Dimensional stability and microleakage of SMART dental composites in primary teeth

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Declaration

I declare that the work represented in this thesis is the result of my own investigations, except where otherwise stated. Information from the published and unpublished work of others has been acknowledged in the text and the relevant references are included.

Aspasia Katsimpali
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Signature

Date 25/7/2019

Dedication

To my mother who is one of the best Paediatric dentists I have come across and that inspired me to become one myself. To my family, friends and colleagues who withstood the constant complaining. Special dedication to my partner Efstathios Kousounis for his patience, love and support.

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Abstract

Dental composites were a revelation when they were introduced into the market. Even though they have kept evolving by becoming mechanically stronger, less technique sensitive and aesthetically appealing, they are still not easy to place and fail due to bacterial microleakage which leads to recurrent caries. Therefore, they have not been the staple material of choice for paediatric patients since they are not as reliable as preformed metal crowns long-term.

AIMS AND OBJECTIVES: The aim of this project was to develop novel SMART composites which will target the paediatric patient. They have antibacterial polylysine and remineralising MCPM incorporated in them which could potentially address the bacterial microleakage problem of existing materials. They are self-etching and can be used as a bulk filling after soft caries removal. Good monomer conversion at depth and dimensional stability are essential for bulk filling and prevention of microleakage respectively and therefore the focus of this work.

METHODS: An FTIR machine was used to measure the monomer conversion at 2 different sample depths (1mm, 2mm) with three different curing times (10s, 20s, 40s) in order to determine the effect of low versus high MCPM and PLS. In addition, mass and volume change was measured and shrinkage was calculated in order to determine the volumetric stability of the SMART composites. To assess microleakage, a dye test was performed in natural primary teeth drilled and directly filled with the SMART composite. This had the highest MCPM and PLS content. Three commercial materials which included Activa (following tooth etching) and two glass ionomer cements were used as comparators.

RESULTS: Antibacterial polylysine and remineralising MCPM had negligible effect on monomer conversion. Volume change (2%) upon water sorption could help balanced polymerization shrinkage (3%). Microleakage of the SMART composite was equivalent to Activa but less than the glass ionomer cements.

CONCLUSION: SMART composites good monomer conversion, volumetric stability, microleakage resistance and ease of placement should enable a more viable and predictable composite restorative paediatric option in the future.

Impact statement

Dental caries is a global problem and the focus rightly has been towards prevention. Early Childhood caries though is on the rise with an astonishing worldwide prevalence of 63% in 5 year-olds that require dental treatment.

This thesis is part of a number of theses aiming to develop a novel dental composite material that surpasses the weaknesses of already existing materials and provides novel dynamic features to the mundane process of restoring a cavity. Bacterial microleakage is the main reason existing composites fail and therefore is addressed by incorporating the antibacterial and remineralising elements into this developing material. Self-etching, antibacterial and remineralising materials exist in the literature but not as yet commercialised. The aim of this work is therefore to produce SMART composites that are commercially viable.

If SMART composites manage to pass the clinical trial phase and become released to the market, they will provide a more aesthetic reliable option for young, anxious paediatric patients who are unable to cooperate for chairside dental treatment. This would gradually have an impact on the number of patients undergoing dental treatment under general anaesthetic. This would lead to major money savings for the NHS that could be spent in other domains in need.

Furthermore, this material is ideal for a large scale national health system dental intervention not only in the UK but in other countries as well. It only requires a dental professional to identify the decayed tooth, basic moisture control with a cotton roll, ideally a hand excavator, the material and a light curing device.

Last but not least, another group of paediatric patients that would benefit from such a material would be that of underdeveloped low income countries that do not offer dental care through their national health systems. It should keep in mind that conventional dentistry as practised in developed countries, is expensive. It requires a lot of expensive technologically savvy gadgets, training and materials in order to restore teeth and this cost is too much in some parts of the world. SMART composites would offer an alternative in such countries where the only solution would be extraction.

Table of Contents

Table of Figures

Table of Tables

Abbreviations

4META	4-Methacryloyloxyethyl trimelitate anhydride
Bis-GMA	Bisphenol-A-glycidyl methacrylate
CQ	Camphorquinone
DMAEMA	2-Dimethyl amino ethyl methacrylate
ECC	Early Childhood Caries
FDI	World Dental Federation
FTIR	Fourier Transform Infrared
GIC	Glass Ionomer Cement
MCPM	Monocalcium Calcium Phosphate Monohydrate
PPGDMA	Poly-Propylene Glycol Dimethacrylate
PLR	Powder Liquid Ratio
PLS	Polylysine
RMGIC	Resin Modified Glass Ionomer Cement
SDF	Silver Diamine Fluoride
TEGDMA	Triethylene Glycol Dimethacrylate
UDMA	Urethane Dimethacrylate
WHO	World Health Organization

1 Literature Review and introduction

This chapter eases the reader into what is dental caries, explores current restorative materials and concepts, highlights the main problems clinicians face and introduces a possible solution.

1.1 Caries

1.1.1 Definition

The term caries or dental decay is used worldwide in describing the process and clinical manifestation of tooth decay (Kidd and Fejerskov, 2004).

1.1.2 Epidemiology

The WHO (World Health Organisation) began constructing a global map with data on the prevalence of caries back in 1969, because caries is considered one of the most common chronic diseases affecting humanity worldwide. It is stated that 60 – 90 % of young people in industrialised countries are affected by tooth decay (Iheozor-Ejiofor et al., 2013), and globally 35% of the general population (including children) present with caries. What is interesting is the fact that caries is a preventable disease. Therefore, since 1985 in the United Kingdom, there have been several surveys of child dental health under the Public Health England (PHE) epidemiology program, to document the problem, plan and organise appropriate public health interventions. According to the latest survey, 12% of three year olds in England have one or more teeth affected by dental decay extending into dentine (PHE, 2013). This percentage rises to 24.7% at five years of age in the same region (PHE, 2016) and reaches an astonishing 46% in fifteen year olds (Vernazza et al., 2016). If caries is left untreated it may affect an individual's everyday life and can be deemed a cause of disability (Listl et al., 2015). Therefore, it comes as no surprise that caries is one of the most common reasons children are hospitalised (Moles and Ashley, 2009). Prevention from an early age would have a great impact on quality of life, since it has been shown that dental caries may affect body weight and growth in pre-school children (Heilmann et al., 2016).

1.1.3 Aetiology

The aetiology of caries can be broken down into three major causes : bacteria, diet and susceptibility.

1.1.3.1 Bacteria

The cariogenic process begins in the biofilm, known as dental plaque, a pellicle that hosts a community of microorganisms which coats all solid surfaces in the oral cavity (Manji and Fejerskov, 1990; Zero et al., 2009).

The tooth is a combination of hard tissues; – enamel, dentine, cementum- which are considered solid surfaces within the oral cavity and are coated with a biofilm. These communities of microorganisms are very metabolically active, and therefore the products of this activity – acids resulting from carbohydrate metabolism- cause constant changes in the pH of the oral cavity. These constant changes play a significant role in the dynamic process of remineralization or demineralization of the hard tooth structures (Marsh, 2006; Takahashi and Nyvad, 2011). When the pH is below the critical pH of 5.5, the hard tooth tissues demineralise (Dawes, 2003) and when it rises again, they will remineralise (Hicks et al., 2004). Biofilms are environment and location sensitive which translates into different sites of the tooth being more susceptible to caries than others. Occlusal surfaces for example, that are

protected from the tongue, the cheeks, abrasive foods or tooth brushing, appear to have a more mature biofilm and are therefore more susceptible in acquiring a carious lesion (Kidd and Fejerskov, 2004).

In the past, three hypotheses were proposed regarding the role of bacteria in the aetiopathogenesis of dental caries. The first hypothesis was the specific plaque hypothesis, which suggested that specific bacteria in the dental plaque – mainly mutans streptococci and lactobacilli - contained in the dental plaque were considered the sole cause of caries (Loesche, 1976). In contrast, proponents of the non-specific plaque hypothesis advocated that the total bacteria community in the dental plaque were involved in initiating and sustaining the caries process (Theilade, 1986). Advancements in our knowledge of caries microbiology and the mechanism of dysbiosis, resulted in the proposal of the ecological plaque hypothesis (Marsh, 1994) which is considered the most accepted theory of caries pathophysiology. This states that the biofilm's cariogenic ability is affected by the balance of microbial interactions among biofilm bacteria and with their environment. This sensitive bacterial homeostasis is dynamic, meaning that any change can affect it topically and predispose specific sites to dental decay. For example the presence of Actinomyces spp. and Streptococcus Mutans which are both acid producers disturbs this homeostasis, enhances the demineralization process and hence the dental decay (Takahashi and Nyvad, 2011).

1.1.3.2 Diet

The increased consumption of carbohydrates either in the form of solid food or drinks is associated with increased dental caries. WHO suggested that a 10% decrease in the intake of sugar may be enough to lower dental caries (Moynihan and Kelly, 2014). In general, it should be kept in mind that sugar intake has a lifelong impact and caries have a significant link with obesity which affects more than 340 million children and adolescents worldwide (WHO, 2015).

1.1.3.3 Susceptibility

Saliva is a major factor in caries formation and progression. A new pathway of genes affecting the immune system via saliva - the flow, buffering capacity or consistency- and therefore indirectly caries is currently being explored. Sequencing is apparently the future. What is of interest to paediatric dentists is that heritability seems to mainly apply on the primary dentition (Werneck et al., 2010).

1.1.4 Prevention

As mentioned before, even though caries affect such a large amount of the population, it is a preventable disease. In June 2014, Public Health England published the third edition of a prevention toolkit, called "Delivering better oral health: an evidence based toolkit for prevention" to emphasize the importance of prevention and provide evidence-based guidance to dentists for the preventative management of caries. Fluoride intake is the most significant factor in preventing dental caries, since it interferes with the mineral loss or gain of hard dental tissues. The toolkits' aim is to maximise the daily intake and impact of fluoride through simple everyday interventions. According to the toolkit, all children should use fluoridated toothpaste with a minimum 1000ppm of fluoride concentration when they are younger than six years old; above six

years of age, the use of toothpastes containing higher fluoride concentrations (1450ppm) is recommended. Brushing should take place twice a day, always under supervision and rinsing with water is not recommended in order not to wash out the fluoride (PHE, 2014).

It is well established that fluoride in constant low concentrations is beneficial in preventing dental caries and in light of this, water fluoridation has been taken up in some countries such as the USA (AAPD, 2008). Parts of the UK have fluoridated water as well and certain schools offer fluoridated milk (Yeung et al., 2015), but there is still room for improvement. The cost of setting up and sustaining a national water fluoridation is immense but very small in comparison to the cost of restorative dentistry that could potentially be avoided. The substantial cost and presence of insufficient evidence in order to determine the effect of water fluoridation in preventing dental caries have been successful in putting a halt in such national wide interventions. On the other hand, there is also no evidence to stop already existing water fluoridation programs (Iheozor-Ejiofor et al., 2013). Therefore, governments should reconsider.

1.1.5 Management of Dental Caries

Although caries can be reversible in the first stages of the disease, the formation of a cavity has a significant clinical importance since it marks the time when prevention failed and a restoration must take place. Many different schools of thought exist such as complete caries removal, partial caries removal, stepwise excavation of caries and the “seal it in” concept. The evidence supporting the “seal it in” concept is increasing. What this concept entails is the partial removal of soft caries, and the hard caries are left behind in order to avoid a pulp exposure, which could escalate the management to include root canal treatment (Ricketts et al., 2013). There are more advantages in a single visit partial caries removal than two visits in primary teeth (Ribeiro et al., 2012). The carious dentine left behind and sealed, appears to be less infected than conventional dentine following caries removal and therefore removing it would not be necessary (Maltz et al., 2013). What is crucial for partial caries removal to be successful, is not the caries removal technique (partial or complete), nor the material used (Casagrande et al., 2013). It is the maintenance of a bacteria-tight seal, ensuring that any residual bacteria are rendered metabolically inactive, which allows the caries process to become arrested. In order to aid clinicians to attempt successfully sealing, a great variety of materials exist today such as amalgam, glass ionomer cements, resin composites, resin modified glass ionomer cements and preformed metal crowns (PMC).

1.1.6 Caries: a problem without borders

Dental caries is definitely a problem both for each individual affected and for the public as a whole. First of all, the function of a person is affected, meaning that everyday tasks such as chewing food adequately and eating a variety of food in terms of texture (hard, soft, chewy like, etc.) is affected. In addition, attendance to work and performance can be influenced if in acute pain from dental decay affecting one or more teeth. Last but not least, participation in

social events may be avoided due to the inability to smile as a result of compromised aesthetics which can lead to isolation.

It is evident that dental caries can have a significant impact in a persons'- adult or child- quality of life (QOL). According to the World Health Organisation (WHO) quality of life is defined as "an individual's perception of their position in life in the context of the cultural and value systems in which they live and in relation to their goals, expectations, standards and concerns" (WHO, 1997). This means that it includes not only the practical aspects such as function and aesthetics but also the child's psychological and social well-being which is most of the times unaccounted for in studies about quality of life.

1.1.6.1 Person

A clinical assessment cannot depict the 100% impact of intraoral conditions on the well-being of a patient, let alone a child who may have difficulty communicating this to an adult (McGrath et al., 2004). Since oral health is age dependent (John et al., 2004), so is oral health related QOL (OHRQOL) therefore differences exist amongst children and adults (Tapsoba et al., 2000). OHRQOL has such an impact that validated, age-specific questionnaires have been developed in an attempt to study and quantify it (Sischo and Broder, 2011). Unfortunately, they focus on frequency or impact of symptoms and do not include questions such as how it makes the child feel.

Paediatric dentists are particularly interested in the Oral Health Related Quality of Life (OHRQOL) and it's correlation with the paediatric patient which in most studies is negative. Even though the majority of the studies come from under-developed countries (such as Brazil), the aforementioned problem is pertinent even in developed countries such as Norway, that may have established prevention programs for years (Dahl et al., 2011). What is of interest is that the socioeconomic status (education of parents, income) of the family of a child, as well as the age of the mother and home conditions (family structure, siblings or not, crowding of house) have an effect on the oral health quality of life of the child (Kumar et al., 2014; Ramos-Jorge et al., 2015). In children the above translates into the appearance of a special kind of caries known as Early Childhood Caries which has a detrimental effect on their QOL (Sara L. Filstrup, 2003; Fernandes et al., 2015).

1.1.6.2 Public

For the Public, caries is an expensive disease to treat. The global economic impact of dental disease was calculated at an astonishing US$422 billion in 2010 with US$298 billion accounting to direct costs and US$144 billion to indirect costs (Listl et al., 2015). In order to understand the number above, note that cancer worldwide costed approximately US$895 billion in 2008 (Society, 2010). The fact that dental disease costs almost half as much, dictates the gigantic economic burden health systems worldwide. In the UK alone, the NHS spent more than 30£ billion for hospital based tooth extractions for children under the age of 18 years-old in 2012 to 2013 (RCS, 2015).

1.1.7 Early Childhood Caries (ECC)

Early Childhood Caries (ECC) is an age specific rampant pattern of caries that affects children between birth and 71 months of age (AAPD, 2016). In literature it is referred to as “nursing caries”, “bottle caries”, “baby bottle tooth decay” and “night bottle mouth”. Only in England, 12% of three year olds have one or more teeth affected by dental decay extending into dentine(PHE, 2013). No pattern has been universally identified in order to be able to recognise which groups in a society are of higher risk, although similar to caries there is an association with the socioeconomic status of the family and the education level of the child’s carer (Psoter et al., 2006). In the past they were related to nocturnal feeding but now the term is age confined and therefore aetiologically does not differ from the very well investigated dental caries.

ECC is a major problem for the young population worldwide and because it affects so many, the International Association of Paediatric Dentistry organised a global summit in Bangkok in November 2018 in order to discuss ECC epidemiology, aetiology, risk assessment, societal burden, management, education, and policies under a global perspective and produced a paper to guide professionals (Tinanoff et al., 2019). The global mean prevalence was derived by 72 worldwide studies conducted between 1998 and 2018 and divided by age group, was 17% for 1 year-olds, 36% in 2 year-olds, 43% in 3 year-olds, 55% in 4 year-olds and 63% in 5year-olds. It is astonishing how the percentage doubles from 12 to 24 months of age and how by the age of five years old almost six out of ten children are affected (Tinanoff et al., 2019).

Due to the young age of the affected patients, caries management can also present considerable behavioural challenges. Treatment must ideally be carried out by trained Paediatric dentists. Speed is key for success, and the aim is not to aesthetically satisfy the parents but to stabilise the mouth and keep the patient free of pain and active infection, deeming them dentally fit. Meeting the above standards can be challenging, and therefore it comes as no surprise that less than 15% of dental caries on patients under the age of five are treated in the United Kingdom (Brown et al., 2006; Duangthip et al., 2017). Due to its complexity, the management of ECC may incorporate various treatment modalities including the conventional invasive approach of “drilling and filling”, the less invasive Atraumatic Restorative Technique (ART) or alternative non-invasive approaches such as the Hall technique using preformed metal crowns (Arrow, 2016; Tedesco et al., 2017) including fluoride varnish, Silver Diamine Fluoride (SDF), chlorhexidine (CHX), xylitol and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Yengopal and Mickenautsch, 2009; Cochrane and Reynolds, 2012; Duangthip et al., 2017). Furthermore, pulp treatment of primary teeth, multiple extractions of primary teeth which may require a space maintainer device, increasing the need for future orthodontic treatment, are also included. All the above techniques may be performed chair-side or require the need for sedation (intravenous or inhalation sedation) and/or general anaesthesia when children cannot cooperate, raising the overall cost. ECC also have an impact in medical expenditures since if left untreated may require hospitalisations, emergency visits, cause growth and development delays and due to the acute or chronic pain dictate the use of painkiller medications and antibiotics.

1.1.8 Secondary/Recurrent caries

The term secondary caries describes a carious lesion in the margins of a restoration which occurred after the initial caries were removed and restored (Mjor, 2005). It is though that when the restoration/tooth interface fails, a gap is created due to microleakage, in which the biofilm extends and results in secondary caries (Kuper et al., 2015). They are the predominant reason composites fail after 24 months (Kuper et al., 2014), a timeframe that is shorter than that of amalgam restorations (Rasines Alcaraz et al., 2014). This may happen due to failure of the operator, non-compliance in oral hygiene from the patient or a combination of the above. A clinical study by Bernardo et al showed that secondary caries incidence in composite restorations is 3 to 4 times higher than in amalgam restorations, has shifted scientific interest towards a material specific problem (Bernardo et al., 2007). Another study that examined primary teeth specifically also indicated secondary caries as a failure reason (Hickel et al., 2005). Many composite properties have been the subject of investigation in order to determine which one encourages the occurrence of secondary caries more. Nedeljkovic et al. (2015) discussed the effect of polymerization shrinkage and microleakage and in 2016 focused more on the ability of a material to hold a buffering ability towards cariogenic bacteria such as *S.Mutans* (Nedeljkovic et al., 2015; Nedeljkovic et al., 2016). This indicates that a composite material with antibacterial properties, and the ability to increase the pH at the restoration/tooth interface, could potentially be resistant to secondary caries. As a result, the longevity of such a composite material would extend hopefully beyond the 2 year limit of detected failure and surpass that of amalgam restorations which have now been phased out.

1.2 Current Restorative Dental Material Options

1.2.1.1 Amalgam

Amalgam has been the “golden” standard material in dental restorations for the last 165 years (Rasines Alcaraz et al., 2014) because it is cheap, easy to use and reliable. In 1819 it was introduced in England and later on in France (Marquez et al., 2000). “An amalgam is an alloy of mercury and one or more other metals. Dental amalgam is produced by mixing liquid mercury with solid particles of an alloy containing predominantly silver, tin and copper. Zinc and palladium may be present in small amounts” (Ronald L. Sakaguchi, 2012).
It dominated the dental material market throughout the 20th century due to its low cost, adequate working time, easy non sensitive handling, forgiving moisture control and longevity. As a material it presents with high compressive strength and sufficient wear resistance. It has been shown that 89,6% of amalgam restorations have a 5 year survival rate and an astonishing 79,2% survive a decade (Opdam et al., 2007).

Another excellent feature of amalgam is its antibacterial properties. It is believed that the corrosive by-products that occur gradually over the course of time, cause plaque inhibition in vitro due to the release of Ag, Cu, Sn and Hg (Orstavik, 1985; Okabe et al., 1987). Another reason this might happen is that they are quite acidic (Sutow et al., 1991). However, these same corrosive by-products presumably seem to precipitate a seal at the restoration/tooth interface therefore, minimizing microleakage (Kuper et al., 2015).

Despite the fact of all the above advantages mentioned due to its silver colour and need for extensive preparation of the tooth to achieve mechanical retention, it appears to have a limited place in the modern, aesthetic and minimally invasive dentistry (Correa et al., 2012; Moncada et al., 2015). Furthermore the lack of adhesion to the dental tissues unavoidably leads to microleakage and therefore secondary caries (Cenci et al., 2004). Although in subgingival caries, compromised isolation situations, high caries risk patients, high stress areas of posterior teeth and budget “herodentistry” amalgam will still remain the material of choice.

In light of the Minamata disaster in Japan, global concerns about the use of mercury in everyday products and its consequences were raised. After the Minamata conference held in 2013, 140 countries signed an agreement for the reduction of mercury pollution through targeted activities. Dental amalgam consists of 42 to 45% of mercury by weight (Roberson TM, 2006) and even though it does not pose a direct risk in the health of patients with amalgam fillings (Ucar and Brantley, 2011; Maserejian et al., 2012) it can cause environmental pollution through its waste via water. In the US alone, more than half of the mercury wastewater pollution is due to dental amalgam (EPA, 2019). Therefore, in Dentistry this translated into phasing down amalgam and replacing it with alternative mercury free materials, possibly by 2030. According to the British Dental Association from July the first 2018, amalgam will be restricted for use in children under the age of 15 and in pregnant and breastfeeding women unless clinically indicated (BDA, 2019).

A Cochrane review published in 2014 states that even though the existing evidence is considered low quality, composite restorations in children have a higher possibility of failure compared to amalgam (Opdam et al., 2007; Rasines Alcaraz et al., 2014). In general, in high caries risk patients amalgam tends to last longer than composite restorations (Opdam et al., 2010).

1.2.1.2 Composites

Composites were developed back in the 50’s and 60’s as an aesthetic alternative to amalgam. On the contrary to amalgam that requires mechanical retention, composites are adhesive restorative materials. Therefore, research is focused on understanding and achieving a stronger, better, reliable and durable bond of the material with the dental tissues. The adhesion along with the fact that composites mimic the colour of the teeth, allows clinicians to perform minimally invasive and aesthetic restorations. The following sections will go into detail about the composition of composites, new innovative additions to their composition such as remineralising and antibacterial agents, their properties and most common reasons they fail.

1.2.1.2.1 Composition

Their composition has changed greatly since they were first introduced from different monomers, to different fillers(Fong et al., 2005; Hosseinalipour et al., 2010; Wu et al., 2010). A description of their main components will follow in the sections below.

1.2.1.2.2 Organic polymer matrix

The organic polymer matrix is a cross linked matrix of dimethacrylate monomers which bond together via the polymerization process and create a bigger molecule (Peutzfeldt, 1997). The most common cross linking dimethacrylate used since 1962 is the 2,2-bis (4(2-hydroxy-3methacryloxy)-phenyl) propane (Bis-GMA) developed by Bowen (Asmussen and Peutzfeldt, 1998; Ferracane, 2011). Property wise, it is considered to have adequate adhesive and mechanical properties and hardens rapidly (Moszner et al., 2008). UDMA is an alternative cross linking dimethacrylate developed by Foster and Walker in 1974 and can be used alone or in combination with the Bis-GMA. It is less viscous than Bis-GMA but also of increased flexibility (Peutzfeldt, 1997). Both Bis-GMA (and UDMA less so) are highly viscous. Therefore, in order to achieve the appropriate consistency low molecular diluents such as TEGDMA or Bis-EMA6 are required to improve the flow of the composite. Low flow translates into more filler being added into the mixture and enhancing some of the properties which declined due to the decreased viscosity (Barszczewska-Rybarek, 2014). 4META (4-methacryloyloxyethyl trimellitate anhydride) is another monomer commonly used in adhesion systems (Van Landuyt et al., 2008). It was introduced in the market as a material for bonding orthodontic brackets in Japan (Chang et al., 2002).

1.2.1.2.3 Inorganic reinforced filler particles

Filler addition is significant in creating a body with bulkiness to the composites and determines some of the properties (Masouras et al., 2008). These fillers fortify the organic polymer matrix and assist in the control of shrinkage due to polymerization. They consist of glass, ceramic, quartz, micro fine silica and nanoparticles. Heavy metal oxides such as barium or zinc are usually included in order to increase the radiopacity of the composite.

According to the shape, size and concentration of the particles contained in the fillers (Sabbagh et al., 2004), composites are classified as:

- Macrofills: particles ranging within 20-30μm
- Hybrid: 5-15% micro fine silica particles ranging within 0.04-0.2μm and larger particles of 2-4μm,
- Microhybrid: contain 70% filler/volume, micro fine silica particles and smaller particles of 0.04-1μm
- Nanofills: particles ranging within 1-100nm (Ferracane, 2011).
- Nanohybrids: nanoparticles and larger particles of 0.4-5μm (Ronald L. Sakaguchi, 2012)
-

1.2.1.2.4 Coupling agents

Coupling agents are needed for the inorganic reinforced filler particles to bind to the organic polymer matrix (Halvorson et al., 2003; Lung and Matinlinna, 2012). In addition, they play a significant role in distributing evenly the stress amongst the matrix and the filler particles and manage to facilitate a hydrophobic environment which leads to minimal water resorption- a crucial factor in restoration longevity. Silanes which are hybrid organic/inorganic compounds are usually used as coupling agents because their methacrylate group links with the organic matrix and their methoxysilane group with the inorganic fillers. The link described is of great significance to the clinical performance of the material (Karabela and Sideridou, 2008). The most

common silane used is the 3-methacryloxypropyltrimethoxysilane (MPTS) on which several papers suggest that silanated particles may cause an increase the bond strength.

1.2.1.2.5 Initiator – Accelerator

Nowadays the majority of composites are light cured though self-cured and dual cured materials are still of use when light curing is not an option (Sideridou et al., 2012). The light cure triggers the dimethacrylate monomers to polymerize and form a cross linked network. The blue light used predominantly today has a wavelength of 465nm. It is understandable that optimizing the process of polymerization is of importance for enhancing the mechanical properties, biocompatibility and color stability of the material (Moon and Shin, 2012). Camphoroquinone (CQ) is the most common photo sensitive composite compound (de Oliveira et al., 2015) which is contained in a 0,1-1% concentration of the light cured materials. It is a substance of yellow color at room temperature (Ronald L. Sakaguchi, 2012) and consists of a bleachable chromophore group which when combined with an amine co-initiator commences the polymerization process by producing free radicals upon activation (Neumann et al., 2006). This phenomenon is called hydrogen abstraction photo-initiation (Asmussen et al., 2009).

The yellow color is the main disadvantage of CQ because it may have an effect on the color and consequently light permeability of the restoration. This obstacle can be partially overcome by a phenomenon known as photobleaching were the surface layer of the material bleaches initially allowing more light to penetrate through (de Oliveira et al., 2015). CQ reaches its peak absorption at 470nm. Diketone PPG (1-phenyl 1,2-propandione is used as an alternative photoinitiator to CQ (Schneider et al., 2009). In self cured composites benzol peroxide (BPO) along with an aromatic tertiary amine activator are the initiator system (Sideridou et al., 2012).

1.2.1.2.6 Pigments and other components

In order for composites to be able to mimic teeth color they contain inorganic iron oxides in quantities which vary depending on the color they need to mirror. New composites containing fluorescent agents are being developed by manufacturers in pursuit of the most natural looking restoration (Ronald L. Sakaguchi, 2012). Although such components excited dentists and took the term tooth mimicking literally by even including enamel anomalies and smoking stains, the majority of patients do not appreciate paying for “stained” fillings.

1.2.1.2.7 Antibacterial agents

1.2.1.2.7.1 Chlorhexidine (CHX)

Chlorhexidine digluconate (CHX) is a disinfectant and antiseptic used widely in all fields of dentistry. Moreover, it is part of prevention and is contained in various toothpastes, mouthwashes, vanishes, gels and sprays. It is classified as a broad spectrum antibacterial agent of low cytoxicity (Zhang et al., 2014) that can decrease the level of *S.Mutans* and subsequently lower the risk of dental decay (Anusavice et al., 2006). CHX is bactericidal (kills bacteria) and bacteriostatic (prevents their growth when present in small concentrations).

Therefore, it was thought that incorporating CHX into dental composites would potentially address the problem of recurrent caries and reduce composite failure. When CHX releasing HEMA composites were developed, it was determined that the CHX releasing mechanism was related to the degree of monomer conversion (Leung et al., 2005). Essentially, the incorporation of CHX into composites had a negative effect on their mechanical properties and specifically in decreasing the wear resistance and strength which resulted in creating a porous surface. Therefore, mesoporous silica nanoparticles were introduced in order to maintain the mechanical properties by enclosing the CHX (Zhang et al., 2014). Such composites containing the nanoparticles with the enclosed CHX were shown to be able to preserve a pH of 6.5- which is above the 5.5 critical pH. This lead to a 10 to 20 times decrease of bacterial activity compared to composites without the nanoparticles (Chang et al., 2002).

1.2.1.2.7.2 Quaternary ammonium compounds (QACs)

Quaternary ammonium compounds were introduced into the consistency of a composite combined with methacrylate monomer and promoted long term antibacterial effects (Cocco et al., 2015). The first QAC to be used in dentistry as an antibacterial agent was methacryloyloxydodecylpridinium bromide monomer (MDPB) which was developed by Imazato in 1994 (Cocco et al., 2015). MDPB presented with bactericidal effects not only before but also after polymerization (Imazato, 2003; Kitagawa et al., 2014). It is usually included into the composition of dentinal adhesive agents rather than the composite (Tziafas et al., 2007). Another QAC called quaternary ammonium dimethacrylate (QAMD) was integrated into composites and appeared to decrease the *S.Mutans* levels and managed to maintain its antibacterial effect after ageing in water for 180 days (Cheng et al., 2012). Benzalkonium chloride is another antibacterial agent of the QAC family that is usually added in acid etchant products and has been used since 1935 (Tezvergil-Mutluay et al., 2011).

1.2.1.2.7.3 Zinc oxide nanoparticles

It is known that zinc oxide possesses antibacterial properties against cariogenic bacteria such as *S.Mutans*. It acts on the membrane of the cells by cutting off the supply and metabolism of sugar in the biofilm (Jones et al., 2008).

1.2.1.2.7.4 Silver nanoparticles

Silver is an antibacterial agent and fairly biocompatible. In small concentrations ranging from 0.3 to 1%, silver particles interfere with the activity of bacteria such as *S.Mutans*, *S.Aureus* and *E.coli* (Burgers et al., 2009; Melo et al., 2013; Cocco et al., 2015). However, dental restorative materials containing silver nanoparticles have poor mechanical properties and present with color instability that compromises aesthetics.

1.2.1.2.7.5 Triclosan

Triclosan was first introduced in 1972 as an antibacterial agent effective against gram positive and gram negative bacteria (Nudera et al., 2007; Rathke et al., 2010). It is therefore regarded as a broad spectrum antibacterial agent

(Kalyon and Olgun, 2001). Triclosan was initially added in personal hygiene products such as deodorants (Wicht et al., 2005) but was recently banned by the American food and drug administration (FDA) for use in soap products in fear of precipitating the already rising antibiotic resistance (Carey and McNamara, 2015). When incorporated in a concentration of 0.3%, it results in disinfection of the tooth cavities (Rathke et al., 2010). Triclosan's mechanism of action is along the fab pathway of bacterial enzymes which results in bacterial growth suspension (Nudera et al., 2007).

1.2.1.2.7.6 E- Polylysine (PLS)

Polylysine is the product of aerobic fermentation of *Streptomyces aldulus*, a gram positive, non-pathogenic bacteria identified in Japanese soil (Hiraki et al., 2003; Shih et al., 2006). A mutant strain than produces four times more PLS has also been isolated (Kahar et al., 2001). It is used commonly in Japan as a food preservative of everyday delicacies such as fish sushi, boiled rice and vegetables, soup stocks and noodles (Shih et al., 2006) and has been approved by the American food and drug administration (FDA) for consumption in concentrations that do not exceed 50 mg per kilo (Ye et al., 2013). Chemically speaking, PLS is homo-polypeptide that has residues of L-lysine and contains charged amino groups that provide the antibacterial properties (Hiraki et al., 2003; Yoshida et al., 2004).PLS has many properties that make her an excellent antibacterial agent such as high water solubility, biodegradability, stability and biocompatibility (Ye et al., 2013). PLS does not absorb through the gastrointestinal track –94%goes through unabsorbed- nor has she been detected in any other organ or tissue (Fukutome et al., 1995; Hiraki et al., 2003). PLS presents as a pale yellow substance with no scent and the faintest bitter taste that does not affect food flavor. PLS's mechanism of action is due cytoplasm abnormalities caused by her absorption through the cell membrane that then strips off (Badaoui Najjar et al., 2009). In the past it has been used as a trojan horse through the membrane for small drugs (Ryser and Shen, 1980). Methods of assessing PLS release are UV spectrometry and high performance liquid chromatography (HPLC).

1.2.1.2.8 Remineralising agents

1.2.1.2.8.1 Calcium Fluoride

Fluoride has a significant role in dentistry because it can incorporate itself in the tooth structure and create fluorapatite which is more resistant to caries acid attacks that hydroxyapatite. When incorporated into a composite material via fluoride reservoirs it can be released slowly indefinitely and remineralise demineralized hard tissues. Composites as such have demonstrated similar flexural strength and elastic modulus as other commercial composite materials (Xu et al., 2007).

1.2.1.2.8.2 Calcium Phosphate

The majority of hard tissues in our body such as bones, cartilages and teeth contain both calcium and phosphate and therefore it is no surprise that the scientific community has examined them with such interest and in a similar manner (Dorozhkin, 2009; Sanchez-Enriquez and Reyes-Gasga, 2013). Materials that contain calcium and phosphate are very biocompatible and are used not only in dentistry as composite ingredients but also in other fields of

medicine (Shadanbaz and Dias, 2012). They also may have the ability to remineralise the surface of the tooth they come into contact with (Sanchez-Enriquez and Reyes-Gasga, 2013) and even precipitate hydroxyapatite formation (Aljabo et al., 2016). The different ratio of calcium and phosphate is the element that differentiates the forms that calcium phosphate comes into such as amorphous calcium phosphate (ACP), dicalcium phosphate anhydrous (DCPA), dicalcium phosphate dehydrate (DCPD) or brushite, monocalcium phosphate anhydrate (MCPA) and monocalcium phosphate monohydrate (MCPM). The solubility of the composite is affected by the different ratio of calcium and phosphate. Unfortunately this release of calcium and phosphate that appears to be so beneficial for the restoration and the restored tooth, lasts only a few months (Zhang et al., 2015). This phenomenon presumably alters the surfaces of both the tooth and the restorative material and therefore can affect the bond between them, compromising adhesion.

1.2.1.2.8.3 Tri-strontium phosphate (TSrP)

Strontium and calcium are very similar property wise and it would come as no surprise if one could replace the other in the formation of hydroxyapatite as they both promote the process of remineralization and could further improve the hydroxyapatite crystal (Thuy et al., 2008). It was demonstrated that when incorporated in dental composites along with MCPM, it enhances hydroxyapatite formation by remineralising the affected dentine and decreases the shrinkage due to polymerization (Panpisut et al., 2016). The explanation could be that when the restoration shrinks due to polymerization and creates a gap, the material stimulates the tooth structure to commence a remineralising process which would result in closing the gap with newly formed hydroxyapatite. The above statement has yet to be confirmed but if true may change the restorative dentistry material market.

1.2.1.2.9 Properties

1.2.1.2.9.1 Polymerization Shrinkage

Polymerization shrinkage is a major disadvantage of dental composites because it compromises the longevity of the restoration by creating marginal gaps which result in secondary caries, restoration and/or tooth fracture, marginal staining and sensitivity (Braga et al., 2005; Boaro et al., 2010). The above complications have a significant impact on the patients budget and psychology and the dentist's credibility. The mechanism of polymerization shrinkage is that as monomers come close to one another and form bonds as part of the polymerization process, there is a reduction in Van Der Waal's volume and the free volume by 1,5 till 5% (Braga et al., 2005; Ronald L. Sakaguchi, 2012). This volume reduction translates into contraction stress of the material which is bonded to the tooth which then debonds and forms a gap (Boaro et al., 2013) with all the consequences that implies. Bacteria and nutrient penetration through these gaps, referred to as microleakage in the literature and has a significant clinical importance because it allows the biofilm to expand within the gap and bacteria to recolonize under the restoration. As a result the longevity of a restoration becomes compromised. The ideal composite material would have zero polymerization shrinkage and would remain 100% into contact with the tooth surface it initially bonded to (Ferracane and Hilton, 2016). Since it is established that that is not a viable clinical

scenario with the restorative materials in hand today, some studies have focused on reducing the shrinkage and resulting microleakage, as much as possible by placing composite in increments, by optimizing the curing light's intensity, angle and time needed to cure, by lowering the concentration of photoinitiators, by introducing non bonded particles into the material in order to relieve the contraction stress, by adding prepolymerised resins into the monomers, by increasing the amount of inorganic fillers and by using high molecular mass monomers (Condon and Ferracane, 2000). In addition, a lot of research has now focused towards incorporating antibacterial substances into the restorative material. Therefore, it is evident that monomer conversion (MC), the inorganic reinforced fillers contained in the material and the molecular weight of the monomers are some of the variables affecting polymerization shrinkage and if manipulated can have an impact on a dental material's properties.

1.2.1.2.9.2 Delay Time

Delay time in this project is defined as the time from first switching on the curing light until initiation of the polymerization chain reaction on the lower sample surface.

1.2.1.2.9.3 Reaction Rate (RR)

Reaction rate is defined as the change in the percentage of monomer conversion over the change in time. It assesses how quickly the material polymerizes and may affect its properties.

1.2.1.2.9.4 Monomer Conversion (MC)

Monomer conversion is the percentage that describes how many carbon-carbon double bonds have become single bonds between monomers (Liaqat et al., 2015). Not 100% of the monomers bond amongst each other during the polymerization process and this leads to unpolymerised monomer residue which has an effect on the properties of the material (Leprince et al., 2013). The degree of monomer conversion ranges from 55 to 75% (Silikas et al., 2000). The desirable is to have a high degree of monomer conversion because the monomer residue compromises not only the mechanical properties of the material but also the biocompatibility of it (Sideridou et al., 2012). Monomer residue is toxic for the pulp and may lead to pulp inflammation and necrosis. As mentioned in the previous section, microleakage is in correlation with the percentage of monomer residue (Du and Zheng, 2008; Walters et al., 2016). Factors such as the chemical structure of the monomer mix, the temperature of the sample or the room, the inorganic filler and activator concentration and the initiator system have an impact on the degree of monomer conversion (Asmussen and Peutzfeldt, 2005; Sideridou et al., 2012; Panpisut et al., 2016). The main instrument to quantify monomer conversion is the FTIR (Fourier Transform Infrared) machine which utilizes an attenuated total reflected infrared beam that hits a sample and via a computer software displays a graph from which monomer conversion is calculated live. Other methods of measuring the monomer conversion are the micro hardness differential thermal calorimetry (DCT) and the differential scanning calorimetry (Liaqat et al., 2015).

1.2.1.2.9.5 Flexural Strength

Flexural strength translates into the ability of a material to resist fracture under stress (Chung et al., 2004). Monomer conversion and the inorganic reinforced filler content exert influence upon this property (Ikejima et al., 2003). Clinically, flexural strength of the material matters in order to choose the appropriately strong material for the appropriate tooth. Posterior teeth receive high occlusal loads from the masticatory forces which is an indicator for the use of a material of higher flexural strength. In addition, flexural strength can act as a wear predictor of the material (Thomaidis et al., 2013). Flexural strength can be measured by testing systems such as Instron.

1.2.1.2.9.6 Wear

Wear is defined as the interaction between two surfaces moving into contact that progressively leads to removal of material. Wear of the dentition can be of natural causes and has been calculated to be around 29μm per year for molars and 15μm per year for premolars (Cao et al., 2013). Managing to develop a material with similar wear to the natural wear has proved to be of great difficulty. Amalgam and gold seemed to be working nicely but unfortunately are not tooth coloured, therefore not aesthetic today. Developing a white colored material with similar wear to the natural wear our dentition experiences, seemed almost impossible. Wear resistance in composites is all about the shape, size and distribution of the inorganic reinforced fillers (Oliveira et al., 2012). Smaller sized particles offer smaller shrinkage, better polishing and less wear than bigger sized particles (Antunes et al., 2014). In addition, longer curing times suggest a more crosslinked organic polymer network that can effectively resist wear (Condon and Ferracane, 1997). Furthermore, highly viscous composites seem to have low wear resistance (Cao et al., 2013). Concluding, a composite's composition has a detrimental effect in promoting or inhibiting its wear.

1.2.1.2.9.7 Water sorption

Water sorption is defined as the diffusion of water into the material (Ortengren et al., 2001). Because it affects the volume of the material by causing it's expansion, it could potentially be used to compensate for the polymerization shrinkage (Versluis et al., 2011). Restorative composite materials with hydrophilic components such as mono calcium phosphate monohydrate (MCPM) that can be incorporated into the composite, can deliberately induce this property (Wei et al., 2013; Panpisut et al., 2016). Water sorption can obviously have an effect on the mechanical properties of the composite material (Liaqat et al., 2015) and the tooth filled with that. The increase of volume of the material in the filled cavity leads to internal stresses that build up and may cause the tooth to fracture (Watts et al., 2000; Liaqat et al., 2015).

1.2.1.2.10 Why composites fail?

The success and failure of a composite restoration depends on a number of factors regardless of the material used. A percentage of 1-3% per year is the number of composite restorations that fail (Demarco et al., 2012). Composites as materials are very technique sensitive and require a level of familiarity from the clinician (Tobi et al., 1999). Therefore, it was believed that if a composite restoration failed within a period of 5 years, it was due to a technical mistake

from the clinician and if it failed after the 5 years it was due to recurrent caries (Drummond, 2008). The vicious cycle of failure begins with adhesion failure, which creates a gap in which the biofilm extends. Clinically, this translates into microleakage which subsequently leads to recurrent caries and tooth and/or restoration fracture. It has been suggested that the type of material can dictate the bacterial activity and growth on the tooth surface (Cazzaniga et al., 2015). However, a very recent systematic review done by Askar in 2016 presents inconsistent results on the subject of recurrent caries and different restorative materials (Moraschini et al., 2015; Brouwer et al., 2016). Another reason for failure may be extensive wear which causes a decrease in volume due to abrasion and the occlusal stresses from the masticatory muscles (Boaro et al., 2013). In addition, composites may also fail aesthetically due to discoloration of the margins or even the whole restoration.

1.2.1.2.11 Why is the peripheral seal key to success?

When removing caries in operative dentistry back in Dental school as undergrads, the main principal taught, was that the periphery of the cavity should be caries free in order to be able to bond predictably. In the new age of the “sealing” the caries, this principal still is of upmost importance. Materials and bonding systems have progressed but the enamel and especially the dentine remain structurally complex and vary within the tooth. Furthermore, the complete disinfection of the tooth or the oral cavity is undeniably impossible by nature. The periphery of a cavity usually is key because it acts as a barrier to microleakage which if it occurs, it compromises the adhesion not because the material fails but because the tooth structure fails due to structural changes that affect the material/tooth interface. It is known that bacteria can still be detected in caries bonded dentine but it is not known whether they remain abeyant whilst embedded in the restoration (Alleman and Magne, 2012). Therefore, it makes sense to aim to achieve a peripheral seal in order not to allow new bacteria to recolonize the tooth tissues or reactivate the already embedded bacteria. This can be achieved either mechanically by “capping” the whole tooth with a preformed metal crown or chemically by cementing the material to the tooth predictably.

1.2.1.3 Glass Ionomer Cements (GIC)

Glass ionomer cements were introduced into dentistry in the 70’s. They create a chemical bond with the tooth surface via an acid/base reaction that results into ion exchange (Watson et al., 2014). Their composition consists of a polycarboxylic acid that acts as a matrix, finely ground fluoroaluminosilicate (FAS) that act as fillers, water and tartaric acid (Ronald L. Sakaguchi, 2012). They can be chemically cured, light cured or both. Property wise, glass ionomer cements have low resistance to wear (Peutzfeldt et al., 1997) and have low toxicity. Furthermore, they demonstrate some sort of biocompatibility, due to their thermal compatibility and remineralising potential because of fluoride release (Thuy et al., 2008). Clinically, they are easy to use, not technique sensitive since moisture control does not need to be as strict as in composites, and usually only require a single step for placement. Unfortunately, their inferior mechanical properties have made them suitable for usage only as temporary or interim restorations, as materials indicated for practice of the atraumatic restorative technique (ART)(Smales and Yip, 2000),

clinical scenarios where moisture control is not possible, as liners for pulp protection in deep restorations and as cements for crowns, orthodontic bands and bridges. In paediatric dentistry, they are widely used because the behavior of the young patients may prohibit the use of rubber dam isolation and not allow the placement of other materials in a predictable manner. In addition, preformed metal crowns which are a common restorative option for primary teeth, are cemented with glass ionomer cement.

1.2.1.4 Resin Modified Glass Ionomer Cements (RMGIC)

Resin modified glass ionomer cements were introduced in dentistry a decade later, in the 80's as an improved version of GICs with some composite properties. This was achieved by incorporating a hydrophilic methacrylate monomer into the material along with some free radical initiators. This change into the consistency translated into improved wear resistance and less polymerization shrinkage. In addition, because they presented with good adhesion to teeth surfaces they were characterized as self-adhesive and therefore could be used without prior etching with phosphoric acid (Ronald L. Sakaguchi, 2012). They are used in similar clinical scenarios as the regular GICs described previously.

1.2.1.5 Compomers

Compomers or poly acid modified composites were introduced in the dental world much later, in 1992, as a hybrid of glass ionomer cements and composites (Sidhu and Nicholson, 2016). Their consistency was of a Bis-GMA macromonomer blended with diluents (TEGDMA) as a matrix, 42-67% of inorganic fillers of quartz or silicate, silane as a coupling agent, acidic functional monomers (TCB resin) and modified monomers that release fluoride. In contrary to glass ionomer cements and resin modified glass ionomer cements, compomers contain no water at all. They are usually light cured and set with an acid/base reaction as the monomer absorbs moisture and then fluoride release commences. This water absorbance though has a negative effect on the mechanical properties of the restorative material, which gradually becomes weaker. Over a period of few weeks, its strength ends up being reduced by a significant 40% (Nicholson, 2007). The use of compomers was limited to fissure sealants, cementation of orthodontic bands and restorations in low stress areas (Nicholson, 2007; Ronald L. Sakaguchi, 2012). Today, the most known and widely used compomer, is Activa Kids which claims to have the advantages of both composites and glass ionomer cements and targets the paediatric patient. Activa has become popular amongst paediatric dentists due to its non-technique sensitive, straightforward application and claimed biocompatibility.

1.2.1.6 Preformed Metal Crowns (PMC)

Preformed metal crowns are the material of choice for restoring carious primary molars. They are made either completely out of stainless steel, a combination of stainless steel interior with a white veneer exterior, completely out of glass fibers or 100% white ceramic. Most of the types described above can be done with the conventional technique of prepping the tooth with two slices mesially and distally following local anaesthetic. The stainless steel one's can be also placed with the Hall technique which requires no local

anaesthetic, no tooth preparation, just soft caries removal with a hand excavator. Regardless of the placement technique, they are the most reliable restorative option for primary teeth compared to all other materials available (Yengopal and Mickenautsch, 2009; Innes et al., 2015). In the UK were dentistry is under the national health system (NHS) Hall crowns are a staple. Even though, Hall crowns specifically are fast and easy to place, offer predictable outcomes and are not technique sensitive, they are of silver color which can cause aesthetic concerns to paediatric patients and parents. The silver color also limits their use in posterior teeth and condemns grossly carious anterior teeth to extraction. New white crowns have entered the market but they do not offer the advantages mentioned above and have limited room in paediatric practices or in large public health dental units.

1.3 Why is there a need for a new material, especially in Paediatric Dentistry?

According to the American academy of paediatric dentistry, Paediatric dentistry is " an age defined specialty that provides both primary and comprehensive preventive and therapeutic oral health care for infants and children through adolescence including those with special care need" (AAPD, 2013).

The anatomical differences between primary and permanent dentition, the medical history, the age of the child, their ability to allow and accept local anaesthetic are crucial factors that influence greatly the treatment. Currently the available restorative materials commonly used in adults do not meet paediatric standards of care in terms of behaviour management and anatomical variations.

Infants, children, adolescents and people with special needs require delicate behaviour management in all levels. Communication pathways are more complex and trained specialist paediatric dentists are required in order to successfully manage the patients anxiety and cooperation. The time available to perform some clinical tasks and the mouth opening may be limited and moisture control may not be ideal, enhancing the need for a new and forgiving material.

Unlike common perceptions, primary teeth are not scaled down permanent teeth. They differ in morphology and have enamel, dentine and pulps that vary from the permanent dentition. These anatomical differences not only enable caries to progress faster into the tooth but also dictate the use of different techniques and materials for their management.

Primary teeth are more bulbous- hence why preformed metal crowns are so successful- molars in particular are wider mesiodistally and have wider, flatter contact surfaces between them, closer to the gingiva, whilst incisors are more squared. The enamel is thinner and the prisms are narrower and enamel rods are occlusally inclined. The dentine is thinner in layer, less mineralised, lower in density of dentine tubules which are smaller in diameter and appears to have lower permeability. As a result of the above, the amount of sound tooth structure available in primary teeth is usually limited and therefore adhesion of

the restorative materials can be significantly compromised. Furthermore, the pulps of primary teeth are proportionately larger than those of permanent teeth with an average depth of 2mm of enamel/dentine compared to 6mm in permanent teeth. Consequently, deep cavities are likely to involve the pulp, which complicates treatment and also the restorative materials will not exceed in thickness the 2mm which is favourable for light cured materials.

However, the above morphological differences do not seem to affect the way that materials bond to primary teeth in order to create a seal and how biocompatible these materials are. Fleming et al suggests that the bond strength of resin bonded materials may be reduced in primary teeth due to the more extended prismless layer (which interprets clinically to an increased etching time) and the less mineralised dentine (Fleming et al., 2001). Be that as it may, the data Fleming used was derived from research work done more than 50 years ago and that should be taken into account when assessing such papers.

To conclude, the ideal paediatric restorative material should be easy to place, fast, single stage bulk like placement, dimensionally stable, able to provide a good seal without excellent moisture control, white coloured, radiopaque so leaking can be seen radiographically and come into a child friendly packaging.

1.3.1 SMART dental composites history

The story behind SMART dental composites started back in ~ 2000 when the Eastman Dental Institute received a grant in order to develop dental composites with antibacterial properties in order to address the problem of recurrent caries due to failure of the peripheral seal. The initial idea was a dental material that could release chlorhexidine which was the most widely used antibacterial agent in dental products at the time. The first paper published was by Leung et al in 2005. This described UDMA / TEGDMA / HEMA based dental composites with water sorption induced and diffusion controlled CHX release. Unfortunately, higher water sorption translated into mechanically weak composites.

In 2008, studies investigated whether these composites could be used in bone as well (Main, 2014) since bone and teeth have many similarities as tissues and literature about dental composites being used in vertebral fracture repair existed (Beckman and Smith, 2009). This bone application exploration supported marketing of the bone composite Comp06 in 2011. This deviation also lead to consideration of MCPM and TCP in dental composites which at the time the group were additionally employing in calcium phosphate bone cements.

In 2009, Mehdawi et al published work in which the composites first used by Leung were modified by replacing HEMA with MCPM and TCP (Mehdawi et al., 2009). It was noted, that the MCPM reacted with water and created crystals of low solubility brushite in the bulk of the material. This resulted in an increase of water sorption and material expansion. This new mechanism of water sorption considerably enhanced CHX release and consequently the

antibacterial properties. Sadly, the composites remained mechanically weak and therefore prone to fracture.

In 2016, Aljabo et al published work on much stronger composite formulations. This was achieved through modification of the filler phase (Aljabo et al., 2016). They additionally established that the released CHX accumulates in an apatite layer that forms on material surfaces when placed in simulated body fluids. As this layer dissolves when attacked by bacterial acid this provides a mechanism to respond to bacterial microleakage. In 2015, Walters et al published work in which TEGDMA was replaced by PPGDMA making the materials more cytocompatible (Walters et al., 2015).

Unfortunately, in recent years there have been increasing concerns about anaphylaxis related to CHX. Furthermore, as CHX is considered a drug, its inclusion had the potential to make a dental composite a class 3 device. Under the Food, Drug and Cosmetic Act, all medical devices must be classified in order to assure safety and effectiveness. A class 1 device poses the lowest risk whilst a class 3 the greatest risk(FDA). This caused problems with regulatory bodies which would make commercial mass production difficult. Consequently, a decision was made to seek alternative antibacterial agents.

In 2014, a patent to use PLS as an antibacterial agent instead of CHX, was submitted. The reason PLS was chosen was due to the fact that it can be antibacterial but also compatible with eukaryotic cells. In the literature PLS is not considered a drug but a food preservative, which helps address regulatory concerns raised in the past. Panpisut et al were the first to publish a paper on PLS containing dental composites (Panpisut et al., 2016). Since then, there has been much work in the group optimising a formulation in order to be able to match if not overcome dental composite standards. This work has even discovered new advantages related to PLS enhancing cavity sealing which will be published in due course.

The material within the group is called by the acronym SMART (Self-etching, Mechanically strong, Adhesive, Remineralizing, Tooth mimicking) dental composite (Figure 1-1). It differs from existenting commercial materials in the fact that it contains MCPM as a remineralising agent and PLS as an antibacterial agent in addition to self-adhesion properties. The SMART acronym also includes the initials ART which refer to the atraumatic restorative technique which aims in retaining teeth that would in other circumstances be extracted in children of developing countries with only the use of hand instruments (Smales and Yip, 2000).

In 2018, following ethical approval and approval from the Medicines and Healthcare products Regulatory Agency (MHRA) for a clinical trial which included 7 non restorable primary teeth belonging to 7 children was obtained for a first man safety trial. A month after the fillings were placed, the teeth were extracted under general anaesthetic and were taken to the lab for further testing. Currently, a report is being submitted to the MHRA in order to proceed with the second phase of a clinical trial which will access the SMART composite material’s retention and will involve > 50 primary teeth. In the

meantime, former Eastman graduates working at Khon Ken University in Thailand are attempting to proceed with a separate clinical trial of the SMART composites in order to address their national problem of decay in young children.

Figure 1-1 Graphic summary of novel SMART composites properties through explanation of the abbreviation SMART.

1.4 Aims and Objectives

The aim of the team's effort is to develop a viable restorative paediatric material option which will be dimensionally stable and therefore reliably satisfy current needs and demands of paediatric patients and dentists.

The aim of this project in particular, was to determine the effect of antibacterial polylysine (PLS) and remineralising MCPM on the polymerization and dimensional stability of SMART dental composites and how does that affect microleakage. In addition, since a clinical trial is currently running, it would be of interest to explore if these novel SMART composites are stable enough to warrant CE marking.

In this project the following hypotheses were examined:

1. The effect of antibacterial PLS and remineralising MCPM on the delay time, reaction rate, half time and final monomer conversion of the SMART composites.
2. If the SMART composites exhibited higher final monomer conversion than the commercial comparators.
3. The minimum curing time needed in order to gain high monomer conversion above the 50% cytotoxic limit at varying depths.
4. The volume and mass change of SMART composites over long periods of time while submerged into water at a temperature of 37°C imitating the oral cavity temperature. If there are changes noted, to determine a possible explanation.
5. If the calculated shrinkage from the polymerisation reaction of SMART composites is compensated by the volume change due to swelling.
6. Comparison of all SMART composite formulations to two commercial materials –Activa which is a compomer and the main market competitor and a composite staple, Z250 for the above points mentioned.
7. How much microleakage does the SMART final formulation have compared to four commercial materials (Activa, Z250, Fuji II LC, Fuji IX).

8. If SMART composite formulations are stable enough to warrant CE marking.

Other properties of the same material such as wear, biaxial flexure and bonding strength have been in the center of multiple other theses of the Eastman Dental Institute group in the past.
Hopefully, the SMART composites will open the way for new smart materials for paediatric patients with behavioural problems and others who do not have access to conventional dental facilities that allow the application of sophisticated techniques and materials. They also seem to follow the trend of biocompatibility and dynamic drug release when required, that is seen in today's Medicine.

1.5 Experiments

The following experiments were carried out in pursuance of the above:

- FTIR (Fourier Transform Infrared Spectroscopy)
- Mass and Volume change
- Microleakage test

The data collected from the above were inserted in customized excel spreadsheets and various parameters were calculated and statistically analysed.

2 Materials and Methods

2.1 Materials

The materials used in this project were six formulations of the SMART composites plus a control, Activa™ Kids, 3M™ Filtek™ Z250, Fuji II LC and Fuji IX GP (Figure 2-2). Their composition, method of acquisition, properties and clinical indications will be discussed in detail in this section.

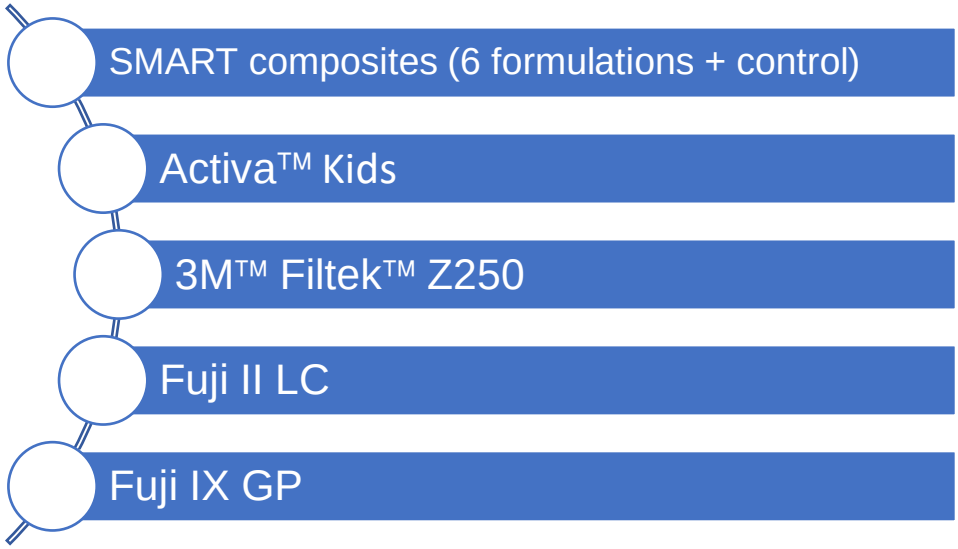

Figure 2-1 Summary of materials used in this thesis that will be described in this section

2.1.1 Composition of SMART composites

SMART composites are composed of a powder part and a liquid part which are mixed together in two different weight ratios (either 3 parts : to 1 or 4 parts : to 1) to create the final product.

2.1.1.1 Powder phase - Fillers

The powder phase strongly affects SMART composite consistency. It consists of three components (Figure 2-2):

1. Glass particles: 60% of glass particles are of 7 μm in size, 30% are of 0.7μm in size and the remaining 10% consist of nanoglass particles.
2. MCPM (Monocalcium Phosphate Monohydrate) : is responsible for the self-etching and remineralising properties of the SMART composites due to its hydrophilic and soluble nature that promotes the brushite formation by releasing calcium phosphate and phosphoric acid. MCPM particles can vary in size. 50μm sized particles were used for the majority of SMART formulations and smaller 10μm sized MCPM particles for a formulation named F5small which will be described below. All formulations used had MCPM in a percentage of either 8% or 4% and 50μm sized particles unless specified differently.
3. E- Polylysine (PLS) : is responsible for the antibacterial properties of SMART composites. As described in the introduction, PLS is the byproduct of fermentation of aerobic bacteria by *Streptomyces albulus*, a gram positive, non-pathogenic microorganism identified in Japanese soil (Hiraki et al., 2003; Shih et al., 2006). Commercially, it is used commonly in Japan as a food preservative (Shih et al., 2006) and has been approved by the American food and drug administration (FDA) for consumption in concentrations that do not exceed 50 mg per kilo (Ye et al., 2013). Chemically speaking, PLS is homo-

polypeptide that has residues of L-lysine and contains charged amino groups that provide the antibacterial properties (Hiraki et al., 2003; Yoshida and Nagasawa, 2003). PLS has many properties that make it an excellent antibacterial agent such as high water solubility, biodegradability, stability and biocompatibility (Ye et al., 2013). It presents as a pale yellow material with no scent nor taste. In general, PLS is a broad spectrum antibacterial agent that inhibits the growth of microbes including yeast and gram positive and negative bacteria (Yoshida and Nagasawa, 2003) by being absorbed through the membrane of the cell causing abnormalities to the cytoplasm (Badaoui Najjar et al., 2009). The formulations in this work incorporated PLS in a percentage of either 4% or 2wt% of the filler phase.

Therefore the powder phase percentage consists of the sum of MCPM (4% or 8%), PLS (4% or 2%) and the rest is the percentage of glass particles. For example, the formulation which consists of 8% MCPM and 4% PLS has a glass particle percentage of 88% (100% - (8%+4%)) of which 60% are of 7 μm in size, 30% are of 0.7μm in size and the remaining 10% consist of nanoglass particles. Only 0.7μm sized glass was used in the single formulation with smaller MCPM particles.

Figure 2-2 Summary of powder components

2.1.1.2 Liquid phase - Monomers

The liquid phase includes all the monomers of the composite which are the following (Figure 2-3):

1. UDMA (Urethane dimethacrylate **$C_{23}H_{38}N_2O_8$**): is the bulk monomer and is the main component of the liquid in a percentage of 72%. It is a high weight molecular base monomer that induces significantly higher monomer conversion by increasing the complete cross-linking and decreasing the drainage of uncured monomer compared to Bis-GMA (Floyd and Dickens, 2006). In combination with PPGDMA it is known as the UP-monomer (Walters et al., 2015).
2. PPGDMA (Poly-(propylene glycol) dimethacrylate **$H_2C=(CH_3)CO(OC_3H_6)n\ O_2CC\ (CH_3)=CH_2$**): is the diluent monomer making the composites' consistency more runny hence easier to manipulate clinically and adaptable into cavities. This was incorporated in a percentage of 24%. In addition, it aids the process of monomer conversion due to its high molecular weight and flexibility and can have a positive effect on depth of cure without the shrinkage disadvantage (Walters et al., 2015) (Liaqat et al., 2015). Furthermore, it eliminates the need for a polymerization activator.
3. 4-META (4- Methacryloxyethyl trimellitate anhydride **$C_{15}H_{12}O_7$**): is in the form of a crystalline powder and is an acidic monomer that promotes adhesion and present at 3wt% of the monomer. It is commonly used in self-etching materials (Chang et al., 2002).

4. CQ (Camphoroquinone): is the photo initiator of the setting process in a percentage of 1%. As described earlier, CQ is the most common photo initiator usually with a tertiary amine as a co-initiator used in light cured materials (Maciel et al., 2018). CQ is yellow in room temperature and this yellow color is her main disadvantage because it may have an effect on the color of the restoration although photobleaching has been used to correct that (de Oliveira et al., 2015).

Figure 2-3 Summary of liquid components

2.1.1.3 Mixing method

The powder and liquid components were mixed in two different ratios (3:1 and 4:1) in the lab facilities of Synergy Devices Ltd by Dr Wendy Xia. The scale up process was carried out using a centrifugal planetary mixer called Speedmixer with a serial number DAC600.2 CM51. The main advantages of using a Speedmixer are the ability to avoid air bubbles from being incorporated into the mixture and interfering with the sufficient wetting of the powder from the liquid.
The mixing procedures involved the following steps:
1. 225 grams of powder were mixed at 1000 rpm for 30 seconds (no Vacuum used)
2. 75 grams of liquid were then added to the powder mix
3. Wetting of the powder with the liquid followed at 2300 rpm for 15 seconds (no Vacuum used)
4. Vacuum was applied for 3 minutes (without any speed) and the pressure was dropped down to around 20 mbar
5. Mixing followed at 1800 rpm for 30 seconds (17 mbar)

All different elements of the powder were weighted individually and mixed on a rubber mixing pad by hand with a stainless steel spatula prior to incorporating the liquid elements. Usually, the composite pastes were created in batches no more than 8 to 10 grams. Between experiments, the pastes where stored in amber glass vials within a refrigerator at 4°C. The resulting paste had a consistency equivalent to the majority of commercial materials available in the market.

2.1.1.4 Different experimental formulations used in this work

In this project, six different formulations were compared in three different groups, plus a control formulation (Table 2-1). The first group consists of the four formulations with high and low MCPM and PLS percentages and same powder liquid ratio, the second group is comprised of two formulations with the same MCPM and PLS percentages but different powder liquid ratios and the last group is composed of two formulations with the same MCPM and PLS

percentages and the same powder liquid ratios but different MCPM particle size (one normal sized MCPM and the other smaller sized MCPM).

Each SMART composite formulation was labelled with three (3) numbers as below:

- # -

MCPM – PLS – Powder Liquid ratio

The first one represents the MCPM percentage (%) , the second one the PLS percentage (%) and the third one the powder liquid ratio.

Table 2-1 Summary of all SMART composite formulations used in this project and their composition.

Formulation	MCPM(%)	PLS (%)	Powder : Liquid ratio
F8	4	2	3
F6	4	4	3
F7	8	2	3
F5	8	4	3
F5 small	8	4	3
F2	8	2	4
Control Hybrid (CH)	0	0	3

2.1.1.5 Comparisons between experimental formulations

As is evident from Table 2-1, the effect of different concentrations of MCPM and PLS (high and low) was to be determined. In addition, one formulation with a different powder liquid ratio was examined in order to investigate the effect of that factor and one with different sized MCPM and glass filler particles than all the rest to assess if MCPM had a different effect when in smaller sized particles. A control was used with zero MCPM, zero PLS and the standard powder liquid ratio of 3:1 in order to be used a baseline.

2.1.1.6 Final experimental formulation

As this project developed with time, F5 which contains high MCPM(8%) and high PLS(4%) was proven to be superior to the rest and therefore became the final formulation and hence referred as Eastman Dental Institute (EDI) composite in the microleakage test. It is available in one chameleon shade and is radiopaque. Different means of packaging were tested prior to being released to the market with a capsule being the most likely candidate. This formulation is the one used in the clinical trials that are being run now in the UK and possibly the one that will take place in Thailand in the future.

2.1.2 Commercial materials used for comparison

All of the commercial materials described below were used as means of comparison because they claim to present similarly desirable properties and indications of use with the novel SMART composites and therefore would be

the main market competitors when SMART composites become commercially available in the future. Table 2-2 summarizes the composition of the commercial materials used for this work.

Table 2-2 Summary of the four commercial materials used and their composition

Commercial material	Type of material	Composition
Activa™ KIDS	Compomer	• 44,6% Diurethane + Other methacrylates + Modified polyacrylic acid • 6.7 % Silica • 0.75% Sodium fluoride
3M™ Filtek™ Z250 (Shade B3)	Composite	• Bis-GMA, UDMA, Bis-EMA • Filler content: 78%wt, combination of silica, zirconia and silica/zirconia cluster, size 10nm - 3.5μm
Fuji II LC	GIC	• Powder: alumino-fluoro-silicate glass • Liquid: 25-50% HEMA, polycarboxylic acid, 1-5% UDMA, 1-5% DMA, <1%CQ • Powder liquid ratio: 3.2/1
Fuji IX GP	Fluoride releasing GIC	• Powder: alumino-fluoro-silicate glass • Liquid: 50% distilled water, 40% polyacrylic acid, polycarboxylic acid • Powder liquid ratio: 3.6/1

2.1.2.1 Activa™ KIDS

Activa is a fairly new restorative material that was launched in 2013 by Pulpdent® Corporation and belongs to the compomer family of materials. Until today, it has received multiple awards with the latest being the dental advisor bioactive restorative award in 2019. It is one of the first dental materials marketed as a bioactive restorative material and therefore is the main market competitor for SMART composites. Activa claims to be a “dynamic” material that responds to PH changes in the mouth and therefore precipitates the remineralization pathway and stimulates the formation of hydroxyapatite by releasing and recharging calcium, phosphate and fluoride. This dynamic property of chemically bonding to the tooth structure just by etching for 10 seconds, without requiring a bonding agent, results in the formation of a good seal without any bacterial microleakage which could lead to failure of the restoration (van Dijken et al., 2019).

In addition, by being a compomer, it is advertised as a hybrid material of glass ionomer and composite resin which holds the advantages of both. It presents sufficient mechanical properties such as fracture and wear resistance and due to a patented rubberised-resin molecule to the Activa resin matrix it is stress and shock absorbent. One of its main advantages, is that it is moisture tolerant hence excellent for use in children where the moisture control can become questionable. In the brochure circulated by the company to dentists, it is a dual cure material which has an initial self-curing time at 37°C of two and a half to

three minutes. It requires a light curing time of just 20 seconds in order to achieve sufficient monomer conversion. It claims to allow curing up to 4mm of thickness even though the paediatric version is more opaque which would not allow light penetration. It has a post polymerization shrinkage of 1.7% and contains 21.8% glass fillers. The clinical scenarios that Activa is indicated for in primary teeth are class I, II, III and IV cavities without any pulp involvement as well as on top of silver diamide fluoride (SDF) treated primary teeth.

Activa also is BPA (Bisphenol A) free. BPA is an industrial chemical compound contained and used in the manufacturing of polycarbonate plastics and epoxy resins and may be contained in some dental resins. Some research emerged claiming BPA may pose potential health risks hence many companies decided to manufacture BPA free products. However, the European Food and Safety authority (EFSA) as well as the United States food and drug administration (FDA) have deemed that BPA is safe for use at current levels (NHS, 2010). Activa is available in the form of a two paste system in an automix syringe with disposable tips and a dispenser and it is available in one shade called pedo shade which is close to an opaque A1. It has a shelf life of two years unopened.

2.1.2.2 3M™ Filtek™ Z250

3M™ Filtek™ Z250 is a microhybrid radiopaque composite resin material by 3M™ that has been used in restorative dentistry since 1992 therefore being a staple of excellence and a mean of comparison after twenty seven years of clinical application. It requires etching and a bonding agent and comes in the form of single dose capsules or traditional syringe. For predictable results, it would require the use of rubber dam isolation since it is not moisture tolerant. Z250 is available in a wide range of shades A1, A2, A3, A3.5, A4, B0.5, B1, B2, B3, C2, C3, C4, D3, incisal and universal dentine. As instructed by the manual for use, Z250 should be placed into cavities in layers and not exceed 2.5mm per layer for light shades and 2.0mm for darker shades since these represent the maximum depth of cure in order for the light to penetrate. Each layer should be light cured for 20 seconds for light shades and 30 seconds for darker shades in order to achieve sufficient monomer conversion. The micro hybrid glass element enables great polishing which is essential in order to avoid plaque deposition and to achieve aesthetic anterior restorations. Furthermore, Z250 is durable enough to be suited for posterior restorations that need to be able to withstand occlusal biting forces. Other clinical indications mentioned by the manufacturer include core build-ups, splinting and indirect restorations such as inlays, onlays and veneers. The monomers contained in Z250 are BIS-GMA, UDMA and BIS-EMA resins and the filler is zirconia/silica. In specific, the inorganic filler loading is 60% by volume (without silane treatment) with a particle size range of 0.01 to 3.5 microns. It also contains methacrylate which may be an allergen for a small amount of the population. Z250 is claimed to have a post polymerisation shrinkage of approximately 2.2%. It has an impressive shelf life in room temperature of three years.

2.1.2.3 Fuji II LC

Fuji II LC is the first light cured glass ionomer restorative material that entered the market back in 1992. It has a place in every dental practice and all dentists

are familiar with its use. Fuji II LC is marketed as a resin modified glass ionomer with the advantages of dual curing, immediate finishing and good aesthetics. Fuji II LC claims to be self-etching hence does not require the use of etching or a bonding agent since it forms a chemical bond with the tooth structure. This chemical bond clinically translates to an excellent seal which is further enforced by a potential remineralising effect due to the rechargeable fluoride release. In addition, Fuji II LC is hydrophilic meaning it does not require excellent moisture control and can be used without rubber dam isolation. Therefore, on the GC website, Fuji II LC is portrayed as ideal for use in paediatric patients. Other favourable qualities include radiopacity and a blue hue which makes the material distinguishable from the tooth structure so it is easily removed. Light curing of 20 seconds is indicated for 2mm deep cavities and if that is exceeded, the material should be placed in layers. As a primary indication of use are class III and V cavities and primary teeth. Secondary indications include to be used as a liner, base or core build up. Because it does not cause crown discoloration it is the material of choice when sealing a root canal treatment prior to composite restoration in maxillary and mandibular incisors. Fuji II LC is available in a powder and liquid hand mix package or in single use capsule form and has variety of eleven shades. It has a shelf life in room temperature of three years for the powder and 2 years for the liquid and single use capsule.

2.1.2.4 Fuji IX GP

Fuji IX GP is a conventional self-curing glass ionomer restorative material. It requires no etch or bonding agent, no moisture control or light source to cure and therefore is marketed towards the paediatric and geriatric patients. Primary indications for permanent teeth include permanent or temporary class I restorations, non-load-bearing Class I and II restorations, Class V and root surface restorations, core build-ups and for use with a composite or an inlay in the immediate or delayed sandwich technique. For primary teeth any type of restoration is indicated. The main packaging options available is single use ready mix capsules of seven different shades that require a mixing time of 10 seconds, allow a working time of 1 minute and 15 seconds and by 3 minutes have completely set. It has a shelf life time in room temperature of three years.

2.2 Methods

Three different experiments types were performed in order to determine whether dimensional stability of the SMART composites is maintained (Figure 2-4). Their methodology will be discussed in detail in this section.

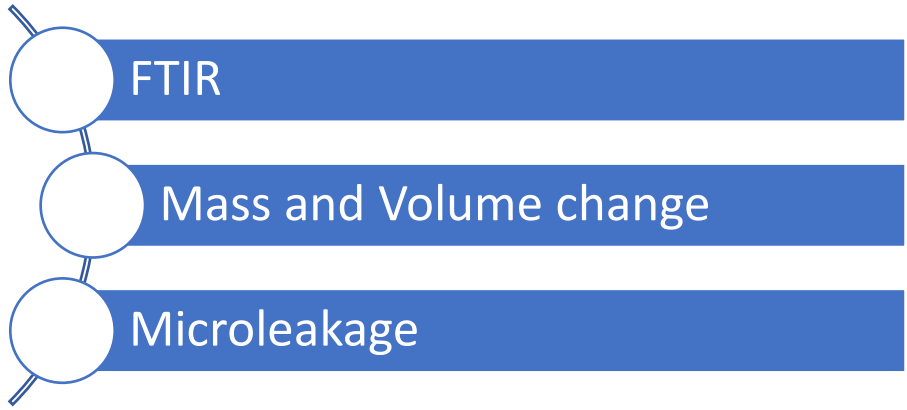

Figure 2-4 Summary of experiments that will be described in this section

2.2.1 FTIR – Fourier Transform Infrared (IR) spectroscopy

2.2.1.1 Background of FTIR

Fourier Transform Infrared (IR) spectroscopy was selected in order to measure in real time the degree of monomer conversion, the rate of the polymerization reaction, the delay time prior to polymerization initiation and the t0.5 time which is the time needed to achieve 50% monomer conversion. All the above have been adequately documented in the literature (Stansbury, 2000; Halvorson et al., 2003) and have been used specifically in dentistry in order to determine the conversion of methacrylate monomers contained in dental composites (Young et al., 2009).

2.2.1.2 Physics behind FTIR

Information on the molecular structure of the material tested, can be provided by measuring the infrared absorbance. When a molecule or atom is exposed to IR radiation, part of the radiation is absorbed by the molecule or atom and part is transmitted through the molecule or atom (passes through), meaning that it switches from one energy level ($E_{initial}$) to another (E_{final}). According to Planck's law (Mehdawi et al., 2009):

$$E_{final} - E_{initial} = hf$$

Equation 1

Where h is the Planck's constant and f is the frequency of the absorbed radiation (Hz).
But

$$f = vc$$

Equation 2

Where v is the wavenumber (cm^{-1}) and c is the velocity of light ($8x10^8$ m s^{-1})
Therefore equation 1 becomes :

$$E_{final} - E_{initial} = hvc$$

Equation 3

The wave length λ (nm) is correlated with frequency f and with Equation 2 gives:

$$\lambda = \frac{c}{f} = \frac{c}{cv}$$

Equation 4

Concluding that Equation 3 can also be given as

$$E_{final} - E_{initial} = h\frac{c}{\lambda}$$

Equation 5

Equation 5 demonstrates that the energy that a molecule absorbs must be equal with the energy needed for molecular transition. The absorbance of energy causes vibration and oscillation of the bonds between the molecules at specific frequencies and changes in the dipole moment of the bond. Different bonds vibrate at different frequencies due to the fact that they absorb different wavelengths of IR radiation.

The FTIR detects these changes in vibration due to the different energy absorbance and plots them as IR radiation versus wavenumber v (cm^{-1}). In this graph that occurs, the peaks represent the different vibrational transitions. There are two regions within the graph depending on the wavenumber that have different uses. From a wavelength of 4000 to 1300 cm^{-1} the vibrations are associated with specific functional groups and from 1300 to 500 cm^{-1} the vibrations are associated with the entire molecule therefore being known as the fingerprint region. In order to be able to identify the different peaks, FTIR spectra from the initial components were obtained.

In this project, in order to calculate monomer conversion (MC) the peak at 1320 cm^{-1} above background at 1335 cm^{-1} was used.

2.2.1.3 FTIR instrumentation set-up

The FTIR machine is composed of the following components (Figure 2-5):

1. An IR radiation source
2. An Interferometer – which measures the ratio of the incident radiation to absorbed/transmitted radiation
3. A Detector – which measures the wave length λ (nm) of the incident and absorbed/transmitted radiation
4. Beam splitter – which divides the IR radiation into two optical beams
5. Two Mirrors – which reflect back the two beams, one is fixed and the other is mobile along the path of the beam
6. A Computer software – which uses the Fourier transform and the Beer Lambert law to covert the interferogram (produced by the recombined beams at the splitter) into a plot of absorbance versus wave length λ (nm)

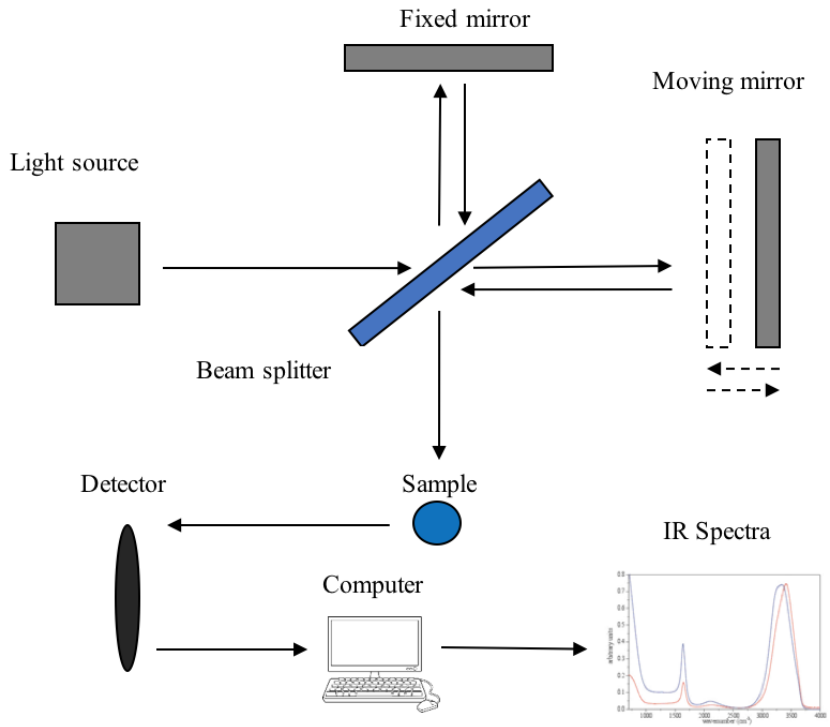

Figure 2-5 Schematic representation of FTIR instrumentation and function

2.2.1.4 Attenuated Total Reflectance (ATR) infrared spectroscopy

The Attenuated Total Reflectance (ATR) is a sampling method for thicker samples who would have very high IR absorption if examined conventionally. ATR FTIR measures the polymerization reaction in the lower surface (bottom) of the sample that is in contact with the ATR diamond. The method differs to the one described above because as the beam passes through the crystal reaching into the sample, it is mainly reflected back through the ATR diamond. It penetrates the sample just by 0.5-5 μm. In addition, measuring double bonds in the lower surface is easier than in the bulk of the sample. Furthermore, ATR allows real time monitoring of the reaction of polymerization.

2.2.1.5 How curing profiles were collected

In this project, a Perkin – Elmer Series 2000 UK FTIR spectrometer, a golden gate diamond ATR attachment (Specac, UK) and the computer software Timebase were used in order to determine monomer conversion at 37°C which is normal body temperature. Some initial samples were tested at 24°C in order to be able to compare with previous relevant work. Each formulation was placed on the ATR diamond in a 10.2mm in diameter and 1mm thick brass ring. In order to achieve the 2mm thick samples, two identical brass rings were stacked on each other. An acetate sheet was placed above the sample to prevent surface oxygen interfering with polymerization reaction. This was pressed against the restorative material with a glass surface in order to ensure 100% contact with the ATR diamond and to avoid air bubbles from forming and obscuring the readings. Each sample was light cured 20 seconds after the initiation of the program Timebase for a period of 10 seconds, 20 seconds and 40 seconds. The decision to start the curing at 20 seconds was for practical reasons because that gave sufficient time to the operator to set up the sample

in the brass ring and return to the computer to initiate the experiment. Every 4 seconds for a total time of 20 minutes, spectra were collected from 700 cm^{-1} to 2000 cm^{-1} with a resolution of 8 cm^{-1}. The six formulations described before (F2, F5, F6, F7, F8, F5small), a control and two commercial materials (Activa Kids and Z250) were tested and every sample was repeated three times (n=3) for 1mm thick samples. Towards the end, because the process was standardized and operational faults were minimised due to becoming familiar with the test steps, repetition per sample for 2mm thickness was reduced to 2.

The 4mm thickness samples turned out to be a challenge. First of all, stacking four brass rings of top of each other and filling them was tricky and gluing might interfere with the polymerization reaction. Furthermore, shrinkage due to polymerisation caused detachment of the sample from the ATR diamond. Data collected were therefore unusable because it was impossible to acquire spectra for sufficient time. Different ways of pressing the material on the diamond were tried but none was deemed consistent enough and since the target of the SMART composites are primary teeth with a maximum cavity depth of 2mm (otherwise pulp may have been involved) a decision was reached, to not proceed further with thicker samples.

2.2.1.6 Interpreting data plotted in Microsoft Excel

An example of how the data was plotted using excel is in Figure 2-6.

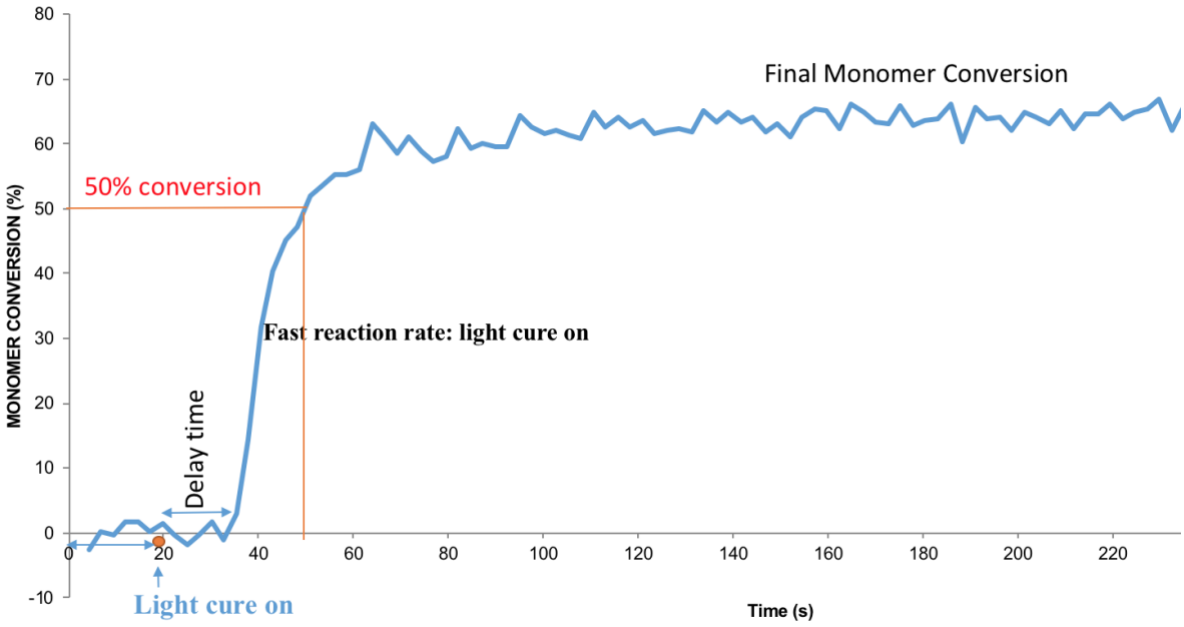

Figure 2-6 Example plot of monomer conversion versus time. Indicated with an arrow is the timepoint that the light cure commences, another annotated arrow dictates the delay time, the slope indicates the reaction rate and the plateau extrapolated to infinite time is the final monomer conversion.

The blue line represents the polymerization reaction over time in seconds. The moment the composite is placed onto the ATR FTIR diamond is the beginning of this graph at t=0 seconds. At t=20 seconds, the light curing began and it is evident that there can be a delay of a few seconds before the polymerization reaction starts. The subsequent slope in Figure 2-6 depicts the fast

polymerization reaction rate which gradually slows down when a plateau value of final monomer conversion is reached.
In the following, delay time, the maximum reaction rate and the half time were used order to visualise effects of different formulation variables.

2.2.1.7 Information collected from the spectra analysis on Microsoft Excel

2.2.1.7.1 Delay time (s)

The delay time was calculated by checking on the graph at which timepoint did the monomer conversion started creating a slope and 20 seconds were subtracted from that time because that is when the curing commenced.

2.2.1.7.2 Reaction Rate (%MC/s)

The reaction rate was calculated by selecting the data after the delay time when the slope begins and before it reaches the plateau using the SLOPE function in excel.

2.2.1.7.3 T0.5 (s)

The half time by definition is the time when 50% monomer conversion is reached. This is calculated by the ratio of 50%monomer conversion versus the reaction rate plus the delay time.

2.2.1.7.4 Final Monomer Conversion (%MC)

The degree of Monomer Conversion (MC) was calculated through the difference in the height of the absorbance of the monomer peaks at a wavelength of 1320 cm^{-1} and above background at a wavelength of 1335 cm^{-1} , before and after curing. The above wavelength range indicates the C-O stretch in the methacrylate that switched to lower wavenumbers (cm^{-1}) during the polymerization reaction. The mathematical formula used is :

$$\% \text{ of MC} = 100\ (1 - \frac{h_t}{h_\circ})$$

Equation 6

Where $h_\circ$ is initial peak height above background and h_t is peak absorbance at time,t.

2.2.1.8 Statistical Analysis of FTIR results

Data were analysed using SPSS version 25 for Mac (IBM, USA). Monomer conversion, delay time, reaction rate and t0.5 were analysed with a Kruskal - Wallis test performed in the three subgroups described above.

2.2.1.9 Calculated Polymerization Shrinkage (φ) and heat (J/cc)

The degree of monomer conversion is proportional to the calculated shrinkage. It was calculated with the mathematical equation below :

$$\varphi = 23C\rho f \sum_i \frac{n_i x_i}{w_i}$$

Equation 7

Where each symbol stands for :

C – monomer conversion (%)
ρ – composite density (g/cm^3)

n_i – the number of C=C bonds per molecule
x_i – mass fraction for each monomer
W_i – molecular weight (g/mol)

The number 23 is derived from the fact that one mole of polymerizing C=C bonds gives 23 cm^3/mol of volumetric shrinkage.

Equation 7 was inserted into an excel spreadsheet, calculations were done and then plotted into a bar chart.

For heat generation the relevant mathematical equation used was:

$$heat\ \left(\frac{kj}{cc}\right) = nf\rho\left(\frac{C}{100}\right)$$

Equation 8

Where each symbol stands for:

C – monomer conversion (%)
ρ – composite density (g/cm^3)
n– the number of C=C bonds per molecule
f – fraction of monomer

Mean monomer conversion of each different curing time (10, 20 and 40 seconds) was used in order to create the plots in excel.
Since the exact composition of Z250 and Activa is not known, polymerization shrinkage or heat could not be calculated with the mathematical Equation 7 and Equation 8.

2.2.2 Mass and Volume change in water

2.2.2.1 Sample Preparation

In order to assess the mass and volume change of the different formulations of the SMART composites and the two commercial materials (Activa and Z250), 1mm diameter discs were created by placing the material within 10.2mm diameter brass rings of 1mm thickness and light curing them for 40 seconds top and bottom, after pressing them on top with an acetate sheet in order to assure maximum polymerization of the entire disc, equal distribution of the material, to avoid voids and surface oxygen interference.
Each of the six SMART composite formulations, the control and two commercial materials (Activa and Z250) had three discs made and tested, giving a total of 27 discs. After 24 hours, the discs were removed from the brass rings with light hand pressure in order not to break them, excess was trimmed away. Each one was weighted using a four figured Metler Toledo AG204 analytical balance equipped with a density kit in dry conditions and wet conditions submerged in 0.1% soapy water (Sodium Dodecyl Sulphate). The above adheres to the Archimedes' principle. Between different timepoints, each disc was stored separately in a sterile tube which contained 10ml of deionised water (dH2O, MicroPure, Barnstead, Thermo Scientific, Hemel Hempstead, UK) and subsequently placed in an incubator at 37°C imitating the human body temperature. Each disc was weighted in dry (upon being blotted dry carefully) and wet conditions using the four figured balance

mentioned above and a density kit at 2 hours, 4 hours, 6 hours, 24 hours, 48 hours, 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. Each time the discs were weighted in wet conditions, the temperature of the soapy water was recorded.

2.2.2.2 Data calculations

All the data was inserted into an excel spreadsheet where the density, the mass change and volume change were calculated and plotted into the three subgroups described previously.

The equations to calculate the mass and volume percentage change respectively were the following:

$$\Delta M(\%) = 100\frac{(M_t - M_0)}{M_0}$$

Equation 9

$$\Delta V(\%) = 100\frac{V_t - V_0}{V_0}$$

Equation 10

where M_t and V_t represent the mass and volume at timepoint t following immersion and M_0 and V_0 stand for the initial mass and volume recorded at t=0.

2.2.2.3 Statistical analysis of Mass and Volume results

Data were imported to SPSS software version 25 for Mac (IBM, USA) and after determining the homogeneity of the data by a levene test, a one way analysis of variance (ANOVA) test was performed. For post hoc comparison, depending on the result of the levene test, a Tuckey's test would be performed if the data was homogenous therefore a parametric test needed, or a Dunnett's T3 test for non-homogenous data that would require a non-parametric test.

2.2.3 Microleakage test

The ISO/TS 11405:2015 protocol was followed using primary teeth (E's, D's and C's) instead of third permanent molars. The teeth were pulled from the Eastman Biobank with ethical approval reference number 1304.

2.2.3.1 Teeth collection, selection and preparation

Primary carious molars were collected from general anaesthetic sessions taking place at the MacMillan Cancer Centre (MCC) during September 2018- less than 6 months prior to testing as the ISO states (See appendix for copy of information leaflet and consent form used). Prior to the procedure of extracting the teeth, parents and patients were given two leaflets one with language relevant to parents and one written for the appropriate age of the patient which stated the purpose of the collection of teeth and brief information. Consent was obtained from all patients and out of two copies obtained one was placed in the patient notes and one filled in the Eastman Biobank database were a unique identifying number was assigned. Following extraction, they were cleaned from soft tissue residue and the one's that fit the inclusion criteria were then placed in 1% chloramine T for two days (maximum one week) in order to be disinfected according to ISO protocol 3696:1987.

Inclusion criteria comprised of selecting teeth with as many sound surfaces as possible, without completely damaged crowns. After that, they were stored in deionised water which was changed every two weeks in order to avoid any bacterial growth, inside a refrigerator with a i.e. nominal temperature of 4°C (ISO 3696:1987, grade 3). Before proceeding with the experiment, they were removed from the refrigerator for four hours so they would obtain room temperature. In order to control the angle and depth of cavity and to aid the operator, some the teeth were mounted in a holder of acrylic in the form of discs. In others that presented with enough tooth structure that they could be hand held, were sealed with acrylic that was paramount to be able to extend over the roots and leave no cementum exposed in order to avoid dye penetration from there that would obscure the sample.

2.2.3.2 Adhesion to enamel test

Round cavities of 2mmX2mm (diameter and depth) dimensions were drilled in the sound surfaces. The maximum sound surfaces were the occlusal, buccal, lingual/palatal, mesial and distal. A high speed handpiece under running water with a cylindrical diamond burr of 1.6mmX4mm (diameter and depth) were used for this purpose. In the presence of carious lesions, soft caries were removed with a hand scaler until a hard surface remained. Each cavity was thoroughly dried for 10 seconds with a three in one dental syringe and filled as a bulk (without any layering) with the materials in Table 2-3 and below:

- EDI composite: directly without etch and/or bond, cured for 20 seconds
- Fuji II LC: directly without etch and/or bond, cured for 20 seconds
- Fuji IX: directly without etch and/or bond, chemical self-polymerization
- Activa: etched for 10 seconds, cured for 20 seconds

Table 2-3 Summary of the placement process for each material used in the microleakage test.

Material	Etch	Bond	Curing t
EDI composite	X	X	20 seconds
Fuji II LC	X	X	20 seconds
Fuji IX	X	X	20 seconds
Activa	10 seconds	X	20 seconds

In total, 10 cavities per material were restored as described in Table 2-3, resulting in 10 samples per restorative material.
The curing was done using a DemiPlus light curing unit from Kerr with a light intensity of approximately 1500 mW/cm2. The curing light was measured using a Bluephase curing light meter by ivoclar vivadent in order to assure the appropriate intensity and to have accurate results.
A conical yellow diamond polishing bur friction grip 862C/010 Extra Fine 5/Pk under running water mounted on the high speed handpiece was used to remove the excess material covering the interface between the tooth structure and the restorative material and polish the restorations.
Then, the teeth were placed in deionised water in an incubator at 37°C- to simulate the human body- for a period of four weeks. At two weeks' time, the deionised water was drained and replaced with fresh.
At the four week time point, since the cementum was already covered by the acrylic, the crowns of the teeth that remained uncovered were coated with two coats of nail varnish to prevent microleakage that was not related to failure of the restorative material but to dental decay or enamel defects. The nail varnish covered all of the crown of the primary tooth except from the restorations and a perimeter of 1mm around them.
After the nail varnish had set, all primary teeth were immersed in a 1% methylene blue staining solution for four consecutive hours. Following that, they were washed with deionised water in order to wash out the excess dye.
All forty (40) restorations were cross-sectioned with a long diamond burr under running water on a high speed handpiece and photos of each were taken using a pluggable 200 X 2M USB Digital Microscope 8LED digital magnifier. Blue tac was used to stabilise each tooth so that the fillings could be photographed. Images were captured and saved on a laptop computer by utilizing the MicroCapture Measurement Software.
All 40 images were assigned a randomized number using the randomization table seen in Figure 2-7.

Table of Random Numbers

36518	36777	89116	05542	29705	83775	21564	81639	27973	62413	85652	62817	57881
46132	81380	75635	19428	88048	08747	20092	12615	35046	67753	69630	10883	13683
31841	77367	40791	97402	27569	90184	02338	39318	54936	34641	95525	86316	87384
84180	93793	64953	51472	65358	23701	75230	47200	78176	85248	90589	74567	22633
78435	37586	07015	98729	76703	16224	97661	79907	06611	26501	93389	92725	68158
41859	94198	37182	61345	88857	53204	86721	59613	67494	17292	94457	89520	77771
13019	07274	51068	93129	40386	51731	44254	66685	72835	01270	42523	45323	63481
82448	72430	29041	59208	95266	33978	70958	60017	39723	00606	17956	19024	15819
25432	96593	83112	96997	55340	80312	78839	09815	16887	22228	06206	54272	83516
69226	38655	03811	08342	47863	02743	11547	38250	58140	98470	24364	99797	73498
25837	68821	66426	20496	84843	18360	91252	99134	48931	99538	21160	09411	44659
38914	82707	24769	72026	56813	49336	71767	04474	32909	74162	50404	68562	14088
04070	60681	64290	26905	65617	76039	91657	71362	32246	49595	50663	47459	57072
01674	14751	28637	86980	11951	10479	41454	48527	53868	37846	85912	15156	00865
70294	35450	39982	79503	34382	43186	69890	63222	30110	56004	04879	05138	57476
73903	98066	52136	89925	50000	96334	30773	80571	31178	52799	41050	76298	43995
87789	56408	77107	88452	80975	03406	36114	64549	79244	82044	00202	45727	35709
92320	95929	58545	70699	07679	23296	03002	63885	54677	55745	52540	62154	33314
46391	60276	92061	43591	42118	73094	53608	58949	42927	90993	46795	05947	01934
67090	45063	84584	66022	48268	74971	94861	61749	61085	81758	89640	39437	90044
11666	99916	35165	29420	73213	15275	62532	47319	39842	62273	94980	23415	64668
40910	59068	04594	94576	51187	54796	17411	56123	66545	82163	61868	22752	40101
41169	37965	47578	92180	05257	19143	77486	02457	00985	31960	39033	44374	28352
76418												

Figure 2-7 Table of random numbers used to randomize sample microleakage images

Following that, the interface between the restorative material and the tooth cervically (meaning towards the root) and coronally (meaning towards the occlusal surface/incisal edge) were scored on a excel spreadsheet using the scoring system from the ISO/TS 11405:2015 protocol as described below:

0 – no penetration
1- penetration into the enamel
2- penetration into dentine, not including pulpal floor of cavity
3- penetration into dentine, including pulpal floor of cavity

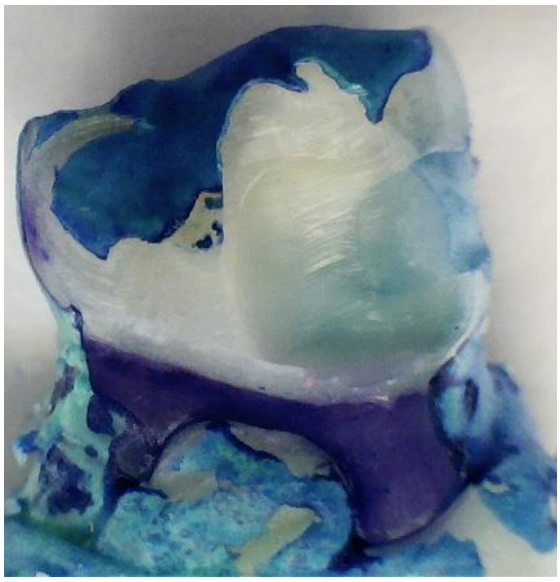

Figure 2-8 Picture of primary molar with restoration in place indicating the coronal and cervical interface taken with USB digital microscope.

Two observers were trained via a PowerPoint presentation that included images of 0,1,2 and 3 microleakage. Subsequently, the two observers marked the randomized images on an excel spreadsheet and on the samples that different scores were noted, a new score was appointed in agreement of both observers. Subsequently, the data was plotted in a Excel spreadsheet for plotting and interpretation and statistical analysis was carried out using SPSS software.

2.2.3.3 Statistical analysis of microleakage results

Data were imported to SPSS software version 25 for Mac (IBM, USA). Due to the statistically small number of samples it would be prudent to assume that data would follow a normal distribution. For that reason, a Kruskal-Wallis test was performed initially separately for the coronal and cervical interfaces and then combined together to assess any significance amongst formulations and coronal and cervical interface microleakage score. Since more than one test were performed, a decision to use the adjusted significant p value was made in order to eliminate the probability of errors occurring by chance due to the increased test number. This adjusted significant p value occurred after using the Bonferroni correction which is a post hoc adjustment for non-parametric statistical tests. In addition, Plum ordinal regression analysis was used in order to establish if different levels of microleakage (0,1,2,3) on the coronal and cervical interfaces can be significantly distinguished.

In addition, Cohen's κappa (κ) test was run in order to determine if there was agreement between the two observers on the microleakage score obtained from 40 randomised images depicting 40 coronal tooth/material interfaces and 40 cervical tooth/material interfaces. There are two existing guidelines to aid interpretation of how good is agreement between the observers, one by Landis and Koch (1977) and an adapted version of that one by Altman (Ashby, 1991). A summary can be seen in the table below:

Table 2-4 showing Cohen's kappa test interpretation according to the two existing guidelines by Landis and Koch (1977) and the modified Landis and Koch by Altman (1991).

kappa value	Strength of agreement	
	Landis and Koch (1977)	**Altman (1991)**
<0.00	Poor	-
0.00 - 0.20	Slight	Poor
0.21 - 0.40	Fair	Fair
0.41 - 0.60	Moderate	Moderate
0.61 - 0.80	Substantial	Good
0.81 - 1.00	Almost Perfect	Very good

The Table 2-4 was used to determine the level of agreement between of the two observers when scoring the coronal and cervical interfaces for microleakage.

3 Results

3.1 FTIR – Fourier Transform Infrared (IR) spectroscopy

The results will be presented in the order the events during curing occurred which can be seen in Figure 2-6. First, delay time followed by reaction rate and half time and last the final monomer conversion.

3.1.1 Delay Time (s) of four formulations with high and low MCPM and PLS

Delay time is the time between the initiation of curing at t=20 seconds until the polymerization reaction starts.

Figure 3-1 shows the different delay times in each different thickness (1mm, 2mm). Samples of 2mm thickness seem to have a longer delay time than 1mm thickness. At 1mm thickness, all four SMART composite formulations have similar delay times to the two commercials. The two formulations containing low PLS (2%) have much shorter delay times than the two formulations containing high PLS(4%).

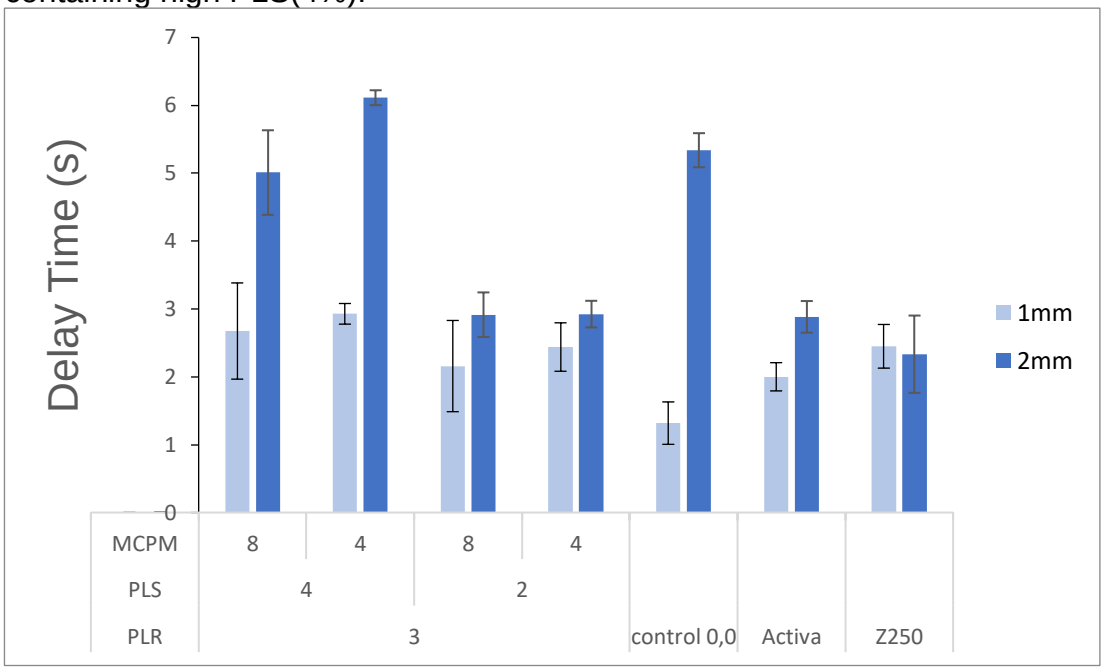

Figure 3-1 Plot of the delay time of four SMART composite formulations, the control and two commercial materials at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.2 Reaction rate (MC%/s) of four formulations with high and low MCPM and PLS

Reaction rate describes how quickly the material polymerizes.

Figure 3-2 shows the different reaction rates in each different thickness (1mm, 2mm). As expected, in three out of four SMART composite formulations and Z250 the reaction rate is higher for the thinner 1mm sample compared to the 2mm thick one. Activa on the other hand, demonstrates the same reaction rate irrespectively of thickness of sample. The low PLS(2%) and low MCPM (4%) SMART composite formulation and the control demonstrate the opposite and show a higher reaction rate at 2mm thickness. The high PLS(4%) and high MCPM (8%) SMART composite formulation seems to have the least discrepancy between 1mm and 2mm thickness samples and has a sufficiently high reaction rate in both.

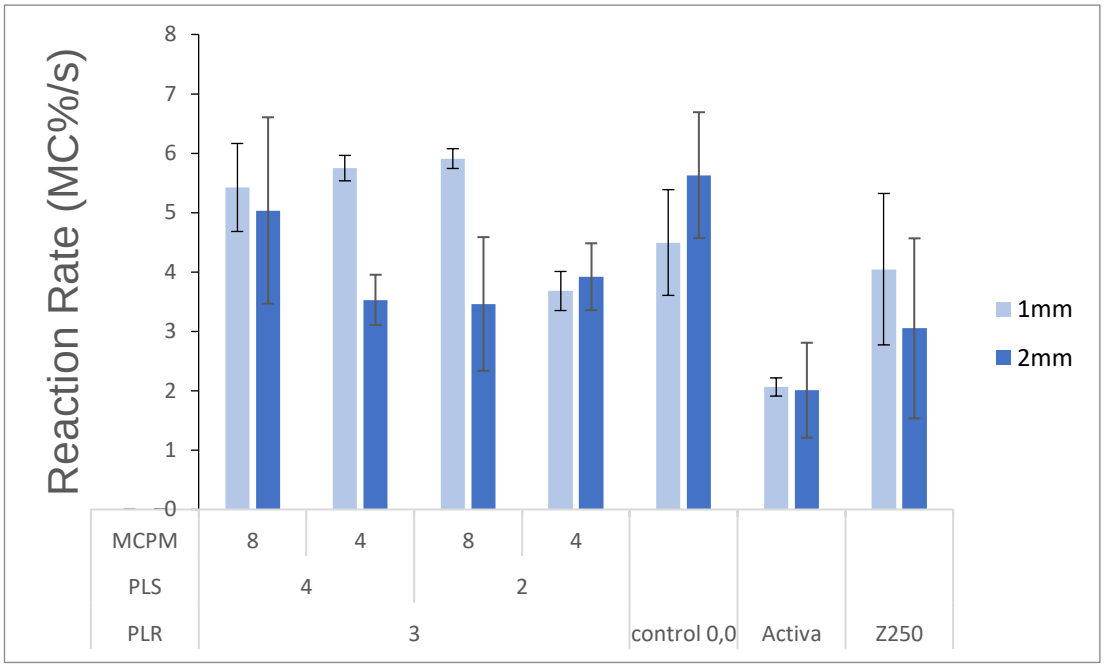

Figure 3-2 Plot of the reaction rate of four SMART composite formulations, the control and two commercial materials at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.3 T0.5(s) of four formulations with high and low MCPM and PLS

T0.5 is the half time were 50% of the total monomer conversion was reached. Figure 3-3 shows the different half times in each different thickness (1mm, 2mm) and the samples of the three different curing times have been added all together and their means have been plotted. All four SMART composite formulations, the control and Activa have a shorter half time at 1mm thickness than 2mm, except Z250. Activa shows a higher half time in both thicknesses compared to all other materials. The two formulations with high MCPM(8%) seem to perform almost identically in both thicknesses.

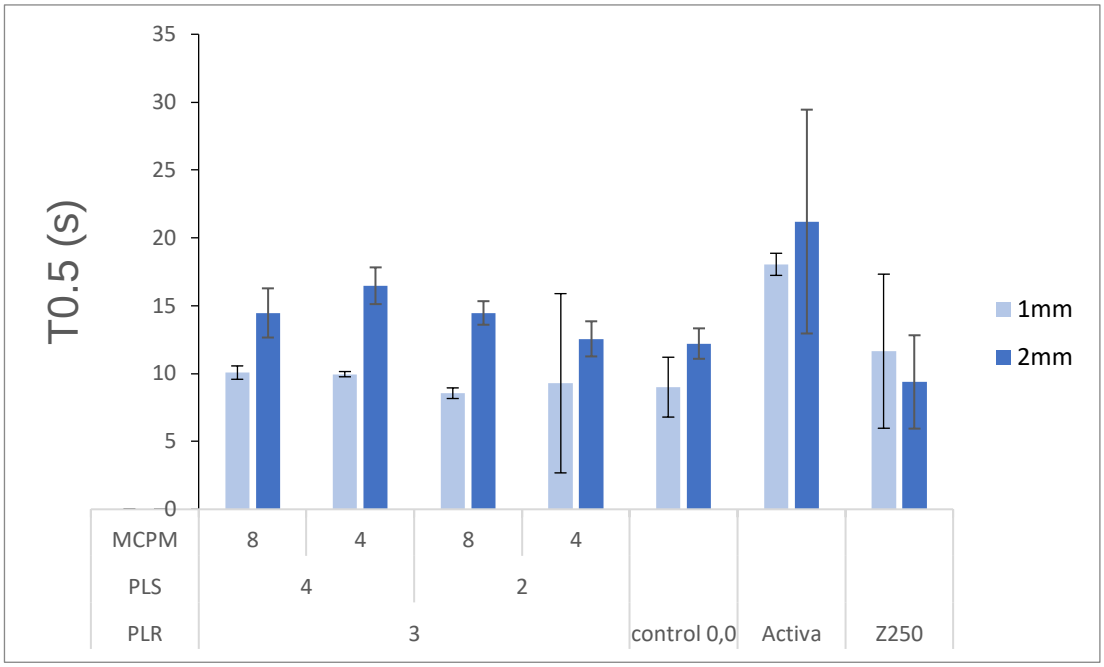

Figure 3-3 Plot of the half time of four SMART composite formulations, the control and two commercial materials at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.4 Monomer Conversion (MC %) of four formulations with high and low MCPM and PLS

Figure 3-4 shows the monomer conversion(%) for samples of 1mm thickness in the three different curing times of 10, 20 and 40 seconds. All four SMART composite formulations are demonstrating a higher monomer conversion than the two commercials which are very similar to the control. The minimum monomer conversion of a SMART composite is 67% of the formulation with low MCPM and low PLS cured for 10 seconds and is definitely higher than 50% which is the safety level in order to avoid toxicity. Generally, at a curing time of 10 seconds, the monomer conversion is lower than the monomer conversion at 20 and 40 seconds of curing time. The bars representing the 20 and 40 seconds curing time are almost identical for Activa. All four SMART composite formulations, the control and Z250 show an increase in monomer conversion when cured for longer. For a curing time of 40 seconds, the two formulations with the high MCPM(8%) reach the highest monomer conversion of 83%. The formulation with low MCPM (4%) but high PLS (4%) exhibits a peak monomer conversion of 85% at 40 seconds curing time surpassing all other SMART composite formulations.

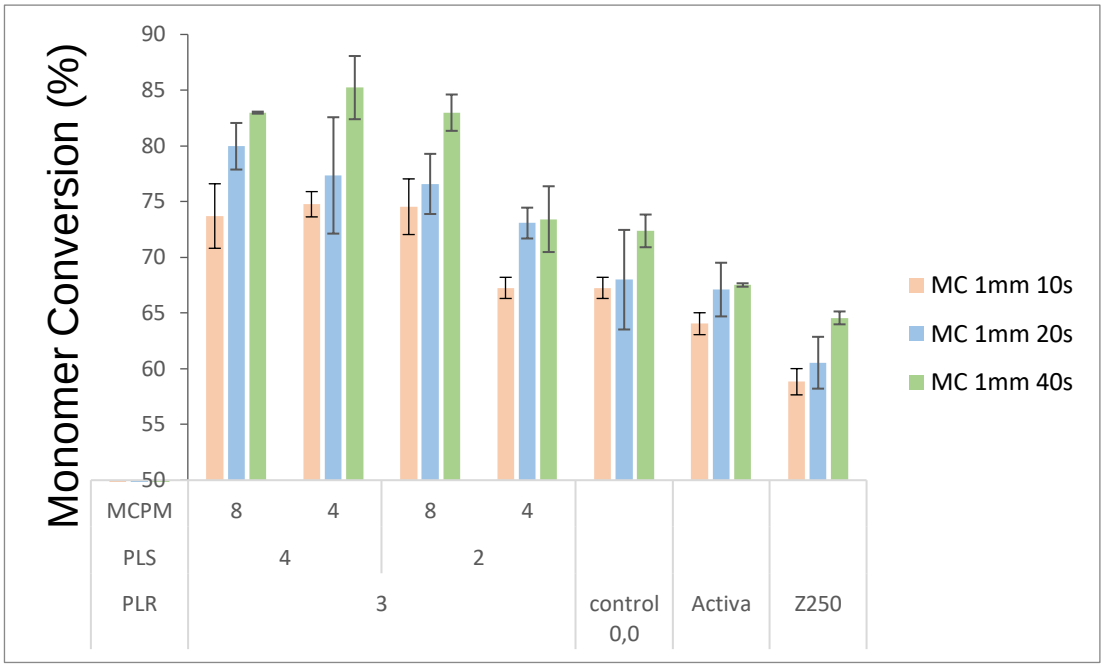

Figure 3-4 Plot of the monomer conversion of four SMART composite formulations, the control and two commercial materials at 1mm (n=3) thickness samples after curing for 10, 20 and 40 seconds (N.B. y axis starts from 50% because that is the minimum percentage of polymerization that a material has to reach in order not to be toxic). Error bars are 95% CI (n=3).

Figure 3-5 shows the monomer conversion(%) for samples of 2mm thickness in the three different curing times of 10, 20 and 40 seconds. Similarly to the 1mm thickness described in Figure 3-5, at the 2mm thick samples the four SMART composite formulations demonstrate a higher monomer conversion than the two commercial materials. As expected, at 10 seconds curing time the monomer conversion is lower than at 20 and 40 seconds with an average close to 70% for the SMART composite formulations and 61% and 59% for Activa and Z250 respectively. At 20 seconds, the monomer conversion amongst the SMART formulations varies without an evident pattern and the formulation with the low MCPM (4%) and high PLS(4%) reaches a 72% which is very similar to Activa which has 70%. At 40 seconds curing time, all four SMART composites consistently reach monomer conversions ranging from 81 to 84%.

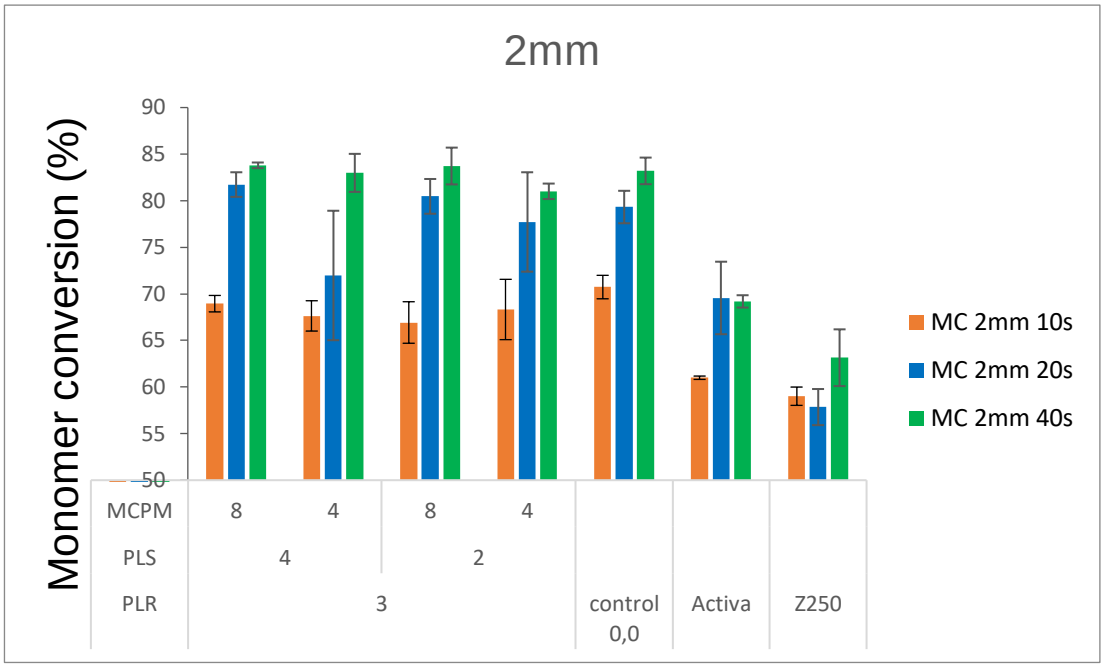

Figure 3-5 Plot of the monomer conversion of four SMART composite formulations, the control and two commercial materials at 2mm (n=2) thickness samples after curing for 10, 20 and 40 seconds (N.B. y axis starts from 50% because that is the minimum percentage of polymerization that a material has to reach in order not to be toxic). Error bars are the standard deviation (n=2).

In both 1mm and 2mm thickness samples, at 10 seconds curing time the monomer conversion average is close to 70%. At 20 seconds, results were less consistent. At 40 seconds, irrespectively of the thickness all SMART composite formulations demonstrate a consistently high monomer conversion exceeding 80%. The two formulations with low MCPM (4%) behaved in a less predictable manner than the two with the high MCPM 8%).

3.1.5 Delay time (s) of two formulations with different MCPM particle size (50 µm versus 10µm)

Figure 3-6 demonstrates that formulations with different particle sizes follow the same pattern of shorter delay time at 1mm thickness and longer at 2mm. The normal sized MCPM particle formulation demonstrates a higher delay time of more than 5 seconds at 2mm thickness compared to the 3.4 seconds of the one with the smaller sized particles.

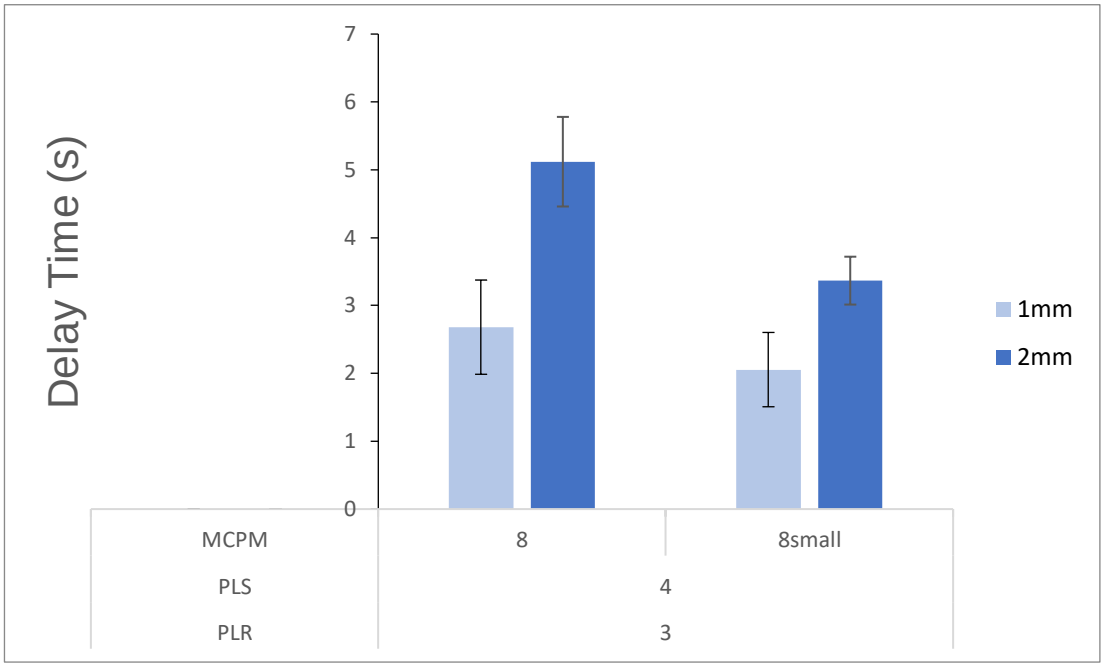

Figure 3-6 Plot of the delay time of two SMART composite formulations with conventional (50µm) and small (10µm) sized MCPM particles respectively, at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.6 Reaction Rate (%MC/s) of two formulations with different MCPM particle size (50µm versus 10µm)

In Figure 3-7 both formulations show an equivalent reaction rate of almost 5.5% at 1mm thickness samples. At 2mm thickness, the formulation with the small sized MCPM particles shows a reaction rate of 3% compared to the other one which has a reaction rate of 5%.

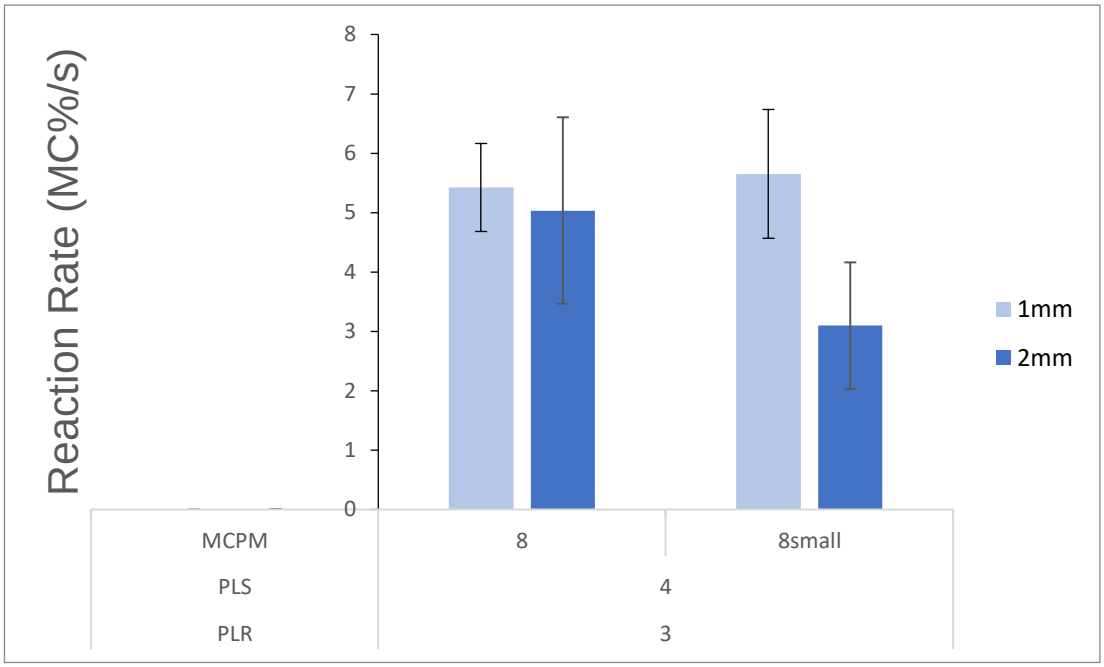

Figure 3-7 Plot of the reaction rate of two SMART composite formulations with conventional (50μm) and small (10μm) sized MCPM particles respectively, at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.7 T0.5 (s) of conventional and small MCPM particle size formulations (50μm versus 10μm)

In Figure 3-8, both formulations show the same pattern of an almost identical half time of 10 seconds at 1mm thickness, to an increased half time by approximately 33% when the sample thickness doubles. The formulation containing the smaller sized MCPM particles demonstrates a higher half time by 2 seconds at 2mm thickness samples compared to the formulation with the normal sized MCPM particles but the error bars are overlapping.

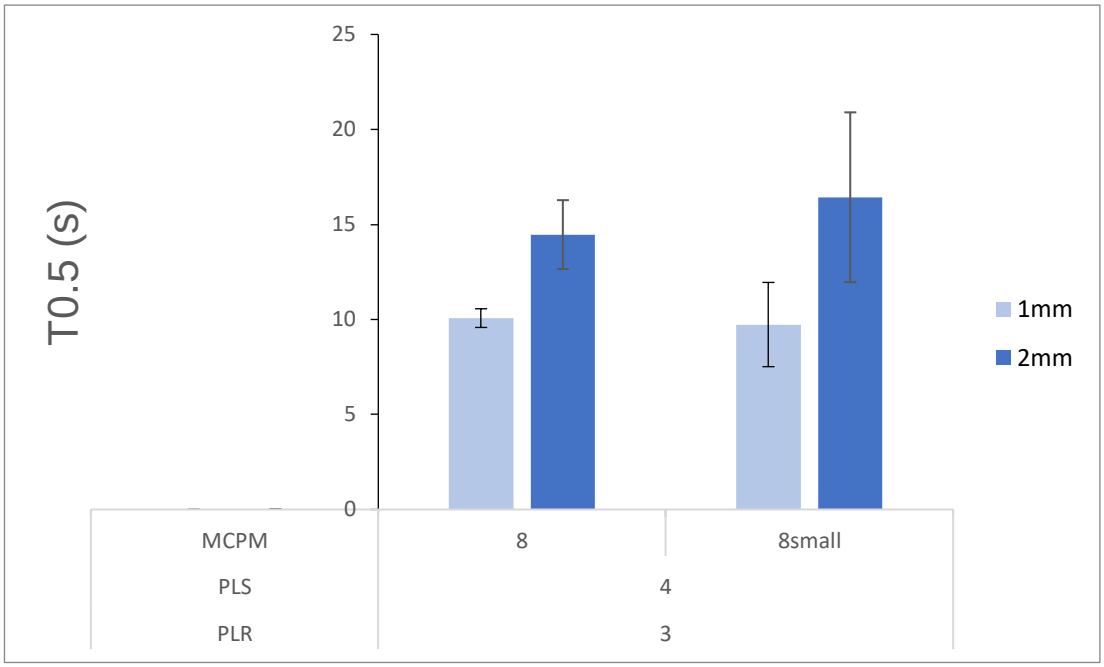

Figure 3-8 Plot of the half time of two SMART composite formulations with conventional (50μm) and small (10μm) sized MCPM particles respectively, at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.8 Monomer conversion (MC%) of two formulations with different MCPM particle size (50 μm versus 10μm)

Figure 3-9 shows the monomer conversion(%) for samples of 1mm thickness in the three different curing times of 10, 20 and 40 seconds. At 10 seconds of curing time, the MCPM particle size does not seem to have an effect on monomer conversion. At 20 seconds curing time the formulation containing small sized MCPM particles peaks with an 83% monomer conversion just 2% higher than the formulation with conventional sized MCPM. At 40 seconds of curing time the SMART composite formulation containing the small MCPM particles demonstrates a lower monomer conversion of 74% compared to the 83% of the one with normal sized particles.

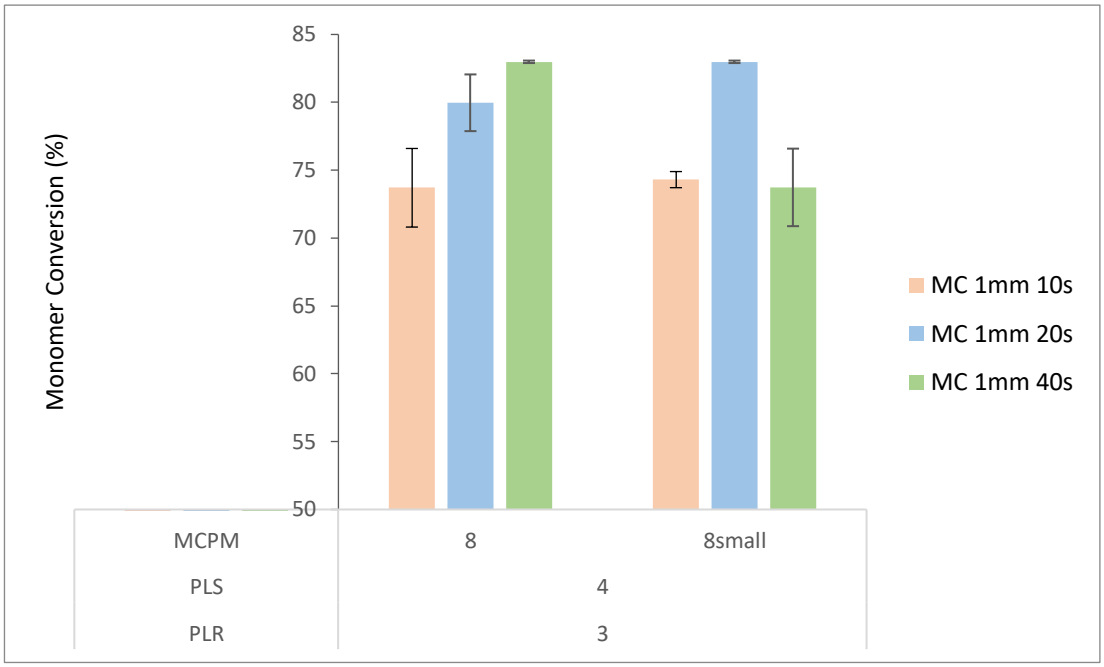

Figure 3-9 Plot of the monomer conversion of two SMART composite formulations with conventional (50μm) and small (10μm) sized MCPM particles respectively, at 1mm thickness samples after curing for 10, 20 and 40 seconds. (N.B. y axis starts from 50% because that is the minimum percentage of polymerization that a material has to reach in order not to be toxic). Error bars are 95% CI (n=3).

Figure 3-10 shows the monomer conversion(%) for samples of 2mm thickness in the three different curing times of 10, 20 and 40 seconds. As one would expect, on this graph monomer conversion is getting higher as the curing time gets longer in both formulations. The SMART composite formulation with the normal sized MCPM particles shows a 13% difference between conversions at 10 and 20 seconds whilst the SMART composite formulation with the smaller sized MCPM particles shows a 7% difference between conversions at 20 and 40 seconds. At 40 seconds the conventional MCPM sized formulation seems to reach the same monomer conversion percentage of 83% which is equal to the monomer conversion of the 1mm thickness samples cured for 20 seconds whereas the small sized MCPM particle one demonstrates an unexpected increase of 9% as the thickness doubles. As is evident from the error bars, the small sized MCPM formulation was less consistent at 2mm thickness samples.

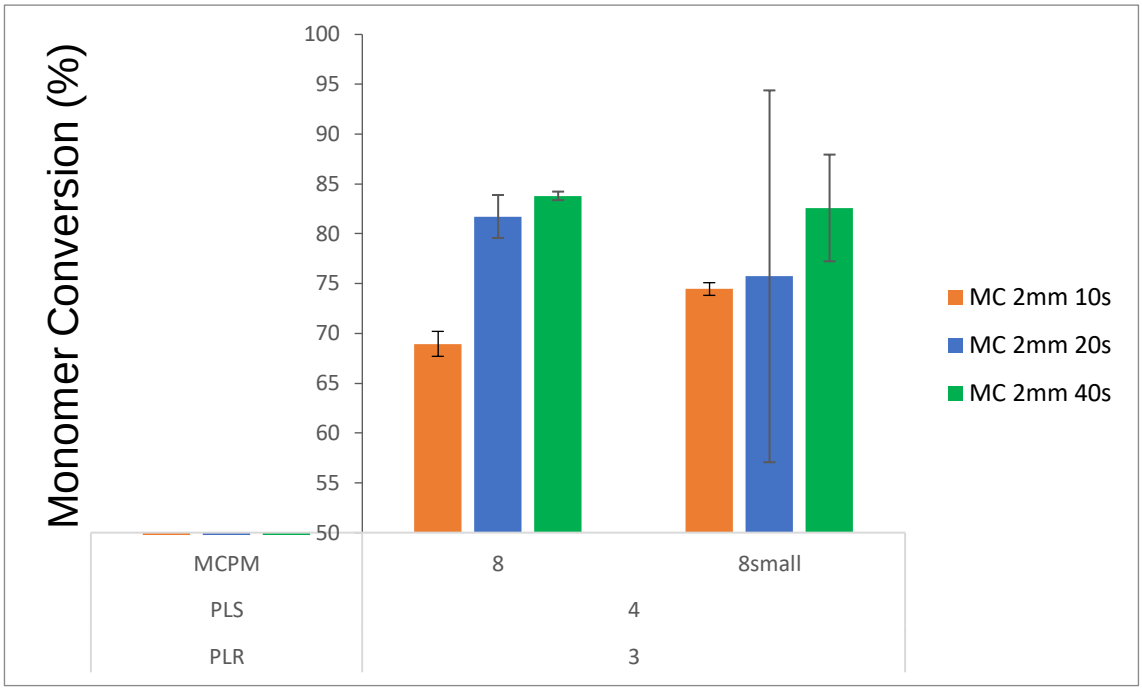

Figure 3-10 Plot of the monomer conversion of two SMART composite formulations with conventional (50μm) and small (10μm) sized MCPM particles respectively, at 2mm thickness samples after curing for 10, 20 and 40 seconds. (N.B. y axis starts from 50% because that is the minimum percentage of polymerization that a material has to reach in order not to be toxic). Error bars are the standard deviation (n=2).

3.1.9 Delay time (s) of two formulations with different powder liquid ratio

Figure 3-11 shows the different delay times in each different thickness (1mm, 2mm) and the samples of the three different curing times have been added all together and their means have been plotted. Both formulations present the same pattern with an expected longer delay time at 2mm sample thickness which does not exceed 4 seconds. Due to the overlapping error bars no other safe conclusion can be drawn.

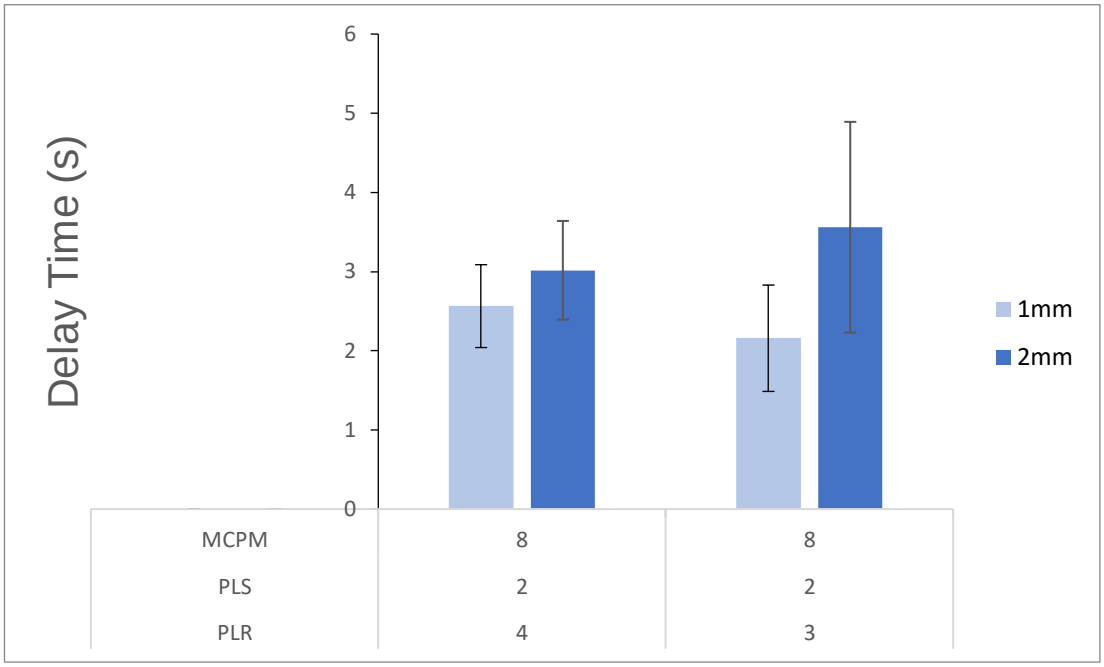

Figure 3-11 Plot of the delay time of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.10 Reaction rate (%MC/s) of two formulations with different powder liquid ratio

Figure 3-12 shows the different reaction rates in each different thickness (1mm, 2mm) and the samples of the three different curing times have been added all together and their means have been plotted. The reaction rate seems to be higher at 1mm thickness samples than 2mm. The highest rate of 6% monomer conversion is reached by the formulation of 3:1 powder liquid ratio at 1mm thick samples.

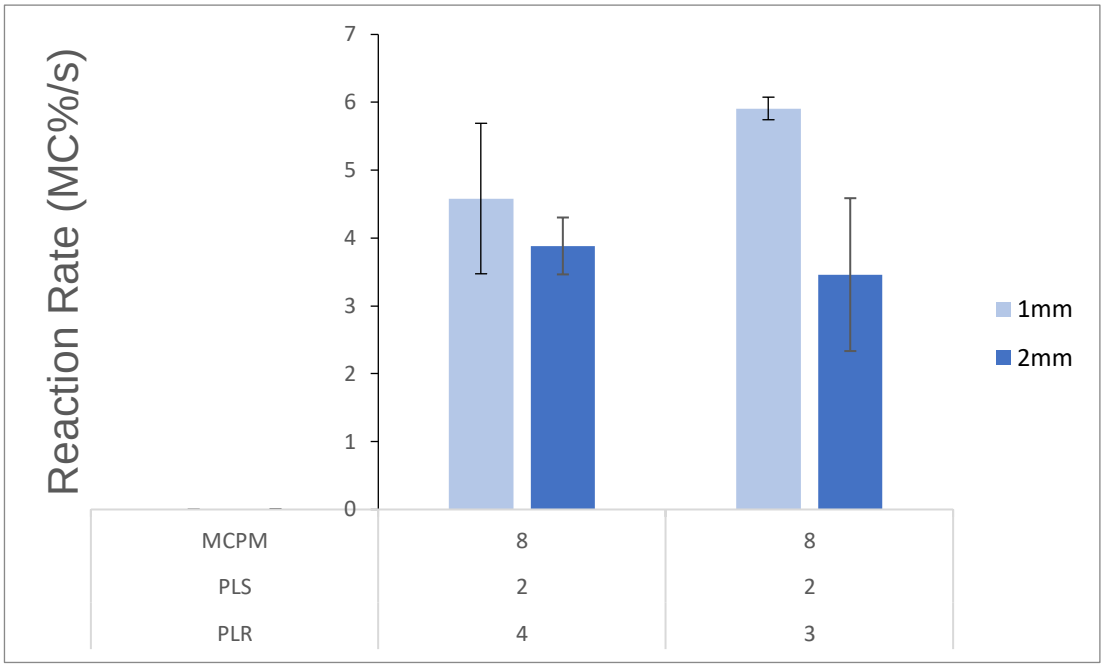

Figure 3-12 Plot of the reaction rate of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.11 T0.5 (s) of two formulations with different powder liquid ratio

Figure 3-13 shows the different half times in each different thickness (1mm, 2mm) and the samples of the three different curing times have been added all together and their means have been plotted. At 1mm thickness, the formulation that reaches 50% monomer conversion the fastest is the one with 3:1 powder liquid ratio whist at 2mm thickness it is the 4:1 with a difference of just a second.

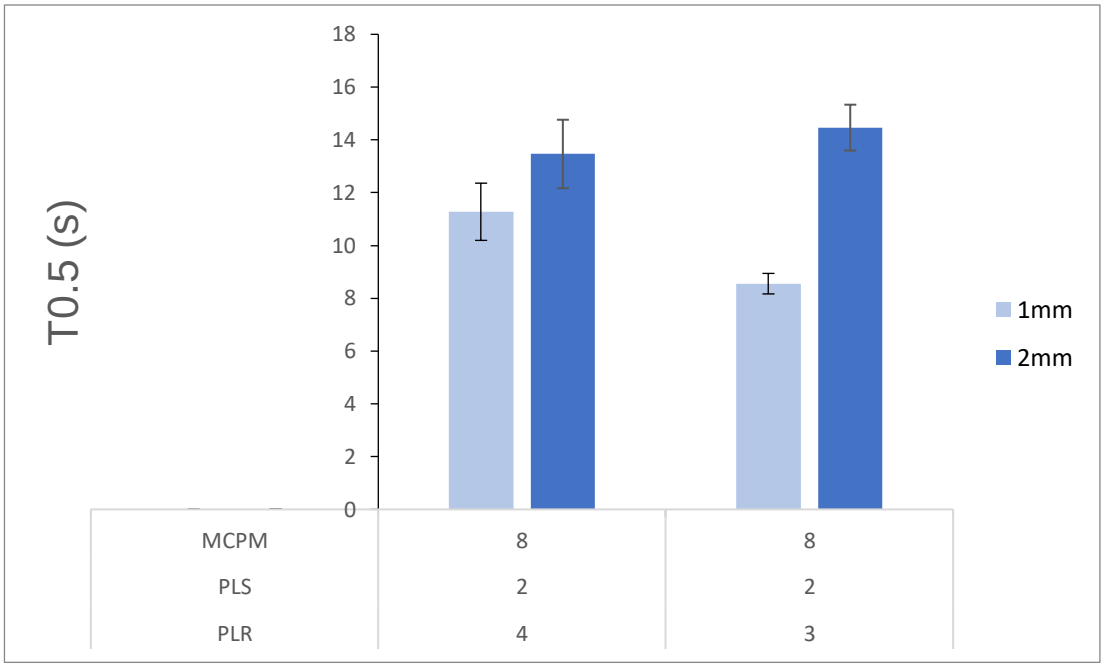

Figure 3-13 Plot of the half time of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 1mm (n=9) and 2mm (n=6) thickness samples regardless of the curing time 10, 20 and 40 seconds. Error bars are 95% CI.

3.1.12 Monomer conversion (MC%) of two formulations with different powder liquid ratio

Figure 3-14 shows the monomer conversion(%) for samples of 1mm thickness in the three different curing times of 10, 20 and 40 seconds. The formulation with the powder liquid ratio of 4:1 shows an expected trend with the monomer conversion percentage raising higher as the curing time becomes longer. The formulation with a powder liquid ratio of 3:1 demonstrates an expected peak of an 83% monomer conversion at 40 seconds whereas it only reaches an 77% of monomer conversion at 20 seconds of curing time. The same formulation is more consistent in providing a predictably high monomer conversion above 75% regardless of the curing time. The formulation with the 4:1 powder liquid ratio demonstrated lower monomer conversions of 64% and 67% at 10 and 20 seconds of curing time and peaks with 85% at 40 seconds curing time which is higher by 2% than the formulation with 3:1 powder liquid ratio.

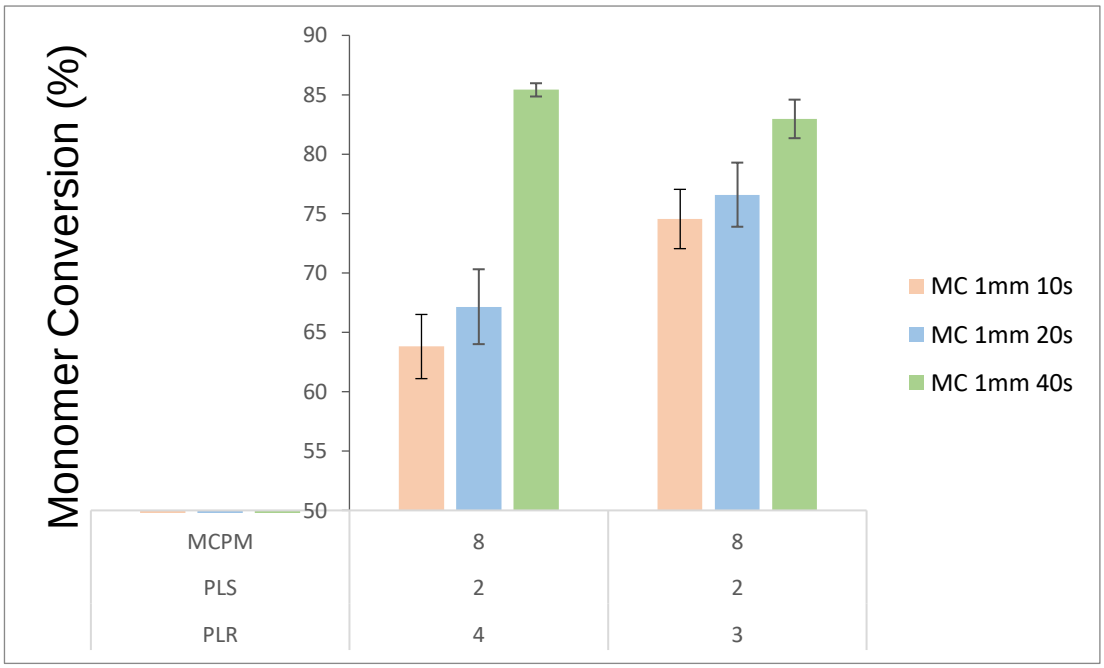

Figure 3-14 Plot of the monomer conversion of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 1mm thickness samples after curing for 10, 20 and 40 seconds. (N.B. y axis starts from 50% because that is the minimum percentage of polymerization that a material has to reach in order not to be toxic). Error bars are 95% CI (n=3).

Figure 3-15 shows the monomer conversion(%) for samples of 2mm thickness in the three different curing times of 10, 20 and 40 seconds. At 2mm thickness, both formulations demonstrate the same pattern with the monomer conversion percentage rising as the curing time extends. At 10 seconds curing time both show a monomer conversion just above 65% and then spike to approximately 80% at 20 seconds curing. At 40 seconds curing time, they reach a monomer conversion high of 82% and 84% respectively. The formulation with the 4:1 powder liquid ratio reaches almost the same monomer conversion percentage at 40 seconds regardless of the thickness whilst the one with 3:1 powder liquid ratio performs the same at 20 seconds curing time. At 40 seconds curing time, thickness does not seem to affect the monomer conversion since the percentages at 1mm and 2mm are almost the same.

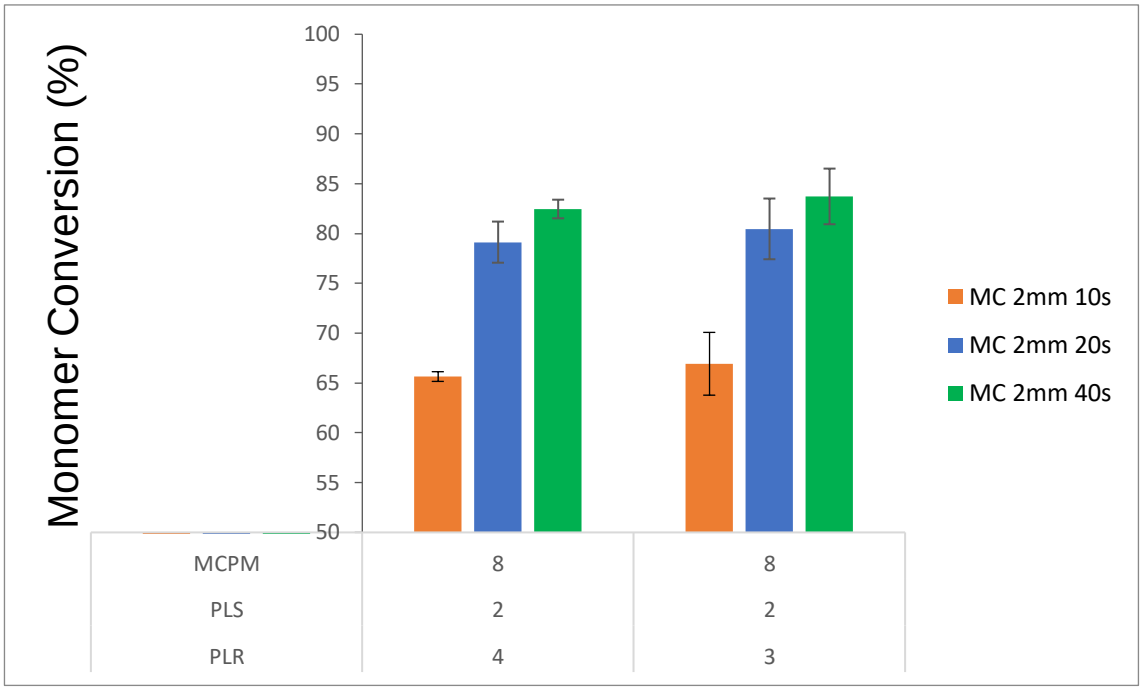

Figure 3-15 Plot of the monomer conversion of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 2mm thickness samples after curing for 10, 20 and 40 seconds. (N.B. y axis starts from 50% because that is the minimum percentage of polymerization that a material has to reach in order not to be toxic). Error bars are the standard deviation n=2).

3.1.13 Statistical Analysis

Monomer conversion, delay time, reaction rate and t0.5 were analysed with a Kruskal - Wallis test.

A pairwise comparison of all the SMART composite formulations against the control and two commercials per thickness was run to assess delay time, reaction rate and half time. Monomer conversion was examined separately for each thickness and curing time.

In Figure 3-16 for 1 mm thick samples, the delay time was significantly lower between the control and two formulations with high MCPM (F8 p=0.049, F5 p=0.021). The formulation with 4:1 powder liquid ratio compared to the 3:1 powder liquid ratio of the control demonstrated a significantly higher delay time (F2 p= 0.007). In addition, the only commercial than showed significance was Z250 that had a significantly higher delay time than the control (p= 0.047).

control-F8	-32.833	10.255	-3.202	.001	.049
control-F5	-35.278	10.255	-3.440	.001	.021
control-z250	-36.861	11.466	-3.215	.001	.047
control-F2	-38.333	10.255	-3.738	.000	.007

Figure 3-16 Table generated by SPSS when running a Kruskal-Wallis test for all tested materials at 1mm thickness for delay time. The orange column is the adjusted significance of the material pairs of the first column.

In Figure 3-17, the only pair that showed significance at 2mm thickness for delay time was Z250 with the control (p=0.019).

z250-control	31.500	9.077	3.470	.001	.019

Figure 3-17 Table generated by SPSS when running a Kruskal-Wallis test for all tested materials at 2mm thickness for delay time. The orange column is the adjusted significance of the material pairs of the first column.

In Figure 3-18 for 1mm thick samples, the reaction rate between Activa and the two SMART formulations containing high PLS (F5 p= 0.005, F6 p≤ 0.0005) was significantly lower. In addition the SMART formulation containing high MCPM and low PLS (F7) demonstrated a higher reaction than Activa (p≤ 0.0005) and the formulation containing low MCPM and low PLS (F8 p=0.003). The formulation containing low MCPM and low PLS also exhibited significantly lower reaction rate than the formulation with low MCPM and high PLS (F6 p=0.009) and the formulation containing the small sized MCPM particles (F5small p=0.022).

Activa-F5	-43.667	11.482	-3.803	.000	.005
Activa-F5 small	-48.444	11.482	-4.219	.000	.001
Activa-F6	-50.889	11.482	-4.432	.000	.000
Activa-F7	-53.944	11.482	-4.698	.000	.000
F8-F5 small	35.167	10.269	3.424	.001	.022
F8-F6	37.611	10.269	3.662	.000	.009
F8-F7	40.667	10.269	3.960	.000	.003

Figure 3-18 Table generated by SPSS when running a Kruskal-Wallis test for all tested materials at 1mm thickness for reaction rate. The orange column is the adjusted significance of the material pairs of the first column.

In Figure 3-20 for 1mm thick samples, only Activa had a significantly higher half time than the SMART formulation containing high MCPM and low PLS (F7 $p \leq 0.0005$), the control ($p=0.008$) and the formulation containing the small sized MCPM particles (F5small $p=0.021$).

F7-Activa	52.389	11.483	4.562	.000	.000
control-Activa	-42.444	11.483	-3.696	.000	.008
F5 small-Activa	39.556	11.483	3.445	.001	.021

Figure 3-20 Table generated by SPSS when running a Kruskal-Wallis test for all tested materials at 1mm thickness for half time. The orange column is the adjusted significance of the material pairs of the first column.

In Figure 3-21 Activa demonstrated a significantly higher half time than Z250 at 2mm ($p=0.002$).

z250-Activa	36.750	9.081	4.047	.000	.002

Figure 3-21 Table generated by SPSS when running a Kruskal-Wallis test for all tested materials at 2mm thickness for half time. The orange column is the adjusted significance of the material pairs of the first column.

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of MC is the same across categories of group.	Independent-Samples Kruskal-Wallis Test	.007	Reject the null hypothesis.

10s

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of MC is the same across categories of group.	Independent-Samples Kruskal-Wallis Test	.005	Reject the null hypothesis.

20s

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of MC is the same across categories of group.	Independent-Samples Kruskal-Wallis Test	.006	Reject the null hypothesis.

40s

Figure 3-22 Table generated by SPSS when running a Kruskal-Wallis test for all tested materials at 1mm thickness at each curing time for monomer conversion. The significance is noted in the relevant column but there was no adjusted significance.

For monomer conversion there was significance (see Figure 3-22) noted when comparing all materials at 1mm thickness for 10, 20 and 40 seconds curing time but there was no adjusted significance and therefore no conclusions can be drawn.

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of MC is the same across categories of group.	Independent-Samples Kruskal-Wallis Test	.095	Retain the null hypothesis.

10s

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of MC is the same across categories of group.	Independent-Samples Kruskal-Wallis Test	.378	Retain the null hypothesis.

20s

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of MC is the same across categories of group.	Independent-Samples Kruskal-Wallis Test	.222	Retain the null hypothesis.

40s

Figure 3-23 Table generated by SPSS when running a Kruskal-Wallis test for all tested materials at 2mm thickness at each curing time for monomer conversion. The significance is noted in the relevant column but there was no adjusted significance.

In Figure 3-23 it is evident that at 2mm thickness regardless of curing time there is no significant differences noted amongst all tested materials.

Due to the results generated by statistics showing no significance, a decision was made to not proceed further with factorial analysis.

3.1.14 Calculated Polymerization Shrinkage (φ) and heat (J/cc)

Calculated polymerization shrinkage and heat are proportional to monomer conversion and therefore the plots below follow identical trends as the ones noted in the equivalent monomer conversion plots.
The mean monomer conversion of each different curing time (10, 20 and 40 seconds) was used in order to create the plots below.

3.1.15 Calculated Polymerization Shrinkage (φ) and heat (J/cc) of four formulations with high and low MCPM and PLS

All four SMART composite formulations show the same pattern of a rising 0.5% percentage of calculated shrinkage as the curing time is extended (Figure 3-24). As expected, the higher polymerization achieved at 40 seconds curing time is also responsible for higher shrinkage. In all formulations though it does not exceed 4%.

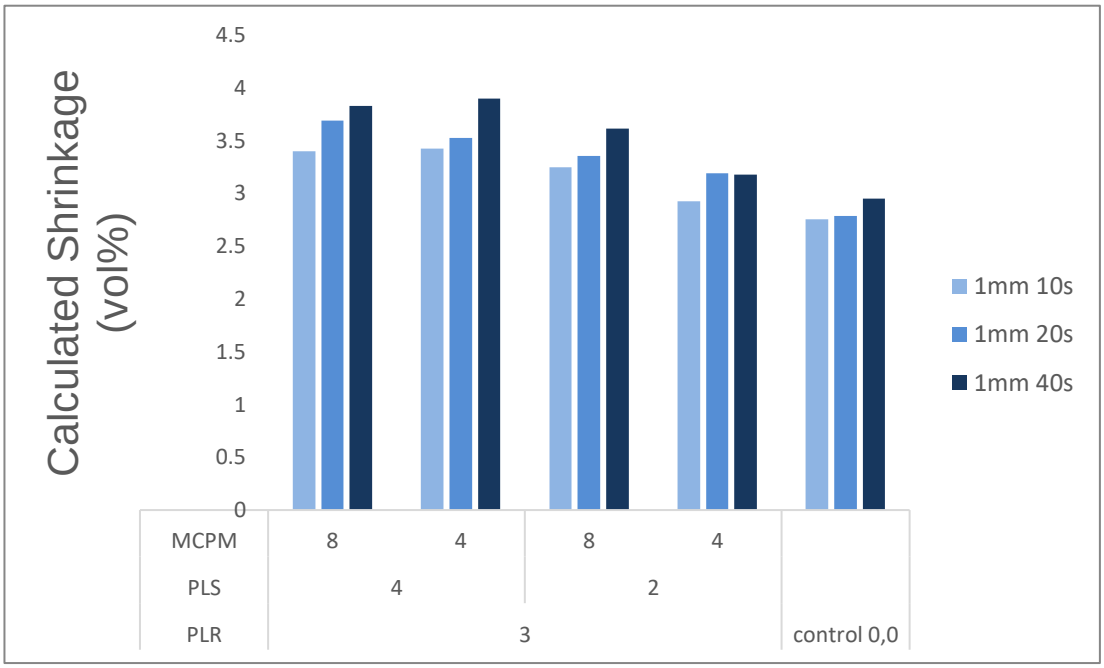

Figure 3-24 Plot of the calculated shrinkage of four SMART composite formulations and the control at 1mm thickness samples after curing for 10, 20 and 40 seconds.

All four SMART composite formulations show the same pattern of a rising 15J/cc of heat as the curing time is extended. As expected, the higher polymerization achieved at 40 seconds curing time generates the most heat since it is an exothermic reaction and it should be taken into account when there is pulp proximity of the restoration (see Figure 3-25).

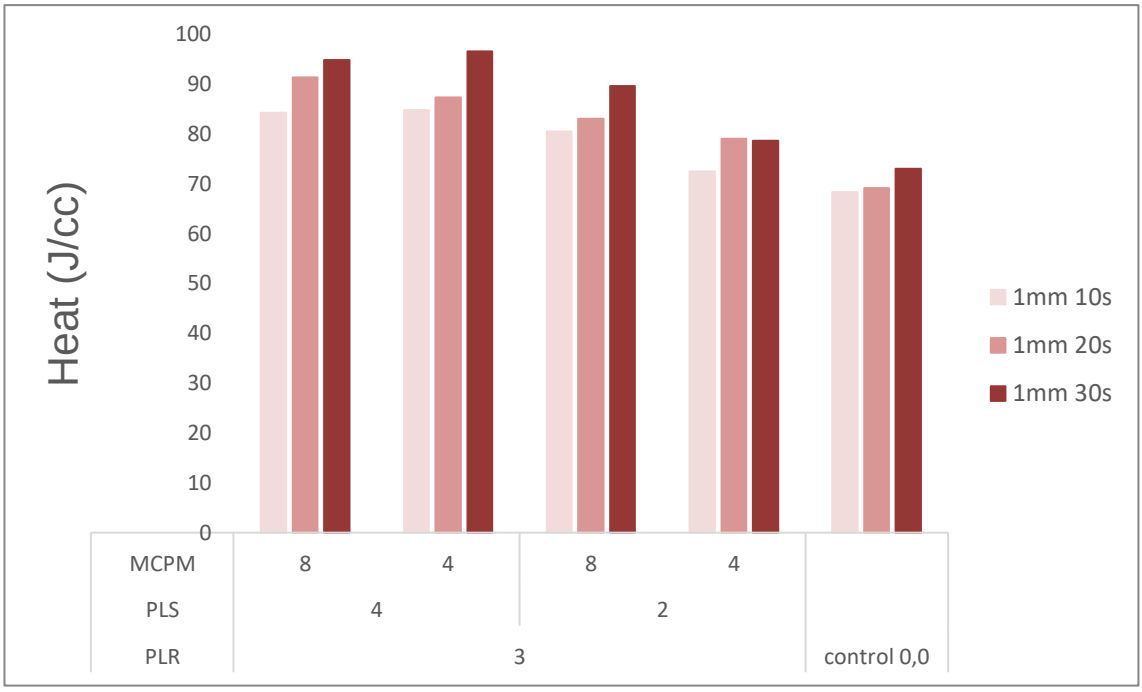

Figure 3-25 Plot of the heat generated from the polymerization reaction of four SMART composite formulations and the control at 1mm thickness samples after curing for 10, 20 and 40 seconds.

As described earlier, the higher calculated shrinkage, ranging from 3.5% to 4% is noted at 40 seconds curing time and it is approximately the same in 1mm and 2 mm thickness samples (see Figure 3-26).

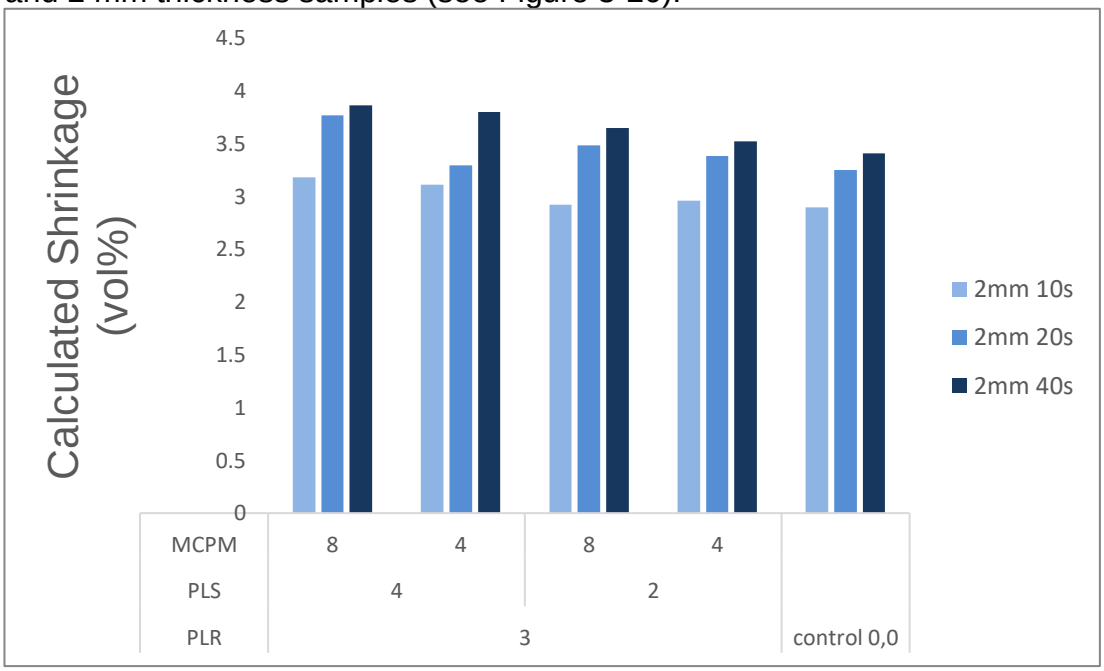

Figure 3-26 Plot of the calculated shrinkage of four SMART composite formulations and the control at 2mm thickness samples after curing for 10, 20 and 40 seconds.

Not surprisingly, heat generation in Figure 3-27 is greater at 40 seconds curing time with the difference between 10 seconds and 40 seconds curing time of 2mm thickness samples, rising to more than approximately 20% instead of 15% at 1mm thickness samples.

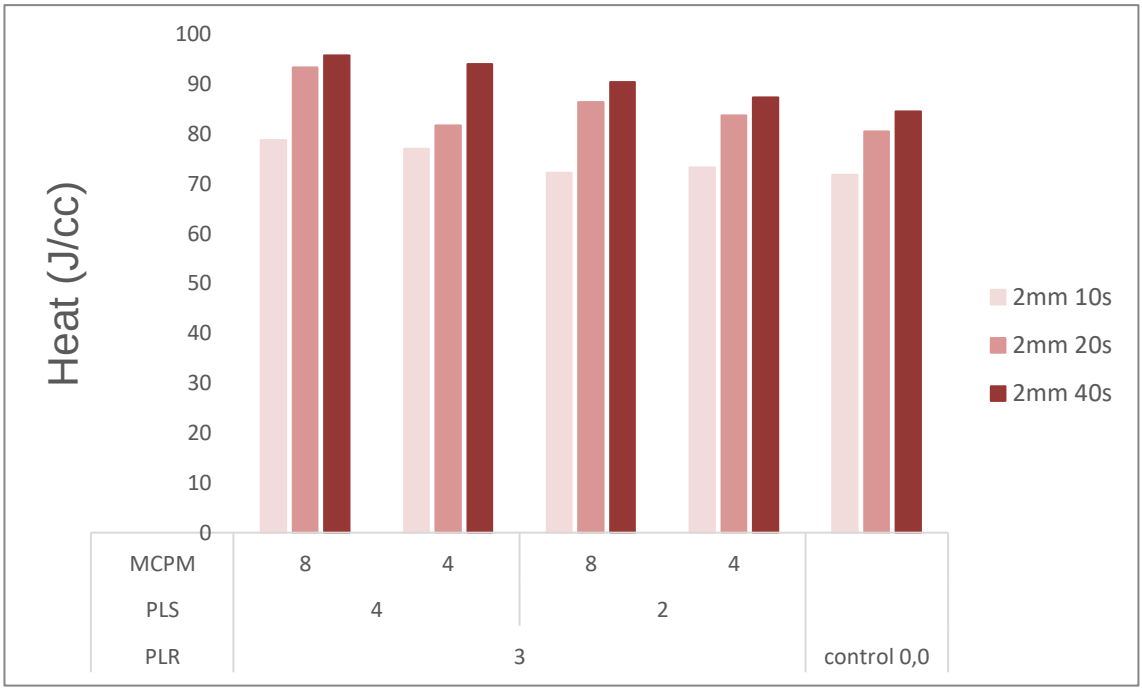

Figure 3-27 Plot of the heat generated from the polymerization reaction of four SMART composite formulations and the control at 2mm thickness samples after curing for 10, 20 and 40 seconds.

3.1.16 Calculated Polymerization Shrinkage (φ) and heat (J/cc) of two formulations with different MCPM particle size (50μm versus 10μm)

In Figure 3-28 it is clear that the formulation with the conventional sized MCPM particles performs in the expected pattern of higher calculated shrinkage at longer curing times. On the contrary, the formulation with the small sized MCPM particles has a peak of shrinkage at 20 seconds curing time and then at 40 seconds curing time, returns to the same 3.1% of calculated shrinkage it had when cured for 10 seconds.

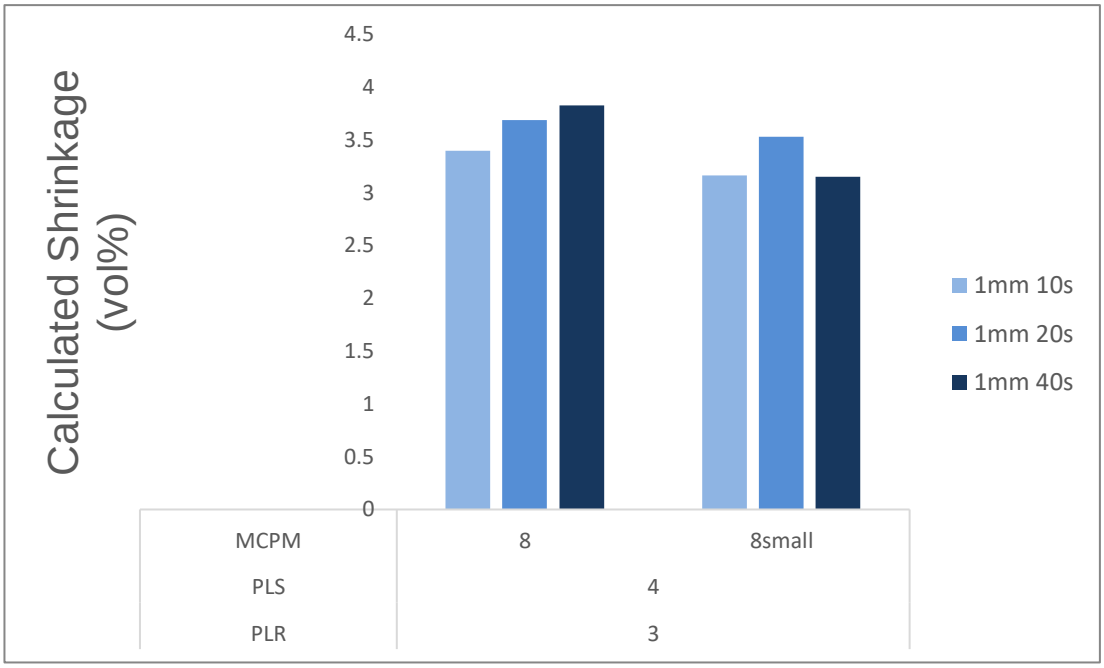

Figure 3-28 Plot of the calculated shrinkage of two SMART composite formulations with conventional (50μm) and small (10μm) sized MCPM particles respectively, at 1mm thickness samples after curing for 10, 20 and 40 seconds.

In Figure 3-29, due to the mathematical equation used to calculate heat generation, inevitably heat generation follows an identical to the calculated shrinkage manner. Both formulations generate heat ranging from almost 80J/cc to 95J/cc.

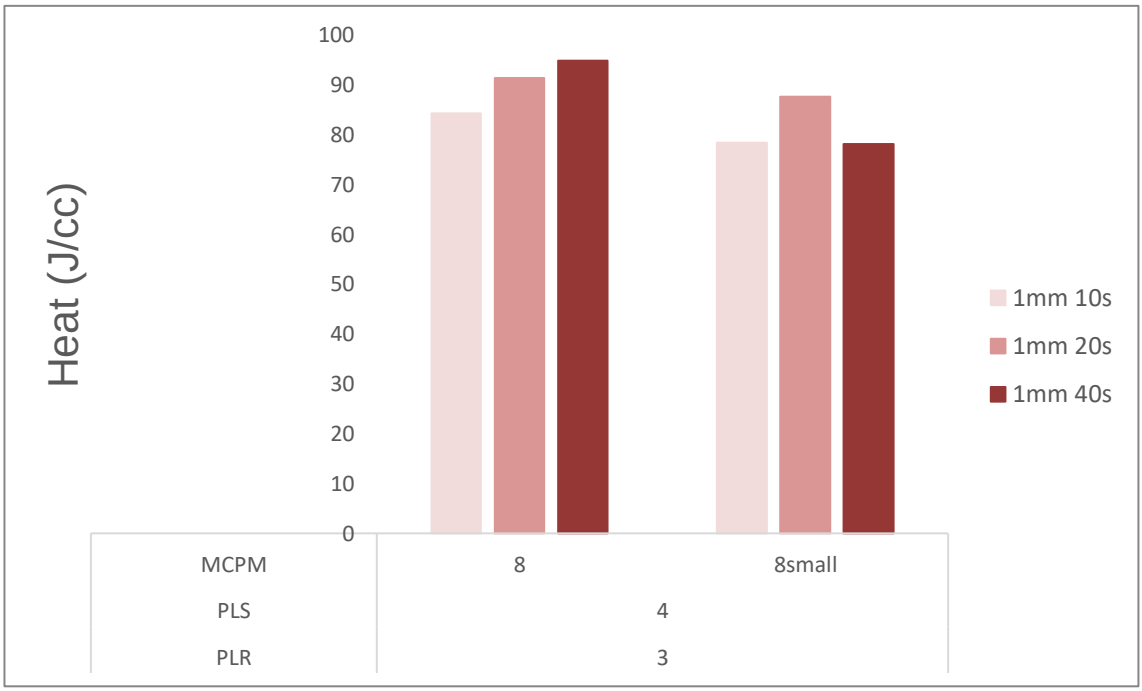

Figure 3-29 Plot of the heat generated from the polymerization reaction of two SMART composite formulations with conventional (50μm) and small (10μm) sized MCPM particles respectively, at 1mm thickness samples after curing for 10, 20 and 40 seconds.

Figure 3-30 shows the calculated shrinkage at 2mm thick samples. There are no surprises since greater calculated shrinkage is notes at 40 seconds curing

time for both formulations. Compared to 1mm thick samples, the formulation with the conventional sized MCPM particles demonstrates a 0.2% drop in calculated shrinkage at 10 seconds curing time.

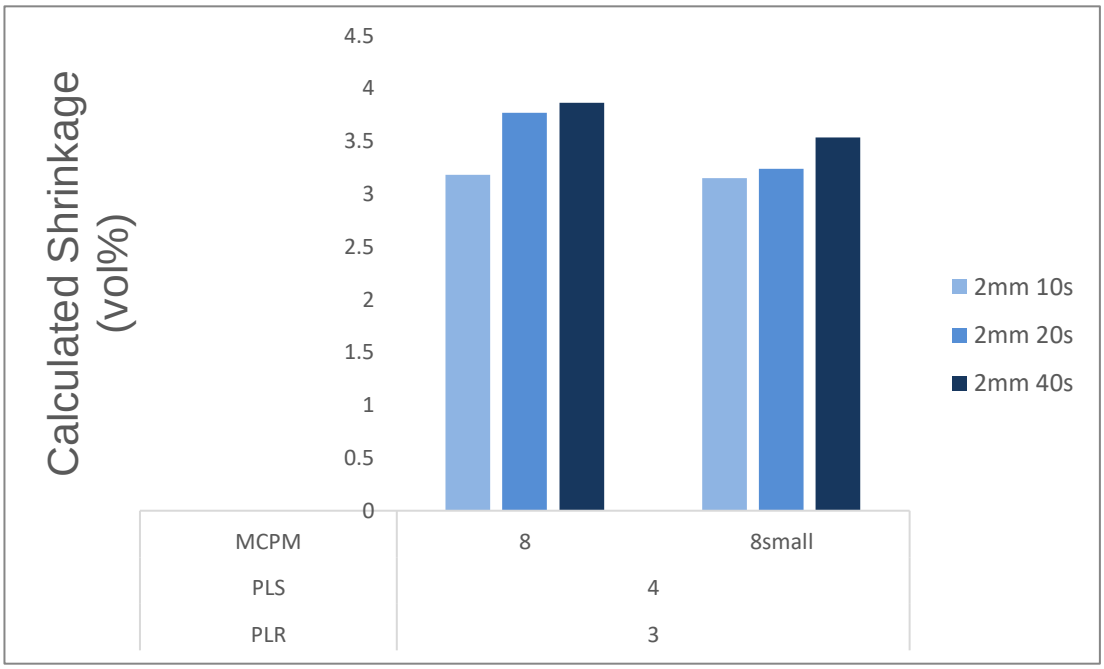

Figure 3-30 Plot of the calculated shrinkage of two SMART composite formulations with conventional (50µm) and small (10µm) sized MCPM particles respectively, at 2mm thickness samples after curing for 10, 20 and 40 seconds.

Predictably, the heat generation in Figure 3-31 is equivalent to the calculated shrinkage one described previously With the formulation containing the conventional sized MCPM particles generating more heat at 20 and 40 seconds curing time compared to the one with small sized MCPM particles.

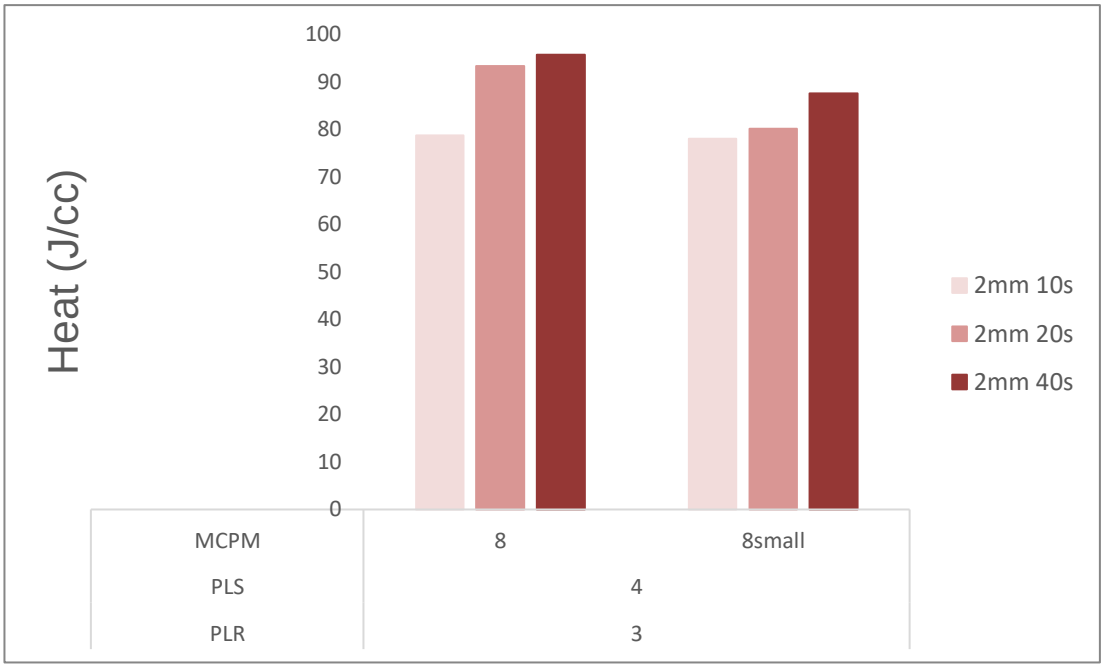

Figure 3-31 Plot of the heat generated from the polymerization reaction of two SMART composite formulations with conventional (50μm) and small (10μm) sized MCPM particles respectively, at 2mm thickness samples after curing for 10, 20 and 40 seconds.

3.1.17 Calculated Polymerization Shrinkage (φ) and heat (J/cc) of two formulations with different powder liquid ratio

In Figure 3-32, higher powder liquid ratio seems to lowers the percentage of calculated shrinkage at 10 and 20 seconds of curing time whereas at 40 seconds curing time, the bars of the two formulations are almost equal at 3.5%.

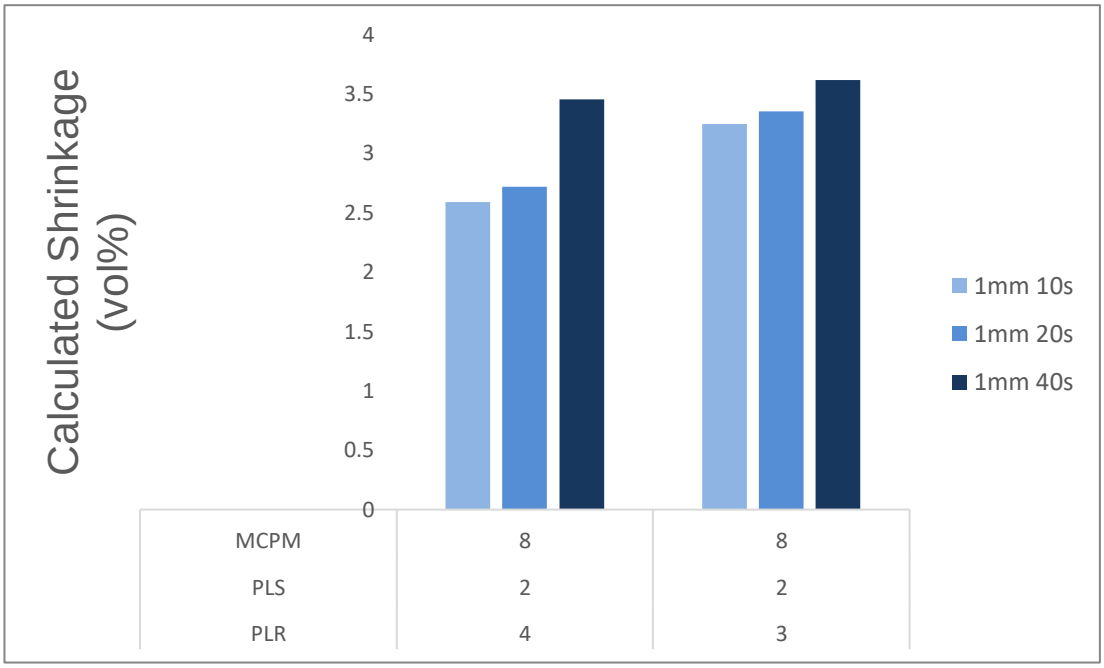

Figure 3-32 Plot of the calculated shrinkage of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 1mm thickness samples after curing for 10, 20 and 40 seconds.

In Figure 3-33 the formulation with the higher powder liquid ratio generates less heat at 10 and 20 seconds curing time and only shows a 5J/cc discrepancy at 40 seconds curing time with the other formulation.

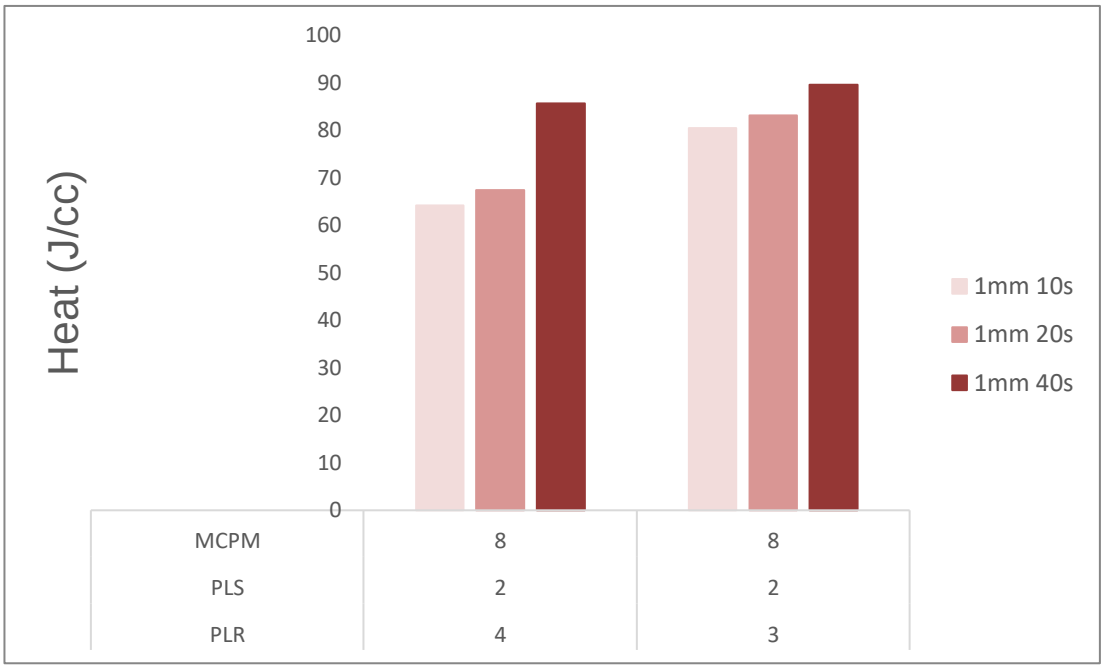

Figure 3-33 Plot of the heat generated from the polymerization reaction of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 1mm thickness samples after curing for 10, 20 and 40 seconds.

At 2mm thickness samples, as seen in Figure 3-34 the formulation with the 4:1 powder liquid ratio seems to always have 0.2% less calculated shrinkage at 10, 20 and 40 seconds of curing time compared to the one with 3:1 powder liquid ratio. As at 1mm thick samples, the formulation with 4:1 powder liquid ratio seems to shrink less compared to the other one.

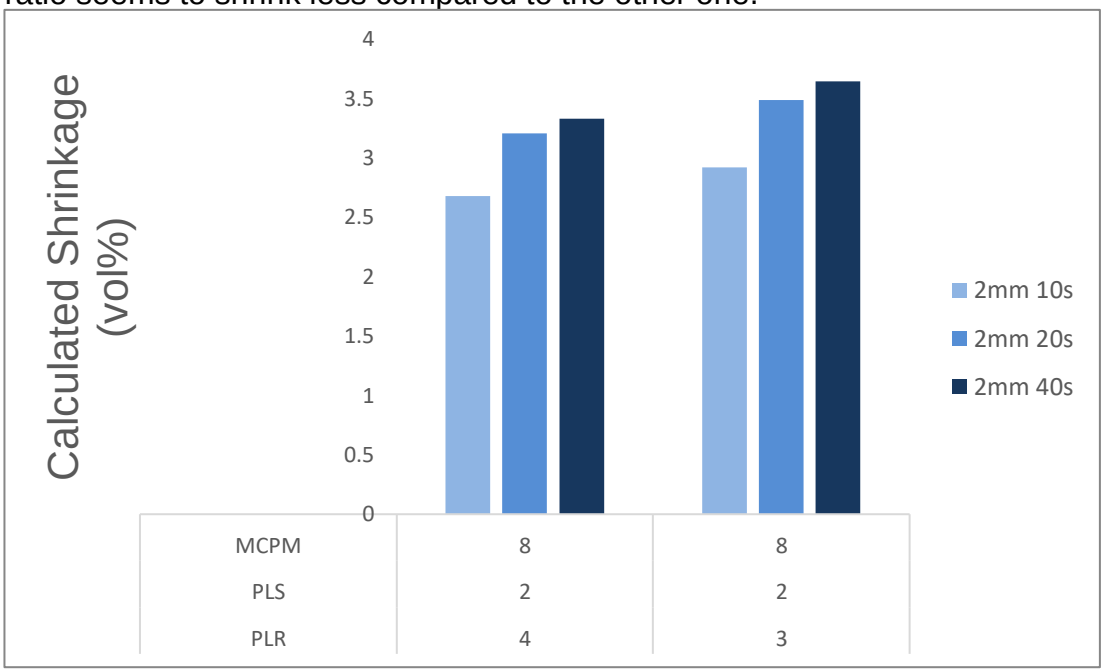

Figure 3-34 Plot of the calculated shrinkage of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 2mm thickness samples after curing for 10, 20 and 40 seconds.

Not surprisingly in Figure 3-35, at 2mm thickness the highest generation of heat of 82.5J/cc and 90.4J/cc respectively for the formulations with 4:1 and 3:1 powder liquid ratio is noted at 40 seconds curing time. Generally the

formulation with the 3:1 powder liquid ratio generates slightly more jouls/cc of heat than the other formulation in all three curing times.

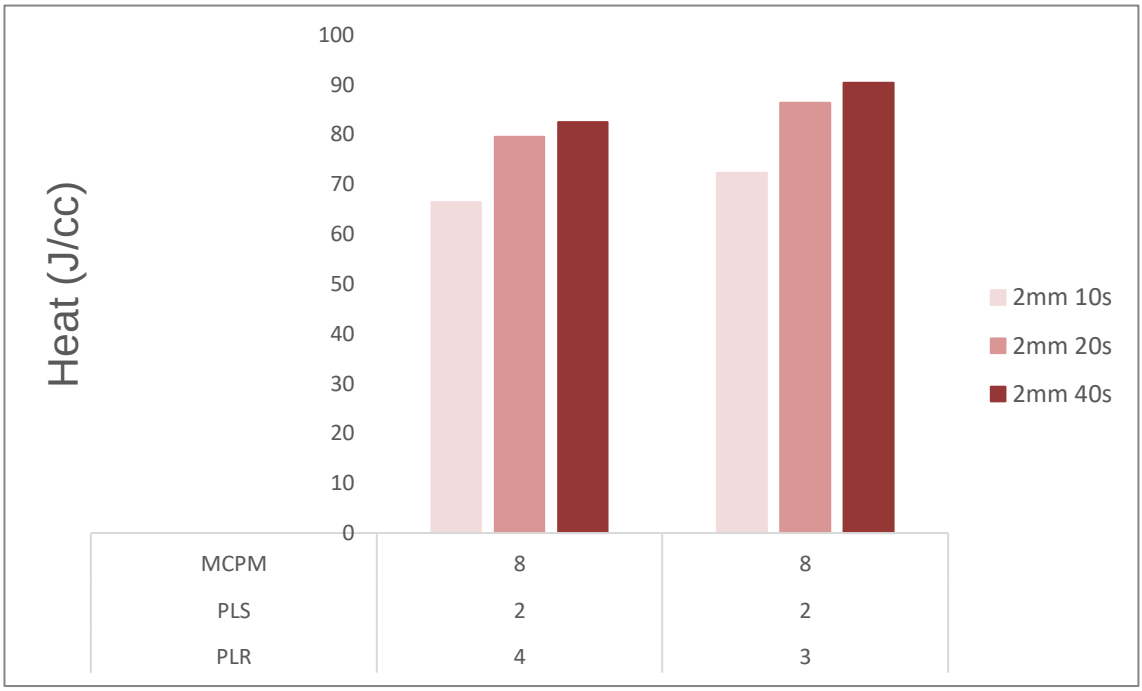

Figure 3-35 Plot of the heat generated from the polymerization reaction of two SMART composite formulations of 4:1 and 3:1 powder liquid ratios respectively, at 2mm thickness samples after curing for 10, 20 and 40 seconds.

3.2 Mass and Volume change

The graphs below occurred following the plotting of the weight of 3 discs per formulation in dry and wet conditions in a period of 5 weeks. Following each mass and volume plot is an average mass and volume plot of the last four timepoints in order to increase confidence in the results following the overshoot noted in all materials. An overshoot is when the material absorbs water which results to mass and volume expansion and then releases components into the liquid it is immersed which causes a decrease in mass and volume.

3.2.1 Mass and Volume change of four formulations with high and low MCPM and PLS

Figure 3-36 shows the percentage of mass change versus the square root of time in hours according to the mathematical equation previously described in the materials and methods chapter. The same colour markers represent the two formulations with the same PLS percentage high or low and the filled and unfilled markers represent the two formulations with the same MCPM percentage high or low. It is noted that all four formulations do not exceed 1.5% of mass change. Following that spike at 6 hours, all four tend to reach a plateau of approximately 1% mass change at 48 hours which is maintained till the end of the 5 weeks. Formulation F5 which contains high MCPM and high PLS and is the final formulation, had the most gradual mass change compared to the other three reaching its maximum 1.1% at 48 hours. At the last two timepoints in the plateau phase, it is clear that F7 and F5 which both contain high MCPM have a lower mass change in comparison to F6 and F8 which contain low MCPM.

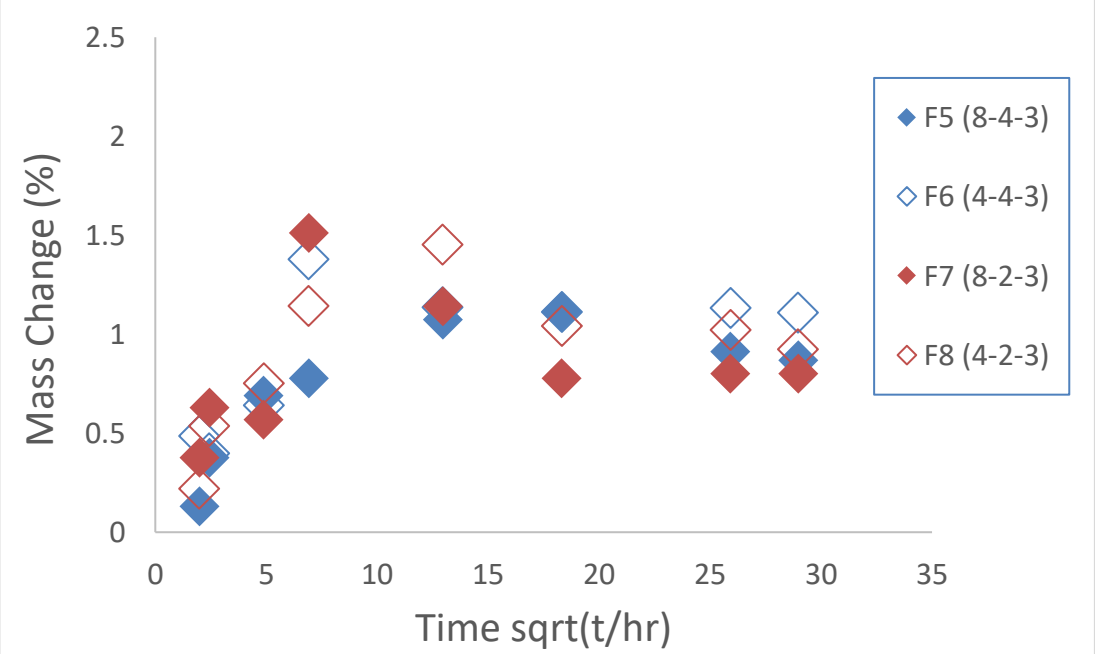

Figure 3-36 Plot showing the percentage of Mass change versus the square root of time for the four formulations with high and low MCPM and PLS.

Each bar on the graph in Figure 3-37 represents the percentage of mass change of the average mass change of the last four timepoints of four SMART composite formulations, a control and two commercial materials (Activa and

Z250). Activa and Z250 have a greater mass change of 2.2% and 1.8% respectively compared to all the SMART composite formulations whose mass change percentage varies from 0.88% to 1.12%. From the SMART composite formulations, the control which has no MCPM and no PLS has the smallest percentage of mass change of 0.58%. Both formulations containing low MCPM (4%) have a bigger mass change percentage than the one's with high MCPM (8%).

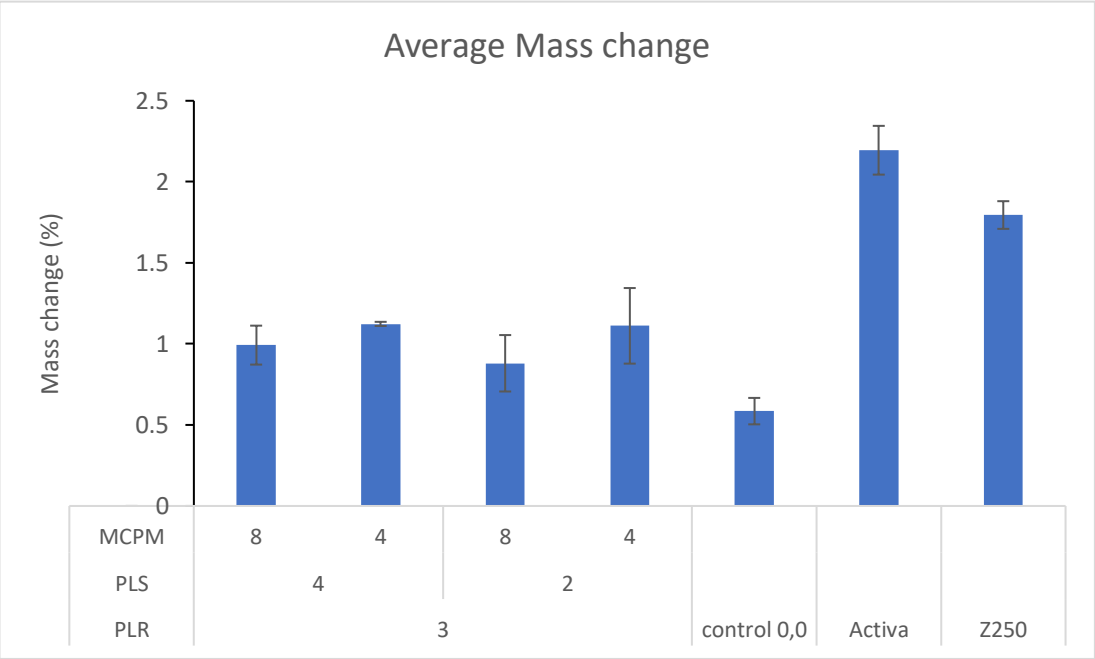

Figure 3-37 Plot showing the percentage of the average mass change of the last four timepoints for the four formulations with high and low MCPM and PLS, a control and two commercial materials. Error barss are the standard deviation of the last four timepoints.

Figure 3-38 shows the percentage of volume change. The same colour and shape code that was described before applies. Unlike the mass change plot, there are no evident general patterns that the formulations follow. F5 with high MCPM and high PLS percentage seems to always have a greater volumetric change than F8 which has low MCPM and low PLS except from the timepoint of 6 hours. F6 which contains high PLS and F8 which contains low PLS seem to be alternating between them in the different timepoints. F5 reaches the highest volumetric change of 3% out of all four formulations in week 4. And then seems to drop to 2% in week 5.

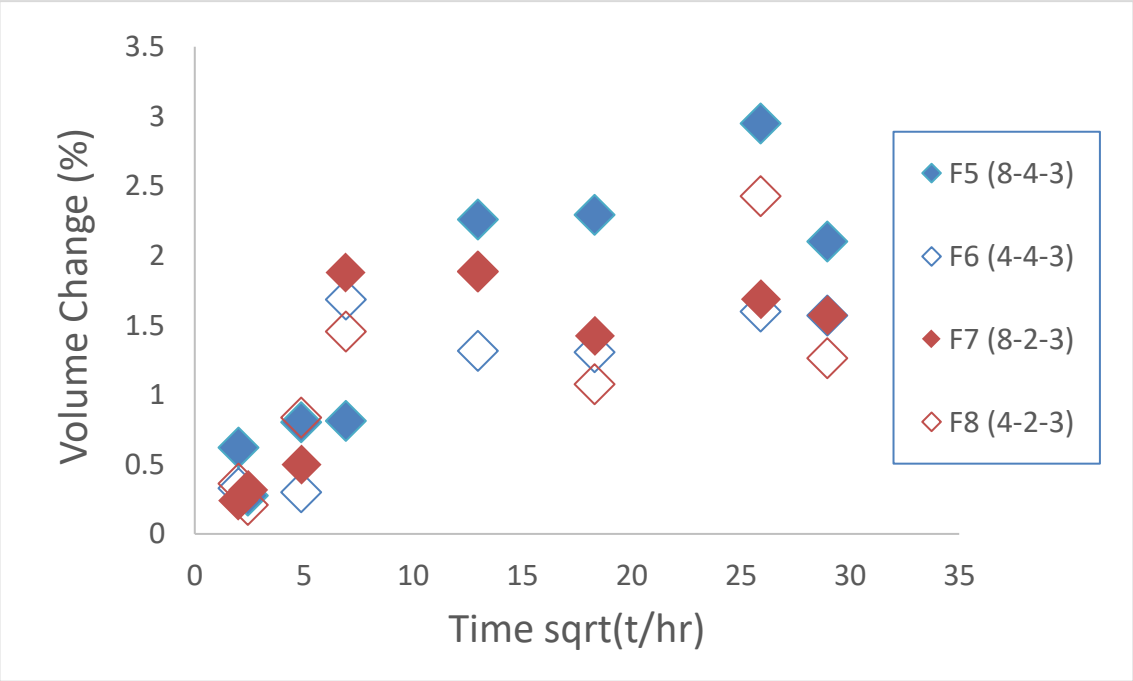

Figure 3-38 Plot showing the percentage of Volume change versus the square root of time for the four formulations with high and low MCPM and PLS.

Each bar in Figure 3-39 represents the percentage of volume change of the average volume change of the last four timepoints of four SMART composite formulations, a control and two commercial materials (Activa and Z250). The error bars compared to the average mass change graph described earlier are larger.

The control which contains no MCPM or PLS seems to almost have no volume change. Activa and Z250 each have a 1.36% and 1.64% of volume change which is lower than all four SMART composite formulations. The one containing high MCPM and high PLS has the largest volume change percentage of 2.4% and is followed by the formulation containing low MCPM and low PLS with a volume change of 1.7%. The other two formulations are range between the two with 1.45% and 1.64% each. No evidence of a pattern is visible.

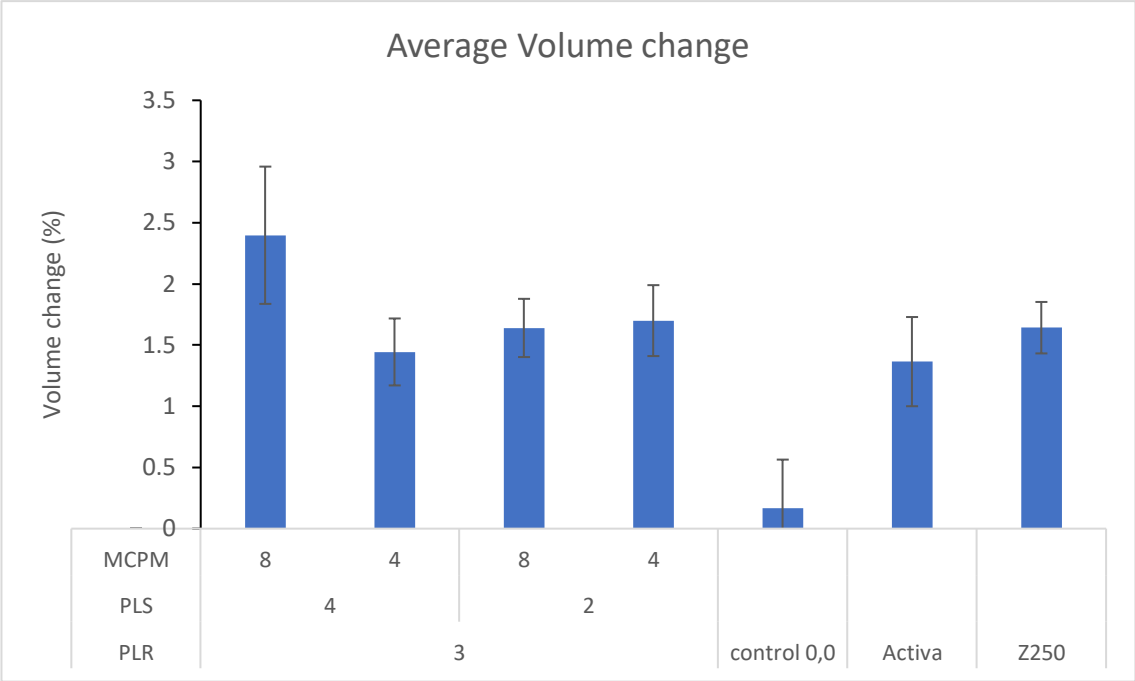

Figure 3-39 Plot showing the percentage of the average volume change of the last four timepoints for the four formulations with high and low MCPM and PLS, a control and two commercial materials. Error bars are the standard deviation of the last four timepoints.

3.2.2 Mass and Volume change of two formulations with different MCPM particle size

Figure 3-40 shows the percentage of mass change versus the square root of time in hours according to the mathematical equation previously described in the materials and methods chapter in the two formulations that have the same percentage of MCPM and PLS but differ in the size of MCPM particles. The smaller marker represents the formulation with the smaller sized MCPM particles. Both F5 and F5 small seem to have a similar increase in mass change up to 6 hours into the experiment and then they demonstrate deviating courses. F5 reaches a maximum of 1.1% mass change and then drops to almost 1% while F5 small keeps noting an increasing mass change with a maximum of 2.25% at week 5. F5 small displays almost double the mass change percentage in comparison to F5 in week 5 where the experiment ends.

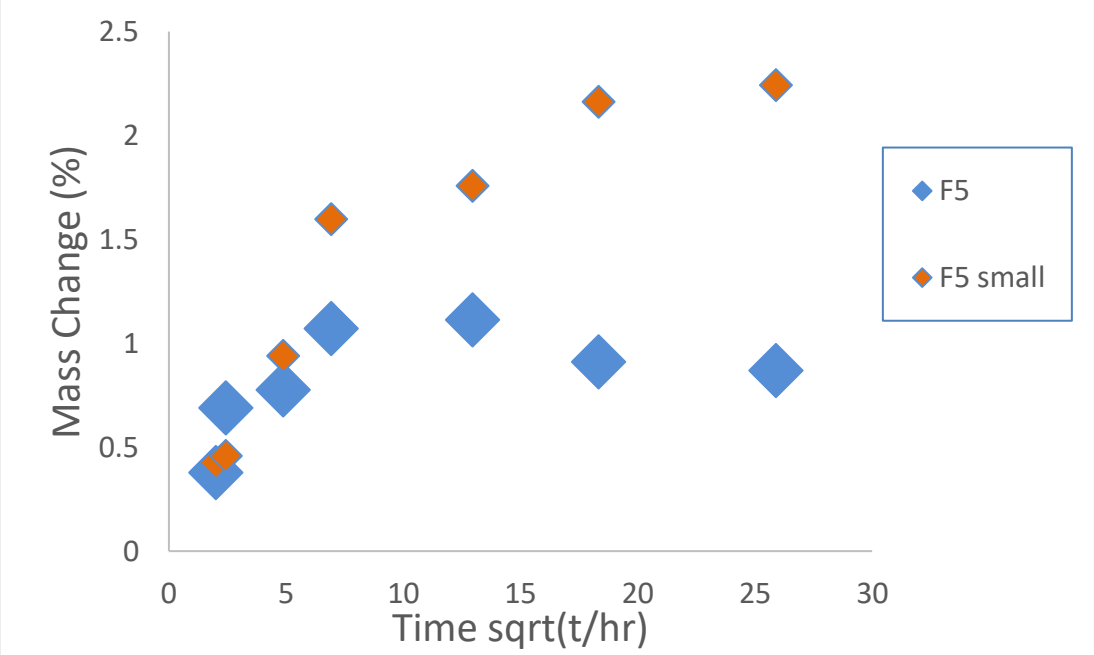

Figure 3-40 Plot showing the percentage of Mass change versus the square root of time for the two formulations with normal and small MCPM particles.

Each bar in Figure 3-41 represents the percentage of mass change of the average mass change of the last four timepoints versus the square root of time for the two formulations with normal and small MCPM particles.
The orange bar represents F5 small which contains the smaller sized MCPM particles. It is evident that F5 small has double the percentage of the average mass change (almost 2%) compared to F5 (1%).

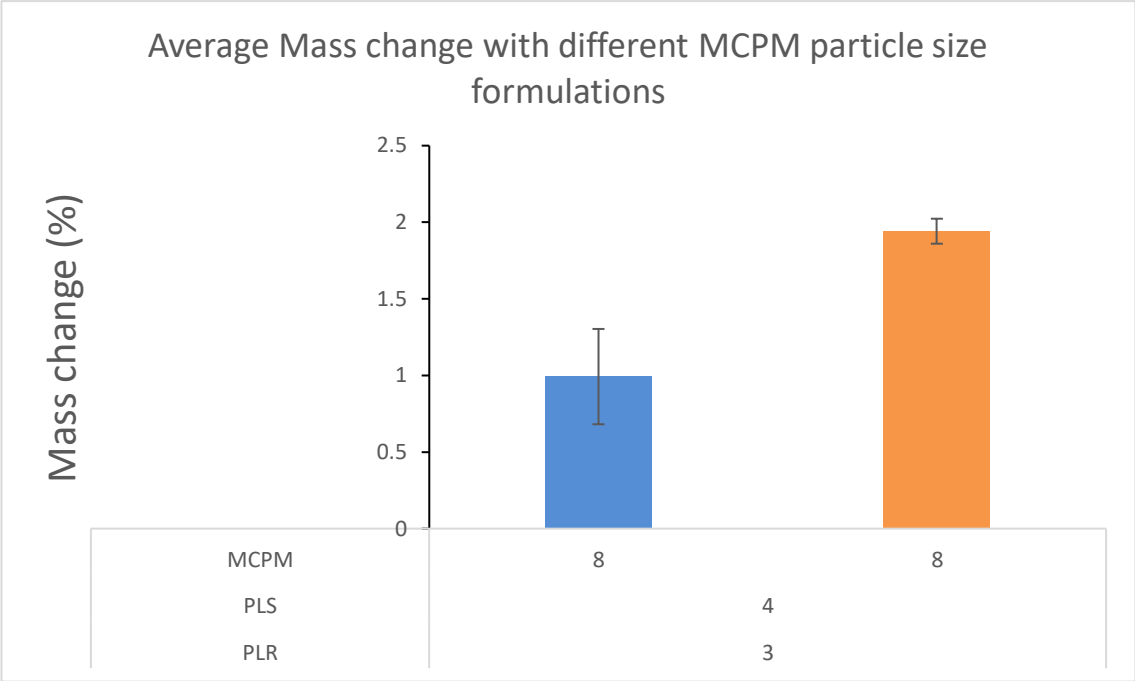

Figure 3-41 Plot showing the percentage of the average mass change of the last four timepoints for the two formulations with conventional (50μm) and small (10μm) sized MCPM particles. Error are the standard deviation of the last four timepoints.

Figure 3-42 is showing the percentage of volume change versus the square root of time in hours according to the mathematical equation previously described in the materials and methods chapter in the two formulations that have the same percentage of MCPM and PLS but differ in the size of MCPM particles. The same marker code as previously described applies here. As seen and in the mass change graph, F5 and F5 small demonstrate a similar small increase of 1% in volume change up until 6 hours. Following that timepoint and up until week 5, they both increase by marking parallel courses with a stable difference of 1% in volumetric change with F5 small being higher. F5 reaches a maximum volumetric change of 3% and F5 small of 4.3% in week 5.

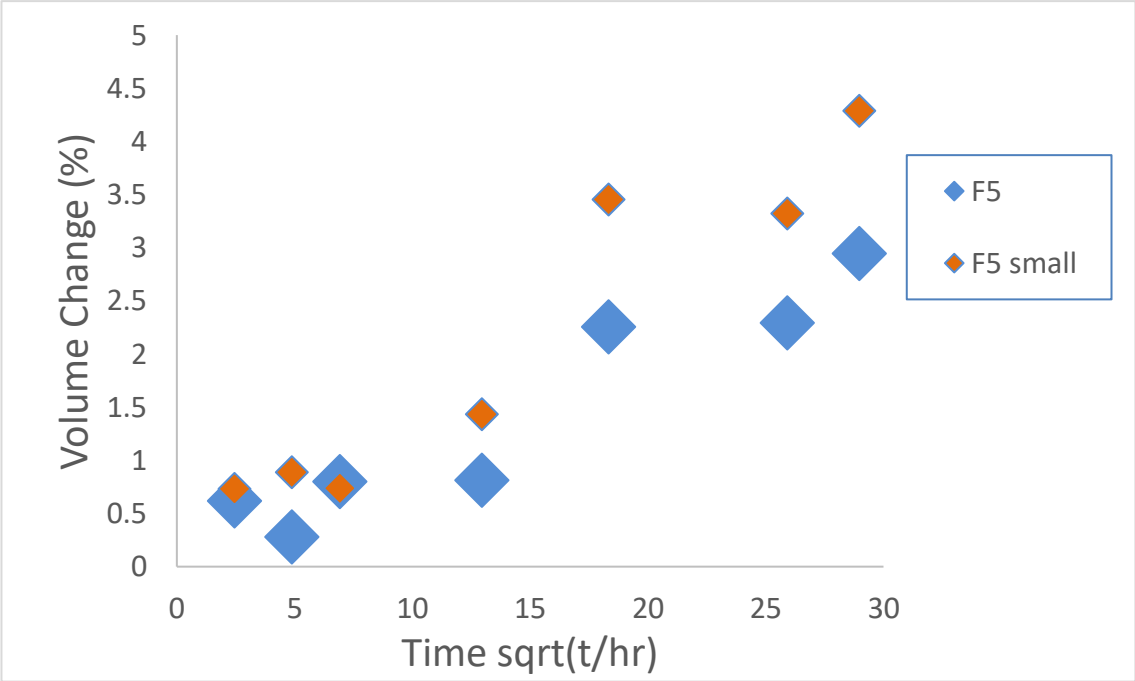

Figure 3-42 Plot showing the percentage of Volume change versus the square root of time for the two formulations with conventional (50µm) and small (10µm) sized MCPM particles.

Each bar in Figure 3-43 represents the percentage of volume change of the average volume change of the last four timepoints versus the square root of time for the two formulations with normal and small MCPM particles.
As previously mentioned, the orange bar represents F5 small which contains the smaller sized MCPM particles. F5 small has a 64% greater average volume change compared to F5 with a percentage of 3,8% volume change compared to 2.4% . This pattern is similar to the average mass change pattern seen in the previous graph.

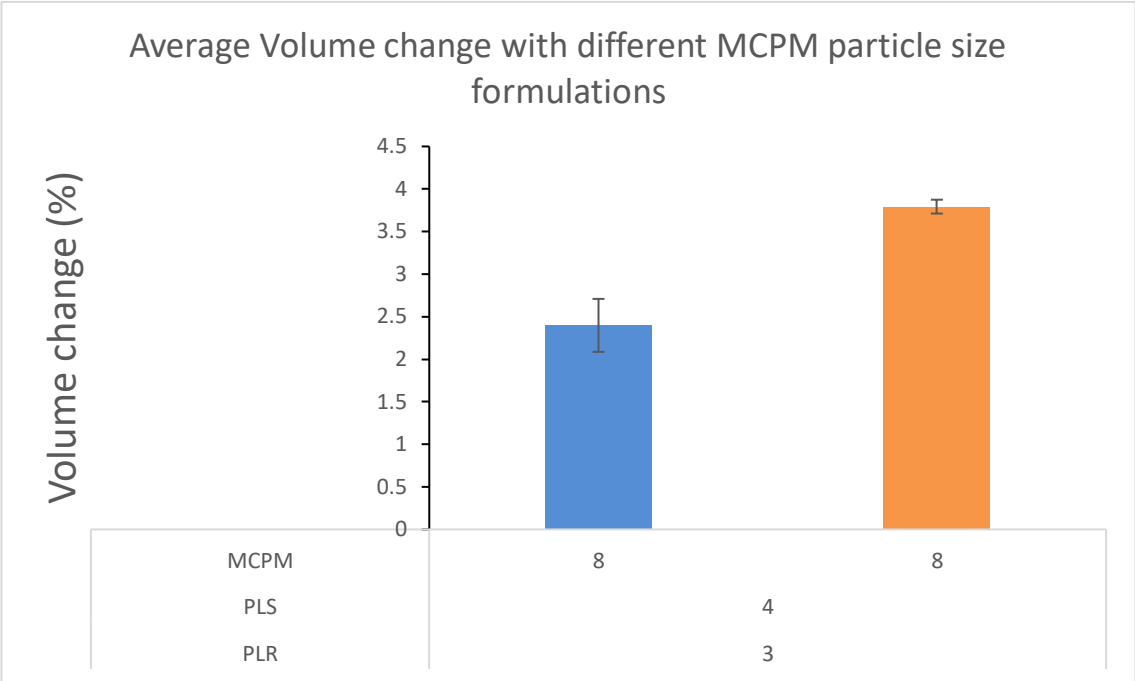

Figure 3-43 Plot showing the percentage of the average volume change of the last four timepoints for the two formulations with conventional (50µm) and small (10µm) sized MCPM particles. Error bas are the standard deviation of the last four timepoints.

3.2.3 Mass and Volume change of two formulations with different powder liquid ratio

Figure 3-44 is showing the percentage of mass change versus the square root of time in hours according to the mathematical equation previously described in the materials and methods chapter in the two formulations that have the same percentage of MCPM and PLS but differ in the powder liquid ratio. There is no evident pattern present. Three to one (3:1) powder liquid ratio seems to have a more expected trajectory as previous formulations resulting in a plateau of 0.8% mass change in week 5. Four to one (4:1) powder liquid ratio has an irregular pattern ranging from a maximum of 1.2% in week 2, drops to 0.6% in week 4 and rises again to 0.8% in week 5 where this experiment ended.

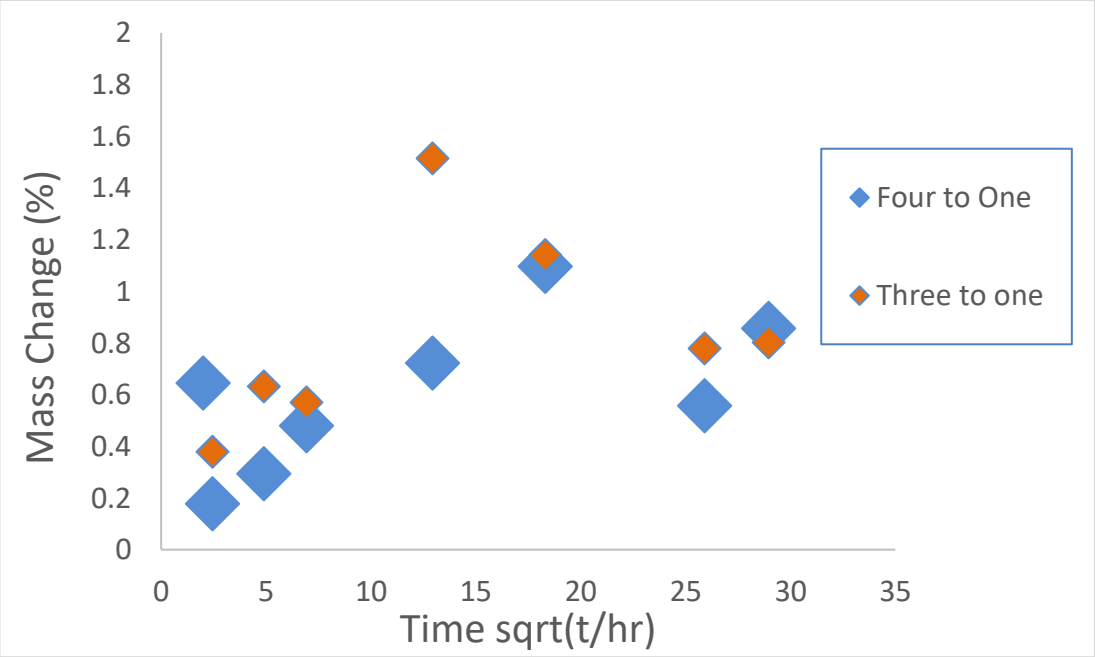

Figure 3-44 Plot showing the percentage of Mass change versus the square root of time for the two formulations with 4:1 and 3:1 powder liquid ratio.

Each bar in Figure 3-45 represents the percentage of mass change of the average mass change of the last four timepoints versus the square root of time for the two formulations with 4:1 and 3:1 powder liquid ratio. The lower powder liquid ratio (3:1) presents a higher average mass change of 0.88% compared to the 0.75% of the higher powder liquid ratio (4:1).

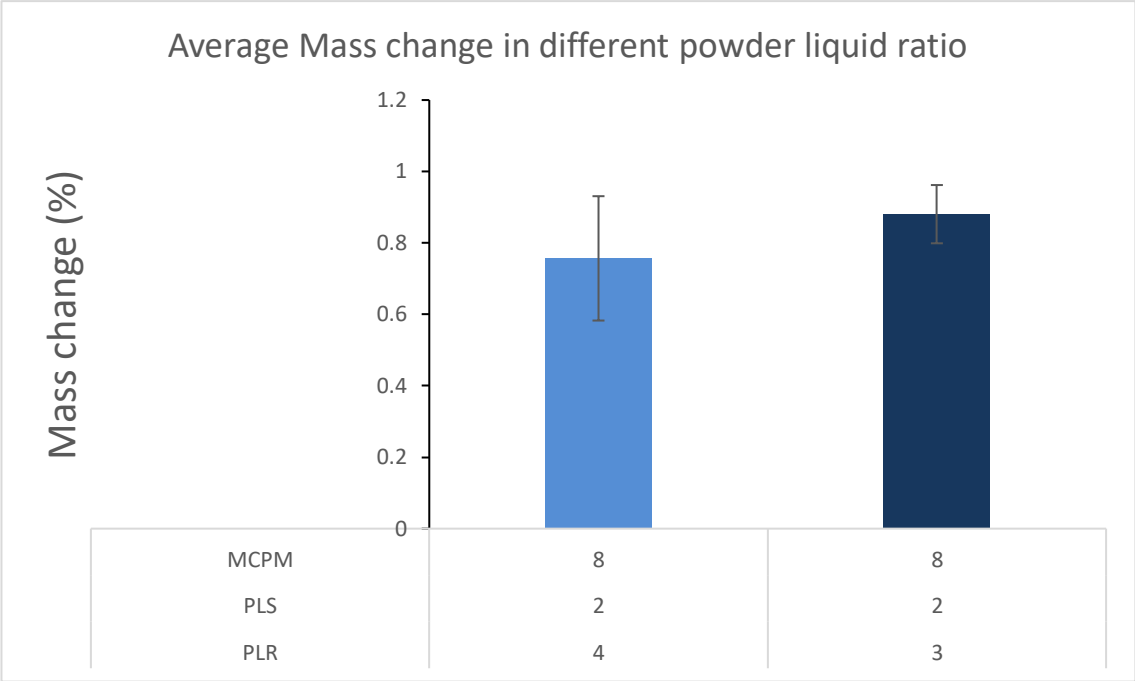

Figure 3-45 Plot showing the percentage of the average mass change of the last four timepoints for the two formulations with 4:1 and 3:1 powder liquid ratio. Error bars are the standard deviation of the last four timepoints.

Figure 3-46 is showing the percentage of volume change versus the square root of time in hours according to the mathematical equation previously described in the materials and methods chapter in the two formulations that have the same percentage of MCPM and PLS but differ in the powder liquid ratio. As in the mass change graph, there is no evident pattern present in this volume change graph. Except in week 1, in all other time points the formulations with the 4:1 ratio demonstrates a higher volumetric change with a maximum of 3.3% volume change in week 5. The formulation with the 3:1 ratio after week 1 presents consistently half the volume change of the 4:1 ratio with a final reading of 1.7% volume change in week 5. This value could be considered a plateau for the 4:1 formulation after week 1.

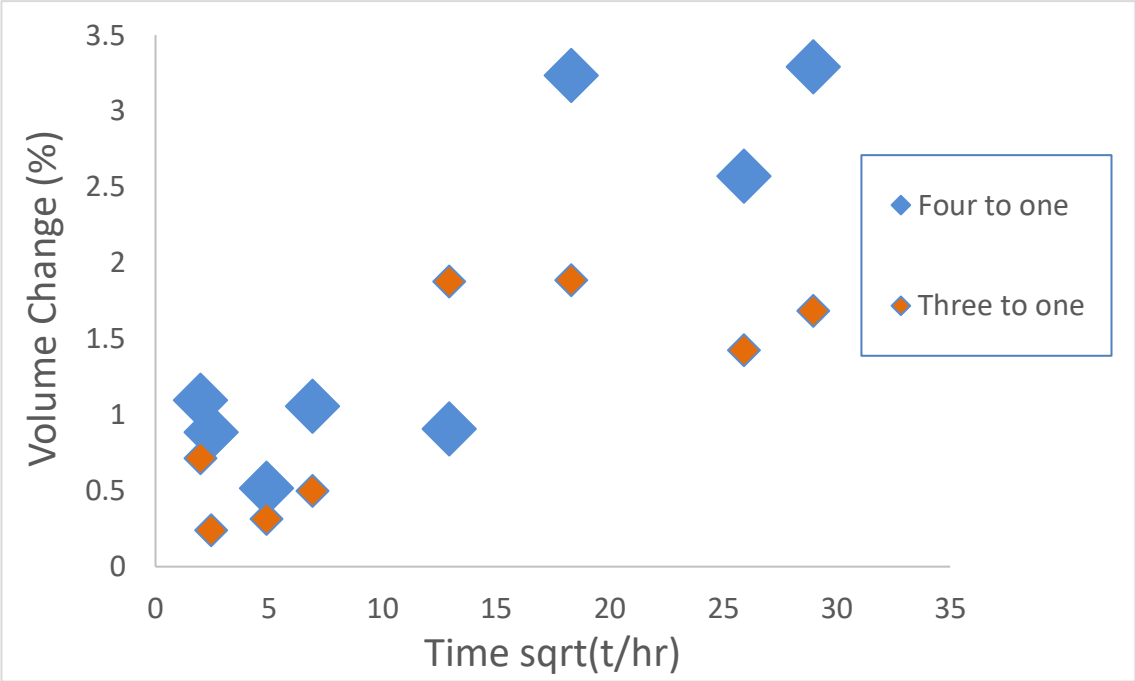

Figure 3-46 Plot showing the percentage of Volume change versus the square root of time for the two formulations with 4:1 and 3:1 powder liquid ratio.

Each bar in Figure 3-47 represents the percentage of volume change of the average volume change of the last four timepoints versus the square root of time for the two formulations with 4:1 and 3:1 powder liquid ratio. Unlike the average mass change where the formula with the 3:1 powder liquid ratio had a higher mass change than the formula with the 4:1 powder liquid ratio, when it comes to volume change the opposite is noticeable. The formula with the 4:1 powder liquid ratio has an average volume change of 2.8% which is higher than the 1.63% of the formula with the 3:1 powder liquid ratio.

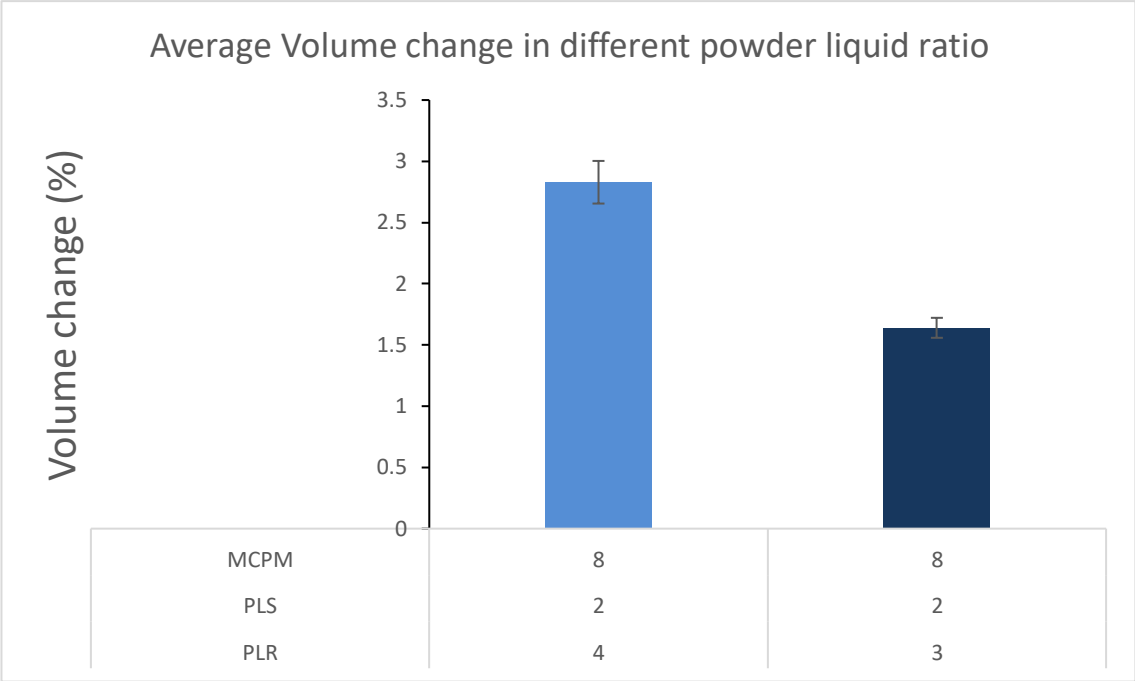

Figure 3-47 Plot showing the percentage of the average volume change of the last four timepoints for the two formulations with 4:1 and 3:1 powder liquid ratio. Error bars are the standard deviation of the last four timepoints.

3.2.4 Statistical analysis

An attempt for a 2 variable factorial analysis via a custom excel spreadsheet was made by using the average mass and volume change of the last 4 timepoints (1,2,4 and 5 weeks). Neither MCPM nor PLS seemed show any significant effect.

One way ANOVA test was run using the mass and volume data from the last timepoint (week 5) in order to detect if MCPM and/or PLS have a significant effect.

A Levene test was run to determine if the mass and volume data are homogenous. Both data sets were homogenous with a Levene value of 2.401 (p=0.059) and 1.576 (p=0.201) respectively. Therefore, parametric statistical tests were used and continued to run a one way ANOVA with a Tuckey post hoc analysis due to the homogeneity. The one way ANOVA would indicate if any of the formulations differed significantly from any another formulation.

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
MASS	Based on Mean	2.401	8	18	.059
	Based on Median	.598	8	18	.768
	Based on Median and with adjusted df	.598	8	7.653	.758
	Based on trimmed mean	2.214	8	18	.077

Figure 3-48 Table generated by SPSS after running the Levene test exhibiting the Levene value of the average mass change of the last timepoints indicating homogeneity of the data.

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
VOLUME	Based on Mean	1.576	8	18	.201
	Based on Median	.325	8	18	.946
	Based on Median and with adjusted df	.325	8	8.647	.935
	Based on trimmed mean	1.430	8	18	.250

Figure 3-49 Table generated by SPSS after running the Levene test exhibiting the Levene value of the average volume change of the last timepoints indicating homogeneity of the data.

SPSS also generated two multiple comparison tables, one for Mass and one for Volume that include the p values of significance amongst different pairs of materials.

In general, the formulation containing the small sized MCPM particles demonstrated a significant higher volume change than all SMART composite formulations and commercial materials and a significantly higher mass

change than just all SMART composite formulations. Table 2-2 shows the p values of significance for each formulation.

Table 3-1 showing p values of significance between the SMART composite formulation with small sized MCPM particles against all other SMART formulations, the control and two commercial materials.

Composite Formulation	Mass change p values	Volume change p values
Control Hybrid (0-0-3)	0.003	<0.0005
F5 (8-4-3)	0.018	0.043
F6 (4-4-3)	0.074	0.027
F7(8-2-3)	0.012	0.003
F8 (4-2-3)	0.026	0.001
F2(8-2-4)	0.002	0.043
Activa	-	0.001
Z250	-	0.003

When analysing mass change, other than the pairs of materials in Table 3-1 that demonstrated significance, significance was also noted in various pairs of SMART formulations versus the two commercial materials. Their p values are included in Table 3-2.

Table 3-2 showing p values of significance between different pairs of materials. The arrows indicates if this significant mass change was higher (⇑) or lower (⇓) within the pair.

Composite Formulation pair of materials	Mass change p values
Control Hybrid (0-0-3) versus Activa (⇓)	0.013
Control Hybrid (0-0-3) versus Z250 (⇓)	0.042
F5 (8-4-3) versus Activa (⇓)	0.065
F7(8-2-3) versus Activa (⇓)	0.044
F2(8-2-4) versus Z250 (⇑)	0.027
F2(8-2-4) versus Activa (⇓)	0.008
Activa versus Z250 (⇑)	0.042

When looking at volume changes the only other material pairs that demonstrated any significance were the control that was significantly lower

than the high PLS and high MCPM formulation (p=0.045) and the formulation with the 4:1 powder liquid ratio (p=0.028).

3.3 Microleakage

Dye microleakage of the novel SMART composite final formulation and 3 commercial materials in the coronal – the more incisally located restoration/tooth interface- and the cervical – the more cervically located restoration/tooth interface are described in the pie charts in Figure 3-50 and Figure 3-51.

3.3.1 Coronal interface

The four pie in Figure 3-50 show the level of microleakage observed at the coronal interface for each material. The EDI SMART material showed zero microleakage in 80% (n=8) of the samples and only 20% (n=2) presented with a microleakage score of 1 which indicates that the dye had penetrated into enamel. Activa followed with 50% (n=5) zero microleakage and out of the 50% that presented with a degree of microleakage, in one sample the dye had penetrated into dentine. Fuji II LC only had one sample with zero microleakage and the rest ranged from 40% (n=4) scoring 1, 20% (n=2) scoring 2 and 30% (n=3) scoring 3 which indicates dye penetration to the pulp chamber. Fuji IX samples presented with a 100% microleakage out of which 40% (n=4) was limited to enamel and 60% (n=6) extended into dentine with two samples reaching the pulp chamber. Overall, it can be observed that the EDI SMART composite and the Activa show similarities with regards to the level of microleakage and much less when compared to the GICs.

Figure 3-50 Pie charts indicating the microleakage score of each material at the coronal interface with a score of 0 microleakage being indicated with white and score 3 microleakage, which is the worst, with dark blue.

3.3.2 Cervical interface

The four pie charts in Figure 3-51 the level of microleakage observed at the cervical interface for each material. 60% (n=6) of the EDI SMART composite samples presented with zero microleakage on the cervical surface. From the remaining 40% (n=4) only one showed penetration of the dye into dentine. In Activa, 90% (n=9) showed zero microleakage and only one sample had dye penetrating into the enamel. Fuji II LC and Fuji IX performed exactly the same, with 100% (n=10) of the samples having dye penetrating into dentine out of which only two did not reach the pulp chamber.

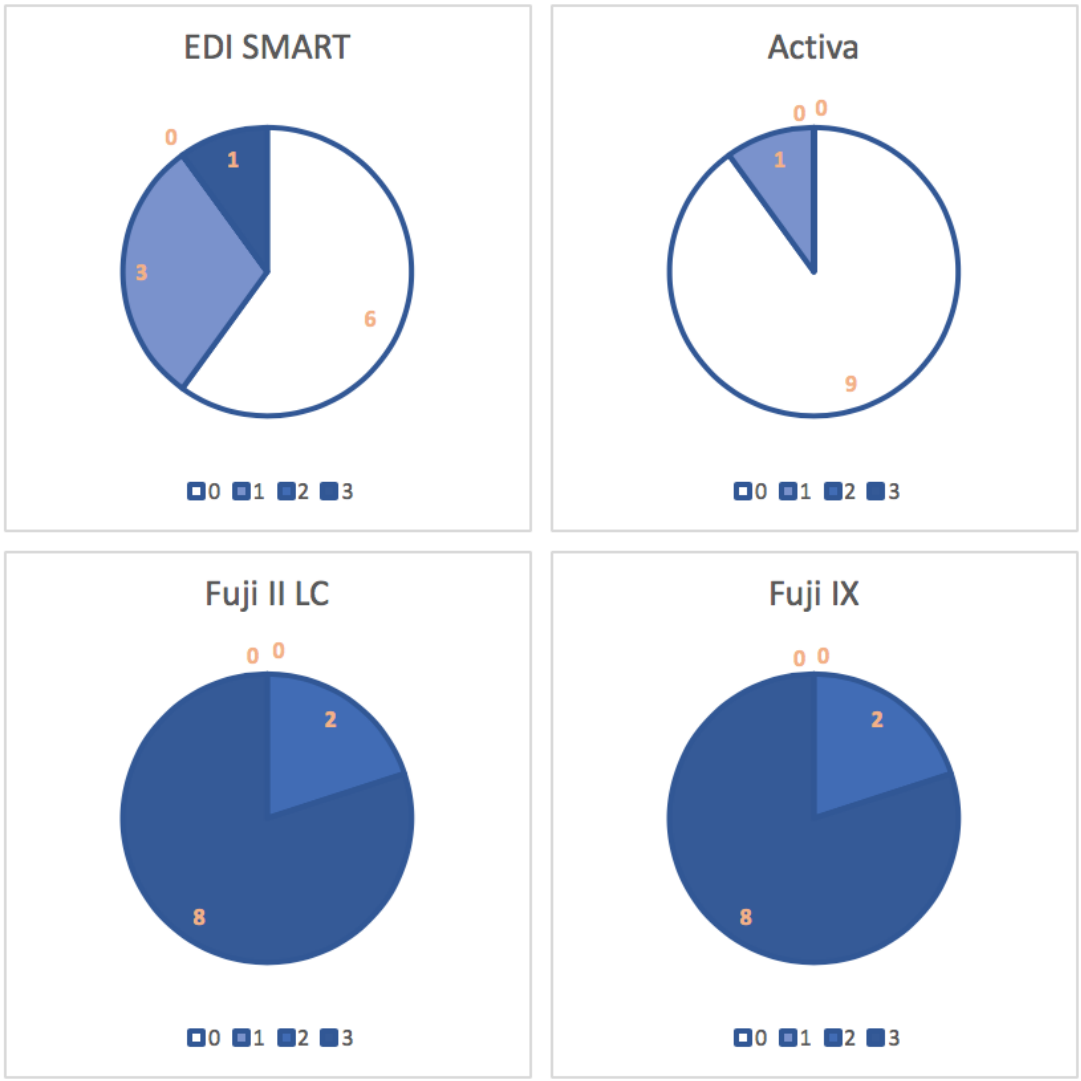

Figure 3-51 Pie charts indicating the microleakage score of each material at the cervical interface with a score of 0 microleakage being indicated with white and score 3 microleakage, which is the worst, with dark blue.

3.3.3 Statistical analysis

3.3.3.1 Plum Ordinal Regression

Following Plum ordinal regression test in SPSS, the parameter estimates table below was generated in order to predict what microleakage score would each material get. Due the statistically small sample size it would be impossible to predict which samples from each material would fall into level 3. Whereas it would be very clear if they consisted of level 0 and 1. It is observed that there is no significant difference noted on the coronal interface but on the cervical interface there is a significance between level 3 and level 0 (p= 0.001) and level 3 and level 1 (p=0.005).

Parameter Estimates

		Estimate	Std. Error	Wald	df	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Threshold	[matetrial = 1]	-6.319	1.720	13.498	1	.000	-9.690	-2.948
	[matetrial = 2]	-2.902	1.249	5.399	1	.020	-5.349	-.454
	[matetrial = 3]	.277	.883	.098	1	.754	-1.453	2.007
Location	[microleakagecoronal=0]	-1.674	1.485	1.270	1	.260	-4.585	1.237
	[microleakagecoronal=1]	-.325	1.124	.084	1	.772	-2.528	1.877
	[microleakagecoronal=2]	.800	1.209	.438	1	.508	-1.570	3.170
	[microleakagecoronal=3]	0[a]	.	.	0	.	.	.
	[microleakagecervical=0]	-4.969	1.550	10.278	1	.001	-8.006	-1.931
	[microleakagecervical=1]	-5.757	2.030	8.046	1	.005	-9.736	-1.779
	[microleakagecervical=2]	.821	1.241	.438	1	.508	-1.610	3.253
	[microleakagecervical=3]	0[a]	.	.	0	.	.	.

Link function: Logit.

a. This parameter is set to zero because it is redundant.

Figure 3-52 Parameter estimates table were significance between the different levels of microleakage is depicted in the relevant column.

3.3.3.2 Kruskal-Wallis test

A Kruskal-Wallis test demonstrated that the distribution of microleakage in the coronal interface is not the same across different materials. EDI SMART composite performed significantly better at the coronal interface than Fuji II LC ($p=0.004$) and Fuji IX ($p=0.001$) which was evident in the pie charts described previously. Activa is significantly better than Fuji IX ($p=0.042$) but showed no other significance. As expected, the pairwise comparison of EDI with Activa and Fuji II LC with Fuji IX did not show any significance.

The same test was done to assess the distribution of microleakage in the cervical interface. EDI SMART composite performed significantly better than Fuji II LC ($p=0.004$) and Fuji IX ($p=0.004$) and so did Activa with $p \leq 0.0005$ respectively for each GIC.

It was also decided to run a Kruskal-Wallis test on the combined coronal and cervical interface scores which also confirmed the pattern of EDI SMART composite and Activa being significantly better microleakage wise to Fuji II LC and Fuji IX ($p \leq 0.0005$) as shown in Figure 3-53.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Activa-EDI	.825	7.040	.117	.907	1.000
Activa-Fuji II LC	-34.500	7.040	-4.901	.000	.000
Activa-GI IX	-35.575	7.040	-5.054	.000	.000
EDI-Fuji II LC	-33.675	7.040	-4.784	.000	.000
EDI-GI IX	-34.750	7.040	-4.936	.000	.000
Fuji II LC-GI IX	-1.075	7.040	-.153	.879	1.000

Figure 3-53 Table generated from Kruskal-Wallis test in SPSS for the combined microleakage score indicating which material pairs show significance.

3.3.3.3 Cohen's κappa (κ) test

Cohen's κappa (κ) test was run in order to determine if there was agreement between the two observers on the microleakage score obtained from 40 randomised images depicting 40 coronal tooth/material interfaces and 40 cervical tooth/material interfaces. On the coronal interface a κ=0.569 (p≤0.0005) occurred, resulting in the level of agreement between the two observers to be moderate according to both the Landis and Altman guidelines as described on Table 2-4 showing Cohen's κappa test interpretation according to the two existing guidelines by Landis and Koch (1977) and the modified Landis and Koch by Altman (1991). in the materials and methods chapter.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.569	.099	5.877	.000
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Figure 3-54 SPSS image showing the κappa value (κ) and its significance at the coronal interface.

On the cervical interface, a κ=0.645 (p≤0.0005) occurred which results to a substantial level of agreement amongst the 2 observers according to the Landis and Koch guidelines or good according to the Altman guidelines. Therefore it is evident that the observers agreed more on the cervical interface by 65% than 57% on the coronal interface.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.645	.093	6.587	.000
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Figure 3-55 SPSS image showing the κappa value (κ) and its significance at the cervical interface.

From SPSS the following Crosstabulation tables (Figure 3-56, Figure 3-57) were generated showing where the two observers agreed the most. The most important numbers to focus upon are the count and the expected count. When the count exceeds the expected count, it means that the two observers are in good agreement. For example in Figure 3-56 regarding the coronal interface, the count for zero microleakage is 13 whereas the expected count is 6.6 meaning that there was indeed good agreement amongst observers.
The data in the two crosstabulation tables (see Figure 3-56 and Figure 3-57) confirm what was found in the PLUM ordinal regression analysis which is that the two observers agree more on scoring zero and three microleakage on both interfaces than other combinations. This also makes sense logically due to the fact that zero and three are the two extremes when it comes to scoring microleakage.

1RST OBSERVER * 2ND OBSERVER Crosstabulation

			2ND OBSERVER				
			0	1	2	3	Total
1RST OBSERVER	0	Count	13	3	3	0	19
		Expected Count	6.6	7.1	3.8	1.4	19.0
		% within 1RST OBSERVER	68.4%	15.8%	15.8%	0.0%	100.0%
		% within 2ND OBSERVER	92.9%	20.0%	37.5%	0.0%	47.5%
		% of Total	32.5%	7.5%	7.5%	0.0%	47.5%
	1	Count	1	9	0	1	11
		Expected Count	3.9	4.1	2.2	.8	11.0
		% within 1RST OBSERVER	9.1%	81.8%	0.0%	9.1%	100.0%
		% within 2ND OBSERVER	7.1%	60.0%	0.0%	33.3%	27.5%
		% of Total	2.5%	22.5%	0.0%	2.5%	27.5%
	2	Count	0	1	4	0	5
		Expected Count	1.8	1.9	1.0	.4	5.0
		% within 1RST OBSERVER	0.0%	20.0%	80.0%	0.0%	100.0%
		% within 2ND OBSERVER	0.0%	6.7%	50.0%	0.0%	12.5%
		% of Total	0.0%	2.5%	10.0%	0.0%	12.5%
	3	Count	0	2	1	2	5
		Expected Count	1.8	1.9	1.0	.4	5.0
		% within 1RST OBSERVER	0.0%	40.0%	20.0%	40.0%	100.0%
		% within 2ND OBSERVER	0.0%	13.3%	12.5%	66.7%	12.5%
		% of Total	0.0%	5.0%	2.5%	5.0%	12.5%
Total		Count	14	15	8	3	40
		Expected Count	14.0	15.0	8.0	3.0	40.0
		% within 1RST OBSERVER	35.0%	37.5%	20.0%	7.5%	100.0%
		% within 2ND OBSERVER	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	35.0%	37.5%	20.0%	7.5%	100.0%

Figure 3-56 Crosstabulation table of the coronal interface microleakage scores generated by SPSS.

1RST OBSERVER * 2ND OBSERVER Crosstabulation

			2ND OBSERVER				
			0	1	2	3	Total
1RST OBSERVER	0	Count	12	1	1	0	14
		Expected Count	4.9	1.8	2.1	5.3	14.0
		% within 1RST OBSERVER	85.7%	7.1%	7.1%	0.0%	100.0%
		% within 2ND OBSERVER	85.7%	20.0%	16.7%	0.0%	35.0%
		% of Total	30.0%	2.5%	2.5%	0.0%	35.0%
	1	Count	1	3	0	0	4
		Expected Count	1.4	.5	.6	1.5	4.0
		% within 1RST OBSERVER	25.0%	75.0%	0.0%	0.0%	100.0%
		% within 2ND OBSERVER	7.1%	60.0%	0.0%	0.0%	10.0%
		% of Total	2.5%	7.5%	0.0%	0.0%	10.0%
	2	Count	1	1	3	3	8
		Expected Count	2.8	1.0	1.2	3.0	8.0
		% within 1RST OBSERVER	12.5%	12.5%	37.5%	37.5%	100.0%
		% within 2ND OBSERVER	7.1%	20.0%	50.0%	20.0%	20.0%
		% of Total	2.5%	2.5%	7.5%	7.5%	20.0%
	3	Count	0	0	2	12	14
		Expected Count	4.9	1.8	2.1	5.3	14.0
		% within 1RST OBSERVER	0.0%	0.0%	14.3%	85.7%	100.0%
		% within 2ND OBSERVER	0.0%	0.0%	33.3%	80.0%	35.0%
		% of Total	0.0%	0.0%	5.0%	30.0%	35.0%
Total		Count	14	5	6	15	40
		Expected Count	14.0	5.0	6.0	15.0	40.0
		% within 1RST OBSERVER	35.0%	12.5%	15.0%	37.5%	100.0%
		% within 2ND OBSERVER	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	35.0%	12.5%	15.0%	37.5%	100.0%

Figure 3-57 Crosstabulation table of the cervical interface microleakage scores generated by SPSS.

4 Discussion

This project investigated the dimensional stability of novel SMART dental composite resin materials and compared their performance with commercially available restorative materials used for restoring primary teeth in young patients. In addition, such a dental material that required no special equipment other than a curing light plus a hand excavator could be used in low income countries that face severe structural impediments and the majority of the population cannot afford access to dental care. Dimensional stability was examined as soon as polymerization was taking place and allowed the calculation of shrinkage was calculated since the consistency of SMART composites is known. In addition, mass and volume stability in dry and wet conditions was also examined along with a final microleakage test in natural primary teeth in order to test the material ex vivo.
The overall aim was to identify whether a tooth mimicking, non-technique sensitive, mechanically strong material that needed no etch or bonding agent in order to bond with carious primary teeth and provide a sufficient seal making it impossible for the caries to progress, by maintaining its dimensional stability in the oral cavity conditions when placed as a bulk filling.

4.1 FTIR – Light curing kinetics

The sequence of events during FTIR experiments can be interpreted with light curing kinetics. Firstly, the delay time was examined, then the reaction rate, then the half time and concluded with the final monomer conversion.
Higher delay time translates into longer curing times needed to achieve good monomer conversion. This would potentially extend the working time in the oral cavity. In paediatric patients it is preferable to be as quick as possible. As a mean of reference, a long delay time for a composite would exceed 10 seconds. Delay time is proportional to the sample thickness. Higher delay time was anticipated at 2mm thickness can be explained by the Beer-Lambert law and the photobleaching phenomenon. The Beer-Lambert law states that the quantity of light absorbed by a substance dissolved in a fully transmitting solvent is directly proportional to the concentration of the substance and the path length of the light through the solution (Beer, 1852). An increase in the depth of the cavity has a negative impact on the monomer conversion degree since it tampers with the light penetration by limiting its intensity. Photobleaching is the bleaching effect observed on the top layer of uncured composite resins during light curing, allowing the light to penetrate into deeper layers. Slower bleaching increases the delay time.

Both the commercial products and four SMART composite formulations exhibited the same low delay time of less than 3 seconds with 1mm thick samples. With 2mm thick samples, the two formulations containing high PLS demonstrated higher delay times exceeding 5 seconds compared to the two formulations containing low PLS which had a delay of 3 seconds similar to the two commercially available materials. This could be explained by the difference in refractive index between monomers and fillers. Even though these composites were professionally mixed their particles cannot be evenly distributed. The normal sized MCPM particle formulation demonstrated a higher delay time of more than 5 seconds at 2mm thickness compared to the smaller sized particle formulations that exhibited delay times of 3.4 seconds indicating some sort of effect. On the other hand formulations with different

powder liquid ratios were not consistent and the overlapping error bars do not allow to deriving any safe conclusions.

Reaction rate is closely correlated to the sample thickness. A thicker sample causes the reaction rate to slow down. All SMART composite formulations had a higher reaction rate than the commercially available materials indicating that they required shorter light curing times. As expected, the 1mm thickness samples demonstrated higher reaction rates compared to the 2mm thick samples in all tested formulations with the exception of the SMART composite formulations containing low MCPM and low PLS and the control. Different MCPM particle size or different powder liquid ratio did not have an effect on reaction rate.

Half time is important because it indicates when all the monomers in dimethacrylates have at least one methacrylate group polymerised. After this conversion, the polymerization reaction slows down as the setting process changes from formation of linear chains into crosslinking reaction. At 1mm and 2mm thickness samples Activa demonstrates a higher half time of 18 and 21 seconds respectively compared to all other SMART composite resin formulations and Z250 which ranged between 10 and 15 seconds. This might be explained by Activa being more translucent due to its shade. The only exception was the SMART formulation with 4:1 powder liquid ratio that exhibited a 14 second half time at 2mm thick samples.

Following delay time and reaction rate, the reaction reaches a final monomer conversion which is a key factor to study, control and manipulate. Good monomer conversion makes composite resins great restorative materials but also can break them since it can be their Achilles' heel and lead them to failure. There is a plethora of studies about how monomer conversion affects the mechanical properties of dental composites, their ability to be biocompatible with the pulp and gingiva which are in close proximity and their aesthetics since it impacts colour stability (Schroeder and Vallo, 2007; Demarco et al., 2012; Walters et al., 2016).

FTIR – Fourier Transform Infrared (IR) spectroscopy employed in this thesis has been a method of choice for quantifying monomer conversion as it allows the calculation of the actual polymerisation in real time circumstances (Ilie et al., 2014) and it is fairly straightforward to operate (Shin and Rawls, 2009).
The degree of monomer conversion is susceptible to many factors from which some of them can be controlled and others cannot.

First and foremost, the final conversion of SMART dental composite resins should be taken into consideration as it affects the cytotoxicity of the material. It is well known in the literature that the monomers incorporated in dental composites are may exert cytotoxic effects to the pulp and gingival tissues due to the potential diffusion of unbound residual monomers that may emerge either due to the incomplete polymerization process or gradual decomposition of the composite resin matrix overtime (Ferracane and Greener, 1986). Following water sorption, unpolymerised monomers can be released and be responsible for a cytotoxic effect (Goldberg, 2008). Composites with higher

degree of monomer conversion release less free monomers and therefore are less cytotoxic. The critical percentage of monomer conversion is 50% because it is considered the minimum conversion in order to avoid cytotoxic related consequences (Lempel et al., 2016). All tested materials achieved 50% monomer conversion including the commercial products, however, SMART composites had higher monomer conversion potentially suggesting their greater biocompatibility.

All four SMART composite formulations and the control exhibited higher monomer conversions than the commercially available materials irrespective of sample thickness indicating their potential for bulk placement (Walters et al., 2016) and enabling easier application in children. The size of MCPM did not have an effect on monomer conversion because conversion is a monomer reaction and therefore more dependent on the liquid phase. Higher powder liquid ratio, however, did demonstrated lower monomer conversions of ~70% at 10 and 20 seconds curing time which agrees with Aljabo's findings since he used a ratio 5:1 and reached ~70% conversion (Aljabo, 2015). A possible explanation is eater light scattering slowing activation of the polymerization process at lower levels.

Other factors that might affect the degree of monomer conversion are the light curing time and the light power (Liu et al., 2013). In this thesis, all SMART composite formulations and commercial products were cured for 10, 20 and 40 seconds in order to determine the minimum curing time required to cure a specific thickness. As expected, all six SMART composite formulations showed higher monomer conversions when cured for longer (Rueggeberg et al., 1993).

Amongst the SMART composite resin formulations, sample thickness was the most important factor affecting the final monomer conversion rather than powder liquid ratio or MCPM particle size. According to the Beer-Lambert law in conjunction with the photobleaching phenomenon that was described earlier, an increase in the depth of the cavity can have a negative impact on the monomer conversion degree since it hampers light penetration. That is the reason that the 2mm thick samples demonstrated the same high monomer conversion as the 1mm thick samples when cured for longer. In deeper cavities, it is assumed that incremental layering and curing each layer rather than bulk material placement might mitigate this effect and thereby enable higher monomer conversions. In addition, 1mm thickness samples of SMART composite formulations of high MCPM had a high monomer conversion in both 20 and 40 seconds curing time indicating that 20 seconds were sufficient enough and 40 seconds of curing were not needed. At 2mm thick samples, on the other hand, the formulations containing the lower MCPM provided more reproducible results at 40 seconds curing time. The above may indicate that MCPM enhances the polymerisation and provides higher monomer conversions.

Temperature was another factor that affected the final monomer conversion and can be divided into the environmental temperature, the airconditioned room temperature were the experiments took place, the composite

temperature at the time of the experiment and the temperature of the surface of the ATR diamond. Although, the ATR diamond was set at 37°C to imitate the oral cavity, these external temperature parameters might have affected some of the results demonstrating low monomer conversions ranging from 60% to 70% in the SMART composite formulations 2mm thick samples cured for 10 seconds. Such monomer conversion values are consistent with cooler temperatures.

These temperature factors might explain the discrepancies 2mm thickness samples demonstrated in monomer conversion, evident by the larger error bars. What also might have had a positive impact, is the temperature rise generated by the polymerisation chain reaction which might be true for samples achieving monomer conversions in the high 80% since it has been proven that pre heated composites demonstrate higher monomer conversions (Awliya, 2007).

In general, heat generation due to the polymerisation chain reaction is dependent upon factors such as sample thickness, method of placement (bulk or incremental), shade of material, location of measurement (more heat at the centre than the corner(Kim et al., 2015)) and method of heat measurement (calculated, thermocouples, thermography) (Dobrzynski et al., 2019). Dentine though acts as an insulator and in just 1mm thickness above the pulp chamber, can decrease significantly pulp chamber roof temperatures (Miletic et al., 2009). The SMART composite formulations placed as a bulk filling generated heat ranging from 80-100 j/cc which is 42-52 °C. The highest values were noted in the thicker and cured for longer samples as expected and correspond with the existing literature(Kim et al., 2015; Janeczek et al., 2016). If instead of a highspeed handpiece, a laser was used to prepare the cavities, it has been shown that the temperature to the pulp chamber did not increase significantly in vitro (Geraldo-Martins et al., 2005; Krmek et al., 2009).

4.1.1.1 General FTIR limitations

Since statistical analysis did not show any significant differences between the SMART composite formulations other than their superior monomer conversion against commercial materials, it would be prudent to read into the small discrepancies detected in the plots. These might have been products of systematic errors on different days. Room temperature, ATR diamond temperature, operator body temperature when handling the material, composite temperature, FTIR machine calibration, unavoidable voids when creating the composite discs, time differences when switching on the curing lamp are some of things that might have caused the discrepancies visible in the results section. In addition, by default the FTIR machine collects spectra only every 4 seconds which immediately indicates a +/- error of at least 2 seconds.

4.1.1.2 Calculated shrinkage based on the final monomer conversion

Calculated shrinkage ranged from 3% to 4% amongst all SMART composite resin formulations which is what is expected from current commercial dental composite resin products (Schneider et al., 2010). These formulations contained PPGDMA instead of TEDGMA because Walters et al (2016) has

demonstrated that this change resulted in higher monomer conversions with lower polymerization shrinkage. These values are slightly higher than 2-2.5% that have been recorded for Z250 (Filho et al., 2007; Aljabo, 2015). According to its manufacturer, a polymerization shrinkage of 1.7% may be achievable with Activa, which seems to be the lowest polymerization shrinkage of all tested materials. Their smaller shrinkage may be attributed to the use of monomers of higher molecular weight (Anseth et al., 1996). According to the mathematical Equation 7 used to calculate shrinkage, higher monomer content would generate higher shrinkage due to increased cross linking. The SMART formulation with the 4:1 powder liquid ration showed lower shrinkage than the one with the 3:1 when cured for 10 or 20 seconds. These differences though were not statistically significant.

4.2 Mass and Volume changes

Dental restorations are destined to survive in the adverse environment of the oral cavity, challenged by higher environmental temperatures (37°C) and the constant presence fluids. This constant immersion, encourages a phenomenon known as water sorption which can alter their mechanical and physical properties and increase their mass and volume. This same phenomenon is responsible for the "biocompatible" property of new materials because it promotes the remineralisation and demineralization process enabling the release and absorbance of molecules. In the SMART composite material, the two components that might be involved in such a process are MCPM and PLS. As noted, MCPM and PLS due their hydrophilic nature promote water sorption which then in turn increases the mass and volume (Panpisut et al., 2016).

What was interesting when looking at the mass and volume plots is that all SMART formulations and commercial materials demonstrated a peak at 24 hours and then reached a plateau value which was maintained until the end of the experiment. This peak likely due to initial absorption of water that then precipitates later release of PLS and MCPM in the SMART composites (Dakkouri, 2015; Panpisut et al., 2016). It is not clear if anything is released from the commercial materials or if it is just the beginning of the interface degradation.

Although, this increase in mass and volume is known to reduce the mechanical properties of the material, it is also welcome because it can compensate for the inevitable polymerization shrinkage and may enhance the materials' antibacterial properties justified by the PLS and MCPM release.

All SMART composite formulations demonstrated a volumetric change of 2% which if deducted from their 3% polymerization shrinkage discussed previously leaves a 1% unaccounted for. This, may result in a gap at the restoration/tooth interface which may then lead to microleakage and long-term failure due to secondary caries. The ideal would be for the volume change to match the polymerization shrinkage (Aljabo, 2015; Walters et al., 2016). If the volumetric change was higher than the polymerization shrinkage, then the tooth would be at risk of fracture. Activa demonstrated an average volume change of 1.3% which if deducted from the claimed polymerization shrinkage of 1.7% leaves a 0.5% possible gap which is smaller than the one by SMART composites. Z250 responded in a similar manner with 1.6% volume change and an average 2.2% polymerization shrinkage.

The small discrepancies noted amongst different SMART composite formulations containing high and low MCPM and PLS are too subtle to have an effect in their clinical performance as restorative materials. Their consistency is a product of many previous experimentations discussed in previous theses.

Mass and volume change can also be in indicator of voids in the material. Voids are of clinical relevance since they weaken the mechanical properties of

the material and pose a weak link that can compromise a restoration. When a sample disc has no air bubbles, the expected volume change should be double the expected mass change (Aljabo et al., 2016) due to the mathematical equation below:

$$V = \frac{\rho_2}{\rho_1} M$$

Equation 11

Where V is the volume change, M is the mass change, ρ_2 is the density of the sample which is typically ~2 and ρ_1 the density of the water equal to 1g/cm$_3$ at 4°C and 0.993316g/cm$_3$ at 37°C. With no voids volume change should therefore be approximately double that of mass change.

In this study, Z250 demonstrated a mass change of 1.79% and volume change of 1.6% and Activa had a mass change of 2% and volume change of 1.3% suggesting the existence of pores in the material. SMART composites on the other hand with a mass change of 1% and volume change of 2% suggests few voids present in the sample. Aljabo when testing similar composites noticed a mass change ranging from 0.5 - 7% and volume change of 1-13% (Aljabo, 2015).

4.3 Microleakage

Dental caries have crossed all geographical and social boundaries and have especially plagued young children. Preformed metal crowns have been the most reliable primary tooth restoration simply because they provide a successfully strong seal. Composites on the other hand have been used with caution because they are known for shrinking upon polymerization which causes a gap that can be infiltrated by bacteria – a phenomenon known as microleakage- and cause secondary caries. Composite microleakage since, has been the centre of many laboratory and clinical studies in order to understand it and avoid it. Many methods to detect and quantify microleakage have been explored especially by endodontists such as dye penetration, dye diffusion, bacterial and endototoxin infiltration, radioisotope penetration and electrochemical or 3D evaluation (Jafari and Jafari, 2017). Dye penetration using methylene blue dye was employed in this thesis because it was straightforward, had a small cost even though it has been known to show variable results. In order to reduce this, an ISO protocol was employed. Furthermore, as done in other studies, the coronal and cervical restoration/tooth interfaces were examined separately (Omidi et al., 2018).

The rationale behind this was that due to the enamel layer being thinner cervically, the cervical restoration/tooth interface would present with higher microleakage score than the coronal one (Gerdolle et al., 2007). This was not confirmed by the statistical analysis of the results. The SMART composite formulation presented similar microleakage to the Activa in both interfaces, though, both demonstrated significantly less microleakage than the two glass ionomer cements (Fuji II LC and Fuji IX) with a $p \leq 0.0005$ respectively. The reasons behind this might be the formation of an apatite layer. This apatite layer occurs due to SMART hydrophilic components (MCPM and PLS). These promote water sorption which allows the release of composite components into the mouth that use apatite to then precipitate from oral fluids. That was

demonstrated by the overshoot phenomenon visible in Figure 3-36, Figure 3-40 and Figure 3-44. Fuji IX has demonstrated some evidence of a remineralising properties in the literature (Six et al., 2000; Paiva et al., 2014) which were not confirmed in our experimental work. If some irregular mineralisations occurred, they did effectively prevent microleakage.

4.4 General comments

Following this in depth analysis of the monomer conversion, delay time, reaction rate, half time, polymerization shrinkage, heat generation due to polymerization, mass and volume change in wet and dry conditions of the four SMART composite formulations, the control and the two commercial materials (Activa and Z250) the question that arose is this: Do all these discrepancies noted amongst SMART composite formulations affect their clinical performance?

The answer is NO. Despite the fact that they differ in consistency, their performance in basic dental composite tests is adequate enough and even surpasses the already commercialised materials used as comparisons. What was also proven, is that the SMART composites maintain their beneficial self-etching, antibacterial and remineralising properties without being highly sensitive to factors such as consistency, operator errors, room and material temperature which makes them reliable enough to be manufactured in a large scale and for use in different countries with different climates. In addition, the microleakage results demonstrated the significant superiority of the final SMART composite formulation against the glass ionomer cements (Fuji II LC, Fuji IX) compiling more evidence towards their favour, in light of the second phase of the clinical trial about to commence.

In addition, when lab studies take place, scientists focus on significances of $p \leq 0.05$. If such significances are not detected, they will not be clinically detectable either. Since no such significances were noticed in this project, there was no need in doing more repetitions per sample to find them.
What is certain is that this thesis and the group are on the right path of actually producing a material with immediate clinical application in a market that desperately needs it.

4.5 Future work

As this project developed, focus in understanding the failure of dental composites shifted from the dental material and its properties, to the tooth surface that it bonds. This surface undergoes changes related to caries progressing, external factors such as the biofilm interfering, the conditioning before adhesion takes place and the close contact with the filling material. When going through literature related to adhesion, the proteolytic enzymes called matrix metalloproteinases (MMPs) and their roles in degrading the hybrid layer at the adhesion site of composite and dentine (Mazzoni et al., 2006; Carrilho et al., 2007) and in organising and remineralising the dentine matrix (Chaussain-Miller et al., 2006) became apparent. Therefore, the key to manipulating the tooth surface lay on the factors inhibiting or promoting MMPs action. Self-etching adhesives have been known to activate MMPs (Mazzoni et al., 2006). CHX on the other hand has been known to decrease MMP

production (Perdigao et al., 2013).Therefore, future work could focus upon assessing if SMART composites self-etch due to MCPM and antibacterial properties promote or inhibit MMP action.

5 Conclusions

The aim of this project was to determine the effect of antibacterial polylysine (PLS) and remineralising MCPM on the polymerization and dimensional stability of SMART dental composites and how does that affect microleakage. In conclusion:

1. Antibacterial PLS and remineralising MCPM do not have a significant effect on the delay time, reaction rate, half time and final monomer conversion of the SMART composites.
2. All SMART composite formulations tested had higher final monomer conversions than the commercial comparators.
3. The minimum curing time needed in order to achieve good final monomer conversion above the 50% cytotoxic limit is 20 seconds regardless of the thickness when having a maximum depth of 2mm.
4. The volume and mass change of SMART composites over long periods of time while submerged into water at a temperature of 37°C imitating the oral cavity temperature do not exceed calculated shrinkage. This may be an indicator of lower fracture risk.
5. The volume change of SMART composites compensates for the calculated shrinkage following mathematical calculations.
6. The overshoot noted in the mass change of SMART composites indicates the release of composite components into the oral cavity.
7. The final SMART composite formulation (containing 8% MCPM and 4% PLS) demonstrates a significantly lower dye microleakage than the two commercial GICs (Fuji II LC and Fuji IX).
8. SMART composites can be placed as a bulk filling without requiring etch or a bonding agent and provide an adequate seal.
9. SMART composites are stable enough to warrant a CE marking.

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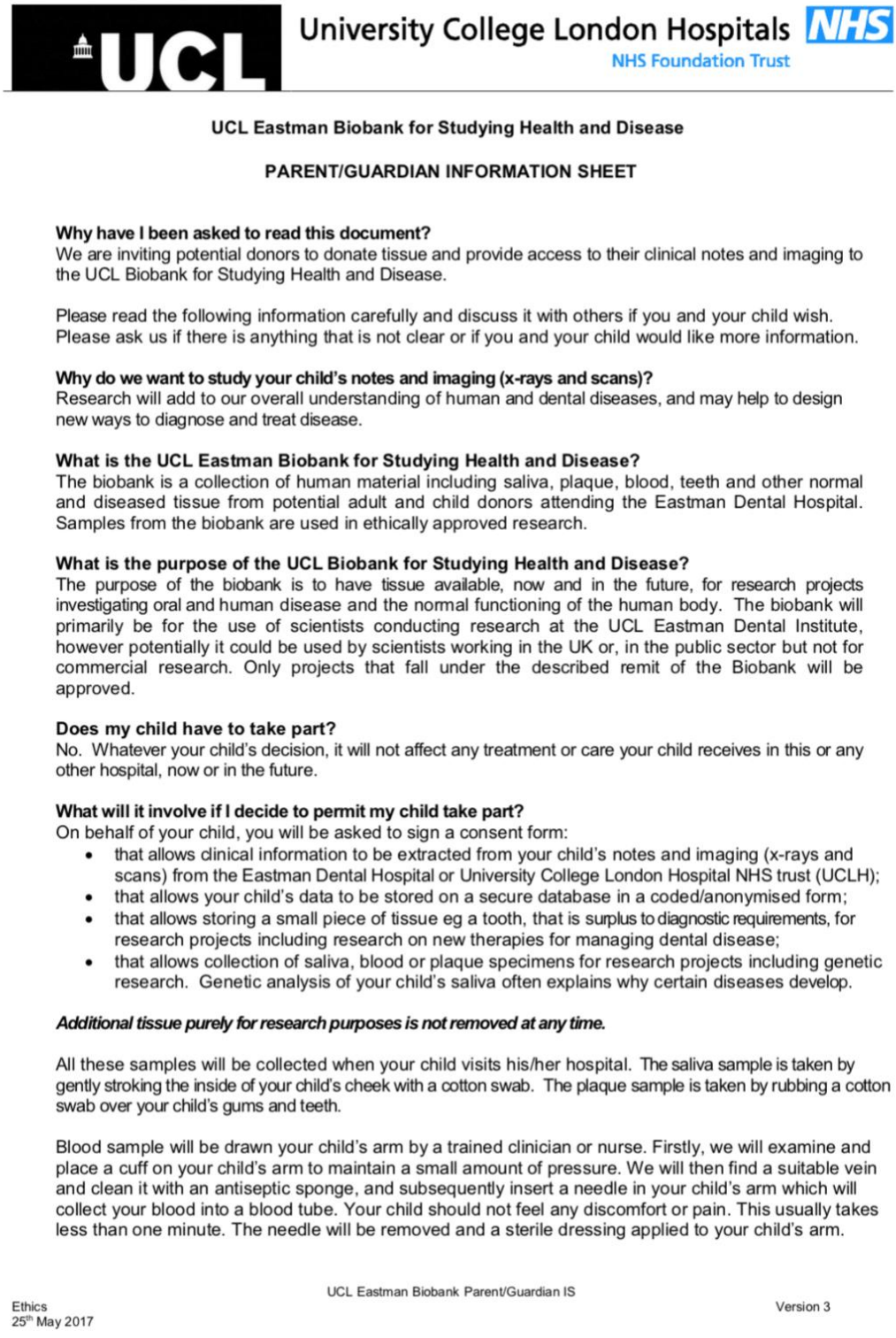
7 Appendix



University College London Hospitals NHS
NHS Foundation Trust

UCL Eastman Biobank for Studying Health and Disease

PARENT/GUARDIAN INFORMATION SHEET

Why have I been asked to read this document?
We are inviting potential donors to donate tissue and provide access to their clinical notes and imaging to the UCL Biobank for Studying Health and Disease.

Please read the following information carefully and discuss it with others if you and your child wish. Please ask us if there is anything that is not clear or if you and your child would like more information.

Why do we want to study your child's notes and imaging (x-rays and scans)?
Research will add to our overall understanding of human and dental diseases, and may help to design new ways to diagnose and treat disease.

What is the UCL Eastman Biobank for Studying Health and Disease?
The biobank is a collection of human material including saliva, plaque, blood, teeth and other normal and diseased tissue from potential adult and child donors attending the Eastman Dental Hospital. Samples from the biobank are used in ethically approved research.

What is the purpose of the UCL Biobank for Studying Health and Disease?
The purpose of the biobank is to have tissue available, now and in the future, for research projects investigating oral and human disease and the normal functioning of the human body. The biobank will primarily be for the use of scientists conducting research at the UCL Eastman Dental Institute, however potentially it could be used by scientists working in the UK or, in the public sector but not for commercial research. Only projects that fall under the described remit of the Biobank will be approved.

Does my child have to take part?
No. Whatever your child's decision, it will not affect any treatment or care your child receives in this or any other hospital, now or in the future.

What will it involve if I decide to permit my child take part?
On behalf of your child, you will be asked to sign a consent form:

- that allows clinical information to be extracted from your child's notes and imaging (x-rays and scans) from the Eastman Dental Hospital or University College London Hospital NHS trust (UCLH);
- that allows your child's data to be stored on a secure database in a coded/anonymised form;
- that allows storing a small piece of tissue eg a tooth, that is surplus to diagnostic requirements, for research projects including research on new therapies for managing dental disease;
- that allows collection of saliva, blood or plaque specimens for research projects including genetic research. Genetic analysis of your child's saliva often explains why certain diseases develop.

Additional tissue purely for research purposes is not removed at any time.

All these samples will be collected when your child visits his/her hospital. The saliva sample is taken by gently stroking the inside of your child's cheek with a cotton swab. The plaque sample is taken by rubbing a cotton swab over your child's gums and teeth.

Blood sample will be drawn your child's arm by a trained clinician or nurse. Firstly, we will examine and place a cuff on your child's arm to maintain a small amount of pressure. We will then find a suitable vein and clean it with an antiseptic sponge, and subsequently insert a needle in your child's arm which will collect your blood into a blood tube. Your child should not feel any discomfort or pain. This usually takes less than one minute. The needle will be removed and a sterile dressing applied to your child's arm.

Figure 7-1 Copy of information leaflet given to parents/guardians prior to donating teeth for the UCL Eastman Biobank (page 1 of 2)

We may ask for any of the above samples on more than one occasion but you can refuse at any time without giving a reason and without your child's medical care being affected.

What are the advantages and disadvantages of my child taking part?
There are potentially no advantages to your child if they participate in research but it may help others in the future. Your child will not receive a financial reward. Donated tissue is considered to be a 'gift' to the biobank.

Will my child's information be kept confidential?
Yes. All information about your child and his/her tissue samples will be treated with the strictest confidence. All information about your child would be coded so that it cannot be traced back to your child by the researcher. Results from genetic studies will be placed on a database to which only authorised individuals have access. Before having access to your child's clinical information and/or tissue samples, researchers must agree to conditions which safeguard your child's confidentiality.

Are my child and I going to receive feedback on the sample(s) my child donated?
We do not expect significant health-related findings from the studies which are approved by the UCL Eastman Biobank to use your donated teeth, plaque, saliva or blood. You and your child will therefore not be informed of the test results including genetic tests. Donating saliva, blood or plaque will have no impact on your medical insurance.

Family members
It is sometimes useful to have saliva, plaque or even blood samples from family members as this allows us to study the impact of genetic and environmental factors on the development of a disease. Therefore in some instances we may ask you to consent to allow us to contact your child's family members (parents/grandparents/cousins etc). You will also be given the Information Sheets to pass on to your child's relatives.

What if there is a problem or I require further information?
If you and your child would like further information or you have concerns about this research at any time you can:

- discuss it with your child's dentist or nurse
- email us on eastmanbiobank@ucl.ac.uk, we will try to answer your and your child's questions
- Normal NHS complaints procedures will apply.

What will happen if I do not want my child to carry on with the study?
You and your child are free to withdraw your consent at any time. This means that researchers will no longer be able to access any of your child's notes and images, and your child's tissue samples in the biobank will be destroyed. You and your child do not have to give a reason for changing your mind. However, if the data has already been used it is not always possible to recall it.

What will happen to any samples that my child gives?
Your child's samples will be used in a variety of studies and may include detailed analysis of your child's DNA to compare healthy DNA with the abnormal DNA found in patients with various diseases, or analysis of the bacteria and other organisms found in your mouth. Samples and data may be given for research purposes to other approved researchers in UK.

What will happen to the results of the study?
The results of our research will be published on our website https://www.ucl.ac.uk/eastman/research/departments/clinical-research/biobank, in dental or medical journals.

Who is organizing and funding UCL Biobank for Studying Health and Disease?
The biobank is overseen by the Research & Development Unit of UCL and UCLH. The cost of operating the biobank is funded by UCL Eastman Dental Institute.

Who has reviewed the project?
The UCL Biobank for Studying Health and Disease has been given a favourable ethics opinion for conduct in the NHS by the NRES Committee Yorkshire and the Humber – Leeds East.

Many thanks for taking the time to read this information.

Figure 7-2 Copy of information leaflet given to parents/guardians prior to donating teeth for the UCL Eastman Biobank (page 2 of 2).

University College London Hospitals NHS
NHS Foundation Trust

UCL Eastman Biobank for Studying Health and Disease

PARENT/GUARDIAN CONSENT FORM

For further information please email eastmanbiobank@ucl.ac.uk

DONOR NAME:

HOSPITAL NUMBER: **DONOR D.O.B:**

If you wish to participate, please complete this section by initialling the boxes and then signing at the bottom of the page.

Please initial box

1. I confirm that I have read and understood the relevant information sheet, version 3 dated 25th May 2017, and have had sufficient opportunity to ask questions. ☐
2. I give permission for my child's clinical data including imaging from any hospitals that my child attend, to be stored on a database in an anonymised format, and made available for ethically approved research, in the UK. ☐
3. I give permission for my child's tissue (surplus to diagnostic requirements) from any hospitals that my child attend to be made available for ethically approved research, including genetic analysis, in the UK. ☐
4. I give permission for my child's saliva to be made available for research, including genetic analysis, in the UK. ☐
5. I understand that all data and samples of my child will be coded for anonymity and all research projects will be approved by an ethical committee. ☐
6. I understand that my child's participation is voluntary, and can be withdrawn at any time without giving a reason. This will not affect my child's medical treatment or legal rights. ☐
7. I understand that my child and I will not receive feedback on the findings from my child's donated sample(s) ☐

CONSENT FOR BLOOD DONATION

In addition to the above, we may also ask for blood samples from your child. Please indicate below if you agree for blood samples of up to 50ml (6-8 teaspoons) to be taken from your child. If a blood sample for research purposes is taken from your child we will do our best to take it either when your child is under anaesthetic or when a blood sample is collected for diagnostic tests, however this may not always be possible. We may ask for blood on a number of separate occasions, but you or your child may refuse any time without giving a reason, and this will not affect your child's medical treatment or legal rights.

My child wish / do not wish to donate blood for research

Parent/Guardian signature:

Print full name: Date: / /

Relationship to child:

Staff signature:
Institution:

Print name: Date: / /

Figure 7-3 Copy of the consent form parents/guardians have to sign in order to donate teeth at the UCL Eastman Biobank.