Development of Antibacterial-releasing Dental Composites with High Strength and Dentine Bonding

Thesis submitted by

Saad Liaqat

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Eastman Dental Institute

Division of Biomaterials and Tissue Engineering

University College London

256 Gray’s Inn Road

London

WC1X 8LD

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Dedicated to

My family and friends
Declaration

I, Saad Liaqat 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.
Acknowledgements

Prima facie, I am grateful to the God for the good health and wellbeing that were necessary to complete this thesis.

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Abstract
The thesis aim is to develop a dental composite with (1) high conversion/depth of cure to provide good strength, (2) water sorption to compensate shrinkage and promote antibacterial release, and (3) added re-mineralising components and acidic monomers to enhance bonding.

Conversion/shrinkage, depth of cure, water sorption, and antibacterial (polylysine & chlorhexidine) release into distilled water versus simulated body fluid were assessed using FTIR, ISO 4049 (scraping test), gravimetric studies, and UV spectroscopy respectively.

Flexural strength/modulus was assessed up to 6 months of water storage. Similarly bonding to moist ivory and human dentine was assessed via a push out and shear bond test.

Factorial analysis was used to analyze the data. The results showed that a major factor affecting the conversion, and shrinkage was sample thickness. Similarly a major factor affecting the depth of cure was duration of light cure. In water sorption studies the major factors enhancing water sorption were use of distilled water, and polylysine. The chlorhexidine release was enhanced by the use of distilled water, while a higher polylysine release percentage was seen with lower levels of drug in the filler phase. The strength and modulus were decreased with the addition of reactive fillers. Lastly, adhesion was improved with the use of adhesive, and acidic monomers.

The materials produced could potentially reduce bacterial micro leakage, which is the most common reason for failure.
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List of abbreviations

ATR FTIR  Attenuated total reflectance fourier transform spectroscopy

BFS  Biaxial Flexural Strength

Bis-EMA  Ethoxylated bisphenol-A dimethacrylate

Bis-GMA  Bisphenol A glycidyl methacrylate

HA  Hydroxyapatite

CaP  Calcium Phosphate

CHX  Chlorhexidine

GIC  Glass ionomer cement

DMPT  Dimethyl para toluidine

E  Young's Modulus

EDX  Energy dispersive X-ray

MDP  10-Methacryloyloxydecyl Dihydrogen Phosphate

GF  Glass fibre

GP  Glass powder

HEMA  Hydroxyethyl methacrylate

MCPM  Monocalcium phosphate monohydrate

PLR  Powder to liquid ratio

SEM  Scanning electron microscopy

TCP  Beta-tricalcium phosphate

TEGDMA  Tri(ethylene glycol) dimethacrylate

$T_g$  Glass transition temperature

UDMA  Urethane dimethacrylate
UV  Ultraviolet-visible spectroscopy
RMGIC  Resin modified glass ionomer cement
4-META  4-Methacryloxyethyl trimellitic anhydride
ACP  Amorphous calcium phosphate
MDPB  Methacryloyloxydodecyl pyridinium bromide
QPEI  Quaternary ammonium polyethylene imine
GPDM  Glycerol dimethacrylate ester of phosphoric acid
SBF  Simulated body fluid
DW  Distilled water
CQ  Camphorquinone
PLS  Polylsine
DOC  Depth of cure
TB  Trypan blue
A  Apatite
IA  Acid etching followed by Ibond
IAn  Only Ibond application
InA  Only acid etching
InAn  No dentine pre-conditioning with acid or Ibond
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW
1. Introduction and Literature Review

In the following sections the literature surrounding this project will be discussed briefly. The sections below will cover literature surrounding dental caries, dentine characterisation, restorative materials, adhesive systems, and properties of composites that will be assessed in the following chapters. The potential problems and shortfalls with current materials will be identified in addition to project scope and aims.

1.1. Dental Caries

1.1.1. Introduction

Dental caries, commonly known as tooth decay, is one of the most common diseases affecting people of all ages. Dental caries causes destruction of dental hard tissues due to the production of acidic by-products by bacterial fermentation of carbohydrates (Selwitz et al., 2007). Dental caries, if left un-treated, can cause damage that can be biological, and physical. In order to stop caries advancement and restore structure and function of the tooth, the damaged dental structures are repaired using different restorative materials.

1.1.2. Aetiology of dental caries

Caries is a complex process, with many factors involved in the progress of the disease. The major etiological factors involved are dental plaque (biofilm), dietary (carbohydrates), and salivary factors.

Dental plaque is a very organised biofilm both structurally and functionally (Marsh, 2006). In dental caries, acidogenic bacteria like *streptococcus mutans* and *lactobacilli* dominate the disease process. Although the dental biofilm is known to be important for caries development, most of the bacteria present are not an etiologic factor.
Another factor involved in the caries process is dietary carbohydrates. Frequent consumption of simple carbohydrates, is mainly linked with increased caries risk. This is usually consumed in the form of dietary sugars (Mobley et al., 2009). These carbohydrates are required by bacteria for the production of intracellular and extracellular polysaccharide matrices. The later aids bacterial adhesion to tooth surfaces.

Lastly, saliva can play a major role in dental caries. Saliva primarily works to minimise the plaque accumulation through its cleansing action. It also acts as a continuous source of minerals to help in reducing enamel solubility. It can act as an antibacterial and buffering agent. Significant decline in local pH results in increased solubility of hydroxyapatite due to a change in the chemical equilibrium of the tooth surface (Jawed et al., 2012). This disruption of equilibrium leads to caries initiation.

In order to prevent dental caries, the course of biofilm development can be controlled by either mechanical or chemical methods. Mechanical methods include tooth brushing, flossing, and professional scaling. Chemical methods involve use of antimicrobials and antiseptics such as chlorhexidine (Tariq et al., 2012).

1.2. Dentine

One of the aims of this project was to characterise dentine from alternate sources. The alternate source of dentine in this project was ivory. In the following section both human and ivory dentine will be described in detail.

1.2.1. Human Dentine

Dentine is considered as the most abundant mineralised tissue in the tooth (Kinney et al., 2003). The properties of dentine (chemical, structural and mechanical) can be highly variable. Understanding factors controlling these properties are key for gaining good bonding.
1.2.1.1. Structure of Human Dentine

Dentine lies below the enamel and surrounds the pulp chamber and root canals. Structurally dentine consists of dentinal tubules that radiate in an outward direction from the pulp to cementum or enamel on the outside. Dentinal tubules are enfolded in peri-tubular dentine and inter-tubular dentine, which contains collagen fibres (Zhang et al., 2014b).

1.2.1.2. Composition of Human Dentine

Dentine is composed of 70 % mineral phase, 20 % organic matrix, and 10 % water by weight (Goldberg et al., 2011). Dentine is considered as a mineralised connective tissue. In its composition as well as its mode of formation, dentine shows numerous similarities with bone, but also certain differences. The dentine organic phase determines its morphology and is believed to be instrumental in the structure of the mineral phase.

1.2.1.3. Types of Dentine

Dentine can be sub-divided into three different types subject to age and in response to stimulus. The dentine formed by odontoblasts during tooth growth is called primary dentine. The primary dentine is composed of straight tubules (Cajazeira Aguiar and Arana-Chavez, 2007). After tooth eruption, the odontoblasts continue to divide and to secrete dentine matrix producing secondary dentine. The secondary dentine is deposited at a slower rate and has more random tubule direction. Furthermore, a third type of dentine called tertiary dentine may be placed in specific areas of the dentine–pulp interface. There are two subtypes of tertiary dentine. That laid down by odontoblasts in response to an appropriate stimulus, is called reactionary dentine. The second subtype is called reparative dentine. This is formed by odontoblast-like cells, in the dental pulp after the loss of the original odontoblasts (Arana-Chavez and Massa, 2004). The different dentine types are shown in figure 1-1.
1.2.2. **Ivory Dentine**

Ivory tusk is a hard, smooth, yellowish white structure. Tusks consist of a peripheral component, the cementum, and a main core of dentine.

1.2.2.1. **Structure of Ivory Dentine**

Structurally, ivory resembles reinforced concrete. The tubules present in ivory look like cylinders or are filled in to form rods. In a matrix of particles and ground substance they look like the metal rods in the pebbles and cement of concrete (Locke, 2008). The tubules are laid in the matrix, and stack one above the other axially to form micro-laminae, usually in the radial/axial plane. The transverse section of ivory shows dentine with Schreger lines and an outside cementum layer (figure 1-2).
1.2.2.2. Composition of Ivory Dentine

Ivory dentine is an inorganic / organic composite composed of a hydroxyapatite (HA)-like mineralised component (60–70 weight %) in which about 20 % of the Ca$^{2+}$ ions are replaced by Mg$^{2+}$, embedded within an organic matrix (type I collagen, 30 weight %). Most of the remaining content is water (Jakubinek et al., 2006).

The structure and composition of the ivory varies in different mammals. Ivory, by description, is the dentine of large teeth, but teeth became large in response to many different evolutionary stimuli (Locke, 2008). The ivory varies considerably among different mammal groups, considering their mechanical necessities.

1.3. Current Restorative Materials

Today, teeth can be filled with gold, porcelain and amalgam, or tooth-coloured materials such as glass ionomer cements and composite. The location and extent of the decay, cost of filling material, patients' insurance coverage, and dentist's recommendation assist in determining the type of filling used.
1.3.1. **Dental amalgam**

Dental amalgams have successfully served the profession for over a century. It was considered as the restorative material of choice, mainly because it is easy to apply, has good strength, durability and low cost. The main drawbacks associated with amalgam restorations were secondary caries formation, restoration fracture, and marginal deficiencies (Shenoy, 2008). Amalgam restorations, however, are increasingly being replaced because of the concerns with mercury toxicity, and poor aesthetics.

1.3.2. **Dental composites and adhesives**

Dental composites are considered as one of the big achievements of modern biomaterials (Cramer et al., 2011a). It replaces and restores the biological tissues. Dental composites are tooth coloured restorative materials. Dental composites usually contain a resin matrix, and inorganic fillers. The fillers can be glass or other reinforcing fillers. The matrix is mainly formed from high molecular weight monomers such as urethane dimethacrylate (UDMA), and bisphenol A-diglycidyl methacrylate (Bis-GMA) (Tsitrou et al., 2014). Fillers are added to increase strength, reduce polymerisation shrinkage and heat generation (Schneider et al., 2010a). A silane coupling agent is used to augment the bond between these two components and to aid filler distribution. An initiator and activator are usually added to begin and later control the polymerisation process when external energy (light) is applied. The composite adhesives have similar chemistry to the composite but usually without filler added.

1.3.2.1. **Chemistry of Materials used in Dental Composites**

The composites are made up of a resin and a filler phase. The chemical structures of a few individual components used in composites are given in Figure 1-3.
1.3.2.1.1. Monomer (Organic) matrix used in dental composites

The organic matrix makes up the body of the composite and is formed by polymerisation of dimethacrylate monomers, through a free radical addition reaction. The organic matrix also contains diluent co-monomers plus polymerisation initiator and activator systems.

1.3.2.1.1.1. Bisphenol A diglycidyl methacrylate (Bis-GMA)

Bis-GMA (2, 2-bis [4- (2-hydroxy-3-methacryloyloxypropyl) phenyl] propane) is formed by the reaction of Bisphenol-A and glycidyl methacrylate. It is a high molecular weight (MW =
monomer, containing two aromatic rings with suspended hydroxyl groups (─OH─). The existence of the double rigid aromatic group and hydrogen intermolecular bonding of hydroxyl groups are responsible for the high viscosity of the monomer (Van Landuyt et al., 2007, Pfeifer et al., 2011). Consequently, the glass transition temperature (Tg) is enhanced and the degree of monomer conversion reduced (Pereira et al., 2002). Due to its high molecular weight, Bis-GMA provides low polymerisation shrinkage and rapid hardening (Shalaby and Salz, 2006).

1.3.2.1.1.2. Urethane Dimethacrylate (UDMA)

Urethane Dimethacrylate (1, 6-bis-[2-methacryloyloxyethoxycarbonylamino]-2,4,4-trimethylhexane) is often used in light-cure systems. UDMA is used either with or as an alternative to Bis-GMA monomer. UDMA is an aliphatic high molecular weight monomer with two imine groups (─NH─). These, through intermolecular hydrogen bonds, can associate with carbonyl groups (C=O). Such intermolecular hydrogen bonds are responsible for the high viscosity (Sideridou et al., 2002). The imine group though, produces weaker hydrogen bonds than the hydroxyl group (─OH─) of Bis-GMA (Barszczewska-Rybarek and Jurczyk, 2015). In addition, the degree of conversion of UDMA is increased by the existence of flexible aliphatic chains. Moreover, the UDMA polymer shows lower water sorption and releases less unreacted species compared to Bis-GMA (Goncalves et al., 2008). This has been attributed to greater polymerisation, and a lower water affinity of the urethane group (─NHCOO─) of UDMA than the hydroxyl group of Bis-GMA. Moreover, UDMA is less cytotoxic than Bis-GMA, in vitro (Goldberg, 2008). In adhesives, UDMA is used on its own or in a blend with triethylene glycol dimethacrylate (TEGDMA) and Bis-GMA. Its main dissimilarity from the latter is its flexibility, and reduced viscosity as the ether bonds in UDMA allow easy rotation as compared to the two bulky aromatic rings in Bis-GMA (Van Landuyt et al., 2007).
1.3.2.1.1.3. Triethylene glycol dimethacrylate (TEGDMA)

Most dental resinous materials contain high quantities of the diluent monomer triethylene glycol dimethacrylate (TEGDMA). TEGDMA is an aliphatic and hydrophilic monomer with much lower viscosity than UDMA, and Bis-GMA (Pereira et al., 2005). This lower viscosity aids in achieving high filler loading in composites. Its glass transition temperature (Tg) is similar to UDMA, and lower than Bis-GMA. This can result in a higher degree of conversion (2.5 times more than Bis-GMA) (Morgan et al., 2000). TEGDMA shows water affinity, which is mainly attributed to the presence of ether linkages (C-O-C).

TEGDMA is usually used in combination with Bis-GMA or UDMA, mainly to reduce their viscosity. This admixture will result in resins with higher conversion rate. High conversion will, however, increase the polymerisation shrinkage (Floyd and Dickens, 2006).

1.3.2.1.2. Initiator and Activator system

Dental composite resins are cured through a free radical addition polymerisation reaction. Free radicals are generated either by photo or chemical activation. Photo activated systems mainly use visible light and camphorquinone (CQ) as a free radical photo initiator with a tertiary amine, such as N, N-dimethyl-p-toluidine (DMPT) as activator.

Camphorquinone is sensitive in the range of 360-510 nm, with peak absorbance at 468 nm (blue light). Camphorquinone is a crystalline powder at room temperature, with limited solubility in water. The main drawback of CQ is its characteristically yellowish-brown colour. CQ is usually used in small amounts (0.03–1%). Despite this it still influences the colour of the adhesive resin considerably (Van Landuyt et al., 2007). Although, the yellow colour partially fades after curing, the remaining yellow colour may possibly cause problems in colour matching.
1.3.2.1.3. Inorganic Filler Coupling Agent

The inorganic filler is often a fluoroalumino silicate glass. A broad range of particle sizes is generally employed to maximize filler content (see section 1.3.2.3.2). The importance of a coupling agent is to provide bonding between monomer phase and the filler phase of the composite to allow the transfer of stress from their matrix to higher modulus filler particles (Halvorson et al., 2003). Different coupling agents like titanates and zirconates have been used as coupling agents but the most common coupling agents are organosilanes such as γ-methacryloxypropyl trimethoxysilane (Anusavice et al., 2012). ‘Silanation’ improves the composite resistance to hydrolytic degradation and enhances mechanical properties through better distribution and stress transmission from flexible monomer matrix to the stiffer and stronger inorganic fillers (Spitznagel et al., 2014).

1.3.2.2. Current Composite Adhesive Systems

Historically, the main problem associated with composites was micro-leakage (Yamazaki et al., 2006). This is particularly important in case of dentine bonding. Microleakage causes marginal leakage and induces post-treatment sensitivity. It can also result in marginal staining. If not treated in time secondary caries can develop beneath the restoration, which will cause pulpitis and eventually pulp necrosis (Tronstad, 2008).

Micro-leakage can occur because of four possible causes: first factor contributing in micro-leakage is polymerisation shrinkage, second factor is lack of self-sealing mechanism of adhesive resins with tooth structure, third factor contributing is the difference in the coefficient of thermal expansion between composite and tooth, and last factor is occlusal loading (Charlton, 1995).

Traditionally, enamel bonding showed good strengths, and had few failures as compared to dentine bonding (Burrow et al., 2008). Dentine bonding is considered more complex. There
are various factors that made bonding to dentine complex, and weak. First, dentine contains more water and less mineral than enamel. Second, the presence of a smear layer makes it difficult for the adhesive to properly wet, and spread across the dentine surface. Even with good wetting the polymerisation shrinkage will pull away the smear layer and cause gap formation. Smear layers are formed on hard tissues whenever they are cut with rotary or hand instruments. The smear layer is a layer of microcrystalline and organic particle debris that is found spread on tooth surfaces after instrumentation (Pashley, 1991). Lastly, the presence of fluids in the tubules will make the bonding less stable between dentine and composite (Tay and Pashley, 2003).

1.3.2.2.1. Chemistry of common monomers used in dental adhesives

1.3.2.2.1.1. 2-Hydroxyethyl methacrylate (HEMA)

2-Hydroxy ethyl methacrylate (HEMA) is commonly used in dental adhesives systems as a solvent and adhesion-promoting agent due to its ability to interact with hard tooth structures. HEMA is an aliphatic low molecular weight monomer, with wide biomedical uses (Lee and Mooney, 2001). HEMA is hydrophilic in nature and this property is attributed to the existence of an OH group. The surfactant-like properties of HEMA aids penetration of the adheisve into water filled tubules and thereby at low levels helps improve bonding. Adhesives with high levels of HEMA, however, are more prone to water contamination. HEMA when uncured absorbs water. This can lead to monomer dilution and thereby halt the polymerisation process (Tay and Pashley, 2003). After polymerisation, poly-HEMA also attracts water and creates hydrogels which weaken the mechanical strength of the polymer (Hosaka et al., 2010).

1.3.2.2.1.2. 4-Methacryloxyethyl trimellitic anhydride (4-META)

4-Methacryloxyethyl trimellitic anhydride (4-META) is an acidic monomer. 4-META is used currently in adhesive systems mainly because of its adhesion promoting and de-mineralising properties (Van Landuyt et al., 2007). 4-META is a crystalline powder. After addition of water
to 4-META powder, a rapid hydrolysis reaction will occur to form 4-MET. After hydrolysis two carboxylic groups are produced which are attached to the aromatic group. These carboxylic groups provide acidic and therefore de-mineralising properties. Additionally, these acidic groups increase the wetting properties. Furthermore, 4-MET is able to establish an ionic bond with calcium in hydroxyapatite. The aromatic group, however, is hydrophobic and will partially counteract the acidity and the hydrophilicity of the carboxyl groups.

This monomer is highly soluble in acetone, moderately soluble in ethanol, but has little solubility in water (Moszner et al., 2005). Nevertheless, ethanol is not a suitable solvent for this monomer, as esterification of the carboxylic groups with the hydroxyl group can occur, particularly in acidic conditions. (Van Landuyt et al., 2007).

1.3.2.2.1.3. Glycero-phosphate dimethacrylate (GPDM)

The phosphorus-containing monomer, glycero-phosphate dimethacrylate (GPDM), is capable of etching enamel and dentine (Zimmermann, 2006). In addition, this monomer also promotes diffusion into the acid-conditioned and underlying sound dentine. GPDM is used as a monomer in some of the present self-etching enamel-dentine adhesives (e.g Optibond XTR).

The chemical structures of a few commonly used adhesion promoting monomers are given in figure 1-4.
1.3.2.2.2. Classification of Dental Adhesives

Dental adhesives can be classified in two different ways; first its development with time, and second on the underlying adhesion strategy.

The first and second generation of adhesives were developed in 1960s, and were used in practice till 1970s. These two generations of adhesives relied on bonding to the smear layer, and did not recommend the use of acid etching. The resultant bond strengths were very weak. As a result they were unable to prevent marginal leakage and staining (Nazarian, 2011, Swift, 2002).

The third generation was developed in 1980s. This introduced the use of separate primer and acid etching to allow better penetration of tubules. This results in relatively better adhesion strengths than the first two generations, but the problems of marginal staining, and failure of bonding at the margins with dentine limits its success (Swift, 2002).

The fourth generation came into practice in early 1990s. These adhesives were able to bond with etched dentine, and penetrated tubules. They formed a hybrid layer (consists of collagen with adhesive resin) which gives high strength, and better dentine sealing. The bond strength

Figure 1-4 Chemical structures of common monomers used in adhesives.
with these adhesives were comparatively better than the previous generations, and they showed significantly less micro-leakage. However, due to the number of steps involved in its application, this adhesive system was considered very technique sensitive (Kugel and Ferrari, 2000). The steps include acid etching followed by separate primer and adhesive application. This generation showed the same issues of marginal staining.

In mid 1990s fifth generation adhesives were developed. In these the primer, and adhesive were combined in one bottle to reduce the number of steps. The adhesives in this generation showed high strengths, but there were still some issues such as poorly controlled surface wetness, adhesive placement, and etching (Stalin and Varma, 2005).

In late 1990s and early 2000s the concept of self-etching primers was introduced in the sixth generation. Primers consists of acidic or hydrophilic monomers dissolved in solvent such as acetone, ethanol, or water. This was a big step in the adhesive industry. In this class the acidic monomers (eg 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), glycerophosphate dimethacrylate (GPDM), and 4-methacryloyloxyethy trimellitate anhydride (4-META) were incorporated into the primer and placed on the dentine, and enamel immediately after cavity preparation (Yaseen, 2009). A separate etching step was eliminated. This system also reduced the post-operative sensitivity (Singh, 2008). The major issue was its reduced bond strength compared with earlier fourth and fifth generations.

In mid 2000s the seventh generation was developed. The concept of all in one was introduced, which combined the etchant, primer, and adhesive into one bottle. They have similar marginal properties with sixth generation. This includes the Ibond total etch (Heraeus Kulzer, Germany) which was used in this project. The Ibond total etch consists of UDMA, 4-META, glutaraldehyde, acetone, water, photo-initiators, and stabilisers. The 4-META helps in the de-
mineralisation of dentine, and as a result improved bonding with the tooth structure. This generation provided good bond strength and sealing (Knobloch et al., 2007).

Adhesives can also be classified based on the underlying adhesion strategy as “etch & rinse,” or “self-etch” adhesives (figure 1-5).

'Etch-and-rinse' adhesives consists of a separate etch-and-rinse stage. This type of adhesive is considered as the most effective approach to achieve stable bonding to enamel (Cardoso et al., 2011). Usually an acid (mostly 30-40% phosphoric acid gel) is applied and rinsed off. The acid conditioning step is followed by a priming step and application of the adhesive resin, making it a three-step application procedure. Simplified two-step etch-and-rinse adhesives combine the primer and adhesive resin into one (De Munck et al., 2005a). The primers in three-step etch & rinse systems usually contain 2-hydroxyethyl methacrylate (HEMA), a polyalkenoic acid, initiators and solvent (water, acetone and / or ethanol). The adhesive resin often contains Bis-GMA, HEMA, tertiary amines (both for light-cure and self-cure initiators) and a photo-initiator (Van Meerbeek B, 2003).

‘Self-etch’ adhesives are based on the use of non-rinse acidic monomers that condition and prime dentine at the same time. This approach is favoured by the clinician as it eliminates the rinsing phase. This not only lessens the clinical application time, but also significantly reduces the technique-sensitivity. For 2-step self-etch adhesives, the primer contains acidic monomers, HEMA, hydrophilic dimethacrylates (TEGDMA), photo-initiators and water. The bonding adhesive may contain MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, photo-initiators, silanated colloidal silica, surface-treated NaF. The one-step self-etch adhesive Ibond contains UDMA, 4-META, glutaraldehyde, acetone, water, photo-initiators, and stabilisers (Van Landuyt et al., 2007).
1.3.2.3. Indications, Classification, and Drawbacks of Dental composites

1.3.2.3.1. Indications of composites

Development of dental composites started back in late 1950s, with the experiments of Bowen on epoxy resins reinforced with fillers (Drummond, 2008). After that composites were progressively being used as restorative materials, core build-up materials, inlays, onlays, crowns, cavity liners, and pit and fissure sealants (Ferracane, 2011). With time, extensive studies have enabled some major improvements in filler and polymer matrix compositions as well as curing and handling characteristics (Bayne et al., 1998, Lim et al., 2002, Watts et al., 2003, Lu et al., 2005, Xu et al., 2004, Drummond, 2008).

Dental composites are usually custom-made for particular restorative requirements. They can be used as a filling material, as cement, and sealant, etc. Composites can also be differentiated on the basis of their consistency and flow properties. They can be either flowable with low
filler content (Bayne et al., 1998), or packable with high filler to monomer ratio (Choi et al., 2000).

### 1.3.2.3.2. Classification of composites

Composite materials can be further divided by the filler sizes. Subdivisions include *macrofill* with particle size ranging from 10-50 μm (Ferracane, 2011). Larger filler size enables higher filler loading which can enhance strength. However, the surface smoothness and polishing is not good. Next one in this class is called *microfill*, with particle size ranging from 40-50 nm (Ferracane, 2011). The important changes that were made in this class of composite were the nano sized fillers, which helped to improve long term aesthetics and polishing properties as compared to *macrofill* composite. However, the low filler content reduced composite strength, and increased polymerisation shrinkage (Ferracane, 2011).

To address these problems of *macrofill* and *microfill* composites, *hybrid* composites with particles of different size ranges, were developed. Hybrid composites are further divided into *minifill*, with particles of sub-micron size 0.4- 1 μm, and a portion of 40 nm (Bayne et al., 1994). The second hybrid composite is called *midfill*, with a combination of particles. Some of the particles are greater than 1 μm, while the rest are 40 nm sized fumed silica. The recent development in this class is called *nanofill* (Association, 2006), with particles ranging from 5-100 nm. Many manufacturers have further modified their microfill composites, with nano sized particles, and pre-polymerised fillers to form a new group called *nanohybrids* (Ilie and Hickel, 2009a, Association, 2006). All of these composites possess different mechanical properties and have different uses (Ferracane, 2011).

### 1.3.2.3.3. Drawbacks of composites

The main drawbacks for most composite restorations are polymerisation shrinkage, complex adhesive procedures for bonding to dentine and brittle fracture (Schneider et al., 2010b).
Composites also have no antibacterial properties and tend to accumulate more biofilm and plaque in vivo than other restorative materials (Zalkind et al., 1998, Beyth et al., 2007). In order to overcome these problems, in this thesis experimental composites are produced containing calcium chelating (4-META), and hydrophilic (HEMA) monomers for dentine bonding. Also included are antibacterial agents to combat infection and calcium phosphates that may encourage water sorption induced swelling and dentine re-mineralisation. All these features together could help prevent bacterial micro leakage.

1.3.3. Conventional and resin modified glass ionomer cement

Glass ionomers were introduced to dentistry over 25 years ago. Glass ionomer cements, are made using calcium, strontium aluminosilicate glass powder (base) combined with an aqueous poly acid solution. Glass ionomers possess certain distinctive properties that make them useful as adhesive and restorative materials. These properties include chemical adhesion to tooth structure, anti-cariogenic properties because of fluoride release, and biocompatibility (Nagaraja Upadhya and Kishore, 2005). One of the drawbacks is their potential sensitivity to water at the early stage of setting. Also their flexural strength is low and can decline further if exposed to saliva during the early stage of setting (Ana et al., 2003).

In order to overcome setting and low mechanical problems associated with conventional GICs, resin modified glass ionomers (RMGIC) were developed. Simplistically, RMGICs are a hybrid of glass ionomers and composite resin, and thus contain acid-base and polymerisable components (Berzins et al., 2010). RMGICs are usually made of basic ion-leachable glass powder and a water-soluble polymeric acid such as poly (acrylic acid). In addition, they contain organic monomers, typically 2-hydroxyethyl methacrylate (HEMA), and an associated initiator system (McLean et al., 1994).
Resin-modified glass-ionomer cements can be cured by light and / or chemical activation of monomer polymerisation. Light curing is through the use of conventional dental curing lamp that emits light at a wavelength centred on 470 nm (Nicholson and Czarnecka, 2008). There is also a second reaction involving the polyacid and basic glass. In Fiji II LC (GC America) and Vitrema (3M ESPE) this acid base reaction is much slower than the polymerisation. It is also much slower than in a conventional GIC. This has been achieved by reducing the reactivity of the glass phase, lowering the water content and also through addition of a silane coupling agent. FTIR has also shown that the set materials require water sorption to extend the process (Young, 2002). The polyacrylic acid protons liberate metal ions and fluoride from the glass, forming a silica hydrogel around the glass surface. The rising aqueous phase pH causes polysalt precipitates to form from the migrating ions, which act as cross-links to the polyacrylic acid chains (Berzins et al., 2010).

RMGICs, as with composites, suffer from polymerisation shrinkage which may allow bacterial microleakage (Bryant and Mahler, 2007). Furthermore, cytotoxic HEMA is known to be leached from some resin-modified glass-ionomers (Nicholson and Czarnecka, 2008). Additionally, RMGICs have low flexural strength (< 80) MPa (Sulaiman et al., 2007) when compared with composites. They can, however, adhere to dentine without the use of a composite adhesive through ionic interactions between carboxylic acid groups in the polyacrylic acid and hydroxyapatite in dentine. In this project, the acidic monomer 4-META is added instead of HEMA into experimental composites to enable similar ionic bonding possibilities. The 4-META contains two carboxylic acid groups that have the potential to de-mineralise dentine and promote bonding with the tooth structure.

1.3.4. Compomers

Compomers were devised in an attempt to combine the aesthetics of traditional composite resins and fluoride releasing ability of GIC cements. They are a further group of tooth coloured
restoration materials for replacing tooth structure damaged by dental caries (McLean et al., 1994). Composers are very similar to composites. They contain the same bulky macromonomers, such as bis-glycidyl ether dimethacrylate (Bis-GMA) or its derivatives and/or urethane dimethacrylate, which are mixed with diluents, such as triethylene glycol dimethacrylate (TEGDMA). These polymer systems are filled with non-reactive inorganic powders, such as quartz or a silicate glass, for example Strontium aluminium fluoro silicate glass (Eliades et al., 1998). Composers, however, contain some added acidic monomers like TCB, which is a di-ester of 2-hydroxyethyl methacrylate with butane tetra carboxylic acid. The acidic monomer TCB contains two methacrylate groups as well as two carboxyl groups. The former can crosslink with other methacrylate terminated resins when initiated through radical polymerisation while the later groups can undergo acid-base reaction to form a salt with metal ions and water (Hes et al., 1999). Additionally, reactive glass powder similar to that in glass-ionomer cements are also present (Nicholson, 2007).

Compomers are advocated for similar clinical applications as conventional composites. These include Class II (Qvist et al., 2004) and Class V (Chinelatti et al., 2004, Demirci et al., 2005) cavities, as fissure sealants (Gungor et al., 2004), and bonding agents for the retention of orthodontic bands (Williams et al., 2005). The major drawbacks remain adhesive agent requirement for adhesion, and higher microleakage than an RMGIC. The aim of this project, however, is to assess if these problems can be addressed through combined use of adhesive monomers with reactive calcium phosphates, and antibacterial agents within a composite.

1.4. Recent Advances in Dental Composites

Since their development many changes have been made to the composition of composites. Some of the additives added to composites in recent years are discussed below.
1.4.1. Calcium Phosphate Containing Composites

Many studies have included calcium phosphates into composites to provide re-mineralising action. The calcium phosphate has to be more soluble than hydroxyapatite in order to be released from the set composite (Vallittu, 2012). Generally, lower ratio of calcium to phosphate correlates with higher aqueous solubility. At physiological pH, solubility increases in the order hydroxyapatite, Ca_{10}(PO_4)_6OH_2 < tricalcium phosphate, Ca_3(PO_4)_2 ~ amorphous calcium phosphate, Ca_{3y}(PO_4)_{6-8}·nH_2O < dicalcium phosphate, CaHPO_4 < mono calcium phosphate, Ca(H_2PO_4)_2 (Young, 2010).

1.4.1.1. Amorphous calcium phosphate

Amorphous calcium phosphate (ACP) has been used in many biomaterials as coatings or in cements and composites (Combes and Rey, 2010). Composites containing ACP have been shown to release calcium and phosphate ions, especially in an acidic environment. These ions can take part in re-mineralisation of enamel (Langhorst et al., 2009, Tung, 2004). The low solubility of ACP, however, may hamper calcium phosphate release (Uskoković and Desai, 2013). Furthermore, poor wetting between the monomers and filler as a result of lack of effective coupling agents could limit filler loading and reduce strength (O’Donnell et al., 2009). The maximum biaxial flexural strength (BFS) of these ACP composites rarely exceed 50 MPa (Skrtic and Antonucci, 2011). The following studies address if these problems can be overcome by combining more soluble mono calcium phosphate mono hydrate (MCPM) with β- tri calcium phosphate (TCP) in experimental composites. The concept is that surface MCPM could dissolve but that that in the bulk would react with the TCP and absorbed water producing a new mineral (brushite) of greater volume than the original phosphates and thereby fill gaps produced by component release.
1.4.1.2. Micro and Nano calcium phosphate composites

Composites with micro amorphous calcium phosphate particles of about 1–55 μm in size have been produced (Langhorst et al., 2009, Dickens et al., 2003). These composites released supersaturating levels of calcium (Ca\(^{2+}\)) and phosphate (PO\(_4^{3-}\)) ions and re-mineralised tooth lesions in vitro (Dickens et al., 2003, Langhorst et al., 2009). Recently, ACP nanoparticles of about 100 nm in size were synthesised via a spray-drying technique (Xu et al., 2010, Xu et al., 2004). Composites containing ACP nanoparticles with high specific surface areas were found to release high levels of Ca\(^{2+}\) and PO\(_4^{3-}\) while having mechanical properties nearly two-fold greater than those of previous CaP composites (Xu et al., 2004, Xu et al., 2010, Cheng et al., 2012a). With nano filler particles, however, filler loading in composites is restricted (Rothon, 2003).

1.4.1.3. MCPM and TCP filled composite

Mono calcium phosphate monohydrate (MCPM) and tri calcium phosphate (TCP) were previously incorporated in various systematically varying dental composites (Mehdawi et al., 2009). When combined with water, these two compounds react via hydrogen ion exchange and re-precipitate as brushite (di calcium phosphate di hydrate, CaHPO\(_4\) • 2H\(_2\)O) or the anhydrous form, monetite (Hofmann et al., 2006). In the composites of Mehdawi et al., addition of water-soluble MCPM fillers encouraged water sorption into the set resin materials, which in turn enhanced the release of chlorhexidine and some calcium phosphate species. The absorbed water was shown to promote reaction between MCPM and β-TCP and brushite formation within the polymerised methacrylate. Unfortunately the problem with Mehdawi et al’s study was the low strength of the composite. An aim of this study is to address if this low strength (~60 MPa) may be overcome by partial replacement of the reactive calcium phosphate with more conventional glass fillers in experimental composite formulations.
1.4.2. **Antibacterial Containing Composites**

The lack of antimicrobial agents in dental composite means there will be plaque formation on the composite surface. The standard silica-based filler added to dental composites has no antibacterial action (Imazato, 2003). Furthermore, Bis-GMA, TEGDMA and UDMA do not kill or inhibit growth of *S. Mutans* (Hisamitsu, 1989). Antibiotic use in dental composites is not recommended because of the potential for increasing bacterial drug resistance but various other antimicrobial agents approved for intraoral use have been incorporated into restoratives. The introduction of antimicrobial was either through dissolution into the resin or addition as insoluble particles. Much early research focussed upon the former (Imazato, 2003).

Formulations that either release antimicrobial agents or have the antimicrobial bound in the material have both been produced. For release to occur the antibacterial agent must dissolve readily in water and be able to diffuse from the set composite when placed under aqueous conditions. Release, however, can cause problems such as creation of voids in the matrix phase and strength reduction. The non released agent’s action is usually through direct contact of composite surface antimicrobials with the bacteria. Non release results in these materials having better mechanical properties than antimicrobial-releasing composites. With bound antibacterial agents, however, there may be little benefit once a biofilm has formed. (Beyth et al., 2014).

1.4.2.1. **Flouride addition**

To provide antibacterial action, strontium fluoride or ytterbium fluoride filler additions have been considered. These modified composites, however showed a rapid decline in fluoride release within a day. As a result frequent external application of neutral fluoride was necessary to maintain the high fluoride release (Xu and Burgess, 2003, Yap et al., 1999). Other flourides added into the monomer phase include acrylic-amine-bifluoride (HF) salts (Hicks et al., 2003), methacryloyl acid-fluoride (Wiegand et al., 2007) and acrylic-amine-BF3 It has been found,
however, to be more difficult to release fluoride from composites than from RMGICs (Tanagawa et al., 1999).

1.4.2.2. Metal addition

Other studies suggested adding Ag$^+$ to the filler of resin composite restorative materials may provide protection from secondary caries. Ag$^+$ supported particles incorporated in dental composites showed long lasting inhibitory effect against *S. Mutans*. However, Ag$^+$ brought down the strength of the composites, and caused discoloration of the material (Yoshida et al., 1999). Furthermore, Ag$^+$ has potential adverse effects on the gingival tissue (Kawashita et al., 2000). Other groups have added zinc oxide as an antibacterial agent in composites. With increase in zinc oxide concentration composites antimicrobial activity significantly increased. An observed problem with zinc oxide addition, however, can be a decrease in shear bond strength (Spencer et al., 2009).

1.4.2.3. Organic antibacterial addition

Organic antimicrobial agents that have been added to the composite resin phase include triclosan. The triclosan modified composite reduced the numbers of *Lactobacilli*, but failed to reduce the overall viable counts in a cavity. It was therefore suggested that triclosan-containing materials can be beneficial for the reduction, but not for the eradication of microorganisms (Wicht et al., 2005). Other studies showed that addition of benzalkonium chloride to a composite enhanced its antimicrobial properties, without significantly decreasing the bond strength (Sehgal et al., 2007). A further study proved that incorporation of 2.5 % cetylpyridinium chloride in a composite enabled antimicrobial activity without varying diametral tensile strength. However, the composite safety, and clinical performance is yet to be investigated (Al-Musallam et al., 2006). Furthermore, chitosan at 0.12 wt % in a composite showed promising antibacterial activity (Elsaka, 2012).
Other filler modification includes 12-methacryloyloxydodecylpyridinium bromide (MDPB) addition. Studies have shown that the MDPB is not released from the material, could inhibit dental plaque formation but gave no reduction in the mechanical properties (Imazato et al., 2003). Other resin modifications include quaternary ammonium polyethylene imine (QPEI) addition. The composite containing QPEI showed a strong antibacterial and antibiofilm effect on biofilms. However, more work is needed to assess its clinical benefit in the oral cavity (Beyth et al., 2010, Othman et al., 2002, Cheng et al., 2012b, Gordon et al., 2011).

1.4.2.3.1. Chlorhexidine diacetate salt hydrate (CHX)

Chlorhexidine (CHX) is a cationic broad-spectrum antimicrobial agent belonging to the bis (biguanide) family. It is effective against most bacteria and fungi. CHX at high levels is harmful, but it can be used is small amounts. It is used in many products, like mouthwashes and storage solutions of contact lens. Chlorhexidine mechanism of action involves destabilisation of the outer bacterial membrane (Denyer and Maillard, 2002). Chlorhexidine remains the most effective and gold standard antiplaque and anti-gingivitis agent with effectiveness comparable to antibiotics (Lewis, 2010).

Chlorhexidine has been included into GICs (Hook et al., 2014), RMGICs (de Castilho et al., 2013) and composites (Anusavice et al., 2006b). A problem, however, is very limited release because the chlorhexidine remains either chemically or physically bound (Palmer et al., 2004b). With GICs and RMGICs, positively charged CHX interacts with the negatively charged polyacid which prevents release. In composites their hydrophobicity can limit CHX release.

CHX-containing dental composites have been studied widely (Leung et al., 2005, Cheng et al., 2012c). One study showed that higher levels of chlorhexidine could reduce the degree of composite polymerisation, leading to a greater loss of organic components and higher chlorhexidine release rates (Anusavice et al., 2006b). Addition of hydrophilic components
such as HEMA (Leung et al., 2005) or MPCM (Mehdawi et al., 2009) could enhance water sorption and thereby increase CHX diffusion through the matrix phase.

In another study, CHX was added with calcium phosphate or calcium fluoride nanoparticles into nanocomposites. The results showed that the added CHX greatly reduced biofilm formation. It also reduced acid production, and metabolic activity of the bacteria (Cheng et al., 2012c). In a more recent study, CHX encapsulated in mesoporous silica nanoparticles (MSNs) was included in dental composites. This encapsulation enabled inhibition of oral biofilm without compromise of materials’ mechanical properties and surface integrity (Zhang et al., 2014a).

1.4.2.3.2. Polylysine (PLS)

Polylysine (ε-poly-L-lysine) is a naturally occurring small homopolymer of L-lysine, and is produced by bacterial fermentation. ε-Poly-L-lysine (PLS) is mainly used to preserve packed food for its wide-ranging antimicrobial action against Gram-negative and Gram-positive bacteria, yeasts, and molds (Ye et al., 2013a). In addition, PLS is water soluble, and has little toxicity (Chakraborti, 2009).

ε-Polylysine typically contains 25-30 L-lysine linkages. Epsilon (ε) refers to the linkage of the lysine molecules. Normally, peptide bonds are linked by the alpha-carbon group, while in lysine, amino acids are molecularly linked by the epsilon amino group, and the carboxyl group. The antibacterial activity of PLS is mainly due to the disruption of the bacterial cell membrane (El-Sersy et al., 2012).

The chemical structures of antibacterial agents used in experimental composites are given in figure 1-6.
i. ε-Polylysine (Antibacterial agent)          j. Chlorhexidine diacetate (Antibacterial agent)

Figure 1-6 Chemical structures of antibacterial agents.

1.4.3. Self-Adhesive Composites

Dentine bonding is considered technique sensitive (Magne et al., 2008, Van Meerbeek et al., 2005). Currently, there is a drive in dental research towards combining the adhesive and composite into one self-adhesive restorative composite. This will make the restoration process easier for the clinician (Poitevin et al., 2013).

Recently, self-adhesive composites have been developed (Poitevin et al., 2013). These composites bond to the tooth surface without the use of conventional bonding systems. Most of these composites have included an acidic monomer (Pinna et al., 2015). The acidic group is able to chelate with calcium in the tooth. Upon monomer group polymerisation this enables bonding between the composite and hydroxyapatite. The most common acidic monomers that have been added to adhesive systems are glycerophosphate dimethacrylate, and 4-methacryloyloxyethy trimellitate anhydride. Glycero-phosphate dimethacrylate has been added to some of the currently available composites like Vertise flow (Pinna et al., 2015, Wei et al., 2011a). In this project acidic monomer 4-META was added to composite formulations. This will help in developing a self-adhesive composite for dental restorations.
1.5. Properties of Dental Composites

1.5.1. Polymerisation Shrinkage and Heat Generation

1.5.1.1. Polymerisation Process

Composites polymerise through free radical addition polymerisation. This means there will be no by-products at the end of the polymerisation reaction (Fouassier and Lalevée, 2012). In light cure resin composites, usually both a photo initiator and amine activator are added. When blue light is directed onto the material, these react to form free radicals, which initiate the reaction. As the reaction continues, the double bonds on both ends of the monomers are opened, enabling propagation of reaction and subsequently cross linking. Termination of the polymerisation reaction occurs when either the free radicals get entrapped in the cross linked structure, or one radical reacts with another radical (Andrzejewska, 2001). These reaction steps (Activation, Initiation, Propagation, and Termination) are given below.

\[
\begin{align*}
A + B & \rightarrow 2I^* & \text{Free-radicals generation} \\
I^* + M & \rightarrow M^* & \text{Initiation} \\
M_n^* + M & \rightarrow M_{n+1}^* & \text{Propagation} \\
M_n^* + M_n^* & \rightarrow P & \text{Termination}
\end{align*}
\]

The free radicals after generation (I*) attack the monomer C=C double bond, breaking it open to create a monomer free radical (M*) that can bond to other monomers leading to the formation of larger polymer chains. For termination, chain radicals may be consumed in pairs to produce a “dead” polymer. Rate constants for all stages of the polymerisation reaction are represented by \(k_d\), \(k_p\) and \(k_t\).
1.5.1.2. Polymerisation Shrinkage and Heat Generation

Polymerisation shrinkage is considered an important property for the long term success of the restoration. Many factors are responsible for polymerisation shrinkage in a dental composite after restoration. These include restorative procedure, light intensity, cavity design, polymerisation characteristics, type of monomers used, and filler loading (Cramer et al., 2011a).

The observed magnitude of polymerisation shrinkage can also depend on the methods used for its measurement (Ensaff et al., 2001). Furthermore the results obtained can vary between operators. Comparison of the published work on shrinkage can therefore be difficult.

Some of the commonly used methods include: use of mercury dilatometer, optical methods, bonded disk method, gas pycnometer, and wall-to-wall shrinkage (De Melo Monteiro et al., 2011). Each method has its own short comings, and there is no single method that explains all the issues that surrounds polymerisation shrinkage.

The calculation of shrinkage, and heat generation from the monomer conversion is another way to predict the shrinkage. The results for commercial materials obtained in this thesis were calculated theoretically from conversion, and estimated composition. The results were in close agreement with the literature values. Therefore, in this study polymerisation shrinkage, and heat generation for experimental composites were also calculated from the monomer conversion.

Shrinkage is generally proportional to heat generation and are both proportional to the number of polymerising methacrylate groups (Kuehn et al., 2005, Ferracane, 2005, Tarle et al., 2002). After polymerisation the molecules are more closely packed together, which leads to bulk contraction of the composite, and a reduction in volume (Braga and Ferracane, 2004). The shrinkage and heat generation is directly proportional to the number of double bonds
converting. High polymerisation shrinkage and heat generation is usually associated with low molecular weight monomers (Atai et al., 2005).

In order to overcome polymerisation shrinkage and heat generation, fillers and high molecular weight monomers are used in composite formulations (Cadenaro et al., 2008). Normally reduction in monomer percentage would reduce the polymerisation shrinkage. This, however, will make the material stiffer, and will compromise the composite-tooth interface. Current composite materials shows a polymerisation shrinkage in the region of 1-4 % by volume (Ferracane, 2005, Schmidt et al., 2011, Watts and Al Hindi, 1999).

1.5.2. Degree of Conversion

Review of the literature suggests the use of various different direct and indirect methods for the determination of degree of conversion of dental composites (Poggio et al., 2012). The most commonly used indirect methods include micro-hardness, use of differential thermal calorimetry (DTC), and differential scanning calorimetry (DSC) (Santana et al., 2009). These techniques measure the relative rate of polymerisation, rather than the absolute degree of monomer conversion. FTIR and Raman are the commonly used direct methods for determination of degree of conversion. These methods are simple to use, and directly quantify the amount of un-reacted monomers. In this study monomer conversion was assessed using FTIR.

Cured composites are made up of inorganic fillers blended in a complex cross linked organic matrix (Anusavice et al., 2012). In resin composites there is a partial conversion of double bonds which means there is still some un-reacted monomer groups (Chung and Greener, 1990, Yoon et al., 2002, Daronch et al., 2005). The degree of conversion is the percentage of carbon-carbon double bonds converted into single bonds. One of the reason that causes the monomer to be un-reacted is its low mobility. Rapid polymerisation after light curing results in the
development of a complex polymer network which leads to reduced movement of some of the monomer molecules (Price et al., 2011). Insufficient levels of monomer conversion can cause initiator leaching, less biocompatibility, reduced mechanical and wear properties, and colour instability (Du and Zheng, 2008). On the other hand high monomer conversion means high polymerisation shrinkage and heat generation. This means if more double bonds are converted to single bond this will result in more cross linking in polymer matrix, and more heat generation resulting in a brittle composite (Stansbury et al., 2005).

A number of factors contributes in deciding the degree of conversion. This includes monomers chemistry, percentage of diluent monomers, fillers concentration, temperature, polymerisation initiators and inhibitors, and light curing units (Yoshida and Greener, 1994, Ferracane and Greener, 1986, Sideridou et al., 2002, Amrouche-Korichi et al., 2009, Halvorson et al., 2003, Peutzfeldt and Asmussen, 2005).

1.5.3. Depth of Cure

Light is scattered by composites, and can penetrate only to a small distance. The depth of cure is governed by how far light can penetrate into the material (Leprince et al., 2012). It is important because it determines the thickness of layers of composite that the clinician can place. The depth of cure of composite depends on a number of factors. This includes monomer chemistry, (its size and composition), fillers addition, colour, translucency, and initiator systems (Kanehira et al., 2012).

The depth of cure determination of the external surface of a restoration can usually be evaluated through simple methods (Agrawal et al., 2015). One issue lies in the determination of degree of cure of the inner layers. The rather inaccessible inner layers makes the determination of cure in deep layers difficult. Extrapolation on the basis of external surface conversions may not be accurate.
It has been shown that inadequate cure due to improper polymerisation results in a reduction of physical properties (Nandini, 2010). The residual monomers may irritate the pulp tissues, and predispose plaque accumulation (Moore et al., 2008). The variables studied in the following thesis have provided an idea about which factors most affect the depth of cure.

The depth of cure is measured through a number of techniques. Some of the commonly employed methods are micro-hardness measurements, measurement using FTIR spectroscopy, micro-Raman technique, optical microscopy, and scraping test (ISO 4049) (Kanehira et al., 2012). Each method has its own shortcomings, and does not address the entire process completely. In this study the results obtained using ISO scraping test were in line with the commercial materials.

Depth of cure can be increased by time for light cure, intensity of curing unit, and distance of the light source from the composite surface (Nomoto et al., 2006). Currently, there is no clearly defined curing depth. It depends largely on the testing method. A number of methods being used to test the depth of cure include conversion of double bonds using Raman or FTIR, hardness measurements at different depths, colour dye indicators, optical distinction and scraping of material (Atmadja and Bryant, 1990, Kanehira et al., 2012). The most used method is the one described in ISO 4049 (Fan et al., 2002). In this method depth of cure is determined by scraping the un-cured soft composite material from the bottom of a cured composite cylindrical sample, and then measuring the height of the remaining cylindrical column at different points. The depth of cure is then given as half this thickness. This is due to the observation that composite materials may appear hard even at conversions below 50% especially those containing Bis-GMA (Aljabo et al., 2015a). All the measurements should be above 1.5 mm in order for the composites to pass ISO requirements.
Adequate depth of cure is very important for clinical success of the restoration. Unsatisfactory cure at the deeper parts of restoration will result in inadequate bonding of composite to tooth structure in deep cavities (Alto et al., 2006). There will be loss of marginal seal and adaptation, which will result in micro-leakage, sensitivity, and recurrent caries.

1.5.4. Water Sorption (Mass and Volume Changes)

Addition of calcium phosphates and other leachable components to composites can cause changes in their hygroscopic properties (Wei et al., 2013). These materials have the ability to absorb water molecules into the composite (McCabe and Rusby, 2004). In this process water molecules diffuse into the composite, and gradually occupy the free volume. This will cause plasticisation of the matrix phase and expansion (Wei et al., 2013). Some of the water molecules get bound inside the composite matrix, and allows absorption of more water molecules. These bound water molecules have the potential to disturb the inter-chain hydrogen bonding. This will result in expansion and plasticisation of the material, and may affect long term mechanical and wear properties (Musanje et al., 2001, Bastioli et al., 1990, Göhring et al., 2002).

The water sorption can be beneficial to an extent, if it compensates for the polymerisation shrinkage during the polymerisation reaction (Versluis et al., 2011). The amount of expansion varies from one material to another which can have high clinical impact especially inside closed cavities. It can also cause serious problems eg tooth cracking if over expansion occurs. Hygroscopic expansion creates non-functional stresses inside the tooth structure that will radiate in an outward direction along the walls of the tooth structure (Watts et al., 2000). Research has shown that such over-compensation can cause cusp fractures, and internal cracks in materials (Van Dijken, 2002). Therefore balance between shrinkage and expansion is important for the clinical success of the restoration.
In this project calcium phosphate in the form of acidic mono calcium phosphate monohydrate (MCPM), and basic tri calcium phosphate (TCP) were added as the filler phase. Calcium phosphate is expected to help in re-mineralisation of the decayed tooth structure, and may additionally aid in compensation of polymerisation shrinkage through promotion of water sorption induced expansion. MCPM alone is acidic, and has the potential of promoting a high amount of water uptake, that can result in reduced mechanical properties. To compensate MCPM high water uptake, TCP was added in equal amount. TCP will react with MCPM and bind the water molecules in brushite crystals (Mehdawi et al., 2013b).

1.5.5. Drug Release (Chlorhexidine and Polylysine)

Recently Drug/medical device products have been studied extensively. Use of drug delivery and combination products have been used clinically to deliver drug locally in cardiovascular diseases, cancer, orthopaedics, dental applications, and diabetes (Wu and Grainger, 2006).

Chlorhexidine has been added to dental materials in previous studies for its antibacterial properties (Mathew et al., 2013, Leung et al., 2005). Studies have shown that even a small CHX release of 2 % in filler phase could significantly reduce the bacterial counts of *Streptococcus Mutans* (Cheng et al., 2012a, Xia et al., 2014). Additionally it could help in the reduction of biofilm formation, metabolic activity in the biofilm, lactic acid formation, and viability of biofilms (Xia et al., 2014). Studies have shown that CHX can kill the bacteria in the first 4 weeks of its release. After that the CHX release was exhausted, and the biofilm developed again (Cheng et al., 2012a). The decline in CHX effect with time may be due to a decrease in CHX release, and bacteria protection with in the growing biofilm.

Antibacterial agents such as chlorhexidine have been incorporated in glass ionomer cements and resin modified glass ionomer cements for many years (Ray and Seltzer, 1991, Nicholson, 1998). Several *in vitro* studies, using an agar diffusion assay (Fraga et al., 1996, de Castilho et
and in vivo studies (de Castilho et al., 2013) have been carried out to evaluate the antimicrobial properties of chlorhexidine containing GICs and RMGICs. In other studies, chlorhexidine was added at concentrations between 1 to 10 wt % (Palmer et al., 2004a, Hoszek and Ericson, 2008). The authors reported an increase in antibacterial activity of the resultant materials against different bacterial species including *S. Mutans* and *L. acidophilus*. In another study, the antibacterial activity of resin modified cements against *S. Mutans* was improved upon addition of 5 wt % of chlorhexidine diacetate, in vitro (de Castilho et al., 2013, TÜRKÜN et al., 2008). In a further study, the antibacterial activity of a conventional GIC against *S. Mutans* and *L. Casei* was also enhanced upon addition of chlorhexidine at concentrations 1, and 2 wt % (Deepalakshmi et al., 2010). In one in vivo study, carious teeth restored with a conventional GIC containing 1 wt % chlorhexidine, discovered lower counts of *S. Mutans* and lactobacilli in both infected and affected dentine than a glass ionomer without chlorhexidine (Frenckken et al., 2007).

Use of chlorhexidine, and other drugs in dental composites has also been previously documented in the literature (Leung et al., 2005, Anusavice et al., 2012). Chlorhexidine was added into various experimental dental composites due its low minimum inhibitory concentrations against oral bacteria and ability to inhibit metalloproteinases (MMPs) (Gendron et al., 1999, Leung et al., 2005). Composites with early release of chlorhexidine might reduce the need for extensive caries affected tissue removal as advocated in modern tooth restoration procedures (Splieth et al., 2001).

The release of antimicrobial drugs has gained particular attention recently. As compared to systemic drug delivery, a key feature in local drug delivery is that high doses of drugs can be targeted to specific sites in quick time, without any serious systemic drug toxicity (Vasilev et al., 2009). Different drugs show varying levels of release, which depends on the local environment. Some materials release high doses of antimicrobials very quickly, but the activity
lasts only for a short term. On the other hand some materials are slow drug-releasing systems, and take time to reach a level of effectiveness. The drugs released must act before bacteria develop any protective extracellular matrix (Vasilev et al., 2009).

To combat bacterial micro-leakage, and caries progression, in this thesis antimicrobials such as chlorhexidine and polylysine have been added to the composite formulations. The first aim was to provide a steady release of drug beneath the restoration that can with time help in prevention of bacterial micro-leakage and recurrent caries formation. The second idea was that by adding these antimicrobials natural tooth structure could be better preserved. This would be achieved by only excavating the soft carious tooth structure, and leaving the hard carious lesions to self repair. With the combined effect of antimicrobials and re-mineralising agents, the decayed tooth structure may be more likely to re-mineralise (Splieth et al., 2001).

1.5.6. Mechanical, Chemical, and Microscopic Characterisation

A number of mechanical, chemical, and microscopic properties of composite and dentine were studied in this project. The mechanical properties tested for composite include biaxial flexural strength, and modulus. Additionally, in the case of dentine three point bending strength, modulus, and strain were studied. Chemical characterisation of dentine was carried out using Raman spectroscopy. The microscopic analyses were carried out using SEM. The properties tested included characterisation of composite surface for quantification of apatite layer, and to see the interface between dentine and composite.

The resin based filling materials ideally should have sufficient mechanical properties to withstand the stresses generated due to masticatory forces. The composite materials in the restored tooth cavity are under the influence of constant compressive, tensile, and shear stresses (Santhosh et al., 2008, Klautau et al., 2011). Flexural stress combines all these stresses.
Therefore, determining the flexural strength of dental composites is a suitable way to assess the mechanical performance of filling materials.

The common mode of failure of dental composite is brittle fracture (Drummond, 2008). This can be due to the propagation of the pre-existing cracks under various stresses. These cracks can also be generated by the incorporation of air bubbles during material mixing / placement, or may be due to the polishing procedure (Van Noort, 2013). The micro-structural imperfections could also lead to cracking in the material (Van Noort, 2013, Weir et al., 2012).

The ISO suggests the use of a 3 point bend test (uniaxial flexural strength) for the determination of mechanical properties of resin based materials. However, due to superior advantages of biaxial over uniaxial flexural strength (Huang and Hsueh, 2011), in this study BFS was determined for the dental composites.

Biaxial flexural strength, and modulus testing is considered more accurate and advantageous over three point, tensile, and compressive test (Palin et al., 2003b, Williams et al., 2002). Usually circular discs are prepared of the desired composite, and placed on a knife edge ring support. In biaxial testing the maximum loading occurs in the central loading area, thus eradicating any edge effect (Palin et al., 2003b). A further issue is that the standard dental light guns irradiate a circular area of 8 mm diameter. With the 15 mm long, 1 mm wide bars used in the ISO 4049 three point bend test much of the light is therefore not irradiating the sample. To irradiate the whole material the light has to be placed at multiple points along the bar. Conversely through small circular motion of the light gun it is possible to fully cure the 10 mm diameter, 1 mm thick biaxial flexural strength discs without use of multiple point exposure.

Three point bending strength, modulus and strain usually utilise rectangular bar specimens. This test produces tensile stresses underneath the specimen surface (Mosaddeghi et al., 2003). The samples prepared for three point test are usually not clinically representative, and since the
samples are much bigger in size than the curing tip, multiple repeated curing is performed. This may result in inconsistencies in final polymerisation. However, the above problems occur only in composite materials. In this thesis, three point bending test was performed for dentine samples, so the above issues didn’t come into consideration.

Chemical modifications on dentine surfaces after various acid etching times, were investigated using Raman spectroscopy. Due to its ability to detect specific molecules, and its high laser lateral resolution of approximately 1 μm, Raman can be used to characterise specimen both chemically and structurally (Yang et al., 2009). Raman can be used both as a qualitative and quantitative tool. A Raman spectrum is obtained by plotting scattered intensity vs. Energy. Each peak corresponds to a specific molecule (McCreery, 2005). The main drawback with Raman is fluorescence, which can complicate the spectra and reduce peak intensities in some cases.

The characterisation of dentine, hybrid layer, and composite surface was carried out using scanning electron microscopy (SEM). SEM can provide high quality images in quick time. It can also allow the operator to work on long depth of field. It can be linked to an EDX detector, to analyse the elements present on the surface of specimens (Goldstein et al., 1981). There are a few limitations with conventional SEM. First the SEM requires vacuum compatible specimens. Secondly, the SEM can potentially damage the sample, and limit its use for further analysis. Lastly, sample dimensions, and preparation is very important (Egerton et al., 2004).

1.5.7. Adhesion of Dental Composite

Adhesion to dentine depends on a number of factors, and usually involves bonding through different mechanisms. Adhesion of a material to a substrate can be mechanical, chemical, physical, or a combination of all of these (Van Noort, 2013).
Mechanical adhesion is the simplest form of adhesion. It can result from the surface roughness, and irregularities. These irregularities are sites for mechanical interlocking of the material with the dentine structure. In mechanical adhesion there is no molecular interaction between the material and substrate (Van Noort and Barbour, 2013).

The second type of adhesion is physical adhesion. This type of bonding occurs when polar molecules are in close proximity, so they develop a dipole interaction. Usually the bonding forces are very small. This type of bond is weaker than the covalent and ionic bond. Another feature of this type of adhesion is that bonding is very fast, and reversible (Van Noort and Barbour, 2013).

Chemical adhesion occurs when a molecule in the material surface chemically (ionic, or covalent) interacts with the substrate to form a strong bond. This type of adhesion is very strong as it involves molecular interactions (Van Noort, 2013).

Bond strengths can be calculated using a variety of tests (Van Meerbeek et al., 2003). The commonly used tests to assess the adhesion of a material include shear, tensile, push out, micro-shear, and micro-tensile (Scherrer et al., 2010, Fusayama et al., 1979).

One of the main aims of this project was to develop a composite that can self-bond to dentine surfaces. For this purpose a number of experimental composite formulations were developed with varying levels of adhesion promoting monomers. Two adhesion tests were used to check bond strength. These were push out and a shear bond test.

**1.5.7.1. Push out test**

Push out test is an appropriate method to measure the bond strengths between composite and dentine. In push out test shear stresses are generated parallel to the composite-dentine interface, which is a better simulation of the stresses occurring in clinical environment (Chen et al., 2013).
The sample preparation for push out test is the same as clinical application, and the sample size mimics the *in vivo* conditions (Mazzitelli and Monticelli, 2009).

Push out test had been widely used to determine the bond strengths of root canal sealers, and posts to dentine (Goracci et al., 2004, Gancedo-Caravia and Garcia-Barbero, 2006, Sly et al., 2007). In this study push out test was carried out using ivory dentine. Previously push out bond strength of glass fibre posts to ivory dentine had been carried out (Thanjal, 2011).

The conventional methods of bond strength determination (Shear or tensile) give high bond strengths like push out test, but did not account for the micro leakage (Mahdi et al., 2013). The confined spaces in push out test accounts more for micro leakage as compared to the shear bond. Similarly the confined spaces exhibit a high C-factor (ratio of bonded surfaces to un-bonded surfaces), which will enhance a better flow of material, and reduced internal stresses in the material (Thanjal, 2011). These confined spaces provides a more favourable clinical simulation. The reduction in bond strengths in push out test is usually due to the production of internal stresses at the dentine-composite interface, produced during polymerisation shrinkage.

### 1.5.7.2. Shear bond test

Shear bond test had been widely used in literature for the screening of new dental restorative materials (Blatz et al., 2003, Derand et al., 2005, De Munck et al., 2005b). It is the most common in vitro test for the determination of bond strengths between composite and dentine (De Munck et al., 2005b). The method used for shear bond test become very popular, because of its relatively easy setup. The shear bond test has less alignment problems as compared to other bonding tests like micro-tensile (Armstrong et al., 2010, Shimada et al., 2002). The stresses developing during shear bond test are mostly tensile, that causes failure of the bond between composite and dentine (Dai et al., 2005).
1.6. Project Aims and Objectives

1.6.1. Aims

The aim of this study was to develop a self-adhesive, antibacterial and calcium phosphate-containing composite with appropriate properties for potential use as a restorative material in both load, and non-load bearing areas.

The properties characterised included degree of monomer conversion, depth of cure, polymerisation shrinkage and heat generation, mass and volume changes, polylysine and chlorhexidine release, biaxial flexural strength and modulus, shear and push out bond strength, surface characterisation of composite and dentine, and interface analysis. Additionally, the study also aimed at the development of an ivory dentine model that can be used as an alternative for human dentine. The small size of the human tooth means that for extensive studies a large number are required. These can have widely varying levels of mineralisation, disease, age etc. Conversely, the large tusk size enables testing of a massive number of composite formulations with a single ivory source. This will aid in reducing the variability issues associated with bond tests that employ human dentine.

1.6.2. Objectives

The objectives of this project were to compare current commercial dental composites (2 packable, and 1 flowable) with those of new materials (48 experimental composites) containing potentially antibacterial, re-mineralising and adhesion promoting components. Properties investigated include:

(a) Degree of monomer conversion, polymerisation shrinkage, and heat generation determined using FTIR.

(b) Depth of cure of composite cylinders measured using a standard ISO scraping test.
(c) Mass and volume changes upon water or simulated body fluid (SBF) immersion quantified using gravimetrical analysis.

(d) Chlorhexidine, and Polylysine release into storage solution (water or SBF) quantified using UV spectroscopy.

(e) Biaxial flexural strengths, modulus obtained using a universal testing machine

(f) Push out test, and shear bond adhesion to human and ivory dentine. (Additionally, human and ivory dentine three point bending strength, strain and modulus were determined with a universal testing machine, chemical analysis of dentine was carried out using Raman, and microscopic analysis was performed with SEM to justify the use of ivory).

(g) Composite surface hydroxyapatite precipitation and interface bonding to dentine analysed using scanning electron microscopy.

1.7. Null Hypotheses

1. The null hypotheses in chapter 3 are that there are no significant differences in the following properties of three commercial, and two new experimental composites:

   - Degree of monomer conversion at 1 or 4 mm depth.
   - Polymerisation shrinkage
   - Heat generation.
   - Depth of cure assessed using an ISO method with 20 or 40 s cure.
   - Mass or Volume change over 12 weeks.
   - Biaxial flexural strength or Modulus over 6 months.
   - Bond strength (push out or shear) to dentine assessed using various dentine pre-conditionings.

2. The null hypotheses in chapters 4, 5, 6, 7, and 8 are that there are no significant differences in the following properties of experimental composites containing 4-
META instead of HEMA, polylysine at 5 instead of 0.5 wt %, chlorhexidine at 5 instead of 0 wt %, and calcium phosphates at 20 or 10 instead of 0 wt %.

- Degree of monomer conversion at 1 or 4 mm depth (Chapter 4).
- Polymerisation shrinkage or Heat generation (Chapter 4).
- Depth of cure assessed using an ISO method with 20 or 40 s cure (Chapter 4).
- Mass or Volume change over 12 weeks (Chapter 5).
- Drug release (Chlorhexidine or Polylysine) over 6 weeks (Chapter 6).
- Biaxial flexural strength or Modulus over 6 months (Chapter 7).
- Bond strength (push out or shear) to dentine assessed using various dentine pre-conditionings (Chapter 8).
CHAPTER 2

MATERIALS AND METHODS
2. Materials and Methods

This section outlines all the materials (commercial and experimental) used throughout the project. A brief summary of materials and methods is also given at the beginning of each chapter.

2.1. Materials

2.1.1. Commercial Materials

A number of commercially available materials were used to compare with the experimental composites. Details are given in table 2-1.
Table 2-1 Details of commercial materials (composites, adhesive system, and etchant) investigated. Description and component information was supplied by manufacturers.

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Product Code</th>
<th>Fillers Content (Wt %)</th>
<th>Fillers Size (µm)</th>
<th>Shade</th>
<th>Components</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z250</td>
<td>3M</td>
<td>202458</td>
<td>78</td>
<td>0.01-3.5</td>
<td>B3</td>
<td>Bis-GMA</td>
<td>Zirconia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UDMA</td>
<td>Silica</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bis-EMA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TEGDMA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre-mixed syringe - light cure</td>
</tr>
<tr>
<td>Gradia Direct</td>
<td>GC Europe</td>
<td>105936</td>
<td>77</td>
<td>0.85</td>
<td>P-A2</td>
<td>UDMA</td>
<td>Fluoro-alumino-silicate glass</td>
</tr>
<tr>
<td>Posterior</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dimethacrylate</td>
<td>Silica</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Co-monomers</td>
<td>Pre-polymerised filler</td>
</tr>
<tr>
<td>Vertise Flow</td>
<td>Kerr</td>
<td>111040 2</td>
<td>70</td>
<td>0.020 – 20</td>
<td>A1</td>
<td>Bis-GMA</td>
<td>Pre-polymerised filler</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HEMA</td>
<td>Ytterbium fluoride</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GPDM</td>
<td>Barium glass</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Colloidal silica</td>
</tr>
<tr>
<td>Ibond Total Etch</td>
<td>Heraeus Kulzer GmbH</td>
<td>FBOH1 000</td>
<td>1-5</td>
<td>--</td>
<td>--</td>
<td>UDMA</td>
<td>Nano-fillers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4-META</td>
<td>Pre-mixed bottle – light cure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HEMA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glutaraldehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Acetone, Water</td>
<td></td>
</tr>
<tr>
<td>Ibond Etch 35 Gel</td>
<td>Heraeus Kulzer GmbH</td>
<td>110818 3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Orthophosphoric acid 35 %</td>
<td>Pre-mixed Gel syringe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td></td>
</tr>
</tbody>
</table>

2.1.2. Monomers used in experimental composite formulations
A number of monomers were used to mix with the filler phase. A list of monomers is given in Table 2-2
Table 2-2 Details of monomers used throughout this project. Molecular weight information from manufacturers.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
<th>Supplier</th>
<th>Product code</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDMA</td>
<td>Urethane dimethacrylate</td>
<td>DMG Dental</td>
<td>100112</td>
<td>470</td>
</tr>
<tr>
<td>TEGDMA</td>
<td>Tri ethylene glycol dimethacrylate</td>
<td>DMG Dental</td>
<td>100102</td>
<td>228</td>
</tr>
<tr>
<td>HEMA</td>
<td>Hydroxyethyl methacrylate</td>
<td>DMG Dental</td>
<td>100220</td>
<td>130</td>
</tr>
<tr>
<td>4-META</td>
<td>4-Methacycloxyethyl trimellitate anhydride</td>
<td>Polysciences</td>
<td>17285</td>
<td>286</td>
</tr>
</tbody>
</table>

2.1.3. Fillers used in experimental composite formulations

The inorganic filler phase of the experimental composites consists of silanated glass particles and fibres, calcium phosphates, and antibacterial agents. The details are given in table 2-3.
Table 2-3 Details of filler materials used throughout this project. Information from manufacturer.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
<th>Manufacturer</th>
<th>Product Code</th>
<th>Size (μm)</th>
<th>Silanated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass (Contains Particles and Fibres)</td>
<td>Barium-boro-alumino-silicate glass particles (GP)</td>
<td>DMG Dental</td>
<td>680326</td>
<td>~ 7</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Silane coated boro-silicate glass fibre (GF)</td>
<td>MO-SCI</td>
<td>0322201-S</td>
<td>~ 15 * 300</td>
<td>Yes</td>
</tr>
<tr>
<td>MCPM</td>
<td>Mono Calcium Phosphate Monohydrate</td>
<td>Himed</td>
<td>MCP-B26</td>
<td>~ 53</td>
<td>No</td>
</tr>
<tr>
<td>TCP</td>
<td>β–Tri Calcium Phosphate</td>
<td>Plasma biotal</td>
<td>SSB210907</td>
<td>~ 53</td>
<td>No</td>
</tr>
<tr>
<td>CHX</td>
<td>Chlorhexidine diaceta salt hydrate</td>
<td>Sigma-Aldrich</td>
<td>1001447866</td>
<td>~ 44</td>
<td>No</td>
</tr>
<tr>
<td>PLS</td>
<td>ε-Polylysine</td>
<td>Handary SA</td>
<td>28211-04-3</td>
<td>~ 74</td>
<td>No</td>
</tr>
</tbody>
</table>

2.1.4. Initiators, and Activators

Initiators and activators used in this project are given in Table 2-4.

Table 2-4 Details of initiator, and activator used throughout this project.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
<th>Manufacturer</th>
<th>Product Code</th>
<th>MW</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CQ</td>
<td>Camphorquinone</td>
<td>Alfa Aesar</td>
<td>10120023</td>
<td>166.22</td>
<td>Initiator</td>
</tr>
<tr>
<td>DMPT</td>
<td>N,N - Dimethyl-p-Toluidine</td>
<td>Sigma Aldrich</td>
<td>15205BH</td>
<td>135.21</td>
<td>Activator</td>
</tr>
</tbody>
</table>
2.2. Methods

A brief description of each method is outlined below.

2.2.1. Apparatus Required for Material Preparation

Monomers were handled carefully to prevent contact dermatitis. Latex gloves were used while handling monomers. A white laboratory coat was worn to prevent contamination of the clothes.

A number of instruments were used to work with monomers and fillers. All viscous monomers and fillers were handled with metal spatulas. For diluent monomers, glass pipettes were used.

All weighing of monomers and fillers were done using a four figure OHAUS Pioneer Series of analytical and precision balances. After weighing, all the monomers and fillers were mixed in a Speed Mixer™ DAC 150.1 FVZ (Synergy Devices Ltd) using disposable jars and lids. The mixer was operated for 20 s at 3000 rpm.

The mixed composites were stored in amber glass bottles in a fridge at 4 °C for a maximum of 1 month.

2.2.2. Commercial Materials Sample Preparation

Commercial materials were handled according to maker’s instructions. The materials were pre-mixed, and supplied in 3-4 g cartridges.

Samples were prepared in different sizes according to ISO standards and test requirements. All the composites were light cured for 40 s on top and bottom using a Demi Plus LED Dental Curing Light (Kerr Dental Supply), for which the power output was 1000 mWcm².
2.2.3. **Composite Sample Preparation**

2.2.3.1. **Monomer Preparation**

Monomers were mixed with the initiator and activator in amber glass bottles. Total amount was between 10 and 12 g. Mass of each constituent was weighed to an accuracy of 0.0001g. The order of weighing was started from the components with smallest amount to largest amount. Viscous monomers like UDMA were added in last. Before adding the UDMA, all other components in monomer phase were mixed for 10 minutes using a magnetic stirrer at room temperature. After UDMA addition the monomer mixture was stirred for a further 15 minutes. This gave a stock monomer mix that could be stored at 4 °C for up to one month with no effect on the curing kinetics of the end mix. The magnetic stirrer was removed before storing the monomer mix.

2.2.3.2. **Filler Preparation**

All fillers were stored at room temperature in air tight plastic containers. To prevent introduction of any moisture into the containers or contamination of the bulk containers, small amounts of fillers sufficient for 3-5 mixes were stored in small plastic containers for daily use.

2.2.3.3. **End mixing**

Each component (monomer and fillers) was weighed into a plastic jar. First, the fillers were weighed starting from the components with smallest amount to largest amount. Monomer mixture (from section 2.2.3.1) was slowly added to filler mix in the end.

A plastic lid was tightened onto the jar, and the jar was placed in the speed mixer for final mixing (20 at 3000 rpm, see above). The final paste was then transferred to amber glass bottles for storage in a fridge at 4 °C.
2.2.4. Factorial Analysis

Factorial analysis is the main method of analysis that has been used to analyse the following data. This method allows the effect of more than one variable to be investigated at the same time. Factorial analysis provides freedom to investigate the effect of various variables on the measured outcome whilst reducing the number of samples. Detailed interpretation of a factorial experiment will be described in the following section.

2.2.4.1. Two Level and Three Variable Design

The full factorial design for two level and three variable, namely the $2^3$ design, consists of eight possible runs. Graphically, we can represent the $2^3$ design by the cube shown in Figure 2-1. The arrows show the direction of increase of the factors. The numbers ‘1’ through ‘8’ at the corners of the design box reference the ‘Standard Order’ of runs.

![Figure 2-1 A $2^3$ two-level, full factorial design with factors $a_1$, $a_2$, $a_3$.](image)

For each test 3 variables $a_1$, $a_2$, $a_3$, with high ($F = +1$) and low ($F = -1$) possible values were chosen. In the full factorial design, formulations with every likely combination of these
variables is investigated (Table 2-5). Overall, an experiment involving 3 variables has 8 possible different formulations to be tested. For each variable, 4 of the samples will have low variable values and the other 4 high variable values.

$V_1$ to $V_4$ all have $F$ for the first variable equal to $+1$ but two each of $+1$ and $-1$ for variable 2 and 3. The effect of variable 1 can therefore be acquired by comparing the average results for sample $V_1$ to $V_4$ with that for $V_5$ to $V_8$. Similarly, comparing the average results for samples $V_3$, $V_4$, $V_7$ and $V_8$ with that of $V_1$, $V_2$, $V_5$ and $V_6$ gives the effect of variable 2, and comparing average results of $V_1$, $V_3$, $V_5$ and $V_7$ with that of $V_2$, $V_4$, $V_6$ and $V_8$ gives the effect of variable 3. Factorial analysis also allows quantification of interaction effects between the variables. Strong interaction effects, though, complicate understanding of the effects of variables and prevent fitting of simple kinetic equations. The size of these interactions is given by $a_{12}$, $a_{13}$, $a_{23}$ and $a_{123}$.

Table 2-5 Sample combination for a two level factorial experimental design involving three variables. $+1$ and $-1$ refer to high and low values of the variable respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Variable 1 ($a_1$)</th>
<th>Variable 2 ($a_2$)</th>
<th>Variable 3 ($a_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_1$</td>
<td>$+1$</td>
<td>$-1$</td>
<td>$+1$</td>
</tr>
<tr>
<td>$V_2$</td>
<td>$+1$</td>
<td>$-1$</td>
<td>$-1$</td>
</tr>
<tr>
<td>$V_3$</td>
<td>$+1$</td>
<td>$+1$</td>
<td>$+1$</td>
</tr>
<tr>
<td>$V_4$</td>
<td>$+1$</td>
<td>$+1$</td>
<td>$-1$</td>
</tr>
<tr>
<td>$V_5$</td>
<td>$-1$</td>
<td>$-1$</td>
<td>$+1$</td>
</tr>
<tr>
<td>$V_6$</td>
<td>$-1$</td>
<td>$-1$</td>
<td>$-1$</td>
</tr>
<tr>
<td>$V_7$</td>
<td>$-1$</td>
<td>$+1$</td>
<td>$+1$</td>
</tr>
<tr>
<td>$V_8$</td>
<td>$-1$</td>
<td>$+1$</td>
<td>$-1$</td>
</tr>
</tbody>
</table>
2.2.4.2. Factorial Equations

To know the concept behind factorial analysis, in this section the equations involved in factorial design are briefly described. With 3 variables at 2 levels each there would be 8 samples. A suitable factorial expression would then be (Ho and Young, 2006):

\[
\ln P = <\ln P > + F_1 a_1 + F_2 a_2 + F_3 a_3 + F_1 F_2 a_{1,2} + F_1 F_3 a_{1,3} + F_2 F_3 a_{2,3} + F_1 F_2 F_3 a_{1,2,3}
\]  

(2-1)

F terms equal +1 or –1 and a terms indicate level of effect of variables or interactions. \(<\ln P>\) is the arithmetic mean of all ‘\ln P’ values. \(2a_i\) is obtained using (Mehdawi, 2009):

\[
2a_i = <\ln P >_{F_i=+1} - <\ln P >_{F_i=-1}
\]  

(2-2)

<\ln P>_{F_i=+1} and <\ln P>_{F_i=-1} are the arithmetic mean values of \(\ln P\) for all four samples with \(F_i\) equal to +1 and -1 respectively. Interaction terms are calculated using:

\[
2a_{ij} = <\ln P >_{F_i F_j=+1} - <\ln P >_{F_i F_j=-1}
\]  

(2-3)

\[
2a_{ijk} = <\ln P >_{F_i F_j F_k=+1} - <\ln P >_{F_i F_j F_k=-1}
\]  

(2-4)

For this thesis the above “\(a\)” terms were calculated using equation 2-2, 2-3 and 2-4 with excel. To enable easy calculation and comparison of terms, spreadsheets were set up that could analyse and compare a wide range of data simultaneously. For each study “\(a\)” would be calculated \(n\) times. \(n\) is the number of specimens of each of the eight formulations allowing determination of a mean result and standard deviation, SD. 95 % confidence interval error bars were calculated assuming C.I = 1.96 × S.D/√\(n\) where \(n\) is sample number. In this thesis these are provided on graphs as error bars. None overlapping error bars then indicate significant difference at 95% confidence. It should be noted that the exponent of <\ln P> equals the geometric mean of P. This can be calculated in excel using the function GEOMEAN. If the variables are significant and interaction terms small, the “\(a\)” values can be converted into average percentage changes \(\delta\) by using equation 2-5.

\[
\delta = 100(\exp(\pm 2a_i) - 1)
\]  

(2-5)
2.2.5. **Fourier Transform Infrared Spectroscopy (FTIR)**

2.2.5.1. **Background**

FTIR stands for Fourier Transform Infrared. In this thesis, FTIR was selected for measuring the polymerisation rate and degree of conversion. FTIR use for measuring degree of conversion is well documented in literature (Halvorson et al., 2002, Stansbury et al., 2005). When IR radiation is passed through a sample some radiation is absorbed (Stuart, 2005) due to changes in vibration of molecular bonds. This provides evidence about molecular structure (Kačuráková and Wilson, 2001).

2.2.5.2. **Theory of Infrared Spectroscopy**

When a molecule or atom absorbs IR radiation, it gains energy as it undergoes transition from one energy level \( (E_{\text{initial}}) \) to another \( (E_{\text{final}}) \). According to Planck’s law, the energy of transition and the frequency of absorbed radiation \( f \) (Hz) are correlated by equation (2-6) (Mehdawi, 2009):

\[
E_t = hf
\]  
(2-6)

Where \( E_t \) is the energy of transition \( (E_{\text{final}} - E_{\text{initial}}) \) and \( h \) is Planck’s constant.

Since \( f = \nu c \), where \( \nu \) and \( c \) are the wavenumber \( (\nu) \) (cm\(^{-1}\)) and velocity of light \( (8 \times 10^8 \) m s\(^{-1}\)), equation (2-6) can be replaced by equation (2-7) (Mehdawi, 2009):

\[
E_t = h\nu c
\]  
(2-7)

The wave length \( (\lambda) \) (nm) is correlated with frequency \( (f) \) by the following equation:

\[
\lambda = \frac{c}{f}
\]  
(2-8)

Therefore, equation (2-6) can also be given as (Mehdawi, 2009):

\[
E_t = \frac{hc}{\lambda}
\]  
(2-9)

The above equations (2.6-2.9) are also valid for Raman and UV spectroscopy.
Energy absorbed by a molecule must match exactly that required for a molecular transition. The molecular bonds oscillate and vibrate at specific frequencies, behaving like springs (Colthup, 2012). On absorption of energy from IR radiation, the vibrational energy and amplitude of the vibrations are enhanced. This will result in bond stretching or bending. In order to detect these changes in FTIR spectra, the vibrational motion should be accompanied by change in dipole moment at both ends of the vibration.

The FTIR spectrum is displayed as a plot of IR absorbance versus wavenumber (cm\(^{-1}\)). Peaks in the spectrum correspond with different vibrational transitions. Generally the FTIR spectrum can be categorised into two regions. The 4000-1300 cm\(^{-1}\) region is usually associated with specific functional groups, while the 1300-500 cm\(^{-1}\) is known as the fingerprint region and associated with vibrations of the entire molecule. The schematic diagram of FTIR setup is given in figure 2-2.

![Schematic representation of FTIR theory.](image-url)
2.2.5.3. Degree of Conversion and Curing Profile

The attenuated total reflectance (ATR) FTIR method used in this project measures the reaction in the lower surface of the material in contact with the ATR diamond. It is less easy to determine conversion of double bonds in the bulk of materials.

For determination of conversion and cure profiles, spectra were obtained at room temperature (23 °C) using an FTIR spectrometer (Perkin-Elmer, UK) with a temperature controlled golden gate diamond ATR attachment (Specac, UK) and using Timebase software. 1 mm, and 4 mm thick composite pastes, within 10 mm diameter brass rings were light cured on the ATR diamond for 40 s. The upper surface of the composite was covered by acetate sheet to inhibit surface oxygen hindering the polymerisation process. Spectra were obtained every 4 s for 20 minutes from 700 cm\(^{-1}\) to 2000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\). Each formulation was repeated six times.

Monomer conversion, C, was calculated using equation 2-10 (Main, 2013).

\[
C(\%) = \frac{100(h_0 - h_t)}{h_0}
\]  
(2-10)

Where \(h_0\) and \(h_t\) were taken as peak absorbance at 1320 cm\(^{-1}\) above the background level at 1335 cm\(^{-1}\) initially and after a time \(t\) (Leung et al., 2005).

2.2.5.4. Heat Generation and Shrinkage

Heat generation and shrinkage were calculated from monomer conversion (Li et al., 2009). Both these properties are directly proportional to monomer conversion. With one mole of C = C polymerisation typically 57 kJ of heat is generated (Kuehn et al., 2005). This will also tend to provide a volumetric shrinkage of 23 cm\(^3\) (Rueggeberg and Tamareselvy, 1995). To calculate
the composite shrinkage $V_s$ and heat generation $\Delta H$ after light curing for each formulation (six repeats), the following equations were used respectively (Main, 2013).

$$V_s (\%) = 23N \times 100$$ (2-11)

$$\Delta H (kJ / cc) = 57N$$ (2-12)

$N$ is the number of moles of reacted bonds per unit volume in each of the formulations. This can be calculated using equation 2-13a (Main, 2013).

$$N = [M]C \rho_{comp} \left( \sum \frac{n_i x_i}{W_i} \right)$$ (2-13a)

Where $C$ is the final fractional monomer conversion calculated from FTIR, $\rho_{comp}$ is the density of composite, and $[M]$ is the total monomer mass fraction. $N_i$, $W_i$ and $x_i$ are number of $C = C$ bonds per molecule, molecular weight (gmol$^{-1}$) and mass fraction of monomer $i$ respectively. $\Sigma$ indicates a sum over all the monomers in the liquid phase.

It should be noted that composite density was calculated assuming an ideal mixture of monomers and fillers. The method does not take into consideration any volume changes occurring due to voids formation. Assuming the composite material behaves ‘‘ideally’’, and is non-porous, density can be calculated using a simple rule of mixtures (Equation 2-13b).

$$\frac{1}{\rho_{comp}} = \frac{f_m}{\rho_{monomer}} + \frac{1-f_m}{\rho_{filler}}$$ (2-13b)

$\rho_{monomer}$ and $\rho_{filler}$ are the combined densities of monomer and filler phase. $F_m$ is the mass fraction of monomer.

The shrinkage and heat generation can be calculated from the above equations (2-11, and 2-12). An example of data fitting was performed for an experimental composite below. The number of moles of reacted bonds per unit volume $N$ for a composite with UDMA : TEGDMA
3:1, with 4-META at 5 wt % in monomer phase, powder liquid ratio of 4:1, monomer conversion of 50 %, and density $\rho$ of 2.26 g cm$^{-3}$ can be calculated as:

$$N = (1 - 0.80) \left( \frac{50}{100} \right) (2.26) \left[ \left( \frac{0.6975}{235} \right) + \left( \frac{0.2325}{143.5} \right) + \left( \frac{0.05}{304.2} \right) \right]$$

$N = 0.00108$

$Vs (\%) = 23(0.00108) \times 100$

$Vs (\%) = 2.49$

$\Delta H (kJ/cc) = 57(0.00108)$

$\Delta H (kJ/cc) = 0.06$

### 2.2.6. Depth of Cure (DOC) Measurement

#### 2.2.6.1. Background

The depth of cure of light activated dental composites was considered as important to the clinical success of these materials soon after these materials were introduced (Moore et al., 2008). The depth of cure of light activated composites has been studied considerably for more than 25 years, but there are still controversies about the DOC of light activated composites. A number of different techniques have been used to measure the DOC of polymerised resin composites versus a wide range of different variables (eg distance of the light source from the specimen surface, light power output, material chemistry, sample thickness etc). One of these techniques includes scraping away the unset material and measuring the remaining specimen, measuring top and bottom hardness and measuring top and bottom degree of conversion of double bonds in the polymer (Moore et al., 2008). The scraping technique has been codified as the depth of cure measure in the ISO standard for dental resins 4049. In this project DOC was measured according to ISO 4049.
2.2.6.2. Sample Preparation

Six specimens of each composite (three each for 20 s, and 40 s cure), 4 mm in diameter and 6 mm deep, were condensed into stainless steel moulds. The moulds were filled with the composite, every care was taken to exclude air bubbles. After slightly overfilling the moulds, the moulds were pressed against two glass slides to allow the excess material to be displaced. The glass slides were then removed, and the material irradiated for 20 s, or 40 s from the top surface.

After light activation, the specimens were immediately removed from the moulds and the uncured material scraped away with a plastic spatula. The length of the remaining material was measured with a digital calliper in three places, and an average length was obtained. This value was divided by two to obtain the ISO 4049 depth of cure (DOC). Average values and standard deviations were calculated for the ISO DOC for each material.

If all three values for materials are > 1.5 mm, the material has complied with the ISO standard (Fan et al., 2002).

2.2.7. Raman Spectroscopy

2.2.7.1. Background

Raman spectroscopy is another vibrational spectroscopy method used to determine the quantum energy levels present within a material and hence to determine the molecular bonds present (Wilson et al., 2012). Raman and FTIR spectroscopy differ in some key fundamental ways. Raman spectroscopy works on changes in molecule polarisability, while IR works with changes in the molecule dipole moment. Secondly, Raman spectroscopy calculates relative frequencies at which sample scatters radiation, while IR spectroscopy on the other hand measures absolute frequencies at which a molecule absorbs radiation. Lastly, Raman is
sensitive to homo-nuclear molecular bonds, while FTIR is sensitive to hetero-nuclear functional groups.

2.2.7.2. Theory of Raman Spectroscopy

Photons interact with matter at a molecular level by absorption or scattering. The latter can either be elastic Rayleigh or in-elastic Raman scattering (Matousek and Parker, 2006). Raman scattering take place when there is energy exchange between photon and system, which results in system consequently decaying to vibrational energy levels either above or below to that of the original state. This frequency shift resultant to the difference in energy between the incident and scattered photon is called Raman shift. This Raman shift can be up or down shift relative to the original state, depending on whether the system has gained or lost vibrational energy. These phenomenon of down and up shifting are named the Stokes and anti-Stokes lines. Raman spectrum is obtained by plotting Raman shift from the incident laser energy versus detected number of photons. Each material has its own vibrational modes, and a specific Raman spectra.

Lasers are used as a photon source due to their highly monochromatic nature, and high beam fluxes. This is essential as the Raman effect is weak, normally the Stokes lines are \( \sim 10^3 \) times weaker than the Rayleigh scattered component (Aravindan, 2014) (figure 2-3).
Similar to IR spectroscopy, Raman spectra can provide both quantitative and qualitative information. The position of different peaks gives evidence about the chemical bond of the molecule, whereas the intensity of the peak gives the concentration of the bond type.

2.2.7.3. Raman Instrumentation

The Raman spectrometer comprises of an optical confocal microscope, a laser excitation source (632.8 nm), and a detector. The sample is first placed on a glass slide under the microscope lens, and brightened with visible light on focused area. After focusing the image on a computer using Raman software (Lab Spec), the light source is turned off. The sample is excited with laser source. The scattered photons are then passed through a pin hole aperture to a Michelson interferometer from which the Raman shifted regions are inferred. Rayleigh scattered radiation is stopped from reaching the detector by selective filters. The data from the interferometer gives a plot of intensity vs Raman shift.

For a clear spectrum with little noise the area of the sample to be mapped should preferably be flat. The laser beam only characterises the surface of the material, and the resultant spectra will only represent material surface chemistry.
2.2.7.4. Raman Resolution

In Raman spectroscopy, once again it is the distance travelled by the moving mirror in the Michelson Interferometer that determines the wavelength resolving power of the instrument. The spatial resolution of the Raman microscope is limited by the optics of the microscope; the slit size and the ‘pin hole’ aperture size. In most cases this will be around 5 μm, and so features smaller than 1 μm (for example small particles of filler) will not be shown as distinct in a Raman map. In this project, the increment for each spectrum in the mapped areas was 2 μm. A Lab Ram spectrometer instrument (Horiba Jobin Yvon, France) was used to generate all Raman spectra in this project. A schematic diagram is given in figure 2-4.

2.2.7.5. Mapping with Raman

Dentine samples (ivory, human) were examined using Raman. Samples were placed on a glass slide, and areas of 40 μm² examined using a 50x magnification lens. A He-Ne laser source of 632.9 nm was used to excite the sample. Wavenumber resolution was 2 cm⁻¹ while the confocal hole was set at 150 μm. Spectra were recorded at 100 points within the chosen area (using 4 μm² step increments). The spectra for each point were generated in the range of 800-1800 cm⁻¹ by averaging four spectra each of 10 s acquisition. Background was subtracted from all spectra prior to normalisation using the intensity at 1670 cm⁻¹. For each dentine type and / or treatment time with phosphoric acid gel six areas were examined and final average spectra then obtained.
2.2.8. Mechanical Testing

A number of mechanical testing methods were used to characterise the composite and dentine samples. These include:

2.2.8.1. Biaxial Flexural Strength Testing

In this project biaxial flexural testing has been used to characterise the mechanical properties of composites. Composite materials are quasi-brittle (De Borst, 2002), so the compressive strength of composite materials will be greater than the tensile strength. When subject to bending, the bottom edge of a test specimen is in tension. The failure of the specimen is governed therefore by the tensile strength of the material.

2.2.8.1.1. Background

Biaxial flexural testing has been generally used for the determination of mechanical properties of dental materials. It has some advantages over other uniaxial flexural testing (3-point bend,
4-point bend) (De Borst, 2002). In 3-point bend, the maximum stress occurs at one point and in 4-point bend there is a region of uniform tensile stress between the loading points. In biaxial testing the stress state is more complex, and the maximum stress is focused on a small volume on the underside of the specimen. The volume over which the maximum stress acts is larger in 4-point than in 3-point bending and both are larger than that for biaxial testing. This larger volume which is subjected to the maximum stress will result in more flaws, and making 3 and 4-point less reproducible than biaxial testing.

One of the disadvantages of uniaxial testing is that they often require larger specimens requiring more material at more expense. Rectangular beam specimens can also be difficult to manufacture, increasing the possibility of flaws. Another disadvantage is the possibility of edge failures due to the likelihood of manufacturing producing flaws at the edges of beam specimens, while biaxial samples do not suffer from edge failures in the same way. Also the samples preparation in biaxial testing are easy to prepare. One other advantage of biaxial testing for dental materials, is that it is more representative of the occlusal stress state.

There are a number of biaxial setups available. They include ball-on-ring, ball-on-three-ball, and piston-on-ring (Ban and Anusavice, 1990). In this project a ball-on-ring test is used. Although the biaxial method is not perfect in terms of geometry, the benefits of better reliability and reproducibility, easiness of specimen preparation, ability to use moulds that are cheap and disposable and small specimen size, are thought to be sufficient justification for using biaxial testing over 3-point or 4-point bending.

2.2.8.1.2. Poisson’s Ratio

Poisson’s ratio $\nu$ is the inverse ratio of transverse to axial strain. It usually occurs when a material is compressed in one way under load that results in material expansion in other directions perpendicular to direction of applied compression. This is called the Poisson effect.
In biaxial testing, the material will expand and contract out of the plane of loading when it is subjected to bending stresses. The in-plane bend relative to out of plane bend is called Poisson’s ratio. Polymer based materials (e.g., G composites) exhibit viscoelastic behaviour (Lakes, 1998). If stiffness varies with time the Poisson’s ratio will vary (Nakayama et al., 1974).

In the biaxial test method used in this project, the crosshead speed is 1 mm/min, and so strain rates are relatively low. It is reasonable therefore to use a value for Poisson's ratio more similar to that calculated by low strain rate testing. For this reason a Poisson’s ratio of 0.3 has been used throughout the biaxial test measurements.

2.2.8.1.3. Sample Preparation for Biaxial Testing

Six brass rings were placed onto an acetate sheet on a glass block. 1 mm thick discs of 10 mm diameter were prepared by placing composite pastes in the brass rings. One disc requires around 0.2 - 0.3 g of paste to fill it (depending on the Powder to Liquid ratio, PLR). The filled rings were covered instantly with another sheet of acetate and topped with a second glass block. The weight of the glass block was enough to expel excess material from the rings. These were then cured using a Demi Plus LED (output intensity 1,000 mW/cm²) for 40 s from both sides. The acetate sheets, and brass rings were removed after curing. The edges of each discs were examined, and smoothed using a sharp razor blade (Figure 2-5).

*Figure 2-5 Biaxial disc specimen after being removed from brass ring mould, after smoothing of edges with a razor blade. The split in the ring enables easier removal of the disc*
2.2.8.1.4. Storing in Water

Six discs were prepared for each formulation. Each disc was individually placed in sterilin tubes after 3 hours post-curing, and stored in 10 ml of distilled water. The tubes along with discs were stored at 37 °C for 24 hours, 1 month, 3 month, and 6 month prior to testing.

2.2.8.1.5. Testing Apparatus

Six discs were used of each formulation to determine biaxial mechanical strength. The biaxial flexural strength (BFS), was found using ball-on-ring method. In this method, a hydrated disc specimen was placed on a knife edge ring support (radius 4mm) and then loaded by a spherical tip in an Instron 4505 Universal Testing Machine (Instron, USA) (Figure 2-6). The load and central deflection of the disc were recorded and plotted on a load vs. Deflection graph from which the maximum load at fracture and the pre-fracture slope were determined to find the biaxial flexural strength (Chen et al., 2011).

BFS ($\sigma$) was measured using equation 2-14 (Timoshenko et al., 1959).

$$\sigma = \frac{L_{\text{max}}}{t^2} \left[ (1 + v) \left( 0.485 \ln \left( \frac{a}{t} \right) + 0.52 \right) + 0.48 \right]$$  \hspace{1cm} (2-14)

Where $\sigma$ is biaxial flexural strength (MPa), $L_{\text{max}}$ is maximum load (N), $a$ is support radius (mm) and $t$ is average thickness of specimen (mm).
Figure 2.6 Schematic of biaxial flexure test. Disc specimen is placed on a ‘knife edge’ circular support. The load cell tip (ball bearing) is lowered onto the specimen at 1 mm/min and the corresponding load vs. Displacement is recorded (Main, 2013).

2.2.8.2. Elastic Modulus

The elastic modulus is used to characterise elastic materials by measuring its stiffness. It is simply the ratio of the stress to strain along an axis.

The elastic modulus (E) was found from the same specimen that were tested for BFS. It was determined from the slope of the load deflection plot from a biaxial test using below equation (Higgs et al., 2001).

\[ E = 0.502 \frac{dF}{dw} \left( \frac{a^2}{h^3} \right) \]  

(2-15)

(h is the sample thickness, \(dF/dw\) is the gradient of load versus central deflection, and \(a\) is the support radius).

2.2.8.3. Three Point bending test

The three point bending flexural test in this project was used to calculate the bending strength, modulus and strain of dentine (sample preparation details given in section 2.2.14.3). Strength was assessed using a “3-point bending” jig. This consisted of two support rollers 5.0 mm in
The centres of support rollers were 10.0 mm apart. Load was applied at the midpoint, between the supports by means of a third roller 3 mm in diameter (Figure 2-7).

The load was applied with a 1 kN load cell, at a cross head speed of 1 mm/min using a computer-controlled universal testing machine (Instron 4502, Bucks, U. K). The modulus $E$, 3-point bending strength $\sigma$, and strain $\varepsilon$ were determined using equations 2-16, 2-17 and 2-18 (Temenoff, 2008, Chang et al., 2010).

\[
E = \frac{mL^3}{4bh^2} \quad (2-16)
\]

\[
\sigma = \frac{3FL}{2bh^2} \quad (2-17)
\]

\[
\varepsilon = \frac{6Dh}{L^2} \quad (2-18)
\]

$F$ is the maximum load on the load deflection curve, $L$ is the length of support span, $b$ is the width of tested specimen, $h$ is the thickness of tested specimens, $m$ is the gradient (i. E., slope) of the initial straight-line portion of the load deflection curve, and $D$ is the maximum displacement of the tested specimen from its original position to the point of highest load.

For assessment of dentine strength, modulus, and strain, ivory and human dentine were cut into rectangular sections of 15 x 5 x 2 mm. The ivory samples were stored dry at room humidity / temperature or in distilled water for 24 h at room temperature. Cut human dentine specimens were all stored in 0.2 % thymol at 4 °C before testing. Specimen thickness was checked using digital Vernier calliper (Moore & Wright, West Yorkshire, UK). Thirteen samples were tested for each type of dentine.
2.2.8.4. Data Fitting for Three Point test

Data from 3-point test were fitted to a Weibull type expression (n = 13) (Xia et al., 2014):

\[ P_f = 1 - \exp\left(\frac{\sigma}{\sigma_\theta}\right)^m \]  

(2-19)

\( m \) – Weibull shape parameter, \( \sigma \) – bending strength, (which can be replaced by modulus, or flexural strain) of each specimen and \( \sigma_\theta \) – Weibull scale parameter. When \( \sigma = \sigma_\theta \), \( P_f = 63.2 \% \).

\( P_f \) was defined as \((i - 0.5) / n\), where \( n \) is the number of specimens and \( i \) is the rank of a specimen in a list when strength, modulus or strain are ordered from lowest to highest values.

Rearranging and taking double logs of equation (2-19) gives:

\[ \ln\ln\left[\frac{1}{1-P_f}\right] = m \ln \sigma - m \ln \sigma_\theta \]  

(2-20)

Weibull parameters were calculated from the slope and intercept of the left hand side of equation (2-20) plotted versus \( \ln \sigma \).
2.2.9. **Water sorption (Mass and Volume Changes) Measurements**

2.2.9.1. **Density Measurement**

Based on Archimedes’ principle, the sample mass in air and following immersion in water can be used to calculate the density ($\rho_a$) of a sample via equation (2-21a):

$$\rho_t = \left[ \frac{M_t}{M_t - M_w} \right] \rho_w \quad (2-21a)$$

Where, $M_t$ and $M_w$ are the sample mass (g), at time $t$ in air and water respectively, while $\rho_w$ (g/cm$^3$) is the density of water at the temperature of measurement.

The volume of the sample at time $t$, $V_t$ (cm$^3$), could then be calculated using equation (2.21b):

$$V_t = \frac{M_t}{\rho_t} \quad (2-21b)$$

2.2.9.2. **Mass and Volume Changes**

The water sorption (mass and volume) study was done for all commercial and experimental composites. The measurement were carried out gravimetrically using a four-figure balance (OHAUS Pioneer Series) with attached density kit. Each composite disc was placed in upright position using a sterilin bottle, so that both of its surfaces comes in contact with storage solution of 15 ml. Two different solutions were used for storing the discs. Distilled water (DW) was used as required in the ISO 4049 standard (ISO, 2009) for testing of dental composites. Simulated body fluid (SBF) was used as in BS ISO 23317:2012 (ISO, 2012). This standard is used to assess if bone repair materials have the potential to promote hydroxyapatite precipitation. As this SBF is similar in composition to simulated dentinal fluid (Lin et al., 2011) it was used in this thesis to address if the materials could induce hydroxyapatite precipitation for dentine remineralisation. Artificial saliva, was not used as it is less able to promote remineralisation (Dakkouri, 2015). The composition of the SBF used is listed in table 2.6.
Table 2.6 The reagents used for the preparation of 1 litre of SBF.

<table>
<thead>
<tr>
<th>Reagent Name</th>
<th>Amount</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>8.035 g</td>
<td>99.5 %</td>
</tr>
<tr>
<td>Sodium hydrogen carbonate</td>
<td>0.335 g</td>
<td>99.5 %</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.225 g</td>
<td>99.5 %</td>
</tr>
<tr>
<td>Di-potassium hydrogen phosphate trihydrate</td>
<td>0.231 g</td>
<td>99.0 %</td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate</td>
<td>0.311 g</td>
<td>98.0 %</td>
</tr>
<tr>
<td>Hydrochloric acid solution</td>
<td>39 ml</td>
<td>----</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.292 g</td>
<td>95.0 %</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>0.072 g</td>
<td>99.0 %</td>
</tr>
<tr>
<td>Tris-hydroxymethyl aminomethane</td>
<td>6.118 g</td>
<td>99.0 %</td>
</tr>
</tbody>
</table>

After putting discs in storage mediums the tubes were then incubated at 37 °C for 2, 6, 24, 48 hrs and 1, 2, 4, 6, 12 weeks. At each time point, the discs were removed, blotted dry, re-weighed, and placed in fresh storage solution in new sterilin tubes (Mehdawi et al., 2009). The percentage volume and mass change at each time was finally determined from equation (2-22) and (2-23) respectively (Mehdawi et al., 2013b):

\[
% \, Volume \, Change = \frac{100[V_c - V_0]}{V_0}
\]  
(2-22)
\[ \% \text{Mass Change} = \frac{100(M_t - M_0)}{M_0} \]  

\( V_t \) and \( M_t \) are the volume and mass at time \( t \) after immersion, while \( V_0 \) and \( M_0 \) are the initial volume and mass respectively. Six samples of each formulation were used in each solution.

### 2.2.10. Drug Release study

Drug release study was performed through UV-Vis Spectroscopy. In below section, the theory of UV-Vis spectroscopy is described. Followed by that its use in chlorhexidine and polylysine release determination is outlined.

**2.2.10.1. Ultraviolet-visible Spectroscopy**

In this project ultraviolet-visible spectroscopy is used for detection and quantification of chlorhexidine and polylysine release into distilled water and simulated body fluid.

**2.2.10.1.1. Theory of UV Spectroscopy**

UV-visible light has energy values \( \sim 100 \text{ kcal / mole} \) and wavelengths of 200-800 nm (Yadav, 2013). In UV-Vis spectroscopy electron transitions from ground state to a higher energy state occurs when ultraviolet and visible radiation interacts with matter. The ultraviolet region is between 200-380 nm and the visible region between 380-800 nm.

The intensity of incident (\( I_i \)) and transmitted (\( I_t \)) light through homogenous absorbing systems (figure 2-8) at a given monochromatic wavelength is given by the Beer-Lambert law (Feigenbrugel et al., 2005):

\[ A = -\log \left( \frac{I_t}{I_i} \right) = kcl \]
Where $A$ is the absorbance, while $l$ is the light path length (cm), $c$ is the concentration of absorbing species (mol L$^{-1}$) in solution (distilled water, SBF) and $K$ is the molar absorptivity (L mol$^{-1}$ cm$^{-1}$). At a given wavelength, the molar absorptivity (molar extinction coefficient) for any absorbing species is a constant.

![Diagrammatical representation of Beer-Lambert law.](image)

**Figure 2-8 Diagrammatical representation of Beer-Lambert law.**

### 2.2.10.1.2. UV Spectroscopy Instrumentation

The Ultraviolet-visible spectrometer (Figure 2-9) consists of a deuterium lamp (for UV light) for the range of 160-375 nm and tungsten lamp (for visible light) in the range 360-1000 nm. The light beam passes through the monochromator via a slit. After entering the monochromator the light is reflected via a series of mirrors to a diffraction grating, which through rotation allows specific wavelengths to pass. The monochromatic light then passes through an exit slit into a beam splitter, which splits the light in two beam different beam paths. One beam is allowed to pass through the reference cell, and the second passes through the sample cell. The difference between the two measured at the detector provides the absorbance due to the sample.
2.2.10.2. Chlorhexidine release

The absorbance of the storage solutions, used in mass and volume studies, was obtained at each time point using a UV spectrometer (Unicam UV 500, Thermo- Spectronic®, UK) for a period of up to 6 weeks. For determination of the standard calibration curve of chlorhexidine diacetate (CHX) in distilled water, a stock solution of 1000 ppm CHX was prepared. For determination of the standard calibration curve of chlorhexidine diacetate (CHX) in SBF, a stock solution of 50 ppm CHX was prepared due to its lower solubility in this fluid. Both stock solutions were serial diluted to 20, 10, 5, 2.5, and 1.25 ppm. A 1 cm path length quartz cuvette was filled with distilled water and the UV spectrum recorded between 200 and 400 nm with a resolution of 1 nm. UV spectra for the calibration solutions were then recorded and the spectrum of distilled water subtracted. Absorption maximum of CHX in this thesis were found at 231 and 255 nm. By plotting concentration versus absorbance, the calibration curve for CHX was obtained (Figure 2-10).
The amount of CHX released ($R$ in grams) was then calculated using equation (2-25) (Mehdawi et al., 2009):

$$R = \frac{A}{g} V$$

(2-25)

$A$ is the absorbance at 255 nm, $g$ is the gradient of a calibration curve of absorbance versus CHX concentration, and $V$ is the storage solution volume (15 ml). The % cumulative amount of drug release ($\% R_c$) at time $t$ was then given by equation (2-26) (Mehdawi et al., 2009):

$$\% R_c = \frac{100 \sum R_t}{w_c}$$

(2-26)

$w_c$ is the weight of CHX incorporated in each formulation in grams. Six repeat runs were performed for each sample (at every time point) in both distilled water and SBF.

2.2.10.3. Polylysine Release
Polylysine (PLS) was measured in the same way as mentioned earlier for chlorhexidine release through UV-Vis spectroscopy. However, since polylysine itself does not absorb light in the high UV or visible region it cannot readily be quantified directly with UV-Vis spectroscopy. Nevertheless, the concentration of polylysine in aqueous solutions can be determined indirectly with the help of Trypan blue (TB) assay (Grotzky et al., 2010).

2.2.10.3.1. **TB Assay**

Trypan blue (TB) is a large oligoanionic dye comprising two azo groups (R–N=N–R\(^9\)) and four sulfonate groups (M. Wt of TB = 872.88 g mol\(^{-1}\)). Dilute aqueous solutions of TB are blue with an absorption maximum at \(\lambda_{\text{max}} = 580\) nm (Grotzky et al., 2010). The TB assay is based on the poly-cationic character of PLS in acidic aqueous solutions. PLS (M. Wt of Lysine = 146.19 g mol\(^{-1}\)) binds ionically with the anionic dye TB, and causes precipitation of the dye. This results in concomitant decrease in the intensity of the blue colour of the solution. Under the experimental settings used, there is a linear relationship between the absorbance at \(\lambda_{\text{max}} = 580\) nm and the PLS concentration in the solutions between 1 and 9 ppm. Three different reagent solutions of TB assay were prepared using distilled water, SBF, and 0.02 2-morpholinoethanesulfonic acid (MES) / 0.03 sodium chloride (NaCl) buffer solution. Reagent solutions were prepared at 8000 ppm, and from that diluted down to 80 ppm to be mixed later with PLS serial dilutions.

2.2.10.3.2. **PLS determination**

For determination of the standard calibration curve of PLS, a stock solution of PLS was prepared at 10,000 ppm in distilled water and SBF. The stock solution was serial diluted to 20, 18, 14, 10, 8, 4, and 2 ppm. To determine PLS, the serial dilutions of PLS were mixed with the three different reagent solution of TB (80 ppm) in equal amounts. So after mixing of two solutions the final TB concentration will be 40 ppm, and the serial PLS dilutions will be 10, 9,
7, 5, 4, 2, and 1 ppm respectively. After mixing the final mix was incubated for an hour at 37 °C, after which it was cooled down to room temperature for another hour. Lastly, the mix was centrifuged at 13,000 rpm for 20 min to sediment the precipitate. While pipetting the supernatant care was taken to avoid pipetting the precipitate.

A 1 cm path length disposable plastic cuvette was filled with distilled water and the UV spectrum recorded between 200 and 800 nm with a resolution of 1 nm. UV spectra for the calibration solutions were then recorded and the spectrum of distilled water subtracted. Absorption maximum of PLS was found at 580 nm. By plotting concentration versus absorbance, the calibration curve for PLS was obtained (Figure 2-11) and the gradient noted through linear regression. The % drug release, and the amount of PLS released in grams were calculated with the equations mentioned for CHX release. Six repeat runs were performed for each sample in both distilled water and SBF.

![Polylysine calibration curve (absorption 580 nm).](image)

*Figure 2-11 Polylysine calibration curve (absorption 580 nm).*
2.2.1.1. Data Interpretation for Water sorption and Drug release test

The mass and volume changes, and drug release results were plotted as a function of square root of time (SQRT/min) to enable clear understanding of the trends at both early and late time points. Initial intercept, gradient, and maximum or final numbers were determined and analysed using factorial analysis.

Initial intercept (y-intercept) was calculated by using the intercept function in excel. The data point’s chosen for calculating intercept in water sorption studies (mass, and volume changes) was between 48 hours to 6 weeks, while in drug release studies (CHX, and PLS) was between 48 hours to 4 weeks.

Similarly, the gradient was calculated by using the slope function in excel. The data point’s chosen for calculating gradient in water sorption studies was between 48 hours to 6 weeks, while in drug release studies was between 48 hours to 4 weeks.

Lastly, the maximum or final number was taken from the raw data of water sorption, and drug release studies for each composite. In case of water sorption the final mass, and volume changes were at 12 weeks, while in case of final chlorhexidine, and polylylysine release were at 6 weeks time point.

To explain the main factors or variables affecting the degree of conversion, mass and volume changes, biaxial flexural strength, modulus, push out and shear bond strength, and drug release the geometric mean result (calculated using the function geomean in excel) of all samples with a given variable at its high value was divided by that obtained for all samples with the same variable at its low value. This ratio, and its natural logarithm ($a_i$) will equal one and zero respectively if the given variable has on average no effect. If $a_i$ is of greater magnitude (either positive or negative) the variable has a more significant effect.
Throughout this project, the error bars refer to 95% confidence interval of the mean (C.I) assuming C.I = 1.96×S.D/√n.

2.2.12. Adhesion Testing (Bond strength measurements)

In this project bonding of composite to dentine was measured by two different methods. The first one was a Push out testing which was performed only with ivory dentine. The second test used was shear bond assessment. Shear bond was assessed using ivory and human dentine.

2.2.12.1. Push out test

In the push-out test, a flat indenter is used to apply load to the top surface of composite restoration.

2.2.12.1.1. Theory

The different steps involved in a classic push-out test for a composite bonded to dentine (without any internal stress) under conditions of stable crack propagation, are shown in Figure 2-12.
The resulting load/displacement curve is given in figure 2-13. When a compressive load is applied to the top surface of composite as shown in the figure, shear stresses ‘τ’ are introduced at the interface with a maximum value occurring at the region near the top face. When the load reaches $P_i$, the shear stress reaches its critical limits, and debonding initiates, causing a change in slope in figure 2-13 (Chandra and Ghonem, 2001). This is called initial debonding. Once debonding occurs, the shear stress in the debonded region declines. This results in the maximum stress being shifted away from the top face when load is increased, and results in

---

**Figure 2-12** Debonding sequence in push out test (Chandra and Ghonem, 2001).
partial debonding $P_d$. $P_d$ represents any load in the middle of $P_i$ and $P_{\text{max}}$. When the applied load reaches $P_{\text{max}}$, the shear stress reaches to its maximum limits at the bottom face, and this will result in complete debonding. After that the entire length of the composite filling is pushed out of the dentine which is represented by $P_f$ in figure 2-13. This results in a sudden drop in the load as shown in Figure 2-13.

![Figure 2-13 Typical push out test curve with progressive debonding (Chandra and Ghonem, 2001).](image)

The interfacial push out stress $\tau$ can be calculated using equation 2-27 (Zhou et al., 2001).

$$\tau = \frac{P}{\pi dl} \tag{2-27}$$

Where, $P$ is the applied load, $d$ is the diameter of the composite filling, and $L$ is the length (thickness) of the specimen.
2.2.12.1.2. Push out Instrumentation

The push out tests was performed using ivory dentine with varying dentine conditionings (details given in section 2.2.14.4), and composite on Instron 4502, U.K (figure 2-14). The tests were conducted at a cross-head speed of 0.5 mm per min, using a 50 kN load cell. The maximum displacement to reach before the cross head return to its original position was 1.5 mm. The maximum load required for complete debonding was noted, and interfacial stress measured. Comparison was made for different commercial and experimental composites. For each formulation there were six samples.

![Push out test with Instron on ivory dentine filled with composite.](image)

2.2.12.2. Shear bond test

2.2.12.2.1. Background

The shear bond strength is measured by dividing the applied load by the bonded cross-sectional area. The shear bond is a more accurate model for replicating class II, and V cavities. The push out is more suitable model for class I occlusal cavities. Furthermore there is very limited
literature on push out bonding strength, so to compare experimental composites bonding strengths with that of literature values, shear bond strength was performed.

2.2.12.2.2. Theory and Instrumentation

In shear bond testing the stress at interfaces generates from the force vector component parallel to the cross section. Whereas, normal stress, on the other hand, arises from the force vector component perpendicular to the material cross section (Bird et al., 2007).

The shear bond test was done according to ISO 29022:2013. Shear bond strength was determined using an Instron Universal testing machine with a “Flat-edge shear fixture” jig. The jig consisted of a metal holder with an adjustable screw to secure the specimen and an adjustable blade, which was used to shear the tube from the dentine (figure 2-15).

A 1 kN load cell at cross head speed of 1 mm/min was used. The load at break was recorded and the bond strength $\tau$ calculated using equation 2-28, in which $F$ is the load at break, and $A$ is the bonded area of the cylinder. Each composite formulation there were six samples.

$$\tau = \frac{F}{A} \quad (2-28)$$
2.2.13. Scanning Electron microscopy

All scanning electron microscopy (SEM) images were captured using Phillip XL-30 (Eindhoven, The Netherlands).

2.2.13.1. Theory SEM

SEM can be regarded as a high magnification microscope. It uses a focused beam of electrons to generate images of specimen surfaces. The electron beam after generation, is passed through a high voltage which will accelerate the beam. After that, the accelerated beam is passed through a series of apertures and electromagnetic lenses to produce a thin beam of electrons. Lastly, the beam passes through scan coils, that will scan the surface of the specimen, and an image is obtained on a computer.

2.2.13.2. Imaging Dentine and Interface

To visually assess micro-gap formation due to composite shrinkage, human and ivory dentine blocks of 3 and 5 mm depth were produced containing cavities of 3 mm diameter (details about
sample preparation are given in section 2.2.14.5). After drilling, the cavities were washed and then treated in the four different dentine pretreatment methods discussed in section (2.2.14.5). Following dentine conditioning the cavities were restored by placing composites in 2 mm layers and curing as above. After 24 hours in distilled water at room temperature, restored cavities were cut in half vertically to expose the interface on two sides and from top to bottom of the restoration. The surface of ivory, and human dentine were polished using P4000 silicon carbide paper. Specimens that were to be imaged were mounted onto stubs with fast setting epoxy adhesive, and sputter coated with gold / palladium using Polaron E5000,UK for 90 s at 20 mA. After coating, microscopic images of interfaces and dentine were taken using a Scanning electron microscope. The voltage was set at 5 kV for imaging.

2.2.14. Ivory and Human Dentine preparation

Ivory is a hard, smooth, substance, composed primarily of dentine, that constitutes the tusks. Ivory tusk consists of hydroxyapatite which gives strength and rigidity and collagen for flexibility, growth, and repair. Ivory dentine is composed of mainly pure dentine, with a thin layer of cementum on outside. Ivory tusks were used in this project as they allowed testing of large numbers of samples in a standardised way. This would not have been possible using human dentine.

2.2.14.1. Dentine Collection and Storage

Ivory dentine was obtained from the lower third of a full length tusk. The tusk was from Africa and provided by the U. K Border Agency, Heathrow airport under the Convention on International Trade in Endangered Species for research purposes (CITES Reference 08/2012). The tusks were stored at room temperature and humidity.
Non-curious adult human dentine was collected from sound human teeth through the UCL Eastman Bio-bank after ethical approval and patient consent (Bio-bank ID number 1304). The teeth were stored in a 0.2 % thymol solution at 4 °C for up to 4 weeks prior to use.

2.2.14.2. Dentine cutting, grinding, polishing

Ivory tusk and human teeth were mounted in slow setting viscous self-curing epoxy resin, after cutting into small sections using accutom-50 precision cut-off machine.

Grinding and polishing was done using Sturers LaboPol-5. A series of grind papers were used. The grinding was started from P120 paper, and followed by P500, P1000, P2400, and finished with P4000 grind paper. The final surface was even and smooth when visually inspected.

2.2.14.3. Dentine preparation for mechanical testing

For assessment of dentine strength, modulus, and strain, ivory and human dentine were cut into rectangular sections of 15 x 5 x 2 mm. Specimen thickness was checked using digital vernier calipers (Moore & Wright, West Yorkshire, UK). Strength was assessed using a “3-point bending” jig.

2.2.14.4. Dentine preparation for Adhesion testing

For shear bond test cut ivory cubes (~1x1x1 cm) were placed in water for 24 hours at 37 °C. Thereafter they were kept in small sealed containers to reduce water evaporation and used within 48 hours. Ivory cubes and 0.2 % thymol stored human teeth cut vertically in half, were embedded in slow-setting viscous self-curing resin such that dentine tubules were perpendicular to the top resin surface. To assess bond strength, composite pastes were poured in 2 mm increments into a brass tube of 3 mm internal diameter, and 6 mm long placed on the surface of the dentine. The end of the tube in contact with dentine was chamfered at 45 degrees to reduce its contact area. Each 2 mm increment was cured for 40 s.
The push out test was undertaken on ivory. Ivory was cut parallel to the direction of dentineal tubules, to give blocks of 33 x 30 x 5 mm sections (Figure 2-16). The cut sections were placed in distilled water for 24 h in an incubator at 37 ºC, and then left to dry at the same temperature for a further 24 h. This provided a slightly moist dentine and was found to improve reproducibility.

Twelve holes were then drilled into cut blocks of 3 mm in diameter and 5 mm deep. Finally, cavities were fully filled with either the commercial or experimental composite pastes. Each composite sample after restoration was cured for 40 s from both sides. The samples were then stored in an incubator at 37 ºC for 24 h prior to push out test.

![Ivory block and drilled holes](image)

*Figure 2-16 Photographs of ivory tusk cut into rectangular block and cylindrical holes after drilling.*

### 2.2.14.5. Dentine preparation for Interface and Micro-gap checking

To visually assess dentine-composite interace, and micro-gap formation due to composite shrinkage, human and ivory dentine blocks of 3 and 5 mm depth were produced containing cavities of 3 mm diameter. After drilling, the cavities were washed and then treated using four different dentine cavity pretreatments:
1) Ibond application and light cure for 20 s as per manufacturer’s instructions, or
2) Acid etch application for 20 s followed by water rinsing, gentle drying and Ibond application and cure, or
3) Acid etch for 20 s, rinse and dry, or
4) No acid or Ibond treatment.

Following treatment the cavities were restored by placing composites in 2 mm layers and curing as above. After 24 hours in distilled water at room temperature, restored cavities were cut in half vertically to expose the interface on two sides and from top to bottom of the restoration. The surface of ivory, and human dentine were polished as mentioned in earlier section (2.2.14.2), and sputter coated with gold / palladium before taking microscopic images of interfaces using a Scanning electron microscope.
CHAPTER 3

CONTROL AND COMMERCIAL MATERIALS
3. Control and Commercial materials

3.1. Introduction

Current dental composites have shown improved properties, which has enabled clinicians to place composites in a multitude of case types (Leprince et al., 2013). The properties that are extensively studied include strength, modulus, shrinkage, and bonding to dentine (Ilie and Hickel, 2009a, Ilie and Hickel, 2009b). The strength and modulus of composites range from 70 to 180 MPa, and 3 to 10 GPa respectively (Ferracane, 2011). This wide range of values is in part a result of changing the filler phase. Generally higher filler loads make composites stronger and stiffer.

Several studies address polymerisation shrinkage. High molecular weight monomers like Bis-GMA, modified UDMA, and traditional UDMA are used to reduce the shrinkage. Reducing monomer conversion reduces shrinkage (Naoum et al., 2012, Goracci et al., 2014) but as a result un-cured monomer may leach from the set material. Current composites have been shown to have shrinkage between 1 and 5 vol % (Ferracane, 2005).

The majority of composites currently in the market are designed for bulk filling. The bulk filling composites described in this chapter include Z250 and Gradia. These two materials were selected because of their wide use in dental practice, and different base monomers (Bis-GMA in Z250, and UDMA in Gradia). Both materials are supplied in pre-mixed cartridges, and are highly viscous with filler loading of approximately 80 wt % (Watanabe et al., 2008a, Uhl et al., 2006). The monomer conversion of Z250 and Gradia were 50 %, and 45 % respectively (Palin et al., 2003a, Bracho-Troconis et al., 2008). The mechanical properties of these bulk composites are studied widely. In terms of strength, Z250 showed highest strength of up to 180 MPa among commercial materials (Lien and Vandewalle, 2010, Mitra et al., 2003). In
comparison Gradia is considered weaker, with average strength around 80 MPa after 24 hrs storage in DW (Blackham et al., 2009).

The self-adhesive and flowable composite studied in this project was Vertise flow. This is considered as the latest class of composites. These composites are similar in composition to bulk filled composite, but with less filler content (Ferracane, 2011). Additionally, they have some acidic monomer in the formulations that helps provide self-adhesive properties. Due to their weak mechanical properties, they are not prescribed for bulk filling, and can be used only in low stress bearing areas.

The aim of this chapter was to characterise commercial flowable (Vertise flow), and bulk filling composites (Z250, Gradia), and compare the results with those of two basic experimental composites. The experimental control composites studied in this project are both bulk filled composites and identical except that one has the hydrophilic monomer HEMA (5 wt %), and the other de-mineralising 4-META (5 wt %). Their filler phase consists of 100 % glass particles. The purpose of these control composites is to make comparisons between commercial composites, and provide benchmark properties for the subsequent development of new composites.

FTIR analysis provides information on the chemical changes during light curing of the composites. The properties obtained using FTIR data included monomer conversion, polymerisation shrinkage, and heat generation. The latter two were determined theoretically using conversion data from FTIR. Depth of cure was measured using ISO 4049 (scrapping test). Mass and volume changes were determined using a four figure balance, and density kit. Biaxial flexural strength (BFS) and modulus were obtained using an Instron testing machine. Adhesion of composites to ivory dentine was initially assessed using a push out test. Later, adhesion tests
were done using shear bond test on human and ivory dentine. Finally, the interface of composite and dentine were checked for micro-gaps using SEM.

3.2. Aims and Objectives

The main aim of this project was to compare and characterise commercial and control composites. By characterising these composites, target properties for new experimental dental composites can be outlined.

FTIR spectra before and after cure provide the percent monomer conversion. The conversion data can be used further to calculate theoretical shrinkage and heat generation.

Depth of cure is assessed using an ISO 4049 test method.

Mass and volume changes are carried out using a density kit connected to a four figure balance. The mass and volume changes are determined over a period of 12 weeks. Two parallel studies are carried out for each composite in distilled water (DW) and simulated body fluid (SBF).

Mechanical properties are determined using a universal testing machine (Instron). The properties determined include biaxial flexural strength, and Young’s modulus. Possible relations between curing and mechanical properties will be discussed.

Adhesion related properties are determined first using a push out test and ivory dentine. The ivory dentine will provide freedom in terms of sample size, and reproducibility. Secondly, the materials are tested using a shear bond method, with ivory and human dentine. Use of the later enables comparison of the results with literature and helps to confirm the validity of using ivory.

Finally, interfaces between dentine (human and ivory) and composites will be assessed using scanning electron microscopy. This will help to quantify the interface layer, and enable measurement of any micro-gaps formed after polymerisation shrinkage.
3.3. Materials and Methods

In this chapter, three commercial, and two experimental composites were compared and investigated. The details are given in table 3.1.

*Table 3-1 Commercial and control materials to be investigated with manufacturers and type explanation.*

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer/Region</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek™ Z250</td>
<td>3M ESPE, USA</td>
<td>Bulk Filling Dental Composite</td>
</tr>
<tr>
<td>GC Gradia Direct posterior</td>
<td>GC Corporation, Europe</td>
<td>Bulk Filling Dental Composite</td>
</tr>
<tr>
<td>Vertise™ Flow</td>
<td>Kerr Corporation, Italy</td>
<td>Self-Adhering Flowable Composite</td>
</tr>
<tr>
<td>C-HEMA</td>
<td>Experimental Composite</td>
<td>Bulk Filling Dental Composite</td>
</tr>
<tr>
<td>C-4META</td>
<td>Experimental Composite</td>
<td>Self-Adhering Bulk Composite</td>
</tr>
<tr>
<td>Ibond Total Etch</td>
<td>Heraeus Kulzer GmbH, Germany</td>
<td>Adhesive</td>
</tr>
<tr>
<td>Ibond Etch 35 Gel</td>
<td>Heraeus Kulzer GmbH, Germany</td>
<td>Acid etchant</td>
</tr>
</tbody>
</table>
The detailed composition of each commercial material is provided in detail in chapter 2 of this thesis. The control composites were prepared with the following compositions (see table 3.2).

*Table 3-2 Control materials monomer and filler phase composition.*

<table>
<thead>
<tr>
<th>Material</th>
<th>Powder to liquid ratio</th>
<th>Monomer Phase</th>
<th>Filler Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-HEMA</td>
<td>4:1</td>
<td>UDMA 69.75 wt %, TEGDMA 23.25 wt %, HEMA 5 wt %, CQ 1 wt %, DMPT 1 wt %.</td>
<td>Silane treated glass particles 100 wt %</td>
</tr>
<tr>
<td>C-4META</td>
<td>4:1</td>
<td>UDMA 69.75 wt %, TEGDMA 23.25 wt %, 4-META 5 wt %, CQ 1 wt %, DMPT 1 wt %.</td>
<td>Silane treated glass particles 100 wt %</td>
</tr>
</tbody>
</table>

The composition of commercial materials were provided from published literature and manufacturer’s information.

The changes in intensity of spectral peaks that were involved in free radical addition polymerisation were used to determine the degree of conversion using FTIR (n=6). The samples were prepared in 1 mm, and 4 mm thickness and cured for 40 s from top. Polymerisation shrinkage and heat generation were determined using conversion data, and equations given in chapter 2 of this thesis. Depth of cure was performed according to ISO 4049 scraping technique (n=6) after 20 s and 40 s cure. Specimens mass, and volume changes were determined using density kit, and four figure balance (n=6) in DW and SBF up to 12 weeks.

The biaxial flexural strength and modulus of composite discs (1mm thickness, 10 mm diameter) were determined after 24 hrs, 1, 3, and 6 months storage in DW at 37 °C (n=6). The push out test was done using ivory dentine. Each test was repeated six times. Shear bond strength to ivory and human dentine was carried out using ISO 29022:2013, each sample was repeated 6 times. The interface study was carried out for all composites using both dentine types, and by using different dentine conditionings.
3.4. Results

3.4.1. Conversion

Individual monomers, and glass filler spectra are given in figure 3-1. A number of peaks can be identified. Each peak corresponds to different functional groups. The important peaks that can be seen in all monomers includes 1716 cm\(^{-1}\) that corresponds to C=O stretch. This peak is slightly more intense with UDMA than with the rest of the monomers. The 1636 and 1400 cm\(^{-1}\) peaks are caused by C=C stretch, and a C-H attached to C=C respectively in un-polymerised methacrylate; UDMA, TEGDMA, and HEMA. A UDMA specific peak due to N-H deformation can be seen at 1528 cm\(^{-1}\). The region between 1608-1612 cm\(^{-1}\) corresponds to C=C, which is present in aromatic carbon rings in Bis-EMA, and Bis-GMA. The peaks that were seen in all monomers were 1452 cm\(^{-1}\) corresponding to aliphatic C-H vibrations, 1296 and 1320 cm\(^{-1}\) due to C-O stretch, and 1160 cm\(^{-1}\) due to C-O-C stretch. The spectra of glass showed a strong peak mainly at \(~\) 988 cm\(^{-1}\).
Figure 3-FTIR spectra for monomers used in commercial and control composites. An example spectrum of a glass filler is also given.
Example FTIR spectra before and after composite cure for 40 s are given in figure 3-2. Monomers and filler peaks can be seen. The changes after curing can be noted in the region of 1300-1350 cm\(^{-1}\). The intensity of 1320 cm\(^{-1}\) peak due to C-O stretch above background at 1350 cm\(^{-1}\) can be used to measure the conversion of the monomer. In this case the intensity is decreased by \(\sim 50\%\) on curing. The above mentioned method was used for all composites to measure the monomer conversion.

![Figure 3-2 Sample FTIR spectra of Z250 commercial composite before and after light curing for 40 s.](image)
Monomer conversion at the bottom of the composite discs 1 mm, and 4 mm in depth after curing for 40 s are given in figure 3-3. The final monomer conversion was calculated using equation 2-10. The commercial bulk filling composites showed lower conversion than the experimental bulk composites and commercial flowable composite. The conversions at 4 mm depth were on average 10 % less than that at 1 mm. Experimental composite C-HEMA, and C-4-META showed higher conversion of ~ 80 % and ~ 70 % at 1 mm and 4 mm depth, followed by Vertise flow ~ 65 % and ~ 55 % at 1 mm and 4 mm depth. The lowest conversions were noted with Gradia and Z250 (~ 45 % and ~ 40 % at 1 mm and 4 mm depth).

![Figure 3-3 Conversion in percentage of commercial and experimental composites at depth of 1 mm and 4 mm (Error bars are 95 % confidence interval, n=6).](image-url)
3.4.2. Polymerisation Shrinkage and Heat Generation

Polymerisation shrinkage and heat generation were measured theoretically using conversion data (details given in section 2.2.5.4). The exact amounts of the monomers in commercial materials were not disclosed by the manufacturers, the shrinkage results were calculated assuming equal quantities of each monomer. Monomer conversion is directly proportional to polymerisation shrinkage and heat generation. So, for example if monomer conversion is low the shrinkage and heat generation associated with it will be less, and vice versa.

The shrinkage at the bottom of 1 mm and 4 mm deep specimens of commercial bulk filling composites were ~ 2.5 and 2 % respectively. The experimental composite showed a shrinkage of ~ 4.2 % and 3.5 % at the bottom of 1 mm and 4 mm thick samples. The highest shrinkage was associated with flowable composite Vertise flow with a shrinkage of ~ 5 and 4.2 % at the bottom of 1 mm and 4 mm deep samples (see figure 3-4).

![Figure 3-4 Volumetric shrinkage in percentage of commercial and experimental composites at depth of 1 mm and 4 mm (Error bars are 95 % confidence interval, n=6).](image_url)
The same trends can be seen in case of heat generation. Bulk filled commercial composites showed less heat generation at the bottom of 1 mm, and 4 mm thick samples than experimental bulk filled composites, and the commercial flowable composite (figure 3-5).

Figure 3-5 Heat generation of commercial and experimental composites at depth of 1 mm and 4 mm (Error bars are 95% confidence interval, n=6).
3.4.3. Depth of Cure

Depth of cure after 20 or 40 s light exposure were measured using ISO 4049 method outlined in section 2.2.6.2 (see figure 3-6). All composites showed a depth of cure between 2.28-2.36 mm after 20 s cure. The cure depth was increased further after 40 s cure to 2.45-2.49 mm. The maximum depth of cure was seen in case of Z250, while the rest of the composites showed no significant difference in depth of cure.

![Graph showing depth of cure for different composites after 20 and 40 s cure](image)

Figure 3-6 Depth of cure using ISO scraping technique of composites after curing of 20 s and 40 s (Error bars are 95% confidence interval, n=6).
3.4.4. Mass and Volume Changes

Mass and Volume changes were measured over a period of 12 weeks in both distilled water (DW), and simulated body fluid (SBF).

3.4.4.1. Mass Changes

Mass changes over 12 weeks in DW and SBF for all commercial and experimental composites plotted versus square root of time are given in figure 3-7 and 3-8. Maximum mass change was calculated using equation 2-23. The maximum mass change in DW was 1.3 ± 0.1 %, and 1.1 ± 0.1 % in SBF at 12 weeks. Initial 0.4-0.6 % increase occurs in first 24 hours. The mass changes for all composites continued to increase up to 6 weeks. It was assumed that equilibrium was reached between 6-12 weeks. The mass changes were slightly higher in the case of C-HEMA and Vertise flow composite than C-4META, Z250, and Gradia.

![Graph showing mass change of control experimental and commercial composites over 12 weeks in DW.](image)

*Figure 3-7 Mass change of control experimental and commercial composites after storing in DW over a period of 12 weeks (Error bars are 95 % confidence interval, n=6). It was assumed that equilibrium was reached by 6 weeks.*
Figure 3-8 Mass change of control experimental and commercial composites after storing in SBF over a period of 12 weeks (Error bars are 95 % confidence interval, n=6). It was assumed that equilibrium was reached by 6 weeks.
3.4.4.2. Volume Changes

Volume changes over 12 weeks in DW and SBF for all commercial and experimental composites plotted versus square root of time are given in figure 3-9 and 3-10. Maximum volume change was calculated using equation 2-22. Volume change in DW was 2.2 ± 0.2 %, and 1.6 ± 0.2 % in SBF. Initial 0.5-1.0 % increase occurs in first 24 hours. Final volume changes for C-HEMA and Vertise flow was higher than the rest of the bulk filled composites. It was assumed that final equilibrium was reached between 6-12 weeks. In SBF on average the volume changes were less than in DW.

![Volume Change Graph](image)

*Figure 3-9 Volume change of control experimental and commercial composites after storing in DW over a period of 12 weeks (Error bars are 95 % confidence interval, n=6). It was assumed that equilibrium was reached by 6 weeks.*
Figure 3-10 Volume change of control experimental and commercial composites after storing in SBF over a period of 12 weeks (Error bars are 95% confidence interval, n=6). It was assumed that equilibrium was reached by 6 weeks.
3.4.5. Mechanical Properties

3.4.5.1. Biaxial Flexural Strength

Biaxial flexural strength for commercial and experimental control composites were checked over a period of 6 months (details given in section 2.2.8.1.3). The flexural strengths are given in figure 3-11.

![Biaxial flexural strength of commercial and experimental composites after 24 hrs, 1, 3, and 6 month storage in DW. Error bars represent 95 % CI, n=6.](image)

Figure 3-11 Biaxial flexural strength of commercial and experimental composites after 24 hrs, 1, 3, and 6 month storage in DW. Error bars represent 95 % CI, n=6.

Early strengths were assessed after 24 hrs storage in DW. The initial strength of Z250, C-HEMA, and C-4META were comparable with values of 158, 167, and 168 MPa respectively. The initial strengths of Vertise flow and Gradia were significantly lower than other bulk filled composites with values of 125, and 77 MPa respectively. There was a similar pattern of strength reduction with time seen in Z250, C-HEMA, and C-4META. With these three materials decline in strength between 24 hours and 1 month was much greater than that between 1 and 3 months.
or 3 and 6 months. With Gradia, and Vertise, more comparable decrease in strength between each time point was seen. It was seen that all changes in strength with time (commercial and experimental) could fit well to the following equation 3.1.

\[
\ln \left( \frac{S_t - S_f}{S_0 - S_f} \right) = -0.50 \left( \frac{t}{hr} \right)^{0.5} \quad (R^2=0.95)
\]  

(3.1)

Subscripts \( t, 0 \) and \( f \) indicate strength \( (S) \) at time \( t \), initially (as \( t\rightarrow0 \)) and finally (\( t\rightarrow\infty \)) when the composite appeared to have reached equilibrium water sorption. Fitting of equation 3.1 gave initial and final strengths for Z250 of 156 and 104 MPa. Its maximum strength reduction was therefore 33 \% (52/156 MPa). Gradia, and Vertise flow gave similar percentage reduction to Z250. The experimental composites showed a slightly higher predicted maximum percentage reduction of 39 \pm 1 \% (see table 3-3) (see also Appendix 1).

Table 3-3 Fitting of Equation 3-1 on biaxial flexural strength. Fractional reduction in strength at 6 months is also given.

<table>
<thead>
<tr>
<th>BFS</th>
<th>( S_0 )</th>
<th>( S_t )</th>
<th>( S_0-S_t )</th>
<th>Fraction reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradia</td>
<td>80</td>
<td>54</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>Z250</td>
<td>156</td>
<td>104</td>
<td>52</td>
<td>33</td>
</tr>
<tr>
<td>Vertise Flow</td>
<td>134</td>
<td>89</td>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>C-HEMA</td>
<td>169</td>
<td>101</td>
<td>68</td>
<td>40</td>
</tr>
<tr>
<td>C-4META</td>
<td>173</td>
<td>108</td>
<td>65</td>
<td>38</td>
</tr>
</tbody>
</table>
3.4.5.2. Young’s Modulus

Modulus for commercial and experimental control composites were checked over a period of 6 months in DW at 37 °C. The moduli (calculated using equation 2-15) are given in figure 3-12.

![Figure 3-12 Young’s Modulus of commercial and experimental composites after 24 hrs, 1, 3, and 6 month storage in DW. Error bars represent 95% CI, n=6.](image)

Initial moduli were assessed after 24 hrs storage in DW. The initial modulus of Gradia was lowest (2.4 GPa), followed by Vertise flow (2.9 GPa), Z250 (3.7 GPa), C-HEMA (4 GPa), and highest in case of C-4META (5.5 GPa). It was seen that all changes in modulus with time (commercial and experimental) could fit well to the following equation 3-2.

\[
\ln\left(\frac{E_t - E_f}{E_0 - E_f}\right) = -0.10 \left(\frac{t}{\text{hr}}\right)^{0.5} \quad (R^2=0.95)
\]  

(3-2)
Subscripts t, 0 and f indicate modulus (E) at time t, initially (t→0) and finally (t→∞) when the composite appeared to have reached equilibrium water sorption. Fitting of equation 3-2 gave initial and final moduli for Z250 of 4 and 3 GPa. Its maximum moduli reduction was therefore 23 % (1/4 GPa). Gradia had similar predicted maximum percentage reduction. C-4-META had higher reduction at 28 %, followed by Vertise flow at 47 %, and C-HEMA at 49 % at 37 °C (see table 3-4) (see also Appendix 1).

Table 3-4 Fitting of Equation 3-2 on to Young’s modulus over 6 months and calculated maximum fraction reduction in modulus.

<table>
<thead>
<tr>
<th>Modulus</th>
<th>S₀</th>
<th>S₉</th>
<th>S₀-S₉</th>
<th>Fraction Reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradia</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>Z250</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>Vertise Flow</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>C-HEMA</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>49</td>
</tr>
<tr>
<td>C-4META</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>28</td>
</tr>
</tbody>
</table>

3.4.6. Adhesion Properties

Adhesion of composites to ivory dentine was first measured using a push out test. Later both bonding to human and ivory dentine were assessed using a shear bond test.
3.4.6.1. Push out bond strength

Push out test was performed according to method outlined in section 2.2.12.1. From the combined results the biggest factor increasing bond strength was use of the adhesive Ibond. In case of commercial composites with Ibond use the bond strengths were between 33-43 MPa, while the experimental composites gave values between 42-50 MPa. With acid etching and without Ibond use, increased average bond strengths were seen in both flowable Vertise flow, and C-4META composite. The bond strength was between 26-35 MPa. With all the other bulk filled composites (C-HEMA, Z250, and Gradia) the bond strengths with acid treatment were between 14-20 MPa. Without using any dentine pre-treatment the flowable Vertise flow, and experimental self-adhesive C-4META composite bond strengths were between 23-30 MPa. Bond strengths for the rest of bulk filled composites were between 9-10 MPa (see figure 3-13).

![Figure 3-13 Push out bond strength of each composite to ivory dentine using the following dentine preconditionings: IAV (Acid etching followed by Ibond adhesive), IAnV (Only Ibond application), InAV (Only 35 % phosphoric acid etching), InAnV (No dentine pre-treatment). Error bars represent 95 % CI, (n=6).](image_url)
3.4.6.2. Shear bond Strength

Shear bond strength was calculated using equation 2-28. From the combined results the biggest factor increasing bond strength was use of the adhesive Ibond. In case of commercial composites with Ibond use (with or without acid etching) the bond strengths were between 30-35 MPa, while the experimental composites were between 35-45 MPa. With acid etching and without Ibond use increased average bond strengths were seen in both flowable Vertise flow, and C-4META composite. The bond strength was between 22-30 MPa. With all the other bulk filled composite the bond strengths with acid treatment and without Ibond use were between 11-16 MPa.

Without using any dentine pre-treatment the flowable Vertise flow, and experimental self-adhesive C-4META composite bond strengths were between 18-22 MPa. Bond strengths for the rest of the bulk filled composites were between 6-9 MPa (see figure 3-14). The results obtained by shear bond test were systematically slightly lower with ivory compared with human dentine.

Figure 3-14 Shear bond strength of each composite to ivory dentine (V), and human dentine (H) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment). Error bars represent 95 % CI, (n=6).
3.4.7. Interface and Micro-gap determination

Dentine interfaces with composites were assessed using both ivory and human dentine (section 2.2.13.2). There was no obvious difference between bonding of composites to ivory versus human dentine as assessed by SEM. In this section therefore, only bonding of composites to human dentine will be shown. With each composite there are four different dentine pre-conditioning possibilities (details given in section 2.2.14.5). These include IA (Acid etch 20 s followed by Ibond application, and curing for 20 s), IAn (Ibond application, and curing for 20 s), InA (Acid etch for 20 s), InAn (No acid or Ibond application).
3.4.7.1. Z250

The interface of Z250 with dentine showed a relatively intact bond with the use of IA. The interface layer was well defined, with a thickness of ~ 10 µm. IAn showed a similar interface with Z250, but the interface layer was less defined. Using only acid etching (InA) showed peeling of the interface layer away from the Z250 surface. This can possibly be associated with polymerisation shrinkage, creating a micro-gap of ~ 5-10 µm. Additionally, the acid etched interfacial layer of the dentine can be seen to be ~ 10 µm thick. This suggests that interface layer is more pronounced when acid etching is used. With no dentine pre-conditioning, there was no bonding present between dentine and composite. Additionally, the gaps were ~ 5-10 µm (figure 3-15).

![Images of interfaces with different dentine pre-conditionings]

*Figure 3-15 Interface (I) of Z250 composite (Ct) to dentine (Dt) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment).*
3.4.7.2. Gradia

The interface of Gradia with dentine showed a comparatively strong bond with IA. The interface layer was well defined, with a thickness of ~10 µm. Additionally, some air bubbles, and pores can be seen in the composite. IAn showed a similar interface to IA, but the interface layer was less defined. Using only etching (InA) showed a micro-gap of ~5 µm. The interface gap was less pronounced as compared to that with Z250. With no dentine preconditioning, there was no bonding present between dentine and composite. Additionally, the gaps were of ~10 µm wide (figure 3-16).

![Figure 3-16 Interface (I) of Gradia composite (Ct) to dentine (Dt) using the following dentine preconditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment).](image)
3.4.7.3. Vertise Flow

The interface of Vertise flow with dentine showed a relatively intact bond with IA as compared to other dentine pre-treatments. The interface layer is difficult to differentiate, with the dentine and composite surfaces showing a complete union. Some air bubbles were visible in the interface layer, and cracking in the dentine surface can be seen. The interface layer has a thickness of ~ 5-10 µm. IAn showed a similar interface to IA. In some areas, minor peeling of the interface layer can be noted, possibly because of high polymerisation shrinkage associated with Vertise flow. Using only acid etching (InA) showed a micro-gap of less than 5 µm in few areas. Areas of bonding can be seen, with less defined interface layer. With no dentine pre-conditioning, there was a micro-gap of ~ 5 µm. In some areas bonding between composite and dentine can be seen (figure 3-17).

![Figure 3-17 Interface (I) of Vertise Flow composite (Ct) to dentine (Dt) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment).](image-url)
3.4.7.4. C-HEMA

The interface of C-HEMA with dentine showed similarities with Z250. The C-HEMA composite showed a relatively intact bond with IA as compared to other dentine pre-treatments. The interface layer was well defined, with a thickness of ~ 10-15 µm. IAn showed a similar interface to IA, but the interface layer was less defined. Using only acid etching (InA) showed peeling of the interface layer away from the C-HEMA surface. This can possibly be associated with polymerisation shrinkage, creating a micro-gap of ~ 5-10 µm. With no dentine pre-conditioning, there was no bonding present between dentine and composite. Additionally, the gaps were of the size ~ 5-10 µm (figure 3-18).

![Figure 3-18 Interface (I) of C-HEMA composite (Ct) to dentine (Dt) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35% phosphoric acid etching), InAn (No dentine pre-treatment).](image-url)
3.4.7.5. C-4META

The interface of C-4META with dentine showed a thick interface layer when both Ibond and acid etch was used. The interface layer was well defined, with a thickness of ~ 15-20 µm. IAn showed a comparable interface to IA, but the interface layer was less defined. Additionally, air bubbles can be seen in composite surface. Using only acid etching (InA) showed minor cracking in some areas of composite. A thick interface layer of ~ 10 µm can be seen. The bonding was intact along the whole length of dentine and composite. When no dentine pre-conditioning was used, areas with intact bonding can be seen. The micro-gaps were of reduced thickness, with maximum thickness of ~ 2 µm (figure 3-19).

![Image](image_url)

*Figure 3-19 Interface (I) of C-4META composite (Ct) to dentine (Dt) using the following dentine preconditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35% phosphoric acid etching), InAn (No dentine pre-treatment).*
3.5. Discussion

The above study has compared three commercial (2 bulk filled, and 1 flowable composite), and two experimental bulk filled composites, with C-4META composite having self-adhesive properties.

3.5.1. Conversion, Shrinkage, Heat Generation, and Depth of Cure

Experimental composites (C-HEMA, C-4META) and flowable composite (Vertise flow) final conversion were higher than that of the bulk filled commercial composites. Incomplete conversion is usually associated with un-reacted monomers in the polymer matrix, which has the potential to dissolve in the wet oral environment (Ribeiro et al., 2012, Urban et al., 2007). This can reduce the longevity of the composite filling. The above conversion data for commercial composites Z250, Gradia, and Vertise flow were comparable to that noted in literature using FTIR (Palin et al., 2003a, Bracho-Troconis et al., 2008, Eliades et al., 2013, Czasch and Ilie, 2013).

The high monomer conversion associated with experimental composites seen in figure 3-3 suggested that there will be less un-cured monomer in the final matrix. High monomer conversion is usually associated with improved mechanical, and biological properties of the composites (Cramer et al., 2011b). In addition high cure is usually associated with less leaching of monomers, and greater biocompatibility (Gupta et al., 2012a, Mousavinasab, 2011). Moreover, if there is a high percentage of un-cured monomer present in the set matrix, it can cause plasticisation of the polymer phase (Catelan et al., 2014). That may be the main cause of reduced mechanical properties observed in case of Gradia.

The minimum degree of conversion that can provide a clinically stable restoration has not been precisely defined (Nandini, 2010). However, values between 55-65 % conversion is considered relatively safe (Kraemer et al., 2008, Galvão et al., 2013). The experimental composite studied
in this study showed a conversion between 70-80 %, while the bulk commercial composites were between 40-50 %.

The composition of composites, especially the monomers used, has a significant effect on the final conversion. The filler content has very little role in monomer conversion. Bis-GMA has high intermolecular interactions, making it highly viscous, and less mobile as compared to other monomers like UDMA, TEGDMA, and Bis-EMA (Pereira et al., 2002). Therefore composites with Bis-GMA as bulk monomers are associated with reduced monomer conversion as in the case of Z250.

UDMA, and TEGDMA on the other hand are more flexible, have low molecular weight and viscosity (Gajewski et al., 2012). This can help in enhanced mobility during the polymerisation process, and as a result increased conversion. The above can explain the high conversion associated with experimental composites.

Higher glass transition temperature of Bis-GMA -8 °C (Charton et al., 2007) as compared to UDMA -35 °C (Sideridou et al., 2002), and TEGDMA -83 °C is usually associated with low monomer conversion (Charton et al., 2007, Sideridou and Achilias, 2005). The high glass transition temperature makes the monomer more viscous, and as a result mobility of the monomer is reduced. This will results in low mechanical properties. The high glass transition temperature of Bis-GMA can explain the low conversion seen in the case of Z250. The higher monomer conversion observed with Vertise flow having Bis-GMA in its formulation may be due to low percentage of Bis-GMA. Secondly, it contains additional reactive diluent monomer HEMA, that can react with the residual C=C bonds of di- and multi-functional monomers that become immobilised in the set polymer. Both of these factors help to increase conversion seen in Vertise flow.
Monomer conversion is also dependant on the refractive indices of the polymer matrix and the filler addition (Howard et al., 2010). High conversion is usually more feasible when there is a match between the refractive indices of the polymer and fillers. Light transmission is very important for high conversion. The refractive index of typical barium, and zirconium based glasses is 1.55, which is very close to Bis-GMA 1.54 (Khatri et al., 2003). On the other hand the refractive index of TEGDMA is 1.46. TEGDMA acts as a common diluent for Bis-GMA. Addition of TEGDMA to Bis-GMA based resins, not only decreases its viscosity, but also lowers the refractive index of the resin. This will lead to a mismatch between the fillers and monomers. This could be one explanation behind lower conversion of Z250. In Gradia mismatch between refractive indices of UDMA 1.48 and fillers 1.55 can also lead to a lower monomer conversion (Khatri et al., 2003). In case of Vertise flow the Bis-GMA ratio is reduced to obtain higher conversion.

Polymerisation shrinkage and heat generation was calculated from the material composition, and degree of conversion (details given in section 2.2.5.4). Both of these properties are directly proportional to average molecular weight per methacrylate group, and monomer volume fraction. The exact amount of monomers in commercial composites Z250, Gradia, and Vertise flow were not disclosed by the manufacturer. The shrinkage, and heat generation values were therefore estimated assuming equal quantities of each monomer. The values obtained for commercial materials Z250, Gradia, and Vertise flow were comparable to what was found in literature. For this reason the calculated method is thought to be one of the way to estimate polymerisation shrinkage, and heat generation.

Polymerisation shrinkage is dependent on the type of monomer used in the composite. As discussed in chapter 2 of this thesis one mole of C=C polymerisation gave a volumetric shrinkage of 23 cm$^3$/mol (Regnault et al., 2008). Larger monomers like Bis-GMA require less methacrylate groups per unit volume to be converted to give a hardened polymer (Ferracane,
2008). Therefore the shrinkage and heat generation associated with hardening is lower. This can explain the low shrinkage and heat generation of Z250. The calculated shrinkage values obtained in this study is in agreement with literature (Eliades et al., 2013, Kleverlaan and Feilzer, 2005, Braga and Ferracane, 2004, Boaro et al., 2013). In case of experimental composites, which were UDMA, and TEGDMA based, the calculated shrinkage was much higher than Bis-GMA based commercial bulk filled composite as shown in figure 3-4, and 3-5. The reason behind this can possibly be because the polymerisation process continues to a much greater extent in UDMA, and TEGDMA based composites. This will result in generation of high heat, and internal stresses (Charton et al., 2007). The lower shrinkage in Gradia can possibly be due to the addition of pre-polymerised fillers in the monomer phase (Naoum et al., 2012, Tanno et al., 2011). The pre-polymerised fillers make monomer movement difficult in set matrix, and can lead to less conversion of monomers to polymer. This can result in less shrinkage, and heat generation. It has been found in literature that pre-polymerised fillers reduce shrinkage associated with polymerisation process.

The shrinkage values obtained for Vertise flow (4 %) at 1 mm depth is in agreement with what was seen in literature previously (Wei et al., 2011b). The higher shrinkage associated with Vertise flow can be attributed to the high monomer content, and reduced filler loading (70 wt %). Increasing the filler content is associated with reduced polymerisation shrinkage. This can explain why bulk filled commercial composites showed less shrinkage than the flowable composite.

The depth of cure is considered an important physical property of composites (De Camargo et al., 2009). In this study depth of cure of composites were measured according to ISO 4049. The depth of cure depends on many factors, which includes monomer, and filler composition. It also depends on the colour, translucency, and the initiator systems used in the composite (Kanehira et al., 2012). Some other factors that have contributed to attainable depth of cure
include intensity of the light source, duration of the light activation, and distance of the light source from the composite.

In ISO 4049 the depth of cure is determined by scraping the un-polymerised composite material from the bottom of the composite cured cylindrical sample, and measuring the remaining depth of hard cured specimens with a digital calliper. The final depth of cure is half of the thickness of cured sample.

All resin composites tested in this study fulfilled the ISO requirements of 1.5 mm minimum depth of cure. However, studies showed that the ISO scraping method data may overemphasise depth of cure particularly in Bis-GMA based composites (Kanehira et al., 2012).

Depth of cure is related to level of monomer conversion. Beer Lambert law can be used to explain the decrease in level of conversion with depth due to reduction in light intensity. It can be mathematically expressed in equation 3-3.

\[
\frac{I}{I_0} = 10^{-\varepsilon[CQ]h}
\]

Where \(I_0\) is intensity of incident light; \(I\) is intensity of transmitted light; \(\varepsilon\), molar extinction coefficient of CQ \((46 \text{ cm}^{-1}/ \text{ (mol/L)})\) (Chen et al., 2007). [CQ] concentration of CQ in the experimental composites \((0.024 \text{ mol L}^{-1})\); \(h\), sample depth (mm).

From this expression the transmitted light intensity at a depth of 4 mm is estimated to be 0.36 times that seen at the top surface. CQ to some extent enables higher transmission of light by photo bleaching. The mismatch in refractive indices of monomer and filler can also contribute to reduced light transmission.

The conversion of bulk filled commercial composites were noted above to be less than 50 % while the depth of cure associated with these composites is between 2.25-2.35 mm, and 2.45-2.50 mm at 20, and 40 s cure (figure 3-6). This may be overstating the results from
biocompatibility point of view as the potential hazardous un-cured monomers can leach into oral environment, and may have cytotoxic effects.

This study showed a similarity between depths of cure of all composites. The only significant difference was associated with doubling the curing time from 20 to 40 s. There was on average a 0.2 mm increase in curing depth after light activation for 40 instead of 20 s. This was seen previously in literature, with studies reporting 16-23 % increase in depth of cure as a result of doubling the curing time (Scotti et al., 2013, Hwang et al., 2002).

3.5.2. Mass and Volume Changes

In the literature most of the resin composites have shown water sorption in aqueous solution to some extent. In conventional composites the water sorption was mainly associated with the resin phase. On absorption of water, various chemical, biological, and mechanical changes could be seen. The release of un-reacted monomers from the composites upon water sorption is considered a serious problem. These un-reacted monomers can leach into the oral environment, and can have cytotoxic effects (Ak et al., 2010). Additionally, these un-reacted monomers cause plasticisation of the polymer matrix, and encourage water sorption catalysed hydrolytic degradation, that results in reduced mechanical properties (Liu et al., 2011, Park et al., 2009).

The resin composites used in this study would be used in a wet oral environment. Therefore, evaluation of their water sorption properties is considered extremely important. The ISO 4049 standards for measuring water sorption of resin based composites indicates that, the sample should be of 1 mm thickness, and 15 mm diameter. To cure samples of this diameter, multiple overlapping of curing light is required. In this study, samples of 1 mm thickness, and 10 mm diameter were prepared, which was closer to the diameter of the light curing tip (8 mm). The samples could be cured in a single step. The ISO 4049 suggested storage of samples for 1 week.
In this study, a prolonged water sorption of composites over a period of 12 weeks storage in aqueous solution were performed.

Mass increase after 12 weeks immersion in water for experimental (C-HEMA, C-4META), and commercial composites (Z250, Gradia, and Vertise flow) was $1.2 \pm 0.2\%$ (figure 3-7). This was comparable to what was found in literature (Anttila et al., 2008, Kleverlaan and Feilzer, 2005, Boaro et al., 2013). The mass changes in simulated body fluid was $1.0 \pm 0.2\%$ for all composites as seen in figure 3-8. Similarly, the volume increase in water of all composites was $2.0 \pm 0.2\%$ (figure 3-9), while in SBF it was $1.4 \pm 0.2\%$ (figure 3-10). There was only a minor reduction in water sorption in SBF stored samples, possibly because of high concentration of ions in the SBF (El-Ghannam et al., 2005, Mehrali et al., 2014).

Water sorption mainly occurs in the polymer matrix, and cross linking is expected to play a major role. Cross linking seems to play a role in kinetics of water sorption (diffusion coefficient), and has very limited role in final water sorption per unit volume (Örtengren et al., 2001).

The slightly higher mass, and volume changes in flowable Vertise flow, and C-HEMA can be attributed to the hydrophilic monomers present in the resin phase. Monomers like GPDM, and HEMA present in both composites have affinity for water molecules (Qamar, 2012). These monomers on absorbing water can result in swelling of the polymer matrix. In case of other bulk composites no such monomers are present, so limiting its water sorption.

There are two possible explanations behind the water sorption of these materials. Firstly, when material is placed in aqueous solution, it will absorb water, and expand the composite. In this case the volume increase will be equal to that of original sample plus the volume of aqueous solution. The second scenario is that upon absorption of aqueous solution, the water is dragged
into the porosities present in the composite. In that case the mass will increase, but volume of material will remain un-changed.

The volume increase in water shown in this study for all commercial and experimental composites were almost double that of mass change. That suggests expansion of composite due to water (Wei et al., 2011b).

3.5.3. Flexural Strength, and Modulus

Dental composites should have sufficient mechanical properties to withstand the masticatory forces. The composite materials are exposed to tensile, shear, and compressive stresses when in use. The flexural stress combines all of the three stresses. Therefore, measuring flexural strength is the proper way to evaluate the mechanical performance of composite materials.

In this study the experimental composites C-4META, and C-HEMA showed highest strengths of ~165 MPa. In case of commercial composites Z250 showed a strength of ~160 MPa. This was comparable to that of the experimental composites, and to values observed in literature (Anttila et al., 2008, Ersoy et al., 2004). Vertise flow, and Gradia showed a lower strength as compared to other studied composites with initial values of ~125, and 80 MPa respectively as shown in figure 3-11. Similar results were found in literature previously (Blackham et al., 2009, Ferracane, 2011).

The high values obtained for the two experimental, and commercial Z250 composites can be attributed to the higher filler loading, and possibly due to the selection of larger particles. Both these increase the strength of composites (Ersoy et al., 2004). In addition to filler size, there are other factors which affect the mechanical properties of composites. These include filler type, and shape. For example, the spherical shape of the particles in Z250, was found to increase the packing, which is expected to increase the strength (Wei et al., 2011b).
In case of Gradia, similar filler loading to that of experimental composites, and Z250 was seen, but the early strength of Gradia was almost half of the above composites. This drastic decline in Gradia strength can be attributed to the presence of pre-polymerised fillers, which disturbs the stress distribution from the matrix to the filler particles. Also the lower conversion of Gradia, can be one of the reasons of reduced strength.

The strengths of Vertise flow observed after 24 hrs were comparable to what was observed in literature (Ferracane, 2011). The lower strength of Vertise flow can be possibly due to two factors. (1) Lower filler loading than other bulk filled composites, and (2) Pre-polymerised fillers, that will reduce the strength in the same way as explained in case of Gradia.

The initial decline in strength from 24 hrs to 1 month, can possibly be due to water sorption. Water molecules may fill up the pores, and can also plasticise the polymer matrix. Between 1 month to 3 month, and then 6 month, the rate of decline was less, possibly because of the material having reached its maximum absorption potential, and maximum polymer plasticisation.

The moduli of commercial composites were found to be between 2.3 to 3.5 GPa in literature (Czasch and Ilie, 2013, Boaro et al., 2013), and for experimental between 4 to 5.5 GPa at 24 hrs storage in water (figure 3-12). There was a steady decline in moduli of all composites over a period of 6 months, with values coming down to 2-3 GPa for commercial, and 2.9 to 4.5 GPa for experimental composites.

The relatively low modulus of Z250 compared to experimental composites can be due to lower monomer conversion in Z250, which would decrease the amount of polymer cross linking, and results in pulling apart of the intertwined polymer chains. In case of Gradia the same explanation can be used, with an additional decline made possible by addition of pre-polymerised fillers. It was noted previously that pre-polymerised fillers can reduce the moduli.
of composites if added to filler phase (Blackham et al., 2009, Lu et al., 2006). In case of Vertise flow with pre-polymerised fillers, the modulus was higher as compared to Gradia. This can be explained by the relatively good conversion of Vertise flow to that of Gradia.

The higher moduli in case of C-HEMA, and C-4META composite can be attributed to higher monomer conversion which will result in more monomers converted to polymer, and strong polymer cross linking. This cross linking will make the composite stiffer, and makes it difficult for the polymer chains to be pulled apart.

### 3.5.4. Adhesion and Interface of Composites to Dentine

In this study adhesion of composites to dentine was first assessed using a push out test. In the initial stage ivory dentine was used. The ivory dentine made it possible to test large number of samples with good reproducibility. The push out test was a good model for class I cavities. Due to limited literature of the use of push out test on composites, and also on the use of ivory, a shear bond test was subsequently performed both on ivory and human dentine. Dentine source had only minor effect on bond strengths. Additionally, the trends obtained in push out test were confirmed by the shear bond test.

Although there has been much criticism of the reproducibility of the shear bond test, it is still commonly used for dentine adhesion studies (Burke et al., 2008). From the above, the biggest factor increasing bond strength was use of the adhesive Ibond (figure 3-13, and 3-14). The values obtained for human dentine using Ibond are in agreement with previous studies using un-etched human dentine (Krifka et al., 2008). In the presence of water the anhydride group in the 4-META within Ibond is hydrolysed to provide two carboxylic acid groups. It is proposed that these may partially de-mineralise the dentine to allow some micro-mechanical interlocking, but in addition enable a chemical bond with calcium in remaining hydroxyapatite. Furthermore, it may bond with basic amino acid groups in the collagen. After solvent
evaporation upon air drying, adhesive polymerisation additionally provides chemical bonds with the monomers in the composite (Nicolae et al., 2014). The Ibond UDMA hydrophobicity aids intermixing with the composites.

Upon acid etching of the human dentine, the above results showed the adhesive forms a much thicker interface layer. This, however, caused only a small increase in the average bond strength consistent with previous studies (Watanabe et al., 2008b). The interface layer thickness observed above was comparable to the depth of acid etching previously observed with phosphoric acid use (Wang et al., 2007). This layer will consist largely of a mixture of residual hydroxyapatite crystallites and collagen fibrils with adhesive monomers replacing displaced unbound water (Breschi et al., 2008a). The solvents, hydrophilic monomers and low viscosity, aid adhesive penetration into water filled collagen and tubules. Acid etching also enables greater penetration of adhesives into tubules which may potentially further enhance interlocking between the adhesive and dentine (Oliveira et al., 2003b). The lack of any significant difference in the bond strengths for ivory with lower density of tubules instead of human dentine, suggests this mechanism of bonding had limited additional benefit when Ibond was employed.

The very low bond strength of the conventional composites (Z250, Gradia and C-HEMA) to un-etched human dentine could be due to lack of any mechanism for chemical or micromechanical bonding. The greater bond strengths obtained with Vertise flow is in agreement with previous studies (Ozel Bektas et al., 2013). This has been attributed to both lower viscosity as a result of lower filler content and the addition of glycerol phosphate dimethacrylate (GPDM) (Vichi et al., 2013, Moszner et al., 2005) which can form ionic bonds to calcium. The comparable bond strength for the higher viscosity C-4META composite suggests the ionic interactions may be more important in this case than filler loading.
The increased bond strength after etching of human dentine for the C-HEMA composite but lack of improvement upon using ivory could suggest that this composite may require penetration into tubules to improve bonding. This may have been improved by the addition of the hydrophilic monomer HEMA (Van Landuyt et al., 2008b). With Gradia and Z250 improved bonding to etched ivory may be a consequence of these materials having some weak micro-mechanical interaction with highly de-mineralised collagen. Vertise flow may be able to bind un-etched ivory less well due to lack of hydroxyapatite. This problem may have been reduced with the C-4META composite by enhanced acidity (Moszner et al., 2005) and therefore increased etching and chemical interaction.

The advantages of higher filler content in the 4-META composites compared with Vertise Flow include increased strength and lower shrinkage of the composite upon polymerisation (Weinmann et al., 2005, Braga et al., 2005, Kleverlaan and Feilzer, 2005). The SEM images show that when the bond strength is low the material can upon polymerisation generate a gap between the dentine and composite. The size of the gaps observed in figure 3-15, 3-16, 3-17, 3-18, and 3-19 were comparable with what might be expected from the known sample dimensions and composite shrinkages upon polymerisation. These are typically ~3 % for conventional composites with around 80 wt % filler (Braga and Ferracane, 2004) but 4.5 % for Vertise flow with 70 wt % filler (Wei et al., 2011b). The higher filler content, in combination with moderate bond strengths would explain the limited gaps when C-4META was employed.
CHAPTER 4

CONVERSION, POLYMERISATION
SHRINKAGE, HEAT GENERATION, AND
DEPTH OF CURE OF NOVEL DENTAL
COMPOSITES
4. Conversion, Polymerisation shrinkage, Heat generation, and Depth of cure of Novel Dental Composites

4.1. Introduction

As discussed in previous chapters, the main problems associated with current composites are the low degree of conversion, depth of cure, and high polymerisation shrinkage. All these properties significantly affect the longevity of a restoration in the oral environment. The lessons learned from the properties of commercial composites discussed in chapter 3, were employed to design new composite formulations that can overcome the deficiencies associated with current composites.

One area of composite improvement discussed in this chapter includes monomer conversion. This is considered very important, as the amount of un-cured monomers can have a potential negative effect on mechanical properties and adverse effects on surrounding tissues (Gupta et al., 2012b). The other area of composite improvement discussed in this chapter includes polymerisation shrinkage, and heat generation. Shrinkage can be reduced by using high molecular weight monomers, and increasing the filler content. High polymerisation shrinkage is a major cause of micro-gaps formation, which can lead to recurrent caries (Mante et al., 2012).

This chapter will also discuss the depth of cure of composite formulations. Usually, poor depth of cure means un-cured monomer present in the lower surface of the restoration that may lead to cytotoxicity, and reduced mechanical properties. Poor depth of cure and shrinkage effects can be compensated to some extent by placing the composites in small increments (Nanjundasetty et al., 2013). The depth of cure can also be improved by better matching of monomer and filler refractive indices.
The aim of this chapter was to investigate the effects of systematically varying composition, sample thickness, and curing time on final conversion, shrinkage, heat generation, and depth of cure of experimental composites. Degree of conversion was obtained through FTIR, whilst the shrinkage and heat generation were determined theoretically from FTIR conversion. Depth of cure was determined through ISO 4049. The variables tested in conversion, shrinkage, and heat generation studies included polylysine level (5 or 0.5 wt %), HEMA replacement with 4-META, chlorhexidine level (5 or 0 wt %), sample thickness (4 mm or 1 mm), and calcium phosphate (CaP) level (20, 10, or 0 wt %). In case of depth of cure studies, similar variables were tested, except for sample thickness which was replaced by curing time variation (40 s vs 20 s). The systematically varying levels of fillers, and monomers, along with curing time, and sample thickness, will help in formulating a novel dental composite.
4.2. Aims and Objectives

This chapter aims to determine the conversion, polymerisation shrinkage, heat generation, and depth of cure of novel dental composites, and compare with current commercial composites discussed in chapter 3.

Conversion will be determined using FTIR. Polymerisation shrinkage and heat generation will be determined theoretically using conversion, monomer volume fraction, average molecular weight of monomers present in each formulation, and number of methacrylate groups present in each monomer. Depth of cure will be determined using the ISO 4049 scraping test.
4.3. Materials and Methods

A series of preliminary experiments were carried out in the start of this project that gave an idea about the optimal level of various fillers and monomers that are described in this thesis. A detail of variables tested, and its effect on the mechanical and adhesion properties are given in Appendix 7.

A total of 24 different formulations were tested for their conversion, shrinkage, heat generation, and depth of cure. They were divided into two groups. The basic formulation consists of UDMA: TEGDMA 3:1 containing CQ and DMPT (both 1wt %) added to a powder containing 5 wt % fibres and glass particles. The details are given in Appendix 2.

The variables investigated in studies of composite monomer conversion, shrinkage, and heat generation included: sample thickness (4 or 1 mm), adhesive monomer 4-META or HEMA, polylysine level (5 or 0.5 wt %), chlorhexidine level (5 or 0 wt %), and CaP level (20, 10, or 0 wt %). All the samples were cured for 40 s. With depth of cure studies the same variables were tested, except the first variable was curing time (40 or 20 s) instead of sample thickness. The sample size was 3 for all techniques.

To analyse all data three variable (thickness or cure time, 4-META vs HEMA or PLS level), two level factorial analysis was initially undertaken for each wt % of CaP and CHX. With this factorial analysis it was possible to determine the level of effect of the first three variables, and any interaction effects. Subsequently the average effect of the first three variables was obtained in order to additionally assess the effects of CaP and CHX level. Details of experimental methods, and factorial analysis are given in chapter 2 of this thesis.
4.4. Results

For each property tested in this chapter actual experimental data obtained either experimentally, or theoretically, factorial analysis, and average effects of each variable on each property will be provided below.

4.4.1. Monomer Conversion and Polymerisation Shrinkage

Figure 4-1 shows representative FTIR spectra for formulations with reactive fillers before and after light cure of 40 s. All the changes observed upon light exposure were characteristic of methacrylate monomer polymerisation. The 1320 cm\(^{-1}\) peak heights before and after cure was measured to obtain conversion. This corresponds to C-O bond stretching in the polymerising methacrylate group. The spectra also shows monomer/polymer peaks at 1710 cm\(^{-1}\) (C=O stretch), 1640 cm\(^{-1}\) (C=C stretch), 1528 cm\(^{-1}\) (N-H deformation), 1455 cm\(^{-1}\) (C-H bend), and 1160 cm\(^{-1}\) (C-O-C stretch). The spectra also showed the presence of TCP, and MCPP at 1005/940 cm\(^{-1}\) and 1040 cm\(^{-1}\) respectively due to phosphate (P-O) stretching.

![FTIR Spectra](image)

Figure 4-1 Representative FTIR spectra of an experimental composite before and after 40 s light curing. The specific formulation has PLR 4:1, glass powder 55 wt %, glass fibre 5 wt %, MCPM 5 wt %, TCP 5 wt %, CHX 5 wt %, and PLS 5 wt %.
A representative figure of percent monomer conversion plotted against time (s) is given in figure 4-2. In these studies the composite paste was placed on the FTIR diamond and spectra generated for ~ 40 s before light exposure of 20 or 40 s. Upon light application there is sudden increase in monomer conversion. Rapid reaction continues for ~ 20 s before tending towards a maximum.

![Representative FTIR profile of an experimental composite showing monomer conversion after light is applied on the composite discs from the top surface. The specific formulation has PLR 4:1. The powder consists of glass powder 75 wt %, glass fibre 5 wt %, MCPM 5 wt %, TCP 5 wt %, CHX 5 wt %, and PLS 5 wt %. Sample thickness was 1 mm.](image)
The conversions at 5 minutes from the start of cure of all formulations (calculated according to equation 2.10) are given in figure 4-3. This was between 50-80 % with highest conversion noted with 1 mm thickness, and least conversion noted with 4 mm samples.

Assuming small changes in the filler density with varying composition has negligible effect on the volume fraction of monomer, the polymerisation shrinkage and heat generation will be directly proportional to conversion. The estimated polymerisation shrinkage calculated theoretically (details in section 2.2.5.4) for all formulations using polymerisation levels at the 2 different depths are given in figure 4-3. The shrinkage values estimated using polymerisation levels at 4 mm were ~ 2.5 %, while those using 1 mm depth conversions were ~ 3.5 %.

![Figure 4-3 Monomer conversion and Polymerisation shrinkage with PLS (5 or 0.5 wt %), adhesive monomers (4-META or HEMA), and sample thickness (4 mm or 1 mm) for all formulations with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).](image-url)

*Figure 4-3 Monomer conversion and Polymerisation shrinkage with PLS (5 or 0.5 wt %), adhesive monomers (4-META or HEMA), and sample thickness (4 mm or 1 mm) for all formulations with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).*
Factorial analysis (details in section 2.2.4.2) (figure 4-4) confirmed reducing sample thickness increased monomer conversion and shrinkage. Adding 4-META instead of HEMA or reduced polylysine level also increased conversion and shrinkage but to a lesser extent. Interaction effects between these three variables (thickness, adhesive monomer or PLS level) were negligible in comparison with the level of the effect of all the variables. Furthermore, the lack of variation in the “a” values with altering the level of CaP or CHX shows these additional variables had little effect on the level of effect of thickness, adhesive monomer or PLS. The small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ and error bars not crossing zero confirm that the individual variables are all having significant effects on monomer conversion and shrinkage.

Figure 4-4 Factorial analysis showing the effect of variables on monomer conversion and polymerisation shrinkage. $a_1$ corresponds to effect of thickness 4 mm vs 1 mm $a_2$ to 4-META monomer vs HEMA, and $a_3$ to Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure shows that interaction effects with calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, or chlorhexidine levels 5 wt % vs 0 wt % are small.
The average results for the first 3 variables are provided in figure 4-5. The average conversion for formulations with 4 mm thickness was ~ 50 %, as compared to ~ 70 % with 1 mm thickness. The average conversion for 4-META, and HEMA formulations were ~ 60 %. Similarly, the average conversion with PLS (5 or 0.5 wt %) was ~ 60 %.

From figure 4-5 the effect of first 3 variables on shrinkage was also calculated. The average shrinkage for formulations estimated using 4 mm thickness conversion was ~ 2.7 %, as compared to ~ 3.7 % upon using the 1 mm thickness conversion values. The average shrinkage for 4-META, and HEMA formulations were ~ 3.2 %. Similarly, the average conversion with PLS (5 or 0.5 wt %) was also ~ 3.2 %.

From this figure, it is possible to see a systematic upward trend in conversion and shrinkage with decreasing CaP level. With CaP level going from 0-20 wt % there was a decline in conversion and shrinkage of 7-10 %. The effect of CHX was not significant.

![Figure 4-5 The average effect of sample thickness, 4-META replacement with HEMA, and PLS levels on final monomer conversion with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C.I of the mean (n=6).](image-url)
4.4.2. Heat generation

The heat generation was calculated theoretically from conversion and polymerisation shrinkage (section 2.2.5.4). The heat generation is 0.0175 times the calculated shrinkage values. The heat generation is affected by the same variables as that of conversion and shrinkage.

The heat generation values for samples using conversions at a thickness of 4 mm were ~ 0.06 kJ/cc, while using 1 mm thickness values gave ~ 0.08 kJ/cc.

4.4.3. Depth of cure

Depth of cure of experimental composite formulations obtained using ISO 4049 are given in figure 4-6. The values obtained for depth of cure of experimental formulations passed the minimum ISO requirement of 1.5 mm minimum thickness (details about depth of cure given in section 2.2.6.2). The values obtained were between 1.55-2.5 mm with greater depths being achieved with 40 s cure and lower CaP level.

Figure 4-6 Depth of cure with PLS (5 or 0.5 wt %), adhesive monomer (4-META or HEMA), and sample curing (40 s or 20 s) for all formulations with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).
Factorial analysis (figure 4-7) confirmed that increasing curing time to 40 s increased depth of cure. Addition of 4-META instead of HEMA and reducing level of PLS also slightly increased depth of cure. Interaction effects between these three variables (curing time, adhesive monomer or PLS level) were negligible in comparison with the level of the effect of all the variables. Altering the level of CaP from 0-20 wt % decreased $a_1$. In comparison, CaP level had negligible effect on $a_2$, and $a_3$. Furthermore, the lack of variation in the “$a$” values with altering the level of CHX shows this additional variable had little effect on the level of effect of curing time, adhesive monomer or PLS. The small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ suggests that the individual variables are all having significant effect on depth of cure. Decreasing the Calcium phosphate (MCPM + TCP) levels from 20 to 10 wt % significantly increased the effect of cure time whilst changing from 10 to 0 wt % or the Chlorhexidine level from 5 wt % to 0 wt % gave less of an interaction effect.

![Figure 4-7](image)

*Figure 4-7 Factorial analysis describing the effect of each variable, and interactions associated with combination of variables on depth of cure. $a_1$ corresponds to effect of light curing duration 40 s vs 20 s, $a_2$ corresponds to 4-META monomer vs HEMA, and $a_3$ corresponds to polylysine (PLS) levels 5 wt % vs 0.5 wt % on the depth of cure.*
The average results for the first 3 variables are provided in Figure 4-8. The average depth of cure for formulations with 40 s cure was ~ 2 mm, as compared to ~ 1.7 mm with 1 mm thickness. The average depth of cure for 4-META, and HEMA formulations were ~ 1.9 mm. Similarly, the average depth of cure with PLS (5 or 0.5 wt %) was ~ 1.9 mm. From this figure, it is possible to see a systematic upward trend (~ 22 %) in depth of cure with decreasing CaP level. The effect of CHX was not significant.

*Figure 4-8 The average effect of curing time, 4-META replacement with HEMA, and PLS levels on depth of cure with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C.I. of the mean (n=6).*
4.5. Discussion

4.5.1. Degree of Conversion

The degree of conversion is an important property that affects the performance of dental composites. The mechanical, and biological properties of resin based composites generally improve with increase in monomer conversion. The improved mechanical properties will reduce the chances of material failure under masticatory loads (Dejak and Młotkowski, 2014). Additionally, high conversion can substantially reduce the cytotoxic effects associated with the release of un-reacted monomers (Ergun et al., 2011).

The monomer conversion for experimental formulations in this study was in the range of 50-80 % upon photo-initiation as shown in figure 4-3. Generally, the monomer conversion of methacrylate based dental materials are lower (36-69 %) (Emami and Söderholm, 2003, Kim et al., 2010). The degree of conversion for commercial composites reported in chapter 3 of this thesis was between 36-66 %. The high degree of conversion in the above experimental composites in this thesis could be attributed to the incorporation of diluent co-monomers (TEGDMA, HEMA, and 4-META) in addition to the bulk monomer UDMA (Prakki et al., 2007). The aliphatic monomer UDMA used in this study allows more flexibility, and movement in the polymer matrix than the aromatic monomer Bis-GMA used in some commercial composites like Z250 (Sideridou et al., 2002). All the co-monomers (TEGDMA, HEMA, and 4-META) increase the hydrophilicity and wetting ability to dentine. These co-monomers reduce the viscosity of monomers, and increase the flexibility of the polymer chains. These factors will reduce the glass transition temperature of the resin matrix, and increases the mobility of the reactive monomers, which will lead to increase in monomer conversion (Cramer et al., 2011a). Additionally the longer curing time of 40 s in this study, compared to 20 s which is usually used for curing of commercial composites can be one of the reasons for enhanced monomer conversion (Discacciati et al., 2004).
In this study higher conversion was seen with samples thickness of 1 mm, as compared to 4 mm (figure 4-5). The low conversion associated with thicker samples, suggests the possibility of un-cured monomers at critical areas in a restoration such as composite-dentine interface. With thicker samples the amount of un-cured material at the bottom is much higher. With time the un-cured monomer can leach into the surrounding tissues, and cause cytotoxic effects. As discussed in chapter 3, the transmitted light intensity at a depth of 4 mm is estimated to be 0.36 times that seen at the top surface. Therefore, a reduced conversion was expected at depths of 4 mm. CQ photo bleaching may mitigate this problem to some extent.

Other studies have suggested that the addition of reactive fillers could potentially reduce the rate of methacrylate polymerisation (Cramer et al., 2011a). This effect is more pronounced when there is mismatch of refractive indices in filler (β-TCP 1.62, MCPM 1.52, CHX 1.66, Glass particles 1.55), and monomer (1.48) phase (Shortall et al., 2008). Additionally, higher filler loading may reduce the wetting ability of the monomer systems, and cause polymerisation inhibition due to incorporation of increased air bubbles in the final mix. These factors can explain the low monomer conversion especially seen with the addition of CaP reactive fillers.

The relatively small levels of CHX, and PLS in this study had only small effects on the final conversion. Previous studies showed contradicting results. With one study, increase in conversion with CHX addition was attributed to its stabilising effect on free radicals inhibiting the termination of the polymerisation reaction (Cadenaro et al., 2009). In another study, addition of CHX to un-filled dental resins resulted in a decrease in monomer conversion (Anusavice et al., 2006a).

The relatively higher, or comparable degree of conversion for experimental composites than commercial materials, could indicate their greater suitability for clinical applications. High monomer conversion is usually associated with improved biocompatibility as the un-reacted
monomers are less likely to penetrate into dental tissues, and initiate an inflammatory reaction (Goldberg, 2008).

There are some disadvantages that could be associated with high polymerisation including shrinkage. As mentioned earlier in this chapter, the rate of conversion is directly proportional to the rate of shrinkage (Stansbury et al., 2005). This could mean a high shrinkage for experimental composites in this study than the commercial bulk composites. However, this shrinkage in experimental composite could be compensated by volumetric expansion due to water sorption. This expansion to compensate shrinkage will be discussed further in below sections, and in chapter 5 (Mass and volume changes). A more detailed explanation of factors controlling conversion is given in chapter 3 of this thesis.

4.5.2. Polymerisation Shrinkage and Heat Generation

The values obtained in this study for shrinkage, and heat generation were comparable to commercial bulk filled composites Z250, and Gradia (Figure 3-4, and 3-5). The factors that reduced the shrinkage and heat generation to a greater extent were sample thickness, and CaP addition (figure 4-4). As discussed previously shrinkage, and heat generation are directly related to conversion, and conversion is affected by depth (thickness) of composites. In case of thicker samples less conversion is seen. For thick layer placement of composite, this might be a mechanism of reduced shrinkage and heat generation in lower sections due to less monomer conversion while maintaining a higher conversion at the top surface. Previous studies have also found reduced polymerisation shrinkage with increase in thickness of the samples (Świderska et al., 2014). This suggests that there may be more contraction in the top composite surface than the lower surface. The higher shrinkage and heat generation in thin samples can be explained by large number of photons on the top surface (Son et al., 2014). All these factors explain the decrease in shrinkage and heat generation in thick samples (4 mm).
Similarly, the addition of fillers is usually associated with the reduction in shrinkage and heat generation. The reduction in shrinkage, and heat generation seen with CaP addition, and other fillers (CHX, PLS) can be due to a mismatch in the refractive indices of filler and monomer phase (Moser et al., 2014).

The polymerisation shrinkage can have serious outcomes on the longevity of the restorations. Figure 4-9 shows one of the problems (micro-gap formation) that is associated with the current composites upon light curing. The micro-gaps can be formed after composite shrinks upon cure, thus pulling itself away from the dentine surface. As shown in the figure 4-9, some of the composite resin tags are pulled away from the dentinal tubules, while the rest have blocked the dentinal tubules. These micro-gaps provide pathways for the bacteria to penetrate into the deep tooth structures, as well as along the interface (Spencer et al., 2012). The ultimate result will be recurrent caries, loss of restoration, or tooth structure. In this study some of the polymerisation shrinkage, and heat generation issues may be addressed and compensated by the water sorption associated with the CaP expansion, and with the use of acidic monomer (4-META). CaP reaction with water may cause expansion. It could also dissolve and re-precipitate along the composite-dentine interface to reduce any gaps caused by polymerisation shrinkage. Alternatively, the 4-META through its de-mineralising action on dentine can allow the material to penetrate into the tubules, and make a stronger inter-locking hybrid layer. This study also advocates the incremental filling of the composite in thin layers, to avoid the potential cytotoxicity of the un-cured monomers in deep layers.
Figure 4-9 Scanning electron microscopy image of interface (I) between dentine (Dt), and composite (Ct). The image is showing post light cure shrinkage, with composite resin tags pulling the composite out of the dentinal tubules. Composite filled tubules can also be seen.

4.5.3. Depth of Cure

The main factors that affected the depth of cure of experimental materials were the use of reactive fillers CaP, and curing time (20 s vs 40 s) (figure 4-6, 4-7, and 4-8). The reduction in depth of cure with the addition of CaP was ~ 5 %. This reduction with high levels of CaP can be attributed to the mismatch in refractive indices of filler, and liquid phase, which results in decreased depth of cure. It was shown in the literature that the filler addition, especially the CaP, increases the translucency of the material, making it difficult for the light to penetrate into thick samples (Kutz, 2011). This low translucency with CaP can also explain the decrease in depth of cure. The obtained values in this study were on average less than the commercial bulk
composites which can also be related to the addition of various fillers to the experimental composites.

As seen from the literature and results shown, the depth of cure is influenced by the duration of light cure (Alto et al., 2006, Yue et al., 2009, Sa'di Shirshab Thiab et al., 2009). The results obtained for depth of cure in this study with 20 s cure was ~ 20% less than that of 40 s cure. This effect was observed in almost all formulations. More conversion means more polymer matrix formation, and increased depth of cure. A previous increase of 16-23% in monomer conversion was noted in literature with doubling the curing time (Scotti et al., 2013). The results obtained in this study were in agreement with the values noted in literature (Hwang et al., 2002).

The ISO method has its own drawbacks, and it had been reported in literature that this method sometimes overstates the results from biocompatibility point (Kanehira et al., 2012). More detailed analysis is needed to confirm the ultimate depth of cure of these materials.
CHAPTER 5

M ASS AND V OLUME C HANGES (W ATER S ORPTION) OF D ENTAL C OMPoSITEs
5. Mass and Volume Changes (Water Sorption) of Dental Composites

5.1. Introduction

As described in chapter 1 of this thesis the main cause of composite failure is recurrent caries which can be exacerbated by polymerisation shrinkage (Mjor, 2005, Condon and Ferracane, 2000). The resultant micro-gaps can provide a passage for bacteria to penetrate along the margins of the restoration. This can lead to marginal staining, and restoration failure (Sarrett, 2005).

This study assesses the changes in mass, and volume of experimental composites upon immersion in distilled water (DW), or Simulated Body Fluid (SBF). The variables tested included use of either adhesive monomer 4-META or HEMA, polylysine level (5 or 0.5 wt %), chlorhexidine level (5 or 0 wt %), and CaP level (20, 10, or 0 wt %). Discs of 1 mm thickness, and 10 mm diameter were made.

5.2. Aims and Objectives

This chapter aims to determine the water sorption, and expansion associated with novel dental composites, and compare with the current commercial composites discussed in chapter 3. Additionally, volumetric studies will determine which experimental formulations have appropriate expansion to closely compensate polymerisation shrinkage. This will be determined using a density kit attached to a four figure balance.
5.3. Materials and Methods

48 different formulations were tested. These were divided into two groups. The details are given in Appendix 3. The variables tested for mass and volume changes included: storage medium (DW or SBF), adhesive monomer 4-META or HEMA, polylysine level 5 wt % or 0.5 wt %, chlorhexidine level 5 wt % or 0 wt %, and CaP level (20, 10, or 0 wt %). All the samples were 1 mm thickness, and 10 mm diameter, and cured for 40 s on both sides. The samples were then stored in 15 ml of storage solution at 37 ºC for 2, 6, 24, 48 hrs and 1, 2, 4, 6, 12 weeks. At each time point, the discs were removed, blotted dry, re-weighed, and placed in fresh storage solution in new sterilin tubes.

A three variable and two level factorial design was used to analyse the data. A detail of experimental method, and factorial analysis is given in chapter 2 of this thesis.
5.4. Results

5.4.1. Mass Change of Experimental Composites

Representative mass changes plotted against square root of time are given in figure 5-1. All the formulations shown in this figure have fixed amount of polylysine, and chlorhexidine at 5 wt %. Additionally the monomer phase has adhesive monomer 4-META at 5 wt % instead of HEMA.

There was initially fast mass increase in the first 24 hrs. Mass change was then proportional to the square root of time up to 6 weeks. Equilibrium was assumed to be reached for all formulations by 12 weeks. The final mass increase with 20 wt % calcium phosphate was higher (5-6 %) than with 10 wt % (3-4%), and 0 wt % (1-2 %). It was also higher in distilled water (~1% higher) than in SBF. All other formulations indicated very similar trends and are provided in appendix 2. To enable comparison of all data simultaneously, the gradient and intercept of mass change versus square root of time using data between 48 hours and 6 weeks were obtained (details given in section 2.2.11).

Figure 5-1 Representative mass change plotted against square root of time. The variables shown in figure are Distilled water (DW) or simulated body fluid (SBF), and CaP levels 0, 10, or 20 wt % (n=6). The equilibrium was assumed to be reached by the 6 weeks point.
5.4.1.1. Intercept of mass versus square root of time

The early 24 hour mass change estimated from the intercept of percent mass change on the y-axis (details about intercept given in section 2.2.11) plotted against square root of time is given in figure 5-2. CaP level has an obvious effect on this intercept. The average intercept was ~ 1.5, 0.75 and 0.5 % with 20, 10 and 0 wt % CaP respectively. The relative effects of other variables are more clearly observed using the factorial analysis below.

Figure 5-2 Initial mass changes measured by intercept of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).
Factorial analysis of intercept data (figure 5-3) shows the magnitude of a terms for the 3 different variables were dependent upon both CHX and CaP level. A significant mass increase with 0 wt % CaP samples stored in DW instead of SBF was observed. The large interaction effects and error bars crossing zero, however, indicate that the variables associated with $a_1$, $a_2$ and $a_3$, had generally only small effects. The figure also show, however that there are complicating interaction effects between the first 3 variables and Chlorhexidine levels 5 wt % vs 0 wt % or Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %.

![Figure 5-3 Factorial analysis of initial mass changes. $A_1$ indicates the level of effect of Distilled water vs SBF, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ shows the effect of Polylysine (PLS) at 5 wt % vs 0.5 wt %. Error bars crossing the 0 line indicate when variables have no significant effect.](image-url)
Average early mass change is provided in figure 5-4. On average the early mass change in DW (~ 0.8 %) was higher than SBF (0.5 %). Similarly the initial mass change with 5 wt % PLS (~ 0.8 %) was higher than with 0.5 wt % PLS (~ 0.6 %). From this figure, it is possible to also see a systematic upward trend in intercept with increasing CaP level. Average intercepts were 0.4, 0.8 and 1.2 with 0, 10 and 20 wt % CaP respectively. The effect of increasing CaP was greater in SBF than water, with HEMA instead of 4 META and with high PLS. The average intercept for all formulations containing 5 wt % CHX was 0.6, as compared to 0.8 with 0 wt % CHX. The variable 4-META vs HEMA in comparison had negligible effect.

Figure 5-4 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on early mass change with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C. I of the mean (n=6). None overlapping error bars indicates significant difference.
5.4.1.2. Gradient of mass versus square root of time

The final gradient (details about gradient given in section 2.2.11) of mass versus square root of time for all variables increased systematically with increase in CaP level. The gradient for all formulations with 0, 10, and 20 wt % CaP was on average ~ 0.04, 0.08, and 0.12 wt % / hr$^{0.5}$ respectively. Similarly a minor increase in gradient was seen with formulations containing HEMA, PLS 5 wt %, and samples stored in DW. The addition of chlorhexidine showed no effect on the mass gradient (figure 5-5). On average the mass gradient for DW stored formulations were between 0.05-0.17 wt %/hr$^{0.5}$, and SBF stored formulations between 0.02-0.15 wt %/hr$^{0.5}$.

![Figure 5-5 Final gradient of mass increase of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).](image-url)
Factorial analysis of final mass gradient (figure 5-6) shows all the first three variables had a significant effect. Using equation 2-5 in section 2.2.4.2, the “a” values can be converted into average percentage change. These show an increase of ~ 40 % with the use of DW in place of SBF. Similarly an increase of ~ 35 % was seen with the use of 5 wt % PLS instead of 0.5 wt % PLS (a3). A decline of ~ 20 % in mass gradient was seen with the use of 4-META compared to HEMA (a2). The change in level of CaP from 0-20 wt % decreased a1 and a2 but had no effect on a3. CHX only affected a1 when the CaP content was 0. The small interaction terms a12, a13, a23, and a123 confirm that the individual variables are all having significant effects on mass gradient.

Figure 5-6 Factorial analysis of final gradient of mass increase versus square root of time. A1 corresponds to effect of Distilled water vs SBF, a2 corresponds to effect of 4-META vs HEMA, and a3 corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effects with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, or Chlorhexidine levels 5 wt % vs 0 wt %.
The average results for the first 3 variables are provided in figure 5-7. The mass gradient for formulations stored in DW was between 0.05-0.13 wt %/hr^{0.5}, as compared to 0.03-0.11 wt %/hr^{0.5} with SBF. The average mass gradient for 4-META, and HEMA formulations were between 0.04-0.13 wt %/hr^{0.5}. Similarly with 5 wt % PLS the mass gradient was 0.05-0.15 wt %/hr^{0.5}, as compared to 0.04-0.10 wt %/hr^{0.5} with 0.5 wt % PLS. From figure 5-7, it is possible to see a systematic upward trend in mass gradient with increase in CaP level. The average gradient for all formulations with 0, 10, and 20 wt % CaP was ~ 0.04, 0.08, and 0.12 wt %/hr^{0.5} respectively. The average gradient with CHX addition removal caused a small decline in gradient.

Figure 5-7 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on final gradient of mass increase with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C. I of the mean (n=6).
5.4.1.3. Final mass change

The maximum mass change (details about maximum change given in section 2.2.11 at 12 weeks for all formulations (figure 5-8) showed a systematically upward trend with the increase in CaP level. On average with 20 wt % calcium phosphate the mass change was ~ 4-7 %, with 10 wt % it was ~ 2.5-4.5 %, and with 0 wt % final mass change at 12 weeks was ~ 1-2.5 %.

The maximum final mass change seen in formulations stored in DW (2.5-7 %) was higher as compared to SBF (1-5.5 %). Similarly with the PLS 5 wt % the final mass change (2-7 %) was higher than 0.5 wt % PLS (1-5 %). With the use of 4-META the final increase was less than HEMA. CHX showed no effect on final mass change.

![Final Mass Change Graph](image)

*Figure 5-8 Final mass increase of all formulations at 12 weeks with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).*
Factorial analysis of maximum mass increase at 12 weeks (figure 5-9) suggests an increase of ~ 30% (see equation 2-5) with the use of DW instead of SBF. Similarly with the use of 5 wt % PLS an increase of ~ 40% was seen as compared to 0.5 wt %. A decline of ~ 20% in mass gradient was seen with the use of 4-META compared to HEMA. Increase in CaP from 0-20 wt % decreased both $a_1$ and $a_2$ but increased $a_3$. CHX had no effect on the $a$ terms for the first 3 variables. The small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ confirm that the individual variables are all having significant effects on final mass increase.

Figure 5-9 Factorial analysis of final mass increase of formulations at 12 weeks with variables, $a_1$ corresponds to effect of Distilled water vs SBF, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effects with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, or Chlorhexidine levels 5 wt % vs 0 wt %.
The average results for the first 3 variables are provided in figure 5-10. An evident increase in final mass was seen with the use of HEMA, DW, and PLS 5 wt % in place of 4-META, SBF, and 0.5 wt % of PLS respectively. From this figure, it is possible to see a systematic upward trend in final mass change with the increase in CaP level from 0-20 wt %. The final mass increase for all formulations with 0, 10, and 20 wt % CaP was ~ 2, 3.5, and 5 % respectively. The CHX addition had no significant effect on the first 3 variables. The final mass increase at 12 weeks for all formulations were between 1.3-6.1 %.

![Figure 5-10](image-url)  
*Figure 5-10 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on final mass increase at 12 weeks with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C. I of the mean (n=6).*
5.4.2. Volume Change of Experimental Composites

Representative volume changes plotted against square root of time are given in figure 5-11. All the formulations shown below have fixed amount of polyllysine, and chlorhexidine at 5 wt %. Additionally the monomer phase contains adhesive monomer 4-META at 5 wt % instead of HEMA. All the other formulations volume change plotted versus square root of time are given in appendix 2.

The volume increase with 20 wt % calcium phosphate in DW was highest ~ 12 % as compared to ~ 8 % in SBF. Similarly the final volume change at 12 weeks for 10 wt % CaP in DW was ~ 7.5 %, and in SBF it was ~ 5 %. With 0 wt % CaP in DW the final change in volume was ~ 4 %, while in SBF the change was ~ 2 %. The maximum change in volume can be seen in initial 24-48 hrs, followed by a slow increase in volume till 6 weeks. The equilibrium was assumed to be reached for all formulations by 12 weeks.

![Figure 5-11 Representative volume change plotted against square root of time. The variables shown in figure are Distilled water (DW) or simulated body fluid (SBF), and CaP levels 0, 10, or 20 wt % (n=6). The equilibrium was assumed to be reached by the 6 weeks point.](image)
5.4.2.1. **Intercept of volume versus square root of time**

The initial volume change can be obtained by determining the intercept (details about intercept given in section 2.2.11) of percent volume change data plotted against square root of time for each formulation. From figure 5-12 it can be seen that CaP level has strong effect on the intercept with average intercept of ~ 3, 1.5, and 1 % with 20, 10, and 0 wt % CaP respectively. The other factor that is having an effect is SBF. With the use of SBF the initial volume change was less (~ 1.5 %) as compared to DW (2 %).

![Graph showing intercept of volume versus square root of time with different formulations and conditions.](image-url)

*Figure 5-12 Initial Volume changes measured by intercept of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).*
The factorial analysis of intercept data (figure 5-13) shows a significant volume increase with 0 wt % CaP samples stored in DW as compared with SBF. Otherwise, the relatively large error bars and interaction effects suggest that the first 3 variables have largely negligible effects on the initial volume change. Changing the levels of CaP from 0-20 wt %, and CHX from 0-5 wt % also had only minor effects on a2, and a3. The large interaction terms a12, a13, a23, and a123 confirm that the individual variables are not having significant effects on final mass increase.

Figure 5-13 Factorial analysis of initial volume changes shown by intercept of formulations with variables, a1 corresponds to effect of Distilled water vs SBF, a2 corresponds to effect of 4-META vs HEMA, and a3 corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effects with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, or Chlorhexidine levels 5 wt % vs 0 wt %.
The average results for the first 3 variables are provided in figure 5-14. On average the initial volume change in case of DW (~ 1.2 %) was only higher than SBF (1 %). Similar average effect was seen with the use of PLS. From this figure, it is possible to also see a systematic upward trend in intercept with increasing CaP level. Average intercepts were 0.6, 1.2 and 2.0 with 0, 10 and 20 wt % CaP respectively. The effect of increasing CaP was greater in SBF than water, with HEMA instead of 4 META and with high PLS. Addition of CHX at 5 wt % reduced the intercept of all formulations on average from 1.2 to 1.0. The variable 4-META vs HEMA in comparison had negligible effect.

![Graph](image)

*Figure 5-14 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on initial volume change intercept with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C. I of the mean (n=6).*
5.4.2.2. Gradient of volume versus square root of time

The final volume gradient (details given in section 2.2.11) for all variables increased systematically with increase in CaP level. Similarly a minor increase in gradient was seen with formulations containing HEMA, PLS 5 wt %, and samples stored in DW in place of 4-META, PLS 0.5 wt %, and SBF stored samples. The use of chlorhexidine showed no changes on the volume gradient (figure 5-15). On average the volume gradient for DW stored formulations were between 0.06-0.30 wt %/hr^{0.5}, and SBF stored formulations between 0.02-0.20 wt %/hr^{0.5}.

![Figure 5-15 Final gradient of volume increase of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).](image-url)
Factorial analysis of final volume gradient is given in figure 5-16. By using equation 2-5 in section 2.2.4.2, the “a” values can be converted into average percentage change. The result suggests an increase of ~50% with the use of DW in place of SBF ($a_1$). Similarly, an increase of ~35% was seen with the use of 5 wt % PLS instead of 0.5 wt % PLS ($a_3$). A decline (average of 30%) in volume gradient was seen with the use of 4-META compared to HEMA ($a_2$). Change in CaP from 0-20 wt % decreased the level of effect of variables 1, and 2. There was no effect seen on $a_3$ with the addition of CaP. CHX addition also had no effect on the first 3”a” terms. The small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ confirm that the individual variables are all having significant effects on final mass increase.

![Figure 5-16](image_url)

*Figure 5-16 Factorial analysis of final gradient of volume increase of formulations with variables, $a_1$ corresponds to effect of Distilled water vs SBF, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effects with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, or Chlorhexidine levels 5 wt % vs 0 wt %.*
The average results for the first 3 variables are provided in figure 5-17. The volume gradient for formulations stored in DW was between 0.09-0.24 wt %/hr$^{0.5}$, as compared to 0.05-0.19 wt %/hr$^{0.5}$ with SBF. The average volume gradient for 4-META, and HEMA formulations were between 0.05-0.22 wt %/hr$^{0.5}$. Similarly with 5 wt % PLS the volume gradient was 0.08-0.26 wt %/hr$^{0.5}$, as compared to 0.05-0.18 wt %/hr$^{0.5}$ with 0.5 wt % PLS. From figure 5-17, it is possible to see a systematic upward trend in volume gradient with increase in CaP level. The CHX addition had no significant effect on the first 3 variables.

![Figure 5-17](image-url)

*Figure 5-17 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on final gradient of volume increase with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C. I of the mean (n=6).*
5.4.2.3. **Final Volume change**

The maximum volume change (details in section 2.2.11) at 12 weeks for all formulations (figure 5-18) showed a systematically upward trend with the increase in CaP level. On average with 20 wt % calcium phosphate the volume change was ~ 7-12 %, with 10 wt % it was ~ 4-8 %, and with 0 wt % final volume change at 12 weeks was ~ 2-4 %. The maximum final volume change seen in formulations stored in DW (3.5-12.5 %) was higher as compared to SBF (1.5-9 %). Similarly with the PLS 5 wt % the final volume change (1.5-12.5 %) was higher than 0.5 wt % PLS (1.2-8.9 %). With the use of 4-META the final increase was less than HEMA. CHX showed no effect on final mass change.

![Figure 5-18 Final percentage Volume increase of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).](image-url)
Factorial analysis of maximum mass increase in 12 weeks (figure 5-19) suggests an increase of ~ 50% with the use of DW instead of SBF. Similarly with the use of 5 wt% PLS an increase of ~ 30% was seen as compared to 0.5 wt%. A decline (~ 20%) in mass gradient was seen with the use of 4-META compared to HEMA. The change in levels of CaP from 0-20 wt % decrease the level of effect of variables $a_1$, and $a_2$. There was no effect seen on variable $a_3$ with the addition of CaP. The CHX showed no effect on the first 3 variables. The small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ confirm that the individual variables are all having significant effects on final volume increase.

![Figure 5-19 Factorial analysis of final volume increase of formulations with variables. $a_1$ corresponds to effect of Distilled water vs SBF, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polylysine (PLS) levels 5 wt% vs 0.5 wt%. The figure also shows the interaction effects with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt%, or Chlorhexidine levels 5 wt% vs 0 wt%.](image-url)
The average results for the first 3 variables are provided in figure 5-20. The evident increase in final volume was seen with the use of HEMA, DW, and PLS 5 wt % in place of 4-META, SBF, and 0.5 wt % of PLS respectively. From this figure, it is possible to see a systematic upward trend in final volume change with the increase in CaP level from 0-20 wt %. The final volume increase for all formulations with 0, 10, and 20 wt % CaP was ~ 3, 5, and 8 % respectively. The CHX addition had no significant effect on the first 3 variables. The final volume increase at 12 weeks for all formulations were between 2-10 %.

![Figure 5-20](image.png)
5.5. Discussion

Most composite materials exhibit water sorption to some extent upon immersion in aqueous solution (Ito et al., 2005). In the conventional composites this water sorption is mainly due to the monomer phase (Sideridou et al., 2003). Various physico-chemical, mechanical, and biological changes occur upon absorption of water into these materials. The water sorption can lead to the release of un-reacted monomers from the composite materials, which can have cytotoxic effect, and promote growth of cariogenic bacteria (Hegde et al., 2012, Bakopoulou et al., 2009). Additionally too much water sorption can lead to plasticisation of the polymer matrix, which results in a decline in mechanical properties (Ito et al., 2005, Han and Drzal, 2003).

The experimental formulations designed in this study are primarily developed for dental purposes. Evaluation of their water sorption properties was therefore very important. The long term behaviour of these composites in DW, and SBF will help in deciding balanced formulations for clinical use.

The mass and volume changes in this study were carried out using Archimedes’ principle, which is commonly employed (Prasad et al., 2014, Dhal and Mishra, 2013, De Melo Monteiro et al., 2011). The method outlined in ISO 4049 for the assessment of water sorption of dental composites, indicate the use of 1 mm thick, and 15 mm diameter samples. To cure such samples, multiple light cure overlapping is required, which prolongs the light exposure time. In this study the use of 1 mm thick, and 10 mm diameter sample reduces this issue. The material diameter is very close to the curing tip diameter of 8 mm, therefore maximum cure is achieved in less time.

The ISO 4049 suggests one week storage of samples in aqueous medium. In this study the prolonged duration of 12 weeks was considered important because of the presence of various
reactive fillers and hydrophilic components, which could encourage water sorption, and could affect the chemical, physical, and biological properties over a prolonged time.

Mass and volume changes for composites increased significantly in the first 24-48 hrs (figure 5-2, and 5-12). The changes seen initially in mass and volume can be attributed to the presence of water soluble MCPM (Mehdawi et al., 2009). MCPM has the ability to increase the internal osmotic pressure upon dissolution in water (Abou Neel et al., 2010). If water replaces the MCPM, composite mass would decline, but the volume would remain constant. Similarly if the absorbed water expands the polymer chains, then both the composite mass and volume would increase. Moreover, the addition of these fillers would decrease the wetting property of the monomers, and would results in more air bubbles in the final mix (Abou Neel et al., 2010). Upon immersion of these composites in water, rapid filling of the pores will occur. This would result in an increase of the composite mass, but would not cause an increase in volume. These were the few possibilities that could occur with the addition of CaP to the composites.

In this study most of the time the volume increase was associated with mass increase. It suggests that the material on submersion in water would expand the polymer chains. However, it’s difficult to predict exactly and there may be a combination of processes going on internally that results in the increase in mass and volume.

The aim behind the addition of CaP, particularly MCPM was to provide a soluble source of Ca and P to promote the self-healing of decayed tooth structure (Young et al., 2009). Initially in the first 24-48 hrs, MCPM (Ca/P 0.5:1) will be released from the surface. This would leave behind holes, polymer matrix, and insoluble TCP (Ca/P 1.5:1) (Mehdawi et al., 2009, Abou Neel et al., 2010). The loss of MCPM is because of its high solubility, it may be lost additionally as phosphoric acid, with some hydrogen ions from polymer degradation products. Most of the
MCPM loss is from the material surface. The remaining MCPM that is in the core could react will TCP to form brushite. This can be shown in the equation (5-1) below:

\[
\text{Ca (H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O} + \beta\text{-Ca}_3 (\text{PO}_4)_2 + 7\text{H}_2\text{O} \rightarrow 4\text{CaHPO}_4\cdot2\text{H}_2\text{O}
\]  

(5-1)

This equation shows that 1g of MCPM requires 0.5g of water in order for the reaction to proceed. Most of the MCPM is converted into brushite after reacting with TCP. The remaining un-reacted MCPM will be released to promote re-mineralisation. The mass and volume increase at later stages were at a slow rate. These changes could cause polymer swelling (Mehdawi et al., 2013b). This would result in increase in volume of the sample.

The diffusion controlled water sorption can be described by a standard Fickian equation with an additional \( \Delta M_0 \) term (equation 5-2) (Fu and Kao, 2010, Leung et al., 2005).

\[
\Delta M = \Delta M_0 + \Delta M_\infty \sqrt{\frac{2Dt}{\pi l^2}}
\]  

(5-2)

\( 2l \) is the sample thickness, \( D \) is the diffusion co-efficient of water sorption, \( t \) is time, \( \Delta M_\infty \) is the maximum change in the solution, and \( \Delta M \) represents the change in mass or volume in solution. The added \( \Delta M_0 \) term takes account of the early burst release from the surface (intercept on y-axis).

In this study the relatively lower intercept, gradient, and final number with SBF was seen as compared to distilled water (figure 5-2 to 5-20). This means that initial burst increase, subsequent steady diffusion controlled mass increase afterward, and maximum final increase in mass and volume were lower in SBF than in DW. This could be explained by a reduced osmotic gradient between the water droplets present inside the polymer matrix (containing dissolved calcium and phosphate), and the outside storage solution (Keraliya et al., 2012). When CaP is added into the formulation the effect of ions in the medium is reduced as there are now ions in the material to balance those outside. This shows that the water sorption of the
experimental formulations in this study is an osmotically driven process. Water diffusion into the composites may slow after the first 24 hours due to the reactive fillers precipitating to form brushite of lower aqueous solubility and or slowing of release of hydrophilic components (Breschi et al., 2008b, Yiu et al., 2006).

The final mass and volume changes observed earlier reached an equilibrium by the end of experiment (12 weeks). The final changes were always higher than the initial values. This could be due to the additional volume of the water bound to brushite, which exceeds the mass and volume of any component being released.

Hydrophilic monomers such as HEMA and hydrolysed 4-META have greater affinity for water than the viscous bulk monomers like UDMA (Bakopoulou et al., 2009). The above studies show HEMA encourages slightly more water sorption than 4-META (figure 5-10). 4-META has a molecular weight of 304 compared with 18 g/mol for a water molecule. For 5 wt% 4-META to be fully hydrolysed in the samples 0.3 % of water therefore needs to be absorbed. Once this has occurred, the hydrophilic acid groups could bind with the calcium phosphates and / or attract further water sorption. HEMA has hydrophilic hydroxyl groups that will bind to water molecules, and enhance water sorption (Yiu et al., 2004). This water sorption plasticises polyHEMA, reduces its T_g, and thereby increases its chain mobility. Moreover, adding HEMA or 4-META may reduce the density of crosslinks in the polymer matrix. This will also increase water diffusion, and swelling of the polymer matrix.

In the above study, PLS addition encouraged water sorption into the dental composites. PLS solubility is high in both DW, and SBF (Gao et al., 2011). Water sorption was increased with raising the level of PLS in the filler phase. PLS contains -NH_3^+Cl^- groups which will dissociate in aqueous solution to form cationic polymers that draw in more water. PLS and CaP both work synergistically to increase water sorption. In comparison, increasing CHX concentration from
0 wt % to 5 wt % in filler phase had no significant effect on both the mass and volume change (figure 5-10, and 5-20). This may be a consequence of its more limited solubility in water than PLS.

The role of TCP in this whole process is to balance the high solubility associated with MPCM, by preventing its dissolution (Abou Neel et al., 2010). The above study has shown that maximum water sorption is proportional to the CaP percentage in the filler phase.

To summarise the above discussion, the mass and volume increase in all experimental formulations was mainly controlled by the reactive CaP (MPCM, TCP) level, storage solution type, followed by other variables. The final volume change was almost double that of mass change for most of the formulations. Similarly the increase in DW was almost 50 % higher than SBF.

Water sorption can be beneficial to an extent. The absorbed water could result in material expansion, which with some materials may have a beneficial effect of reducing the micro-gaps between composite-tooth restoration interfaces, which arise upon polymerisation shrinkage (Parolia et al., 2014). Thereby, increasing the longevity of the restoration. Therefore, this volumetric expansion could play a vital role in reducing bacterial micro leakage, and recurrent caries (Lu et al., 2004, Al-Saleh, 2009). Excessive volumetric expansion is not desirable. As this would create un-necessary stresses on the tooth structures, and could cause cracking, or fracture of the weakened cusps. More work is desired in this regard to develop a balance composite with controlled volumetric expansion.
CHAPTER 6

ANTIBACTERIAL DRUG RELEASE
(CHLORHEXIDINE, AND POLYLYSINE),
AND QUANTIFICATION OF APATITE
PRECIPITATION ON COMPOSITE SURFACES
6. Antibacterial Drug Release (Chlorhexidine, and Polylysine), and Qualitative Analysis of Apatite like Precipitation on Composite Surfaces

6.1. Introduction

Currently, the main current cause of composite failure is polymerisation shrinkage, which enables recurrent caries, and ultimately tooth loss (Bohaty et al., 2013). This chapter aims to assess antibacterial release, and re-mineralisation capability of the experimental composite formulations in the former chapters. These properties of dental composites could promote re-mineralisation of de-mineralised dentine, and combat any bacterial infection either at interface or beneath the restoration.

This study assesses chlorhexidine, and polylysine release from the new formulations described in the last 2 chapters upon immersion in distilled water (DW), and simulated body fluid (SBF). It additionally, addresses if the release of calcium phosphate (CaP) will promote apatite like precipitation on the composite surfaces in SBF.

The variables tested in chlorhexidine release studies included storage solution (water or SBF), 4-META vs HEMA, polylysine level (5, or 0 wt %), and CaP levels (20, 10, or 0 wt %). The same variables were tested in polylysine release investigations but with an additional variable of chlorhexidine level (5, or 0 wt %). The variables that were assessed in qualitative analysis of apatite (A) like precipitation on composite surface studies included CaP level (20, 10, or 0 wt %), and storage time in SBF (24 hrs, 1 week, 1 month, and 3 month). In this study CHX, PLS and 4-META content were all 5 wt%.

Discs of 1 mm thickness, and 10 mm diameter were made, and stored in either DW or SBF. Each composite disc was placed in upright position using a sterilin bottle, so that both of its
surfaces were in contact with the 15 ml of storage solution. Chlorhexidine, and polylysine release into storage solutions were determined using UV spectroscopy for a maximum period of 6 weeks. Precipitation of apatite like precipitation on the surface of composite discs were assessed with scanning electron microscopy (SEM) for a maximum period of 3 months.

6.2. Aims and Objectives

The aim of this study was to determine drug release (chlorhexidine, and polylysine) from experimental composites upon storing in either DW or SBF. In addition, this study will assess apatite like precipitation on the surface of composites (with 4-META, CHX and PLS all at 5 wt %) upon varying CaP levels. Chlorhexidine, and polylysine release into DW and SBF will be assessed through UV spectroscopy. The morphology of the apatite like precipitate on composite discs will be observed using SEM.

6.3. Materials and Methods

A total of 24 different formulations were tested for chlorhexidine release. They were divided into two groups. The details are given in Appendix 4. The variables tested for chlorhexidine release includes: storage medium (DW or SBF), adhesive monomer 4-META or HEMA, polylysine level 5 wt % or 0.5 wt %, and CaP level (20, 10, or 0 wt %).

A total of 48 different formulations were tested for polylysine release. They were divided into two groups. The details are given in Appendix 4. The variables tested for polylysine release includes: storage medium (DW or SBF), adhesive monomer 4-META or HEMA, polylysine level 5 wt % or 0.5 wt %, chlorhexidine level 5 wt % or 0 wt %, and CaP level (20, 10, or 0 wt %).

Three formulations were used in assessment of apatite like precipitate on the surface of composites. The variables tested include storage time in SBF (24 hrs, 1 week, 1 month, or 3
months), and CaP levels (20, 10, or 0 wt %) whilst 4-META, CHX and PLS were fixed at 5 wt %.

All the samples were 1 mm thick, and 10 mm diameter, and cured for 40 s on both sides. The samples were subsequently stored in 15 ml of storage solution at 37 °C for the periods given in chapter 2.

Filler characterisation was carried out using SEM. Each filler was attached individually to an adhesive tape, which was then attached to a stub. The specimens were then visualised under SEM.

A three variable and two level factorial analysis was performed to analyse the drug release data. Details of the experimental method, and factorial analysis is given in chapter 2.

6.4. Results

For each of the properties tested in this chapter actual experimental data, a full factorial analysis, and average effect of individual variable on each property will be provided in below sections.
6.4.1. Chlorhexidine Release

Representative chlorhexidine (CHX) release data from experimental composites plotted against square root of time are given in figure 6-1. All the formulations shown below have fixed amount of CaP at 10 wt %, and polylysine at 5 wt %. The variables that are shown in figure 6-1 are 4-META versus HEMA, and DW versus SBF. Chlorhexidine release plotted versus square root of time for all the other formulations are given in appendix 3.

The maximum chlorhexidine release was seen when samples were stored in DW. The formulations with 4-META had slightly higher release than those with HEMA. The maximum chlorhexidine release at 6 weeks in DW was 9 % with 4-META, and 8 % with HEMA. Similarly, the maximum CHX release in SBF at 6 weeks was 3 % with 4-META, and 2 % with HEMA. The CHX release in DW and SBF was proportional to square root of time as expected for a diffusion controlled process after an initial burst release in the first 24 hours.

Figure 6-1 Representative chlorhexidine release plotted against square root of time. The below examples have fixed amount of CaP (10 wt %), and polylysine (5 wt %) in the filler phase. The variables shown in the figure are storage medium (DW vs SBF), and adhesive monomer 4-META vs hydrophilic monomer HEMA (n=6). The 4 weeks represent the point where it was assumed that equilibrium was reached.
6.4.1.1. Intercept of chlorhexidine versus square root of time

The initial CHX burst release was obtained from the intercept of percent CHX release (details about intercept given in section 2.2.11) data plotted against square root of time for each formulation using data between 48 hours and 6 weeks. From figure 6-2 it can be seen that the variables affecting the initial burst release are DW versus SBF, and 4-META versus HEMA. With DW the intercept was between 1-2.5 %, while in SBF it was between 0.2-0.7 %. With formulations with 4-META the initial burst release (0.5-2.5 %) was higher than with HEMA (0.2-2.3 %). There was no obvious systematic effect of CaP, and polylysin level on the initial CHX release.

![Figure 6-2 Initial burst chlorhexidine release of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %). Error bars represent 95 % CI, (n=6).]
The factorial analysis figure 6-3 shows the magnitude of a terms for the 3 different variables. Using equation 2-5 in section 2.2.4.2, the “a” values can be converted into average percentage change. From the figure it can be seen that the burst release of CHX in DW was ~ 80 % higher than SBF (a1). The other variable that increased the initial burst release was the use of 4-META, which on average increased the release by ~ 20 % as compared to HEMA. Changing the level of CaP from 0-20 wt %, had no systematic effect on a1, a2, or a3. On average the initial CHX release is not significantly affected by variable 3. Only a1, and a2 are significant with the rest of variables showing no significant effect because of high interaction terms a12, a13, a23, and a123.

![Graph](image)

*Figure 6-3 Factorial analysis of initial burst chlorhexidine release of all formulations. A1 indicates the level of effect of Distilled water vs SBF, a2 corresponds to effect of 4-META vs HEMA, and a3 shows effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %.*
The average early CHX release for the first 3 variables is given in figure 6-4. On average the early CHX release in DW (~ 1.5 %) was higher than SBF (~ 0.4 %). Similarly the initial CHX release with 4-META formulations (~ 1.0 %) was higher than HEMA containing formulations (~ 0.75 %). Increasing CaP levels (0-20 wt %) had no systematic effect. Similarly, altering polylysine level had no effect on the early CHX release.

![Figure 6-4](image)

*Figure 6-4 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on initial burst chlorhexidine release intercept with formulations containing varying levels of calcium phosphate. Error bars are 95 % C. I of the mean (n=6).*
6.4.1.2. Gradient of chlorhexidine versus square root of time

The final gradient of chlorhexidine release (details about gradient given in section 2.2.11) versus square root of time for all variables increased systematically with increase in CaP level. The average gradient in DW for formulations with 0, 10, and 20 wt % CaP was ~ 0.08, 0.15, and 0.25 wt % / hr^{0.5} respectively. Similarly, the average gradient in SBF for formulations with 0, 10, and 20 wt % CaP was ~ 0.03, 0.05, 0.06 wt % / hr^{0.5} respectively. Furthermore, with high polylysine level (5 wt %), the gradient was increased particularly when HEMA was employed instead of 4-META.

![Figure 6-5 Final gradient of chlorhexidine release of all formulations with 5 or 0.5 wt % PLS, adhesive monomers (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %). Error bars represent 95 % CI, (n=6).]
Factorial analysis of final chlorhexidine gradient (figure 6-6) shows all the first 3 variables had a significant effect. By using equation 2-5 in section 2.2.4.2, the “a” values can be converted into average percentage change. These show an increase of ~ 60, 40, and 30 % with the use of DW instead of SBF, 4-META as opposed to HEMA, and 5 instead of 0.5 wt % polylysine in the formulations respectively. Increasing CaP from 0-20 wt % caused a systematic increase in $a_1$ but had negligible effect on $a_2$, and $a_3$. Small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ suggests that individual variables have significant effect on gradient of CHX release.

Figure 6-6 Factorial analysis of final gradient of chlorhexidine release of formulations with variables, $a_1$ corresponds to effect of Distilled water vs SBF, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %.
The average results for the first 3 variables are given in figure 6-7. The average CHX release gradient for formulations stored in DW was on average ~ 0.15 wt %/hr\(^{0.5}\), but ~ 0.05 wt %/hr\(^{0.5}\) with SBF. The gradient with 4-META formulations was on average ~ 0.11 wt %/hr\(^{0.5}\) as compared to ~ 0.06 wt %/hr\(^{0.5}\) with HEMA. Similarly with 5 wt % PLS the gradient was ~ 0.11 wt %/hr\(^{0.5}\), while with 0.5 wt % PLS it was ~ 0.07 wt %/hr\(^{0.5}\). From figure 6-7, it is possible to see a systematic upward trend in CHX release gradient with increase in CaP level. The average gradient for all formulations with 0, 10, and 20 wt % CaP was ~ 0.05, 0.1, and 0.15 wt % / hr\(^{0.5}\) respectively. The effect of CaP on the gradient was more pronounced in DW (0.06-0.27 wt %/hr\(^{0.5}\)) as compared to SBF (0.03-0.06 wt %/hr\(^{0.5}\)).

![Figure 6-7](image)

Figure 6-7 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on final gradient of chlorhexidine release with formulations containing varying levels of calcium phosphate. Error bars are 95% C.I of the mean (n=6). None overlapping error bars indicates significant difference.
6.4.1.3. Final Chlorhexidine release

The maximum chlorhexidine release (details about final release given in section 2.2.11) at 6 weeks for all formulations (figure 6-8) showed a systematically upward trend with the increase in CaP level. On average with the use of CaP 20 wt % the final release in DW was ~ 8-12 %, while in SBF it was ~ 1.5-3.5 %. With 10 wt % CaP in distilled water the final release was ~ 4-8 %, and 1.2-3 % in SBF. In case of 0 wt % CaP the final release was 2-4 % in DW, and 1-2 % in SBF. The use of 4-META, and polylysine at 5 wt % in place of HEMA, and 0.5 wt % polylysine respectively increase the final release to a smaller extent.

Figure 6-8 Final Chlorhexidine release of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %). Error bars represent 95 % CI, (n=6).
Factorial analysis of final chlorhexidine release in 6 weeks (figure 6-9) suggests an increase of ~ 60, 35, and 30 % with the use of DW, 4-META, and 5 wt % polylysine instead of SBF, HEMA, and 0.5 wt % polylysine (see equation 2-5). The change in levels of CaP from 0-20 wt % increase the level of effect of the variable associated with $a_1$. There was no effect seen on $a_2$ and $a_3$ with the addition of CaP. Small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ confirm that individual variables have significant effect on final release of CHX.

Figure 6-9 Factorial analysis of final chlorhexidine release of formulations, $a_1$ indicates effect of Distilled water vs SBF, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %. 
The average results for the first 3 variables are provided in figure 6-10. The evident increase in CHX release was seen with the use of DW, 4-META, and polylysine level 5 wt % in place of SBF, HEMA, and 0.5 wt % of polylysine respectively. Similarly, an upward trend was noted in final CHX release with the increase of CaP level from 0-20 wt %. The final average CHX increase for all formulations with 0, 10, and 20 wt % CaP was ~ 2.2, 4.2, and 6 % respectively. The final level of CHX release was much higher in DW, as compared to SBF.

![Figure 6-10: The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on final chlorhexidine release with formulations containing varying levels of calcium phosphate. Error bars are 95 % C. I of the mean (n=6).](image-url)
6.4.2. Polylysine release

Representative examples of Polylysine (PLS) release plotted against square root of time are given in figure 6-11. All the formulations shown below have 4-META in the monomer phase, and were tested in DW. Additionally, they have fixed amount of chlorhexidine at 5 wt %. The variables that are shown in figure 6-11 are CaP level (20, 10, or 0 wt %), and polylysine (5 wt %, or 0 wt %). Polylysine release plotted versus square root of time for all the other formulations are given in appendix 3.

The polylysine release showed early fast diffusion controlled release in the first 48 hrs, followed by slower diffusion controlled release up until 6 weeks. The final release was dependent on polylysine, and CaP levels in the formulations. The release with 20 wt % CaP was ~ 87 %, and 57 % with 0.5, and 5 wt % of polylysine respectively. With 10 wt % CaP the release was ~ 80 %, and 56 % with 0.5, and 5 wt % of polylysine. Similarly in case of 0 wt % CaP the final release was ~ 73, and 52 % with 0.5, and 5 wt % of polylysine respectively.

![Graph showing polylysine release](image)

Figure 6-11: Representative polylysine release plotted against square root of time. The below examples were stored in DW with fixed amounts of chlorhexidine, and 4-META at 5 wt % in filler and monomer phase respectively. The variables shown in figure are polylysine level (5 or 0.5 wt %) and CaP level 0, 10, or 20 wt % CaP (n=6). It was assumed that equilibrium was reached by 4 weeks.
6.4.2.1. Intercept of polylysine versus square root of time

The early burst polylysine release (details about intercept given in section 2.2.11) can be accessed from the intercept of percent polylysine release data plotted against square root of time between 48 hrs and 6 weeks. From figure 6-12 it can be seen that CaP level has a strong effect on the early burst release but primarily only when the PLS level is low. Average intercepts of ~ 25-50 % are observed with 20 wt % CaP, ~ 20-45 % with 10 wt % CaP, and 20-35 % with 0 wt % CaP. The other factor that is having a major effect is polylysine level. At 0.5 wt % the initial burst release was ~ 40 %, with 5 wt % but ~ 25 % with 5%. There was no evident change in intercept noted with other variables including DW vs SBF, and 4-META vs HEMA. CHX level (5 or 0 wt %) did not affect the level of effect of the first 3 variables.

![Figure 6-12 Initial burst polylysine release measured by intercept of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).]
The factorial analysis of intercept data is provided in figure 6-13. By using equation 2-5 in section 2.2.4.2, the “a” values can be converted into average percentage change. The factorial analysis shows that with the use of DW the initial polylysine release was ~ 15 % higher than in SBF. The major effect was seen with polylysine level. With 0.5 wt % the intercept was increased by ~ 60 % as compared to 5 wt % for all formulations. Variable 2 on average showed no effect. Altering the CaP level from 0-20 wt % caused a systematic increase in the magnitude of a3, but had negligible effect on a1, and a2. The CHX addition also had negligible effect on a1, a2, and a3.

Figure 6-13 Factorial analysis of polylysine burst release. A1 corresponds to effect of Distilled water vs SBF, a2 corresponds to effect of 4-META vs HEMA, and a3 corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows interaction effects between Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt % and a3. Error bars crossing the 0 line for all other terms suggests a3 and CaP are only variables with a significant effect.
The average intercept for each of the first 3 variables are provided in figure 6-14. With the use of DW the initial PLS release was a fraction higher than SBF (~ 30 % with DW, as compared to ~ 25 % with SBF). The biggest change was noted with the addition of 0.5 wt % PLS. The PLS release was between 32-45 % with 0.5 wt % PLS, and 22-25 % with 5 wt % PLS. The adhesive monomer variation had no effect on intercept. The addition of CaP (0-20 wt %) showed a minor upward trend with all average results but was most evident with 0.5 wt % PLS. Chlorhexidine addition did not significantly change the initial PLS burst release.

Figure 6-14 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on initial burst polylsine release intercept with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C.I of the mean (n=6).
6.4.2.2. Gradient of polylysine versus square root of time

The PLS release final gradient (details about gradient given in section 2.2.11) for all variables showed a systematically upward trend with an increase of CaP level (0-20 wt %). Higher gradient was seen with 0.5 wt % polylysine instead of 5 wt%. On average the gradient with 0.5 wt % PLS was between 1.10-1.45 wt % / hr$^{0.5}$ and 0.85-1.20 wt % / hr$^{0.5}$ with 5 wt % PLS. The variables DW vs SBF, and monomer change had no obvious effect on gradient of PLS release. Similarly, the CHX addition did not affect the PLS gradient (Figure 6-15).

![Figure 6-15 Final gradient of polylysine release of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).]
Factorial analysis of final PLS gradient (figure 6-16) suggests an increase of \( \sim 10\% \) with the use of DW, as compared to SBF \( (a_1) \) but this was primarily when the CaP content was high. Similarly a decline \( \sim 30\% \) in PLS gradient was seen with the use of high PLS level 5 wt % as compared to 0.5 wt % \( (a_3) \). The variable 2 showed no effect. CaP increase from 0-20 wt %, made \( a_3 \) less negative and increased \( a_1 \). The CHX addition showed no significant effect on the first 3 “a” terms. Error bars are crossing the 0 line, suggesting the variables having no significant effect, except for variable \( a_3 \) which showed significant effect.

Figure 6-16 Factorial analysis of final gradient of polylysine release of formulations with variables, \( a_1 \) corresponds to effect of Distilled water vs SBF, \( a_2 \) corresponds to effect of 4-META vs HEMA, and \( a_3 \) corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows that interaction effects with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, or Chlorhexidine levels 5 wt % vs 0 wt % are small.
The average results for the first 3 variables of PLS gradient are provided in figure 6-17. The evident increase in PLS gradient was observed with the use of 0.5 wt % PLS. The average gradient for all formulations with 0.5 wt % PLS was higher (~ 1.3 wt %/hr$^{0.5}$), as compared to 5 wt % PLS (~ 1.0 wt %/hr$^{0.5}$). A minor upward trend in average PLS gradient was seen with increase in CaP level only with water or high PLS (figure 6-17). There was no effect seen of any other variable on the PLS gradient.

Figure 6-17 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on final gradient of polylysine release with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C.I of the mean (n=6).
6.4.2.3. Final PLS release

The final PLS release (details in section 2.2.11) at 6 weeks for all formulations (figure 6-18) showed that with low level of PLS (0.5 wt %), the final PLS release was between 70-90 %.

With high levels of PLS (5 wt %) the release was between 50-60 %. A small increase in release was seen in formulations stored in DW (5 %) as compared to SBF. With the use of 4-META or HEMA no effect on final release of PLS was noted. CaP increase (0-20 wt %) caused a minor upward trend in PLS release with all 3 variables, while chlorhexidine level did not change the final PLS release.

![Final Polylysine release](image)

*Figure 6-18 Final polylysine release of all formulations with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and storage medium (Distilled water or SBF) with varying levels of calcium phosphate (20, 10, or 0 wt %), and chlorhexidine (5 or 0 wt %). Error bars represent 95 % CI, (n=6).*
Factorial analysis of maximum PLS release in 6 weeks (figure 6-19) suggests an increase of ~ 5 % with the use of DW as compared to SBF ($a_1$). Conversely with the use of 0.5 wt % PLS an increase of ~ 40 % was seen in comparison to 5 wt % PLS ($a_3$). A decline (~ 5 %) in final PLS release was seen with the use of HEMA instead of 4-META ($a_2$) (See equation 2-5, and 2-6). Change in CaP from 0-20 wt % increased $a_1$ but had negligible effect on $a_2$ and $a_3$. The CHX showed negligible effect on the first 3 “a” terms. The small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ confirm that the individual variables are all having significant effects on final PLS release.

*Figure 6-19 Factorial analysis of final polylysine release of formulations with variables, $a_1$ corresponds to effect of Distilled water vs SBF, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows that interaction effects with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, or Chlorhexidine levels 5 wt % vs 0 wt % are small.*
The average final release for the first 3 variables are compared in figure 6-20. A significant increase was seen with the use of 0.5 wt % polylysine (~ 80 %) as compared to 5 wt % polylysine (~50 %). No evident changes were seen with other variables on the final PLS release (figure 6-20). From this figure, it is possible to see a systematic upward trend in final PLS release with the increase in CaP level from 0-20 wt %. The CHX addition at 0 wt % results in a small increase in final polylysine release (~ 5 %) as compared to 5 wt %.

![Figure 6-20](image)

*Figure 6-20 The average effect of storage medium (water/SBF), 4-META replacement with HEMA, and PLS levels on final polylysine release with formulations containing varying levels of calcium phosphate, and chlorhexidine. Error bars are 95 % C. I. of the mean (n=6).*
6.4.3. Qualitative Analysis of Apatite like Precipitation on Surface of Composites

SEM images of the surface of experimental composite formulations with 0, 10, and 20 wt % CaP are given in figure 6-21, 6-22, and 6-23 respectively. The samples were immersed in SBF for 24 hrs, 1 week, 1 month, or 3 months.

With 0 wt % CaP there was no apatite (A) like precipitate seen on the surface at any time point. The surface of the composite contained evident glass particles. Additionally some spherical pores could be seen on the surface at all time points (figure 6-21).

Figure 6-21 Representative SEM images for experimental composite formulations with 0 wt % Calcium phosphate immersed in SBF for 24 hrs, 1 week (wk), 1 month (Mh), and 3 month (Mh). Pores (P), and composite glass particles (CG) can be seen.
With 10 wt % CaP at 24 hrs no apatite (A) like precipitates were seen. The number of pores, however, were enhanced. When samples were stored for 1 week, an apatite like layer was seen covering ~ 70 % of the surface. The layer further increased in thickness, and coverage after 1 month, and 3 months of storage in SBF (figure 6-22). All the surfaces showed small number of porosities but with apatite like precipitation on the surface the number of porosities was reduced.

Figure 6-22 Representative SEM images for experimental composite formulations with 10 wt % Calcium phosphate immersed in SBF for 24 hrs, 1 week, 1 month, and 3 month. Pores (P), apatite like (A) precipitate, and composite glass particles (CG) can be seen.
With the addition of 20 wt % CaP, apatite like precipitation after 24 hrs in SBF was seen. At 1 week almost 90 % of the surface was covered with apatite. The apatite like layer further increased in thickness and coverage after 1 month, and 3 months of storage in SBF (figure 6-23).

Figure 6-23 Representative SEM images for experimental composite formulations with 20 wt % Calcium phosphate immersed in SBF for 24 hrs, 1 week, 1 month, and 3 month. Pores (P), apatite like (A) precipitate, and composite glass particles (CG) can be seen.

6.4.4. Characterisation of fillers used in experimental composites

The various fillers used in composite formulations were characterised using scanning electron microscopy (figure 6-24). From figure 6-24, it could be noted that the particles of chlorhexidine diacetate were attached to each other, and appeared like a big lump on SEM. The Polylysine particles were globular in shape with oily appearance. The glass fibers under SEM looks like rods, while the glass particles crystals were either dispersed separately, or united together to
form a bulbous structure. Similarly, MCPM consists of needle like particles dispersed all over the surface, while $\beta$-TCP SEM showed platelet shaped particles.

Figure 6-24 Microscopic structures of individual components used in filler phase.
6.5. Discussion

6.5.1. Chlorhexidine release

Release of CHX from composite discs stored in DW was linear with the square root of time for most of the study period as expected for a diffusion controlled process (figure 6-1). In SBF, CHX released from the bulk may at later times become entrapped within the apatite layer that forms (Aljabo et al., 2015b). CHX, may bind to the anions in the SBF, forming salts of low solubility that precipitate with the apatite (Mehdawi et al., 2009). The final release from the composite disc in SBF was therefore much lower than in DW (figure 6-8).

As seen in the chapter 5, CHX had very little effect on the mass and volume changes of the composite. This would be possibly be explained by it being of low hydrophilicity and upon release being replaced by water of similar density.

The addition of CaP (MCPM, and TCP) had no effect on the initial burst release of CHX from the material as seen in figure 6-3, and 6-4. This would suggest that most of the initial release is from the surface of the material, and is not reliant upon water sorption into the material bulk. Later on the CHX release is substantially influenced by the addition of CaP. This could be explained by CaP increasing water sorption. Water diffusion into the material is most rapid in the initial 24-48 hrs. This is most likely becoming bound in the brushite phase (Babaei et al., 2013, Mehdawi et al., 2009). Despite water sorption then slowing with time the CHX release remained linear with the square root of time for several weeks. A possible reason could be that the brushite forms channels through the polymer matrix within the composite that will allow faster CHX diffusion. Formulations with high levels of CaP, showed higher water sorption and faster CHX release from the composite. As shown in the above results the amount of CHX release in DW with 20 wt % CaP was much higher (12 % in 6 weeks) than for the 0 wt % CaP formulation (4 % in 6 weeks) (figure 6-10).
As seen from the results the addition of PLS at 5 wt % increased the final CHX release by 30 %, as compared to 0.5 wt % PLS. However, there was no effect seen of PLS on the initial CHX release. Both PLS and CaP encouraged water sorption. PLS has the potential to draw in more water into the polymer, and cause expansion (Shan et al., 2009). When more water is attracted to the polymer it will enable solid CHX particles to dissolve in the composite bulk and subsequently allow its release from the core of the material.

The addition of 4-META monomer to the resin phase encouraged CHX release at all times as compared to HEMA. The increase in final CHX release noted with the addition of 4-META might be explained by the cationic nature of CHX. The positively charged CHX might interact with negatively charged 4-META. This interaction could aid dissolution of CHX in absorbed water and thereby enhance its release from the material.

The diffusion controlled CHX release can be described by a Fickian equation 6-1 (Fu and Kao, 2010, Leung et al., 2005).

\[
\Delta M = \Delta M_0 + \Delta M_\infty \sqrt{\frac{2D_t}{\pi l^2}}
\]  

(6-1)

2l is the sample thickness, D is a CHX diffusion co-efficient, t is time, M_∞ is the maximum change in the solution, and \Delta M represents the change in cumulative drug in solution. The added \Delta M_0 term takes account of the early burst release from the surface (intercept on y-axis).

Early fast release of CHX is very important to remove the residual bacteria (Schroeder et al., 2012), and the early colonisation of the gaps between a restoration and tooth structure (Mehdawi et al., 2009). This could be particularly helpful if the CHX then gets trapped within these gaps and in the apatite layers. CHX through its antibacterial effect, and interfering with the endogenous enzymatic degradation of the hybrid layer, should increase the longevity of the restoration (Tezvergil-Mutluay et al., 2011).
6.5.2. Polylysine release

PLS is industrially produced by *Streptomyces albulus* (Yoshida and Nagasawa, 2003). It is a homo-poly amino acid with peptide bonds between α-carboxyl, and ε-amino groups. PLS has shown broad antimicrobial action (Ye et al., 2013b). It is highly water soluble, edible, biodegradable, and is nontoxic to humans (Shih et al., 2004). The use of PLS is well documented in cosmetics, food, and pharmaceutical industries (Yu et al., 2009). In this study PLS was incorporated as an antibacterial in the filler phase, to add antibacterial properties to experimental composites. The Lysine present in PLS is a positively charged amino acid. The cationic nature of PLS helps in the inhibition of cell membranes of a wide range of bacteria, fungi, and yeasts (Carmona-Ribeiro and de Melo Carrasco, 2013). Examination of the literature suggests, however, that PLS has not previously been used in dental composites.

In the above study the PLS is dispersed as particles rather than dissolved in the polymer matrix phase of the composite. Its release is therefore dependent upon water sorption to aid its dissolution and diffusion through the polymer matrix. A high water sorption might therefore mean more PLS will be released into the surrounding solution. The above PLS study showed a small average increase in the initial, as well final release of PLS with CaP filler addition (figure 6-12 to 6-20). As explained earlier for CHX release, the CaP may promote the release of drugs from the material through encouraging water sorption. As PLS, however, is itself highly hydrophilic, the need for added CaP to increase water sorption induced release may have been reduced.

Upon submersion in DW, the composite absorbs more water than in SBF. This might be expected to result in increased volumetric expansion, and thereby enhanced release of PLS from the material. Unlike CHX, however, PLS release in SBF was almost equal to that in DW. A possible reason for the differences might be that unlike CHX, PLS is highly soluble in both DW, and SBF (Sakai et al., 2004, Shan et al., 2009). It was observed that with low PLS, high
calcium phosphate and use of SBF there could be some slight reduction in PLS release. This might be a consequence of low levels of the released PLS becoming entrapped within the precipitating apatite like layer. In this case the positively charged PLS could interact with the apatite like layer, as has been seen to occur with CHX.

A major increase (60 %) in percentage PLS release with the reduction in PLS from 5 down to 0.5 wt % PLS might be explained by the Higuchi expression (6-2) (Mehdawi et al., 2009, Otsuka et al., 2010, Zhou et al., 2014).

\[
(K_{0.5})^2 = \frac{DC_s(2C_o-C_s)}{4l^2C_o^2}
\]  

(6-2)

Where \(D\) is the diffusion co-efficient, \(C_0\) 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, and is equal to the gradient of fractional release versus the square root of time. For \(C_s,<<C_o\) this simplifies to (6-3).

\[
(K_{0.5})^2 = \frac{DC_s}{2l^2C_o}
\]  

(6-3)

As \(C_s\) is constant this indicates that the drug release gradient should be inversely proportional to its level in the material. Conversely with \(C_o = C_s\) the gradient will be independent of drug concentration. In the above study the results are between these 2 extremes.

However, it should be noted that the average cumulative PLS release in \(\mu g/ml\) will from the above data approximately 7 times higher for formulations with 5 wt % PLS than 0.5 wt % PLS.

There was no effect seen of the monomers (4-META vs HEMA), and CHX level (5 or 0 wt %) on the final PLS release (figure 6-20). It means that PLS release is dependent on water sorption, and drug concentration processes.
6.5.3. Apatite like precipitation on composite surface

The use of SBF as a storage medium for dental and medical purposes had been widely studied in the past decade (Zhou et al., 2014, Santos et al., 2001, Fong, 2004). This has led to the development of an ISO 23317:2007 standard for the preparation of SBF. Qualitative analysis of apatite like precipitation was carried out in this study using SBF (Paital and Dahotre, 2009, Pecheva et al., 2004).

The apatite like precipitates shown by the SEM were comparable to previous studies (Brundavanam et al., 2013, Wang et al., 2008, Santos et al., 2001). Previous works have shown that the MCPM usually dissolves from the surface of reactive filler composites in the first 24 hrs after placement in water (Mehdawi et al., 2009). This results in lowering of the pH of the storage solution. As the pH of the solution decreases, the solubility of apatite increases. Below pH =4 brushite can precipitate instead of apatite as its solubility becomes less than that of apatite (Bohner, 2000). One possible explanation behind the formation of apatite, and not brushite in the presence of CHX, MCPM, and TCP is that TCP, and CHX helps in the buffering of solution in addition to providing extra calcium.

The SEM images showed that a more rapid, and thicker apatite like precipitate was seen with high level of CaP. This precipitation could be beneficial for the re-mineralisation of the tooth structure, as well filling of the micro-gaps associated with polymerisation shrinkage.
CHAPTER 7

MECHANICAL PROPERTIES (BIAXIAL FLEXURAL STRENGTH AND YOUNG’S MODULUS) OF COMPOSITES
7. Mechanical Properties (Biaxial Flexural Strength and Young’s Modulus) of Composites

7.1. Introduction

As was discussed in the introduction (chapter 1), composite failure can occur as a result of brittle fracture due to low strength and high modulus. Much has been done to improve the mechanical properties of composites. The BFS of current commercial materials range from 70 to 180 MPa (Boaro et al., 2013, Sabbagh et al., 2004), while the modulus ranges between 3 to 10 GPa (Boaro et al., 2013, Czasch and Ilie, 2013). Mechanical property improvement was made possible through both monomer and filler optimisation. Generally, composites having higher filler are stronger and stiffer (Ersoy et al., 2004, Blackham et al., 2009). Many modern day composites have similar mechanical properties to amalgam, and are far superior to glass ionomers (Shenoy, 2008).

This chapter’s aim was to see if the previously described composites with re-mineralising, antibacterial, and self-adhesive components, also had mechanical properties comparable to current commercial composites. The properties of commercial composites discussed in chapter 3, indicate the strength range to aim for.

Experimental composites with varying levels of fillers and monomers as in earlier chapters were prepared. The variables investigated again included CHX level (5 or 0 wt %), 4-META replacement with HEMA, PLS level (5 or 0.5 wt %), CaP level (20, 10, or 0 wt %), and storage time of samples in DW (24 hrs, 1, 3, or 6 months). Samples were prepared of 1 mm thickness, 10 mm diameter, and light cured for 40 s on both sides. All the samples were then stored in DW for the periods mentioned above. Both BFS, and Modulus were determined using an Instron Universal testing machine, using a load cell of 1 kN, and a cross-head speed of 1 mm/min.
7.2. Aims and Objectives

The aim of this chapter was to develop and compare the long term BFS, and modulus of novel dental composites with the current commercially available materials.

Biaxial flexural strength and modulus for a series of formulations will be tested using an Instron universal testing machine.

7.3. Materials and Methods

BFS, and Young’s modulus of a total of 24 different formulations were determined. A detailed list of formulations is given in Appendix 5.

The variables tested for BFS, and modulus studies includes: storage time in DW (24 hrs, 1, 3, or 6 month), adhesive monomer 4-META or HEMA, polylysine level 5 wt % or 0.5 wt %, chlorhexidine level 5 wt % or 0 wt %, and CaP level (20, 10, or 0 wt %). A three variable and two level factorial analysis was performed to analyse the data for each CaP level.

The samples were prepared as 1 mm thick, and 10 mm diameter discs. All the samples were light cured for 40 s on both sides. Each formulation and time point had 6 sample replicates.

7.4. Results

For each of the properties tested in this chapter experimental data obtained either experimentally, or theoretically, factorial analysis and the average effect of each variable on each property will be provided in sections below.
7.4.1. Biaxial Flexural Strength (BFS) of Experimental Composites

The representative load / deflection graph obtained by a computer connected to the load cell is given in figure 7-1. The figure 7-1 (a) is representative of composites with only glass particles in the filler phase. With the addition of other reactive fillers the profile changes as shown in figure 7-1 (b).

*Figure 7-1* Representative load / deflection graph of experimental composite formulations with (a) 100 wt % glass particles in filler phase, and (b) glass particles 65 wt %, along with additional fillers CaP 20 wt %, glass fibres 5 wt %, CHX 5 wt %, and PLS 5 wt % in the filler phase.
The biaxial flexural strength for experimental composite formulations (calculated using equation 2-14) containing varying levels of CHX (5 or 0 wt %), PLS (5 or 0.5 wt %), CaP (20, 10, or 0 wt %), and monomers (4-META or HEMA) tested after 24 hrs, 1, 3, and 6 month storage in DW are given in figure 7-2.

The amount of decrease seen in BFS with the addition of 5 wt % CHX was ~ 5 MPa as compared to 0 wt % CHX. Similarly an increase of ~ 8 MPa seen with low levels of PLS (0.5 wt %) instead of high levels of PLS (5 wt %). The CaP addition brings down the BFS for all formulations. The decline in strength with 20, and 10 wt % CaP was ~ 10 MPa, and ~ 5 MPa respectively as compared to 0 wt % CaP. With the addition of 4-META to the monomer phase the BFS was increased on average by ~ 10 MPa for all materials.

The BFS was highest after 24 hours, with a marked decline seen after 1 month. Between 1 and 6 months a small decline in BFS was noted for all composites. The average BFS for all formulations at 24 hrs were between 105-140 MPa. Those at 1 month were between 85-120 MPa, at 3 month between 78-115 MPa, and at 6 month between 70-110 MPa for all composite formulations.

Figure 7-2 BFS with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and 5 or 0 wt % CHX for all formulations with varying levels of calcium phosphate (20, 10, or 0 wt %), tested at 24 hrs, 1, 3, and 6 months. Error bars represent 95CI, (n=6).
Factorial analysis of BFS data (figure 7-3) shows the magnitude of a terms for the 3 different variables were dependent on both CaP level and storage time. Using equation 2-5 in section 2.2.4.2, the “a” values can be converted into average percentage change. An increase of ~8, 7, and 10 % was seen in strength with the use of CHX 0 wt %, 4-META, and PLS 0.5 wt % instead of 5 wt % CHX, HEMA, and 5 wt % PLS respectively. The change in levels of CaP from 0-20 wt %, and storage time from 24 hrs to 6 months had only minor effects on a terms for the other 3 variables. Interaction effects were generally smaller than the effects of the first 3 variables.

Figure 7-3 Factorial analysis describing the effect of each variable, and interactions associated with combination of variables on biaxial flexural strength (BFS). $a_1$ corresponds to effect of chlorhexidine 5 wt % vs 0 wt %, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, and time of storage 24 hrs, 1, 3, and 6 month in distilled water.
The average effect of the first 3 variables along with the effect of CaP (0-20 wt %), and storage time (24 hrs-6 months) on these variables are given in figure 7-4. From the figure evident decline in BFS was seen with CHX, and PLS high level (5 wt %) with average strengths of (75-125 MPa), as compared to low levels (0 or 0.5 wt %) which showed strengths of (85-135 MPa). Similarly the BFS with 4-META formulations were ~ 10 MPa higher than HEMA formulations. The CaP addition, and storing samples for longer time both decreased the BFS. The decline in BFS was proportional to addition of CaP fillers. The average BFS for all formulations with 0 wt % CaP was ~ 125, 110, 100, and 95 MPa after 24 hrs, 1, 3, and 6 months respectively. With 10 wt % CaP it decreased to ~ 120, 105, 90, and 85 MPa after 24 hrs, 1, 3, and 6 months respectively. Similarly with 20 wt % CaP it was ~ 117, 95, 85, and 75 MPa after 24 hrs, 1, 3, and 6 months respectively.

Figure 7-4 The average effect of CHX levels, 4-META replacement with HEMA, and PLS levels on BFS with formulations containing varying levels of calcium phosphate, tested at 24 hrs, 1, 3, and 6 months. Error bars are 95 % C. I of the mean (n=6).
7.4.2. **Young’s Modulus of Experimental Composites**

The Young’s modulus for experimental composite formulations containing varying levels of CHX (5 or 0 wt %), PLS (5 or 0.5 wt %), CaP (20, 10, or 0 wt %), and monomers (4-META or HEMA) tested after 24 hrs, 1, 3, and 6 month storage in DW are given in figure 7-5.

The amount of decrease seen in modulus with the addition of 5 wt % CHX was ~ 0.5 GPa as compared to 0 wt % CHX. Similarly an increase of ~ 0.75 GPa was seen with low levels of PLS (0.5 wt %) instead of high levels of PLS (5 wt %). The CaP addition brings down the modulus for all formulations. The decline in modulus with 20, and 10 wt % CaP was ~ 1 GPa, and ~ 0.75 GPa respectively as compared with 0 wt % CaP. With the addition of 4-META to the monomer phase the modulus was increased on average by ~ 1 GPa for all materials.

The Young’s modulus of composites declined with storage time. After 24 hrs immersion in DW the modulus of experimental composites were between 4-6 GPa. After 1 month the modulus was between 3-5 GPa, at 3 month was between 2-4 GPa, and at 6 month was between 1.5-3.5 GPa for all the experimental composites. With large error bars associated with modulus data, some of the variables showed no effect on the final results.

![Figure 7-5 Modulus with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and 5 or 0 wt % CHX for all formulations with varying levels of calcium phosphate (20, 10, or 0 wt %), tested at 24 hrs, 1, 3, and 6 months. Error bars represent 95 % CI, (n=6).](Figure 7-5 Modulus with 5 or 0.5 wt % PLS, adhesive monomer (4-META or HEMA), and 5 or 0 wt % CHX for all formulations with varying levels of calcium phosphate (20, 10, or 0 wt %), tested at 24 hrs, 1, 3, and 6 months. Error bars represent 95 % CI, (n=6).)

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Factorial analysis (figure 7-6) showed no significant change in modulus associated with the first 3 variables. CaP (0-20 wt %), and storage time (24 hrs-6 months) had no effect on the a terms for the first 3 variables. The large interaction effects and error bars crossing zero, however, indicate that the variables associated with $a_1$, $a_2$ and $a_3$, had generally only small effects.

Figure 7-6 Factorial analysis describing the effect of each variable, and interactions associated with combination of variables on Young’s modulus. $a_1$ corresponds to effect of chlorhexidine 5 wt % vs 0 wt %, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Polylysine (PLS) levels 5 wt % vs 0.5 wt %. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 20, 10, 0 wt %, and time of storage 24 hrs, 1, 3, and 6 month in distilled water.
The average effect of the first 3 variables along with the effect of CaP (0-20 wt %), and storage time (24 hrs-6 months) on these variables are given in figure 7-7. From the figure evident decline in modulus was seen with CHX, and PLS high level (5 wt %) with average modulus of (2.1-4.5 GPa), as compared to low levels (0 or 0.5 wt %) which showed modulus of (2.8-5 GPa). Similarly the modulus with 4-META formulations were ~ 1 GPa higher than with HEMA formulations. The CaP addition, and storing samples for longer time both decreased the modulus. The decline in modulus was proportional to the level of CaP fillers, and storage time. The average modulus for all formulations with 0 wt % CaP was ~ 5, 4.7, 4.1, and 3.7 GPa after 24 hrs, 1, 3, and 6 months respectively. With 10 wt % CaP was ~ 4.8, 4.1, 3.7, and 2.8 GPa after 24 hrs, 1, 3, and 6 months respectively. Similarly with 20 wt % CaP was ~ 4.3, 3.5, 3.2, and 2.3 GPa after 24 hrs, 1, 3, and 6 months respectively.

![Figure 7-7](image-url)
7.5. Discussion

7.5.1. Biaxial flexural strength

In this study the incorporation of reactive fillers bring down the BFS, but the final BFS at 6 months were either comparable or in some cases better than the commercial composites. These fillers will additionally provide the extra properties to the new experimental formulations. The long term storage (up to 6 months) of these CaP, PLS, and CHX containing composites prior to testing was considered very important. These reactive fillers had the potential to promote absorption of water, and could also be released from the material. The results shown at 6 month suggested that even after the release of various drugs, and water sorption the experimental composite maintained good mechanical properties. This would be crucial for the long term success of these composites.

In this study a decline in strength of composite stored in DW was noted with time. The decline in strength was more pronounced in the initial 1 month. After 1 month to 3 month, and then at 6 month the rate of decline was very small (figure 7-2, and 7-4). The reduction in the rate of decline could be explained by the reduction and/or cessation of water sorption, and decrease in the release of reactive fillers CaP with time (Mehdawi et al., 2009). Additionally the brushite formed will potentially bind any unbound water, and decrease the availability of the free water that could cause plasticisation of the polymer matrix.

The decline in strength was seen with the addition of CaP fillers. This could be due to soluble MCPM encouraging high water sorption, which is the key factor in reducing the BFS of composites (figure 7-3). This would occur because of material plasticisation, and disruption of the polymer matrix. Similarly the release of fillers upon water sorption could also be a factor responsible for decline in strength. Additionally, with the addition of these fillers the refractive indices mismatch between the filler and monomer increase, which will result in low conversion,
and ultimately reduced mechanical properties. The lower reduction in BFS of experimental composites without CaP, could be a result of high conversion and cross linking.

Similarly a decline in strength with the use of 5 wt % PLS, and CHX was seen. This could be explained by the high water sorption, and solubility of PLS, and CHX in DW that would encourage more water sorption. The water absorbed will expand the polymer chains, and replace the fillers in the polymer matrix. As a result the strength will go down. This decline in strength with PLS, and CHX could also be explained by the poor wetting ability of the monomer with the addition of these fillers. The poor wetting could increase incorporation of air bubbles during the composite mixing, that could be considered as weak points for the propagation of cracks.

The relatively small increase with 4-META monomer over HEMA, could be explained by the hydrophilicity of the HEMA monomer. HEMA being a hydrophilic monomer has the potential to attract more water as compared to 4-META. This will results in high plasticisation of the polymer matrix, and a decline in mechanical properties. 4-META, however, might also bind to the calcium phosphate fillers and act in a similar way to a silane coupling agent.

7.5.2. Young’s modulus

Modulus is considered a very important mechanical property for dental composites (Xavier et al., 2010a, Santos Jr et al., 2013). Modulus is a measure of the stiffness of a material. A high modulus indicates that the material is brittle, while low modulus suggests flexibility with in the material (Xavier et al., 2010b). The same factors that increased or decreased the BFS, influenced the modulus. The modulus noted for all experimental materials were either lower, or comparable to commercial composites. A lower modulus aids energy absorption and reduces brittle fracture.
The decrease in modulus with high CaP, PLS, and CHX could possibly be due to the mismatch between the refractive indices of filler and liquid phases (figure 7-5). This mismatch will cause a lower conversion, which will in turn reduce the modulus of the materials.

As seen in chapter 3 the commercial, and control composite lack cross linking in the polymer matrix. This will allow the entangled polymer chains to be pulled apart. In case of experimental material studied in this study the high cross linking will prevent the polymer chains from breaking apart as readily.

The composite discs that were stored for a longer time in DW absorb more water, and therefore greater reduction in the modulus of the materials. A similar decline in modulus was noted with the addition of hydrophilic monomer HEMA (figure 7-7). Both of these decline in modulus could be explained by water sorption of the material. More water in the polymer matrix will cause more plasticisation, and flexibility of the polymer chains, hence reduction in modulus.

Similarly, the reduction in modulus upon adding CaP, and subsequent submersion in DW could be due to lack of reactive filler matrix bonding, and high porosity respectively. Whilst reduction in strength is disadvantages, decline in modulus will increase resilience and energy absorption.
CHAPTER 8

DETERMINATION OF DENTINE (IVORY AND HUMAN), CHEMISTRY, MECHANICAL PROPERTIES, AND ADHESION TO COMPOSITES (PUSH OUT, SHEAR BOND, AND INTERFACE STUDIES)
8. Determination of Dentine (Ivory and Human), Chemistry, Mechanical properties, and adhesion to composites (Push out, Shear Bond, and Interface studies)

8.1. Introduction

Dental caries, otherwise known as tooth decay, is one of the most prevalent chronic diseases of people worldwide (Selwitz et al.). The caries progression will leads to tooth decay, and cavitation. The cavities formed in tooth must be removed and replaced by a filling material to restore tooth function and prevent continuing decay. Currently, composites are considered as material of choice for restoring tooth structure. Unfortunately composites are technically more difficult to place and have higher failure rates primarily due to secondary caries beneath the restoration (Bernardo, 2007, Mehdawi et al., 2013a, Krifka et al., 2012). This occurs because upon set the composites shrink, damaging the bond between the tooth and composite and allowing bacteria to penetrate between the micro-gaps that are formed.

Traditionally, the most reliable composite bond has been achieved by first acid etching the dentine (Erhardt et al., 2004). This provides a porous hydroxyapatite depleted surface collagen mesh and opens up aqueous fluid filled dentine tubules. Flowable hydrophilic dentine primers and adhesives can penetrate and upon polymerisation physically interlock with both collagen and tubules (Wang and Spencer, 2002). The adhesives can additionally contain acidic monomers that can form ionic bonds with the dentine. Furthermore, the adhesive is able to chemically bond with the viscous hydrophobic composites.

In an attempt to increase simplicity and reduce complexity, in the last decade, a major drive has been towards “single step” adhesives that may bond without etching. Their clinical success, however, has been variable. More recently, “flowable” composites, that have the potential to
bond without etching or adhesive, have also been produced (Frankenberger et al., 2002). These flowable composites, however, can have high shrinkage enhancing micro-gap formation. Furthermore, their low strength limits the clinical situations in which they may be applied. In this study the acidic monomer 4-META will provide the self-adhesive property to experimental composites that can bond to dentine without the use of any acid, or adhesive to dentine.

Many studies have used extracted human teeth to evaluate the adhesive strength of restorative materials. Human teeth, however, are difficult to obtain. Other problems include their small size, variability with age and disease level (Cardoso et al., 2008, Earl et al., 2010, Jeon et al., 2011), infection hazard (De Munck et al., 2012), and ethical issues (Skene, 2002). Alternative dentine models to quantify and provide better understanding of factors affecting bonding are therefore required. One of the aims of this study was to assess if ivory is a good replacement model. The study additionally compares the dentine bonding of various experimental composites with and without a single step adhesive and / or acid etching. The composites include conventional bulk filled composites in addition to a new viscous composite with potential for self-bonding to dentine.

This study was performed to characterise two different dentines (ivory and human), that can be used to test the adhesion of novel dental composites. Additionally, this study will assess the micro-gaps between composites with 0, 10, 20, and 40 wt % CaP, and dentine surfaces.

### 8.2. Aims and Objectives

The aim of this study was to develop an alternate ivory dentine model. Additionally, this study also aimed at the assessment of the self-adhesive property of experimental composites with CaP and acidic monomer 4-META. Lastly, the aim of this study was to determine the interface between experimental composites and dentine using various dentine pre-conditionings.
Ivory and human dentine was analysed chemically, microscopically, and mechanically using Raman, SEM, and 3 point bend test. Adhesive monomer 4-META, and varying levels of CaP were incorporated into the composite formulations. Adhesion properties of these formulations to dentine were assessed through push out, and shear bond strength test. Lastly, micro-gaps between composite and dentine were visualised using SEM.

8.3. Materials and Methods

A total of 32 different formulations were used for push out strength determination. The detailed list of formulations is given in Appendix 6. The variables tested included use of Ibond vs No Ibond, 4-META vs HEMA, use of phosphoric acid etching vs No acid etching, and CaP levels (40, 20, 10, or 0 wt %). Push out test was carried out using ivory dentine. The composite pastes were put into 3 mm wide, and 5 mm deep holes, and cured from both sides for 40 s (n=6).

A total of 64 different formulations were tested for shear bond strength determination. The detailed list of formulations is given in Appendix 6. The variables tested included use of Ibond vs No Ibond, 4-META vs HEMA, use of phosphoric acid etching vs No acid etching, CaP levels (40, 20, 10, or 0 wt %), and dentine type (ivory or human). For shear bond tests composite pastes were poured in 2 mm increments into a brass tube of 3 mm internal diameter, and 6 mm long placed on the surface of the dentine (ivory and human). The samples were cured for 40 s (n=6).

Micro-gap formation after composite restoration of 3 mm diameter cavities in dentine was assessed by SEM. The formulations tested in this study were having fixed amounts of PLS, and CHX at 5 wt % in filler phase, and 5 wt % of 4-META in monomer phase. The variable used for interface studies included CaP level (40, 20, 10, or 0 wt %). All formulations were assessed using human and ivory dentine. As there was no significant difference seen in interfaces with
both dentines, however, for the purpose of clarity only human dentine data will be shown in this chapter.

Both the bond strength and micro-gap formation for each formulations were assessed with and without use of acid pre-treatment and / or the adhesive Ibond.

The mechanical properties for dentine were fitted to a Weibull’s type expression. The details are given in chapter 2 (Materials and Methods). A three variable and two level factorial analysis was performed to analyse the push out and shear bond data.
8.4. Results

For each property tested in this chapter a full factorial analysis, actual experimental data obtained either experimentally, or theoretically, and average effect of each variable on each property will be provided in below sections.

8.4.1. Dentine Characterisation

Dentine characterisation microscopically, chemically, and mechanically will be described in this section.

8.4.1.1. Dentinal Tubules

Representative SEM images of acid etched human dentinal tubules near the dentino-enamel junction, and ivory are provided in Figure 8-1. With human dentine the dentine tubule density (number/mm²) decreased progressively from approximately 15,000 near the crown to 30,000 near the pulp. With ivory the tubule density was ~ 10,000 mm². The ivory dentinal tubules were smaller in size ~ 2 µm, as compared to human dentinal tubules of ~ 5 µm.

Figure 8-1 Ivory and Human dentinal tubules. The amount of tubules present in human dentine is almost doubled that of ivory dentine.
8.4.1.2. Chemical Analysis of Dentine

The normalised average spectra (details in section 2.2.7.5) of ivory and human dentine were practically identical between 1200 and 1800 cm\(^{-1}\). This range included the 1670 cm\(^{-1}\) amide I, 1453 cm\(^{-1}\) amide II, and 1260 cm\(^{-1}\) amide III peaks due to collagen (Figure 8-2a and 2b). Below 1200 cm\(^{-1}\) both the hydroxyapatite (961 cm\(^{-1}\)) and \(\beta^-\text{CO}_3\)\(^2-\) (1073 cm\(^{-1}\)) peaks were more intense for the human dentine. After acid etching with 37 % phosphoric acid for 20, 60 and 120 s both the ivory and human dentine hydroxyapatite and carbonate peaks declined (Figure 8-2c). The 20 s etched human dentine was almost identical to that of ivory except for having a much stronger carbonate peak.

Normalised intensity at 961 cm\(^{-1}\) for both ivory and human dentine could be described well by an equation (8-1) of the form:

\[
\ln\left[\frac{I_t - I_f}{I_0 - I_f}\right] = -0.03t
\]  

(8-1) \(R^2 > 0.99\)

Where \(I\) is intensity and subscripts \(t\), \(0\) and \(f\) indicate times \(t\), initial and final. This indicates the time for half maximum surface reaction is 23 s \((-\ln (0.5))/0.03\) for both ivory and human dentine. For ivory and human dentine \(I_0\) values were 1000 and 500 and \(I_f\) values 190 and 105 respectively.
Figure 8-2 Chemical analysis of dentine using Raman spectroscopy. Raman spectra of (a) ivory, and (b) human dentine both after 0, 20, 60, or 120 s of acid etching. (c) Normalised intensity at 961 cm\(^{-1}\) is plotted against etching time in seconds (n=5).
8.4.1.3. Mechanical Properties of Dentine

\( P_f \) (Eq 2-19) was plotted versus bending strength, modulus, and flexural strain for ivory and human dentine in Figure 8-3.

*Figure 8-3 \( P_f \) plotted against specimen (a) three point bend strength, (b) modulus, and (c) flexural strain of ivory (dry & wet) and human dentine (wet).*
Mean results and Weibull parameters (details given in section 2.2.8.4) obtained by fitting equation 2-20 are provided in Table 8-1. The high $R^2$ values indicated the Weibull distribution described strength variation particularly well but could also fit modulus and strain data. The Weibull strength scale parameter, $\sigma_\theta$, and mean strength were significantly higher for human dentine as compared to ivory dry and wet. With dry ivory, the reduced shape parameter, $m$, and greater 95 % CI, indicated a broadened distribution of strengths compared with wet ivory or human dentine. Conversely, the Weibull scale parameter and mean modulus, increased in the order wet ivory < human wet dentine < dry ivory. Flexural strain increased in the order dry ivory < wet ivory < human wet dentine. Modulus and strain shape parameters, were not significantly affected by dentine type.

**Table 8-1** Weibull scale $\sigma_\theta$ and shape $m$ parameters obtained upon fitting equation (2-20) to three point strength, modulus, and flexural strain data.

<table>
<thead>
<tr>
<th></th>
<th>Weibull Parameter</th>
<th>Ivory Dry</th>
<th>Ivory Wet</th>
<th>Human Dentine Wet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bending Strength</strong></td>
<td>$\sigma_\theta$</td>
<td>38</td>
<td>36</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>$m$</td>
<td>3</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.97</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>34</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
<td><strong>95 % CI</strong></td>
<td></td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Bending Modulus</strong></td>
<td>$\sigma_\theta$</td>
<td>1.7</td>
<td>1.0</td>
<td>1.2</td>
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<td></td>
<td>$m$</td>
<td>6</td>
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<tr>
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<td>$R^2$</td>
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<td>0.90</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
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<td>0.9</td>
<td>1.2</td>
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<tr>
<td><strong>95 % CI</strong></td>
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<tr>
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<td>$\sigma_\theta$</td>
<td>2.7</td>
<td>4.7</td>
<td>7.2</td>
</tr>
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<tr>
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<td>$R^2$</td>
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<td>0.96</td>
<td>0.90</td>
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<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>2.5</td>
<td>4.3</td>
<td>6.7</td>
</tr>
<tr>
<td><strong>95 % CI</strong></td>
<td></td>
<td>0.3</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>
8.4.2. Push out Bond Strength

The push out strength (details in section 2.2.12.1) for experimental formulations are given in figure 8-4. The main effects seen on the bond strength were the use of Ibond and 4-META. With the use of Ibond the bond strength with 4-META formulations was between 26-40 MPa. With HEMA and Ibond use it was between 23-36 MPa. When no Ibond was used the bond strength for 4-META formulations was ~ 15-30 MPa, and that of HEMA formulations ~ 8-14 MPa. Acid etching had negligible effect on the bond strength when Ibond was used. Without Ibond, in the case of 4-META formulations, there was no major effect seen of acid treatment. The average values were between 15-30 MPa. In case of HEMA formulations, the average bond strengths with acid treatment were between 11-14 MPa, and 8-9 MPa with no acid treatment. Increased level of CaP (0-40 wt %) caused a downward trend in bond strength irrespective of the other 3 variables.

![Figure 8-4 Push out strength of experimental composite with variables Ibond vs no Ibond, adhesive monomer (4-META vs HEMA), and acid etching vs no acid etching. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 40, 20, 10, 0 wt %. All formulations contained a fixed amount of CHX 5 wt %, glass fibres 5 wt %, and PLS 0.5 wt %. Error bars represent 95 % CI, (n=6).](image)

Error bars represent 95 % CI, (n=6).
Factorial analysis of push out test (figure 5-6) shows all the first three variables had a significant effect. Using equation 2-5 in section 2.2.4.2, the “a” values can be converted into average percentage change. The factorial analysis showed an average increase in bond strength of ~ 90% with Ibond use instead of no Ibond application. Similarly an increase of ~ 50% was seen with formulations with 4-META instead of HEMA. A small increase of ~ 15% was seen with acid etching. The large interaction effects a_{12}, and a_{13} are because the effects of variables 2 and 3 are greater with no Ibond. An increase in level of CaP from 0-40 wt % decreased a_2 and made a_{12} less negative but had less effect on a_1 and a_3. Large interaction term a_{12} shows the strong interaction effect associated with variable a_1, and a_2. Smaller interaction terms a_{13}, a_{23}, and a_{123} suggests that individual variables have significant effect on push out bond strength.

Figure 8-5 Factorial analysis describing the effect of each variable, and interactions associated with combination of variables on Push out test. a_1 corresponds to effect of Ibond vs No Ibond, a_2 corresponds to effect of 4-META vs HEMA, and a_3 corresponds to effect of Acid etch vs No Acid etch. The figure also shows some interaction effects with Calcium phosphate (MCPM + TCP) levels 40, 20, 10, 0 wt %.
The average results for the first 3 variables are provided in figure 8-6. The result showed that evident increase in bond strength was seen with the use of Ibond, and 4-META in place of HEMA. In this case, taking averages hides the interaction effect of 4-META with versus without Ibond. The acid etching caused a minor increase in bond strength. The CaP addition declined the strength for all formulations. The decline became increasingly more pronounced with 20 and then 40 wt % CaP. With 10 wt % CaP only a minor decline in bond strength was seen as compared to 0 wt % CaP.

![Graph showing bond strength](image)

**Figure 8-6** The average effect of Ibond / No Ibond, 4-META replacement with HEMA, and Acid etching vs No Acid etching on Push out strength, with formulations containing varying levels of calcium phosphate (40, 20, 10, or 0 wt %). Error bars are 95 % C. I of the mean (n=6).
8.4.3. Shear Bond Strength

The shear bond strength (details in section 2.2.12.2) for experimental formulations are given in figure 8-7. The main effects seen on the bond strength were with Ibond and 4-META. With the use of Ibond the bond strength with 4-META formulations was between 24-38 MPa. With HEMA and Ibond use it was between 22-35 MPa. When no Ibond was used the bond strengths for 4-META formulations were ~ 12-27 MPa, and those of HEMA formulations ~ 5-14 MPa. Acid etching did not affect the bond strength when Ibond was used. Without Ibond in all formulations, there was an increase in shear bond strength noticed with acid etching. In case of 4-META the increase was ~ 7 MPa, while ~ 5 MPa seen with HEMA. Increased level of CaP (0-40 wt %) showed a small systematic decline in bond strengths. The use of dentine (human vs ivory) did not affect the bond strength in any cases.

![Figure 8-7 Shear bond strength of experimental composite with variables Ibond vs no Ibond, adhesive monomer (4-META vs HEMA), and acid etching vs no acid etching. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 40, 20, 10, 0 wt %, and dentine type (Ivory (I) vs Human (H)). All formulations contained a fixed amount of CHX 5 wt %, glass fibres 5 wt %, and PLS 0.5 wt %. Error bars represent 95 % CI, (n=6).](image-url)
Factorial analysis (figure 8-8) showed an increase in bond strength ~ 80 % (see equation 2-5) with Ibond use instead of no Ibond application. Similarly an increase of ~ 40 % was seen with formulations with 4-META instead of HEMA. A small increase of ~ 20 % was seen with acid etching as compared to no acid etching. The $a_{12}$ and $a_{13}$ interactions are due to the 2$^{nd}$ and 3$^{rd}$ variables having much greater effects when no Ibond is used. The change in levels of CaP from 0-40 wt %, and dentine type (ivory vs human) had only minor effects on $a_1$, $a_2$, and $a_3$ values. Small interaction terms $a_{12}$, $a_{13}$, $a_{23}$, and $a_{123}$ suggests that individual variables have significant effect on shear bond strength.

Figure 8-8 Factorial analysis describing the effect of each variable, and interactions associated with combination of variables on Shear bond test results. $a_1$ corresponds to effect of Ibond vs No Ibond, $a_2$ corresponds to effect of 4-META vs HEMA, and $a_3$ corresponds to effect of Acid etch vs No Acid etch. The figure also shows the interaction effect with Calcium phosphate (MCPM + TCP) levels 40, 20, 10, 0 wt %, and dentine type (Ivory (I) vs Human (H)).
The average effect of each variable was calculated by comparing the means of various formulations. The figure 8-9 showed that evident increase in bond strength was seen with the use of Ibond, acid etching, and 4-META in place of no Ibond, no acid etching, and HEMA respectively. The effects of CaP addition were complex. On average the formulations with 10 wt % showed slightly better bonding than with no CaP. Further increase in CaP level (20, and 40 wt %), however, caused decline in bond strength. The decline in bond strength was more pronounced with 40 wt % CaP. A small increase in bond strength was seen with the use of human, as compared to ivory dentine.

Figure 8-9 The average effect of Ibond / No Ibond, 4-META replacement with HEMA, and Acid etching vs No Acid etching on Shear bond strength, with formulations containing varying levels of calcium phosphate (40, 20, 10, or 0 wt %) tested on both ivory (I), and human (H) dentine. Error bars are 95% CI of the mean (n=6).
8.4.4. Interface between Composites and Dentine

8.4.4.1. Interface between Composite with 0 wt % CaP and Dentine

The SEM images of interfaces were comparable with ivory and human dentine. With 0 % CaP, and with the use of Ibond adhesive (with acid and no acid etching) a thin intact interface layer of 2-5 micron was typically observed between the dentine and composite (figure 8-10 IA, IAn). Additionally some air bubbles can be seen on the composite surface.

With acid etched dentine and no adhesive (Ibond), the experimental material with 0 % CaP could form a weak interface with micro-gaps ~ 5-10 microns. With no acid etching or adhesive (Ibond) similar micro-gaps of the size ~ 10 microns were seen (figure 8-10 InA, InAn).

![Interface images](image)

Figure 8-10 Interface (I) of experimental composite with 0 % calcium phosphate (Ct) to dentine (Dt) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment). All composites have fixed amounts of PLS, CHX, and 4-META at 5 wt % in filler and monomer phase.
8.4.4.2. Interface between Composite with 10 wt % CaP and Dentine

With 10 wt % CaP an intact interface was seen with most of the dentine pre-conditioning methods. Using Ibond adhesive (with acid and no acid etching) a thick intact interface layer of 10-15 microns was seen with IA, and a thin 2-5 micron was typically observed with IAn, between the dentine and composite (figure 8-11 IA, IAn). Additionally some air bubbles, and cracks can be seen on the composite surface.

With acid etched dentine and no adhesive (Ibond), the experimental material with 10 % CaP could form a thick interface of ~ 5 microns. With no acid etching or adhesive (Ibond) micro-gaps of the size ~ 2-5 microns were seen (figure 8-11 InA, InAn).

Figure 8-11 Interface (I) of experimental composite with 10 % calcium phosphate (Ct) to dentine (Dt) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment). All composites have fixed amounts of PLS, CHX, and 4-META at 5 wt % in filler and monomer phase.
8.4.4.3. Interface between Composite with 20 wt % CaP and Dentine

With 20 wt % CaP an intact interface was seen with most of the dentine pre-conditionings. Using Ibond adhesive (with acid and no acid etching) a thin intact interface layer of 2-5 microns was seen between the dentine and composite (figure 8-12 IA, IAn). Additionally some air bubbles, and cracks can be seen on the composite surface.

With acid etched dentine and no adhesive (Ibond), the experimental material with 20 % CaP could form a thin interface of ~ 2 microns. With no acid etching or adhesive (Ibond) micro-gaps of the size ~ 2-5 microns were seen (figure 8-12 InA, InAn).

![Figure 8-12 Interface (I) of experimental composite with 20 % calcium phosphate (Ct) to dentine (Dt) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment). All composites have fixed amounts of PLS, CHX, and 4-META at 5 wt % in filler and monomer phase.](image_url)
8.4.4.4. Interface between Composite with 40 wt % CaP and Dentine

With 40 % CaP, and with the use of Ibond adhesive (with acid and no acid etching) a thick intact interface layer of 15-20 microns was typically observed between the dentine and composite. In case of IAn an intact interface was seen with a much thinner interface layer < 2 microns (figure 8-13 IA, IAn). Additionally some air bubbles, and cracks can be observed on the composite surface.

With acid etched dentine and no adhesive (Ibond), the experimental material with 40 % CaP could form a weak interface with micro-gaps ~ 8 microns. With no acid etching or adhesive (Ibond) micro-gaps of the size ~ 10 microns were seen (figure 8-13 InA, InAn).

![Figure 8-13 Interface (I) of experimental composite with 40 % calcium phosphate (Ct) to dentine (Dt) using the following dentine pre-conditionings: IA (Acid etching followed by Ibond adhesive), IAn (Only Ibond application), InA (Only 35 % phosphoric acid etching), InAn (No dentine pre-treatment). All composites have fixed amounts of PLS, CHX, and 4-META at 5 wt % in filler and monomer phase.](image-url)
8.5. Discussion

8.5.1. Dentine Characterisation

Microscopic analysis showed that ivory tubules were smaller in size, but comparable in shape to those in human dentine. The tubule density was lower in ivory as compared to human dentine. Sizes observed were comparable with those observed previously with human dentine (Gupta et al., 2011, Poggio et al., 2013, Chandra et al., 2012). Tubule density increasing in human dentine closer to the pulp has been previously reported (Chng et al., 2002).

The Raman spectra observed above for dentine and acid etched dentine were comparable with those observed previously for intact and carious dentine. Earlier studies additionally showed that etch depth was ~8 micron with 15 s etch of non-carious dentine but that this could be doubled if the dentine was carious (Wang et al., 2007). The Raman microscope used in the above study provides the chemistry of the top 10-20 micron thick surface layer and is therefore ideal for assessing changes in chemistry upon acid etching. The relative levels of surface collagen and hydroxyapatite in the above new study suggests that chemically both 20 s etched human dentine and un-etched ivory are a good model for carious dentine. 20 s etched ivory would then be a model for etched carious dentine. The above new results also show acid clearly dissolves the carbonate associated with hydroxyapatite. Carbonate is formed by dissolution of carbon dioxide (e. G from carbonated drinks) in saliva and is able to replace phosphate ions in hydroxyapatite (Ignjacović et al., 2012). This increases the solubility of the hydroxyapatite (Pan and Darvell, 2010). The lack of carbonate in ivory is consistent with lack of contact with food and saliva.

For the human tooth there is a continual mineralisation and de-mineralisation process (Habelitz et al., 2014). The dentine used above was obtained largely from adults. In adult teeth mineral content is likely to be enhanced. Mineral level can increase with age due to stimulation of the
dental pulp as a response to trauma or caries (Cooper et al., 2014). Additionally increasing absorption of fluoride (e.g., from toothpaste) can enhance mineral content through reducing hydroxyapatite solubility (Shen et al., 2011, Tschope and Meyer-Lueckel, 2012). This suggests ivory is chemically a suitable model for teeth of younger rather than older patients.

The above bending strength of the human dentine is comparable with that observed in the literature (between 15 (McKittrick et al., 2010), and 200 MPa (Hayashi et al., 2008, Plotino et al., 2007, Vollenweider et al., 2007, Imbeni et al., 2003)). The broad range of values observed was a consequence of the large number of factors that affect this property. Strength has been shown to increase if the tubules are aligned parallel to the direction of applied force (Imbeni et al., 2003) or if the dentine is heated (Hayashi et al., 2010). A reduction in tubule density would also enhance strength. Changes in chemistry, including hydroxyapatite and water content or collagen structure, might also modify strength. Collagen consists of rigid rod like triple helices with crosslinks that bind them into fibrils. Water can penetrate between the helices and expand the fibrils (Van Raaij and Mitraki, 2013). Hydroxyapatite precipitates around the fibrils (Wang et al., 2011) stabilising the collagen against excessive water sorption induced expansion and attack by enzymes. The above new studies suggest that enhancing water content of the ivory has little effect on strength. This might be a consequence of the plasticising effect of excessive water sorption being compensated by ionisation of the collagen enhancing forces of attraction between the collagen fibrils. The higher strength of the human dentine compared with wet ivory could be a consequence of the higher hydroxyapatite content limiting water sorption and thereby compensating the higher density of tubules.

Increasing water content of collagen can reduce dry modulus of dentine from ~5 GPa down to a few MPa (Wenger et al., 2007). Increasing volume density of the tubules will decrease modulus but addition of rigid hydroxyapatite (modulus ~100 GPa) enhances modulus (Roop Kumar and Wang, 2002). The above observed reduction in modulus of the ivory upon
hydration will be due to plasticisation of the collagen. The higher modulus of human dentine compared with wet ivory could be primarily due to the increased hydroxyapatite.

From the above study it is clear that water sorption enhances the flexural strain for ivory but that it is even higher for the more mineralised human dentine. A possible explanation could be that in water, positive and negative charges may form (e.g. lysine and aspartic acid amino acids respectively) in collagen providing inter-fibril interactions. The precipitation of hydroxyapatite could further enhance these ionic interactions. Stronger ionic attractions between the collagen fibrils would enable them to be pulled further apart before breaking (Profeta, 2014).

8.5.2. Variables affecting bond strength of experimental composites

The major effects seen in this study was the use of Ibond, acidic monomer 4-META, and acid etching (figures 8-6, and 8-9). The use of adhesive (Ibond) with and without acid etching improved the bond strengths of all formulations. This could be explained by the presence of 4-META in the Ibond. The anhydride 4-META in the presence of water would form two carboxylic acid groups (Peumans et al., 2005). These acid groups will cause further de-mineralisation of the dentine and provide a bigger area for mechanical interlocking (Van Landuyt et al., 2007). Similarly the 4-META would also form an ionic bond with calcium in the remaining hydroxyapatite (Van Landuyt et al., 2007). Furthermore, the carboxylic acid will interact with the basic amino acids of collagen to form a chemical bond. The solvent evaporation and adhesive polymerisation in Ibond will additionally provide chemical interactions with the monomers in composite (Kumar and Wang, 2002). The low viscosity, and hydrophilicity of the Ibond adhesive will additionally allow a better penetration of the adhesive into dentinal tubules (De Munck et al., 2005b). The high bond strengths with Ibond in the above study could be due to the above mentioned possibilities. However, as bonding seems to be
improved with the adhesive application. This usually complicates the restorative procedure by increasing the number of steps involved in cavity filling.

The use of 4-META in dental adhesives is well documented (Van Landuyt et al., 2007, Ikemura and Endo, 2010). In this study incorporation of 4-META to the monomer phase of experimental composites improved the bond strength under varying dentine pre-treatments. A particularly significant effect was seen with 4-META when no acid etching was used. The materials with 4-META were able to bond to dentine, and maintained a good bond strength as compared to experimental formulations with HEMA, and commercial composites. The 4-META could form a weak ionic interaction with the calcium in the hydroxyapatite (Ikemura et al., 2003) resulting in improved bond strength. The hydrophilic monomer HEMA used in this study also improved bonding with acid etching (figures 8-4, and 8-7). This could be due to better penetration of the monomers into the wet dentineal tubules (Van Landuyt et al., 2008a).

Acid etching with phosphoric acid has been used in dentistry for decades (Mazzoni et al., 2006). Acid etching will open up the tubules, and will allow better penetration of the materials into the tubules (Oliveira et al., 2003a). In this study the acid etching improved the bond strength of experimental composites. The acid etching roughens the dentine surface (Aguilar-Mendoza et al., 2008). This roughening of the dentine provides a greater surface area for the material to bond to dentine.

The CaP addition had very little effect on bond strengths of composites. The minor decline, especially with high CaP (40 wt %) could be due to the poor wetting of the filler phase with the monomers increasing material viscosity (figures 8-5, and 8-8). This makes it difficult for the material to penetrate into the tubules. Additionally with high CaP, the final composite mix contains a large amount of porosities. This could further decrease the bond strengths of
experimental composites. These porosities are weak links in the composite for cracks propagation, and hence provide weak bond strengths.

### 8.5.3. Dentine-Composite interfaces

The interface between dentine and composites is considered very important for the long term success of restorations in the oral environment (Sadan, 2008, De Munck et al., 2005b, Piocon et al., 2001). The main purpose of this study was to evaluate the various factors that could affect the bond strength, and see the effect of those variables on the dentine composite interface under SEM. As discussed earlier the type of dentine (ivory, human) did not affect the bond strength. So for the purpose of simplicity, in this thesis interface study was carried out using human dentine.

The effect of 4-META is already established in chapter 3, which provided self-adhesive property to experimental composites. Similarly no effect of the PLS, and CHX were found in preliminary work (unpublished data). So in this study 4-META monomer was used, along with fixed levels of PLS, and CHX.

One of the purposes of this project was to determine the effect of reactive fillers CaP (0, 10, 20, and 40 wt %) on the interface between figures 8-10 to 8-13.

With the use of Ibond adhesive an intact interface was seen in all experimental formulations. The results suggested that with Ibond use only and no acid etching, usually a thin interface was seen. The adhesive was able to penetrate into the dentinal tubules, and formed strong material / dentine interlocking.

Similarly with the use of acid etching with phosphoric acid with or without Ibond, a thick hybrid layer was seen between composite and dentine in most of the experimental formulations. This layer could possibly contain particles from composite, and dentine debris from acid etching (Eick et al., 1997, Hashimoto et al., 2003). With Ibond use in combination with acid
the interface layer was much thicker, and intact along the whole length of bond between composite and dentine. In the case of no Ibond use and acid etching, gaps were seen with 0, and 40 wt % CaP. In the case of 10, and 20 wt % CaP a relatively more intact layer was seen.

The micro-gap formation in the case of 0 wt % CaP, with and without acid etching could be explained by the lack of re-mineralising CaP fillers that would have precipitated along the interface, and reduced the micro-gap formation. The acid etching had provided opening of the tubules but is not sufficient enough in this case to provide a strong interlock. The micro-gaps in 40 wt % CaP with and without acid etching could be linked to poor wetting ability of the monomer due to high reactive filler content. This makes the composite paste too dry, and difficult to flow, and penetrate into the dentinal tubules.

The relatively strong interface of 10, and 20 wt % CaP with and without acid etching suggested that these composites have better flow properties, which would have enabled them to penetrate into the tubules. Also the wetting ability of the monomers to fully cover the fillers is enhanced in this case.

This shows that CaP addition at 10, and 20 wt % CaP could be ideal for an intact interface. The reduction in micro-gaps associated with these intermediate levels of CaP would be beneficial to combat the bacterial micro-leakage associated with polymerisation shrinkage. This will in the long run increase the longevity of the restoration.
CHAPTER 9

CONCLUSIONS AND FUTURE WORK
9. Conclusions and Future Work

This chapter summarises the main conclusions and future work necessary to further characterise the properties of high strength, re-mineralising, antibacterial, and self-adhesive dental composites.

9.1. Conclusions

The depths of cure of the experimental control formulations and commercial materials examined in chapter 3 were of similar magnitude. Despite this, the levels of conversion were much higher for the experimental and flowable composites than with Gradia and Z250. For the later two materials conversion could be below 50 % even in thin samples. The mass and volume change of the commercial and control formulations were all ~1 wt % or 2 vol % and only slightly higher in water compared with in SBF. Mechanical strengths of the experimental control composites were comparable or better than the commercial materials. The major factor increasing the bond strength was the use of adhesive Ibond. The control with 4-META, however, had better adhesion properties than all the other formulations especially when there was no Ibond use. Despite the higher shrinkage and modulus of this new 4-META-containing material it enabled reduced microgap formation. It is therefore a promising self adhesive composite.

In chapters 4 to 8, CaP, CHX and PLS were added to the control composites in chapter 3. Although CaP addition decreased conversion and depth of cure, all experimental materials had > 50 % monomer conversion even at 4 mm depth. Furthermore, depth of cure was also always above the required ISO 4049 level of 1.5 mm. CaP addition of 20 wt % caused approximately a five fold increase in maximum mass and volume change. This enhanced water sorption substantially increased CHX release but had a lesser effect on PLS release kinetics. Furthermore, use of SBF instead of DW caused a large reduction in CHX release but not PLS
release. PLS release rates were in all cases higher than those of CHX. Increasing CaP level also raised the rate of apatite-like precipitation on the surfaces of materials submerged in SBF. Although CaP addition also caused a reduction in material strength, with 20 wt % CaP or less the results remained above those of Gradia. Chemically, ivory was similar to acid etched human dentine and gave similar bond strengths with all commercial and experimental materials. High CaP could reduce initial bonding to dry ivory and human dentine but intermediate levels could reduce microgap formation.

The experimental materials with moderate levels of CaP, CHX, PLS and 4-META show promise as dental composites that can promote apatite-like formation, antibacterial release and good bonding to dentine both with and without use of an additional adhesive.
9.2. Future work

Whilst the materials monomer conversion, polymerisation shrinkage, depth of cure, heat generation, water sorption, drug release, mechanical properties, adhesive properties, hydroxyapatite precipitation properties, and dentine interface were characterised, there are other areas of material development that need exploration. The areas for future investigations will be described in this section.

1. Polymerisation shrinkage was determined theoretically in this thesis, but should be further determined using ISO 17304:2013. This method determines the densities of un-polymerised paste and polymerised discs by measuring their mass in air and water. This will validate the theoretical shrinkage results.

2. Surface characterisation of composite, and quantification of apatite can be further characterised using Raman spectroscopy. Raman will not only identify the various functional groups present in the composites, but will also determine the chemical changes induced on the composite upon immersion in DW, and SBF. In the second stage, surface apatite quantification can be done through EDX. That will identify the Ca: P ratio, and from the ratio of these two elements the nature of the precipitate layer will be determined. Preliminary studies have been carried out on experimental composites using Raman, and EDX. The results confirmed the presence of apatite on the surface but further detailed analysis is needed.

3. It has been suggested that the apatite like precipitate hinders the release of CHX into the SBF. This will be proved by analysing the precipitate layer for any entrapped CHX using UV-Spectroscopy.

4. Ideally the composite materials should not be toxic to the pulp cells, and surrounding oral tissues. In the future biocompatibility of the composite formulations will be tested. The effect of altering levels of reactive fillers, antibacterial agents, and monomers will be
determined. In this project the levels of leachable agents from dental composites have been inferred from the level of conversion. Monomers leaching could be measured using High Performance Liquid Chromatography (HPLC). This will involve analysing the solution in which composite was stored after light curing.

5. The re-mineralisation properties of the dental composites described in this project should be further explored. The composite materials could be attached to de-mineralised/decayed human dentine, and left in SBF for 24 hrs, 1 week, 1, and 3 month. After each time point, the amount of minerals content in dentine would be examined. This can be done through Raman mapping, and EDX which will detect any possible increase in calcium and phosphate contents.

6. The antibacterial activity of the formulations developed in this project could be assessed in future using a Constant Depth Film Fermenter (CDFF). The composite materials activity against oral biofilms could be determined.

7. The long term adhesion properties of the experimental composite with human and ivory dentine will be assessed in the next stage. This will be determined by long term storage of samples in DW, or SBF, and measuring the bond strengths afterward. This will give us an idea about the long term stability of the restoration in oral cavity.

8. The initial interface study between composite and dentine was done through SEM. In the next stage a detailed analysis of the composite-dentine interface should be carried out using micro-CT, and Atomic Force Microscopy (AFM). The later will eliminate the high vacuum that is associated with the SEM, which can potentially affect the interface.
CHAPTER 10

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High strength re-mineralizing, antibacterial dental composites with reactive calcium

DOCTOR OF PHILOSOPHY, University College London.

MEHDAWI, I., NEEL, E. A. A., VALAPPIL, S. P., PALMER, G., SALIH, V., PRATTEN, J.,

MEHRALI, M., MOGHADDAM, E., SEYED SHIRAZI, S. F., BARADARAN, S.,
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CHAPTER 11
APPENDICES
11. Appendices

11.1. Appendix 1 (Control and Commercial materials)

Figure 11-1 Change in biaxial flexural strength of control and commercial materials calculated by using equation 3-1 plotted against square root of time / months. The corresponding parameters are given in table 3-3.

Figure 11-2 Change in modulus of control and commercial materials calculated by using equation 3-2 plotted against square root of time / months. The corresponding parameters are given in table 3-4.
11.2. Appendix 2 (Conversion, Shrinkage, Heat generation, and Depth of cure)

11.2.1. Group A (4-META formulations)

The Group A formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, 4-META 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-1.

Table 11-1 Summary of group A formulations used for Conversion, Polymerisation shrinkage, Heat generation, and Depth of cure.

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<tr>
<th>Formulations</th>
<th>G. Particles (wt %)</th>
<th>G. Fibres (wt %)</th>
<th>MCPM (wt %)</th>
<th>TCP (wt %)</th>
<th>CHX (wt %)</th>
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* Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polyclysine.
11.2.2. Group B (HEMA formulations)

The Group B formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, HEMA 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-2.

*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.

*Table 11-2 Summary of group B formulations used for Conversion, Polymerisation shrinkage, Heat generation, and Depth of cure.*

<table>
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<th>Formulations</th>
<th>G. Particles (wt %)</th>
<th>G. Fibres (wt %)</th>
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11.3. Appendix 3 (Mass and Volume changes)

11.3.1. Group A (4-META formulations)

The Group A formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, 4-META 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-3.

Table 11-3 Summary of group A formulations used for Mass and Volume changes in either Simulated body fluid (S) or Distilled water (W).

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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.
Figure 11-3 Mass changes versus square root (Sqrt) of time (hr) for experimental formulations (1-8). Error bars are estimated 95 % CI with n=6.

Figure 11-4 Mass changes versus square root (Sqrt) of time (hr) for experimental formulations (9-16). Error bars are estimated 95 % CI with n=6.

Figure 11-5 Mass changes versus square root (Sqrt) of time (hr) for experimental formulations (17-24). Error bars are estimated 95 % CI with n=6.
Figure 11-6 Mass changes versus square root (Sqrt) of time (hr) for experimental formulations (25-32). Error bars are estimated 95 % CI with n=6.

Figure 11-7 Mass changes versus square root (Sqrt) of time (hr) for experimental formulations (33-40). Error bars are estimated 95 % CI with n=6.

Figure 11-8 Mass changes versus square root (Sqrt) of time (hr) for experimental formulations (41-48). Error bars are estimated 95 % CI with n=6.
Figure 11-9 Volume changes versus square root (Sqrt) of time (hr) for experimental formulations (1-8). Error bars are estimated 95% CI with n=6.

Figure 11-10 Volume changes versus square root (Sqrt) of time (hr) for experimental formulations (9-16). Error bars are estimated 95% CI with n=6.

Figure 11-11 Volume changes versus square root (Sqrt) of time (hr) for experimental formulations (17-24). Error bars are estimated 95% CI with n=6.
Figure 11-12 Volume changes versus square root (Sqrt) of time (hr) for experimental formulations (25-32). Error bars are estimated 95 % CI with n=6.

Figure 11-13 Volume changes versus square root (Sqrt) of time (hr) for experimental formulations (33-40). Error bars are estimated 95 % CI with n=6.

Figure 11-14 Volume changes versus square root (Sqrt) of time (hr) for experimental formulations (41-48). Error bars are estimated 95 % CI with n=6.
11.4. Appendix 4 (Chlorhexidine and Polylysine release)

11.4.1. Chlorhexidine release

11.4.1.1. Group A (4-META formulations)

The Group A formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, 4-META 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-5.

*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.*

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<th>Formulations</th>
<th>SBF(S) / Water(W)</th>
<th>G. Particles (wt %)</th>
<th>G. Fibres (wt %)</th>
<th>MCPM (wt %)</th>
<th>TCP (wt %)</th>
<th>CHX (wt %)</th>
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11.4.1.2. Group B (HEMA formulations)

The Group B formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, HEMA 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-6.

Table 11-6 Summary of group B formulations used for Chlorhexidine release in either simulated body fluid (S) or Distilled water (W).

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<th>G. Fibres (wt %)</th>
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<th>TCP (wt %)</th>
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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.
Figure 11-15 Chlorhexidine release versus square root (Sqrt) of time (hr) for experimental formulations (1-12). Error bars are estimated 95% CI with n=6.

Figure 11-16 Chlorhexidine release versus square root (Sqrt) of time (hr) for experimental formulations (13-24). Error bars are estimated 95% CI with n=6.
11.4.2. Polylysine release

11.4.2.1. Group A (4-META formulations)

The Group A formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, 4-META 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-7.

Table 11-7 Summary of group A formulations used for Polylysine release in either Simulated body fluid (S) or Distilled water (W).

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<th>Formulations</th>
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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.
11.4.2.2. Group B (HEMA formulations)

The Group B formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, HEMA 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-8.

Table 11-8 Summary of group B formulations used for Polylsine release in either simulated body fluid (S) or Distilled water (W).

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Figure 11-17 Polylysine release versus square root (Sqrt) of time (hr) for experimental formulations (1-8). Error bars are estimated 95 % CI with n=6.

Figure 11-18 Polylysine release versus square root (Sqrt) of time (hr) for experimental formulations (9-16). Error bars are estimated 95 % CI with n=6.

Figure 11-19 Polylysine release versus square root (Sqrt) of time (hr) for experimental formulations (17-24). Error bars are estimated 95 % CI with n=6.
Figure 11-20 Polylysine release versus square root (Sqrt) of time (hr) for experimental formulations (25-32). Error bars are estimated 95 % CI with n=6.

Figure 11-21 Polylysine release versus square root (Sqrt) of time (hr) for experimental formulations (33-40). Error bars are estimated 95 % CI with n=6.

Figure 11-22 Polylysine release versus square root (Sqrt) of time (hr) for experimental formulations (41-48). Error bars are estimated 95 % CI with n=6.
11.5. Appendix 5 (Biaxial Flexural Strength and Young’s Modulus)

The formulations assessed for BFS, and Modulus consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, 4-META or HEMA 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-9.

Table 11-9 Summary of formulations used for Biaxial flexural and Young’s modulus of experimental composites.

<table>
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<th>Formulations</th>
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<th>G. Fibres (wt %)</th>
<th>MCPM (wt %)</th>
<th>TCP (wt %)</th>
<th>CHX (wt %)</th>
<th>PLS (wt %)</th>
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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.
11.6. Appendix 6 (Push out and Shear bond test)

11.6.1. Push out test

11.6.1.1. Group A (4-META formulations)

The Group A formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, 4-META 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-10.

Table 11-10 Summary of group A formulations used for Push out test.

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<th>Formulations</th>
<th>Ibond (I) / No Ibond (NI)</th>
<th>Acid (A) / No Acid (NA)</th>
<th>G. Particles (wt %)</th>
<th>G. Fibres (wt %)</th>
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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.*
11.6.1.2. Group B (HEMA formulations)

The Group B formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, HEMA 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11.

Table 11-11 Summary of group B formulations used for Push out test.

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<tr>
<th>Formulations</th>
<th>Ibond (I) / No Ibond (NI)</th>
<th>Acid (A) / No Acid (NA)</th>
<th>G. Particles (wt %)</th>
<th>G. Fibres (wt %)</th>
<th>MCPM (wt %)</th>
<th>TCP (wt %)</th>
<th>CHX (wt %)</th>
<th>PLS (wt %)</th>
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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.

11.6.2. Shear bond test

11.6.2.1. Group A (4-META formulations)

The Group A formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, 4-META 5 wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-12.
### Table 11-12 Summary of group A formulations used for Shear bond test.

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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β-Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.

#### 11.6.2.2. Group B (HEMA formulations)

The Group B formulations consists of a monomer, and a powder phase. The powder and liquid ratio is 4:1. The monomer phase is made of UDMA 75 wt %, TEGDMA 25 wt %, HEMA 5
wt %, DMPT 1 wt %, and CQ 1 wt %. The detail powder phase compositions in each formulations are given in table 11-13.

Table 11-13 Summary of group B formulations used for Shear bond test.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Ivory (ID) / Human dentine (HD)</th>
<th>Ibond (I) / No Ibond (NI)</th>
<th>Acid (A) / No Acid (NA)</th>
<th>G. Particles (wt %)</th>
<th>G. Fibres (wt %)</th>
<th>MCPM (wt %)</th>
<th>TCP (wt %)</th>
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*Contents are given in weight percentage, G. Particles; Glass particles, G. Fibres; Glass fibres, MCPM; Mono calcium phosphate monohydrate, TCP; β – Tri calcium phosphate, CHX; Chlorhexidine diacetate salt hydrate, PLS; Polylysine.
11.7. Appendix 7 (Preliminary work)

11.7.1. BFS formulations

11.7.1.1. Series one adhesive formulations

Series one adhesive contained two groups of monomer. 1st with UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA at 5 wt %, with CQ and DMPT both at 1 wt %. The filler consisted of equal masses of MCPM and β-TCP along with glass fibres, CHX and polylysine. The glass particle used was IF2019. 2nd monomer contained UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA Phosphate at 5 wt %, with CQ and DMPT both at 1 wt %. The filler content was the same except the glass particle DMG 7 µm was used instead of IF2019. All formulations had powder (filler) / liquid (resin or monomer phase) mass ratio (PLR) of 4:1. The variables used in series one formulations are given in table 11-14.

Table 11-14 Series one variables.

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<tr>
<th>VARIABLES</th>
<th>IF2019 / DMG</th>
<th>POLYLYSINE (Wt %)</th>
<th>HEMA / HEMA Phosphate</th>
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11.7.1.2. Series two adhesive formulations

Series two resins consisted of UDMA, and TEGDMA in a fixed ratio of 3:1, 4-META at 5 wt %, with CQ and DMPT both at 1 wt %. The filler contained varying quantity of MCPM and β-TCP along with glass fibres, CHX and 2-AEMA. The glass particle used was DMG 7 µm in all formulations. All formulations had powder (filler) / liquid (resin or monomer phase) mass ratio (PLR) of 4:1. The variables used in series two formulations are given in table 11-15.
Table 11-15 Series two variables.

<table>
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<tr>
<th>VARIABLES</th>
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<th>CHX (Wt %)</th>
<th>2-AEMA (Wt %)</th>
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11.7.1.3. Series three adhesive formulations

Series three resins consisted of UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA or HEMA Phosphate at 5 or 10 wt %, with CQ and DMPT both at 1 wt %. The filler contained varying type of CHX (Diacetate vs Base) along with glass fibres, and calcium phosphate. The glass particle used was DMG 7 µm in all formulations. All formulations had powder (filler) / liquid (resin or monomer phase) mass ratio (PLR) of 4:1. The variables used in series three formulations are given in table 11-16.

Table 11-16 Series three variables.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>HEMA / HEMA Phosphate</th>
<th>Wt % HEMA / HEMA Phosphate</th>
<th>CHX Diacetate / CHX Base</th>
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</thead>
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11.7.2. Push out formulations

11.7.2.1. Series one adhesive formulations

Series one adhesive contained two groups of monomer. 1st with UDMA, and TEGDMA in a fixed ratio of 3:1, 4-META at 5 wt %, with CQ and DMPT both at 1 wt %. The filler consisted of high (20 wt %) and low (5 wt %) each of MCPM and β-TCP. The glass fibres and CHX diacetate were at fixed level. The glass particle used was DMG 7 µm. 2nd monomer contained UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA Phosphate at 5 wt %, with CQ and DMPT both at 1 wt %. The filler content was the same as 1st group. The PLR was 4:1 in both groups. The variables given used in series one formulations are given in table 11-17.
Table 11-17 Series one variables.

<table>
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<th>VARIABLES</th>
<th>4-META/HEMA Phosphate</th>
<th>TCP/MCPM (Wt %)</th>
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11.7.2.2. Series two adhesive formulations

Series two adhesive contained four groups of monomer. 1st with UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA at 5 wt %, with CQ and DMPT both at 1 wt %. 2nd with UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA at 10 wt %, with CQ and DMPT both at 1 wt %. 3rd with UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA Phosphate at 5 wt %, with CQ and DMPT both at 1 wt %. And last with UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA Phosphate at 10 wt %, with CQ and DMPT both at 1 wt %. The filler content was the same for all groups and consisted of equal masses of MCPM and β-TCP along with glass fibres and CHX diacetate. The glass particle used was DMG 7 µm. All formulations had powder (filler) / liquid (resin or monomer phase) mass ratio (PLR) of 4:1. The variables given used in series two formulations are given in table 11-18.

Table 11-18 Series two variables.

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<th>Wt % HEMA / HEMA Phosphate</th>
<th>Tx / NO Tx H₃PO₄</th>
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</table>
11.7.2.3. Series three adhesive formulations

Series three monomer consisted of UDMA, and TEGDMA in a fixed ratio of 3:1, HEMA at 5 wt %, with CQ and DMPT both at 1 wt %. The filler contained varying level of polylysine (PLS), and glass fibres. The glass particle used was DMG 7 µm in all formulations. All formulations had powder (filler) / liquid (resin or monomer phase) mass ratio (PLR) of 4:1. The variables given used in series three formulations are given in table 11-19.

Table 11-19 Series three variables.

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<th>GLASS FIBRES (Wt %)</th>
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11.7.3. Results

11.7.3.1. Biaxial flexural strength Results

The strength results of three series of experiment are discussed below:

11.7.3.1.1. Series one results

The strength results showed that:

- The glass particles IF2019 and DMG 7µm were nearly comparable strength of around 170 MPa.
- Polylysine high 5 wt % in the formulation decrease the strength by approximately 10-15 MPa as compared to low 0.5 wt %.
- The addition of adhesive HEMA Phosphate in monomer phase decease the strength of formulations by approximately 20 MPa as compared to HEMA.
- The high percentage of glass particles (IF2019 and DMG 7 µm) causes increase in the modulus of composite formulations.

The biaxial strength, and factorial analysis of series one are shown in figure 11-23, and 11-24 respectively.
Figure 11-23 BFS of series one formulations (n=6).

Figure 11-24 Factorial analysis of series one formulations. $a_1$ is Glass variable with no significant effect. $a_2$ is Polylysine (5 wt% / 0.5 wt%) and $a_3$ is (HEMA / HEMA Phosphate). Strength increases on average by 20 and 12 MPa respectively on raising Factor $a_3$ or reducing Factor $a_2$. 
11.7.3.1.2. Series two results

The strength results showed that:

- High MCPM / TCP 10 wt % in formulations decrease the biaxial strength of formulations by about 15 MPa approx. as compared to 0 wt % in formulations.

- Chlorhexidine high 5 wt % in the formulation decrease the strength by approximately 20 MPa as compared to low 0 wt %.

- The addition of 2-AEMA 5 wt % in filler phase decrease the strength of formulations by approximately 18 MPa as compared to 0 wt % 2-AEMA. The high percentage of glass particle DMG 7 µm causes increase in the modulus of composite formulations.

The biaxial strength, and factorial analysis of series two are shown in figure 11-25, and 11-26 respectively.
Figure 11-25 BFS of series two formulations (n=6).

Figure 11-26 Factorial analysis of series two formulations. $a_1$ is MCPM / TCP, 10 or 0 wt %. $a_2$ is chlorhexidine (5 or 0 wt %) and $a_3$ is 2-AEMA (5 or 0 wt %). Strength decreases on average by 20 and 18 MPa respectively on raising Factor $a_2$ and $a_3$ respectively.
11.7.3.1.3. Series three results

The strength results showed that:

- High level of HEMA and HEMA Phosphate 10 wt % decrease the average strength of formulations by about 8 MPa as compared to 5 wt %.
- There was no significant difference in between chlorhexidine acetate and base on strength.
- The use of adhesive monomers HEMA, and HEMA Phosphate show similar effects on strength. The only drawback of HEMA Phosphate is its phase separation into Phosphate, and HEMA over time. So properly mixing is advised before HEMA Phosphate use.

The biaxial strength, and factorial analysis of series three are shown in figure 11-27, and 11-28 respectively.
Figure 11-27 BFS of series three formulations (n=6).

Figure 11-28 Factorial analysis of series three formulations. $a_1$ is adhesive monomers HEMA, and HEMA Phosphate with no significant effect. $a_2$ is percentage of adhesive monomer (5 or 10 wt %), and $a_3$ is (CHX diacetate or CHX base). Only $a_2$ has slight effect on strength i.e. low percentage of adhesive monomer increase strength.
11.7.3.2. Push out Adhesion test

The adhesion results of three series of experiment are discussed below:

11.7.3.2.1. Series one results

The adhesion results showed that:

- The formulations with 4-META shows good adhesion as compared to HEMA Phosphate. On an average the debonding force increase by 110 N with 4-META.

- High level of MCPM / TCP 20 wt % each decreases the debonding force of adhesion by about 95 N as compared to low level of MCPM / TCP 5 wt %.

- The most significant result was the effect of treatment with 37 % phosphoric acid for 20 s. The adhesion result shows an increase in debonding force by about 155 N with acid treatment.

Push out bond strength, and factorial analysis of series one are shown in figure 11-29, and 11-30 respectively.
Figure 11-29 Push out bond strength of series one formulations (n=6).

Figure 11-30 Factorial analysis of series one formulations along with error bars showing 95% confidence interval. \( a_1 \) is 4-META / HEMA Phosphate, \( a_2 \) is MCPM / TCP (5 or 20 wt%) and \( a_3 \) is treatment / no treatment with 37% phosphoric acid. Adhesion increases on average by 110 N with 4-META, 95 N with 5 wt% MCPM / TCP, and 155 N with phosphoric acid treatment.
11.7.3.2.2. Series two results

The adhesion results showed that:

- The formulations with HEMA, and HEMA Phosphate show nearly similar effects on adhesion and they show no significant effect.
- High level of HEMA / HEMA Phosphate 10 wt %, and low level 5 wt % shows nearly similar debonding force.
- The most significant result was the effect of treatment with 37 % phosphoric acid for 20 sec. The adhesion result shows an increase in debonding force by about 400 N with acid treatment.

Push out bond strength, and factorial analysis of series two are shown in figure 11-31, and 11-32 respectively.
Figure 11-31 Push out bond strength of series two formulations (n=6).

Figure 11-32 Factorial analysis of series two formulations along with error bars showing 95% confidence interval. $a_1$ is HEMA / HEMA Phosphate, $a_2$ is % HEMA / HEMA Phosphate (5 or 10 wt %), and $a_3$ is treatment / no treatment with 37% phosphoric acid. $a_1$ and $a_2$ are not significant. Adhesion increases on average by 400 N with phosphoric acid treatment.
11.7.3.2.3. Series three results

The adhesion results showed that:

- Polylysine in 5 wt % high levels shows good effect on adhesion. The 0.5 wt % polylysine bring down the debonding force by about 100 N as compared to 5 wt % polylysine.
- There was no effect of high and low levels of glass fibres on the adhesion test.
- As mentioned earlier the most significant result was the effect of treatment with 37 % phosphoric acid for 20 s. The adhesion result shows an increase in debonding force with acid treatment. This shows the importance of phosphoric acid treatment before composite restorations.

Push out bond strength, and factorial analysis of series three are shown in figure 11-33, and 11-34 respectively.
Figure 11.33 Push out bond strength of series three formulations (n=6).

Figure 11.34 Factorial analysis of series three formulations along with error bars showing 95 % confidence interval. \(a_1\) is polylysine (0.5 or 5 wt %), \(a_2\) is glass fibres (5 or 20 wt %) and \(a_3\) is Tx / No Tx with 37 % phosphoric acid.
CHAPTER 12

LIST OF CONFERENCE PRESENTATIONS, AND PUBLICATIONS
12. List of Conference Presentations, and Publications


8. S Liaqat., L Bozec., PF Ashley., & AM Young. High-Strength, Low-Shrinkage Composite with Potential to Self-Bond to Dentine. Proceedings of International Association of Dental Research, 93rd IADR/AADR/CADR General Session Boston, Massachusetts, USA, 11-14/03/2015.


