Thesis

DEVELOPMENT OF A NOVEL ANTIBACTERIAL AND REMINERALISING DENTAL COMPOSITE FOR PAEDIATRIC DENTISTRY

Thesis submitted by

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Declaration

I hereby certify that the work embodied in this thesis is the result of my own investigations and work.

Abeer A Alshami

Acknowledgment

My deepest gratitude first goes to my supervisors Dr Paul Ashley and Dr Anne Young for their excellent guidance and invaluable assistance which enable me to complete my thesis successfully. I would also like to thanks, Dr Graham Palmer and Dr Wendy Xia for their help in my experimental works, for which I am much obliged.

Dedication

This work is lovingly dedicated to my parents; for their support, encouragement, and constant love which have sustained me throughout my life. To my sister, for all the support she gave me during these hard years. Finally, my little family, my husband and my son for their unconditional love.

Abstract

The aim of this study was to develop new high strength dental composites with antimicrobial release, using primarily high molecular weight monomers for reduced shrinkage but additional inclusion of components that might promote dentine adhesion and remineralisation.

Urethane dimethacrylate : poly(propylene glycol) dimethacryate (3:1) was mixed with 5 wt% adhesive monomer (2-hydroxyethyl methacrylate or methacrylate phosphate). This was combined with glass particles mixed with chlorhexidine diacetate (CHX, 5 or 10 wt%) and mono and tri calcium phosphate (CaP 10 or 40 wt%) in a powder to liquid ratio of 4:1

Light activated monomer conversion was assessed by FITR. Biaxial flexural strength of set discs (10 mm diameter, 1 mm thick, n=8) was determined after 24 hours in distilled water and compared with a commercial control (Gradia). CHX release (24 hours, 2 weeks, and 4 weeks) was assessed using UV spectrometry.

The monomer conversion of experimental formulations was 71% and 81% with 5 and 10 wt% CHX respectively and not affected by other variables. Gradia conversion was 50%. New material strengths ranged between 130 and 180 MPa being lower with higher CHX and CaP. Varying adhesive monomer type had negligible effect. Gradia strength was 70 MPa. Over 4 weeks, CHX release was 2.9 - 7.1 wt% and mass increase 1.2 - 3.9 wt%.

In conclusion, experimental composites containing antibacterial, remineralising and adhesion promoting components have been produced with flexural strengths comparable with commercial materials and higher monomer conversion.

Key words: composite, restoration, chlorhexidine, HEMA, polymerisation, shrinkage

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List of Abbreviations

BP	Benzoyl peroxide
β-TCP	β-Tricalcium phosphate
BFS	Biaxial flexure strength
CDFF	Constant Depth Film Fermentor
CHX	Chlorhexidine diacetate
CQ	Camphorquinone
Cap	Reactive calcium phosphate (MCPM, β-TCP): nanosilic-
DCPD	Dicalcium phosphate dihydrate (brushite)
DCPA	Dicalcium phosphate anhydrate (monetite)
DMPT	N,N-dimethyl-p-toludine
HEMA	2-Hydroxyethylmethacrylate
HEMA Ph	2-Hydroxyethylmethacrylate phosphate
ISO	International organisation of standardisation
LED	Light emitting diodes
MCPM	Monocalcium phosphate monohydrate
RMGICs	Resin modified glass ionomer cements
MPS	3-Methacryloxypropyltrimethoxysilane
2MP	Bis [2-(methacryloyloxy)ethyl] phosphate
MIC	Minimum inhibitory concentration
TEGDMA	Triethylene glycol dimethacrylate
PLR	powder monomer liquid phase mass ratio
UDMA	Urethane dimethacrylate

Chapter One: Literature Review

1 Introduction

1.1 Caries: an introduction

Dental caries is one of the most common diseases afflicting mankind, and has been estimated to affect up to 40% of UK adults. The UK government spends millions every year on the prevention and treatment of dental caries. Around 175 million pounds has been spent on the placement of direct restoration by the NHS in both England and Wales since the 90's (Dental Practice Board, 1996). Caries adversely affects and progressively destroys the tissues of the tooth, including the dental pulp, leaving teeth unsightly, weakened and with impaired function (Davies *et al*., 2011;Marya *et al*., 2010).

1.1.1 The Epidemiology of Dental Caries

Dental caries is probably one of the most common chronic diseases; it has affected humans since prehistoric times. The prevalence of this disease has increased due to the dietary changes that occurred in human life styles though there is now evidence that this trend has peaked and begun to decline in certain segments of the population of Western Europe, New Zealand, Unites State, and Australia. The main cause of the decrease is the addition of fluoride to toothpastes, and also the addition of trace amounts of fluoride ions to public drinking water. This decline in developed countries has been prominent in the upper classes and middle classes, while the lower socioeconomic and rural classes have retained a high prevalence of tooth decay (Kidd and Fejerskov 2004; Kidd *et al*., 2002).

Dental caries has been studied extensively during the last 60 years in both Europe and North America. Studies have been very useful in determination of the extent of the need for, and effectiveness of, dental treatment. The most common epidemiological measure of caries is the DMF; this is a measure of the number of teeth that are diseased, missing, or filled. Since 1973, national surveys of dental caries in children (one series directed decennially by the Office of Population Censuses and Surveys and another, using analogous methods, coordinated regionally on a regular basis by the British Association for the Study of Community Dentistry) have produced a comprehensive record of trends in caries experience of children in England and Wales (Davies *et al.,* 2011; Kidd *et al*., 2002). Between 1973 and 1993 a decline in caries experience of 55% in primary teeth of 5-year-old children, 75 per cent in permanent teeth of 12-year-old, and 74 per cent in 14-year-old children, was documented.

Data from the NHS Dental Epidemiology Programme for England (British Association for the Study of Community Dentistry) indicates that the mean number of decayed, missing or filled primary teeth (dmft) in 5-years-old in great Britain is 1.11 (2007-2008), and DMFT of permanent teeth in 11 year old in England and Wales is 7.4 (2008 – 2009). In the UK, only 14% of teeth with dentine decay in 5 years old are restored (NHS DEP) and if caries is not treated affected teeth are likely to become non-vital and may require extraction. The number of children who required hospital admissions for caries treatment rose by 66% between 1997 and 2006 (Moles and Ashley 2009). A great part of this failure is due to the difficulties faced by dentists in treating young children.

1.1.2 The Aetiology of Caries

Dental caries is an infectious microbiological disease of the teeth that results in localized dissolution and destruction of the calcified tissues (Kidd and Beighton 1996; Kidd and Fejerskov 2004). The evidence for the role of bacteria in the genesis of caries is overwhelming. Animal and human models have been used in an extensive series of studies. The presence of fermentable carbohydrates (Banerjee *et al*., 2001) such as sucrose, glucose, fructose and cooked starch, and the present of the biofilm on the teeth support the metabolism of acidogenic microorganisms (*mutans streptococci* and *lactobacilli*) (Banerjee *et al*., 2004), results in the release of acidic substances which lead to dissolution of the carbonated hydroxyapatite crystal lattice of enamel, cementum and dentine. The greater the demineralisation, the more cavitations occur on the tooth surface (Featherstone 2000; Kidd 2004). It is now established that the aetiology of caries is no longer as simplistic; it involves the complex interaction of these various factors within the dental plaque biofilm.

1.1.3 The pathogenesis of caries

Demineralisation and remineralisation are dynamic processes affected by both tooth structure and the oral environment. The mineral composition of enamel and dentine, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, is at equilibrium at neutral pH (6-7). Hydroxyapatite, however, will start to dissolve when the pH of the local environment declines below a critical level (for hydroxyapatite ~ pH 5.5) (Dawes 2003). This phenomenon is known as demineralisation and normally occurs every time sugary food is ingested with the presence of bacteria (Pecharki *et al*., 2005)

The demineralisation process, however, is reversed by the buffering effect of hydroxyapatite dissolution products and the presence of sufficient Ca^{2+} and PO_4^{3-} in the surrounding environment. Environmental pH neutralisation above the critical level enhances precipitation of Ca^{2+} and PO_4^{3-} within demineralized tooth structures. This phenomenon is known as remineralisation (Pecharki *et al*., 2005).

The earliest visible sign of enamel caries is the “white spot lesion”. On a smooth surface lesion, it is classically triangular in shape. It follows the direction of the enamel prisms and can be thought of as multiple individual lesions each at different stages of progression. The central traverse, where the lesion is deepest, is the oldest and most advanced part of the lesion where the biofilm is thickest. The shape of the lesion and the activity of the lesion entirely reflect the specific environmental conditions of overlying biofilm.

Occlusally, purely because of the sloping fissure walls and direction of enamel prisms, the lesion assumes an undermining character. This explains why in the advanced lesion, where there appears to be a small hole in the tooth, something apparently so small on the surface can be large when entered with the bur. Following exposure of dentine to the mass of bacteria in the cavity, the dentine is decomposed by the action of acids produced by microorganisms and this is known as the zone of destruction. Underneath this, tubular invasion of bacteria is frequently seen which is called the zone of penetration because the tubules have become penetrated by microorganisms. Beyond this is an area of demineralised dentine which does not yet contain bacteria. When a lesion progresses rapidly, something called the dead tract may form when the odontoblast processes have been destroyed without producing tubular sclerosis (Kidd 1999).

The defence reaction of tubular sclerosis and tertiary dentine formation continue as a response to these destructive processes. Both processes reduce the permeability of the

dentine, although tertiary dentine is less well mineralised than primary or secondary dentine, and contains irregular dentinal tubules (Kidd 1998). At this stage, removal of caries from the cavity and placing a filling to seal the area arrests the progression of the lesion and encourages the two reparative processes. When dead tracts have formed and odontoblast have been destroyed, new odontoblasts can form fibroblasts in the pulp and lay down dentine. If destruction continues, eventually the pulp will become inflamed.

1.1.4 Management of caries

A necessary first step in preventing dental caries in children is to understand the caries risk. Unfortunately dentists often see children when it is too late and caries is established. In primary teeth, enamel cavities or lesions that have progressed into the superficial dentine layer are usually treated by simple restorations involving amalgam, composites or glass ionomer cement. Because the enamel and dentine layers are thinner in primary teeth, the reality is that by the time caries is diagnosed, more invasive operative procedures are often necessary.

1.2 Current Restorations:

1.2.1 Amalgam

Despite the dental material revolutions in the last century, amalgam is still the most widely used dental material in many parts of the world (Berry *et al*., 1998). This material, which consists of mercury and alloy particles of different compositions and form, is cost effective in the management of dental caries, although lacking many of the features of an ideal dental filling material. (Downer *et al*., 1999; Mackert, Jr. and Wahl 2004). Amalgam has been used for over 150 years. The continued use of amalgam, however, has become a controversial topic in recent years with concerns being raised about its safety, particularly in the relation of mercury release during removal, placement carving and, in the longer term, during chewing (Engel 2011; Merchant 2011). There are also concerns regarding the safety of disposing of amalgam capsules after use. There remains however, considerable evidence to support its safety as a dental material, and no apparent evidence to indicate an association with any systemic disease.

Amalgam is an alloy of mercury with other metals. High copper alloys are most commonly used nowadays with 12-13% copper (by weight) in addition to silver, tin and zinc. The high amount of copper helps eliminate the weak gamma 2 phase, which was very corrodible.

Amalgam has many advantages; it is cheap, strong, easy to handle, wear resistant, has low microleakage and good longevity. However, its major limitations include poor aesthetics, inability to bond to tooth structure and mercury hazard to operator, associate staff and possibly to patients.

1.2.2 Composite

Dental composites were developed in the 1960s (Bowen 1963) and represented at the time a big revolution in clinical dentistry materials. They contain filler particles which are usually a type of glass or silicon dioxide - the greater the filler, the better the physical properties, although this has to be balanced by reduction in clinical handling. The improved performances of resin composites have encouraged more clinicians to select resin-based composites for posterior restorations as an alternative to amalgam (Jordan and Suzuki 1991; Ottenga and Mjor 2007; Roulet and Noack 1991; Suzuki and Leinfelder 1993) because the adhesive technology allows limited removal of tooth substance beyond that required to eliminate caries and undermined enamel. Lack of electrical conductivity and elimination of galvanism was a great advantage over amalgam fillings. Composites also incorporate an initiator to initiate polymerisation and this may be mediated by chemical or by light activation (460-480 nm).

The physical, mechanical, and aesthetic properties and the clinical behaviour of composites depend on their structure. Three main components of dental composites are the organic matrix, inorganic fillers accelerator and initiator. The latter two together allow curing to take place in the presence of inhibitors (eg. hydroquinone monomethyl ether). The inhibitors maximise the product storage life and provide colour stability and eliminate the effect of UV light on the amine compounds in the initiator system that might cause discolouration in the medium long term (Del Nero and de la Macorra 1999; Peutzfeldt 1997). The predominant base monomer used in commercial dental composites has been bis-GMA, which due to its high viscosity is mixed with dimethacrylates, such as TEGDMA, UDMA or other monomers. In attempts to have a clear clinical indication for the existing commercial composites, classification criteria were developed, most of them based on filler system. These criteria are primarily based on the amount of inorganic filler fraction in volume per cent or the particular

filler size. Young's modulus of elasticity and intrinsic surface roughness, however, has also been taken into consideration. Composites can be divided into traditional, hybrids containing a mixture of ground glass and microfill particles, and microfill composites. The microfills are further divided into subclasses including a characterization of the type of prepolymerzied resin fillers incorporated. The shape of fillers are taken to account also, since the spherical fillers can be incorporated in higher amount in composite than irregular fillers of the same size and result in higher wear rate (Venhoven *et al*., 1996). It has been proved that microfill composites have the more ideal aesthetic qualities due to their excellent polishability and capacity to retain surface smoothness over time (Venhoven, de Gee, Werner, & Davidson 1996). However, it's also accepted that, due to their poor mechanical properties, these materials are contraindicated for stress-bearing restorations, such as incisal edges and moderate to large stress bearing restorations in occlusal contact with opposing (Ferracane and Mitchem 1994; Fujishima *et al*., 1995). Recently, another classification system was introduced based on the filler volume fracture and filler size, distinguishes between densified composites, microfine composite, miscellaneous composite, and fibre-reinforced composite. The densified composites were subdivided into classes, midway-filled (<60 vol %) and compact-filled (>60 vol %) with classification of ultrafine (<3μm) and fine (>3 μm) within each category as a function of the mean particle size of the filler (Ferracane 2011).

Monomers can be mixed with low level of peroxide initiators and amine activators such as benzoyl peroxide (BP) and dimethyl paratoluidine (DMPT). The methacrylate based composites set via free radical initiated polymerization (Sideridou *et al*., 2008). Alternatively, camphorquinone (CQ) and an amine can be used (Ogunyinka *et al*., 2007;Sideridou et al. 2004a). Blue light (470 nm) exposure is used in this case to activate polymerization. New modern narrow wavelength light sources include blue lasers and light emitting diodes (LED). Levels and rates of polymerisation of methacrylate can be measured using spectroscopic methods such as Raman and Fourier Transform Infrared (FTIR). During light exposure low levels of free radicals are generated. Polymer chains then form which bind the monomers together. For monomethacrylates chains are linear. With dimethacrylate systems all monomers molecules can potentially be bounded within polymer molecules with just 50% methacrylate conversion (Burdick *et al*., 2003). Light curable composite are supplied as single component formulations. This is considered as a major advantage as it eliminates any chance of irreproducibility that might occur during mixing.

1.2.2.1 The organic matrix:

The organic matrix constitutes the body of the composite and is produced by polymerization of dimethacrylate monomers, via a free radical reaction. Along with dimethacryalte monomers, Bis-GMA (BisphenolAdiglycidylmethacrylate), and Urethane dimethacrylate are widely used in formulation of the dental composite. In addition the organic matrix contains diluent monomers and polymerisation initiator and activator systems as it will be mentioned below.

1.2.2.2 Urethane dimethacrylate:

Urethane dimethacrylate (UDMA) (Figure 1-1) 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). These types of intermolecular hydrogen bonds are responsible to some degree for the high viscosity and relatively high glass transition temperature (T_g) of the monomer. This imine group however, produces weaker hydrogen bonds than the hydroxyl group (–OH–) of Bis-GMA (Hoszek *et al*., 2005). Therefore, the viscosity and glass transition temperature (T_g) of UDMA are much lower than Bis-GMA and hence on curing, exhibit higher degree of conversion (Frencken *et al*., 2007).

However, the degree of conversion of UDMA is considerably higher due to the presence of flexible aliphatic chains. The UDMA polymer shows lower water sorption and releases less untreated species compared to Bis-GMA (Karanika-Kouma *et al*. 2001). This has been explained by the lower water affinity of the urethane (–NHCOO–) group of UDMA than the hydroxyl group of Bis-GMA. In addition, UDMA has been proved as less cytotoxic than Bis-GMA, *in vitro (*Vermeersch *et al.,* 2005*a)*.

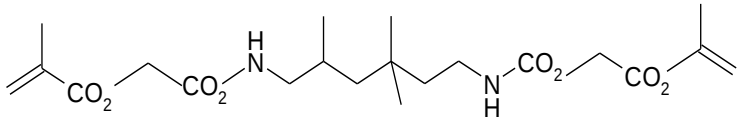


Figure 1-1 The chemical structure of UDMA (molecular weight 470 g mol-1).

1.2.2.2.1 Bisphenol A diglycidyl methacrylate:

Bisphenol A diglycidyl methacrylate (Bis-GMA) was initially used by Bowen and named after him (Bowen's Resin) (Weerheijm *et al*., 1993; Weerheijm *et al*., 1999) (Figure 1-2). It is a high molecular weight monomer, which has two aromatic rings with pendant hydroxyl groups (─OH─). This double rigid aromatic group and hydrogen intermolecular bonding of hydroxyl groups are responsible for the high viscosity of the monomer (Takahashi *et al*., 2006; Botelho 2003). Consequently, the glass transition temperature (T_g) is activated and the degree of monomer conversion will be reduced (Takahashi *et al.,* 2006). Moreover, the presence of pendant hydroxyl groups account for some inevitable water sorption after curing (Palmer *et al*., 2004)

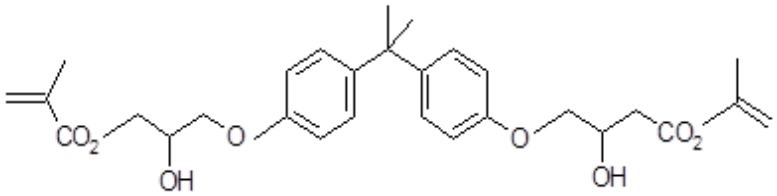


Figure 1-2 The chemical structure of Bis-GMA (molecular weight 510.6 g mol-1)

1.2.2.3 Diluent comonomers:

In order to reduce the high viscosity of base monomers and enhance handling properties of composites, low molecular weight monomers are introduced to the resin phase. The most commonly used diluent monomers are triethylene glycol dimethacrylate (TEGDMA) and 2-hydroxyethylmethacryalte (HEMA)

1.2.2.3.1 Triethylene glycol dimethacrylate (TEGDMA):

Triethylene glycol dimethacrylate (TEGDMA) is an aliphatic and hydrophilic monomer with less viscosity and glass transition temperature (T_g) and higher degree of conversion compared to UDMA and Bis-GMA (Frencken *et al.,* 2007)(Figure 1-3). The water affinity of TEGDMA is mainly attributed to the presence of ether linkages (C-O-C) (Matalon *et al*., 2006).

Although the degree of conversion of resin composites increases upon addition of TEGDMA (Kielbassa *et al*., 2003), polymerisation shrinkage and water sorption (Kielbassa *et al.,* 2003) are adversely affected. TEGDMA is still widely used within most current dental composites regardless of its high polymerisation shrinkage.

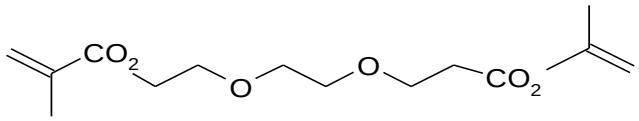


Figure 1-3 The chemical structure of TEGDMA (molecular weight 286.3 g mol-1).

1.2.2.3.2 2-Hydroxyethylmethacryalte (HEMA):

2-Hydroxyethylmethacryalte (HEMA) is an aliphatic low molecular weight monomer, which is commonly used in biomedical applications (Figure 1-4). HEMA is widely used in dental adhesive systems as a solvent and adhesion-promoting agent (Trachtenberg *et al*., 2009). The hydrophilic nature of HEMA makes it attractive for use in the formulation of bioactive dental composites that release remineralising (Dawes 2003) or antibacterial agents to reduce bacterial microleakage. After polymerisation however, poly (HEMA) has high water sorption (Chen *et al*., 2003). This affinity for water is attributed to the presence of the hydroxyl group (─OH) on HEMA molecules.

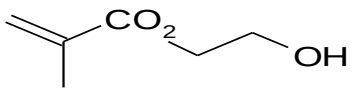


Figure 1-4 The chemical structure of HEMA (molecular weight 130.14 g mol-1).

1.2.2.4 Initiator/ activator system:

In general, dental composite resins are cured through a free radical polymerization reaction where free radicals are generated through two systems: chemically activated and photoactivated.

1.2.2.4.1 The chemically activated system

This system uses benzoyl peroxide (BP) (Figure 1-5) which is activated by a tertiary amine, such as N,N-dimethyl-p-toludine (DMPT) (Figure 1-6). Dental composites cured by this method are supplied as a two paste system, where one of the pastes contains the initiator and the other the activator. When these two pastes are mixed together, free radicals will be formed from the chemical reaction between peroxide-amine systems, to induce the polymerization process (Aiuchi *et al*.,2008).

1.2.2.4.2 Photo activated systems

In the beginning, UV light was used to cure the dental composites, but this provided a limited depth of curing which compromised the composite structure. In addition, UV light exposure can induce tissue damage. It has therefore been replaced with high intensity visible (blue) light sources which are more compatible with living tissue. These include halogen lights (tungsten-quartz) and more recently light emitting diodes (LED) and lasers. Curing of dental composites by either LED or halogen light sources produces comparable degrees of monomer conversion. The emission spectra of LED light units, however match the absorption spectrum of Camphorquinone (CQ) (Goto *et al*., 2006), better than the broader spectrum of the halogen light. Furthermore, LED light sources produce lower temperature

rise within the composite upon polymerisation (Ginebra *et al*., 2006). This can decrease the possibility of thermal stress on pulpal tissues particularly within deeper restorations.

This system works by visible light and Camphorquinone (CQ) (Figure 1-7) as a free radical photoinitiator with a tertiary amine, such as DMPT as co-initiator. The Camphorquinone (CQ) absorbs light between 400-500 nm (maximum at 470 nm) to form an activated state complex with association of the tertiary amine. This complex subsequently breaks down to produce free radicals, which will start the polymerisation reaction.

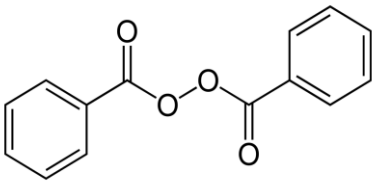


Figure 1-5 BP (C14H10O4) (molecular weight 242.23 g mol-1)

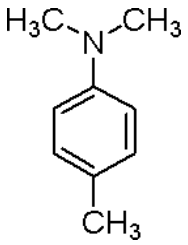


Figure 1-6 DMPT (C9H13N) (molecular weight 135.21 g mol-1)

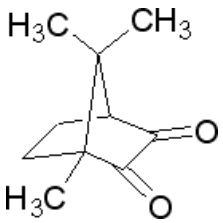


Figure 1-7 CQ (C10H14O2) (molecular weight 166.22 g mo-1)

1.2.2.5 Inorganic filler:

The inorganic filler phase of composites consists of silanated glass, fibres and quartz particles. Ideally, around 80 wt% is incorporated in the resin matrix phase, mainly to enhance physical properties and decrease polymerisation shrinkage (Bohner 2000).

The filler particles are categorised according to their size into macrofillers or microfillers. The macrofillers are obtained through mechanical grinding and milling of larger glass or quartz particles to sizes ranging from 0.2 to 20 μm. The microfillers, such as pyrogenic silica particles are prepared through heat treatment of silicon chloride ($SiCl_4$) and have an average particle size of 0.04 μm.

1.2.2.6 Silane bonding:

The silane coupling agent acts as a bond between the resin matrix and the inorganic filler phase of the composite. This type of adhesion plays a major role and decisive effect on several properties of the dental composites for example;

1.2.2.6.1 Silorane

Silorane is a ring-opening, silicon based monomer which is composed of siloxan and oxirane. The ring opening character of the monomer reduces polymerisation shrinkage below 1% of the overall volume (Figure 1-8).

Several studies have been performed on this monomer and its clinical properties. Palin *et al (*2005), Ilie and Hickel (2006) showed that microleakage was significantly lower for a silorane material than other composites (Z250, 3M ESPE). Al-Boni *et al* (2010) also showed that low shrinkage silorane system had lesser microleakage than other methacrylate resin based composite (Amelogen, Filtek Z250). In another study, Krifka *et al* (2012) found that Filtek

Silorane, a silorane-based composite resin, exhibited the best marginal seal in comparison with methacrylate based composite.

Silanation will enhance the composite resistance to hydrolytic degradation and maintain good mechanical properties (Lee *et al*., 2007; Regnault *et al*., 2008). Besides that, it also improves filler wetting by hydrophobic monomers, which subsequently will enhance the level of filler that can be incorporated within the composite

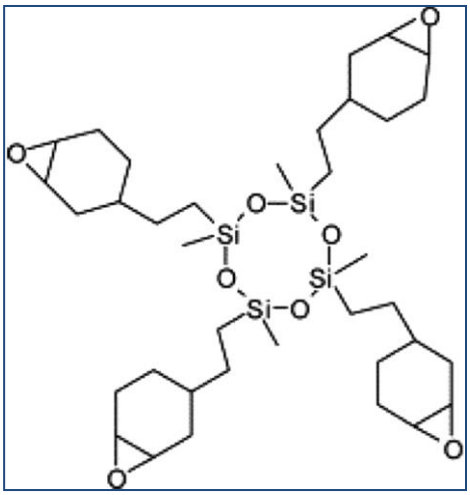


Figure 1-8 the chemical structure of Silorane

1.2.3 Glass Ionomer cements

Glass ionomer cements were first developed in the 1970s and have undergone significant modification since then. Their major advantage over other restorative materials is the fluoride release and the ability to adhere directly to dentine. Disadvantages are poor mechanical properties (Okuyama *et al*., 2006). They have traditionally contained fluoroaluminosilicate glass. This contains aluminium and silica, in addition to fluoride. Other elements include calcium and strontium. The liquid component is polyacrylic acid. Setting occurs by the acid attacking the surface of the glass, forming a gel layer with resultant cross-linking as the material sets (Ab-Ghani *et al*., 2007; Smith 1998). Although the reaction starts fast it can continue at a slow rate for several days (Young *et al*., 2004). It's believed that fluoride release inhibits caries formation. The average fluoride release rate from GIC samples at two days is approximately half that at one day and continues to decline thereafter (Okuyama *et al.,* 2006). Glass ionomers also have the same coefficient of thermal expansion as tooth and minimal setting contraction. Therefore it has been reported that glass ionomers provide a good marginal seal and reduced microleakage.

Water is a crucial component of glass ionomer cements. It provides initial fluidity and enables transport of ions through the polymer matrix but then becomes bound in the polyacrylate salts structures upon setting of the material. It has been found in some formulations that a fixed number of molecules of water are required for each polyacid group reacting and the additional water sorption by set materials from the surroundings may drive the polyacid neutralisation process closer to completion (Young 2002; Young *et al*., 2004).

Glass ionomer is supplied as a separate powder and liquid which the clinician needs to mix. This might lead to reduced mechanical properties (Fleming *et al*., 2003). Alternatively, they

are available in capsules which could improve the reproducibility of the mixing process (De Moor *et al*., 1996)

It is well known that glass ionomer cements have poor mechanical properties compared with other composite and hybrid resin fillings. Their flexural strength is (10-30) MPa which makes them an unsuitable restoration for adult posterior teeth and limits their use a permanent filling. Although recently improved GICs have high compressive strength (100-300) MPa they wear more than composites (Piwowarczyk *et al*., 2002a). According to ISO 9917 for water–based dental cements, all materials should fall between 70 MPa if used as a liner / base or luting material and 130 MPa for restorative cement.

The mechanism of glass ionomer adherence to the tooth surface is a combination of micro-mechanical attachment and chemical bonding (Van Meerbeek *et al*., 2003). The carboxyl group interacts with the calcium ions of hydroxyapatite in both enamel and dentine. Conditioner use enhances the adhesive properties of the GIC. This is typically 10 % polyacrylic acid solution. (Pereira *et al*., 2002). The glass ionomer cement bond is considered more durable. This could be due to the presence of ionic bonds and crosslinks between polyacrylate calcium ions which can break and reform, while covalent bonds in a polymerized methacrylate cannot reform once it breaks (Sidhu *et al*., 2004a; Sidhu *et al*., 2004b; Sidhu 2011).

Fluoride release from glass Ionomer is limited in its effect. There is an initial high burst of fluoride release due to the high concentration of fluoride that exists in the matrix immediately after the setting reaction is complete. During the initial acid dissololution of the powder particle edges, a large amount of fluoride becomes part of the reaction product matrix. This fluoride diffuses quickly from the matrix exposed on the surface of the material and is only slowly replaced by fluoride diffusing from greater distances in the matrix below the surface or

by fluoride diffusing from the particles into the matrix for the first time. Therefore, the long-term fluoride release is at much lower rates.

1.2.4 Resin adhesives

Adhesive materials are non-filler monomers, which enhance the adhesion procedure between the tooth surface and composite filling. However, the strength of adhesion varies from enamel to dentine due to the differences in their structures. The unit of structure in dental enamel is hydroxyapatite crystals which make the bonding between methacrylate and hydroxyapatite easier, more straightforward and more reproducible. In generally, this involves acid etching with 37% of phosphoric acid gel for thirty seconds. Rinsing and drying will leave a microscopic rough surface enhancing methacrylate monomers to penetrate into it. Upon polymerisation with the light cure, a micromechanical bond will be formed (Van Landuyt *et al*., 2007).

Dentine hydrophilicity is considered as a challenge in adhesion and bonding mechanisms due to dentine structure which consists of water (10%), collagen (20%) and hydroxyapatite (70%) (Perdigao *et al*. v n, 2013). During tooth preparation, cutting of the tooth structure with a rotary instrument, a smear layer will be formed and by etching with 37% of phosphoric acid for 10-20 seconds and rinsing this layer will be removed. This etching process will also dissolve the surface hydroxyapatite from dentine inner surface. Methacrylate primer is therefore often applied to acid etched dentine prior to a low viscosity resin adhesive. Lowering the viscosity of fluid will improve its ability to spread over and penetrate dentinal tubules (De *et al*., 2005a;Van *et al*., 2005). In general, the solvent may be water, ethanol and/or acetone and it must be eliminated by air-drying before monomer polymerisation. Beside the solvent the primer also contain adhesion promoting monomers / polymers with acidic phosphate and / or carboxylate group.

Adhesive resins systems have been divided into:

1) three step technique etch and rinse as above. This is the most commonly employed technique by clinicians because of formation of a hybrid layer of intertwined collagen and polymethacrylates

2) two step etch and rinse,

3) two steps without rinsing (self-etch)

4) one step (self-etch) (Van Meerbeek *et al*., 2003). With self-etch resins phosphoric acid is not used. Instead the resin contains acidic monomers or polymers. These types of adhesive are not rinsed after application but chelate with calcium in hydroxyapatite to form an ionic bond similar to that in GICs.

The most commonly used methods for assessing dental adhesive bond strength include shear (Piwowarczyk *et al*., 2007) and micro tensile tests (De *et al*., 2005; Van Meerbeek *et al*., 2003). Results produced, however, have been highly variable for a wide number of reasons and are rarely comparable between groups. In general, bond strength to dentine has a significant reduction upon reducing the number of stages and steps (De *et al*., 2005)

1.2.5 Hybrid restorative materials

GICs have significant problems. In order to overcome these, researchers developed hybrid materials. These were a combination of conventional GICs and composites and can be divided into compomers (or acid modified composite) and resin modified glass ionomer cements (RMGICs)

1.2.5.1 Resin modified glass ionomer cements

RMGIC bond properties to enamel and dentine are higher than those of conventional GICs (Inoue *et al*., 2003; Palmer *et al*., 1999). This type of restoration has hydrophilic properties due to the presence of water in its composition and hydroxyl ethyl methacrylate (Smith 1998). Resin modified glass ionomer cements have the advantage of being able to set in moist environments but improper mixing of the liquid and powder components together can lead to diffusion of HEMA molecules. This will raise the concerns of the amount of HEMA which could be used in dental restoration because of the higher potential of allergy (Lan *et al*. 2003; Michelsen *et al*., 2003; Mine *et al*., 2008; Palmer *et al.,* 1999). Therefore, the manufacturers supply resin modified glass ionomer cements (RMGIC) in capsules. (Lan *et al*., 2003; Mine *et al.,* 2008). RMGIC has a slower reaction than conventional glass ionomer cements due to the use of more compatible fillers which reduce its reactivity and by adding a silane coupling agent which leads to the polyacid reaction occurring after polymerisation (Young *et al*., 2004).

A further problem with early RMGICs was excessive water sorption due to the hydrophilicity of (HEMA) leading to pressure on and potentially cracking of the tooth (Smith 1998). In more modern materials water sorption has been better controlled (Cefaly *et al.,* 2006). Moreover, some RMGICs contain chemical cure initiators that ensure regions not exposed to light are fully polymerised soon after formulation mixing (eg potassium persulphate and ascorbic acid

in Vitremer). Modern RMGICs achieve better controlled water sorption by greater monomer crosslinking caused by raised polymerisation and dimethacrylate inclusion (Cefaly *et al.,* 2006)

In more recent studies, further enhancement of RMGIC mechanical properties has been gained through the development of multi-arm polyacids with several attached methacrylate groups. These would increase crosslinking even more (Xie *et al.,* 2007). Ideally sufficient water should be absorbed (~ 3 vol%) to just enable the associated material expansion (Sidhu *et al.,* 2004) to overcome the polymerisation shrinkage. Additionally this water sorption should be sufficient to drive the polyacid neutralisation process to completion (Young *et al*., 2004). Regarding fluoride release from modern resin modified glass ionomer cements; early fluoride release can be comparable to that of conventional glass ionomer cement. This may be due to an increase of the glass fluoride content (Xu and Burgess 2003a). Although RMGIC couldn't replace amalgam and composite filling in posterior permanent teeth; they have been proven in primary molars (Qvist *et al*., 2004)

1.2.5.2 Compomers (acid- modified composite)

In many studies it has been shown that compomers have lower flexural strength than composites (Piwowarczyk *et al.,* 2002; Chung *et al*., 2004; Gomec *et al*., 2005; Janda *et al*., 2006). According to the ISO 4049 (polymer- based restorative materials) compomers have sufficient strength to be used as restorative materials but still there are concerns due to the declination of the flexural strength and mechanical properties with time (Rodrigues Filho *et al*., 2006). Despite the significant increase in strength in the first twenty four hours due to water sorption (Piwowarczyk *et al*., 2002) and continuing polymerisation (Young *et al*., 2004), compomers flexural strength is lower than the clinician's expectation (Meyer *et al.*

1998; Nicholson 2007; Piwowarczyk *et al.,* 2002). According to the ISO 1491 requirement compomers strength are within the acceptable level (120- 260 MPa) but as it is mentioned above compomers are lower than composite strength wise (Gomec *et al.,* 2003)

Compomers are similar to composites but have more monomers/polymers with acidic chemical groups which will increase the water sorption and then react with additional inorganic particulate phase that contains the fluoride particle (Meyer *et al.,* 1998; Nicholson 2007). Water sorption by compomers is higher than in composites. Swelling may compensate the shrinkage during polymerisation (Huang *et al*., 2002) but water sorption is not enough to enhance the high level of acid / glass reaction in the bulk of the material (Young *et al*., 2004). Manufacturers supply it as a paste which contains monomer / polymers and it is polymerised by using light activation.

Compomers release fluoride in comparable amount with conventional glass ionomer cements (Meyer *et al.*,1998), however, in the early stages its release is quarter the amount of fluoride release from the conventional glass ionomer cement.

1.3 Current Problems with current aesthetic restoration

1.3.1 Composite

Di-methacrylate-based resin composites still demonstrate some negative or questionable aspects: wear resistance, surface roughness, handling property, proximal contact adhesion and contouring or sculpturing, marginal adaptation and polymerisation shrinkage (Inoue *et al.,* 2003; Kidd & Beighton 1996; Peutzfeldt and Asmussen 1996). The excessive wear loss of composite fillings could be noticeable under the enamel margins, or proximal contact in class II restorations, which may lead to open proximal contacts (Piwowarczyk *et al.,* 2002;

Rodolpho *et al*., 2006). This phenomenon may arise from a combination of factors, including polymer or filler composite, filler size, and filler-polymer matrix binding quality, especially in early resin composite systems (Kusy and Leinfelder 1977).

Polymerisation shrinkage is an inherent property of resin restorative materials. During polymerisation, the formation of the polymer network results in formation of a denser structure, causing changes in volume which lead to shrinkage (Bowen 1963) – this causes microleakage Microleakage may be defined as the passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it (Kidd 1976).

Many laboratory techniques have been developed to study marginal permeability at the interface between tooth and restoration. These include the use of dyes, radioactive isotopes, air pressure, bacteria, neutron activation analysis and artificial caries techniques. The results of these studies emphasise that margins of restorations are not fixed, inert and impenetrable borders, but 'dynamic micro-crevices' which contain a busy traffic of ions and molecules.

When monomers in proximity react to establish a covalent bond, the distance between the two groups of atoms is reduced and there is a reduction in free volume, both of which translate into volumetric shrinkage. The magnitude of volumetric shrinkage experienced by a composite is determined by its filler volume fraction and the composition and degree of conversion of the resin matrix. Shrinkage values reported for BisGMA (5.2%) and TEGDMA (12.5%) are substantially higher than those displayed by typical composites, which range between 2 and 3%. This difference is due to the fact that in hybrid composites, approximately 60% of the volume is occupied by filler particles. Microfilled composites, though their inorganic content is typically about 40 vol%, have shrinkage values similar to hybrids, due to the presence of pre-polymerized composite particles, sometimes referred to

as ‘organic fillers’, which render them similar to hybrid composites in terms of the actual volume fraction of polymerising resin.

1.3.2 Glass ionomer cement

In clinical investigations the 50% survival time for GICs in primary teeth was found to be less than 4 years on average. Use of GICs instead of amalgam, however, significantly reduced progression of caries on adjacent teeth (Qvist *et al*., 2004b; Qvist et al., 2004c). In class V restorations (gingival and non-load bearing) average annual failure rates for GICs were better than obtained with resin adhesive (De *et al.,* 2005; Peumans *et al*., 2005). In contrast, there is strong evidence suggesting that the use of GICs as a liner beneath a composite may prevent recurrent caries (Rodolpho *et al.,* 2006). Fluoride– releasing, tooth adhesive GICs therefore play a major role in restorative dentistry but their use could be greatly extended if their mechanical properties strength could be raised.

1.3.3 Resin Modified Glass Ionomer Cement

One main potential problem of RMGICs is low surface hardness and wear resistance (Peutzfeldt 1997; Xie *et al*., 2000). This may be primarily due to the low level of crosslinking arising with use of high levels of monomethacrylates. Additionally, the fast polymerisation kinetics designed to overcome HEMA toxicity concerns may have resulted in more but shorter, faster - wearing polymer molecules. Furthermore, reaction of the glass particles may interfere with the silane bond between the matrix and filler phase. A further problem with early RMGICs could be excessive water sorption due to the hydrophilicity of poly (HEMA) leading to pressure on and potentially cracking of the tooth (Smith 1998).

1.3.4 Compomers

Compomer flexural strengths and moduli at 24 hours are typically lower than those of composites (Chung *et al*., 2005; Janda *et al.,* 2006; Piwowarczyk *et al.,* 2002). Although compomer mechanical properties can meet the ISO 4049 requirements they tend to decline more with time than those of composites (Rodrigues *et al*., 2006). This is most likely due to higher water sorption and fluoride release of the compomers. Compressive strengths (120-260 MPa) of compomers have also been found to be lower than those of composites (Gomec *et al*., 2005; Xu and Burgess 2003).

1.4 History of restorations with antibacterial properties

1.4.1 Introduction

Dental biofilm can be established on any surface intra-orally, that could be on the tooth or restoration surface. This biofilm can initiate recurrent caries at the interface zone in resin restoration. Therefore, it was well observed that composite resin compared to other restoration has the higher level of cariogenic biofilms (Beyth *et al.,* 2007; Moura *et al.,* 2004; Svanberg *et al.,* 1990; Vermeersch *et al*., 2005). This may be explained by the limitation of antibacterial properties in the current dental composite used. In addition some studies have proven that some resin monomers act as a perfect media for cariogenic bacteria to grow (Hansel *et al*., 1998)

In order to be able to assess the antibacterial effect of a dental restoration, a measurement of minimum inhibitory concentration (MIC) is used. It is one of the mostly common used methods (Musanje *et al*., 2001). Direct contact and agar diffusion tests are also commonly used because this test is readily available, inexpensive and widely approved as a simple screening method (Slutzky *et al.,* 2006; Tobias *et al.,* 1988). Usually it has been used in evaluation of the antibacterial activity of materials that release water-soluble components in the surrounding medium. It is also used to quantify the ability of materials to inhibit the growth of bacteria facing the contact surface. In agar diffusion; the materials are inserted onto agar plates which have been inoculated with intraoral bacteria, mainly *stretoccocus* and *lactobacillus* species.

Another test introduced to measure the activity of biofilms in dental restoration is the Constant Depth Film Fermentor (CDFF). This test was originally developed to mimic the media in oral cavity by growing microbial biofilm in the laboratory, under controlled

conditions. This model is particularly suitable for studying antibacterial properties of restoration materials as it enables biofilms similar to those in the oral cavity to be grown (Wilson 1996)

Several studies have been carried out to assess the antibacterial activity of dental restorative materials, mostly through the agar diffusion assay, direct contact tests (Matalon *et al.,* 2004; Slutzky *et al.,* 2006; Tobias 1988), measurement of minimum inhibitory concentration (MIC) (Imazato *et al*., 2002) and inhibition of bacterial growth in biofilm models such as the Constant Depth Film Fermentor (CDFF) (Leung *et al*. 2005; Mehdawi *et al*., 2013)

In the last ten years, most have been done on antimicrobial aesthetic restorations which have the ability to inhibit the bacterial growth in different methods, which could be by adding direct antimicrobial agent or indirect addition. These techniques will be reviewed in the next part of this chapter which will include also the mode of action of different antibacterial actions and chemical components.

1.4.2 Composites with antibacterial properties

Different antibacterial agents, such as chlorhexidine, triclosan, benzalkonium chloride (BAC) and fluoride, have been introduced in both commercial and experimental dental composites.

1.4.2.1 Chlorhexidine:

Chlorhexidine is considered as a potent antimicrobial agent, which is commonly used in dentistry, mainly in periodontal diseases. Subsequently, chlorhexidine was introduced to resin restorations such as composites, GIC and adhesives. The mechanical and antibacterial properties of commercial dental composites containing various chlorhexidine salts have been evaluated (Jedrychowski *et al.,* 1983). Formulations incorporating either chlorhexidine gluconate or chlorhexidine dihydrochloride inhibited the growth of tested bacterial strains, in an agar diffusion assay, for up to four days. Whereas chlorhexidine gluconate reduced mechanical properties, chlorhexidine dihydrochloride could be added at levels of 10 % without an observable effect.

Experimental dental composites, containing hydrophilic monomer (HEMA) with 10 wt% chlorhexidine diacetate added have been formulated (Wilson and Wilson 1993). With these materials, the degree of conversion increased significantly upon addition of chlorhexidine (Leung *et al.* 2005). In this study, it was observed that the polymerisation rate decreased upon raising HEMA wt%, whereas the rate of chlorhexidine release and volumetric expansion increased. Composites containing 50 or 90 % HEMA / chlorhexidine in the monomer phase both had reduced oral biofilm growth on their surfaces up to one week in a CDFF when compared with controls without chlorhexidine or commercial fluoride - releasing materials. After 10 weeks in a CDFF, bacterial penetration into a bovine dentine cylinder restored by these experimental composites was also substantially reduced when compared with a commercial composite.

In one contrary study, a dental composite containing chlorhexidine exhibited no antibacterial effect when tested against *S. mutans* in an agar diffusion assay or direct contact test. The release of chlorhexidine, however, was marginally close to its minimum inhibitory concentration. This might be due to the use of chlorhexidine base which has lower aqueous solubility when compared with chlorhexidine salts (e.g: diacetate and gluconate). Additionally, with use of conventional hydrophobic composite formulations there may have limited water sorption. This may be required to plasticise regions to enable faster drug diffusion through polymer chains.

Antibacterial properties of composites often rely upon water sorption to promote release of an antibacterial agent. This strategy can produce effective antibacterial composites, which suppress bacterial growth in both planktonic culture (Jedrychowski, Caputo, & Kerper 1983) and a biofilm model. Water sorption and active drug release, however, often lead to deterioration of mechanical properties (Jedrychowski *et al.*, 1983). Furthermore, release of soluble antibacterial from resin matrix may be characterized by high initial release, followed by rapid drug release rate decline (Wilson & Wilson 1993). Therefore, new approaches and techniques have to be developed for the production of an effective antibacterial restorative material.

1.4.2.2 Triclosan

Triclosan (2,4,4-trichloro-2-hydroxidiphenilethere) (Figure1-10) is considered as a wide spectrum antibacterial agent (Kolenbrander 2000) which has the ability to inhibit bacterial growth by interrupting their enzymatic activities (Wierzbicka *et al.,* 1987). It is a widely used antibacterial agent within toothpastes, detergents and cosmetic products.

Triclosan was initially promoted as an antibacterial component of toothpaste. Subsequently, it was introduced into commercial dental composites and its anti-bacterial activity was investigated against *lactobacilli* (Badet and Thebaud 2008). This study showed that a composite containing 1 wt% triclosan has a significant inhibition of bacterial growth when a direct contact test was used (Badet & Thebaud 2008). On the other hand, another study on a dental composite containing 0.3% triclosan, no antibacterial activity against the same bacteria, either in an agar diffusion assay or in a direct contact test was observed. In this study it was proposed that triclosan is released at levels below its minimum inhibitory concentration due to its limited solubility in water (Sehgal *et al.,* 2007).

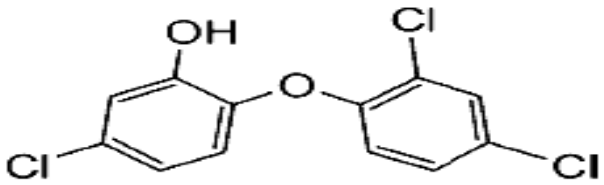


Figure 1-9 Triclosan chemical structure (C12H7Cl3O3) (molecular weight 289.5 g mol-1)

1.4.2.3 Benzalkonium chloride

Benzalkonium chloride (BAC) is a wide spectrum quaternary ammonium antibacterial agent (see Figure 1-10), which acts as antibacterial agent with the ability to induce the antibacterial action through attraction to the negatively charged bacterial membrane (Allmyr *et al*., 2006) (Beyth *et al.*, 2007; Sehgal *et al.,* 2007). BAC is broadly used as a preservative in ophthalmic solution and nasal spray. Its effect on the antibacterial and mechanical properties of dental restorative materials (mainly in light cured dental composite) has been evaluated at levels of 0.25%, 0.75%, 1.25%, 1.75%, 2.5%, and 5% (wt) (Saito *et al*., 2007). It was reported that this type of antibacterial agent has a significant effect as an inhibitor against *S. mutans* and *S.sorbinus*, in an agar diffusion assay and direct contact test (Saito *et al.,* 2007). This antibacterial activity was enhanced upon raising benzalkonium chloride content. The mechanical properties of the composite were not affected by incorporation of benzalkonium chloride (Othman *et al*., 2002).

In contrast, one study has shown no significant effect of BAC on Escherichia coli and Staphylococcus aureus. (Khajavi *et al*., 2007)

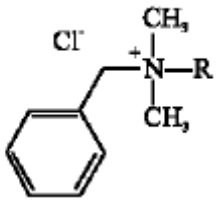


Figure 1-10 Chemical structure of benzalkonium chloride (BAC) (C22H40ClN)(molecular weight 339.99 g mol-1) .

1.4.2.4 Fluoride - releasing dental composites:

In order to develop a new fluoride releasing composite, different techniques have been developed in the last decade. These include incorporation as inorganic water-soluble salts, addition to glass fillers, bonding to a resin component and as an organic fluoride salt (Han *et al.,* 2002; Dionysopoulos *et al*., 2013). Fluoride is well known as an ion suitable for tooth remineralisation enhancement and this will increase resistance to demineralisation by cariogenic bacteria (Bowden 1990; Han et al. 2002; Itota *et al*. 2002; Kameyama *et al*. 2002). The inorganic water soluble salts, sodium fluoride (NaF) and strontium fluoride (SrF_2) added to the resin phase of a composite (Sales *et al*., 2003) are washed out easily leaving porous structures and reduced mechanical properties (Arends and van der Zee 1990; Rawls 1987; Xu and Burgess 2003). Conversely, ytterbium trifluoride (YF_3) and fluoride - leaching glass fillers have provided composites with adequate mechanical properties despite release of fluoride (Boeckh *et al.,* 2002; Itota *et al.,* 2002)

Fluoride - releasing monomers based on acrylic-amine-hydrofluoride salts have been incorporated into the resin phase of dental composites (Rawls 1987). These monomers

released fluoride through an ion exchange mechanism, which, it was proposed, might have less effect on mechanical properties (Ling *et al.,* 2009).

New fluoride-releasing monomers based on a dimethacrylate monomer and ternary zirconium fluoride chelate were introduced; however, formulation of a dental composite with sustained fluoride release in addition to good mechanical properties is difficult (Ling *et al*., 2009). The success of developing dental composites with sustained fluoride release is considerably less compared with the percentage that has been released from glass ionomer and compomer (Boeckh *et al*., 2002; Bowden 1990; *Itota et al.,* 2002)

Furthermore, the amount of fluoride released from dental composites has generally been insufficient to produce significant antibacterial action. For example, even a dental composite containing 15.6% ytterbium trifluoride did not affect the growth of *S. mutans* in an agar diffusion test (Bapna *et al*., 1988).

1.4.2.5 Silver - containing dental composites:

Silver has been added to the filler phase in composite due to it is potent antibacterial efficacy (Ryu *et al.,* 2012) and its high biocompatibility (Kang *et al.,* 2009; Park *et al*., 2009). Dental composite containing silver showed a bacteriostatic effect against three strains of oral streptococci *(Streptococcus oralis, Streptococcus sanguinis and Streptococcus mitio)*

Silver has been applied widely as an antibacterial coating or filler for surgical catheters , bone cements and wound dressings (Alt *et al.,* 2004; Rupp *et al*., 2004) The bactericidal effect of silver ions has been attributed to their interference with bacterial enzymatic activities (Jelinek *et al.,* 2013; Liu *et al*., 2013). Silver - containing glass fillers have been shown to exhibit antibacterial effects against three strains of oral streptococci (*Streptococcus oralis, Streptococcus sanguinis* and *Streptococcus mitior*) that are commonly isolated from the surfaces of dental composites (Lansdown 2002; Yamamoto *et al.*,1996).

One disadvantage of using silver as an antibacterial agent in dental resins is the colour stability (van der Burgt and Plasschaert 1985). Those containing silver zeolite for example exhibited extensive discoloration following 24 h in a storage solution. The composite containing 10% silver-apatite, however exhibited better colour stability.

The incorporation of other silver compounds such as silver-zirconium phosphate or silver-silica gel into dental composites has also been reported (Syafiuddin *et al.,*1997). Dental composites containing either 5 % silver-zirconium phosphate or 7 % silver-silica gel inhibited the growth of *S. mutans (*Yamamoto *et al.,*1996*)*

1.4.2.6 Antibacterial pre-polymerized resin fillers:

The antibacterial property of a filler system consisting of pre-polymerised resin fillers with immobilized MDPB (PPRF) have been investigated (Imazato *et al.,* 2003). This filler system contained milled pre-polymerised methacrylate and antibacterial MDPB monomers (at 15.8 wt%) with glass silica particles. After 18 h contact with this filler growth of *S. mutans* was completely inhibited. The authors reported some elution of unpolymerised MDPB (~ 1 µg/ml) following 24 h immersion in water but since this is approximately 16 fold less than the MIC of MDPB against *S. mutans* , direct contact between particles and bacteria was the most likely mode of antibacterial action.

In further studies, an experimental dental composite with 17.9 wt% of the above PPRF, suppressed accumulation of *S. mutans in vitro* (Ebi *et al*., 2001). This was attributed to interference with bacterial adhesion, glucan synthesis and bacterial growth. In addition, the authors reported no elution of unpolymerised MDPB. Furthermore, incorporation of this filler had no effect on either surface roughness or hydrophobicity, which is known as contributing factors affecting adhesion of bacterial biofilms to surfaces.

1.4.3 Dental adhesives with antibacterial properties

1.4.3.1 Introduction

Incomplete infiltration into the demineralized collagen network within the hybrid layer is a major problem with current dental adhesives (Yuan *et al.,* 2007). This will lead to what is known as nanoleakage (Sano *et al.,* 1995). It increases penetration of fluids and bacterial byproducts with subsequent degradation of the resin adhesive (Wang and Spencer 2003) and collagen fibrils (Pashley *et al*., 2004). Ultimately, this will lead to deterioration of bond strength (Koshiro *et al.,* 2004), and subsequently increase the risk of microleakage and secondary caries (Banerjee *et al*., 2001; Banerjee *et al.,* 2000).

Another problem reported with adhesive systems can be incorporation of a smear layer that harbours bacteria. One solution could be a dentine-bonding agent with antibacterial activity. Inclusion of specific components, such as glutaraldehyde and acidic comonomers that can provide the dental adhesives with some antibacterial activity as discussed below

1.4.3.2 Glutaraldehyde:

Glutaraldehyde (Figure 1-12) is a well - known fixative agent with effective disinfectant properties (McDonnell and Russell 1999) that is frequently used for tissue fixation and disinfection of medical and dental equipment. Glutaraldehyde was initially introduced into dental adhesives to maintain the integrity of collagen fibrils within the hybrid layer. This was expected to promote bond strength (Cilli *et al*., 2009) and minimise the risk of postoperative hypersensitivity (Chermont *et al.,* 2010). The maximum level of glutaraldehyde added to dental adhesives is usually 5 %.

Figure 1-11 Chemical structure of glutaraldehyde (C5H8O2) (molecular weight 100.12 mol-1)

One *in vivo* study showed that dental adhesives containing glutaraldehyde could eliminate bacteria from dentinal tubules (Yu *et al.,* 2010). Furthermore, cured dental adhesives containing glutaraldehyde showed strong inhibition against a variety of bacteria including known cariogenic species, *S. mutans*, *Streptoccocus sorbinus, L.casei* and *A. viscosus* (Cilli *et al*., 2009; *Yu et al*., 2010). In the former studies, since an agar diffusion assay was used, the antibacterial activity was induced through release of glutaraldehyde. Glutaraldehyde, however, is known to induce toxic and mutagenic effects, which has given major concern regarding its use in clinical applications (Takigawa and Endo 2006)

1.4.3.3 Acidic co-monomers:

Co-monomers containing phosphoric and carboxylic acid groups are incorporated into primers of self-etching adhesive systems, mainly to help in dentine demineralisation and facilitate resin infiltration in the absence of an additional etching step (Tay and Pashley 2001). Some studies have shown that un-cured self-etching primers have antibacterial activity (Elsaka and Elnaghy 2012). Using agar diffusion assay, the growth of *S. mutans* and *A. viscosus* was inhibted by self-etching primers (Cehreli *et al*., 2003; Kitasako *et al*., 2004) . These authors reported complete elimination of *S. mutans* after 30 seconds contact with self-etching primers. Using a model cavity test and an agar diffusion assay, a self-etching

primer solution was also observed to inhibit growth of *S. mutans*. This antibacterial activity was attributed to lower pH values of un-cured self-etching primers (Kuramoto *et al.,* 2005; Ye *et al.,* 2007).

This immediate antibacterial property of un-cured self-etching adhesives could contribute to eradication of residual bacteria in the cavity. This antibacterial activity however, can be significantly reduced after light curing or buffering by dentinal fluid (Ye *et al.,* 2007).

1.4.3.4 Antibiotics:

Incorporation of antibiotics into dentine bonding agents has also been tried. Vancomycine or metronidazole has been added to the dentine bonding system based on 4-META/MMA-TBB (4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane) (Kudou *et al*., 2000). The effect of adding vancomycine at 1, 2, and 5 wt% and metronidazole at 1 wt% on inhibition of six bacterial strains and tensile bond strength to dentine were investigated. The authors reported that resin adhesive containing vancomycine at 1-5 wt% was effective in inhibition of the growth of all tested streptococci and actinomyces strains in an agar diffusion assay. The adhesive resin with 1 wt% metronidazole however, exhibited milder antibacterial activity, which was limited to three bacterial strains. Furthermore, tensile bond strength of the bonding agent was not affected by addition of either vancomycine or metronidazole. The lower release of antibiotics however, may promote the development of resistant bacterial strains. Therefore, antibiotic incorporation is not an ideal strategy to develop dental adhesives with antibacterial activity.

1.4.3.5 Fluoride:

Several fluoride releasing dentine adhesives are available for clinical application (Han *et al.* 2002a;Han *et al*., 2002b; Kameyama *et al.,* 2002b; Okuyama *et al.,* 2006). These adhesives released fluoride to restoration margins and the hybrid layer. The fluoride releasing adhesives may inhibit development of secondary caries through enhancing tooth resistance to demineralization (Dubroc *et al*. 1994; Xu & Burgess 2003). Some reports, however, have indicated that fluoride - releasing adhesives have limited ability to inhibit secondary caries (Bapna *et al*.,1988; Marya *et al.,* 2010) or maintain bond strength to dentine.

1.4.3.6 Chlorhexidine

Deterioration of bond strength between adhesives and dentine has been partially attributed to enzymatic degradation of demineralised collagen fibrils, which are unprotected by adhesive resin. This collagenolytic activity is mediated through endogenous enzymes known as matrix metalloproteinases (MMPs) (Gendron *et al*., 1999). Chlorhexidine has been reported to act as an inhibitor of MMPs (Van *et al*., 2003) and thereby helps maintain the integrity of the hybrid layer (Carrilho *et al*., 2007; Hebling *et al*., 2005). This stabilising effect of chlorhexidine on the hybrid layer has promoted many studies of chlorhexidine – containing dental adhesives (Carrilho *et al.,* 2007). One *in vivo* study showed that surface treatment of dentine with 2% chlorhexidine solution could preserve the bond strength of dental adhesives for up to 14 months (Hebling *et al.,* 2005). In a further *in vitro* study, similar chlorhexidine pre-treatment was found to decrease the deterioration in composite bond strength after 6 months storage in artificial saliva (Carrilho *et al*., 2007). In one recent study, a dental adhesive system used in association with either 0.2 or 2 wt% of chlorhexidine digluconate, showed less decline in bond strength following 6 months storage using *in vivo* like conditions (Carrilho *et al*., 2007; Stanislawczuk *et al*., 2009; Zhou *et al*., 2009).

The preservation effect of chlorhexidine on the marginal seal of dental composites is considered as a significant property that should further promote use of this antibacterial agent in developing antibacterial restorative materials.

1.5 Demineralisation and remineralisation of tooth structure:

The carious process results from the loss of normal equilibrium between remineralisation and demineralisation and an overall increase in the latter. Therefore, providing extra sources of calcium and phosphate in the oral environment may help increase the process of tooth remineralisation.

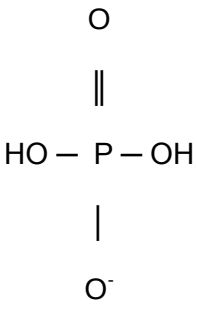
1.5.1 Calcium phosphates:

Calcium phosphates include various salts of tribasic phosphoric acid (H_3PO_4). $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-} ions can all be formed through progressive removal of H^+ ions from this acid. Their natural occurrence in skeletal tissues and teeth makes them of particular interest to both clinicians and biomedical scientists. These compounds are highly biocompatible and osteoconductive materials (Goto *et al.,* 2006) and widely used as bone substitutes and as carriers for drug delivery (Ginebra *et al.,* 2006).

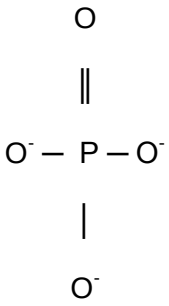
Several calcium phosphate species are known to dissolve in neutral or basic solution and re-precipitate as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$ which is similar in structure to apatite found in bone and teeth. The solubility of calcium phosphate phases in aqueous solution is an important property and mainly correlated with the calcium (Ca) / phosphorous (P) ratio. Generally the higher the Ca/P ratio, the lower the solubility. At physiological pH, the solubility of calcium phosphate species for example decreases in the order MCPM > DCPD = DCPA > OCP > β-TCP > HA (Table 1-1) (Figure 1-13)

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

Table 1-1 The main Calcium phosphates arranged according to Calcium (Ca) and phosphorus (P) ratio (Bohner *et al*., 2006)



(a) MCPM anion ($Ca(H_2PO_4)_2.H_2O$) (molecular weight 252 g mol^{-1}).



(b) β-TCP anion ($Ca_3(PO_4)_2$) (molecular weight 310 g mol^{-1}).

Figure 1-12 (a) and (b): The chemical structure of MCPM and β-TCP.

1.5.1.1 Calcium phosphate dental composites:

In order to formulate restorative materials with remineralising activity, many studies have incorporated soluble calcium phosphate species within dental monomers. If these phosphate fillers are more soluble than hydroxyapatite they can be released from the set resin and re-precipitate within a tooth lesion.

Amorphous calcium phosphate (ACP) in particular, has been extensively studied. Additionally, composites containing dicalcium phosphate anhydrate (DCPA), tetracalcium

phosphate (TetCP) and monocalcium phosphate monohydrate (MCPM) have been investigated.

1.5.1.2 Amorphous calcium phosphate:

Amorphous calcium phosphate (ACP) has been incorporated up to 40%, in various methacrylate dental monomers. Upon immersion in water, the set ACP composites release calcium and phosphate that increases upon raising filler mass fraction (Skrtic *et al*., 1996) and filler particle size (Lee *et al.,* 2007). Furthermore, this release can advantageously be enhanced upon lowering the pH of the storage media. The levels of calcium and phosphate release from ACP composites were sufficient to promote tooth remineralisation, *in vitro (*Cheng *et al.,* 2012; Skrtic and Antonucci 2007*)*. These composites however, exhibited lower biaxial flexure strength than the base polymer or conventional glass filled composites. This was attributed mainly to the tendency of the ACP filler particles to agglomerate within the composite and increase water sorption (Regnault *et al.,* 2008)

Several studies therefore, have been carried out to improve the mechanical properties, for example through enhancing interaction between the filler and resin matrix, ACP hybridization with glass fillers, reduction in water sorption (Skrtic & Antonucci 2007) , or lowering of filler particle size. The maximum biaxial flexure strengths of these wet ACP composites, however, achieved to date is ~ 50 MPa, which is in the range of glass ionomer cements. Therefore, the ACP composites are not suitable for use as restorative material in stress bearing areas.

ACP composites however, have sufficient strength to be considered as dental adhesives or liner/base materials. In one study, the shear bond strength of an experimental ACP composite to dentine was 18 MPa (Schumacher *et al*., 2007). Upon water storage, some

decline in this strength was observed but the failure mechanism also changed from adhesive to adhesive/cohesive. Recently, ACP composites have been commercialised as an adhesive cement and pit and fissure sealant. With commercial ACP orthodontic adhesive (Aegis Ortho) the shear bond strength was 7 MPa, which was comparable with that of a commercial RMGICs type orthodontic adhesive but approximately half that of a conventional resin adhesive (Uysal *et al.,* 2010).

In the last five years, a lot of work has been done introducing chlorhexidine as an antimicrobial agent in restorative dentistry and calcium phosphate as a remineralising agent. Some studies showed weak strength with slightly increase in adhesion properties. Others used the chlorhexidine as conditioning just before placing the final filling; on the other hand different types of calcium phosphate were used. In table 1-2 is a summary of those studies;

Author*/* year	Aim of the study	Results
Cheng L *et al* (2012)	The objectives of this study were to develop nanocomposites containing amorphous calcium phosphate (ACP) or calcium fluoride (CaF(2)) nanoparticles and CHX particles, and investigate Streptococcus mutans biofilm formation and lactic acid production for the first time.	Adding CHX fillers to ACP and CaF(2) nanocomposites greatly increased their *antimicrobial* capability. ACP and CaF(2) nanocomposites with CHX that were inoculated with S. mutans had a growth medium pH>6.5 after 3 d, while the control commercial composites had a cariogenic pH of 4.2. Nanocomposites with CHX reduced the biofilm metabolic activity by 10-20 folds and reduced the acid production, compared to the controls. CFU on nanocomposites with CHX were three orders of magnitude less than that on commercial *composite*. Mechanical properties of nanocomposites with CHX matched a commercial *composite* without fluoride

Author*/* year	Aim of the study	Results
Cheng L, *et al* (2012)	The objective of this study was to develop antibacterial and mechanically strong nanocomposites incorporating a quaternary ammonium dimethacrylate (QADM), nanoparticles of silver (NAg), and nanoparticles of amorphous calcium phosphate (NACP).	Flexural strength and elastic modulus of NACP+QADM, NACP+NAg, and NACP+QADM+NAg matched those of commercial composites with no antibacterial property ($p>0.1$). The NACP+QADM+NAg *composite* decreased the titer counts of adherent S. mutans biofilms by an order of magnitude, compared to the commercial composites ($p<0.05$)
Rodrigo Stanislawczuk *et al* (2011)	This study evaluated the effect of modified tetracycline on the resin-dentin bond strength (μTBS), silver nitrate uptake (SNU) and solution homogeneity (SH) of two adhesives.	The μTBS has no significant difference among groups for the SB adhesive ($p > 0.05$). As regards SNU, significant differences were observed for both adhesives ($p < 0.05$). Lower % of SNU was observed for PB and SB when CH and MI was used in comparison with DO($p < 0.05$)

Auther/ year	Aim of the study	Results
Claro-Pereira D *et al* (2011)	The aim of this study was to compare, in situ, the initial dental plaque formation on a recently developed silorane-based composite resin, Filtek Silorane, and on a widely used methacrylate-based composite resin, Synergy D6, and to relate possible differences to surface free energy, hydrophobicity and type of organic matrix.	In contrast to previous in vitro studies, the present in situ study found no statistically significant differences with respect to bacterial adhesion between Filtek Silorane and Synergy D6, despite the differences found for surface free energy and hydrophobicity.
Auther/ year	Aim of the study	Results
Borges *et al*	The purpose of this study	There were no statistically significant

(2011)	was to provide information regarding the marginal adaptation of composite resinonlays in primary teeth previously treated with 1% sodium hypochlorite (NaOCl) (pulp irrigant) using two different resin lutingagents	differences (p>0.05) among the groups. The relative risk test revealed that some groups were more apt to have a presence of gaps than others.
Milward Pj *et al* (2011)	The aim of this paper was to develop experimental compomers with enhanced properties, based on adhesive monomers vinyl phosphonic acid and pyromellitic dianhydride glycerol dimethacrylate, and to compare their properties to those of commercially	Based on the results of this study, higher amounts of vinyl phosphonic acid (VPA), pyromellitic dianhydride glycerol dimethacrylate (PMGDM) and reactive glasses render the material with enhanced fluoride release and adhesion with properties similar to glass-ionomers whereas their decrease gives properties similar to conventional dental *composite resins*with improved properties such as strength and wear resistance

	available products.	
Singla M *et al* (2011)	This study evaluated the effect Off 2% chlorhexidine cavity disinfectant on microleakage in class II cavities restored with light cured composites using a single bottle adhesive in an in vitro mode	*Chlorhexidine* cavity disinfectants produced significantly higher microleakage while restoring the cavities using a self-etching single bottle adhesive.
Hiraishi N April 2010	The purpose was to investigate the chlorhexidine release and the antibacterial effect of chlorhexidine-incorporated PMMA-based luting cement	Super-Bond C&B that contained 3.0 and 4.0% of chlorhexidine exhibited prolonged release of chlorhexidine for 5 weeks, whereas those containing 1.0 and 2.0% depleted at 1 week and 2 weeks, respectively.
H.S.Siso *et al* (2009)	The current study evaluated the influence of KTP (Potassium-Titanyl-Phosphate) laser	There were no significant differences among the four groups at the gingival surface ($p>0.05$). Microleakage at the occlusal margins of all the groups was

	irradiation, 2% chlorhexidine gluconate and Clearfil Protect Bond on the microleakage of Class V composite restorations.	compared; differences between the KTP laser and chlorhexidine gluconate group and the KTP laser and Clearfil Protect Bond group were found to be statistically significant ($p<0.05$).
Hiraishia N (2008)		Using 2% *chlorhexidine* gluconate did not interfere with the microtensile bond strength of glass ionomer cements and *composite*.

Table 1-2 Summary of latest studies using CHX in resin restoration

1.6 The need to develop a new dental material for paediatric dentistry

There is strong evidence showing that invasive types of dental procedures are the most anxiety provoking in dentistry (Oosterink *et al*., 2008). A study in 2002, found that children with many carious lesions at the age of five years are likely to be dentally anxious at 10 years of age, probably because they have had pain and other negative treatment experiences (Raadal *et al*., 2002). This anxiety results in avoidance behaviour only detectable by a compulsory examination system such as school visits. However, it is usually associated with high caries rate and the need for oral rehabilitation (Eitner *et al*., 2006). Indeed, several studies reported the strong relationship between dental anxiety and avoidance of dental care (Arnrup *et al*., 2003). Furthermore, dental anxiety has been reported to decrease with repeated exposure to dental treatment, possibly due to habituation Therefore, different techniques have been established.

Historically dentists were taught to remove all infected dentine but identification of all diseased tissue can be difficult. Furthermore, complete removal of caries increases the risk of pulpal exposure, pain, tooth weakening and ultimately tooth loss (Banerjee *et al.,* 2000; Kidd 1999). Moreover, there is increasing evidence to suggest that bacterial action may be halted by “restoration sealing”. This is used in the more tooth conservative approach (Banerjee *et al.,* 2001). The atraumatic restorative technique (ART) was originally developed for regions of the world with no access to dentists and associated infrastructure eg dental chair, electricity, light

gun, compressed air etc (Mehdawi *et al*., 2013; Molina *et al*., 2009; Takahashi *et al.,* 2006). Moreover, it is also favoured by children due to reduced use of local anaesthetics / drills and greater procedure simplicity (Molina *et al.,* 2009; Peumans *et al*., 2005). It uses hand instruments to remove carious tissue and then a glass ionomer cement (GIC) to seal the cavity.

Therefore In this study, we addressed the need to develop a novel composite with antimicrobial and remineralising properties to improve the quality of dental treatment for anxious child for paediatric dentistry using ART approach.

Chapter Two: Aim & objectives

2 Aims and objectives

2.1 Aim

The study aim was formulation of composites with high monomer conversion, high mechanical strength, early release of antimicrobial agent and remineralising properties, without compromising the standard values of mechanical and biological properties of resin restorations.

2.2 Hypothesis

Our hypothesis was that the addition of water-soluble MCPM fillers should encourage water sorption into the set resin materials, which in turn should enhance the release of chlorhexidine and calcium phosphate species. Subsequently, however, the absorbed water should promote reaction between MCPM and βTCP and brushite formation within the polymerised methacrylate. As this reaction can bind water and encourage reprecipitation of less soluble species in material regions from which components have been released, it was also anticipated that it might limit the reduction in strength normally associated with water sorption and component release (Figure 2-1)

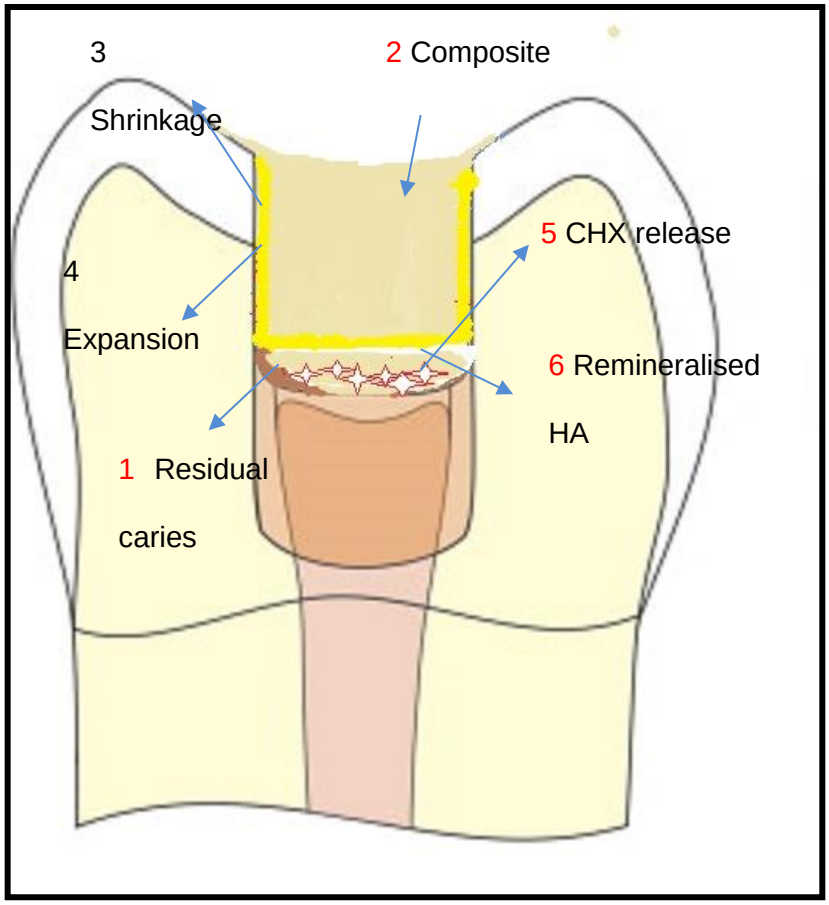


Figure 2-1 Schematic diagram explaining the hypothesis of this study

2.3 Objectives

To formulate and test systematically varying dental composites containing UDMA base monomer, 25 wt% PPGDMA diluent, and 5 wt% of adhesive methacrylate dental resins (HEMA or HEMA phosphate). These are combined with 80 wt% filler containing 10 or 40 wt% reactive calcium phosphate fillers (MCPM and β-TCP) and 5 or 10 wt% chlorhexidine diacetate dispersed in a conventional dental glass.

- Poly (propylene glycol) Dimethacryate monomer was introduced due to its high molecular weight which subsequently should ensure lower shrinkage and less heat generation per mole of monomer conversion than standard TEGDMA diluent.
- Chlorhexidine diacetate was added to to provide antibacterial properties
- Mono calcium phosphate and tri calcium phosphate reactive filler were included to enhance remineralisation
- Hydrophilic adhesive monomers (HEMA & HEMA phosphate) would enhance water sorption to promote swelling to compensate polymerisation shrinkage

Chapter Three: Materials used

3 Materials

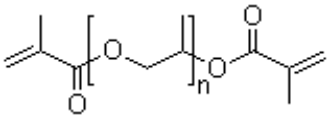
3.1 Monomer

3.1.1 Urethane Dimethacrylate (UDMA)

It is high molecular weight monomers which was developed and introduced in commercial materials to overcome the limitations of BisGMA-based systems. As an example, formulations based on urethane dimethacrylate (UDMA; MW=470 g/mol) became increasingly common, due to this monomer's low viscosity and high flexibility in relation to BisGMA (Floyd 2006). UDMA copolymers in general present higher flexural strength, elastic modulus and hardness (Tanimoto *et al.*, 2005).

In general, shorter chain monomers with lower molecular weight have greater polymerisation shrinkage per mole of monomer conversion. A high molecular weight monomer reduces the polymerisation shrinkage, due to lower concentrations of C=C. Reducing shrinkage minimizes marginal contraction gaps, microleakage, marginal staining and caries recurrence, whilst also dissipating and reducing functional stresses across the restorative-tooth interface (Braga *et al.,* 2005). For all materials used in this study see (Table 3-3)

3.1.2 Poly (propylene glycol) Dimethacrylate



$(C_4H_5O)\cdot(C_3H_6O)_n\cdot(C_4H_5O_2)$

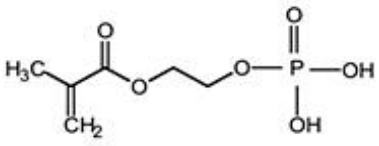
Figure 3-1 The chemical structure of PPGDMA

PPGDMA has a flexible long polypropylene glycol (PPG) chain in between two methacrylate functional groups. Its low viscosity improves handling properties of the UDMA.

3.1.3 Hydroxyethyl methacrylate

Hydroxyethyl methacrylate (HEMA) is a hydrophilic monomer which has the ability to improve degree of conversion, decrease viscosity and improve handling properties.

3.1.4 Hydroxyethyl methacrylate phosphate



C6H10O6P

Figure 3-2 The chemical structure of HEMA phosphate

HEMA-phosphate (also called MEP, 2-methacryloyloxyethyl dihydrogen phosphate) (Figure 3-2), can be hydrolytically unstable (Salz *et al*., 2005).

In aqueous solutions, it can partially hydrolyse into HEMA and strongly acidic phosphoric acid. Adhesive systems that contain this monomer may therefore be quite acidic. (Table 3-1)

3.2 Commercial composite used

3.2.1 Gradia

Gradia Direct is classified as a micro-fine hybrid resin composite with prepolymerised resin filler. These so-called colloidal systems, like Gradia Direct, are known in the clinical situation to have much-appreciated advantages in terms of esthetics, polishability, and wear resistance. The average particle size is 0.85 microns. The material itself uses urethane dimethacrylate (UDMA) and dimethacrylate co-monomers for its matrix. (Table 3-2)

Gradia Direct uses a combination of camphorquinone and amine as the catalyst for photoactivation. Light activation can be carried out with quartz halogen, plasma, or LED curing lights

3.2.2 3M™Filtek Z250

Filtek Z250 is a hybrid resin composite with particle size distribution from 0.01μm to 3.5μm with an average particle size of 0.6μm resin filler. The new resin system of 3M™Filtek™ Z250 Universal Restorative consists of 3 major components. In Filtek Z250 restorative, the majority of TEGDMA has been replaced with a blend of UDMA (urethane dimethacrylate) and Bis-EMA (Bisphenol A polyethey- lene glycol diether dimethacrylate). Both of these resins are of higher molecular weight and therefore have fewer double bonds per unit of weight. The high molecular weight materials also impact the measurable viscosity. (Table 3-2)

Monomer	Abbreviations	supplier	Product code	MW	% wt
Urethane Dimethacrylate	UDMA	Esstech	X850 0000	470	68%
Poly(propylene glycol) Dimethacrylate	PPDdma	Esstech	04380-250	660	20%
Hydroxyethyl methacrylate/ phosphate	HEMA HEMA phosphate	Esstech	X9687044	130	5% or 5%

Table 3-1 Types of Monomer used in experimental; composite

Composite	Resin	Filler	Fillers by Wt %	Fillers by Vol%	Fillers Size
Filtek Z250	Bis-GMA Bis-EMA UDMA	Zirconia/silica	78	60	0.01-3.5µm
Gradia Direct	UDMA	Silica Prepolymerized filler	73	64	0.85µm

Table 3-2 Commercial composite used in this study adapted from (Watanabe *et al*., 2008)

3.3 Fillers

3.3.1 Chlorhexidine

Chlorhexidine is known as a gold standard for chemical plaque control. It is anti-plaque, anti-inflammatory and bactericidal or bacteriostatic at low versus high concentration. Chlorhexidine diacetate used in this study has a molecular weight of 625.6 g/mol (Figure 3-3). Reported bacterial resistance to chlorhexidine is rare. Chlorhexidine can be effective against ninety five percent of oral bacteria (Tirali *et al*., 2013)

The mode of antibacterial action of CHX is reduction of membrane formation and modification of bacterial cell walls, causing bacterial cell lysis and prevention of bacterial adherence to teeth. This is facilitated by electrostatic forces, since chlorhexidine is positively charged, while the phosphate and carboxyl groups of bacterial cell walls carry negative charges. Binding of CHX to phosphate and carboxyl groups of bacterial cell walls causes disruption of the osmotic barrier and interference with membrane transport.

CHX can be adsorbed on enamel and soft tissue and has a high intraoral substantively (Addy and Dowell 1986) in the mouth (Schwach-Abdellaoui *et al*., 2000). Prolonged retention at bacteriostatic levels would explain the effectiveness of CHX. *In – vitro*, chlorhexidine- treated enamel prolongs the lag phase of bacterial division of adherent organisms. When delivered orally, systemic toxicity and microbial resistance is reduced, and supra infections do not usually occur.

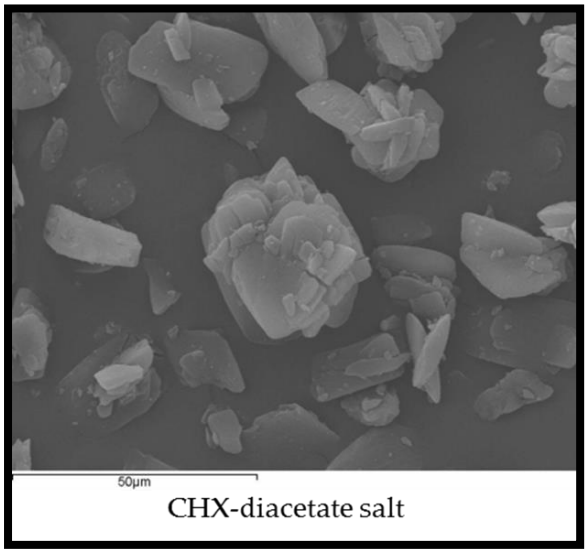


Figure 3-3 SEM images of CHX particles

3.3.2 Inorganic filler

In the following experimental composites, silanated glass was used as the major component of the filler phase (Figure 3-4). Ideally, around 80 wt% is incorporated in the resin matrix phase, mainly to enhance physical properties and decrease polymerisation shrinkage (Bohner 2000)

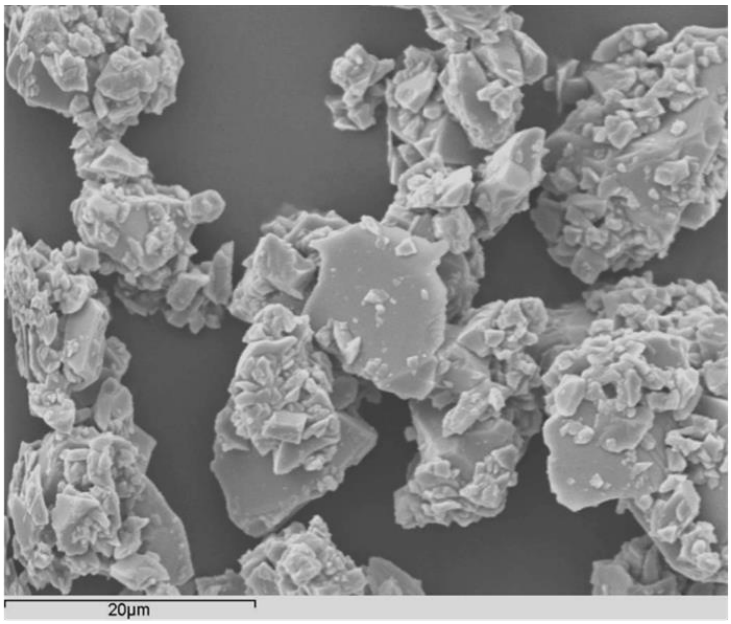


Figure 3-4 IF 2019 glass 5µm

3.3.3 Calcium phosphate

In this study MCPM and TCP was used. (figure 3-5 and figure 3-6)

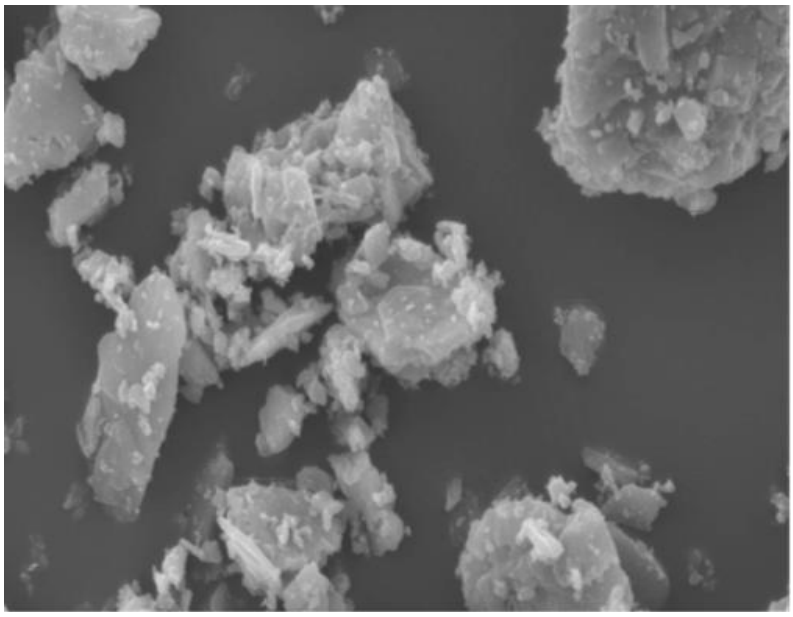


Figure 3-5 SEM image for MCPM

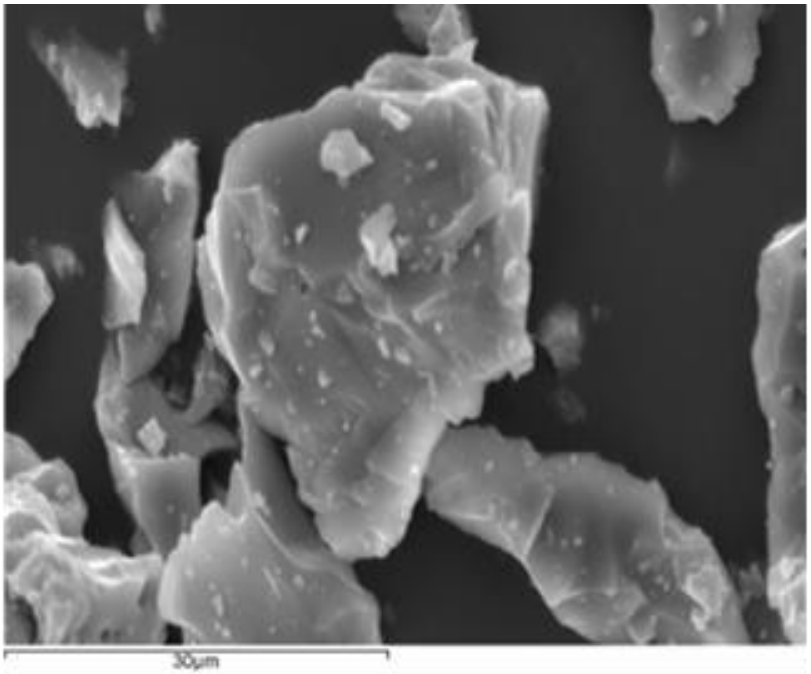


Figure 3-6 SEM image TCP

Abbreviation	Chemical name	Supplier	MW	Properties
CHX	Chlorhexidine-diacetate	S-Aldrich		Antibacterial, increases the setting time
MCPM/b-TCP(CaP)	Monocalcium phosphate ,β tri calcium phosphate	Himed	310/252	For brushite and hydroxyapatite formation, Decrease brittlness, release of Ca and PO_4 ions
IF2019-Glass particle	Silanized barium boro alumino silicate glass	Sci-pharm		Decreases polymerisation heat and shrinkage, Increases strength
UDMA(Base monomer)	Urethane dimethacrylate	Esstech	470	High molecular weight monomer ensures low shrinkage / heat generation, cross linking provides strength and wear resistance
PPG DMA	polypropylene-glycol-dimethacrylate	Polysciences	660	Flexible, cross linking monomer that improves handling by lowering viscosity
HEMA	Hydroxyethyl methacrylate	Esstech	130	Low viscosity monomer that improves conversion

				and handling
HEMA Phosphate	Hydroxyethyl methacrylate phosphate	Esstech	210	Low viscosity monomer – adhesion properties
CQ	camphorquinone	S-Aldrich	166	Source of free radicals
DMPT(accelerator)	N,N-dimethyl -p-toluidine	S-Aldrich	135	Stabilizes free radicals

Table 3-3 List of materials, companies' names and properties

Chapter Four: Methods

4 Methods

4.1 Specimen manufacture:

For all experimental formulations, filler and monomers were weighed using a four-figure balance. Initiator (CQ and DMPT) were dissolved in the monomers (UDMA, PPG, and HEMA or HEMA phosphate) and combined with filler material. The mixture was loaded in to 10mm diameter spilt brass ring. The samples were fully covered by an acetate sheet (to decrease air inhibition of the polymerisation process) and a glass microscope slide, which was pressed gently to express excess material. Light cure of samples was initiated by irradiation for 60 seconds with a blue light-curing unit (460-480 nm), for which the power output was 1000 mW/cm^2. The samples were removed from the moulds, excess material removed to get smooth edges and then stored at room temperature until usage (Figure 4-1). Eight samples were developed from each formula for biaxial flexure strength (BFS) test and were left in distilled water for 24 hours before testing. While five samples for each individual formula were developed for testing monomer conversion, mass changes and CHX release tests.

In all studies PLR was 4:1. Variable factors were:

1) high and low MCPM and TCP (5 or 20 wt% of each) (reactive CaP phase)

2) CHX at 5 or 10 wt% of the powder phase,

3) 5 wt% HEMA or HEMA Phosphate

Formulations with 0 wt% of CaP or CHX were used as a control

Figure 4-1 sample preparation

4.1.1 Series 1 Formulations

For control formulations, monomer mixtures were prepared using 68 wt% UDMA, 25 wt% PPG and 5 wt% HEMA (sample F1) or HEMA phosphate (sample F5). This

was combined with 1 wt% DMPT, 1 wt% CQ and 80 wt% glass powder. Z250 Composite and Gradia were additionally tested in this control group.

4.1.2 Series 2 Formulations

For series 2, monomer mixtures were prepared using 68 wt% UDMA, 25 wt% PPG, 1 wt% DMPT, 1 wt% CQ and 5 wt% HEMA. In this group, 5 or 10 wt% CHX, 5 or 20 wt% β-TCP and 5 or 20 wt % MCPM were introduced with 50, 55, 80 or 85 wt % non reactive fillers (glass) (Table 4-1).

	Powder				Liquid
	4				1
	CaP		CHX	Glass	Monomer mixture
	MCPM	β-TCP			68% UDMA
F2	5%	5%	5%	85 %	25% PPG
F3	5%	5%	10%	80%	5% HEMA
F4	20%	20%	5%	55%	1% CQ
F5	20%	20%	10%	50%	1 DMPT

Table 4-1 Powder and liquid concentrations for series 2 formulations

4.1.3 Series 3 Formulations

Monomer mixtures were prepared using 68 wt% UDMA, 25 wt% PPG and 5 wt %, HEMA Phosphate and 1 wt% DMPT, 1 wt% CQ. In this group reactive fillers such as 5, 10 wt% CHX, 5, 20 wt % β-TCP and 5, 20 wt % MCPM were introduced, finally adding 50, 55, 80, 85 wt % non reactive fillers (glass). (Table 4-2)

			Powder		Liquid
			4		1
	CaP		CHX	Glass	Monomer mixture
	MCPM	β -TCP			68% UDMA
					25% PPG
F7	5%	5%	5%	85%	5% HEMA Phosphate
F8	5%	5%	10%	80%	1% CQ
F9	20%	20%	5%	55%	1 DMPT
F10	20%	20%	10%	50%	

Table 4-2 Powder concentrations based on factorial design

4.2 Degree of conversion and polymerisation reaction profile

4.2.1 Fourier transform infrared spectroscopy

Infrared (IR) spectroscopy is a chemical analytical technique that uses the intensity of infrared light to determine the functional groups in compounds. The infrared light can be classified according to the wavenumber into far infrared (10-200 cm^{-1}), mid infrared (200-4000 cm^{-1}) and infrared (4000-12800 cm^{-1}). The mid infrared region provides greater information on molecular structures; hence, it is most commonly used. FTIR spectroscopy has been used in this study to measure the rate and level of monomer conversion of the novel composite being formulated.

4.2.2 Principles of infrared absorption:

When a molecule or atom absorbs IR radiation, it gains energy as it undergoes transition from one energy level ($E_{initial}$) to another (E_{final}). According to Planck's law, the energy of transition and the frequency of absorbed radiation *f* (Hz) are correlated by equation (4.1):

$$E_t = hf \qquad \text{Equation 4-1}$$

Where E_t is the energy of transition (E_{final}- $E_{initial}$) and *h* is Planck's constant.

Since $f = vc$, where *v* and *c* are the wavenumber (*v*) (cm^{-1}) and velocity of light (8×10^8 m s^{-1}), equation (4.1) can be replaced by equation (4.2):

$$E_t = hvc \qquad \text{Equation 4-2}$$

The wavelength (λ) (nm) is related to frequency (*f*) by the following equation:

$$\lambda = c/f \qquad \text{Equation 4-3}$$

Therefore, equation (4.2) can also be given as:

$$E_t = hc/\lambda \qquad \text{Equation 4-4}$$

Energy absorbed by a molecule must match exactly that required for a molecular transition. When a molecule absorbs Infrared radiation, it will be excited to a higher vibration energy state. IR absorption depends on molecular vibrations. This can be 1) a stretching vibration due to change in bond length 2) a bending vibration caused by change in bond angle.

In order to observe 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 generally displayed as a plot of IR absorbance versus wavenumber (cm^{-1}) (see for example Figure 4-2). Peaks in the spectrum correspond with different vibrational transitions. Generally the FTIR spectrum can be classified into two regions. The absorption between 4000-1300 cm^{-1} is mainly due to vibrations associated with specific functional groups, while the second region between1300-500 cm^{-1} is known as the fingerprint region and associated with vibrations of the whole molecule.

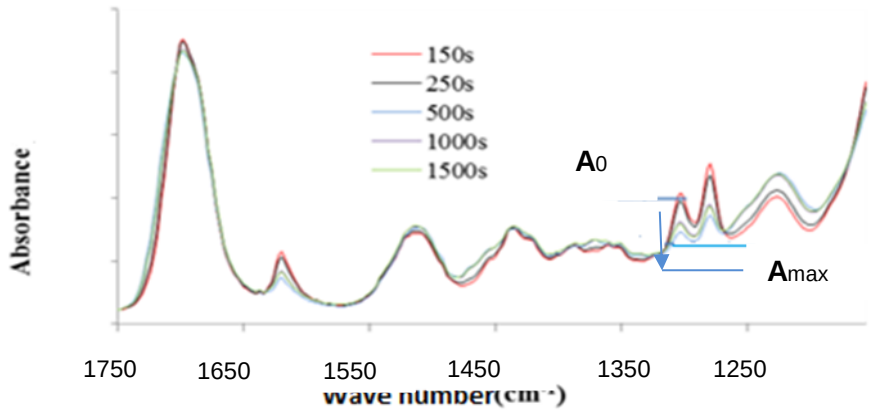


Figure 4-2 Example FTIR spectra of setting composite

4.2.3 Fourier transforms infrared spectroscopy instrumentation:

FTIR instruments consist of a light source, interferometer and detector. The interferometer contains a beam splitter and two mirrors.

The light beam that includes all frequencies of IR radiation is split into two optical beams via the beam splitter. The beams are reflected back to the splitter by the mirrors. One mirror is stationary while the second is allowed to move along the path of light beam. The time required for light to travel from mirrors to splitter will be different for both beams and dependent on the wavelength. The reflected beams are recombined at the splitter and the resultant used to produce an interferogram. After interaction with the sample, the resultant interferogram can be converted to absorbance versus wavenumber through computer software and Fourier transformation.

4.2.3.1 Attenuated total reflectance (ATR) -FTIR:

Before generating any FTIR spectra background was scanned first without any sample on the ATR diamond piece so that the ratio of light intensity through the instrument with and without the sample could be calculated by the computer. Chemical reactions of different formulations of composite containing CHX, MCP and TCP were then monitored and the reactant and end products identified by comparison of spectra of pure compounds.

To investigate reaction kinetics each formulation was mixed and loaded immediately into a metal mould (10 mm diameter and 1mm thickness) on the centre diamond of a golden gate^{TM} heated ATR top-plate (Specac Ltd, UK) in an FTIR spectrometer (Perkin Elmer series 2000, UK). The temperature was kept at 37 ºC (to simulate normal human body temperature) and number of samples was five for each formula. The top surface of the sample was then covered with acetate sheet to prevent oxygen inhibition of the polymerisation. FTIR spectra of the sample in contact with the diamond were obtained with resolution set at 4 cm^{-1} and wave number range between 600 and 2000 cm^{-1}. Number of scans was fixed at 8 and the total run time was 20 minutes for all specimens.

For a simple single reaction change in absorbance versus wavenumber has the same profile irrespective of time. For the methacrylate polymerisation reaction high absorbance change is observed at 1320 cm^{-1} (C-O stretch in the monomer) and also 1700 cm^{-1} (C=O stretch). The degree of monomer conversion was quantified through change in the absorbance due to the monomer peak at 1320 cm^{-1} (C-O stretch) above the background at 1335 cm^{-1}. % degree of monomer conversion was calculated using equation (4-5)

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

Equation 4-5

$\mathbf{A}_0$ and $\mathbf{A}_t$ are the peak height due to the C–O bond stretch peak at 1320 cm^{-1} initially and at time t after start of the experiment respectively.

4.2.4 Heat Generation and Shrinkage (Polymerisation):

Polymerisation heat and shrinkage can be calculated from molecular weight (*Mw*) of monomers, powder liquid ratio (*PLR*) and final conversion levels (*C*) of the different formulations. One mole of polymerising C=C bonds typically generates 57 kJ of heat (Lewis 2006) and gives volumetric shrinkage of 23 cm^3 (Kobayashi *et al*., 1998). The total heat generation and shrinkage due to the composite curing process can therefore be estimated using equations 4-6, 4-7, 4-8, 4-9 and 4-10

$$vol(\%) = 23N * 100$$

Equation 4-6

$$Heat(kJ/cc) = 57N$$

Equation 4-7

N is the number of moles of reacted bonds per unit volume. This can be estimated using

$$N = mCr_{comp}\sum_i \left(\frac{n_i x_i}{W_i}\right)$$

Equation 4-8

m is the total monomer mass fraction and *C* the final fractional monomer conversion calculated from FTIR. ∑ indicates a sum over all the monomers in the liquid phase. n_i, W_i and x_i are number of c=c bonds per molecule, molecular weight (g/mol) and mass fraction of monomer *i* respectively. PPG, UDMA and HEMA molecular weights were taken as 560, 470 and 130 g/mol. Assuming the formulation density, ρ_{comp} (g/cm^3), behaves "ideally" it can be estimated using a simple rule of mixtures

$$1/\rho_{comp} = m/\rho_{monomer} + (1-m)/\rho_{filler}$$

Equation 4-9

$\rho_{monomer}$ and ρ_{filler} are the average densities of the monomer mixture and filler and m is the mass fraction of monomer. The filler density was similarly calculated using

$$1/P_{filler} = m_G/p_G + m_T/p_T + m_M/p_M$$ **Equation 4-10**

Where G, T and M represent glass, TCP and MCPM with densities of 3.0, 3.15 and

2.22 g/cm^3 respectively.

4.3 Mechanical behaviours:

4.3.1 Biaxial flexural strength

In this study, biaxial flexural strength was determined. Flexural strength is obtained from the maximum stress experienced in the material at failure when subjected to bending load. The rationale for using this type of test was based on possible applications of this composite as dental material which can be subjected to flexural stresses during mastication (Chung *et al.*, 2004). The biaxial flexural test was chosen over the uniaxial test in this study. In this test the disc shaped composite is supported near its periphery by a continuous ring shaped structure and later loaded by a coaxially located ball. In addition the disc shape is circularly symmetrical; the stress field is equibiaxial in the central region in which it is at a maximum. Therefore, this method will minimise the effect of the test specimen edge preparation compared to uniaxial test because the generated stresses are lowest at the test specimen edges (Figure 4-3).

For strength testing 8 discs of each composition of 10 cm diameter and approximately 1mm thickness were prepared and their thickness accurately measured to 0.001 mm. Samples were tested after 24 hours dry, and then composite compositions were hydrated in distilled water for 24 hours at 37º C just prior to testing. Again after one week after immersion. Flexural strength was

determined using the Instron Model 4505 Universal Testing Machine provided with a load cell of 1kN and the crosshead speed was fixed at 1 mm/min. Before load application, each sample was measured for thickness **t** (mm) at three different points and the average thickness was obtained.

The hydrated disc specimen was placed on the knife edge ring support (radius a = 4mm) and then loaded by the spherical tip in an Instron mechanical tester. The maximum load (kN) at fracture, P and load versus central displacement gradient, dP/dw were determined. From the maximum load and the average thickness of the composite, the strength for each sample was calculated using the following formula by Timoshenko (theory of plates and shells);

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

Equation 4-11

Wher

σ is biaxial flexural strength.

P is maximum load.

a is support radius.

t is average thickness of specimen.

Ω is poisson's ratio= 0.3

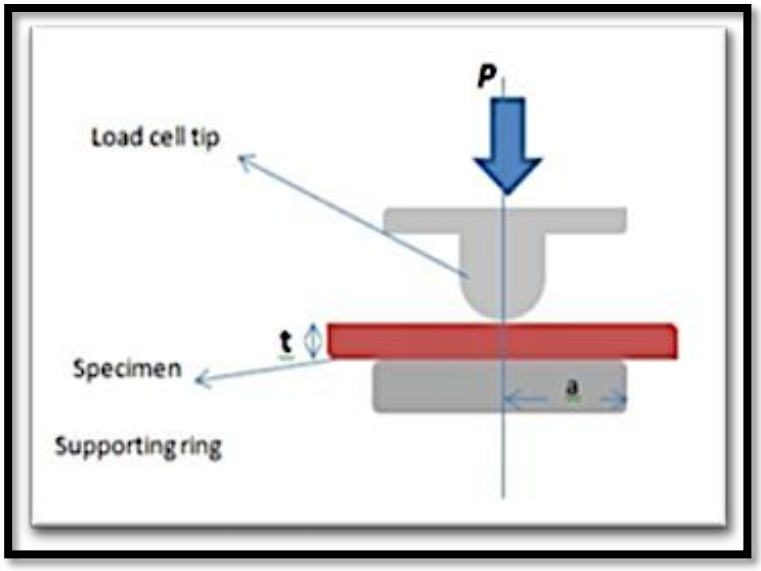


Figure 4-3 Schematic diagram of biaxial test

4.3.2 Biaxial flexural modulus

Elastic modulus is a measure of stiffness of the material. This can be experimentally determined from the slope of a stress-strain curve created during tensile tests conducted on a sample of the material and which is known by Young modulus.

The Instron machine produces data in pound (lbs) and inches, so the data needed to be converted to Newton (N) and millimeters (mm). From the load -displacement data, a graph was plotted for each sample (Load against displacement) to determine the slope required for the calculation of modulus based on the following equation;

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

Equation 4-12

Where :

$\Delta P/\Delta W$ = change in force/change in displacement

= gradient of the force displacement curve.

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

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

4.3.3 Toughness

Toughness can be determined by measuring the area underneath the stress-strain curve and its energy needs for mechanical deformation per unit volume prior to fracture. The explicit mathematical description is

$$\frac{\text{energy}}{\text{volume}} = \int_0^{\epsilon_f} \sigma \, d\epsilon$$ **Equation 4-13**

Where;

- ϵ is strain
- ϵ_f is the strain upon failure
- σ is stress

4.3.4 Resilience

Resilience is the ability of a material to absorb energy when it is deformed elastically. It is given by

$$U_r = \frac{\sigma_y^2}{2E}$$ **Equation 4-14**

Where;

- σ_y Biaxial flexural strength
- E Young's elastic modulus

4.4 Chlorhexidine Release profiles

The absorbance of different storage solutions was obtained at each time point using UV spectrometry (UV) for a period of up to 4 weeks. For determination of a calibration curve, standard solutions were prepared in water. CHX solutions of 20, 10, 5, 2.5, and 1.25 ppm were made. 1 cm path length quartz cells were used. The spectrum was recorded between 200 - 400 nm. Maximum absorption for the CHX standards was found at 231 and 255 nm (Figure 4-4).

Five specimens of each formulation were prepared, were placed in water (5 ml at 37°C). Samples were removed and replaced in fresh medium at 2, 6, 24 and 48 hours, and 1, 2, 3, and 4 weeks. Samples were stored at 37°C prior to CHX quantification.

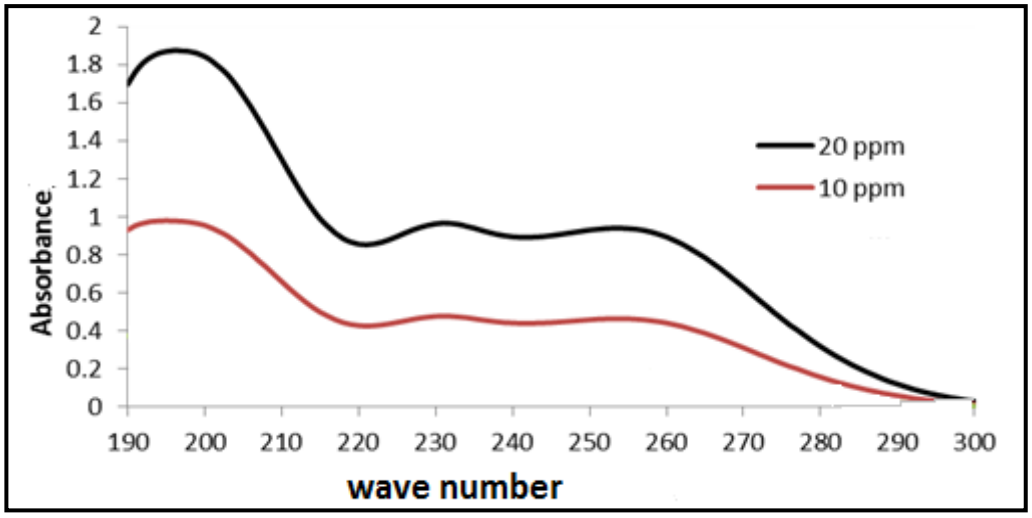


Figure 4-4 Absorbance vs wave number cm $^{-1}$ for CHX standards

The CHX concentration calibration curve was obtained by plotting the absorbance at 230 nm and 255 nm against the concentration of CHX in samples (Figure 4-5). Each of the storage samples were diluted with distilled water (dilution factor recorded) and analysed using UV spectrometry. The absorbance at 230 nm and 255 nm were recorded and analysed using Microsoft Excel 2010.

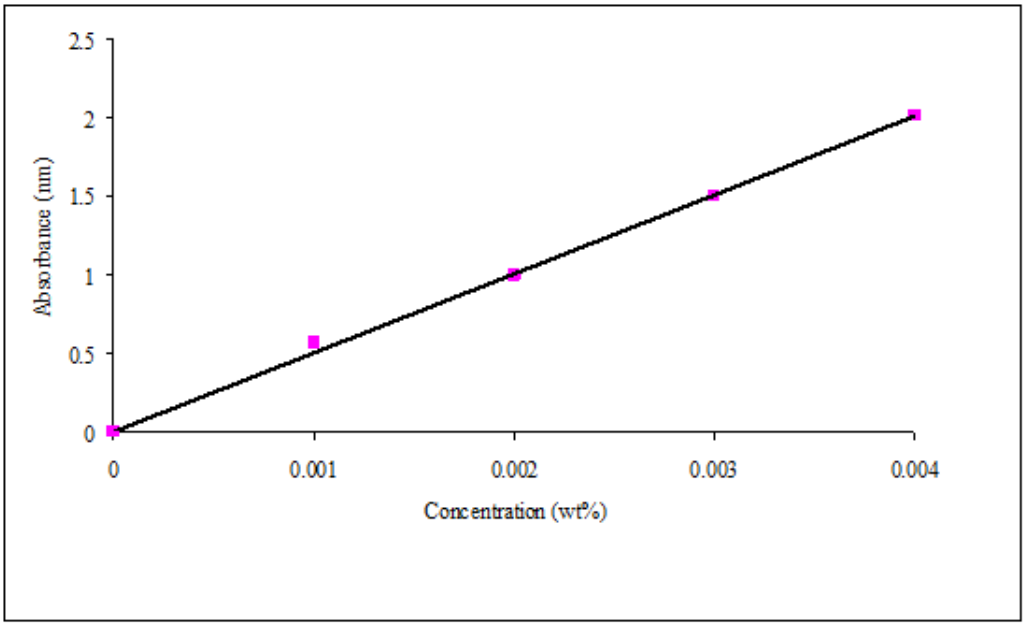


Figure 4-5 Chlorhexidine diacetate calibration curve (absorption at 255 nm).

The amount of CHX released (D, in grams) from the specimen was calculated

Equation 4-15

Cumulative mass of CHX release in gram (C_D) at time "t" was calculated using

$\boldsymbol{C_D = 0tDt}$ Equation 4-16

6

The percentage release (P (%)) at time "t" was calculated using $\mathbf{P} = \frac{C_D}{C_M} \times \mathbf{100}\%$

Equation 4-17

$\boldsymbol{D = \left(\frac{A}{k}\right) \times V_s}$ **Equation 4-15**

$\boldsymbol{C_D = \sum_0^t D_t}$ **Equation 4-16**

$\mathbf{P} = \frac{C_D}{C_M} \times \mathbf{100}\%$ **Equation 4-17**

Where

A = absorbance due to CHX at 255 nm,

k = gradient of the calibration curve for CHX.

V_S = storage solution volume (ml).

C_M = original CHX mass in specimen.

4.5 Mass changes

For each composition, five discs were used for the mass and volume change test. All composites were individually coded and immersed in 10ml of distilled water in a sterilin tube. The tubes were then incubated at 37°C. At different time points 2, 4, 6, 24 hours, 48 hours, one week, two weeks, 3 weeks and 4 weeks. Each sample was removed from the sterilin tube and blotted dry on tissue paper. The storage solutions were kept aside for UV analysis to determine drug release profile. The disc was then weighed using a four figure balance and re-immersed in a new sterilin tube containing fresh 10ml distilled water stored at 37°C.

$$\Delta m\ (\%) = 100\ \times \frac{mt - m0}{m0}$$

Equation 4-18

4.6 Microstructure study of experimental composites

In this study, the effect of the monocalcium phosphate, tri calcium phosphate and chlorhexidine on microstructure of composites were investigated using a scanning electron microscope (SEM). SEM uses a beam of highly energetic electrons to examine objects on micron scale. A stream of electrons is formed under high vacuum (by electron guns). This stream is accelerated towards the specimen with a positive electrical potential while being confined and focused using metal apertures and magnetic lenses into a thin, focused, monochromic beam. Each sample is irradiated by the beam and interactions occur inside the irradiated sample, affecting the electron beam. These interactions and effects are detected and transformed into an image.

4.7 Data Analysis

In order to assess mechanical behaviour, chlorhexidine release and mass changes at each formula, data were analysed by comparing the mean and standard deviation. The effect of the chlorhexidine, Calcium phosphate, HEMA and HEMA phosphate on development composite was analysed using univariate analysis of variance for all variables and simple t test where appropriate

4.7.1 Simple T test

This test is used for comparing the means of two samples, even if they have different numbers of replicates. In simple terms, the t-test compares the actual difference between two means in relation to the variation in the data (expressed as

the standard deviation of the difference between the means). In this study, the T test where used with series one formulation (Control group)

4.7.2 Univariate Analysis of Variance

Univariate analysis is the simplest form of quantitative (statistical) analysis, it is explores each variable in a data set, separately. It looks at the range of values, as well as the central tendency of the values. It describes the pattern of response to the variable as it's describes each variable on its own.

In this study, eight samples were made for each formula for testing the effect and changes of CHX, CaP, HEMA, HEMA phosphate on the mechanical behaviour on the new development composite any evidence of differences between them at the significance level 0.05. Five samples were made for testing the same variables on monomer conversion, CHX release and mass changes using SPSS 2012 (Windows software) (see Appendix 1)

4.7.3 Factorial analysis

To quantify the effects of different variables on the degree of conversion, BFS, CHX release and mass changes, results were fitted to a factorial expression

$$\mathbf{lnP=ln<P>+F1a_1+F2a_2+F3a_3+F1F2a_{12}+F1F3a_{13}+F2F3a_{23}+F1F2F3a_{123}}$$

Equation 4-19

ln represents natural logarithm. Fi takes values of -1 or +1 when the variable, i, has its low and high value respectively. a_i quantifies the effect of the three variables (HEMA/ Hp, CaP % and CHX %) on the property P. a_{12}, a_{13}, a_{23} and a_{123} are 2 and 3 variable interaction terms. It can be shown that

$$\langle PH \rangle / \langle PL \rangle = \exp(2_{ai})$$ **Equation 4-20**

<PH> and <PL> are the geometric mean property values of all samples with the variable, i, at its high or low value respectively (Mehdawi *et al.,* 2009) (see Appendix 2). The effect of a variable was considered significant if its 95% confidence interval was smaller than the magnitude of a_i and interaction effects were small in comparison.

Throughout this study, error bars refer to 95 % confidence interval of the mean (CI) assuming CI= 1.96×SD/√n. In factorial analysis plots of a_i error bars not crossing zero indicate the effect of a variable or interaction is significant.

Chapter Five: Results

5 Results

5.1 Setting Kinetics and Final conversion

In table 5-1 a summary of all formulations used is provided

CODE	UDMA	PPGDMA	HEMA	HEMA Ph	βTCP	MCP	CHX	Glass	PLR
F1	68	25	5	0	0	0	0	100	4/1
F2	68	25	5	0	5	5	5	85	4/1
F3	68	25	5	0	5	5	10	80	4/1
F4	68	25	5	0	20	20	5	55	4/1
F5	68	25	5	0	20	20	10	50	4/1
F6	68	25	0	5	0	0	0	100	4/1
F7	68	25	0	5	5	5	5	85	4/1
F8	68	25	0	5	5	5	10	80	4/1
F9	68	25	0	5	20	20	5	55	4/1
F10	68	25	0	5	20	20	10	50	4/1

Table 5-1 Composition of experimental composites

5.1.1 Conversion Profile:

Figure 5-1 provides example cure profiles. Polymerisation began soon after the light was turned on for all samples (~20s from the start of the experiment).

Commercial composites exhibited much lower final conversion than the experimental materials.

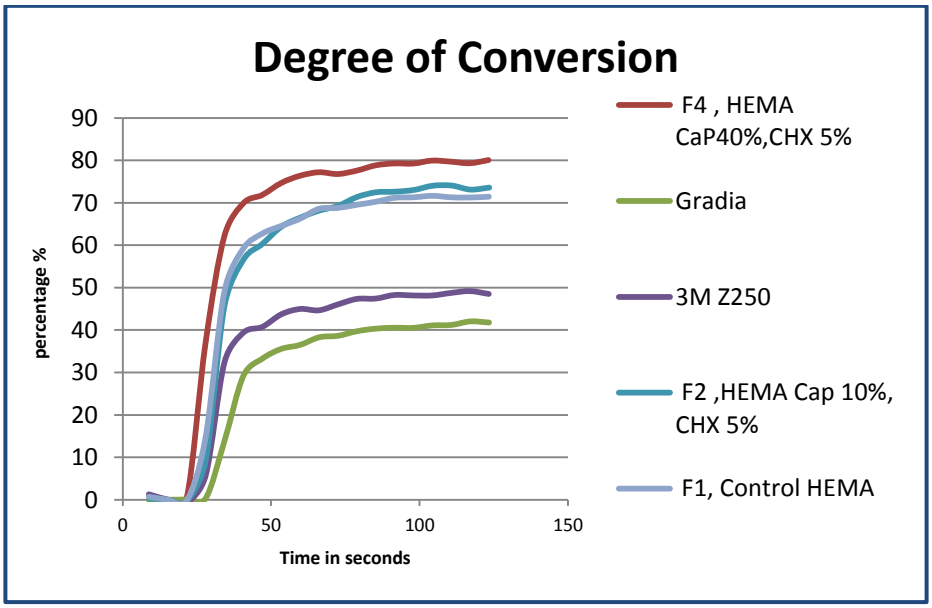


Figure 5-1 Degree of monomer conversion of example experimental and commercial (Gradia - 3M Z250) composites. Both commercial samples showed less than 50% monomer conversion while experimental samples had monomer conversions between 70% and 80%

5.1.2 Final monomer conversions

In series one, the experimental composites final level of polymerisation was statistically higher ($P<0.05$) than commercial composites (Gradia and Z250) (Figure 5-2). Commercial composites (Gradia and Z250) final monomer conversions were 50 ±2% and 42 ± 3% respectively (n=5). There was no significant difference in final conversion between F1 with HEMA and F6 with HEMA phosphate ($P<0.05$) (simple T test).

Figure 5-3 illustrates the final monomer conversion of all experimental composites. Results varied between 71% - 81%. For each pair of formulations with the same CHX and CaP level (e.g F2 and F7) no significant effect of changing HEMA to HEMA phosphate was observed.

Statistical analysis, however, showed that both increasing CHX and CaP caused a significant increase in conversion (3 and 7% respectively) with no interaction effect. This was for both series 2 and 3.

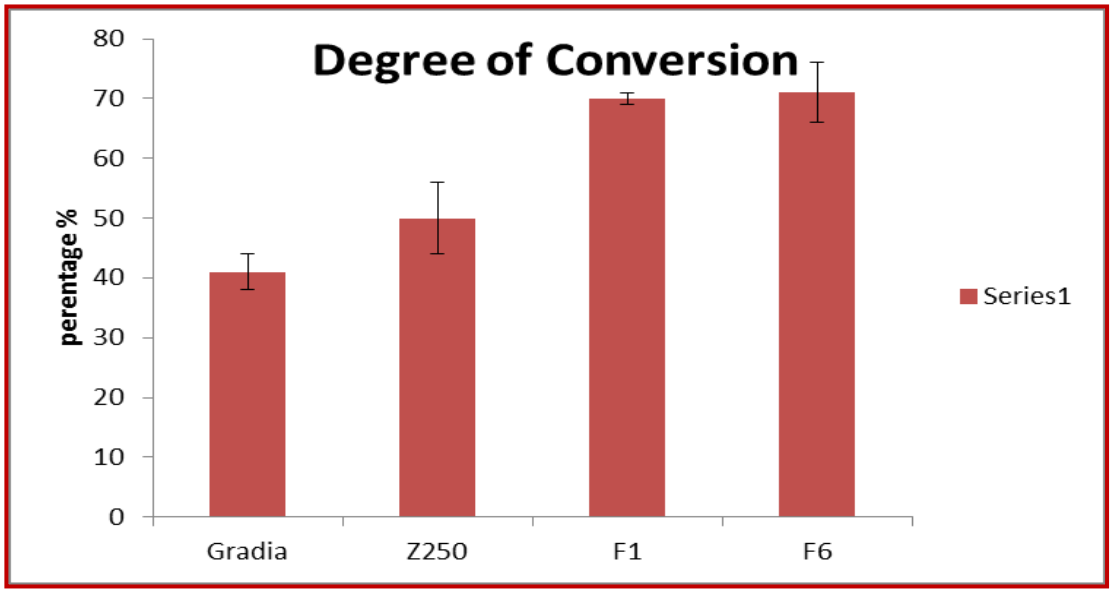


Figure 5-2 Degree of monomer conversion of experimental control composites (filled only with glass) and commercial composites. Errors bars shown are 95% CI, (n =5)

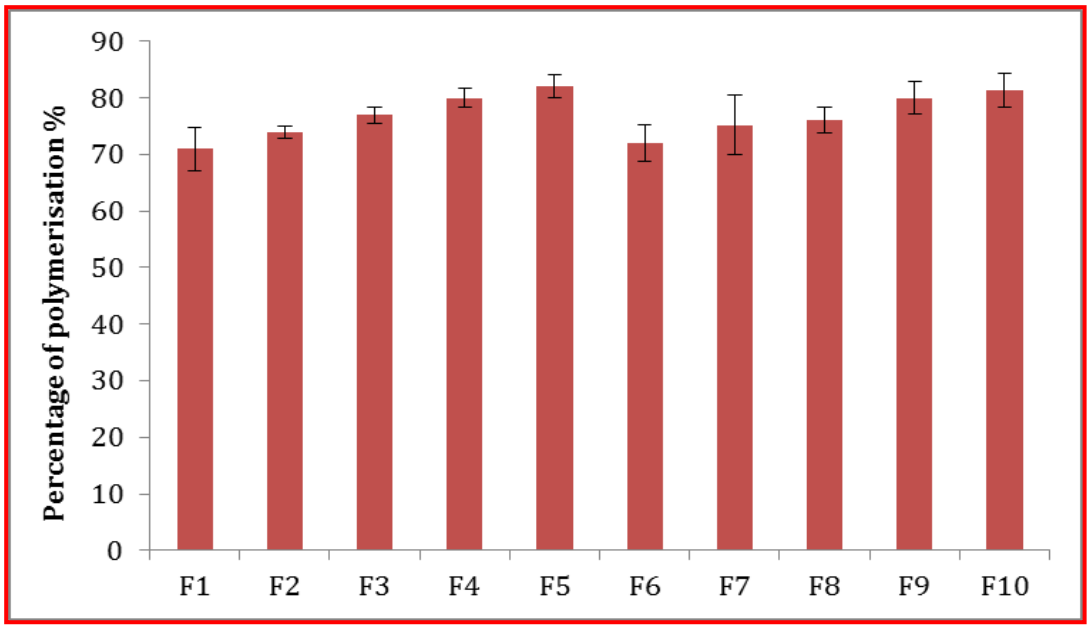


Figure 5-3 Final degree of monomer conversion of all experimental composite series one, two and three. Errors shown are 95% CI, (n=5)

5.1.3 Volume shrinkage and heat generation

Polymerisation reaction produces heat and increase in volume that is proportional to the number of reacting bonds in a given volume. PLR, final conversion, and type of monomer used therefore all have an effect on the polymerisation heat and shrinkage. Higher PLR and monomer molecular weight, or reduced final conversion, will decrease both. The amount of heat generated and level of shrinkage calculated using equations 4-6, 4-7, 4-8 and 4-9 are provided in Table 5.2. Samples which have high level of CHX and high CaP (F5 and F10) show higher percentage of volume shrinkage.

Formulas					**% shrinkage(V) (cc/g)**	**Heat (H)(kcal/g)**
	HEMA	HEMA phosphate	CaP	CHX		
F1	5	0	0	0	3.0	0.0752
F2	5	0	10	5	3.2	0.0784
F3	5	0	10	10	3.1	0.0816
F4	5	0	40	5	3.2	0.0848
F5	5	0	40	10	3.4	0.0912
F6	0	5	0	0	3.0	0.0736
F7	0	5	10	5	3.2	0.0767
F8	0	5	10	10	3.1	0.0777
F9	0	5	40	5	3.2	0.0818
F10	0	5	40	10	3.3	0.0828

Table 5-2 Calculated heat and shrinkage for all experimental composite and commercial product

5.2 Mechanical behaviour

It was noticed that whenever CHX was added to the mixture it made sticky which made it difficult to handle. Adding the CaP had a different effect which made the samples more putty-like with gritty texture.

5.2.1 Biaxial flexural strength

Figure 5-4 illustrates that the biaxial flexural strength of control composites (series one) containing HEMA, F1, or HEMA phosphate, F6. After 24 hours in deionised water, both had statistically greater strength than Z250 or Gradia ($P<0.05$)

For a fixed level of CHX and CaP, changing from HEMA to HEMA phosphate had no significant effect (Figure 5-5). Furthermore, average results on changing this monomer were not significantly different. Increasing CHX and CaP, however, both significantly decreased strength on average by 10 and 14% respectively

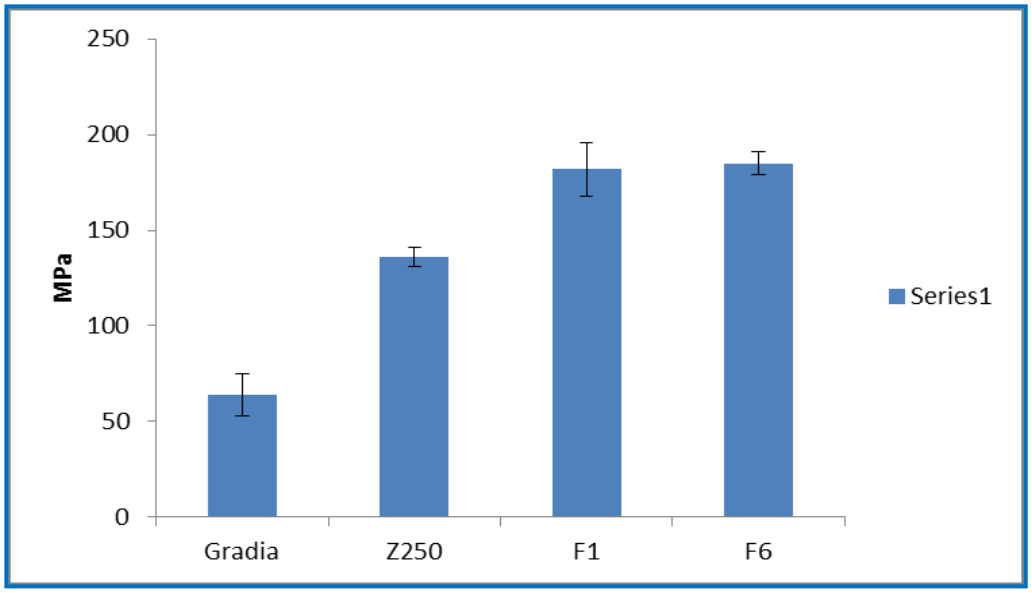


Figure 5-4 Biaxial flexural test of control group with 5% HEMA (F1) or 5% HEMA Phosphate (F6) and commercial composites after 24 hours in deionised water (series one). Errors bars shown are 95% CI, (n =8).

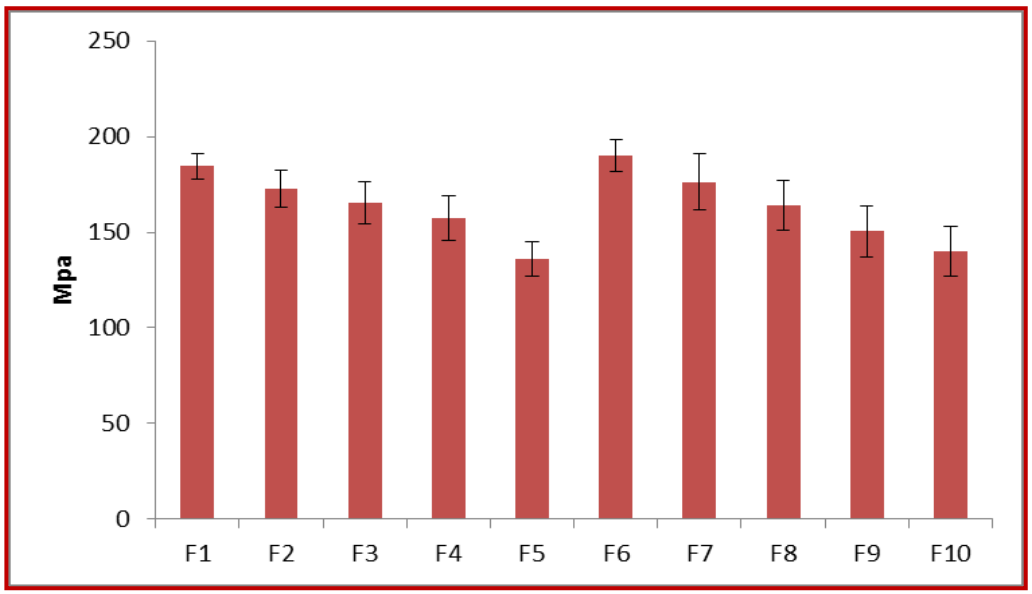


Figure 5-5 Biaxial flexural strength after 24 hours in deionised water of series two and series 3 Errors bars shown are 95% CI, (n =8)

5.2.1.1 Summary of biaxial strength

Upon immersion in deionised water there was a significant decline in strength for all experimental materials over a week ($P < 0.05$) (Table 5-3). The greatest rate of decline, however, occurred in the first 24 hours.

	HEMA	HEMA phosphate	CaP	CHX	Dry		24 h/W		1week/W	
F1	5	0	0	0	191	±5	182	±3	178	±7
F2	5	0	10	5	182	±7	172	±9	164	±4
F3	5	0	10	10	176	±4	164	±8	157	±5
F4	5	0	40	5	170	±8	157	±11	143	±6
F5	5	0	40	10	154	±6	136	±9	129	±2
F6	0	5	0	0	194	±7	185	±4	176	±3
F7	0	5	10	5	183	±6	175	±12	167	±5
F8	0	5	10	10	173	±2	165	±11	159	±6
F9	0	5	40	5	169	±3	155	±12	143	±2
F10	0	5	40	10	158	±8	131	±14	124	±3

Table 5-3 Biaxial flexure strength for series one, two and three experimental composites, before and following 24 h and 1 week immersion in deionised water. The errors represent 95% C.I of the mean (n=8).

5.2.2 Biaxial flexural modulus

In Figure 5-6 elastic modulus of the control samples are provided. Statistically, there were no significant differences comparing F1 or F6 with commercial composites or each other.

In series two and three (Figure 5-7), only F5 with both high CHX and CaP has a significantly lower modulus than the control F1. Additionally in series three only F10 is significantly different from the control F6 ($P < 0.05$)

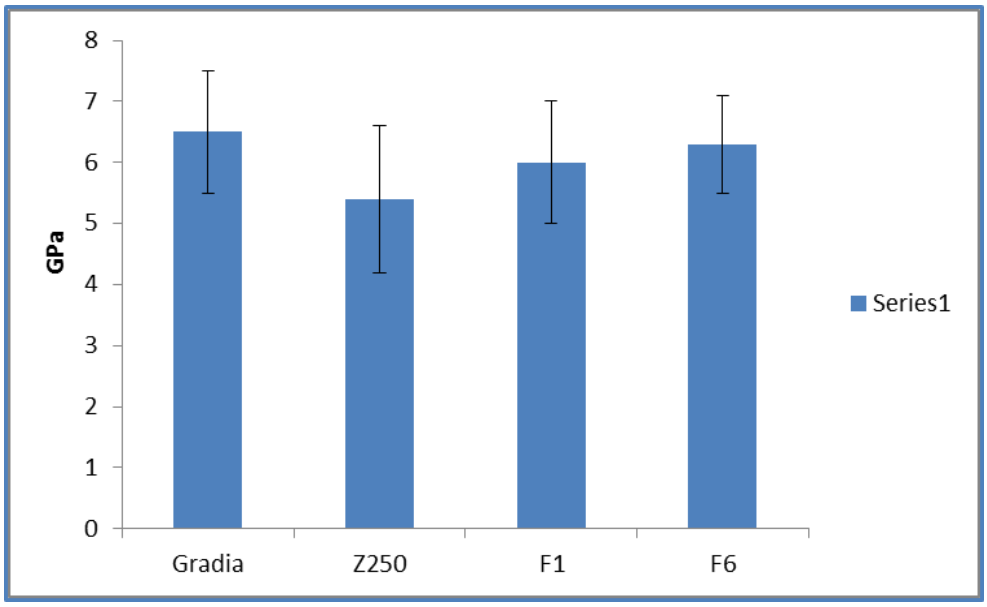


Figure 5-6 Elastic Modulus on control group without reactive filler with 4:1 PLR, 80% glass 5% HEMA,5% HEMA Phosphate experimental composite and commercial composite after 24 hours in deionised water (series one). Errors bars shown are 95% CI, (n =8).

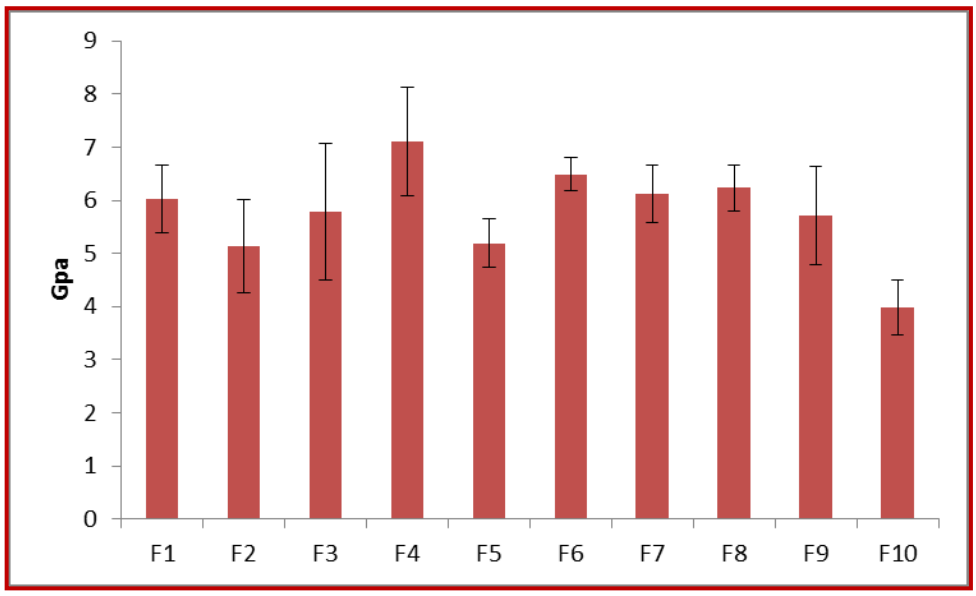


Figure 5-7 Elastic modulus of series 2 after 24 hours in deionised water. Errors bars shown are 95% CI, (n =8).

5.2.3 Toughness

In series one (Figure 5-8), both commercial composite (Gradia and Z250) had not shown any significant higher resistance to fracture than the experimental control group F1 and F6

The CaP content had the largest effect on the fracture behaviour of the material and toughness. More CaP led to a less brittle failure mode. Figure 5-9 explains the difference in the fracture behaviour of the low and high level of CaP, in series two and three. As the low CaP content formulations behave in a brittle manner at failure, the ability to carry load drops off suddenly as the material cracks. In contrast, the high level of CaP results in a non-brittle failure. However, this effect was statistically not significant, as both CaP and CHX have no effect on toughness

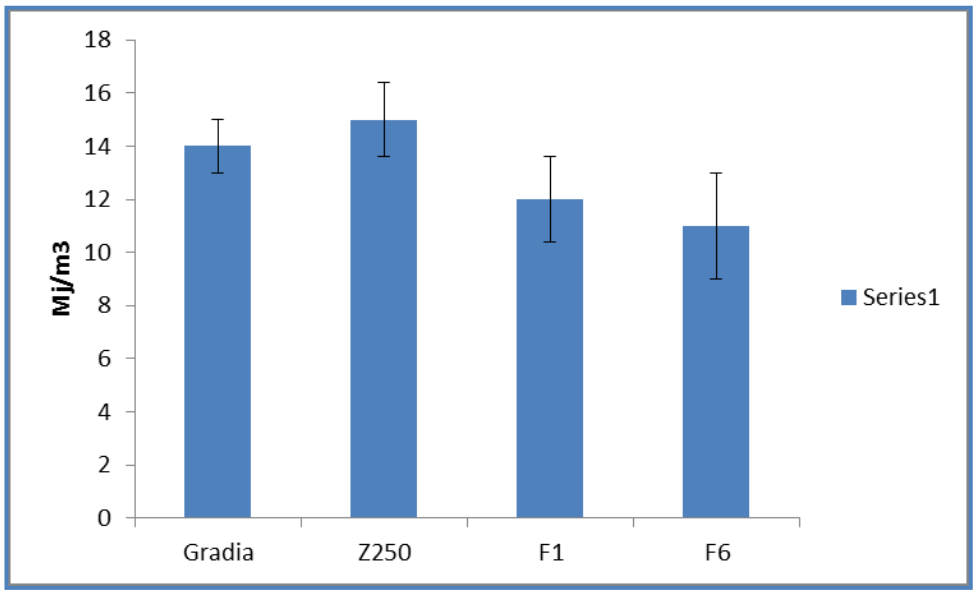


Figure 5-8 Toughness on control group without reactive filler with 4:1 PLR, 80% glass 5% HEMA,5% HEMA Phosphate experimental composite and commercial composite after 24 hours in deionised water (series one). Errors bars shown are 95% CI, (n =8).

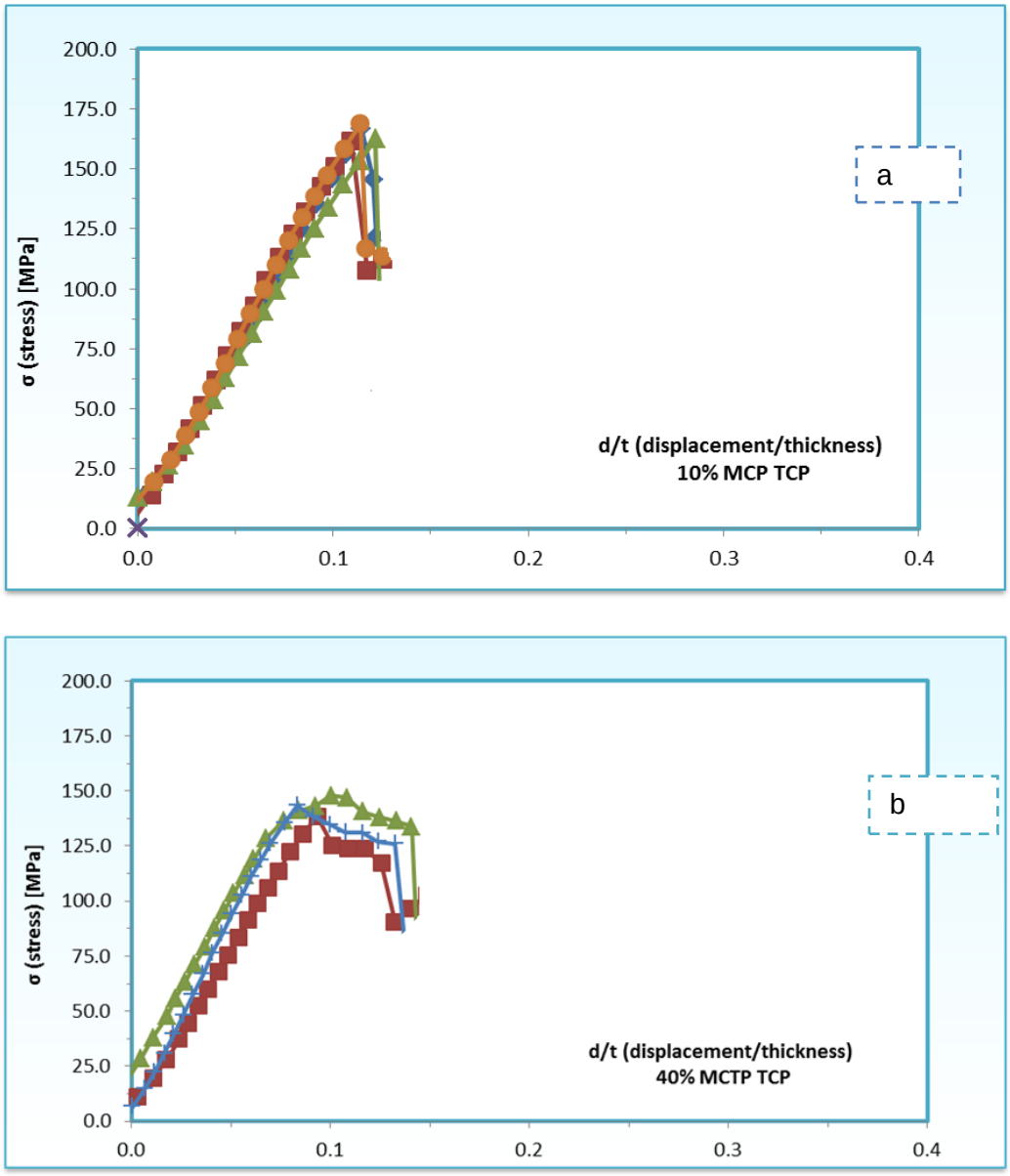


Figure 5-9 Samples with low CaP as (a) showed less area under the curve while samples with high CaP (b) showed larger area under the curve

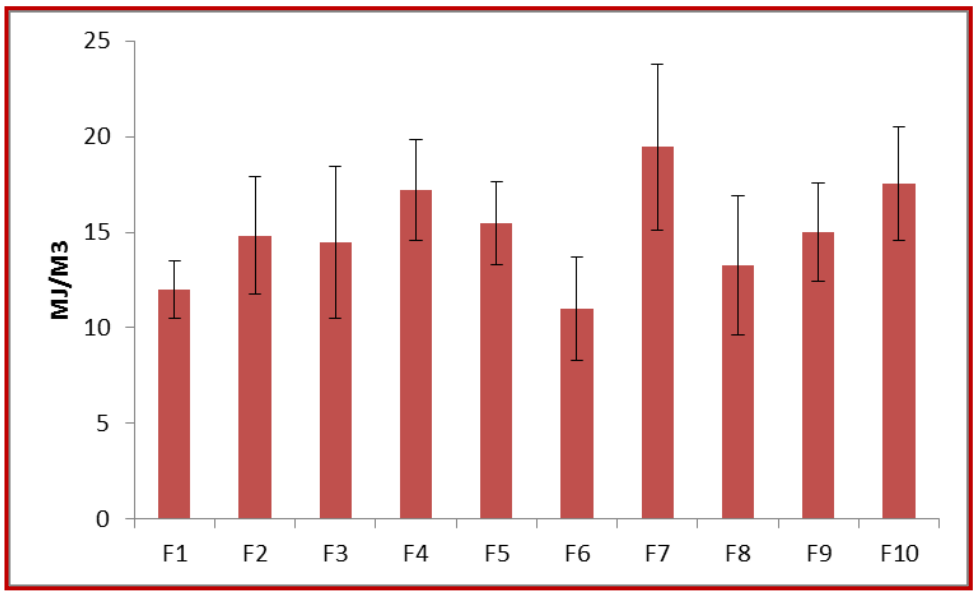


Figure 5-10 Effect of 5% HEMA, 5%, 10% CHX and 10%, 40% CaP(series two) on toughness after 24 hours in deionised water Errors bars shown are 95% CI, (n =8).

5.2.4 Modulus of resilience

With the control group (Figure 5-11); there were no significant differences between commercial and experimental composite modulus of resilience (F1, F6). Furthermore, factorial analysis showed interaction effects were larger than any effect of a variable.

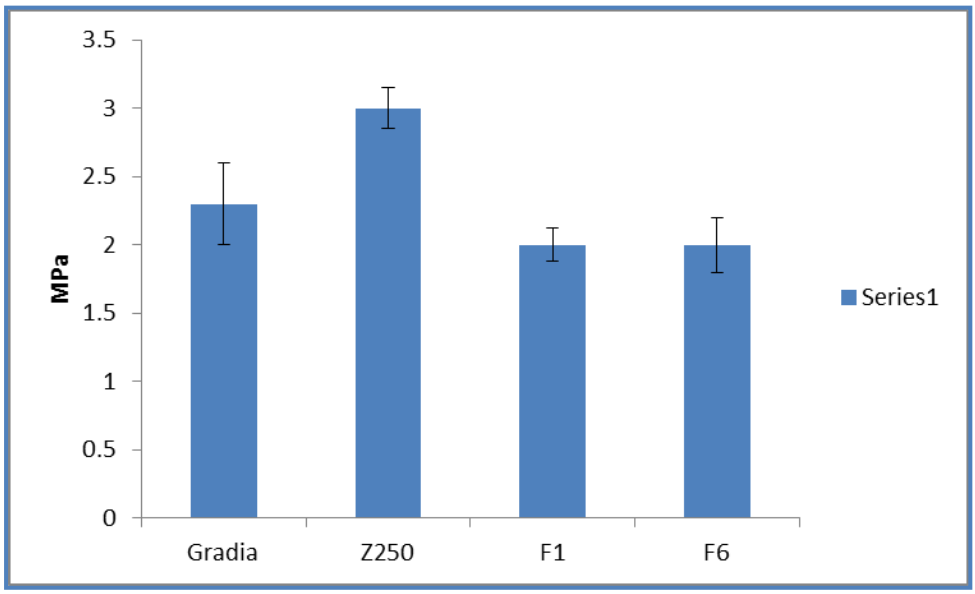


Figure 5-11 Resilience of control group without reactive filler with 4:1 PLR, 80% glass 5% HEMA,5% HEMA Phosphate experimental composite and commercial composite after 24 hours in deionised water (series one). Errors bars shown are 95% CI, (n =8).

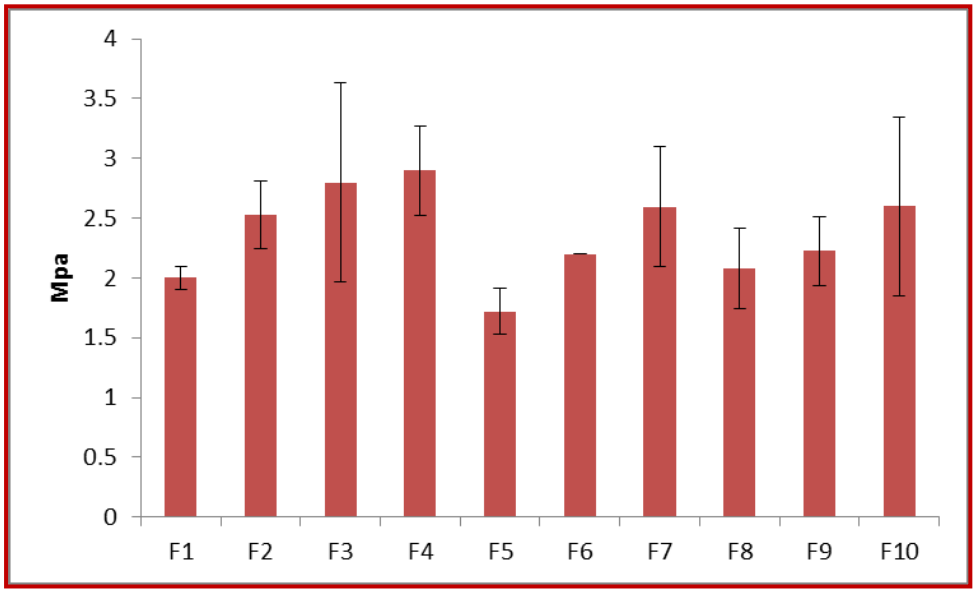


Figure 5-12 Effect of 5% HEMA, 5%, 10% CHX and 10%, 40% CaP on resilience. Errors bars shown are 95% CI, (n =8).

5.3 Chlorhexidine Release

Specimens were placed in distilled water. Storage solutions were analysed using UV spectroscopy, after 2, 4, 6, 24 hours, 48 hours, one week, two weeks, 3 weeks and 4 weeks.

5.3.1 Chlorhexidine in water

Cumulative CHX release was linear to the square root of time as expected with minor burst release in the first hours (Figure 5-13). Release rate therefore decreases with time. At week one, CHX release from F5 and F10 were 1.2 and 1.6 % respectively. At 4 weeks these numbers increased to 5.1 % and 7.2 % respectively (Figure 5-13)

Changing HEMA to HEMA phosphate, increasing CHX and increasing CaP all significantly enhanced CHX percentage release. Interaction effects were insignificant (Figure 5-14).

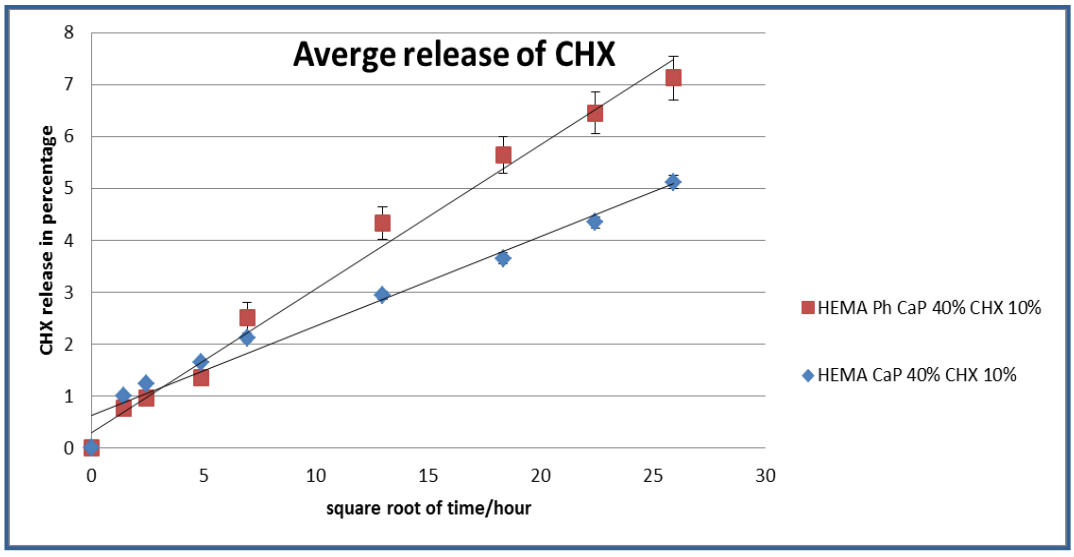


Figure 5-13 Chlorhexidine release into distilled water as a function of the square root of time/hours (SQRT) for composites F5 and F10 with 4:1 PLR, 40% wt CaP, 10% wt CHX. Errors bars shown are 95% CI, (n =5).

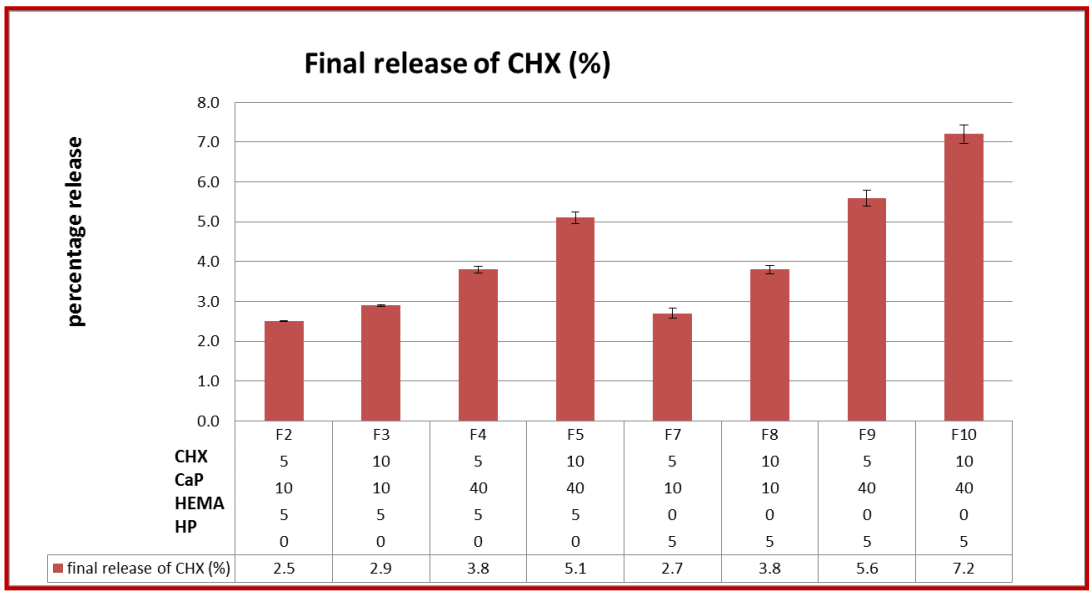


Figure 5-14 Final Chlorhexidine release in 4 weeks into distilled water 10ml with 4:1 PLR, 5% HEMA, 5%HEMA phosphate, 10%, 40%wt CaP, 5%, 10%wt CHX with experimental composite. Errors bars shown are 95% CI, (n =5)

5.4 Mass change:

Specimens were placed in distilled water and mass determined at after 2, 4, 6, 24 hours, 48 hours, one week, two weeks, 3 weeks and 4 weeks. Cumulative mass increase was linear to the square root of time as expected for a little burst increase in the first hours for both F10 and F5 (Figure 5-15). Increase rate decreases with time. Initial burst release (24hrs) was 0.6 % for both samples. At week one, F5 or F10 increased in the mass by 2.5 or 2.6 % respectively. At 4 weeks these numbers increased to 3.6 % and 3.9 % respectively (Figure 5-15)

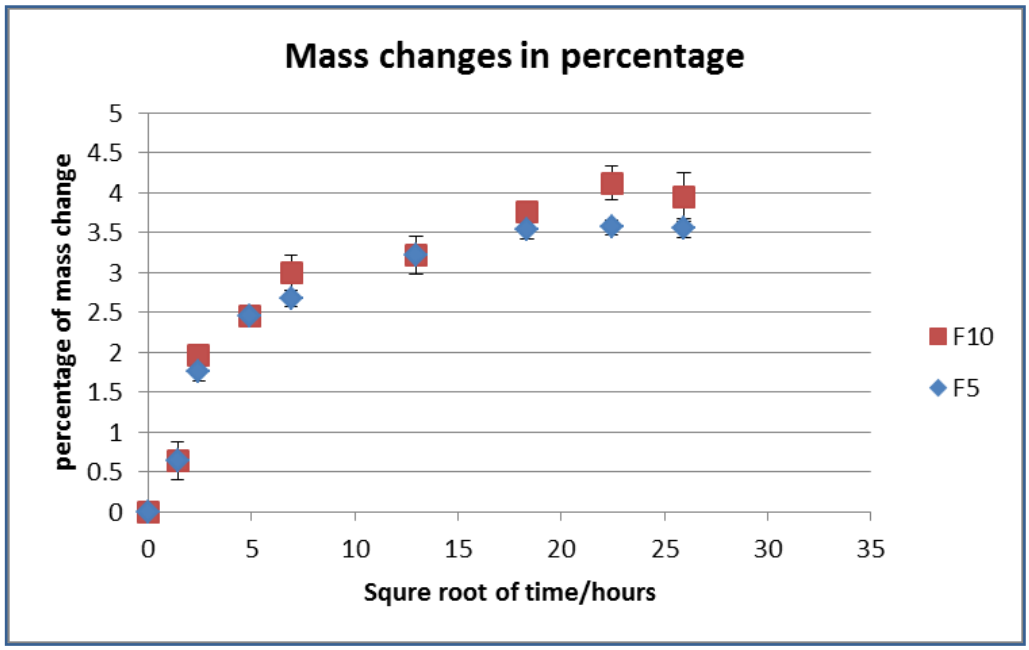


Figure 5-15 Samples mass change into distilled water as a function of the square root of time/hours (SQRT) for composites F5 and F10 with 4:1 PLR, 40% wt CaP, 10% wt CHX. Errors bars shown are 95% CI, (n =5).

5.4.1 Final mass change

Figure 5-16 shows maximum mass increase obtained by each experimental composite. These ranged from 1.2 % to 3.9 %. Changing HEMA to HEMA phosphate, increasing

CHX and increasing CaP all significantly increased final mass. Factorial analysis demonstrated that the CaP had the biggest effect.

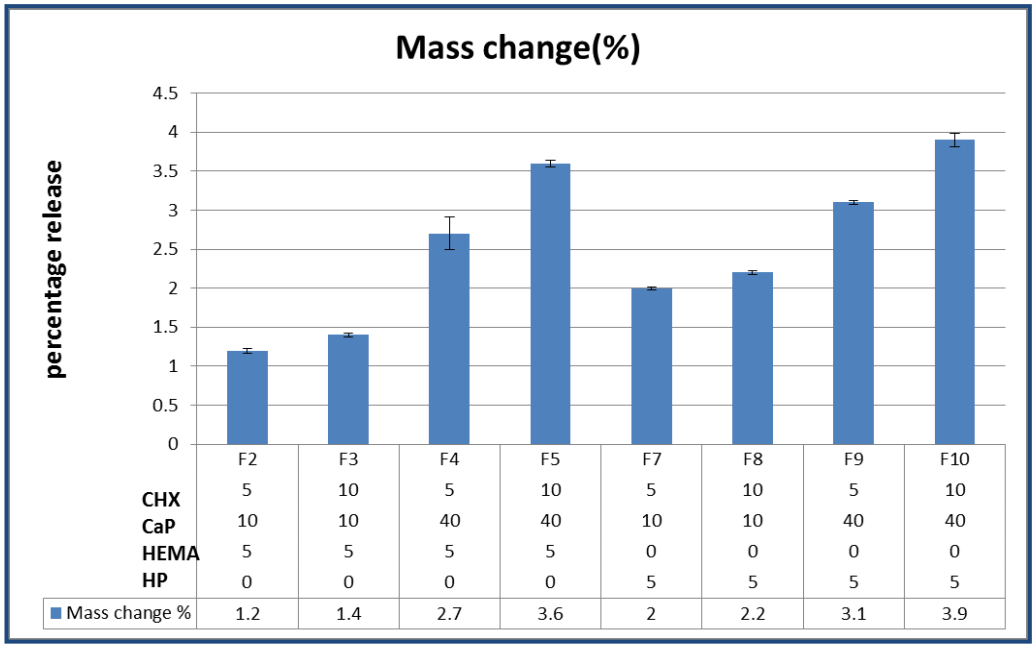


Figure 5-16 Final mass changes in 4 weeks into distilled water 10ml as a function of the square root of time (SQRT) with 4:1 PLR, 5% HEMA, 5%HEMA phosphate, 10%, 40% wt CaP, 5%, 10% wt CHX with experimental composite. Error bars shown are 95% CI, (n =5

5.5 Microstructure study of experimental composite

The top and fracture surface microstructure of the cured composite was observed under a scanning electron microscope (SEM) at different magnifications. Specimens were scanned dry and after being immersed in deionised water for different time periods.

5.5.1 Dry specimen (surface)

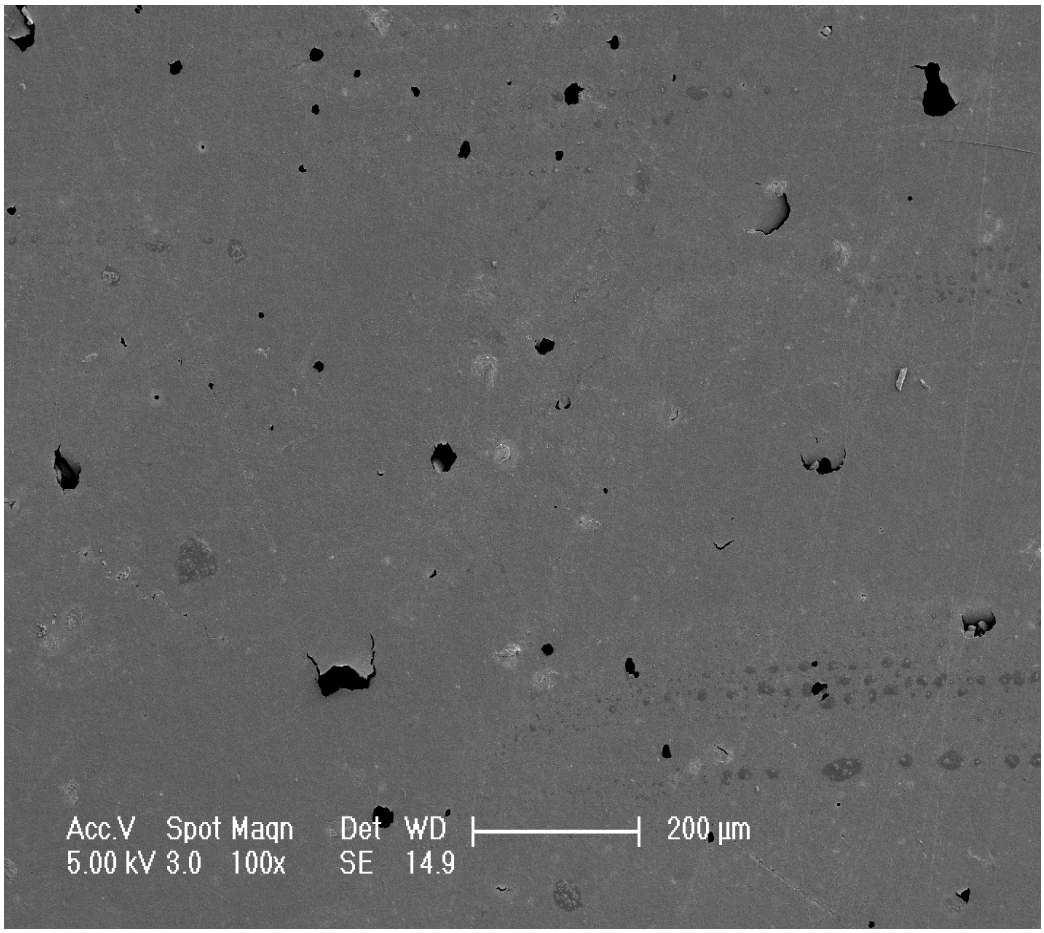


Figure 5-17 Scanning Electronic imaging of dry specimen (surface) with 4:1 PLR, 5% HEMA , 40% wt CaP, 10% wt CHX (F5)

In Figure 5-17 and 5-18 the surface and core of composite (F5) at x200 magnification showed a homogenous surface with small areas of porosity and voids. This was characteristic of all dry samples (featureless).

5.5.2 Dry specimen (Core composite)

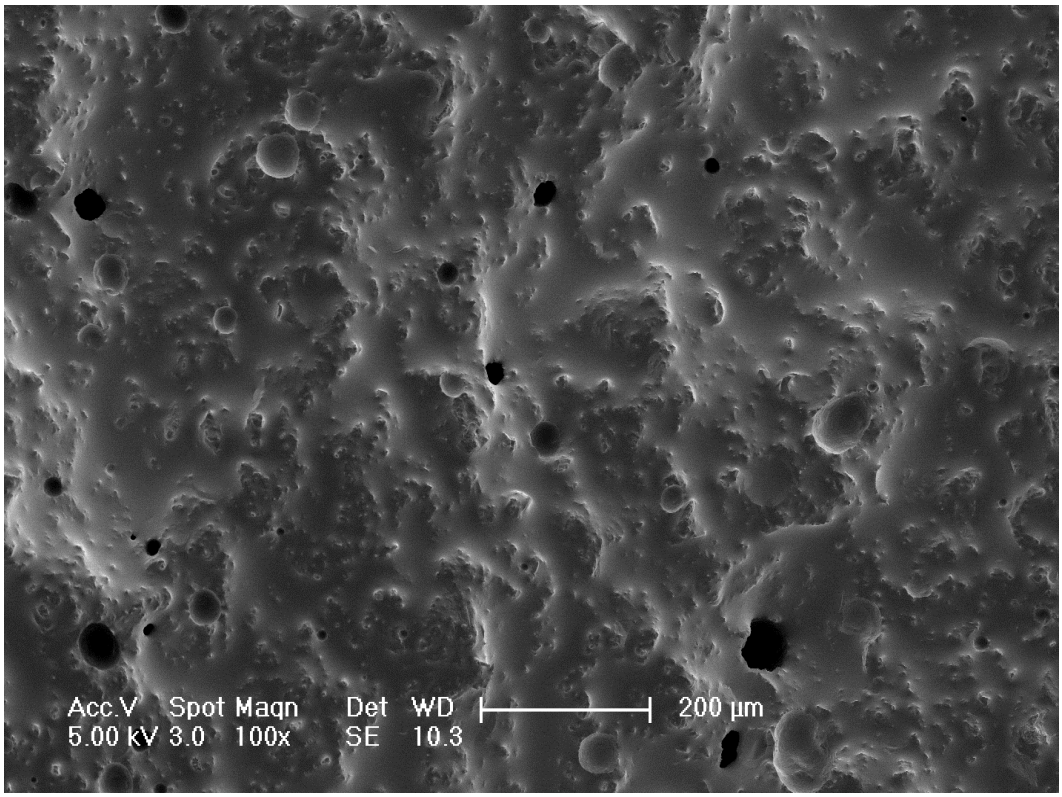


Figure 5-18 Scanning Electronic imaging of dry specimen (fractured) with 4:1 PLR, 5% HEMA , 40% wt CaP, 10% wt CHX (F5)

5.5.3 Water specimen (7 and 14 days)

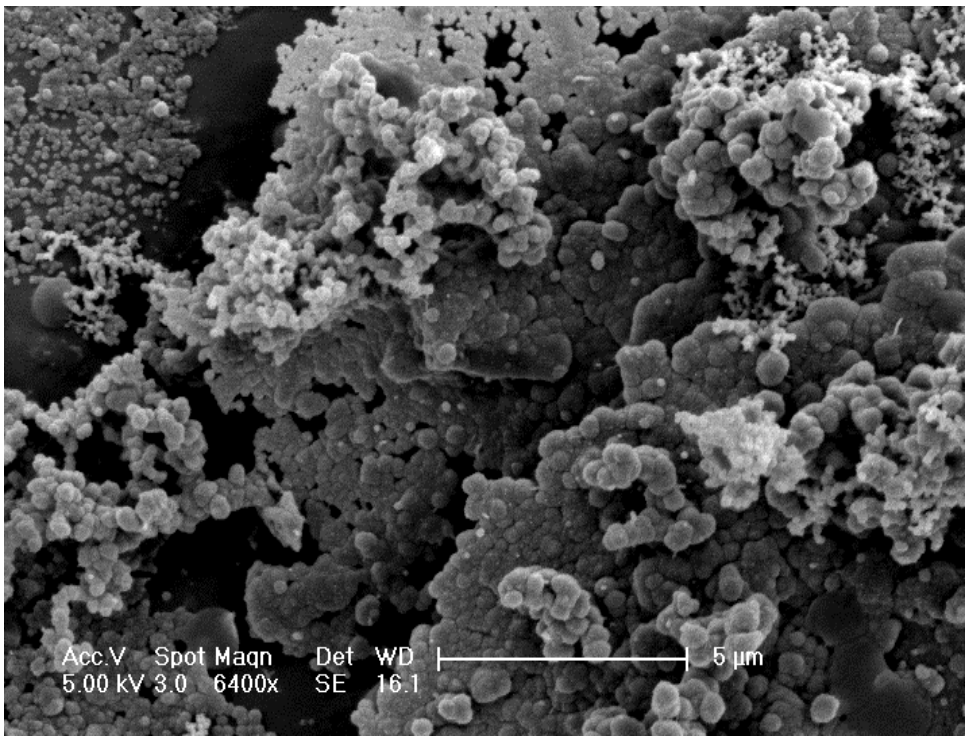


Figure 5-19 SEM image after 7 days in deionised water with 4:1 PLR, 5% HEMA , 40% wt CaP, 10% wt CHX with experimental composite

After placement in water samples F1-F4 and F6-F9 had no obvious new features by SEM. With F5 and F10, however, surfaces were largely covered by layers of spheres which looks like hydroxyapatite balls of between 0.1 µm -1µm diameter (see for example Figure 5-19). These features were more extensive at 14 days (Figure 5-20) compared with at 7 days. Additionally at 14 days some needle – like crystals could also be observed on these samples.

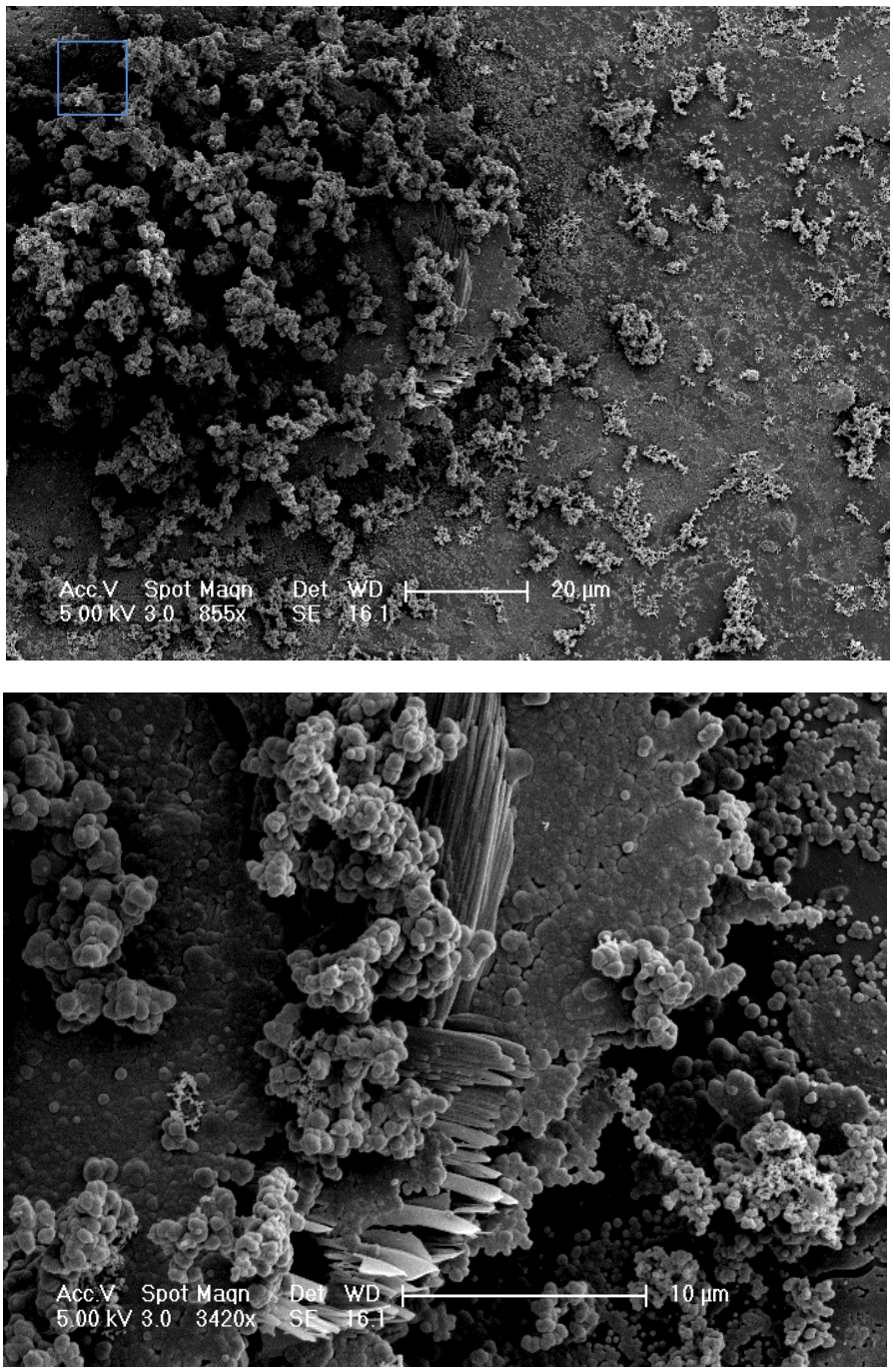


Figure 5-20 SEM image 14 days in deionised water with 4:1 PLR, 5% HEMA , 40% wt CaP, 10% wt CHX with experimental composite. This image shows layers of sharp crystals that could be Brushite and early formation of what looks like HA in (a) surface and (b) higher magnification of fractured composite

After 14 days in deionised water, the sample showed thicker layer of what looks like HA with more nucleation. In addition, this sample showed more homogeneous texture of formation of HA in some areas. Furthermore, it showed the characteristics of small crystals (needle – like appearance) of what looks like brushite (Figure 5-20)

Chapter Six: Discussion

6 Discussion

6.1 Degree of Conversion

The degree of conversion is a fundamental characteristic that affects the performance of resin based restorative materials. The physical, mechanical and biological properties of these materials generally improve with increasing degree of monomer conversion (Demarco *et al*., 2012; Rho *et al*., 2013). Studies have reported that the higher the physical and mechanical properties the less probability of material failure under masticatory loads. They also improve the efficiency of bonding at tooth/restoration interface, hence lowering the risk of bacterial microleakage and recurrent caries (Kidd 1976, Blazic *et al*., 2004; Gomec *et al*., 2005)

Furthermore, higher degree of conversion can substantially reduce the possibility of cytotoxic effects upon release of unreacted monomers which subsequently lead to post-operative hypersensitivity (Goldberg 2008; Tseng *et al*., 2007). In addition, the degree of monomer polymerisation could have an impact on the release of reactive fillers (Skrtic and Antonucci 2007); the higher the polymerisation, the lower the drug release obtained. This may be linked to the higher crosslinking with high monomer conversion (Moszner *et al*., 2005).

According to the literature reviews, both direct and indirect techniques have been used in measuring the degree of monomer polymerisation. Microhardness for example, has been used widely as an indirect measurement of the degree of monomer conversion (Santini *et al.*, 2012; Sebra *et al*., 2007). Indirect assessment has also been carried out using differential thermal calorimetry (DTC) (Sebra *et al*., 2007), and differential scanning calorimetry (DSC) (Atai *et al*. 2007; Viljanen *et al.,* 2007), However, these techniques measure relative polymerisation rate rather than the absolute degree of

conversion. In contrast, NMR, Fourier transform infrared (FTIR) and Raman spectroscopies have been commonly applied for direct quantification of monomer conversion measurement and degree of polymerisation (Ilie *et al*., 2013; Richard-Lacroix and Pellerin 2013; Wang and Spencer 2003)

In this study, high monomer conversion was obtained (Figure 5-3) due to high monomer flexibility. Average final conversion of experimental composite was as high as 72-82% compared to Z250 and Gradia which only had 50 ±2, 46 ± 3 respectively. This could prevent the leakage of monomer into the underlying pulp tissues and reduce hypersensitivity reactions or inflammation. Moreover, composite with high monomer conversion usually has better mechanical strength (Palin *et al*., 2003). Furthermore, degree of monomer conversion could have an impact on release kinetics of bioactive substances (e.g: CHX as in this study), as resin permeability and water sorption generally decrease upon raising the degree of conversion (Braga *et al*., 2005a). In contrast, Z250 had low monomer conversion while still maintained a good quality of mechanical properties. This could be due to the monomer type, which is the key in determining the final possible level of conversion. UDMA is less rigid and more flexible than Bis-GMA which is the main bulk in the monomer of Z250. This lowers the glass transition temperature (Tg) for better handling and high conversion with more cross-linked polymer at a given temperature. On the other hand, Gradia's main monomer is UDMA which is the main bulk of experimental composite in this study with the addition of silica prepolymerised filler (Watanabe *et al.*, 2008). Despite of the similarity of monomers between experimental composite and Gradia, experimental composite has higher monomer conversion. This could be due to the addition of the diluent monomer PPGDMA and HEMA to the composite.

In general, the reaction is initiated by light-activated break down of the CQ into free radicals that are stabilised by the DMPT. These initiate the formation of monomer

radicals and a chain reaction. The reaction is terminated when free radicals combine. Once the light source is removed, free radicals are no longer generated and the termination step causes the reaction to slow. Previous results suggest that CHX may stabilise the free radicals slowing the termination step. This was explained by the presence of amine groups which as in DMPT (Leung *et al.*, 2005;Mehdawi *et al*., 2013). Addition of PPGDMA and UDMA leads to crosslinked structures that may reduce molecular diffusion rates, slowing in particular the termination step thereby enhancing the overall reaction rate (Elliott *et al.,* 2001;Sharifi *et al.,* 2008).

In contrast, it was clear adding chlohexidine to the formulations will increase the level of polymerisations by (10-14) as it was reported in previous study (Leung *et al*., 2005)

In this study, there were no significant effects of HEMA and HEMA phosphate on degree of conversion as both series showed comparable results and there were no significant differences

6.2 Mechanical Behaviour

One of the aims in this study was to improve the quality of adhesive restoration without compromising the mechanical properties to withstand the stresses of the masticatory forces or residual internal stresses which occur during curing (Heo *et al.* 2013; Karmaker *et al.,* 2007; Oliveira *et al*., 2012)

According to the International organisation of standardisation (ISO) ISO4049:2009 recommendations, strength should be evaluated using a three- point bending test (uniaxial flexure strength) (Chung, Yap, Chandra, & Lim 2004;Ikejima et al., 2003). However, in this study biaxial strength has been used. This test offers many

advantages over the three-point bending test. The former test requires preparation of samples of large dimensions (25×2×2 mm^3) and therefore, multiple curing with small light sources. This leads to samples with inhomogeneous polymerisation and therefore enhanced variability of the observed results (Yamazaki *et al*., 2008) (Van *et al.,* 2007). In addition, the test is sensitive to the presence of flaws at the edges of samples. These flaws are unavoidable, in specimens with such large dimensions. The biaxial flexure strength test however, requires preparation of smaller sample size that could be cured in a single curing step. Furthermore, the test results are less dependent upon presence of flaws (Jivraj *et al.,* 2006).

In the present study, the mechanical properties of the newly developed formulations were further evaluated using biaxial flexural strength, toughness and modulus of resilience. This has previously been used with resin based restorative materials (Hatton *et al.,* 2006; Tyas 2006)

For better understanding of the effect of reactive calcium phosphate fillers and CHX on the mechanical properties, series one formulations were studied without any reactive filler. In series two and three, reactive fillers were added.

To enhance the mechanical properties of new generations of composite, different researchers have focused on silanisation of fillers (Wasson and Nicholson 1993) changing filler fraction and particles size and shape, a heat treatment, incorporation of fibers (Knight 1994;Tyas *et al*., 1989), nanoparticles (Ikejima *et al*., 2007) and ceramic whiskers (Firoozmand *et al.,* 2013; Bowen 1963; Xu *et al.,* 2002). Recently, addition of silica that has been fused onto silicon carbide or silicon nitride whiskers, was found to enhance mechanical properties whilst maintaining the ion release activity of calcium phosphate based dental composites (Xu *et al.,* 2002; Xu and Quinn 2001). The silica nanoparticles were fused to the whiskers through heat treatment. This was mainly to

facilitate their silanisation and retention in the resin matrix phase and thereby increase the mechanical properties of the composite.

In the present study, unreactive fillers are defined here as silica based materials that are largely biologically inert. The silane coupling agent used has one functional group that can adhere to the glass surface and a methacrylate group that can polymerise with the monomer. This provides a chemical bond between the polymer and filler phases in the cured material allowing loads to be transferred between the two phases – improving the strength of the composite (Yoshida *et al.*, 2002)

In current study there was a significant increase in biaxial strength of experimental composite comparing with commercial composite. This might be explained by the presence of the dilutent monomer PPGDMA and high level of conversion.

All the studied formulations additionally exhibited a much greater decline in biaxial flexure strength after 24 h of immersion in distilled water (see table 5-3). This is presumably due to the soluble MCPM encouraging high water sorption, which is considered a key factor reducing composite mechanical properties (Ortengren *et al.,* 2000). This occurs via plasticisation and disruption of the polymer matrix (Abou Neel et al., 2010; Mehdawi *et al.,* 2013; Regnault *et al.,* 2008). Filler release arising upon water sorption will also, however, be a contributory factor causing decline in strength (Mehdawi *et al.,* 2013). The lack of further deterioration in strength between 24 h and one week of water immersion is likely due to both cessation of water sorption and substantial reduction in calcium phosphate release rate during this period. Furthermore, the formation of brushite potentially binds water and reduces the amount of free water that could plasticize the polymer network.

The ability of a notched specimen to resist fracture and crack propagation is defined as fracture toughness. Thus, the fracture toughness has become an important criterion in dental materials longevity (Watanabe *et al.,* 2008). Fracture toughness is a function of several factors including crosslink density, materials homogeneity, network flexibility, molecular weight and free volume. Fracture is a component in the previously mentioned modes of failure. These values are dependent on the physical properties and chemical composition of the individual components of the restorative material (Kim *et al.*, 2000). A material which has high fracture toughness has the ability to better resist crack initiation and propagation. Thus, the property of fracture toughness becomes one of the highest recommendations in resin restoration (Kim and Okuno 2002).

Identifying the types of forces that can cause a crack is crucial to the understanding of fracture mechanics. A force or load may lead to the development of a crack and this is described, in engineering terms, in three modes: Mode I is an opening mode, Mode II is the sliding mode, and Mode III is the shearing mode (Watanabe *et al.,* 2008). What were concerned about in this experiment were Mode I and Mode II as they are related to dental materials.

One of the down sides of this study was not using the glass fibers as it has reported in several studies previously, the benefits of adding glass fibres over using the glass alone in composite preparation (Asakawa *et al.,* 2013; Chong *et al.,* 2007; Kim *et al.,* 2011; Rashidan *et al.,* 2010). However, initially the idea in the current study was not to use the fibre glass and count on formulas contained high CaP%. Assuming the addition of CaP to the experimental composite will increase the volume surface in particular β-TCP (Lopes 1998), which should create residual stresses. This will lead to a possible compressive stresses field around crack tip occurred and extra energy will be required to extend the crack, leading to the higher fracture toughness of the composites

compared to other composite without CaP reactive fillers (Lopes 1999). But this result was not significant in this current study.

6.3 Chlorhexidine Release

Increasing CaP filler mass fraction substantially enhanced the release of chlorhexidine (Figure 5-14). This could be attributed to higher water sorption upon raising filler wt%. Despite the fact that most of the water has been absorbed in the first 24 hours, the release of this drug also continued for several weeks. Brushite crystals and blades structure were formed and precipitated as it shown in SEM images. A possible explanation is that precipitation of brushite gives a composite structure with channels through the polymer that allow drug diffusion.

Early release of chlorhexidine from the experimental composite in both series (two and three) was proportional to the square root of time as expected for a diffusion-controlled process (see Figure 5-13), but with time, the amount of CHX released was less. This could be possibly because of drug being trapped in the centre bulk of the materials (Leung *et al.,* 2005). In this study, chlorhexidine release had limited effect on mass changes because its percentage mass is relatively low and it is likely to be replaced in the bulk of the material by water of similar density.

In comparison, several studies with chlorhexidine releasing composite (Leung *et al.,* 2005; Pallan *et al*., 2012; Tirali *et al.,* 2013) showed comparable results with the current experimental formulations. It showed less amount of percentage release than expected, this was between 2.5 wt% and 7.1 wt% in accumulative average in four weeks (see figure 5-13). This could be explained by the high level of polymerisation, which did not enhance drug release. Furthermore, there was one study which reported

the correlation between HEMA and CHX release increase (Leung *et al*., 2005). On raising HEMA content in the Leung study, water sorption increased, which increased CHX release. However, in current study only 5 wt% of HEMA was used, but high amount Calcium Phosphate was introduced, but this substantially decreases composite strength. In other study (Mehdawi *et al.,* 2009), the addition of reactive calcium phosphate fillers rather than increasing HEMA content could be a more appropriate approach to encourage chlorhexidine release from matrix resins, particularly with the remineralisation benefits of the calcium phosphate filler, which is relevant to what have been done in current study.

As with ion release (see above), formulations with higher water sorption exhibited faster chlorhexidine release. Increased release of chlorhexidine from methacrylate based polymers has also previously been observed upon raising resin hydrophilicity (Huang *et al*., 2002; Leung *et al.,* 2005; Mehdawi *et al.,* 2009).

Fast early release of chlorhexidine is important to eliminate residual bacteria and/or early colonisation of the gaps between a tooth and restoration and this could be particularly useful if the drug can then be trapped within such gaps. In addition, the release of chlorhexidine from new composite formulations could potentially maintain the durability of bond strength through interfering with endogenous enzymatic degradation of the hybrid layer (Hashimoto *et al.,* 2000; Hebling *et al*., 2005).

Finally, replacing HEMA with HEMA phosphate in series three had increased the CHX release as it was significant difference this could be contributed to high water sorbtion with HEMA phosphate which will eventually enhance release. The break down into phosphoric acid could cause the greater water sorption.

6.4 Mass change

It is well known that resin based materials have high a potential water sorption upon immersion in aqueous medium. In conventional materials this property is controlled predominantly by the resin phase (Boaro *et al*., 2013; Sideridou *et al*. 2004). When water is absorbed into the material structure, it can induce various changes in the physico-chemical, mechanical and biological properties. On water sorption, the resin based restorative materials will release unreacted monomers, which could induce cytotoxic effects (Reichl *et al.,* 2006) and promote growth of cariogenic bacteria. Furthermore, water sorption, if excessive, can lead to plasticization and hydrolytic degradation and a decline in the mechanical properties (Braga *et al.,* 2005; Ferracane 2006)

The adhesive formulations in the present study were developed primarily for tooth restoration. Evaluation of their behaviour in a wet environment was therefore extremely important. In addition, through water sorption studies, it was possible to understand better the chemical, mechanical and antibacterial properties of various formulations.

The mass change was measured using Archimedes' principle, which is a commonly applied method (Ruttermann *et al.,* 2007; Ruttermann *et al*., 2011). The ISO standards 4049 for assessment of resin based restorative materials indicate that, for water sorption studies the samples should be of 1 mm thickness and 15 mm diameter. To cure samples with such diameter, an overlapping light is required, which prolongs the required time of light exposure. In the current study, however, samples of 1 mm thickness and 10 mm diameter were used, which was closer to the diameter to the light tip (8 mm). They could therefore be cured in a single step. The time of storage according to ISO standards 4049 is one week compared with 4 weeks in the present study. The prolonged period was considered important to study because all studied

formulations had hydrophilic elements, which could encourage water sorption, and associated changes over a more prolonged period.

The mass increase in all studied formulations was mainly controlled by the reactive calcium phosphate (MCPM/β-TCP) filler content, followed by the chemical structure of the resin matrix. On increasing of reactive filler mass fraction both mass and volume increases were substantially enhanced and by adding hydrophilic monomers wt% HEMA/ HEMA phosphate subsequently will increase the drug release.

The maximum mass increase was faster and greater in samples which had 40% wt CaP due to higher water sorption. Despite, using two different hydrophilic monomers, there were significance differences in both series two and three (see Figure5-19). On the other hand, samples which had 10% wt Cap declined approximately by 5% in both series.

In a previous study, the average maximum mass increase was mainly affected by the percentage of CaP with decreasing the MCPM level having a greater effect than increasing the HEMA content (Mehdawi *et al.*, 2009)

Water sorption can induce mass expansion in the material, which with some restoration materials may have a benefit in reducing gaps at tooth/restoration interfaces that arise upon polymerisation shrinkage (Braga *et al.,* 2005; Oliveira *et al.,* 2012). Furthermore, the expansion could relieve the stress created by polymerisation shrinkage; thereby help maintain the integrity of the tooth/restoration interface. Therefore, the volumetric expansion could play an important role in lowering the risk of bacterial microleakage, recurrent caries and enhance longevity of the restoration. As this is one of the aims in this study, however, excessive mass expansion could result in cracking and fractures of weakened tooth structures. Expansion of some formulations in this study might be considered excessive comparing it with ISO.

6.5 Microstructure study (Scanning electronic microscope)

The gap following leakage at tooth restoration interfaces could be exposed to a continuous de- and re-mineralisation process (Gao *et al*., 2001). The acid production by cariogenic bacteria can diffuse to tooth tissue, resulting in the partial demineralisation of tooth structure. If the rate of demineralisation is higher than the rate of remineralisation, the net result is the gradual loss of mineral content that will lead to an irreversible carious cavity (Aoba 2004). On the contrary, if the rate of remineralisation or crystal growth exceeds the rate of mineral loss, the demineralised tooth structure can be retained with the re-precipitation of mineral contents (Featherstone, 2004a, 2009).

It is generally accepted that the precipitation of calcium phosphate compounds in aqueous solutions mainly include dicalcium phosphate dihydrate (DCPD or brushite), octacalcuim phosphate (OCP) and hydroxyapatite (HA) (Koutsoukos and Nancollas1981; Zapant 1981; Lu and Leng, 2005). HA is considered the most thermodynamically stable in physiological environment while OCP and DCPD have been regarded as the precursors of HA. However, DCPD precipitation rate is the highest among the calcium phosphate phases (Aoba, 2004).

The limitation of this study was that the amount of HA formation on the surface of the various dental composite formulations was not analysed or confirmed by Raman and EDX. However, in previous published data Raman spectra were used to determine the phosphate peak and confirmed the precipitation of calcium phosphate ions on similar materials to this study (Piyaphong Panpisut, 2013)

The surface of composite showed signs of early formation of what looked like hydroxyapatite. This is an unexpected finding as samples were stored in deionized

water only. In previous studies the same features were seen after storage in body stimulated fluid (BSF) (Han et al., 2013;Li *et al.,* 2006). The surface of composite (F5) showed the characteristics of small crystals (needle – like appearance) (see Figure 5-22). This is expected finding as increased acidity will lead to form brushite cement (Baroud *et al*., 2006;Bohner 2000)

When comparing the SEM images of each formulation, it was apparent that very few precipitations were seen in F2, F3, F4, F7, F8, and F9, while, F5 and F10 showed higher precipitation. A possible explanation for this might be that MCPM/TCP promotes water sorption into the dental composite, thus enhancing the release of ions. Moreover, the CHX released on the surface may also enhance the HA crystallisation process. Future studies for this investigation are therefore recommended.

Formation of HA on composite surface will enhance the remineralisation phenomena and therefore formation of reparative dentine and subsequently may decrease the sensitivity and microleakage

Chapter Seven: Conclusion & further work

7 Conclusion

Novel CHX-releasing calcium phosphate-filled methacrylate-based dental materials have been developed that could be used as a permanent filling in paediatric dentistry with high monomer conversion. These new formulations have high degree of monomer conversion, high strength that is suitable for several clinical applications. Through the addition of MCPM, water sorption by the polymerised materials is encouraged, increasing CHX release. Brushite formation within the polymer is encouraged through the further addition of β-TCP, providing a novel means to control water sorption and ion release. MCPM and β-TCP are precipitated within 24 h after placement of the polymerised formulations in water, which could be advantageous for early dentine remineralisation. This calcium phosphate was observed to re-precipitate as a mixture of brushite and hydroxyapatite on the material surface and fracture line. The level of calcium phosphate release required for remineralisation processes would depend upon the thickness of the demineralized dentine layers but could be increased by raising the amount of excess MCPM, e.g. by increasing the calcium phosphate, CHX release increased, as it was reported in previous study, it inhibited the bacteria formation on both agar plates and in biofilms (Leung *et al.,* 2005; Mehdawi *et al*., 2009; Mehdawi *et al.*, 2013) using an in vitro model for assessment of cell compatibility.

In conclusion, experimental composites containing antibacterial, remineralising and adhesion promoting components have been produced with higher flexural strengths and higher monomer conversion than commercial materials.

7.1 Further work

7.1.1 Adhesion properties of HEMA and HEMA phosphate:

Adhesion between the tooth and the composite is very important. Adhesion properties of HEMA, HEMA phosphate will be assessed through push out test. Increase adhesive properties of the composite will prevent the leakage of composite and will ensure good bonding with the dentine

7.1.2 Antibacterial studies:

Antibacterial release of different antimicrobial will be assessed through UV and HPLC. Subsequently effectiveness of antibacterial CHX will be studies through various techniques of microbiology (such as MIC, agar diffusion test, etc.) the ability of CHX to be trapped under restoration

7.1.3 Cell compatibility of CHX:

Effect of CHX on odontoblast will be assessed through cell compatibility test and compared with other commercial composite

7.1.4 Mechanical Properties:

The formulations being developed in this project require further assessment of mechanical properties for more prolonged time. In addition, other mechanical properties such as modulus of elasticity and surface hardness should also be evaluated. Furthermore, alternative approaches to improve the mechanical properties could be tried (e.g.: incorporation of glass fibres or addition of strontium compound). Increase toughness of the material is desirable in bone as it will not allow the material to break abruptly and even if small cracks appear, it can heal or the gap will be filled with remineralising CaP. Surface topography with and without fracture will be assessed

through SEM and Raman at different time period in different solution.

7.1.5 Shelf life of the monomer mixtures:

Monomer mixture will be assessed for cure profile at different period of time and temperature, thus giving us an idea of the stability of the monomer mixture at different time and temperature, particularly the HEMA phosphate

7.1.6 Microstructure study of experimental composite

Further studies needs to be done using Raman to prove the formation of hydroxyapatite in composite surface

Chapter Eight: References

8 References

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9 Appendix 1

9.1 Statistical analysis

9.1.1 Univariate Analysis of Variance for polymerisation (FTIR)

HEMA = HEMA

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	10
	1	CHX high	10
CaP_low_high	0	CaP low	10
	1	CaP high	10

a. HEMA = HEMA

Tests of Between-Subjects Effects[a]

Dependent Variable: FTIR

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	136.200[b]	3	45.400	8.486	.001
Intercept	121992.200	1	121992.200	22802.280	.000
CHX_low_high	39.200	1	39.200	7.327	.016
CaP_low_high	39.200	1	39.200	7.327	.016
CHX_low_high * CaP_low_high	57.800	1	57.800	10.804	.005
Error	85.600	16	5.350		
Total	122214.000	20			
Corrected Total	221.800	19			

a. HEMA = HEMA

b. R Squared = .614 (Adjusted R Squared = .542)

HEMA = HEMA P

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	10
	1	CHX high	10
CaP_low_high	0	CaP low	10
	1	CaP high	10

a. HEMA = HEMA P

Tests of Between-Subjects Effects[a]

Dependent Variable: FTIR

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	117.200[b]	3	39.067	19.533	.000
Intercept	124188.800	1	124188.800	62094.400	.000
CHX_low_high	88.200	1	88.200	44.100	.000
CaP_low_high	16.200	1	16.200	8.100	.012
CHX_low_high * CaP_low_high	12.800	1	12.800	6.400	.022
Error	32.000	16	2.000		
Total	124338.000	20			
Corrected Total	149.200	19			

a. HEMA = HEMA P

b. R Squared = .786 (Adjusted R Squared = .745)

T-Test

HEMA = HEMA P

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
FTIR	8.0	8	14.000	3.7796	1.3363
	10.0	8	18.625	2.1339	.7545

a. HEMA = HEMA P

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
FTIR	Equal variances assumed	.991	.336	-3.014	14
	Equal variances not assumed			-3.014	11.051

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
FTIR	Equal variances assumed	.009	-4.6250	1.5346
	Equal variances not assumed	.012	-4.6250	1.5346

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
FTIR	Equal variances assumed	-7.9163	-1.3337
	Equal variances not assumed	-8.0007	-1.2493

a. HEMA = HEMA P

T-Test

HEMA = HEMA P

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
FTIR	7.0	8	19.038	3.7842	1.3379
	9.0	8	15.250	2.3755	.8399

a. HEMA = HEMA P

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
FTIR	Equal variances assumed	2.383	.145	2.398	14
	Equal variances not assumed			2.398	11.775

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
FTIR	Equal variances assumed	.031	3.7875	1.5797
	Equal variances not assumed	.034	3.7875	1.5797

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
FTIR	Equal variances assumed	.3995	7.1755
	Equal variances not assumed	.3384	7.2366

a. HEMA = HEMA P

T-Test

HEMA = HEMA

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
FTIR	3.0	8	14.250	3.2842	1.1611
	5.0	8	16.350	2.8998	1.0252

a. HEMA = HEMA

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
FTIR	Equal variances assumed	.145	.709	-1.356	14
	Equal variances not assumed			-1.356	13.789

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
FTIR	Equal variances assumed	.197	-2.1000	1.5490
	Equal variances not assumed	.197	-2.1000	1.5490

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
FTIR	Equal variances assumed	-5.4222	1.2222
	Equal variances not assumed	-5.4270	1.2270

a. HEMA = HEMA

T-Test

HEMA = HEMA

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
FTIR	2.0	8	15.088	3.6651	1.2958
	4.0	8	15.750	1.9086	.6748

a. HEMA = HEMA

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
FTIR	Equal variances assumed	5.110	.040	-.453	14
	Equal variances not assumed			-.453	10.537

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
FTIR	Equal variances assumed	.657	-.6625	1.4610
	Equal variances not assumed	.659	-.6625	1.4610

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
FTIR	Equal variances assumed	-3.7960	2.4710
	Equal variances not assumed	-3.8954	2.5704

a. HEMA = HEMA

```
UNIANOVA CHX BY CHX_low_high CaP_low_high
  /METHOD=SSTYPE(3)
  /INTERCEPT=INCLUDE
  /SAVE=PRED RESID
  /PLOT=PROFILE(CHX_low_high*CaP_low_high)
  /CRITERIA=ALPHA(0.05)
  /DESIGN=CHX_low_high CaP_low_high CHX_low_high*CaP_low_high.
```

9.1.2 Univariate Analysis of Variance for BFS

HEMA = HEMA

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA

Tests of Between-Subjects Effects[a]

Dependent Variable: BFS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5933.943[b]	3	1977.981	11.522	.000
Intercept	814130.114	1	814130.114	4742.561	.000
CHX_low_high	1626.382	1	1626.382	9.474	.005

CaP_low_high		4098.112	1	4098.112	23.873	.000
CHX_low_high * CaP_low_high	*	209.449	1	209.449	1.220	.279
Error		4806.611	28	171.665		
Total		824870.668	32			
Corrected Total		10740.554	31			

a. HEMA = HEMA

b. R Squared = .552 (Adjusted R Squared = .505)

9.1.3 Univariate Analysis of Variance for Elastic modulus

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA

Tests of Between-Subjects Effects[a]

Dependent Variable: EM

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15.776[b]	3	5.259	4.702	.009
Intercept	1002.400	1	1002.400	896.322	.000
CHX_low_high	4.278	1	4.278	3.825	.061
CaP_low_high	3.990	1	3.990	3.568	.069
CHX_low_high * CaP_low_high	7.508	1	7.508	6.713	.015
Error	31.314	28	1.118		
Total	1049.490	32			
Corrected Total	47.090	31			

a. HEMA = HEMA

b. R Squared = .335 (Adjusted R Squared = .264)

HEMA = HEMA P

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA P

Tests of Between-Subjects Effects[a]

Dependent Variable: EM

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	30.438[b]	3	10.146	15.798	.000
Intercept	898.880	1	898.880	1399.619	.000
CHX_low_high	3.511	1	3.511	5.467	.027
CaP_low_high	20.801	1	20.801	32.389	.000
CHX_low_high * CaP_low_high	6.125	1	6.125	9.537	.005
Error	17.983	28	.642		
Total	947.300	32			
Corrected Total	48.420	31			

a. HEMA = HEMA P

b. R Squared = .629 (Adjusted R Squared = .589)

9.1.4 Univariate Analysis of Variance for Toughness

HEMA = HEMA P

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA P

Tests of Between-Subjects Effects[a]

Dependent Variable: BFS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9924.858[b]	3	3308.286	16.634	.000
Intercept	759312.453	1	759312.453	3817.711	.000
CHX_low_high	899.940	1	899.940	4.525	.042
CaP_low_high	9021.603	1	9021.603	45.359	.000
CHX_low_high * CaP_low_high	3.315	1	3.315	.017	.898
Error	5568.979	28	198.892		
Total	774806.290	32			
Corrected Total	15493.837	31			

HEMA = HEMA

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA

Tests of Between-Subjects Effects[a]

Dependent Variable: Toughness

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.508[b]	3	6.503	.717	.550
Intercept	7549.133	1	7549.133	832.553	.000
CHX_low_high	.113	1	.113	.012	.912
CaP_low_high	15.263	1	15.263	1.683	.205
CHX_low_high * CaP_low_high	4.133	1	4.133	.456	.505
Error	253.889	28	9.067		
Total	7822.530	32			
Corrected Total	273.397	31			

a. HEMA = HEMA

b. R Squared = .071 (Adjusted R Squared = -.028)

HEMA = HEMA P

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA P

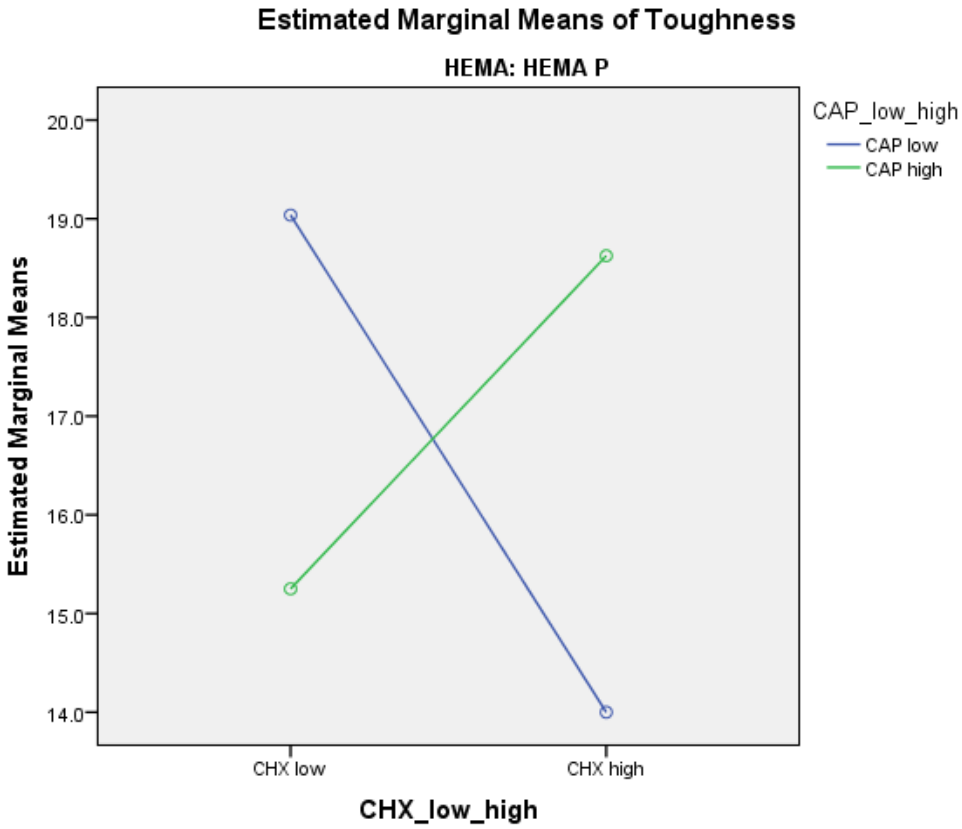
Tests of Between-Subjects Effects[a]

Dependent Variable: Toughness

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	148.471[b]	3	49.490	5.102	.006
Intercept	8954.565	1	8954.565	923.104	.000
CHX_low_high	5.528	1	5.528	.570	.457
CaP_low_high	1.403	1	1.403	.145	.707
CHX_low_high * CaP_low_high	141.540	1	141.540	14.591	.001
Error	271.614	28	9.700		
Total	9374.650	32			
Corrected Total	420.085	31			

a. HEMA = HEMA P

b. R Squared = .353 (Adjusted R Squared = .284)



HEMA = HEMA P

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
Toughness	7.0	8	19.038	3.7842	1.3379
	9.0	8	15.250	2.3755	.8399

a. HEMA = HEMA P

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Toughnes	Equal variances assumed	2.383	.145	2.398	14

s	Equal variances not assumed			2.398	11.775

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Toughness	Equal variances assumed	.031	3.7875	1.5797
	Equal variances not assumed	.034	3.7875	1.5797

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Toughness	Equal variances assumed	.3995	7.1755
	Equal variances not assumed	.3384	7.2366

a. HEMA = HEMA P

```
T-TEST GROUPS=Formula(8 10)
  /MISSING=ANALYSIS
  /VARIABLES=Toughness
  /CRITERIA=CI(.95).
```

T-Test

HEMA = HEMA P

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
Toughness	8.0	8	14.000	3.7796	1.3363
	10.0	8	18.625	2.1339	.7545

a. HEMA = HEMA P

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Toughness	Equal variances assumed	.991	.336	-3.014	14
	Equal variances not assumed			-3.014	11.051

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Toughness	Equal variances assumed	.009	-4.6250	1.5346
	Equal variances not assumed	.012	-4.6250	1.5346

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Toughness	Equal variances assumed	-7.9163	-1.3337
	Equal variances not assumed	-8.0007	-1.2493

a. HEMA = HEMA P

9.1.5 Univariate Analysis of Variance for Resilience

HEMA = HEMA

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA

Tests of Between-Subjects Effects[a]

Dependent Variable: Resilience

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.883[b]	3	2.628	8.678	.000
Intercept	171.588	1	171.588	566.647	.000
CHX_low_high	.090	1	.090	.298	.589
CaP_low_high	7.703	1	7.703	25.438	.000
CHX_low_high * CaP_low_high	.090	1	.090	.298	.589
Error	8.479	28	.303		
Total	187.950	32			
Corrected Total	16.362	31			

a. HEMA = HEMA

b. R Squared = .482 (Adjusted R Squared = .426)

HEMA = HEMA P

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	16
	1	CHX high	16
CaP_low_high	0	CaP low	16
	1	CaP high	16

a. HEMA = HEMA P

Tests of Between-Subjects Effects[a]

Dependent Variable: Resilience

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.268[b]	3	.423	1.346	.279
Intercept	171.588	1	171.588	546.349	.000

CHX_low_high	.015	1	.015	.049	.827
CaP_low_high	.090	1	.090	.288	.596
CHX_low_high * CaP_low_high	1.163	1	1.163	3.702	.065
Error	8.794	28	.314		
Total	181.650	32			
Corrected Total	10.062	31			

a. HEMA = HEMA P

b. R Squared = .126 (Adjusted R Squared = .032)

9.1.6 Univariate Analysis of Variance for CHX

HEMA = HEMA

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	10
	1	CHX high	10
CaP_low_high	0	CAP low	10
	1	CAP high	10

a. HEMA = HEMA

Tests of Between-Subjects Effects[a]

Dependent Variable: CHX

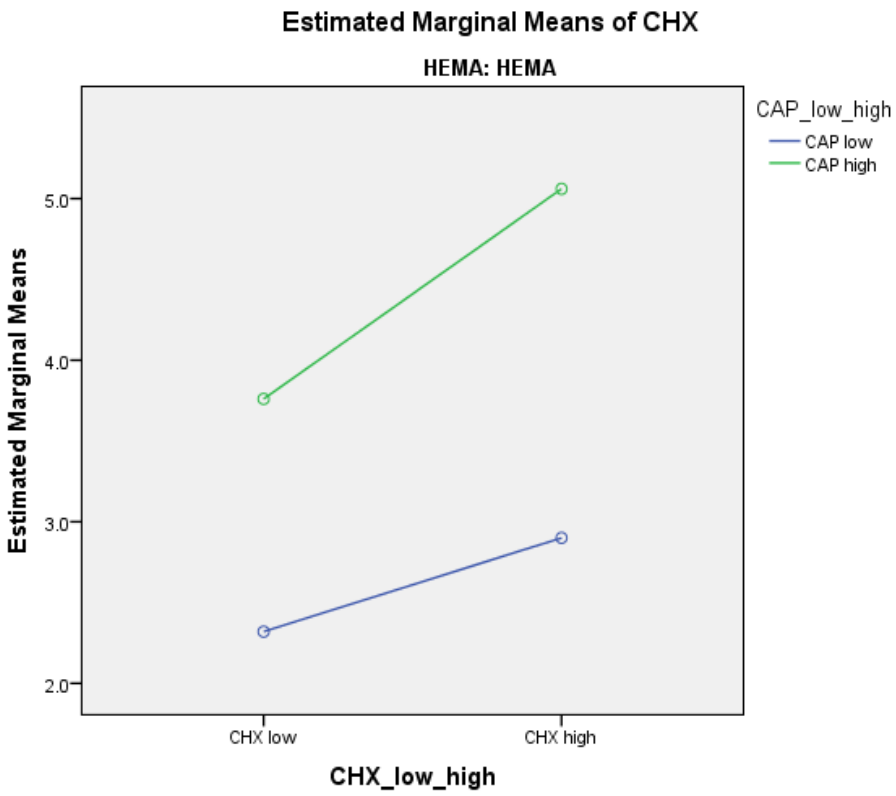
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21.266[b]	3	7.089	124.363	.000
Intercept	246.402	1	246.402	4322.842	.000
CHX_low_high	4.418	1	4.418	77.509	.000
CaP_low_high	16.200	1	16.200	284.211	.000

CHX_low_high * CaP_low_high	.648	1	.648	11.368	.004
Error	.912	16	.057		
Total	268.580	20			
Corrected Total	22.178	19			

a. HEMA = HEMA

b. R Squared = .959 (Adjusted R Squared = .951)

Profile Plots



HEMA = HEMA P

Between-Subjects Factors[a]

	Value Label	N

CHX_low_high	0	CHX low	10
	1	CHX high	10
CaP_low_high	0	CaP low	10
	1	CaP high	10

a. HEMA = HEMA P

Tests of Between-Subjects Effects[a]

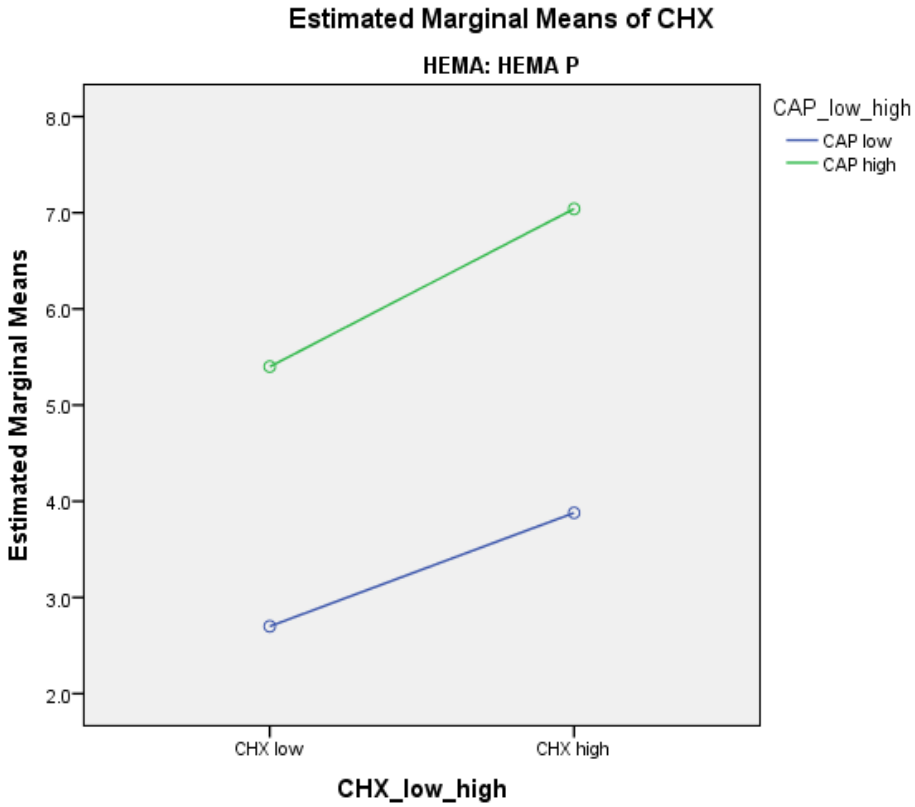
Dependent Variable: CHX

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	53.130[b]	3	17.710	272.459	.000
Intercept	452.201	1	452.201	6956.931	.000
CHX_low_high	9.941	1	9.941	152.931	.000
CaP_low_high	42.925	1	42.925	660.377	.000
CHX_low_high * CaP_low_high	.265	1	.265	4.069	.061
Error	1.040	16	.065		
Total	506.370	20			
Corrected Total	54.170	19			

a. HEMA = HEMA P

b. R Squared = .981 (Adjusted R Squared = .977)

Profile Plots




```
T-TEST GROUPS=Formula(2 4)
  /MISSING=ANALYSIS
  /VARIABLES=Toughness
  /CRITERIA=CI(.95).
```

T-Test

HEMA = HEMA

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
CHX	2.0	8	15.088	3.6651	1.2958
	4.0	8	15.750	1.9086	.6748

a. HEMA = HEMA

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
CHX	Equal variances assumed	5.110	.040	-.453	14
	Equal variances not assumed			-.453	10.537

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
CHX	Equal variances assumed	.657	-.6625	1.4610
	Equal variances not assumed	.659	-.6625	1.4610

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
CHX	Equal variances assumed	-3.7960	2.4710
	Equal variances not assumed	-3.8954	2.5704

a. HEMA = HEMA

```
T-TEST GROUPS=Formula(3 5)
  /MISSING=ANALYSIS
  /VARIABLES=Toughness
  /CRITERIA=CI(.95).
```

T-Test

HEMA = HEMA

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
CHX	3.0	8	14.250	3.2842	1.1611
	5.0	8	16.350	2.8998	1.0252

a. HEMA = HEMA

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
CHX	Equal variances assumed	.145	.709	-1.356	14
	Equal variances not assumed			-1.356	13.789

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
CHX	Equal variances assumed	.197	-2.1000	1.5490
	Equal variances not assumed	.197	-2.1000	1.5490

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
CHX	Equal variances assumed	-5.4222	1.2222
	Equal variances not assumed	-5.4270	1.2270

a. HEMA = HEMA

```
UNIANOVA MASS BY CHX_low_high CAP_low_high
  /METHOD=SSTYPE(3)
  /INTERCEPT=INCLUDE
  /SAVE=PRED RESID
  /PLOT=PROFILE(CHX_low_high*CAP_low_high)
  /CRITERIA=ALPHA(0.05)
  /DESIGN=CHX_low_high CAP_low_high CHX_low_high*CAP_low_high.
```

9.1.7 Univariate Analysis of Variance for Mass change

HEMA = HEMA

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	10
	1	CHX high	10
CaP_low_high	0	CaP low	10
	1	CaP high	10

a. HEMA = HEMA

Tests of Between-Subjects Effects[a]

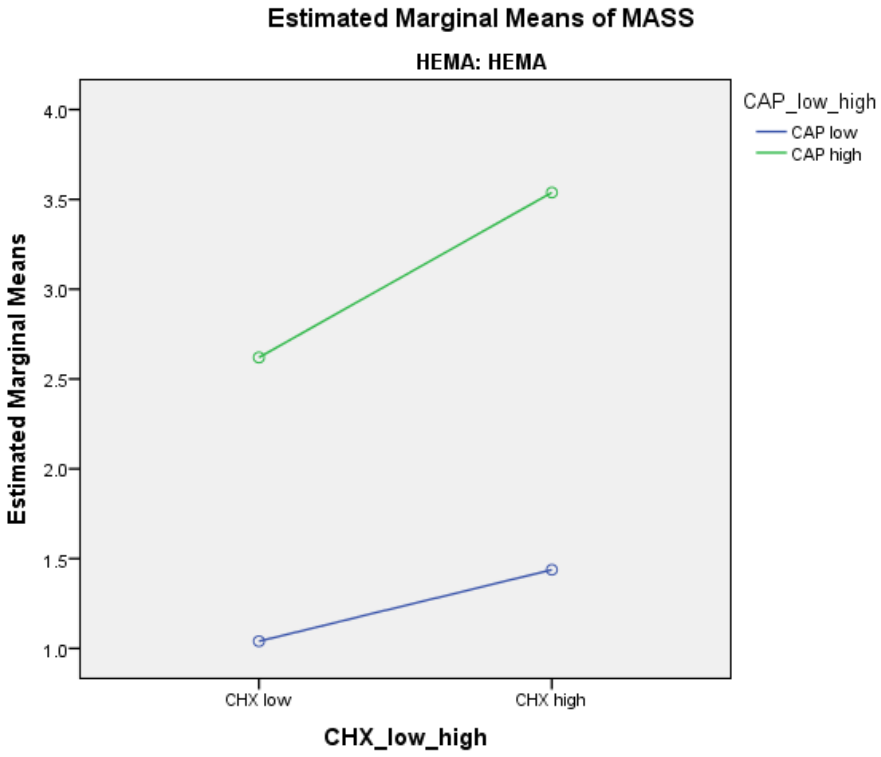
Dependent Variable: MASS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.431[b]	3	6.477	212.463	.000
Intercept	93.226	1	93.226	3058.082	.000
CHX_low_high	2.165	1	2.165	71.013	.000
CaP_low_high	16.928	1	16.928	555.289	.000
CHX_low_high * CaP_low_high	.338	1	.338	11.087	.004
Error	.488	16	.030		
Total	113.144	20			
Corrected Total	19.919	19			

a. HEMA = HEMA

b. R Squared = .976 (Adjusted R Squared = .971)

Profile Plots



HEMA = HEMA P

Between-Subjects Factors[a]

		Value Label	N
CHX_low_high	0	CHX low	10
	1	CHX high	10
CaP_low_high	0	CaP low	10
	1	CaP high	10

a. HEMA = HEMA P

Tests of Between-Subjects Effects[a]

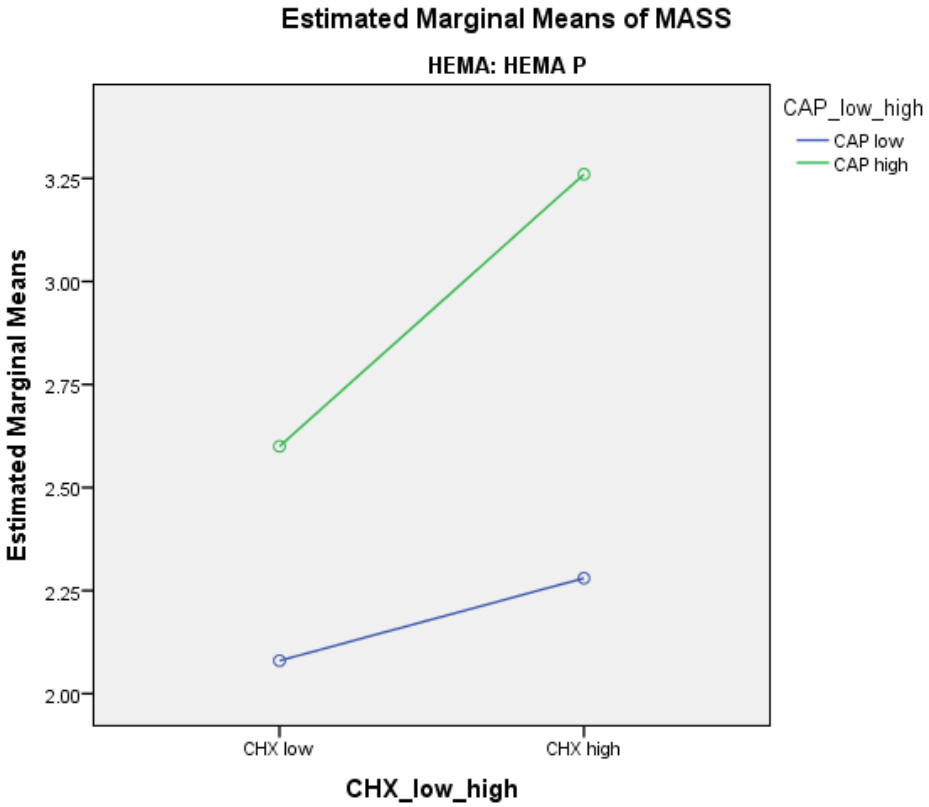
Dependent Variable: MASS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.002[b]	3	1.334	37.573	.000
Intercept	130.561	1	130.561	3677.761	.000
CHX_low_high	.925	1	.925	26.042	.000
CaP_low_high	2.813	1	2.813	79.225	.000
CHX_low_high * CaP_low_high	.265	1	.265	7.451	.015
Error	.568	16	.036		
Total	135.130	20			
Corrected Total	4.570	19			

a. HEMA = HEMA P

b. R Squared = .876 (Adjusted R Squared = .852)

Profile Plots


Estimated Marginal Means of MASS
HEMA: HEMA P
CAP_low_high
CAP low
CAP high
Estimated Marginal Means
3.25
3.00
2.75
2.50
2.25
2.00
CHX low
CHX high
CHX_low_high

T-Test

HEMA = HEMA

Group Statistics[a]

Formula	N	Mean	Std. Deviation	Std. Error Mean
3.0	8	14.250	3.2842	1.1611
5.0	8	16.350	2.8998	1.0252

a. HEMA = HEMA

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Mass change	Equal variances assumed	.145	.709	-1.356	14
	Equal variances not assumed			-1.356	13.789

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mass change	Equal variances assumed	.197	-2.1000	1.5490
	Equal variances not assumed	.197	-2.1000	1.5490

Independent Samples Test[a]

	t-test for Equality of Means
	95% Confidence Interval of the Difference

		Lower	Upper
Mass change	Equal variances assumed	-5.4222	1.2222
	Equal variances not assumed	-5.4270	1.2270

a. HEMA = HEMA

```
T-TEST GROUPS=Formula(2 4)
  /MISSING=ANALYSIS
  /VARIABLES=Toughness
  /CRITERIA=CI(.95).
```

T-Test

HEMA = HEMA

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
Mass change	2.0	8	15.088	3.6651	1.2958
	4.0	8	15.750	1.9086	.6748

a. HEMA = HEMA

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Mass change	Equal variances assumed	5.110	.040	-.453	14
	Equal variances not assumed			-.453	10.537

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mass change	Equal variances assumed	.657	-.6625	1.4610
	Equal variances not assumed	.659	-.6625	1.4610

Independent Samples Test[a]

	t-test for Equality of Means

		95% Confidence Interval of the Difference	
		Lower	Upper
Mass change	Equal variances assumed	-3.7960	2.4710
	Equal variances not assumed	-3.8954	2.5704

a. HEMA = HEMA

```
T-TEST GROUPS=Formula(8 10)
  /MISSING=ANALYSIS
  /VARIABLES=Toughness
  /CRITERIA=CI(.95).
```

T-Test

HEMA = HEMA P

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
Mass change	8.0	8	14.000	3.7796	1.3363
	10.0	8	18.625	2.1339	.7545

a. HEMA = HEMA P

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Mass change	Equal variances assumed	.991	.336	-3.014	14
	Equal variances not assumed			-3.014	11.051

Independent Samples Test[a]

	t-test for Equality of Means

		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mass change	Equal variances assumed	.009	-4.6250	1.5346
	Equal variances not assumed	.012	-4.6250	1.5346

Independent Samples Test[a]

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Mass change	Equal variances assumed	-7.9163	-1.3337
	Equal variances not assumed	-8.0007	-1.2493

a. HEMA = HEMA P

```
T-TEST GROUPS=Formula(7 9)
  /MISSING=ANALYSIS
  /VARIABLES=Toughness
  /CRITERIA=CI(.95).
```

T-Test

HEMA = HEMA P

Group Statistics[a]

	Formula	N	Mean	Std. Deviation	Std. Error Mean
Mass change	7.0	8	19.038	3.7842	1.3379
	9.0	8	15.250	2.3755	.8399

a. HEMA = HEMA P

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
Mass	Equal variances assumed	2.383	.145	2.398	14

change	Equal variances not assumed			2.398	11.775

Independent Samples Test[a]

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
Mass change	Equal variances assumed	.031	3.7875	1.5797
	Equal variances not assumed	.034	3.7875	1.5797

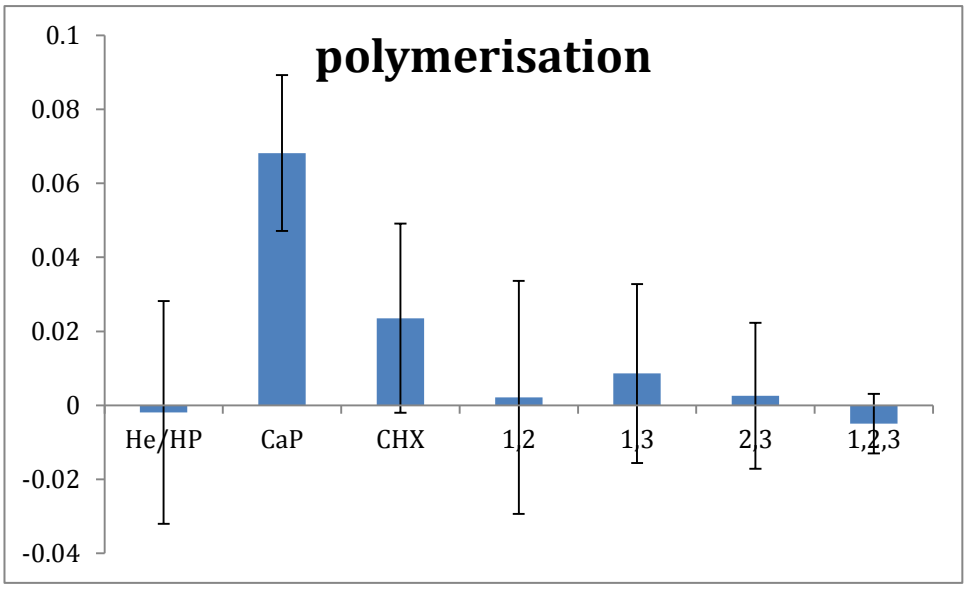
Independent Samples Test[a]

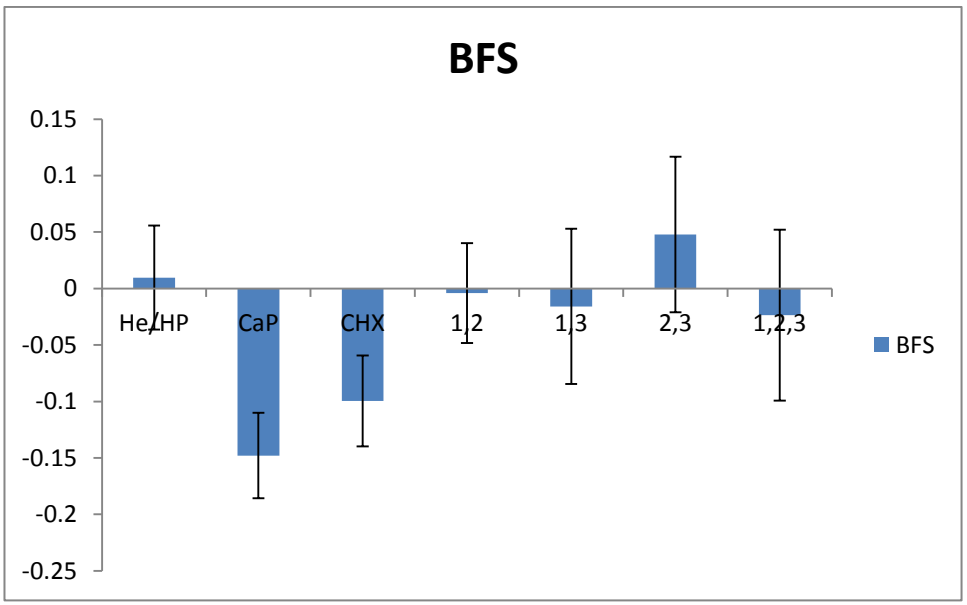
		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Mass change	Equal variances assumed	.3995	7.1755
	Equal variances not assumed	.3384	7.2366

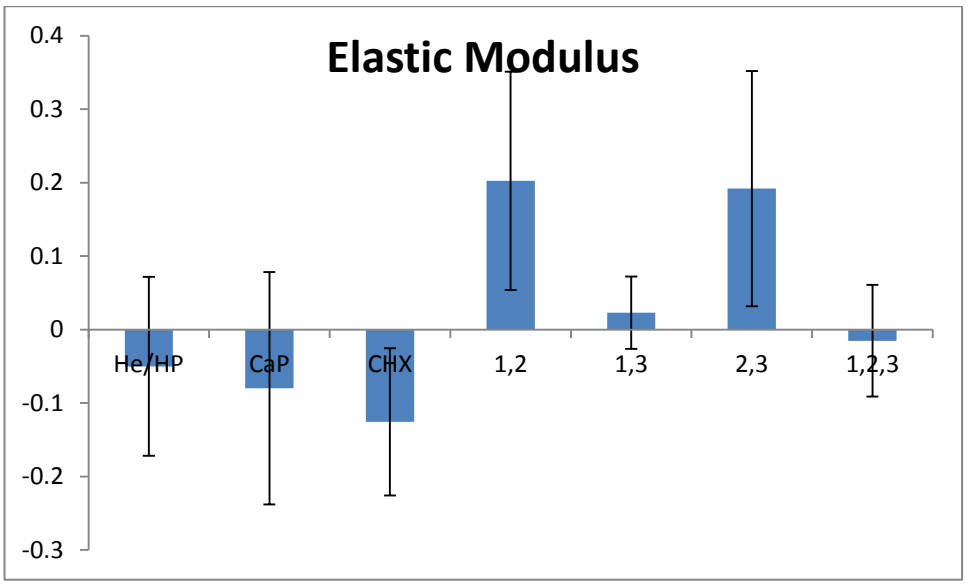
a. HEMA = HEMA P

10 Appendix 2

10.1 Factorial design analysis






Elastic Modulus
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0.3
0.2
0.1
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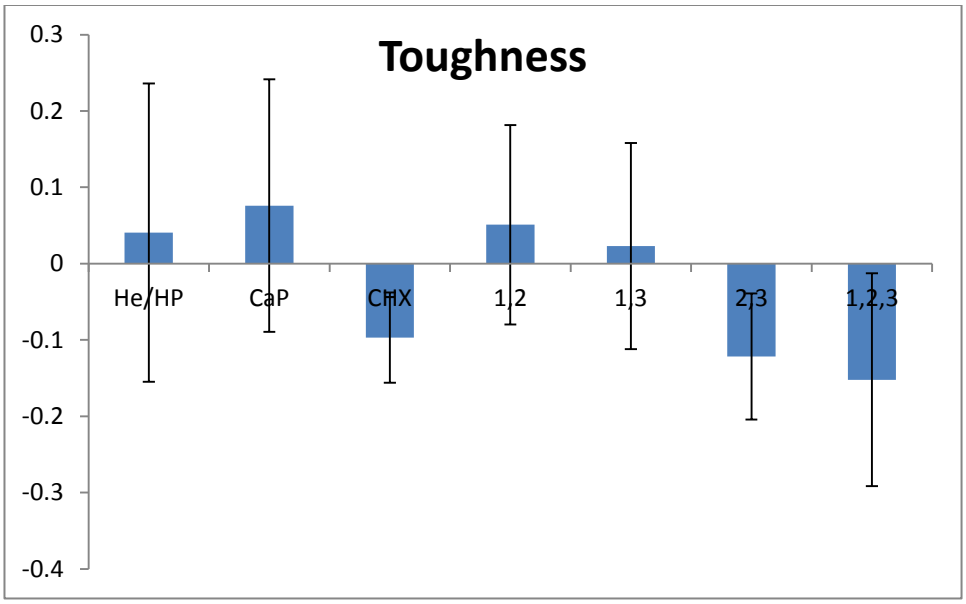
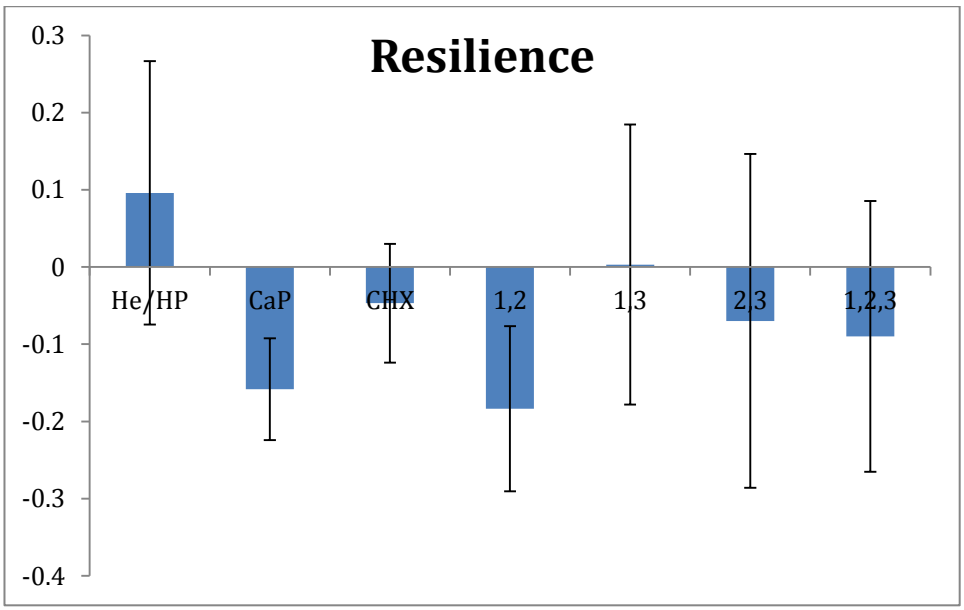
-0.2
-0.3
He/HP
CaP
CHX
1,2
1,3
2,3
1,2,3


Toughness
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
He/HP
CaP
CHX
1,2
1,3
2,3
1,2,3


Resilience
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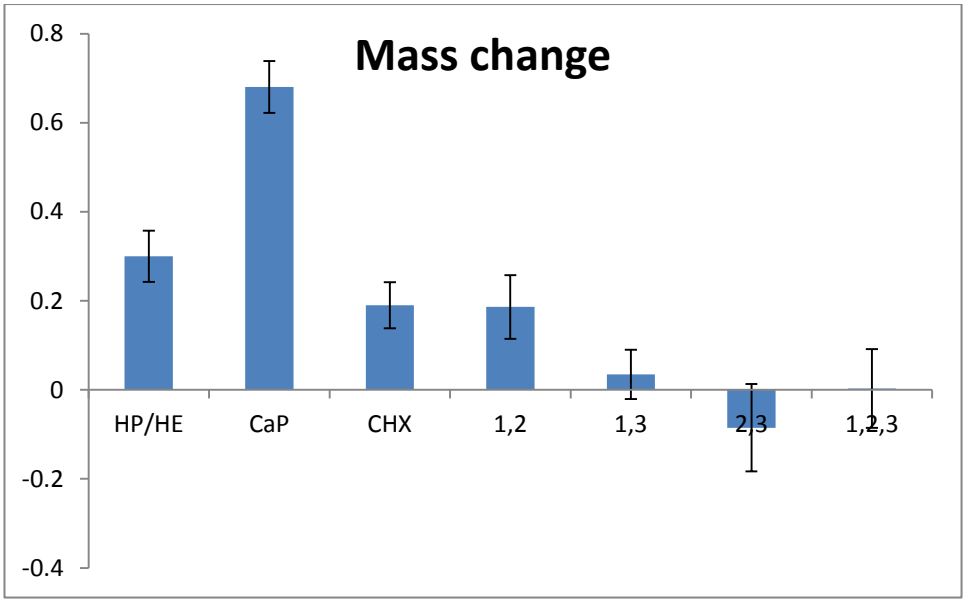
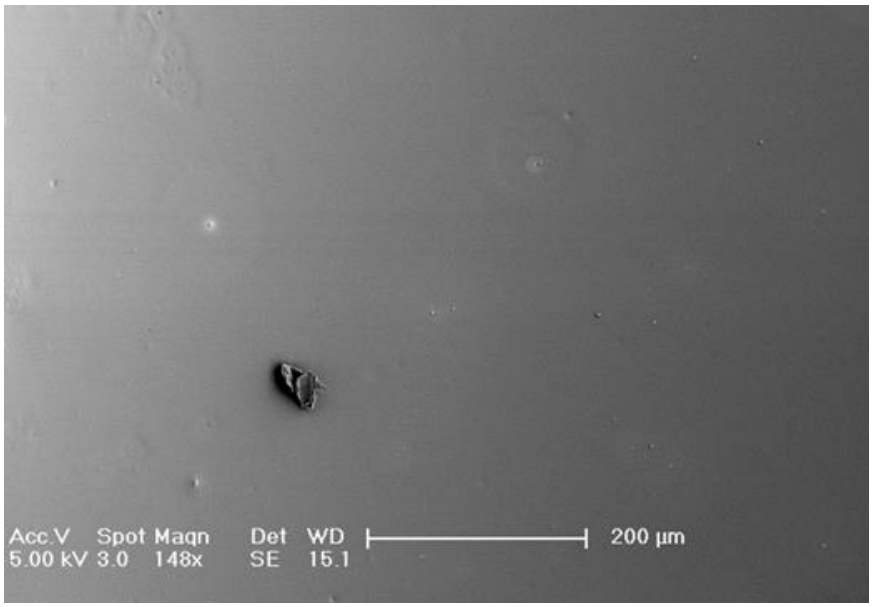
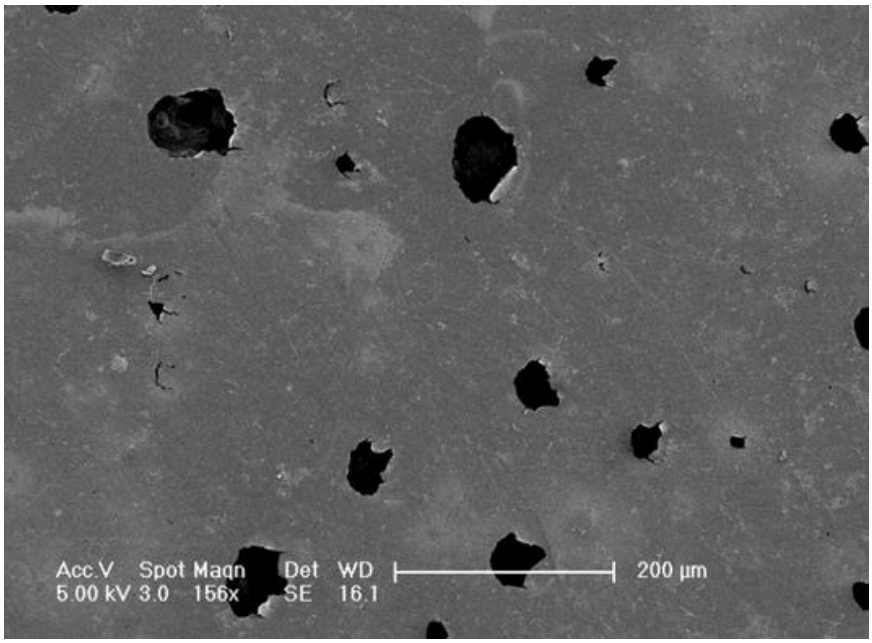
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
He/HP
CaP
CHX
1,2
1,3
2,3
1,2,3

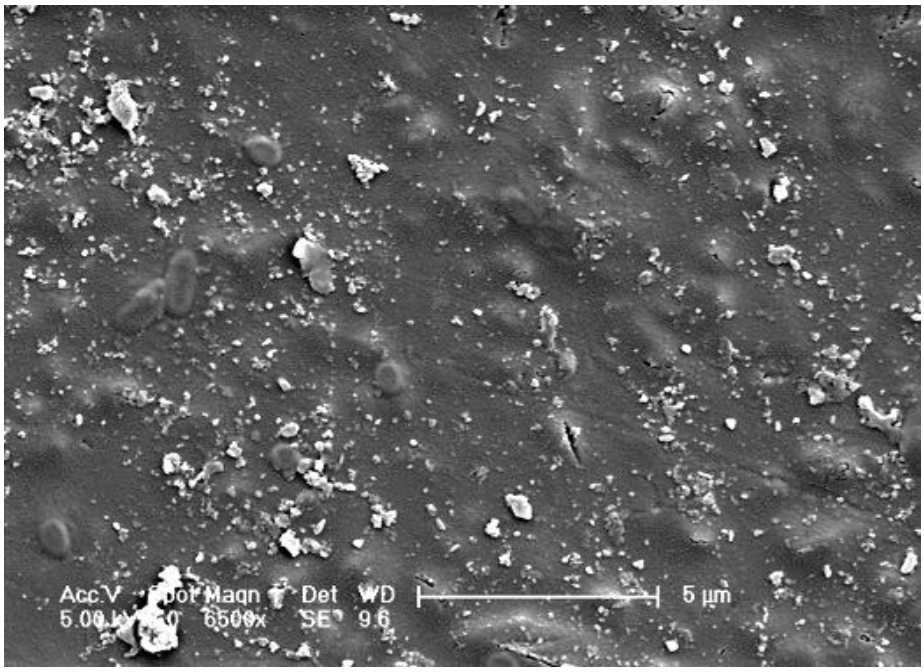

Mass change
0.8
0.6
0.4
0.2
0
-0.2
-0.4
HP/HE
CaP
CHX
1,2
1,3
2,3
1,2,3

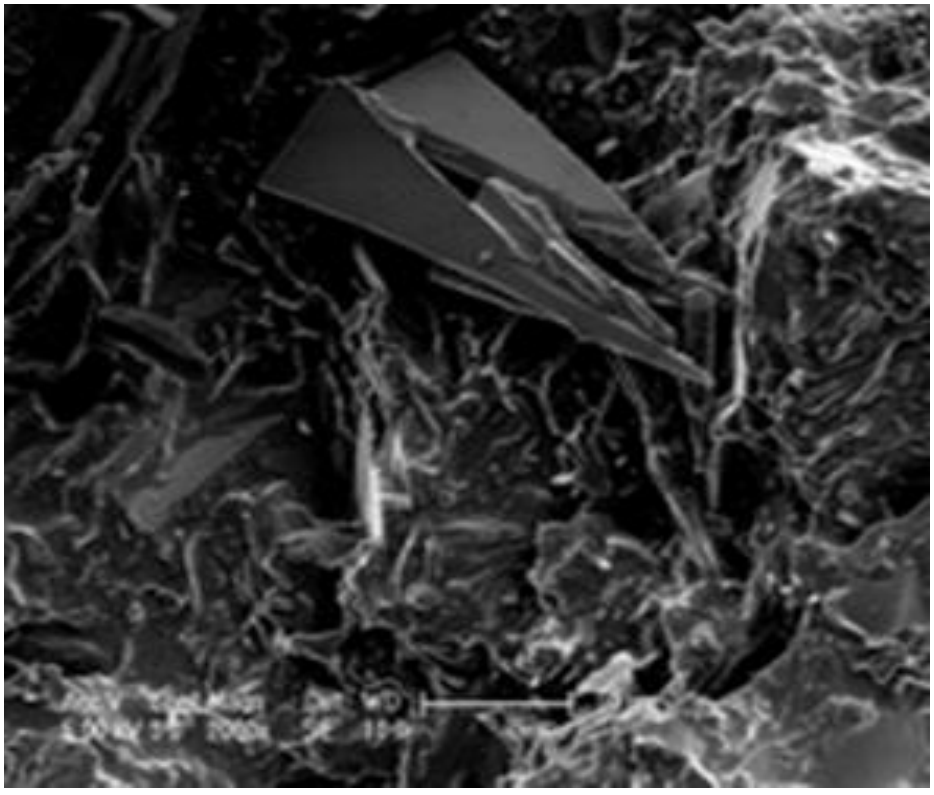
11 Appendix 3

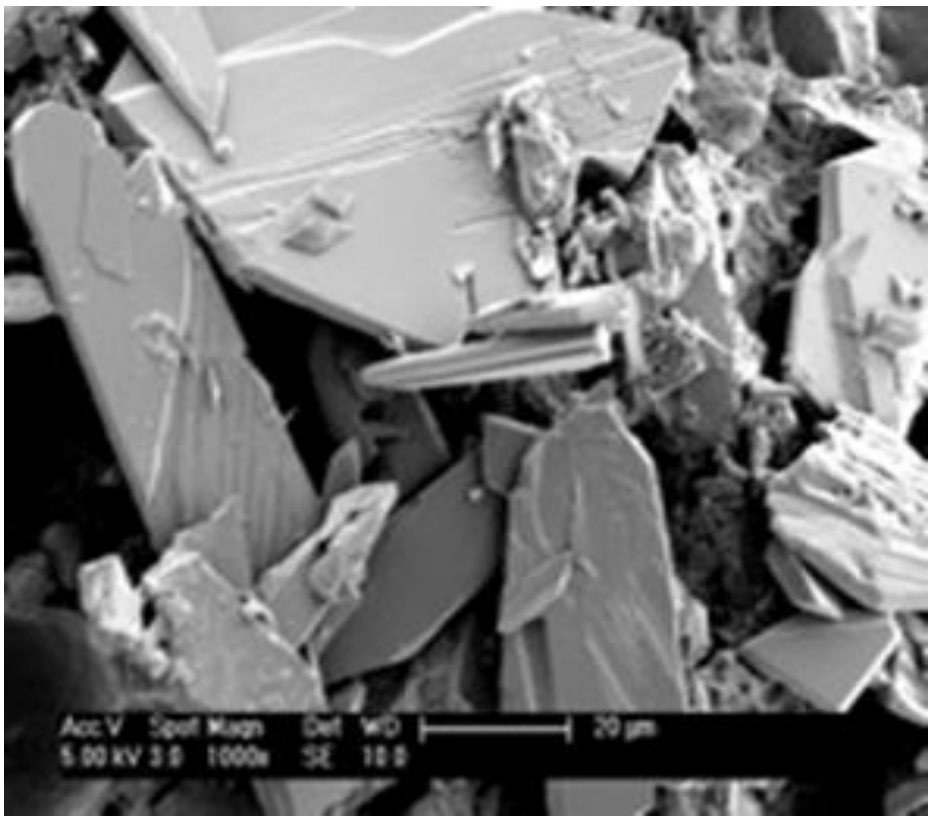
11.1 SEM images






Acc.V Spot Magn Det WD
5.00 kV 3.0 6500x SE 9.6
5 µm

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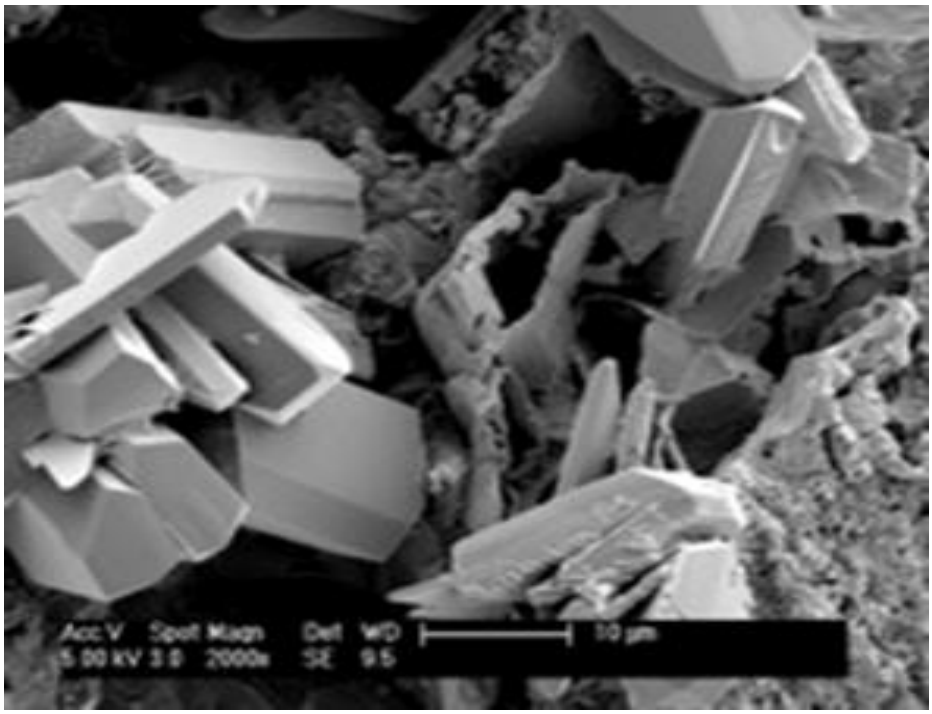
5.00 kV 3.0 1000x SE 10.0


Acc.V Spot Magn Det WD 10 µm
5.00 kV 3.0 2000x SE 9.5

Re Audit of Follow-up Procedures for Patients who Did Not Attend Appointments in the Paediatric Department at Eastman Dental Hospital, University College London

Abeer Alshami

Supervised by:

Adèle Johnson

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List of Abbreviations

Word or phrase	Abbreviation
Did not attend	DNA
University College Hospital	UCH
General medical practitioner	GMP
General dental practitioner	GDP
Community dental services	CDS
Hospital dental services	HDS
British Society of Paediatric Dentistry	BSPD
New patients	NP
General Anaesthesia	GA
Decayed, missed, and filled teeth	DMFT
British Dental Association	BDA
Behaviour management	BM
Medical problems/special needs	med/spec
Orthodontics	ortho
Gingival problems	gingival

Abstract

Background:

Attending dental appointment it's very important for patient, dentist and hospital. Failure to attend dental appointment (DNA) may have a negative impact on child's health and my raise safeguarding issues and concerns. Beside these concerns failing to attend dental appointment has been considered as a waste of the National Health Services (NHS) financial resources. So it was the need to establish a pathway to ensure all patients are followed up within the department of paediatric dentistry in Eastman Dental Hospital.

Aim: To establish robust procedures to ensure that all patients who DNA their appointments are not lost to follow up.

Standard: The standard set for this audit was 100% of patients should be followed up.

Method: A six month, retrospective audit of patients who DNA'd appointments within the department of paediatric dentistry. Patients were divided into new patients (NP), treatment/follow up (Tx/FU), and general anaesthesia (GA) groups.

1 Background

1.1 Introduction

Dental caries is one of the common diseases afflicting mankind, and has been estimated to affect up to 40% of UK adults. The UK government spends millions every year on the prevention and treatment of dental caries. 175 million pounds has been spent on the placement of direct restoration by the NHS in both England and Wales since the 90's (Dental Practice Board, 1996).

Caries adversely affects and Dental caries is probably one the most common chronic diseases; although it has affected humans since prehistorical times. The prevalence of this disease has increased due to the dietary change that occurs on human life style but now there is evidence of that this trend peaked and began to decline in certain segments of the population of Western Europe, New Zealand, Unites State, and Australia. The main cause of the decrease is addition of the fluoride to toothpastes and it's attributed to the addition of trace amount of fluoride ion to public drinking water. This declined in the developed countries has been prominent in the upper classes and middle classes, while the lower socioeconomic and rural classes have retained a high prevalence of tooth decay(Kidd and Fejerskov C35-C38;Kidd et al. 8-13).

progressively destroys the tissues of the tooth, including the dental pulp, leaving teeth unsightly, weakened and with impaired function (Marya et al. 41-45;Davies et al. 5-11).

Dental caries has been studied extensively during the last 60 years in both Europe and North America in which studies have been very useful in determination of extent of the need for, and effectiveness of, dental treatment. The most common epidemiological measure of caries is the DMF; this is a measure of the number of teeth that are diseased, missing, or filled. Since 1973, national surveys of dental caries in children, one series directed decennially by the Office of Population Censuses and Surveys and another, using analogous methods, coordinated regionally on a regular basis by the British Association for the Study of Community Dentistry, have produced a comprehensive record of trends in caries experience of children in England and Wales (Davies et al. 5-11;Kidd et al. 8-13). Between 1973 and 1993 a decline in caries experience of 55 per cent in deciduous teeth of 5-year-old children, 75 per cent in permanent teeth of 12-year-old, and 74 per cent in 14-year-old children, was documented.

Data from the NHS Dental Epidemiology Programme for England (British Association for the Study of Community Dentistry) indicates that the mean number of decayed, missing or filled primary teeth (dmft) in 5-years-old in great Britain is 1.11 (2007-2008), and DMFT of permanent teeth in 11 year old in England and Wales is 7.4 (2008 – 2009). In the UK, only 14% of teeth with dentine decay in 5 years old are restored (NHS DEP) and if caries is not treated affected teeth are likely to become non-vital and may require extraction. Number of children who required hospital admissions for caries treatment rose by 66% between 1997 and 2006 (Moles and Ashley E14-E19). Therefore, it can be seen that attending dental appointments is considered an important aspect of maintaining child's health.

In 1998 a study in Sweden founded that the mean DMFT of children who missed dental appointment at the age of 18 was significantly higher than those who attended their appointments in the same age group (Skaret et al., 1998).
According to the National Diet and Nutrition Survey it was proven that Dental attendance patterns were vary with age, social class, and educational qualifications. (Hinds and Gregory, 1995) Preschool children of all ages were more likely to be taken to the dentist if they came from non-manual households; their mothers had secondary education qualifications and were themselves regular dental attenders. Other factors which have been associated with broken appointments include; patient dentist relationship, length of time between appointments, time and day of the week, weather conditions, urgency of the appointment and patient satisfaction. It has also been observed that patient who fail to attend appointments have shown the same behavior in the past (Almog et al., 2003).

There were so many studies investigating the reasons why parents failed to take their children to attend dental appointment, among these studies there was a study carried out in Sweden which concluded the reasons in to 1) being overloaded in daily life this was especially a problem for single parents where they felt overwhelmed by the amount of work and responsibilities, and they also complained that they were too busy to even remember to call and cancel an appointment. 2) Lack of dental healthcare traditions where parents themselves did not consider oral health care important and did not attend check-ups, therefore dental care for children fell low in priority. 3) A lack of trust in the dental healthcare system where parents felt that the dental team showed no empathy or understanding when scheduling appointments. 4) A lack of parental confidence where parents found it difficult and challenging to persuade their children to

attend dental appointments. (Hallberg et al., 2008). A study was conducted in the Eastman Dental Centre, in New York, to compare a new automated appointment confirmation system with manual confirmation strategies. The new system allowed patient details to be uploaded a day before the appointments were scheduled for, and then generated an automated, pre-recorded, interactive voice message, which allowed parents to either confirm, cancel, or leave a voice mail for the clinician. The techniques were conducted over a period of twelve months, and results showed a significant drop in the percentage of patients who failed to attend (Almog et al., 2003). By introducing this method to the appointment system a better patients attendance out comes will be obtained and better performances.

According to the British Dental Association patient failing to attend was a real problem and waste for hospital resources, it has been showed that dentists in England had a loss which was equivalent of two weeks a year due to patients not attending appointments. Therefore there was a suggestion of charging patients with a fee for not attending their appointments, to decrease the rate of failed appointments. But this action was highly criticised. (British Dental Association, 2011).

1.2 Safeguarding and Neglect

Dental neglect is defined, as "the persistent failure to meet a child's basic oral health needs, likely to result in the serious impairment of a child's oral or general health or development" (Haris et al., 2009). While dental neglect is considered a more focused type of neglect, it is occasionally an early indicator of a broader general neglect. Although failing to attend dental appointments is *not* an immediate indicator of dental neglect, its persistence may raise suspicions.

Signs and symptoms of maltreatment may be either physical or psychological. These may be observed on the child himself, or in the parent-child-interaction. These effects have both short and long term impacts on the child's health, development and well-being. Psychological effects may lead to anxiety, depression, anti-social behaviours', drug abuse, and may affect inter-personal relationships as well. While physical maltreatment may result in scarring or lifelong disability, which in extreme cases may be fatal (NICE clinical guideline, 2009).

It is the responsibility of every person who interacts with children to be able to recognize the signs and symptoms of child abuse and maltreatment, from receptionists, nurses, therapists, to dentists. Physical signs of maltreatment include,

bruises in areas of the body not common to have bruises in children, such as behind the ears, on the cheeks, or in young children who are not mobile, multiple bruises or bites, bone fractures when there is no medical reason (NICE clinical guideline, 2009).

When it comes to identifying dental neglect, dental and *non*-dental findings should be taken into consideration. Caries is a very common disease as already mentioned, with prevalence of 39.5% in 5-year olds in Great Britain (British Association for the Study of Community Dentistry, 2006), but it is also known that the disease is multi-factorial, and that the disease level is *not* necessarily an indication of maltreatment. Other factors which contribute to the level of decay in children go beyond just frequency of sugary intake, poor oral hygiene, bacteria level, but also lack of parental knowledge regarding the disease and its aetiology, parents own fear and anxiety of the dentist which therefore prevents them from taking their children for dental care. Furthermore, these factors may be intensified by poverty and/or stress (Haris et al., 2009).

According to NICE guidelines, "Consider neglect if parents or carers fail to administer essential prescribed treatment for their child, if parents or carers repeatedly fail to attend essential follow-up appointments that are necessary for their child's health and wellbeing, if parents or carers have access to but persistently fail to obtain NHS treatment for their child's dental caries (tooth decay), and if parents or carers fail to seek medical advice for their child to the extent that the child's health and wellbeing is compromised, including if the child is in ongoing pain" (NICE clinical guideline, 2009).

The Children's Act 1989 states "Whether neglect is willful or not, it is essential to remember that the welfare of the child is the paramount consideration" (HM Government, 1989). In 2006 it was reported, "The primary aim of intervention is not to blame the family, but to ensure that children receive the support needed to safeguard their welfare. A feature of particular concern is the failure of parents to respond to offers of acceptable and appropriate treatment" (American Academy of Paediatrics Committee on Child Abuse and Neglect, 2006, Harris et al., 2006).

When dental neglect has been identified, there are 3 recommended strategies of

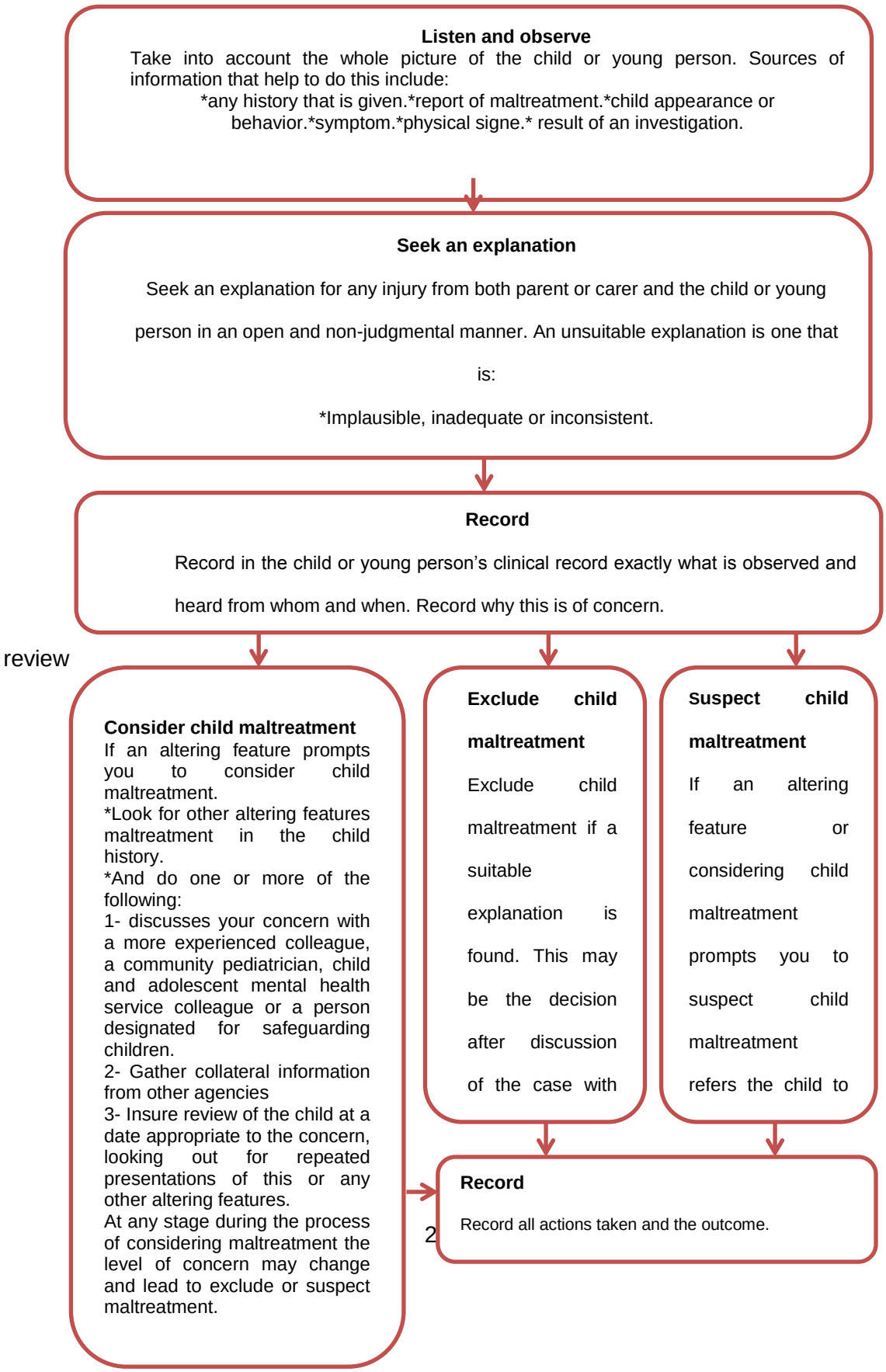
intervention as suggested by the BSPD (Haris et al., 2009). The first is preventive dental team management with dental professionals relieving any pain the child is experiencing. The second strategy is preventive multi-agency management. Here, if concerns continue, or situations worsen with a child, then the dental team has the responsibility to seek parental consent to consult other professionals who have contact with the child, to see if concerns are shared. If they are then a meeting should take place to formulate a joint plan of action. The final strategy is a child protection referral. This is when there is a suspicion that the child is undergoing significant harm from dental or general abuse, and then a child protection referral should be made after local protection procedures.

Every NHS trust, dental practice and community clinic must have a clear pathway to follow and a policy in place whenever there are concerns regarding the safeguarding of a child. The NICE guidelines published in 2009 suggested following the process as shown in figure1.

Figure 1. NICE flow chart to follow any concerns to children's safeguarding

In the NHS time is valuable and a huge amount of pressure exists and heavy workloads. In private practice DNA's also have financial implications but here fees may be applied for missed appointments. The child may suffer directly from a missed appointment for example following dental trauma, or information may be missed like attendance at an emergency clinic for pain and / or swelling.

Locally, in the Department of Paediatric dentistry – systems loosely exist for new patients in that they can DNA twice but are then discharged and for GA patients they get a second appointment too but if they DNA twice then they are removed from the waiting list following discussion with a Consultant. When it comes to treatment or



review

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patients – the action taken varies widely from another appointment in the post, a telephone call, to a letter asking the parent / carer to contact. This means that outcomes vary and potentially in all 3 groups children may be lost to follow-up.

1.3 Aim and Objectives

The aim therefore of this audit was to examine, and establish robust procedures to ensure that all patients who DNA'd an appointment at the Eastman Dental Hospital, UCLH Paediatric Dental Department, were not lost to follow up.

The gold standard was that ***all*** patients who did not attend their appointments (DNA) in all three groups were followed up appropriately and not lost in the system.

2 Materials and methods

2. 1 Sample selection

This was a retrospective audit looking at patient clinical notes, divided in to three groups according to type of appointment. Data was collected over a six month period, from the 1st January until June 31st, 2012.

2.2 Data collection

Lists of DNA'd patients who their appointments in the six month period selected were needed to be able to request the notes from medical records for all three patient groups.

Information gathered included patients details, type of patient, referral source, dental diagnosis, staff grade, DNA outcome and other findings on the form shown in figure 2.

Medical records provided the notes, and the data extraction form was completed for each patient and kept in a folder. The completed data forms were fed into a scanner where information was transferred in to the Excel database created.

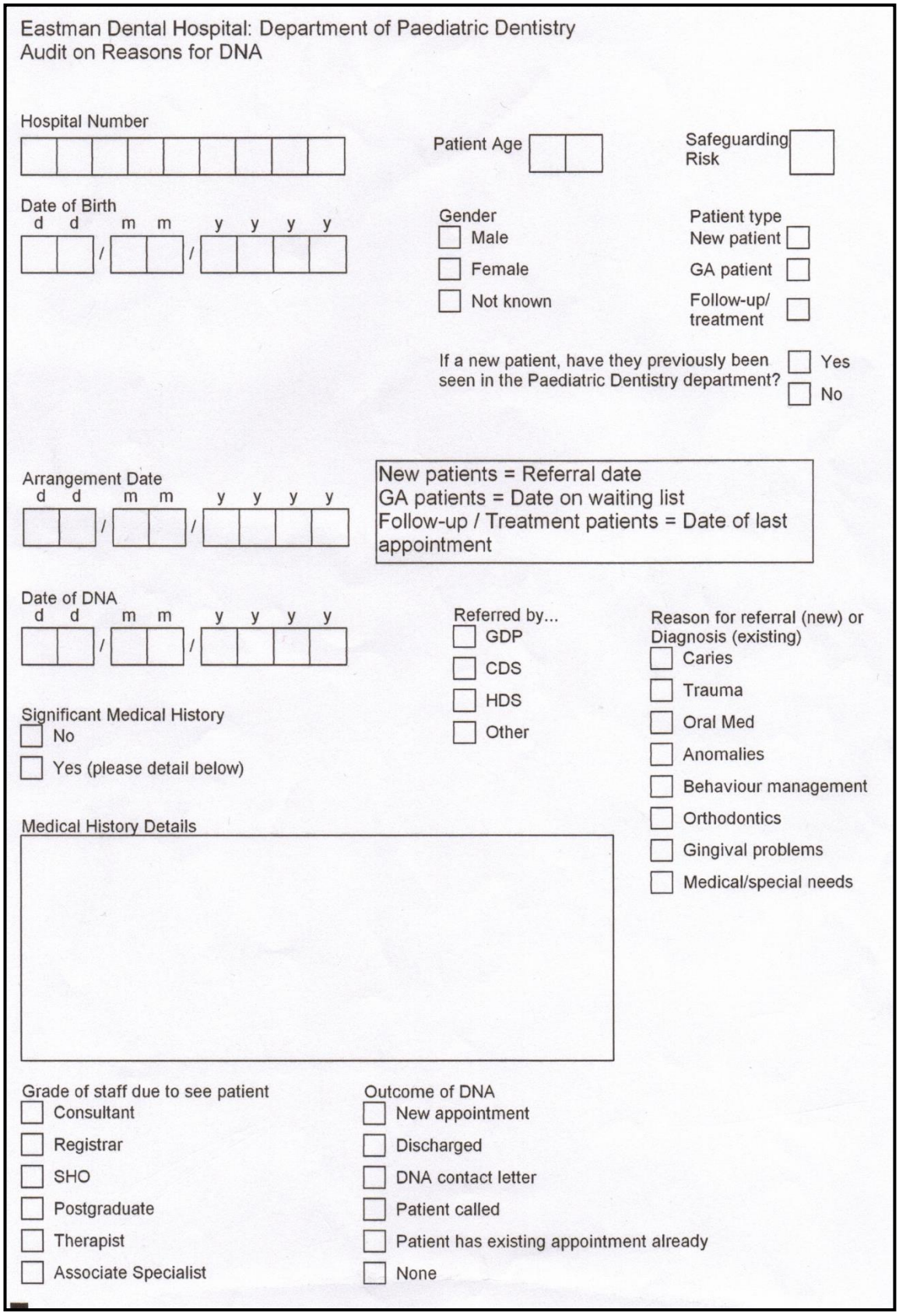

Figure 2. Audit data extraction form.

In 2011, the first cycle of this audit was done by Ebtesam Alzain who was a graduate student in paediatric department at the Eastman Dental Hospital. She focused initially on the DNA'd patients in all three groups and established a protocol to track all patients data. This was obtained by having an action plan to be followed in each category of the DNA's patients.

3 First Cycle Result

- **All patients**

3.1 Sample

In the six months period investigated, initially, the total number of patients who had evidently supposedly DNA their appointments in all three groups was 754 patients. However, the number of patients who were potentially eligible for inclusion in each group, and the number of notes actually retrieved are illustrated in table 1 below.

	New patient	Treatment/follow up	General anaesthesia
Total number of pt potentially eligible	**136**	**575**	**43**
Total number of notes retrieved	**133**	**351**	**33**
Final number of DNA patients	**133**	**260**	**4**

Table 1. Total number of patients who were potentially eligible for inclusion in each group, number of notes retrieved & final numbers.

The results showed that the total number of patients who failed to attend their appointments in all three groups was 397. In the new patient group, all 133 notes retrieved were for patients who actually DNA their dental appointment.

In the treatment/follow up group, out of the 575 patients potentially eligible for inclusion, 37 patients had repeated DNA, and out of the 351 notes retrieved, 91 had no obvious DNA and therefore were excluded, ending up with a sample of 260 notes, who actually did not attend their appointments.

In the general anaesthesia group, out of the 33 patient notes retrieved, 29 patients did not DNA but actually cancelled their appointments according to what was written in the notes. If a patient cancels an appointment in the 24 hours before their surgery this is logged on the system as a DNA but for this audit these patients were not true DNA's. The total number who actually DNA their appointment was 4 patients.

The total number of patients who did not attend their appointments in the three patient groups is shown in figure 3.

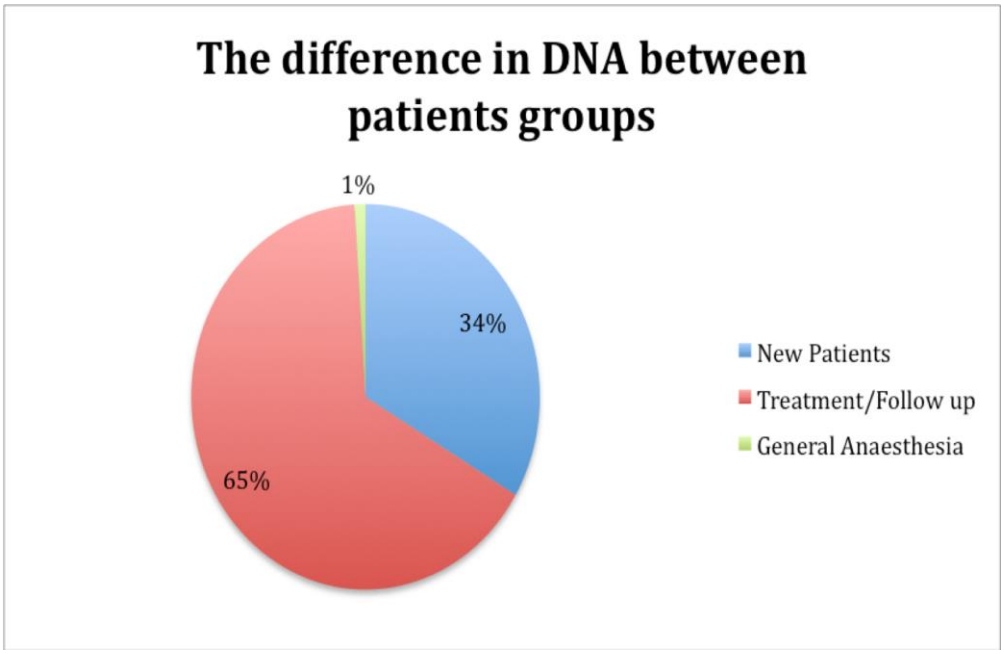

Figure 3. Total number of patients who DNA appointments in *all* three patient groups.

The overall age range of patients that did not attend their appointments was 1 - 18 years old with an average age of 10.3 years. There were 5 patients who were known to be a safeguarding risk. The ratio of male to female patients who did not attend their appointment was M: F = 1.1:1. Results also showed that, 44 (11%) patients had a significant medical history.

As to the patients' referral source, results showed that the majority of referrals were from general dental practitioners (GDP), followed by other referrals such as, internal or from specialists. Then, they were followed by referrals from community dental services (CDS). The fewest referrals were from hospital dental services (HDS). These results are displayed in figure 4.

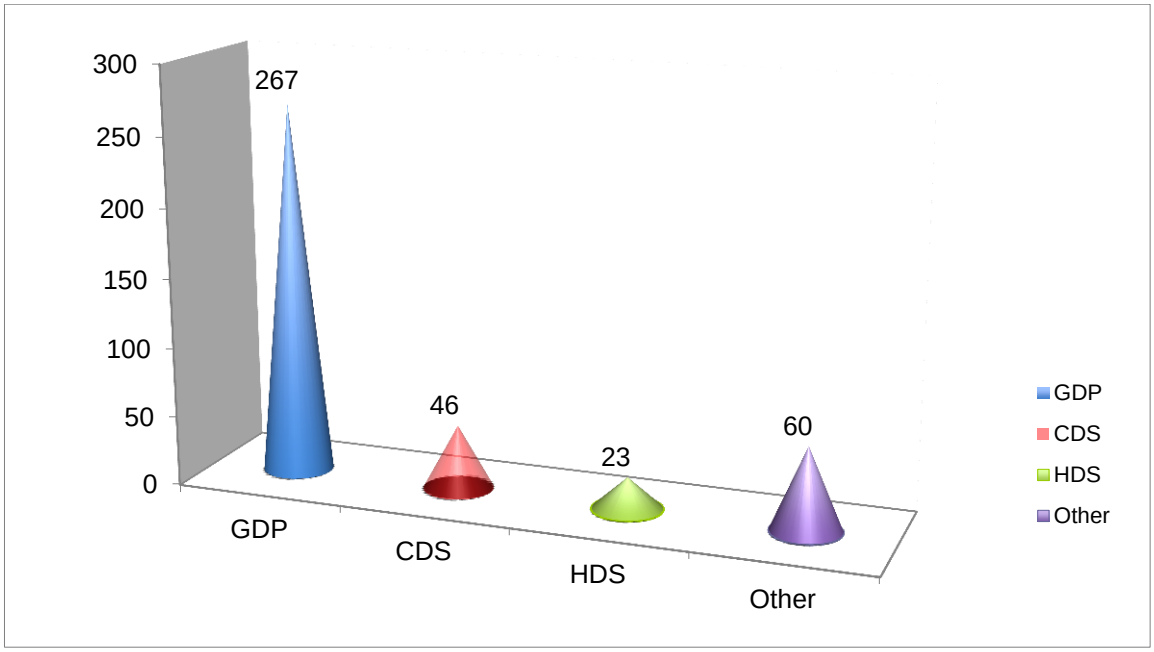

Figure 4. Referral source to *all* patient groups

Reasons for referrals varied as shown below.

Diagnosis	Number of referrals (%)
Caries	**126 (31.7%)**
Trauma	**81 (20.4%)**
Anomalies	**54 (13.6%)**
BM	**7 (1.7%)**
Ortho	**16 (4%)**
Oral medicine	**11 (2.7%)**
Gingival problems	**2 (0.5%)**
Med/special needs	**1 (0.25%)**
Caries & BM	**62 (15.6%)**
Caries + anomalies	**15 (3.7%)**
Caries + med/spec.	**2 (0.5%)**
Caries + BM + ortho	**1 (0.25%)**
Caries + anomalies + ortho	**2 (0.5%)**
Caries + trauma + BM	**1 (0.25%)**
Caries + ortho	**3 (0.75%)**
Caries + oral medicine	**1 (0.25%)**
Trauma + med/spec.	**2 (0.5%)**
Anomalies + med/spec.	**1 (0.25%)**
Anomalies +ortho	**4 (1%)**
Anomalies + BM + gingival	**1 (0.25%)**
BM + ortho	**4 (1%)**

Table 2. Reasons for referrals for *all* patients who DNA appointments

Results showed that different grades of staff saw patients who DNA their appointments and they were different in each of the three different patient groups, these are illustrated in the following.

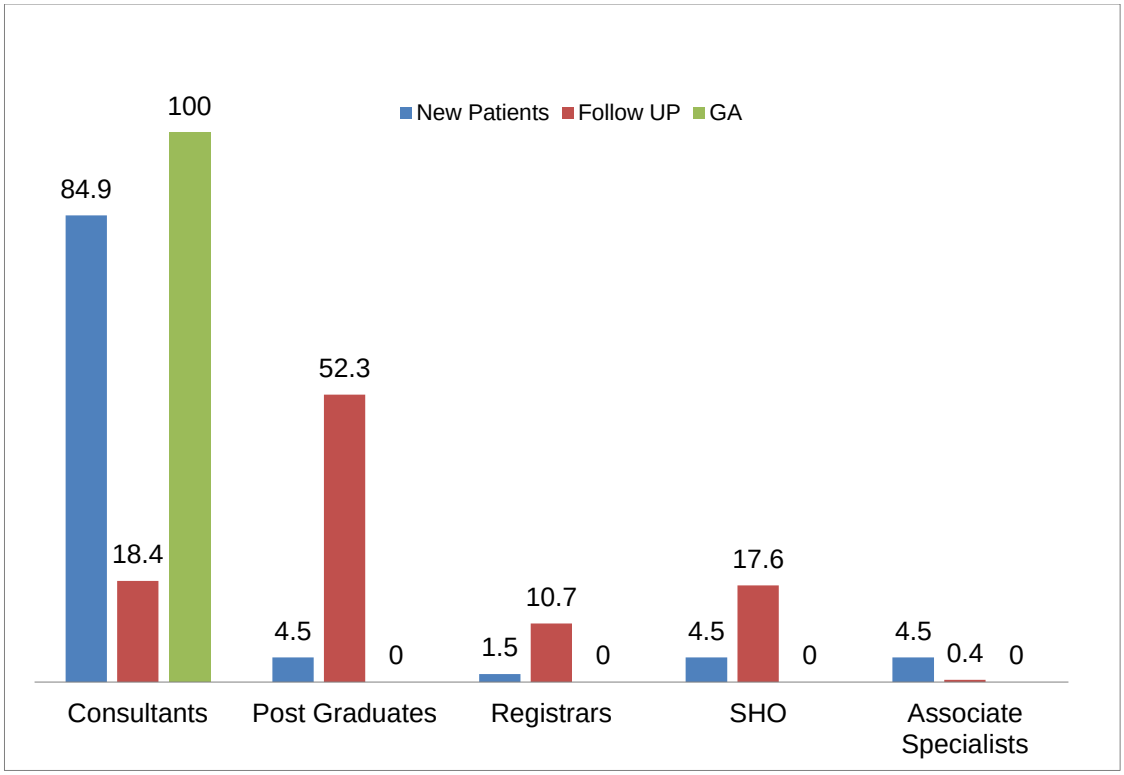

Figure 5. Prevalence of grade of staff who had DNA in each group

As to the outcome of patient failed appointments, results revealed that clinician's varied with regards to their action taken upon DNA, theses outcomes depending on patient groups are summarised in figure 6.

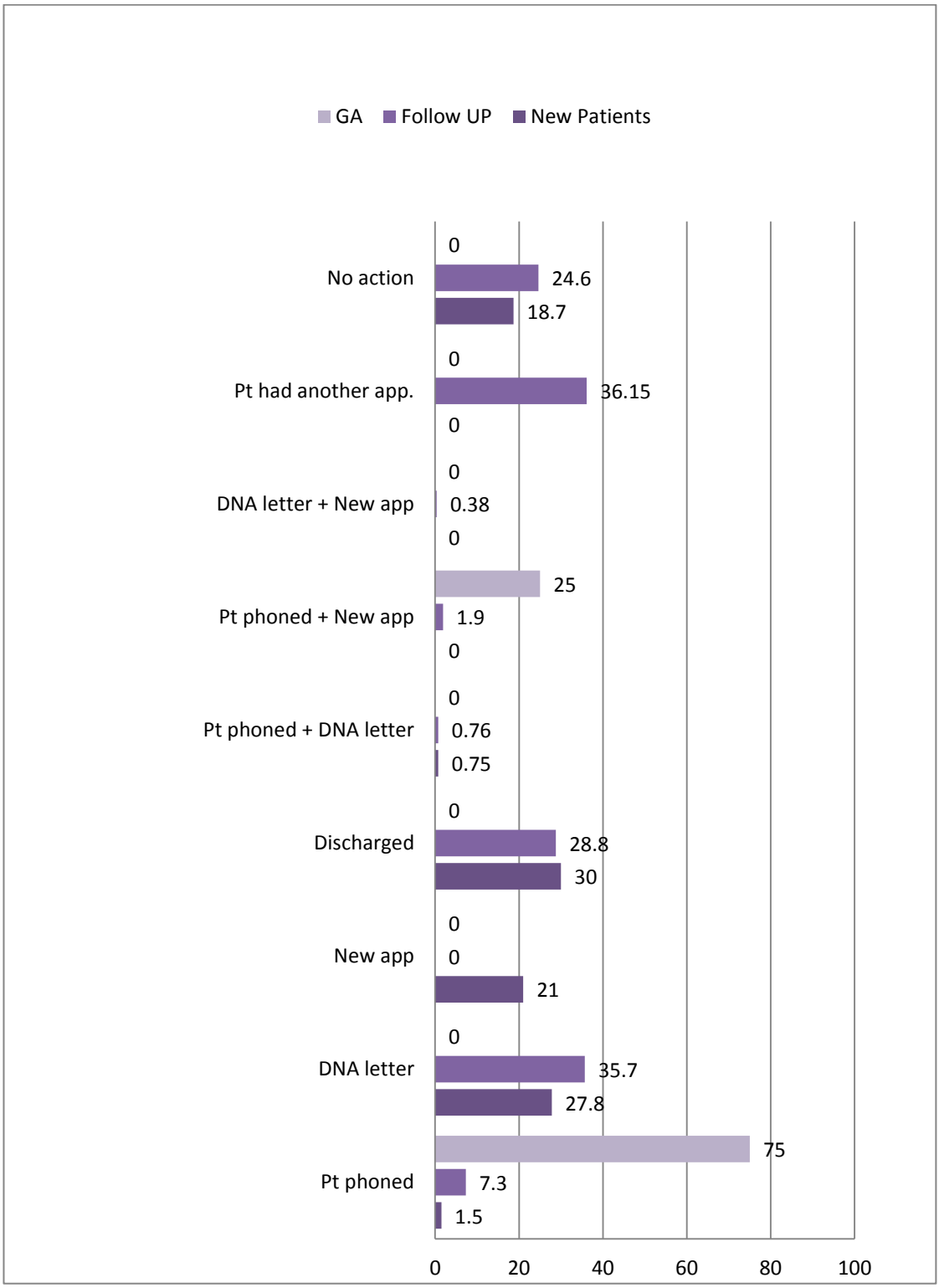

Figure 6. DNA outcome in *all* patient groups (%).

Results showed that, according to the notes, when considering the 3 individual groups as one, in 89 cases (22.4%) clinicians did not take any action following a missed appointment.

The prevalence of patients with significant medical history, whether they were at a known safeguarding risk and the main diagnosis for those children who DNA'd and no action was taken are shown in table 3.

DNA with No action	Medical history	Safeguarding risk	Main diagnosis
89	**8**	**1**	**(17) Trauma** **(29) Caries**

Table 3. The frequency of different parameters in relation to patients who DNA'd and no action was taken.

As to the distribution of no action taken according to the patient group, results are illustrated below.

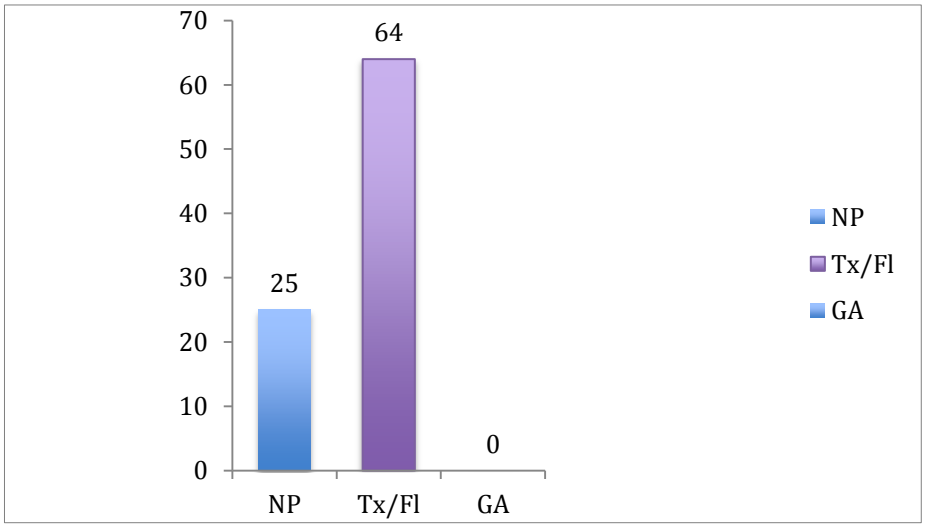

Figure 7. Frequency of no action taken after a DNA according to patient group.

The frequency of DNA with no action taken according to grade of staff is shown in figure 8.

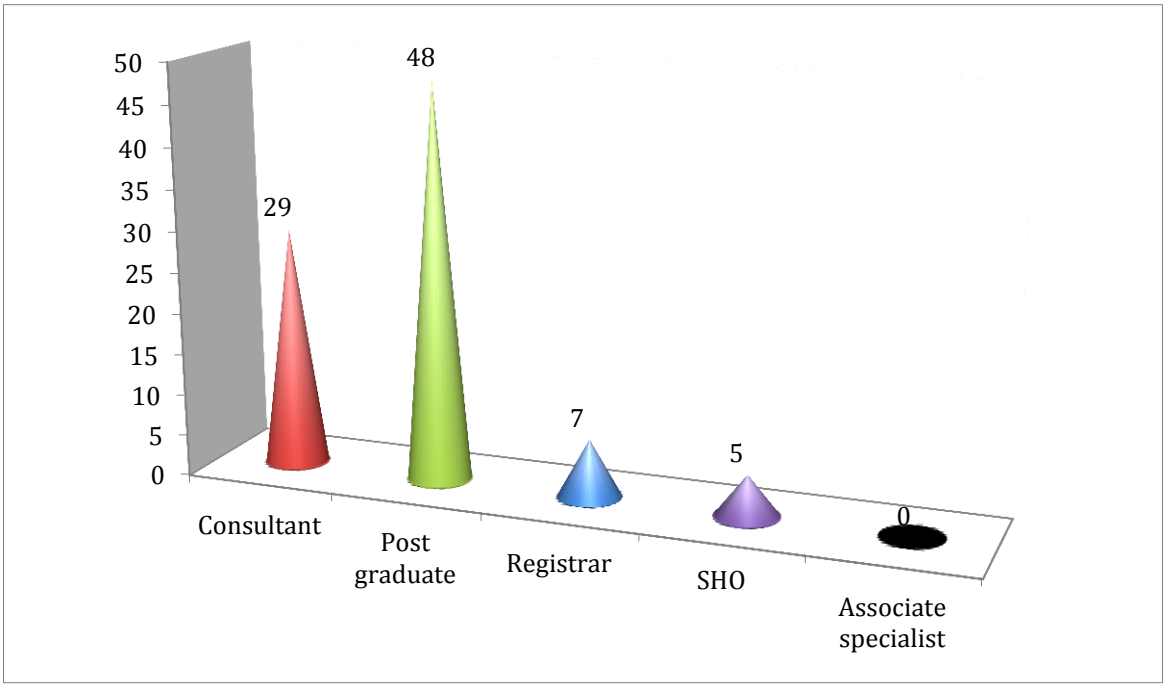

Figure 8. Frequency of DNA with no action taken according to grade of staff.

Action plan

A suggested protocol would be to have a system that would allow all patients who are due for appointments within 24 hours or 48 hours receive a text message to remind them of their coming appointment. The text message would have a message guiding parents to text back if unable to attend or wish to cancel the appointment. This would give reception the opportunity to book in patients who need appointments in the slots of patients who cancelled theirs. This however is not widely available at present.

The results of this audit will be presented to the paediatric department staff at a meeting soon and clinician feedback will be sought with regards to finalising the 3 proposed pathways. After that, the protocols will be distributed to ensure that all patients, in the three patient groups identified, are followed up. This protocol / system will be implemented for one year, followed by a re-audit.

Another audit will be carried out to look into the 89 patients who DNA'd and were lost to follow up.

If patients do DNA, then according to the patient group, the provisional action in each of the three groups will be as shown ***(to be confirmed following staff meeting),***

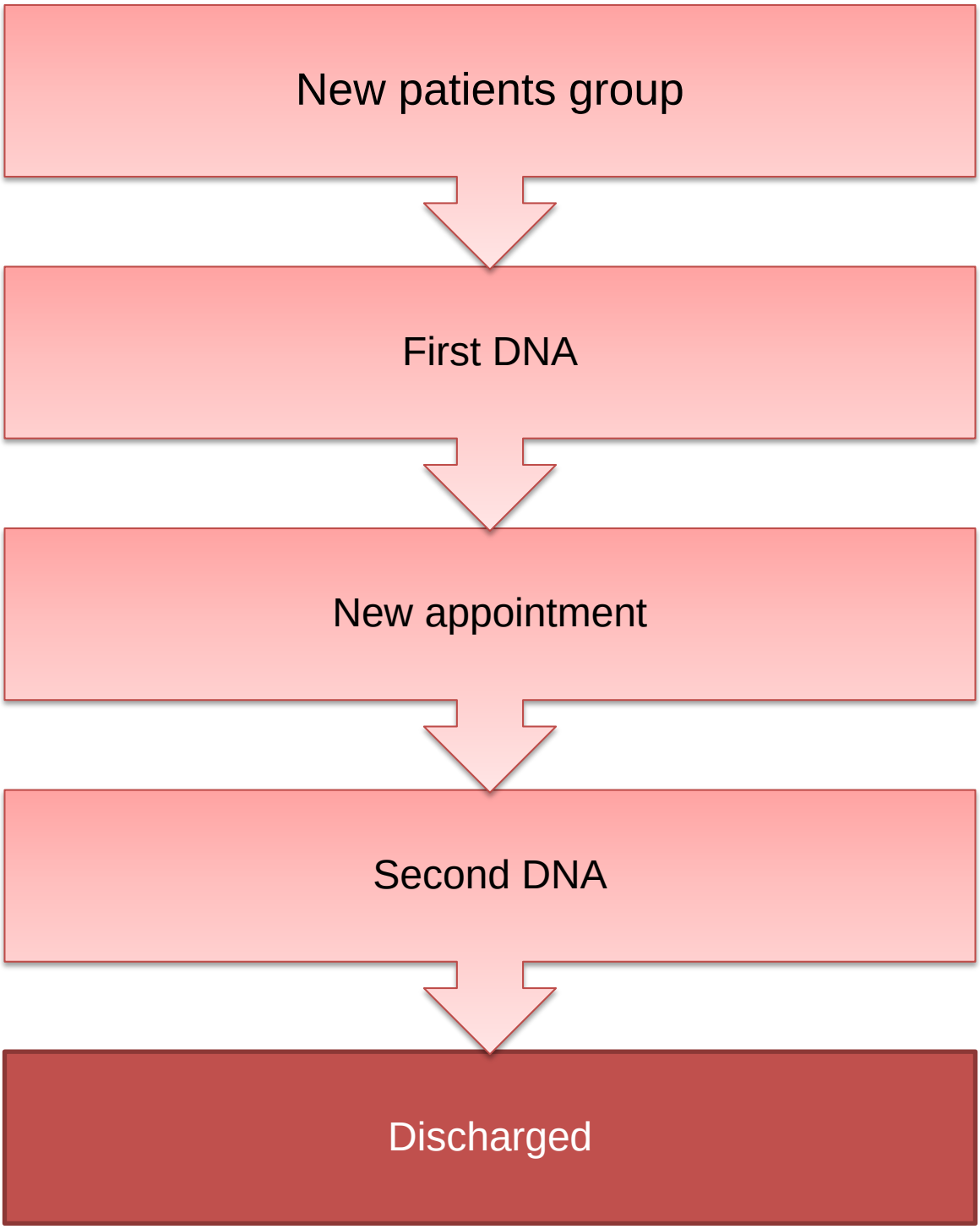

Figure 9. Action plan for patients who DNA in the new patient group.

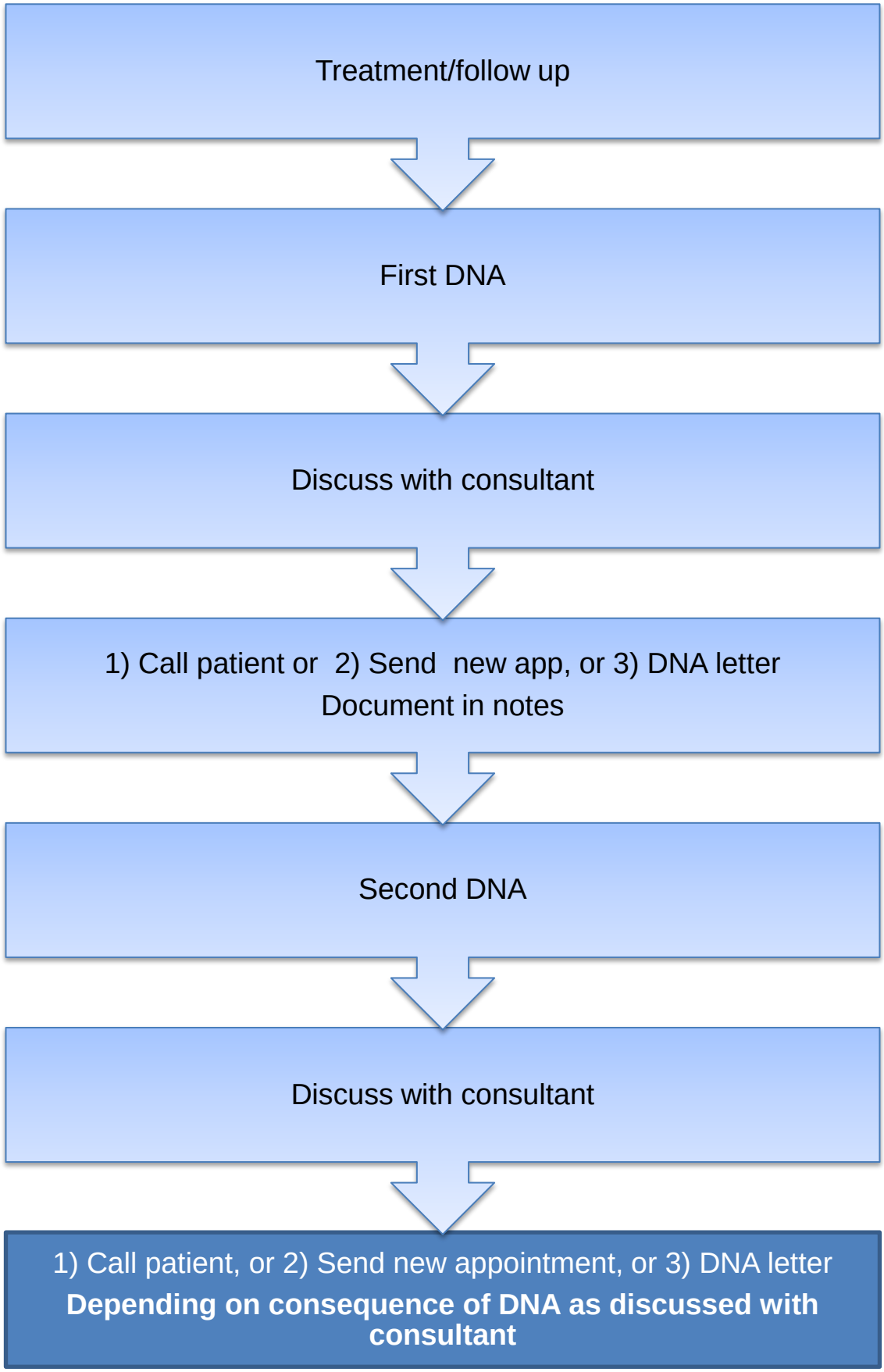

Figure 10. Action plan for patients who DNA in the Treatment/follow up group.

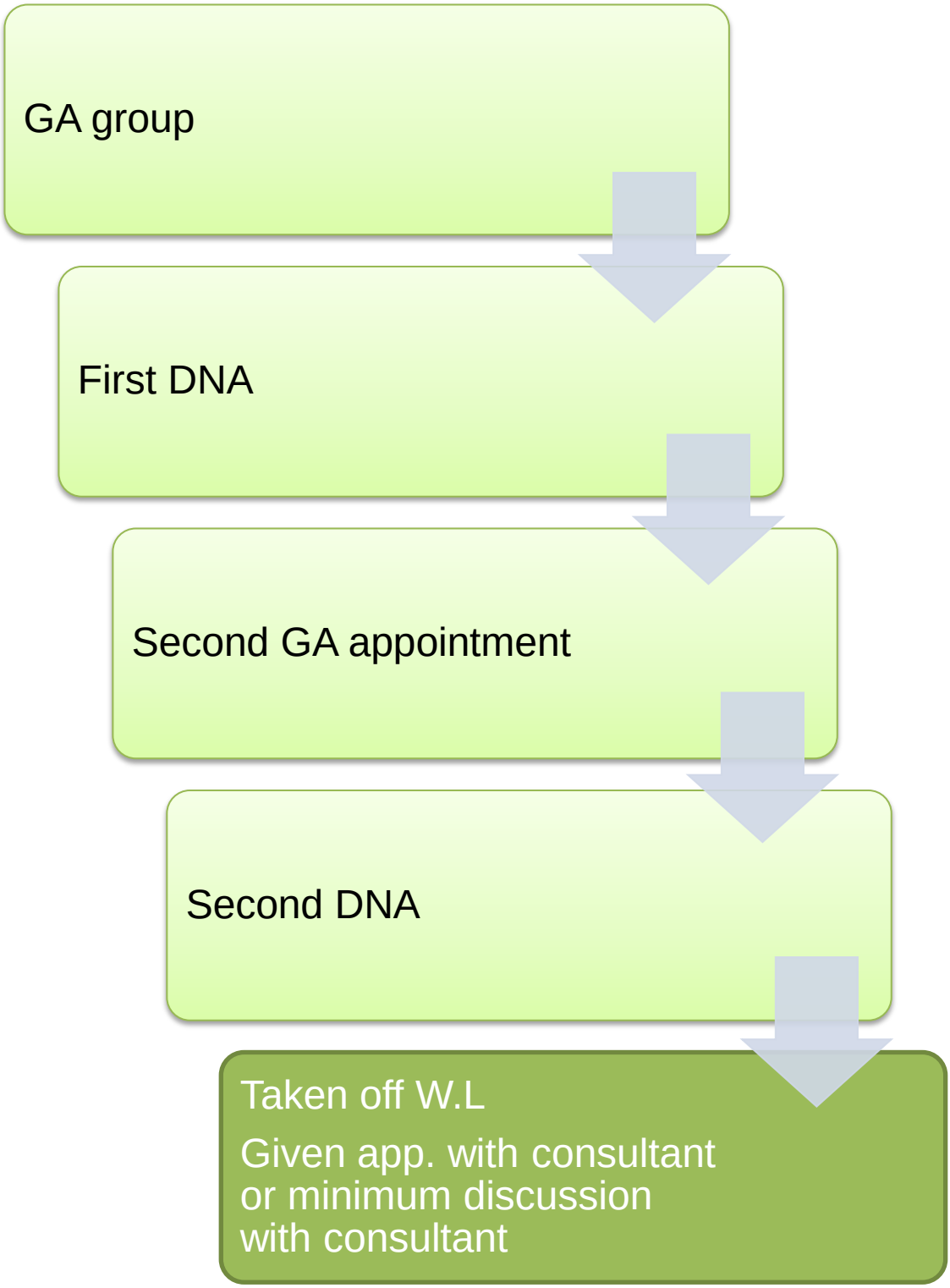

Figure 11. Action plan for patients who DNA in the general anaesthesia group

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CASE NUMBER: 1

Age of child at start of treatment: 5 years and 7months

CASE SUMMARY

A healthy 5 year old boy was referred by his GDP for treatment of grossly carious primary teeth. In the referral, the dentist mentioned that Z.K was too anxious for dental treatment at his practice.

Clinical and radiographic examinations revealed severe early childhood caries affecting almost all of the primary dentition. Although he was anxious, he allowed examination, and appeared cooperative. Utilizing different non-pharmacological and pharmacological behaviour management techniques, he allowed completion of preventive and comprehensive dental treatment successfully under local anaesthesia and inhalation sedation. Z.K is on 3 months periodic review programme to ensure good oral health.

PATIENT DETAILS

- Initials: Z.K
- Gender: Male
- Age at start of treatment: 5 years, 7 months
- Age at last review: 7 years, 2 months

PRE-TREATMENT ASSESSMENT

HISTORY OF PRESENTING COMPLAINT(S)

- Pain when eating food on upper right side and occasionally at night for 6 months
- Controlled with paracetamol when required
- History of facial swelling URQ
- History of antibiotic given (Amoxicillin)

RELEVANT MEDICAL HISTORY

- Nil relevant

DIET HISTORY

- Eats sweets and biscuits daily
- Drinks milk, water and apple juice

TEETH BRUSHING

- Brush once a day with children toothpaste (unsupervised)

FAMILY HISTORY

- No history of dental anomalies

DENTAL HISTORY

- Regular attendee at GDP
- History of dental treatment under local anaesthesia (LA)

CLINICAL EXAMINATION

Extra-oral:

- Face: symmetrical, no swelling
- No palpable lymph nodes
- No tenderness of muscle of mastication
- Normal tempromandibular joint movement
- Normal mouth opening
- Behaviour: Anxious

Intra-oral:

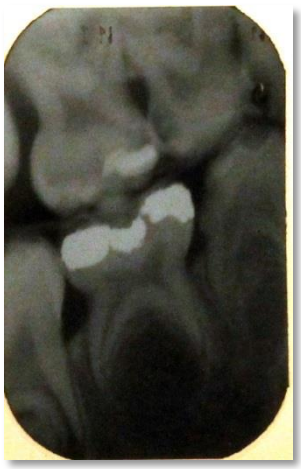
- ❖ Teeth present
    - ✓ URE, URD, URC, URB, URA, ULA, ULB, ULC, ULD, ULE
    - ✓ LRE, LRD, LRC, LRB, LRA, LLA, LLB, LLC, LLD, LLE
    - ✓ LR6 and LL6 partially erupted

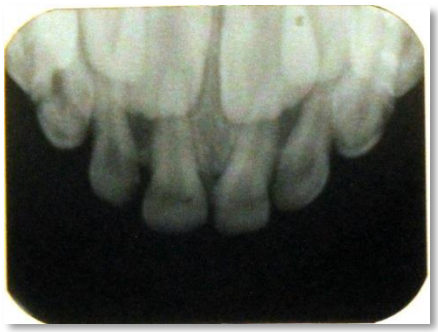
- Visible plaque accumulation on most surfaces of the teeth (60% score)
- Generalised mild gingivitis
- Localised buccal abscess URE area and glass ionomer
- Amalgam filling LRE
- Caries affecting most teeth surfaces
- Well aligned upper and lower arches with spacing in the upper but not lower arch

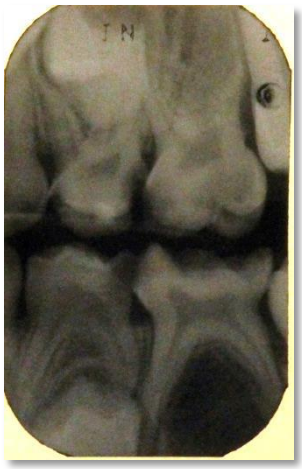
Orthodontic assessment

- Primary dentition stage
- Mesial step primary molar relationship
- Upper and lower arches well aligned
- Skeletal: class I base

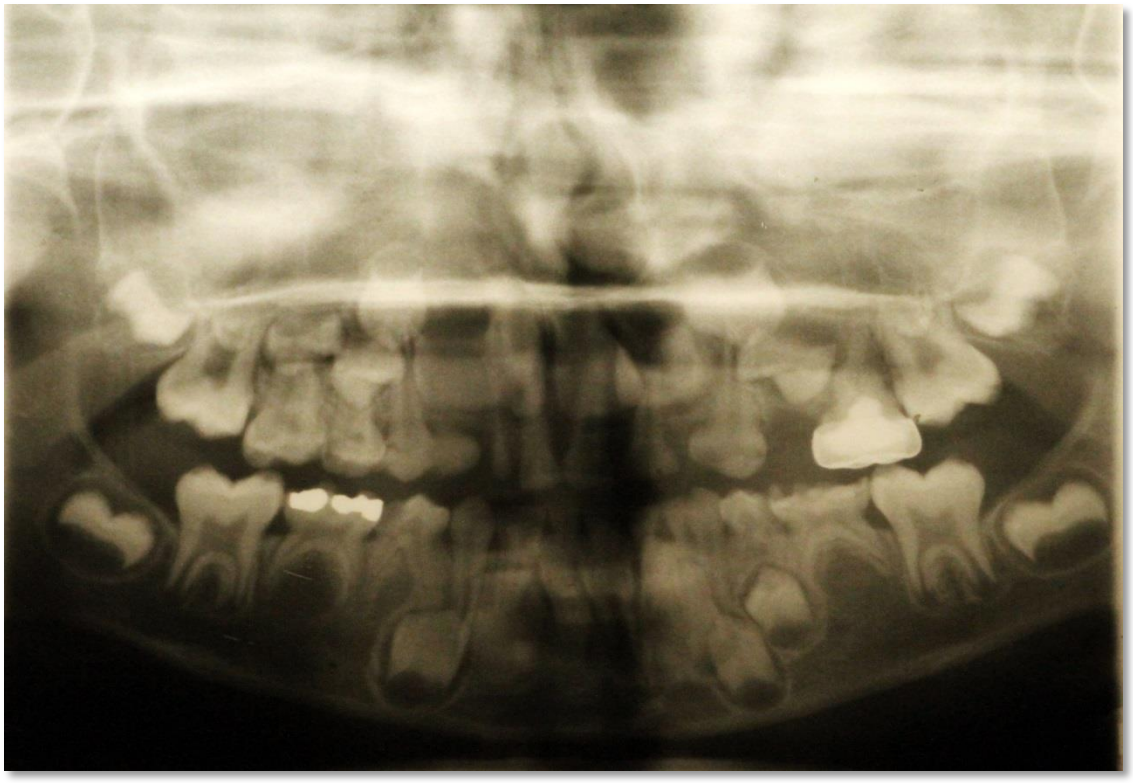
RADIOGRAPHIC EXAMINATION







- Bitewing radiographs were taken to assess caries and bifurcation involvement (3.03.12)

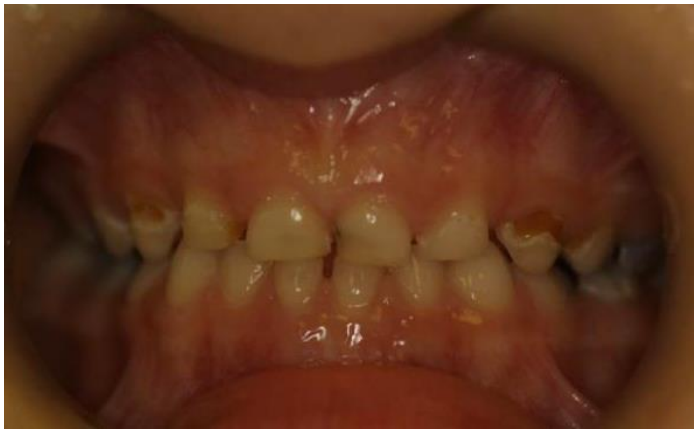


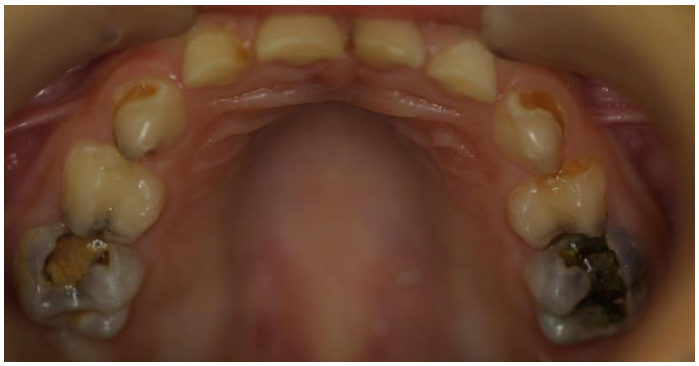
- Orthopantomogram (OPG) was requested to assess
    - Dentition development and presence of permanent successors
    - OPG was taken on the 3rd visit (7.06.12)to confirm suspected hypodontia

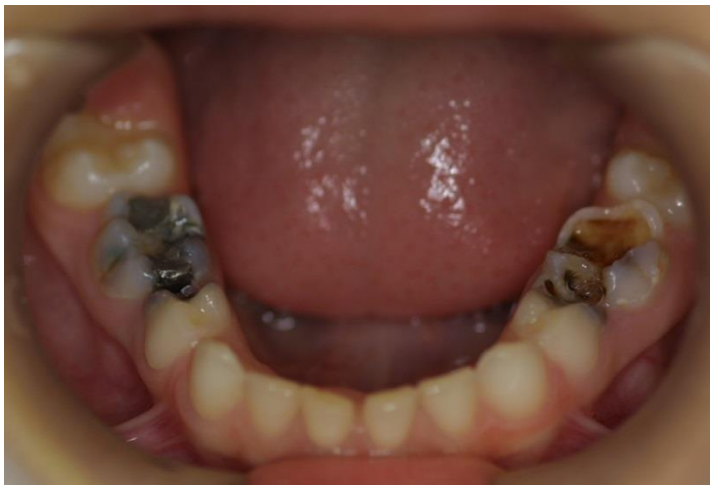
RADIOGRAPHIC FINDINGS:

- Quality of OPG, vertical bitewings and upper anterior occlusal : Diagnostically acceptable
- Proximal caries into enamel : URA,URB, ULA and ULB
- Caries into dentine : URC, ULC
- Restorations on LRE and URE
- Occlusal and proximal caries into pulp: URD,ULD,LRD and LLD
- Caries into pulp : URE,ULE
- Potential crowding in the upper arch
- Missing UL5, LL5, LR4 and LR5

PRE-TREATMENT PHOTOGRAPHS: EXTRA AND INTRA ORAL (3.03.12)







DIAGNOSTIC SUMMARY

- Early childhood caries
- Generalised gingivitis
- Hypodontia UL5,LL5,LR4 and LR5
- Anxious child

AIMS AND OBJECTIVES OF TREATMENT

- Acclimatise Z.K to dental treatment and instil a positive dental attitude to dental health
- Relieve sources of pain and infection
- Establish a preventive regimen consistent with the Department of Health preventive toolkit (third edition).
- Incorporation of nitrous oxide inhalation sedation as a pharmacological behavioural management technique, due to amount of treatment
- Restore aesthetic and function of carious teeth
- Monitor the occlusion of developing dentition and treat as necessary

TREATMENT PLANE

visits	Prevention	Treatment	Others
1 **3.03.12**	• Oral hygiene instructions (OHI) • Plaque score (PI) 60% • Fluoride advice • Fluoride application • Issuing diet sheet	• Examination , charting and radiographs • Treatment plan formulation	• Anxiety Scale measurement Frankl (2) • Introduction to local anaesthesia and restorative procedures + inhalation sedation • Clinical photographs
2 **23.03.12**	• OHI • PI 40% • Diet analysis and advice • Prophylaxis polishing	• Composite restorations on URB and ULB • Composite restoration URA and ULA • Temporisation of primary molars with temporary fillings IRM • URE temporisation ledrmix + IRM	• Nitrous oxide inhalation sedation (IS) • LA
3 **7.06.12**	• OHI • PI 33% • Patient had pain on ULD	• ULC : Labial restoration • ULE : Ferric Sulphate Pulpotomy and preformed metal crown (PMC) • ULD : Extraction	• LA • IS
4 **16.06.12**	• PI 20%	• URC : Labial restoration • URD : Extraction	• Frankl (3) • LA

		• URE : Extraction	• IS
5 **23.06.12**	• Pl 10%	• LLE: Ferric Sulphate pulpotomy and PMC • LLD: Extraction	• LA • IS
6 **30.06.12**	• Plaque score • OH has improved • PI 10%	• LRD : Extraction • LRE : restoration and preformed metal crown	• Frankl (4) • LA • No IS needed

KEY STAGES IN TREATMENT PROGRESS

Prevention

- Stressed to the parents the importance of following the oral hygiene instructions and the caries preventive measure
- Oral hygiene instructions
  - ✓ Brushing twice a day with parental help
  - ✓ Brushing last thing at night and on one other occasion
- Spiting after brushing but not rinsing
- Diet advice
  - ✓ Reducing the amount and the frequency of sugar and limiting it to meals time
  - ✓ Not to consume sugar more than four times a day
- Plaque score and polishing with paste and rubber cup
- Fluoride advice
  - ✓ 1450 ppm fluoride toothpaste
  - ✓ Application of fluoride varnish 22,600 ppm every 3-4 times yearly
- Fissure sealant all 6's when they erupt

Behaviour management

- Non-pharmacological techniques:

- ✓ Tell-Show-Do, enhanced control, distraction, and positive reinforcement

- Pharmacological technique
  - ✓ Inhalation sedation: Nitrous oxide 30% and 70% O2
  - ✓ Local analgesia :
    - ➢ Infiltration of 2% lignocaine with 1:80,000 epinephrine local anaesthetics with restoration
    - ➢ 4% Articaine with 1:100,000 epinephrine infiltration was used to provide more profound analgesia in all extractions

Restorative and Extraction:

All restorative treatment were completed using rubber dam isolation

- ✓ Anterior Composite restoration on URC,URB,URA,ULA,ULB and ULC
- ✓ Pulpotomy with 15.5% ferric sulphate & PMC ULE, LLE
- ✓ Extraction URE,URD,ULD,LLD and LRD

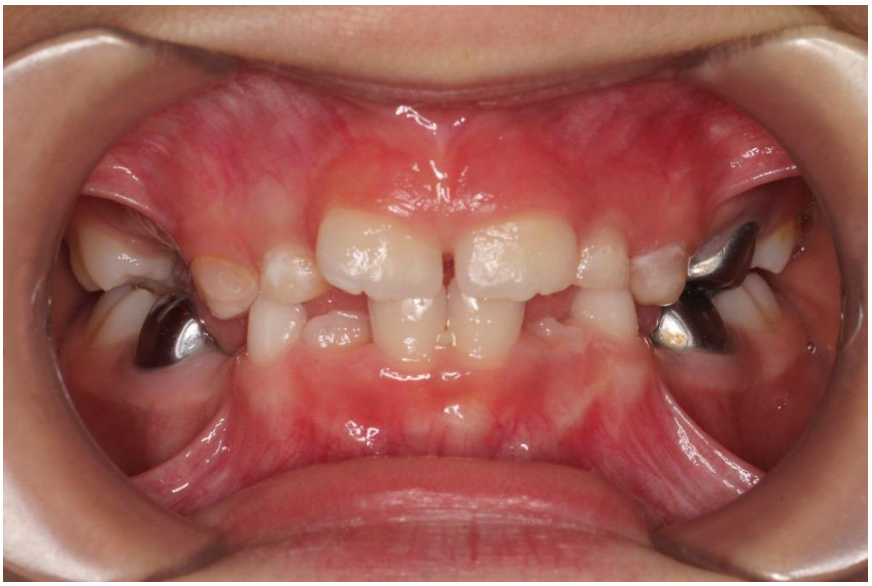
- Some changes in the treatment plan were made, mainly delaying extraction of URE as patient came in visit 3 complaining of pain on ULQ
- Treatment completed over six months period

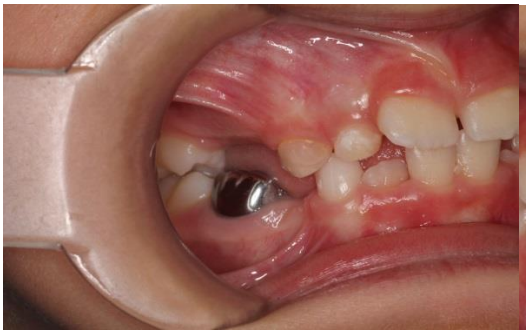
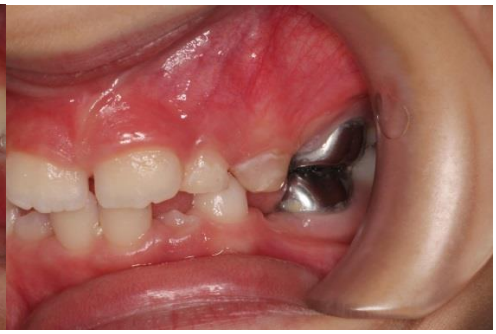
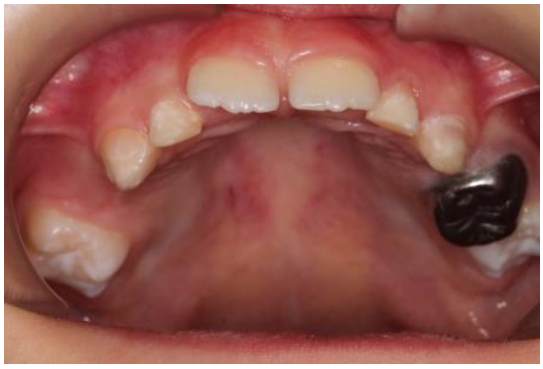
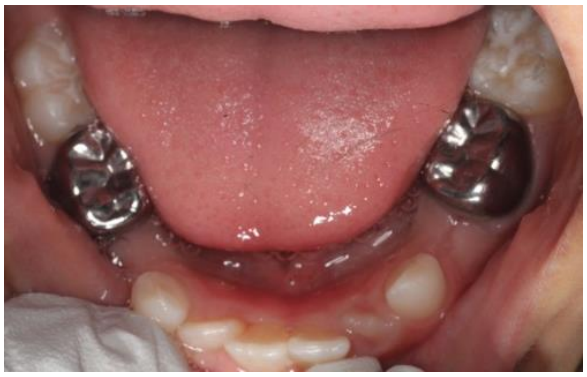
Review: (3, 9, 12, 18 months)

First review was 3 months following treatment completion. The caries risk assessment indicated low disease activity. Therefore, the recall interval adjusted into 6 months period. The following were considered:

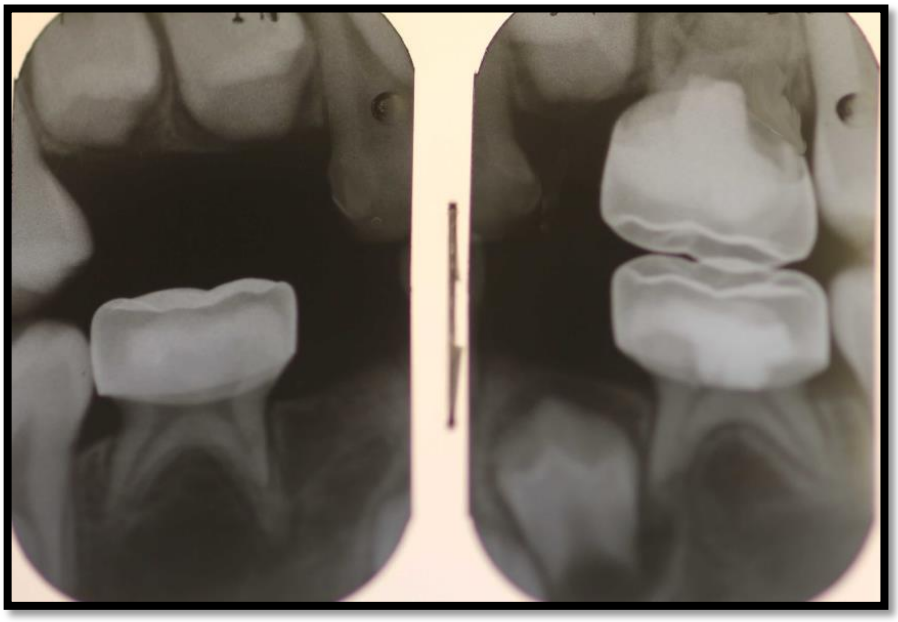
- Review of restorations
- Reinforcing preventive advice
- Application of fluoride varnish
- Resin fissure sealants on all 6's
- Monitoring the eruption of permanent dentition
- Upper and lower centrals incisors were erupted

POST-TREATMENT EXTRA ORAL AND INTRA ORAL PHOTOGRAPHS: last review visit (27/07/14)



POST-TREATMENT RADIOGRAPHS: (27/07/14)



LONG TERM TREATMENT PLAN AND FUTURE CONSIDERATIONS

- Regular long-term diet monitoring and reinforcement of oral hygiene practice
- Z.K is already Hypodontia clinic waiting list regarding missing UL5, LL5, LR4 and LR5
- Periodic review of the restorations and pulpotomies with radiographic assessment
- Monitoring the eruption pattern of the permanent teeth
- Monitoring retained primary molars (infraocclusion)

DISCUSSION

Behaviour Management

Z.K had an amalgam restoration under LA with his GDP; unfortunately this experience increased his anxiety towards dental treatment. In addition, he

experienced episodes of dental pain mainly at night and whilst eating that was controlled with pain killers.

Sedation will not necessarily convert an uncooperative child into a co-operative one. However, it can help to alleviate anxiety, improve a child's tolerance of invasive procedures, and increase the child's ability to cope with prolonged treatment. In this case, Z.K was traumatised from a previous dental experience which made treatment under local anaesthesia quite difficult to obtain. Therefore, inhalation sedation with nitrous oxide was introduced as the flow was set initially very low then slowly the dose was titrated to 30 %-70% nitrous oxide and oxygen respectively [1]

Furthermore, the mother was present during the dental treatment acting as passive observer. This was helpful in facilitating the treatment and reducing Z.K's anxiety.

Several non-pharmacological behaviour management techniques were used in this case aiming to create positive dental attitude and long-term interest in prevention, and maintaining oral health. Tell-show-do is a widely used technique and easy to use. Using simple language was implemented to familiarise Z.K with new procedures. Distraction, with the help of the dental nurse, and positive reinforcement were also used. Explaining the duties of parents and dental nurse before starting treatment was important in achieving successful treatment.

Z.K's behaviour was initially negative towards dental treatment but improved through visits to definitely positive according to the Frankl rating scale.

Prevention

Prevention plays a major role in the management of dental caries. Prior to formulating a prevention plan, it is essential to understand the patient's caries risk [2]. Z.K was a high caries risk patient, so he was approached with a comprehensive prevention treatment plan that includes plaque control and tooth brushing, practical diet advice, and fluoride therapy.

Restorative Management

Restorations and extractions of damaged teeth were necessary to re-establish oral health and prevent further dental pathology.

Resin- based Fissure Sealant

Pit and fissure sealants have been described as materials which are placed in order to obliterate the fissures and remove the sheltered environment in which caries may thrive. In this case all permanent molars were sealed as soon as they erupted

Resin-based Composite Restorations

In this case ULC, ULB, URA, ULA, URB and URC were restored with composite resin as these teeth exhibited no pulpal involvement and the size of the cavities were adequate for intra-coronal restoration. Composite restoration is a technique sensitive procedure. Thus, rubber dam isolation was employed.

Pulp Therapy using Ferric Sulphate

Pulpotomy is defined as the clinical procedure involving the removal of the inflamed and infected coronal pulp tissue while maintaining vital healthy radicular pulp. It has been reported that inflammation of the pulp precedes carious exposure. In addition, primary teeth with caries involving more than half bucco-lingual intercuspal distance or more than 50% of the dentine thickness have increased risk of pulp inflammation[3]. Accordingly, in this case pulpotomy was indicated to be performed on the ULE and LLE. Clinically, these teeth did not show any signs of infection, such as intra-oral swelling, sinus, pathological mobility, or pain on palpation of the teeth or the buccal sulcus.

In this case 15.5% ferric sulphate was used as pulpotomy medicament. On contact with blood in the pulp chamber, a ferric ion-protein complex is formed promoting haemostasis [4] Ferric sulphate gained wide popularity as a potential replacement of formocresol which is associated with concerns regarding toxicity and possible carcinogenicity. Despite the high reported clinical and radiographic success of ferric sulphate pulpotomy, there is no absolute evidence of its superiority to other pulpotomy medicaments.

Preformed Metal Crowns (Stainless Steel Crowns SSCs)

In high caries risk patients not maintaining a satisfactory oral hygiene, the British Society of Paediatric Dentistry guidelines indicate the use of PMCs. It is the restoration of choice when extensive multi-surface restoration is required, and following endodontic treatment. Several studies reported higher success rates when using PMCs compared to other restorative materials [5]. Furthermore, clinical experience still favours PMCs in restoring primary molars in the above mentioned situations compared to alternatives [6]. Aiming to achieve the best coronal seal and greater longevity, all primary molars with pulp therapy were restored with FMCs in this case.

Extraction of Primary Molars

A. Z.K had localised abscess, pain, and extensive caries associated with URE and non- restorable D's. Extraction was unavoidable due to their poor restorability and long term prognosis. Z.K coped well with the extraction under local anaesthesia and inhalation sedation

Space maintenance was not indicated in this case. The upper arch was well-aligned and extractions of these teeth are unlikely to result in space loss or substantial crowding. In addition, as Z.K is high-caries risk caries recurrence risk was deemed to outweigh the space loss risk if would it be as Z.K considered a hypodontia patient

Lessons Learnt

• Z.K presented initially with caries affecting most of his teeth and poor oral hygiene. Empathic approach was useful at initial consultation helping to obtain full dental history from parents in a non-judgmental manner. This approach was also useful in motivating Z.K and parents to follow prevention instructions for now then moving to adult dentition

• It is important in these cases to spend time with parents educating them about the importance of prevention in maintaining dental health in the long term.

- Z.K presented with hypodontia, therefore it was necessary to change his attitude toward dental treatment as he would need more dental treatment in the future.

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3. KASSA, D., DAY, P., HIGH, A., and DUGGAL, M. 2009. Histological Comparasion of Pulpal Inflammation in Primary Teeth with Occlusal or Proximal Carries. International Journal of Paediatric Dentistry, 19, p. 26-33.

4. RODD, HD., WATERHOUSE, P.J., FUKS, AB., FAYLE, SA., and MOFFAT, MA. 2006. Pulp Therapy for Primary Molars; British Society of Paediatric Dentistry. International Journal of Paediatric Dentistry. 16 Suppl,

5. KINDELAN, S., DAY, P., NICHOL, R., WILLMOTT, N., and FAYLE, S. 2008. UK National Clinical Guidelines in Paediatric Dentistry: Stainless Steel Preformed Crowns for Primary Molars. International Journal of Paediatric Dentistry, 18, p. 20-28.

6. INNES, NP., PICKETTS, DN., and EVANS, DJ. 2007. Preformed Metal Crowns for Decayed Primary Molar Teeth. Cochrane Database Systematic Review, 24, (1): CD005512.

CASE NUMBER: 2

Patient’s age at start of treatment (8 years, 5 months)

CASE SUMMARY

An 8 year-old boy with Axenfeld-Rieger's syndrome (ARS) was referred by his GDP to the new patient clinic regarding missing permanent teeth. T.D had been diagnosed with ARS syndrome, with ventricular septal defect (VSD) which was surgically corrected in his early years.

Examination, radiographs and history were carried out. He was diagnosed with caries, malformed teeth (conical / microdont teeth), infraoccluded primary molars and hypodontia. Due to the complexity of his condition a multidisciplinary hypodontia clinic visit was needed for planning considering the fact that T.D was being bullied because of the appearance of his teeth. After liaising with his paediatrician, treatment was carried out under LA which included fissure sealants, fillings, upper overdenture and lower resin bonded bridge.

T.D and his twin brother live with a foster family, and at each visit his social worker was updated with the progress of his treatment

PATIENT DETAILS

Initials: T.D
Gender: Male
Age at start of treatment: 8 years, 5 months
Age at last review: 11 years, 2 months
Follow up over 30 months

PRE-TREATMENT ASSESSMENT

HISTORY OF PRESENTING PATIENT'S COMPLAINT(S)

- No history of dental pain or abscesses at presentation. However, T.D was concerned about his appearance and his foster mother mentioned he was being bullied at school due to his missing teeth

RELEVANT MEDICAL HISTORY

- ARS: vision and hearing impairment and ventricular septal defect which was corrected in his early years
- Medication: multivitamins
- T.D wears hearing aids

SOCIAL HISTORY

- T.D and his twin brother who has the same condition lived with a foster family for the last 4 years and recently they were adopted by the same family and they became the legal guardians

DENTAL HISTORY

- Regular attendee at general dental practitioner (GDP)

- No history of operative treatment
- Tooth-brushing twice daily with children toothpaste (unsupervised)

CLINICAL EXAMINATION

Extra-Oral Examination:

- Hard tissue: prominent supraorbital ridges, broad nasal bridge and prominent forehead
- Maxillary hypoplasia/prognathic profile
- Thick glasses and hearing aids
- Soft tissue, TMJ and lymph nodes: nothing abnormal detected
- Behaviour : positive toward dental treatment (Frankl Scale:3)

Intra-Oral Examination:

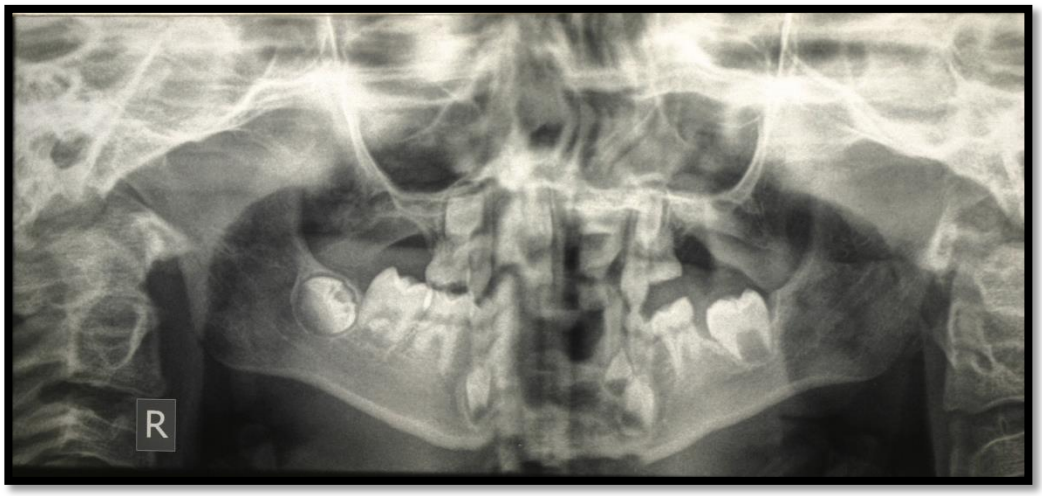
- Teeth presented intra-orally :
  - ✓ URD, URC, UR1, UL1,URC, URD
  - ✓ LR6, LRE, LRD, LRC, LLC, LLD, LLE and LL6
- Good oral hygiene
- Caries on LLD and LRD
- Conical UR1,UL1
- Infraoccluded primary molars (LRE ,LRD ,LLD ,LLE)
- Hypomineralised permanent molars

Oral Hygiene

- Good oral hygiene, no visible plaque deposits on teeth (Simplified plaque index score)

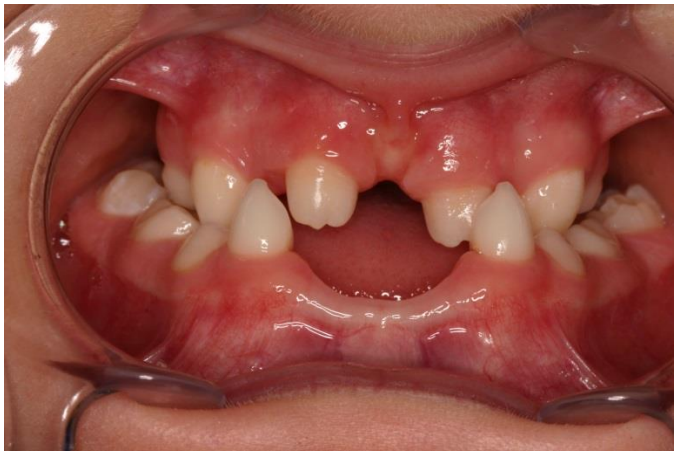
Orthodontic Assessment

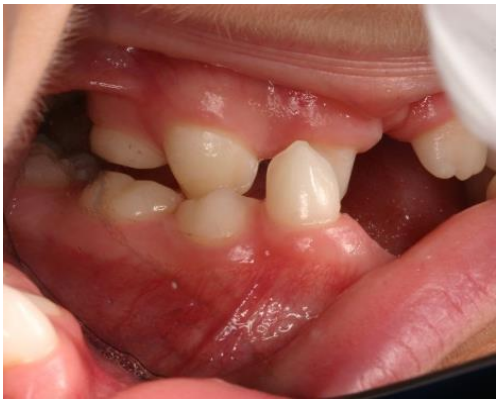
- Mixed dentition stage
- Midline diastema (6mm space between upper central incisors)
- Molar Relationship: right and left cross bite
- Upper and Lower Arch: anterior segment spacing
- Skeletal: Class III base

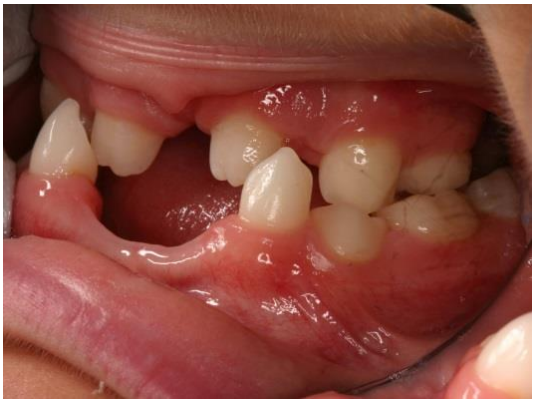


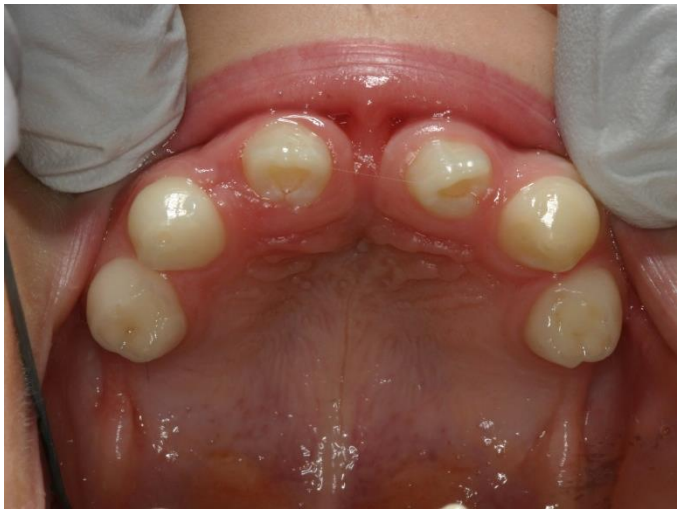
- DPT (16.03.11):
  - ✓ was diagnostically acceptable in the right and left side but not in the middle section presumably because of patient movement
  - ✓ Hypodontia :
    - ➢ UR2, UR3, UR5, UR6,UR7, UL2, UL5, UL6 and UL7
    - ➢ LR5,LR2,LR1, LL1, LL2, LL5 and LL7

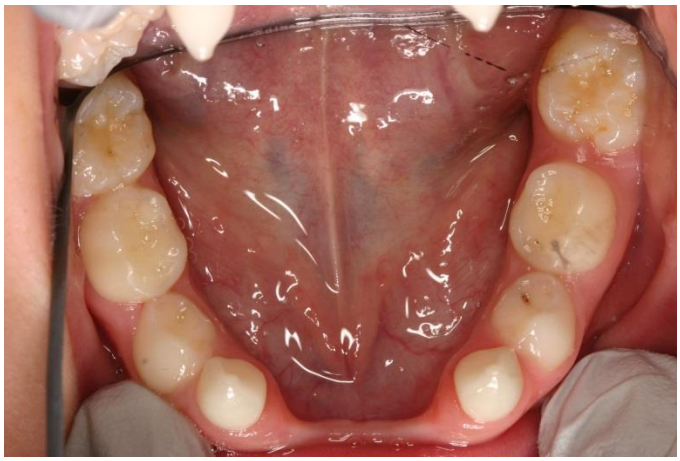
PRE-TREATMENT PHOTOGRAPHS: EXTRA and INTRA-ORAL (16/03/ 2011)











DIAGNOSTIC SUMMARY

- Caries in primary teeth LLD,LLE
- Infraoccluded LLD,LLE,LRD and LRE
- Severe hypodontia (Oligodontia)
- Upper anterior spacing
- Skeletal: Class III base

Motivation

- Good
- continuous support from his foster mother to carry on treatment

AIMS AND OBJECTIVES OF TREATMENT

1. Rehabilitation of function and aesthetics
2. Cosmetically improve appearance of anterior teeth
3. Management of caries primary teeth
4. Monitoring of developing dentition
5. Oral health maintenance and preventive programme

TREATMENT PLAN

Visit	Treatment and prevention provided
1 **16.03.11**	• Examination and charting • Oral hygiene instructions (OHI) • Issue diet sheet • Refer to hypodontia clinic
2 **11.05.11**	• OHI ,Simplified Plaque Index (PI), diet analysis & advice • Prophylaxis and fluoride application (F) • Composite restoration LLD,LLE • Silicon impression for construction resin bonded bridge (RBB) replacing LR2,LR1,L1,L2 and using LRC and LLC as abutment
3 **27.5.11**	• Resin fissure sealant on ✓ LR6,LRE,LRD, LLD,LLE,LL6 ✓ URD,UR1,UL1,ULD • Cementation of lower RBB • Upper primary impression for special tray • Patient was satisfied and happy with the bridge

4 **3.06.11**	• Silicon impression using constructed special tray • Shade guide
5 **23.6.11**	• Bite registration • Prosthodontic consultation with regard to upper over denture: ✓ No need for facebow registration ✓ Anterior teeth should be aligned in to class I
6 **12.7.11**	• Teeth try in
7 **29.7.11**	• Insertion • Post insertions instructions
8 **9.2.12**	• Periodic review ✓ No significant findings ✓ PI, OHI & F

9 **27.9.12**	• Periodic review ✓ No significant findings ✓ PI, OHI & F
10 **11.1.13** Review in hypodontia clinic	• Recommended to be reviewed in a year
11 **29-01-2013** Emergency appointment	• Pt misplaced denture • New impression for constructing upper denture which took several visits
16 **13.2.14**	• Periodic review ✓ No significant findings ✓ PI • OHI & F • Clinical photographs were taken

- Review every 6 -12months:
    - Periodic review of restorations
    - Review and reinforce oral hygiene measures and diet advice
    - Review upper overdenture and how is he coping with it
    - Monitor eruption of permanent teeth
    - Monitor infra-occluded primary teeth and any signs of ankeylosis

KEY STAGES IN TREATMENT PROGRESS

Liaison with paediatrician and cardiologist

T.D's physicians were contacted regarding his condition and there were no concerns regarding dental treatment under local anaesthesia (LA)

- ✓ Social worker was contacted and updated regularly with T.D progress

Prevention

- Plaque score 10% and subsequently 0%
- Oral hygiene instruction:
  - Brush twice daily with fluoridated toothpaste that contains at least 1,350 ppm fluoride
  - Brush last thing at night and first thing in the morning
  - Spit out after brushing and not to rinse
- Diet analysis and advice:
  - Issuing diet sheet
  - Sweets should be consumed at mealtimes as a dessert rather than between meals.
  - Sugars should not be consumed more than four times per day.
- Fluoride varnish application (2.26% F)

Behaviour management

- Non-pharmacological behaviour management techniques including tell-show-do and positive reinforcement were used during the provision of preventive care.
- Local anaesthetic (a pharmacological behaviour management technique) was utilised to provide the planned comprehensive dental treatment (Infiltration of 2% lignocaine with 1:80,000 epinephrine)

Caries management

Treatment performed under LA and rubber dam:

- Occlusal composite restoration for LLD and LLE
- Resin fissure sealant of LRD,LRE,LR6,LLE,LL6, UR1 and UL1

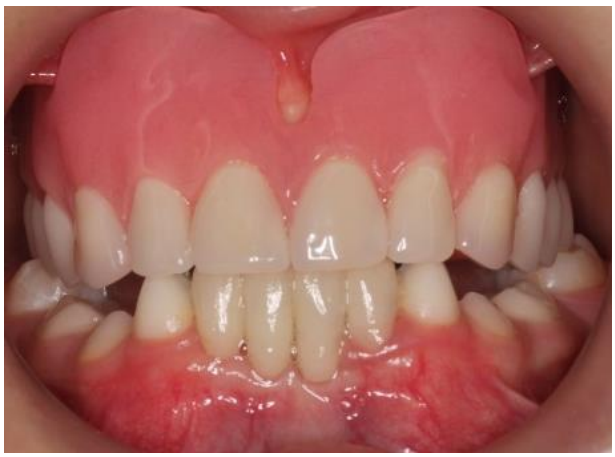
Aesthetic management

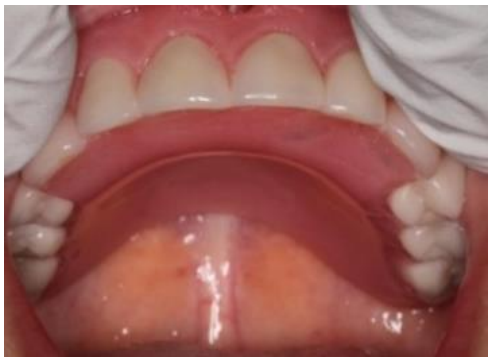
- Construct an upper over denture to improve:
    - aesthetic
    - correct occlusion
    - upper lip support
- Construct fixed fixed resin bonded bridge on lower anterior segment

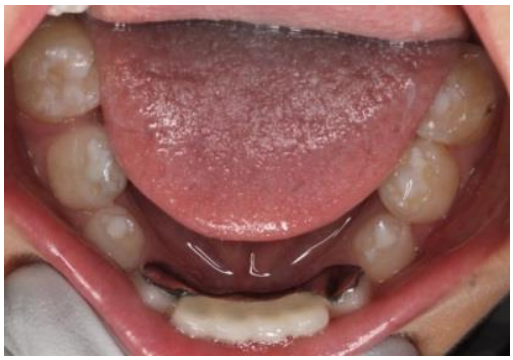
Review

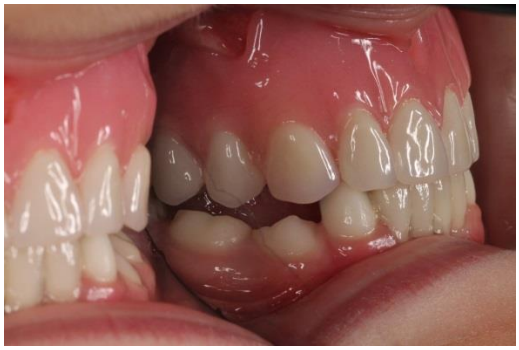
Periodic reviews every 6 months were done over 3 years, they involved monitoring of restorations, enhance oral hygiene, preventive and dietary advice, and fluoride varnish application (2.26% F).

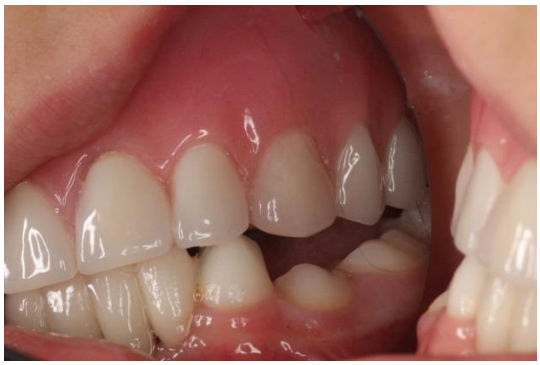
POST-TREATMENT PHOTOGRAPHS: EXTRA AND INTRA ORAL (last review 13.2 2014)











LONG TERM TREATMENT PLAN AND FUTURE CONSIDERATIONS

- Periodic review of restorations
- Review of oral hygiene and reinforcement of oral hygiene practice
- Monitor the development and eruption pattern of permanent dentition
- Monitor infraoccluded primary molars
- Referral to hypodontia clinic at appropriate timing to formulate a future plan to replace missing teeth. Possible treatment options include:
    - Partial dentures
    - Resin bonded bridge

- Implant

DISCUSSION AND REFLECTION

Axenfeld- Rieger's syndrome (ARS) (# OMIM 180500)

Axenfeld Rieger's syndrome (ARS) is a rare genetic disorder characterized by malformations of the anterior chamber of the eye (goniodysgenesis) it may be accompanied by a spectrum of dental, craniofacial, and somatic anomalies. Its frequency in the general population has been estimated to be 1 per 200,000. When only the eyes are affected, the condition is termed the Rieger anomaly [1]
At present, two different genes encoding transcription factors (PITX2 and FOXC1) are known to cause the alterations observed in the ARS. In addition, at least two genetic loci involve genes that have not yet been characterized (13q14 and 16q24). Furthermore, two other putative genes have been implicated (PAX6 and MAF) [1]

Clinical manifestation of ARS [2]

- Ocular : Goniodysgenesis and glaucoma
- Cranio -facial: Maxillary hypoplasia/prognathic profile, mandibular hypoplasia, hypertelorisim, prominent supraorbital ridges, telecanthus, broad nasal bridge, protrusive lower lip/recessive upper lip and enlarged sella turcica
- Dental: Hypodontia, hypoplasia, taurodontia, microdontia
- Associated systemic conditions: congenital heart defect, kidney malformations, retarded bone growth

Congenital heart defects are common in this syndrome and are often the clinical manifestations leading to diagnosis. Many different defects can occur but the most frequently reported cardiac malformations include ventricular septal defect.

Dental problems are hypoplasia or hypomineralisation of enamel, this common defect can increase the risk for dental caries (as is the case with T.D). Other dental problems including delayed tooth eruption and aberrant tooth shape, hence close monitoring of the development of the occlusion and radiographic examination should be undertaken at an appropriate age to exclude hypodontia[3]

Behaviour management

Several non-pharmacological behaviour management techniques like tell-show-do and positive reinforcement were used with him during the preventive care visits with the aim of creating positive dental attitude and long-term interest in prevention and maintaining oral health. T.D's hearing and vision impairment made communication challenging; however, improvement in his general behaviour toward dentistry was noticed over subsequent visits, his rating in Frankl scale improved definitely positive. However, T.D was shy and withdrawn at the first few visits, he refused photographs to be taken but subsequently agreed.

Restorative treatment (hypodontia)

Management of hypodontia requires a multi-disciplinary care which involves the close working relationship of a committed team contributing their individual expertise to achieve an optimum outcome for the patient and family. Early orthodontic and restorative interception is highly recommended for the best long-term treatment planning. After referring T.D to the hypodontia clinic, construction of maxillary overdenture was recommended. This was to improve aesthetics and provide better lip support than ordinary removable partial denture (RPD) [4]. In addition, resin bonded bridge was constructed for the lower anterior segment instead of RPD as children often cope better with fixed appliances rather than removable especially in the lower arch.

Infraoccluded primary molars

Retention of primary molar teeth where the permanent successor is absent is important in cases of hypodontia since they retain bone in an area which may be a site for future transplantation or implant therapy. The prevalence of infraocclusion is 1-9%. Altered pulp pathology will determine the most appropriate management but

may involve pulp therapy and restoration with pre-formed metal crowns. Where restoration for caries is not necessary, an infraoccluded tooth that is static may have onlays placed in order to facilitate cleaning as well as preventing food packing and its squeal. If seen early enough it may be prudent, in conjunction with orthodontists, to remove the primary molar tooth where there is no successor to achieve and optimum outcome. The longevity of the primary dentition, where there are no permanent successors, is uncertain [5.] In this case, over the review period the infraoccluded teeth were in the same level without any signs of ankyloses. Therefore, the suggested treatment was to monitor.

Preventive care and Recall

T.D had multiple carious lesions with hypomineralisation enamel defects. Therefore it was important to establish preventive regimen aiming to prevent the progress of the disease and reduce the risk of development of further caries. An evidence-based preventive approach was utilised for T.D

He was placed on a 6 months recall visits, and with implementation of behaviour management techniques, he received diet advice, oral hygiene instructions, and fluoride therapy [6]

LESSON LEARNT

- I have learned that careful planning of treatment while liaising with other colleagues is essential for successful management of these children. This requires clear pre-treatment and early liaising with orthodontics and prosthodontics in hypodontia clinic
- It is essential for these patients and parents to be educated about oral hygiene and regular recalls to maintained all remain teeth
- It was rewarding experience, seeing this young man regaining his confident and self-steam and how has it implied on his attitude toward dental treatment.

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6. Department of Health and British Association for the Study of Community Dentistry (2014). Delivering better oral health: an evidence-based toolkit for prevention. Third Edition. London. Department of Health

CASE NUMBER: 3

Patient’s age at start of treatment: 10 years, 4 month

CASE SUMMARY

C.L a 10 year old boy, who was referred by A&E for management following severe dentoalveolar trauma, which resulted in avulsion of maxillary permanent central incisors. C.L received emergency treatment at a General Hospital where replantation and splinting of the teeth was performed.

Subsequently he presented one week later with tenderness to percussion and mobility. Pulp extirpation and root canal treatment were carried out, currently, 3 years following the accident C.L has a restored dentition that is functionally and aesthetically acceptable. However, UR1 has a poor prognosis.

This case will be monitored aiming to preserve the affected teeth on the medium term, the bone and mucogingival structures on the long term and provide a definitive long term treatment options.

PATIENT DETAILS

Initials: C.L

Gender: Male.

Age at the start of treatment: 10 years, 4 month.

Age at the last review: 13 years, 5 months.

PRE-TREATMENT ASSESSMENT

PATIENT COMPLAINT

- Pain associated to upper front teeth.

HISTORY OF PRESENTING COMPLAINTS

- Trauma of anterior teeth on the 12/7/2011 at 04:30 pm when patient hit a lamp post while he was on his bicycle in front of his house
- UR1 and UL1 were avulsed and both were fractured
- The avulsed UR1 had half root fracture and couldn't be found
- Chin and lower lip were abraded
- Teeth were immediately stored in milk and patient attended Accident and Emergency department at General Hospital where
    - ✓ Posteroanterior X ray view was taken to roll out any skull injuries. No neurological signs or symptoms were detected.
    - ✓ Amoxicillin 250 mg TDS was given
    - ✓ Tetanus booster was given

- ✓ Around 6:20 pm teeth were replanted under local anaesthesia, teeth were splinted with resin composite. Patient was referred to the paediatric department

- Patient was seen in the department as emergency on 17/7/2011

RELEVANT MEDICAL HITORY

- Mild asthma controlled with salbutamol inhaler.

DENTAL HISTORY

- Regular attender at general dental practice.
- No previous dental treatment.

CLINICAL EXAMINATION

Extra-Oral Examination:

- Lower lip swelling
- Upper lip and chin abrasion
- No lymphadenopathy.
- No muscles tender to palpation.
- Tempromandibular joint (TMJ): no pain, no deviation in closing.
- Behaviour :
  - At first visit he was complaining of sore mouth and pain in his teeth which subsequently increased his anxiety level (Frankl scale: 2). Through visits he was cooperative , well – motivated and looking forward to improve the aesthetics of his teeth

Intra-Oral Examination:

- UR1 enamel and dentine fracture (E+D#) mesially
- UL1 E+D# mesially
- UR2 E+D# incisally
- UR1 and UL1 tender to percussion, grade II mobility

- Poor resin composite splint (chipped interproximally btween UR2,UR1, UL1and UL2)

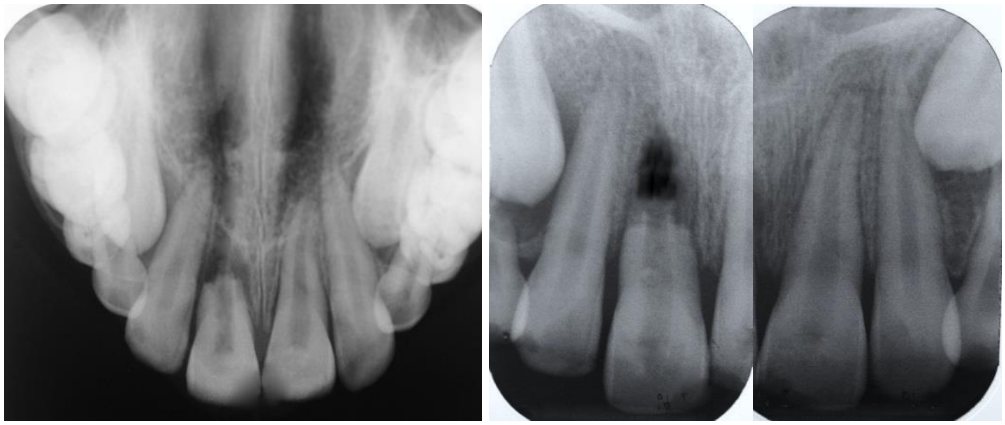
Vitality test:

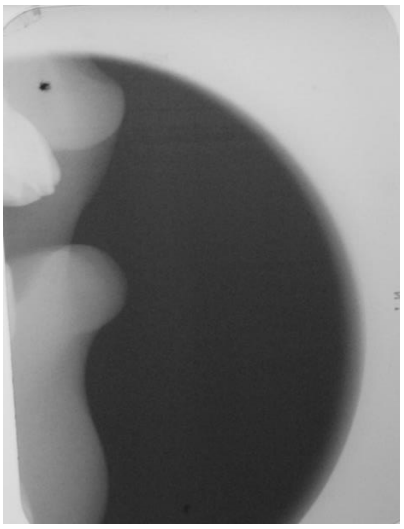
- ✓ UR1,UL1 negative
- ✓ UR2 positive

- Poor oral hygiene (Simplified plaque index) PI : 76%
- Mixed dentition stage.
- Orthodontic assessment:
  - Class I incisors
  - Class I molars relationship both right and lift
  - Well aligned upper and lower labial segments

RADIOGRAPHIC EXAMINATION

Periapical radiographs for UR1, UL1 and Upper anterior occlusal radiograph, to assess the dental trauma.



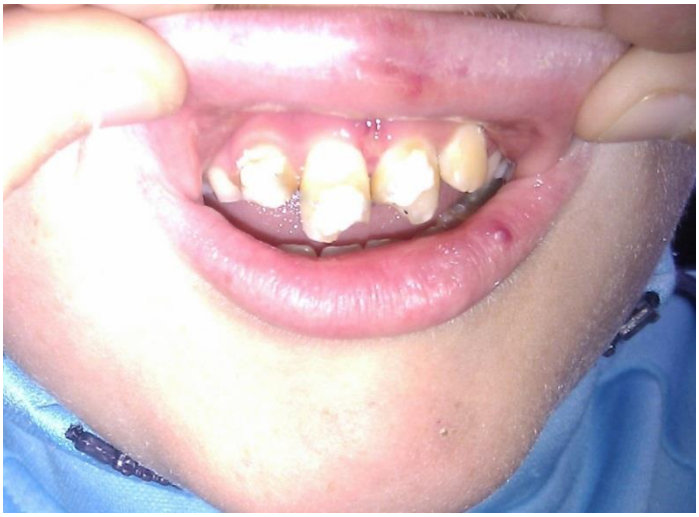


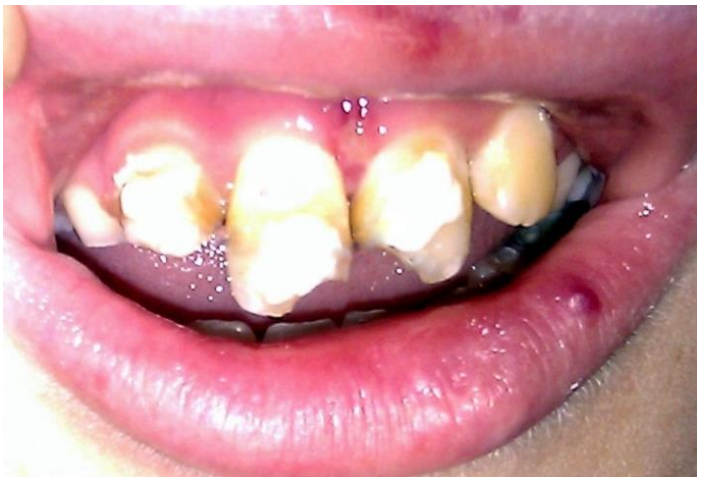
12-7-2011

RADIOGRAPHIC FINDINGS:

- UR1: enamel dentine fracture and missing half of the root,
- UL1: Enamel dentine fracture, radiolucency suggesting inflammatory resorption, no signs of root fracture and closed apex with complete root development.
- Soft tissue X-ray was taken to exclude any tooth fragment which could be impeded in the lower lip

PRE-TREATMENT PHOTOGRAPHS: EXTRA-ORAL AND INTRA-ORAL





Note: C.E was in pain and quite anxious therefore obtaining more pictures was difficult

DIAGNOSIS SUMMARY

Hard tissue

- UR1:
  - Avulsion
  - E +D#
  - Half root fracture and couldn't be found

- UL1:
    - Avulsion
    - E+D#
- UR2: E+D#
- Caries free dentition.

Soft tissue

- Upper lip abrasion

AIMS AND OBJECTIVES OF TREATMENT

- Restore function and aesthetic of the traumatised incisors.
- Eradicate infection and stabilise inflammatory resorption by performing pulp extirpation.
- Monitor periodontal ligament of the injured teeth for signs of root resorption.
- Preserve teeth in the medium term, aiming to maintain the bone level in the long term.
- Conserve the upper permanent central incisors for as long as possible
- Improve OH
- Educate patient and parents about the poor long term prognosis of these teeth, time required for treatment and the available future treatment options.

TREATMENT PLAN

1- Immediate re-splinting of UR1 and UL1 using orthodontic wire and resin composite
2- Pulp extirpation of UR1 and UL1
3- Restoration of fractured teeth with composite resin restorations.

4- Monitor the periodontal ligament of the injured teeth for signs of root resorption or bone replacement
5- Monitor adjacent teeth
6- Provide preventive advice and oral hygiene instructions.

KEY STAGES IN TREATMENT PROGRESS

- **First visit 12/7/2011**
    - Examination, radiographs, clinical photographs.
    - Pulp extirpation on UR1 and UL1 under local anaesthesia (Infiltration of 2% lignocaine with 1:80,000 epinephrine) and dry dam
    - Estimation working length and $Ca(oH)_2$ dressing on UR1 and UL1
    - Pulp sensibility testing (ethyl chloride and electric pulp testing) for UR2 and UL2 which responded positively
    - Splinting UR1 and UL1 for 4 weeks
    - Flowable composite using L-Pop on UL1, UR1 and UR2 to cover the exposed dentine

- **4 weeks visit 18/7/2011**
    - Splint removal
    - Pulp sensibility testing (ethyl chloride and electric pulp testing) for UR2 and UL2 gave positive responses.
    - UR1 grade II mobility
    - $Ca(0H)_2$ change UR1and UL1, Sodium hypochlorite as irrigation and working length

- composite restoration on UR1,UR2 and UL1

- **3 months visit (14/11/2011)**
  - Pulp sensibility testing UR2 and UL2 gave positive response
  - No signs of bone replacement
  - UR1 grade II mobility
  - UR1and UL1 $Ca(0H)_2$ change
  - Mouth guard was constructed and advised to wear during sports

- **6 months visit (6/2/2012)**
  - UL1 obturation performed using performed with (Obtura-II system)
  - UR1 obturation performed using MTA
  - post-operative periapical radiographs UL1, UR1 showed satisfactory root filling

- Review visits at 6, 12 and 24 showed no signs or symptoms of infection, both clinically and radiographically.

- **Review visits at 36 months (6/8/2014)**
  - Disclouration UL1
  - Grade II mobility UR1
  - Poor OH which lead to moderate gingivitis

- Patient now is under annual review visits.

Prevention and oral Hygiene improvement

Prevention

- Oral hygiene instruction:
    - Brush twice daily with fluoridated toothpaste that contains at least 1,350 ppm fluoride
    - Brush last thing at night and first thing in the morning
    - Spit out after brushing and not to rinse
- Fluoride varnish application (2.26% F)

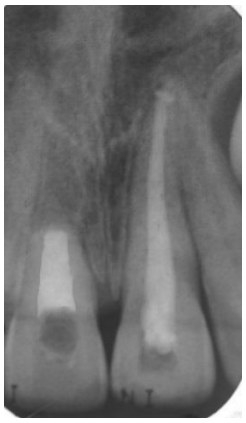
At last review patient was referred to the hygienist clinic for oral hygiene instructions, dietary advice, and fissure sealant and fluoride application.

Long term restorative options

- Periodic recalls to review traumatised teeth and evaluate the degree of mucogingival defect which may occur if bone replacement happens
- Bleaching if any traumatised teeth developed discolouration
- Future treatment options
    - Partial removable denture
    - Resin bounded bridge
    - Auto-transplantation
    - Implant

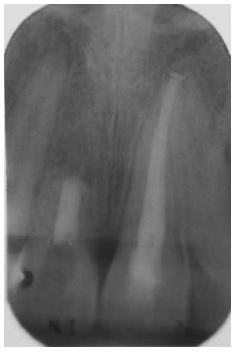
POST-TREATMENT RADIOGRAPHS

PA 6 months post trauma (6/2/2012)



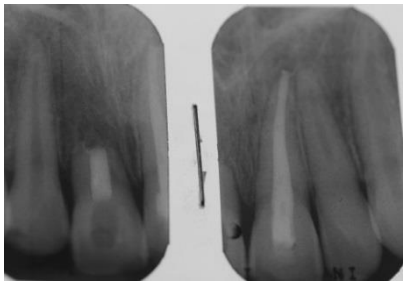
- UL1: Well condensed Gutta-percha (GP).
- Length of GP: to the root apex.
- UR1 well condensed MTA
- No signs of periapical lesions, pathology and bone replacement

PA 24 months post trauma (30/8/2013)



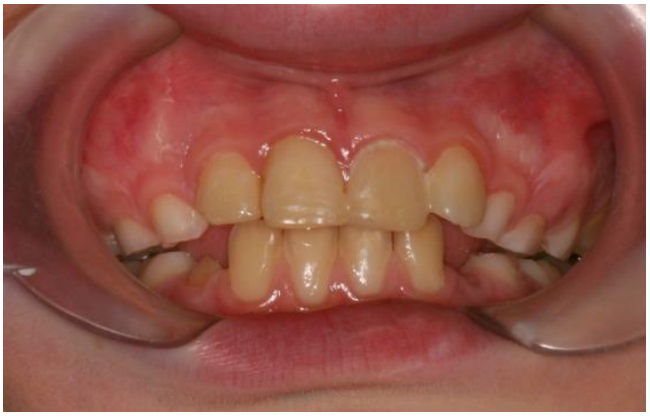
- No signs of periapical lesions or pathology or bone replacement
- No occlusion disturbance

Last Review 3 years post trauma (6/08/2014)

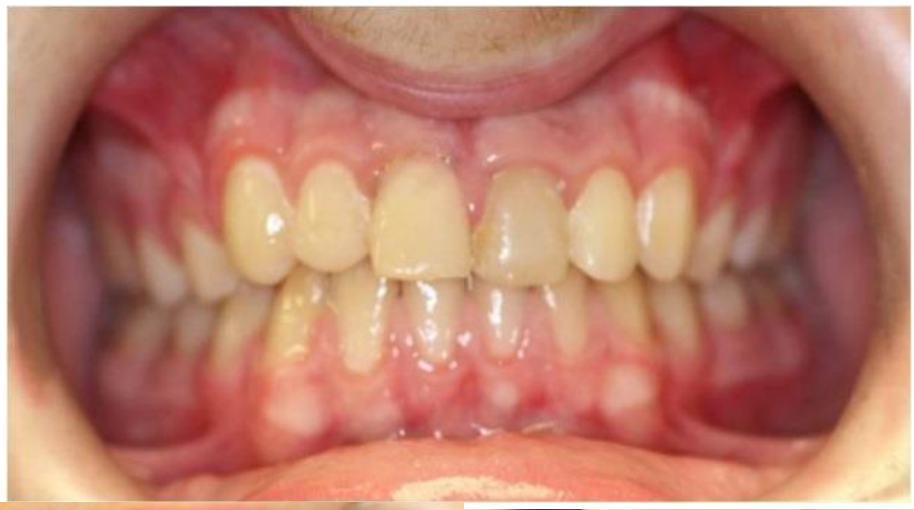


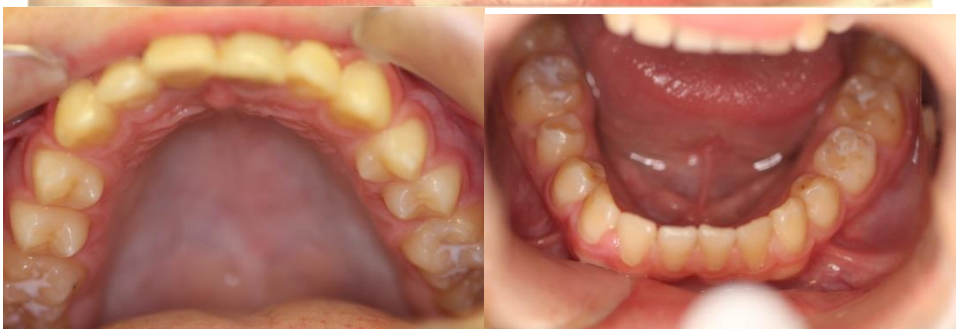
- No signs of periapical lesions or pathology or bone replacement

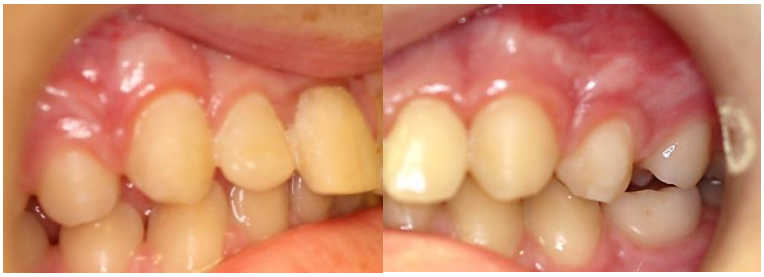
INTERMEDIATE PHOTOGRAPHS: 12 MONTHS POST TRAUMA 30/8/2012



POST-TREATMENT PHOTOGRAPHS: EXTRA-ORAL AND INTRA-ORAL 3 YEARS POST TRAUMA (6/8/2014)







DISCUSSION AND REFLECTIONS

Avulsion injuries

Introduction

Avulsion represents the one of the most severe displacement injuries, and comprises 0.5-3% of the traumas affecting the permanent dentition. Numerous studies have shown that this injury is one of the most serious dental injuries, and the prognosis is very much dependent on the actions taken promptly after the avulsion. Replantation is in most situations the treatment of choice, but cannot always be carried out immediately. An appropriate emergency management and treatment plan are important for a good prognosis [1].

In C.L case, teeth were restored in milk for two hours. According to the IADT, this is considered as non-favourable outcome for mature roots, as the majority of cases ended with bone replacement [2]. However, C.L avulsed teeth did not suffer any signs of bone replacement even after 3 years of follow up.

Management in Clinic

Choice of treatment is related to the maturity of the root (open or closed apex) and the condition of the periodontal ligament cells. The condition of the cells is depending on the storage medium and the time out of the mouth, especially the dry time, as this is critical for survival of the cells. After a dry time of 60 min or more, all periodontal ligament (PDL) cells are nonviable[1,2]

What made this case challenging are the extra-oral time, the root fracture of UR1 and the maturity of the roots. All these will have an impact on the prognosis of these teeth. In this case the goal in delayed replantation was, in addition to restoring the tooth for aesthetic, functional and psychological reasons, to maintain alveolar bone contour [3]

Splinting

It is considered best practice to maintain the repositioned tooth in correct position, provide patient comfort and improve function. Current evidence supports short-term, flexible splints for splinting of replanted teeth. Studies have shown that periodontal and pulpal healing is promoted if the replanted tooth is given a chance for slight motion and the splinting time is not too long [1]. In this case, 4 weeks of splinting using orthodontic wire and composite resin was carried out, following the IADT recommendation for a closed apex tooth with < 60 minutes extra orally.

Endodontic treatment

Root canal treatment is indicated with closed apex teeth, the ideal time to begin treatment is 7–10 days post-replantation. Calcium hydroxide is recommended as an intra-canal medication for up to 1 month followed by root canal filling with an acceptable material as long it's free of any signs or symptoms of persisting infection. C.L started endodontic treatment 7 days after accident and calcium hydroxide has been successfully used to form an apical calcific barrier, high success rates in the range of 79−96% have been reported [4]. Despite such success rates, however, calcium hydroxide has its limitations associated with the long treatment time needed. There is also an increased risk of loss of coronal seal and tooth fracture owing to prolonged dressing with calcium hydroxide [4]. Additionally, the calcific barrier formed has been found to be porous and often containing small amounts of tissue. Therefore, Mineral trioxide aggregate (MTA) has recently emerged as a material suitable to overcome the problems associated with calcium hydroxide. It offers good sealing ability, biocompatibility, antibacterial properties and the ability to induce apical hard tissue formation. In this case, MTA was the material of choice for UR1 due to wide opened end, therefore it was difficult to obtain apical barrier using regular $Ca(OH)_2$. In contrast, UL1 was filled with gutta-percha after apical barrier formation within six months from trauma due to the root development. MTA provided optimum sealing for a very short root which subsequently increased the chance of tooth discolouration [6].

Outcomes of replanted avulsed teeth

Despite the delay in replantation, maturity of roots and root fracture results were beyond expectation. The teeth remain functional and symptom free over 36 months. C.L was pleased with the outcome; however UR1 has a poor prognosis due to the short root

Lessons learnt

- It is very important to be realistic about the long term prognosis of such cases and to educate the patient and the parents about the different trauma outcomes and treatment options especially in case the tooth is lost in the future
- It is very challenging managing child behaviour immediately after trauma
- Choosing final root canal restoration by weighing the benefits and the disadvantages of each dental material which will end in patient's best interest

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