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Biodentine™ as a Root Filling Material in Immature Permanent Teeth- a preliminary *in Vitro* study

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Dedication

I dedicated this thesis with special thanks to my wife, Norah for her endless love, support, understanding and patience.

Many thanks to my Father whose footsteps I have always followed. Without him, none of this would have been possible.

My deepest regards to my Mother for her support, encouragements, and prayers all along the way.

Special thanks for my daughter Shaikah and my son Othman for being here with me, as they provide me with love when I look at their eyes.

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Author's Declaration

I, Yasir O. BinAhmed, declare that the work contained within this thesis is my own work.

The contributions to this dissertation have already been acknowledged but can be listed as follows:

- The study was designed with the help of Professor Timothy Watson and Professor Marie-Therese Hosey.
- All statistical analyses were carried out by Dr. Martyn Sherriff.

Abstract

This thesis is the preliminary study of Biodentine™, when used as a root filling material, using a randomised controlled *in vitro* design.

Aims: this thesis aimed to evaluate the fracture resistance (compressive strength) of simulated immature teeth after filling the root canals with a calcium tri-silicate cement (Biodentine™) or gutta-percha. Furthermore, record the type of fracture in simulated immature teeth when loaded to failure.

Background: Root canal filling materials in immature teeth alter the flexural strength of dentine leading to fracture under compressive loads. And so children with their root filled teeth commonly undergo further tooth fracture. In this regard, Biodentine™ may offer an alternative to current conventional materials.

Design: Following pilots study, twenty extracted mandibular first premolar teeth were randomised into two groups: Biodentine™ and gutta-percha. All teeth were prepared to size 110 K-file at the apex and had Biodentine™ placed as the apical seal (5mm). In one group thermoplastize gutta-percha was placed as far as the cementoenamel Junction, in the other, Biodentine™ is used to fill the canal to the CEJ. Both groups receives composite as coronal restoration.

Results: There was no significant difference in the fracture resistance (compressive loading) between the Biodentine™ and gutta-percha group. [Two sample t-Test ; 95% CI (-587 to

39.55) and P=0.08]. Crown fracture was the most common type of fracture in both groups. In the Biodentine™ group [n=8, 64%] and in the gutta-percha group [n=7, 57%].

Conclusions: This study suggests that Biodentine™ should be further investigated in vitro in the treatment of non-vital immature teeth.

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1 Introduction

The main goal in paediatric dentistry is to provide a good quality dental care to children, to prevent pain and infection, and to restore the child's oral health to optimum level.

Trauma to primary and permanent teeth is a relatively common reason for referral in dental practice. There is ongoing research into the management and specifically into the dental materials used to treat these injured teeth (DiAngelis *et al,* 2012). Some of the conventional materials dry-out the tooth, making the tooth more prone to future fracture.

An immature tooth is the tooth that is not fully formed, particularly the root apex. A vital pulp is necessary for the development and maturation of the tooth root. Following trauma, teeth can become non-vital. If the tooth loses vitality, the maturation process will cease leaving a tooth with wide open apex, and thin canal walls. The treatment of immature non-vital tooth is root canal treatment that is complicated by the absence of an apical constriction against which the root filling material is condensed. Moreover, it has been observed that non-vital immature teeth fracture with minimal trauma. Therefore, even forces generated during normal chewing may lead to the fracture of the immature root canal treated teeth.

Recent advances in dental materials, specifically root canal fillings, could improve the long term prognosis of the teeth of children who have sustained a dentoalveolar trauma. This thesis is the preliminary *in vitro* investigation of one of these, namely, Biodentine.

2 Literature Review

2.1 Dental trauma

Dental trauma is not only distressing and painful for the child but it is also a challenge for the paediatric dentist who must manage it in such a way as to give the best possible, aesthetically pleasing clinical outcome. Dental trauma can affect the child's appearance if tooth loss has occurred. This may have an impact on the child's socialization; affecting their psychological development (Sgan-Cohen *et al*, 2005). Indeed, the social impact of dental trauma has become a common finding and as a consequences social functioning, emotional balance, and well being of the child may be impaired (Lee and Divaris, 2009).

There are several consequences following dental trauma that may lead to tooth loss. Pulp necrosis and inflammatory root resorption may be seen in young patients with either delayed or insufficient treatment following dental injury (Al-Nazhan *et al*, 1995). Pulp necrosis reported to be the most common sequelae (Andreasen *et al*, 1985) there are several signs that may indicate non-vital tooth: coronal discoloration; external inflammatory root resorption; periapical infection; and arrested root development.

Inflammatory root resorption is a pathological process that leads to progressive loss of root structure (Finucane and Kinirons, 2003). Therefore, the paediatric dentist's goal is to intervene as soon as possible to prevent tooth loss and to try to achieve a good aesthetic and functional outcome. This intervention is in the form of root canal therapy.

2.1.1 Prevalence of dental trauma

The prevalence of traumatic dental injuries is high worldwide. Oral injuries represent 18% of all injuries that occur in preschool children (Petersson *et al*, 1997). The dental injuries are the commonest of all facial injuries. In the UK, one in five children had experienced traumatic dental injuries to their permanent anterior teeth before leaving school (Chadwick *et al*, 2006).

2.1.1.1 Prevalence according to age

Many children have immature teeth at the time that they sustain dental trauma. Authors agree that the maxillary central incisor, commonest injured tooth, is not fully formed until at least 11 years of age (Mcdonald *et al*, 2010).

The prevalence of children sustaining dental trauma to their incisors increased with age from five percent at the age of eight years to thirteen percent at the age of fifteen years. The National Children's Dental Health Survey in 2003 reported that eleven percent of children sustained accidental injuries to their permanent incisors at the age of 12 while it was 13% for 15 years old (Chadwick *et al,* 2006).

2.1.1.2 Prevalence according to tooth affected

The most frequently affected tooth by dental trauma in the permanent dentition is the maxillary central incisor, followed by the maxillary lateral incisor and mandibular central incisor (Shulman and Peterson, 2004).

2.1.1.3 Prevalence according to type of injury

Crown fractures are the most frequent dental injuries in permanent teeth (Kaste *et al*, 1996).

2.1.1.4 Prevalence according to the cause of injury

Young children are more prone to dental injuries due to falls, collisions, and being struck by hard object during physical activities. On the other hand, contact sport, road traffic accident and assaults are the main causes of dental injuries in teenagers (Rodd and Chesham, 1997).

2.1.2 Consequences of dental trauma to oral tissue

Andreasen defines injury as an interruption in the continuity of tissues, and healing as the re-establishment of that continuity. This can happen either by tissue repair, where the healed tissue restores the continuity, or by tissue regeneration, where the anatomy and function are both restored.

Dental tissues have marked capacity for regeneration compared to other tissues in the body. Moreover, injuries to the pulp and periodontal ligament may show regeneration or tissue repair with fibrous scar and bony tissue.

There are several factors that tissue regeneration depends on. First of which, is the presence of tissue-specific cells population at the site of injury. Therefore, the absence of these cells at the site of injury can lead to tissue repair rather than regeneration. Ankylosis of the teeth is an example of tissue repair where the tissue-specific cells are absent (PDL). Second, is creating the best environment for tissue specific cells to migrate into the wound site. Therefore, if an avulsed tooth is incompletely repositioned leading to damage to the periodontal ligament cells, this may result in in-growth of the bone and PDL-derived cells into the pulp canal. The third factor is the presence of bacteria or foreign body contamination, which may lead to inflammation at the wound were repair rather than regeneration occurs. This happen due to the formation of non-specific tissue cell when inflammation is presents that result in repair (Andreasen *et al*, 2007).

As Paediatric dentist we can control the second and the third factors, thus by preventing the development of infection and creating the best environment for healing. Therefore, using a root filling material with good physical and antibacterial properties is essential.

The teeth have natural defence mechanisms against trauma. One of these defence mechanisms is periodontal ligaments. The main function of the periodontal ligaments is to support the tooth in the socket during function. Moreover, following trauma the periodontal ligament acts as cushion that absorbs the impact of trauma. However, if the trauma force exceeds the absorptive capacity of the periodontal ligaments then injury to the tooth and supporting structure may be observed (Anderasen *et al*, 2007).

There are many complications that can occur following dental trauma to the permanent teeth. The most common sequalae is pulp necrosis that usually occurs as a result of the disruption in the neurovascular supply at the apex of the tooth (Lauridsen *et al*, 2012). The risk of pulp necrosis varies widely with each type of injury.

In uncomplicated crown fractures there are evidence that 0-6% of the teeth may undergo pulp necrosis and this will increase to 25% when associated with luxation injuries (Robertson *et al*, 2000). Moreover, the risk of pulp necrosis is 20 – 44 % in root fracture teeth (Andreasen *et al*, 2004).

In severe luxation and avulsion where damage to the periodontal ligament occurs, that may result in root resorption (Finucane and Kinirons, 2003). There are different types of root

resorption that may occur; the commonest in relation to trauma are inflammatory external and internal root resorption. The type is determined by: exposure of the dentinal tubules; the content of the pulp, whether ischaemic, sterile, necrotic, or infected; presence of adjacent cementoblast; and viability of the periodontal ligament cells (Finucane and Kinirons, 2003). The key treatment of inflammatory root resorption is to control of infection through root canal treatment.

2.2 Causes of devitalisation of teeth other than trauma

Trauma is the most common cause of loss of vitality in anterior teeth followed by dental caries. In the dental caries case, when the caries is deep that may insult the pulp with bacterial by products that will cause reversible inflammation, if not treated will move on to irreversible inflammation and finally pulp necrosis.

The prevalence of dental caries in the United Kingdom among twelve years old children is high. The National Children's Dental Health Survey in 2003 reported that 33.4% of pupils were found to have experience of caries, having one or more teeth which were decayed to dentinal level, extracted or filled because of caries (Pitts *et al*, 2006).

There are other conditions that may lead to loss of vitality such as dens invaginatus; which is a developmental dental anomaly affecting the anterior teeth, most commonly maxillary incisors (Alani and Bishop, 2008).

2.3 Management of non-vital teeth

In the United Kingdom, Mackie *et al* (2010) published a guideline on the management of non-vital immature teeth. The permanent maxillary central incisor normally erupts at seven to eight years with complete root formation at ten to eleven years (McDonald *et al*, 2010). Therefore, the occurrence of trauma or deep caries at that age in children leads to a need for root canal treatment of permanent teeth with open apices.

The biological aim of root canal treatment is to prevent or eliminate apical periodontitis. This is achieved by chemo-mechanical debridement of the root canal space followed by root canal filling (Nair, 2006).

2.3.1 The problems associated with non-vital tooth with immature apex

There are multiple challenges that face a paediatric dentist in treating non-vital immature tooth:

1. The roots of these teeth are thin with a higher susceptibility to fracture (Andreasen *et al*, 2002). Indeed, Stormer *et al* (1988) claimed that 60% of all endodontically treated teeth with immature root formation have had cervical root fractures due to minor impacts.
2. The infected root canal space cannot be disinfected with the standard root canal protocol with the aggressive use of endodontic files.

3. Once the microbial phase of the treatment is complete, filling the root canal is difficult because the open apex provides no barrier to prevent the root filling material from projecting beyond the tooth into the periodontal tissue.

The use of a root filling material that reinforces the root against fracture could help in overcoming the problems associated with immature tooth.

2.3.2 Root filling materials used in the management of non-vital immature teeth

There are many dental materials that can be used to root fill teeth; each one has its advantage over the other.

The role of a root canal filling material is to seal the canal. If the coronal seal fails the ideal root canal filling should prevent microleackage, thus preventing re-infection (Hirsch *et al*, 1979). In addition, a root filling material should be easy to use, dimensionally stable, bactericidal (or at least prevent bacterial growth), biocompatible, radiopaque, sterile, easily removed and should not stain tooth structure (Grossman, 1966).

2.3.2.1 Calcium hydroxide

Calcium hydroxide material was introduced into endodontics by Hermann in 1920. Calcium hydroxide has many clinical applications in dentistry due to its widely known properties.

2.3.2.1.1 Properties of calcium hydroxide

Calcium hydroxide paste is very alkaline. In other words, it has a high pH (approximately 12.5–12.8). It is this alkalinity that gives its antibacterial property (Farhad and Mohammadi, 2005). Calcium hydroxide achieves its main action through the ionic dissociation of calcium ions and hydroxyl ions to subsequently affect vital tissues: the induction of hard-tissue deposition and antibacterial properties (Rehman *et al*, 1996).

One of the useful clinical characteristics of calcium hydroxide is that it has low solubility. This allows more time for it to take effect when it comes into contact with fluids and vital tissue (Spangberg and Haapasalo, 2002). Moreover, calcium hydroxide has a mineralization effect when used in pulp capping and in apexification procedures by inducing calcified barrier (Eda, 1961).

Because of the high pH of calcium hydroxide, a superficial layer of necrosis occurs in the pulp to a depth of up to two millimetres (Estrela and Holland, 2009). Beyond this layer, only

a mild inflammatory response is seen and a hard tissue may be formed. Provided the area is free from bacteria (Estrela *et al,* 1995). The hydroxyl group in the calcium hydroxide provides the alkaline environment and this is responsible for the formation of active calcification and repair. The high pH not only neutralizes the lactic acid secreted from osteoclasts, thus preventing dentine minerals from dissolution, but also activates the alkaline phosphatase enzyme. This has an important role in the formation of hard tissue (Estrela *et al*, 1995).

2.3.2.1.2 Viscosity of the calcium hydroxide mix

The viscosity of the mix of calcium hydroxide used as root canal medicament may affect the antimicrobial activity of the calcium hydroxide paste. Behnen *et al* (2001) reported that a thick mixture of calcium hydroxide and water resulted in a significant reduction in antibacterial activity against E. faecalis in dentine tubules when compared to a thin mix available in some commercial products.

2.3.2.1.3 Advantages of calcium hydroxide

Antibacterial activity is one of the major advantages of calcium hydroxide; this activity is through the release of the hydroxyl ions in an aqueous environment, which is highly oxidant free radical. The lethal effect of hydroxyl ion on the bacteria is through multiple mechanisms, damaging the DNA, denaturing the protein, and damaging the cytoplasmic membrane (Estrela *et al*, 1994).

Reports show that calcium hydroxide is well tolerated and sufficiently biocompatible if extruded in the periapical area that it will resorb (Briseno and Willershausen, 1992). Pissiotis and Spangberg (1990) evaluated mandible bone reactions of guinea pigs to implants of hydroxyapatite, collagen, and calcium hydroxide, alone or in different combinations, over a period of 16 weeks. Findings revealed that no major inflammatory reactions occurred in any of the implant combinations. Indeed, the calcium hydroxide and collagen implants were partially or totally resorbed and replaced by bony tissue.

Calcium hydroxide is also used as intra-canal medicament to control internal resorption (Haapasalo and Endal, 2006). Furthermore, it controls inflammatory root resorption (Majorana *et al*, 2003). It is recommended by the International Association of Dental Traumatology in 2012, that any teeth with a necrotic pulp following luxation injury should be dressed with calcium hydroxide.

2.3.2.1.4 Disadvantages of calcium hydroxide

Calcium hydroxide requires time to induce an apical barrier; from 6-18 month in case of apexification (Mackie *et al*, 1988). Moreover, the length of time to induce the apical barrier makes the risk of failure higher due to patient compliance toward subsequent appointment attendance as well as the delay in receiving the definitive treatment.

Moreover, the apical barrier that calcium hydroxide forms, is incomplete due to vascular inclusions, these tunnels may allow bacterial invasion through the barrier (Cox *et al*, 1996; Nair *et al*, 2008).

The long-term of use of non-setting calcium hydroxide increases the risk of cervical root fracture (Rosenberg *et al*, 2007; White *et al*, 2002; Cvek, 1992). Calcium hydroxide increases the risk of cervical root fracture in immature teeth. This is due to the loss of the inorganic and organic component of the dentine (Andreasen *et al*, 2002). Rosenberg and colleagues (2007) in a vitro study found that the microtensile fracture strength of dentine was reduced by 23% to 43.9% following introduction of calcium hydroxide into the root canal for between 7 to 83 days further. Cvek (1992) reported that the incidence of cervical root fracture in immature teeth dressed with calcium hydroxide ranges from 28-77%. The frequency of fracture depends on the stage of root development: the highest risk of fracture is in immature teeth.

Calcium hydroxide cannot be used as permanent root filling material due to its solubility in tissue fluids. Therefore, there are few studies that measure the suitability of calcium hydroxide as an intra-canal filling material. A study by McMichen *et al* (2003) confirms that the solubility of calcium hydroxide sealers in water is more than other sealers.

These physical properties and clinical outcomes conclude that calcium hydroxide cannot be used as permanent root filling material, although it can be used as inter-canal medicament to provide favourable environment to healing.

The use of non-setting calcium hydroxide in immature teeth to produce apical barrier for many years has been the gold standard (Mackie, 2010). It has been shown that an apical barrier formation will be formed in 74% to 100% with the use of this technique (Sheehy and Roberts, 1997). The average time to achieve barrier formation is 6 months (Mackie, 2010). However, there are many studies that (Rosenberg *et al*, 2007; White *et al*, 2002; Cvek, 1992) show that long term treatment with calcium hydroxide weakens the dentine, and subsequently leads to cervical root fracture.

2.3.2.2 Gutta-percha

Gutta-percha is the most commonly used root filling material. It is made from the dried juice of the Taban tree; it was first introduced by Sir Jose d'Almeida to the Royal Asiatic Society of England in 1843 and then, in the late 1800s, it was introduced to dentistry (Swain, 1890).

2.3.2.2.1 Properties of gutta-percha

Gutta-percha considered to be an isomer of natural rubber known as trans-polyisoprene and it is harder, less elastic and more brittle than natural rubber. Whereas natural rubber is mainly amorphous, the gutta-percha is 60% crystalline, this fact accounts for the difference in the mechanical properties (Friedman *et al*, 1975).

Crystalline gutta-percha has two forms: the alpha and the beta phase. The alpha phase comes from the natural tree. The beta phase is the processed gutta-percha that is used in dentistry (Cohen and Hargreaves, 2006).

Gutta-percha forms are interchangeable depending in the temperature of the material. The alpha form is less subject to shrinkage which is used in thermoplasticized obturating system. On the other hand, the beta form is usually found in the gutta percha cones or points that are used in the cold compaction techniques (O'Brien, 2008).

The gutta-percha cones contain 20% gutta-percha, 60-75% zinc oxide and the remaining 5-10% consists of wax, resin (plasticizer), and heavy metal sulfate (Friedman *et al*, 1975; Cohen and Hargreaves, 2006).

2.3.2.2.2 Advantages of gutta-percha

Gutta-percha points are weak bactericidal agents (Moorer and Genet, 1982). Many workers have attempted to improve the bactericidal activity. Martin and Martin (1999) introduced a new formulation of gutta-percha that contained iodoform (Medicated Gutta-Percha). Within the filled root canal, the iodine/iodoform depot in the Medicated Gutta-Percha cone is a biologically active agent that inhibits microbial growth. The iodoform is centrally located within then gutta-percha and takes about 24 hours to leach to the surface. “The iodoform remains inert until it comes in contact with tissue fluids that activate the free iodine” (Martin and Martin, 1999). However, a *in vitro* study done by Shur and colleagues in 2003 showed that the Medicated Gutta-Percha only had a bacteriostatic effect on specific bacteria, not all of the species in the root canal system.

One of the advantages of gutta-percha is that it’s easy to use and can be inserted and removed from the root canal system, relatively simply compared to other materials.

2.3.2.2.3 Disadvantages of gutta-percha

Gutta-percha doesn't bond to dentine; it has to be used in conjunction with a sealer to achieve a good apical seal.

Gutta-percha also exhibits a degree of tissue irritation. This is related to the high content of zinc oxide: a known irritant (Das, 1981). Gutta-percha is not dimensionally stable. Even though it is thought that when it is compressed with force its volume is reduced, studies have shown that it is actually compacted, not compressed, and that there are increased volumetric changes due to heating (Schilder *et al*, 1974). Unfortunately, warmed gutta-percha also shrinks as it returns to body temperature. Schilder *et al* (1974) recommended that vertical pressure need to be applied to compensate for volume changing as cooling take place in all warm gutta-percha techniques.

Allergic reactions to gutta-percha have also been a concern to clinicians as the material contains rubber. The American Association of Endodontists (1998) stated that due to the chemical similarity between natural rubber and gutta-percha, patient with history of latex allergy should be patch tested before initiating the obturation phase.

2.3.2.3 Mineral Trioxide Aggregate

Mineral Trioxide Aggregate (MTA) was discovered in 1993 by Torabinejad and co-workers (Torabinejad *et al*, 1994). MTA is a material that has many uses in dentistry. It has many ideal characteristics, non-toxic, non-carcinogenic, non-genotoxic, biocompatible, insoluble in tissue fluids, and dimensionally stable. It's been recommended for pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apex, repair of root perforation, and root canal filling material (Torabinejad and Pitt Ford, 1996; Simon *et al,* 2007; Felippe *et al*, 2007; Ribeiro, 2008).

MTA has been recognized as a bioactive material (Enkel, 2008) that is hard tissue conductive (Moretton *et al*, 2000), hard tissue inductive, and biocompatible. Furthermore, the presence of moisture should not affect its sealing ability (Torabinejad and Pitt Ford, 1996).

2.3.2.3.1 Chemical properties of Mineral Trioxide Aggregate

MTA powder contains fine hydrophilic particles that set in the presence of moisture. The main components of MTA are calcium and silica, as well as bismuth oxide (Asgary *et al*, 2005; Camilleri *et al*, 2005; Asgary *et al*, 2006; Belio-Reyes *et al*, 2009).

There are two forms of MTA in the market; gray (GMTA) and white (WMTA). The WMTA was introduced when it was reported that the GMTA caused tooth discolouration. GMTA basically consists of dicalcium and tricalcium silicate and bismuth oxide, whereas WMTA is primarily composed of tricalcium silicate and bismuth oxide (Camilleri *et al*, 2005).

When MTA powder is mixed with water, calcium hydroxide and calcium silicate hydrate are initially formed and then eventually transform into a poorly crystallized and porous solid gel (Camilleri, 2007). The formation of calcium hydroxide initially gives the MTA its alkalinity after hydration. Camilleri (2008) believed that calcium hydroxide is formed from dicalcium and tricalcium silicate, after mixing MTA powder with water, whereas Dammaschke *et al* (2005) reported that calcium hydroxide is a product of tricalcium aluminate hydrogenation.

Bismuth affects calcium hydroxide precipitation after MTA hydration (Camilleri, 2007). Because bismuth oxide dissolves in an acidic environment, it has been suggested that placing MTA in an acidic environment such as inflammatory tissues might result in the release of bismuth oxide (Camilleri, 2007). This might decrease MTA's biocompatibility because bismuth oxide does not encourage cell proliferation in cell culture (Camilleri *et al*, 2004).

2.3.2.3.2 Advantages of Mineral Trioxide Aggregate

Materials used to root fill teeth may be in contact with the periodontium and periapical tissue, therefore it must be biocompatible and non-toxic. A meta-analysis evaluating the apical sealing, and biocompatibility of silver amalgam, super EBA (reinforced zinc oxide cement), intermediate restorative material (IRM) and MTA as retrograde filler materials, showed that MTA is the most compatible (Fernández-Yáñez *et al*, 2007).

A study done by Torabinejad and colleagues in 1995, to compare the setting time, compressive strength, and solubility, reported that MTA have low or no solubility. Moreover, there are differences in solubility with different types of MTA (Islam *et al,* 2006), with increased solubility reported in a long term study (Fridland and Rosado, 2005). However, after a thorough analysis of the literature, in my opinion MTA have a low solubility.

Mineral Trioxide Aggregate has good sealing ability. This has been confirmed in numerous dye leakage studies (Torabinejad *et al*, 1994; Martell and Chandler, 2002). On the other hand, Tobon-Arroyave *et al* (2007) found that there is more leakage and dye penetration in WMTA when placing the samples in dye before complete setting.

There are many factors that may affect the MTA leakage studies, the thickness of the dentinal walls, the dye pH, the type of the dye, pre-treatment with chelating agents, the

tooth storage medium before the experiment, and the setting status of MTA before its placement in the dye, that might explain the different results in the two studies.

Yan and colleagues (2006) investigated the dentine-bond strength of MTA after immersion in 5.25% sodium hypochlorite, 2% chlorhexidien, and Glyde file prepration (combination of EDTA and carbamide peroxide in a water-soluble base) for two hours. The result shows that the bonding strength of MTA to dentine is reduce significantly with glyde file preparation; however, this is not relevant clinically because none of the materials is left in the tooth for two hours during clinical procedure.

Interestingly, studies have suggested that mineral trioxide aggregate induce hard tissue formation by promoting osteoclast activity. Sarkar and colleagues in 2005, placed root filled teeth with MTA in contact with PBS (synthetic tissue fluid composed of a neutral phosphate buffer saline solution) for 2 months. They reported that MTA leached some ions in different proportions. Moreover, they found that there was a white layer between MTA and the root canal walls. This white layer was composed of calcium, phosphorus, and oxygen in similar properties to hydroxyapatite.

2.3.2.3.3 Disadvantages of Mineral Trioxide Aggregate

The mean setting time of MTA is approximately 165 min (Torabinejad *et al*, 1995). This long setting time is one of the major drawbacks of the material. Many researchers have tried to overcome this disadvantage. In a study by Wiltbank and colleagues (2007) the addition of accelerators improved the setting time but the sealing ability and biocompatibility were not evaluated.

Another disadvantage of MTA is the gray discolouration of the teeth. This usually happens with GMTA at the cervical area of the tooth. The introduction of WMTA was to overcome this but despite this there are still reports that it can occur (Moore *et al*, 2011).

2.3.2.4 Biodentine™ (calcium silicate-based material)

There are very few studies of Biodentine™ at the present time in the published literature. Biodentine™ was developed by Septodont's Research Group as a new class of dental material which could conciliate high mechanical properties with excellent biocompatibility, as well as a bioactive behaviour. Several years of active and collaborative research between Septodont and several universities led to a new calcium-silicate based formulation, which is suitable as a dentine replacement material whenever original dentine is damaged.

A new experimental silicate-based restorative cement has been developed, put on the market under the name of Biodentine™ (Septodont, Saint Maur des Fosses, France). It is a calcium-based cement, similar to ProRoot MTA (Torabinejad *et al*, 1995; Camilleri *et al*, 2005) and Portland's cements (Camilleri *et al*, 2006).

2.3.2.4.1 Properties of the different components of Biodentine™

The main component of the powder is a tri-calcium silicate, with the addition to the powder of calcium carbonate and radiopaquer. The liquid is a solution of calcium chloride with a water reducing agent (Table 2.1). It is dispensed in a fixed powder:liquid proportion, and mixed with an amalgamator for 30 seconds (Figure2.1).



Figure 2.1 Biodentine™ putty-like consistency

The setting reaction leads to a gel structure, which allows possible ionic exchanges. Compared to other silicate- based cements, this material presents two advantages: i) a faster setting time of about 12 minutes and ii) higher mechanical properties. These physico-chemical properties associated with the biological behaviour (Laurent *et al*, 2008) suggest that it may be used as a permanent dentine substitute.

Component	Property
POWDER	
Tri-calcium silicate	**It is the main component of the powder. It regulates the setting reaction.**
Di-calcium Silicate	**Second core material**
Calcium carbonate and Oxide	**Its role is similar to the fillers.**
Iron Oxide	**It gives the shade**
Zirconium dioxide	**Provide the radio-opacity to the cement.**
LIQUID	
Calcium chloride	**Is an accelerator.**
Water reducing agent	**It can reduce the viscosity of cement.**

Table 2.1 The component of Biodentine™ (Scientific file, Septodent)

2.3.2.4.2 Setting reaction

The reaction of the powder with the liquid leads to the setting and hardening of the cement. The hydration of the tri-calcium silicate leads to the formation of a hydrated calcium silicate gel and calcium hydroxide (Taylor *et al*, 1997). The cement located in inter-grain areas has a high level of calcite content (Figure 2.2).



Figure 2.2 Biodentine™ after setting (Scientific file, Septodent)

The tri-calcium silicate hydration is achieved by dissolution of tri-calcium silicate and precipitation of calcium silicate hydrate. Layers of calcium silicate hydrated gel surround the unreacted tri-calcium silicate grains, which are relatively impermeable to water. Due to permanent hydration of tri-calcium silicate the C-S-H will form, which gradually will fill the space between the grains of tri-calcium silicate. The following formula shows the complete hydration reaction (Taylor *et al*, 1997; Allen *et al*, 2007).

$$2(3CaO.SiO_2) + 6H_2O \Rightarrow 3CaO.2SiO_2.3H_2O + 3Ca(OH)_2$$

2.3.2.4.3 Advantages of Biodentine™

Biodentine™ and MTA have the same main component namely, tri-calcium silicate. Septodont improved the physico-chemical properties (short setting time, high mechanical strength) which make Biodentine™ clinically easy to handle and compatible (Camilleri *et al*, 2007). The Sealing ability of this biomaterial was also assessed to be equivalent to glass-ionomers, without requiring any specific conditioning of the dentine surface. Leakage resistance and mechanical strength improves over the first weeks after placement (Scientific file, Septodent).

Moreover, reactionary dentine formation has been demonstrated in rats. As such the material exhibited a high quality and quantity of protective dentine stimulation especially in indirect pulp capping. In the case of direct pulp capping and pulpotomy in pigs, the compatibility with the pulp enables a direct contact with fibroblasts and produced only limited inflammatory response compared to controls. Formation of a regular and dense dentine bridge is histologically demonstrated within one month (Scientific file, Septodent).

Besides the usual endodontic indications of this class of calcium-silicate cements (repair of perforations or resorption, apexification, root-end filling), Biodentine™ has been evaluated for its restorative properties in comparison to composite (Z100, 3M ESPE). In a three year follow-up, randomized clinical study in 400 patients, Biodentine™ was suitable as a permanent dentine substitute and temporary enamel substitute. Restoration of deep or large crown carious lesions provides a very tight seal, without post-operative sensitivity and

insures the longevity of restorations in vital teeth. Biodentine™ has also achieved 100% success in direct pulp capping in adults presenting with a healthy pulp (Scientific file, Septodent).

The main challenges with immature non-vital teeth is the difficulty in disinfecting the root canal without instrumentation, as well as the absence of an apical barrier against which root canal material can be condensed. Biodentine™ offer a solution for these challenges as it does not require an apical barrier and it has bactericidal activity due to its alkalinity. However, recent study has reported that the Biodentine can significantly reduce the dentine flexure strength if aged in direct contact with dentine (Sawyer *et al*, 2012).

2.3.2.4.4 Disadvantages of Biodentine™

Biodentine™ is a newly developed material that is under investigation by Septodent Company. There are several ongoing studies to assess its physical properties. One of the disadvantages for Biodentine™ as a root filling material is the short setting time. It has a setting time of 12 minutes (Goldberg *et al*, 2009); this will give short time for the material to be condensed into the canal; compared to MTA which has a three to four hours setting time (Torbinejad *et al,* 1995). Perhaps, more importantly, the high alkalinity of hydrated Biodentine might induce denaturing of the organic component of dentine leading to a

reduced flexural strength of dentine and thus an increased likelihood of fracture (Atmeh *et al*, 2012).

2.4 Potential uses of new materials as root filling for immature non-vital teeth

The materials that are currently used as root filling material in immature teeth are thought to weaken the dentin and reduce its flexure strength. Moreover, most of these root filling materials do not reinforce the tooth.

There are some studies that have suggested that root canal filling materials reinforce the root (Trope and Ray 1992; Lertchirakarn *et al,* 2002). On the other hand, numerous studies show that root canal filling material did not increase the fracture resistance of the tooth (Apicella *et al*, 1999; Zandbiglari *et al*, 2006). There is still no enough evidence to support specific root filling materials as agent that might reinforce the tooth.

BioAggregate materials have been used for retrograde root filling, repair of perforation, and apexification in immature non-vital teeth (Park *et al*, 2010). In a *vitro* study by Tuna *et al* (2011), the long-term fracture resistance of human immature teeth restored with BioAggregate, mineral trioxide aggregate, and calcium hydroxide was assessed. They

reported that the teeth restored with BioAggregate had a higher fracture resistance. However, in this study the sample was not standardised.

There is newly developed material, Resilon™ that consists of core material and resin based sealer. Resilon™ has the ability to penetrate the dentinal tubules of the canal wall and simultaneously develop a strong bond between the core material and the sealer (Shipper and Trope, 2004). As such, this material may reinforce the root and increase the fracture resistance. Indeed, the ability to increase fracture resistance have been proven by some studies (Hammad *et al,* 2007), but not in others (Hanada *et al*, 2010). Resilon™ requires an apical stop to be condensed against, however, in non-vital immature teeth the apical stop in absent.

The ideal material that can be used in immature tooth will be the material that can reinforce the root and increase the fracture resistance.

2.5 Normal bite force

In humans, the maximum biting force ranges from 150 to 250 Newtons in the incisor region (Carlsson, 1974). The maximum biting force is generated when the maxillary and mandibular teeth are in contact. However, the actual time for the teeth to be in contact is 15-30 minutes per day (Anderson and Picton, 1957).

Immature non-vital teeth are at a higher risk of fracture compared to mature teeth due to incomplete root development which results in thinner dentine walls. Therefore, they are more vulnerable to fracture under normal biting forces.

2.6 *In vitro* studies

2.6.1 Methodology

Fracture resistance is the loss of energy or strength resulting in fracture. Fracture resistance testing is the application of continuous loading to a test specimen in order to determine how it performs under loading.

In vitro studies utilise several methods to measure the fracture resistance. One of these methods is based on the application of forces that increase gradually until the tooth fracture. The application of consistent force for a given number of cycles is another way to measure the fracture resistance.

Causwels and colleagues (2010) used a gradually increasing compressive force to measure the fracture resistance. On the other hand, Mannocci *et al* (2002) used cyclic loading to measure the fracture resistance. When comparing both studies, the use of cyclic loading to measure the fracture resistance more closely simulates the natural loading imposed by mastication. Therefore, cyclic loading is a better method for measuring fracture resistance.

A relatively common method for testing the fracture resistance is the Staircase method. Staircase method was first introduced by Dixon and Mood (1948) for testing explosives. The Staircase method has been popularized by Little (1975) for fracture resistance testing. The Staircase method utilizes a simple protocol in which a specimen is tested at a given starting force for a specified number of cycles or until failure, whichever comes first. If the specimen survives, the stress level is increased for the next specimen; likewise the stress is decreased if the specimen fails. This is applied for all samples, followed by the use of Dixon and Mood's equations to calculate the mean fatigue strength and standard deviation for a given number of cycles (Pollak *et al*, 2005).

With regard to the direction of force application this can be either in a perpendicular, axial, or angled direction to the long axis of the tooth. In real life the tooth is subjected to forces

from different angles. The normal masticatory forces are multidirectional and applied on larger area over time (Fernandes *et al*, 2003). Furthermore, maxillary and mandibular incisors contact at an average angle of 130^{o} in class I occlusion (Pene *et al*, 2001). Therefore, the applied force should simulate the average angle of contact between these teeth if the normal life situation is to be reproduced.

2.6.2 Root filling materials used

There are many studies that compare the ability of different root filling materials to strengthen immature teeth. Andreasen *et al* (2006) suggested the replacement of long-term calcium hydroxide treatment by one visit MTA apexification. Despite the advantages of MTA, no studies proved that it strengthens the immature teeth. In a study by Wilkinson and colleagues (2007) they evaluated the fracture resistance in teeth with different root filling materials. They reported that composite resin was the only material that has significantly higher fracture resistance than gutta percha and Resilon^{TM}. However, composite resin in not used for root canal filling because it is difficult to handle in root canal.

Many previous studies analysed the fracture resistance and the long-term effect of root filling materials on different sample types such as sheep, bovine, or human teeth. The difference in structure and shape between sample types may be the reason for the inconsistency in the results of these studies (Cauwels *et al*, 2010).

3 Aims and hypothesis

3.1 Aims

The aims of this *in vitro* study were to:

a. To evaluate the fracture resistance (compressive strength) of simulated immature teeth after filling the root canals with a calcium tri-silicate cement (Biodentine™) or gutta-percha.

b. To record the type of fracture in simulated immature teeth when loaded to failure.

3.2 Hypotheses

The null hypotheses are:

1. Biodentine™ as root filling material in simulated immature teeth does not improve the fracture resistance in comparison to gutta-percha.
2. There is no specific type of fracture in simulated immature teeth.
3. There was no difference in respect to fracture type between the two groups.

4 Pilot Study

4.1 Aims of the pilot study

a. To develop the method of ensuring a uniform sample of extracted teeth in respect to tooth selection and tooth preparation.
b. To determine the best method of determining the maximum compressive strength endured by each sample prior to fracture.
c. To gain operator experience with the research tools.

4.2 Materials

4.2.1 Tooth sample

The sample consisted of sixteen sound human premolar teeth extracted for orthodontic purposes. The inclusion criteria for the selected teeth are listed in the table below (Table 4.1); any tooth that didn't meet these criteria was excluded. All the teeth were examined under electronic microscope to exclude those with cracks or decalcifications (Figure 4.1).

Inclusion criteria
1. Caries free
2. No restorations, enamel defect, nor tooth surface loss
3. Single rooted with single canals and straight root anatomy
4. Tooth length ranged from 20-24mm (measurement of the teeth done using a Dial gauge)

Table 4.1 Inclusion criteria for the study sample



Figure 4.1 Extracted tooth buccal and lateral view

All teeth were stored in distilled water in a refrigerator for no longer than one month and cleaned using a prophylaxis rubber cup in a slow-speed hand-piece and water-pumice slurry.

4.2.2 Weight

Each tooth was weighted in grams using an electronic digital weight scale which had machine error of 0.0001 gram. The importance of the weight is to ensure that the same amount of tooth tissue was lost due to preparation irrespective of obturation material. Therefore, to facilitate the difference in weight between the beginning and the end of preparation, weight was calculated. Any individual tooth that was found to have a difference in weight of more than 10% following preparation was excluded from the study. Each tooth included in the pilot study was then given a unique identifier (ID) based on the letters of the alphabet (A, B, C, etc.), (Table 4.2).

Sample ID	Tooth length in millimeter	Occlusal table width in millimeter		Root width at CEJ in millimeter		Weight before in grams	Weight after in grams	Weight loss in percentage
		Mesio-Distal	Bucco-Lingual	Mesio-Distal	Bucco-Lingual			
A	20.9	6.8	5	4.5	7	1.113	1.054	5.28
B	20.2	6.6	4.7	4.3	6	0.967	0.943	2.47
C	23.7	6	5.7	4.5	6.8	1.153	1.114	3.43
D	22.8	6.7	5	3.9	6.4	1.023	1.006	1.64
E	21.3	6	4.6	4.2	6.8	1.034	0.997	3.51
F	21.8	6.2	5.3	4.6	5.8	0.996	0.945	5.13
G	21	5.7	4.4	4.7	5.7	1.087	1.029	5.38
H	23	5.9	4.3	4.7	6	1.000	0.959	4.14
I	22	6.4	4.9	4.3	5.6	0.941	0.879	6.16
J	20.9	6.6	4.5	4.5	6.9	0.861	0.847	1.40
K	23.9	5.5	4.3	5	7	1.231	1.142	7.22
L	21.1	5.7	5.2	4.2	5.9	1.083	1.035	4.82
M	22.2	5.7	5.2	4.2	5.9	0.820	0.808	1.51
N	22.1	5.7	4.6	4.9	6.5	0.773	0.7179	7.18
U	22.3	6.1	4.2	4.1	6.1	0.936	0.914	2.24
P	21.4	6.3	4.9	4.2	5.8	0.947	0.920	2.85

Table 4.2 Tooth ID, tooth length, occlusal table width, and root width at cementoenamel junction

4.2.3 Teeth preparation

4.2.3.1 Coronal part preparation

The access cavity was cut with a high-speed hand piece using a 330 bur and a rose-head bur in a slow hand-piece. The aim was to produce an access cavity that was minimal with complete de-roofing of the pulp champer. The same bur type was used in all teeth, to standardize the access cavity.

4.2.3.2 Radicular part preparation

The pulp was extirpated using barbed broaches after negotiating the pulp canal with size 15 K-file. This was followed by preparation using K-files from size 15,20,25,30,35,40,45,50, and 55 respectively. Three milliliters of 5.2% sodium hypochlorite (NaOCl) was used between each file. The length was recorded at the length of the file when it was visualized at the apical foramen.

The canal was further prepared using a gates glidden, starting with size one, followed by size two, then size three and finally size four. The size of Gates Glidden was chosen because it best simulated the canal size in immature teeth (The size four gates glidden corresponds to

size 110 k-file). Gates glidden size three was used to open the apex (to simulate an immature apex), of diameter three millimeter (Figure 4.2).

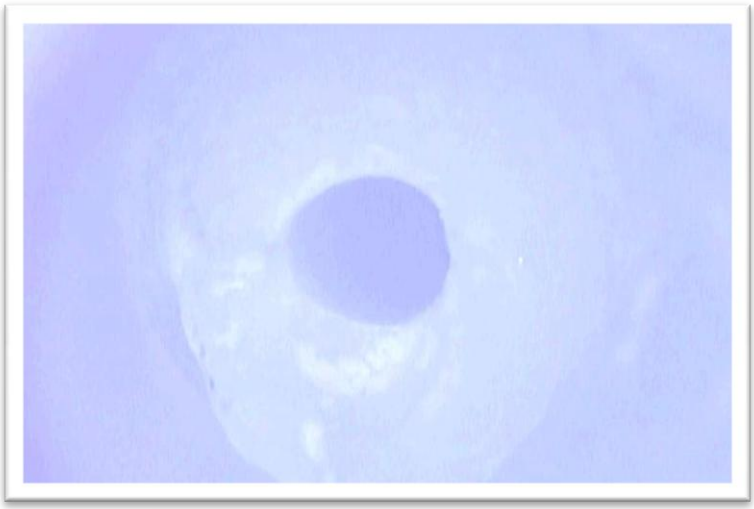


Figure 4.2 Apex of the tooth under electronic microscope view after radicular preparation

4.2.4 Allocation of the samples to two groups

The teeth were randomized into two groups (Biodentine™ group and gutta-perch group) according to the length (Table 4.3) using the toss of a coin. The other teeth in the sample (n=8) were excluded due to the presence of enamel cracks seen under microscopic examination following tooth preparation.

Biodentine™ (n=5)		Gutta-percha (n=5)	
Sample ID	**Length in millimetre**	**Sample ID**	**Length in millimetre**
L	**21mm**	**B**	**21.1mm**
F	**22.3mm**	**E**	**22.3mm**
D	**22.8mm**	**H**	**22.8mm**
A	**20.9mm**	**M**	**21.4mm**
Mean	**21.75**	**Mean**	**21.9**
Standard deviation	**0.946**	**Standard deviation**	**0.787**

Table 4.3 The randomization of the samples into two groups according to the tooth length

<table>
<tr><td colspan="2">Sound premolars (n= 8)</td></tr>
<tr><td colspan="2">Pumice prophylaxis</td></tr>
<tr><td colspan="2">Access cavity preparation</td></tr>
<tr><td colspan="2">Root canal preparation</td></tr>
<tr><td colspan="2">Randomisation</td></tr>
<tr><td>Biodentine™ filling the root canal to the CEJ (n=4)</td><td>Biodentine filing the apical part of the canal – 4mm (n=4)</td></tr>
<tr><td></td><td>Gutta-percha used to fill the canal to the CEJ (n=4)</td></tr>
<tr><td colspan="2">37% Phosphoric acid-etch and rinse (n= 8)</td></tr>
<tr><td colspan="2">Application of adhesive agent (n=8)</td></tr>
<tr><td colspan="2">Composite restoration (n=8)</td></tr>
<tr><td colspan="2">Mounting the tooth in resin block (n=8)</td></tr>
<tr><td colspan="2">Static loading – application of compressive force until fracture (n=8)</td></tr>
<tr><td colspan="2">Microscopic evaluation (n= 8)</td></tr>
</table>

Table 4.4 Overview of the pilot study

4.2.5 Canal obturation

4.2.5.1 Canal obturation in the Biodentine™ group

The tooth canals were dried using paper points. Biodentine™ (Septodont, Saint-Maur-des-Fosses, France) was carried to the canal using an amalgam carrier and then condensed into the canal as far as the CEJ. Following this, the tooth was wrapped with a wet towel to provide a humid environment. The teeth were stored at 37°C and 100% humidity for 48 hours; this is to allow the Biodentine™ to set completely.

4.2.5.2 Canal obturation in the gutta-percha group

The tooth canal was dried with paper points. A four millimetre apical plug of Biodentine™ (Septodont, Saint-Maur-des-Fosses, France) was condensed into the apical third of the canal using endodontic pluggers after carrying the Biodentine™ to the canal with an amalgam carrier. The pluggers are premeasured (aligned against the side of the tooth) to the correct length. Following the condensation of Biodentine™ the tooth was wrapped in a wet towel to provide a humid environment. The teeth were stored at 37°C and 100% humidity for 48 hours; this is to allow the Biodentine™ to set completely.

Afterwards, Zinc oxide based sealer (AH Plus sealer, Dentsply) was mixed according to manufacturer's instructions and applied to the canal walls using a paper point. The canals were backfilled to the level of the CEJ with gutta-percha (ObturaII, Sybron Endo) using the Elements Obturation Unit (Gutta Percha Plugs, Sybron Endo). The gutta-percha was vertically compacted with pluggers to the level of CEJ. Excess sealer was removed from the chamber with a dry cotton pellet.

4.2.6 Coronal restoration

The coronal restoration was placed after 48 hours to allow the radicular restoration to completely set. Before starting the coronal restoration, the level of the radicular filling was checked by periodontal probe (measuring the crown length). The first step was, to etch the coronal cavity with 37% phosphoric for ten seconds. A bonding agent (XP Bond, Dentsply) was applied afterwards using a micro-brush, and then thin dried with air. It was light cured using a light cure unit (Ortholux™ Luminous Curing Light, 3M, USA). Light cured Composite (HFO Enamel Plus, GDF GmbH, Germany) was used to fill the cavity. The excess composite was removed before curing with plastic instrument. The complete setting of the composite was accomplished using a light cure unit (Ortholux™ Luminous Curing Light, 3M, USA). The Figure 4.3 shows the final restorative component of each group.

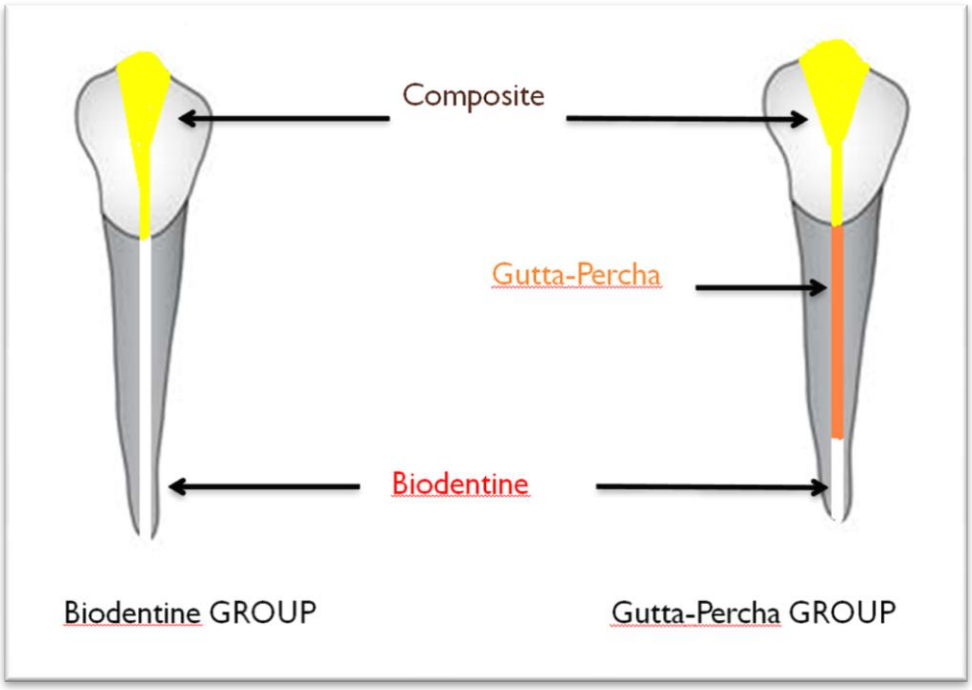


Figure 4.3 The restoration component of the Biodentine™ and gutta-percha tooth

4.2.7 Mounting the samples

The tooth was attached to compound wax for ease of manipulation. To simulate the periodontal ligament each tooth was dipped into silicone (Metrosil) up to two millimeters below the CEJ (Figures 4.5 and 4.6). The thickness of the silicone was 0.2-0.3 millimeters, similar to the thickness of the periodontal ligament (Figure 4.5). The root of each tooth were then mounted the root of each tooth in a resin block using a plastic mould. The long axis of the tooth was mounted in a perpendicular angle to the resin block to simulate axial forces as closely as possible. The mounting was to the level of two millimeters below the CEJ (Figure 4.6). All the samples were covered with a wet towel afterwards, to provide humidity for twelve hours, and allow complete set of the resin block without drying out the teeth (setting time of a resin block is twelve hours). Adjustments to the blocks were done using abrasive discs to remove excess resin (Figure 4.6).



Figure 4.4 Silicon material



Figure 4.5 Root covered with silicon to simulate periodontal ligaments

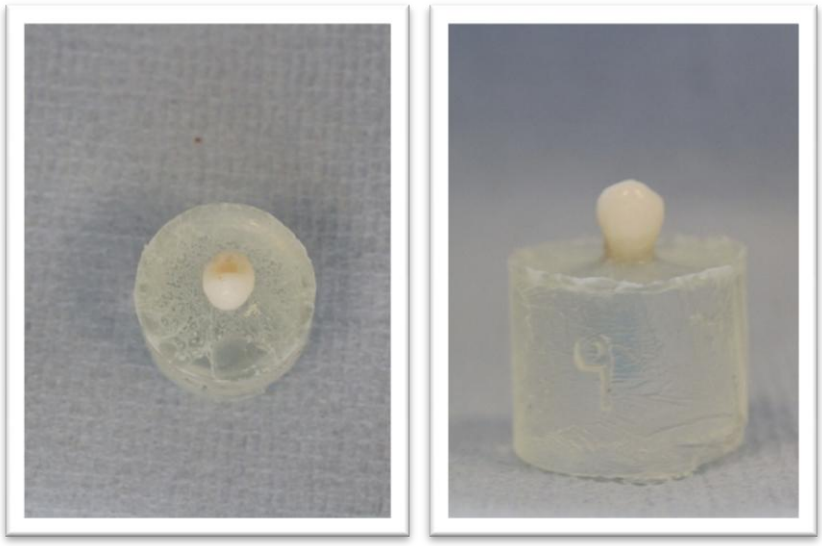


Figure 4.6 The tooth mounted in the resin block

4.2.8 Static loading test

To decide on the best method of determining the maximum compressive strength endured by each sample prior to fracture a static loading test was used. A static loading is based on the application of gradually increasing compressive force. An INSTRON machine (5569A, Univeral testing machine, Instron limited, High-Waymcombe, UK) was used to apply the force starting from zero Newtons to failure (Figure 4.7). The load was applied to the buccal cusp of the tooth. This was used to measure whither the teeth fractured within the known range of the normal human bite-force. The test was applied to both groups.



Figure 4.7 INSTRON machine

4.2.8.1 Results for the static loading test

For the Biodentine™ group the first sample to undergo the test was sample " I", which fractured at 382.34 Newtons, followed by sample "L" that was fractured at 1100.01 Newtons. Further details are shown in Table 4.5, 4.7 and Figure 4.8. The mean force was 1148 Newtons (382.34-1783N, SD=584.41).

Sample ID	Group	Force at which tooth fracture in Newtons
I	Biodentine	382.34
L	Biodentine	1100.01
A	Biodentine	1329.27
D	Biodentine	1783
Mean		1148.65
Standard deviation		584.41

Table 4.5 The static loading test for Biodentine™ group

For the gutta-percha group the sample “B” fractured at 405 Newtons. Further details are shown in Table 4.6, 4.7 and Figure 4.8. The mean force was 782 Newtons (405-1390N, SD=427.30).

Sample ID	Group	Force at which tooth fracture in Newtons
B	Gutta-percha	405
C	Gutta-percha	736
E	Gutta-percha	1390
H	Gutta-percha	598
Mean		782.25
Standard deviation		427.30

Table 4.6 The static loading test for gutta-percha group

For the samples as a whole the maximum compressive load in the static loading ranged from 382- 1783 Newtons. Table 4.7 confirms that the teeth in the sample, irrespective of root canal filler endured a mean force of 965.37 Newtons before they fractured.

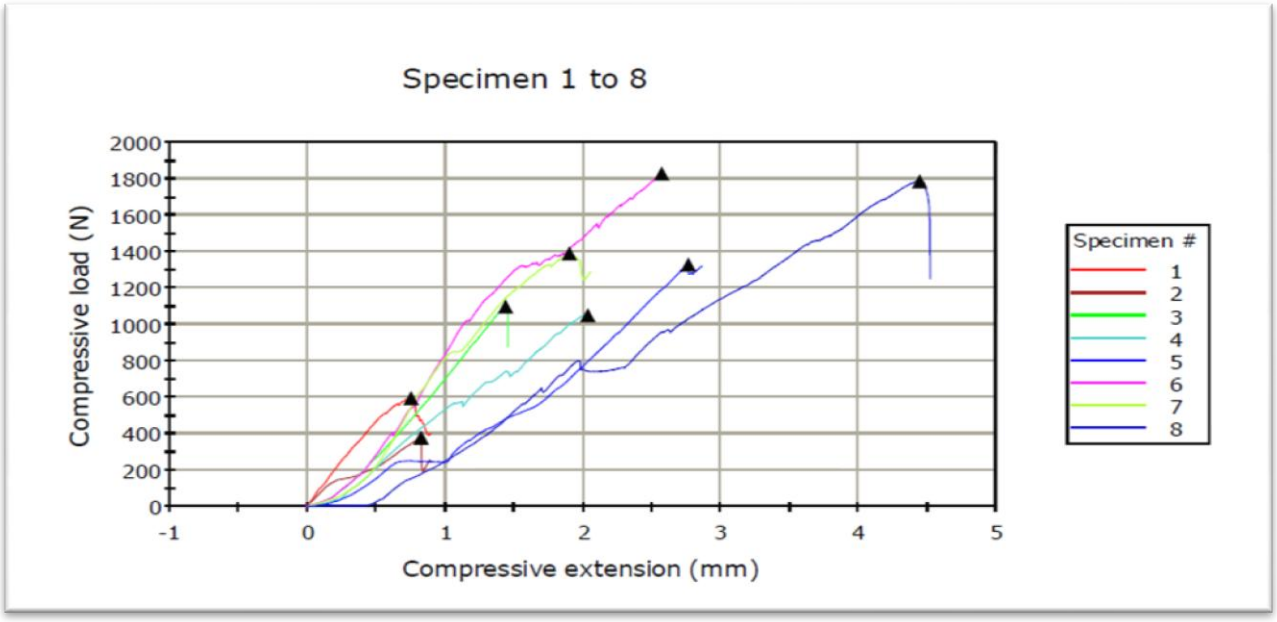


Figure 4.8 The maximum compressive load for each sample in relation to compressive extension

Specimen number (n=8)	Tooth ID	Maximum compressive load in Newtons
1	H	598
2	I	382
3	L	1100
4	B	405
5	A	1329
6	C	736
7	E	1390
8	D	1783
Mean		965.37
Standard deviation		181.31

Table 4.7 Specimen number, tooth ID, and maximum compressive load under static loading

4.3 Discussion

There are many different variables in relation to tooth dimensions:

1. Occlusal table width mesiodistally and buccolingually.
2. The length of the tooth.
3. The width of the root at the cementoenamel junction mesiodistally and buccolingually.

Randomization of the samples with all these variables is not possible. Therefore, the length of the tooth was selected since this was deemed to be the most relevant, especially given the direction of the force applied in the real life situation.

The maximum comprehensive loads varied widely in both groups and were outwith the normal biting force. The normal biting force is around 200 Newtons in the incisor region (Hellsing, 1980). This suggests that the application of force around this value should be used in the main study. Even though, it should be kept in mind that during traumatic injury greater forces might be applied. Furthermore, these forces are unlikely to be in axial direction. Therefore, for the purpose of this preliminary study the application of force that are within the normal bite range, and that are similar to other studies (Abdul Salam *et al*, 2006) were considered to be the most appropriate.

Some authors have used a cyclic loading test (Abdul Salam et al, 2006). This test is based on the application of a constant force that is repeated for a predetermine number of cycles. So,

unlike the static loading test it simulates not just ‘one bite’ but repeat biting and chewing. This test was thought to more closely simulate the real life environment and so it was decided to use this rather then static loading for the main study.

4.3.1 Summary of changes to the main study

1. The use of tooth length to randomise the samples.
2. Introduce the use of a cyclic loading test to evaluate the fracture resistance (compressive strength) of simulated immature teeth after root filling.

5 Main study

5.1 Aim of the main study

The aim of the main study was to:

a. To evaluate the fracture resistance (compressive strength) of simulated immature teeth after filling the root canals with a calcium tri-silicate cement (Biodentine™) or gutta-percha.
b. To record the type of fracture in simulated immature teeth when loaded to failure.

5.2 Hypotheses

The null hypotheses are:

a. Biodentine™ as root filling material in simulated immature teeth does not improve the fracture resistance in comparison to gutta-percha.
b. There is no specific type of fracture in simulated immature teeth.
c. There was no difference in respect to fracture type between the two groups.

5.3 Materials

The main study had two parts; the first was the cyclic loading and the second was the recording of the site of fracture when the teeth were subsequently fractured.

5.3.1 Tooth selection

5.3.1.1 Cyclic loading – Part one

A new sample of twenty sound human premolar teeth extracted for orthodontic purposes were included in the sample. The measurement for length and weight were conducted as previously mentioned in the pilot study. The teeth were given numerical identification (ID), (Table 5.1).

5.3.1.2 Fracture type – Part two

The sample used to report on the fracture type was obtained from the unfractured teeth used in the cyclic loading test above (n=15). These teeth were then subjected to compressive load and fracture type was recorded.

Tooth ID	Tooth length in mm	Weight before in grams	Weight after in grams	Weight loss in percentage
1	21.3	0.950	0.900	5.20
2	22.6	0.905	0.850	5.50
3	21.5	0.875	0.830	5.14
4	23.6	0.964	0.890	7.67
5	21.5	1.085	1.075	0.90
6	24	1.056	0.980	7.60
7	23	1.120	1.070	4.46
8	20	0.798	0.750	6.01
9	23	1.195	1.180	1.25
10	24	0.968	0.890	9.80
11	20	0.890	0.850	4.49
12	24	1.115	1.050	5.82
13	22	1.065	1.020	4.22
14	21.7	1.055	0.950	9.95
15	22	0.975	0.960	1.53
16	23	1.455	1.430	1.03
17	24	1.075	1.030	3.25
18	21.4	0.840	0.810	3.57
19	22.1	1.075	1.030	3.25
20	22.3	0.996	0.945	5.13
Mean	22.35	1.022	0.974	4.78
Standard	0.276	0.032	0.033	2.62

Table 5.1 The sample ID, tooth length, weight before and after tooth preparation, and weight loss after the tooth preparation

5.3.2 Teeth preparation

The coronal and radicular preparation exactly follows the same protocol that has been reported in the pilot study. The details of length and weight and the uniformity of the final tooth sample are shown in table (Table 5.1). The mean length was 22.35 millimeters (20-24, SD= 0.276). The mean weight was 1.022 grams (0.798-1.455,SD= 0.032). The mean weight after preparation was 0.974 grams (0.750-1.430,SD= 0.033).

5.3.3 Randomisation of the samples to two groups

The teeth were randomised into two groups (Biodentine™ group and gutta-perch group) according to the length (Table 5.2) using the toss of a coin. The mean length of GP was 22.36mm (20-24, SD= 0.389) and the Biodentine™ mean length was 22.31mm (20-24, SD= 0.414).

Gutta Percha (n=10)		Biodentine™ (n=10)	
Tooth ID	**Length in millimetre**	**Tooth ID**	**Length in millimetre**
6	24mm	17	24mm
12	24mm	10	24mm
7	23mm	9	23mm
16	23mm	4	23.6mm
2	22.6mm	18	21.4mm
15	22mm	20	22mm
13	22mm	19	22.1mm
1	21.3mm	3	21.5mm
14	21.7mm	5	21.5mm
8	20mm	11	20mm
Mean	22.36mm	Mean	22.31mm
Standard deviation	0.389	Standard deviation	0.414

Table 5.2 The randomization of samples into two groups according to the tooth length

5.3.4 Canal obturation in Biodentine™ group and gutta-percha group

The same obtruration techniques were used in pilot study were followed in both groups.

5.3.5 Coronal restoration

The same coronal restoration protocols that have been used in the pilot study were followed in both groups.

5.4 Methods

5.4.1 Cyclic loading test

The cyclic loading test was applied for each group independently. The force used range from 200 to 250 Newtons. The first sample was tested at a given starting force using the Endur TEC (Endura TEC ELF Model 3300, Bose, Eden Prairie, MN, USA) machine for 200000 cycles (200000 'bites') or until failure, whichever comes first. Once the first sample survived the force, the stress level was increased by five percent for the next sample; contrariwise, the stress was decreased by five percent if the sample failed. This was applied for all samples

consequently. The five percent incremental increase was used is because the normal bite-force ranges from 200-250 Newtons with the sample of 10 teeth, the five percent will cover the whole range.

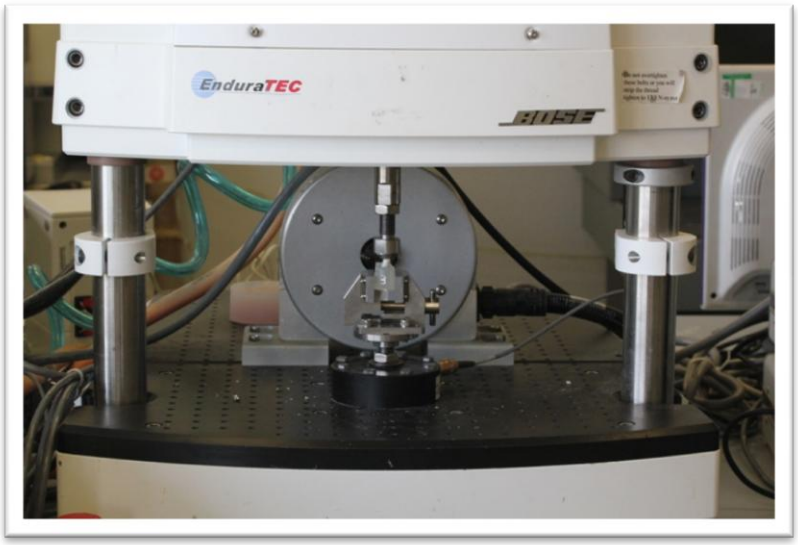


Figure 5.1 Endura TEC machine

5.4.2 Static loading after cyclic loading

The samples that survived the test underwent compressive loading to failure and the maximum load was recorded.

5.5 Type of fracture

The type of fracture was recorded after examining the sample under a microscope following failure. They were classified into crown fracture, root fracture, and crown-root fracture.

5.6 Statistical analysis

Data is analysed using Stata 12.0 and significance is pre-determined at P =0.05.

5.6.1 Static loading after cyclic loading

A two sample t-Test was used to determine whether the fracture resistance (compressive strength) in Biodentine™ improved in comparison with gutta-percha group. In the analysis only compressive failure (Newtons) following tooth fracture will be considered.

5.6.2 Type of fracture

The Pearson Chi-square test was used to determine any difference in the type of fracture in simulated immature teeth when loaded to failure.

6 Results

6.1 Cyclic loading test

6.1.1 Cyclic loading test in the Biodentine™ group simulating normal biting-force

The first five samples survived the test after application of force staring from 200Newtons with five percent incremental increase up to 243Newtons. The sixth sample subjected to cyclic loading failed at 180962 cycles with compressive force of 255 Newtons. The seventh, eighth, and ninth sample survived the cyclic loading. The Tenth sample fractured at 197600 cycles with compressive force of 282 Newtons. The following Table 6.1 shows the complete details for the cyclic test results.

Sample ID	Cyclic loading force	Survive/Failure	Number of cycles
4	200N	Survive	200000
10	210N	Survive	200000
9	220N	Survive	200000
3	231N	Survive	200000
20	243N	Survive	200000
5	255N	Failure	180962
17	243N	Survive	200000
18	255N	Survive	200000
11	268N	Survive	200000
19	282N	Failure	197600

Table 6.1 Cyclic loading test in the Biodentine™ group using force between 200-280 Newtons

6.1.2 Cyclic loading test in the gutta-percha group simulating normal biting-force

The first and second samples survived the test after application of force of 200, and 210 Newtons respectively. The third sample fractured after the application of 220Newtons for 140853 cycles. The fourth, fifth, sixth, and the seventh samples all survived the cyclic loading for 200000 cycles. The eighth sample subjected to cyclic loading fractured at 192546 cycles with comprehensive force of 255 Newtons. Likewise; the ninth sample fractured after the application of 243Newtons for 200000 cycles. The last sample (tenth) survived the test after the application of 231 Newtons. The following Table (6.2) shows the results of the cyclic loading test in the gutta percha group.

Sample ID	Cyclic loading force	Survive/Failure	Number of cycles
16	200N	Survive	200000
1	210N	Survive	200000
12	220N	Failure	140853
13	210N	Survive	200000
2	220N	Survive	200000
15	231N	Survive	200000
7	243N	Survive	200000
14	255N	Failure	192546
6	243N	Failure	200000
8	231N	Survive	200000

Table 6.2 Cyclic loading test in the gutta-percha group using force between 200-280 Newtons

6.1.3 Static loading following cyclic loading test in the Biodentine™ group

The mean compressive force was 1692.6 Newtons (1279-1794N, SD=294.53) for the Biodentine™ group. The following table (Table 6.3) shows a detailed description of the results.

Sample ID	Group	Force at which tooth fracture in Newtons
4	Biodentine	1767
10	Biodentine	2232
9	Biodentine	1689
3	Biodentine	1279
20	Biodentine	1794
17	Biodentine	1747
18	Biodentine	1696
11	Biodentine	1337
Mean		1692.6
Standard deviation		294.53

Table 6.3 Static loading after cyclic loading test in the Biodentine™ group to produce fracture

6.1.4 Static loading following cyclic loading test in the gutta-percha group

For the Gutta-Percha group the mean compressive force was 1418.5 Newtons (1115-1831N, SD= 263.14). Further details are shown in Table 6.4.

Sample ID	Group	Force at which tooth fracture in Newtons
16	Gutta-percha	1115
1	Gutta-percha	1601
13	Gutta-percha	1831
2	Gutta-percha	1179
15	Gutta-percha	1326
7	Gutta-percha	1602
8	Gutta-percha	1276
Mean		1418.5
Standard deviation		263.14

Table 6.4 Static loading after cyclic loading test in the gutta-percha group to produce fracture

6.2 Statistical analysis of the static loading after cyclic loading test

After data analysis using the two sample t-Test there was no significant difference in the fracture resistance (compressive loading) between the Biodentine™ and gutta-percha groups. Therefore, the study failed to reject the null hypothesis (P>0.05, n=15). Table 6.5 shows the two sample t-Test of the difference in compressive strength following a fatigue regime between gutta-percha and Biodentine™ reinforced teeth. Moreover, the raw data, mean value, and associated 95 % confidence interval are detailed in Figure 6.1.

Two-sample t-test with equal variances

Group	Observation	Mean	Std. Err.	Std. Dev.	95% conf. Interval	
GP	7	1418.571	99.45922	263.1444	1175.203	1661.939
Biodentine	8	1692.625	104.135	294.5383	1446.385	1938.865
Combined	15	1564.733	78.77433	305.0917	1395.779	1733.687
difference		-274.0536	145.1652		-587.6639	39.55675

diff= mean (GP) - mean (Biodentine) t = -1.8879

P = 0.0816

Table 6.5 Two sample t-test of the difference in compressive strength following a fatigue regime between gutta-percha and Biodentine™ reinforced teeth

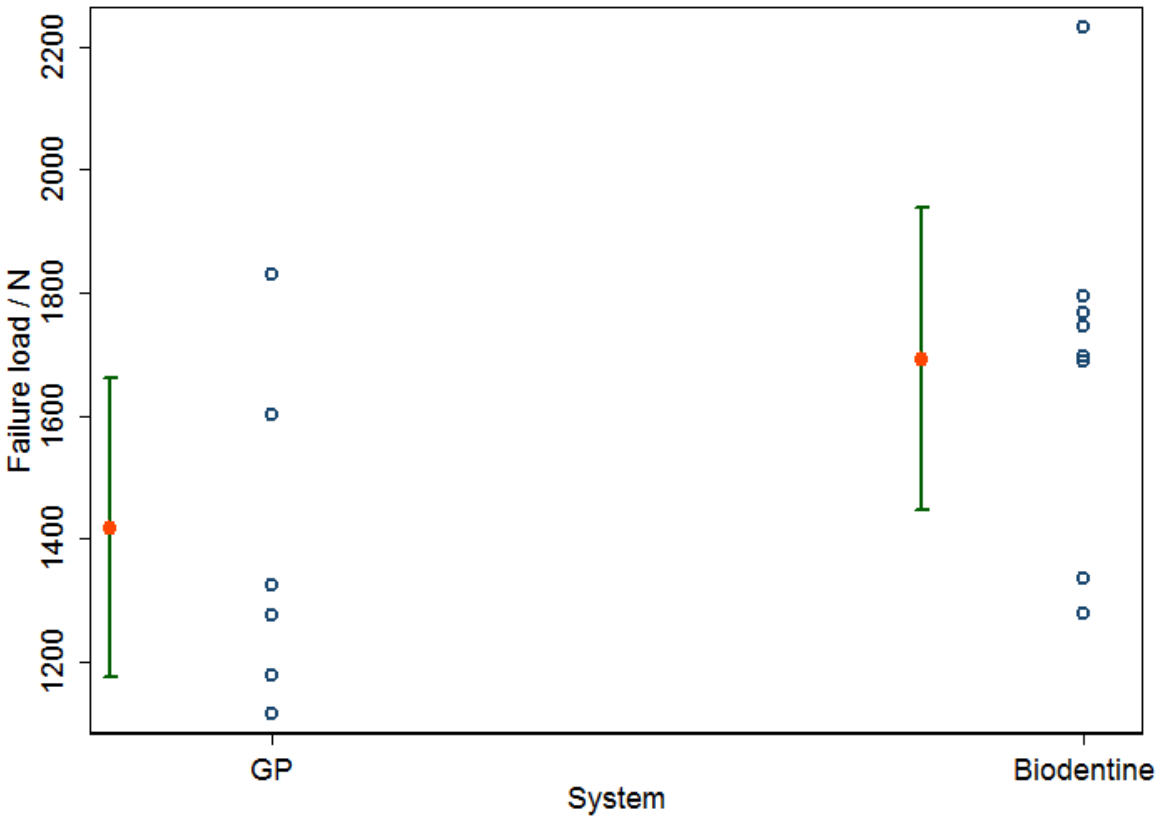


Figure 6.1 The raw data, mean value, and associated 95 % confidence interval

6.3 Type of fracture

6.3.1 Biodentine™ group

In fourteen teeth of the Biodentine™ group the fracture type was recorded. Further details are shown in Table 6.6 and Figure 6.2.

Sample ID	Part of study	Type of force applied	Group name	Fracture type
15	Main study	Cyclic loading – static loading	Biodentine	Crown-Root
27	Main study	Cyclic loading – static loading	Biodentine	Crown
24	Main study	Cyclic loading – static loading	Biodentine	Crown
29	Main study	Cyclic loading – static loading	Biodentine	Crown
13	Main study	Cyclic loading – static loading	Biodentine	Crown
14	Main study	Cyclic loading – static loading	Biodentine	Crown
3	Main study	Cyclic loading – static loading	Biodentine	Crown-Root
4	Main study	Cyclic loading – static loading	Biodentine	Crown

Table 6.6 Type of fracture in Biodentine™ group

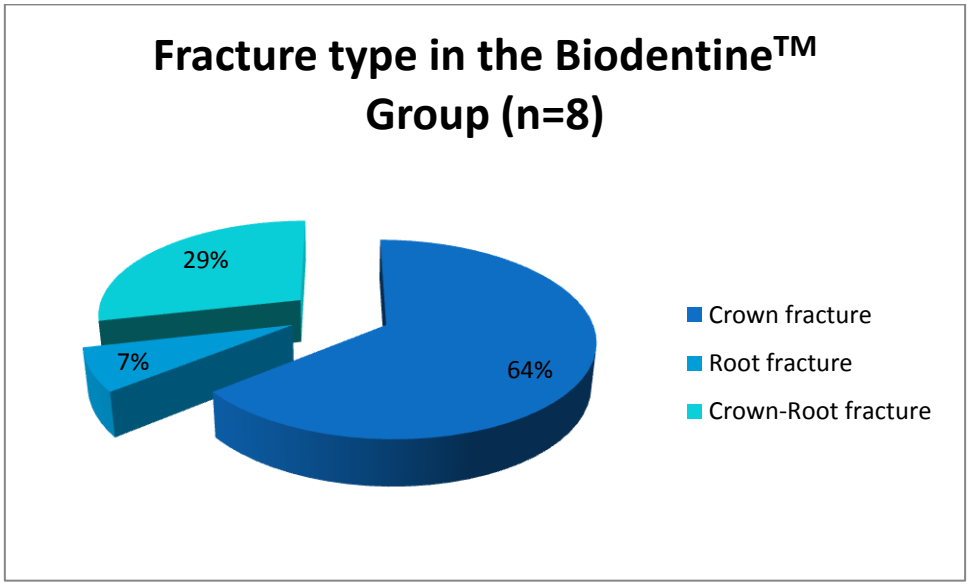


Figure 6.2 fracture types in the Biodentine™ group

6.3.2 Gutta-percha group

The type of fracture was recorded in fourteen teeth in the gutta-percha group. Further details are shown in Table 6.7 and Figures 6.3.

Sample number	Part of study	Type of force applied	Group	Fracture type
12	Main study	Cyclic loading – static loading	Gutta-Percha	Crown
11	Main study	Cyclic loading – static loading	Gutta-Percha	Crown-Root
21	Main study	Cyclic loading – static loading	Gutta-Percha	Root
2	Main study	Cyclic loading – static loading	Gutta-Percha	Crown
19	Main study	Cyclic loading – static loading	Gutta-Percha	Crown-Root
1	Main study	Cyclic loading – static loading	Gutta-Percha	Crown
22	Main study	Cyclic loading – static loading	Gutta-Percha	Crown

Table 6.7 Type of fracture in gutta-percha group

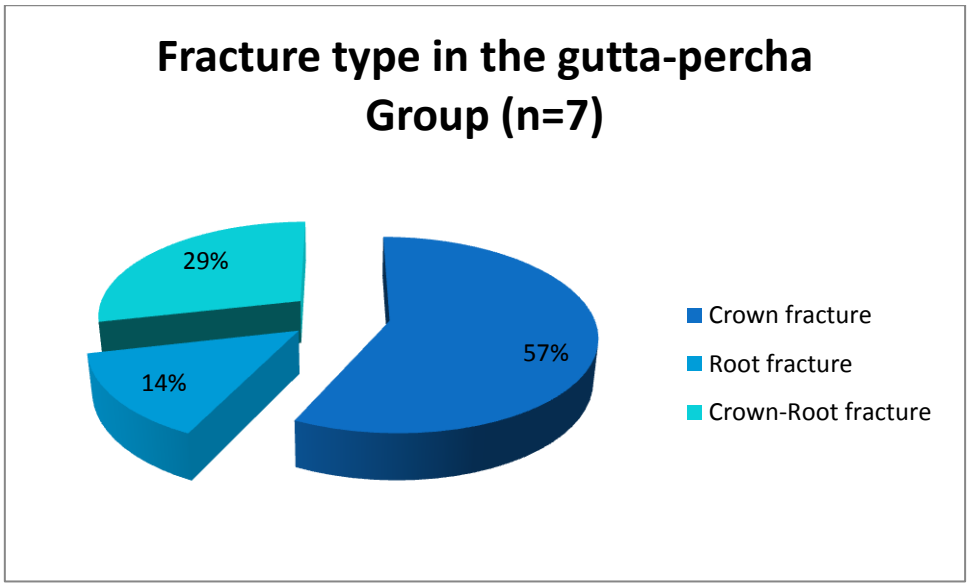


Figure 6.3 Fracture types in the gutta-percha group

6.3.3 Statistical analysis of the type of fractures

There was no significant difference in the type of fracture between the gutta percha and Biodentine™ group, after statistical analysis using Pearson Chi-square (P<0.05) (Table6.8, Figure 6.4).

Failure type		System	
		Gutta Percha	Biodentine
Cervical root fracture	Number of samples	1	0
	Standard deviation	0.467	0.533
Crown Fracture	Number of samples	4	6
	Standard deviation	4.667	5.333
Crown-Root Fracture	Number of samples	2	2
	Standard deviation	1.867	2.133
P = 0.512			

Table 6.8 Pearson Chi-square for the type of fracture in Biodentine™ and gutta-percha group

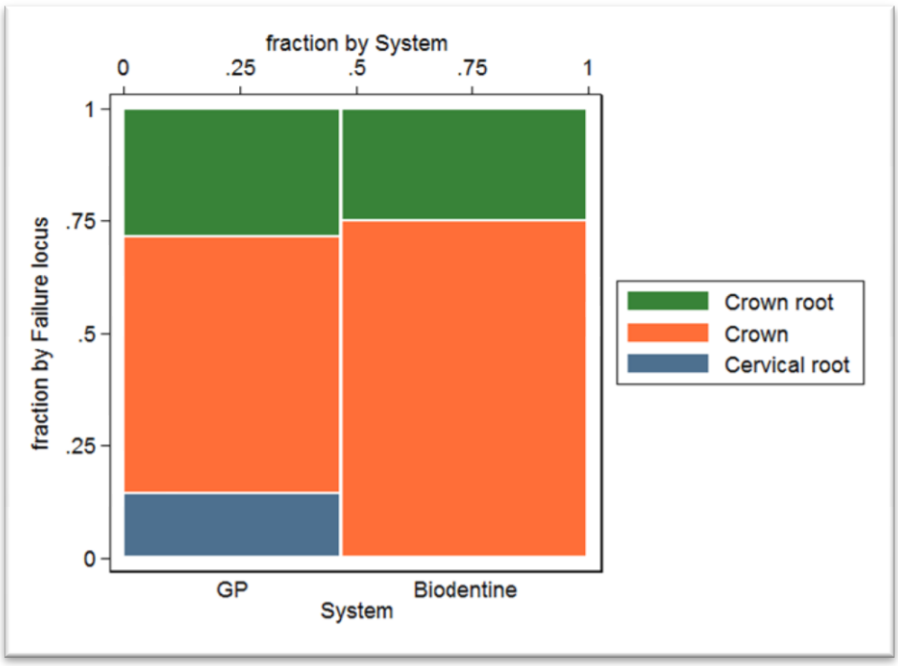


Figure 6.4 Type of fracture in Biodentine™ and gutta-percha group

7 Discussion

There were no significant differences in fracture resistance (compressive strength) of simulated immature teeth after filling the root canals with a calcium tri-silicate cement (Biodentine™) or gutta percha. Moreover, there is no difference in respect to fracture type between the two groups.

7.1 Fracture resistance studies

There are many studies that investigate the ability of root filling materials to reinforce immature non-vital root. It was observed that there are few studies that investigate the effect of endodontic root filling materials on fracture resistance.

Biodentine™ is a newly developed material and its clinical application includes: dentine replacement as coronal restoration, repair of root perforation and vital pulp therapy. The use of silicate-based cement (Biodentine™) as a root filling material has not been studied yet.

Furthermore, Biodentine is a commercially available material; the findings of this study may have a potential negative feedback on the clinical application of this material. Even thought it isn't licensed as a root canal filling material.

Most of endodontically treated teeth lose a large amount of the coronal dental structure as a result of the original traumatic injury and subsequent access cavity preparation. This will lead to a massive reduction in their structural stability (Alomiri *et al*, 2010). Therefore, the amount of force that endodontically treated teeth can sustain before fracturing is much less than that of vital non-root filled tooth.

In this study, the cyclic loading was applied in order to mimic actual masticatory forces. Moreover, the anterior teeth are more susceptible to horizontal traumatic forces due to their inclination (Mehta and Millar, 2008; Fernandes and Dessai, 2001). However, the application of horizontal forces causes a significantly higher stress concentration within dentine than forces that are more parallel to the long axis of the tooth. The aim of this study was to evaluate the effect of root filling materials in reinforcing the tooth. Therefore, vertical loading was applied to investigate the root filling material effect.

The cyclic loading was performed on the immature root filled teeth for 200000 cycles, which represent less than one year of masticatory function (Kelly, 1997). Therefore, further studies

should consider extending the period of evaluation over a larger number of total loading cycles to achieve failures solely by cyclic loading test.

There was no significant difference in the fracture resistance (compressive loading) between the Biodentine™ and the gutta-percha group in the study. The coronal component of the tooth may lead to this insignificant finding. Moreover, there are several studies reported that silicate based cement decreases the fracture strength over time (Hatibovic-Kofman *et al,* 2008; Sawyer *et al*, 2012). Therefore, incorporation of the time factor in future studies should be considered.

In this study the fracture types were classified to crown, crown-root and root fracture. The fracture type we allocated to oblique, vertical and horizontal group in other studies (Michael et al, 2010). The classification used was chosen as it represent normal clinical situation where the other classification is more subjective.

There was no significant difference in the type of fracture between the gutta-percha and the Biodentine™ group. The most common type of fracture in both groups was crown fracture; this may be explained by the use of the same coronal restoration in both groups.

7.2 Limitations of the study

7.2.1 Sample selection

The ideal way to standardise the samples is using extracted teeth from the same patient. However, this method was not applicable in this study due to the difficulty in achieving teeth that fit the inclusion criteria.

Many previous studies have utilized sheep teeth (Andreasen *et al*, 2006); or human teeth (Lertchirakarn *et al,* 2011) to analyse the fracture resistance of immature non-vital teeth and the long term effect of root filling materials in reinforcing the teeth. The lack of standardisation and different test parameters used contributed to the conflicting results in the literature.

Although other studies have used the facio-lingual dimension of the tooth at CEJ for randomisation (Wilkinson *et al*, 2007), in this study the tooth length was used to randomise the samples because the tooth length is the essential factor in vertical loads studies (Abdul salam *et al*, 2006).

7.2.2 Access cavity standardisation

The access cavity preparation has a great influence on the fracture resistance of the endodontically treated tooth. In previous studies attempt have made to standardised the access cavity either by using a metal template (Michael *et al*, 2010) or by mounting the handpiece (Wilkinson *et al*, 2007). However, due to dimensions and size variation between the samples the standardisation by these previously mentioned methods was not accurate. Therefore, weighting the tooth before and after preparation was used in this study. This will measure the actual amount of lost dental structure following preparation. There are no up-to-date published studies that used tooth weight for standardisation of the sample.

7.2.3 Periodontal ligament simulation

In this study attempt was made to reproduce the resiliency of periodontal ligament through using silicon material. This method was used in other studies to simulate the periodontal ligament and showed greater influence on the fracture type rather than the fracture value (Soares *et al*, 2005). However, correct actual resiliency of the periodontal ligament was difficult to achieve due to difficulty in controlling the material thickness.

7.2.4 Mounting of the sample

There were two millimetre gap between the mounting material and the cementoenamel junction. The purpose of this gap is to simulate the physiological distance found clinically between the bone crest and the cementoenamel junction.

Resin was used to mount the teeth during testing, which sets by exothermic reaction that may affect the dental structures. However, both materials that are commonly used, resin and stones, set by exothermic reaction.

7.3 Type of fracture

There are several limitations in general for experimental studies. The strength of dentine is highly affected by the age of the patient, pulp condition before extraction, and the storage medium that may affect the fracture pattern (Fernandes and Dessai, 2001). Moreover, the duration between the time of extraction and the study has a direct effect on the dentine flexural strength.

All teeth used in this study were extracted for orthodontic purposes. Thus, the collected teeth were from the same age group. In addition, the same storage medium was used to preserve all the teeth and time interval between the time of their extraction and the study

was no more than one month. Therefore, all aforementioned factors were taken into consideration in order to keep variables in this study to minimum.

7.4 Future work

This study is the first study that investigates the use of Biodentine as root filling material. Therefore, it can be considered as pilot study for future research in this topic area.

There are several factors need to be considered in the future work. The use of more standardised samples by having the teeth from the same subject i.e a matched paired design. Furthermore, the effect of time needs to be incorporated in to the study as the long term effect of the silicate-based cement materials on the flexural strength of dentine have been reported (Sawyer *et al*, 2012). Moreover, the use of non-setting calcium hydroxide dressing should be considered as an intermediate filling before placing the final root canal filling since this mimic the clinical situation.

Furthermore, to make sure that the fracture resistance is affected only by the type of material used as root canal filling, the quality of root canal treatment needs to be assessed radiographically to eliminate other variables such as voids.

8 Conclusion

This preliminary *in vitro* study found no difference in fracture resistance (compressive strength) of simulated immature teeth after filling the root canals with calcium tri-silicate cement (Biodentine™) compared to gutta-percha. Moreover, there is no difference in respect to fracture type between two groups. However, due to the limitations in the study and its preliminary nature the results should be interpreted with caution.

Biodentine™ is a newly developed material that shows promising clinical outcomes due to its physical properties. However, further research is required to investigate its promising properties as a root filling material.

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