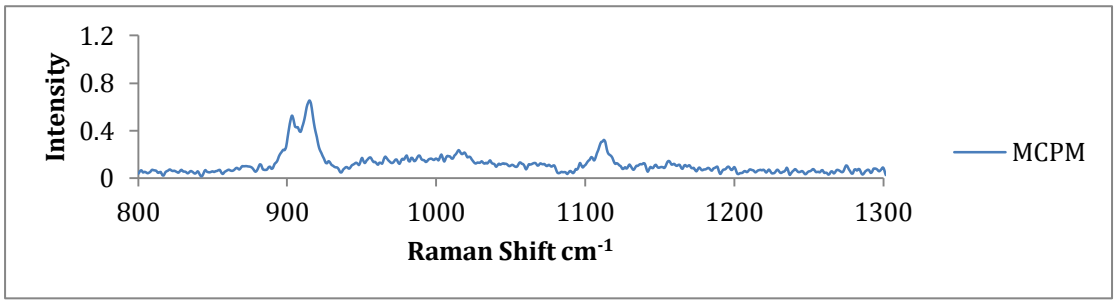
Figure 3.14 shows the Raman spectrum for the dry samples before immersing them in water or SBF.

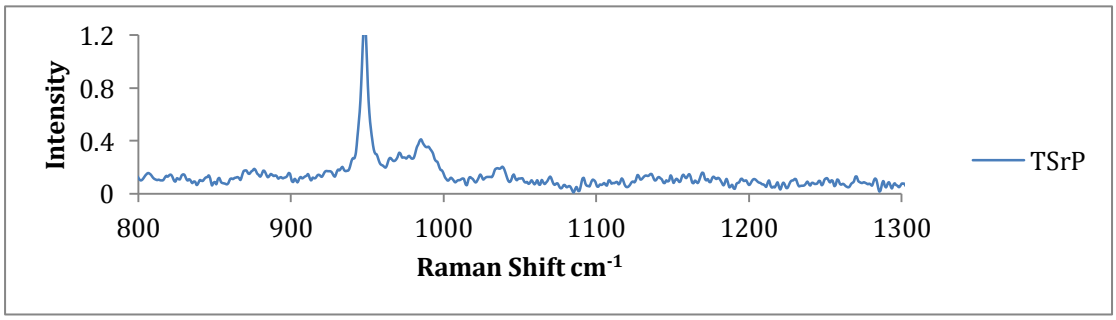
All CaP and SrP formulations were immersed in deionized water or SBF for two weeks and then they were analysed using Raman microscopy.

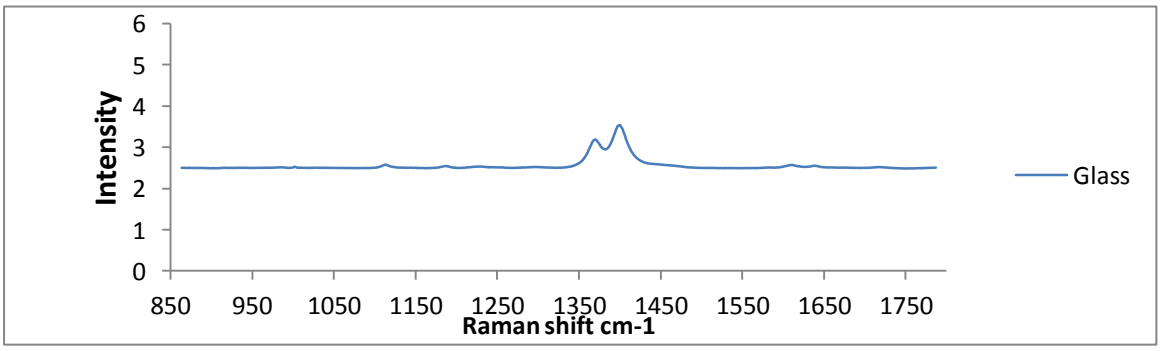
Figure 3.15 provides representative Raman spectra of CaP samples after soaking them in deionized water and SBF for 2 weeks. Very sharp peak was observed at 960 cm^{-1} on the specimen that was kept in SBF, while no peak was determined at 960 cm^{-1} in the ones that were kept in water. In addition, peaks of CHX were clearly detected in the water sample.

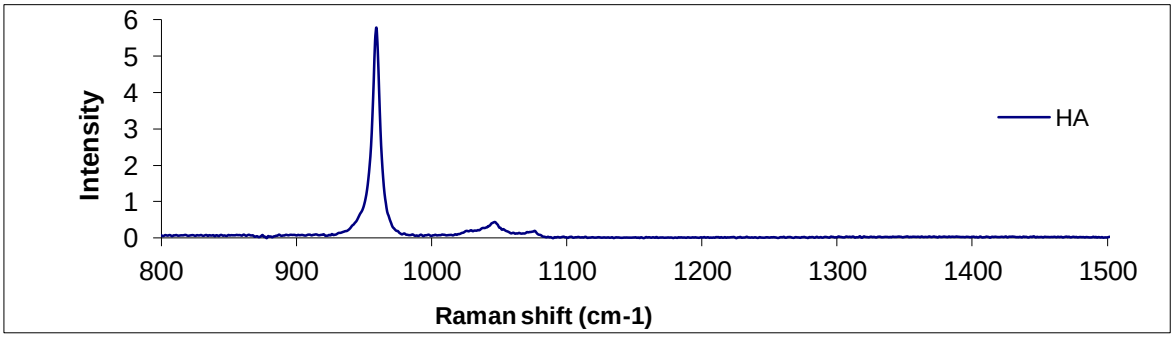
Figure 3.16 demonstrates Raman spectra of SrP specimens after immersing them in distilled water and SBF for 2 weeks. Similar results have been observed in those samples compared to the CaP ones.


1.2
0.8
0.4
0
Intensity
800
900
1000
1100
1200
Raman Shift cm-1
TCP


1.2
0.8
0.4
0
Intensity
800
900
1000
1100
1200
1300
Raman Shift cm-1
MCPM


1.2
0.8
0.4
0
Intensity
800
900
1000
1100
1200
1300
Raman Shift cm-1
TSrP


6
5
4
3
2
1
0
Intensity
850
950
1050
1150
1250
1350
1450
1550
1650
1750
Raman shift cm-1
Glass


6
5
4
3
2
1
0
Intensity
800
900
1000
1100
1200
1300
1400
1500
Raman shift (cm-1)
HA

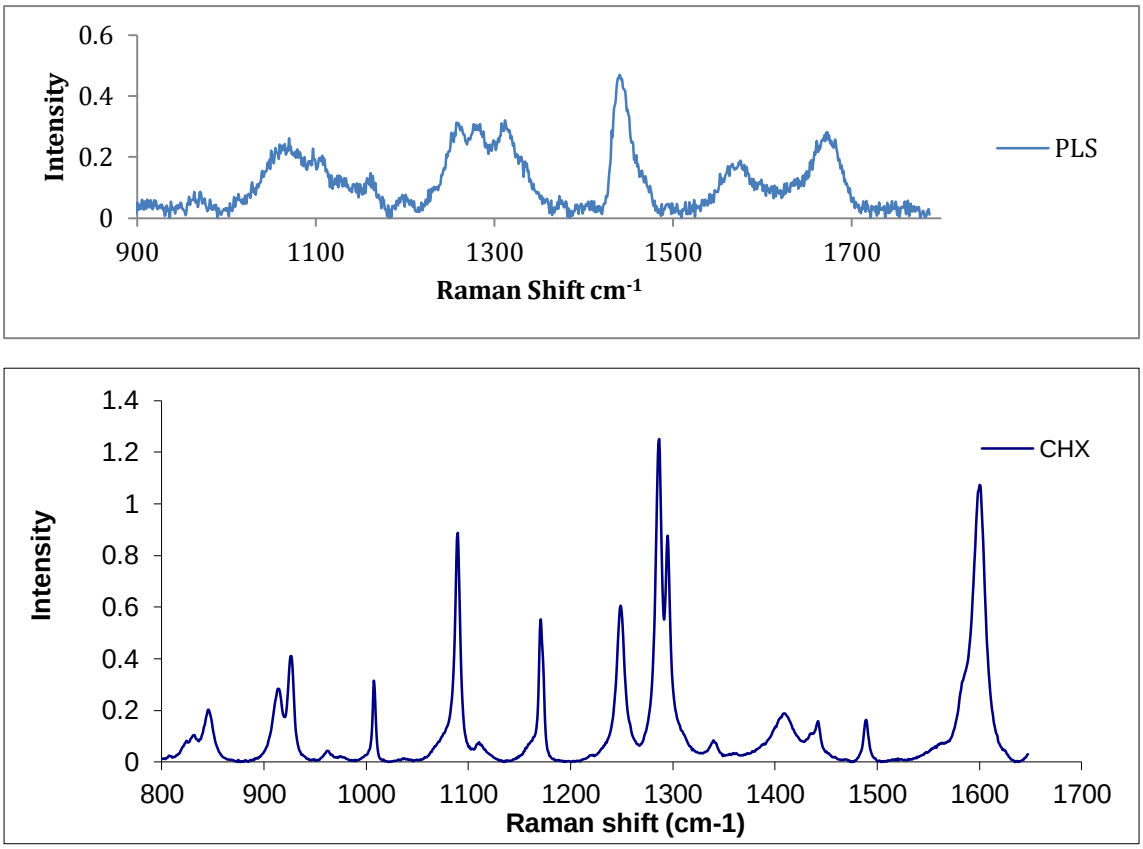
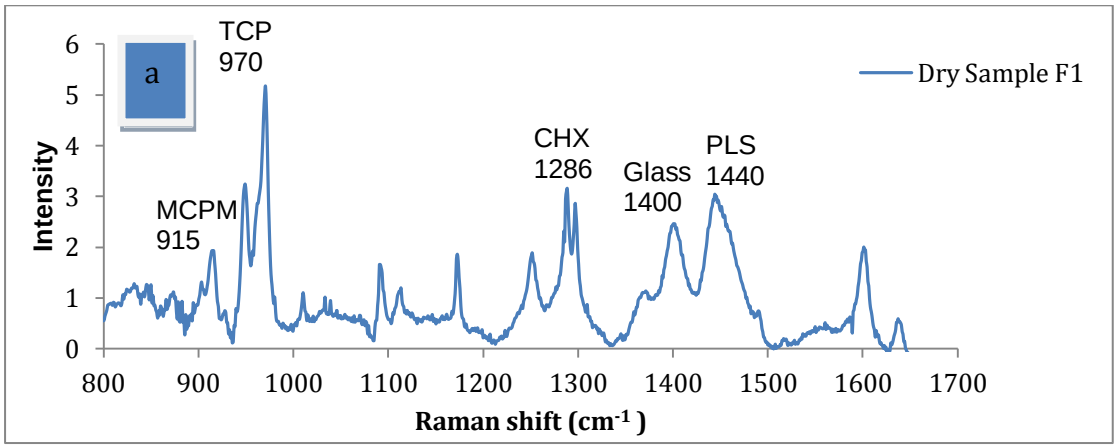


Figure 3.13: Standard Raman Spectra of the different components of experimental composite and the phosphate bond in HA. glass (1400 cm^{-1}), TSrP (947 cm^{-1}), TCP (949, 969 cm^{-1}), MCPM (902, 913, 1113 cm^{-1}), PLS (1437, 1577, 1673 cm^{-1}) and CHX (1286, 1598 cm^{-1})



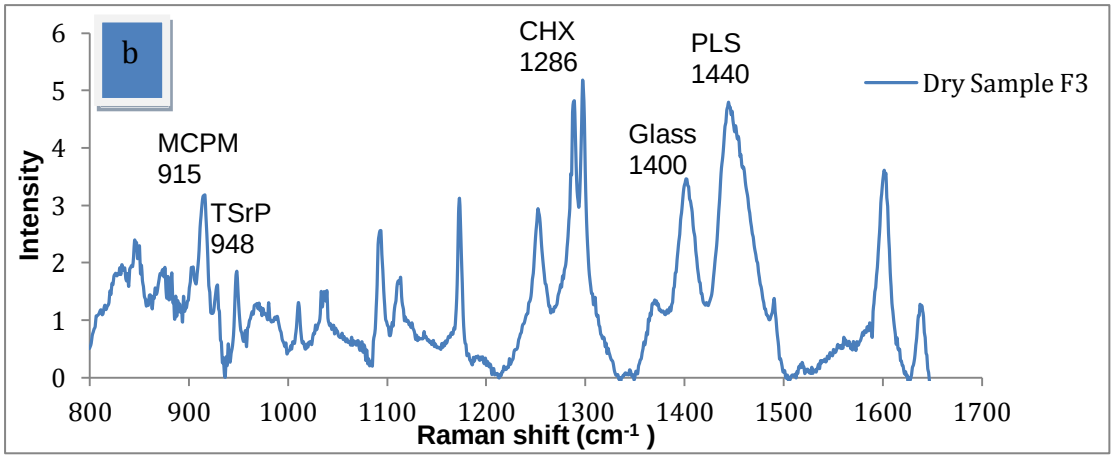


Figure 3.14: Representative Raman of F1 (a) and F3 (b) before soaking them in solution. They show a spectrum of all components with different wave numbers.

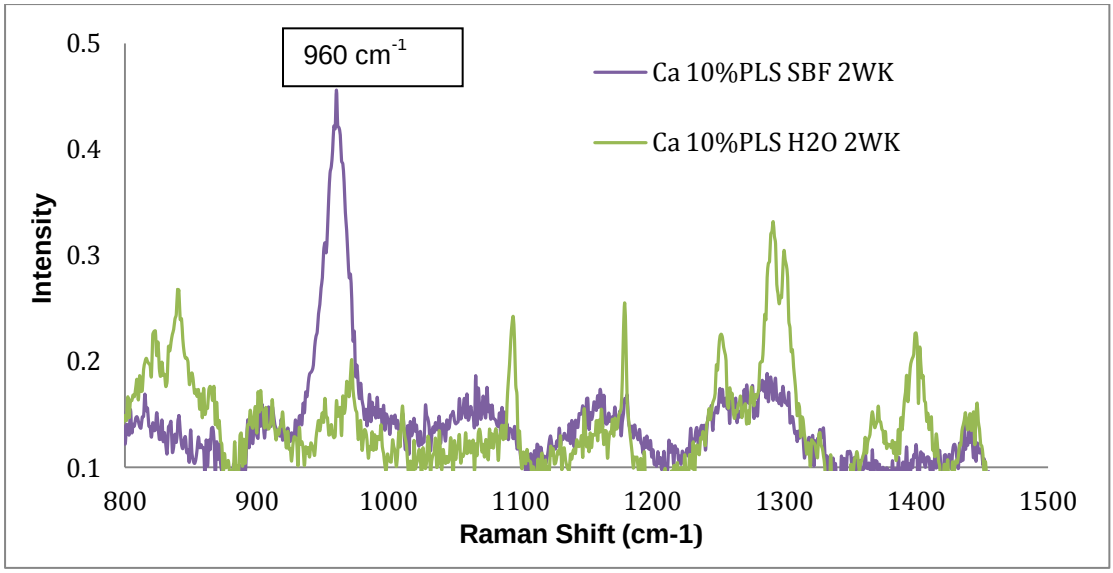


Figure 3.15: Representative Raman spectra recorded for CaP samples after soaking them in deionized water and SBF for 2 weeks.

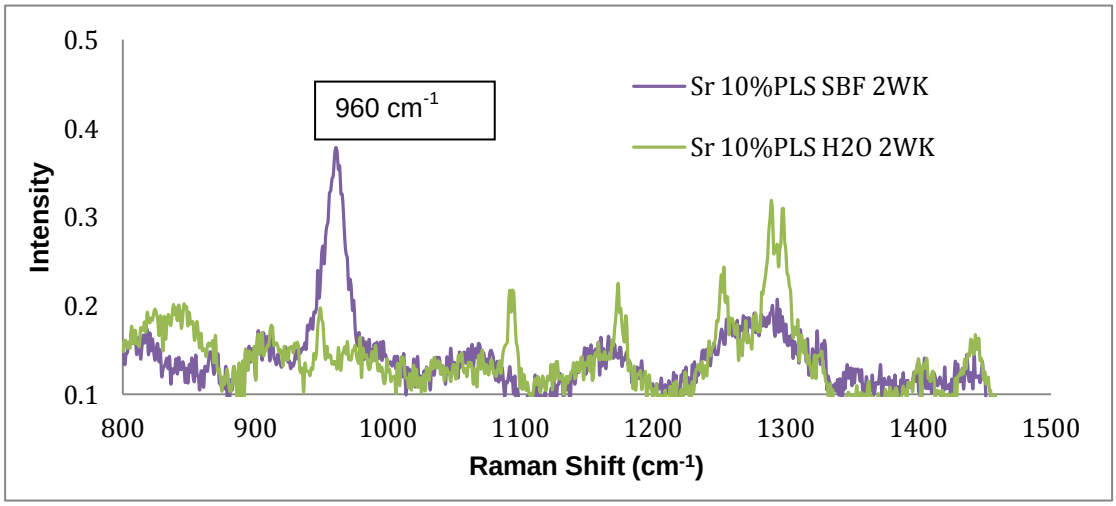
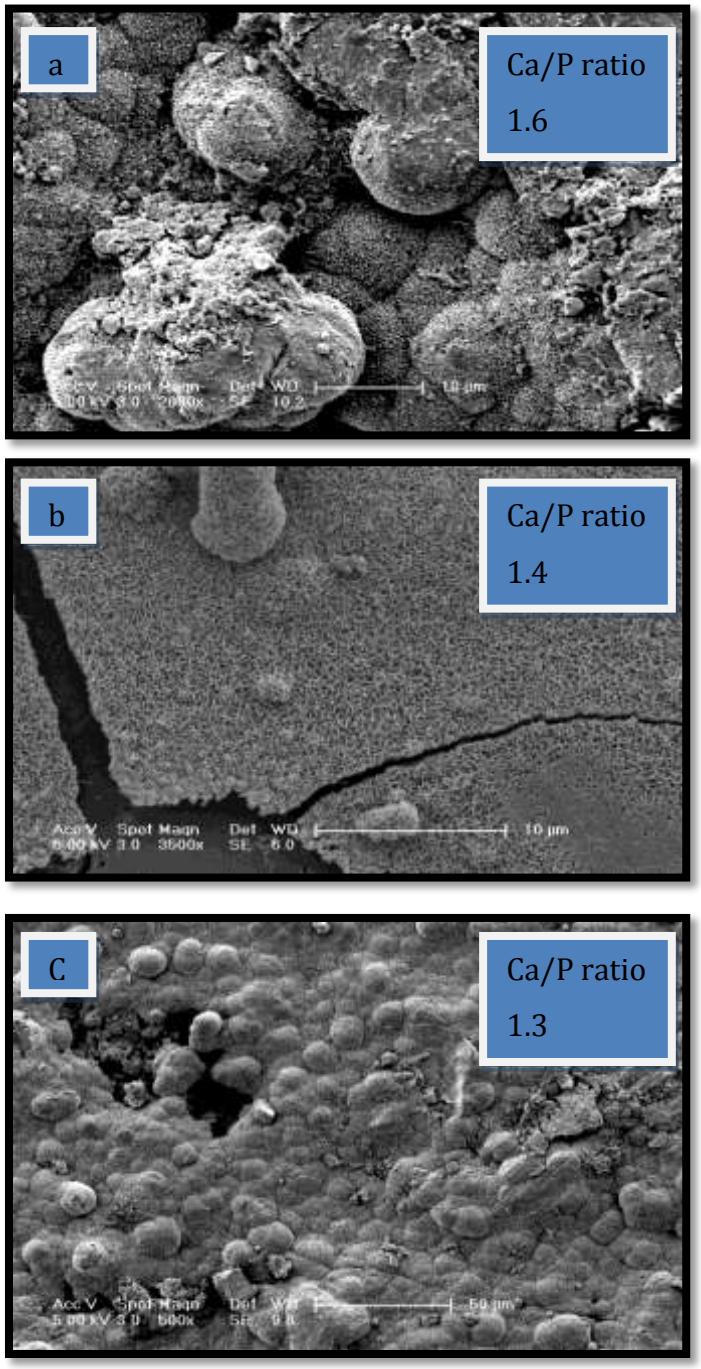


Figure 3.16: Representative Raman spectra recorded for SrP samples after soaking them in deionized water and SBF for 2 weeks.

3.7 Energy-Dispersive X-ray Spectroscopy (EDX)

EDX experiment was carried out to confirm the Ca/P ratio that was precipitated on the composite surface. Figure 3.17 provides multiple images with different magnifications of the composite surfaces and shows the Ca/P ratios on different areas of the samples



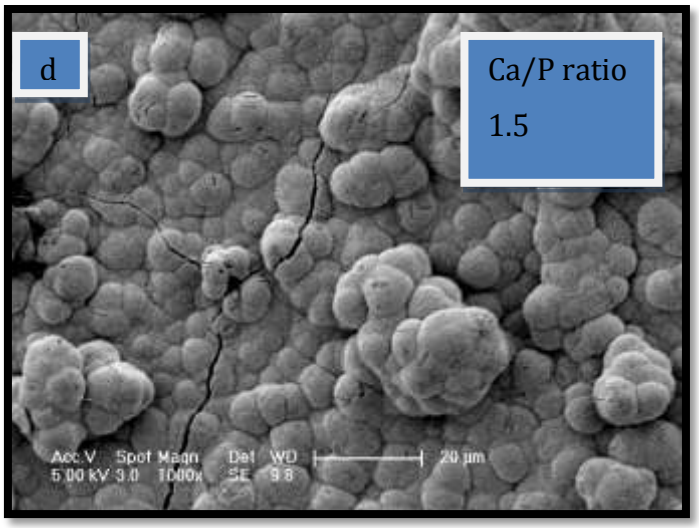


Figure 3.17: Representative SEM images of the samples containing either TCP or TSrP, They illustrate a precipitation layer that looks like honeycomb as in (a) mesh(b) globules with different sizes as in (c) and (d).

3.8 Biaxial Flexural Test

3.8.1 Biaxial Flexural Strength

Figure 3.18 demonstrates a representative stress strain plot of the commercial composite and one of the composite formulations.

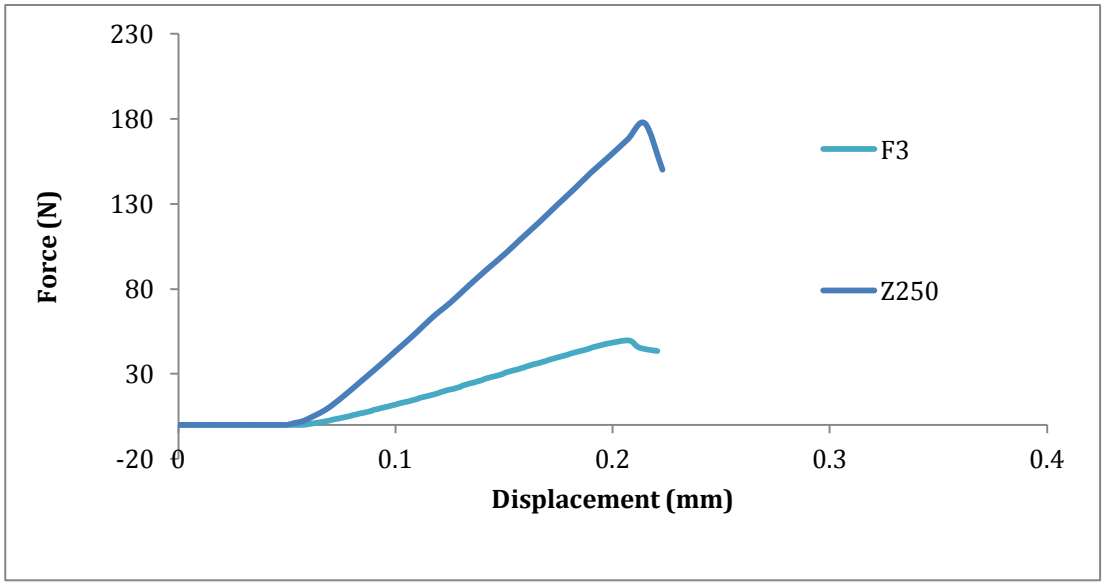


Figure 3.18: Stress-strain Curve of experimental composite and commercial one.

Figure 3.19 shows the biaxial flexural strength of all formulations at three time points (24h, 2wk, 3M). The biaxial flexural strength of the experimental dental composites was lower than the control composites (glass and Z250). Generally, the strength decreased with longer immersing time for all formulations.

Univariate analysis of variance showed that PLS and time were the only factors giving significant effect ($P < 0.001$). (Appendix 7.5)

Average results for samples with high PLS (10%) were 78, 52, 36 MPa at 24h, 2 weeks and one month respectively. Conversely with low PLS the results were 98, 69 and 44 MPa respectively.

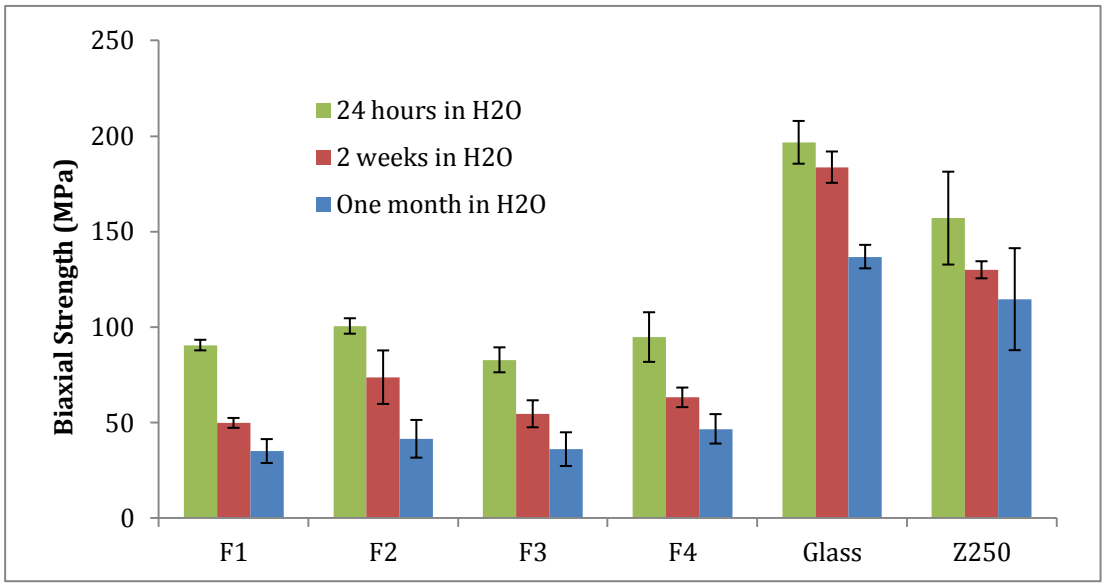


Figure 3.19: Biaxial Flexural Strength of all composites at three different time intervals. The error bars are standard deviations with n=6.

3.8.2 Biaxial Flexural Modulus

The modulus of elasticity describes the stiffness of the material and it is measured from the slope of the elastic region of the stress-strain graph (Anusavice et al., 2012).

The data in figure 3.20 showed that all experimental formulations exhibit significantly lower modulus than glass control samples and Z250.

Average results for specimens with high PLS were 2, 1.5 and 0.9 GPa at 24h, 2 weeks and 4 weeks respectively, whereas with low PLS the results were 3, 2.1 and 1.2 GPa respectively.

Univariate Analysis of variance confirmed the significant effect of PLS and time on the elasticity of the material with $P<0.001$. (Appendix 7.5)

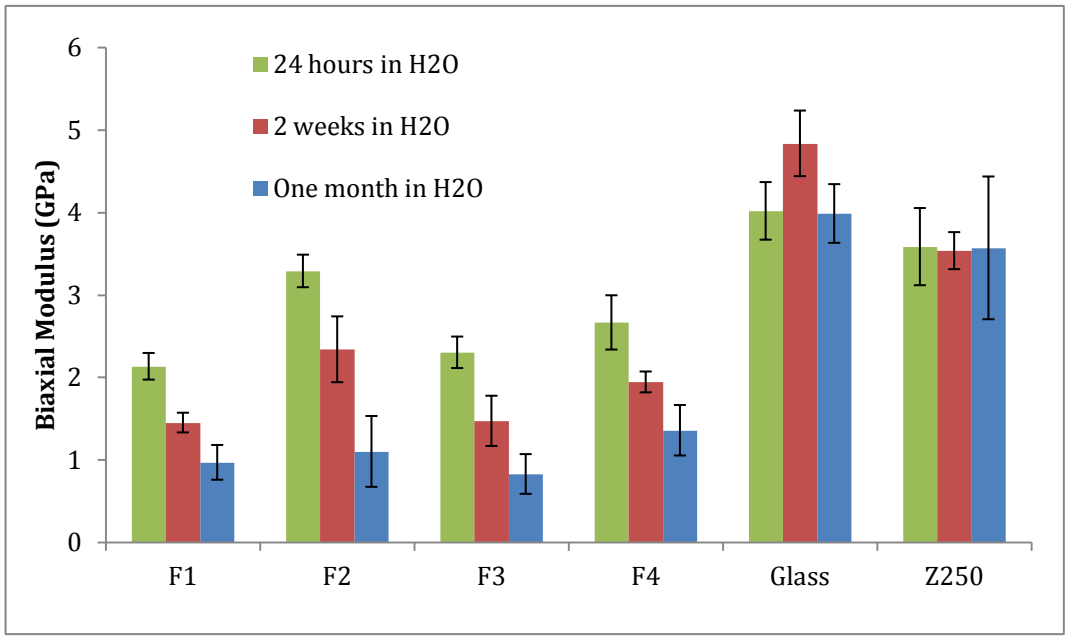


Figure 3.20: Average results of all composite samples. The error bars are standard deviation with n=6.

Chapter Four

Discussion

4 Discussion

The basic structure of dental composite is resin matrix, inorganic fillers and coupling agent. The content of monomer, type, size, distribution of the fillers as well as bonding among matrix and fillers have a significant effect on the properties of this filling material and on the release of remineralising and different antimicrobial agents.

Remineralising and antibacterial releasing dental composites have been developed in the current study. The effects of TCP replacement with TSrP and addition of two different antimicrobial, one of them (PLS) with different percentages, on composite polymerisation and water sorption were first assessed. Several experiments were subsequently carried out to demonstrate the remineralising and antibacterial releasing ability of these formulations. Finally the effects of additives on the mechanical properties were assessed.

In this study, the liquid monomer phase is composed of UDMA, TEGDMA, HEMA, CQ and DMPT, while the powder phase consists of reactive fillers and non-reactive filers. The reactive fillers are MCPM, TCP, TSrP, CHX and PLS whereas the non-reactive filler is glass. After mixing, all specimens were tested after soaking in deionized water or SBF for different time points.

All the experimental formulations had high monomer conversions compared to Z250 and a control composite with only glass as the filler phase. Higher monomer conversions were obtained with higher PLS content. In all formulations MCPM played an essential role in promoting water sorption and subsequent diffusion of the remineralising ions and antimicrobial agents from the set materials. Effects of replacing TCP by TSrP on water sorption were minimal. Addition of PLS at higher percentages, however, encouraged greater water sorption and more release of CHX. A calcium phosphate layer precipitated on the composite surfaces that were soaked in SBF. The Ca/P ratio was close to that of mineral in dentine. Whilst high levels of antibacterial and remineralising agents may be beneficial for the prevention of recurrent

caries the levels must be optimised to ensure mechanical properties are not severely compromised.

4.1 Degree of Monomer Conversion

The degree of monomer conversion is an essential property that has an impact on the behaviour of resin based restorative materials. Improvement of the physical, mechanical and biological characteristics of these materials has been enhanced with increasing level of monomer conversion. (Demarco et al., 2012, Rho et al., 2013). It has been stated that the failure of restorative material under masticatory forces diminishes with greater physical and mechanical properties. Furthermore, the high mechanical and physical properties play an important role in improving the effectiveness of bonding at tooth/restoration interface, therefore lowering the risk of bacterial micro-leakage and secondary caries (Blazić et al., 2003, Kidd, 1976, Gömeç et al., 2005).

In addition, it has been proved that the reduction of cytotoxic effect due to the release of unreacted monomers is inversely related with the degree of monomer conversion. Thus, higher degree of conversion helps to reduce the post-operative hypersensitivity (Goldberg, 2008, Tseng et al., 2007).

Furthermore, the release of reactive fillers might be influenced by degree of monomer conversion (Skrtic and Antonucci, 2007). With higher polymerisation less drug release would be expected. This could be related to the higher cross-linking upon greater monomer conversion (Moszner et al., 2005).

According to the literature reviews, there are direct and indirect methods for assessing the degree of monomer conversion. The following techniques are examples of indirect measurement; microhardness (Santini et al., 2012, Sebra et al., 2007) , differential thermal calorimetry (DTC) (Sebra *et al*. 2007), differential scanning calorimetry (DSC) (Atai et al., 2007, Viljanen et al., 2007). These methods measure the relative polymerisation rather than the definite monomer conversion. This is contrary to Fourier transform infrared (FTIR) and Raman spectroscopies, which are considered a direct quantitative

method to measure the level of monomer conversion. The advantage of these two techniques over the other (indirect) ones is that they provide the absolute degree of polymerisation (Ilie et al., 2014, Richard-Lacroix and Pellerin, 2013, Wang and Spencer, 2003).

In the current study, high level of monomer conversion was obtained with high PLS containing formulae but no difference was found between CaP and SrP. The average results of final conversion of experimental dental composites were 73.5% compared to that of Z250 that had only 61%. High conversion can inhibit the leakage of monomer into underlying pulp tissues and decrease the hypersensitivity or inflammatory reaction. Moreover, composites with high polymerisation levels usually show improvement in the mechanical properties (Palin et al., 2003); however the mechanical properties of composite is low in this study. This could be due to the high water sorption and presence of MCPM which enhanced the release of antimicrobial drug and remineralising cations.

Additionally, the release kinetics of bioactive substances can be influenced by the degree of monomer conversion as a result of decline in the water sorption and resin permeability upon raising the degree of conversion (Braga et al., 2005). This contradicts with the results of this study in which higher monomer conversion was obtained with composites containing higher percentage of PLS. The possible explanation of this is a small increase in the monomer conversion has very little effect in comparison with the effect of PLS and water sorption. Presence of 10% of PLS in the composite may suggest the slightly increase in the cross-linking and monomer conversion compared to 2%PLS but the effect is minimal in comparison with effects on water sorption.

In the above study the chemical reaction was initiated upon light activation of CQ. This produces free radicals that are stabilised by the co-initiator DMPT. These form the monomer radicals and a chain reaction which then terminates when free radicals combine. By removing the light source, the generation of free radicals stop and the termination step decelerates the reaction. Slowing the polymerisation reaction, however, has an advantageous effect in providing

time for polymerisation stress relaxation and dissipation of heat of polymerisation. Furthermore, it could leave monomer groups free to enable linkage with other surfaces in case of utilizing the material as an adhesive, base or lining for composites placed on top (Leung *et al.* 2005).

In this study, stabilizing of free radicals might also be enhanced by CHX and PLS which both contain amine groups as in DMPT (Leung *et al.* 2005; Mehdawi *et al*. 2013).

Addition of UDMA causes cross-linked structures that could lead to reduction of the molecular diffusion rates, slowing in particular the termination step thereby improving the overall reaction rate (Elliott et al., 2001, Sharifi et al., 2008).

4.2 Mass and Volume Change

Water sorption tends to be high in the resin based materials after soaking them in aqueous medium. In conventional restorative materials, the resin phase plays an important role in controlling this property through a diffusion–controlled process (Örtengren et al., 2001, Sideridou et al., 2004, Boaro et al., 2013). As a result of water sorption, changes in mechanical, chemical and biological characteristics are expected. When the water is absorbed into the material structure, release of un-reacted monomers takes place; therefore this might cause cytotoxic effects (Reichl et al., 2006) and encourage the growth of cariogenic bacteria. Moreover, if the water sorption is excessive, plasticization and hydrolytic degradation will possibly decrease the mechanical properties of the restorative materials (Braga et al., 2005, Ferracane, 2006).

Clinically, as a result of oral fluid uptake in dental composite restorations, 30% reduction of the yield strength and fracture toughness has been observed (Kalachandra and Wilson, 1992). In this study, the adhesive formulations were produced mainly for tooth restoration so, evaluation of their behaviour in a wet environment was very important. Furthermore, the water sorption studies

provide a chance to understand the chemical, mechanical and antimicrobial properties of different formulations.

Evaluation of the mass and volume changes was assessed using Archimedes' principle, which is a commonly applied method (Rüttermann et al., 2007, Rüttermann et al., 2011). The ISO standards 4049 for evaluation of resin based restorative materials require that for water sorption tests, the thickness and diameters of the specimens should be 1 mm and 15 mm diameter.

Curing the composite samples with this diameter requires an overlapping light, which extends the essential time of light exposure. In this study, however, preparation of samples that measure 1 mm thickness and 10 mm diameter were carried out because it was closer to the diameter to the light tip (8 mm). Hence, curing of them can be applied in fewer steps.

Studying the experimental dental composites for a longer period was considered important to assess the effect of hydrophilic elements that enhance the water sorption and the associated changes over a considerable time. All experimental dental composites exhibited increase of both mass and volume in two different media (deionized water and SBF) compared to the control with glass only. This is mainly controlled by the combination of CaP and different percentages of PLS.

A study undertaken by Aljabo *et al* in 2015 demonstrated around 2.6% increase in mass following immersing a similar composite to F1 but with no PLS in distilled water for 6 weeks, while in this study the percentage has increased to 6% upon adding 10% PLS for the same period of time. Furthermore, Aljabo *et al* proved the role of CaP in mass changes when they compared the water sorption of composites containing different percentages of CaP. They pointed out that addition of 40% CaP increased the mass by 7% in the sixth week compared to 2.6% in 20% CaP containing composite (Aljabo et al., 2015b). Moreover, it has been stated that combining 40% CaP and 5% PLS in one formula had the biggest effect on mass compared to only CaP

containing composite and to sample contained only MCPM (Dakkouri, 2015)(Lama Dakkouri PhD submitted thesis). Table 4.1 summarizes the mass changes of composite formulae in different studies after 6 weeks being soaked in distilled water.

Aljabo study 2015	Aljabo study 2015	Dakkouri study 2015	Dakkouri study 2015	Dakkouri study 2015	This study	This study	This study	This study	This study	This study
40% CaP 10% CHX 0% PLS	20% CaP 10% CHX 0% PLS	40% CaP 0% CHX 5% PLS	40% CaP 0% CHX 0% PLS	20%MCPM 0% CHX 5% PLS	20% CaP 10%CHX 10% PLS	20% CaP 10%CHX 2% PLS	20% SrP 10%CHX 10% PLS	20% SrP 10%CHX 2% PLS		
Mass Change after 6 weeks in water	7.2%	2.6%	3.8%	3.1%	1.7%	6.1%	5.1%	6.7%	4.5%	

Table 4.1: comparison of mass changes of composite samples containing different percentages of reactive fillers in different studies after soaking them in water after 6 weeks.

All these combined results prove the role of CaP, CHX and PLS in increasing the water sorption of composite via increasing the osmotic pressure in the polymer and then swelling of the material. The effect of these reactive fillers was higher than replacing TCP with TSrP in F3 and F4.

Some formulations exhibited decrease in mass in water over longer time. This can be explained by slow filler loss being replaced by water of lower density but same volume, but also water being forced out of the polymer matrix when osmotic pressure was decreased and fillers precipitated as brushite. Other formulations showed steady values of mass and volume by the end of the experiment. The reason behind this might be due to the additional volume of water being bound in low solubility brushite formed by filler reaction and limited further release of any components.

The formulations in the current study provide the necessary ions that promote the water sorption and hence diffusion of calcium, strontium and phosphate ions which remineralise the demineralized tissue. The presence of water is essential for formation of brushite which is considered the precursor of HA (Hofmann et al., 2006). To form brushite, every gram of MCPM requires 0.5 g of water according to the following equation (Mehdawi et al., 2009);

$Ca_3 (PO_4)_2 + Ca (H_2PO_4)_2.H_2O + 7H_2O \rightarrow 4CaHPO_4.2H_2O$ equation 4.1

Regarding the addition of PLS in the filler phase of the composite and the noticeable changes that has been made, this increase in mass and volume could be due to porosities, which were created during mixing therefore diffusion of calcium and phosphate ions and then formation of the remineralising layer.

Moreover, the increase in mass with addition of the reactive fillers is due to the hydrophilicity of the MCPM and PLS inducing greater water sorption. As the TSrP and TCP are relatively water insoluble and hydrophobic changing the cation was expected and observed to have only minor effects on water sorption.

The above results show that faster and greater early water sorption is encouraged by higher levels of hydrophilic PLS. With high PLS, however, at later times the observed decrease in mass could be a consequence of both the PLS and extra water being released from the composite.

Volumetric expansion in the material generated by water sorption can be an advantage in reducing the gaps at tooth–restoration interfaces that arise upon polymerisation shrinkage (Braga, Ballester et al. 2005) (Oliveira et al., 2012). Moreover, another beneficial effect of this volumetric expansion is to alleviate the stress created by polymerisation shrinkage; therefore maintaining the integrity of the tooth–restoration interface. These advantages provide the volumetric expansion an important role in reducing the risk of bacterial microleakage and then recurrent caries. On the other hand, cracking and fractures of tooth structure can result from an excessive volumetric expansion. The ISO standards 4049 stated that the proper volume expansion should be 2% to compensate the polymerisation shrinkage; however it was around 6% in this study, this would be considered as an excessive expansion.

4.3 Antimicrobial Release

In order to control the number of acid producing microbes and thus the secondary caries, all formulations in this study included antibacterial agents; chlorhexidine and polylysine.

4.3.1 CHX Release in water

Chlorhexidine was included into the filler phase of different dental restorative materials in several studies to obtain the anti-bacterial effect (Leung et al., 2005, Mehdawi et al., 2013). It has been proved that the addition of 2% of CHX into the filler phase can decrease the number of cariogenic bacteria *Streptococcus Mutans* (Xia et al., 2014, Cheng et al., 2012). Furthermore, this reduction in cariogenic bacteria would retard the formation of biofilm, the metabolic activity and consequently the viability of biofilms (Xia, Razi et al. 2014). In the study of Cheng *et al* in 2012, it has been stated that the bacteriocidal activity of this drug

can be continuous until one month from the release. After 4 weeks, the diffusion diminishes and the bacteria can grow again.

In this study, the level of CHX release in deionized water was linear with the square root of time in all time points. This can be explained by Fick's equation (Leung et al., 2005).

$$\Delta M / M_{\infty} = 2\sqrt{\frac{Dt}{\pi l^2}} \qquad \text{equation 4.2}$$

2l is the sample thickness, *D* is CHX diffusion coefficient, *t* is time, M_{∞} is the maximum release of drug into solution, and Δ*M* represents the change in cumulative drug in solution.

The primary discharge of antibacterial agents might be due to the absorption of water promoted by, MCPM in particular, that has more solubility than β-TCP. This early diffusion could have an effect on the microorganisms and colonization of the bacteria in the gaps at tooth-restoration interface particularly if this drug is entrapped inside the gaps and apatite layer. It has also been stated that the released chlorhexidine from experimental composites might play a role in increasing the durability of bond strength through inhibiting endogenous enzymatic degradation of the hybrid layer (Carrilho et al., 2007).

In this study, the release of CHX in CaP formulae was higher than the SrP formulae. A possible explanation for the increase in CHX release with using CaP instead of SrP could be an increased water sorption. This enhanced water sorption could be balanced by enhanced mass loss resulting in negligible changes in mass.

Furthermore, it was noticed that addition of different levels of PLS (10% and 2%) played an important role in encouraging the release of CHX. In the third month,

for example, 10% wt PLS increased the release of CHX from 13.7% to 23.2% in the CaP and from 11.1% to 19.7% with the SrP containing formulae.

The role of PLS was confirmed by comparing the CHX release in this study to another study, which was done by Aljabo *et al* in 2015. Aljabo *et al* did not include the PLS in the composite formulae but added the same percentage of CHX and CaP used in this study. They found around 11.9% CHX release after 12 weeks (Aljabo et al., 2015a) of soaking the samples in deionised water whereas 23.2% of CHX release was detected upon adding 10%PLS in the above thesis. A possible reason could be because of the ability of PLS to attract water into the polymer which leads to expansion of the material (Shan et al., 2009). Consequently, dissolution of CHX takes place and then the release from the bulk of the material.

As shown in figure 3.14 and 3.15, CHX peaks were clearly detected after soaking the samples in water for two weeks. This confirms that the CHX is still diffusing from the sample into the water as illustrated in figure 3.6.

4.3.2 PLS Release in water

In the current study the release of PLS took place in two stages. The first stage is the burst release in the first few hours. This kind of release may be a consequence of the drug being released initially primarily from the composite surface. Subsequently the second stage release can be explained by the Higuchi equation

$$(K_{0.5})^2 = \frac{DC_S(2C_0 - C_S)}{4l^2C_0^2} \qquad \text{equation 4.3}$$

Where D is the diffusion co-efficient, C_o is the initial resin drug concentration, C_s is the solubility of the drug in the resin matrix, and l is the sample thickness. $K_{0.5}$ is a rate constant related with early diffusion controlled drug release.

When $C_0 > > C_S$, the gradient of the drug release decreases with increasing C_0 and the equation simplifies to

$$(K_{0.5})^2 = \frac{DC_S}{2l^2 C_0} \qquad \text{equation 4.4}$$

This equation suggests that release is inversely proportional to the concentration of the PLS in the samples as was observed in later times of the study.

In the above result the percentage of PLS that was forced out from the composite was low when the PLS concentration was high. The possible reason of this low release could be because of the amount of water absorbed is not sufficient to dissolve the PLS and consequently it is trapped within the polymer. This result here is almost similar to the percentage of PLS release from brushite cements where PLS interacts with MCPM and prevents PLS from being released (N.A Ismail, 2014). In addition reactions may take place in the bulk of the material between PLS, MCPM and TCP or TSrP making less soluble products that can inhibit the diffusion of PLS from the composite.

4.4 Calcium Phosphate Layer Formation

Raman Spectroscopy, SEM and EDX were utilized to prove the formation and precipitation of the remineralising layer on the experimental composite surfaces. In this study, Raman spectroscopy, SEM and EDX have been used to prove the formation of apatite layer. All samples which were soaked in the water did not exhibit any apatite layer formation, only those which were immersed in SBF demonstrated this layer. This observation could be due to the effect of ions which were present in the SBF medium. This apatite precipitate has an advantage in remineralising the dental tissue and filling the gaps that are caused by polymerisation shrinkage.

Elemental test (EDX) was carried out on the surfaces of these specimens to obtain the Ca/P ratio. When the area is fully covered by precipitate the Ca/P, ratio is close to that expected for hydroxyapatite. If the surface is not fully

covered, the Ca/P ratio is reduced. This could be because the material Ca/P ratio is lower than that of HA. Alternatively there might also be some formation of other calcium phosphates eg calcium deficient HA or octacalcium phosphate with Ca/P = 1.3. On the other hand, Sr was not detected in the apatite layer on the composite surface. The possible explanation of this result could be due to the formation of Sr substituted brushite in the bulk of the composite rather than the surface.

4.5 Mechanical Properties

4.5.1 Biaxial Flexural Strength

Flexural strength is defined as " the maximum stress that a material can resist before failure when subjected to bending load" (Chung et al., 2004a). The flexural strength includes tensile, compressive and shear stresses, therefore it is suitable to use the flexural strength measurements to assess the mechanical properties of dental composites. Clinically, dental composite restorations undergo significant masticatory forces with an extensive amount of several stresses which involve tensile, compressive and shear (Li et al., 2002). High flexural strength is an essential property for these restorations which are subjected to high mechanical forces (Feilzer et al., 1995, Ilie and Hickel, 2009)

As mentioned in the results section, all experimental dental composites revealed poorer mechanical properties than the control ones (glass and Z250). In addition, longer soaking time in deionized water exhibited a massive decline in the biaxial strength. The best explanation of this observation is the combination of the hydrophilic fillers (MCPM, CHX, PLS) which have an essential role in promoting high water sorption; therefore it is considered a crucial factor in decreasing the mechanical properties of the composites. By replacing TCP with TSrP, the strength was similar and no significant difference noticed which suggests the important role of MCPM. (Arima et al., 1995, Neel et al., 2010, Regnault et al., 2008). Another factor that could be part of the cause is the great amount of reactive filler release upon water sorption.

Biaxial Flexural strength of composites containing 20% CaP, 10%CHX and 0% PLS was 118 MPa after one month of soaking them in water (Aljabo et al., 2015c). This result is considered significantly higher than the BFS in this study, which was 46.7MPa in same composites containing 10% PLS.

This strongly suggests the addition of highly hydrophilic antimicrobial agent (polylysine) had a detrimental effect on the mechanical properties of the experimental dental composites. This can further be seen from the fact that strengths of F1 and F3 were also lower compared to F2 and F4 with reduced PLS.

Furthermore, continuous fall in strength might also be as a result of water sorption, constant filler dissolving and chlorhexidine and PLS release. The effect of chlorhexidine and PLS concentrations on mechanical properties could be attributed to formation of holes upon drug release.

The British Standard EN ISO 4049:2009, Dentistry-Polymer-based restorative materials verifies that the flexural strength of polymer based direct restorations that include the occlusal surfaces of teeth should be greater than 80 MPa.

The average results of flexural strength of experimental dental composites in this study were all below 80 MPa except F2 and F4 which showed 98 MPa following 24 h soaking in deionized water. However all of these figures declined dramatically after longer immersion time (2 weeks and 1 month). Consequently, the strength of dental composite restorations is not matching the ISO test and hence the long term prognosis is affected.

It has been stated that the enhancement of mechanical properties of new generations of dental composites has been obtained through improvements in the chemical bond between the resin matrix and inorganic filler via coupling agent use (Xu et al., 2002a, Sakaguchi and Powers, 2012, Anusavice et al., 2012, Mehdawi et al., 2013). Many researchers have thought to silanize the fillers (Wasson and Nicholson, 1993), to change their fraction, particle size and

shape, to involve heat treatment, to add in fibers(Knight, 1994, Tyas et al., 1989), nanoparticles (Ikejima et al., 2003) and ceramic whiskers (Firoozmand et al., 2013, Xu et al., 2002b, Bowen, 1963a).

Recently, it has been found that there is an advantage of adding silica which has been fused onto silicon carbide or silicon nitride whiskers in improving mechanical properties at the same time as keeping the ion release activity of calcium phosphate based dental composites (Xu and Quinn, 2001, Xu et al., 2002b). When the heat treatment was used to fuse the silica nanoparticles to the whiskers, the effect was helpful in facilitating the silanization and retention in the resin matrix phase and in that way increases the mechanical properties of the composite.

In numerous researches, it has been stated the advantages of adding glass fibers over using the glass only in composite preparation but this fiber glass has not been included in the formulations of this study (Asakawa et al., 2013, Chong et al., 2007, Kim et al., 2011, Rashidan et al., 2010).

4.5.2 Modulus of Elasticity

Modulus of elasticity is considered an important feature of any composite material because it describes the rigidity of the material. Clinically, there is a demand for dental composites with a range of moduli. Class V cavities, for example, require a restorative material with a low modulus to be able to flex with the tooth. However, a relatively higher modulus is needed to restore posterior teeth where it is important to be able to overcome the masticatory forces (Junior et al., 2007) .

As shown in the results chapter, the moduli of elasticity of experimental composites are lower than the commercial and control ones. A possible explanation of this could be due to adding high percentages of reactive fillers which can generate a more flexible polymer network than the commercial and

control materials; hence the modulus is low. Another reason for this could be the prolonged time of soaking the material in the deionised water. This could affect the behaviour of the material and make it more plasticized and flexible. Moreover, all the fillers that have been added caused porosities in the composite and therefore more water sorption which also can affect the modulus.

Chapter Five

Conclusion and Future Work

5 Conclusion and Future Work

5.1 Conclusion

Remineralising and antibacterial releasing dental composites have been developed in the current study. TCP was replaced with TSrP and addition of two different antimicrobial, one of them (PLS), with different percentages. Several experiments have been carried out trying to prove the remineralizing and antibacterial releasing ability of these formulations without compromising the mechanical properties. In all formulations the MCPM played the essential role in water sorption and therefore diffusion of the remineralizing ions and antimicrobial agents and no difference was observed between TCP and TSrP. Addition of PLS with different percentages encouraged more release of CHX and enhanced the precipitation of Ca and PO_4 ions. Remineralizing layer was precipitated on the composite surfaces that were soaked in SBF and proved by Ca/P ratio which helps in remineralizing demineralized dental tissue. Higher monomer conversions were obtained with higher PLS formulations compared to Z250 and glass. On the other hand, the mechanical properties of these dental composites were affected by addition of different reactive fillers. To sum up, this work proved that replacing TCP by TsrP had no effect on the properties of dental composite and addition of MCPM and different percentages of PLS played the important role in generating remineralising and antibacterial releasing dental composite; however high percentages of antimicrobial drugs decreased the strength of the material dramatically.

5.2 Future Work

In this study the water sorption, antimicrobial release, hydroxyapatite precipitation, monomer conversion and mechanical properties have been

characterized. Many other aspects of the material need further investigations. In the following paragraphs there are some suggestions for some future work.

1. Ideally the composite material should not affect the dental tissue cells and surrounding oral tissue; therefore the biocompatibility of the formulations will be assessed. The effect of changing percentages of reactive fillers which include remineralising agents and antimicrobial drugs as well as the monomer will be determined. In addition, Monomers leaching can be measured by using Performance Liquid Chromatography (HPLC). This will include testing the medium in which composite is stored after light curing.

2. Furthermore, the remineralising property explained in the current study should be further explored. These formulations can be tested by attaching them to demineralized or decayed human dentine, then left in SBF and assessed at different time points. The amount of minerals in the human dentine then would be analysed at different time points. EDX will help to show the possible increase in calcium and phosphate levels.

3. Constant Depth Film Fermenter (CDFF) can be applied to further assess the antimicrobial activity of the formulations generated in this project.

4. The adhesion properties of the experimental dental composite with human dentine can be investigated in the next stage. This will be checked by long term soaking of specimens in distilled water or SBF and measuring the strength of bond afterward. This will provide an idea about the long term stability of the restoration in the oral cavity.

5. Although adding two antimicrobial agents improved the water sorption, the percentages added were so high and affected the mechanical properties of the composite. It is suggested to reduce the percentages of these drugs to generate stronger and more durable composite.

Chapter Six

References

6 References

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Chapter Seven

Appendix

7 Appendix

7.1 Post Hoc Tests for monomer conversion

Multiple Comparisons

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
F1	F2	4.06667*	.62302	.000	1.7934	6.3400
	F3	-.30000-	.62302	1.000	-2.5733-	1.9733
	F4	4.33333*	.62302	.000	2.0600	6.6066
	z250	15.33333*	.62302	.000	13.0600	17.6066
	Glass	4.66667*	.62302	.000	2.3934	6.9400
F2	F1	-4.06667-*	.62302	.000	-6.3400-	-1.7934-
	F3	-4.36667-*	.62302	.000	-6.6400-	-2.0934-
	F4	.26667	.62302	1.000	-2.0066-	2.5400
	z250	11.26667*	.62302	.000	8.9934	13.5400
	Glass	.60000	.62302	1.000	-1.6733-	2.8733
F3	F1	.30000	.62302	1.000	-1.9733-	2.5733
	F2	4.36667*	.62302	.000	2.0934	6.6400
	F4	4.63333*	.62302	.000	2.3600	6.9066
	z250	15.63333*	.62302	.000	13.3600	17.9066
	Glass	4.96667*	.62302	.000	2.6934	7.2400
F4	F1	-4.33333-*	.62302	.000	-6.6066-	-2.0600-
	F2	-.26667-	.62302	1.000	-2.5400-	2.0066
	F3	-4.63333-*	.62302	.000	-6.9066-	-2.3600-
	z250	11.00000*	.62302	.000	8.7267	13.2733
	Glass	.33333	.62302	1.000	-1.9400-	2.6066
z250	F1	-15.33333-*	.62302	.000	-17.6066-	-13.0600-
	F2	-11.26667-*	.62302	.000	-13.5400-	-8.9934-
	F3	-15.63333-*	.62302	.000	-17.9066-	-13.3600-
	F4	-11.00000-*	.62302	.000	-13.2733-	-8.7267-
	Glass	-10.66667-*	.62302	.000	-12.9400-	-8.3934-
Glass	F1	-4.66667-*	.62302	.000	-6.9400-	-2.3934-
	F2	-.60000-	.62302	1.000	-2.8733-	1.6733
	F3	-4.96667-*	.62302	.000	-7.2400-	-2.6934-
	F4	-.33333-	.62302	1.000	-2.6066-	1.9400
	z250	10.66667*	.62302	.000	8.3934	12.9400

7.2 Univariate Analysis of Variance for mass and volume after two weeks

Source	Type III Sum of Squares	Mean Square	Sig.
Corrected Model	187.672[a]	26.810	.000
Antimicrobial	170.959	170.959	.000
Ca_Sr	1.654	1.654	.022
Medium	6.225	6.225	.000
Antimicrobial * Ca_Sr	2.454	2.454	.006
Antimicrobial * Medium	5.362	5.362	.000
Ca_Sr * Medium	.664	.664	.136
Antimicrobial * Ca_Sr * Medium	.354	.354	.272
Error	9.076	.284	
Corrected Total	196.748		

Source	Type III Sum of Squares	Mean Square	Sig.
Corrected Model	14.742[a]	2.106	.015
Antimicrobial	6.972	6.972	.003
Ca_Sr	2.756	2.756	.056
Medium	.042	.042	.808
Antimicrobial * Ca_Sr	2.652	2.652	.060
Antimicrobial * Medium	1.560	1.560	.145
Ca_Sr * Medium	.756	.756	.307
time * Medium	.000	.	.
Antimicrobial*Ca_Sr* Medium	.002	.002	.955
		.	.
Error	22.412	.700	
Corrected Total	37.154		

7.3 Univariate Analysis of Variance for CHX release

Source	Type III Sum of Squares	Mean Square	Sig.
Corrected Model	445.000[a]	148.333	.000
Antimicrobial	405.000	405.000	.000
Ca_Sr	39.200	39.200	.002
		.	.
		.	.
Antimicrobial * Ca_Sr	.800	.800	.600
Error	44.800	2.800	
Corrected Total	489.800		

7.4 Univariate Analysis of Variance for PLS release

Source	Type III Sum of Squares	Mean Square	Sig.
Corrected Model	1549.800[a]	516.600	.000
Antimicrobial	1548.800	1548.800	.000
Ca_Sr	.800	.800	.780
Antimicrobial * Ca_Sr	.200	.200	.889
Error	158.000	9.875	
Corrected Total	1707.800		

7.5 Univariate Analysis of Variance for Strength and Modulus

Source	Type III Sum of Squares	Mean Square	Sig.
Corrected Model	36766.550[a]	3342.414	.000
Antimicrobial	2528.663	2528.663	.000
Ca_Sr	90.731	90.731	.381
time	33276.896	16638.448	.000
Antimicrobial * Ca_Sr	39.122	39.122	.564
Antimicrobial * time	196.407	98.204	.435
Ca_Sr * time	284.925	142.463	.301
Antimicrobial * Ca_Sr * time	349.806	174.903	.231
Error	6983.085	116.385	
Corrected Total	43749.635		

Source	Type III Sum of Squares	Mean Square	Sig.
Corrected Model	37.058[a]	3.369	.000
Antimicrobial	6.249	6.249	.000
Ca_Sr	.257	.257	.137
time	28.211	14.105	.000
Antimicrobial * Ca_Sr	.326	.326	.095
Antimicrobial * time	.618	.309	.074
Ca_Sr * time	.288	.144	.289
Antimicrobial * Ca_Sr * time	1.110	.555	.011
Error	6.808	.113	
Corrected Total	43.866		