The effect of Monocalcium Phosphate and Polylysine on SMART composite self-bonding to dentine.

Submitted by

Dimitra Saliakelli

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Eastman Dental Institute

Department of Paediatric Dentistry

University College London

256 Gray's Inn Road London WC1X 8LD

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Declaration

I certify that this project is the result of my own investigations and work.

Dimitra Saliakelli

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Abstract

Introduction

Despite the great effort made for prevention against caries, this is still a significant public health issue that affects a large number of the population worldwide. Restoration of carious teeth in children can be challenging and often the complex techniques used render the successful completion of the treatment extremely difficult.

Aims and objectives. This study investigates the impact that different levels of hydrophilic remineralising Monocalcium Phosphate Monohydrate (MCPM) and antimicrobial polylysine (PLS) agents have on the bond strength of new experimental and potentially self-adhesive composites to ivory and human dentine.

Methods and materials

Experimental composite formulations with low versus high levels of MCPM and PLS components were tested. Differences in morphology, mineralisation and caries level of teeth, all affect bond strengths with composites. The great diversity that human teeth exhibit, in addition to the limited supply, has forced the need for model systems. Ivory dentine was therefore used for initial assessment of the bonding strength, of multiple materials under different conditions, before testing optimised formulations in extracted human teeth.

For the tests performed in ivory: The experimental composite formulations were placed and light cured in cylindrical cavities drilled fully through ivory blocks (depth 5mm and hole diameter 3mm). Different conditions tested included presence or not of etch and/or bond and storage condition which included or submerged in Simulated Body Fluid (SBF) or artificial saliva. The bond strength was assessed using a push-out test in a Shimadzu machine. In human dentine: Human teeth were cross sectioned into 2mm discs of dentine and the soft caries was removed, but not into hard dentine. The composite restorations were carried out without any tooth conditioning, so that the experiments mimic the way this material may be used.

Results

The results in ivory showed that increased levels in both antibacterial and remineralising agents allowed for moderate bonding irrespective of the previous conditioning of dentine. Halving MCPM and PLS simultaneously increased initial (control) bond strength to untreated dentine (from 11 - 8 MPa). Dentine pre-treatment increased this to 14-16 MPa but benefits were minimal with low additive levels. Average bond strength (of all formulations/dentine conditioning/ medium type) halved within 1 week in medium from 11 to 5 MPa, but reached the initial levels of 10 MPa (of when the samples were stored in dry environment for 24 hours) by 30 days. In human teeth, the bond strength following dry storage for 24 hours (control bond strengths) was 7-9 MPa and comparable with those using ivory, but extremely variable following 1 week in Artificial Saliva (9-35 MPa). In 30 days in Artificial Saliva, the bond strength reached magnitudes of 6.5 MPa to 8.0 MPa similar to those of ivory dentine in 30 days in liquid medium.

MCPM, PLS and their ratio could affect the bond strength**.** MCPM / PLS = 2 increased the bond strength to untreated dentine for samples stored in dry storage for 24 hours.

Conclusion

Factors affecting bond strength are complex but SMART composites with 4% instead of 2% PLS and MCPM/PLS = 2 can improve bonding.

Impact statement

Dental caries remains a significant public health issue. In children, the management of caries can be difficult and often the majority of the non - pharmacological behavioural management techniques (such as Tell – Show – Do, positive reinforcement, modelling, desensitisation) as well as the conscious sedation (Inhalation Sedation) prove to be insufficient. Several factors including the use of local anaesthetic, tooth preparation for caries removal prior to the restoration, dental anxiety due to lack of previous experience especially in children of a very young age, complex and technique sensitive materials, have increased the likelihood of the treatment being carried out under General Anaesthesia and a significant number of teeth being extracted. In the UK, a large number of children receive treatment under General Anaesthesia, which not only consumes a significant amount of NHS resources, but also poses some risk on children's health which should not be underestimated.

The current materials that are available in the market and are being widely used for caries management have several disadvantages. The use of amalgam is gradually being reduced and therefore it needs to be replaced by another material. Glass Ionomer restorative materials can be used as a temporary solution before a final restoration is provided and composites require lengthy appointments due to the use of etch and bond, while proper isolation and good co-operation is essential for the optimal restoration to be carried out. Preformed metal crowns used with the Hall technique can be an alternative to composite restorations. The preformed metal crowns can be the material of choice when an extensive cavity presents and caries removal might lead to pulp exposure. They are also recommended in cases where patients with limited co-operation are being treated, where the extraction under General Anaesthesia might be the only alternative. However, for the provision of a preformed metal crown two appointments are normally required. One for the placement of separators, in order to create adequate space and another a couple of days to one week later for the crown to be fitted. The preformed metal crowns are often not accepted by the patient and the parents due to the silver colour, as this is causing aesthetic concerns. As the superiority of a dental material against another in paediatric patients had not yet been proved, the necessity of a novel material with optimal properties came into existence.

Development of a strong composite material that simplifies tooth restoration in children, would decrease the need for tooth extraction and / or General Anaesthesia. Remineralising and antibacterial action of a restorative material would eliminate the need for complete caries removal. This could be very helpful in managing carious lesions in the paediatric population. Additionally, self-etch and seal characteristics would aid ease of placement. For this reason,

experimental SMART composites were developed with the addition of Monocalcium phosphate (MCPM) and Polylysine (PLS).

The necessity of assessing the bond strength of those experimental composites led to the decision of executing experiments using ivory dentine in order to obtain preliminary results, followed by bond strength tests in human teeth. The samples were collected amongst teeth that were extracted mainly under General Anaesthesia and once consent had been obtained by the child's parents or legal guardian.

The results showed significant difference on the bond strength in different levels of MCPM, PLS and their ratio. These results can be used for future research projects on the field of novel composites which can lead to a restorative material with enhanced properties. Children's experience on the way composite restorations are currently being carried out will positively change and this may reduce the number of teeth being extracted as well as the necessity for restorative treatment under general anaesthesia.

List of abbreviations

ACP	Amorphous calcium phosphate
AS	Artificial Saliva
Bis GMA	Bisphenol A-glycidyl methacrylate
Ca	Calcium
CAD/CAM	Computer - aided design and computer – aided manufacturing
CHX	Chlorhexidine diacetate
CQ	Camphoroquinone
ECC	Early childhood caries
HEMA	2-Hydroxyethylmethacrylate
HV	Vickers Hardness
ICCMS	International Caries Classification and Management System
ICDAS	International Caries Detection and Assessment System
ISO	International Organisation for Standardisation
MCPM	Monocalcium phosphate monohydrate
MDPB	Methacryloyloxydodecylpyridinium bromide
MPa	Mega Pascal
MS	Mutans Streptococci
MSNs	Mesoporous silica nanoparticles
NHS	National Health Service
PHE	Public Health England
PO_4	Phosphate
PLR	Powder to liquid mass ratio
PLS	Polylysine
PPGDMA	Poly (propylene glycol) dimethacrylate
RMGICs	Resin modified glass ionomer cements
SBF	Simulated Body Fluid
SMART material	Self – adhesive, Mechanical, Antibacterial, Remineralising, Teeth like
TEGDMA	Triethylene glycol dimethacrylate
TTCP	Tetracalcium phosphate
UDMA	Urethane dimethacrylate
WHO	World Health Organisation

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1 Chapter - Introduction

1.1 Dental Caries

1.1.1 Epidemiology

Dental Caries is one of the most common childhood diseases globally. In early years of life, it is usually known as Early Childhood Caries (ECC). According to the World Health Organisation (WHO) 60%-90% of pre-school children and nearly 100% of adults are affected by dental caries worldwide (Petersen and Ogawa, 2016a).

In the UK, according to Public Health England (PHE), 12% of 3 -year olds have caries into dentine. For these children, 89 % of the affected teeth remained untreated (*Dental public health epidemiology programme*, 2014). A contemporaneous survey in the United States, carried out by the National Health and Nutrition Examination Survey from 2011 to 2012, revealed that approximately 23% of 2–5 year olds had dental caries in primary teeth (Dye et al., 2015). Additionally, the same data showed that approximately 10% of children of that age group had untreated dental caries. Although dental caries starts in childhood, it can continue in adulthood and will affect the elderly population as well.

Generally, dental caries is correlated to socioeconomic factors. Its rates are higher in deprived areas (Jain et al., 2007) and its prevalence is rising in developing nations compared to the developed ones. The two major factors that lead to caries distribution around the world are the frequency in consumption of refined products and the implementation of fluoride preventive measures. As the industrialised countries have reduced the import of sugar, the highest amount of exported sugar from the developing countries is now being consumed by themselves. Therefore, in the developing countries, where caries prevalence was low in first place, as they become more industrialised and get easier access to refined products, an increase in caries prevalence has been noted. On the other hand, the use of fluoride that is gaining ground worldwide, has resulted in a downward trend in caries activity in many industrialised countries.

Dental caries is expensive to treat and it is well known that in industrialised countries 5%-10% of health care expenditures are spent in the field of oral health, with its treatment becoming cost prohibitive in many parts of the industrialising world (Widström and Eaton, 2004; Meyerhoefer et al., 2014).

1.1.2 Aetiology

Dental caries is a multi-factorial disease with bacteria (oral microbiota), diet (fermentable carbohydrates) and salivary factors being the key players in the development of this process.

1.1.2.1 Diet

According to recent studies (Moynihan and Kelly, 2014), when sugar intake in the diet is less than 10% energy, there is a possibility of caries reduction. It is important here to note that it is not only the quantity of sugars that one consumes in his/her diet but also the frequency. Therefore, the consumption of sugars in between the main meals or sipping from sugary beverages during the day for a prolonged period, can have a deleterious impact on the individual's dental health. The time in which the bacteria of the oral microbiota are in contact with sugars is increased and it therefore leads to an extensive time of acid by-products release. This is subsequently increasing the risk of caries development through the demineralisation process.

In very young children the consumption of milk without the teeth being brushed afterwards by the parents can lead to caries development that has a characteristic appearance affecting primarily the buccal surfaces of the maxillary incisors. Children often have milk on demand in order to sleep or relax. This can be either bottle milk or human milk. According to WHO breastfeeding is very beneficial for the children up to the age of two. However, the human milk can also cause carries which is linked to prolonged breastfeeding including nocturnal feeding during sleep (Tham *et al.*, 2015).

1.1.2.2 Bacteria/oral biofilm/oral microbiota

Oral microbiota refers to the group of microorganisms that colonise the oral cavity after birth. (Wilson, 2004) These microorganisms, that can be bacteria, viruses, yeasts etc, are in a mutualistic relationship with the host, as they benefit from a warm and nutritional environment, while at the same time they protect the host from commensal bacteria attack (Devine et al., 2015).

These bacteria are held in contact with the tooth surface through their adherence to the acquired pellicle, which is an acellular, bacteria-free organic film that is deposited on teeth,

occupying a critical position between the enamel surface and the dental biofilm. The pellicle consists of salivary derivatives such as proteins, bacterial products, food and cervicular fluid (Scannapieco, 1994). It forms within seconds after saliva initially contacts the external layer of the tooth, and it protects the tooth from extrinsic acidic activity which can cause enamel demineralisation. When bacteria get attached to the pellicle a biofilm is formed, called dental plaque. Failure to remove this biofilm by regular tooth brushing can lead to oral diseases such as gingivitis, periodontitis and dental caries.

The “specific plaque hypothesis” suggested that specific bacteria are responsible for caries. Streptococcus mutans (MS) along with streptococcus sobrinus play a key role. These species have the ability to adhere to the acquired pellicle and produce acidic metabolites which increases the risk of carious cavity formation (Forssten et al., 2010). Several studies have shown a connection between MS levels in mothers and their children. Mothers are considered the principal source of MS for their infants (Caufield and Walker, 1989) possibly suggesting that the infants’ immune system “shows a preference” for maternally-derived strains allowing them to colonise the oral cavity. The initial acquisition of MS occurs during a clearly marked period of the child’s biological development known as “window of infectivity”. Studies suggest that MS colonises the oral cavity only after teeth eruption occurs as it requires a non-desquamating surface. The average age at which initial acquisition of MS occurs is therefore 26 months (Caufield et al., 1993). Along with MS, Lactobacilli can increase caries progression, while Actinomyces appears with cementum caries. The above-mentioned bacteria known for their acidogenic nature, can create acidic by-products after metabolising fermentable carbohydrates.

More recently, the involvement of many more bacteria in caries formation has been described by the “extended caries ecological hypothesis”. This proposes that many different acidogenic and aciduric bacteria of the oral microbiota, are involved in the caries process. These include, in addition to mutans streptococci, lactobacilli and actinomyces, many other aciduric strains of non-mutans streptococci, bifidobacteria and yeasts (Takahashi and Nyvad, 2011).

Lactic acid is the basic end-product from sugar metabolism (Takahashi, 2005) and is considered to be the main acid involved in caries formation. This process results in lowering the pH in the oral cavity, below the critical 5.5 pH. This leads to calcium, phosphate and hydroxide ions to be released from the tooth enamel (González-Cabezas, 2010).

Remineralisation of enamel though can occur after the dental plaque is disturbed and/or the pH has returned to a more neutral level. If there is a concentration of the above-mentioned ions still available in the saliva, they can be reabsorbed by the enamel surface (González-Cabezas, 2010). This is a continuous process (deconstruction- reconstruction) and is described by a dynamic equilibrium between de and remineralisation that normally in early stages of caries can maintain the integrity of the tooth structure. When the balance is shifted towards demineralisation, caries starts to develop with a subsequent cavity on the tooth surface (Peters, 2010; Pitts *et al.*, 2017).

1.1.2.3 Saliva

Saliva flow rate, composition in organic and inorganic components and buffering capacity are of paramount importance in caries control. After consuming fermentable carbohydrates, the pH in the dental plaque drops below the so-called critical pH (5.5 for enamel and 6.0 for dentine) and demineralisation occurs. Saliva has an important function against caries as it cleanses the tooth surfaces and neutralises the pH. Saliva flow rate is also important. The higher the saliva flow is, the faster clearance and neutralisation occurs. Additionally, salivary proteins are paramount in protection against diseases in which bacteria play a key role, such as caries (Ar *et al.*, 2009).

As the salivary flow increases, its concentration of protein, sodium, chloride and bicarbonate increases substantially. The increase in the concentration of bicarbonate has the ability to neutralise the plaque acids, with a subsequent increase on the plaque pH, therefore it inhibits demineralization. As salivary pH rises, the concentration of calcium in stimulated saliva rises as well. With respect to the inorganic phosphate, while its concentration in saliva inevitably falls on stimulation, the fact that $H_2PO_4^-$ reacts and turns into PO_4^{-3} leads to a final slight increase in PO_4^{-3} which balances the fall in total phosphate (Edgar et al., 1994).

From the above we can conclude that the stimulated saliva is supersaturated in respect to calcium and phosphate which moves the balance in favour of the remineralisation of the tooth structure (Larsen and Fejerskov, 1989).

1.1.3 Caries Management

1.1.3.1 Caries risk assessment

As all children are in a potential risk of developing dental caries, predicting the risk of dental caries development in childhood is of paramount importance for the patients, their families and also for the clinicians. Treating caries, especially when it comes to children can be challenging. When all the other techniques of both pharmacological and non-pharmacological behaviour management are deemed inefficient and impractical, the treatment is often carried out under General Anaesthesia (Campbell et al., 2012; 'UK National Clinical Guidelines in Paediatric Dentistry', 2002). In England, more than 26.000 children between the age of 5 and 9 were admitted to the hospital in 2017 – 2018 due to dental caries. This figure is double the number of the children admitted to the hospital for tonsillitis (Royal College of Surgeons, 2018). According to a recent study, children that undergo dental treatment under general anaesthesia are in higher risk of having poor oral hygiene and developing dental anxiety as they get older (Haworth et al., 2017).

Caries risk assessment is a useful tool which according to the **International Caries Classification and Management System** (ICCMS) can ascertain the patients' risk on developing caries and categorise them as of low, medium and high risk. That is based on a careful examination and recording of the patient's exposure to all the risk factors that are known to cause caries or increase the likelihood of caries development (Ismail *et al.*, 2015).

Such risk factors are:

1) Medical history including radiation in the head and neck as it is associated with hyposalivation and xerostomia. (Glenny *et al.*, 2010)
2) Diet- Consumption of sugared snacks and drinks (Lim *et al.*, 2008)
3) Insufficient fluoride intake (Marinho et al., 2003)
4) Poor oral hygiene (frequency, timing of brushing and flossing) and plaque accumulation (Doméjean et al., 2011)
5) Existence of deficient restoration (Goldberg *et al.*, 1981)
6) Socio-economic status (Jain, Shankar and Ramaiah, 2007)
7) Mother's caries experience (Dye et al., 2011)
8) Active carious lesions (Ismail *et al.*, 2015)

1.1.3.2 Diagnosis

Early and accurate caries diagnosis is of paramount importance for its management. Several techniques have been used aiming to determine this widely spread, painful and costly to treat disease. Some of these techniques are not in use any more while others are gaining ground in the field of caries diagnosis. A few examples of these techniques are:

1) Clinical examination and ICDAS (International Caries Detection and Assessment System) is the first step in caries diagnosis. ICDAS is a coding classification system for visual carious lesions diagnosis with codes from 0 to 6, with 0 being a sound enamel and 6 an extensive carious cavity into dentine.

2) Radiographic examination has always been an important diagnostic tool for caries. For the areas that cannot be directly visualised and assessed for caries activity such as intradentally, the well-known bitewing radiographs have been vastly used.

3) Light fluorescence is being widely used in caries detecting devices (Virajsilp *et al.*, 2005). When an incident ray of light interacts with a carious tooth it gets absorbed and is reemitted as visible light of lower frequency. DIAGNOdent and Spectra are two examples of these devices.

4) Optical Coherence Tomography is a new trend in caries diagnosis. It uses a low-coherence broadband near infrared light source. As the light produced gets reflected from the sample it gets collected within a Michelson or Mach-Zehnder interferometer, which produces excellent spatial resolution (~20 μm) and real-time images. Although this technique has been successfully used in vitro, there is still an ongoing research for its promising future appliance in vivo. (Tsang and Sharma, 2018)

5) Visual- tactile is a conventional method used in caries diagnosis. Along with the obvious colour change on the surface of a tooth due to caries development, a difference in the touch can normally be noted. This technique is not being used any more as the probe can cavitate initial lesions which by applying preventative measure could be remineralised and arrested.

6) Teeth separation is another way of visually diagnosing caries in areas that cannot normally be visualized. The use of orthodontic separators for a short period of time can give access to the proximal areas where caries presence may be suspected.

1.1.3.3 Prevention

1.1.3.3.1 Fluoride

According to Hippocrates, preventing is better that treating. When it comes to dental caries prevention, the main preventative measure is fluoride (Buzalaf *et al.*, 2011; Petersen and Ogawa, 2016b). Fluoride is a naturally occurring element that can be found in rocks and soil (*Fluoride - NHS*, no date). Fluoride is absorbed through the gastrointestinal tract and is distributed in our body via plasma. 50% of it is up taken by the calcified tissues and the rest is excreted in urine. Fluoride works in two ways. It inhibits demineralisation and it enhances remineralisation (Ten Cate, 1992). When the pH drops in the dental plaque biofilm due to the acid attack from acidogenic bacteria following the consumption of fermentable carbohydrates, in favourable conditions where remineralisation prevails, fluoride is being released from saliva and plaque and consequently gets absorbed by the crystals at enamel surface, protecting it from demineralisation. When the acid attack is very strong and manages to partially demineralise enamel, the available fluoride ions along with calcium ions are absorbed by enamel making its structure stronger than before as less carbonates are now present. According to recent studies, fluoride is more effective against caries when used topically rather than systemically, as it may increase the risk of skeletal and dental fluorosis (Hellwig and Lennon, 2004). Fluoride can be found in toothpastes, gels, varnishes and mouthwashes and should be used by the different age groups and caries risk patients as appropriate to ensure increased effectiveness without exposing them to any general health risks (Marinho *et al.*, 2003).

1.1.3.3.2 Fissure sealants

Fissure sealants were introduced in dentistry in 1960 and since then they have been used as a protective coating which is placed on the pits and grooves of the newly-erupted posterior teeth. First permanent molars are very susceptible in pit and fissure caries even before the teeth are fully erupted due to their anatomy, which promotes the biofilm formation and allows its retention. By placing fissure sealants, the occlusal surfaces of those teeth become smoother and the oral hygiene becomes more effective as the surfaces are smoother and can

easily get cleaned, while the growth of the biofilm is disturbed as it cannot get access to nutrients (*Explanation of what fissure sealants are - NHS Health Scotland*, no date). Therefore, the risk of developing pit and fissure caries is reduced (Ahovuo-Saloranta *et al.*, 2017).

Sealants are available in opaque, clear and colour-reversible, photosensitive ones. Resin sealants of different generations can be used, including those of 4th generation that contain fluoride. Equally, Glass Ionomers release fluoride for a prolonged period of time after application and they can also be placed in partially erupted molars (Naaman et al., 2017) when a necessary and essential isolation for the application of resin composite in fissure sealants is not possible. They are divided into chemically and light cured ones, with the latter being resin-modified. Glass Ionomers are not sensitive to moisture due to their hydrophilic properties when compared to the hydrophobic resin- based fissure sealants. No difference was detected between the caries- preventive effect of glass ionomer and resin-based fissure sealants (Mickenautsch and Yengopal, 2011). Resin based sealants showed high retention in 2 years (Muller-Bolla et al., 2006) and 11%-51% caries reduction at 24 and 48 months when compared to no sealants, whereas no clear conclusion could be drawn for the glass ionomer sealants.

Although we are all aware of the significant importance of caries prevention, there are still many cases where the oral health promotion has failed. According to a cross-sectional based study in Wales exploring the reasons that oral health promotion and prevention may have failed, only 9% of the parents asked, claimed that fluoride varnish has been topically applied to their children's teeth. 34% believed that caries 'runs in families', while 27% mentioned that it was bad luck that their children had caries. However, the vast majority of the parents would find leaflets or information on a website regarding caries very useful (Karki et al., 2011). This combination of insufficient or even non-existent prevention, along with lack of information can lead to the formation of carious teeth with significant consequences for the patient which becomes more of an issue when it comes to children.

1.1.3.3.3 Silver Diamine

Silver diamine is a liquid that has been used to arrest caries as it is an inexpensive material and is not technique sensitive. It is a painless and non-invasive procedure which can be carried out simply under cotton roll isolation. In countries such as Japan, Brazil and Argentina it has been used as a caries arrest drug for more than 50 years. It normally contains fluoride in 38% concentration (44.800ppm), and silver. Silver diamine acts in two ways: it enhances remineralisation on the carious lesions and it inhibits the growth of cariogenic bacteria. One the disadvantages of this material there is the black discolouration that is often caused on the

tooth surface following its application. It is therefore being used mostly in cases where a patient's challenging behaviour does not allow for a composite restoration and there is no indication for general anaesthesia or this option is not available (Mansouri and Maryam, 2018).

1.2 Current Restorative Materials

When a carious lesion progresses and it is no longer at the initial stage where remineralisation can be promoted and therefore a cavity has formed, its restoration is indicated. There are four categories of biomaterials being used in dentistry as restorative materials: metals, ceramics, polymers and composites.

1.2.1 Metals

Metals and alloys are very useful in restorative dentistry due to their unique characteristics. Their strength, stiffness and longevity make them ideal for long term dental applications especially when they are placed in stress-bearing areas, while the fact that they are ductile and malleable provides them with elastic or plastic behaviour. This property makes them ideal for being placed and condensed into a cavity. They can be cast, drawn or machined. As a result, they serve as good dental restorations. Finally, the fact that they can be polished, prevents surface roughness which can accumulate dental plaque.

1.2.1.1 Amalgam

One of the most commonly used materials in the metal category is amalgam. Dental amalgam is an alloy of liquid mercury mixed with solid particles of mainly silver, tin, copper while zinc and palladium can exist in smaller quantities. In addition to the above-mentioned characteristics, amalgam is easy to use, it is not technique sensitive and it is cost effective. (Greig, 2012).

Amalgam is a material easy to use, can last for a long time and is cost-effective therefore it supports the financial stability of the health system and makes dental treatment affordable to the patients. On the other hand, its silver colour forbids its usage in aesthetically important areas. Moreover, amalgam can suffer corrosion and can cause galvanic action, while it is not ideal for areas where the tooth structure has been severely weakened. It releases mercury vapor and particles and can have a negative impact on the environment from its disposal (Mutter, 2011).

Following the Minamata disease, many countries signed the Minamata Convention on Mercury in 2013 which requires a phase - down in the use of dental amalgam as a mercury added product. Minamata disease is a neurological condition caused by severe mercury poisoning, which was first discovered in Minamata Bay in Japan in 1956 caused by affected fish. The patients developed numbness in arms, limps and around the mouth, accompanied by slurred speech, disturbance in vision, deafness, dysphagia and in severe cases mental confusion was observed along with drowsiness and choreiform movements (Mcalpine and Araki, 1958). The Minamata Convention on Mercury is an international treaty designed to protect human health and the environment from mercury emissions and releases and mercury compounds. The Convention was approved by 140 countries and was signed on 10 October 2013 at a Diplomatic Conference held in Kumamoto, Japan. The Convention is named after the Japanese city of Minamata where the incident of mercury poisoning occurred.

Mercury is a chemical element that belongs to metals. It is a heavy, silvery white, liquid metal. Compared to other metals, it is a poor conductor of heat but a fair conductor of electricity. It does not react with the majority of acids but it tends to dissolve many metals such as gold and silver to form amalgams. It has seven stable isotopes with Hg202 to be the most abundant and the Hg194 the longest with a half live of 444 years. A conventional dentistry amalgam alloy commonly consists of silver (65%), tin (29%), copper (8%) and other trace metals being dissolved in mercury. It is extremely toxic and can be absorbed through the skin and mucous membrane and it can also be inhaled in the form of vapour. The most toxic forms are usually organic compounds which can cause both acute and chronic poisoning. It can be treated but the research on mercury poisoning and treatment is limited at the moment (Bernhoft, 2012).

In the UK, there is the intention to phase out amalgam on environmental grounds by 2030. In terms of its usage as a dental material, the need of developing an alternative material to amalgam, before it is fully banned from dental treatment has been raised (*National plan to phase down use of Dental Amalgam in England Contents*).

According ding to the last regulations, from the 1st of July 2018, dental amalgam "shall not be used for dental treatment of deciduous teeth, of children under 15 years and of pregnant or breastfeeding women, except when deemed strictly necessary by the dental practitioner based on the specific medical needs of the patient" (Article 10(2) EU Regulation (EU) 2017/852 on Mercury).

1.2.1.2 Preformed metal crowns

Another way of restoring carious teeth is by using a metal crown instead of filling the carious cavities. Preformed metal crowns are used in Paediatric dentistry for the management of various dental pathologies such as caries, as a final restoration following a root canal treatment on primary molars (i.e pulpotomy or pulpectomy) and for the management of enamel and dentine defects (i.e enamel hypoplasia / hypomineralisation and dentinogenesis imperfecta). They are also known as stainless steel or nickel chrome crowns. They can be used on a conventional way that requires tooth preparation under local anaesthetic for the crown to fit, or alternatively using the Hall Technique in which no caries removal and tooth preparation is required, and therefore no local anaesthetic is needed, given that there is no associated periapical pathology and the tooth is asymptomatic. Often the use of separators for the short period of one week is necessary to create enough space intradentally for the crown fit. The advantages of the use of preformed metal crowns is the great sealing they offer, the protection of the tooth against caries until its exfoliation, the acceptance from patients and parents and the easy technique for dentists (Innes et al., 2007). On the other hand, the poor aesthetics, the existence of metal and sometimes nickel allergy can exclude its use from the treatment plan on caries management (Innes et al., 2007). Despite its advantages, this option is mainly available in specialist centres and therefore the vast majority of children with caries will still be treated by general practitioners. In this case a caries restorative material has to be used, unless the tooth is unrestorable or there is lack of co-operation which will eventually lead to tooth extraction.

1.2.2 Ceramics

Ceramics are inorganic metal oxides. Their properties include hardness, stiffness, but they are poor thermal and electrical conductors. Their translucency and opacity can be modified to comply with the aesthetic requirements and they can be cast or machined. In dentistry they are used in crowns, denture teeth and as fillers for resin matrix composite filling materials. Porcelain, which is a type of ceramics, consists of the following: kaolin, quartz and feldspar processed by firing at a high temperature. The metal-ceramic restorations belong to this category and they have the same, above - mentioned composition. They can be constructed in the lab by the technicians or they can be computer- designed and manufactured (CAD-CAM). As for their disadvantages it is important to mention that in addition to the decreased toughness and low plastic behaviour that makes them brittle, micro cracks can occur during their fabrication which by chewing can lead to the extension of those cracks. This is a

phenomenon called “slow crack growth” and which can dramatically reduce the survival rate of a ceramic restoration. (Greig, 2012)

1.2.3 Polymers

A polymer is a molecule that consists of many monomers and it can be made of the same or many different monomers. They are usually composed of hydrocarbons or silicon compounds. In dentistry they are used in sealants, cements, orthodontic space maintainers, impressions, root canal filling materials, denture bases, athletic mouth guards and composites. They are formable, translucent or opaque, with low density, hardness, stiffness, functional ability and melting point. They are also poor conductors of electricity and thermal energy. (Greig, 2012)

1.2.4 Composites

Dental composites were initially produced in 1940’s and in the beginning, they were chemically activated (Hervás García *et al.*, 2006). This was followed by composites which were light-cured using light in ultraviolet wavelengths, while nowadays the light used for their activation is in the visible wavelengths (Hervás García *et al.*, 2006). As many countries have minimised the use of amalgam due to Minamata Convention on mercury, composites are gaining ground as a dental restorative material. Their properties, however, are very different.

Composites are thermal and electrical insulators, are formable, can be machinable and have low solubility (*Craig’s Restorative Dental Materials - E-Book* -). They can be bonded to enamel and dentine after those surfaces have been appropriately prepared. In dentistry, they are used as sealants, intracoronal and extracoronal restorations, veneers, denture teeth, cements, core build ups and provisional restorations. By combining those two different classes, we get a material with properties that cannot be found in those classes individually. For example, ceramics are not packable, but when added to a polymer, the final material exhibits this property. In addition, polymers that are not stiff or stable, when they are combined with a ceramic, the created material presents with enhanced abilities of stiffness and stability. Composites can be either translucent or opaque to serve multiple aesthetical requirements. Oxidation and aging, however, can alter the colour of the composite undermining the aesthetic result of the restoration. (Örtengren *et al.*, 2001)

Composites are bonded to the tooth structure, therefore there is no necessity for a specific shape and size cavity that would increase the mechanical retention, as happens with amalgam restorations. This minimises the amount of often healthy tooth structure that needs to be

prepared and eventually lost, for the composite restoration to be placed and retained. The success and longevity of a composite restoration relies on the chemical bond that develops between the tooth structure and the material itself which is up to date is facilitated by the use of a bonding agent (Shenoy, 2008).

The major disadvantage of composites however, is the polymerisation shrinkage which occurs upon setting in the tooth (Bowen, 1963). This leads to defective areas on the margins of the restoration where micro-leakage results in bacterial intrusion. As this occurs, discolouration, secondary caries, inflammation with sensitivity or pulpal pain (reversible or irreversible pulpitis), and eventually apical periodontitis are the most often referred signs and symptoms of a defective and often failed composite restoration.

1.2.4.1 Composite composition

Composites may be defined as combinations of at least two chemically different materials with a distinct interface. Dental resin composites consist of a resin matrix (organic phase), inorganic filler particles (dispersed phase), filler-matrix coupling agent (interface), and minor additions including polymerization initiators, stabilizers, and colouring pigments (Zimmerli *et al.*, 2010; Alrahlah, 2013). The polymer, which is commonly a matrix of dimethacrylate monomers, functions as a matrix for the particles, whereas the particles reinforce the strength of the material. The monomers could be methacrylate monomers, low shrink methacrylate monomers and low-shrink silorane monomers. The two main bulk monomers that have been commonly used are 2,2-bis[4(2-hydroxy-3-methacryloxy-propyloxy)- phenyl] propane (Bis-GMA) and urethane dimethacrylate (UDMA) (Greig, 2012). As for the bisphenol A glycidyl methacrylate (Bis-GMA) which was incriminated for an oestrogen-like effect, studies have shown that it was not detected in the blood, therefore no systemic exposure to it cannot be justified (Rathee et al., 2012). Triethylene glycol dimethacrylate (TEGDMA) and Poly (propylene glycol) dimethacrylate (PPGDMA) are monomers that have also been used to composites. TEGDMA is a low viscosity monomer that has been added to composite materials to reduce viscosity and increase mobility during polymerisation, and subsequently favours the material's conversion. Its reduced viscosity is associated to the absence of strong intermolecular interactions such as hydrogen bonding. The addition, TEGDMA increases the material's water sorption which can negatively affect the mechanical strength of the restoration (Gajewski *et al.*, 2012). PPGDMA, which is a high molecular weight diluent monomer, was used instead of TEGDMA and as showed on a recent study it increased the conversion by 1.1 and 1.2 times at 1 and 4 mm respectively. In the same study, experimental formulations with

PPGDMA exhibited lower shrinkage compared to those containing TEGDMA (Walters *et al.*, 2016).

This inorganic filler can be glass, microfine silica, ceramic particles or nanoparticles. They are often radiopaque. Conversely, polymers exhibit low radiopacity. As a consequence, high filler volume fraction composites show highest radiopacity. Composites are though less radiopaque when compared to amalgam. The differences in the diameter of the filler particles can provide better packing properties to the composite and enable higher filler content.

The coupling agent allows the bonding between the organic and the inorganic compounds during setting. The most widely used agents are organic silicons known as silane (Greig, 2012).

The initiator-accelerator system activates the polymerisation of composite. An example is camphorquinone which gives to the uncured composite a yellow colour which bleaches after composite restoration being light cured (Greig, 2012).

1.2.4.2 Classification

Composites can be classified according to the size of particles, type of fillers and viscosity.
By size, they can be divided into macrofills, hybrid/ microhybrid and nanocomposites (Greig, 2012). Macrofills were the first composites to be introduced in dentistry with particles diameter of 20 to 30 μm. They were opaque and had low resistance to wear. Nanocomposites is another type with smaller particle size. With oxide nanoparticles being the vastly used type of nanomaterial in nanocomposites, these composites exhibit novel properties due to the small size of their particles (nanoparticles). They contain particles that are all nanometer sized (1-100nm). They are below the visible light; therefore, they provide highly translucent composites. Nanofilled composites have similar wear resistance and physical properties similar to those of hybrid composites and can be used for both anterior and posterior restorations (Mitra, Wu and Holmes, 2003).
Hybrid composites are composed of two different types of fillers. Those that are of 0.6 μm, or greater, and those of 0.05 diameter, or smaller (Sideridou *et al.*, 2009). They have good wear resistance properties and they are ideal for stress-bearing surfaces. In the disadvantages, we can point out that their surface can become rough with the time. Microhybrid composites on the other side, are produced by mixing particles of size 0.04 to 1 μm with microfine silica. They contain 60%-70% filler by volume. Nanohybrid composites consist of nano-sized particles added in microhybrid composites which become gradually dull with the years due to the smaller size of the particles. It has been reported that nanohybrid composites exhibit inferior

properties when compared to nanofilled ones, whereas they appear to have similar properties to microhybrid composite materials (Moraes *et al.*, 2009). Lastly, microfilled composites are dimethacrylate resins with 30%-50% by volume silica fillers. They are ideal for class 3 and 5 restorations as they can give a better aesthetic result (Ferracane, 2011). They exhibit higher water sorption and thermal expansion (and sometimes more shrinkage) than microhybrids and nanocomposites.

Based on their viscosity, composites can be classified as packable and flowable. Packable composites are very viscous materials that can be compressed into a dental cavity. The filler loading is 50%-70% by volume. They can be light cured in a deeper level, they are radiopaque so they can be assessed radiographically and they have lower polymerisation shrinkage when compared to other types. On the other hand, flowable composites are low viscosity materials, ideal for low stress-bearing areas. Their filler loading is around 37%-53% by volume which can lead to greater polymerisation shrinkage. They are easy to handle (Baroudi and Rodrigues, 2015).

1.2.4.3 Polymerisation and shrinkage

Polymerisation of composite initiates once they are exposed to the visible blue light. The composite becomes harder within seconds under the appropriate visible light but this process continues for 24 hours after. With some materials there can remain a thin layer of unpolymerised composite lying above the polymerised material. This is caused by oxygen inhibition. The light needs to penetrate the entire depth of the composite restoration and this can be influenced by a number of factors. The initiator should be available in a sufficient concentration, while the size and the shape of the particles plays a significant role in the degree of polymerisation shrinkage – stress (Satterthwaite *et al.*, 2012). Other study has showed that the reduction on the size of the silica filler increases the polymerisation conversion rate on the floor of the cavity (Fujita et al., 2011). Polymerisation is also associated with the shade of the composite formulation and for those in darker shade longer polymerisation time is required (Ferracane *et al.*, 1986).

During polymerisation, the matrix phase undergoes a volumetric contraction, known as polymerisation shrinkage and stiffens. While this happens, the fact that the composite is constrained by the adhesives to the enamel and dentine of the tooth cavity, produces stress within the composite. This is something that can be tackled by using a higher molecular weight monomer (Carvalho *et al.*, 2015) with lower shrinkage and by placing the composites in increments into the dental cavity (Bicalho *et al.*, 2014)

1.2.4.4 Water sorption induced expansion

Following polymerisation, composites undergo expansion due to water sorption from liquids that are present in the oral cavity, mainly saliva that is in constant contact with the teeth and the restorations. The water diffuses into composite and causes expansion. This process can be slow to reach the maximum expansion level. The water sorption is important as it alters the dimensions of a composite restoration and increases the radial pressure. It can compensate polymerisation shrinkage but this takes time. This is typically within four weeks with a hydrophobic resin composite. Overly hydrophilic composites, however, can cause expansion greater than polymerisation shrinkage (Bowen et al., 1982). Other studies that have tested composites of different hydrophilicity on the shrinkage stress on both wet and dry conditions have shown that the shrinkage can be reversed by the presence of water and that this phenomenon was strongly related to the hydrophilic properties of the material. (Park and Ferracane, 2014)

1.2.4.5 Water sorption induced solubility

The water diffusion can also lead to erosion of the composite material and release of unreacted monomer. This typically occurs within 1 week and causes mass loss of the material. Small, hydrophilic molecules are generally eluted quicker than larger ones. TEGDMA is the main monomer released from dental composites, while monomers such as 2,2-bis [4-(2-hydroxy-3-methacryloyloxypropoxy)-phenyl]-propane (Bis-GMA) and urethane dimethacrylate (UDMA) may also be eluted into water. These released monomers are cytotoxic and can potentially be irritants and allergens for the patient (Mousavinasab, 2011).

1.2.4.6 Mechanical Strength

Composite reach close to maximum mechanical strength within a few minutes of light exposure and can therefore be immediately adjusted and polished. Similar to other composite structures, the type and composition of the resin matrix as well as the filler particles have strong influence on the mechanical properties. Water sorption and solubility, however, generally reduces the mechanical properties of the material longer -term (Ito *et al.*, 2005). In addition, stress cracks in the polymer, can decrease strength through debonding of the filler (Drummond, 2008).

When it comes to strength, there are different types such as tensile, shear and compressive and flexural. Tensile strength is the ability of a material to resist a pulling force (Wang *et al.*, 2003). Tensile strength is particularly important for composites as they are more brittle than ductile materials. High tensile strength materials withstand loads causing elongation. Compressive strength is the ability of a material or structure to withstand loads that tends to reduce material size (Wang *et al.*, 2003). In other words, compressive strength resists compression (being pushed together), whereas tensile strength resists tension (being pulled apart) – Figure 1. Shear strength is the ability of a material to resist forces that can cause the internal structure of the material to slide against itself. The shear strength is the load that an object is able to withstand in a direction parallel to the face of the material, as opposed to perpendicular to the surface – Figure 2.

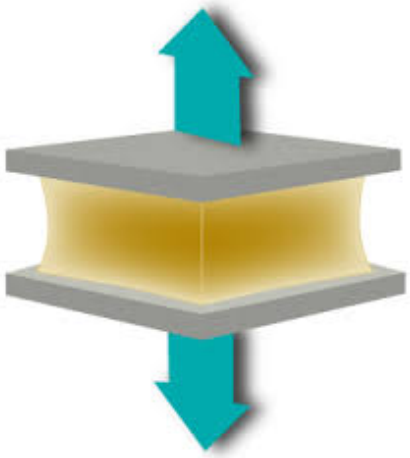


Figure 1.1: Tensile Strength

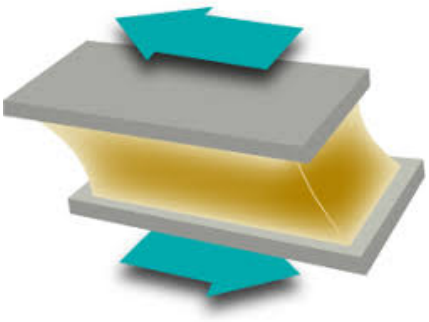


Figure 1.2: Shear Strength

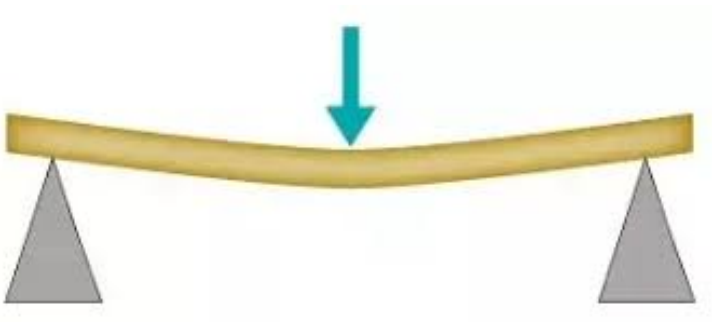


Figure 1.3: Flexural strength

Clinically, composite restorations are subjected to complex mastication forces with a considerable amount of flexural stresses. The flexural strength of a material is the maximum stress that it can resist before failure when subjected to bending load – Figure 3. The required flexural properties are highly dependent on the clinical applications. For restorations that are subjected to large masticatory stresses, high flexural strength is desired (Chung *et al.*, 2004).

Temperature can affect composites' strength. According to a study carried out in 1981, the ultimate strength of all the tested materials decreases linearly with increasing temperatures above 20°C. The strength of the material with 0.45 volume fraction filler was greater than the strengths of the materials containing 0.55 volume fraction filler. The lowest strength was exhibited by a 0.55 volume fraction material, which contains a small percentage of very large particles (Draughn, 1981).

Comparing to composites, polymers have low strength and stiffness, metals have intermediate strength and stiffness but high ductility, and ceramics have high strength and stiffness but are brittle. In ceramic matrix composites, the objective is often to increase the toughness rather than the strength and stiffness; therefore, a low interfacial strength bond is desirable (Weber, Shaw 3rd. C.F. and Petering, 1987).

1.2.4.7 Modulus

Stiffness indicates how adequately a material resists deformation. Elastic modulus (also known as modulus of elasticity, sometimes referred to as Young's modulus) is a quantity that measures the resistance of an object or a substance to be deformed elastically (i.e., non-permanently) when a stress is applied to it. Elastic modulus is the ratio of stress, below the proportional limit, to the corresponding strain. It is the measure of rigidity or stiffness of a material. In terms of the stress-strain curve, the modulus of elasticity is the slope of the stress-

strain curve in the range of linear proportionality of stress to strain. The greater the modulus is, the stiffer the material, or the smaller the elastic strain that results from the application of a given stress. Composites' elastic modulus is around 850 MPa, while for enamel is 1300 MPa and for dentine is 1600MPa (Chun and Lee, 2014). The modulus is an important design parameter used for computing elastic deflections.

1.2.4.8 Wear resistance and surface hardness

When restoring a cavity with composites, the aim is to sufficiently replace enamel and dentine and the purpose they serve. Composite that replaces enamel needs to exhibit higher hardness and wear resistance, close to that of enamel (274 MPa) whereas dentine that absorbs forces from mastication has hardness around 65 HV. Composites' hardness ranges between 80-124 HV which is lower than ceramics (400 HV) or zirconia (1250 HV). It is though, higher than that of dental amalgam (Chun and Lee, 2014). Hardness is the ability of a material to resist in a plastic deformation.

Composites experience surface wear as they come in contact with food and opposing teeth during the mastication process. Although composites are ideal for anterior teeth restorations where they serve aesthetical purposes and where the forces they sustain are minimal, caution needs to be taken when composites are placed in posterior teeth where the occlusal forces and the lateral excursive contacts are higher than in anterior segment.

1.2.4.9 Composite bonding to tooth structure

1.2.4.9.1 Bonding agents

The use and longevity of a conventional composite restoration relies on the application of adhesive agents. These are solutions of resin monomers with both hydrophilic and hydrophobic groups. The hydrophilic group increases the wettability of the dental tissues and the hydrophobic group allows the interaction and co-polymerisation with the restorative materials. More specifically, the monomers currently being used in bonding agents are the hydroxylethyl methacrylate, known as HEMA and the Bisphenol glycidyl methacrylate (bis-GMA). HEMA is the hydrophilic component that serves as the wetting agent and bis-GMA is the hydrophobic component (Migliau, 2017).

From the first to the eighth generation of bonding agents, the aim has always been the optimal bonding of the composite material to the tooth structure. Phosphoric acid was initially used to etch enamel and dentine, before the primer and subsequently the bonding agent were applied. This technique allows for hybrid layer to be formed, offering higher bond strength and better sealing, however, it is a complex and time - consuming technique. The fifth - generation agents were a single - bottle material which reduced the working time but it increased the water degradation compared to the fourth-generation agents. Adding the etchant into the primer was then introduced in the self- etching primer and adhesive agents which exhibited inadequate bonding to enamel, possibly due to insufficient etch preparation of it. Therefore, when this technique is being used, enamel needs to be additionally etched with phosphoric acid. For dentine bonding the results were satisfactory. Later on, the acid, the primer and the adhesive were combined in one single material. This favours the dentists for less technique sensitive and time-consuming procedures, but at the same time it didn't show enough good results in bond strength when compared to all other bonding agents that are available in the market. In 2010 the eighth-generation bond agents were developed. They contain nanosized fillers (cross-linking silica particles) of 12nm diameter which allows for higher bond strength when compared to bond agents of 6^{th} and 7^{th} generation. (Kamble *et al.*, 2015)

1.2.4.9.2 Factors that affect the bonding

Several factors, that are mentioned below can affect the quality of the adhesion. In addition to those, the etching and rinsing of the dental surface can undermine the adhesion of the bonding agent. In case of over drying the tooth structure after the etching, the dentine collagen may collapse leading to inability of the resin monomer to diffuse into it (Migliau, 2017). On the other hand, when the tooth surface is left very wet, the adhesive agent may dilute in the presence of water, thus the bond strength will be negatively affected (Migliau, 2017). It is important to mention, that over-etching the tooth surface can have a deleterious effect on the quality of the adhesion. In this case the etchant agent will go very deeply into dentine where possibly the monomer will not be able to reach, leaving behind unsupported areas and flow movement into the dentinal tubules. The former can cause degradation of the interface over time and the latter is the main reason of post-operative sensitivity that many patients complain of.

1.2.4.9.3 Use of tooth surface conditioning in a composite restoration

Traditionally a composite restoration is carried out by initially etching the tooth surface commonly with a 36% phosphoric acid for 10-30 seconds which is subsequently rinsed off. This allows the removal of the smear layer that is formed during the tooth preparation as well as the increase in porosity of collagen matrix and the exposure of dentinal tubules. This is followed by the use of a hydrophilic dentine primer and adhesive agent that penetrates in depth the aqueous fluid filled tubules and results in physical interlock with collagen and the tubules (Migliau, 2017). Finally, the resin composite is placed in the dental cavity and is chemically bonded to the adhesive agent previously used. The idea of flowable composites is based on simplifying this technique as it does not require the use of an adhesive agent. Although it is simple in use and less time consuming, flowable composites exhibit high shrinkage and low strength limits, therefore they can only be used in low stress- bearing areas (Baroudi and Rodrigues, 2015).

1.2.5 Glass Ionomer Cements

Glass ionomer cements are water based and self-adhesive materials used for restorations in dentistry. Conventional glass ionomers consist of a filler, which is a glass (base) called fluoroaluminosilicate and a liquid which is a water-based solution of polymers or copolymers of acrylic acid. Modern glass Ionomers can be found in two types: a) Conventional glass ionomers in which the setting reaction is an acid-base reaction and b) resin-modified glass ionomers in which there are additional monomers and polymerisation setting reaction.(Sidhu and Nicholson, 2016)

1.2.5.1 Conventional glass ionomer

In conventional glass ionomers the initial set might be achieved within 3-4 minutes but an extra 24-hour period at least is required for the setting to be complete They exhibit low solubility, high opacity, lower bond strength to dentine when compared to resin composites and elastic modulus similar to that of dentine. The bond strength achieved with enamel ranges between 2.6 MPa to 9.6 MPa while in dentine is much lower (1MPa – 4 MPa).

1.2.5.2 Resin-modified glass ionomer

As conventional glass ionomers had the disadvantage of the short working time, the idea of resin-modified ionomers was conceived and they were introduced in the dental field in the late 1980's. They consist of fluoroaluminosilicate glass and an aqueous polycarboxylic acid solution in which a limited amount of a hydrophilic methacrylate monomer, similar to those in dental composite adhesives is added. This leads to a photo-initiated reaction in addition to the chemical one that as well occurs. This type of glass ionomer is ideal for low stress-bearing areas, such as erosions in cervical areas. They can provide an enhanced aesthetic result when compared to the conventional ones. Patients experience minimal postoperative sensitivity when this type of restoration is used, as no etching is required for its application something that protects the collagen from collapsing. On a 3 year retrospective study on the survival rate of modified glass ionomer on class II restorations on primary molars (Webman *et al.*, 2016), a success rate of 97.42% was achieved.

1.2.5.3 Clinical indications of glass ionomer and resin modified

Glass ionomer and resin modified glass ionomer are both being used in low stress-bearing areas. They are ideal for liner and base when it comes to extensive cavities and they are considered the optimal material in atraumatic restoration technique. They are also the material of choice in many cases in paediatric dentistry when behaviour management is an issue and neither proper isolation nor a long-time procedure can be achieved.

1.2.5.4 Properties of glass ionomer and resin modified glass ionomer

Glass Ionomers have generally less optimum physical and mechanical properties. It is important to mention though that modified glass ionomers have a low modulus at the beginning of their curing, which reaches its maximum when the material is eventually fully set. This means that the material is initially very elastic, hence when placed as a base or liner under a composite restoration it releases the stress that composite undergoes due to the polymerisation shrinkage.

Regarding the thermal expansion, modified glass ionomer displays thermal expansion similar to that of dentine, something that does not occur with resin composite. Furthermore, low thermal conduction makes glass ionomer a more ideal material for liner and base as it protects the pulp from thermal shock.

Another characteristic of this material is the fluoride release. This mainly occurs during the first day of the placement of the restoration followed by a fall in fluoride release in the subsequent days. These materials can also act as a fluoride reservoir by capturing available fluoride from dental substances rich in fluoride such as toothpastes and fluoride varnishes. During this essential process the available fluoride can be absorbed by the tooth making glass ionomer particularly advantageous in high caries risk patients as it can potentially assist in caries prevention. (Mousavinasab and Meyers 2009). However, this proposed ability of glass ionomer to uptake fluoride and subsequently release it, hence enhancing caries prevention seems to be more complicated in reality and negatively affected by the maturation of the restoration even after one month of application. (Sidhu and Nicholson, 2016)

1.2.6 Compomers

Compomers, also known as poly acid-modified composites are a recent product being used for teeth restoration. It consists of poly acid-modified monomers and fluoride-releasing silicate glasses in approximately 40%-60% by volume. They are formulated without any water. They are hydrophobic materials and they combine the benefits of composites and glass ionomers. Compomers are set by an initial light cured process which is followed by a subsequent chemical reaction. The fluoride release is less when compared to this of glass ionomers as well as the fluoride uptake by toothpastes and other materials. They appear to be inferior to composites due to their inability to bond to hard tooth tissue, lower flexural modulus of elasticity and higher wear rates. They are being used in paediatric dentistry due to fluoride being realised from the restoration and simple handling with recurrent caries being the main reason indicating the replacement of the restoration. On a study carried out in 2010 regarding the use of compomers on restoring class II cavities in primary dentition, the authors conclude that compomers can be used except in teeth with pulpotomy or pulpectomy. Comparing to composites, the procedure requires less steps but patients' co-operation is still very important. (Marks et al., 2010)

1.2.6.1 Activa ^{TM} KIDS

Activa is a restorative dental material that has been available in the market since 2013. It is a bioactive material with enhanced remineralising properties. As it is a compomer, it combines the characteristics of both composite and glass ionomer materials, as mentioned above. Its main advantage is the moisture tolerance, which makes it extremely useful for use in paediatric patients. For its application, etching of the tooth structure for 10 seconds without any

subsequent bonding agent is required, followed by 20 seconds of light curing which allows for sufficient curing in up to 4mm depth. The clinical indications of Activa include the restoration of Class I, II, III and IV cavities and but also its placement on the top of surfaces previously treated with silver diamine fluoride.

1.3 Restorative challenges in Paediatric Dentistry

The restoration of carious teeth in paediatric patients can be very challenging. That can be due to lack of past dental experience or existing dental anxiety because of previous unpleasant or painful dental experience (Skeie et al, 2004). Pain and dental anxiety are strongly associated (Costa et al., 2012) and very often lead to avoidance behaviours for dental treatment (Arnrup et al., 2003). The differences in anatomy between primary and permanent teeth is another factor that indicates revised approach as for instance the smaller width of enamel-dentine can easily lead to iatrogenic pulp exposure.

Therefore, for paediatric dentistry a material that would not provoke anxiety when caries management and teeth restoration is indicated. The use of local anaesthetic and tooth preparation can be significantly stressful and traumatic for children and often renders the restoration of a carious lesion unfeasible. Therefore, the extraction of the tooth might be the only treatment that can be provided, often under General Anaesthesia.

The ideal material should give the clinician the chance to use it in an atraumatic way, avoiding for instance the administration of local anaesthetic or drilling tooth structure. It should not require many steps for its application, something that could turn the long-lasting dental procedures to short appointments. Eventually it should be placed in a bulk instead of increments without though compromising the long-term quality of the restoration, which will again reduce the duration of a dental visit.

Traditionally, the restoration of a carious lesion was performed only when hard to probing dentine could be detected (non-selective removal to hard dentine). This technique, was based on the theory that the presence of healthy dentine is necessary for the termination of the carious process. However, this was proven to increase the risk of pulpal exposure as well as tooth weakening. Additionally, the identification of healthy or infected dentine cannot be easily assessed. Therefore, new techniques in caries management have been established, based on research and better understanding of caries process. Selective caries removal is one of these techniques (Schwendicke *et al.*, 2015) that minimises discomfort, pain and anxiety during dental procedure as well as the potential risk of pulp exposure. Additionally, it

maximises the longevity of the tooth as it suggests the removal of enough soft dentine that will allow enough space for a bulk restoration to be carried out without weakening the tooth.(Banerjee et al., 2017)

All these lead to the need of developing a novel composite material, self- adhesive to dentine, with remineralising and antimicrobial properties which will enhance the qualities of restorations performed under selective caries removal.

1.4 Novel Composites

1.4.1 Antimicrobial Composites

The main reason that still leads to the failure of a restoration is the so-called secondary caries on the adjacent surfaces of a restoration. (Murray et al., 2002). Composite restorations exhibit a high level of cariogenic biofilm formation of their surfaces as has been proved from many studies (Beyth et al., 2007). The polymerisation shrinkage of the composite along with the ability of Streptococcus Mutans to get into the gaps on the margins of the composite restoration lead to the development of caries.(Kermanshahi et al., 2010) This evidence is indicative of the need to maintain the connection surface between the tooth and the restoration sound as this will determine the long- term success of the restoration (Donmez et al., 2005).

Therefore, apart from creating a composite with low polymerisation shrinkage, an antimicrobial releasing composite would be beneficial and could partially handle the above-mentioned problem, something that would improve the longevity of a composite restoration. Therefore, a series of antimicrobial agents are being used in several resinous dental materials in commercial or experimental level, such as fluoride, chlorhexidine, triclosan, zinc oxide, benzankonium chloride, polylisine, MDPB etc. As the evidence for the antimicrobial enhancement of the commercially available composites due to addition of fluoride remains ambiguous, the need for new antibacterial agents appeared.

1.4.2 Antimicrobial agents

1.4.2.1 Chlorhexidine

Chlorhexidine is a widely known antimicrobial agent that is being used in dentistry especially for treating diseases such as gingivitis or following a periodontal treatment. Chlorhexidine was embodied to resin restorations such as adhesives, composites and glass ionomers. The bacterial inhibitory action of chlorhexidine salts added into composites has been proved with tests that were carried out on agar plates. (Jedrychowski et al., 1983) Several studies explored the behaviour of chlorhexidine- containing dental composites (Riggs et al., 2000; Anusavice et al., 2006; Cheng *et al.*, 2012). It has been showed that the amount of chlorhexidine released after the placement of such a restorative material is influenced by the level of cross- linking between the polymer chains (Riggs et al., 2000). Higher crosslinking reduces water sorption and drug release but increases polymerisation shrinkage and strength. Although the presence of chlorhexidine has a positive antimicrobial effect, the fact that it remains immiscible with the dental monomers, it leads to aggregates in the matrix which upon their dilution in presence of water leave the area porous and deteriorates wear resistance. Thus, staining can be caused.

Similar results were found in another study carried out in which chlorhexidine- releasing HEMA based composites were produced. (Leung *et al.*, 2005) In this study, after the addition of hydrophobic dimethacrylates, UDMA and TEGDMA an increase in the levels of monomer cross- linking was noted which subsequently could control the release rates of chlorhexidine.

Chlorhexidine containing composites are more effective in preventing surface biofilm formation when compared to fluoride-releasing composites. Sufficient expansion due to water sorption could have a positive effect on micro leakage control by balancing polymerisation shrinkage, however, the strength of the materials was low (Leung *et al.*, 2005). It is important to highlight that recent studies have demonstrated an increasing antibiotic resistance to chlorhexidine and some severe hypersensitivity reactions (Kampf, 2016; Pemberton and Gibson, 2012). In addition to that, the decrease in the material's mechanical properties and the surface integrity has led to experiments where chlorhexidine was released from dental composites using mesoporous silica nanoparticles (MSNs) which has improved the previously reported poor physical and mechanical properties. However further investigated in the field is required. (Zhang *et al.*, 2014)

1.4.2.2 Triclosan

Triclosan is a wide spectrum antibacterial agent which was incorporated in toothpastes due to its potential ability to inhibit bacterial development. In a study carried out in 2010 the antibacterial effect of triclosan against Streptococcus mutans, Actinomyces viscosus and Lactobacillus casei, was tested by adding 0.3wt% triclosan into a resin composite matrix. The results showed a reduction in all three cariogenic bacteria, indicating that triclosan could be effectively used in the protection of a restored dental cavity from bacterial infection (Rathke et al., 2017). More recent studies have shown that the addition on triclosan methacrylate in composite can decrease the viability of the cells in the biofilm and also alter its architecture in terms of thickness and surface roughness, suggesting that Triclosan can be used as an antimicrobial monomer in restorative dentistry (Araujo, de Paula, 2018). However, its use was banned by the US Food and Drug Administration FDA in antiseptic soaps due to lack of efficacy. Additionally, in recent epidemiological studies, triclosan was found to increase the risk of asthma, allergies and spontaneous abortions rate and negatively affect fecundity (Weatherly and Gosse, 2017).

1.4.2.3 Benzyalkonium chloride

Benzalkonium chloride is an organic salt and is a quaternary ammonium compound. Due to its antimicrobial activity, it is being used in many products such as eye drops, hand sanitisers, shampoos and mouthwashes. Its bactericidal action is attributed to the disruption of the intermolecular interactions. Studies have shown that the incorporation of benzalkonium chloride into resin composites can increase the antimicrobial effect of the material without having a detrimental effect on its mechanical and physical properties. The release of this agent was continuous and generally constant over the study period of 240 days.(Othman et al., 2002). However, more studies are needed to explore the possibility of their future clinical application.

1.4.2.4 Methacryloyloxydodecylpyridinium bromide

The above-mentioned antimicrobial agents base their action on their release in presence of a wet environment. However, the downside of this is that those agents are being released over a limited period of time and they have detrimental effects on the quality of the restoration. With this being a reality, Imazato et al proposed the idea of the “immobilised bactericide” which

quickly attracted the attention of the dental society. The innovative concept behind this was that antibacterial monomers would be strongly attached, with for instance covalent bonding, to the conventional methacrylate monomers and they would be co- polymerised. Their antibacterial action would be performed once the bacteria come in contact with the material, no more requiring the release of the antibacterial agent. (Imazato *et al.*, 2003) The well-known methacryloyloxydodecylpyridinium bromide (MDPB) which was produced from quaternary ammonium, was successfully incorporated into a dental adhesive agent and has been commercially available since 2004 (Cocco et al., 2015). Regarding the cytotoxicity of this material, experiments run in mouse fibroblasts, odontoblast- like cells and human pulpal cells, showed that MDPB is an acceptable component for dental use.

Since then, many studies were carried out and experimental composites containing MDPB were produced, revealing that this composite could inhibit the attachment, the glucan synthesis and the growth of bacteria in its surface (Imazato *et al.*, 1999; Namba *et al.*, 2009). Additionally, a recent study proved that MDPB composite could inhibit root caries in presence of biological artificial caries (Yoshikawa *et al.*, 2007). The addition of MDPB proved that when it is added in specific concentration it has no effect on the curing performance of the composite meaning that MDPB has an excellent curing ability. (Cocco *et al.*, 2015)

1.4.2.5 Polylysine

Polylysine is a lysine homopolymer and has a yellow colour and a bitter taste. ε-Polylysine (ε-poly-L-lysine, EPL) is typically produced as a homopolypeptide of approximately 25–30 L-lysine residues. The proposed mechanism of antimicrobial action of the εPL was concluded to be an electrostatic interaction with the microbial cell surface, followed by the disorganisation of the outer membrane. This eventually causes cytoplasmic components to leak out of the bacterial cell. ε-Poly-L-lysine is used as a natural preservative in food products. It has been stated that under aerobic conditions ε-Polylisine is produced by Streptomyces albulus ssp which was primarily isolated from Japanese soil. It is biodegradable, water soluble and environmentally safe. ε-Polylisine was tested and proved to be safe and biocompatible.(https://www.thermofisher.com/blog/food/support-for-%C9%9B-poly-lysine-as-natural-antibacterial-preservative/)

It is noted that it is not absorbed by the GI tract as 94% of it is being excreted in faeces. Polylysine remains stable regardless of the pH changes and can inhibit the growth of several micro-organisms including yeast, fungi, Gram positive and negative bacteria. In multiple

studies it was found to cause a reduction in the number of streptococcus mutans and aerobic oral microflora. (Yoshida and Nagasawa, 2003; Shukla et al., 2012).

In dentistry, polylysine is exclusively being used at the Eastman Dental Institute by the research team of the Biomaterials department as an antibacterial agent in the production of the new experimental composite formulations. A major advantage of polylysine is its high hydrophilicity which enables its release from hydrophobic composites (Panpisut *et al.*, 2016). Furthermore, the ability of polylysine to promote deep penetration of composites into dentine tubules and carious dentine was recently discovered (Alkhouri, PhD thesis, in preparation). This enabled effective sealing of the cavity which reduced enzyme activated collagen degradation.

1.4.3 Self- adhesive composites

On an experimental composite produced in a recent study, adhesive monomers 4Meta and PMDM were incorporated into CaP containing composites. These adhesive monomers diffuse into dentinal tubules and due to capillarity, they spread across them. Therefore, the less viscous an adhesive agent or flowable composite is, the easier and more effectively this micro-mechanical retention occurs. Ionic bonding also develops between these monomers and calcium in the tooth hydroxyapatite. The adhesion properties of these composites were tested in ivory dentine due to its similarities to human dentine. The results showed that these two adhesive monomers enhanced the shear bond strength that could reach 26MPa. This could be further improved by adding an adhesive agent (iBond) and could reach 30 MPa. (Aljabo, 2015; Liaqat et al., 2015).

1.4.4 Remineralising Composites

1.4.4.1 Calcium phosphates

Calcium phosphates consist of calcium (Ca^{2+}) and phosphate ions (PO_4^{3-}) in different ratios. Generally, solubility deceases as Ca/P ratio increases. They dissolve in basic solutions then precipitate as hydroxyapatite that exhibits great similarities to the apatite of the teeth. It is considered therefore as a biocompatible material. (Goto *et al.*, 2006). Presence of calcium and phosphate in a dental composite could potentially lead to the remineralisation of the damaged carious tooth structure (Reynolds, 2008). Therefore, many compounds including amorphous calcium phosphate ACP, tetracalcium phosphate TTCP and mono dicalcium phosphate have been added to composites as fillers.

1.4.4.2 Amorphous calcium phosphate

Amorphous calcium phosphate ACP when added to a methacrylate matrix can be released as calcium and phosphate ions in an aqueous environment.(Skrtic et al., 1996) Although this can enhance tooth structure remineralisation the fact that ACP aggregates in particles can jeopardise the mechanical stability of the composite and deteriorate the already existing problem of all composites which is their low strength and toughness. (Regnault et al., 2008)

1.4.4.3 Tricalcium phosphate

Tricalcium phosphate (TCP) is a calcium salt of phosphoric acid with the chemical formula Ca3(PO4)2. It is a white solid of low solubility. Most commercial samples of "tricalcium phosphate" are in fact hydroxyapatite. MCPM and β-TCP have been added together in dental composites. The β-TCP enables more control over the MCPM dissolution and composite water sorption. Highly soluble MCPM on the surface of the material dissolves but in the bulk, it reacts with the β-TCP to form less soluble, water-binding brushite (dicalcium phosphate dihydrate) crystals. (Aljabo, 2015)

In the above study, remineralisation potential was generally assessed through calcium and phosphate release determination (Mehdawi *et al.*, 2009). Predicting the release levels required to promote remineralisation, however, is complex and dependent upon many other parameters. Alternatively, ability of the composites to promote HA deposition in simulated

dentinal fluid has been studied (Aljabo, 2015). This feature, apart from providing remineralisation of adjacent collagen, could also potentially enable the closure of marginal gaps between the material and tooth and reduce bond deterioration over time.

1.4.4.4 Monocalcium phosphate monohydrate

Monocalcium phosphate monohydrate (MCPM) is an inorganic compound with the chemical formula Ca(H2PO4)2. It can be found as food additive in animals' food as it provides them with the essential calcium and phosphorus for the development of healthy bones and skeleton as well as a functional nervous system and it is also used as a leavening agent that makes baked food to rise. It is a white or grey hydrophilic powder that exhibits good solubility in water and enhances water sorption. As it easily dissolves, it can make the dentinal fluid supersaturated and that promotes hydroxyapatite precipitation. A study carried out in the field of remineralising and antibacterial composites revealed that MCPM was a key player in providing remineralising ions and apatite precipitation. It can also enhance antibacterial agent release. (Alqadi, 2016). Furthermore, when employed with polylysine there is evidence that it enhances tubule sealing which inhibits enzyme activity (Alkhouri, PhD thesis in preparation).

1.5 Summary

Dental caries exhibits a multifactorial nature and still remains a significant public health issue, while its management can be extremely challenging in specific age groups. Children, due to lack of previous dental experience may present with increased anxiety, which often leads to failure in restorative treatment, and subsequently to the extraction of the affected tooth. A new material that would allow the provision of a simple restoration of a carious cavity, without the necessity of local anaesthetic administration or caries removal by drilling the tooth, would allow caries management in young children in an efficient and quick way. In this field, SMART composite is a revolutionary experimental material that aims to combine self - adhesion to the cavity with remineralisation of the carious dentine and release of an antimicrobial agent. Several studies have been carried out in order to determine a material with, amongst others, optimal monomer conversion, low polymerisation shrinkage, apatite precipitation and resistance in mechanical wear (Kangwankai *et al.*, 2017). Although previous studies have been carried out at the Eastman Dental Institute on the bond strength of novel experimental composites (Liaqat *et al.*, 2015), the wide range of the different agents and their various combinations that have been tried over the time render further tests in the field necessary. Development of a strong composite material that reduces the need for drilling and simplifies

the tooth restoration in paediatric patients, would minimise the need for tooth extraction and / or treatment under general anaesthesia. SMART composites have therefore been developed with MCPM and PLS. Together those promote effective dentine seal by producing long and extensive resin tag formation in carious dentine (Alkhouri, PhD thesis in preparation) but their effect on bonding requires elucidation.

1.6 Aims and objectives

The aim of this study is to assess the effect of varying remineralising MCPM and antibacterial and collagen binding PLS levels on novel composite bonding to ivory and to carious human dentine.

The objectives of this study:

1. Bonding to human caries is affected by the tooth heterogeneity. Furthermore, obtaining sufficient teeth can be challenging. Therefore, ivory was used which was a helpful material to address these issues. However, sample size needs to be large and differences in structure (lower tubule density and apatite content) considered.

2. A control test was initially carried out for both ivory and human dentine. In that test, the samples were placed in a dry storage, following the composite restorations, for 24 hours. In order to simulate real conditions of the oral cavity, the control test results were compared against the bond strength that the experimental formulations achieved when the ivory and the human teeth samples were emerged in a liquid medium. This allowed us to understand how each material behaves in a liquid medium and how the bond strength is affected by the time.

3. Executing the same experiments, using the same formulations and under the same conditions in human teeth, gave us the chance to compare the bond strength results of ivory with those of human dentine. As human teeth can be a challenge to collect and use as a sample due to extensive caries, small size and unusual morphology, the results taken from human teeth were much less than those given by ivory blocks and further experiments need to be carried out.

The null hypothesis of this study was that the levels of MCPM and PLS do not affect the bond strength of the novel experimental composite formulations in the different conditions that the tests were carried out.

2 Chapter - Materials and Methods

2.1 Introduction

In this study, bond strength of six different composite formulations with high versus low levels of remineralising and antibacterial agents are described. The tests were executed initially in ivory blocks, giving the similarities that ivory dentine has with human dentine (Liaqat, 2015), using a push out test. In this test, a flat intender applies load to the top surface of a composite restoration into the ivory dentine holes and the composite is gradually pushed out. Different ways of dentine conditioning as well as storing of the samples were tried prior to the push out test. The dentine underwent either no conditioning or was prepared with acid etch and/or bond, whereas the restored ivory -following the composite placement and light curing - were stored either in a dry environment or in a liquid media for different periods of time. The maximum force was recorded and afterward the maximum strength was calculated using the area of each hole. The results of the above – mentioned test were then compared to those of a push out test, carried out to assess the behaviour of the same materials under the same circumstances, in human dentine. In previous studies, push out bond strength of glass fibre posts to ivory dentine was tested (Thanjal and Tina, 2011)

2.2 Materials and methods

2.2.1 Ivory Dentine Structure

Ivory is a substance derived from the tusks and teeth of several animals, mainly elephants. It is widely used in art and manufacture as well as in dentistry where it serves as a well-known biomaterial due to its similarities to human dentine.

As with human dentine, ivory consists of an inorganic and an organic component. The inorganic phase, which is Hydroxyapatite (HA) - like crystals, forms the 60%-70% of its mass. Approximately 18% of the Ca ions in the HA crystal lattice is substituted by Mg ions (Cui, F. et al, 1994; Su and Cui, 1999). The organic part is type I collagen and accounts for 30% of the ivory mass. There is also 0%- 10% of water.

Recent Raman studies showed that the level of hydroxyapatite in ivory before etching was half that of human dentine and it was reducing by half every 23sec of etching with 37% phosphoric acid. The ivory dentine showed less strength than human dentine (56 MPa) possibly due to this lower lever of hydroxyapatite. In the same study, the average bond

strength, as measured using the push out test in presence of acid etch and Ibond, was calculated as 25-40MPa and 23-37 MPa with Ibond and without acid etch. In cases where no Ibond was used, in formulations with 4-META the average bond strength was 15-30 MPa whereas in those with HEMA it was 8-15MPa. The application of acid etch did not seem to have had a great impact on the bond strength. Additionally, increased level of CaP (0-40% of the filler) caused a downward trend in bond strength. (Liaqat et al., 2015)

Dentinal tubules in ivory run the area from the central pulp to the periphery of dentine-peripheral layer known as cementum (Su and Cui, 1999). Around the tubules, the collagen fibrils are radially organized in two layers and as they are interconnected they create a collagen network. In each layer, the fibrils sit parallel to each other and they are rotated from one layer to the next. Between consecutive collagen molecules, there are spaces, which are known as gap zones, and in which the crystals of the hydroxyapatite are deposited.(Jakubinek et al, 2006). Figure 2.1 shows the differences between ivory and human dentinal tubules.

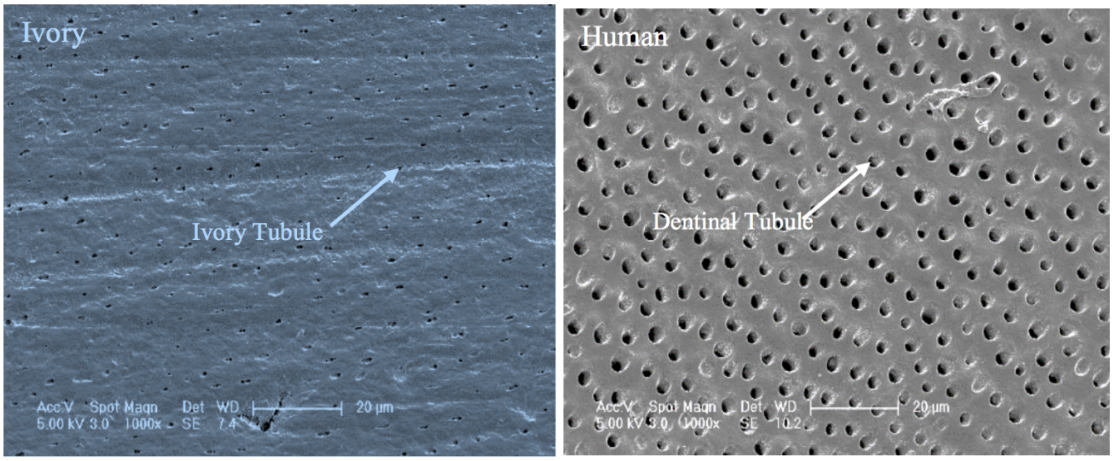


Figure 2.1 - Ivory and human dentinal tubules. The density of dentinal tubules in ivory dentine is half that of human dentine (Saad Liaqat PhD thesis, 2015)

2.2.2 The push out test

In a push out test the material tested is being mechanically pushed out of another material. In dentistry, push out tests have been carried out to measure the strength of different obturation materials (Fisher et al., 2007).

In this study, the push out test was performed using the Shimadzu machine and ivory / human dentine. A 5 mm diameter cylindrical stainless-steel plunger applied a constant compressive load on the composite at a speed of 0.5 mm/min until bond failure occurred. The plunger was only in contact with the composite and there was no contact with the ivory walls. The ivory

disks were placed in such a way to permit the plunger to move uneventfully from one side of the hole to the other. A real-time computer software program gave load versus time while the push out test was executed. The de-bonding of the composite occurred when a sharp decline was noted on the graph and/or there was a complete displacement of the composite out of the ivory disc. Subsequently, the bond strength was calculated in MPa by dividing the load in Newtons by the internal cylindrical area of the ivory hole.

The shear, tensile and push-push out tests can both be used to measure the bond strength of a composite. Shear and tensile tests will give significantly different results because they do not account for polymerisation shrinkage (Mahdi et al., 2013) or subsequent water sorption induced expansion. The push-out test with the use of confined spaces, simulates better the clinical situation of a cavitated tooth. It accounts for dimensional changes and exhibits higher C-factor (ratio of bonded surfaces to un-bonded surfaces), something that allows for better flow of the material and reduced internal stresses in it (Thanjal and Tina, 2011).

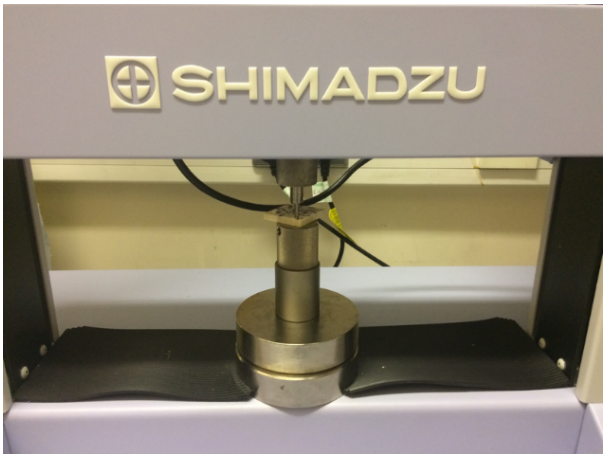


Figure 2.2 - Shimadzu machine used for push out test

2.2.3 Ivory preparation

The ivory was cut to give blocks of 33 x 30 x 5 mm sections as shown on the picture below (Figure 2.3). The holes were then drilled into cut blocks of 3 mm in diameter and 5 mm in height. The cut sections were placed in distilled water for 24 hours in an incubator at 37 ^{0}C, and then left to dry in the same temperature for 24 hours. This provided a slightly moist dentine which was found to improve reproducibility (Liaqat et al., 2015). Eventually, the cavities were fully filled with composite pastes in bulks. Each composite sample, after its placement in the ivory holes was cured for 40 seconds in both sides of the hole. The samples were then stored in an incubator at 37 ^{0}C for 24 hours prior to the push out test. Ivory holes were re-used for the needs of each experiment. Each formulation was tested in five different holes and there was rotation of the formulations in different holes.

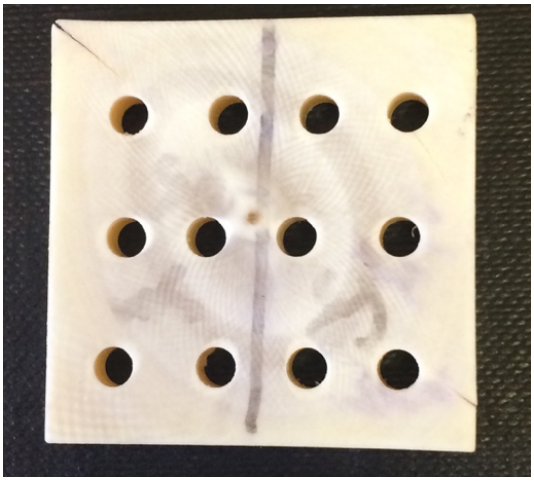


Figure 2.3 - Ivory used for push out test

The ivory was prepared in three different ways:

1) No acid and no bond application
2) Phosphoric acid 35% for 20 seconds followed by water rinsing and gentle air drying
3) Phosphoric acid for 20 seconds followed by water rinsing and gentle air drying. Subsequently iBond was applied, dried with light air spray and then light cured for 20 seconds as per manufacturer's instructions.

The samples were then stored in three different ways and for three different periods of time:

1) In dry environment for 24 hours (used as a control condition)
2) In SBF for 24 hours, 1 week and one month
3) In artificial saliva for 24 hours, 1 week and 1 month

The push out test was then executed and the maximum strength was calculated using the Maximum force required to debond the composite restoration and the internal cylindrical area of the hole.

2.2.4 Teeth collection and preparation

The teeth were extracted mainly under General Anaesthesia and collected in the Eastman Biobank under the 1304 ethical approved protocol. Prior to teeth extraction and collection, the patients' parents/legal guardians were informed about the study and the need for teeth collection verbally and through an information leaflet. Upon participation agreement a consent was signed (appendices – consent form).

Subsequently, the samples were washed under running water and all blood and adherent tissues were carefully removed the day after the extractions. Primary teeth with extensive carious cavities were excluded. Teeth were disinfected with Chloramine T for 2 days (maximum 1 week) and were stored in deionised water in the refrigerator (ISO 3696:1987, grade 3). The water was changed every 2 weeks to prevent bacterial growth on the samples. Prior to the execution of each experiment, the samples were removed from the refrigerator and maintained in the room for four hours in order to obtain room temperature.

The teeth were moulded in epoxy resin. Permanent molars were mainly used. The teeth were subsequently cross sectioned in 2mm width discs into dentine after enamel was removed. From each tooth, 2 sample discs were obtained. In total, 10 teeth were used and 20 samples were collected for this test. Following this, a hole was drilled in the non - carious part of the disc (ensuring that enough dentine was available on the walls) using a diamond bur in high speed under water to create a cavity of 3 – 5mm. This was sufficient to enable the piston of Shimadzu machine to pass through during the push out test. In cases that extensive caries would not allow for a sound cavity to be prepared, caries to firm dentine were removed. The human dentine samples had no conditioning prior to the composite restoration placement which was followed by light curing for 20 seconds. From other studies we know that this gives sufficient monomer conversion in a 2 mm cavity (Katsimpali, DDent project, unpublished thesis in preparation). Control samples were placed in a dry storage in an incubator at 37 ^{0}C for 24 hours prior to the push out test. The rest were submerged in artificial saliva for 24 hours, 1 week and 1 month in the incubator at 37 ^{0}C. The push out test was then executed and the maximum strength was calculated using the Maximum force required to debond the composite restoration and the internal cylindrical area of the hole.

2.2.5 Formulations tested

The experimental formulations consist of a powder and a liquid phase. The powder phase is made of glass particles, MCPM and PLS. Glass particle appear in three different sizes. 60% of glass particles are of 7μm, 30% of 0.7μm and the rest is nanoglass particles. MCPM particle sizes are 50μm with the exception of F5small which is 10μm. MCPM appears in 8% or 4% whereas PLS in 2% or 4%.

The liquid phase consists of 73% UDMA, 24% PPGDMA, 3% 4-META and 1% Camphoroquinone (CQ). UDMA is a monomer with low viscosity and PPGDMA is a diluent monomer with high molecular weight. 4-META is an acidic monomer that enhances self-

adhesion through ionic interactions and CQ is a photo initiator that initiates polymerisation in presence of a light source.

The powder / liquid ratio of 3:1 or 4:1 (see Table 1). The samples were prepared in the lab facilities of Synergy Devices Ltd by Dr Wendy Xia.

Seven experimental composite formulations were tested in this thesis. The variables are given in Table 1.

Table 1 – Experimental formulations used.

Name of Formulation	Remineralising % (Monocalcium Phosphate)	Antibacterial % Polylysine (PLS)	Powder / Liquid Ratio
Control	0	0	0
F2	8	2	4
F5small	8	4	3
F5	8	4	3
F6	4	4	3
F7	8	2	3
F8	4	2	3

F5 small is similar to F5 in the amount of Monocalcium Phosphate, Polylysine and Powder / Liquid Ratio. The Monocalcium phosphate particle size in F5 small is 10 microns, while for all the other formulations is 53 microns.

2.2.6 Equipment

- Ivory pieces 5mm thickness and 3mm diameter
- Human teeth
- Phosphoric acid 35%
- Adhesive (iBond Total Etch)
- Simulated Body Fluid (SBF)
- Artificial Saliva
- Incubator – 37^0C
- Shimadzu Machine
- Dental curing light

Artificial Saliva

Preparation was done according to "Artificial saliva preparation from Macknight-Hane & Whitford 1992 without sorbital (Levine et al, 1987)".

Composition gL^{-1}	
Methyl – p – hydroxybenzoate (Na salt)	2.3
Sodium Carboxymethyl Cellulose	10.00
KCl	0.625
$MgCl_2$. 6 H_2O	0.059
$CaCl_2$. 2 H_2O	0.166
K_2HPO_4 . 3 H_2O	1.040 (1.055)
KH_2PO_4	0.326

Table 2 - Artificial Saliva composition

The pH of the artificial saliva was adjusted to 6.75 with 1.0 M HCl

Simulated Body Fluid

Preparation was done under the ISO 23317:2007(E)

Components and quantity for 1L

1	NaCl	8.035 g
2	$NaHCO_3$	0.355 g
3	KCl	0.225 g
4	$K_2HPO_4 \cdot 3H_2O$	0.231 g
5	$MgCl_2 \cdot 6H_2O$	0.311 g
6	1.0 M HCl	39.0 ml
7	$CaCl_2$	0.292 g
8	Na_2SO_4	0.072 g
9	$((HOCH_2)_3CNH_2)$	6.118 g
10	1.0 M HCl	Appropriate amount for adjusting the pH $\sim$ 7.4

Figure 2.4 - SBF composition

2.3 Experiments

2.3.1 Experiment 1 – Bond strength in ivory dentine – dry storage

Bond strengths of the experimental composites were tested using ivory dentine as in the methods described below. Ivory dentine had no etch / no bond, etch, etch & bond conditioning prior to the composite application. In all cases the method involved:

Step 1: The ivory blocks were submerged in water at 37^0C incubator for 24 hours.
Following this the ivory blocks were semi dried by leaving in the 37^0C incubator for another 24 hours.

Step 2: Three sets of ivory cavities were prepared**:**

a. no conditioning,
b. etched with 35% phosphoric acid for 20 seconds followed by rinsing with water then drying with paper and mild air spray.
c. Etch preparation as in "b" followed by iBond total etch application in the walls of each hole and light cure for 20 seconds each side.

Step 3: The ivory holes were filled in bulks with the above-mentioned composite formulations and light cured for 40 seconds each side. For each formulation 5 repetitions were carried out (5 holes were used for each composite).

Step 4: Subsequently, they were placed an incubator at 37^0C in a dry storage for 24 hours

Step 5: In each experiment, the push-out test was executed using the Shimadzu machine and the maximum force required to de-bond the composite from each hole was recorded. Using the following equation (Strength = Maximum Force / Area) the strength was calculated, where area was the composite-ivory interface equal to π x (diameter of hole) x (depth of hole)

2.3.2 Experiment 2 – Bond strength in ivory dentine – SBF

In this experiment, ivory bond strength was assessed following samples emersion in SBF. Steps 1, 2, 3 and 5 are the same as on Experiment 1.

Step 4: Ivory blocks were placed and kept in SBF, in the incubator at 37^0C, for 24 hours, 1 week or 1 month.

2.3.3 Experiment 3 – Bond strength in ivory dentine – Artificial saliva

In this experiment, ivory bond strength was assessed following samples emersion in Artificial saliva. Steps 1, 2, 3 and 5 are the same as on Experiment 1 and 2.

Step 4: Ivory blocks were placed and kept in Artificial saliva, in the incubator at 37^0C, for 24 hour, 1 week or 1 month.

2.3.4 Experiments 4/5 - Push out test protocol (human teeth) – Pilot study

1. Teeth preparation: Primary (ideally) or permanent teeth disc shaped (2mm thickness) with healthy walls and carious cavity in the middle. The carious cavity should ideally be circular.

2. Measurement of each sample in terms of sample thickness and cavity area.

3. Caries removal to firm but not hard dentine.

4. No dentine preparation prior to composite restoration.

5. Cavities filled with experimental formulations (F5, F6, F7 and F8)

6. Time and type of medium: samples placed for 24 hours in a dry storage (control study) and for 24 hours, 1week and 1 month in artificial saliva.

7. Shimadzu push out test using supplemental equipment for tooth support in the Shimadzu machine (for example a metal flange as shown in the Figure 2.4)

8. Calculation of maximum strength to de-bond the composite restoration from the tooth.

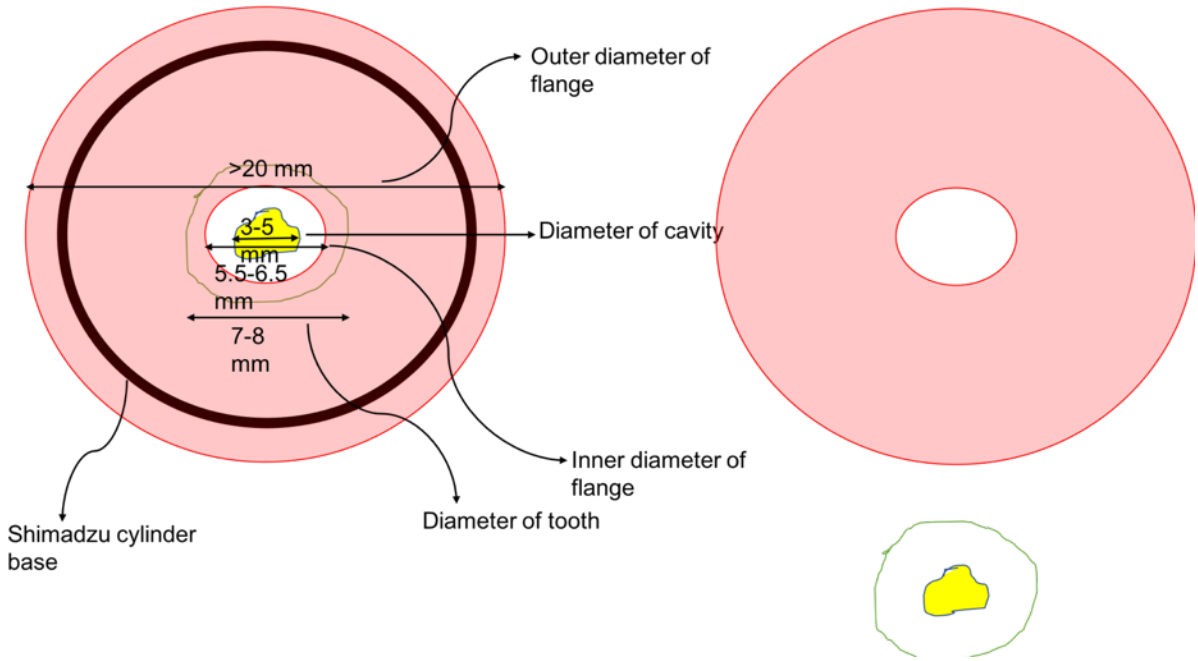


Figure 2.5-Design shows how a cylinder base can be used to support the tooth sample (disc) for the completion of the push out test in Shimadzu machine.

2.3.4.1 Experiment 4 – Bond strength in human dentine – dry storage

Experimental composite formulations were tested in human dentine after samples being placed in a dry environment for 24h without any dentine conditioning.

Method

Step 1: The human teeth samples were submerged in water at 37^0C incubator for 24 hours. Following this the samples were dried and left at 37^0C incubator for another 24 hours.

Step 2: In each sample, the caries was removed with high speed hand piece until no obvious caries was visible. The holes were then dried with gentle air spray and their dimensions were measured.

Step 3: The holes were filled in bulks with the above-mentioned composite formulations and light cured for 20 seconds. For each formulation 5 repetitions were carried out.

Step 4: Subsequently, they were placed in a dry storage in the incubator at 37^0C for 24 hours.

Step 5: Eventually the push-out test was executed using the Shimadzu machine and the maximum force required to de-bond the composite from each hole was recorded.

Step 6: Using the following equation (Strength = Maximum Force / Area) the strength was calculated, where area was the composite-ivory interface equal to π x (diameter of hole) x (depth of hole).

2.3.4.2 Experiment 5 – Bond strength in human dentine – Artificial saliva

Method

Steps 1, 2, 3, 5 and 6 were the same as on Experiment 4.

Step 5: The samples were placed in Artificial saliva in the incubator at 37^0C for 24 hours, 1 week and 1 month.

2.3.5 Experiment 6 - Ivory demineralisation with formic acid (Calibration Curve – Mass loss vs Time)

The aim of this test was to identify how the bond strength is affected when the material is placed into demineralised due to caries dentine, the first step was to replicate the level of demineralisation of dentine in a carious tooth using formic acid and ivory due to its similarity with human dentine. By submerging the ivory into formic acid, the hydroxyapatites react with the formic acid which leads to the demineralisation of the ivory and subsequently to the relevant mass loss. This test allowed us to discover the relationship between mass loss of ivory against time due to its exposure to formic acid of specific volume and concentration. When formic acid ***HCOOH*** reacts with hydroxyapatite ***$Ca_{10}(PO_4)_6(OH)_2$, 4Ca*** are released -which is responsible for the mass loss-. We also have the formation of 6 dicalcium phosphate ***$6CaPHO_4$*** and $2H_2O$. ***$Ca_{10}(PO_4)_6(OH)_2(s) + 8\ H(aq) = 6\ CaHPO_4 + 2\ H_2O + 4\ Ca$***

Method

3 different pieces of ivory (a, b, c) placed in distilled water for 3 weeks, dried with paper and gentle air spray until no obvious humidity was detected and then weighted (0.1mg precision used)

a- Mass: 1.3mg

b- Mass:1.6mg

c- Mass: 0.8mg

Following this each ivory piece was placed separately in 10 ml Formic acid Normality 4. The mass was measured every 30 min the first 6 hours and then intervals were gradually increased as the rate of mass change dropped. When the test had to be paused for a few hours, ivory was kept in water and then before placed in formic acid again was dried as before. Formic acid was changed in 24hours as acid weakened. The pH was checked afterwards in 4 days, 1 week and 2 weeks

2.4 Statistics

For all data shown in bar charts the error bars represent the 95% confidence interval. This was calculated using

$$95\%\mathrm{CI} = \frac{2 \times Standard\ Deviation}{\sqrt{n}}$$

Where n is the sample number. Unless otherwise stated, the sample number was 5.

For the statistical analysis of the results, SPSS program was used. For the majority of the cases, **Kruskal Wallis test** was performed as the data that was collected did not follow a normal distribution. In cases where two samples had to be compared against each other, a **Mann – Whitney U Test** was carried out.

2.4.1 Null hypotheses

The null hypotheses to be tested were that the following had no significant effect on bond strength:

1) Formulation including
   - **a)** MCPM level
   - **b)** PLS level
   - **c)** Particle size
   - **d)** PLR

2) Conditioning of dentine including
   - **a)** none
   - **b)** etching
   - **c)** etch and bond

3) Storage solution or time
   - **a)** dry 24 hours
   - **b)** SBF
   - **c)** AS for
     - **i.** 24 hours
     - **ii.** 1week
     - iii. 1 month

3 Chapter - Results

3.1 Bond strengths to ivory

3.1.1 Experiment 1 - Dry storage

3.1.1.1 Dry storage - No etch or bond

In Figure 3.1 material bond strengths to ivory with neither etch nor with bond are shown. Error bars suggest F2 and F8 exhibited significantly higher bond strength than all other formulations except F5, with F8 achieving the highest of 13MPa. Control, F5 small, F6 and F7 bond strength were all ~7MPa.

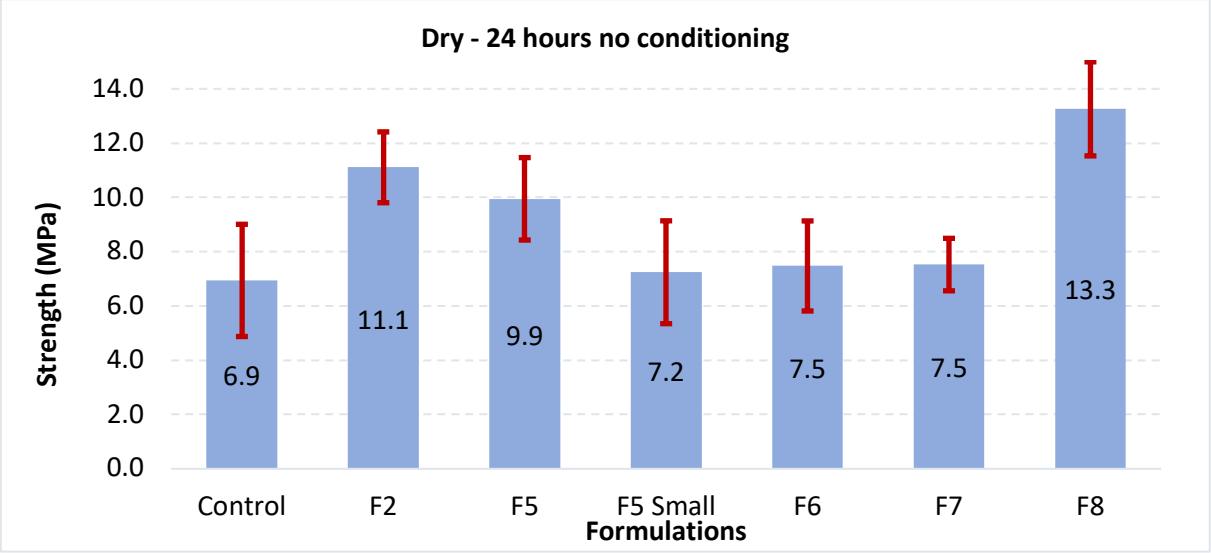


Figure 3.1 Push out test results - Bonding strength of each formulation in ivory with no etch / no bond dentine preparation.

A Kruskal Wallis test confirmed there was statistically significant difference in the data for different formulations (see Figure 3.2). The test showed that there was significant difference between F8 and; Control (P = 0.002), F5 small (P = 0.006), F6 (P = 0.037) and F7 (P = 0.043).

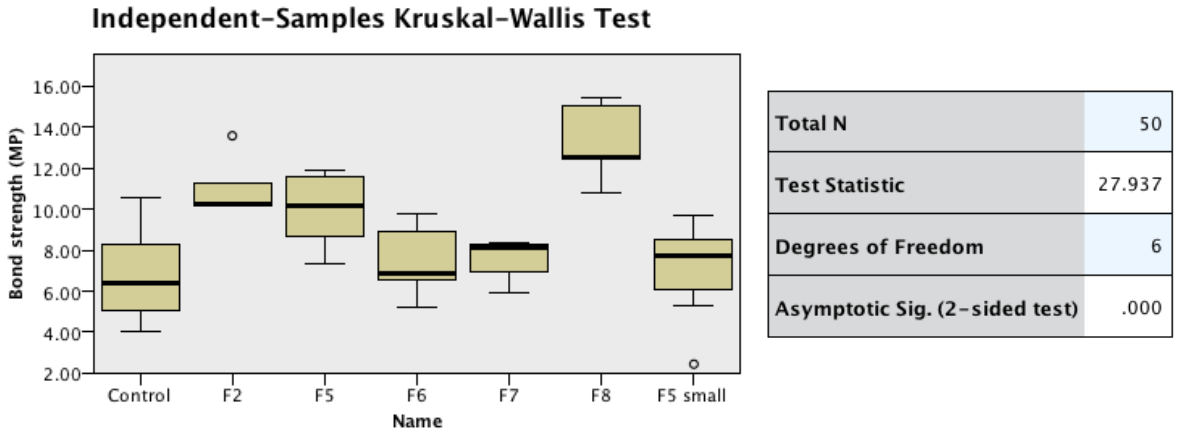


Figure 3.2-Kruskal – Wallis test for bond strength vs formulations (ivory with no etch / no bond dentine preparation, placed in a dry storage for 24 hours prior to the push-out test)

Furthermore, the Mann – Whitney U test confirmed F5 exhibited higher bond strength than the same formulation but with smaller MCPM and glass particles, F5 small (P = 0.009) (Figure 3.3). Additionally, F2 bond strength (with PLR =4) was statistically higher than for the same formulation with PLR=3, F7 (P = 0.008) (Figure 3.4).

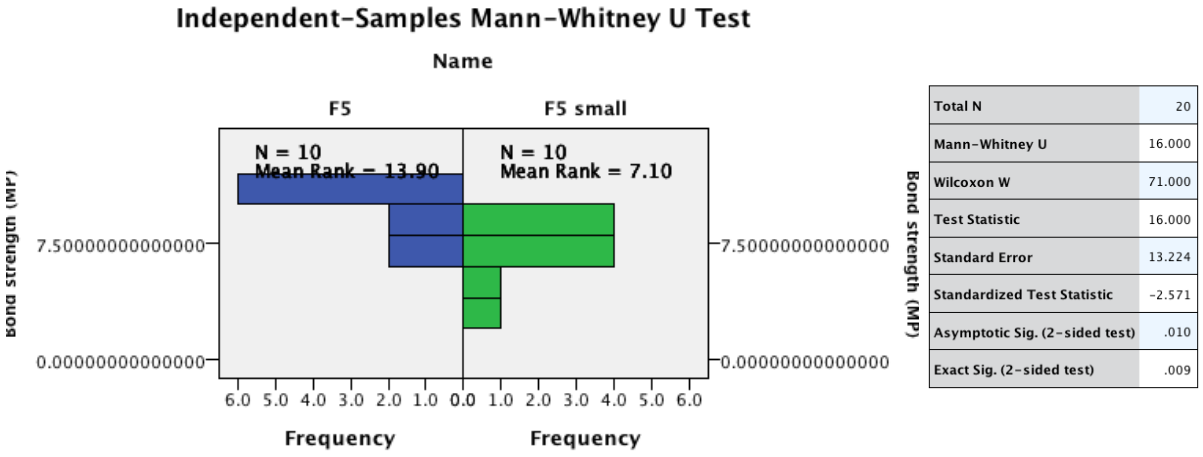
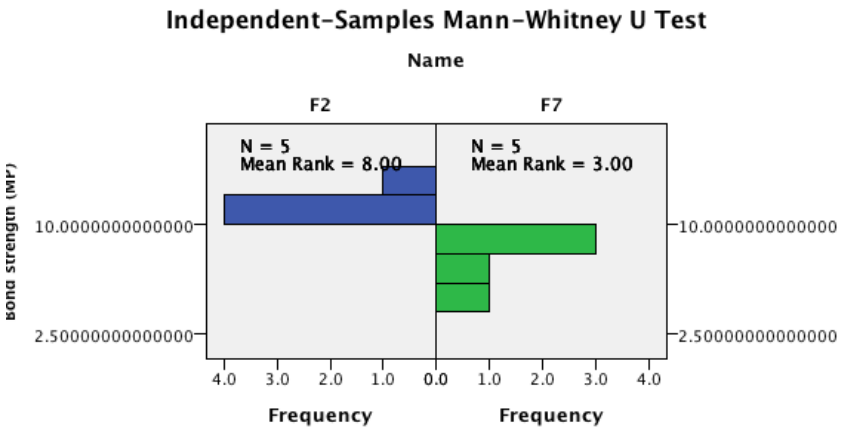


Figure 3.3-Mann-Whitney test of the bond strength of F5 versus F5 small (Ivory with no etch / no bond dentine preparation, placed in a dry environment for 24 hours prior to the push-out test)



Total N	10
Mann-Whitney U	.000
Wilcoxon W	15.000
Test Statistic	.000
Standard Error	4.787
Standardized Test Statistic	-2.611
Asymptotic Sig. (2-sided test)	.009
Exact Sig. (2-sided test)	.008

Figure 3.4-Mann-Whitney test of the bond strength of F2 (PLR=4) versus F7 (PLR=3) (Ivory with no etch / no bond dentine preparation, placed in a dry environment for 24 hours prior to the push-out test)

3.1.1.2 Dry storage - etch no bond

For dry stored samples where the ivory was etched, F8 again exhibited the highest bond strength (14MPa) followed by the rest of the formulations that reached bond strengths of 8-11 MPa (Figure 3.5). The Kruskal Wallis test, however, confirmed the null hypothesis, that there was no significant difference between the formulations (P = 0.124).

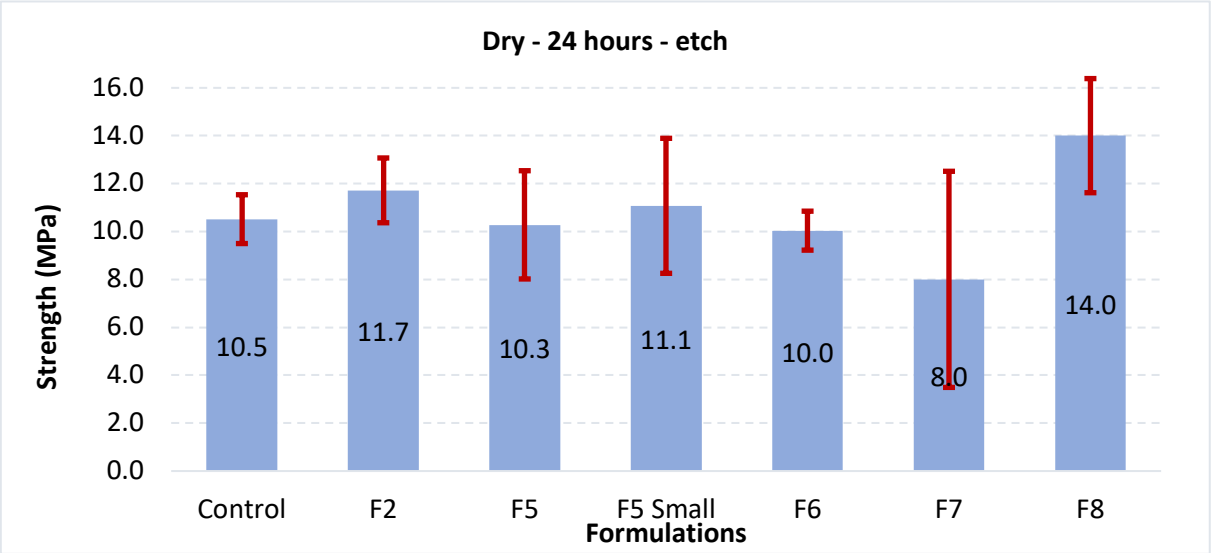


Figure 3.5- Push out test results - Bonding strength of each formulation in Ivory - etch dentine preparation, placed in a dry environment for 24 hours prior to the push-out test

3.1.1.3 Dry storage - etch & bond

Following 24 hours in dry environment with etch and bond conditioning of dentine prior to restorations, all the formulations apart from the control reached bond strengths between 13.9 – 16.6 MPa (Figure 3.6). The Kruskal – Wallis test (Figure 3.7) confirmed significant difference in the bond strength between Control and; F8 (P = 0.008), F2 (P = 0.005), F7 (P = 0.004) and F5 small (P = 0.000). No significant difference was detected between F2, F5, F6, F7, F8 and F5 small.

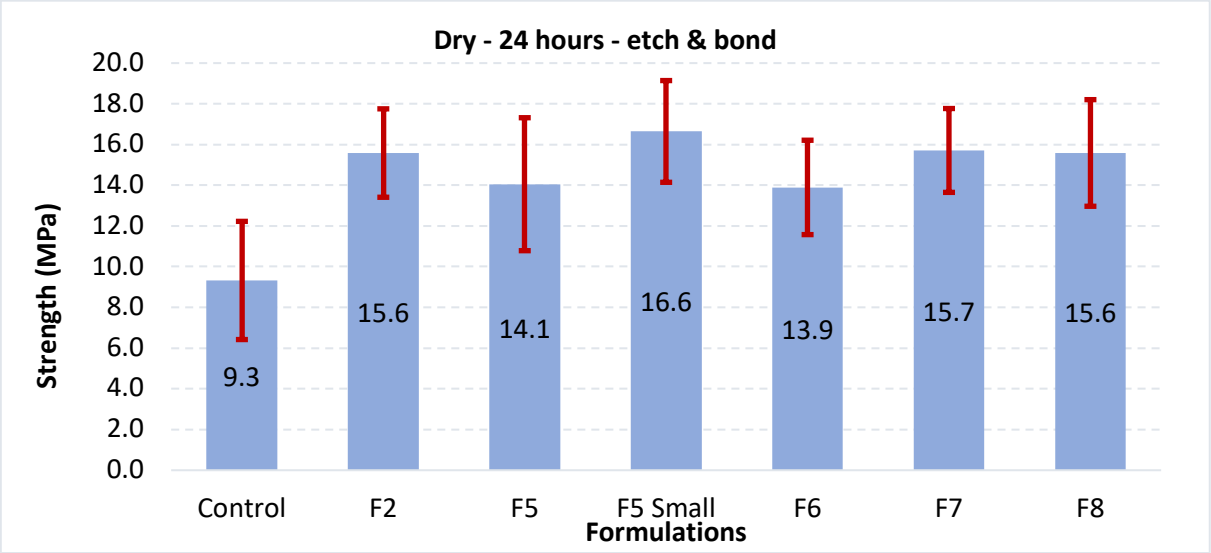


Figure 3.6 Push out test results - Bonding strength of each formulation in Ivory – etch & bond dentine preparation, placed in a dry environment for 24 hours prior to the push-out test

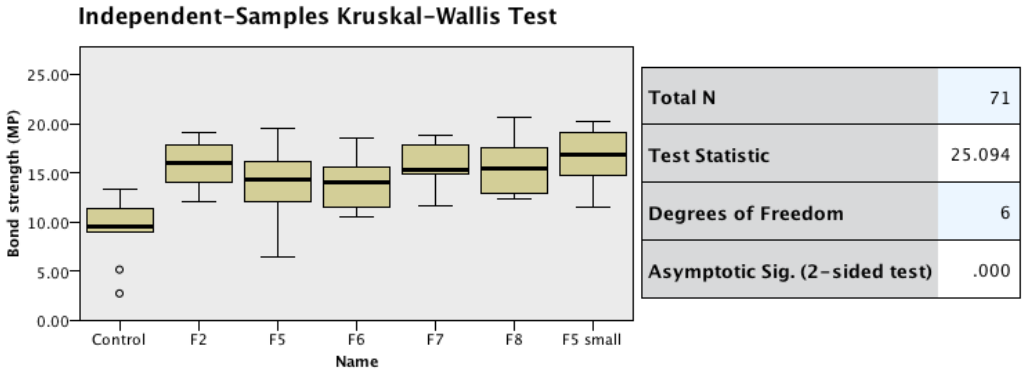


Figure 3.7 - Kruskal – Wallis test for bond strength vs formulations (ivory with etch & bond dentine preparation, placed in a dry environment for 24 hours prior to the push-out test.

3.1.2 Experiment 2 - Storage in SBF

3.1.2.1 No etch or bond

3.1.2.1.1 24 hours in SBF

Figure 3.8 gives bond strengths of seven formulations in ivory with no etch or bond following when 24 hours in SBF. F8 again had the highest bond strength (10.2 MPa). F6 had the lowest (4.2MPa). The rest of the formulations reached bond strengths of 8-10 MPa. The Kruskal Wallis test indicated no significant differences between samples (P = 0.639). Furthermore, Whitney test showed no significant difference between F2 and F6 (P = 0.143).

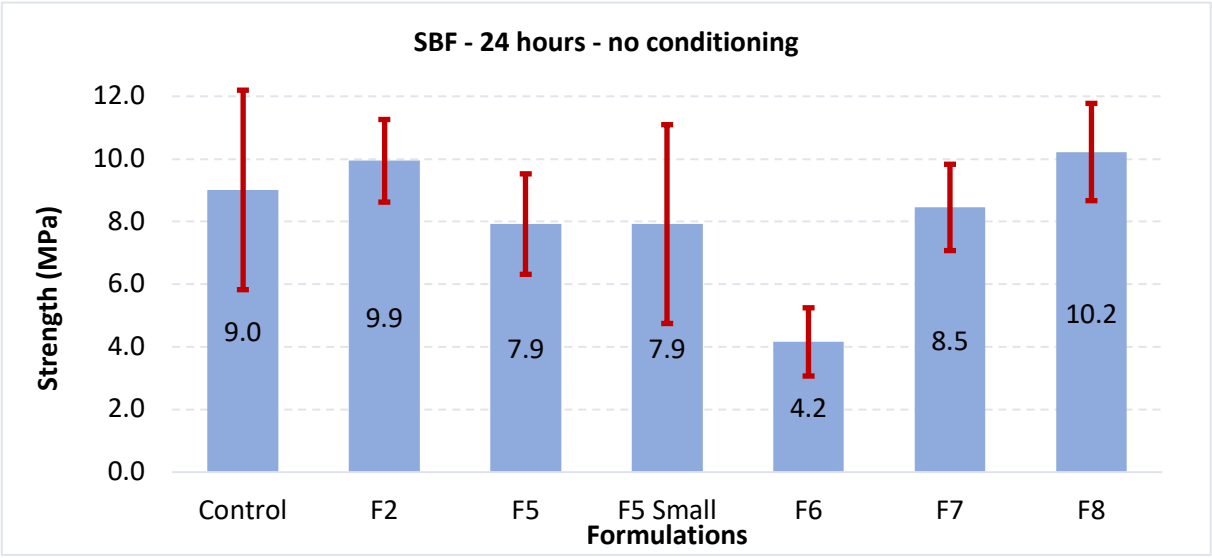


Figure 3.8 Push out test results - Bonding strength of each formulation in Ivory – no etch / no bond dentine preparation, placed in SBF for 24 hours prior to the push-out test

3.1.2.1.2 1 week in SBF

Following 1 day in SBF, Figure 3.9 shows results on the bond strength, with no ivory dentine conditioning, after samples being placed in SBF for 1 week. All the formulations exhibited low bond strengths (0.6 – 3.1MPa). F6 again had the lowest bond strength while F5 small had the highest. A Kruskal Wallis test (Figure 3.10) confirmed the bond strength with F5 small was significantly higher than with F6 (P = 0.024) – Figure 3.10.

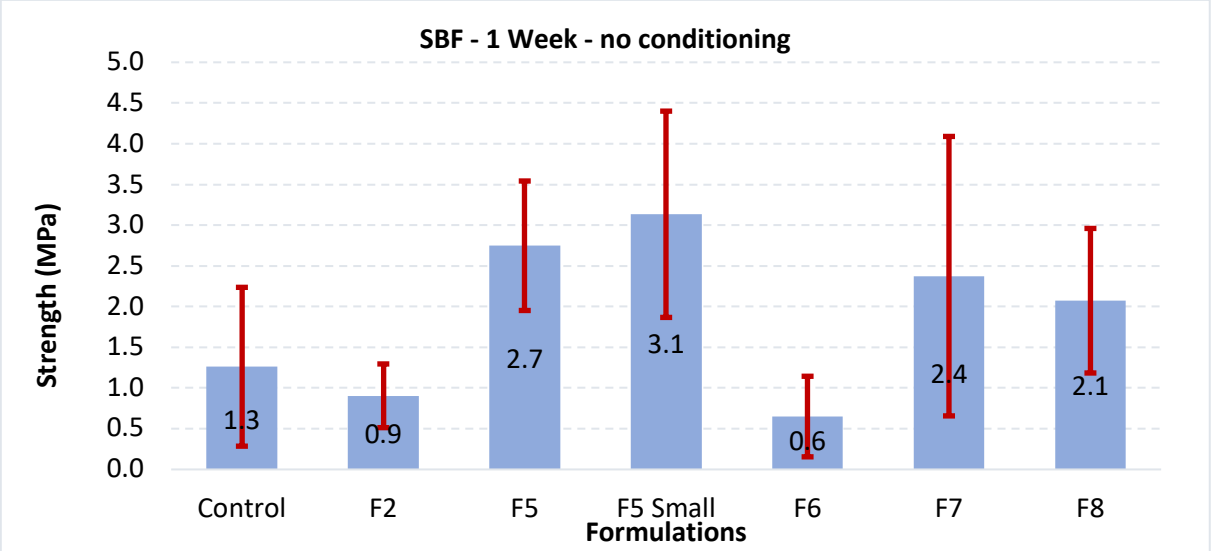


Figure 3.9 - Push out test results - Bonding strength of each formulation in Ivory – no etch / no bond dentine preparation, placed in SBF for 1 week prior to the push-out test

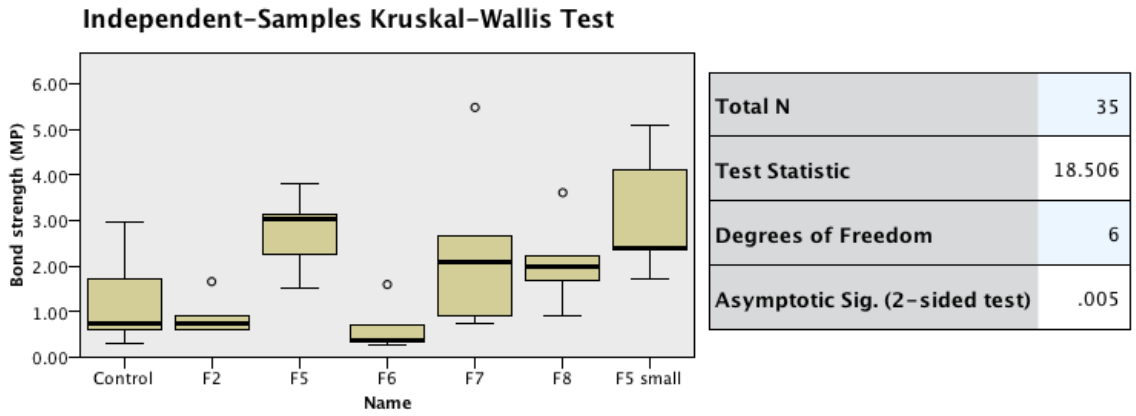


Figure 3.10 - Kruskal – Wallis test for bond strength vs formulations (ivory with no etch / no bond dentine preparation, placed in SBF 1 week prior to the push-out test)

3.1.2.1.3 1 month in SBF

Following 1 month in SBF, F6 exhibited the lowest bond strength (1.7MPa) while all the others reached levels between 4.3MPa and 6.5MPa (Figure 3.11). F5 reached the highest bond strength. A Kruskal Wallis test retained the null hypothesis that there was no significant difference between the samples, although the P was 0.055.

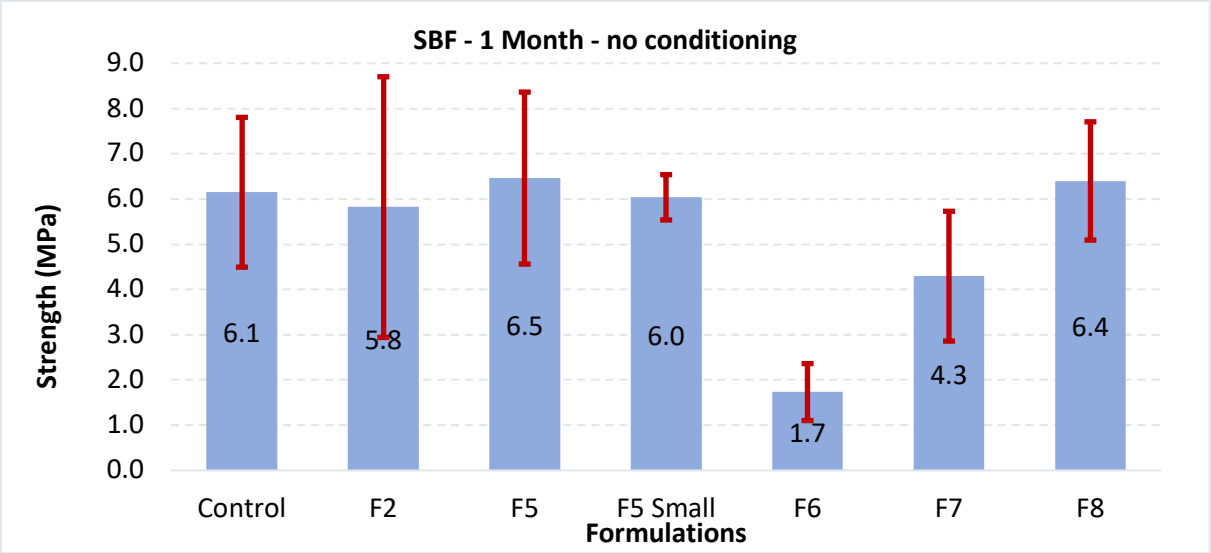


Figure 3.11 - Push out test results - Bonding strength of each formulation in Ivory – no etch / no bond dentine preparation, placed in SBF for 30 days prior to the push-out test

Following this, the different formulations were tested for significantly important difference between them in pairs based on common characteristics. First the formulations with the same powder / liquid ratio were assessed F5, F6, F7, F8 and F5 small. The null hypothesis was rejected as there was significantly important difference between F5 and F6 bond strength (P = 0.018) as shown on the graph below (Figure 3.12).

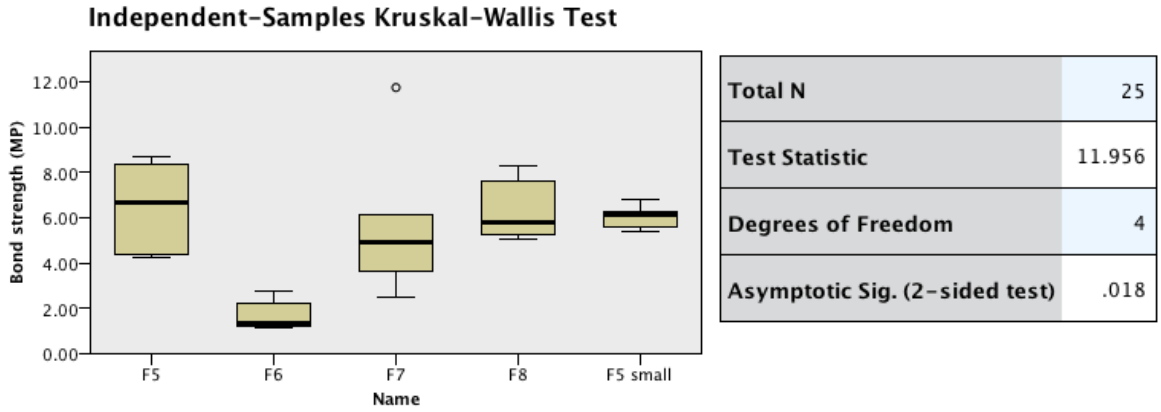


Figure 3.12 - Kruskal – Wallis test for bond strength vs formulations (ivory with no etch / no bond dentine preparation, placed in SBF for 30 days prior to the push-out test)

Subsequently F5 was compared with F5 small as the latter has smaller size particles. The null hypothesis was retained (P = 0.841).

Based on the different powder liquid ratio F2 was compared F7 as they have the same amount of MCPM (8%), the same level of PLS (2%) but different powder / liquid ratio. F2 powder / liquid ratio is 4 whereas F7 is 3. The null hypothesis was retained (P = 1.000)

3.1.2.2 Etch no bond

3.1.2.2.1 24 hours in SBF

Figure 3.13 provides the bond strengths following etch of dentine and restoration storage in SBF for 24 hours. Control, F2, F5, F6 and F8 have the lowest bond strength to the ivory dentine under those conditions. F5 small and F7 reached slightly higher magnitudes of 2.3MPa and 3.7MPa respectively. A Kruskal Wallis test rejected the null hypothesis of no significant difference between Control – F7 (P = 0.008) and F5 – F7 (P = 0.048) (see Figure 3.14).

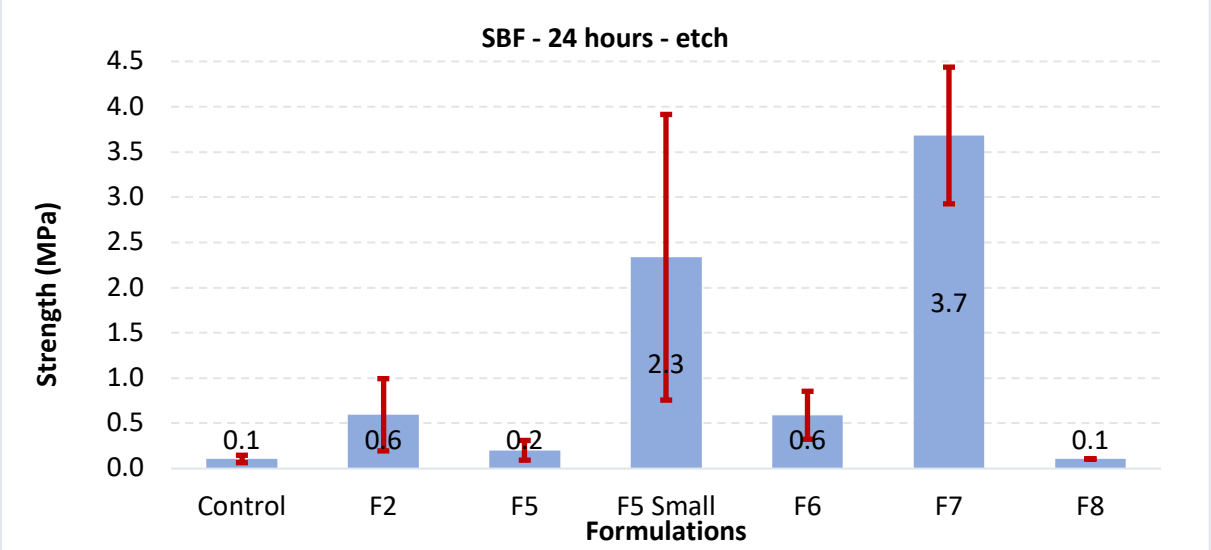


Figure 3.13 - Push out test results - Bonding strength of each formulation in Ivory – etch dentine preparation, placed in SBF for 24 hours prior to the push-out test

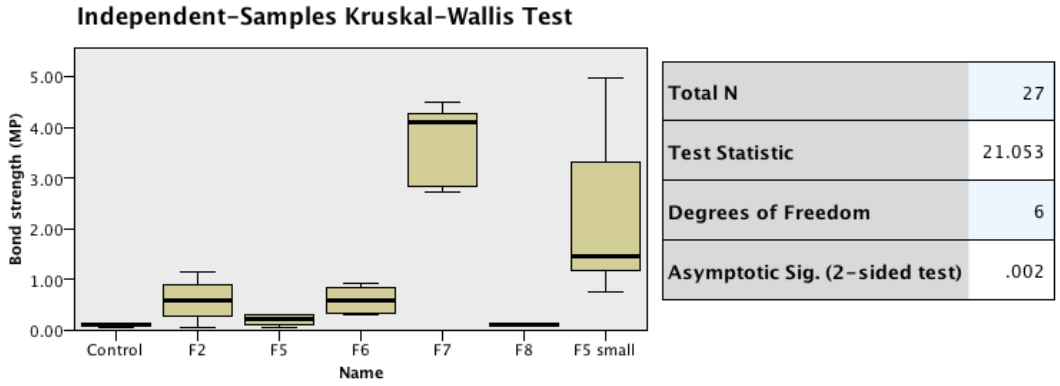


Figure 3.14 - Kruskal – Wallis test for bond strength vs formulations (ivory - etch dentine preparation, placed in SBF for 24 hours prior to the push-out test)

3.1.2.2.2 1 week in SBF

Figure 3.15 shows that F8 reached the highest bond strength (7MPa) for 1 week in SBF following previous dentine conditioning with etch. Control and F2 showed the lowest bond strength (1-2 MPa) while for the rest of the formulations the bond strength ranged between 4MPa – 5MPa.

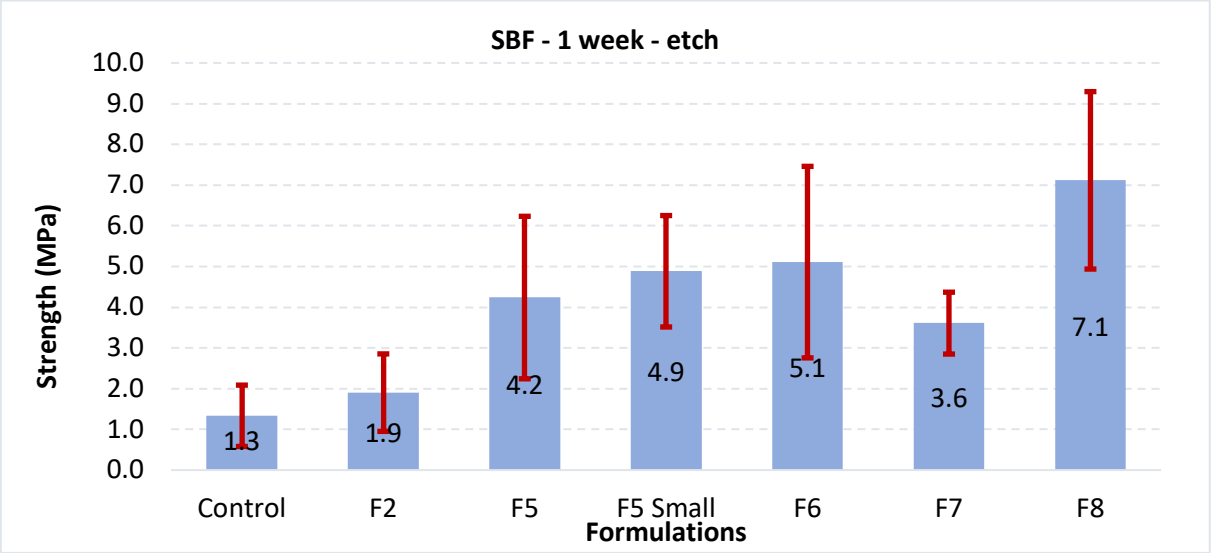
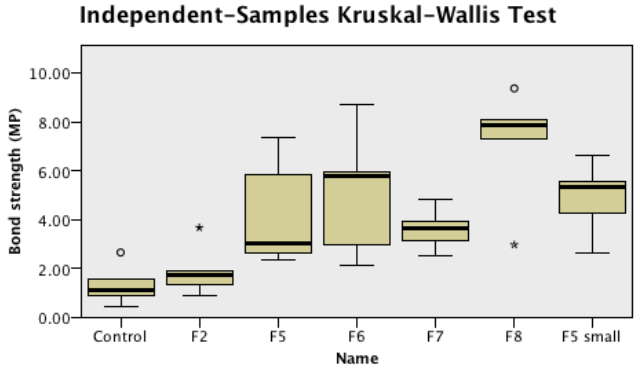


Figure 3.15 - Push out test results - Bonding strength of each formulation in Ivory – etch dentine preparation, placed in SBF for 1 week prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above – mentioned conditions. The test performed rejected the null hypothesis indicating that there is statistically important difference between Control – F8 (P = 0.006) and between F2 – F8 (P = 0.28) as shown in the graphs below (Figure 3.16).



Total N	35
Test Statistic	19.688
Degrees of Freedom	6
Asymptotic Sig. (2-sided test)	.003

Figure 3.16 - Kruskal – Wallis test for bond strength vs formulations (ivory - etch dentine preparation, plac in SBF for 1 week prior to the push-out test)

3.1.2.2.3 1 month in SBF

Figure 3.17 shows that F6 and F7 have the highest bond strength (9 MPa), followed by F2 (6 MPa) when ivory dentine was prepared with etch prior to storage in SBF for 1 month. Control, F5, F5 small and F8 showed the lowest bond strength of 1 – 2 MPa.

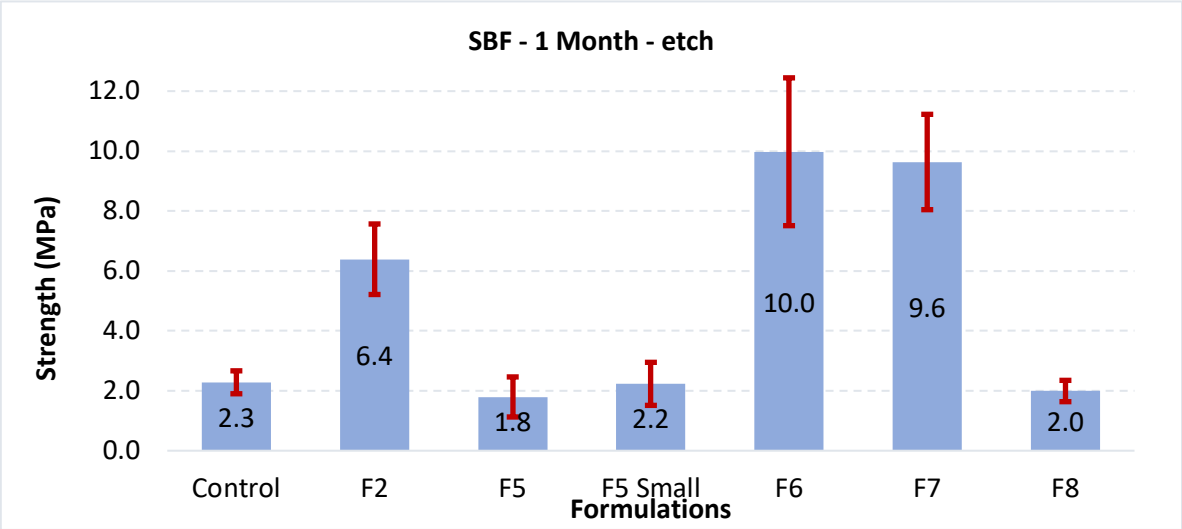


Figure 3.17 - Push out test results - Bonding strength of each formulation in Ivory – etch dentine preparation, placed in SBF for 30 days prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength. The test performed rejected the null hypothesis indicating that is statistically important difference between F5 – F6 (P = 0.009) and between F5 small – F6 (P = 0.045) as presented on the graph below (Figure 3.18).

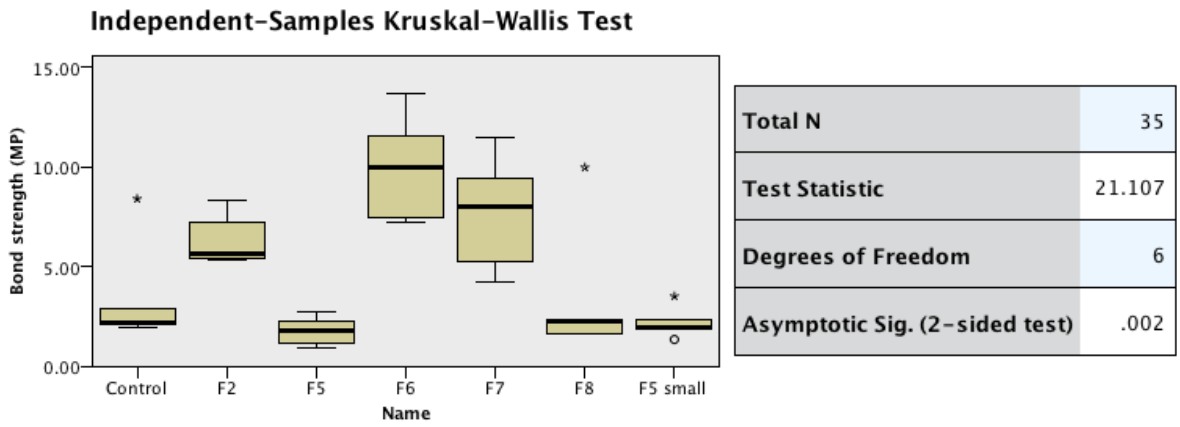


Figure 3.18 - Kruskal – Wallis test for bond strength vs formulations (ivory - etch dentine preparation, placed in SBF for 30 days prior to the push-out test

3.1.2.3 Etch and bond

3.1.2.3.1 24 hours in SBF

Figure 3.19 shows that all the formulations reached low levels of bond strength in presence of etch and bond conditioning for 24 hours in SBF. F8 exhibited the lowest bond strength with the ivory dentine in those conditions (0.8MPa) and F2 the highest (3.2MPa).

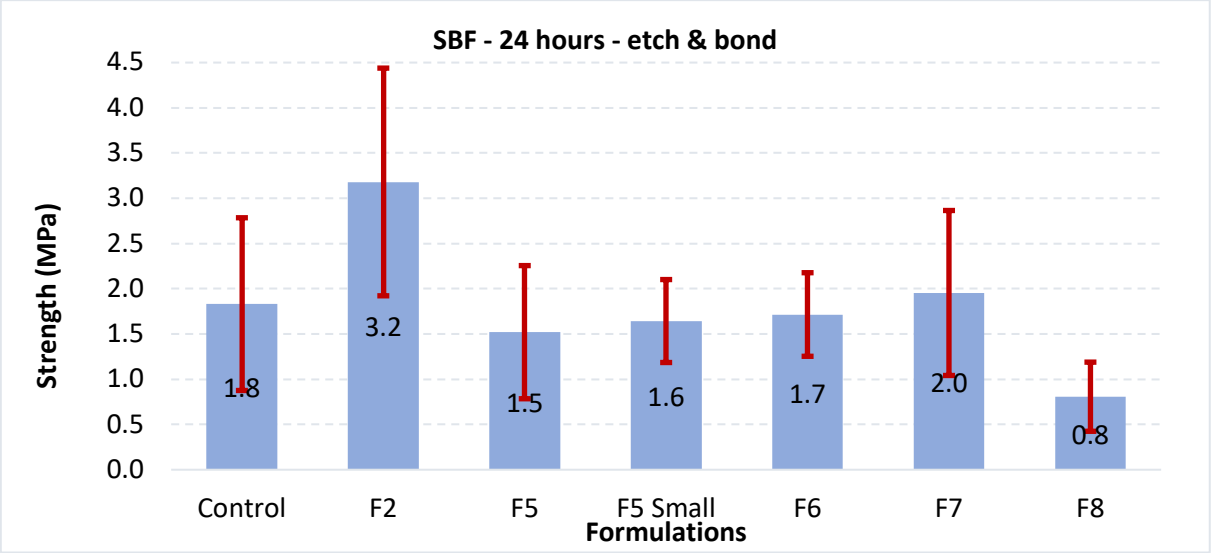


Figure 3.19 - Push out test results - Bonding strength of each formulation in Ivory – etch & bond dentine preparation, placed in SBF for 24 hours prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above - mentioned conditions. The test performed rejected the null hypothesis as there was significantly important difference in the bond strength between F8 – F2 (P = 0.032) as shown on the graph below (Figure 3.20).

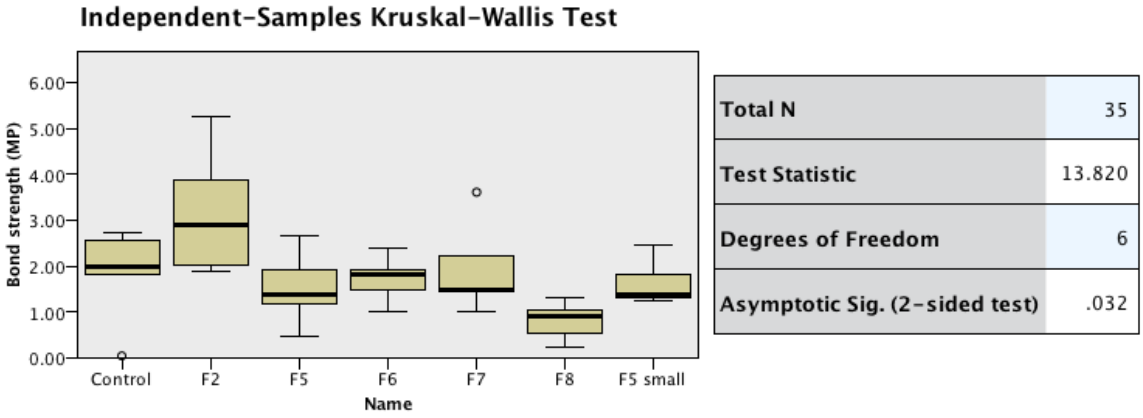


Figure 3.20 - Kruskal – Wallis test for bond strength vs formulations (ivory - etch & bond dentine preparation, placed in SBF for 24 hours prior to the push-out test)

3.1.2.3.2 1 week in SBF

Figure 3.21 represents the bond strengths to ivory dentine when samples were placed in SBF for 1 week with etch and bond dentine conditioning prior to the composite application. Control and F2 showed the lowest bond strength just below 1 MPa, although F2 exhibited the highest bond strength in 1 day. F5 small showed the highest bond strength of 8MPa while the rest of the formulations reached bond strengths between 4MPa and 6MPa. The error bars in the majority of the cases were large due to the results being equally distributed in a wide range.

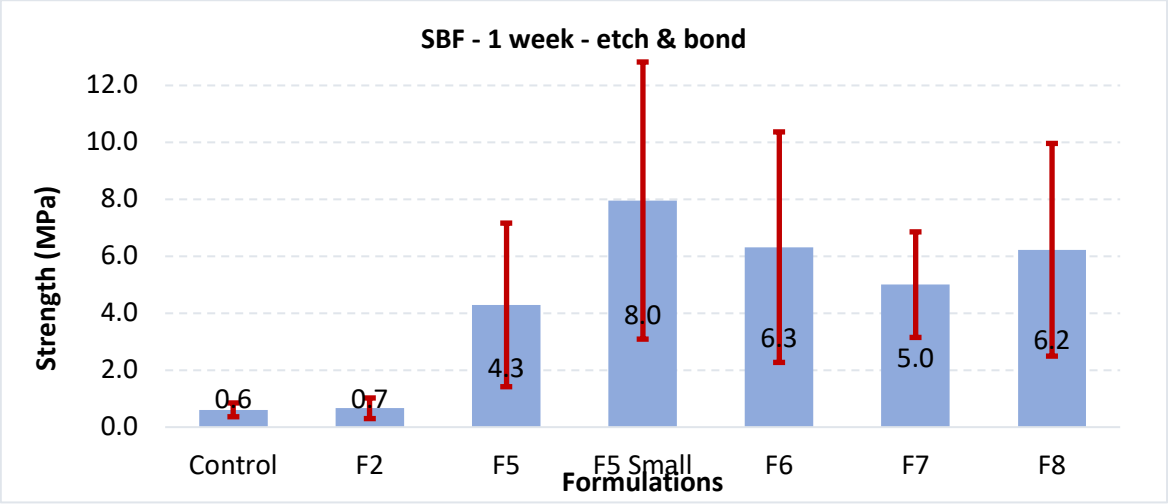


Figure 3.21 - Push out test results - Bonding strength of each formulation in - Ivory – etch & bond dentine preparation, placed in SBF for 1 week prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference. The test performed rejected the null hypothesis (P = 0.001) as presented on the graph below (Figure 3.22). Specifically, there is significantly important difference between Control – F7 (P = 0.045), Control – F8 (P = 0.034), Control – F5 small (P = 0.034), F2 – F7 (P = 0.045), F2 – F8 (P = 0.034) and F2 – F5 small (P = 0.034)

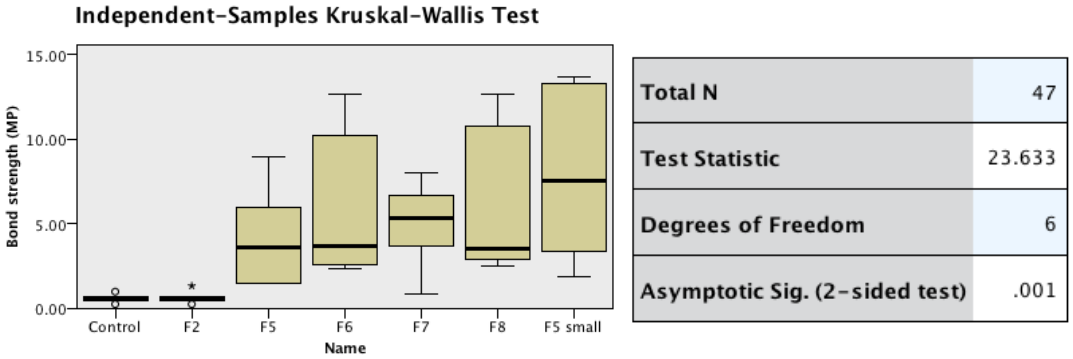


Figure 3.22 - Kruskal – Wallis test for bond strength vs formulations (ivory - etch & bond dentine preparation, placed in SBF for 24 hours prior to the push-out test)

3.1.2.3.3 1 month in SBF

In SBF for 30 days, with the ivory dentine having been treated with etch and bond prior to the composite application F8 reached the highest bond strength close to 12MPa while F5 small and F6 bond strength was half that of F8. Control and F2 exhibited bond strength around 8-9MPa, double that of F5 and F7 (Figure 3.23)

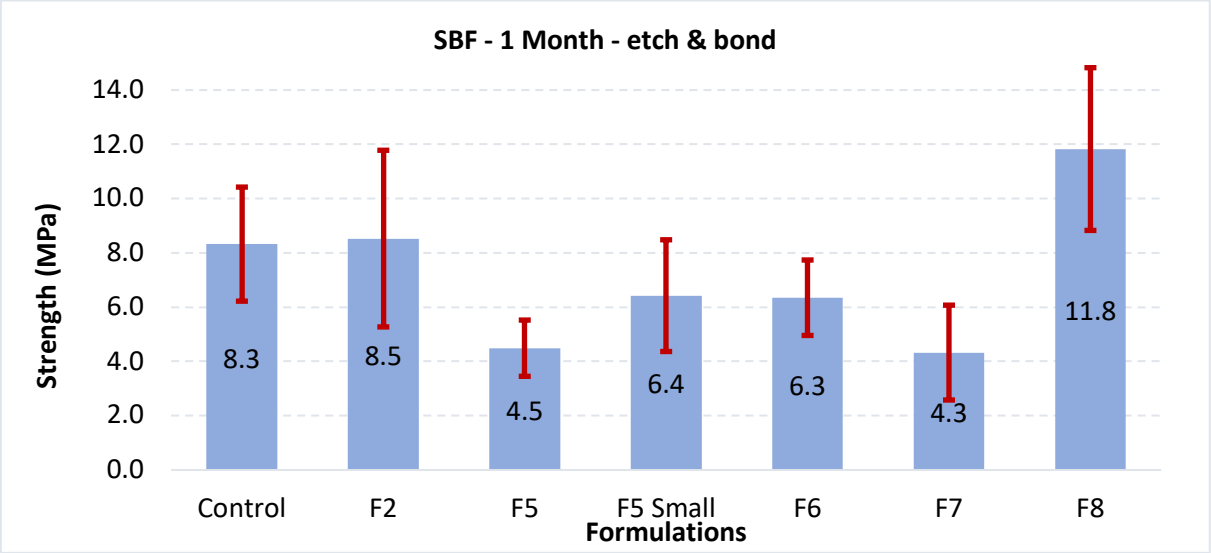


Figure 3.23 - Push out test results - Bonding strength of each formulation in Ivory – etch & bond dentine preparation, placed in SBF for 1 week prior to the push-out test

The Kruskal Wallis test rejected the null hypothesis (P = 0.008) as presented on the graph below (Figure 3.24). Specifically, significantly important difference was detected between F5 – F8 (P = 0.031) and F7 – F8 (P = 0.031)

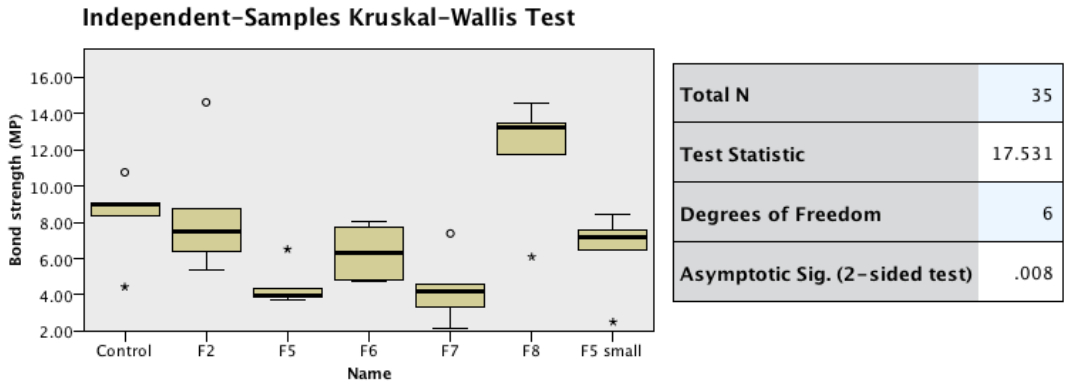


Figure 3.24 - Kruskal – Wallis test for bond strength vs formulations (ivory - etch & bond dentine preparation, placed in SBF for 1 week prior to the push-out test

3.1.3 Experiment 3 - Storage in Artificial saliva

3.1.3.1 No etch no bond

3.1.3.1.1 24 hours in Artificial Saliva

For 24 hours in saliva with no conditioning of the ivory dentine prior to the composite application, F2 and F5 reached the highest magnitudes ~10MPa, while control and F8 exhibited the lowest bond strength to ivory dentine. The bond strength of the other three formulations ranged between 5-7 MPa. (Figure 3.25) The large error bars on F5 and F6 are caused by the fact that the results are being equally distributed in a wide range.

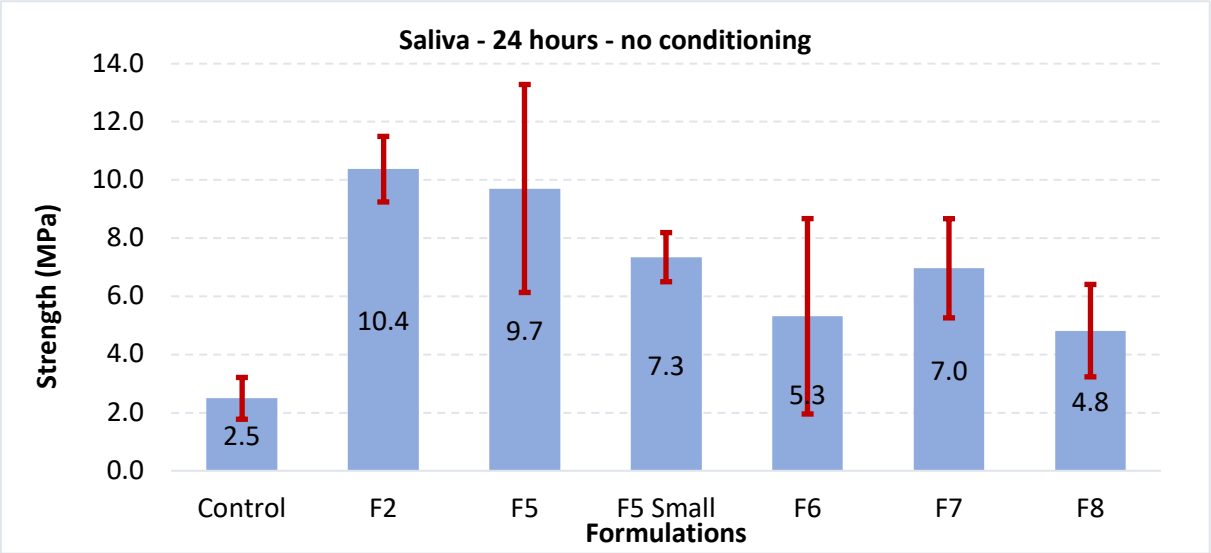


Figure 3.25 - Push out test results - Bonding strength of each formulation in Ivory – no dentine conditioning, placed in Artificial saliva for 24 hours prior to the push-out test

The Kruskal Wallis test retained the null hypothesis as no significant difference was noted between the bond strength of the different formulations (P = 0.2)

3.1.3.1.2 1 week in Artificial Saliva

Figure 3.26 represents the bond strength of six formulations against a control when ivory samples were placed in saliva for 1 week with no conditioning of the ivory dentine prior to the composite application. F6 bond strength reached 8 MPa, the highest magnitude in this test, followed F2, F5 and F5 small which reached roughly 6 MPa. Control, F7 and F8 exhibited the lowest bond strength (2-3 MPa).

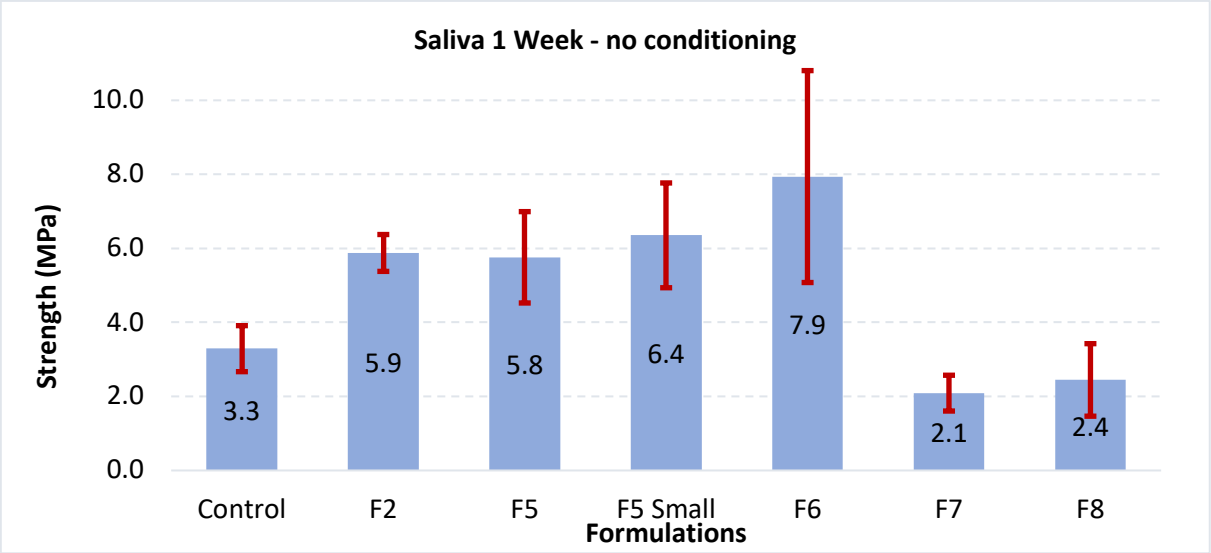


Figure 3.26 - Push out test results - Bonding strength of each formulation in Ivory – no etch / no bond dentine preparation, placed in Artificial saliva for 1 week prior to the push-out test

As expected, the Kruskal Wallis test rejected the null hypothesis (P = 0.003) as shown in the graph below (Figure 3.27). Significantly important difference was noted in the bond strength between F7 – F6 (P =0.20) and F8 – F6 (P =0.35).

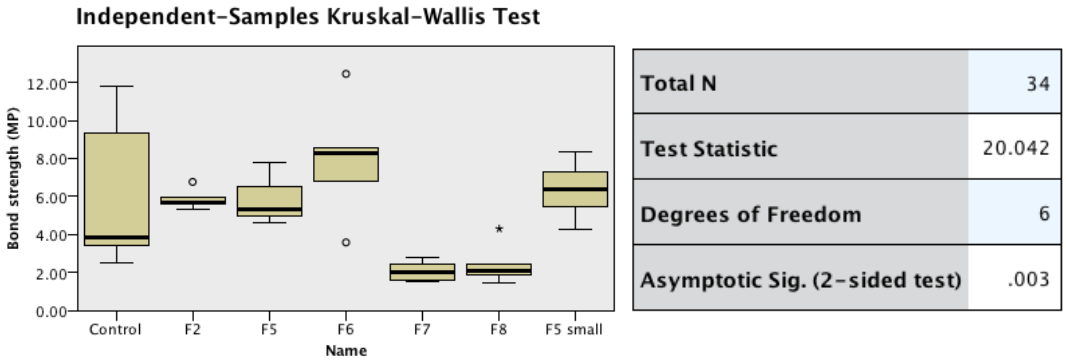


Figure 3.27 - Kruskal – Wallis test for bond strength vs formulations (ivory – no etch / no bond dentine preparation, placed in Artificial saliva for 1 week prior to the push-out test)

3.1.3.1.3 1 month in Artificial Saliva

Figure 3.28 represents the bond strength of six formulations against a control when ivory samples were placed in saliva for 1 month with no conditioning of the ivory dentine prior to the composite application. F6 reached again the highest magnitude in bond strength as well as F5 did, whereas F7 exhibited again the lowest bond strength. F6 appears to have a large error bar, indicating that there is a wide range of results equally distributed.

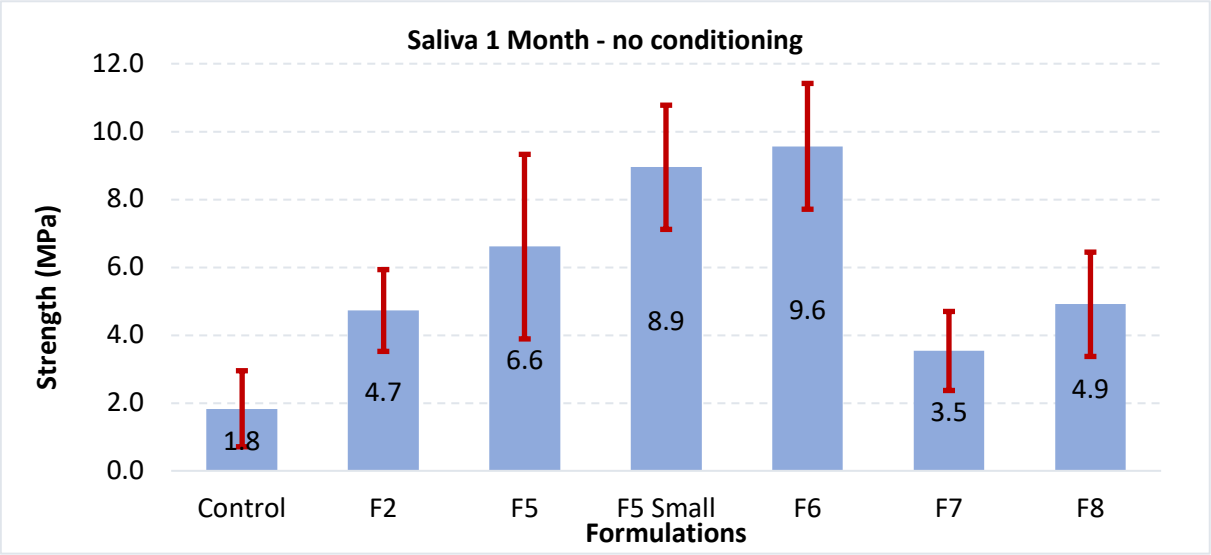


Figure 3.28 - Push out test results - Bonding strength of each formulation in Ivory – no etch / no bond dentine preparation, placed in Artificial saliva for 30 days prior to the push-out test

The Kruskal Wallis test rejected the null hypothesis (P = 0.001) as shown in the graph below (Figure 3.29). Specifically, significantly important difference was detected in the bond strength between Control – F5 small (P = 0.04) and Control – F6 (P = 0.07).

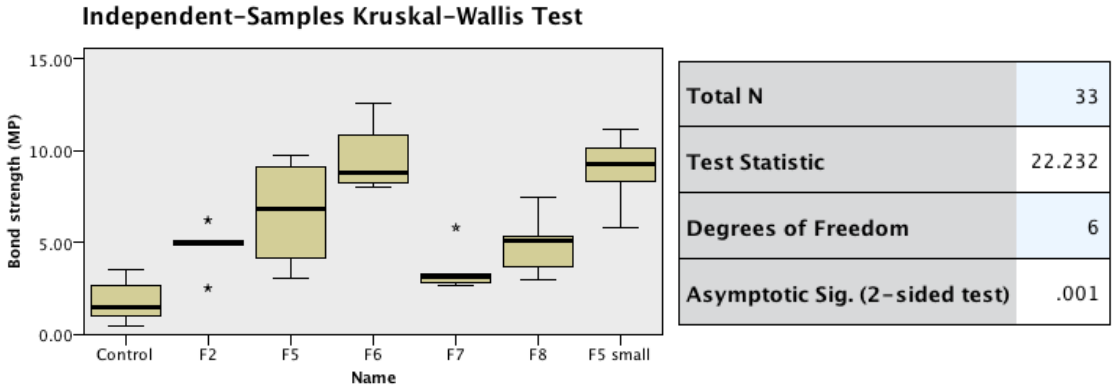


Figure 3.29 - Kruskal – Wallis test for bond strength vs formulations (ivory – no etch / no bond dentine preparation, placed in Artificial saliva for 30 days prior to the push-out test)

3.1.3.2 Etch no bond

3.1.3.2.1 24 hours in Artificial Saliva

In Figure 3.30 the highest bond strength to ivory placed in saliva for 24 hours, with no dentine conditioning was achieved by F5 small (14MPa), followed by F5 and F2 that reached 12 and 11 MPa respectively. The bond strength of the other formulations reached magnitudes of 9 and 10 MPa.

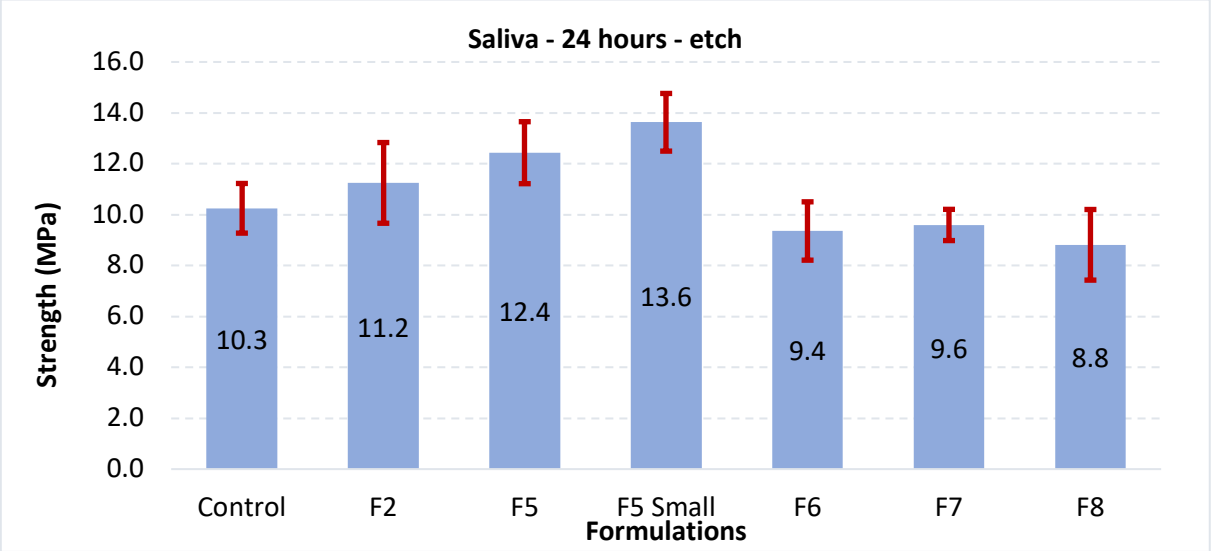


Figure 3.30 - Push out test results - Bonding strength of each formulation in Ivory – etch dentine preparation, placed in Artificial saliva for 24 hours prior to the push-out test

The Kruskal Wallis test rejected the null hypothesis (P = 0.007) as presented in the graph below (Figure 3.31). Specifically, significantly important difference was detected in the bond strength between F6 – F5 small (P = 0.18) and F8 – F5 small (P = 0.18).

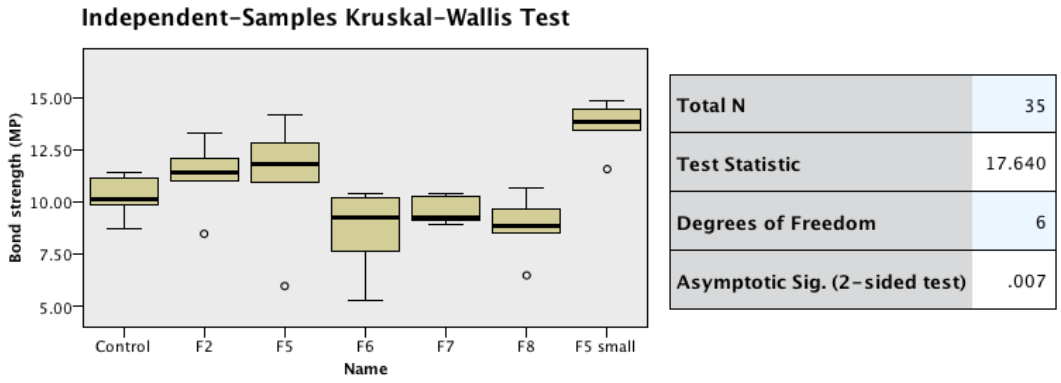


Figure 3.31 - Kruskal – Wallis test for bond strength vs formulations (ivory – etch dentine preparation, placed in Artificial saliva for 24 hours prior to the push-out test)

3.1.3.2.2 1 week in Artificial Saliva

Figure 3.32 represents the bond strength to ivory when samples were placed in saliva for 1 week in presence of etch prior to the composite application. The highest bond strength was noted in F6 and F5 small 9MPa and 8MPa respectively. The other five formulations exhibited lower bond strength around 3 MPa– 4MPa. Control, F5 and F8 have large error bars suggesting that the results were equally distributed in a wide range.

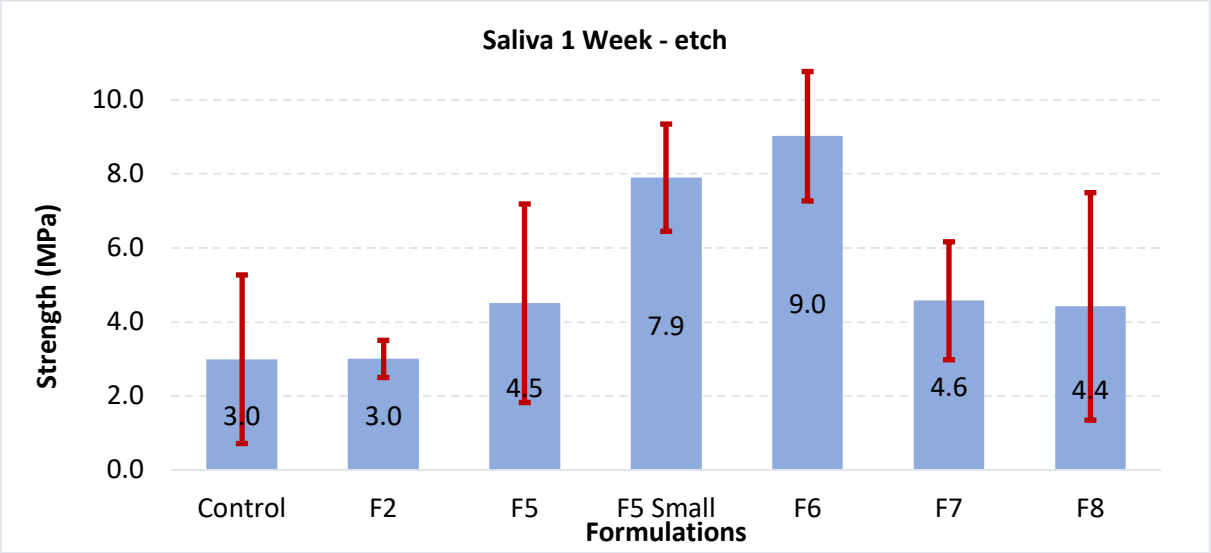


Figure 3.32 - Push out test results - Bonding strength of each formulation in Ivory – etch dentine preparation, placed in Artificial saliva for 1 week prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above - mentioned conditions. The test performed retained the null hypothesis as no significant difference was found in the bond strength between the formulations.

3.1.3.2.3 1 month in Artificial Saliva

In 1 month in saliva, all the formulations exhibited relatively low bond strength, with F5 having the lowest level (0.1MPa). None of the formulations reached bond strength magnitudes higher than 4.5 MPa (F8). The majority of the error bars are large and this test should ideally be repeated for a conclusion to be safely drawn.

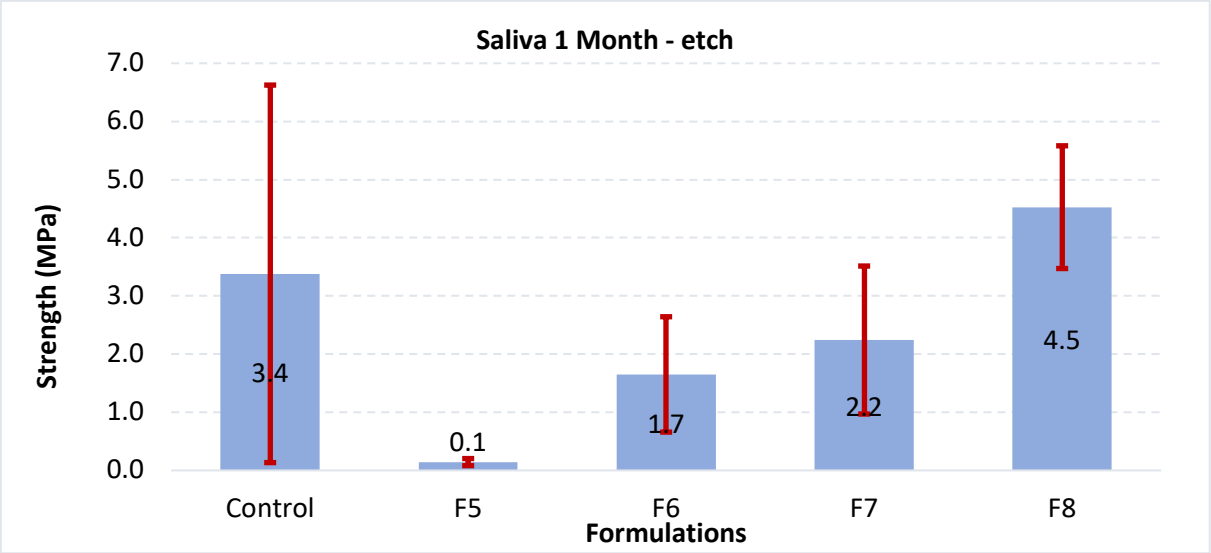


Figure 3.33 - Push out test results - Bonding strength of each formulation in Ivory – etch dentine preparation, placed in Artificial saliva for 30 days prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above - mentioned conditions. The test performed retained the null hypothesis as no significant difference was found in the bond strength between the formulations (P = 0.065).

3.1.3.3 Etch and bond

3.1.3.3.1 24 hours in Artificial Saliva

Figure 3.34 shows that with etch and bond prior to the composite application, control reached the highest bond strength, while for the other six formulations the bond strength ranged between 7-9 MPa.

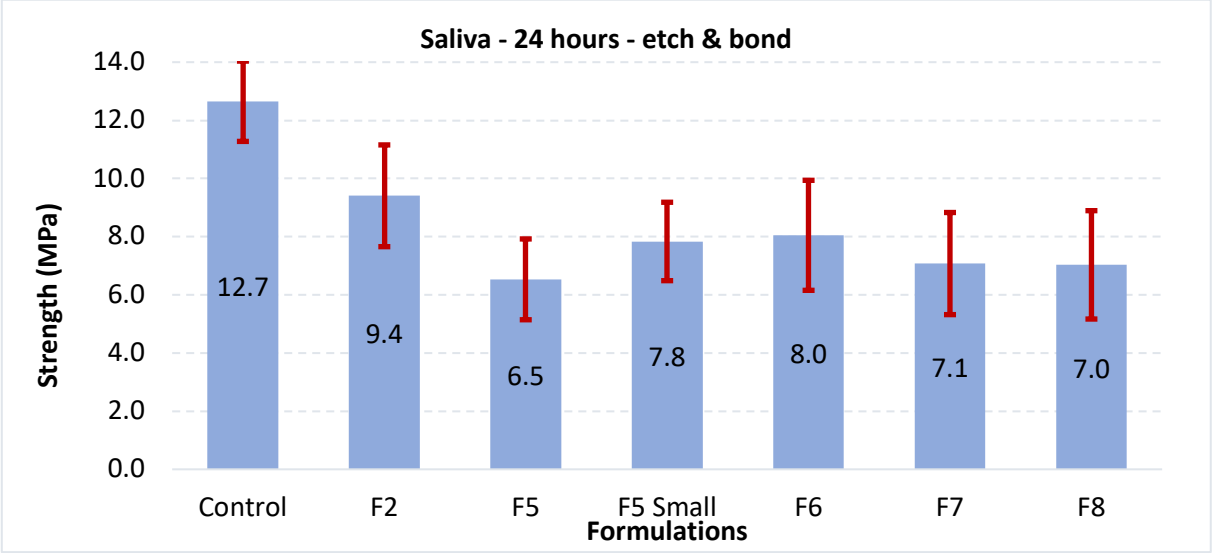


Figure 3.34 - Push out test results - Bonding strength of each formulation in Ivory – etch & bond dentine preparation, placed in Artificial saliva for 24 hours prior to the push-out test

The Kruskal Wallis test retained the null hypothesis as no significant difference was found in the bond strength between the formulations (P = 0.388).

3.1.3.3.2 1 week in Artificial Saliva

Figure 3.35 represents the bond strength of four formulations against a control when ivory samples were placed in saliva for 1 week following etch and bond use. F6 and F8 exhibited the lowest bond strength, below 2 MPa. The bond strength in control formulation was also low with a large error bar. F5 and F7 reached the highest bond strength magnitudes in this test (6 MPa – 7 MPa).

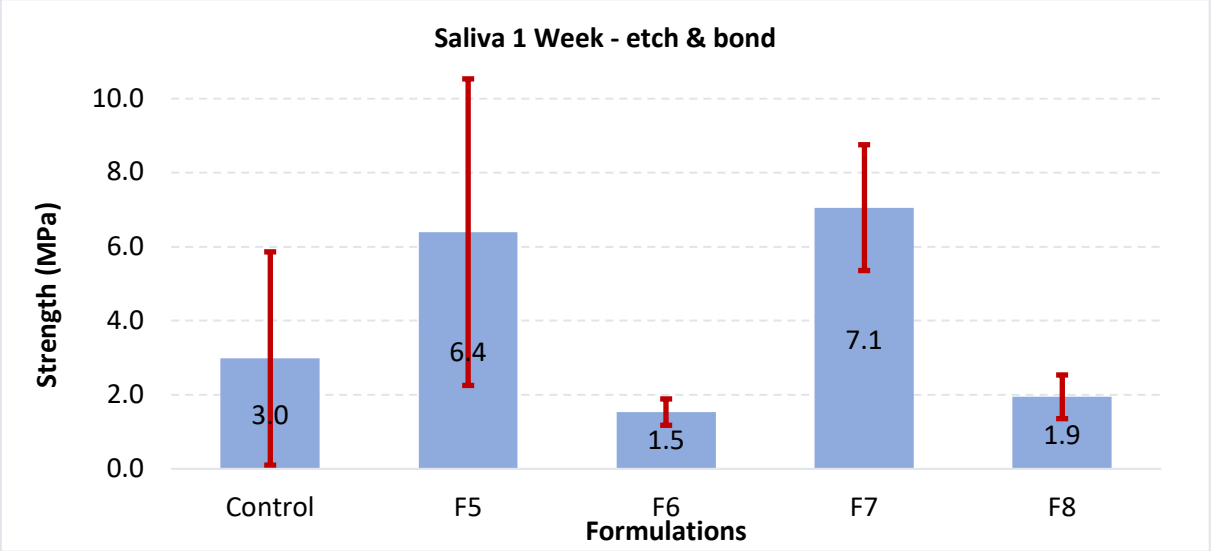


Figure 3.35 - Push out test results - Bonding strength of each formulation in Ivory - etch & bond dentine preparation, placed in Artificial saliva for 1 week prior to the push-out test

The Kruskal Wallis test rejected the null hypothesis (P = 0.034) as showed on the graph below (Figure 3.36). However, no significant difference was found in pairwise comparisons as the adjusted significance was higher than 0.05.

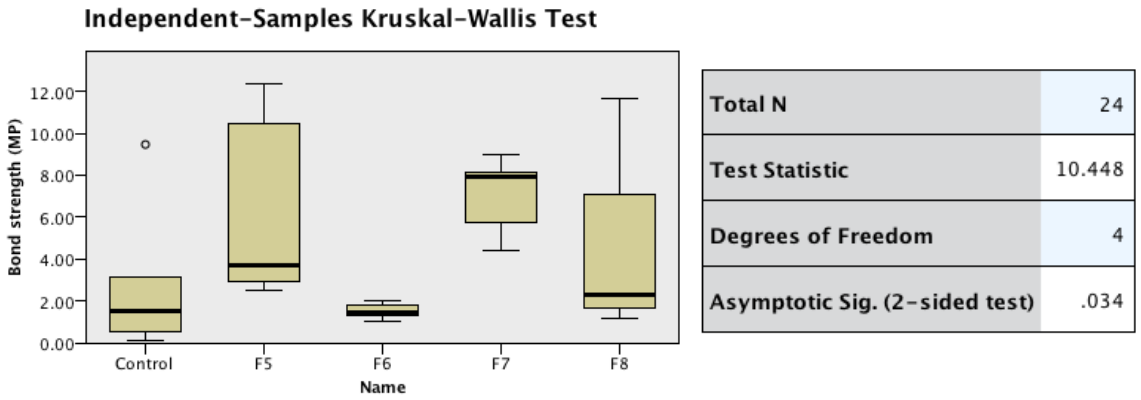


Figure 3.36 - Kruskal – Wallis test for bond strength vs formulations (ivory – etch & bond dentine preparation, placed in Artificial saliva for 1 week prior to the push-out tes

3.1.3.3.3 1 month in Artificial Saliva

Figure 3.37 represents the bond strength of six formulations against a control when ivory samples were placed in saliva for 1 month. Ivory dentine was prepared with etch and bond prior to the composite application. F7 and F8 bond strength reached 7 and 8 MPa respectively, while Control, F5 small and F6 achieved bond strength magnitudes close to 4-5 MPa. F2 and F5 exhibited the lowest bond strength of all (2.5 MPa and 3 MPa respectively).

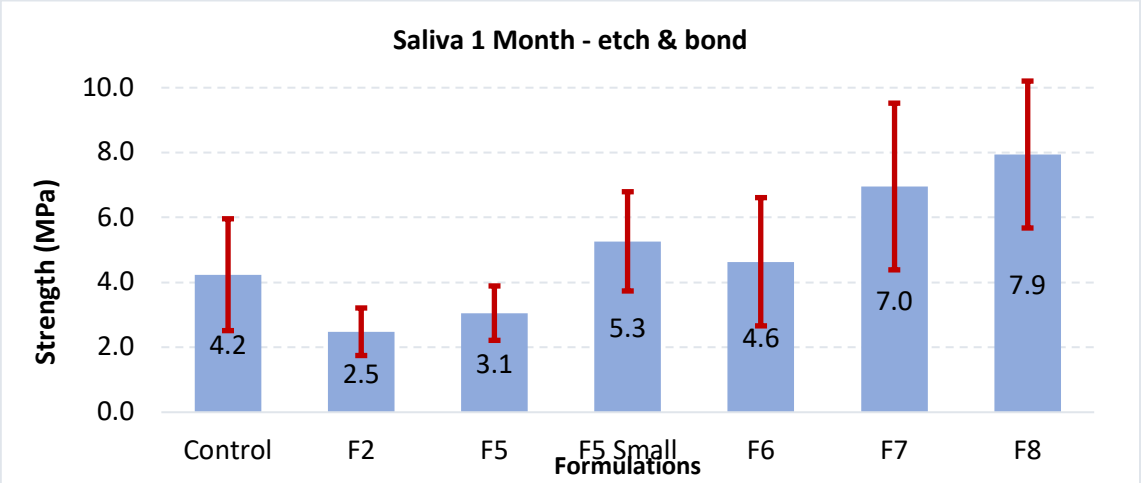
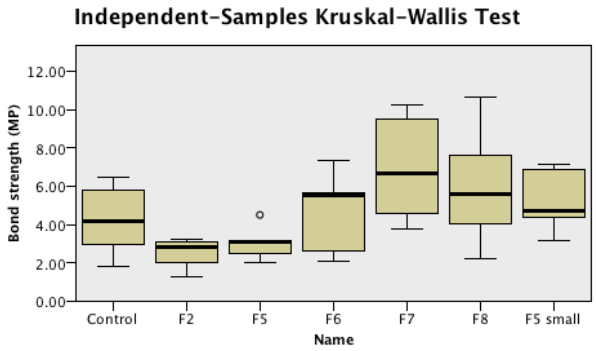


Figure 3.37 - Push out test results - Bonding strength of each formulation in Ivory – etch & bond dentine preparation, placed in Artificial saliva for 30 days prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above - mentioned conditions. The test performed rejected the null hypothesis (P = 0.05) as showed on the graph below (Figure 3.38). However, no significant difference was found in pairwise comparisons as the adjusted significance was higher than 0.05.



Total N	35
Test Statistic	12.614
Degrees of Freedom	6
Asymptotic Sig. (2-sided test)	.050

Figure 3.38 - Kruskal – Wallis test for bond strength vs formulations (ivory – etch & bond dentine prepaation, placed in Artificial saliva for 30 days prior to the push-out test

3.2 Bond Strengths to Human Dentine

3.2.1 Experiment 4 - No etch or bond in dry storage

3.2.1.1 24 hours in dry storage

Figure 3.39 represents the bond strength of four formulations when human teeth samples were placed in a dry environment for 24 hours (control). Human dentine had no etch and no bond preparation prior to the composite application. The graph shows equal levels of bond strength on all formulations with larger error bar on F8.

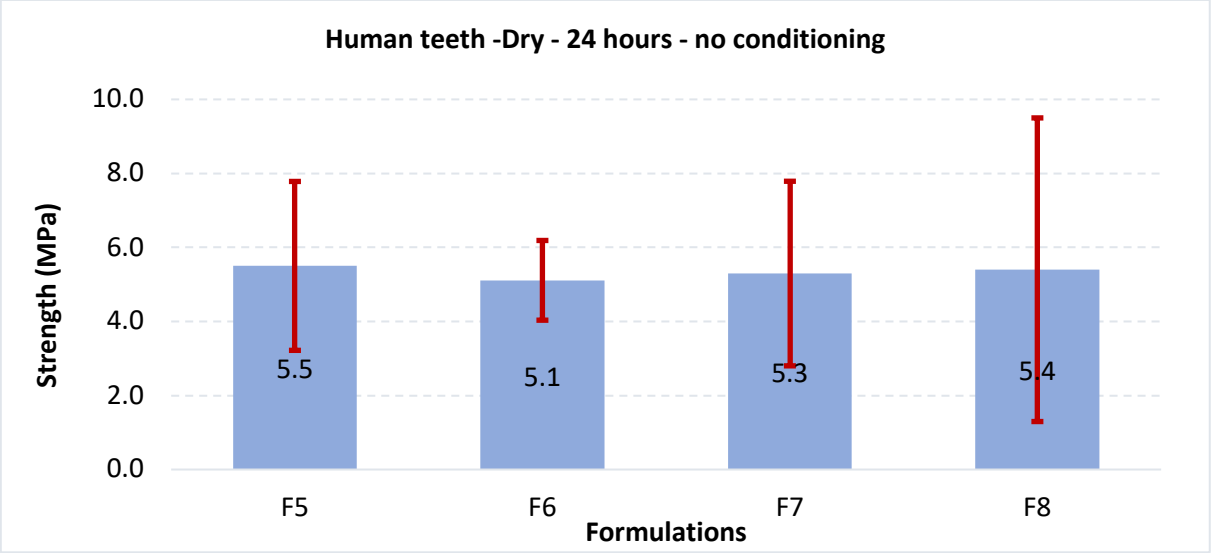


Figure 3.39 - Push out test results - Bonding strength of each formulation in Human dentine– no dentine preparation, placed in a dry storage for 24h prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above - mentioned conditions. The test performed retained the null hypothesis as no significantly important difference was found in the bond strength (P = 0.964).

3.2.2 Experiment 5 - No etch or bond in Artificial saliva

3.2.2.1 24 hours in Artificial Saliva

Figure 3.40 represents the bond strength in human teeth, in saliva liquid medium for 24 hours with no dentine conditioning. F7 appears to have the highest bond strength, whereas F5, F6 and F8 exhibit similar bond strengths around 4-5 MPa.

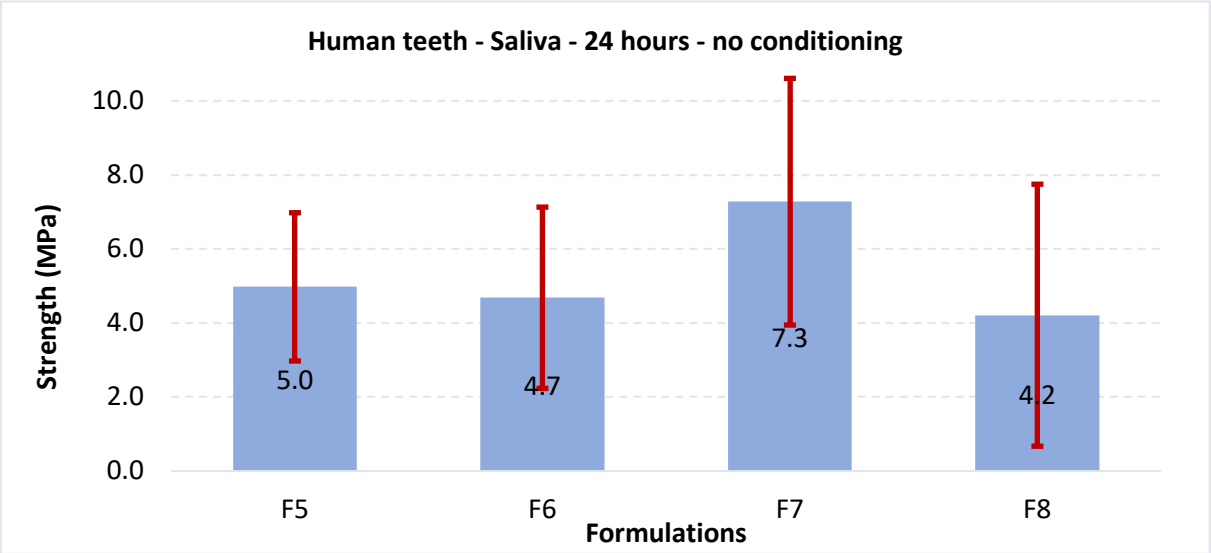


Figure 3.40 - Push out test results - Bonding strength of each formulation in Human dentine – no dentine preparation, placed in Artificial saliva for 24h prior to the push-out test

As expected, the Kruskal Wallis test retained the null hypothesis as no significantly important difference was found in the bond strength between these formulations (P = 0.436)

3.2.2.2 1 week in Artificial Saliva

Figure 3.41 shows that F7 reached the highest level of bond strength (22 MPa). F6 and F8 also reached high magnitudes (19.8 and 12.4 MPa respectively), whereas F5 exhibited the lowest bond strength amongst all (6.5 MPa).

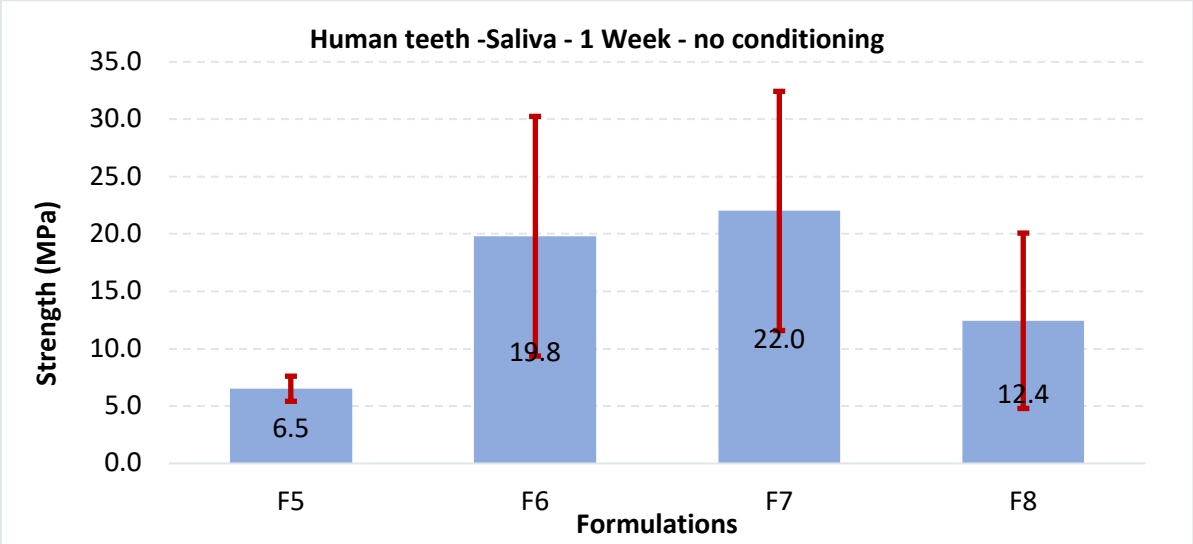


Figure 3.41 Push out test results - Bonding strength of each formulation in Human dentine – no dentine preparation, placed in Artificial saliva for 1 week prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above - mentioned conditions. The test performed retained the null hypothesis (P = 0.103). A Mann Whitney test was performed to compare F7 with F5 as on the graph they appear to have a big difference on the bond strength they have reached but the null hypothesis was retained (P = 0.151)

3.2.2.3 1 month in Artificial Saliva

Figure 3.42 showed that the four formulations, reached similar levels of bond strength to human dentine, that ranged between 6.5 – 8 MPa

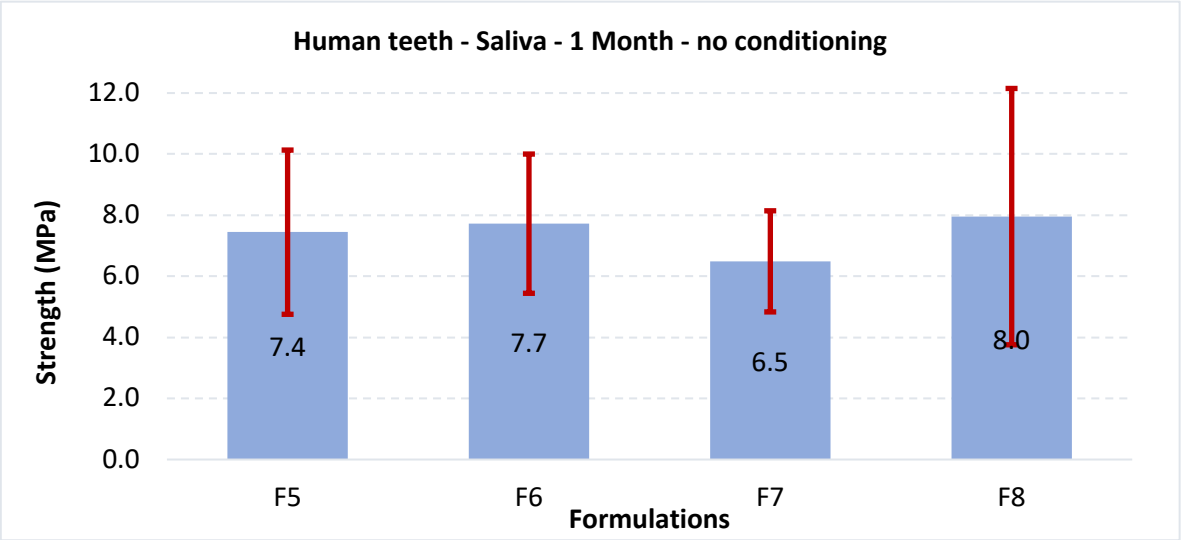


Figure 3.42 Push out test results - Bonding strength of each formulation in Human dentine – no dentine preparation, placed in Artificial saliva for 30 days prior to the push-out test

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength under the above - mentioned conditions. The test performed retained the null hypothesis (P = 0.843).

3.3 Experiment 6 - Ivory demineralisation with formic acid

Calibration Curve – Mass loss vs Time

This triple line graph (Figure 3.43) shows the mass loss of the three different ivory samples with the time. No further drop in the mass was noted after the 30% of the mass of each ivory was lost which occurred after 2 days of the samples being immersed in the formic acid.

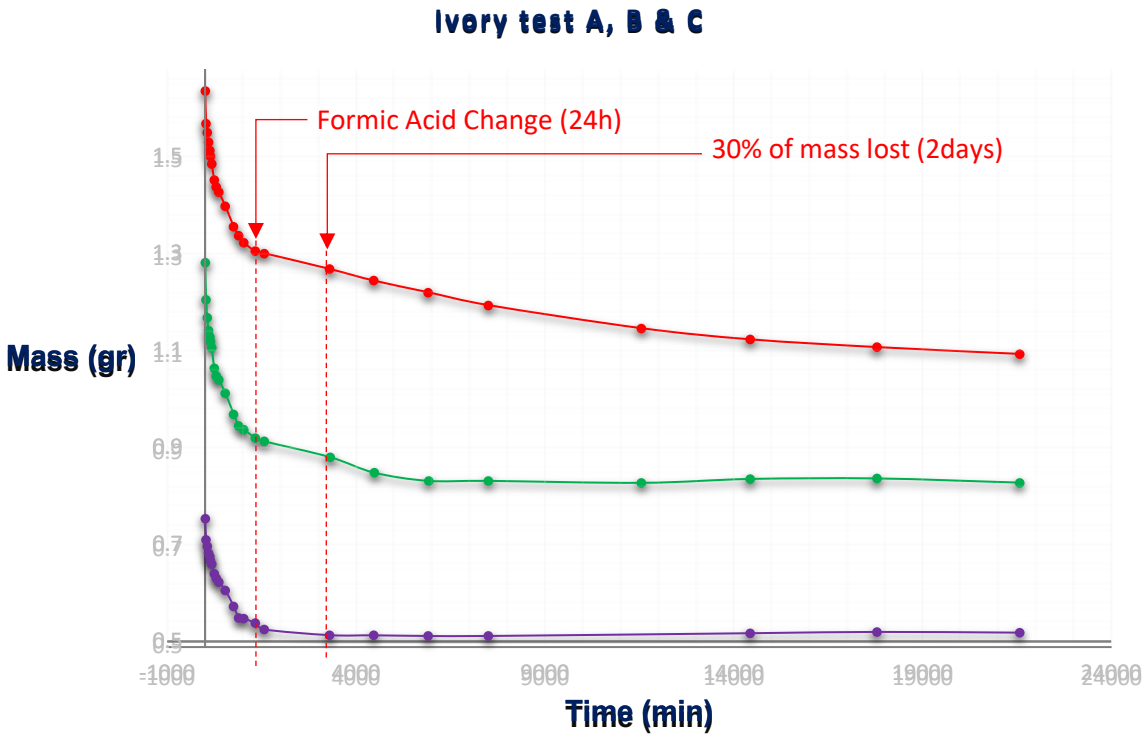
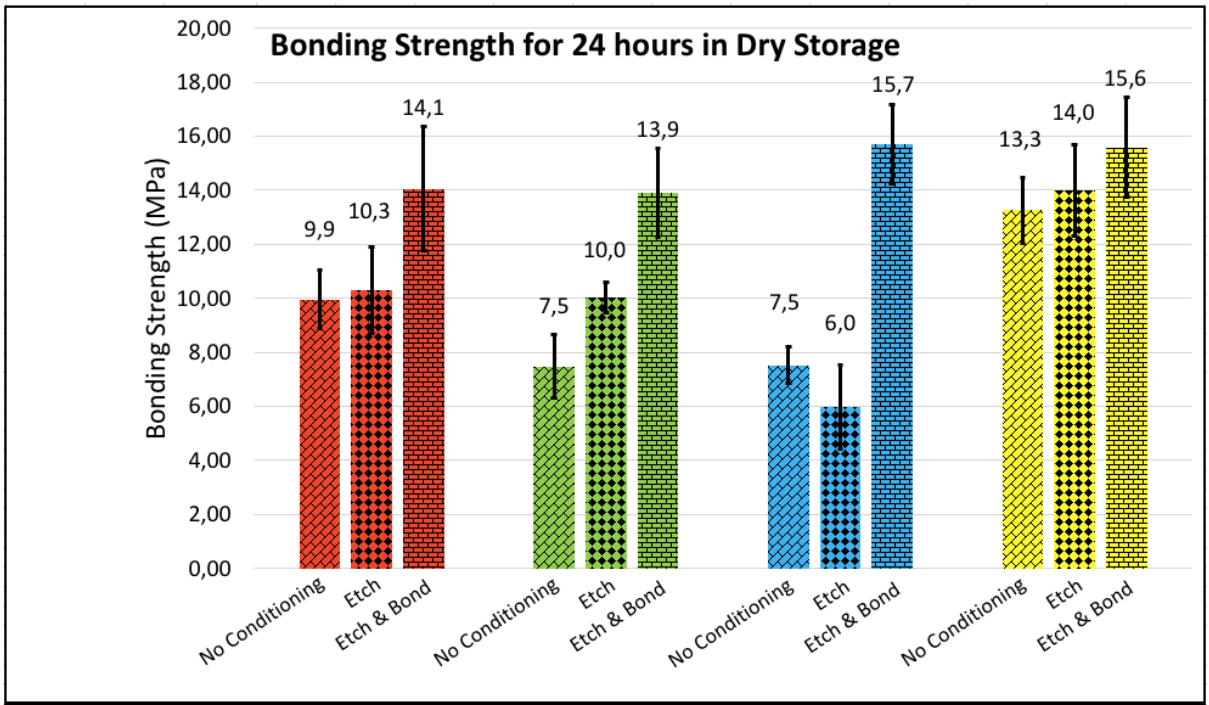


Figure 3.43 - Triple line graph that represents the mass loss of the three different ivory pieces versus exposure time in formic acid

3.4 Additional investigations

3.4.1 Ivory 24 hours in dry storage

Figure 3.44 represents the bond strength of four formulations when ivory samples were placed in a dry environment for 24 hours under three different ways of dentine preparation i.e no etch / no bond, etch, etch and bond. It is noticeable that with no etch / no bond the bond strength of the four formulations is reduced. With no etch / no bond the formulations with MCPM / PLS ratio = 2 (F5 & F8) reached the highest bond strengths and the presence of etch did not affect their bond strength. On F6 and F7 the presence of bond almost doubled the bond strength, while in F8 and F5 increased it by 2 – 4 MPa.

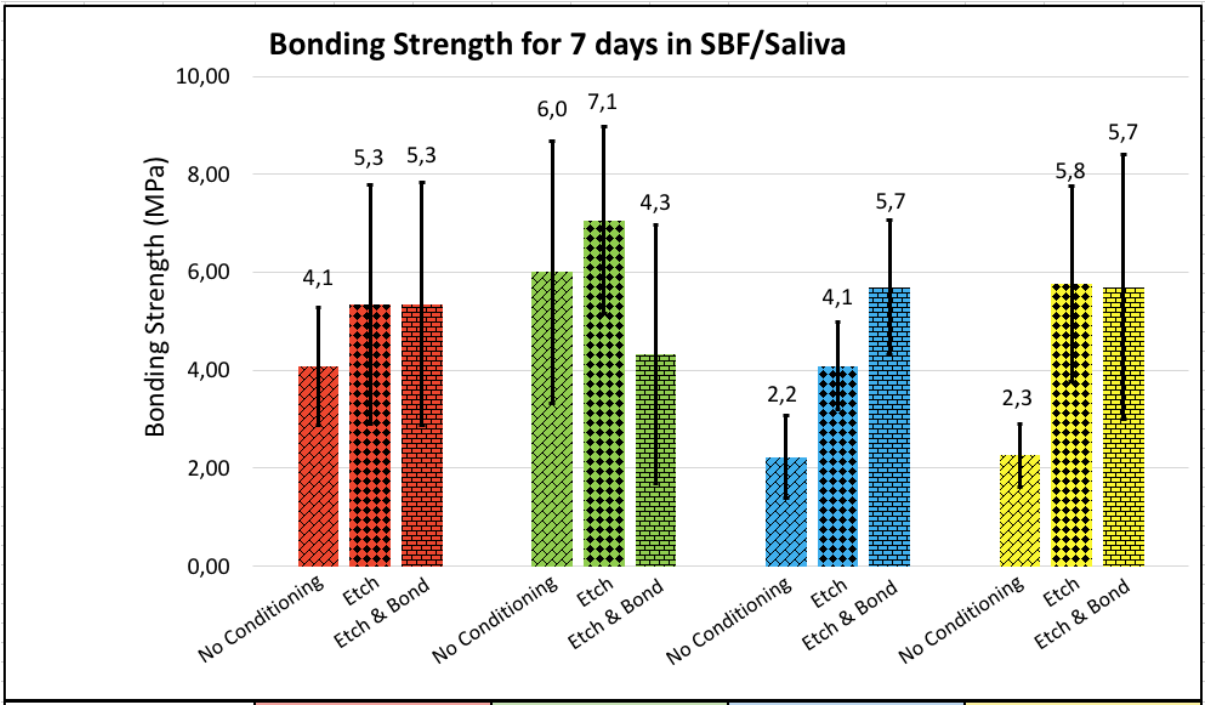


Name	F5	F6	F7	F8
Antibacterial (PLS)	4%	4%	2%	2%
Remineralising (MCPM)	8%	4%	8%	4%

Figure 3.44-Bar chart showing the bonding strength of F5, F6, F7 and F8 for 24 hours in dry environment in all three different ways of ivory preparation (no etch/no bond, etch, etch & bond)

3.4.2 Ivory 1 week in liquid medium (SBF & Artificial Saliva)

Figure 3.45 depicts the bonding strength of F5. F6, F7 and F8 formulation when samples were placed in a liquid medium for 1 week under three different ways of dentine preparation i.e no etch / no bond, etch, etch and bond. Comparing to 1 day in dry storage, the bonding strength is generally reduced with F6 showing the least level of decrease. The formulations with PLS = 4% (F5 & F6) reached higher bonding strength comparing to those with PLS = 2, in absence of dentine conditioning. In F5 and F8 the addition of etch improved the bond strength but the presence of bond did not have any further effect on it.

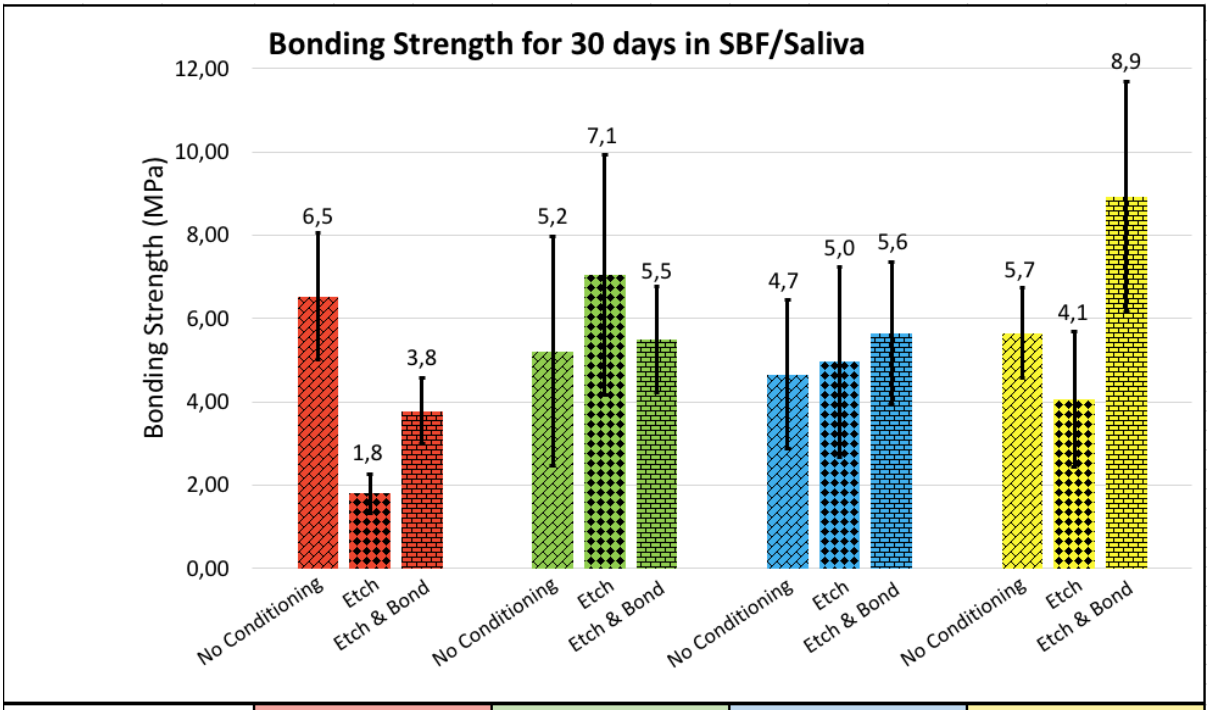


Name	F5	F6	F7	F8
Antibacterial (PLS)	4%	4%	2%	2%
Remineralising (MCPM)	8%	4%	8%	4%

Figure 3.45 -Bar chart showing the bonding strength of F5, F6, F7 and F8 for 1 week in a liquid medium in all three different ways of ivory preparation (no etch/no bond, etch, etch & bond)

3.4.3 Ivory 1 month in liquid medium (SBF & Artificial Saliva)

Figure 3.46 shows the the bonding strength of F5. F6, F7 and F8 formulation when samples were placed in a liquid medium for 1 month under three different ways of dentine preparation i.e no etch / no bond, etch, etch and bond. In absence of dentine conditioning, bonding strength of F5, F7 and F8 shows an increasing trend, while F6 bonding strength reduces even more comparing to 1 day in dry storage and 1 week in liquid medium. F5 reaches the higher value in bonding strength, which is also much higher in abscess of etch / etch and bond while in F8 the addition of bond increases the bond strength.



Name	F5	F6	F7	F8
Antibacterial (PLS)	4%	4%	2%	2%
Remineralising (MCPM)	8%	4%	8%	4%

Figure 3.46 -Bar chart showing the bonding strength of F5, F6, F7 and F8 for 1 month in a liquid medium in all three different ways of ivory preparation (no etch/no bond, etch, etch & bond)

3.4.4 Bond strength vs Time in liquid medium (SBF / Artificial saliva)

Figure 3.47 shows that the bond strength reduced from 24 hours to 1 week, whereas in one month the trend indicates that the bond strength reached levels close to the initial magnitudes. As there were no measurements of the bond strength in different times between 24 hours – 1 week and 1 week – 1 month it is not possible to know how to bond strength changed in these time intervals.

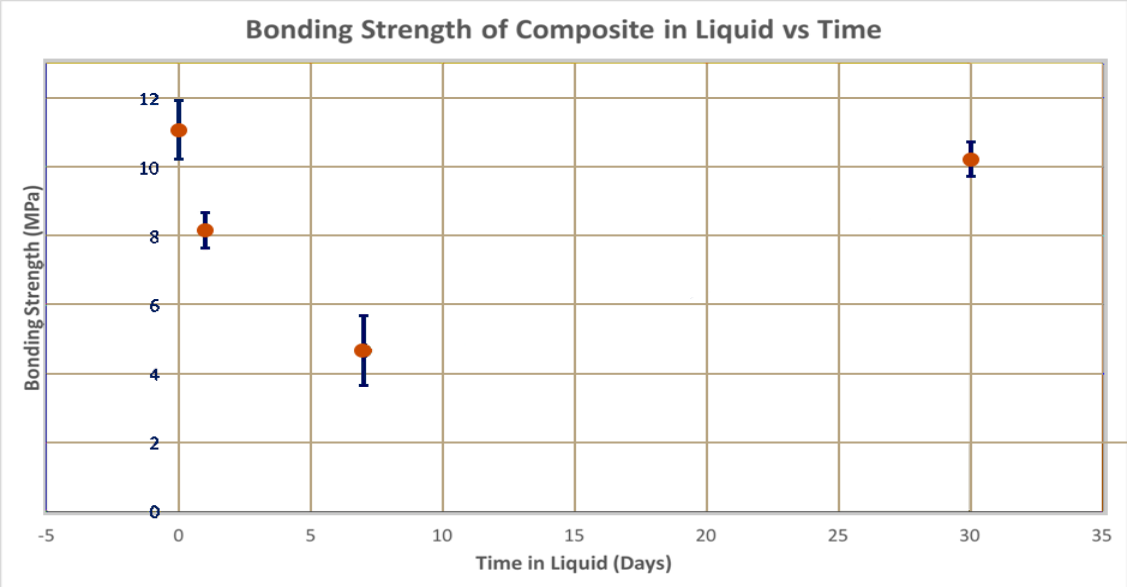
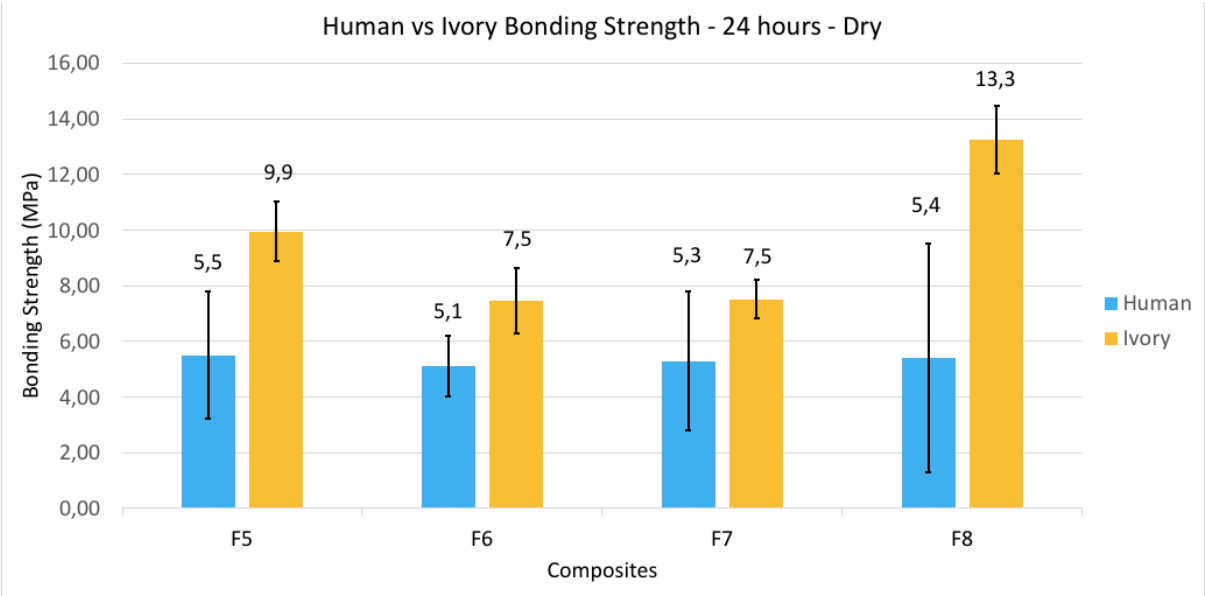


Figure 3.47 - Scatter graph showing the evolution of bond strength versus time in a liquid medium. Each point represents the average of 40 repetitions of F5, F6, F7 and F8 in SBF and Saliva for 24 hours, 1 week and 1 month. The first point shows the one day in dry environment- day 0

3.4.5 Bond strength in Human teeth vs Ivory

3.4.5.1 24 hours in dry storage

Figure 3.48 shows a bar chart that represents the bond strength of F5, F6, F7 and F8 in ivory and human dentine in dry storage without etch or bond conditioning. All formulations showed higher bond strength in ivory, while in human dentine the bond strengths were similar ~ 5 MPa.



Antibacterial (PLS)	4%	4%	2%	2%
Remineralising (MCPM)	8%	4%	8%	4%

Figure 3.48 - Bond strength Human vs Ivory dentine – no dentine preparation, dry storage for 24h prior to the push-out test

3.4.5.2 24 hours & 1 week in saliva

Figure 3.49 shows the difference in bond strength between human and ivory dentine in 1 and 1 week. Dentine had no conditioning prior to the composite application. Human dentine exhibits higher bond strength than ivory in 1 week, however that error bar in this case is large. This could be related to the fact that the results were equally distributed in a wide range of values. In 24 hours, the bond strength in ivory is higher than in human dentine.

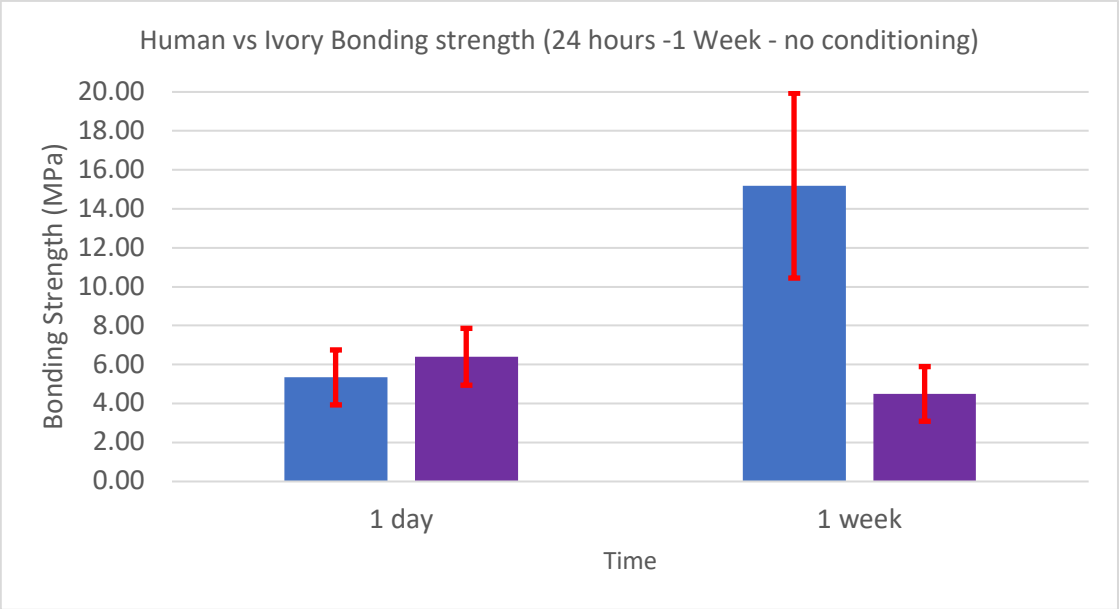
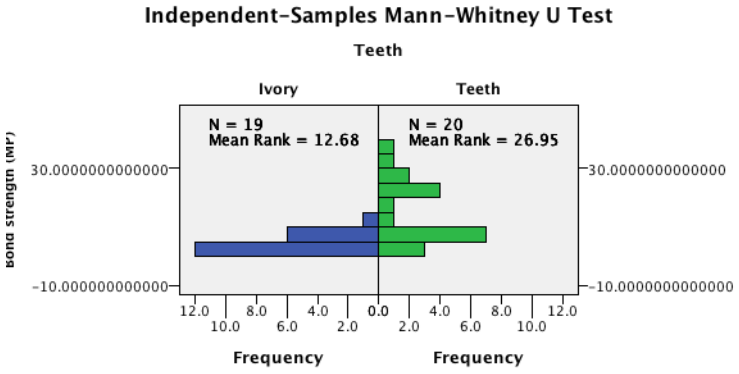


Figure 3.49 - Bond strength in human vs ivory dentine, samples placed in saliva for 1 and 1 week, no dentine conditioning (ivory in purple, human dentine in blue)

A Mann – Whitney test was performed to assess whether there is a statistically important difference in the bond strength between ivory and human teeth when samples are placed for 24 hours in saliva. The test retained the null hypothesis, as no statistically important difference was found (P = 0.247)

A Mann – Whitney test was performed to assess whether there is a statistically important difference in the bond strength between ivory and human teeth when samples are placed for 1 week in saliva. The test rejected the null hypothesis as the bond strength of composite appears to be higher in human dentine (P = 0.000, Figure 3.50)



Total N	39
Mann-Whitney U	329.000
Wilcoxon W	539.000
Test Statistic	329.000
Standard Error	35.590
Standardized Test Statistic	3.906
Asymptotic Sig. (2-sided test)	.000
Exact Sig. (2-sided test)	.000

Figure 3.50 - Mann - Whitney test for bond strength in human vs ivory dentine (no dentine preparation, placed in Artificial saliva for 7days prior to the push-out test)

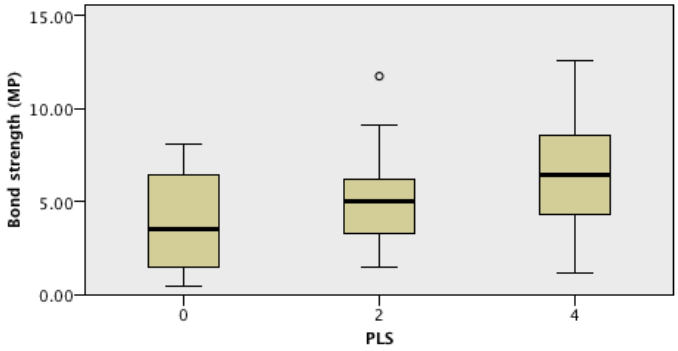
3.4.5.3 1 month in saliva

A Mann – Whitney test was performed to assess whether there is a statistically important difference in the bond strength between ivory and human teeth when samples are placed for 1 month in saliva. The test retained the null hypothesis as no significant difference was detected in the bond strength between ivory and human dentine in the above-mentioned conditions (P = 0.105)

3.4.6 Ivory - bond strength vs PLS

3.4.6.1 No etch / no bond, in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the level of PLS under the following conditions: The samples had no etch / no bond dentine preparation prior to composite application and they were placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. The null hypothesis was retained for 24 hours and 1 week. In 30 days, the null hypothesis is rejected (P = 0.040, Figure 3.51) showing the significantly higher bond strength achieved with 4% PLS comparing to PLS= 0% and higher than PLS= 2% but not statistically important.



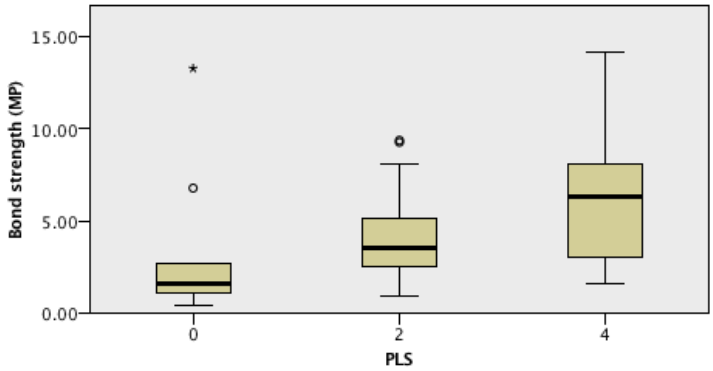
Total N	68
Test Statistic	6.418
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.040

Figure 3.51-Kruskal - Wallis test for bond strength in ivory dentine vs PLS level (no dentine preparation, placed in SBF / Artificial saliva for 30 days prior to the push-out test)

3.4.6.2 Etch (35% phosphoric acid), in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the level of PLS under the following conditions: The ivory dentine was prepared with 35% phosphoric acid prior to the composite restoration. The samples were subsequently placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days.

In 24 hours, the null hypothesis was retained as there was no significance difference between PLS=0, 2 and 4. In 1 week, the test performed rejected the null hypothesis (P = 0.001, Figure 3.52). Specifically, there is significantly important difference between PLS=0 and 4 (P = 0.002). PLS=4 is higher than PLS=2 but the difference is not statistically important. However, the adjusted significance is 0.06 very close to P = 0.05, while the significance is 0.021 so a higher sample number might have shown a significantly important difference.



Total N	70
Test Statistic	13.095
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.001

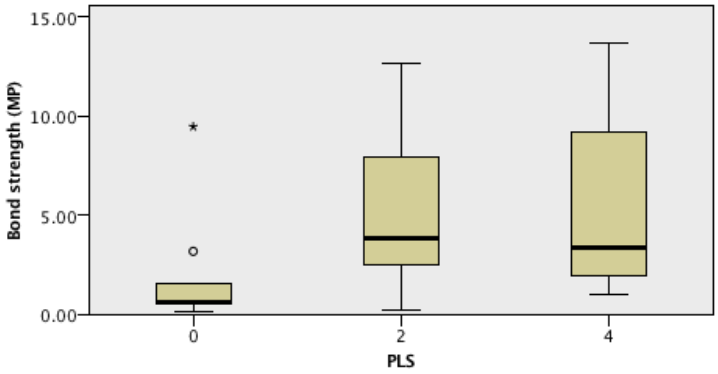
Figure 3.52-Kruskal – Wallis test for bond strength in ivory dentine vs PLS (etch preparation, placed in SBF / Artificial saliva for 7days prior to the push-out test)

In 30 days, the test performed retained the null hypothesis. Although PLS=2 showed higher bond strength, the difference was not statistically important.

3.4.6.3 Etch (35% phosphoric acid) and iBond, in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days.

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the level of PLS under the following conditions: The ivory dentine was prepared with 35% phosphoric acid and iBond application prior to the composite restoration. The samples were subsequently placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. In 24 hours, the null hypothesis was retained as no statistically important difference was detected between the different levels of PLS in presence of etch and bond (P = 0.878)

In 1 week, the null hypothesis was rejected (P = 0.004, Figure 3.53) as there was statistically important difference PLS=0 and PLS=2 (P = 0.005) and also between PLS=0 and PLS=4 (P = 0.005. No significant difference was detected between PLS=2 and PLS=4.



Total N	71
Test Statistic	11.236
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.004

Figure 3.53 - Kruskal – Wallis test for bond strength in ivory dentine vs PLS (etch & bond preparation, placed in SBF / Artificial saliva for 7days prior to the push-out test)

In 30 days, the null hypothesis was retained as no significantly important difference was detected between the different levels of PLS (P = 0.343)

3.4.7 Ivory – bond strength vs MCPM

3.4.7.1 No etch or bond, in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the level of MCPM under the following conditions: The samples had no etch / no bond dentine preparation prior to composite application and they were placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. The null hypothesis was that the level of MCPM does not affect the bond strength. For 1, 7 and 30 days the null hypothesis was retained. No significantly important difference was found in any case of these cases. The Ps for 1, 7 and 30 days where 0.316, 0.445 and 0.154 respectively.

3.4.7.2 Etch (35% phosphoric acid), in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the level of MCPM under the following conditions: The samples were prepared with 35% phosphoric acid and placed for 24 hours in liquid medium (SBF/ Artificial saliva). The test performed retained the null hypothesis indicating that there is no significant difference on the above-mentioned conditions (P = 0.415). In 1 week, the null hypothesis was rejected (P = 0.003, Figure 3.54) with the difference in bond strength between the formulations with 0% MCPM and those with 4% being statistically significant (P = 0.002). In 30 days, the null hypothesis was retained (P = 0.263)

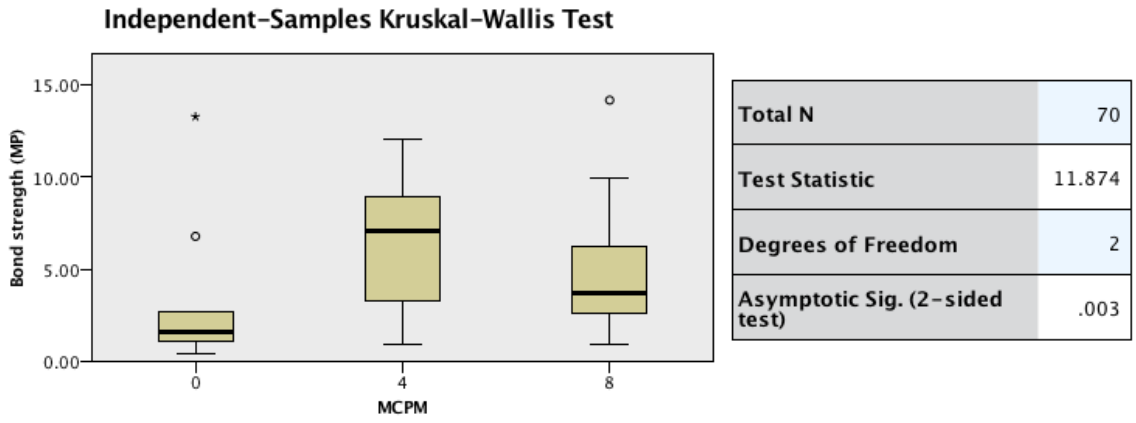
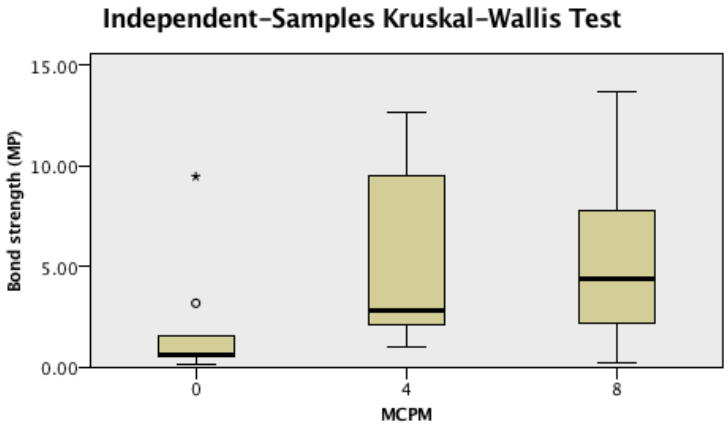


Figure 3.54-Kruskal – Wallis test for bond strength in ivory dentine vs MCPM (etch preparation, placed in SBF / Artificial saliva for 7days prior to the push-out test)

3.4.7.3 Etch (35% phosphoric acid) & iBond, in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the level of MCPM under the following conditions: The samples were prepared with 35% phosphoric acid and iBond and were subsequently placed for 1, 7 and 30 days in liquid medium (SBF/ Artificial saliva). The test performed and the null hypothesis was retained for 1 and 30 days (P = 0.394 and 0.094 respectively). For 1 week, the null hypothesis was rejected (P = 0.003, Figure 3.55) as there was statistically significant difference between MCPM 0% and 4% (P = 0.010) and 0% and 8% (P = 0.003) but not between 4% and 8%.



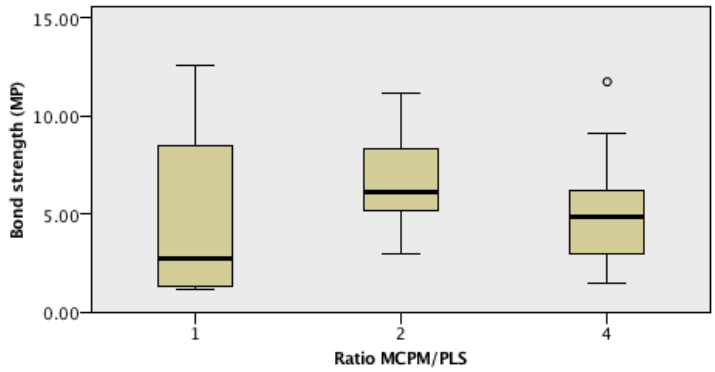
Total N	71
Test Statistic	11.311
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.003

Figure 3.55-Kruskal – Wallis test for bond strength in ivory dentine vs MCPM (etch & bond preparation, placed in SBF / Artificial saliva for 7days prior to the push-out test)

3.4.8 Ivory – bond strength vs MCPM / PLS ratio

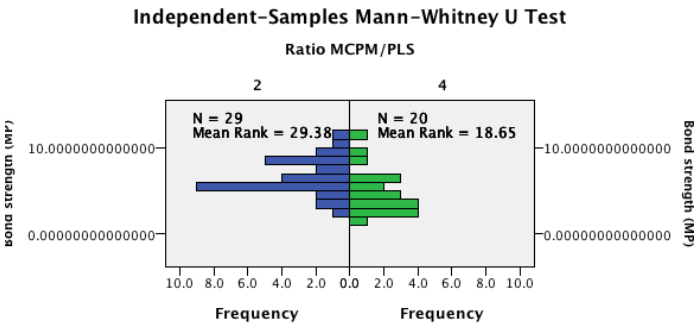
3.4.8.1 No etch / no bond, in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days.

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the MCPM / PLS ratio under the following conditions: No dentine conditioned was carried out prior to the composite application and the samples were placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. The test performed based on the null hypothesis that MCPM / PLS ratio does not affect the bond strength. In 1 day and 1 week, the null hypothesis was retained (P = 0.155 and 0.340). In 30 days, the null hypothesis was rejected (P = 0.046, Figure 3.56). However, in pairwise comparison the adjusted significance was higher than 0.05. In formulations with MCPM / PLS =4 versus MCPM / PLS = 2 although the adjusted significance is 0.064, the significance is 0.021. Possibly with a higher sample number this would indicate a clearly significantly important difference between the two. When compared between each other using a Mann – Whitney test, the null hypothesis was rejected showing a statistically important difference between the MCPM / PLS = 2 and MCPM / PLS = 4 (P = 0.01, Figure 3.57)



Total N	58
Test Statistic	6.173
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.046

Figure 3.56 - Kruskal – Wallis test for bond strength in ivory dentine vs MCPM / PLS ratio (no dentine conditioning, placed in SBF / Artificial saliva for 30 days prior to the push-out test)



Total N	49
Mann-Whitney U	163.000
Wilcoxon W	373.000
Test Statistic	163.000
Standard Error	49.157
Standardized Test Statistic	-2.584
Asymptotic Sig. (2-sided test)	.010

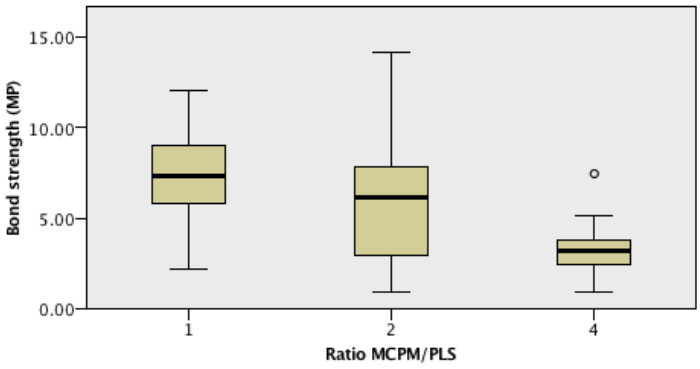
Figure 3.57 - Mann – Whitney test for bond strength. MCPM / PLS = 2 vs MCPM / PLS = 4 (no dentine conditioning, placed in SBF / Artificial saliva for 30 days prior to the push-out test)

3.4.8.2 Etch (35% phosphoric acid), in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the MCPM / PLS ratio under the following conditions: Ivory dentine was prepared with 35% phosphoric acid prior to composite application and the samples were subsequently placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days.

The test showed no significant difference on the MCPM / PLS ratio in 24 hours (P = 0.504).

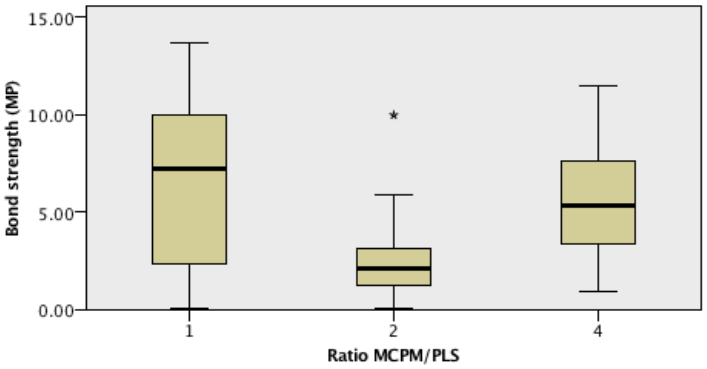
The null hypothesis was rejected in 1 week (P =0.003) as shown in Figure 3.58. In pairwise comparison, formulations with MCPM / PLS = 4 exhibited statistically lower bond strength than those with MCPM / PLS = 2 (P = 0.017) and those with ratio being equal to 1 (P = 0.006).



Total N	60
Test Statistic	11.959
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.003

Figure 3.58 - Kruskal – Wallis test for bond strength in ivory dentine vs MCPM / PLS ration (etch dentine preparation, placed in SBF / Artificial saliva for 7days prior to the push-out test)

In 30 days, the null hypothesis was rejected (P = 0.007) as shown in Figure 3.59. In presence of etch, the bond strength in formulations with MCPM / PLS = 4 is statistically higher than in cases where MCPM / PLS = 2 (P = 0.017)



Total N	48
Test Statistic	9.894
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.007

Figure 3.59-Kruskal – Wallis test for bond strength in ivory dentine vs MCPM / PLS ration (etch dentine preparation, placed in SBF / Artificial saliva for 30 days prior to the push-out test)

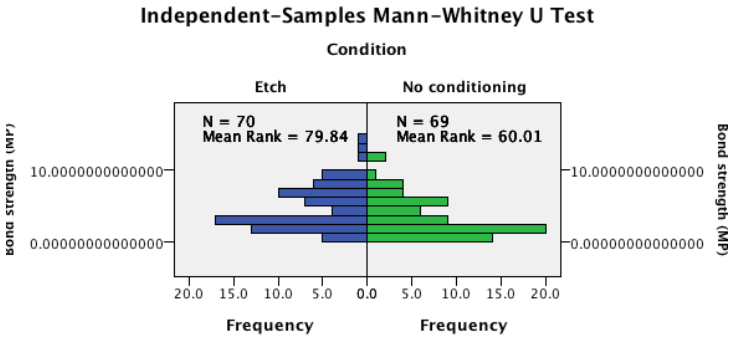
3.4.8.3 Etch (35% phosphoric acid) and iBond, in liquid medium (SBF / Artificial saliva), 1 / 7 / 30 days

A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the MCPM / PLS ratio under the following conditions: Ivory dentine was prepared with 35% phosphoric acid and iBond prior to composite application and the samples were subsequently placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. The test performed to assess the null hypothesis, which in this case was that the bond strength in not affected by the MCPM / PLS ratio on the above-mentioned conditions. In 1, 7 and 30 days, the null hypothesis was retained. The P was 0.289, 0.603 and 0.840 respectively.

3.4.9 No conditioning vs Etch in Bond Strength results

An Independent-Samples Mann-Whitney U Test was performed to assess whether there is a statistically important difference in bond strength in presence of 35% phosphoric acid prior to composite application and placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. The null hypothesis was that there was no difference in the bond strength of the formulations under the above-mentioned conditions. In 24 hours, the null hypothesis was retained (P = 0.665)

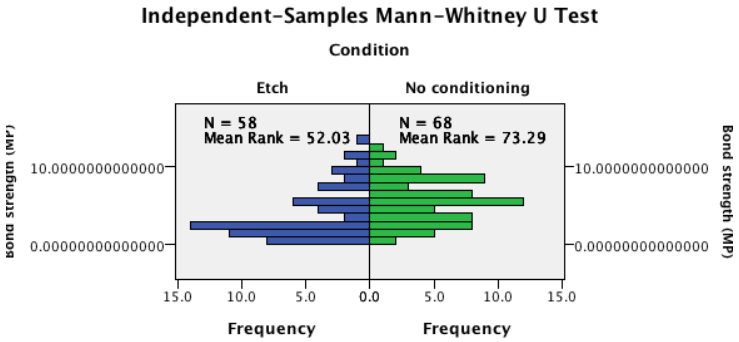
In 1 week, the null hypothesis was rejected (P = 0.004) as shown in Figure 3.60. The graph below shows that the use of etch improved the bond strength in ivory in 1-week experiment.



Total N	139
Mann-Whitney U	3,104.000
Wilcoxon W	5,589.000
Test Statistic	3,104.000
Standard Error	237.372
Standardized Test Statistic	2.903
Asymptotic Sig. (2-sided test)	.004

Figure 3.60 - Mann - Whitney test for bond strength. Etch vs no dentine conditioning in SBF / Artificial saliva for 1 week prior to the push-out test

In 30 days, the null hypothesis was also rejected (P = 0.001) as presented in Figure 3.61, showing that the presence of etch negatively affected the bond strength in the long term.



Total N	126
Mann-Whitney U	1,306.500
Wilcoxon W	3,017.500
Test Statistic	1,306.500
Standard Error	204.296
Standardized Test Statistic	-3.258
Asymptotic Sig. (2-sided test)	.001

Figure 3.61 - Mann – Whitney test for bond strength in ivory dentine. Etch vs no dentine conditioning in SBF / Artificial saliva for 30 days prior to the push-out test

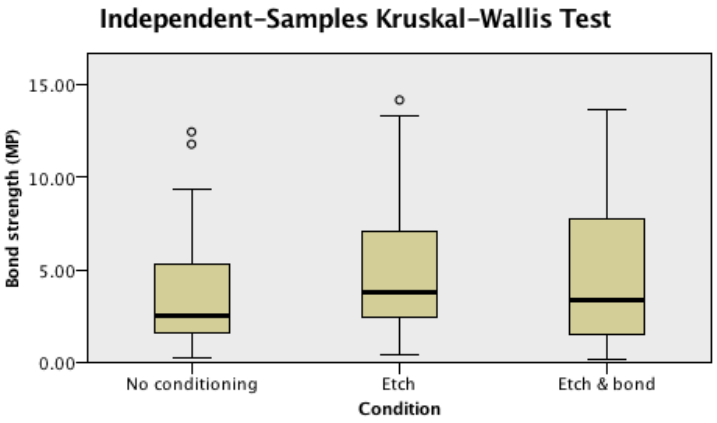
3.4.10 No Conditioning vs Etch and Bond in Bond Strength results

An Independent-Samples Mann-Whitney U Test was performed to assess whether there is a statistically important difference in bond strength in presence of 35% phosphoric acid and iBond prior to composite application and placed in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. The null hypothesis was that there was no difference in the bond strength of the formulations under the above-mentioned conditions. In 24 hours, the null hypothesis was retained (P = 0.077). In 1 week, the null hypothesis was retained (P = 0.210) suggesting that the presence of etch and bond does not significantly affect the bond strength.
In 30 days, the null hypothesis was also retained (P = 0.077).

3.4.11 Bond Strength vs Condiitoning

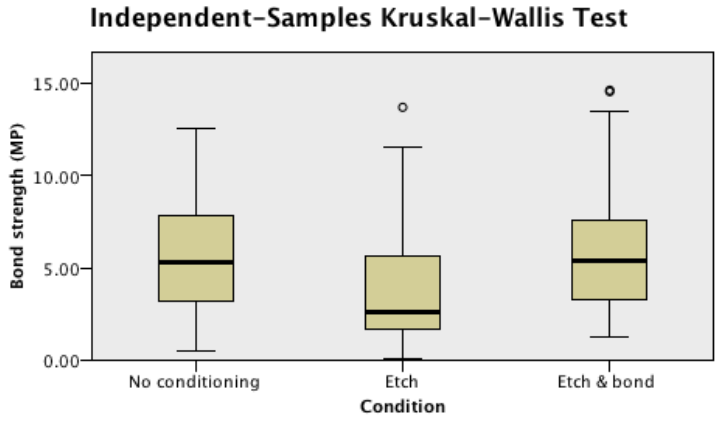
A Kruskal Wallis test was performed to assess whether there is a statistically important difference in the bond strength according to the presence of etch or etch and bond versus no dentine conditioning prior to composite application. The samples were subsequently stored in liquid medium (SBF / Artificial saliva) for 1, 7 and 30 days. The test performed in order to assess the null hypothesis, which in this case was that the bond strength is not affected by the presence of etch or etch and bond.

In 24 hours, the null hypothesis was retained suggesting that there is no significantly important difference in the bond strength in presence or absence of etch, etch and bond (P = 0.197) In 1 week, the null hypothesis was rejected (P = 0.025) as shown in Figure 3.62. Specifically, significantly important difference was detected between no dentine conditioning and etch conditioning (P = 0.020). In 30 days, the null hypothesis was also rejected (P = 0.000). In pairwise comparison, significantly important difference was detected between no conditioning – etch (P = 0.003) and between etch – etch & bond (P = 0.001, Figure 3.63)



Total N	210
Test Statistic	7.355
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.025

Figure 3.62 - Kruskal - Wallis test for bond strength in ivory dentine. Etch, Etch & bond vs no dentine conditioning in SBF / Artificial saliva for 1 week prior to the push-out test



Total N	196
Test Statistic	16.268
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.000

Figure 3.63 - Kruskal - Wallis test for bond strength in ivory dentine. Etch, Etch & bond vs no dentine conditioning in SBF / Artificial saliva for 30 days prior to the push-out test

4 Chapter - Discussion

In this study, the effect that the combination of two agents in different levels - a remineralising (MCPM) and an antimicrobial (PLS) agent - has on the bond strength of experimental composite formulations was examined. Six different experimental composite formulations were tested against a control. Four formulations had the same powder / liquid ratio (F5, F6, F7 and F8) and in pairs they had equal amount of MCPM (F5-F7 8%, F6-F8 4%) and PLS (F5-F6 4%, F7-F8 2%). One formulation had smaller particle size (F5 small) and one had different powder / liquid ratio (F2). As part of the experiment, a push out test was initially carried out using ivory blocks, as ivory dentine exhibits various similarities with the human dentine. Various ways of conditioning the dentine and different medium storages were used. The bond strength was subsequently calculated.

The first test was carried out as a control to assess the behaviour of the different formulations in absence of liquid medium and storage of the restoration dry for 24hours. Although this does not mimic oral conditions, as in oral cavity there is always saliva that covers the teeth and therefore the existing restorations, it might be used as a way to predict which formulations have a mechanism to improve bonding as it provides more reproducible results.

In ivory samples that were placed for 24 hours in dry storage prior to the push out test, the formulation with lower level of both MCPM and PLS (F8) showed increased bonding strength irrespective of the dentine conditioning (etch, etch & bond) prior to the composite restoration.

In all formulations irrespective of MCPM and PLS level, the addition of etch and bond improved the bond strength of the materials. In absence of dentine conditioning, F8 (4% MCPM, 2% PLS) exhibited the highest bond strength, statistically higher than Control (0-0) whereas the formulation with smaller size of particles (F5 small) reached statistically lower bond strength than the equivalent formulation with bigger size particles (F5). This could be related to the fact that smaller size particles produce composites with increased viscosity which would reduce the flow of the material and subsequently the bonding. On the other hand, smaller particles have a larger surface area resulting in more active ingredients which could potentially enhance the bond strength. Under the specific conditions of absence of dentine conditioning and placement of samples in a dry storage, the smaller particle size negatively affected the bond strength.

The effect of powder liquid ratio was also investigated. The formulation with powder / liquid ratio = 4 (F2) reached statistically higher bond strength than the formulation with ratio = 3 (F7)

when all the other additives are kept the same in absence of dentine conditioning and liquid storage medium. Although PLR = 4 would make the formulation more viscous and therefore would negatively affect the bond strength due to reduced flow, the presence of more active ingredients appears to have improved the bond strength in this scenario. In both cases (F2 – F7 and F5 – F5 small) there seems to be a complicating effect caused by the smaller particle size/increased viscosity of the material that needs to be further investigated for a safe conclusion to be drawn.

In presence of etch in dry storage, all formulations exhibited an increase in their bond strength (with the exception of F7 MCPM = 8% and PLS = 25), with F8 reaching the highest magnitude, as also occurred in absence of dentine conditioning. Etch possibly had opened the tubules and therefore created a stronger interlock of the materials to ivory dentine in dry conditions. In presence of etch and bond, the F5 small exhibited the highest bond strength. In dry storage, MCPM / PLS ratio = 2 increased the average bond strengths to untreated ivory dentine. Whilst doubling PLS content reduced average bonding to untreated ivory, it had a complex effect with etched ivory resulting in the same average bond strength.

The bond strength generally reduces when samples are placed in a liquid medium for 1 week. This could be related to the fact that the presence of the liquid medium compromises the bonding strength. This might be due to the fact that because of microleakage, the liquid penetrates between the sample and the composite and thus it reduces the bond strength. Additionally, in 1-week time remineralisation might not have occurred yet, which would have added on the improvement of the bonding strength. When looking into 1-month bonding strength, it is noticeable that the bonding strength shows an increasing trend in the majority of the formulations, which could be associated with the remineralisation effect of MCPM as well as swelling of the materials due to water sorption.

When the samples were placed into a liquid medium (SBF, Artificial saliva) the test showed that the bond strength was reduced in 1 day and 1 week, however, in 1-month time it reached the former magnitudes when no dentine conditioning was performed. In 30 days in liquid medium, although the MCPM did not affect the bond strength, PLS = 4% led to statistically higher bond strength comparing to 0. When compared to PLS = 2%, the bond strength was also higher but no statistical difference was detected. This may be proved with a larger sample number. For the same test, in 7 days with etch dentine conditioning, PLS = 4% achieved bond strength statistically higher than 0% and MCPM = 4% higher than 0%. Bond strength in PLS =4% was also higher than PLS =2% same as MCPM = 4 % higher than MCPM = 8%, although none of them was significantly higher. However, the adjusted significance in both cases was

close to P value = 0.05 and therefore larger sample number may also reveal significant difference in the bond strength between them. When bond was added to the preparation of ivory dentine, in 7 days, PLS = 2% achieved statistically higher bond strength than PLS = 0% and PLS = 4% higher than PLS = 0%. 4% and 2% reached similar levels of bond strength. The presence of PLS appears to improve the bond in comparison to no PLS. Interaction between positively charged amine groups and negatively charged amino acids in collagen is believed to cause attraction between the material and dentine. The PLS can also interact with the negatively charged 4 META that is in the composite. That better results have previously been seen with close to equal molar levels of MCPM and PLS suggest these components act synergistically. MCPM = 4% showed significantly higher bond strength than absence of MCPM as well as MCPM = 8% against no MCPM. MCPM = 8% reached higher magnitudes than MCPM = 4%, but the difference was not significant.

What also appears to affect the bond strength is the MCPM / PLS ratio. In 30 days in liquid medium, without any dentine conditioning, the bond strength of the formulations with MCPM / PLS = 2 is significantly higher than those with MCPM / PLS =4. In presence of etch, when the samples were immersed in a liquid medium for 7 days, the MCPM / PLS = 4 reached significantly lower bond strength than both MCPM / PLS = 1 and MCPM / PLS = 2. However, this was exactly the opposite for 30 days' time. The MCPM / PLS ratio does not seem to be critical on the bond strength when dentine has been previously conditioned with etch and bond. Generally speaking, the etch was proven to improve the bond strength in 7 days but caused a significant reduction on it in 30 days. The presence of etch and bond in the time period of 1 month did not affect the bond strength, which however remained higher in the absence of any dentine conditioning.

The bond strength test was also executed in human teeth without any conditioning of the dentine prior to the composite restoration as the purpose of this novel material is to be used without the addition of etch and / or bond in the procedure. The results showed that the tested formulations reached similar levels of bond strength without any of them being significantly better than the others ~ 5MPa. When comparing to ivory dentine in dry storage, without dentine conditioning, bond strengths in ivory were higher than human dentine. This can be due to the fact that restorations in ivory were perfectly circular versus diverse morphology of carious cavities in human teeth. In 7 days in artificial saliva, F6 (4-4) and F7 (8-2) exhibited very high magnitudes of 19-22 MPa. However, no statistically important difference was detected amongst the different formulations possibly due to the small sample size. Ideally this test should be repeated with a larger sample number for a conclusion to be drawn.

When comparing the results between human and ivory dentine, in the control case (dry environment for 1 day), F7 and F8 (PLS = 2%) showed higher bond strength in human dentine, whereas F5 and F6 (PLS = 4) performed better when placed in ivory dentine. It is interesting to mention that in 7 days, the bond strength in human dentine increases while in ivory dentine a reduction was noted. Statistically, there is a significantly important difference in the bond strength between human and ivory dentine in absence of any dentine conditioning when the samples are placed in a liquid medium for 7 days.

In the experiment carried out to determine the demineralisation of ivory, following 30% of mass loss in all three ivory pieces that was noted in about 48 hours in formic acid, no further drop in their mass was noted indicating that no inorganic component was left.

Clinical relevance of results

The increase in bond strength noted in one – month time in liquid medium (both SBF and artificial saliva) could be possibly associated to the volumetric expansion of the composite and/or to the remineralisation of the ivory dentine which is enhanced by the MCPM. This could potentially be confirmed by assessing the samples under the SEM. If remineralisation is occurring it would be of great clinical importance as it would allow the remineralisation of the demineralised dentine. The level of dentine remineralisation gained in relation to the degree of caries is also important to be determined. The use of MCPM and PLS both enhances remineralisation and also has an antimicrobial effect. Those two conditions would be extremely beneficial in partial caries removal. Eliminating the need for local anaesthetic as well as the necessity for drilling a tooth would help dentists to proceed to the restoration of children's teeth easier than before and it would be an asset in challenging cases in terms of behavioural management (i.e phobic or very young children). The remineralisation of carious dentine in combination with the antibacterial effect of the material will improve the effectiveness of restorative dentistry carried out in children and will reduce the number of teeth being extracted.

The push out experiments in human teeth, although it was a pilot study, showed that the bond strength in human dentine increased from 1 day to 1 week and reached much higher levels comparing to ivory dentine. In 1month a drop was detected in the bond strength which ranged between 6 MPa – 8MPa. This could be due to the differences in human dentine in terms of dentinal tubules density and minerals contest and it is definitely something that needs further investigation.

The mass loss in ivory using demineralisation techniques can be used to simulate dentine being affected by caries in different levels. This can be particularly useful for the initial assessment of the materials' bond strength before being tested in carious human teeth. Human dentine can be different between individuals or even between the teeth of the same person in terms of morphology, minerals content and dentinal tubules. All these can affect the bond strength. When caries is added to that, this great heterogeneity can be a limiting factor in research that attempts to determine the bond strength of a material into human dentine. As the aim of the novel composite is to be placed in the cavity without the need of caries removal into firm dentine, it stands to reason that the materials need to be tested in presence of caries. Therefore, controlled demineralisation of ivory using the results of this experiment can be very useful prior to the test being executed in human teeth.

SBF and artificial saliva simulate the environment of the oral cavity and how the materials behave in a non – dry environment. Only testing the formulations after being stored in a dry environment would not give us adequate information on the behaviour of the materials and would exclude properties such as water sorption and expansion.

Relate to previous study results

Based on previous work done in this field (Liaqat *et al.*, 2015), the initial level of hydroxyapatite in ivory dentine is half that in human dentine. In the same study it was also presented that the surface hydroxyapatite level in ivory decreases by approximately half with every 23s of acid etch. Liaqat et al., 2015 proved that the average bond strength as measured using the push out test in presence of acid etch and Ibond was between 25-40 MPa and 23-37 MPa with Ibond and without acid etch. In cases where no Ibond was used in formulations with 4-META the average bond strength was between 15-30 MPa, whereas in formulations with HEMA it was 8-15 MPa. The application of acid did not seem to have a great impact on the bond strength. Also increased level of CaP (0-40 wt %) caused a downward trend in bond strength.

These results appear to be much higher than the bond strength in the study presented (10-15MPa). This could be associated to a variety of factors. First of all, the formulations tested in this study did not contain HEMA. In presence of 4 – META, which is an acidic monomer that helps with bonding, additional etching of the ivory dentine may have caused a collapse on the ivory collagen, restricting therefore the diffusion of monomers into the intratubular dentine and negatively affecting the bond strength. This leads to the idea that if etching needs to be carried for future research, etching the ivory for 10 instead of 15 minutes may show an increase in the bond strength. The use of bond (iBond) seems to have no particular effect in this study, or even decreasing the bond strength in some cases. An unpublished study from the Eastman

has shown that the SMART composites can achieve a deeper resin tag formation comparing to other market composites and the presence of a bonding agent than forms much shorter resin tags may decrease the materials final bond strength. On the other hand, composite restorations are technique sensitive and any insufficient drying can lead to a dilution of the adhesive agent and subsequently to the weakening of the bond strength. Moreover, the nature of the ivory and the fact that the same ivory blocks have been previously used for similar experiments may have resulted in lower bond strength. Bond strength in ivory therefore appears to be multifactorial and controlling each parameter is challenging.

No previous study has looked at the bond strength in human dentine using the push out test, therefore a direct comparison with other studies was not possible. However, results given from shear bond tests showed that bond strength is affected by the presence of iBond and 4-META. Dentine conditioning with iBond in formulations with 4- META increased the bond strength from 12-27 MPa to higher magnitudes between 24 – 35MPa (Liaqat, 2015). In this study, in which push out test was carried out in human dentine, in absence of any dentine conditioning, the bond strength reached levels up to 22 MPa, while in ivory, the addition of etch and bond improved the bond strength of the materials, in the formulations with low levels of MCPM and PLS. What is interesting to mention is the fact that in Liaqat's study, the presence of etch without use of any bonding agent increased the bond strength, as well as the addition of etch and bond greatly enhanced the bond strength. In this study an increase in bond strength was noticed in 1 week in presence of etch only, while in 1 month it was decreased. In 1 month, in presence of etch and bond, the bond strength was similar to no dentine conditioning.

Study limitations

A significant amount of results in this study were given by tests carried out in ivory dentine. Despite the similarities between the ivory and human dentine, the differences of those two substances may have led to results that do not represent a real clinical situation implicitly. Collecting and testing teeth in such a great number would though have not been possible. The necessity to test all the formulations and get as many and reproducible results as possible was limited by the differences that teeth exhibit in quality and morphology.

On the push - out test, which was performed in human teeth, it was not feasible to use primary molars. The majority of the primary molars that were available for this test were heavily carious. Given the small diameter that the crowns of the primary molars have, after removing the caries the remaining dental tissue was not sufficient to allow a restoration to be performed and the push out test to be carried out. Additionally, the use of a primary molar does not allow

for multiple discs to be taken from each tooth. This is again due to the fact that the primary molar crowns are smaller in the incisal – cervical dimension and therefore, with the removal of the enamel layer, the dentine above the pulp chamber was not enough for multiple samples as it happened on the permanent molars. The use of permanent molars, although it helped us overcome those technical problems and execute the test, it is understandable that the differences between primary and permanent molars including their shape, mineralisation and dentinal tubules may create different behaviour of the material and affect the bond strength. This is something that needs to be addressed especially because the novel composites are designed to be used in paediatric patients who up to the age of 12 still have primary molars that may need to be restored. In case where teeth were carious in a way that they could not have been used after being cross – sectioned horizontally, they were cross - sectioned vertically, which might have affected the bond strength due to the direction of the dentinal tubules.

In addition to that, the push out test does not represent a typical way of a force being applied on a restoration that could cause its debonding in the oral cavity. This is because there are no such cavities that are through and through and in which a force applied could cause the restoration to be pushed out of the cavity from one side to the other.

5 Chapter - Conclusions & Future work

Conclusions

When the samples were immersed in a liquid medium a decrease in bonding strength was noted in 1day and 1 week, followed by an increase in bonding strength in 1month time with results being comparable to those of when the samples were left in dry environment before being tested. From the push out test in human teeth, an increase was noted in the bond strength in 1 week with formulations reaching very high magnitudes of 22MPa followed by a decrease in 1month (6MPa – 8MPa). However, as this was only a pilot study with small sample size without any additional repetitions of the results that seemed out of the norms, further investigation in the field is needed**.** MCPM, PLS and their ratio seem to affect the bond strength in the following ways**:**

- In nearly all formulations irrespective of MCPM and PLS level, the addition of etch and bond improved the bond strength of the materials.
- The formulations with half the amount of MCPM compared to the rest showed improved average bond strengths to untreated ivory.
- Whilst doubling PLS content reduced bonding to untreated ivory, it had a complex effect with etched ivory and decreased the benefit of bonding agent.
- SMART composites with 4% instead of 2% PLS and with ratio MCPM/PLS = 2 showed stronger bonding on average which agrees with other studies that show higher resin tag formation with these formulations (Alkhouri unpublished study).

Future work

Although the results of the experiments on this study gave some answers to the questions / aims that were set in the beginning, further tests that would lead to the ideal material are necessary. On this direction future work could include: push out test on ivory and human dentine to measure the bond strength of commercial products that are widely being used such as Activa, Fuji II and IX and Z250 and compare them with the bond strength of the experimental formulations that were tested for the purposes of this project. Also assessing the effect of higher viscosity on bond strength (F2 formulation) using the push out test could also provide us with useful knowledge on the behaviour of the material. It would also be interested to attempt the push out test in primary molars. As previously explained this was not feasible for this test as the majority of the primary molars we had were grossly carious and therefore the diameter of the disc after cross sectioning the tooth was not enough to support a restoration and following push out test. This may be possible with primary molars less affected by caries, by having a smaller hole diameter that would still though allow the push out test to

be performed and / or by cross sectioning the crown on a different dimension to give a wider area surface.

Using the results of the test on the demineralisation of the ivory with formic acid, knowing the different levels of minerals loss versus time, tests could be carried out on demineralised ivory so that it replicated carious dentine. Before using carious teeth to assess the bond strength of the different experimental materials on them, it would be beneficial to test them on demineralised ivory as a quicker option which can give many and reproducible results because the ivory can be demineralised on a more or less equal manner, while in carious teeth dentine can be affected in different levels which can impact on the bond strength results. Following the completion of the push out test, the samples can be assessed on the SEM where the composite – ivory dentine and subsequently the composite – human dentine interface can be examined for potential hydroxyapatite formation, based on the remineralising potential of the experimental composite formulations.

Future research could focus on storing the samples in liquid media for a longer period of 3 to 6 months and measuring the bond strength. The effect of using etch and / or bond could also be investigated to find out whether their addition to the composite restoration would improve significantly the bonding strength of the material in a longer time interval than 30 days. Additionally, exploring whether the increase in bond strength, which was noted in 1-month time on the ivory is associated to the remineralising effect of the MCPM could be of great interest. Assessing the composite / ivory or human dentine interface under electronic microscope, following the push out test, could provide us with valuable information on the possible hydroxyapatite formation after a period of time that composite restoration is present. Also, demineralising ivory so that it represents a carious cavity could be significant in testing the bond strength of a material in presence of caries as an alternative to inducing caries using streptococcus mutans in the lab (Gama-Teixeira *et al.*, 2007)

Lastly, it would be interesting to restore extracted primary carious teeth with the experimental composite formulations and monitor the cavities for caries progression and the quality of the restorations in different time periods of 1, 3, 6, 9 and 12 months. This would involve visual assessment of the margins of the restoration and a way to assess caries progression, for example with radiographic examination of the restorations if possible.

6 Chapter – References

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7 Chapter - Appendices

Tables of results

Table 3-Bond strength, no etch/no bond preparation, 1 day in dry storage

Formulation	Strength (MPa)
Control	6.9
F2	11.1
F5	9.9
F5 small	7.2
F6	7.5
F7	7.5
F8	13.3

Table 4-Bond strength in ivory, etch dentine conditioning, 1 day in dry storage

Formulation	Strength (MPa)
Control	10.5
F2	11.7
F5	10.3
F5 small	11.1
F6	10.0
F7	8.0
F8	14.0

Table 5-Bond strength, acid & bond preparation, 1 day in dry condition

Formulation	Strength (MPa)
Control	9.3
F2	15.6
F5	14.1
F5 small	16.6
F6	13.9

F7	15.7
F8	15.6

Table 6-Bond Strength, no etch/no bond, 1 day in SBF

Formulation	Strength (MPa)
Control	9.0
F2	9.9
F5	7.9
F5 small	7.9
F6	4.2
F7	8.5
F8	10.2

Table 7-Bond strength, no ecth/no bond, 1 week in SBF

Formulation	Strength (MPa)
Control	1.3
F2	0.9
F5	2.7
F5 small	3.1
F6	0.6
F7	2.4
F8	2.1

Table 8-Bond strength, no etch/no bond, 1 month in SBF

Formulation	Strength (MPa)
Control	6.1
F2	5.8
F5	6.5
F5 small	6.0

F6	1.7
F7	4.3
F8	6.4

Table 9-Bond strength, etch, 1 day in SBF

Formulation	Strength (MPa)
Control	0.1
F2	0.6
F5	0.2
F5 small	2.3
F6	0.6
F7	3.7
F8	0.1

Table 10-Bond strength, etch, 1 week in SBF

Formulation	Strength (MPa)
Control	1.3
F2	1.9
F5	4.2
F5 small	4.9
F6	5.1
F7	3.6
F8	7.1

Table 11-Bond strength, etch, 1 month in SBF

Formulation	Strength (MPa)
Control	2.3
F2	6.4
F5	1.8
F5 small	2.2
F6	10.0
F7	9.6

F8	2.0

Table 12-Bond strength, etch and bond, 1 day in SBF

Formulation	Strength (MPa)
Control	1.8
F2	3.2
F5	1.5
F5 small	1.6
F6	1.7
F7	2.0
F8	0.8

Table 13-Bond strength, etch and bond, 1 week in SBF

Formulation	Strength (MPa)
Control	0.6
F2	0.7
F5	4.3
F5 small	8.0
F6	6.3
F7	5.0
F8	6.2

Table 14-Bond strength, etch and bond, 1 month in SBF

Formulation	Strength (MPa)
Control	8.3
F2	8.5
F5	4.5
F5 small	6.4
F6	6.3

F7	4.3
F8	11.8

Table 15-Bond strength, no etch/no bond, 1 day in saliva

Formulation	Strength (MPa)
Control	2.5
F2	10.4
F5	9.7
F5 small	7.3
F6	5.3
F7	7.0
F8	4.8

Table 16-Bond strength, no etch/no bond, 1 week in saliva

Formulation	Strength (MPa)
Control	3.3
F2	5.9
F5	5.8
F5 small	6.4
F6	7.9
F7	2.1
F8	2.4

Table 17-Bond strength, no etch/no bond, 1 month in saliva

Formulation	Strength (MPa)
Control	1.8
F2	4.7
F5	6.6
F5 small	8.9
F6	9.6
F7	3.5
F8	4.9

Table 18-Bond strength, etch, 1 day in saliva

Formulation	Strength (MPa)
Control	10.3
F2	11.2
F5	12.4
F5 small	13.6
F6	9.4
F7	9.6
F8	8.8

Table 19-Bond strength, etch, 1 week in saliva

Formulation	Strength (MPa)
Control	3.0
F2	3.0
F5	4.5
F5 small	7.9
F6	9.0
F7	4.6
F8	4.4

Table 20-Bond strength, etch, 1 month in saliva

Formulation	Strength (MPa)
Control	3.4
F5	0.1
F6	1.7
F7	2.2
F8	4.5

Table 21-Bond strength, etch and bond, 1 day in saliva

Formulation	Strength (MPa)
Control	12.7
F2	9.4
F5	6.5
F5 small	7.8
F6	8.0
F7	7.1
F8	7.0

Table 22-Bond strength, etch and bond, 1 week in saliva

Formulation	Strength (MPa)
Control	3.0
F5	6.4
F6	1.5
F7	7.1
F8	1.9

Table 23-Bond strength, etch and bond, 1 month in saliva

Formulation	Strength (MPa)
Control	4.2
F2	2.5
F5	3.1
F5 small	5.3
F6	4.6
F7	7.0
F8	7.9

Table 24-Bond strength in human dentine, no etch / no bond, 1 day, dry storage

Formulation	Strength (MPa)
F5	5.5
F6	5.1
F7	5.3
F8	5.4

Table 25-Bond strength in human dentine, no etch / no bond, 1 day, Artificial saliva

Formulation	Strength (MPa)
F5	5.0
F6	4.7
F7	7.3
F8	4.2

Table 26-Bond strength in human dentine, no etch / no bond, 1 week, Artificial saliva

Formulation	Strength (MPa)
F5	6.5
F6	19.8
F7	22.0
F8	12.4

Table 27-Bond strength in human dentine, no etch / no bond, 1 month, Artificial saliva

Formulation	Strength (MPa)
F5	7.4
F6	7.7
F7	6.5
F8	8.0

Consent Forms

University College London Hospitals 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: / /

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.