Calcium silicate cements used as a therapeutic dentine replacement In-vitro and in-vivo studies

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Calcium silicate cements used as a therapeutic dentine replacement: in-vitro and in-vivo studies

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Abstract

Objectives: This research aims to investigate clinically and radiographically the dentine-pulp response to a calcium silicate cement (Biodentine™) compared to a glass ionomer cement (Fuji IX™ GIC) control material following indirect pulp capping in patients with signs of reversible pulpitis and to assess the integrity of the overlying resin composite restoration (N'Durance®) using USPHS and FDI criteria. This research also aims to evaluate the interface between Biodentine™ and the resin composite restoration using micro-shear mechanical testing and to investigate the possibility of reducing the radiation dose of cone beam computed tomography (CBCT) while maintaining an optimised image.

Materials and methods: The randomised controlled clinical trial involved recruiting patients with signs of reversible pulpitis from Kings College Dental Institute at Guys Hospital. A CBCT and periapical radiograph (PA) were taken at baseline followed by minimally invasive (MI) treatment of the carious lesion. The definitive veneering composite restoration was placed one month later. The patients were followed up longitudinally at 6 and 12 months. Another CBCT and PA radiograph were taken at 12 months. The in-vitro study involves micro-shear testing of the bond strengths of resin composite to Biodentine™ vs. glass ionomer cement vs. resin modified glass ionomer cement using an adhesive in self-etch (SE) / total etch (TE) mode after aging the 3 substrates and the bond. Failure modes were characterised and SEM images were analysed. Reducing the CBCT radiation dose was attempted by comparing CBCT scans taken at 360° vs.180° rotation while maintaining accurate linear measurements. Accuracy of measurement was judged against the corresponding measurements taken from the porcine jaw specimens used as a reference standard.

Results: 72 restorations (36 Biodentine™, 36 Fuji IX™) were placed randomly in 53 patients. Clinical success rates for Biodentine™ and Fuji IX™ GIC when used as indirect pulp capping agents were equal (83.3%). CBCT was significantly more effective in detecting periapical radiolucencies compared to

PA radiographs ($p<0.05$). The majority of healed CBCT lesions had received Biodentine™ while the majority that didn't heal received Fuji IX™. With regards to the in-vitro studies, no significant differences were observed between (SE) / (TE) bonding modes ($P=0.42$). With material aging, a significant reduction in micro-shear bond strength occurred between early and delayed time intervals for Biodentine™ ($P=0.001$). Failure modes were primarily cohesive within the material (68.82%). Furthermore, no significant difference between the measurements from 180° or 360° rotations, nor any difference between the two rotations and porcine jaw specimens.

Conclusions: Although no statistically significant difference was detected in the clinical efficacy of Biodentine™ / Fuji IX™ when used as therapeutic indirect pulp capping materials in patients with reversible pulpitis, CBCT showed a significant difference where the majority of healed CBCT lesions had received Biodentine™ while the majority that didn't heal received Fuji IX™. The restorations performed well when assessed using USPHS and FDI criteria. Biodentine™ was found to be weak in its early setting phase. Placing the overlying resin composite is best delayed for at least 2 weeks to allow adequate setting/maturation of the Biodentine™ to withstand sufficiently the contraction forces of the resin composite. This would also allow sufficient time to review the tooth if Biodentine™ was placed on symptomatic pulps. A total etch or self-etch adhesive may be used. Moreover, a CBCT image sufficient to make accurate clinical measurements with a reduced radiation exposure may be obtained by using 180° rotation of the CBCT tube head.

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List of Abbreviations

ALARA: As low as reasonably achievable

AP-I: Activator protein-I

BMP-7: Bone morphogenic protein -7

Bis-GMA: Bisphenol A-glycidyl methacrylate

CARS: Caries adjacent to restorations and sealants

CBCT: Cone beam computed tomography

CDJ: Cemento-dentinal Junction

CGRP: Calcitonin gene related peptide

CONSORT: Consolidated standards of reporting trials

DSP: Dentine sialoproteine

DPP: Dentine phosphoprotein

DSPP: Dentine sialophosphoprotein

EDJ: Enamel-dentine junction

EPT: Electric pulp tester

FDI: Fédération dentaire internationale

FOV: Field of view

GCP: Good clinical practice

GIC: Glass ionomer cement

HBSS: Hanks balanced salt solution

HEMA: Hydroxyethyl methacrylate

$HgCl_2$: Mercuric chloride

HSP: Heat-shock proteins

ICC: Intra-class correlations

Ig: Immunoglobulin

IL-1a: Interleukin-1a

IRM: Intermediate restorative material

LED: Light emitting diode

LTA: Lipotechoic acid

LPS: Lipopolysaccharide

MB: Methacrylate-based

MeHgCl: Methyl mercury chloride

mGy: Milligray

MI: Minimally invasive

MMP-2: Matrix metalloproteinase-2

μSBS: Micro-shear bond strength

MTA: Mineral trioxide aggregate

MT1-MMP: Membrane type-l matrix metalloproteinase

NaOCl: Sodium hypochlorite

NF-KB: Nuclear factor kappa B

NKA: Neurokinin A

PBS: Phosphate buffered saline

PA: Periapical

PAMP: Pathogen associated molecular pattern

PDL: Periodontal ligament

PTF: Pre-test failure

RMGIC: Resin modified glass ionomer cement

ROS: Reactive oxygen species

SB: Silorane-based

SE: Self-etch

SEM: Scanning electron microscopy

TE: Total-etch

TEG-DMA: Triethylene glycol dimethacrylate

TGF-B: Transforming Growth Factor Beta

TIMP-2: Tissue inhabitor of metalloproteinase-2

TLR: Toll-like receptor

TNF-a: Tumour necrosis factor-a

UDMA: Urethane dimethacrylate

USPHS: United States Public Health Service

VHN: Vickers hardness number

ZOE: Zinc oxide eugenol

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CHAPTER 1

Introduction

1.1 Overview

Dental caries is one of the most prevalent chronic diseases of people worldwide, with individuals being susceptible to this disease throughout their lifetime (Selwitz *et al.*, 2007). The treatment of dental caries has high cost implications especially with deep carious lesions approaching the pulp (Ricketts *et al.*, 2006b; Ricketts *et al.*, 2013). Reversible pulpal injury resulting from a deep carious lesion can now be treated effectively using a selective, minimally invasive approach (Tziafas *et al.*, 2000). Indeed the present knowledge about the caries process, the way in which lesions progress, and the biology of the dentine-pulp complex with its defence and degenerative reactions have set the trend for selective, minimally invasive caries removal in the management of such cases rather than the traditional excavation approach which often leads to unnecessary iatrogenic pulp exposure and therefore root canal treatment (Kidd, 2010). Furthermore, the development of bioactive materials such as calcium silicate cements have helped pave the way for this ultraconservative treatment modality where tissues may be sealed and even healed (Jefferies, 2014).

However, reaching a diagnosis of reversible pulpitis is fundamental for the treatment to be successful and this remains a challenge as current clinical and radiographic diagnostic methods do not give an accurate representation of the histological status of the pulp (Dummer *et al.*, 1980).

Conventional periapical radiographs commonly used in such situations are limited by the fact that three-dimensional anatomy of the area is compressed into a two-dimensional image revealing limited aspects of the three-dimensional anatomy due to superimposition. Furthermore, geometric distortion of the anatomic structures being imaged is another contributing problem affecting optimal diagnosis (Patel *et al.*, 2007a). These problems have been overcome by the introduction of small volume cone beam computed tomography (CBCT) which is well established in many fields in dentistry such as implant dentistry, maxillo-facial surgery, orthodontics, and endodontics. CBCT provides images with a high diagnostic quality due to the creation of images in “real time” using multiplanar reformation offering 3 dimensional information which help improve the assessment and diagnosis (Scarfe *et al.*, 2006).

The regular use of CBCT in conservative dentistry has yet to be recognised and if established will mean even more wide spread use of CBCT in dentistry. Due to the increased uses of CBCT, reducing the radiation dose to as low as reasonably achievable (ALARA) is essential and a balance between an optimised diagnostic image and a low radiation dose is desirable.

1.2 Research aims and objectives

The studies carried out and presented in this dissertation have two arms: an in-vivo study in the form of a randomised controlled clinical trial evaluating the use of calcium silicate cement (Biodentine™) as an indirect pulp capping agent in patients with reversible pulpitis, establishing the effectiveness CBCT in diagnosing reversible pulpitis, and evaluating the integrity of the overlying resin composite restoration using different assessment criteria.

The second arm includes two in-vitro studies. As CBCT has been used in the clinical trial, an attempt to reduce the radiation dose while maintaining an

optimised diagnostic image was investigated using an in-vitro animal model. The second study investigated the interfacial characteristics between calcium silicate cement and the overlying veneering resin composite restoration since clinically, calcium silicate cements are used in the so-called “sandwich” / laminate / layered technique. The quality and durability of the adhesive bond between Biodentine™ and the resin composite is of clinical significance with regards to the longevity and predictability of the final laminate restoration (Fig 1-1).

The aims were to evaluate the interaction between Biodentine™ and tooth structure both clinically and radiographically in patients with signs / symptoms of reversible pulpitis to help develop the clinical evidence base required to justify the materials’ use clinically and to assess the overlying resin composite restoration using available assessment criteria. This was preceded by investigating the use of high and low resolution CBCT scans to ascertain which dosage provides an optimised image with appropriate radiation exposure before using CBCT in the clinical trial. In addition, the aim was to assess the physical interfacial characteristics between Biodentine™ and resin composite using micro-shear mechanical testing to determine whether Biodentine™ could be veneered in the same clinical visit as its placement since this would be easier and less time consuming rather than bringing back the patient for a second visit to complete the veneering resin composite restoration.

The objectives of the study are the following:

1. To assess clinically the dentine-pulp response to Biodentine™ and the integrity of the overlying restoration.
2. To assess radiographically the presence of early peri-radicular changes using CBCT.
3. To attempt to reduce the radiation dose of CBCT while maintaining an optimised image by an in-vitro investigation using an animal model.
4. To evaluate the interface between Biodentine™ and resin composite restorative materials using micro-shear mechanical test.

1.3 Structure of the thesis

The second chapter provides a critical review of the literature beginning with a general overview of the dentine-pulp complex and its reactions to most common stimulants such as caries, different dental procedures and materials. Diagnosis of pulpitis is discussed clinically and radiographically and different treatment modalities of pulpitis due to deep carious lesions with a focus on minimally invasive approaches. Different materials used for indirect pulp capping procedures are discussed with a focus on giving a brief overview of each material, why it is used as a pulp capping material, and the dentine-pulp capping material, pulp capping material-overlying restoration interaction. A more in depth discussion about Biodentine™ is then provided followed by an account on different assessment methods for restorative materials. Chapter 3 is dedicated to the first in-vitro study which assessed the physical characteristics of the interface between Biodentine™ and the overlying resin composite restoration using micro-shear testing. In Chapter 4, the second in-vitro study compared the diagnostic yield obtained from high and low resolution CBCT scans in an attempt to reduce the radiation dose of CBCT. Chapter 5 describes the clinical trial assessing the efficacy of Biodentine™ as an indirect pulp capping agent in patients with signs of reversible pulpitis compared to glass ionomer cement as a control both clinically and radiographically and to assess the integrity of the overlying resin composite restoration using the available assessment criteria. Chapter 6 provides a general summary tying together the conclusions of the three studies along with areas ripe for future research. Published papers of these chapters are provided in the appendix along with all documentation associated with the clinical trial.

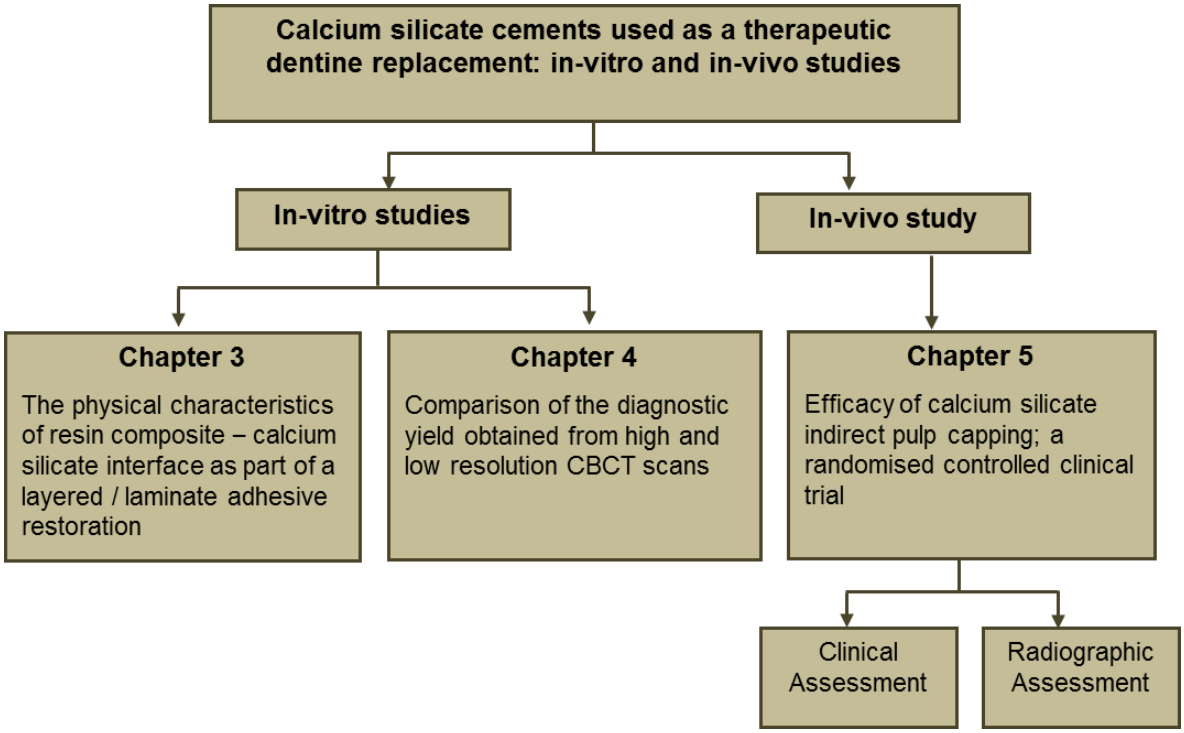
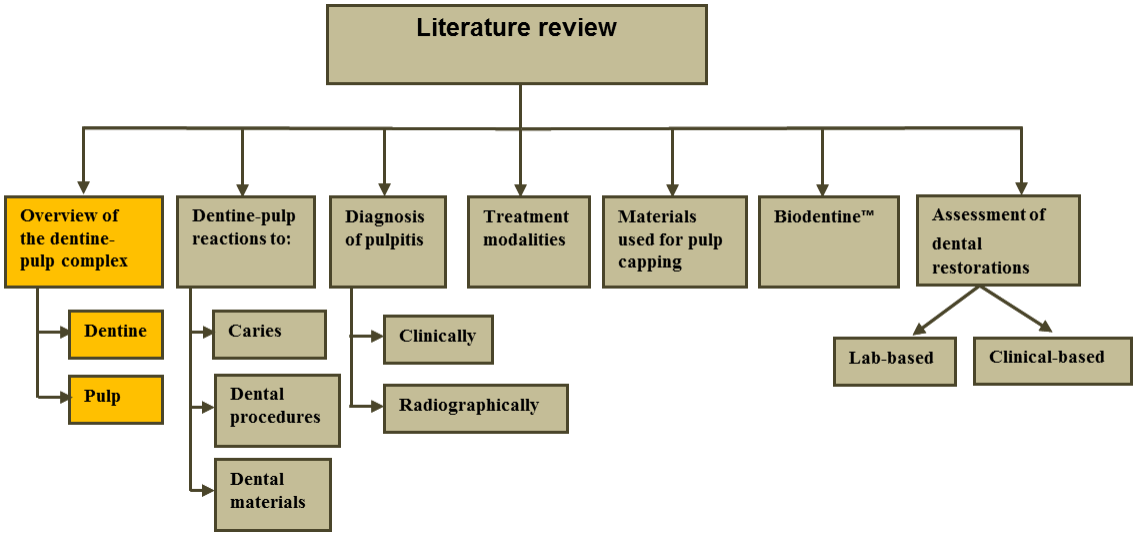


Figure 1-1: Structure of the PhD project.

CHAPTER 2

Literature Review

2.1 Introduction: A brief overview of the dentine-pulp complex



Flowchart of the literature review structure in the thesis.

The dentine and pulp are similar in development, structure, and function (Orchardson and Cadden, 2001). Although they are different anatomically and chemically (Pashley, 1996), they function together as one unit; the “dentine-pulp complex” (Orchardson and Cadden, 2001). A sophisticated interaction between the dentine and pulp start as early as their development. The dentine and pulp originate from the neural crest cells of the dental papilla the common ancestry for both tissues. In the tooth bud, the dental papilla cells close to the epithelial-mesenchymal interface differentiate into *preodontoblasts* whereas the rest of

the dental papilla cells form the dental pulp (Linde and Goldberg, 1993; Balic *et al.*, 2010). Following the withdrawal of the *preodontoblasts* from the mitotic cycle, they differentiate into *polarising odontoblasts* which in turn differentiate into active secretory odontoblast cells (young odontoblasts) and finally into mature and terminally differentiated odontoblasts (Linde and Goldberg, 1993). These mature odontoblasts then recede towards the pulp leaving behind cell process that extend into the dentinal tubules of dentine (Mina, 2014). A more detailed description of the normal structure and physiology of the dentine and pulp which will likely cause tissue reactions in response to clinical treatment of these tissues are provided in the next sections.

2.1.1 Dentine structure and dynamics

Dentine is a porous biologic composite made up of a mineral phase (70% in weight) consisting of apatite crystal particles in an organic extracellular dentine matrix (20% in weight) and water (10% in weight) (Pashley, 1996). The extracellular matrix consists of mainly collagen (Types I, III and V) and around 10% non-collagenous proteins including growth factors, enzymes, proteolipids, polyamines, blood serum proteins, and calcium-binding proteins (Goldberg *et al.*, 2011). This mineralised tissue is traversed by tubules 1-2 μm in diameter extending from the enamel-dentine junction (EDJ) or cemento-dentinal junction (CDJ) to the pulp. They harbour the odontoblastic process and dentinal fluid and are the major channels for diffusion of materials across dentine.

The permeability of dentine is an important property that varies according to age of the tooth, location within the dentine, degree of mineralisation of the tubules, and tissue changes in the dentine. Furthermore, the number of tubules and the type of branching may also affect dentine permeability (Mjör *et al.*, 2002b). The density and diameter of the tubules increase from the DEJ to the pulp, therefore dentine permeability is lowest at the DEJ and highest close to the pulp. Dentine permeability can be generally classified as *transdentinal* movement of substances through the dentine tubules such as fluid shifts in response to

hydrodynamic stimuli or *intradentinal* movement of exogenous substances such as resin into intertubular dentine (Pashley, 1996).

Mantle dentine is the outer layer at the periphery of the tooth in the coronal region beneath the enamel-dentine junction and is the first layer of dentine deposited during dentinogenesis. It is an atubular 150 μm thick layer having few, thin curved tubules (Goldberg *et al.*, 2011). This is because mantle dentine matrix is secreted from young odontoblasts which have not yet fully differentiated and are lacking odontoblastic processes which create patent tubules (Linde and Goldberg, 1993; Tjäderhane *et al.*, 2012). This outer layer is hypomineralised and hence has been proposed to contribute to the elastic properties of teeth by allowing relatively high occlusal loads without fracture of enamel or dentine by dissipating the pressure or forces (Goldberg *et al.*, 2011; Tjäderhane *et al.*, 2012).

Circumpulpal dentine forms the largest part of the dentine and constitutes primary and secondary dentine. *Primary dentine* is formed rapidly during tooth formation until the tooth becomes functional and differs from mantle dentine in that it is formed by mature odontoblasts, the collagen matrix is more compact, and it contains intertubular and peritubular dentine (Tjäderhane *et al.*, 2012). *Intertubular dentine* is the prominent part of primary dentine located between the tubules and formed of a dense fibrous network of collagen with deposited mineral crystals. *Intratubular or peritubular dentine* lines the dentine tubules and is more highly mineralised than intertubular dentine. Peritubular dentine contains few collagen fibrils and is rich in non-collagenous matrix components (Smith, 2012). Peritubular dentine is harder than intertubular dentine due to the lower collagen content and hence can be dissolved more quickly than intertubular dentine by acid-etching agents during dental restorative procedures hence enlarging the opening of the dentinal tubules, making dentine more permeable (Weiner *et al.*, 1999; Luukko *et al.*, 2011).

Secondary dentine forms as soon as contacts between the antagonistic cusps are established and continues throughout life (Goldberg *et al.*, 2011). The tubules of the secondary dentine matrix are continuous with those of the

primary dentine suggesting that the same odontoblasts are responsible for the secretion of primary and secondary dentine. However, down-regulation of the secretory activity of these cells means that the deposition of secondary dentine occurs at a relatively slower pace (Smith, 2012). Furthermore, secondary dentine varies from primary dentine in that the curvature of dentine tubules is more accentuated and the tubular structure may be less regular (Linde and Goldberg, 1993). The deposition of dentine is also uneven with increased deposition in the floor and roof of the pulp chamber especially in molar teeth (Tjäderhane *et al.*, 2012). This leads to a reduction in the pulp volume as well as the height of the pulp horns (Arana-Chavez and Massa, 2004).

Tertiary dentine is secreted in response to external stimuli such as dental caries, tooth wear, trauma, and other tissue injury and can be sub-classified as either reactionary or reparative depending on the extent of external stimuli. Mild stimuli will result in reactionary dentine formation secreted by mature odontoblasts while more severe stimuli will result in reparative dentine formation secreted by a new generation of odontoblast-like cells (Smith, 2012). More details of this are described in the following section.

Aging or the presence of a persistent stimuli such as tooth attrition or dental caries will cause *dentine sclerosis;* obliteration of the dentinal tubules either partially or completely with mineral deposits hence shielding the pulp from irritation by reducing the permeability of dentine. *Physiologic sclerosis* is an acceleration of intratubular dentine formation usually in the apical third of the root as a function of age. *Pathologic sclerosis* represents precipitation of hydroxyapatite and whitlockite crystals within the dentinal tubules as a result of caries or attrition (Luukko *et al.*, 2011).

Interglobular dentine is seen in the region separating mantle and circumpulpal dentine and contains unmineralised organic matrix due to coalescence failure of the growing calcopherites. It is normally seen in coronal dentine however in cases of fluorosis or vitamin D deficiency, interglobular dentine may be seen close to Tomes granular layer in the root (Kagayama *et al.*, 1997; Luukko *et al.*, 2011).

Predentine is a 15-20 μm layer of unmineralised organic matrix lining the pulpal aspect of dentine between the odontoblast layer and the mineralised dentine (the mineralisation front). It is the most recent layer to be deposited by the odontoblasts during dentinogenesis as they recede into the pulp and is narrower in the root than in the crown (Linde and Goldberg, 1993). It consists of collagen type I and II, proteoglycans, glycoproteins, and dentine phosphoprotein (Luukko *et al.*, 2011). The main trunk of the odontoblastic process penetrate this layer of predentine (Linde and Goldberg, 1993).

2.1.2 Pulp histology

The dental pulp is a soft connective tissue uniquely encased within the mineralised dentine with a communication via the apical foramen with the periapical tissues. It is a highly vascularised tissue with abundant myelinated and unmyelinated nerves serving to protect and provide nutrition to the dentine (Goldberg and Smith, 2004).

The odontoblast layer is the outermost layer of the pulp located immediately subjacent to the predentine. It is made up of odontoblast cells responsible for the maintenance of the teeth integrity by the deposition of dentine throughout life (Arana-Chavez and Massa, 2004). There are junctional complexes including desmosomes, gap junctions, and tight junctions that connect adjacent odontoblasts. Desmosomes mechanically join the odontoblasts together while gap junctions provide permeable pathways through which signal molecules can pass between cells. Tight junctions limit the passage of molecules, ions, and fluids between the extracellular compartments of the pulp. These tight junctions are disrupted during dental procedures hence increasing the dentine permeability. The odontoblast processes pass on through this layer to the predentine and into the inner part of the dentine (Fig 2-1) (Luukko *et al.*, 2011). Subjacent to this layer in the coronal pulp is a cell-poor zone which is a narrow zone relatively free of cells and hence called the *cell-free layer of Weil*. It contains blood capillaries, unmyelinated nerve fibres, and the cytoplasmic processes of fibroblasts. Subjacent to it is the subodontoblastic area consisting of the cell-rich zone containing fibroblasts, macrophages, dendritic cells, and undifferentiated mesenchymal stem cells. It has been suggested that this zone

is considered a source of cells that differentiate into secondary or replacement odontoblasts (odontoblast-like cells) upon injury to the primary odontoblasts (Fig 2-1) (Okiji, 2012).

The pulp proper is the central mass of the pulp containing fibroblasts, loose connective tissue, larger blood vessels, and nerves (Luukko *et al.*, 2011). The pulp interstitium occupies the extracellular space and consists of the interstitial fluid and extracellular matrix. It is made up of mainly collagen (Type I and III) with glycoproteins (proteoglycans, fibronectin, laminin, tenascin), hyaluronan, and elastic fibres. Absence of certain non-collagenous proteins in the pulp which are otherwise identified in dentine such as dentine sialoprotein, phosphoprotein, dentine matrix protein 1, and osteocalcin could perhaps explain why the pulp does not mineralise under physiologic conditions (Goldberg and Smith, 2004; Okiji, 2012).

Innervation of the pulp occurs via nerve bundles entering through the apical foramen of the teeth. These can be sympathetic autonomic/efferent nerve fibres mainly projecting to the radicular pulp which innervate the smooth muscle cells of the arterioles providing regulation of the blood flow in the capillary network. When these fibres are stimulated, constriction of the arterioles occur leading to a decrease in blood flow. No sympathetic innervation is seen in the odontoblast and subodontoblast layers (Fried and Gibbs, 2014). Afferent nerve fibres supplied from the maxillary and mandibular branches of the trigeminal nerve conduct sensory impulses which could either be myelinated (*A* fibres) or unmyelinated (*C* fibres). These nerve bundles pass upwards from the radicular pulp into the coronal pulp and fan out under the cell-rich zone into smaller bundles forming the *subodontoblastic plexus of Raschkow* (Walton and Ramachandran Nair, 1995; Yu and Abbott, 2007). From this plexus, the fibres extend between the odontoblasts into the coronal dentine traveling a short distance into the dentinal tubules where they terminate near an odontoblast process (Abd-Elmeguid and Yu, 2009a).

The myelinated *A* fibres are mainly located at the pulp-dentine border in the coronal portion of the pulp and concentrated around the pulp horns. They transmit pain directly to the thalamus, generating a fast sharp pain that is easily

localised. *A* fibres respond to various stimuli through the hydrodynamic effect which depends on the movement of dentinal fluid in the dentinal tubules in response to a stimulus. Normal slow capillary outward movement does not stimulate the nerve endings and hence does not cause pain however sudden desiccation of the dentine or hot/cold stimuli cause fluid movement through the dentinal tubules resulting in pain sensation in a tooth with a viable sensory pulp (Närhi *et al.*, 1991; Ngassapa, 1996; Abd-Elmeguid and Yu, 2009a). Additionally, when saturated sucrose solutions come in constant contact with sensitive dentine, this activates the nerves through osmotic pressure (Abd-Elmeguid and Yu, 2009a).

The unmyelinated *C* fibres are located in the pulp proper extending to the cell-free zone beneath the odontoblast layer. They are influenced by many modulating interneurons before reaching the thalamus resulting in a slow dull ache. The location of the *C* fibres in the core of the pulp may explain both *"referred"* pain and the difficulty in localising pain to a specific tooth because these nerve fibres innervate multiple teeth with multiple pulps. These fibres have less excitability with a higher threshold than the *A* fibres and hence need more intense stimuli to be activated (Närhi, 1990; Walton and Ramachandran Nair, 1995). *C* fibres may also survive during hypoxia because their cell bodies are found in ganglia outside the pulp (Luukko *et al.*, 2011). This explains the pain sensation felt during root canal treatment of a necrotic pulp (Abd-Elmeguid and Yu, 2009a).

Vascularisation of the dental pulp is supplied from the dental artery which is led by the maxillary artery; a branch of the external carotid artery entering the tooth via the apical foramen by way of arterioles along with the nerve bundles (Yu and Abbott, 2007). Additionally, smaller vessels may enter the pulp through lateral or accessory canals. The arterioles branch off to form a capillary network that spreads towards the odontoblast layer forming a capillary plexus in the subodontoblast region (Luukko *et al.*, 2011). This network provides oxygen, nutrients and metabolites to the odontoblasts and waste products are exchanged by diffusion. The waste products are removed by pulp venules (Yu and Abbott, 2007).

Regulation of blood flow is dominated by the autonomic nerve fibres. A relatively high blood flow is normally observed in the pulp compared to other tissues and organs. This is to maintain intraluminal pressure within the pulp vasculature in harmony with the pulp tissue pressure. The relatively high pulp tissue pressure results in the outward flow of fluid in the dentinal tubules which plays a role in diluting toxins and washing away bacteria (Yu and Abbott, 2007).

However, the pulp is particularly sensitive to any circulatory disturbances due to its encapsulated location within the dentine (Dahl and Mjor, 1973). This is discussed in more detail in the following section.

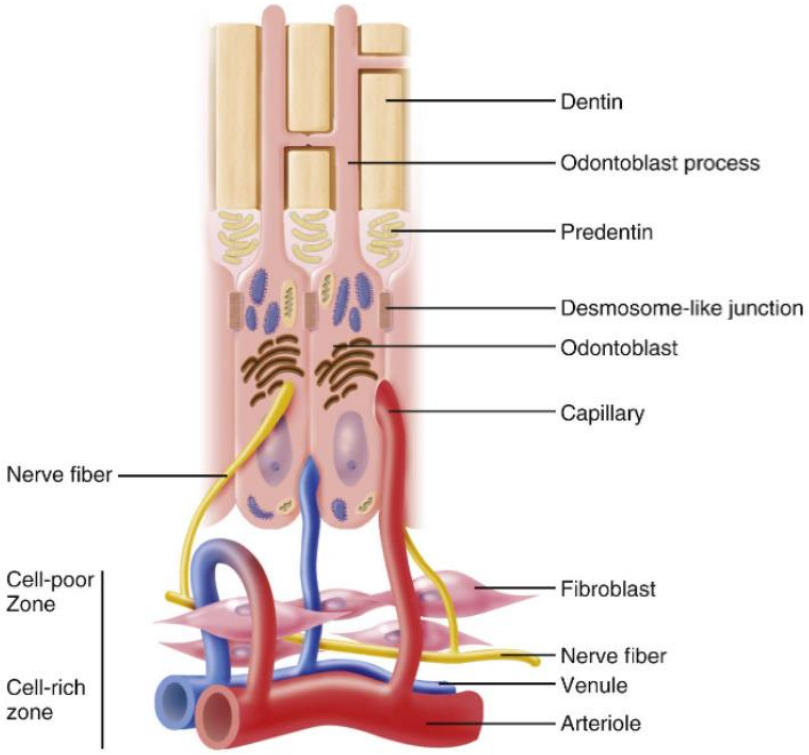
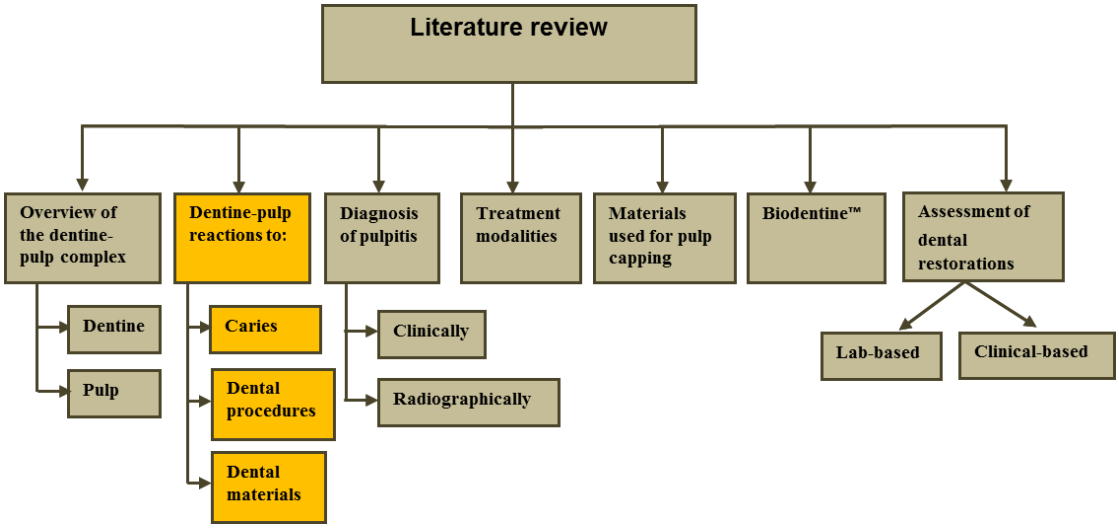


Figure 2-1: Diagrammatic representation of the odontoblast & subodontoblast region of the pulp from (Luukko *et al.*, 2011)

2.2 Dentine-pulp reactions : A biological basis



Flowchart of the literature review structure in the thesis.

2.2.1 Dentine-pulp reactions to caries

“Dental caries is the localised destruction of the susceptible dental hard tissue by acidic by-products from bacterial fermentation of dietary carbohydrates” (Fontana *et al.*, 2010). Signs of caries demineralisation are seen in the hard dental tissues however, the disease process is initiated in the bacterial biofilm that covers the tooth surface (Selwitz *et al.*, 2007). Dental caries is a continuum of disease states of increasing severity and tooth destruction; ranging from sub-clinical sub-surface changes at the molecular level to lesions with dentine involvement either with an intact surface or obvious cavitation followed by pulp exposure and finally a necrotic, infected root canal system with the presence of apical pathosis (Selwitz *et al.*, 2007; Bjørndal, 2008a).

The responses of the dentine-pulp complex are broad depending upon the degree of disease activity and extent of spread with a complex interplay between injury, defence, and repair physiology (Smith, 2002). Unfortunately, the focus of pulp reactions to caries in the literature has been centred on the later stages of disease progression with fewer describing the earliest pulp-dentine reactions to the initial stages of caries. This may be due to a reduction in the proficiency of the available histology methods when these studies were carried out. Enamel caries and pulp reactions cannot be viewed simultaneously in the

same histological section. In ground sections enamel caries can be viewed however pulp tissues are destroyed. Demineralisation of teeth to produce paraffin or EM sections will allow visualisation of the pulp but will eliminate enamel (Langeland, 1987).

2.2.1.1 The early carious lesion

Pulp response to the carious lesion occurs from the very outset. It has been observed that the pulp might react to signals passing through the enamel even before histologic caries reactions can be observed in the dentine (Bjørndal, 2008a). In a series of in-vivo studies where in this early stage, following removal of the cariogenic biofilm left undisturbed for a week on the surface of a tooth, no changes were seen on the enamel clinically even after thorough air-drying. However at the ultra-structural and microscopic level when viewed using polarised light and scanning electron microscopy, there were signs of direct dissolution of the outer enamel surface seen as an enlargement of the intercrystalline spaces due to partial dissolution of the individual crystal peripheries (Holmen *et al.*, 1985a; Holmen *et al.*, 1985b). In a further study at this same initial stage, a pulp response was triggered through the transmission of stimuli from the cariogenic biofilm through the rod/interrod enamel which resulted in a reduction in the odontoblast-predentine region before the start of peritubular dentine mineralisation. Furthermore, reactions in the subodontoblastic layer involved fibroblast-like cells invading the cell-free zone (Bjørndal *et al.*, 1998).

Following two weeks of undisturbed cariogenic biofilm left on the tooth surface, enamel changes can be visible clinically as “white spot” lesions after the teeth were air-dried. After three and four weeks, these lesions can be seen even before the samples were air-dried (Holmen *et al.*, 1985a; Holmen *et al.*, 1985b). In such cases of early enamel demineralisation, the cytoplasm:nucleus ratio of the odontoblast cells is reduced with a reduction in the predentine zone. There is more proliferation of fibroblast-like cells in the cell-free zone (Bjørndal *et al.*, 1998).

As the lesion approaches the EDJ but without extending to it there is an increased growth of the predentine matrix and an increase in collagen type I synthesis with bundles of collagen fibres in the extended predentine zone (Bjørndal *et al.*, 1998). Predentine at this stage is primed to form mineralised dentine and this is evidenced by the signs of enhanced peritubular dentine mineralisation and tubular sclerosis which can be visible in dentine (Lee *et al.*, 2006). The activation of the odontoblast cells to produce reactionary dentine in this very early stage of precavitated enamel lesions suggests that pH gradients in the lesion and the cariogenic plaque complex may play a role as driving forces for the transportation of stimulating molecules in and out of the tissue through the enamel rods or crystal populations (Bjørndal *et al.*, 1998). If the transmission of the cariogenic stimuli across the enamel layer is stopped, signs of arrested remission are apparent throughout the dentine-pulp complex (Bjorndal, 2002).

2.2.1.2 Reactionary dentine formation

When the demineralised enamel is in contact with the EDJ, odontoblasts are characterised as being smaller than normal with loss of their columnar appearance and modified junctional complexes to allow passage of dendritic cells to the predentine-dentine interface (Couve *et al.*, 2014). Increased cellular activity is seen in the cell-free zone and reactionary dentine is deposited by the odontoblasts directly affected by the injury process as it proceeds from the EDJ into the outer then inner dentine (Smith *et al.*, 1995). This modified dentinal matrix is a relatively atubular dentine with altered biochemical properties (Charadram *et al.*, 2012). The acidic by-products of the carious process act indirectly by degrading the dentine matrix liberating bioactive molecules responsible for the stimulation of reactionary dentine formation (Smith *et al.*, 1995). These are mainly growth factors from the transforming growth factor beta (TGF-B) family, including TGF-1, TGF-3, and bone morphogenic protein-7 (BMP-7), although other bioactive molecules which haven't been characterised yet may participate in the signalling process of reactionary dentinogenesis (Goldberg and Smith, 2004).

Dentine sialophosphoprotein (DSPP) is a major mineral phase-interactive acidic matrix protein of dentine secreted by odontoblasts and is fundamental for dentine formation (MacDougall *et al.*, 1997; McLachlan *et al.*, 2003). Matrix metalloproteinase-2 (MMP-2) comes from a family of zinc-dependent endopeptidases and is present in abundance in odontoblasts and dentine (Niu *et al.*, 2011). MMP-2 is implicated in the regulation of the bioavailability and bioactivity of TGF-B (Sloan *et al.*, 1999; Wang M *et al.*, 2006). MMP-2 is synthesised and released in the form of an inactive pro-protein. Inactive pro-protein MMP-2 is activated by a process regulated by membrane type-1 matrix metalloproteinase (MT1-MMP) and tissue inhibitor of metalloproteinase-2 (TIMP-2) which is up-regulated due to caries (Charadram *et al.*, 2012). MT1-MMP and TIMP-2 then bind together to activate the latent pro-protein MMP-2. Active MMP-2 cleaves DSPP to release dentine sialoprotein (DSP), and dentine phosphoprotein (DPP) in an active form to enhance mineralisation by sequestering calcium ions (Fig 2-2) (Charadram *et al.*, 2012).

The modified activity of odontoblasts in reactionary dentinogenesis with its structural rearrangement of dentine and diminished tubularity ensures impediment of the bacteria from progressing through the dentine (Charadram *et al.*, 2012).

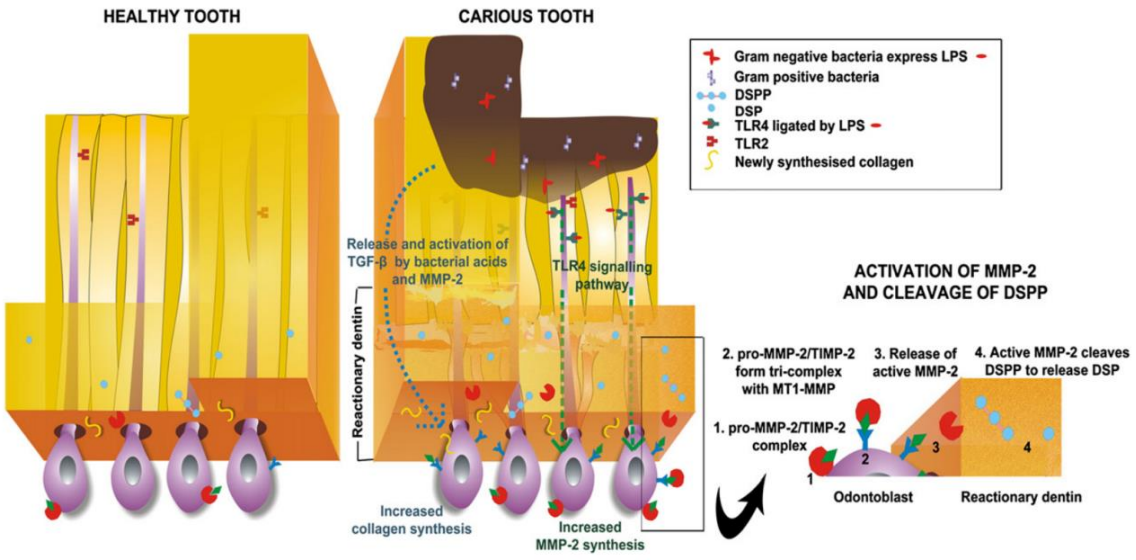


Figure 2-2: Diagramatic representation of the formation of reactionary dentine. Source: (Charadram *et al.*, 2012)

In addition to the formation of reactionary dentine, odontoblasts mediate an inflammatory response at this stage as they possess specific receptors specifically from the toll-like receptor family (TLR1-6 and 9) (Hahn and Liewehr, 2007). These TLRs have the capacity to recognise pathogen associated molecular patterns (PAMPs) such as these present in lipoteichoic acid (LTA), a cell wall component of Gram-positive bacteria mostly present in the initial lesion and lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria present in more advanced lesions (Martin *et al.*, 2002; Goldberg *et al.*, 2008; Farges *et al.*, 2009). As binding between TLRs on the odontoblasts and the bacterial components occur, activation of the nuclear factor kappa B (NF-kB) intracellular signalling pathway which is responsible for the regulation of the molecular inflammatory response follows (Chang *et al.*, 2005). Antimicrobial peptides, chemokines, and pro-inflammatory cytokines such as interleukin-1a, -1b (IL-1a, -b) and tumour necrosis factor-a (TNF-a) are released resulting in the formation of chemotactic gradients leading to the recruitment and activation of immune cells (Veerayutthwilai *et al.*, 2007; Cooper *et al.*, 2010). These immune cells include T- lymphocytes in the initial dentinal caries followed by B-lymphocytes, plasma cells, neutrophils, and macrophages which accumulate initially in the pulp and sub-odontoblastic regions. With caries progression, they extend into the odontoblast layer, and subsequently migrate into the tubule entrance beside the odontoblast process (Cooper *et al.*, 2010).

Similarly, immunoglobulin (Ig)G, IgM, and IgA have been found to accumulate in the odontoblast layer during the incipient lesion and with lesion progression can be seen in the dentinal tubules (Okamura *et al.*, 1980; Okamura, 1985). Nerve fibre sprouting into the reactionary dentine matrix has been found with release of pro-inflammatory neuropeptides such as Substance P, calcitonin gene related peptide (CGRP), neurokinin A (NKA), NKY, and vasoactive intestinal peptide from pulpal nerves causing vasodilation, increased vascular permeability and the development of pain (Couve *et al.*, 2014).

The relationship between the degree of injury that an odontoblast can withstand and still survive is not clear. Correlating caries lesion progression and reactionary/reparative dentinogenesis is still not well identified due to the lack of

chronological information on tissue changes that would distinguish odontoblast survival and renewal (Goldberg and Smith, 2004). Therefore, it is difficult to identify which point exactly during the carious process reactionary dentinogenesis ends and reparative dentinogenesis begins and they are commonly seen superimposed on one another in deeper carious lesions (Fig 2-3) (Bjørndal and Darvann, 1998).

2.2.1.3 Reparative dentine formation

Reparative dentinogenesis follows a more intense insult/injury which odontoblasts cannot withstand leading to their death. A new generation of odontoblast-like cells originating from a precursor population mediate reparative dentine regeneration; an atubular dentine matrix sometimes referred to as osteodentine (Sloan and Smith, 2007). The origin of these odontoblast-like cells has been the focus of many studies and no single cell population from the pulp has been found to be the sole progenitor for odontoblast-like cell differentiation (Sloan and Smith, 2007; Harichane *et al.*, 2011). One potential derivation was suggested to be from progenitor pulp cells present in the cell-rich layer of Höhl adjacent to the odontoblasts. They are stimulated by specific molecular signals without any replication of their DNA. These cells were proposed to be derived from the dividing pre-odontoblasts before their terminal differentiation into odontoblasts (Tziafas, 1995).

Some studies have suggested that odontoblast-like cells originate from perivascular cells migrating out of capillary walls into the surrounding fibrous pulp tissue in response to the degradation of the dentine matrix (Téclès *et al.*, 2005). STRO-1 antigen found on the cell walls of pulp blood vessels and CD146 (a pericyte marker) were suggested to be early markers that infer a possible perivascular niche as a derivative for odontoblast-like progenitors (Miura *et al.*, 2003; Shi and Gronthos, 2003).

Furthermore, a unique population of post-natal dental pulp stem cells (DPSC) with mesenchymal stem cell like qualities have been implicated as progenitors for the formation of odontoblast-like cells (Gronthos *et al.*, 2000; Gronthos *et al.*, 2002). They possess the capacity for self-renewal and have a high proliferative

potential however the origin and precise location of these DPSCs are unknown due to lack of specific markers (Gronthos *et al.*, 2000).

The stem/progenitor cell niches are usually in a quiescent state, however when trauma or injury leading to the death of the post-mitotic odontoblasts occurs, signals are released from the solubilised dentine matrix causing activation of the stem/progenitor cells to proliferate, migrate, differentiate, and deposit a new dentine matrix. Once this has been formed with the layer of odontoblast-like cells covering the injury site, migration of pulp cells ceases (Mitsiadis and Rahiotis, 2004; Sloan and Waddington, 2009). TGF-ß1 and BMP molecules in the solubilised dentine matrix diffuse through the dentinal tubules into the pulp cells along with some pro-inflammatory cytokines stimulating the expression of some of the integrin subunits responsible for facilitating cell migration (Mitsiadis and Rahiotis, 2004). Activator protein-1 (AP-1) is a key regulator of cellular migration, proliferation, and differentiation and has been implicated in enhancing the expression of TGF-ß1, osteocalcin, alkaline phosphatase, and type I collagen (Smith and Lesot, 2001). Notch, nestin, cadherins, and connexins also contribute to the signalling cascade, resulting in odontoblast-like cell differentiation (Mitsiadis and Rahiotis, 2004).

The inflammatory process associated with reparative dentinogenesis is similar to reactionary dentinogenesis but is notably more prevalent and intense (Cooper *et al.*, 2010). The progressive and sequential accumulation of T- and B-lymphocytes, macrophages, neutrophils and plasma cells in the pulp with increased levels of pro-inflammatory gene expression is concomitant with the deepening of the dentin caries lesion and the increase of the bacterial insult (Goldberg *et al.*, 2008). However it should also be noted that although these immune cells play a role in the tissue defence process, they can also cause significant damage due to the release of proteolytic enzymes that degrade the extracellular matrix and cellular contacts. In addition, they release a considerable amount of reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals and degrading enzymes including MMPs affecting DNA, proteins, and lipids leading to further tissue injury and cellular damage (Veerayutthwilai *et al.*, 2007; Cooper *et al.*, 2010).

Key components in pulp inflammation which play a role in the pulp response to disease are the microcirculation and the sensory nerve activity (Olgart, 1991; Yu and Abbott, 2007). During neurogenic inflammation, afferent nerves respond to bacterial antigens by releasing neuropeptides which recruit and activate the immune cells due to the presence of neuropeptide receptors on their cell surfaces (Fristad *et al.*, 2007). The type, duration, and severity of the neurogenic reaction depends on the extent of damage and duration of the inflammation (Fristad *et al.*, 2007). Early stages of acute inflammation cause vasodilation and transudation of fluids causing increased pulpal tissue pressure which stimulates pulp nerves to register pain. Pressure from the increased tissue fluids result in collapse of the thin walled veins and venules in the affected area of the pulp tissue causing localised vascular stasis, ischemia and eventually local cellular death. As the inflammatory process and intra-pulpal pressure changes progress apically and circumferentially by increments total loss of structural integrity of the pulp is achieved resulting in total necrosis (Yu and Abbott, 2007). However it is worth mentioning that increased pulpal tissue pressure encourages absorption of tissue fluid back into the blood hence reducing the pressure. This explains why pulpal tissue pressure in inflamed pulps may be maintained in local regions for long observation periods (Luukko *et al.*, 2011).

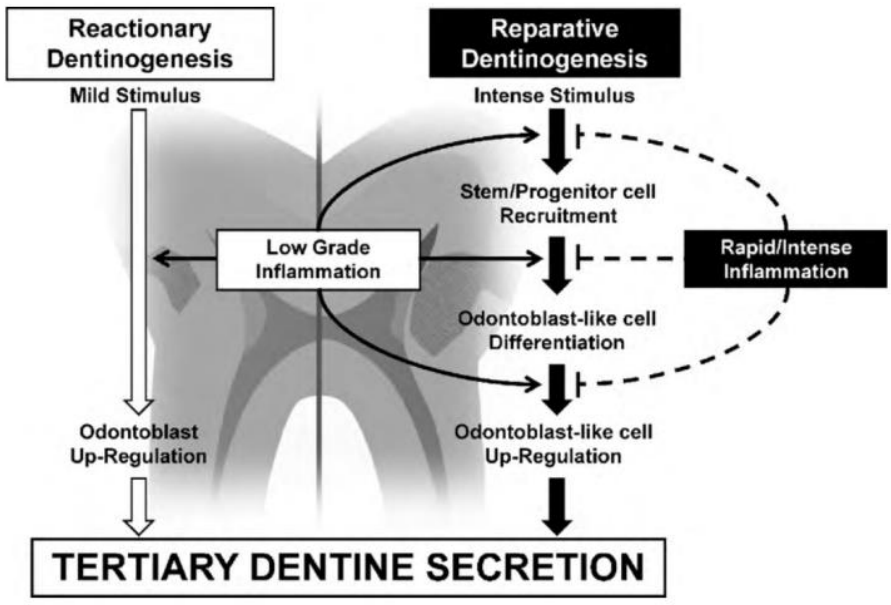


Figure 2-3: Summary of tertiary dentinogenesis. Source: (Cooper *et al.*, 2010)

2.2.2 Dentine-pulp reactions to restorative procedures

Restorative procedures are performed commonly to treat dental caries and these procedures cause significant irritation to the pulp (Deligeorgi *et al.*, 2001). A fundamental requirement for a successful restorative procedure is to cause minimal additional irritation to the pulp so as not to interfere with pulp healing. If a provisional diagnosis of reversible pulpitis is made, a minimally traumatic restorative procedure is desirable to prevent converting the diagnosis to irreversible pulpitis (Fouad and Levin, 2011). This is particularly critical in borderline cases such as teeth with deep lesions but no pulp exposure. However it is generally accepted that the influence of insults on the pulp be it caries, restorative procedures, or materials, are cumulative. With each successive irritation, the pulp has a reduced capacity to remain vital (Fouad and Levin, 2011). Nevertheless, the effect of restorative procedures on the pulp depend on several factors such as the degree of pulp inflammation before treatment, the degree of physical irritation caused by a certain procedure, proximity to the pulp or remaining dentine thickness, permeability of dentine between the area being restored and the pulp, and patient's age (Langeland *et al.*, 1971).

Studying the effect of restorative procedures is difficult to assess as the preparation will either need to be filled with a provisional or permanent restoration which will subsequently have an effect on the pulp hence obscuring the results or left exposed in the oral cavity leading to the accumulation of bacteria and debris. The only way to evaluate this would be to perform the restorative procedure and immediately extract the tooth for assessment (Mjör *et al.*, 2002a).

Studies have been conducted to assess the effect of heat generated during cavity preparations on the pulp. The extent of intra-pulpal heat generated during cavity preparation has been shown to be related to the use of coolants; handpiece rotation speed; size, type, and shape of the cutting instrument;

length of time the instrument is in contact with the dentine; amount of pressure exerted by the handpiece; and cutting technique (Tay *et al.*, 2012).

In a study by Langeland and Langeland (1965) cavity preparations were found to be well tolerated by the pulp provided copious water is used. In an earlier study, infiltration of the pulp with inflammatory cells, most commonly lymphocytes and occasionally PMN leucocytes, was seen with and without using coolants albeit in higher numbers when no coolants were used (Kramer, 1961). Furthermore, the passage of the odontoblast nuclei outwards from the periphery of the pulp into the dentine tubules, “odontoblast aspiration”, was observed more in the groups were no coolants were used during cavity preparation (Kramer, 1961).

One study found that when heat (150°C) was applied to prepared cavities, pulp pathosis was found compared to controls which had not received heat. This was in the form of collagenous tissue on the dentine wall and absence of dentinal tubules. The cell-rich zone was absent from the pulp horns with cellular degeneration and reduced number of cells. The teeth gave no discomfort for the 1 month duration of the study (Nyborg and Brännström, 1968). In another study to determine the threshold for thermal pulp damage, a thermal stimuli ranging from 8.9-14.7°C which more closely resembles that occurring from restorative procedures was applied to heathy teeth which were extracted for histology assessment following a period of clinical monitoring ranging from 68-91 days. An average increase of 11.2°C was not found to damage the pulp as there were no clinical symptoms and no signs of inflammation or reparative processes (Baldissara *et al.*, 1997). This could be due to a heat shock response induced by the synthesis of heat-shock proteins (HSPs) which play a role in anti-apoptotic processes and cell survival. In a study by Amano et al. (2006), rat pulp cells were cultured following exposure to a temperature of 42°C for 30 minutes. The expression of HSP70 was raised and changes in alkaline phosphatase and gap junction proteins were noticed which all returned to normal after a few hours (Amano *et al.*, 2006).

Frictional heat leading to burns on the dentine surface is considered the main cause of an acute pulp response to operative procedures (Kramer, 1961). In one study, it was shown that ultra-coarse grit diamond burs produced the highest increase in intra-pulpal temperature compared to the finer grits with a maximum increase of 2.5°C for fine, 2.6°C for coarse, and 3.2°C for ultra-coarse diamond burs. Additionally, grinding intervals were found to be directly proportional to temperature rise. Fine diamond burs were found to be associated with a short grinding interval and exhibited the lowest temperature elevation (Ottl and Lauer, 1998). The same was found for hand piece rotation speed and pressure where an increase in the handpiece rotations per minute and applied pressure can produce an increased intra-pulpal temperature rise (Hatton *et al.*, 1994).

Excessive air drying of exposed dentine between 20 seconds and 5 minutes causes desiccation leading to aspiration of the odontoblast nuclei into the dentine tubules and a reduction in the odontoblast layer (Brännström, 1960a). Air drying of exposed dentine for 5 minutes causes the same as the above in addition to the deposition of reparative dentine. Inflammatory cells were observed to be the same in the test and control samples (Brännström, 1960b). This was in agreement with another study where pulp inflammation and injury to the cells in the cell rich zone was not found to be associated with the dissolution of the aspired cells (Brännström, 1968). On the contrary, another study found that pulp inflammation was associated with 30 seconds of air drying freshly cut dentine when observed after 19 days, with lymphocytes and PMN leukocyte infiltration. However inflammation ceased after 180 days and the study concluded that pulpal inflammation was reversible and may be due to factors other than air drying (Cotton, 1967).

Light curing units were also found to increase the pulp chamber temperature in the range between 1.4-3.8°C with none of the different types of units exceeding the critical rise of 5.5°C which is thought to cause damage to the pulp (Hannig and Bott, 1999). Light emitting diode (LED) and plasma arc units are associated with less temperature rise compared to Halogen units (Yazici *et al.*, 2006). In another study, halogen unit was associated with an increased intra-pulpal

temperature rise compared to an LED unit, affecting the metabolism of the underlying cultured pulp cells when 0.5 mm-thick human dentine discs were used (de Souza *et al.*, 2009). The decisive factor for temperature rise is the energy absorbed during irradiation rather than the exothermic resin composite polymerisation process which is of secondary importance (Lloyd *et al.*, 1986). Therefore, the potential risk of heat-induced pulp injury increases when using light curing units with high energy outputs compared to ones with lower energy outputs (Hannig and Bott, 1999).

Chemical irritation from the application of etching agents especially strong acids following deep cavity preparations with a remaining dentine thickness less than 300μm has been found to irritate the odontoblast cells enough to stimulate them to secrete reactionary dentine with a persistent pulp inflammatory response (Qvist *et al.*, 1989). In another study, odontoblast displacement into the dentine tubules and pulp inflammation with macrophages and neutrophils present adjacent to the cavity floor was observed when 240μm of remaining dentine was conditioned with 10% phosphoric acid for 15 seconds compared to controls which had been lined prior to etching (Hebling *et al.*, 1999). The outward flow of dentine fluid through the tubules and the presence of plasma proteins are the first line of defence minimising the inward flow of any noxious agents (Pashley, 1996). However, etching deep cavities with remaining dentine thickness of less than 300μm does not prevent the displacement of these noxious agents as dentine permeability is higher allowing them to reach the odontoblasts triggering an inflammatory response associated with tissue disorganisation (de Souza Costa *et al.*, 2002). Therefore the effect of etching on the pulp tissue is dependent on the thickness of the remaining dentine (Pashley, 1991).

Chemo-mechanical gels such as Carisolv™ gel have been developed for the conservative, more selective removal of caries focusing on the removal of irreversibly demineralised and infected carious dentine hence avoiding the unnecessary removal of the reversibly damaged dentine which is especially important to maintain in deep carious lesions where removal of this layer would risk pulp exposure (Banerjee *et al.*, 2000a). Therefore, many studies have focused on evaluating the effect of Carisolv™ gel on the pulp as it is used more

commonly in deep carious lesions when a minimally invasive treatment approach is required. In one study where Carisolv™ gel was applied directly on an exposed pulp for 10 minutes, a very mild pulpal inflammation was observed after one week with irregularities in the odontoblast layer and aspiration of the odontoblasts into the dentinal tubules. In the control teeth which had not received Carisolv™ gel, the same pulpal inflammation was observed but with no irregularities in the odontoblast layer and no aspiration of the odontoblasts. A localised area of haemorrhage was observed. After one month all had subsided in both groups. No statistically significant difference was found between the test and control groups at 1 week and 1 month at the histopathological level. Furthermore, Carisolv™ may have a haemostatic effect on the exposed human pulp (Bulut *et al.*, 2004).

Another study compared the effect of Carisolv™ to physiological saline when applied to the exposed pulps of rat teeth for 30 minutes with an observation period of 1 day to 1 week. Light microscopic examination showed the same cellular response in both test and control teeth with a localised inflammatory reaction seen after 1 day represented mostly by leukocytes, macrophages, dendritic cells, and fibrous tissue formation with dilatation of the blood vessels and small sprouting of the nerves. After 1 week, an inflammatory reaction was still observed in both test and control teeth albeit dominated by macrophages which are normally present in small numbers throughout the pulp instead of leukocytes. This indicates that Carisolv™ has the same safety profile as physiologic saline with the inflammatory reaction being an expected response due to the wound inflicted on the pulp through exposure (Young and Bongenhielm, 2001).

This is in agreement with another study which found the same reaction between Carisolv™ and saline placed on the exposed pulps of rat teeth and an observation period of 1,10, and 20 minutes confirming the biocompatibility of Carisolv™ with the pulp tissue (Dammaschke *et al.*, 2001).

Although Carisolv™ contains sodium hypochlorite (NaOCl) which is known to be hypertonic, alkaline, and caustic causing damage to vital tissues (Tang *et al.*,

2000), it also contains amino acids which dilute the effect of NaOCl by selectively dissolving the outer infected dentine layer containing the irreversibly denatured collagen with limited effect on the affected dentine containing partially/reversibly denatured collagen (Hannig, 1999).

2.2.3 Dentine-pulp reactions to restorative materials

Restorative materials have long been used not only to replace lost or prepared tooth structure, but more importantly to protect and preserve the vitality/sensibility of the pulp (Goodis *et al.*, 2012). However chemicals from restorative materials and bacterial leakage at the tooth-restoration interface have an effect on the pulp (Tay *et al.*, 2012). This is associated with the degree of dentine permeability which in turn is affected primarily by the remaining dentine thickness between the floor of the prepared cavity and the pulp (Fouad and Levin, 2011). Although research has shown that the pulp can tolerate different restorative materials provided that the bacteria and their toxins are excluded from the pulp which indeed is crucial and a key element for pulp survival (Bergenholtz *et al.*, 1982; Cox *et al.*, 1987; Advokaat, 1990), the pulp will still react to noxious stimuli from restorative materials with an inflammatory reaction (Hilton, 2009). Material-related sequelae can either be cytotoxic by destroying the pulp cells or immunosuppressive by reducing the ability of the pulp to respond to a bacterial invasion (Hilton, 2009). Interestingly, it was found that the level of pulp inflammatory activity mediated by restorative materials is identical with and without the presence of bacteria. (Murray *et al.*, 2002c). Pulp reactions to restorative materials used commonly are described below when placed in very deep cavities or directly on the pulp.

Dental amalgam, one of the oldest restorative materials available, has been considered by some to be non-irritating to the pulp, while others consider its high thermal conductivity to be the cause of injury and secondary dentine formation (Swerdlow and Stanley, 1962). Early studies investigating the pulp response to amalgam when placed on non-exposed teeth with different

remaining dentine thickness have shown a reduction in odontoblast numbers with some degenerative changes and dilatation of the blood vessels which all become intensified the deeper the cavity. Suggestions have been made to ensure insulation through the application of a cavity liner beneath amalgam restorations in deep cavity preparations to protect the pulp from irritation maintained by the transmission of thermal stimuli to the pulp (Manley, 1941; Swerdlow and Stanley, 1962; Mjor *et al.*, 1977). Furthermore, ions such as Ag, Cu, Hg released from amalgam have been shown to have a cytotoxic effect on the pulp and the degree of its effect is dependent on the concentrations which reach the pulp via diffusion through the dentine (Wataha *et al.*, 1994). One study assessed the pulp response to low copper amalgam with and without smear layer removal. More pulpal inflammation was seen in the teeth which had the smear layer removed which does not support the idea that toxins produced from the bacteria present in the smear layer causes pulpal irritation. However removal of the smear layer may have improved the seal at the amalgam-tooth interface hence minimising marginal leakage (Jodaikin *et al.*, 1986).

In a more recent in-vitro study, the effects of methyl mercury chloride (MeHgCl) and mercuric chloride ($HgCl_2$) when exposed to human gingival fibroblasts for 24 hours were compared to resin composite components. Higher toxicity was observed with the mercury compounds compared to the resin composite constituents which has been suggested to be due to the ability of the mercury compounds to interfere with the cellular metabolism and function leading to cell swelling and finally necrotic cell death (Reichl *et al.*, 2006). More recent studies assessing the compatibility of amalgam with the pulp tissues are surprisingly scarce.

The use of resin-based materials such as dental adhesives, resin composite, and resin modified glass ionomer cements has been associated with pulp irritation and necrosis especially when placed in direct contact with the pulp or in deep cavities with remaining dentine thickness of less than 0.5mm (Modena *et al.*, 2009). This is because these materials can contain components including bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEG-DMA), camphoroquinone, 2-hydroxyethyl

methacrylate (HEMA) and others, which do not completely polymerise on curing and hence the unreacted monomers may readily permeate dentine into the subjacent pulp inducing an inflammatory response (Goldberg, 2008; Modena *et al.*, 2009).

Light curing for a short period of time or with a low light intensity and the presence of oxygen leads to incomplete conversion of monomers to polymers (Lovelh *et al.*, 1999). Resin monomers may also be leachable if the resin is degraded by esterase from saliva or when hydrolytic degradation occurs (Modena *et al.*, 2009). Etching of dentine causes faster outward movement of the dentinal fluid which may interfere with the complete polymerisation of the fluid resin (Pashley, 1996). Additionally, complete polymerisation may not be achieved due to pulp oedema if placed directly on the pulp (Modena *et al.*, 2009).

Moreover, during light curing of resin composites, heating may reverse the dentin fluid movement which results in the inward movement of the fluid carrying with it the unpolymerised resin through the dentine tubules into the pulp. Furthermore, the application of local anaesthesia including vasoconstrictor may lead to a decrease in the pulp pressure and an increase in the inward flow of dentine fluid which makes the diffusion of the noxious stimuli easier especially these with a smaller molecular size (Pashley, 1996; Hebling *et al.*, 1999).

Murray et al. (2002b) compared the pulp response to exposed and non-exposed cavities restored with resin composite, resin-modified glass ionomer cement, and calcium hydroxide in Rhesus macaca monkeys. Non-exposed cavities had a remaining dentine thickness of 0.5 mm. Bacterial microleakage was observed to be higher in the exposed cavities compared to the non-exposed cavities regardless of which material used. This has been suggested to be due to an increased volume of lost tooth structure and larger material volumes used in the exposed cavities. More severe pulp inflammation was associated with the exposed cavities which may be due to the higher incidence of bacterial microleakage. No severe pulpal inflammation was seen in the uncontaminated

and non-exposed cavity restorations. Tertiary dentine formation was found to be higher beneath the exposed cavities compared to the non-exposed cavities which are explained by the higher magnitude of injury sustained by the pulp following exposure. In the non-exposed cavities, tertiary dentine formation was higher beneath the calcium hydroxide while no difference was found between the resin composite and resin-modified glass ionomer cement (Murray *et al.*, 2002b).

One study reported displacement of the odontoblasts into the dentine tubules with neutrophils and macrophages in the human pulp tissue adjacent to the cavity floor when the remaining dentine thickness was less than 0.5 mm after 7 days observation following application of adhesive resin. After 30 days, reactionary dentine was observed with persistent moderate inflammatory response (Hebling *et al.*, 1999).

In another study, adhesive resin was applied in deep cavities with remaining dentine thickness of 0.5mm and on exposed pulps in human teeth. A persistent inflammatory pulp reaction was observed with particles of resin displaced in the dentine tubules and pulp triggering a foreign body response characterised by macrophages and multinuclear giant cells in addition to irreversible injury to the odontoblasts closest to the cavity floor leading to reparative dentine formation (Gwinnett and Tay, 1998). This was in agreement with another study placing an adhesive resin on exposed human pulps and observed for a short period of time (9-12 days) and a long period of time (53-204 days). During the short term, dilatation and congestion of the blood vessels was demonstrated in addition to a moderate inflammatory response and blanching of the pulp cell nuclei was seen beneath the injury site. Long term observation revealed no evidence of healing with a persistent mild inflammatory pulp response (Pereira *et al.*, 2000). These findings were confirmed in a review by de Souza Costa and others on pulp capping with adhesives where they concluded that adhesives result in inferior pulp healing, chronic inflammation even without the presence of bacteria, and that pulp inflammation due to caries will have decreased capacity for healing (de Souza Costa *et al.*, 2000; Hilton, 2009).

The use of glass ionomer cements directly on the pulp or in deep cavities approaching the pulp has been a subject of controversy. Since the introduction of this material 45 years ago, the biocompatibility of this material has been intensively studied. Earlier studies have shown that glass ionomer cements are related to an increased inflammatory cell infiltrate in the odontoblast layer, more odontoblast aspiration and changes in the odontoblast layer compared to controls when placed in non-exposed very deep cavities in human teeth. However no symptoms were recorded during the observation periods and the changes had mostly resolved towards the end of the experiments (Tobias *et al.*, 1978; Cooper, 1980; Plant *et al.*, 1984). This material was also studied in a number of animal models and these generally reported a mild pulp inflammation comparable to zinc-oxide/eugenol cements (Knibbs, 1988). However one study demonstrated a high prevalence of pulp inflammation and necrosis when glass ionomer cements were placed directly on exposed molar rat teeth (Paterson and Watts, 1981). This was in agreement with another in-vitro study assessing the cytotoxicity of eight different glass ionomer cements by means of pulp cell culture. The authors found that some glass ionomer cements are more cytotoxic than others and concluded that glass ionomer cements should not be placed directly on pulps or near it (Müller *et al.*, 1990). However, great caution should be employed when extrapolating results from in-vitro studies to the clinical situation since the natural protective effect of dentine is eliminated and the individual defence and repair mechanisms which allow toleration of such materials are not present.

Contrary to the above, other animal studies reported no adverse pulp reactions to glass ionomer cement when placed in non-exposed deep cavities when observed over different time periods (Pameijer *et al.*, 1981; Felton *et al.*, 1991). These results are corroborated by more recent studies using improved versions of glass ionomer cements which have shown minimal cytotoxic effects on the pulp. In a study by Six et al. (2000), GIC Fuji IX was placed in deep non-exposed cavities of rat teeth and compared to unfilled cavities as a control. Observation after 8 days revealed few inflammatory cells in both groups, disruption of the odontoblast layer, and dilatation of the blood vessels. The

inflammatory reaction in the glass ionomer group was slightly higher than the control group. After 30 days observation, complete recovery of the pulpal tissue was observed with no disruption of the odontoblast layer. A thick layer of reparative dentine had formed in both groups. The authors concluded that GIC Fuji IX is biocompatible with the pulp and does not induce any harmful effect on the pulp cells (Six *et al.*, 2000).

Another in-vitro study evaluated the cytotoxic effect of glass ionomer, resin-modified glass ionomer, and saline as a control on an odontoblast cell line (MDPC-23). It was found that the glass ionomer cement had the least cytotoxic effect on the cultured cells compared to resin modified glass ionomer which had an intense cytopathic effect on the cultured cells reducing cell metabolism leading to cellular death (de Souza Costa *et al.*, 2003b).

Furthermore, Hume and Mount (1988) studied the effect of glass ionomer cements when placed directly on a sterile tissue culture medium or indirectly through a layer of human dentine. It was found that glass ionomer cement when placed directly had a higher cytotoxic effect while those prepared through dentine had limited or no cytotoxicity (Hume and Mount, 1988).

It can be concluded from these studies that glass ionomer cements are not suitable when placed directly on the pulp however using them as indirect pulp protection / capping agents or as a dentine replacement material in deep cavities is widely accepted (Sidhu, 2011).

The situation is similar with zinc oxide-eugenol formulations which have been used for many years as pulp protection agents, cements, and temporary restorative materials due to their anti-inflammatory and obtundent effect on the pulp (Hilton, 2009). However the cytotoxic effect of eugenol on the pulp is well documented and this is affected by the concentration of eugenol and the remaining dentine thickness (Markowitz *et al.*, 1992). It is reported that thick dentine sections provided better protection to the pulp than thinner sections and this has been suggested to be due to the presence of calcium in the dentine tubules which has a chelating effect on the eugenol limiting its ability to diffuse

through the dentine tubules (Meryon and Jakeman, 1986; Markowitz *et al.*, 1992). Binding of eugenol to the collagen in the organic matrix of the dentine may also play a role in causing a slower diffusion rate (Markowitz *et al.*, 1992).

The effect of zinc oxide-eugenol when placed directly on the pulp was investigated in a clinical study and was found to cause chronic inflammation, no pulp healing, and no dentine bridge formation when observed after 12 weeks compared to complete healing under calcium hydroxide controls (Glass and Zander, 1949). Another clinical study placing zinc oxide-eugenol onto thin residual dentine has shown to result in pulp hyperaemia, inflammatory cell aggregation, reduction in the number of odontoblasts, and the presence of cell nuclei in the tubules (Brännström and Nyborg, 1976). This is due to the higher concentrations of eugenol reaching the pulp when it is placed directly or in close proximity to the pulp with a minimal dentine barrier. Higher concentration of eugenol causes release of superoxide from neutrophils leading to increased tissue damage at the site of inflammation and inhibits neutrophil chemotaxis. However low concentrations released via the dentine tubules may protect the pulp tissue from damage by inhibiting neutrophil function and removing harmful free radicals (Markowitz *et al.*, 1992). Therefore, the maximum benefit of zinc oxide-eugenol may be obtained by avoiding direct contact with the pulp with sufficient intact dentine to allow the eugenol's analgesic and anti-inflammatory effect to predominate over its toxic potential (Markowitz *et al.*, 1992).

Calcium hydroxide cements have long been used in close proximity to the pulp and have been considered as the "gold standard" pulp capping materials for many years due to their antibacterial properties and ability to promote remineralisation (Hilton, 2009). Calcium hydroxide has been reported to release hydroxyl ions which have a bactericidal effect causing damage to the bacterial cytoplasmic membrane, protein denaturation, and DNA damage of the bacterial cells (Farhad and Mohammadi, 2005). The hydroxyl ions are also responsible for the high pH (12.5-12.8) of this material having a caustic effect on the pulp leading to a superficial layer of necrosis (Schuurs *et al.*, 2000). This layer of necrosis consists of three zones: a mummified superficial layer, an intermediate layer where the hydroxyl ions are neutralised, and an apical layer containing

inflammatory cells and macrophages which remove the necrotic debris (Schröder, 1985; Schuurs *et al.*, 2000). The necrosis does not cause permanent damage to the pulp but induces a mildly toxic, irritative effect promoting a sequence of tissue reactions starting with vascular and inflammatory cell migration and proliferation to control and eliminate the irritating agent followed by the repair process which involves migration and proliferation of the mesenchymal and endothelial pulp cells and formation of collagen (Schröder, 1985). The localised increase in calcium ion concentration has been found to increase the expression of mineralisation promoting genes (osteopontin and BMP-2) in the pulp cells contributing to the deposition of minerals in the newly formed collagen (Graham *et al.*, 2006). Furthermore, calcium hydroxide was also found to be capable of solubilising bioactive molecules including TGF-b1 from the dentine matrix inducing the formation of odontoblast-like cells to secrete reparative dentine and dentine bridge formation (Ford and Roberts, 1991; Graham *et al.*, 2006).

The dentine bridge formed by calcium hydroxide has been reported to be often perforated by tunnels and cell inclusions which fail to provide an adequate seal of the underlying pulp tissues against infection due to microleakage. Furthermore, calcium hydroxide is soluble and has been found to disintegrate within 6 months leaving voids beneath the restoration which act as pathways for bacterial infection and subsequently recurrent pulpal inflammation and necrosis (Cox *et al.*, 1995).

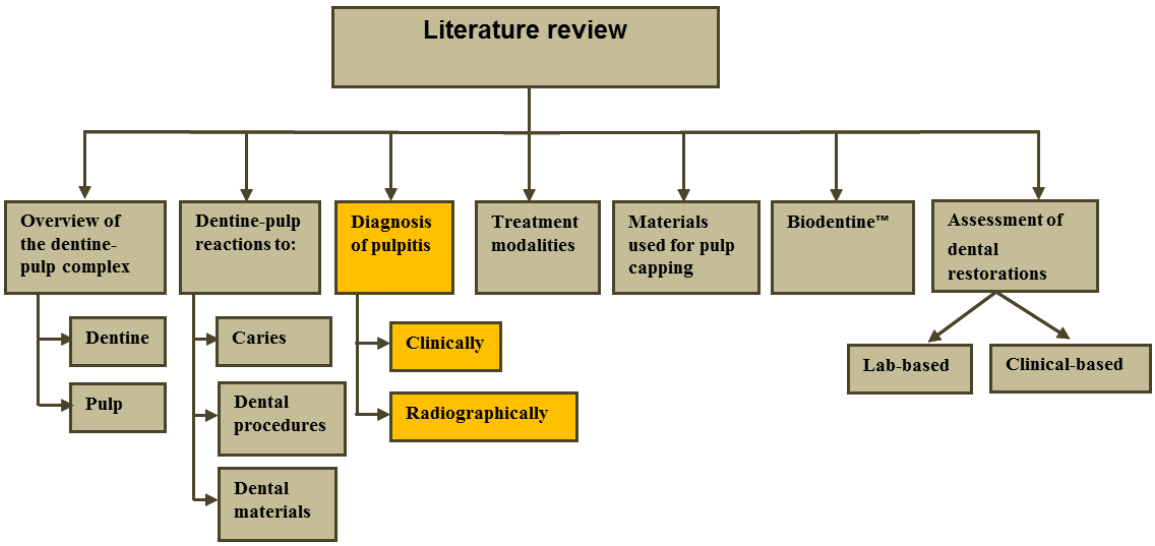
Alternative materials have been developed such as calcium silicate cements which have similar advantages to calcium hydroxide including biocompatibility, antibacterial properties, high pH, and the ability to induce the release of bioactive dentine matrix proteins. However, studies have shown better pulp healing with these materials compared to calcium hydroxide (Hilton, 2009). In a study by Tran et al. (2012) which compared the effect of calcium hydroxide and two calcium silicate materials, mineral trioxide aggregate (MTA) and Biodentine™, when placed directly on rat pulps, the reparative dentine formed was homogenous and in continuity with the primary dentine in the calcium silicate cement materials. The adjacent pulp was normal and free from

inflammatory cells and dentine tubules were well distinguished. A strong expression of osteopontin which regulates the early events leading to reparative dentine formation was observed in the calcium silicate groups. The pulp beneath the calcium hydroxide cement however exhibited a poorly organised reparative structure with many cell inclusions and the necrotic layer was found to be larger with less expression of osteopontin (Tran *et al.*, 2012).

The superior results obtained with calcium silicate cements was confirmed in another study comparing calcium silicate cement (MTA) and calcium hydroxide cement when placed on the pulps of dog teeth. Dentine bridge formation was observed in all the MTA samples with no pulpal inflammation while less dentine bridge formation was found in the calcium hydroxide group with pulp inflammation more likely attributed to microleakage (Faraco and Holland, 2001).

Furthermore, calcium silicate cements are found to be effective in up-regulating the expression of transcription factors such as Runx2 and genes like osteocalcin, alkaline phosphatase, and dentine sialoprotein which help promote the differentiation into odontoblast-like cells responsible for dentine bridge formation. They also increase the secretion of angiogenic factors such as vascular endothelial growth factor, which is important in the process of tissue healing and regeneration (Paranjpe *et al.*, 2010).

2.3 Diagnosis of pulpitis: What we currently have



Flowchart of the literature review structure in the thesis.

Correct pulp diagnosis is the key to predictable treatment with a better outcome. In order to establish a diagnosis, it is necessary to refer to a classification (Baume, 1970). Many attempts in the past have been made to develop classifications for pulp disease (Berman and Hartwell, 2011) however, there is no correlation between clinical signs and symptoms and the pulp histopathology (Dummer *et al.*, 1980; García Aranda, 1990; Martin *et al.*, 2002; Cisneros-Cabello and Segura-Egea, 2005). As the only way to determine the true pulp status is to biopsy it for histological examination which is not practical, a clinical classification has been developed (Berman and Hartwell, 2011). The most recent classification was suggested by the American Board of Endodontics in an attempt to standardise the classifications and establish uniformity in terminology. The following table displays the clinical classification of pulp and periapical disease based on the American Board of Endodontics (AAE, 2009) (Table 2-1).

Table 2-1: Clinical classification of pulp and periapical disease (AAE, 2009).

Pulp Disease	**History**	**Clinical Examination**	**Radiographic Examination**
Normal pulp "Pulp is vital with no inflammation"	Symptom free	"Normal" response to thermal cold testing as compared to healthy control teeth. Tooth may not be histologically normal.	Normal radiographic findings.
Reversible pulpitis "Pulp is vital, any inflammation should resolve and the pulp return back to normal following appropriate management of the etiology"	Short, sharp pain stimulated by hot, cold or sweet stimuli. Few seconds duration and disappears when stimulus is removed. Negative history of spontaneous pain.	Aetiology: exposed dentine, caries, deep restorations. No pulp exposure, sinus, fistula, swelling of periodontal tissues, abnormal tooth mobility, spontaneous pain or sensitivity to percussion. Normal appearance of adjacent gingiva and normal tooth colour. Pulp sensibility tests elicit an exaggerated response which disappears when stimulus is removed.	No radiographic changes in the periapical region.
Irreversible pulpitis (Symptomatic/asymptomatic) "Vital inflamed pulp is incapable of healing and root canal treatment is indicated"	If symptomatic: Dull, throbbing pain, may be acutely sharp. Usually spontaneous but may be exacerbated upon thermal stimulus and lingers for minutes/hours. May also be referred pain. Pain may be accentuated by postural changes such as lying down or bending over. Analgesics are not effective.	Aetiology: Deep caries, extensive restorations, or fractures exposing the pulp tissues. May or may not exhibit pulp exposure, sinus, fistula, swelling of periodontal tissues, abnormal tooth mobility, spontaneous pain or sensitivity to percussion. Pulp sensibility tests elicit an exaggerated response which lingers when stimulus is removed.	May or may not display radiographic changes in the periapical region such as PDL widening or lesion.

Pulp necrosis "Partial or complete death of the dental pulp, necessitating root canal treatment"	No symptoms. May or may not be preceded by symptoms of irreversible pulpitis.	No response to pulp sensibility testing. May or may not exhibit pulp exposure, sinus, fistula, swelling of periodontal tissues, abnormal tooth mobility, and sensitivity to percussion.	May or may not display radiographic changes in the periapical region such as PDL widening or lesion.
Periapical Disease			
Normal apical Tissues		No sensitivity to percussion as compared to a control tooth.	Lamina dura surrounding the root is intact and the periodontal ligament space is uniform.
Symptomatic apical periodontitis "Represents inflammation of the apical periodontium"	Pain on biting.	Sensitivity to percussion.	May or may not be accompanied by radiographic changes
Asymptomatic apical periodontitis "Inflammation and destruction of the apical periodontium that is of pulpal origin"	No symptoms	No sensitivity to percussion.	Periapical radiolucency
Acute apical abscess "Inflammatory reaction to pulpal infection and necrosis"	Rapid onset, spontaneous pain, malaise, fever and lymphadenopathy	Extreme sensitivity to percussion, varying degree of mobility, pus formation and swelling of associated tissues.	There may be no radiographic signs of destruction. But there may be loss of lamina dura and widening of the PDL.
Chronic apical abscess "Inflammatory reaction to pulpal infection and necrosis"	Gradual onset of pain, little or no discomfort.	Not sensitive to percussion but may "feel different". Intermittent discharge of pus through an associated sinus tract.	Periapical radiolucency

Two additional categories have also been added to the pulp disease classification which are: *Previously treated* meaning that the tooth has been endodontically treated and canals obturated with filling materials other than intracanal medicaments, and *previously initiated therapy* indicating that the tooth has been previously treated by partial endodontic therapy such as pulpotomy or pulpectomy (AAE, 2009).

Based on the available classification, a diagnosis can be made. Current available diagnostic tools are still insufficient to provide an accurate evaluation of the histological status of the pulp (Sigurdsson, 2003). Therefore, the diagnosis of the status of the pulp is based on the combination of history, clinical examination, special tests, and radiological examination and not on any one specific test (Gopikrishna *et al.*, 2009).

2.3.1 Clinical diagnostic methods

Listening to the patient's symptoms and taking a comprehensive history is essential as it complements the findings based on the clinical examination and special tests which help reach a diagnosis (Sigurdsson, 2003). The history and clinical examination findings for each pulp classification is described in Table 2-1. Special pulp diagnostic tests are employed to reproduce the symptoms, localise the symptoms, and to assess the severity of the symptoms (Sigurdsson, 2003). An ideal pulp test should be able to do this in a simple, objective, standardised, reproducible, non-painful, accurate, and in-expensive way (Gopikrishna *et al.*, 2009). Pulp testing strategies involve pulp sensibility testing such as thermal or electric pulp testing (EPT) which is based on activating the neural pathway of the pulp initiating a sensory response. This most commonly assesses the integrity of the Aδ nerve fibres in the dentine-pulp complex by briefly applying a stimulus to the outer surface of the tooth. If these fibres are successfully stimulated, the patient will respond by acknowledging a short, sharp sensation / tingling from the tooth (Gopikrishna *et al.*, 2009). A positive response indicates that the nerve fibres are functioning however this does not indicate the status of the blood flow and hence is not a true indicator of the tooth vitality. This is because measurement of nerve viability is open to error

(Chambers, 1982). It has been shown that following trauma, a vital pulp may exist without a viable nerve supply. Furthermore, others reported that nerve fibres are more resistant to inflammation and may remain reactive even though all the surrounding tissues have degenerated. This however is a controversial issue (Chambers, 1982). One thing is for certain and that is pulp vitality testing such as laser doppler flowmetry and pulse oximetry assess the blood circulation of the pulp which is considered more accurate for assessing the pulp vitality. These however are not used routinely in a clinical setting (Berman and Hartwell, 2011). Table 2-2 provides a description of the available clinical diagnostic pulp tests.

Table 2-2: Clinical diagnostic pulp tests.

Method	**Advantage**	**Disadvantage**	**Studies**
Pulp sensibility tests			
Thermal: Fluid movement in the tubules due to thermal stimulation activate sensory receptors in the pulp. ***Cold test*** (Frozen carbon dioxide, refrigerant spray) ***Heat test*** (heated gutta-percha, compound stick, or water)	Does not cause crazing or irreversible damage to the pulp. Easy to use and readily available. Readily available.	May give positive response in partially necrotic multiple rooted teeth. Too much heat may cause irreversible harm to the pulp. Difficult to use in posterior teeth due to limited access.	(Chambers, 1982; Sigurdsson, 2003; Gopikrishna *et al.*, 2009; Berman and Hartwell, 2011)
Electrical: Electric stimulation of the pulp sensory nerves ***Electric pulp test***	Does not cause irreversible damage to the pulp, easy to use and readily available.	Technique sensitive, cannot be used with complete coverage crowns or big metallic restorations.	(Lin and Chandler, 2008; Abd-Elmeguid and Yu, 2009b)

Mechanical: ***Percussion/palpation*** Indirect methods for assessing status of pulp	May identify irreversible pulpitis if associated with +ve response to thermal or electric test.	–ve response does not rule out periapical involvement.	(Sigurdsson, 2003)
Test drilling	Used when full coverage restorations make other tests impossible to carry out.	Damage to the tooth structure.	(Sigurdsson, 2003; Berman and Hartwell, 2011)
Others: ***Selective Anaesthesia***	Used with poorly localised or referred symptoms.	Discomfort from anaesthesia.	(Gopikrishna *et al.*, 2009; Berman and Hartwell, 2011)
Pulp vitality tests			
Laser Doppler flowmetry Measures the flow of blood cells inside the pulp	Objective, non-invasive, accurate, causes no deterioration of the pulp tissue.	Time-consuming, high cost, sensitive to motion, some studies found it to be unreliable as it records signals from periodontal tissues in addition to the pulp.	(Roy *et al.*, 2008; Abd-Elmeguid and Yu, 2009b)
Pulse oximetry Measures oxygen concentration in the blood and pulse rate	Objective, non-invasive, accurate, causes no deterioration of the pulp tissue.	Current devices are too cumbersome and complicated for routine use.	(Munshi *et al.*, 2003; Gopikrishna *et al.*, 2007)
Crown surface temperature (Thermography) Based on the hypothesis that vital teeth are warmer and will rewarm quicker than non-vital teeth.	Objective, non-invasive, causes no deterioration of the pulp tissue.	Technique may be complicated by age changes affecting the blood supply. One study found no difference between vital and non-vital teeth concluding that periodontal tissues are the main source of heat.	(Chambers, 1982; Sigurdsson, 2003)
133Xenon radioisotope Utilizing a radiation probe with 133 xenon radioisotope to	System is light, portable, could be transported with ease,	Expensive, risk of radiation exposure, requires special	(Trope *et al.*, 1986; Sigurdsson,

differentiate between vital vs. non-vital teeth on the basis of blood supply.	and practical.	licencing.	2003)
Dual wavelength spectrophotometry Dual wavelength light source (760 and 850 nm) to determine the oxygen saturation level of the pulpal blood supply. Detects the presence of haemoglobin.	May be useful for determining the inflammatory status of the pulp. Non-invasive, objective, small, portable, and relatively inexpensive. Differs from pulse oximetry in that it does not depend on pulsatile blood flow.	Still not routinely used.	(Nissan *et al.*, 1992; Sigurdsson, 2003)

Although pulp sensibility testing commonly the cold test and electric pulp test are the *de facto* standard employed routinely in dentistry, they have acknowledged limitations. In a study by Petersson et al. (1999), sensitivity, specificity, negative and positive predictive value of the cold test (ethyl chloride), heat test (hot gutta-percha), and electric pulp test (Analytic Technology Pulp Tester®) were calculated and compared to histology sections (gold standard). Sensitivity was 0.83 for the cold test, 0.86 for the heat test and 0.72 for the electrical test. The specificity was 0.93 for the cold test, 0.41 for the heat test and 0.93 for the electrical test. The positive predictive value which is the probability that a positive test result actually represents a disease positive person was 0.89 for the cold test, 0.48 for the heat test and 0.88 for the electrical test. The negative predictive value which is the probability that a person with a negative test result is actually free of disease was 0.90 for the cold test, 0.83 for the heat test and 0.84 for the electrical test. These results indicate that the accuracy of cold test was 86%, heat test was 71%, and electric pulp test was 81% (Hyman and Cohen, 1984; Petersson *et al.*, 1999). Gopikrishna et al. (2007) found similar results with the electric and thermal tests however accuracy of pulse oximetry was found to be 97.5% indicating higher accuracy with this method compared to the other methods used more commonly. False positive results may occur in anxious or young patients

anticipating an unpleasant sensation, partially necrotic multi-rooted teeth, or contact with metallic restorations. False negatives may occur in recently traumatised teeth, patients with incomplete root formation, patients with psychotic disorders or on certain medication or under the influence of alcohol (Gopikrishna *et al.*, 2009). Furthermore, lack of reproducibility is another problem associated with pulp sensibility testing (Chambers, 1982). It has been reported that patients respond differently to pulp tests on different days, and at different hours in the same day (Chambers, 1982). Although pulp vitality testing overcomes the problems associated with pulp sensibility testing, the majority of the methods used are for experimental purposes and not used routinely in dental practice (Abd-Elmeguid and Yu, 2009b).

2.3.2 Radiographic diagnostic methods

The state of pulp health or necrosis cannot be determined using radiographs however certain features should arouse suspicion of degenerative pulp changes: deep carious radiolucencies, deep and extensive restorations, pulp caps, pulpotomies, pulp stones, extensive canal calcification, root resorption, radiolucencies at or near the apex, root fractures, thickened periodontal ligament, and periodontal disease that is radiographically evident (Cohen, 1998). Interpretation of good quality diagnostic radiographs under good illumination and magnification may enable the detection of early pathological changes in or around the tooth denoting a greater probability of pulp involvement (Cohen, 1998). The ability to detect these early and subtle changes is a critical component of pulp diagnosis and outcome assessment. One study found that the pattern, size, and density of bone trabeculae are the best radiographic features for identifying “healthy” teeth. The lamina dura's continuity and shape and the periodontal ligament's width and shape were the most consistent features for diagnosing teeth with non-vital pulps (Kaffe and Gratt, 1988). However current two-dimensional imaging techniques have a limited ability in detecting early changes in the root supporting tissues. Some studies on radiographic diagnosis have shown that lesions confined to the cancellous bone could not be detected using standard periapical radiographs until the cortical plate is partially corroded (Ramadan and Mitchell, 1962; Bender and

Seltzer, 2003a; Bender and Seltzer, 2003b). In contrast, other studies found that artificial periapical lesions confined to cancellous bone could be detected radiographically and that cortical involvement was not necessary for radiographic visualisation of the lesions (Ford, 1984; Lee and Messer, 1986). They argued that widening of the periodontal ligaments observed on the mesial and distal aspects of the affected root are unlikely to result from changes in cortical bone. The same can be said about extraction sockets visible in radiographs despite being confined to cancellous bone. Therefore, there is no reason to consider that cortical bone involvement is a necessary condition for radiographic identification of periapical lesions (Lee and Messer, 1986).

Intra- and inter-examiner agreement on the assessment of the periapical structures has been problematic. In one study, examiners agreed 47%-73% of the time on the success or failure of endodontic therapy and in a follow-up study, they agreed with themselves only 72%-88% of the time (Goldman *et al.*, 1972; Goldman *et al.*, 1974).

Technology has advanced in the field of radiology to include F-speed or Insight (Eastman Kodak, Rochester, NY) conventional film, xeroradiography, digital radiography, subtraction radiography, phosphor images, ultrasound and now CBCT which seem to be promising tools for more accurate diagnosis of periapical changes (Newton *et al.*, 2009).

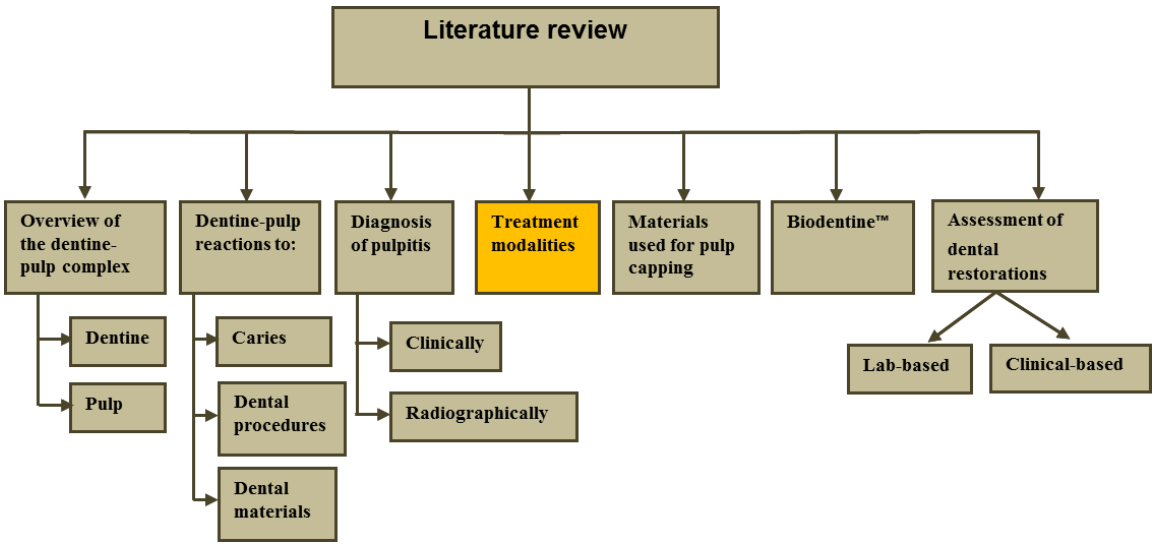
CBCT in particular has been the subject of interest of many studies and has been found to provide more relevant information in the diagnosis of periapical pathosis compared to periapical radiographs (Estrela *et al.*, 2008; Patel *et al.*, 2009a; Paula-Silva *et al.*, 2009; Patel *et al.*, 2011). This is because it overcomes the limitations associated with conventional 2-dimensional imaging techniques: anatomical noise and distortion (Patel *et al.*, 2011). This imaging technique is based on 3-dimensional volume data acquired in a single rotation of the scanner around the patient's head. Depending on the type of CBCT scanner, the cone-shaped x-ray beam and the reciprocating detector, rotate synchronously through 180° and 360° resulting in reduced radiation dose, rapid scan time and image accuracy (Macleod and Heath, 2008). The 3-dimensional

view is provided in three planes simultaneously: axial, sagittal and coronal so any anatomical complexities, lesions and operational errors which may not be seen with conventional radiography due to the 2D image may be identified and seen using CBCT. There is no geometric distortion which is a problem associated with conventional intraoral radiographs and CT scans where the voxels are anisotropic in contrast to CBCT where the voxels are isotropic ensuring that the images produced are geometrically accurate and free from distortion. Anatomical noise is caused by the super imposition of anatomical structures over the object under investigation which would impair visualization of the object and complicate the interpretation of the radiograph which is avoided by the 3-dimensional image provided by CBCT (Patel *et al.*, 2009b).

The reduction in the radiation dose of CBCT is due to the pulsatile action of the x-ray beam so that the patient is exposed to radiation for only a small portion of the overall scan time. A reduction in the radiation dose may also be obtained through x-ray beam collimation using a field of view specific to the patient's needs where only the area of interest is radiated. The field of view can range from small volume CBCT which is focused or limited to a small area with the least radiation dose to craniofacial CBCT scanning a larger area with an increased radiation dose (Durack and Patel, 2012). Reducing the radiation dose may also be obtained by altering the degree of rotation of the tube head from 360° to 180° leading to a reduced number of projections. The amount and type of beam filtration and the kV, mA and voxel size settings are other factors which also play a role in reducing the radiation dose (Durack and Patel, 2012).

Streak artefacts from metallic restorations, and limited soft tissue details remain a disadvantage of this imaging technique (Scarfe *et al.*, 2006; Macleod and Heath, 2008; Patel *et al.*, 2009b). Nonetheless, as costs of CBCT machines go down, and they become more common in dental practice, CBCT may hold the answer to more early and accurate diagnosis of periradicular/periapical pathosis and the evaluation of the healing progress and treatment outcome in addition to resolving the issue of intra- and inter-observer interpretation of the images (Newton *et al.*, 2009)

2.4 Treatment modalities: A minimally invasive approach



Flowchart of the literature review structure in the thesis.

The treatment of deep carious lesions and the related histopathological pulpal changes pose a significant challenge, especially when approaching the pulp as an increased risk of pulp exposure reduces the predictability of the treatment outcome (Barthel *et al.*, 2000; Bjørndal *et al.*, 2010; Dammaschke *et al.*, 2010). Treatment modalities aim to maintain pulp vitality by facilitating healing and repair (Tziafas *et al.*, 2000). Maintaining pulp vitality following intervention is controlled by the degree of odontoblast survival and their ability to initiate a repair response (Murray *et al.*, 2002d). The survival rate of odontoblasts has been found to be linked with the amount of remaining dentine thickness (Murray *et al.*, 2002a). Most cases with deep lesions have reduced dentine thickness, therefore, the protective properties of dentine are lost leading to increased cellular damage (Murray *et al.*, 2002d). Therefore, it is of utmost importance to preserve as much dentine as possible, accomplished using a minimally invasive approach through preferential modification of the deep lesion by sealing the residual caries, encouraging reparative reactions of the dentine-pulp complex to take place (Ricketts, 2001).

Studies found that the selective removal of the heavily infected dentine biomass while leaving affected dentine has favourable results (Banerjee *et al.*, 2000b;

Ricketts *et al.*, 2006a; Maltz *et al.*, 2007; Gruythuysen *et al.*, 2010). One method of achieving this is by indirect pulp protection where carious dentine near the pulp is preserved in order to avoid pulp exposure and is covered with a suitable material. There is no re-entry to the cavity (Bjørndal, 2008b; Gruythuysen *et al.*, 2010). One study has shown that this technique results in a high 3-year survival rate in primary and permanent teeth when examined clinically and radiographically. The survival rate was 96% for 86 primary molars and 93% for 34 permanent teeth (Gruythuysen *et al.*, 2010). In another study, indirect pulp protection in the primary dentition and followed up for 4 years resulted in a high success rate regardless of the material used (Marchi *et al.*, 2006). In addition, another study demonstrated a high clinical and radiographic success rate after 2-year follow up when indirect pulp capping treatment of 48 primary molars was accomplished using an adhesive resin system vs. calcium hydroxide (Falster *et al.*, 2002). Indirect pulp protection in another study had a 94% success rate compared to a 70% success rate with formocresol pulpotomy therapy in primary molars (Vij *et al.*, 2004).

Another method is the stepwise (two-step) excavation technique where re-entry is attempted after different intervals (Bjørndal, 2008b). Although this technique is more time consuming, it is claimed that it overcomes the problem of not knowing when to stop caries excavation when changes in colour of carious dentine are not clear and so the final excavation provides a safer and easier removal of the remaining dry carious dentine (Bjørndal, 2008b). The majority of studies on permanent teeth where conducted using the stepwise excavation technique which demonstrated a high success rate (Leksell *et al.*, 1996; Bjørndal *et al.*, 1997; Bjørndal and Thylstrup, 1998; Maltz *et al.*, 2007; Bjørndal *et al.*, 2010). These studies found that the dentine retained was remineralised and hard when teeth were re-opened. However one could argue if that is the case, then why re-enter? This lacks biological support and would only cause unnecessary risk of pulp exposure (Falster *et al.*, 2002; Maltz *et al.*, 2007). In addition, the change in colour of carious dentine was not found to be linked directly with the bacterial content of dentine and hence has no relevance (Orhan *et al.*, 2008; Lula *et al.*, 2011). One study attempted to tackle this issue by comparing one step indirect pulp treatment and 2-step stepwise excavation

technique and complete caries removal in primary and permanent molars (Orhan *et al.*, 2008). Although the investigators found that none of the treatment modalities eliminated completely the bacteria at the initial excavation, the stepwise excavation technique provided significant bacterial reduction. However, regardless of the technique used, obtaining a tight coronal seal is essential for the success of the procedure (Gruythuysen *et al.*, 2010).

The common issue when conducting indirect pulp capping be it one-step or two-step procedure is the ability to differentiate between infected and affected dentine. These are two distinct histopathological zones characterised by a peripheral caries-infected zone with irreversibly damaged, denatured, softened, necrotic collagen and a deeper caries-affected zone comprising reversibly damaged collagen with a potential to repair under the correct conditions (Green and Banerjee, 2011; Banerjee, 2013). Clinically, the main objectives of the removal of carious dentine tissue are the elimination of infected and necrotic tissue in order to control the progression of the lesion and the removal of softened dentine in order to offer adequate support to the restoration (Bussadori *et al.*, 2011).

Identifying the exact endpoint of caries excavation is not clinically clear (Banerjee *et al.*, 2000b). It is commonly advised that caries removal is conducted until the cavity floor feels “firm”, “sticky and scratchy” and free from “moisture” which is very subjective (Banerjee *et al.*, 2000a; Neves *et al.*, 2011).

Attempts have been made to objectively identify the boundary between infected and affected dentine. “Fusayama” dyes consisting of 1% acid-red solution in a propylene glycol base, were first introduced in an attempt to differentiate between infected and affected dentine by staining the irreversibly denatured dentine and not the remineralisable collagen (Sato and Fusayama, 1976). However research has shown that the dyes stain the deeper collagen in the affected dentine risking over-preparation. Nowadays it is advised to retain the “pink” or “light pink” stained tissues however identifying these colour variations is subjective and is an issue of dispute (Neves *et al.*, 2011).

Other alternatives are dentine solubilising agents used to selectively dissolve carious dentine, such as the sodium hypochlorite-based Carisolv™ (MediTeam,

Göteborg, Sweden) gel which has been reported by studies to be more effective and efficient in selective caries removal (Banerjee *et al.*, 2000a; Munshi *et al.*, 2002). Carisolv™ consists of two main components: 0.5% sodium hypochlorite and a gel containing three amino acids (leucine, lysine and glutamic acid). When the two components are mixed together, chloramine is generated resulting in the chlorination of the partially degraded collagen, disruption of the collagen fibres, and selective softening of the outer infected dentine. Due to the high pH of Carisolv™ which is approximately 11, only the organic phase of dentine is affected (Munshi *et al.*, 2002).

Another dentine solubilising agent is the experimental enzyme-based caries removal gels such as SFC-V and VIII (3M-ESPE, Seefeld, Germany). It consists of pepsin in a phosphoric acid/sodium biphosphate buffer. The phosphoric acid dissolves the inorganic components of the infected dentine, preserving the healthy tooth structure, and gives pepsin access to the organic parts of the carious biomass. Pepsin selectively dissolves denatured but not sound collagen. This method of selective caries removal has been reported to be as effective as Carisolv™ (Clementino-Luedemann *et al.*, 2006).

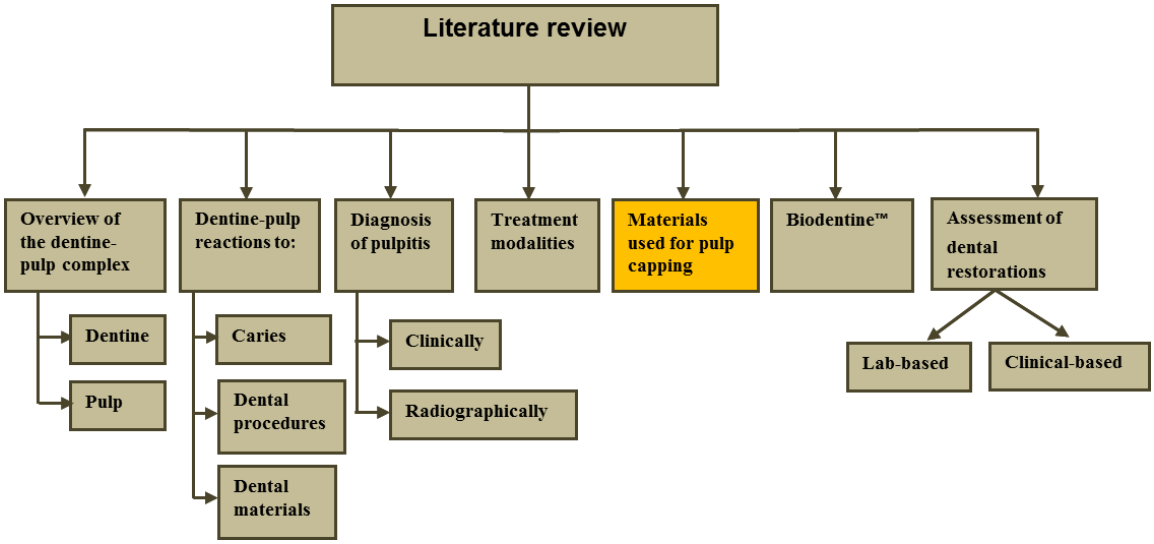
Papacárie® is another enzyme based product which is a papain-based gel employing natural enzymes extracted from papaya husk that act upon the contaminated dentine promoting softening of the dentine (Bussadori *et al.*, 2011). This gel has been reported to be biocompatible, with a neutral pH, and inhibits the growth of bacteria due to the presence of papain (Jawa *et al.*, 2010). In a study on primary teeth, Papacárie® was found to be as effective as the drill in caries removal and more comfortable than the traditional rotatory instruments (Kotb *et al.*, 2009). Another study found it to have a similar clinical efficiency as a chemo-mechanical agent for dentinal caries removal when compared to Carisolv™ (Kumar *et al.*, 2012).

The use of an Er:YAG laser equipped with a laser-induced fluorescence (LIF) feedback system (Key III, Kavo, Biberach, Germany) has also been suggested to be effective in the selective removal of carious dentine. During the carious removal process, fluorescence emitted by the bacterial metabolites present in the carious tissue is continuously measured and when a certain threshold is

reached, the laser device is activated and the carious tissue ablated. The clinical use of a LIF threshold level of 7 has been reported to be an acceptable end point of cavity preparation when the bacterial levels are low. The fluorescence-controlled Er:YAG laser has been found to be an efficient “self-limiting” technique in caries removal (Dommisch *et al.*, 2008).

With emerging technologies, it is expected that these techniques will be more commonly used in dental practice providing increased tissue preservation and pulp protection.

2.5 Materials used for pulp capping procedures



Flowchart of the literature review structure in the thesis.

The increased emphasis to preserve pulp vitality whenever possible in minimally invasive dentistry has led to the development of materials which are biocompatible and bioactive with improved physical, mechanical and aesthetic properties (Hilton, 2009; de Souza Costa *et al.*, 2011). These materials not only serve to protect the dentine-pulp complex from further challenges to the pulp tissue caused by toxicity from restorative materials and bacterial penetration from microleakage, but also aim to regenerate the dentine-pulp complex and promote remineralisation (Modena *et al.*, 2009).

2.5.1 Calcium hydroxide cements

Calcium hydroxide which was first introduced to dentistry by Herman in Germany in 1920, has long been used as a direct and indirect pulp capping agent due to its ability to stimulate dentine bridge formation and promote remineralisation of carious dentine in addition to its biocompatibility and antibacterial properties (Stanley and Lundy, 1972; Stuart *et al.*, 1991; Farhad and Mohammadi, 2005).

Calcium hydroxide is a white odourless powder with low solubility in water which decreases as the temperature rises and is insoluble in alcohol. It is classified chemically as a strong base with its main actions coming from the dissociation of the calcium and hydroxyl ions on contact with aqueous fluids. Hydroxyl ions are responsible for the high alkalinity which accounts for the bactericidal effect while calcium ions play a role in the initiation of the remineralisation process (Farhad and Mohammadi, 2005). Hydroxyl ions have been found to be buffered as they diffuse through the dentine in addition to being affected by adsorption which decreases as the hydroxyl ions diffuse through the circumpulpal dentine because there is insufficient bulk of dentine to significantly buffer or adsorb the ions (Wang and Hume, 1988). Hard setting calcium hydroxide dressings such as Life and Dycal dissolve clinically after 1-2 years leading to microleakage which subsequently compromises the health of the pulp especially with the presence of tunnel defects associated with the dentine bridge (Schuurs *et al.*, 2000). Furthermore, calcium hydroxide does not adhere to dentine and is degraded by etching and rinsing prior to restoration with resin composite. In addition, it cannot withstand the condensation forces of amalgam due to its low compressive strength (Farhad and Mohammadi, 2005). Therefore, sealing / protecting calcium hydroxide with a base material has been suggested prior to the application of resin composite or amalgam and was found to significantly increase the success of indirect pulp capping treatment (Al-Zayer *et al.*, 2003). However placement of a restoration consisting of three materials complicates the technical procedure and is time consuming (Schuurs *et al.*, 2000). More recent calcium hydroxide products contain urethane dimethacrylate with initiators and accelerators, by which they bind to dentine and have a higher resistance to acid dissolution. They are also reported to be stronger and more biocompatible with resin composite (Schuurs *et al.*, 2000).

2.5.2 Glass ionomer cements

Glass ionomer cements (GIC) are another group of materials with many different clinical applications including indirect pulp capping. They consist of a calcium fluoro-alumino-silicate glass powder and an aqueous solution of a poly

(acrylic acid—itaconic acid) copolymer containing tartaric acid (Smith, 1998). Setting reaction involves the acid-base reaction of the polyacrylic acid and glass particles and ions (Al^{3+}, Ca^{2+}) located in the glass network (Nicholson, 1998). Modifications in both components have been made in various brands for both patent and practical reasons (Smith, 1998). GICs have been reported to demonstrate excellent sealing properties and good biocompatibility when placed in close approximation but not directly on the pulp (Hilton, 2009). In addition, they have the ability to chemically adhere to moist dentine through ionic exchange at the interface leading to the formation of a new intermediate dentine-GIC layer approximately 300 μm thick (Zoergiebel and Ilie, 2013). This ionic exchange is triggered at the interface through a diffusion process driven partly by the concentration gradient which exists between the glass-ionomer and the dentine with strontium/ calcium and fluoride ions undertaking apatitic activity in relation to areas in dentine where the calcium ion levels are low (Ngo *et al.*, 2006).

Drawbacks of GICs are within its physical properties as it is susceptible to acid erosion and wear therefore its use lies mainly in the field of dentine replacement in laminate restorations (Davidson, 2006). GICs have been used in both open and closed sandwich restorations with a higher success rate reported for closed sandwich restorations (van Dijken, 1994). This is because in open sandwich restorations, GIC is associated with an increased risk of dissolution due to its susceptibility to early moisture contamination. In addition, the proximal space is exposed to longer acid clearance times which cause a higher erosion rate at the surface. If a GIC lining cement is used, the cement will be stressed continuously by masticatory forces transferred via the overlying restoration resulting in crack formation at the cement-restorative interface followed by fracture of the cement. This is due to the lack of strength of the thin cement layer (van Dijken, 1994). This problem can be overcome by using a restorative cement which is cut back rather than a liner resulting in a more robust restoration with better mechanical properties (Cattani-Lorente *et al.*, 1993). This also has an advantage if an overlying resin composite restoration is used as the increased thickness of the cement reduces the thickness of the resin composite leading to a reduction in the polymerisation shrinkage (Woolford, 1993).

An interaction between GIC and the overlying resin composite restoration was suggested where GIC reduced the hardness of the surface of resin composite adjacent to it up to a distance of 1mm into the thickness of the resin composite. This detrimental interaction was observed when resin composite was placed on fresh GIC therefore, it is recommended to leave the cement to mature as much as possible before the application of the resin composite (Woolford, 1993).

2.5.3 Resin modified glass ionomer cements

Resin modified glass ionomer cements (RMGIC) may also be used as indirect pulp capping agents as they reduce the incidence of post-operative sensitivity, providing protection to the pulp due in part to their self-adhesive nature and their capacity for reducing stress generated by the polymerisation shrinkage of resin composites (de Souza Costa *et al.*, 2011). They have the same acid-base components of GICs but are complemented with monomers, typically HEMA and initiators capable of undergoing photochemical polymerisation (Nicholson and Czarnecka, 2008). These result in enhanced flexural and tensile strength, elastic modulus, and wear resistance (Modena *et al.*, 2009).

The adhesion between RMGIC and dentine includes both a chemical interaction through ionic exchange between the cement and the dentine surface and micromechanical interlocking of the polymer and polyacrylic acid-conditioned tooth surface (Yiu *et al.*, 2004). An “absorption” layer at the interface is developed over time after setting of the RMGIC due to water sorption from dentine to the maturing cement and this layer is observed in deep moist dentine rather than superficial dentine or enamel (Sidhu and Watson, 1998; Yiu *et al.*, 2004). Furthermore, chemical adhesion is present at the RMGIC-restorative interface due to the constituent resin component in RMGICs improving the bond between RMGIC and the overlying resin composite restoration (Chadwick and Woolford, 1993).

Although many histologic studies have demonstrated the cytotoxic effect of RMGICs when placed on/close to the pulp due to the diffusion of HEMA through the dentine into the pulp (do Nascimento *et al.*, 2000; Souza *et al.*, 2006a), clinical studies have shown a high success rate when used as indirect pulp

capping agents (Farooq *et al.*, 2000; Marchi *et al.*, 2006; de Souza Costa *et al.*, 2007; Gruythuysen *et al.*, 2010). Furthermore, in a review by Mickenautsch *et al*. (2010), no significant difference was found in the beneficial outcome to the pulp between the use of hard-setting calcium hydroxide and RMGIC in deep cavities.

2.5.4 Dental adhesives

These materials have been suggested for indirect pulp capping as they are used to enhance retention, reduce microleakage, and decrease post-operative sensitivity of resin composite restorations following the hypothesis that pulps can heal after the placement of an acidic restorative system in deep cavities or even exposed pulps if haemorrhage is controlled before the application of the adhesive system and a hermetic seal against bacterial infiltration is guaranteed (Modena *et al.*, 2009). However increased moisture at the pulp cap site reduces polymerisation of the adhesive hence decreasing adhesion and increasing the availability of the unpolymerised components of the adhesive. These unpolymerised monomers of the adhesive resin can diffuse directly into the pulp at the exposure site as well as diffuse through the dentine tubules causing cytotoxic effects to the pulp tissue (Hilton, 2009). Additionally, resin components reduce the pulp's immune response affecting the ability of the pulp to defend itself against bacterial contamination (Hilton, 2009).

Although clinical and radiographic evaluation of teeth which had undergone pulp therapy with adhesives report a successful outcome with minimal patient symptoms and radiographic signs of failure which appear encouraging, these studies do not demonstrate the biocompatibility of these materials or provide the comprehensive clinical evidence necessary to support their use for pulp capping procedures (de Souza Costa *et al.*, 2000).

2.5.5 Calcium silicate cements

Calcium silicate-based cements have been introduced with a focus mainly in endodontic therapy as a general root filling material however its uses have

expanded to include pulp capping procedures mainly for its excellent biocompatibility, its capacity to induce dentine bridge formation with no or little inflammation to the pulp, better adherence to dentine, and marginal sealing ability (Paranjpe *et al.*, 2010; Jefferies, 2014).

Mineralised trioxide aggregate (MTA) is one such material which is reported to have the greatest pulp healing potential (de Souza Costa et al., 2008; Leye Benoist et al., 2012). MTA is a powder consisting of tricalcium silicate, dicalcium silicate, tricalcium aluminate and bismuth oxide. When mixed with water, calcium hydroxide and calcium silicate hydrate are initially formed and eventually transform into a poorly crystallized and porous solid gel (Parirokh and Torabinejad, 2010). The mean setting time of MTA is 165 ± 5 minutes which is one of its main drawbacks (Parirokh and Torabinejad, 2010). In addition to its slow setting properties, MTA is difficult to handle due to its granular consistency and initial looseness, and once it dries it loses its cohesiveness becoming hard to handle (Ber *et al.*, 2007). It also has limited physical properties, with compressive strengths in the order of 20 to 60 MPa, a value sufficient for their initial clinical use as pulp capping and root filling materials (Jefferies, 2014).

When MTA is placed on moist dentine, calcium ions are released from MTA contributing to the precipitation of hydroxyapatite (HA) at the MTA-dentine interface. HA crystals nucleate and grow, filling the microscopic space between MTA and dentine. Initially, this seal is mechanical but with time, a diffusion-controlled reaction between the apatite layer and dentine leads to their chemical bonding. The result is the creation of a seal at the MTA-dentine interface (Sarkar *et al.*, 2005). In addition to this interfacial layer, another study reported the formation of tag like structures extending from this layer to the dentine tubules indicating that constant formation of the precipitate contributes not only to the formation of the interfacial layer but also to the promotion of an intratubular mineralisation process (Reyes-Carmona *et al.*, 2009).

With regards to the MTA-restorative interface, exposing fresh MTA to a low pH such as during acid etching, was found to weaken the set material's microstructure by disrupting the hydration of the calcium silicates (Kayahan *et*

al., 2009; Giuliani *et al.*, 2010). Therefore it is recommended to delay restorative procedures for at least 96 hours after mixing MTA (Kayahan *et al.*, 2009). On the other hand, milder etching for a shorter period of time may cause selective loss of matrix around the crystalline structures with minimal loss of cement, exposing these crystalline structures and hence encouraging successful adhesion through micro-mechanical retention. Therefore, using a self-etch adhesive system has shown no detrimental effect on the setting reaction of MTA and hence can be used immediately after MTA placement (Tsujimoto *et al.*, 2013). The same has been shown with glass ionomer cements where placement of GIC over MTA after 45 minutes did not affect its setting reaction (Nandini *et al.*, 2007).

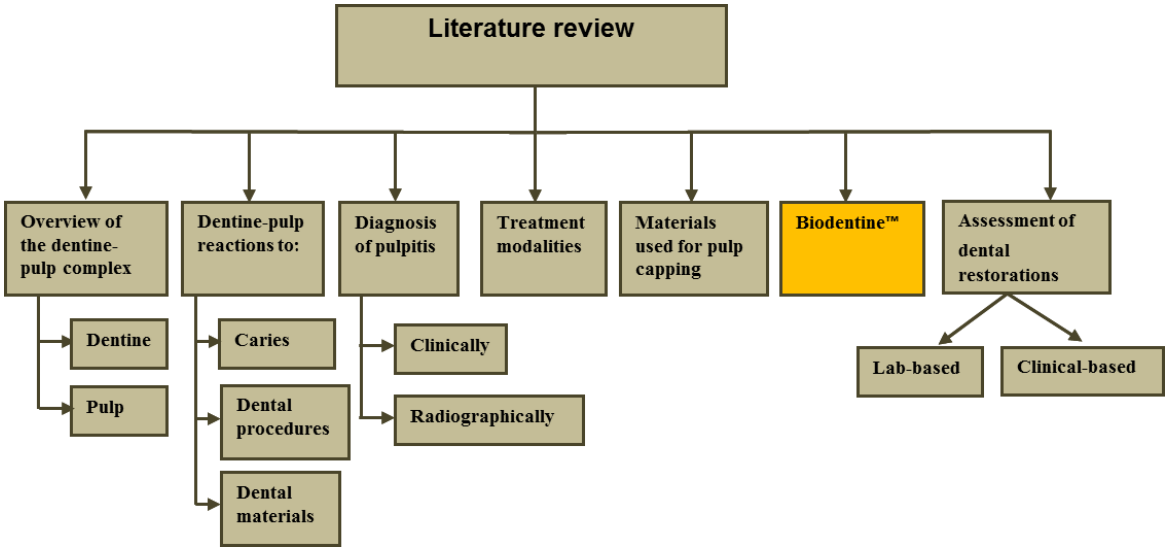
Due to the handling limitations associated with MTA, another calcium silicate cement has been developed marketed under the name of Biodentine™ (Septodont, Saint Maur des Fosse´s, France). This material has the same advantages of MTA yet attempts to overcome the problems associated with it (Han and Okiji, 2011). In addition, unlike MTA, Biodentine™ can be used as a restorative bulk filling material replacing lost dentine (Koubi *et al.*, 2013b). A more detailed description of this material will follow in the next section. Table 2-3 provides a summary of the main advantages and disadvantages of available materials used for indirect pulp capping with clinical studies of the success rates of these materials.

Table 2-3: Commonly used materials for indirect pulp capping procedures

Materials used for indirect pulp capping	**Advantages**	**Disadvantages**	**Clinical success rate as an indirect pulp capping agent**
Setting Calcium hydroxide	Biocompatible, bioactive, antibacterial properties (Stanley and Lundy, 1972; Stuart *et al.*, 1991).	Low compressive strength, soluble in water and acids, no form of chemical or mechanical adhesion (Farhad and Mohammadi, 2005).	83% success rate in primary molars at 2 years (Falster *et al.*, 2002), 96% success rate in primary posterior teeth at 1 year (Al-Zayer *et al.*, 2003), 96% success rate at 12-24 months (Sawusch, 1982), 94% success rate at 6 months (Nirschl and Avery, 1983), 89.8% success rate in permanent teeth at 1 year following stepwise excavation (Bjørndal *et al.*, 2010).
GIC	Biocompatible, chemical bond to tooth structure, fluoride release (Lin *et al.*, 1992).	Potential pulp toxicity when placed directly on the pulp (de Souza Costa *et al.*, 2003b).	94% success rate in primary molars (Vij *et al.*, 2004).
RMGIC	Improved mechanical and physical properties, better cohesive strength, lower modulus of elasticity, improved bond strength to the tooth (Arora *et al.*, 2010).	Leaching of unreacted monomers (de Souza Costa *et al.*, 2003a; Souza *et al.*, 2006b).	93% success rate in primary molars followed up for 2-7 years (Farooq *et al.*, 2000), 94% success rate in primary molars (Hübel and Mejàre, 2003), 93% success rate in primary molars (Marchi *et al.*, 2006), 96% in primary molars and 93% in permanent teeth at 3 years (Gruythuysen *et al.*, 2010).
Dental adhesives	Reducing leakage by providing an effective seal.	Cytotoxic effect on the pulp (de Souza Costa *et al.*, 2000).	96% success rate in primary molars at 2 years (Falster *et al.*, 2002), 100% success rate in primary molars at 2 years (Büyükgüral and Cehreli, 2008).

Zinc Oxide Eugenol (ZOE)	Anti-inflammatory and local anesthetic effects on the pulp in low concentrations (Markowitz *et al.*, 1992).	Eugenol cytotoxic to the pulp, interfacial leakage (Hilton, 2009).	Similar success rate to calcium hydroxide after 5 months in permanent teeth (Fairbourn *et al.*, 1980).
Mineralised Trioxide Aggregate (MTA)	Biocompatible, bioactive, antibacterial properties, high pH (10.2-12.5), bonds to tooth structure (Parirokh and Torabinejad, 2010; Han and Okiji, 2011).	High solubility, long setting time, poor handling properties (Parirokh and Torabinejad, 2010).	93% success rate in permanent teeth at 3 months and 89.6% at 6 months (Leye Benoist *et al.*, 2012).
Biodentine™	Biocompatible, bioactive, antibacterial properties, high pH, bonds to tooth structure, shorter setting time (10 minutes), better handling properties (Laurent *et al.*, 2008; Atmeh *et al.*, 2012; Koubi *et al.*, 2013).	Wears with time, not very radiopaque, not aesthetic so cannot be used in anterior teeth (Koubi *et al.*, 2013).	No clinical trials reporting success rate of indirect pulp capping. The clinical study by (Koubi et al., 2013) does not indicate if Biodentine™ was used as an indirect pulp capping agent.

2.6 Biodentine™: A calcium silicate cement



Flowchart of the literature review structure in the thesis.

2.6.1 Composition and setting reaction

Biodentine™ is produced in the form of a capsulated powder and liquid in separate single-dose units (Table 2-4). Five drops of liquid which is provided in a sealed ampule is added to the capsule containing the powder and the mix is triturated for 30 seconds at 4,000 to 4,200 rpm in a conventional triturator. The Biodentine™ paste is then applied to the tooth without requiring any prior surface treatment (Jefferies, 2014). Setting occurs through a hydration process forming tricalcium silicate hydrate gel and calcium hydroxide (Atmeh *et al.*, 2012). Growth of the gel structure develops through nucleation and growth on the tricalcium silicate surface with calcium carbonate acting as a nucleation site. Crystallisation of the calcium silicate gel continues through hydration until bulk setting is completed in approximately 2 weeks however the initial setting time reported in the product sheet is 9-12 minutes (Bachoo *et al.*, 2013). This short setting time is attributed to the addition of calcium chloride to the mixing liquid, reducing the liquid content, and higher specific surface size of particles (Bachoo *et al.*, 2013; Malkondu *et al.*, 2014).

Table 2-4: Composition of Biodentine™: (Bachoo *et al.*, 2013; Malkondu *et al.*, 2014)

Powder (1g)	Liquid (200 ml)
Tricalcium and dicalcium silicate (main and second core component respectively)	Calcium chloride (accelerator)
Calcium carbonate (acts as a filler)	Water reducing agent (for acceptable flow properties)
Zirconium oxide (for radio-opacity)	Water

2.6.2 Physical, mechanical and chemical properties

Compressive strength is one of the important physical properties of hydraulic cements and Biodentine™ is no exception especially as it is used commonly in vital pulp therapy which requires the ability to withstand external influences e.g. masticatory forces (Malkondu *et al.*, 2014). Biodentine™ is reported to have the capacity to increase in compressive strength until it reaches a similar range with natural dentine (Malkondu *et al.*, 2014). In the first hour, the compressive strength reaches 100 MPa which doubles to 200 MPa after 24 hours. The final compressive strength has been reported to be 300 MPa which is close to the range of natural dentine shown to be 297 MPa (Bachoo *et al.*, 2013). In a study by Grech et al. (2013), Biodentine™ exhibited superior compressive strength compared to a prototype cement, Bioaggregate™, and intermediate restorative material (IRM) which was suggested to be attributed to the low water/cement ratio used in Biodentine™ which is permissible as a water soluble polymer is added to the mixing liquid.

In the same study, microhardness, washout resistance, fluid uptake, sorption and solubility of Biodentine™ were also investigated. Microhardness was performed using a diamond shaped indenter after immersion in Hanks balanced salt solution (HBSS) for 28 days. Vickers hardness number (VHN) for Biodentine™ was found to be significantly higher than the prototype cement, Bioaggregate™, and IRM. Wash out resistance which refers to the tendency of the freshly mixed cement paste to resist disintegration upon early contact with

blood or other fluids has been tested using the basket drop method and was found to demonstrate a very high washout tendency with the loss of material increasing with every drop. Bioaggregate™ and IRM on the other hand showed low wash out tendency. This unfavourable result with Biodentine™ was suggested to be due to the surfactant effect water soluble polymer added to the material to reduce the water/cement ratio. Furthermore, Biodentine™ showed lower fluid uptake and sorption compared to the other materials (Grech *et al.*, 2013).

The bond strength of Biodentine™ to dentine was investigated in a study by Raju et al. (2014) and compared to GIC Fuji IX™. Shear bond strength was tested on 40 extracted non-carious primary and permanent molar teeth with a Universal Testing Machine with the compressive load applied using a knife edge. It was not mentioned whether the specimens were stored in any kind of medium before testing and the mode of failure was not analysed. The mean shear bond strength values were higher for GIC Fuji IX™ which was 6.414 MPa compared to Biodentine™ which was 3.441 MPa. No significant difference was found between primary and permanent teeth for both materials (Raju *et al.*, 2014).

Because Biodentine™ is used in vital pulp treatment among others where a hermetic seal is fundamental, the degree of porosity of the material is important for the overall success of the restoration (Malkondu *et al.*, 2014). One study evaluated the porosity of Biodentine™ compared to Bioaggregate™, a prototype radiopacified tricalcium silicate cement (TCS-20-Zr) and intermediate restorative material (IRM) after immersion for 28 days in HBSS using mercury intrusion porosimetry (Camilleri *et al.*, 2014). The pore diameter of all the materials tested was in the range of 0.01–0.05 μm with the least average pore diameter observed in Biodentine™. The level/percentage of porosity of Biodentine™ and IRM was the lowest compared to the other materials. Porosity is an intrinsic characteristic of tricalcium silicate based cements and occurs as a result of the spaces between the un-hydrated cement grains. Once the material hydrates, these spaces are filled with water and hydration products leading to reduced porosity. Similarly, the porosity is reduced as the cement ages

(Camilleri *et al.*, 2014). In another study, the degree of porosity of Biodentine™ was compared to Pro Root® MTA quantitatively in 3D using a compact micro-CT device. No significant difference was found between the two materials (De Souza *et al.*, 2013).

Microleakage is an important aspect to consider especially when Biodentine™ is used in vital pulp therapy as the tooth is already compromised and leakage may result in failure of the treatment. One study compared the in-vitro marginal integrity of open sandwich restorations placed in class II cavities which were assigned either Biodentine™ or RMGIC (Ionolux, Voco, Cuxhaven, Germany) and covered with resin composite (Koubi *et al.*, 2011). The teeth were subjected to thermo- and mechano-cycling followed by storage in phosphate buffered saline (PBS) for one year. Results of the glucose infiltration analysis showed a similar pattern of leakage between Biodentine™ and RMGIC. This was explained to be due to the formation of hydroxyapatite crystals at the surface forming an increased sealing ability in the Biodentine™ samples (Koubi *et al.*, 2011). This is supported by a similar study comparing microleakage of Biodentine™ and RMGIC (Fuji II LC, GC Corp, Japan) in cervical margins of proximal cavities using silver nitrate solution where no difference was found between the two materials (Raskin *et al.*, 2012). In another study, microleakage was compared stereomicroscopically between Biodentine™, MTA, and GIC used as root end filling materials using dye penetration. Biodentine™ was found to be associated with less microleakage compared to the other materials (Kokate and Pawar, 2012).

2.6.3 Dentine-Biodentine™ interface

Biodentine™ is a biocompatible and bioactive material promoting repair through the deposition of hydroxyapatite on its cement surface in the presence of simulated body fluid. Laurent et al. (2008) were the first to demonstrate the biological properties of Biodentine™ on human pulp fibroblasts. They concluded that Biodentine™ was biocompatible with no cytotoxic or genotoxic effect and does not influence the specific functions of target cells such as mineralisation. Its ability to induce the deposition of reactionary dentine renders this material to

be a potential pulp capping agent. They suggest the need for in-vivo studies to confirm the findings of this study (Laurent *et al.*, 2008). The same authors in another study investigated the ability of Biodentine™ to induce reparative dentine synthesis and modulate pulp cells TGF-b1 secretion in human teeth (Laurent *et al.*, 2012b). Biodentine™ was found to induce odontoblast-like cell differentiation and mineralisation through modulation in TGF-b1 secretion from dental pulp cells similar to MTA and calcium hydroxide (Laurent *et al.*, 2012b). Zanini et al. (2012) studied the biological effect of Biodentine™ on immortalized murine pulp cells and found Biodentine™ to be bioactive increasing cellular proliferation and biomineralisation. They conclude that this material is suitable for dentine-pulp complex regeneration such as pulp capping but confirm the need for in-vivo studies.

The effect of different concentrations of Biodentine™ on the proliferation, migration, and adhesion of human dental pulp cells was evaluated. It was shown that Biodentine™ at 2 mg/ml and 0.2 mg/ml showed a significantly increased proliferation of human dental pulp stem cells, while Biodentine™ at 20 mg/ml had a marked decreased proliferation of cells suggesting cytotoxicity at these levels. On the other hand, Biodentine™ at 0.02 mg/ml concentration was no different than blank controls indicating a minimal effect. It has been suggested that Biodentine™ may play a role in up-regulating the mRNA expression of chemokines and adhesion molecules essential for the tissue regeneration process (Luo *et al.*, 2014).

Remineralisation and increased acid resistance is due to dentine incorporating several elements released from bioactive materials causing chemical and structural modification in dentine (Han and Okiji, 2011). Biodentine™ has been linked to the increased uptake of calcium and silicon ions into the adjacent dentine promoting the biomineralisation ability of this material which has been shown to be more prominent than MTA. This is due to a wider calcium and silicon-rich dentine areas and larger incorporation depths in Biodentine™ explained by the larger amount of calcium and silicon dissolution as confirmed by element mapping and line-scan analysis (Han and Okiji, 2011; Han and Okiji, 2013). The formation of a mineral-rich interfacial layer and a tag-like structure

extending from the interfacial layer to the dentine tubules has been reported in the study (Han and Okiji, 2011; Han and Okiji, 2013). Similar findings were observed in another in-vitro study investigating the interaction between Biodentine™ and dentine compared to GIC using different imaging techniques (Atmeh *et al.*, 2012). It was shown that the dentine/Biodentine™ interface demonstrated tag-like structures in addition to an interfacial mineral infiltration zone which was suggested to be caused by the caustic effect of the alkaline Biodentine™ accompanied by mineral infiltration (carbonate ions) into the inter-tubular dentine (Atmeh *et al.*, 2012).

Camilleri et al. (2014) shed the light on the dentine-Biodentine™ interface from a different perspective using confocal microscopy and scanning electron microscopy. They reported that the environmental conditions in which Biodentine™ is stored has an effect on the sealing ability of the material where dry conditions caused shrinkage of Biodentine™ with cracks developing within the bulk of the material leading to gap formation at the interface potentially allowing passage of micro-organisms (Camilleri *et al.*, 2014). This has significance clinically when used as a pulp capping agent as it may not be placed in moist conditions.

In-vivo studies evaluating the effect of Biodentine™ when used as a pulp capping agent include a study which compared Biodentine™, MTA, and calcium hydroxide placed on pulp exposed primary teeth of pigs and examined histologically after 7, 28, and 90 days (Shayegan *et al.*, 2012). A significant difference in hard tissue formation was found between Biodentine™ and calcium hydroxide at day 7. They concluded that Biodentine™ and MTA are both biocompatible materials for pulp capping. These findings are supported by another similar study comparing Biodentine™, two new nanostructured materials based on active silicate cements, and MTA when used for direct pulp capping of pig teeth and evaluated histologically after 28 days (Popović-Bajić *et al.*, 2013). A favourable therapeutic effect was demonstrated with Biodentine™ and the two new materials which were found to be comparable to MTA in the form of dentine bridge formation, partial or complete closure of the pulp

chamber, and preservation of the integrity of the pulp (Popović-Bajić *et al.*, 2013).

A study by Nowicka et al. (2013) was conducted on human permanent teeth scheduled for extraction. Mechanical exposure of the pulps was carried out and either Biodentine™ or MTA applied followed by clinical and radiographic examination and histologic analysis after 6 weeks. Clinically, all teeth were vital at 6 weeks diagnosed using thermal and electric pulp tests with no symptoms of pain and no radiographic signs of periapical pathology. Histologic evaluation revealed dentine bridge formation with no evidence of inflammation, abscess, or necrosis below the dentinal bridge. Layers of well-arranged odontoblast and odontoblast-like cells were found to form tubular dentine under the osteodentine. No significant difference was found between Biodentine™ and MTA when used as direct pulp capping agents (Nowicka *et al.*, 2013).

Data from randomised long term clinical trials are undeniably more reliable and up to-date there is only one 3-year clinical trial displaying the performance and safety of Biodentine™ as a posterior restoration compared to resin composite (Koubi *et al.*, 2013b). Interim analysis found that this material can be used successfully in bulk for up to 6 months when evaluated using the USPHS criteria however abrasion was the main drawback which increased when left uncovered for more than 6 months. Due to this, the majority of teeth restored with Biodentine™ needed to be covered with resin composite after one year. Pulp vitality was monitored in the study and all teeth remained vital at the interim evaluation (Koubi *et al.*, 2013b).

2.6.4 Biodentine™-restorative interface

In the clinical study conducted by Koubi et al. (2013), Biodentine™ was advocated to be used under resin composite in posterior restorations which supports the major standpoint from which the material was initially developed, in other words as a dentine replacement material. These materials are usually etched with phosphoric acid to enhance the bonding of the dentine replacement material with the overlying composite resin. In one study, the effect of etching on the microstructure, surface micro-hardness and micro-leakage of

Biodentine™ was compared to GIC (Fuji IX) and RMGIC (Vitrebond) when used in an open sandwich restoration (Camilleri, 2013). When etched with 37% phosphoric acid, Biodentine™ exhibited structural and chemical changes with a lower chloride peak and calcium to silicon ratio compared to the non-etched Biodentine™. Significant leakage was observed at the dentine to material interface both when etched and left unprepared. GIC and RMGIC on the other hand showed no chemical or physical changes and no microleakage. The microhardness of all the materials was not affected by etching (Camilleri, 2013).

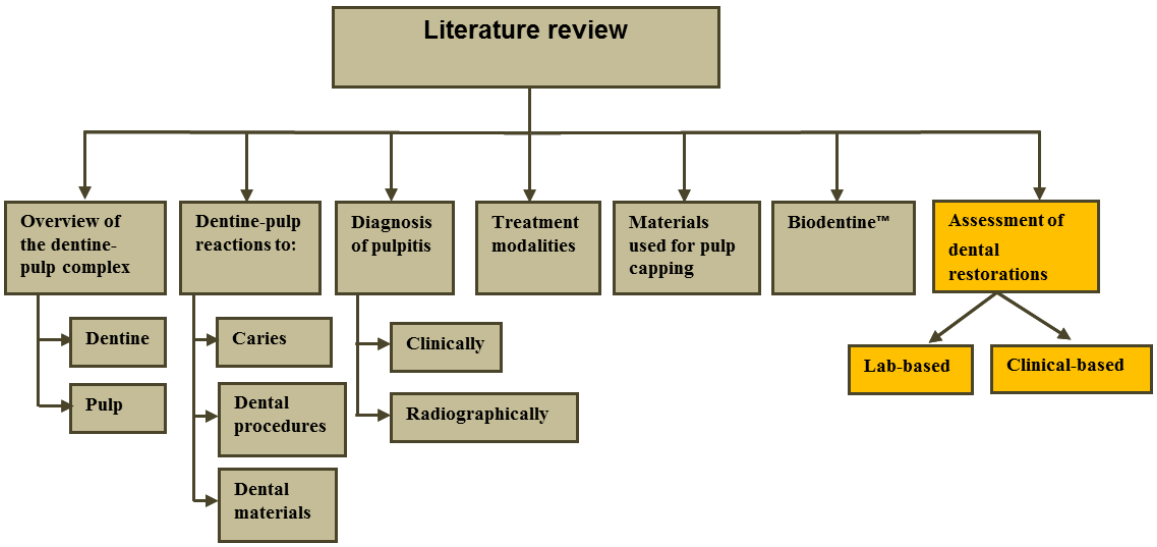
An earlier study by Boinon et al. (2007) investigated the adhesion of Biodentine™ to resin composite when used in sandwich restorations by evaluating the shear bond strength and marginal microleakage. They conclude that etching Biodentine™ with phosphoric acid followed by the application of a silane coupling agent before the adhesive resin produced the highest shear bond strength values and the lowest microleakage (Boinon *et al.*, 2007). This study however had insufficient number of samples for the results to be reliable.

A more recent study evaluated the shear bond strength of methacrylate-based (MB) composite, silorane-based (SB) composite, and GIC to Biodentine™ compared to MTA using a total etch adhesive after leaving the materials to set for 15 minutes and 96 hours respectively (Cantekin and Avci, 2014). Higher shear bond strength values of MB composite to Biodentine™ compared to MTA were found while there was no significant difference in the other groups (Cantekin and Avci, 2014). The study indicated the need to age the material at different time intervals and to consider different bonding protocols and systems. The mode of failure was not identified in the study.

AL-Ashou et al. (2014) conducted a similar study investigating the shear bond strength of Biodentine™ to resin composite, GIC, compomer, and self-adhesive resin cement after leaving Biodentine™ to set for 12 minutes. A total etch adhesive was used before the application of the resin composite and compomer and all specimens were stored for 24 hours before testing. The shear bond strength of Biodentine™ to resin composite, GIC, and compomer was found to exceed the 17-20 MPa range which is required to resist contraction forces to produce a gap-free restorative margin. The shear bond strength to the self-

adhesive cement was below this range. Failure mode analysis for all groups was found to be mixed failure within adhesive material and/or restorative material (AL-Ashou *et al.*, 2014).

2.7 Assessment of dental restorations



Flowchart of the literature review structure in the thesis.

In the era of evidence-based dentistry, decisions made on the appropriate methods for disease treatment and prevention is based on the best available evidence (Pihlstrom and Barnett, 2010). Randomized, controlled clinical trials (RCT) are regarded as the optimal approach to evaluate the performance of dental restorations and hence provide the highest quality of evidence. Although RCTs may be conceptually simple, they are difficult to design, implement, and translate into clinical practice or public health policy (Pihlstrom and Barnett, 2010). Therefore, laboratory studies are performed in an attempt to predict the clinical performance of dental restorations and its effectiveness in routine clinical practice.

2.7.1 Laboratory studies

Correlating laboratory tests with clinical performance is a difficult task and despite all the energy invested into laboratory testing, there are no tests that are truly predictive of long-term clinical performance (Bayne, 2007; Green and

Banerjee, 2011). Clinical trials tend to measure “macro” events which are easily observed with little or no magnification such as restoration colour, caries, smoothness, margin continuity, and patients’ postoperative sensitivity. Laboratory studies on the other hand measure properties of the materials ranging from “macro” to “nano” which may not correlate to the clinical findings (Bayne, 2012). Furthermore, the rate of change observed in a laboratory test is generally an accelerated one compared to the changes observed clinically. It is reported that a laboratory result for a 1-week simulation could predict the relative acceptability of a specific clinical event, occurring over a time period of 3 years (Bayne, 2007). Additionally, clinical challenges are multifactorial with significant interaction among these factors occurring simultaneously. Although laboratory tests are designed to simulate the clinical situation as much as possible for the results to be clinically relevant, in some cases it still does not reproduce the multifactorial environment seen clinically and the control of different variables may be difficult (Sarrett, 2005). Therefore, for a laboratory test to be significant, an outcome level for the test must be assigned that is associated with an acceptable clinical outcome (Bayne, 2007).

There are a great number of laboratory studies evaluating dental restorations in the literature and it is difficult to compare the results even though international organisations for standardising testing protocols have been created (Wang *et al.*, 2003). Although specifications describe in detail the experimental protocol, there still remain aspects which are vague leading to a wide variety of experimental protocols among researchers which affect the outcome (Braga *et al.*, 2010).

Nevertheless, it is without doubt that laboratory tests afford valuable information on the preclinical performance of dental restorations which can provide, to some extent, an estimate on the predictability of their clinical performance (Braga *et al.*, 2010). In general, advantages of ‘laboratory testing’ include, (1) the speed of gathering data on a specific parameter/property, (2) the relative ease of the test methodology commonly used, (3) the possibility (and necessity) to measure one specific parameter, while keeping all other variables constant, (4) to be able to directly compare the performance of a new and/or experimental

material/technique with that of the current 'gold-standard', (5) to be able to test simultaneously many experimental groups within one study set-up, and (6) the ability to use relatively unsophisticated and inexpensive test protocols/instruments (Van Meerbeek *et al.*, 2010).

Many laboratory tests are available to evaluate different properties of dental materials (Table 2-5). The mechanical properties of a dental material are one aspect which involves the ways in which a material responds to load (Bayne, 2007). In the oral environment, restorations are exposed to stresses from mastication action. These forces act on teeth and/or material producing different reactions that lead to deformation or failure of adhesion, which can ultimately compromise their durability over time (Wang *et al.*, 2003). Adhesion analysis of different materials to each other and to tooth structure has been performed by numerous mechanical testing methods (Table 2-6). These testing methods are considered key aspects used to screen new products and study the influence of experimental variables. Adhesive performance may be quantified generally using a "macro" or "micro" set up depending on the size of the bonded area (Salz and Bock, 2010). A "macro" bond strength set up utilises a bonded area larger than 3mm^2 using a tensile, shear, push-, or pull-out protocol. "Micro" bond strength test set ups on the other hand measure a bonded area of 1mm^2 or less (Van Meerbeek *et al.*, 2010). It is outside the scope of this literature review to provide a detailed account on the different testing methods however Table 2-6 summarises each testing method with the advantages and disadvantages of each.

Table 2-5: Biomaterials' properties commonly tested (Bayne, 2007).

Property	Definition	Examples
Physical	Involve motion of electrons, protons, or atoms within the solid such as electrical conductivity of a material.	Thermal conductivity, thermal diffusivity, thermal expansion, reflectivity, radiopacity, density etc.
Chemical	Involve changes in bonding patterns and or hydration states of the atoms or molecules on the surfaces or within the interior of the material.	Water adsorption (onto the surface), water absorption (into the interior), chemical corrosion, electrochemical corrosion, biodegradation and/or new chemical reactions.
Mechanical	Involves the ways in which a material responds to load.	Tensile, diametral compression, compressive, flexural strength, fatigue, hardness.
Biological	Represents interfacial interactions of a material with the hard and soft tissues and which may produce local or systemic responses in the patient.	Bioactivity/biocompatibility (toxicity, sensitivity, mutagenicity) through cell culture tests, tissue culture tests, small animal model tests, or human usage tests in mammals.

Table 2-6: Advantages and disadvantages of different bond strength tests (Salz and Bock, 2010; Van Meerbeek *et al.*, 2010).

Bond strength test	Advantages	Disadvantages
Macroshear bond strength	Ease and speed of use.	Stress distribution is inhomogeneous.
Macrotensile bond strength	More even stress distribution.	Less commonly used.

Push-/pull-out test	Marginal adaptation via SEM analysis and bond strength can be evaluated with the same test specimen. Useful to test the retention of posts luted in root canals.	Minor degree of composite swelling upon water storage can induce a significant amount of friction independent of adhesive performance.
Microtensile bond strength	Better economic use of teeth, better control of regional difference, better stress distribution at the interface, permits testing of small areas.	Labor intensive, technically demanding, difficult to measure very low bond strength (<5MPa), specimens easily dehydrates, specimens easily damaged.
Microshear bond strength	Better economic use of teeth, better control of regional difference, useful for brittle substrates.	Non-uniform stress distribution.

2.7.2 Clinical studies

Clinically, when a dental restoration is placed, it seems reasonable to assume that a subjective assessment of its quality is made by the operator. The criteria used to make such assessments are variable, unreliable, and inconsistent. For this reason, clinical guidelines have been developed upon which judgments could be based (Elderton, 1977). The earliest of these was reported by Gruebbel (1950) which included four criteria for evaluating the quality of dental treatment: restoration of tooth form, faulty margins, fractured filling, and fracture of tooth around filling. There was no explanation of how the examination was conducted and no comparison was made between the assessors. A further study compared the differences between the assessing dentists grading restorations either satisfactory or unsatisfactory (Abramowitz, 1966). Agreement between the two assessing dentists was 74% for 178 restorations and the restorations with “disagreement” was considered no further.

The next group of researchers developed ten criteria for the assessment of amalgam restorations: anatomical carving; marginal ridge relation; contact;

contour; marginal integrity; condensation; occlusion; tissue integrity; postoperative lavage; and surface smoothness. Each of the ten criteria was judged as excellent, acceptable, or unacceptable (Hammons and Jamison, 1967). The description for each criterion was found to be excessively long and open to considerable differences in interpretation by different assessors. Furthermore no information was given to guide in the difference between the category excellent and acceptable and no reference was made to the variability between the assessors (Elderton, 1977). The guidelines for the assessment of amalgam restorations were simplified in a further study to include just four aspects: adaptation; contour; contact and occlusion and the ratings were either satisfactory or not (Lotzkar *et al.*, 1971a; Lotzkar *et al.*, 1971b). Again, a considerable variation was found between the different assessors. The common factor between all these studies was that studying the assessment of quality of dental restorations was not a primary outcome but rather the productivity was.

The first study specifically evaluating the assessment of dental restorations with no regard to the productivity was reported by Cvar and Ryge (1971). They provided a systematic approach to the evaluation of clinical performance of restorative materials known as “Criteria for the clinical evaluation of dental restorative materials” for use by the United States Public Health Service. These criteria are commonly termed “Ryge” or USPHS” criteria which have been designed to measure clinically important features of restorations. Five characteristics are presented: color match, cavo-surface marginal discoloration, anatomic form, marginal adaptation, and caries. A rating scale of either alfa, bravo, charlie, delta, oscar is given for each criterion. It was the intention that two dentists should assess the restoration independently and that in the event of a disagreement, a joint examination is conducted and a final rating agreed between the two examiners. Training of the examiners was an important prerequisite to ensure consistency between the two examiners, and within the same examiner at different time frames, and to prevent drift of judgement over a period of time. Acceptable intra and inter-examiner agreement was defined as 85% (Cvar and Ryge, 2005). Table 2-7 provides an example of the explanation of the rating scale for marginal adaptation.

Table 2-7: Criteria for marginal adaptation (Cvar and Ryge, 2005).

Rating	Criteria
Alfa	The explorer does not “catch” when drawn across the restoration-tooth margin either from tooth to restoration or from restoration to tooth. If a “catch” exists, there is no visible crevice along the periphery of the restoration. The edge of the restoration appears to adapt closely to the tooth structure along the entire periphery of the restoration.
Bravo	The explorer does “catch” and there is visible evidence of a crevice into which the explorer will penetrate, indicating that the edge of the restoration does not closely adapt to the tooth structure. The dentin or base is not exposed, and the restoration is not mobile, fractured, or missing in part or in toto.
Charlie	The explorer penetrates into crevice indicating that a space exists between the restoration and the tooth structure. The dentin or the base is exposed at the periphery, but the restoration is not mobile, fractured, or missing in part or in toto.
Delta	The restoration is mobile, fractured, or missing in part or in toto.
Oscar	Marginal adaptation cannot be assessed due to an excess of restorative material at the margin.

These criteria quickly gained popularity due to its clarity, workability, and lack of complexity and were soon used in many studies worldwide. Due to the development and introduction of many restorative materials, studies began adapting the criteria by extending the number of USPHS categories of direct evaluation to include other parameters of interest such as occlusion, postoperative sensitivity, fracture, retention, and others which were referred to as the modified USPHS guidelines (Appendix 2) (Bayne and Schmalz, 2005). However, authors from different studies did not use the same definitions for assigning these new ratings therefore, it is still a requirement to declare the categories and define the ratings as part of all publications (Bayne and Schmalz, 2005). Additionally, the different modifications in these criteria between studies render comparisons between them difficult. Furthermore, it

was found that detecting early deterioration and differences within the same restoration and between restorations within short time intervals such as 6,12,18 months was difficult because the scale was not very discriminative (Hickel *et al.*, 2006). This limited sensitivity of the Ryge criteria in short term clinical investigations may be misinterpreted by many as acceptable clinical performance (Hickel *et al.*, 2007b).

A new, improved criteria was introduced by Hickel *et al*. (2007) and approved by the FDI World Dental Federation was simultaneously published in the "Journal of Adhesive Dentistry" and "Clinical Oral Investigations" in an attempt to encourage immediate adoption of these criteria in new clinical trials. The authors claim that these criteria have a more sensitive assessment method with enhanced discriminative power compared with the original Ryge criteria. The proposed criteria consist of three categories: aesthetic, functional, and biological with sub-groups for each category (Appendix 3). A score of 1-5 is given for each sub-group with 1 being excellent and 5 needing replacement. The final score in each category is dictated by the highest/most severe score among the sub-group. When summarising the three scores (aesthetic, functional and biological) in one overall rating for the restoration, the highest score is considered the final score (Hickel *et al.*, 2007a).

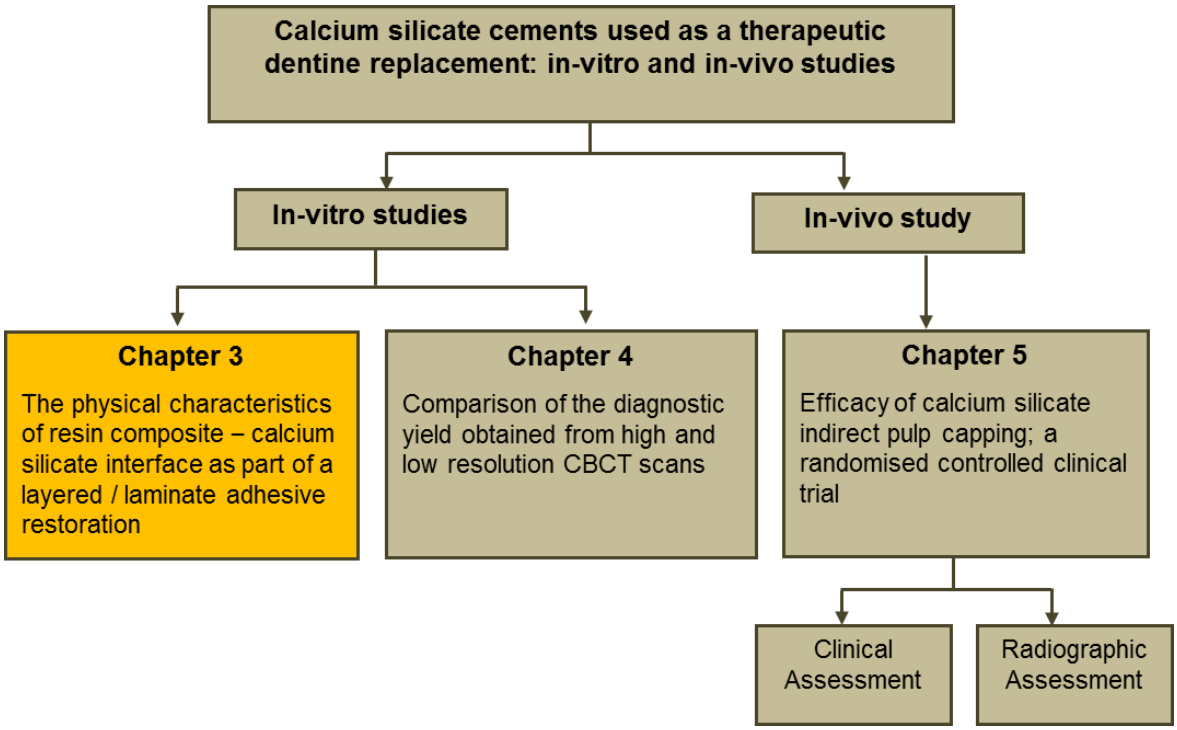
A modification of these criteria was published in 2010 with two major alterations involving staining and proximal contacts (Hickel *et al.*, 2010). The authors emphasise the need for calibration of the examiners for consistency and validity. They also emphasise that not all the clinical criteria may be required and only the criteria necessary to accomplish the objective of the trial should be included. The scores may be reduced from 5 to 4 or even 2 again depending on the purpose of the study (Hickel *et al.*, 2010).

It is evident that these criteria although appearing complex and time consuming at a glance, are feasible during clinical trials as it has been used by a number of studies since it was published (Farag *et al.*, 2011; Rodolpho *et al.*, 2011; Coelho-De-Souza *et al.*, 2012). There is certainly a need for studies comparing the FDI criteria with other existing restorative assessment criteria i.e USPHS

criteria to establish its validity and benefit. There is only one study reported as an abstract in an IADR conference which compares the FDI criteria with the USPHS criteria in the assessment of resin composite restorations placed in posterior deciduous teeth. They found that the FDI method was more sensitive in identifying differences in the resin composite restorations placed in the deciduous teeth (Piva and Coelho-Souza, 2009). More studies are needed in this aspect.

CHAPTER 3

The physical characteristics of resin composite – calcium silicate interface as part of a layered / laminate adhesive restoration



3.1 Introduction

Glass ionomer cements and resin modified glass ionomer cements have long been used as part of a layered/laminate adhesive restoration or the so called "sandwich restoration". Calcium silicate cements such as Biodentine™ (Septodont, St Maure des Fosses, France) are used in a similar way because Biodentine™ is exposed to wear under load with time and has poor aesthetics. Therefore, a second, overlaid veneer of resin composite is required to provide mechanical strength, wear resistance, and improved aesthetics of the definitive restoration (Zhang *et al.*, 2011).

The quality and durability of the adhesive bond between Biodentine™ and the resin composite is of clinical significance with regards to the longevity and predictability of the final laminate restoration. The durability of this bond may be affected by the type of adhesive used (self-etch vs. etch and rinse adhesives). As Biodentine™ has a similar chemical composition to MTA, hydration of Biodentine™ should resemble that of MTA. Therefore, it is assumed that when Biodentine™ is exposed to a low pH such as that of phosphoric acid etch, this could affect the chemical setting of Biodentine™ by disrupting the hydration of tricalcium silicates resulting in weakening of the setting material's microstructure (Lee *et al.*, 2007; Kayahan *et al.*, 2009; Giuliani *et al.*, 2010). Milder etching for a shorter time period may cause selective loss of matrix around the crystalline structures with minimal loss of cement, exposing these crystalline structures and hence encouraging successful adhesion through micro-mechanical retention (Kayahan *et al.*, 2009).

Currently, placing the veneering restoration is a 2-stage clinical procedure, completed ideally within a maximum period of 6 months after placing the initial Biodentine™ bulk restoration, as per manufacturer's recommendations. However, investigating the potential for bonding the veneering restoration at the same visit as placing the Biodentine™ is worthwhile as this would be easier and less time consuming, eliminating the need to bring the patient back for a second visit.

There are many methods used to assess interfacial bond strength between dissimilar restorative materials. Statically, they can be measured using a macro- or micro-test depending on the area of the tested interface (Van Meerbeek *et al.*, 2010). The micro-shear test was used in this investigation allowing simpler specimen preparation with a reduced risk of specimen preparation damage. It eliminates the need to section specimens to obtain sticks or hour-glass specimens which is required for other tests such as the micro-tensile test (Shimada *et al.*, 2002; Placido *et al.*, 2007; Armstrong *et al.*, 2010). Indeed this is necessary with Biodentine™ which is brittle in thin cross section and must be used in bulk to avoid damage to the Biodentine™ samples.

The aim of this in-vitro study was to determine the micro-shear bond strength (μSBS) of a resin composite (N'Durance, Septodont, Louisville, USA) to Biodentine™ using a self-etch adhesive (Scotchbond™ Universal, 3M ESPE, USA) compared to glass ionomer cement (GIC) (Fuji IX™ GP, GC Corporation, Tokyo, Japan) and resin modified glass ionomer cement (RM-GIC) (Fuji II LC, GC corporation, Tokyo, Japan), which are materials that have similar clinical applications to Biodentine™ in terms of being used as provisional bulk restorative materials in deep cavities. The study also aimed to compare the use of the self-etch adhesive in a self-etch mode (SE) and a total-etch (TE) mode while aging the substrates and aging the bond at different time intervals and to identify the specific modes of failure. The null hypothesis was that there is no difference in the μSBS within each substrate (Biodentine™, GIC, and RM-GIC) and when comparing between them using the self-etch and total etch techniques at the different time intervals.

3.2 Materials and Methods

3.2.1 Sample preparation

The materials used are summarised in Table 3-1. Nine hundred and twenty discs of Biodentine™ (n=320), Fuji IX™ (positive control) (n=320) ,and Fuji II LC™ (positive control) (n=280) were fabricated by mixing each material according to the manufacturer's instructions and condensing them into a standardised 3x4 mm cylindrical plastic polymer mold (Fig 3-1). A glass slab was placed on top of the mold so that all the materials set against a smooth surface to ensure standardisation of the sample surface.

Table 3-1: List of materials used in the study.

Material	Manufacturer	Material composition
Tricalcium silicate cement	Biodentine™, Septodont, St Maure des Fosses, France	Powder: Tri-calcium silicate, di-calcium silicate , calcium carbonate and oxide filler, iron oxide, zirconium oxide radiopacifier Liquid: calcium chloride accelerator hydrosoluble polymer water reducing agent.
GIC	Fuji IX™ GP, GC corporation, Tokyo, Japan	Powder: fluoro-alumino-silicate glass, polyacrylic acid powder Liquid: polyacrylic acid, Polybasic carboxylic acid
RMGIC	Fuji II LC, GC corporation, Tokyo, Japan	Powder: Fluoro-alumino-silicate glass Liquid: poly-acrylic acid, 2-hydroxyethyl methacrylate (HEMA) dimethacrylate, camphorquinone, water
Composite	N'Durance®, Septodont, Louisville, USA	The resin based matrix contains approximately 19 wt % of ethoxylated BisGMA, UDMA and the new dicarbamate dimethacrylate dimer acid. The filler system contains approx. 80 wt% (65 vol%) silanated 40 nm ytterbium fluoride, silanated 500 nm barium glass and 10 nm silica. There is approximately 1 wt% of catalyst, inhibitors and pigments.
Self-etch adhesive	Scotchbond™ Universal, 3M, ESPE, USA	MDP phosphate monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, filler, ethanol, water initiators, silane
Etchant	Scotchbond™ Universal, 3M, ESPE, USA	34% phosphoric acid by weight

The samples were divided into two main groups. In the first group, the effect of aging the substrate (Biodentine™, GIC and RM-GIC) on the micro-shear bond strength (μSBS) was investigated. In the second group, the effect of aging the bond on the μSBS was investigated.

The first group (n=440) which investigated the effect of *aging the substrate* on the μSBS was subdivided into:

1. Aging each substrate for “early” time intervals (t=0 min, t=5 min, t=20min, t=24 hours) following which the same adhesive was applied in either SE or TE mode. The first time interval represents the application of the adhesive immediately after setting of each material as stated in the manufacturer's instructions, from the start of mixing. For RM-GIC there were only two time intervals (t=0 min, and t=24 hours) as the material was photo-cured on command.

2. Aging the substrate for a “delayed” time interval (t= 2 weeks, t=1 month, t=3 months, t=6 months) following which the same adhesive was applied in either SE or TE mode.

The bonded samples in both groups were stored in distilled water for 24 hours before being subjected to μSBS testing.

The second group (n=480) which investigated the effect of *aging the adhesive* bond on the μSBS was subdivided into:

1. Aging the substrate for 5 mins following which the same adhesive was applied in either SE or TE mode and the bond then aged in distilled water before testing (t=2 weeks, t=1 month, t=3 months, t=6 months).

2. Aging the substrate for 2 weeks following which the adhesive was applied in either SE or TE mode and the bond aged for the same time intervals as the previous group (t=2 weeks, t=1 month, t=3 months, t=6 months) before being subjected to μSBS testing.

3.2.2 Micro-shear bond strength test

Prior to adhesive resin polymerisation, a 1-mm thick slice of Tygon tubing (Saint-Gobain, USA) with a 0.75-mm internal diameter was placed on the bonded area (McDonough *et al.*, 2002). The adhesive resin was bonded to the discs of Biodentine™/GIC/RM-GIC according to the manufacturer's instructions. Resin composite was condensed into the tube and polymerised for 40 secs (Fig 3-1). All specimens were stored in distilled water at 37˚C for either 24 hours or longer depending on the experimental group, before testing. After the storage period, the Tygon tubes were removed carefully from all specimens using a sharp scalpel (Swann-Morton, Sheffield, England). Pre-test failures (PTFs) were recorded. The specimens were attached to the micro-shear testing device using a fast-setting adhesive (Everbuild Ltd, Leeds, UK). The testing device was attached to a SMAC LAL300 linear actuator (SMAC Ltd., West Sussex, UK). Wire (diameter of 0.2mm) was looped around the resin-composite cylinder and positioned as close as possible to the resin–Biodentine™/GIC/RM-GIC interface (Kikushima *et al.*, 2005). A shear force was applied at a crosshead speed of 1.0mm/min until debonding occurred. The micro-shear shear bond strength (t) was calculated in MPa using the equation $t=F/(\pi R^2)$ where F was the applied load at failure and R was the radius of the resin composite cylinder (Fig 3-1).

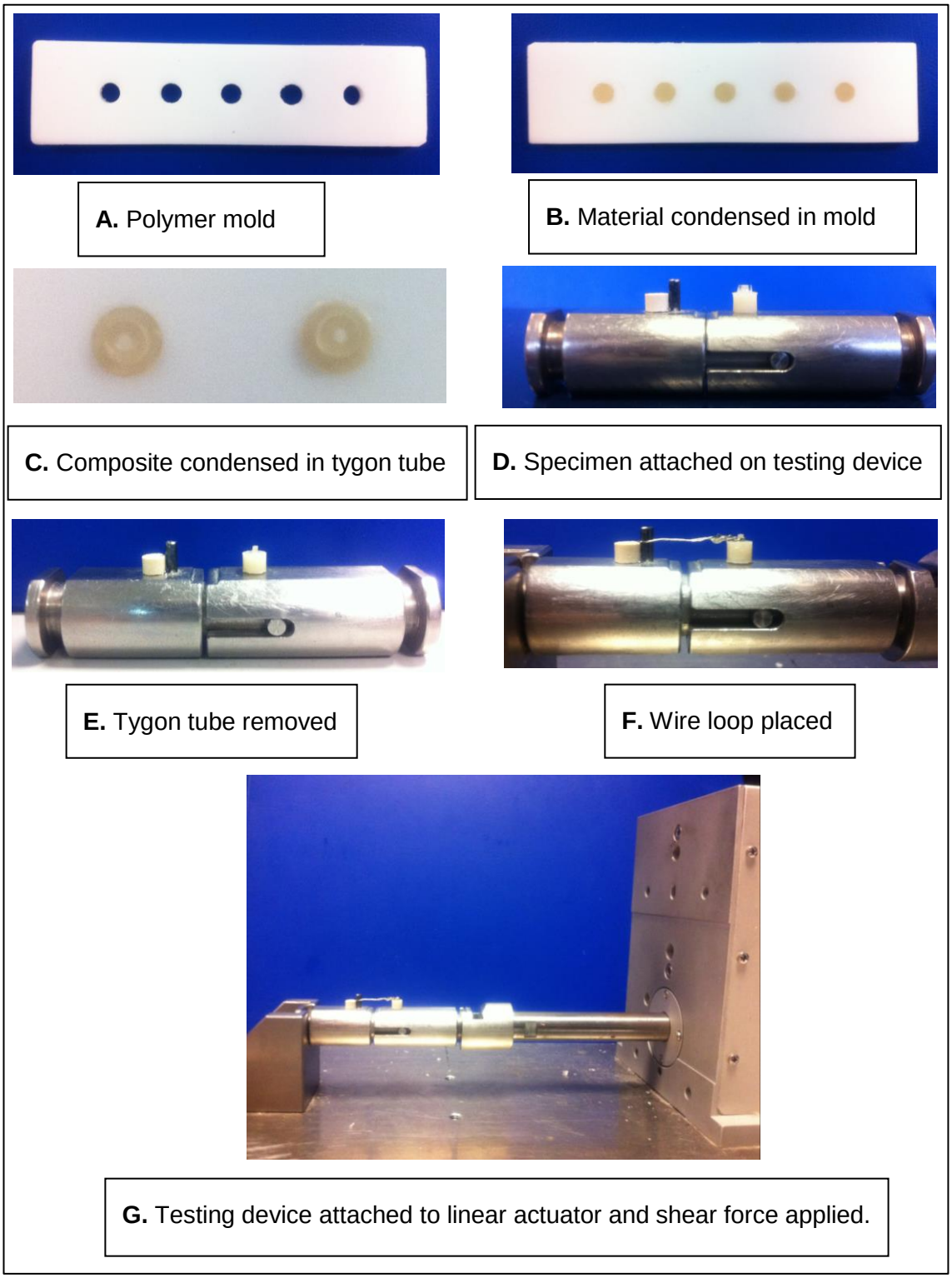


Figure 3-1: Steps of specimen preparation and testing.

3.2.3 Interface examination

After debonding, the fractured surfaces were evaluated using a stereomicroscope (Kyowa Optical Co. Ltd., Tokyo, Japan) with a 60 × 0.75 NA objective to classify the failure modes into one of the following categories: (A) adhesive failure at the interface between resin and Biodentine™/GIC/RM-GIC; (B) adhesive failure at the interface between resin and composite; (C) cohesive failure within resin; (D) cohesive failure within Biodentine™/GIC/RM-GIC; (E) cohesive failure within composite (Fig 3-2). Representative specimens (n=7) chosen randomly from each group were examined additionally using a scanning electron microscope (SEM) (Hitachi S3500, Japan). Prior to SEM observations, the specimens were air dried and gold sputter-coated at 45mA current for 2 minutes (Emitech K550, Kent, England).

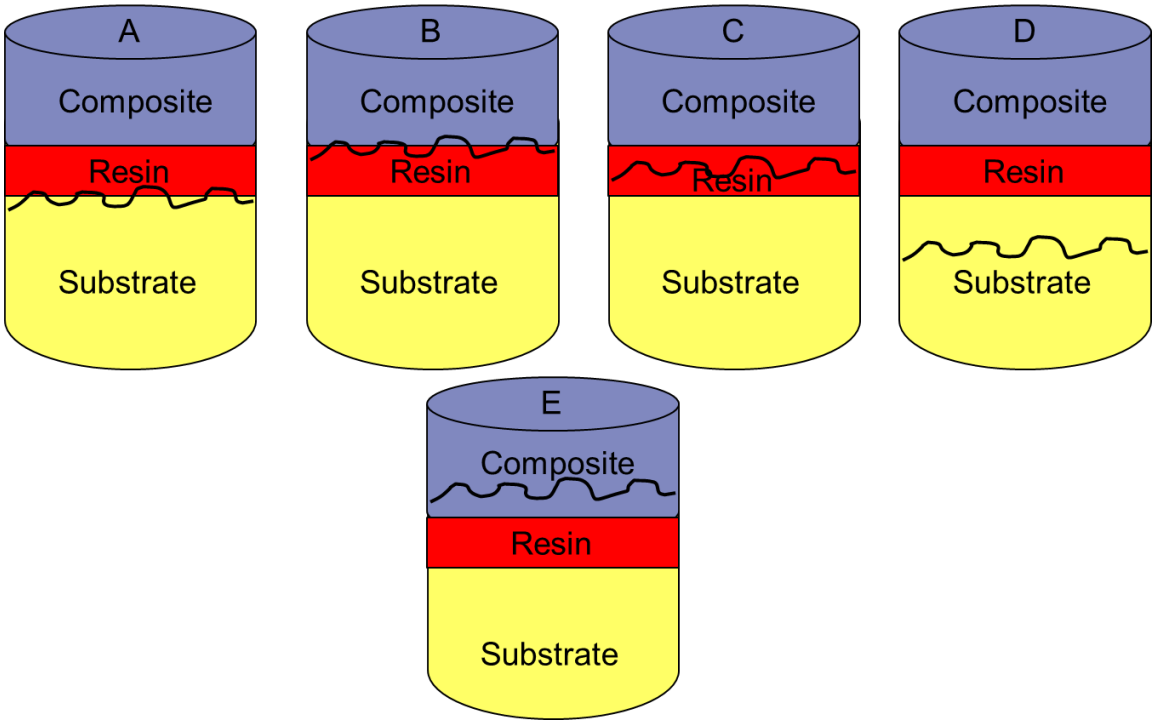


Figure 3-2: Classification of failure modes as described in the text.

3.2.4 Statistical analysis

Descriptive statistics were used to summarise the study characteristics and bond strength for various materials. Percentages were used to present the failure modes for different substrates. The bond strength for the three substrates, namely Biodentine™, GIC and RM-GIC, at different time intervals (both for aging the substrate and aging the bond) were analysed using parametric analysis as the data followed normal distribution. Linear regression models were used to test the significance of various predictors. Significance was predetermined at $\alpha=0.05$. If there was evidence of an interaction effect, the temporal changes were assessed separately for the substrates in the post-hoc analysis which was adjusted for multiple comparisons using the Bonferroni correction. Also, the main effect of substrate and time were tested by including them in the model for its overall significance. All analyses were carried out using Stata/ SE 11.2 for windows (Statacorp LP, USA). Pre-test failures (PTFs) were included in the analysis as “0” values for all groups. Analysis excluding PTFs was only done on the group of aging the substrate for “early” time intervals to compare the results with the ones obtained when including PTFs to determine if there is a difference in the results obtained. Analysis excluding PTFs was not done on all the groups to avoid confusion.

3.3 Results

3.3.1 Results comparing between the effect of including and excluding PTFs in the analysis

The results of the effect of aging the substrate (Biodentine™, GIC and RMGIC) for early time intervals when including and excluding PTFs on the μSBS are summarised in Table 3-2.

More PTFs were found in the Biodentine™ group compared to the other groups which resulted in lower variation in the results and higher mean μSBS values when excluding PTFs (Fig 3-3).

Multivariate linear regression found a significant difference among the three materials (P<0.01). The results were consistent when analyses were performed with and without including the PTF's.

- With PTF's included, the mean bond strength was, in relation to Biodentine, 8.6 MPa higher (95% CI. from 4.4 to 12.9) for GIC and 17.9 MPa higher (95% CI. from 13.7 to 22.1) for RMGIC.

- When PTF's were excluded, the mean bond strength was, in relation to Biodentine, 8.4 MPa (95% CI. from 3.6 to 13.2) higher for GIC and 17.7 MPa higher (95% CI. from 13.2 to 22.3) for RMGIC.

Table 3-2: Mean micro-shear bond strength values for the group of aging the substrate for "early" time intervals including and excluding PTFs.

Substrate	SE/TE	Time interval	No. of samples	Number of PTF's	μSBS excluding PTF's (MPa)	μSBS including PTF's (MPa)
Biodentine	SE	t=0 min	5	3	17.85 ±9.9	7.14±10.9
		t=5 min	5	5	0±0	0±0
		t=20 min	5	0	25.73±4.4	25.73±4.4
		t=24HRS	5	1	18.68±4.6	14.94±9.3
	TE	t=0 min	5	2	11.62±5.8	6.97±7.6
		t=5 min	5	5	0±0	0±0
		t=20 min	5	5	0±0	0±0
		t=24HRS	5	0	24.73±2.3	24.73±2.3
GIC	SE	t=0 min	5	1	16.19±3.5	12.948±7.8
		t=5 min	5	3	16.6±1.8	6.64±9.1
		t=20 min	5	0	19.75±4.7	19.75±4.7
		t=24HRS	5	0	26.23±3.9	26.23±3.9
	TE	t=0 min	5	0	19.75±5.8	19.75±5.8
		t=5 min	5	0	22.41±5.9	22.41±5.9
		t=20 min	5	0	23.74±6.9	23.74±6.9
		t=24HRS	5	1	18.47±8.1	14.77±10.8
RMGIC	SE	t=0min	10	1	26.56±3.8	23.90±9.1
		t=24 HRS	10	0	30.05±6.1	30.05±6.1
	TE	t=0min	10	0	28.14±6.4	28.14±6.4
		t=24 HRS	10	1	31.08±5.5	27.97±11.1

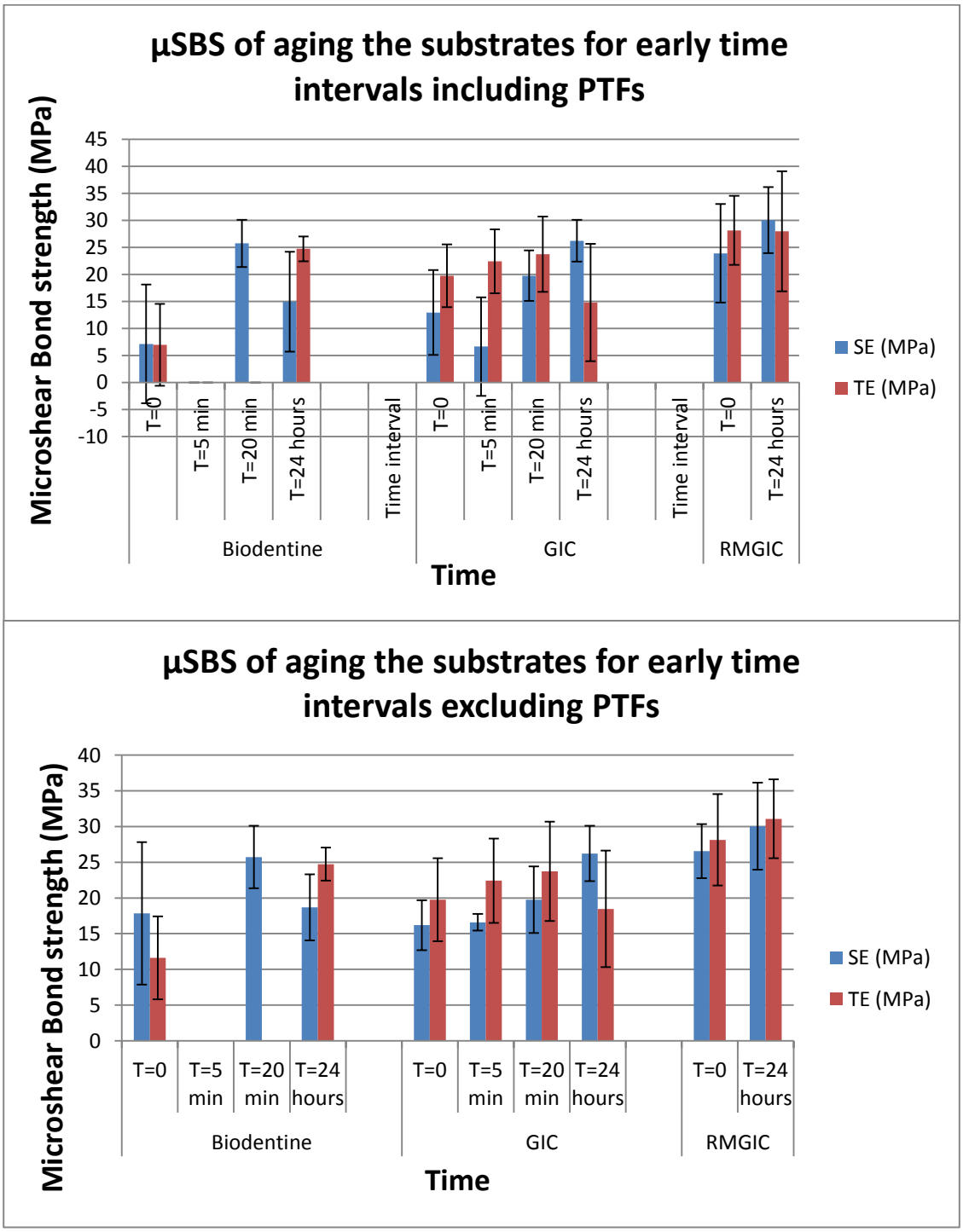


Figure 3-3: Results comparing between the effect of including and excluding PTFs on the mean µSBS values. Error bars depict standard deviations.

3.3.2 Results for all specimens analysed including PTFs

The results of the μSBS to Biodentine™, GIC and RM-GIC are summarised in Fig 3-4. PTFs are presented in the figures and were given a “0” value for statistical analysis.

When taking into account the substrate, a significant increase in the μSBS of GIC and RM-GIC was found compared to Biodentine™ ($P<0.01$). When taking into account the bonding technique, no significant difference was found between the SE and TE adhesive modes ($P=0.42$). When considering the effect of aging the substrate on the μSBS, there was a significant increase in μSBS values in the delayed intervals ($P=0.01$) compared to the early intervals for the Biodentine™ group. Additionally, a high number of PTF’s were observed in the early interval group. No significant difference between the early and delayed intervals were found for the GIC / RM-GIC groups ($P=0.46$, $P=0.51$) respectively.

With regards to the effect of aging the bond on the μSBS, there was no significant difference between the time intervals for aging the bond in both early and delayed aging of the Biodentine™ and RM-GIC groups ($P=0.93$, $P= 0.55$) respectively. However in the GIC groups, there was a significant increase in the μSBS value between aging the bond at 2 weeks and one month time interval in the early aging of the GIC group ($P<0.001$) followed by a decrease in μSBS values which is not statistically significant for the rest of the time intervals. For the delayed aging of the GIC group, there was a significant decrease in the μSBS value when comparing aging the bond at 2 weeks and 1 month ($P=0.0001$) followed by a decrease in μSBS values which is not statistically significant for the rest of the time intervals.

The percentage failure modes are summarised in Table 3-3. The majority of failures (68.82%) occurred cohesively within all the substrates followed by resin-substrate adhesive failures (21.71%). A representative scanning electron micrograph (SEM) of a fractured Biodentine™ sample exhibiting cohesive failure is shown in Fig 3-5.

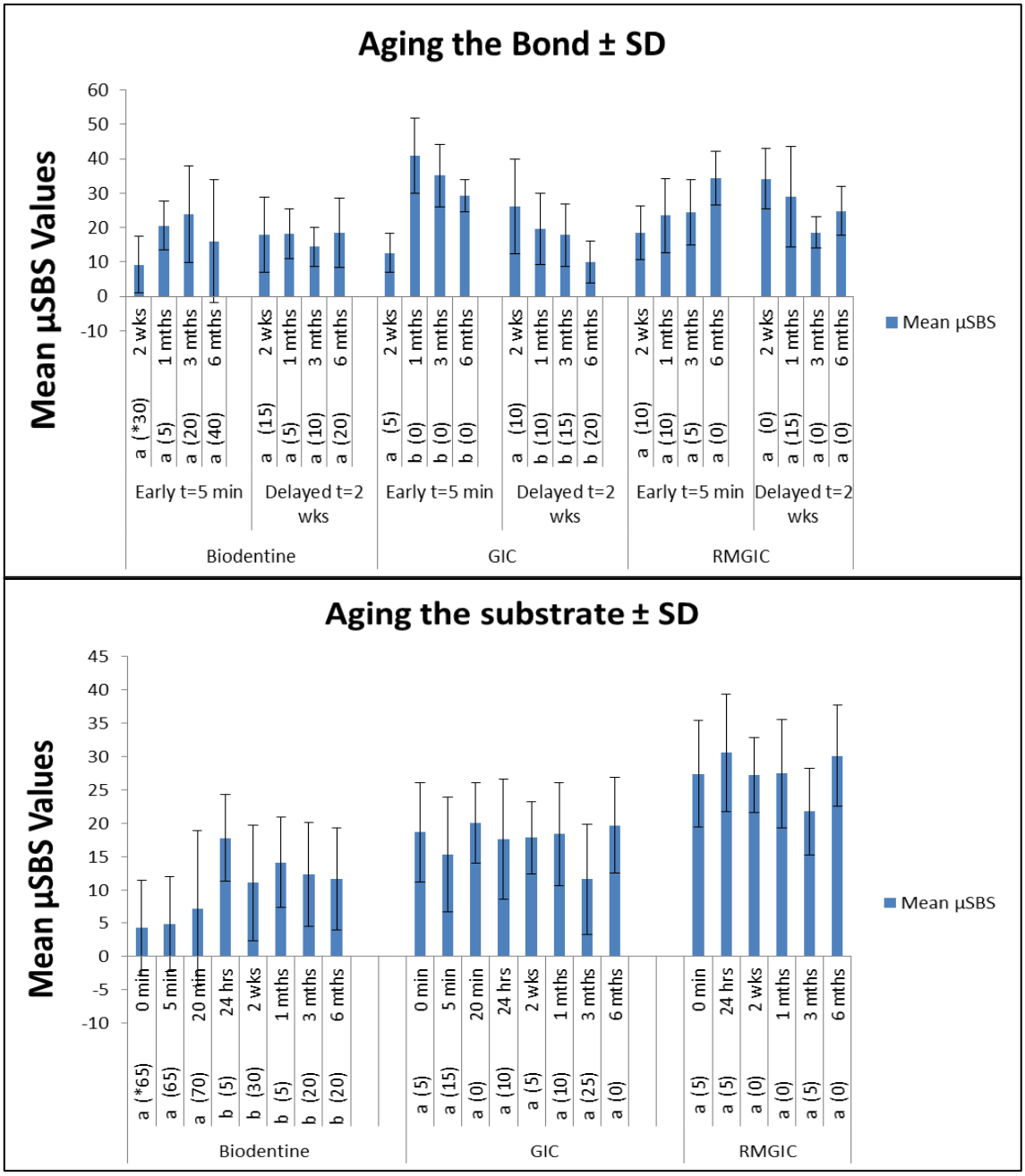


Figure 3-4: Mean µSBS values after aging the substrates and the bond. Bonding modes have been combined. Error bars depict standard deviations. Different letters within each material indicate significant difference in µSBS values between the time intervals. * Percentage of PTFs in parentheses

Table 3-3: Mode of failure of each material.

Failure Mode		Biodentine	GIC	RMGIC	Total
A (Adhesive between resin & substrate)	Count	22	93	80	195
	% within substrate	6.90	30.49	29.20	21.71
A OR C	Count	0	1	0	1
	% within substrate	0	0.33	0	0.11
A OR D	Count	0	0	3	3
	% within substrate	0	0	1.09	0.33
A+D	Count	7	18	19	44
	% within substrate	2.19	5.90	6.93	4.90
B (Adhesive between resin & composite)	Count	0	3	1	4
	% within substrate	0	0.98	0.36	0.45
C (Cohesive within resin)	Count	5	3	6	14
	% within substrate	1.57	0.98	2.19	1.56
D (Cohesive within substrate)	Count	283	179	156	618
	% within substrate	88.71	58.69	56.93	68.82
D+B	Count	1	0	0	1
	% within substrate	0.31	0	0	0.11
E (Cohesive within composite)	Count	1	8	9	18
	% within substrate	0.31	2.62	3.28	2.00
Total	Count	319	305	274	898
	% within substrate	35.52	33.96	30.51	100

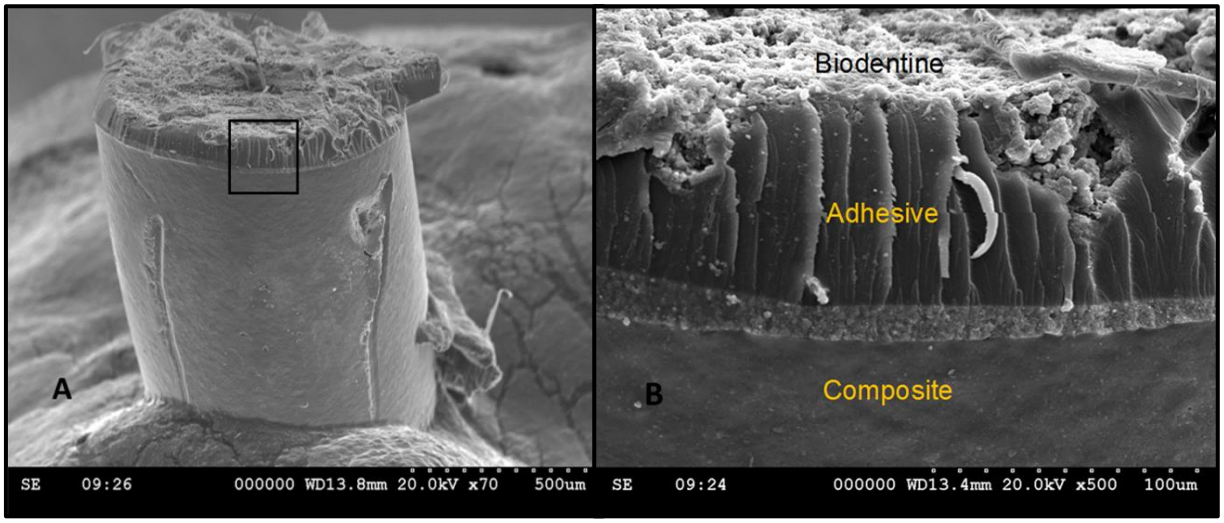


Figure 3-5: (A) SEM image of a self-etch (SE) Biodentine sample representing cohesive failure within Biodentine. (B) The same image magnified at the interface.

3.4 Discussion

Currently, there is limited information in the literature on the interface between Biodentine™ and the overlying adhesive restoration. When comparing the μSBS between Biodentine™, GIC and RM-GIC, the μSBS was the lowest for Biodentine™ and highest for RM-GIC. The high μSBS values for RM-GIC may be attributed to the presence of HEMA in this product which would promote chemical adhesion between the RM-GIC and resin composite (Chadwick and Woolford, 1993). Due to the lack of chemical union between GIC and resin composite, the measured μSBS of GIC would reflect the degree of micromechanical attachment (Chadwick and Woolford, 1993). It is unknown whether a chemical union exists between Biodentine™ and the overlying resin composite restoration, however previous research found the functional monomer 10-MDP, present in the adhesive used in this study, to bind to calcium in tooth structure (Yoshida *et al.*, 2004). Theoretically, it could be assumed that the 10-MDP monomer may bind chemically to the calcium in Biodentine™ hence promoting chemical adhesion in addition to micromechanical attachment.

With regards to aging the substrate, lower μSBS values were found in the early compared to the delayed aging of the Biodentine™ group. Additionally, the majority of pre-test failures (PTFs) were in the early aging period within the Biodentine™ group regardless whether the bond was tested after 24 hours or whether it was tested after aging. All of these PTF's were cohesive within the layer of Biodentine™ closest to the bonded interface. Clinically, this finding must be viewed with caution as this would affect the decision to place the overlying resin composite restoration immediately because the curing contraction of the resin composite may stress the weak Biodentine™ in this early sensitive phase resulting in premature failure. Indeed this is important clinically as Biodentine™ is placed in cavities with a higher C factor as opposed to bonding to one surface which was done in this study. This would highlight the importance of leaving Biodentine™ to mature for a longer time period before the application of the veneering restoration. In this study statistical data showed that the μSBS values of Biodentine™ approached the values of GIC when

composite was bonded to Biodentine™ after 24 hours. However, it takes up to 2 weeks to achieve complete maturation of Biodentine™ and reach its maximum physico-mechanical properties (Bachoo *et al.*, 2013). Therefore clinically, it would be better to leave the material to set for 2 weeks because in addition to the reason stated previously, a follow up appointment would be required as part of efficacious clinical practice to allow monitoring the pulp vitality / response when Biodentine™ is used to treat such deep carious lesions, affecting the pulp.

When considering the effect of aging the bond on the μSBS values of Biodentine™, RM-GIC and GIC, no difference between the different time intervals was found. This is a favorable result as this means the bond does not deteriorate with time up to the 6 month study test period.

In the present study, it was shown that the effect of the bonding technique on the reliability of the bond strength of resin composite to Biodentine™, GIC and RM-GIC was not significant. The consistent use of the same self-etch adhesive in both SE and TE modes eliminated the effect of the addition of another material variable. The similar μSBS values between SE and TE adhesive modes may be due to the porous nature of the Biodentine™ surface which may have nullified the effect of the differences between SE and TE bonding techniques. Furthermore, the acidity from the bonding techniques may have been buffered by the alkalinity of the Biodentine™, also reducing its effect. This result is in line with another study comparing the shear bond strengths of one etch and rinse adhesive and 2 self-etch adhesives to Biodentine™ at 12 min and 24 hour time intervals. No significant differences between all the three adhesive systems at each of the 2 time intervals were found. Higher shear bond strength values were found for the 24 hour interval groups compared to the 12 min time interval group (Odabaş *et al.*, 2013).

In a previous study investigating the effect of etching on Biodentine™ compared with GIC and RM-GIC, structural and chemical changes were found in the etched compared to non-etched Biodentine™, but this did not affect the micro-

hardness of the material. Etching caused surface modifications on the GIC and to a lesser extent on the RM-GIC but with no physical or chemical changes to both materials (Camilleri, 2013).

When assessing the mode of failure, the majority of failures for all materials were cohesive. This finding does not necessarily reflect the true interfacial bond strength between the adhesive resin and the material but rather the cohesive strength of the material itself. The trend toward cohesive failure may be due to the non-uniform stress distribution concentrating in the material substrate leading to its premature failure before the interface itself (Placido *et al.*, 2007). This is an inherent problem associated with micro-shear bond testing where high levels of tensile stress generate below the point of load application and compressive stresses on the opposite side to the point of application (Van Noort, 2008; Armstrong *et al.*, 2010). In addition, the flash of adhesive extending beyond the tested area may have caused a change in the way the stress was distributed during bond testing leading to a distorted nominal bond strength value as the fractured area is usually greater than the interfacial area used to calculate bond strength (Kitasako *et al.*, 1995; Armstrong *et al.*, 2010; Salz and Bock, 2010). However, because of the fine composite cylinder shape with a typical diameter of 0.7 mm, it was impossible to confine the adhesive to the tested area as required by ISO-standard No. 11405 (2003) (Van Meerbeek *et al.*, 2010). An effort to remove the excess adhesive surrounding the test area after removing the Tygon tube and prior to bond testing to try and address this inherent problem was attempted in a pilot study but was found to be ineffective as this compromised the integrity of the fine composite cylinders.

The issue regarding the inclusion or exclusion of PTFs remains to be resolved in the literature as they are not treated in the same statistical manner by different research groups (Scherrer *et al.*, 2010). Including PTFs as zero values in this study stems from the fact that there were many PTFs in the Biodentine™ group and dealing with them as missing data rather than including them in the analysis would result in over-estimating the quality of the bond to this material (Matinlinna and Mittal, 2009). Nevertheless, statistical analysis including and

excluding PTFs was performed on one group of specimens to determine if this would affect the results obtained. As expected, lower variation in the results and higher means were obtained in the group excluding PTFs.

Biodentine™ passes through an initial setting reaction which takes approximately 12 minutes following mixing the powder with the liquid where a hydrated calcium silicate gel structure is formed which has weak physico-mechanical properties. Surface set is achieved at this stage. There is continuous maturation of Biodentine™ where crystallisation of the calcium silicate hydrate gel structure continues for up to two weeks. Bulk set is achieved at this stage with improved physic-mechanical properties (Bachoo *et al.*, 2013). This could explain the trend of increased PTFs in Biodentine™ which are all cohesive in nature when bonding the resin composite at the early setting phase. This also highlights the important role that PTFs play when determining the bond strength of a material and therefore should not be overlooked.

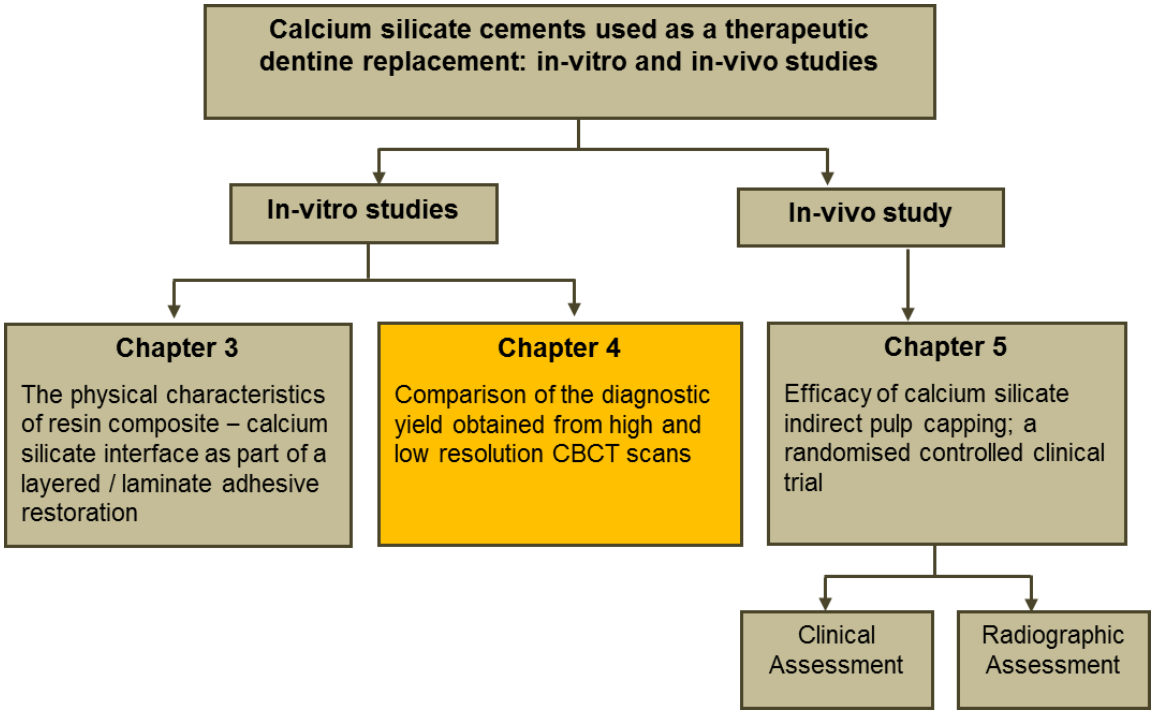
3.5 Conclusion

The null hypothesis stating that there is no difference in the μSBS between SE and TE techniques was accepted. However, the null hypothesis stating there is no difference in the μSBS between the different materials and when applying the bond after different time intervals within each material was rejected.

Biodentine™ is weak in its early setting phase. Placing the overlying composite is best delayed for at least 2 weeks to allow adequate setting/maturation of the Biodentine™ to withstand sufficiently the contraction forces of the resin composite. This would also allow sufficient time to review the tooth if Biodentine™ was placed on symptomatic pulps. A total etch or self-etch adhesive may be used.

CHAPTER 4

An in-vitro comparison of the diagnostic yield obtained from high and low resolution CBCT scans



4.1 Introduction

Cone beam computed tomography (CBCT) has been introduced in response to the high demand for an advanced imaging technique which allows a 3 dimensional view of the oral and maxillofacial region. This technique provides images at a high resolution with lower cost, lower radiation dose, rapid scanning time, ease of handling and accessibility compared to medical CT scans, for improved detection and diagnosis (Patel *et al.*, 2007b; Kamburoğlu *et al.*, 2010). Since the introduction of dedicated dento-maxillofacial CBCT scanners, research has been accomplished and clinical CBCT applications have become more commonplace in dental practice (De Vos *et al.*, 2009). For example, in endodontics, the use of CBCT is becoming more popular as it has been proposed that the technique allows detection of otherwise undetectable apical lesions (Tsai *et al.*, 2012). Studies have also revealed superior diagnostic accuracy in the detection of vertical root fractures (Edlund *et al.*, 2011; Metska *et al.*, 2012), and increased sensitivity in the detection of root perforations allowing earlier diagnosis (Shemesh *et al.*, 2011). The outcome of root canal treatment may also be improved by using this imaging modality (Liang *et al.*, 2011). CBCT may also have a potential use in conservative dentistry helping in improving the accuracy of diagnosis of the condition of the pulp and hence providing the most suitable treatment with a better outcome.

As this diagnostic technology with its increasing applications is being used more commonly in dental diagnostics, a reduction in the radiation dose is essential. The need to keep the radiation dose as low as reasonably achievable (ALARA) is of fundamental importance and the balance between obtaining an optimised diagnostic image and a low radiation dose remains a challenge (Honey and Hogg, 2012).

CBCT scanners are variable in terms of essential exposure parameters including tube current / voltage, exposure time, field of view (FOV) and the extent of rotation of the gantry around the patient's head (Dawood *et al.*, 2012;

Pauwels *et al.*, 2012). This variability results in diverse absorbed radiation doses for patients and is associated with the quality of the image obtained (Martin *et al.*, 1999).

Currently, some CBCT scanners including the Morita Accuitomo (J Morita Corporation, Osaka, Japan) offers a choice of rotation of the gantry at 360° (full rotation of the x-ray tube around the patient's head) or at 180° (half rotation of the x-ray tube around the patient's head). According to the operating parameters used in this study, the absorbed dose for the 360° and 180° rotation is 0.7 and 0.3 mGy, respectively. Although there is an increased radiation dose by approximately more than half with the 360° rotation, higher resolution images are obtained. The aim of this study was to find out if this high resolution image helps improve the accuracy of clinical diagnosis or does it merely expose the patient to a higher radiation dose without diagnostic benefit.

The objective of this study was to investigate the above possibility by comparing linear measurements of clinically relevant anatomical structures viewed on CBCT (Morita Accuitomo) scans taken of sectioned porcine mandibles at 360° (high resolution) and 180° (low resolution) rotation of the tube head to porcine jaw specimens of the same anatomical structures considered as a reference standard.

The null hypothesis was that there is no difference between the measurement accuracy obtained from the CBCT images taken at 360° and 180° rotation of the tube head and when compared to the measurements obtained from the porcine jaw specimens.

4.2 Materials and Methods

4.2.1 Specimen preparation

Twelve fresh porcine hemi-mandibles were sectioned using a handsaw in a vertical and horizontal direction to produce a block section including 2 teeth. This was done to reduce the specimen size to facilitate its placement and stabilisation on the precision saw (Isomet® 1000, Buehler, Lake Buffalo IL). The specimens were sectioned through their mesio-distal long axis using a 600 µm thickness diamond wafering blade (Buehler, USA) with water irrigation (Fig 4-1). The specimens were stabilised in plasticine and a flat glass slab was used to ensure the sagittally sectioned surface of the specimen was parallel with the sagittal CBCT laser.

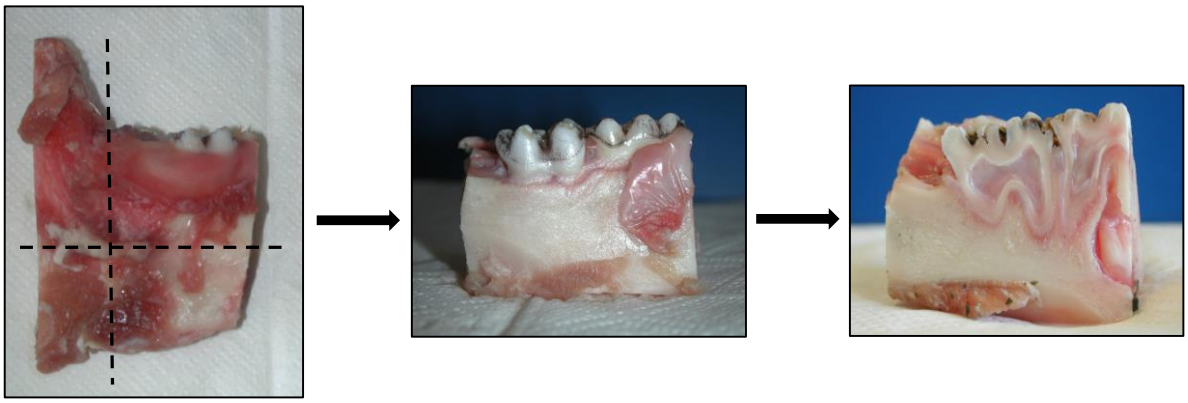


Figure 4-1: The sample was sectioned initially to reduce its size, and then sectioned again in the mesio-distal direction to expose a longitudinal section of the tooth.

4.2.2 Radiographic technique

Each specimen was scanned using the Accuitomo CBCT device (J Morita) at 180° and 360° x-ray tube head rotation while maintaining the following exposure parameters: tube voltage, 60 kV, tube current 2 mA, field of view 40x40 mm, slice angle 0°. All CBCT scans were reformatted to standard manufacturer's settings (0.48 mm slice interval and 0.64 mm slice thickness).

4.2.3 Radiological assessment

The scans were presented using native CBCT software (One-Data viewer, J.Morita) on a 19-inch, 1280 x 1024 pixel TFT-LCD monitor (Hewlett Packard, Houston, USA) with fixed brightness / contrast settings throughout. Six experienced observers (2 radiologists and 4 endodontic specialists), assessed randomised scans and were blinded to whether the image was taken at 360° or 180° tube-head rotation. Each observer measured a series of anatomical structures: dentine thickness (location 1), pulp height from the mesial and distal pulp horn to the apex of the pulp chamber (location 2 and 3 respectively), and periodontal ligament space (PDL) mesially (location 4) and distally (location 5) in each CBCT scan using reference points displayed on a diagram printed from the scan of each specimen to ensure the consistency of the measurement sites for all assessors (Fig 4-2). The measurements were made from the image displayed on the sagittal plane (X-plane). The green sagittal line of the imaging plane was localised to the edge of the sectioned surface of the specimen to ensure the measurements taken on the CBCT image coincided with the measurements taken on the actual reference standard specimen (Fig 4-3). The observers were trained and calibrated before embarking on the assessment. All observers re-took all measurements a second time after 2 weeks to assess intra-operator variability.

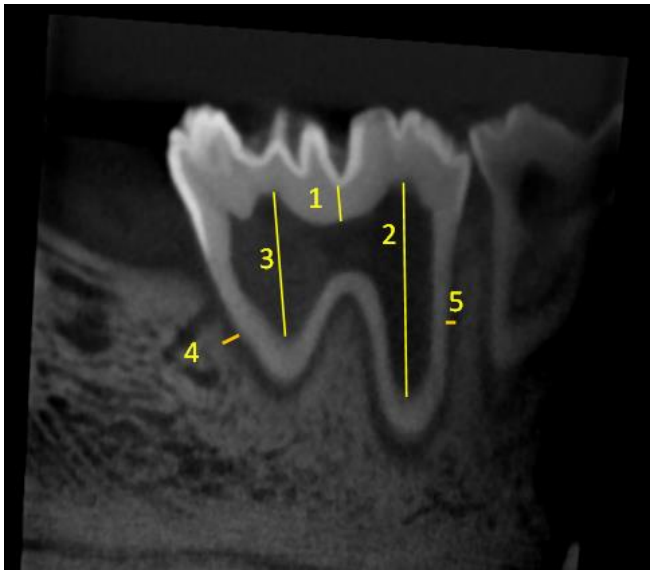


Figure 4-2: Example of the diagram for one of the specimens presented to the observers as a reference for taking measurements on the CBCT scanned image. Written instructions were also included to supplement the diagram for each image.

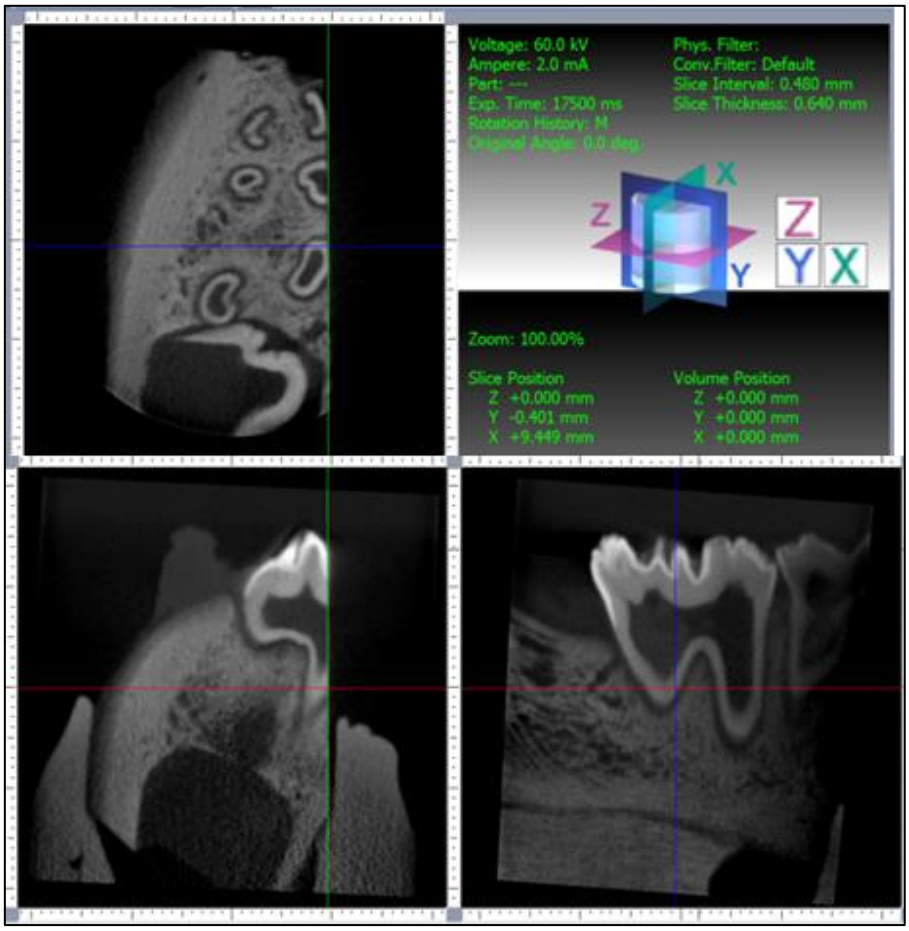


Figure 4-3: The CBCT software One-Data viewer which was used by the observers to take the measurements. The measurements were taken on the lower right image (mesio-distal longitudinal section). Notice the green sagittal line was placed at the edge of the sectioned surface of the sample on the upper and lower left images.

4.2.4 Anatomic assessment

One observer took measurements of the same 5 anatomical structures for each specimen using the same reference points as displayed on a diagram printed from the scan of each specimen to ensure the measurements were taken consistently from the same place and coincided with the measurements taken by the CBCT observers (Fig 4-4). The measurements were taken using a measuring microscope (EZ measurement system, Starett Kinemetric, Laguna Hill, CA) at 7x magnification. The measurements were repeated after 2 weeks to assess intra-operator variability.

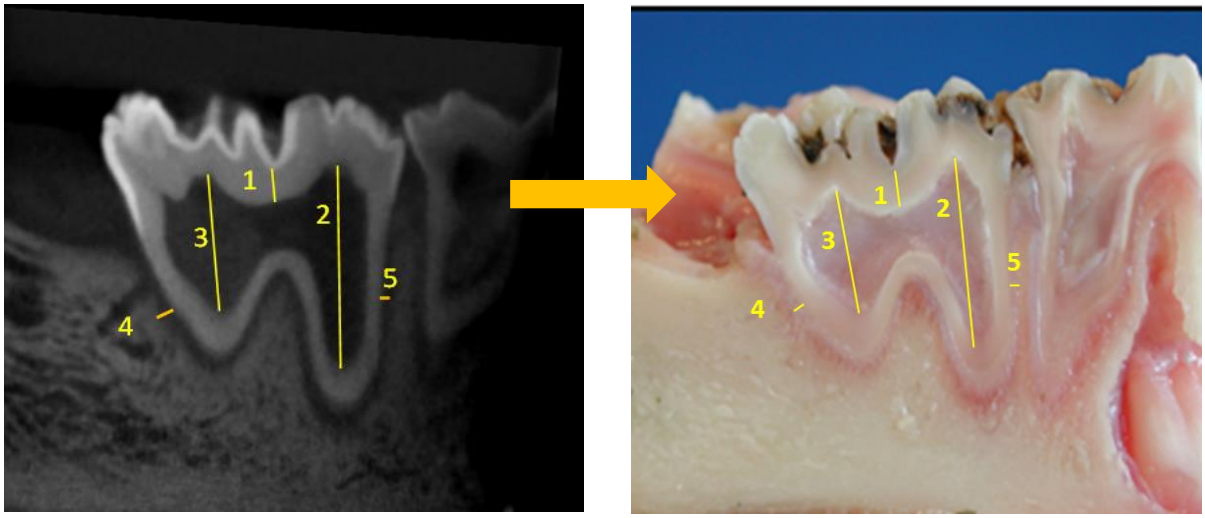


Figure 4-4: Measurements taken on the specimen corresponding to the same measurements taken on the CBCT scan of the specimen.

4.2.5 Statistical analysis

Multilevel modelling was used to assess the agreement between direct measurements of the specimens and each of the CBCT scans (360° vs 180°). Intra-observer reliability (based on two repeats) and measurement of agreement between observers were analysed using intra-class correlations. Six observers took continuous measurements on the CBCT scans for 24 teeth yielding a total of 120 observations per rotation (high/low). This guaranteed 80% power, at the 5% significance level, to detect a difference in the intra-class correlation coefficient of 0.30 and above vs. a null value of 0.10.

4.3 Results

Intra-class correlations (ICC) indicated high intra- and inter-observer agreement in all locations (0.65-0.98 and 0.79-0.98), respectively (Table 4-1).

Table 4-1: Intra-observer and inter-observer agreement. The first line in each location reveals the intra-observer agreement, while the second line reveals the inter-observer agreement. * Confidence interval

	Anatomical reference		**180 Rotation**		**360 Rotation**	
Location	ICC	95% CI*	ICC	95% CI.	ICC	95% CI.
1	0.88	(0.75, 0.99)	0.66 0.79	(0.45, 0.86) (0.62, 0.95)	0.72 0.84	(0.54, 0.90) (0.71, 0.97)
2	0.98	(0.97, 0.99)	0.93 0.96	(0.88, 0.98) (0.93, 0.99)	0.91 0.95	(0.85, 0.98) (0.92, 0.99)
3	0.98	(0.97, 0.99)	0.98 0.98	(0.97, 0.99) (0.97, 0.99)	0.97 0.98	(0.95, 0.99) (0.97, 0.99)
4	0.98	(0.97, 0.99)	0.97 0.97	(0.95, 0.99) (0.96, 0.98)	0.97 0.97	(0.94, 0.99) (0.96, 0.99)
5	0.86	(0.71, 0.99)	0.65 0.79	(0.45, 0.85) (0.63, 0.94)	0.68 0.81	(0.49, 0.88) (0.66, 0.96)

The mixed regression models (Table 4-2) quantified the differences between each rotation with the anatomical measurements of the specimens and between the two rotations.

In location 2, there was a significant difference between rotation at 180° and the anatomical measurements on the specimens. The measure was on average 0.47 mm more with rotation 180° than for the anatomical measurements (P=0.02). The rotation at 180° yielded a measure that was on average 0.33 mm more with rotation 360°. This difference was found to be borderline significant (at the 10% significance level).

In location 3, there was a significant difference between 180° and 360° rotations and the anatomical measurements (P=0.001 and P=0.01 respectively). In both cases, CBCT scans yielded a significant larger measurement. There was no significant difference between the two rotations.

Table 4-2: Differences between the rotations and in relation to the anatomical reference *Significant difference, AR* Anatomical Reference. Raw data presented in appendix 10.

	Percent change	Mean difference	95% CI for mean difference		P-value
Location = 1					
180 vs AR*	-1.5%	-0.03	-0.10	0.04	0.41
360 vs AR	-0.8%	-0.02	-0.09	0.05	0.64
360 vs 180	0.7%	0.01	-0.06	0.08	0.71
Location = 2					
180 vs AR	5.6%	0.47	0.09	0.85	0.02*
360 vs AR	1.7%	0.14	-0.24	0.52	0.46
360 vs 180	-3.7%	-0.33	-0.71	0.05	0.09
Location = 3					
180 vs AR	7.2%	0.60	0.25	0.95	0.001*
360 vs AR	5.5%	0.46	0.11	0.81	0.01*
360 vs 180	-1.6%	-0.14	-0.49	0.21	0.43
Location = 4					
180 vs AR	-2.3%	-0.02	-0.09	0.05	0.60
360 vs AR	4%	0.03	-0.04	0.10	0.37
360 vs 180	6.4%	0.05	-0.02	0.12	0.16
Location = 5					
180 vs AR	-5.1%	-0.04	-0.14	0.07	0.50
360 vs AR	-4.9%	-0.04	-0.14	0.07	0.51
360 vs 180	0.1%	-0.004	-0.11	0.11	0.99

4.4 Discussion

In this study 2 CBCT scans / specimen were taken at 360° and 180° rotations while the remaining exposure parameters where standardised. Although both rotations have the same voxel resolution (0.125mm), the 180° rotation reduces the number of projections, hence reducing the exposed radiation dose. However, this results in a lower resolution image due to the reduced number of slices used for image reconstruction compared to the 360° rotation scan.

This study evaluated the image quality obtained using both rotations by visual measurement of the reproduction of clinically relevant anatomical structures, which is important as detection of pathology correlates with the accuracy of reproduction of anatomy (CEC, 1996; Båth and Månsson, 2007). A limitation to this study was that measurements were taken with reference to a specific anatomic plane, rather than the clinical situation where anatomy would normally be superimposed by other structures. Establishing a 3-dimensional model was not possible in this investigation as this would have made accurate comparisons of measurements taken from CBCT slices and anatomic specimens difficult.

There was no statistically significant difference between the measurements taken with the 180° or the 360° tube-head rotation. Clinically, this could be interpreted as there being no difference in the diagnostic yield between the two scan rotations. This compares favourably with a previous study which compared the effect of using 180° or 360° rotation of the CBCT tube head and periapical radiographs in detecting simulated external inflammatory root resorption lesions in dry human mandibles (Durack *et al.*, 2011). The small volume Accuitomo CBCT scans operating with 360° rotation were no better at detecting the lesions than the same scans taken with 180° of rotation and as such could not be justified in a clinical setting. This study however, was limited to the detection of simulated external inflammatory root resorption lesions and a general consensus on the use of 180° rotation could not be extrapolated from this study.

Another study examined the reduction of effective radiation dose to patients receiving implants by comparing different exposure parameters including 180° / 360° rotation comparison (Dawood *et al*., 2012). It concluded that the diagnostic yield of the 360° CBCT scans was not the highest and that there was a potential for reducing the radiation dose by using lower exposure parameters including the 180° rotation. However, there was no direct comparison between the same image at 180° and the 360° rotation as the comparisons between the rotations were made on different patients.

There were no differences in the measurements between the two rotations and the porcine jaw specimens in most locations in the present study. This is in agreement with a previous study comparing accuracy of linear measurements of alveolar bone taken on Accuitomo CBCT images, Multi slice CT images, and direct measurements on an ex vivo model. No significant difference between the CBCT measurements, multi slice CT measurements and the physical measurements taken directly on the specimen were observed (Loubele *et al.*, 2008). However, the difference found in the present study at location 2 and 3 seem to yield longer CBCT measures, approximately 5.5-7.2% increase compared to the anatomical measurements. Both locations 2 and 3 correspond to the pulp chamber height measured from the pulp horn to the apex of the pulp chamber distally and mesially respectively. This may be due to a consistent error in the interpretation of where the apex was as the anatomical region appeared blurred even in the 360° scan. Decurcio et al (Decurcio *et al.*, 2012) compared measurements of root canal filling on CBCT images and directly on the root specimens. The measurements on the CBCT images were greater than the original root specimens. This increase ranged from 9% to 100%. This has been attributed to the higher density of the root canal filling material which may have produced artefacts causing errors of interpretation (Decurcio *et al.*, 2012).

The intra- and inter-observer agreement in this study was good to excellent with no difference between the two rotations, which demonstrates the consistency of the results obtained. This is in agreement with a study which found the internal

reliability of their CBCT assessors using ICC to be in the range of 0.57-0.87 and the inter-observer agreement to be in the range of 0.72-0.93 (Tsai *et al.*, 2012). This is confirmed in another study where the intra- and inter-observer kappa scores for CBCT were 0.85-0.89 and 0.64-0.67 respectively (Durack *et al.*, 2011). Another found the intra-observer reliability using the Spearman rho statistic to range between 0.89-0.98 (Kumar *et al.*, 2011). Patel *et al.*(2009) found the mean intra- and inter-observer kappa value to range between 0.72 and 0.64 respectively. Although not directly comparable with the ICC results for this study, they do seem to suggest a similar level of agreement.

Within the limitations of the present study, the results demonstrate that when all other exposure parameters are constant, the accuracy of measurements obtained from limited volume CBCT scans with 360° rotation is no different than that from 180° rotation. This might imply that adequate diagnostic images can be obtained using 180° rotation with minimum radiation dose to the patient.

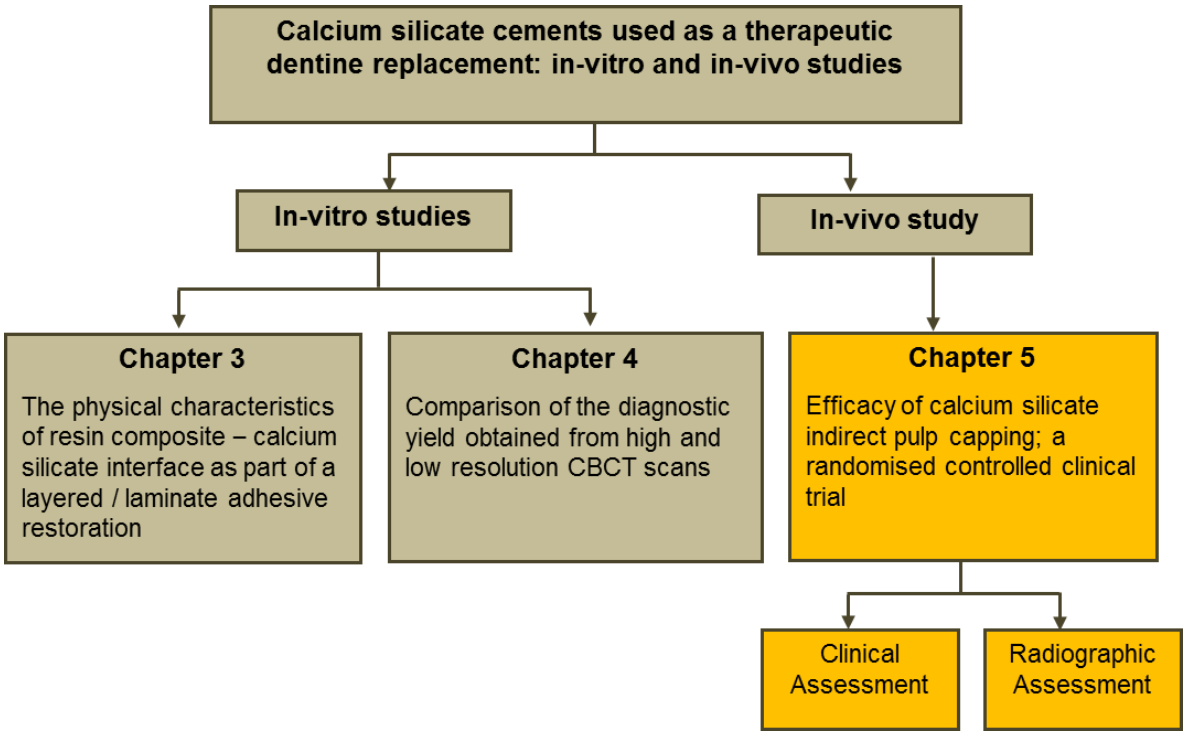
4.5 Conclusion

The null hypothesis stating there is no difference between the measurement accuracy obtained from the CBCT images taken at 360° and 180° rotation of the tube head compared to the porcine jaw specimen measurements obtained from an equivalent anatomical plane, was accepted.

A CBCT scan with detail sufficient to make a sound clinical judgment with reduced radiation dose to the patient may be obtained by using 180° rotation of the CBCT tube head.

CHAPTER 5

Efficacy of calcium silicate indirect pulp capping; a randomised controlled clinical trial



5.1 Introduction

There are several treatment methods available for the management of the pulp in extensively decayed teeth. These range from pulp capping procedures to root canal treatment. Making a treatment decision is significant as this action may be irreversible especially if endodontic therapy is the treatment of choice (McComb, 2001). Indirect pulp capping is the most conservative allowing the pulp to recover and maintain its function and vitality (Tziafas *et al.*, 2000). Furthermore, should the treatment be unsuccessful there is still the opportunity to carry out endodontic therapy. The choice for indirect pulp capping treatment however is dictated by the condition of the pulp. Making a precise diagnosis of the pulp condition has always been clinically challenging as there is no reliable objective method of evaluating the pathological condition of the pulp or the extent of the pulpal inflammation. Identifying reversible or irreversible pulpitis depends upon the patient's descriptions of the subjective symptoms, pulp sensibility testing, and radiographic examinations (Bjørndal, 2002). Additionally, treating such cases with deep carious lesions can prove to be challenging especially when approaching the pulp as an increased risk of pulp exposure reduces the predictability of the treatment outcome (Barthel *et al.*, 2000; Bjørndal *et al.*, 2010; Dammaschke *et al.*, 2010).

Minimally invasive (MI) principles of operative dentistry have encouraged research and development of bioactive materials such as Biodentine™ (Septodont, Saint Maur des Fosses, France) which can be used both for pulp capping and provisional restoration (Hilton, 2009; de Souza Costa *et al.*, 2011). Biodentine™ has been shown to form complete dentine bridge formation with an absence of inflammatory pulp response through the secretion of TGF-b1 from pulp cells that help induce reparative dentine formation (Laurent *et al.*, 2012a; Tran *et al.*, 2012; Zanini *et al.*, 2012; Nowicka *et al.*, 2013). Glass ionomer cements although not bioactive, have been available for a longer period of time and have been used traditionally in similar situations as liners,

sealers, and dentine replacement materials as in the sandwich technique where there is no pulp exposure (Sidhu, 2011). The glass ionomer cement Fuji IX™ has been shown to have good biocompatibility with minimal cytotoxic effect on the pulp cells (Six *et al.*, 2000; de Souza Costa *et al.*, 2003b; Ngo *et al.*, 2006). Fuji IX™ has also been found to contribute to the remineralisation of carious dentine through the release of fluoride and strontium ions into the dentine (Ngo *et al.*, 2006).

Both Biodentine™ and Fuji IX™ are not without limitations. While their therapeutic effect is well known, they are weak materials and are exposed to wear under load with time in addition to their poor aesthetic properties (Davidson, 2006; Bachoo *et al.*, 2013). Therefore, an overlay restoration is required to provide mechanical strength, wear resistance, and improved esthetics. Resin composite is a popular choice as it is aesthetically pleasing and adheres well to glass ionomer cements (van Dijken, 1994) and potentially to Biodentine™ (Bachoo *et al.*, 2013).

Previous investigations found CBCT to be more sensitive than intra-oral periapical (PA) radiographs in detecting the presence of periapical radiolucencies in non-vital teeth requiring root canal treatment (Estrela *et al.*, 2008; Patel *et al.*, 2009a; Paula-Silva *et al.*, 2009; Patel *et al.*, 2011). To date, the ability of CBCT to detect periapical changes in vital teeth diagnosed with reversible pulpitis using the available clinical and intra-oral radiographic methods has not been investigated. This may prove to be important as CBCT in such cases might provide the closest correlation to the histopathological status of the pulp aiding in providing a more accurate diagnosis than the traditional methods. Hence what is believed to be reversible pulpitis might in reality be irreversible pulpitis if a periapical lesion was found in the pre-operative CBCT scan. This would subsequently have an impact on the treatment decision and outcome.

Randomised controlled clinical trials provide the best evidence for the performance of a dental restoration, success or failure of a procedure or technique (Pihlstrom and Barnett, 2010). The “Ryge” or “USPHS” criteria with its many modifications have been used extensively for the assessment of dental restorations in clinical trials (Cvar and Ryge, 2005). The “FDI” criteria were introduced in 2007 with not many studies adopting these criteria to date although they do provide a more sensitive method of assessment with enhanced discriminative power especially useful in short term clinical trials compared to the USPHS criteria (Hickel *et al.*, 2007a). This could be because long term clinical studies which have already used the USPHS criteria are committed to completing their trials using the same criteria (Hickel *et al.*, 2010). Furthermore, the FDI criteria might appear to be complex and time consuming for researchers preventing them from adopting these criteria in clinical trials. Up to date, there is no available information comparing between these criteria in permanent teeth.

This randomised controlled clinical trial following CONSORT guidelines (Appendix 4) aimed to:

1. Investigate clinically and radiographically the dentine-pulp response to calcium silicate cement (Biodentine™) compared to glass ionomer cement (Fuji IX™) used as indirect pulp capping agents in teeth with symptoms of reversible pulpitis.
2. Assess the effectiveness of CBCT to detect early periapical changes associated with teeth diagnosed with reversible pulpitis which would otherwise not be detected using routine PA radiographs and monitor these changes if present over a one year period post-restoration placement.
3. Assess the integrity of the resin composite restoration overlying Biodentine™ compared to Fuji IX™ using both USPHS and FDI criteria.

4. Compare the efficacy of the USPHS criteria compared to the FDI criteria in the assessment of the resin composite restorations overlying both pulp capping agents.

The null hypotheses investigated were:

1. There is no difference clinically and radiographically in the dentine-pulp response between Biodentine™ and Fuji IX™ when used as indirect pulp capping agents in teeth with symptoms of reversible pulpitis.
2. There is no difference in the effectiveness of CBCT and PA radiographs in detecting periapical changes in teeth diagnosed with reversible pulpitis.
3. There is no difference in the integrity of the resin composite restoration overlying Biodentine™ compared to Fuji IX™ using both USPHS and FDI criteria.
4. There is no difference in the efficacy of the USPHS criteria compared to the FDI criteria in the assessment of the resin composite restorations.

5.2 Materials and Methods

5.2.1 Study design and sample size

This single blinded, two-arm, randomised controlled clinical trial compared tri-calcium silicate cement (Biodentine™, Septodont, Saint Maur des Fosse´s, France) as the test material and glass ionomer cement (Fuji IX™ GP, GC Corporation, Tokyo, Japan) as the positive control. The study could not be blinded to the operator placing the restorations due to the different consistency/appearance of the two materials.

The study was reviewed and approved by the London-Westminster research ethics committee (11/LO/1893) and the GSTFT NHS R&D office (Appendix 5 & 6). The study was conducted in compliance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP). Patient information sheets were distributed and informed written consent obtained prior to the implementation of the study (Appendix 7 & 8).

Based on a previous similar study (Falster *et al.*, 2002), the study was designed to have 80% power to detect a difference between the two materials whose proportion of failures were assumed to be 1% and 22% over a period of 1 year. A conventional design would require a sample of 72 restorations to detect the difference at 5% level of significance using the z test for testing two independent proportions. An anticipated loss to follow up of 10% was included in the analysis.

The patients were recruited from clinics at King's College Dental Institute at Guy's Hospital, London, UK. Randomisation was performed centrally by the Biostatistics Unit using tabular randomisation and the materials were coded as Material A and Material B. The randomisation unit was the tooth, and the size of the cavity (one wall affected, two walls, or more) was considered a prognostic factor and was hence taken into consideration during randomisation. Inclusion and exclusion criteria are presented in Table 5-1. Intensity of the pulp symptoms was recorded. Patients' description of sensitivity to hot/cold/sweets lasting for up to 15-20 seconds and settling on its own were considered mild while increased sensitivity lasting for more than minutes and needing pain killers at times were considered severe. Any teeth with symptoms indicating irreversible pulpitis such as dull throbbing pain, sharp spontaneous pain, or pain exacerbated by lying down were excluded. Patients were withdrawn in the case of pulp exposure at baseline requiring conventional root canal treatment.

Table 5-1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Patients male or female over the age of 18 in good general health. 2. A minimum of one deep carious lesion penetrating three quarters or more into the dentine as identified with the PA radiograph. Clinically mICDAS score 4 (Banerjee and Watson, 2011). 3. Clinical symptoms of reversible pulpitis. 4. Positive pulp response to electric pulp test or thermal stimulation. 5. No periapical changes viewed on PA radiographs	1. Clinical symptoms of irreversible pulpitis requiring endodontic treatment. 2. The presence of fistulas or swelling. 3. Mobile teeth or tenderness to percussion 4. Anterior teeth with aesthetic concerns. 5. Pregnant women, in view of requirements for radiographs. 6. Patients younger than 18. 7. Patients unable to give consent.

5.2.2 Interventions

The single clinical operator was trained to ensure standardisation of the MI operative procedures. Methods of clinical assessment included pulp status evaluation using electric pulp test (Kerr Vitality Scanner 2006, SybronEndo, Orange Co., Calif., USA) and thermal test (Roeko Endo-Frost, Coltène/Whaledent, Germany), palpation and percussion tests, along with the presence of signs of inflammation (pain, abscess, sinus tract, and abnormal mobility). PA radiographs were taken at baseline (T0) and assessed to exclude any signs of irreversible pulpitis such as widening of the PDL or lesions. CBCT was also taken at T0 but not assessed at this stage to avoid bias in the diagnosis.

Caries removal was carried out under local anaesthetic and rubber dam isolation using a standardised MI operative protocol: superficial soft infected dentine was excavated using carbon-steel rose-head burs (Ash instruments, Dentsply, Gloucester, UK) in a slow-speed WA56A handpiece (W&H Dentalwerk Bürmoos GmbH, Bürmoos, Austria) and hand excavator instruments, after gaining appropriate access through the cavitated enamel using high speed TA-98 hand-piece (W&H Dentalwerk GmbH, Bürmoos, Austria) under copious irrigation. Deeper caries-infected/affected dentine present more than three quarters the thickness of dentine (Bjørndal, 2008b) was removed using chemo-mechanical gel and hand instruments (Carisolv™, Rubicon Lifesciences, Gothenburg, Sweden) to aid consistency in the quantity of caries removal between the different teeth (Fig 5-1A,B). Residual caries-affected dentine following chemo-mechanical gel treatment was retained on the pulpal aspect of the cavity as any additional pressure with hand excavators will lead to pulp exposure (Fig 5-1C)(Fig 5-2) (Kerkhove *et al.*, 1967).

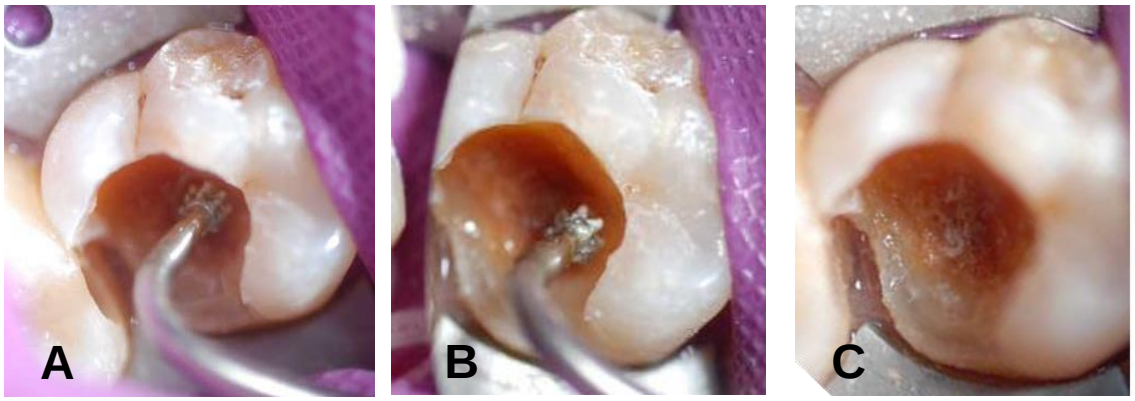


Figure 5-1: (A,B) Removal of deeper caries infected/affected dentine from the upper right 6 using Carisolv™ gel and hand instrument. (C) Residual caries affected-dentine retained on the pulpal aspect of the cavity.

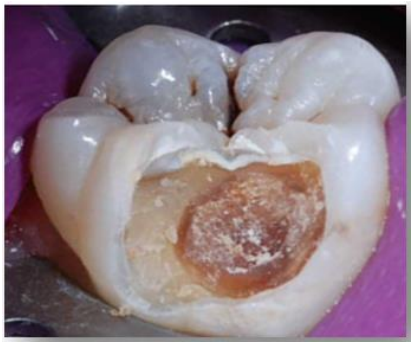


Figure 5-2: Residual caries affected-dentine retained on the pulpal aspect of the cavity on the lower right 6. (Carious fissure subsequently removed).

Material randomisation was done following completion of caries removal and identifying the size of the cavity. Each tooth was fully restored according to the relevant manufacturer's instructions. The definitive resin composite veneer restoration (N'Durance®, Septodont, Louisville, USA) was placed one month after baseline in a "closed sandwich" technique where clinically achievable. A standardised bonding procedure was followed using a total-etch adhesive for both groups, following manufacturer's instructions (Scotchbond™ Universal, 3M ESPE, USA). Follow-up was longitudinal at T1, T6, T12 months (±2 week) intervals. PA radiographs and CBCT were repeated at T12.

5.2.3 Radiographic assessment

Digital PA radiographs using Vistascan phosphor plates were taken with a dental X-ray machine (Heliodent, Sirona, Bensheim, Germany) using a paralleling technique with Rinn film holders permitting standardisation, in addition to small-volume (40 mm^3) CBCT scans at 0.125mm resolution and no dose reduction programmes (Accuitomo, J Morita Corporation, Osaka, Japan). Exposure parameters at T0 and T12 were standardised for each patient. For each tooth, the CBCT scan that best confirmed the presence / absence of PA radiolucency in the sagittal, coronal and/or axial planes was selected, following manipulation of the dataset to optimise slice position, by an experienced clinician (Fig 5-3). The PA and CBCT images were viewed as a Keynote

presentation (Apple, Cupertino, CA, USA) on a laptop computer (MacBook Pro; Apple), with a 15.5-inch backlit LED screen (1680x1050 pixel resolution) in a quiet, dimly lit room (Fig 5-4).

A consensus panel of two trained, calibrated experienced endodontists assessed the CBCT and PA radiographs jointly. The reliability of the consensus panel was evaluated by jointly repeating the assessment of the radiographic images after 4 weeks. The inter-examiner agreement was evaluated by individual randomised assessment of 50% of the PA and CBCT images and repeated after 4 weeks.

The paired images of the roots of each tooth were viewed together by examiners blinded as to which image was taken at T0 / T12 by concealing the crowns of the teeth. Each root was examined for the presence, absence, change (increase/decrease) in size of any PA radiolucency. A *PA radiolucency* referred to widening of the periodontal ligament (PDL) space or a PA lesion. *Widening of the PDL space* was defined as less than double that of the equivalent healthy PDL space as compared to the PDL space of the adjacent healthy tooth. A *PA lesion* was defined as radiolucency associated with the radiographic apex of the root, at least twice the width of the PDL space (Fig 5-5) (Low *et al.*, 2008; Bornstein *et al.*, 2011).

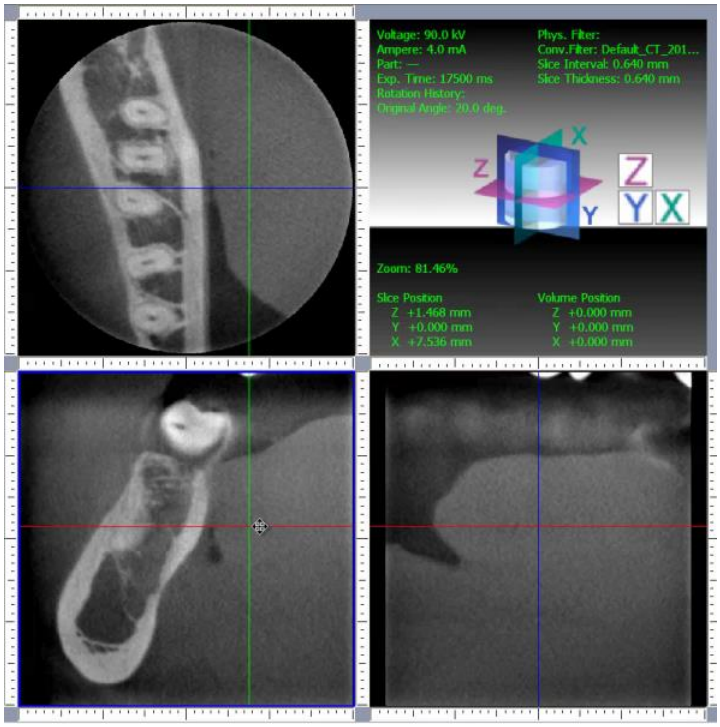


Figure 5-3: Selection of the CBCT slice that best confirmed the presence / absence of PA radiolucency in the sagittal, coronal and/or axial planes.

Figure 5-4: T0 and T12 CBCT images randomised to blind examiners (lower left 6)

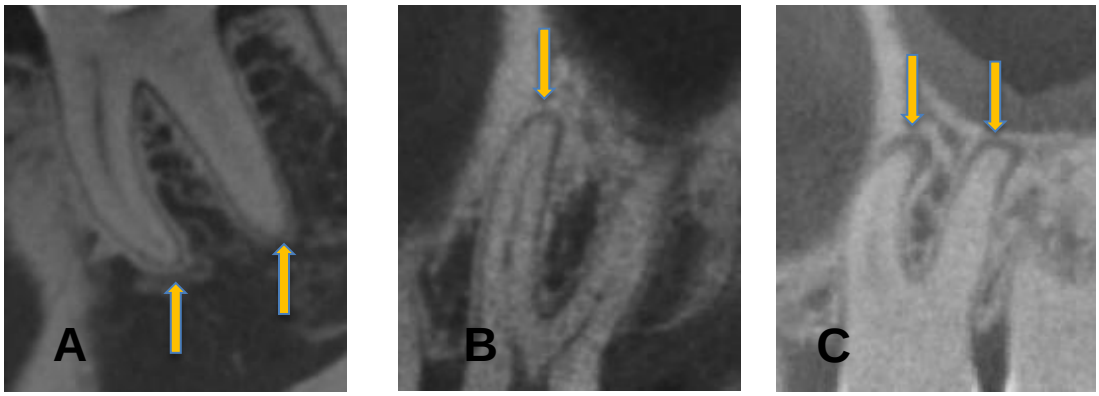


Figure 5-5: (A) Healthy: Normal PDL space (lower left 7). (B) Widening: Increased PDL space less than double the equivalent healthy PDL space (upper left 7). (C) Lesion: Radiolucency associated with the apex of the root at least twice the width of the PDL space (upper left 6).

5.2.4 Clinical assessment of the restoration

In addition to the clinical evaluation of the pulp status during the follow-up visits at T1, T6, and T12 months, assessment of the resin composite restoration overlying Biodentine™ and Fuji IX™ was carried out by two experienced, trained, and calibrated examiners not involved in the operative procedures. Both were blinded to the treatment materials beneath the resin composite restorations. The two examiners recorded the results independently at the same appointment and any disagreement resolved immediately by discussion. Two assessment criteria were used: Modified USPHS criteria based on (Palaniappan *et al.*, 2009) and the FDI criteria (Appendix 2 & 3). The surfaces were dried with air stream before evaluation. Assessment was carried out under ample lighting using a mirror and probe and evaluation of the contact points was done using waxed dental floss in a reproducible manner. A proximal contact point has physiological strength when the dental floss can pass through it and was evaluated for a certain degree of resistance or “snap” effect. No qualitative or quantitative wear measurement was carried out as this was outside the scope of this study and there are many studies available in the literature that discuss this aspect in details. A photograph of the restoration was taken at each follow up visit.

5.2.5 Statistical analysis

The first outcome of the study was a binary variable indicating whether the restored tooth failed to maintain its vitality at T12. Clinical success was evaluated by a positive response to cold test and electric pulp testing, absence of spontaneous pain, negative sensitivity to percussion, absence of sinus/fistula/swelling and abnormal mobility, and absence of PA radiolucencies as determined by PA radiographs. The second outcome was that CBCT scans can either detect or not detect the presence of early PA lesions following IPC in patients with symptoms of reversible pulpitis. The third outcome was that the integrity of the composite resin restoration overlying Biodentine™ is either as good as Fuji IX™ or better or worse. The fourth outcome of the study is that FDI criteria are as effective as or more effective than the modified USPHS criteria.

Descriptive statistics were used to summarise various study variables. Using IBM SPSS Statistics version 22 (IBM, USA) for the clinical results, Chi-square test/ Fisher's exact test was used to assess the association between vitality and other measures (material, extent of cavity, intensity of symptoms, gender). Mann Whitney test was used to test the mean age between vital and non-vital groups. Logistic regression was used to find out the effect of these variables on vitality. If any of the above mentioned measures were significant at liberal 10% level in the univariate analyses, then these were included along with material in the logistic regression model. The final logistic model contained vitality as dependent variable and intensity of symptoms and material as predictor variables. The proportion healed in Bidentine™ and GIC was compared using Z test for proportions.

Radiographic assessment included Kappa analysis to evaluate intra-consensus panel agreement and inter-examiner agreement. The association between the periapical changes in CBCT with various measures (age, gender, vitality, intensity of symptoms, cavity size, and material) were assessed using chi-square test / Mann Whitney test. Further multivariate analysis using logistic

regression was carried out to find out the significant predictors of periapical changes. Sensitivity, specificity, positive and negative predictive values and overall diagnostic accuracy were calculated using CBCT results as reference standard. The significance level was set to $p<0.05$.

With regards to the criteria used to assess the restoration, intra and inter examiner variability was calculated using Fleiss's kappa by considering different ratings for each material. The integrity of the composite restorations overlying both materials assessed using the two systems were summarised using percentages. The efficacy was analysed by comparing the proportion of assessment using USPHS and FDI criteria for Biodentine and GIC. The assessment for each category in both criteria was compared between the materials using Z test for proportions for each rating separately. Statistical significance was considered at 5% level.

5.3 Results

5.3.1 Clinical assessment of the dentine-pulp response to Biodentine™ & Fuji IX™

72 restorations (36 Biodentine™ and 36 Fuji IX™) were placed in 53 patients (21 (39.6%) females and 32 (60.4%) males) with a limit of 2 restorations per person. Age ranged between 18-76 years (median age, 28 years). The majority of restorations (85%) were placed in molars. Clinical success rates for both Biodentine™ and Fuji IX™ were equal (83.3%). 12 teeth had failed to maintain vitality by T12 (6 Biodentine™/ 6 Fuji IX™). Among 53 patients, 5 patients with 6 restorations dropped out of the trial failing to attend subsequent appointments (Fig 5-6). The total number of teeth analysed excluded the drop outs (n=66). An example of two completed Biodentine™ and Fuji IX™ cases are presented in Fig 5-7 and Fig 5-8.

Chi-square test / Fisher's exact test showed no significant statistical differences between pulp vitality and the type of restorative material used (p=0.91), cavity extent (p=0.41), gender (p=0.33), or age (p=0.99). The distribution of the intensity of symptoms between each material group at T0 was not equal (Table 5-2). A significant correlation was found between pulp vitality and intensity of symptoms (p=0.01). In patients suffering from mild reversible pulpitis at T0, 4 teeth (9.75%) became non-vital at T12 whereas in patients suffering from severe symptoms of reversible pulpitis at T0, 8 teeth (32%) became non-vital.

Table 5-2: Clinical assessment of tooth vitality and symptoms distribution for Biodentine™ and Fuji IX™.

Material	**Biodentine™ (n/total)**		**Fuji IX™ (n/total)**		**Total**
Symptoms at T0	Mild	Severe	Mild	Severe	
Vital (at T0)	21/66	10/66	18/66	17/66	66/66
Non-vital (at T12)	3/66	3/66	1/66	5/66	12/66
Lesions at T0	4/60	4/60	3/60	5/60	16/60
Lesions at T12	1/52	1/52	4/52	3/52	9/52
Healed lesions	4/52	1/52	0/52	1/52	6/52
New/progressed lesions	1/52	1/52	4/52	3/52	9/52

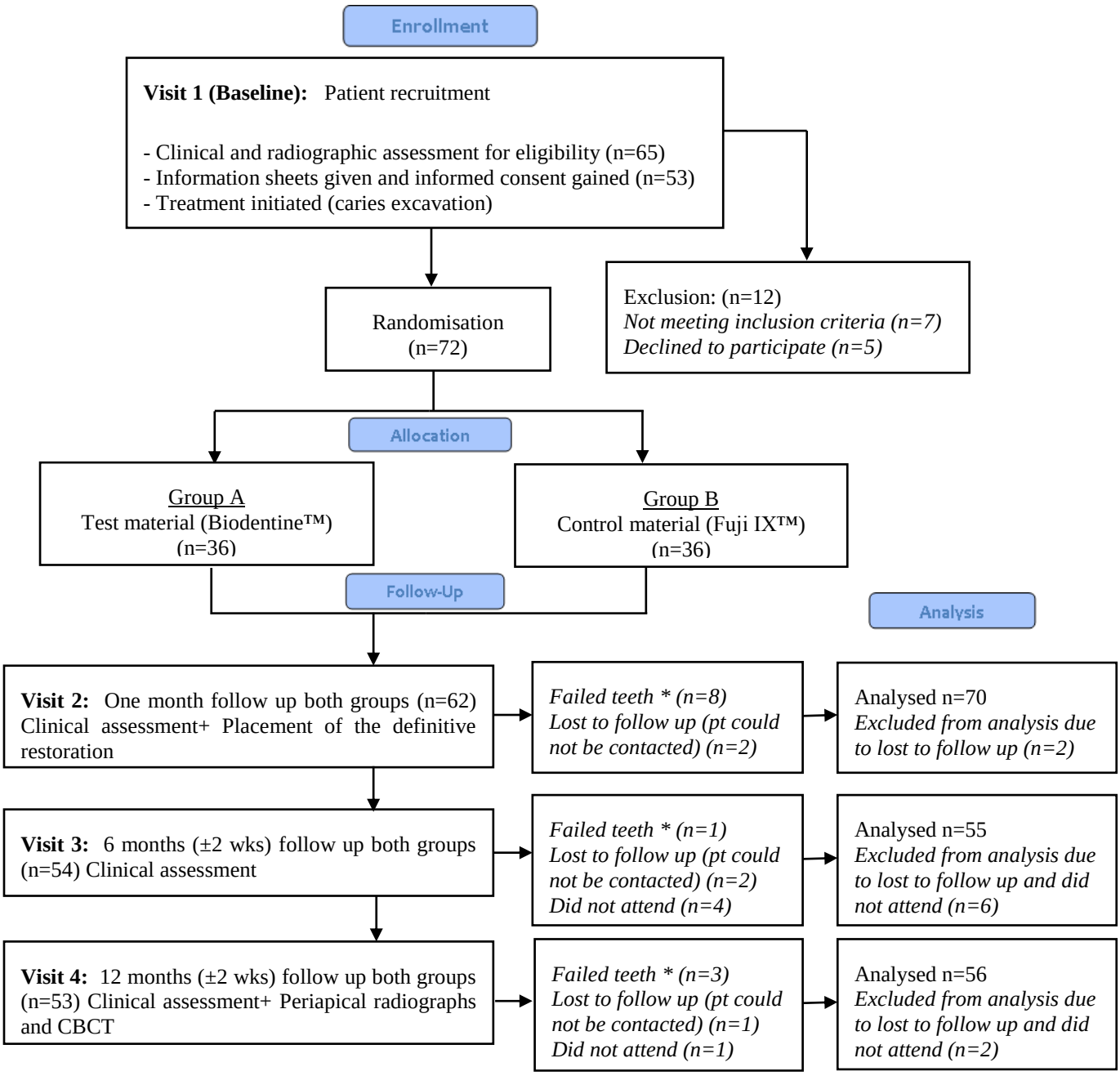


Figure 5-6: Flow diagram indicating patient recruitment and follow up. Adapted from the CONSORT flow diagram. * Failed teeth are ones which developed irreversible pulpitis and underwent root canal treatment.

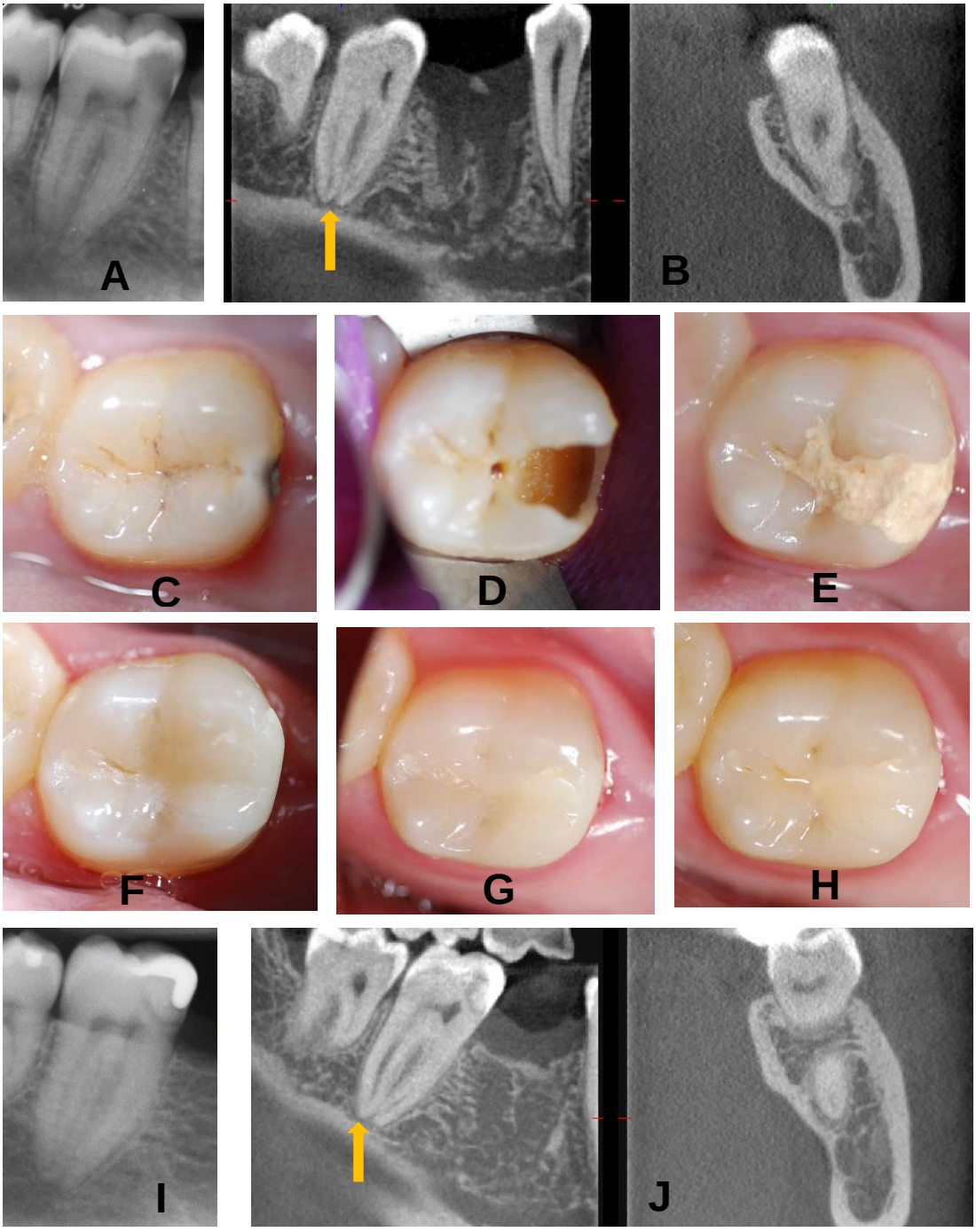


Figure 5-7: (A) Periapical radiograph at T0 revealing large carious lesion on the mesial aspect of the lower right 7 with healthy periapical tissues. (B) CBCT at T0 revealing widening around the apex of the distal root. (C) Clinical photograph of the tooth at T0 before operative intervention. (D) Following caries excavation using MI operative intervention. (E) Biodentine™ application following randomisation. (F) After one month (T1) following cut back of Biodentine™ and placement of resin composite (N'Durance®) restoration. (G) After 6 months (T6). (H) After 12 months (T12). (I) Periapical radiograph at T12 revealing the Biodentine™ restoration and the veneering composite restoration with healthy periapical tissues. (J) CBCT at T12 revealing resolved widening around the apex of the distal root.

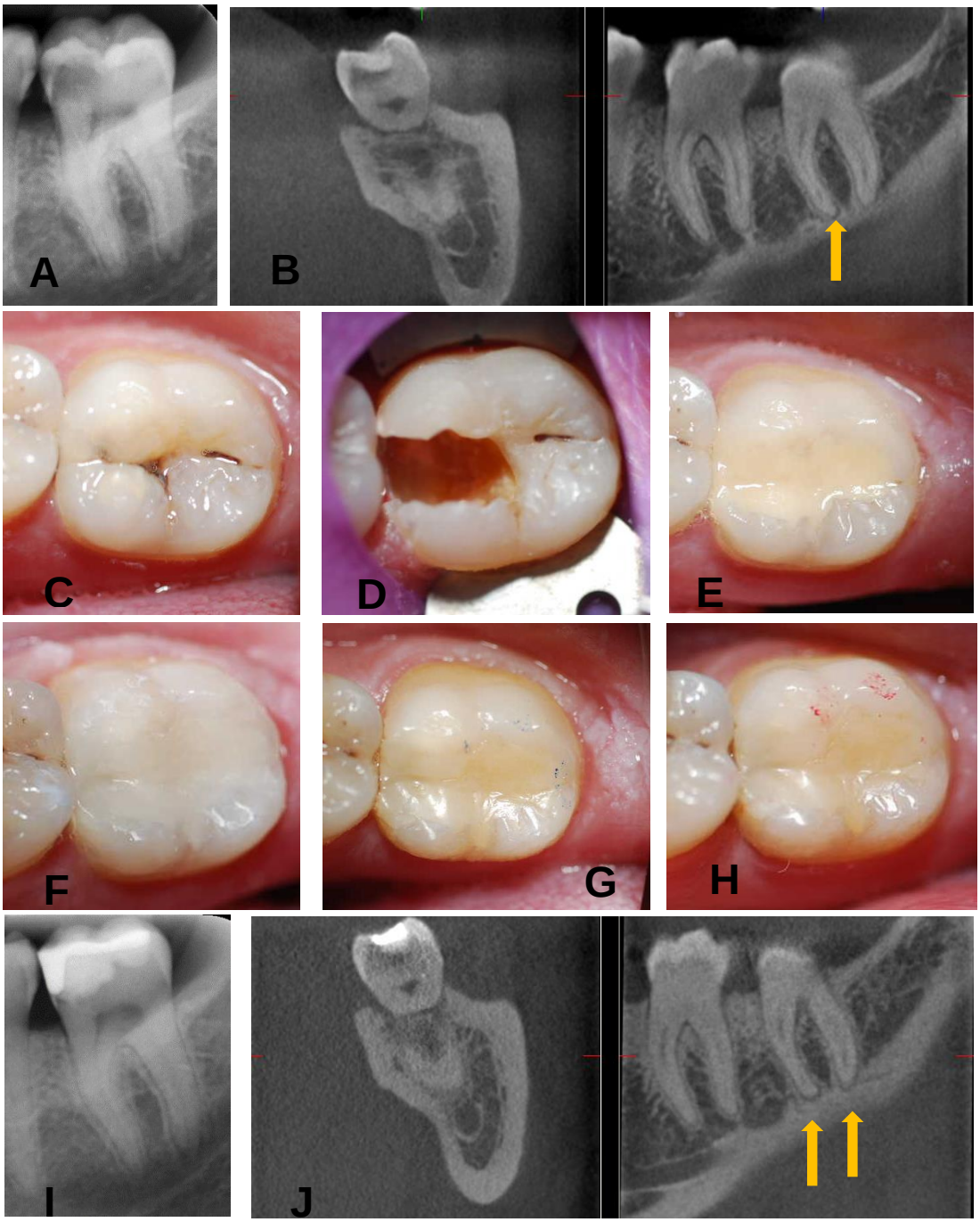


Figure 5-8: (A) Periapical radiograph at T0 revealing large carious lesion on the mesial aspect of the lower left 7 with healthy periapical tissues. (B) CBCT at T0 revealing widening around the apex of the mesial root. (C) Clinical photograph of the tooth at T0 before operative intervention. (D) Following caries excavation using MI operative intervention. (E) Fuji IX™ application following randomisation. (F) After one month (T1) following cut back of Fuji IX™ and placement of resin composite (N'Durance®) restoration. (G) After 6 months (T6). (H) After 12 months (T12). (I) Periapical radiograph at T12 revealing the Fuji IX™ restoration and the veneering composite restoration with widening around the apex of the distal root. (J) CBCT at T12 revealing lesions around the apex of the mesial and distal roots. The tooth is clinically vital with no symptoms and is under observation.

5.3.2 Radiographic assessment

52 paired (T0+T12) CBCT and PA radiographs were analysed. 6 teeth had no T0 and T12 CBCT scans taken, while 8 teeth had no T12 CBCT scan. CBCT was statistically significantly more effective at detecting PA changes compared to radiographs ($p<0.05$). 65.4% / 90.4% of teeth were deemed healthy using CBCT / PA radiographs respectively at T12. Healing/healed rates were 17.3% / 0% (Fig 5-9) while new/progressing radiolucencies were 30.8% / 9.6% with CBCT / PA radiographs respectively (Fig 5-10). The majority of teeth with healing/healed lesions identified using CBCT had received Biodentine™ (71%) while the majority of teeth with new/progressed lesions had received Fuji IX™ (88%). A statistically significant difference ($p=0.02$) between these two proportions was observed (Table 5-2). No statistically significant difference was found in the development of T12 lesions between teeth with mild and severe symptoms within each material ($P>0.05$) (Table 5-2).

51.6% of all teeth had signs of CBCT PA radiolucency at T0 and 26.6% had a PA lesion (Table 5-3). Teeth presenting with a CBCT PA lesion at T0 had a failure rate of 63% whereas teeth with no lesion at T0 had a failure rate of 16%. This was statistically significant ($p=0.02$).

Correlations between CBCT PA changes and symptom intensity, cavity size, material, and patient age were not statistically significant ($p>0.05$).

Kappa values for intra-consensus agreement were 0.68/0.66 for CBCT / PA radiographs respectively and for inter-examiner agreement, 0.53/0.26. Sensitivity, specificity, positive and negative predictive values and overall diagnostic accuracy of PA radiographs were calculated, using CBCT as the gold standard (Table 5-4).

It is worthy to note that an area of radiolucency was observed consistently subjacent to the Biodentine™ restorations while this was less prominent beneath the GIC restorations (Fig 5-11).

Table 5-3: Radiographic assessment including number of teeth and % identified in both CBCT and PA at T0 and T12. (Drop outs excluded from analysis) *Radiolucency includes both widening and lesions.

	CBCT		PA	
	T0 (n=60) N (%)	**T12 (n=52) N (%)**	**T0 (n=60) N (%)**	**T12 (n=52) N (%)**
Healthy	29 (48.3)	34 (65.4)	60 (100)	47 (90.4)
Radiolucency*	31 (51.6)	18 (34.6)	0 (0)	5 (9.6)
Lesions	16 (26.6)	9 (17.3)	0 (0)	2 (3.8)
Healing/healed radiolucency	-	9 (17.3)	-	0 (0)
New/Larger radiolucency	-	16 (30.8)	-	5 (9.6)
Healing/healed lesion	-	6 (11.5)	-	0 (0)
New/Larger lesion	-	9 (17.3)	-	2 (3.8)

Table 5-4: Sensitivity, specificity, positive and negative predictive values and overall diagnostic accuracy of PA compared to CBCT findings as a reference.

Scan	**Sensitivity**	**Specificity**	**Positive Predictive Value (PPV)**	**Negative Predictive Value (NPV)**	**Diagnostic accuracy**
PA	0.24	1	1	0.63	0.66

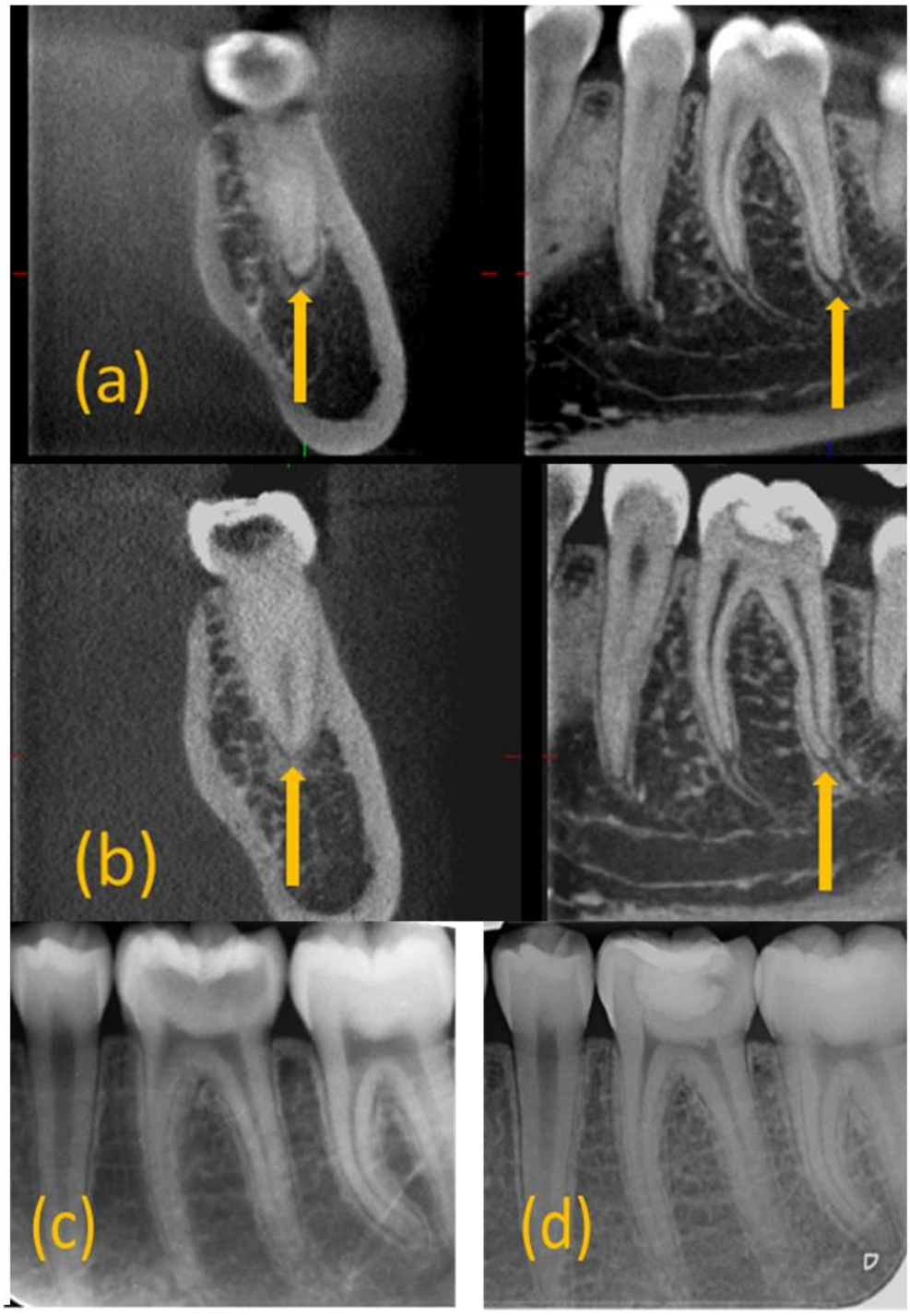


Figure 5-9: Example of healed lesion (a) CBCT at T0 revealing lesion in the distal root of the lower left 6. (b) CBCT at T12 revealing resolved lesion around the distal root of the same tooth.(c) PA radiograph at T0 revealing healthy periapical tissues around the lower left 6.(d) PA radiograph at T12 revealing healthy periapical tissues around the same tooth.

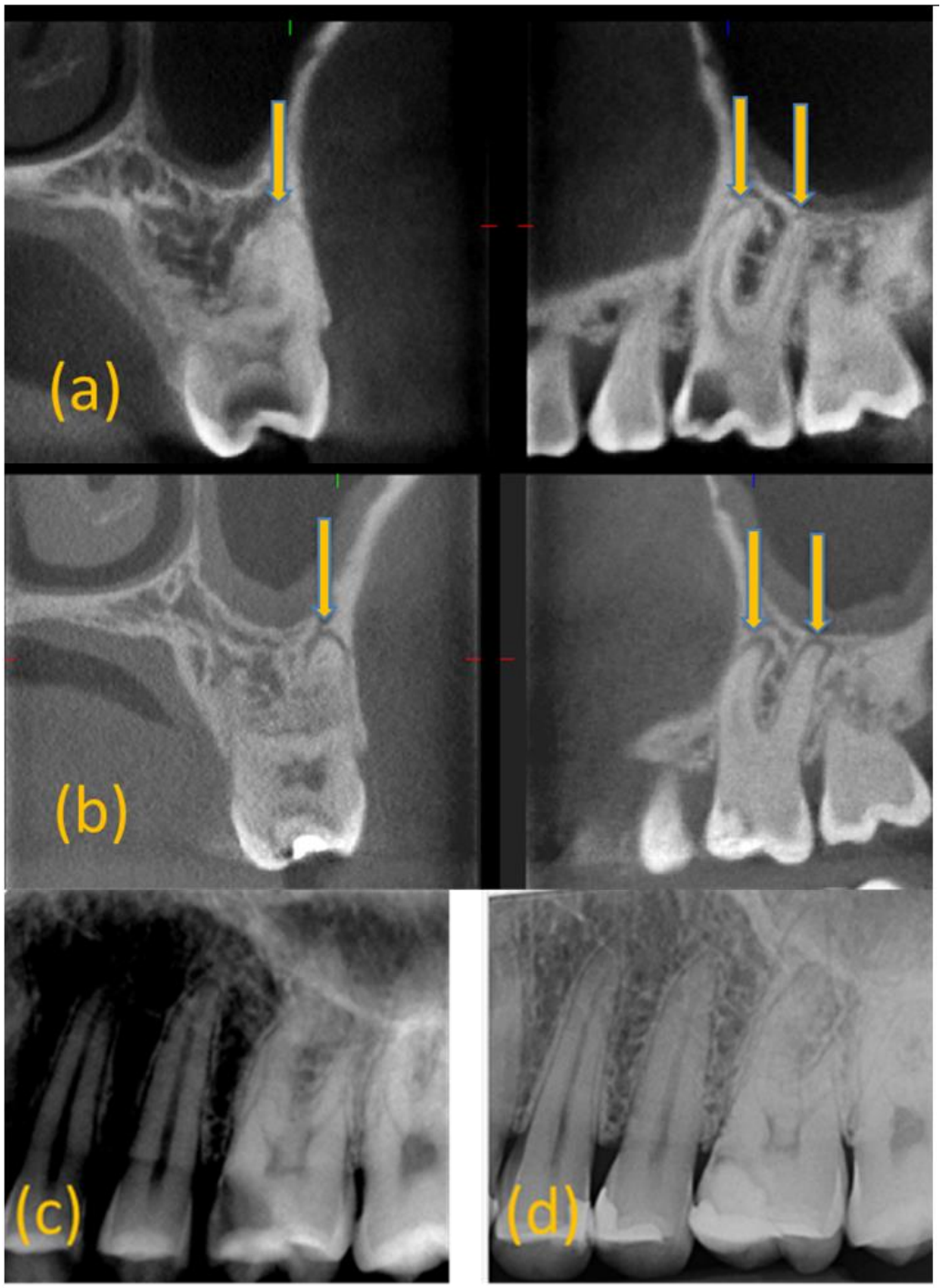


Figure 5-10: Example of new lesion (a) CBCT at T0 revealing healthy periapical tissues surrounding the roots of the upper left 6. (b) CBCT at T12 revealing lesions around the roots of the same tooth.(c) PA radiograph at T0 revealing healthy periapical tissues around the tooth.(d) PA radiograph at T12 revealing slight widening of the PDL around the same tooth.

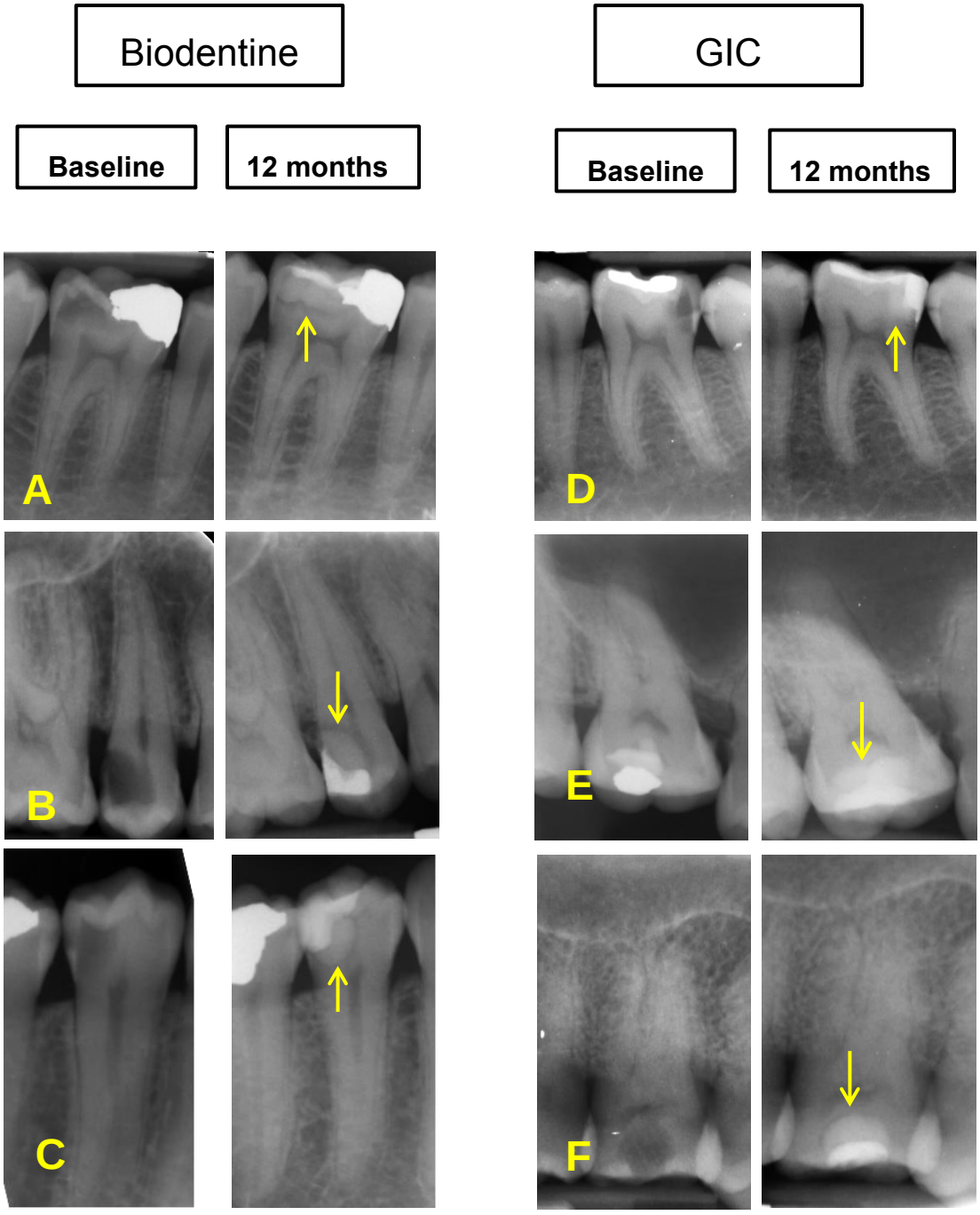


Figure 5-11: Examples demonstrating radiolucent area subjacent to the Biodentine™ restorations (A: lower right 6, B: upper right 5, C: lower right 4) while this was less prominent beneath the GIC restorations (D: lower left 6, E: upper right 6, F: upper left 7). Both experimental groups underwent the same minimally invasive selective caries excavation procedure using Carisolv™gel.

5.3.3 Clinical assessment of the integrity of the restoration

Kappa values demonstrated good intra- and inter-examiner agreement using both assessment criteria for Biodentine™ and GIC (Table 5-5). However, better intra- / inter-examiner agreement was observed for Biodentine™ when compared to GIC. The percentage of assessment rating for all the restorations in each category in the USPHS and FDI criteria for Biodentine™ and GIC are summarised in Tables 5-6 and 5-7. Overall, the integrity of the restoration was excellent to good when assessed using both USPHS and FDI criteria. The p values for testing the proportion of ratings using USPHS and FDI criteria for both Biodentine and GIC were >0.05 at baseline, 6 and 12 months indicating that there is no statistically significant difference in the integrity of the composite restoration overlying Biodentine compared to GIC when judged using USPHS and FDI criteria.

Table 5-5: Intra and inter examiner kappa agreement scores using the USPHS and FDI criteria. The first line denotes intra examiner agreement while the second line reveals the inter examiner agreement.

	USPHS criteria	**FDI criteria**
Biodentine™	0.77 0.97	0.70 0.94
GIC	0.73 0.88	0.58 0.83

5.3.4 Assessment of the efficacy of modified USPHS vs. FDI criteria

There was no statistically significant difference in the efficacy of the USPHS criteria compared with the FDI criteria in the assessment of the Biodentine™ and GIC restorations ($p>0.05$). However there were several observations regarding some of the subcategories in the FDI criteria noted during the assessment of the restorations. In the category “functional properties”, the subcategory “patient’s view” lacked a rating for sensitivity which was found to be a complaint by some of the patients. This could not be recorded in this specific subcategory although it was recorded in the subcategory “Postoperative (hyper)sensitivity and tooth vitality” found in the “biological properties” category. Additionally, in the subcategory “Postoperative (hyper)sensitivity and tooth vitality”, it is not clear whether gingival hypersensitivity can be included in this subcategory which refers to tooth hypersensitivity rather than anything else. Furthermore, in the subcategory “radiographic examination”, no rating was available to note the radiolucent area beneath restorations which was consistent in many of the restorations but could not be recorded. Moreover, in the same subcategory “radiographic examination”, it was unclear what to rate any apical pathology not related directly to the restoration. Similarly, it was unclear what to rate slight gingival inflammation surrounding the tooth from plaque resulting from insufficient oral hygiene and not related to the restoration as the subcategory “periodontal response” in the biological properties referred to changes in the periodontium related to the restoration.

Table 5-6: The percentage of assessment ratings for all the restorations in each category in the modified USPHS criteria for Biodentine™ and GIC at the three follow up visits with p-values indicating no significant difference. A alpha, B bravo, C charlie, D delta. "-" denotes that p values could not be calculated due to very small number. Modified USPHS criteria based on (Palaniappan *et al.*, 2009).

Biodentine (%)													
	Baseline				**6 months**				**12 months**				**P value**
Factor	A	B	C	D	A	B	C	D	A	B	C	D	
Anatomical form	92.9	3.6	3.6	0	92.3	3.8	3.8	0	88.5	7.7	3.8	0	0.96
Secondary caries	100.0	0	0	0	100.0	0	0	0	96.2	0	3.8	0	0.65
Color match	35.7	60.7	3.6	0	34.6	61.5	3.8	0	23.1	73.1	3.8	0	0.90
Retention	100.0	0	0	0	96.2	3.8	0	0	96.2	3.8	0	0	0.54
Marginal adaptation	96.4	3.6	0	0	96.2	3.8	0	0	92.3	7.7	0	0	0.84
Polishability	0	100.0	0	0	0	100.0	0	0	0	100.0	0	0	-
Surface staining	100.0	0	0	0	96.2	0	3.8	0	100.0	0	0	0	0.65
Sensitivity	100.0	0	0	0	96.2	3.8	0	0	88.5	11.5	0	0	0.12
Soft tissue health	96.4	3.6	0	0	96.2	3.8	0	0	96.2	3.8	0	0	1.00
Proximal contact points	94.1	0	5.9	0	88.2	0	11.8	0	87.5	12.5	0	0	0.98
GIC (%)													
	Baseline				**6 months**				**12 months**				**P value**
Factor	A	B	C	D	A	B	C	D	A	B	C	D	
Anatomical	96.6	3.4	0	0	96.4	3.6	0	0	93.3	0	3.4	3.4	1.00

form													
Secondary caries	100.0	0	0	0	96.4	0	3.6	0	96.6	0	3.4	0	0.77
Color match	44.8	55.2	0	0	46.4	50.0	3.6	0	24.1	65.5	10.3	0	0.16
Retention	100.0	0	0	0	100.0	0	0	0	93.1	6.9	0	0	0.33
Marginal adaptation	100.0	0	0	0	89.3	10.7	0	0	93.1	6.9	0	0	0.09
Polishability	100.0	0	0	0	0	96.4	3.6	0	0	100.0	0	0	0.33
Surface staining	100.0	0	0	0	92.9	0	7.1	0	96.6	0	3.4	0	0.32
Sensitivity	100.0	0	0	0	96.4	3.6	0	0	93.1	6.9	0	0	0.54
Soft tissue health	96.6	3.4	0	0	96.4	3.6	0	0	96.6	3.4	0	0	1.00
Proximal contact points	86.7	13.3	0	0	80.0	0	20.0	0	80.0	20.0	0	0	1.00
P-value	0.72	0.98	0.08	-	0.87	0.72	0.34	-	0.88	0.75	0.27	-	

Table 5-7: The percentage of assessment ratings for all the restorations in each category/subcategory in the FDI criteria for Biodentine™ and GIC at the three follow up visits with p-values showing no significant difference between the two materials. 1 excellent, 2 good, 3 satisfactory, 4 unsatisfactory, 5 poor. "-" denotes that p values could not be calculated due to very small number.

	BIODENTINE														
	Factor	**Baseline**					**6 months**					**12 months**				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Esthetic Properties	Surface lustre	96.4	3.6	0	0	0	88.0	11.5	0	0	0	88.5	11.5	0	0	0
	staining surface	100.0	0	0	0	0	96.2	3.8	0	0	0	100.0	0	0	0	0
	staining margin	85.7	14.3	0	0	0	88.5	11.5	0	0	0	76.9	23.1	0	0	0
	color match & translucency	46.4	50.0	3.6	0	0	44.0	52.0	4.0	0	0	38.5	57.7	3.8	0	0
	esthetic anatomical form	85.7	14.3	0	0	0	80.8	15.4	3.8	0	0	80.8	11.5	3.8	3.8	0
Functional Properties	fracture of material & retention	96.4	0	3.6	0	0	88.5	3.8	3.8	3.8	0	92.3	0	0	7.7	0
	marginal adaptation	96.4	3.6	0	0	0	96.2	3.8	0	0	0	88.5	3.8	3.8	3.8	0
	occlusal wear qualitatively	100.0	0	0	0	0	100.0	0	0	0	0	100.0	0	0	0	0
	approximal anatomical form contact point	88.2	0	11.8	0	0	82.4	0	17.6	0	0	75.0	6.3	12.5	6.3	0
	approximal anatomical form contour	89.5	10.5	0	0	0	84.2	10.5	5.3	0	0	88.9	0	5.6	5.6	0
	radiographic	0	0	0	0	0	0	0	0	0	0	84.6	3.8	7.7	0	3.8

	examination														
	patient view	100.0	0	0	0	0	96.2	0	3.8	0	0	88.5	0	3.8	7.7	0
Biological Properties	Post-operative hypersensitivity and tooth vitality	100.0	0	0	0	0	96.2	3.8	0	0	0	88.0	12.0	0	0	0
	recurrence of caries	96.4	3.6	0	0	0	96.2	3.8	0	0	0	96.2	0	0	3.8	0
	tooth integrity (enamel cracks, fractures)	96.4	3.6	0	0	0	96.2	3.8	0	0	0	100.0	0	0	0	0
	periodontal response	92.9	7.1	0	0	0	92.3	7.7	0	0	0	96.2	3.8	0	0	0
	adjacent mucosa	92.9	0	7.1	0	0	92.3	0	7.7	0	0	100.0	0	0	0	0
	oral general health	96.4	3.6	0	0	0	96.2	3.8	0	0	0	100.0	0	0	0	0

GIC

	Factor	**Baseline**					**6 months**					**12 months**				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Esthetic Properties	Surface lustre	100.0	0	0	0	0	85.7	14.3	0	0	0	89.7	6.9	3.4	0	0
	staining surface	100.0	0	0	0	0	96.4	3.6	0	0	0	100.0	0	0	0	0
	staining margin	89.7	10.3	0	0	0	78.6	21.4	0	0	0	75.9	24.1	0	0	0
	color match & translucency	55.2	44.8	0	0	0	57.1	35.7	7.1	0	0	44.8	41.4	13.8	0	0
	esthetic anatomical form	93.1	6.9	0	0	0	78.6	17.9	3.6	0	0	89.7	3.4	3.4	3.4	0
	fracture of material & retention	100.0	0	0	0	0	100.0	0	0	0	0	93.1	0	0	3.4	3.4

	marginal adaptation	100.0	0	0	0	0	89.3	3.6	7.1	0	0	86.2	6.9	3.4	0	3.4
Functional Properties	occlusal wear qualitatively	100.0	0	0	0	0	100.0	0	0	0	0	100.0	0	0	0	0
	approximal anatomical form contact point	81.3	12.5	6.3	0	0	68.8	18.8	12.5	0	0	62.5	12.5	12.5	6.3	6.3
	approximal anatomical form contour	89.5	10.5	0	0	0	83.3	5.6	11.1	0	0	84.2	5.3	5.3	5.3	0
	radiographic examination	100.0	0	0	0	0	100.0	0	0	0	0	82.8	0	10.3	3.4	3.4
	patient view	100.0	0	0	0	0	96.4	0	0	3.6	0	89.7	0	3.4	6.9	0
Biological Properties	Post-operative hypersensitivity and tooth vitality	100.0	0	0	0	0	92.9	7.1	0	0	0	93.1	6.9	0	0	0
	recurrence of caries	100.0	0	0	0	0	96.4	0	3.6	0	0	96.6	0	0	3.4	0
	tooth integrity (enamel cracks, fractures)	100.0	0	0	0	0	100.0	0	0	0	0	96.6	0	0	3.4	0
	periodontal response	96.6	0	3.4	0	0	96.4	0	3.6	0	0	93.1	3.4	0	3.4	0
	adjacent mucosa	96.6	0	3.4	0	0	96.4	0	3.6	0	0	100.0	0	0	0	0
	oral general health	100.0	0	0	0	0	100.0	0	0	0	0	100.0	0	0	0	0
	P-value	0.05	0.76	1.00	-	-	0.61	0.60	1.00	-	-	0.92	0.67	1.00	-	-

5.4 Discussion

In this study, the efficacy of Biodentine™ was compared to Fuji IX™ when used as an indirect pulp capping agent in patients diagnosed clinically and radiographically with reversible pulpitis. Biodentine™ and Fuji IX™ have both been selected as they have similar clinical applications in terms of being used as provisional bulk restorative materials in deep cavities and dentine replacements. In this study it was found that both materials also share a similar success rate of 83.3% at T12 when used as indirect pulp capping agents. Although Biodentine™ and Fuji IX™ are both two classes of water-based cement-type restorative materials with bioactive properties, their interaction and method of remineralisation with the underlying carious tissue is different (Atmeh *et al.*, 2012; Watson *et al.*, 2014). Glass ionomer cements (Fuji IX™) are acidic in nature releasing fluoride and calcium/strontium ions that could replenish the demineralized dentinal tissue ions tipping the balance for apatite reformation (Watson *et al.*, 2014). In Biodentine™, its alkaline caustic effect causes degradation of the dentine collagen leading to the formation of a porous structure that enables the diffusion of high concentrations of calcium, hydroxide, and carbonate ions, leading to increased crystal deposition in the area (Atmeh *et al.*, 2012; Watson *et al.*, 2014).

There are no previous clinical studies comparing these two materials and the only clinical study available of Biodentine™ used as a dentine substitute in deep cavities with no pulp exposure reported a high success rate for the material at 3 years (Koubi *et al.*, 2013a). Although pulp vitality was monitored in this study, its use as a pulp capping agent was not a primary outcome and was not compared to a control.

Symptoms of reversible pulpitis can vary considerably presenting in a range of different intensities from mild to severe symptoms which tip towards irreversible pulpitis. The boundary between severe symptoms of reversible pulpitis and

irreversible pulpitis is difficult to detect as the degree of pain does not necessarily reflect the pulp condition precisely. Evidence does show that the more severe the pain, the worse the histopathosis (Bender, 2000; Aguilar and Linsuwanont, 2011), however that is not always the case (Mejare *et al.*, 2012). Nevertheless, it must be kept in mind that the intensity of symptoms is subjective and patients seeking treatment tend to exaggerate the intensity of pain to emphasise their condition. To avoid this in this study, patients were requested to be precise about their symptoms by explaining how their descriptions might affect the treatment they received. Indeed the results show a significant positive correlation between the intensity of the symptoms and the outcome of the treatment. Teeth which had become non-vital following treatment tend to have suffered from severe symptoms at baseline compared to other teeth.

Making a diagnosis of reversible pulpitis would be assuming there is no apical periodontitis with the aid of periapical radiographs. However, early structural changes in the periapical bone are not visible using periapical radiographs due to a 2-dimensional representation of a 3-dimensional image. CBCT overcomes this disadvantage and indeed 51.6% of the teeth had signs of T0 periapical change when viewed using CBCT and 26.6% had periapical lesions. This is consistent with numerous histology studies demonstrating the presence of chronic inflammation of the pulp with no patient symptoms or clinical/conventional radiographic signs of the true state of the pulpal pathosis (Hilton, 2009).

Out of the 26.6% teeth with periapical lesions at T0, 38% had healed following minimally invasive indirect pulp capping. Although these diseased pulps were inflamed they were vital with a blood supply fundamental for generating pulpal repair and periradicular healing (Torabzadeh and Asgary, 2013). In two previous studies, even teeth clinically diagnosed with irreversible pulpitis and periapical lesions observed in PA radiographs have shown healing of the

periapical lesions following indirect pulp capping (Jordan *et al.*, 1978; Torabzadeh and Asgary, 2013). In another study, vital teeth with periapical radiolucencies observed with PA radiographs and destined for endodontic therapy or extractions were treated by either direct or indirect pulp capping (Moore, 1967). The results showed the pulp capable of repair with absence of periapical radiolucencies and normal vitality after a period varying from 6-36 months. Viable cells were able to effectively repair through the creation of a favourable environment for the cells to complete their function. Vitality of the tooth is an important prerequisite as the cells are the final authority determining the success of such cases (Moore, 1967). General consensus is that periapical involvement demonstrated radiographically indicates total pulp necrosis or irreversible pulpitis, however early periapical pathosis may not necessarily indicate total pulp necrosis and the disease may be limited to the pulp chamber with minimal inflammatory cells and vasodilation in the apical pulpal tissue (Çaliskan, 1995).

In this study the majority of teeth with healed periapical lesions had received Biodentine™ while teeth with new/larger lesions had received glass ionomer cement. Teeth with baseline severe symptoms of reversible pulpitis had received more GIC restorations compared to Biodentine™ as symptom intensity was not considered during the material randomisation. However, the distribution of symptom severity in lesions in the GIC group at T12 was almost equal. This association may be explained by the release of silica ions from Biodentine™ into the underlying dentine, a recognised promoter of remineralisation in addition to its high alkalinity which enhances apatite formation and remineralisation (Watson *et al.*, 2014). Furthermore, its ability to modulate pulp cell transforming growth factor–β1 secretion helps induce reparative dentine synthesis (Laurent *et al.*, 2012a; Zanini *et al.*, 2012; Nowicka *et al.*, 2013). On the other hand, glass ionomer cements may have aggravated a pre-existing compromised situation in the pulp however a histology analysis must be made before a definite explanation can be given. Additionally, this result must be

viewed with care as sample numbers in this situation were low although statistically significant (P=0.02).

The presence of a subjectively noticeable radiolucent area beneath the Biodentine™ restorations, and less obvious beneath the GIC restorations, is an important finding on the T12 PA radiographs and has significant clinical implications. The radiolucent band could be arrested affected dentine due to the minimally invasive technique used in the excavation of caries although if this theory is true then the same extent of radiolucency should have been present beneath the GIC restorations. Another theory could be an effect of the Biodentine™ caustically etching the underlying collagen matrix where mineral deposition may occur in the future. In either case, no re-entry is required and it is important to distinguish this from active caries adjacent to restorations and sealants (CARS) which requires re-entry.

Intra-consensus panel agreement and inter-examiner agreement was moderate to fair in this study. The inter-examiner agreement for CBCT was higher than PA radiographs following a similar trend with previous studies assessing periapical radiolucencies (Sogur *et al.*, 2009; Lennon *et al.*, 2011; Patel *et al.*, 2012). This confirms superior reliability of the CBCT in detecting periapical radiolucency compared to PA radiographs. Both the examiners were experienced in the use of CBCT and detection of radiographic signs of periapical periodontitis. The radiographic techniques and viewing conditions were standardised and viewing sessions were kept as short as practically possible to reduce the likelihood of examiner fatigue. This helps minimise the overall examiner variation due to faults in the radiographic technique, knowledge and judgement as much as possible (Patel *et al.*, 2012). However, unlike previous studies, this study assessed vital teeth which had undergone indirect pulp capping as opposed to root canal treatment and the interpretation of radiographic changes such as widening posed a challenge. Periodontal ligament space (PDL) can demonstrate significant variation, and widening

viewed in CBCT images may be considered an initial sign of disease or just a healthy variation. Therefore “moderate” and “fair” variability in the agreement can be attributed to the difficulty in delineating healthy (slightly wide) PDL space and actual diseased widening. This finding is similar to another study comparing CBCT and PA radiography in the diagnosis of a healthy periapex where the inter-examiner agreement level was fair due to difficulties in identifying variations in the width of the PDL space (Pope *et al.*, 2014). Moreover, it is accepted that inter-examiner agreement is subject to wide variation and can be as low as 25% even between the most experienced examiners (Sogur *et al.*, 2009; Tewary *et al.*, 2011). This reflects the complexity of the decision making process and the diagnostic difficulty when encountering subtle periodontal changes (Lennon *et al.*, 2011). Moreover, joint evaluation was found to reduce observer variation and the findings in this study confirm this (Molven *et al.*, 2002).

Sensitivity of CBCT was found to be twice as high as PA radiographs in detecting periapical changes. Specificity for both imaging techniques was high however it was higher with PA radiographs. This is in agreement with previous studies comparing the two imaging techniques (Stavropoulos and Wenzel, 2007; Estrela *et al.*, 2008; Patel *et al.*, 2009a; Paula-Silva *et al.*, 2009). A histological reference standard to compare the two radiological techniques was not possible due to the nature of this study.

With regards to the integrity of the restoration, no difference was found between Biodentine™ and GIC. The resin composite restorations performed well during the 12 months which is to be expected due to the short observation period. It has been shown that follow-up time needs to be longer, as differences between materials can emerge after more than 10 years (Rodolpho *et al.*, 2011). Furthermore, there was no statistically significant difference in the efficacy of the USPHS criteria compared with the FDI criteria in the assessment of the Biodentine™ and GIC restorations although the FDI criteria is more

comprehensive and contains more detail. This could be due to the short follow up period of one year which is insufficient to detect any changes to the composite restorations. Consideration could be given to modify some aspects in the FDI criteria for improvement. A longer term follow up would be beneficial and is currently ongoing.

5.5 Conclusions

The null hypothesis stating there is no difference in the dentine-pulp response between Biodentine™ and Fuji IX™ clinically was accepted, but not radiographically. The null hypothesis stating there is no difference in the effectiveness of CBCT and PA radiographs in detecting peri-radicular lesions following IPC in patients with symptoms of reversible pulpitis was rejected. Furthermore, the null hypotheses stating there is no difference in the integrity of the Biodentine™ compared to Fuji IX™ restorations using both USPHS and FDI criteria and no difference in the efficacy of the USPHS criteria compared to the FDI criteria in the assessment of the resin composite restorations was accepted.

CBCT detected lesions in teeth diagnosed clinically and using PA radiographs with reversible pulpitis. Although Biodentine™ and Fuji IX™ are both clinically effective when used as therapeutic IPC materials in teeth with reversible pulpitis, CBCT demonstrated a statistically significant difference between the two materials. The majority of teeth with healing/healed lesions identified using CBCT had received Biodentine™ while the majority of teeth with new/progressing lesions had received Fuji IX™. However further studies are needed to establish their effect on the healing dynamics of the PA tissues. The overlying resin composite restoration performed well during 12 months with no difference detected between Biodentine™ and Fuji IX™. Using USPHS or FDI

criteria for the assessment of the restorations were both efficient during the short time recall period however longer term follow up is needed to establish whether FDI criteria is more sensitive than the USPHS criteria.

CHAPTER 6

Summary and suggestions for future work

6.1 Summary

High quality clinical trials provide the best available evidence for decision making and delivering the best care and treatment for patients (Pihlstrom and Barnett, 2010). The aim of this randomised controlled clinical trial was to help develop the clinical evidence base required to justify the use of Biodentine™ compared to GIC as a therapeutic restorative agent for teeth diagnosed clinically and using conventional periapical radiography, with reversible pulpitis. CBCT was used in an attempt to detect early periapical changes in such teeth to help provide a more accurate diagnosis of the pulp status as there is currently no reliable method of identifying objectively the extent of pulp inflammation. Prior to using CBCT in the clinical trial, an in-vitro study using an animal model was conducted in an effort to assess the effect of reducing the radiation dose whilst maintaining an optimised diagnostic outcome, in keeping with the principles of ALARA. This is fundamental as the clinical applications of CBCT are increasing and this imaging technique is being more widely adopted with its lower cost, increasing availability, lower radiation dose especially with small volume CBCT, improved image accuracy, rapid scan time, and lower image artefact (Scarfe *et al.*, 2006).

As Biodentine™ is exposed to clinical wear under load with time and has poor aesthetics, an overlying veneer of resin composite is required to provide mechanical strength, wear resistance, and improved aesthetics of the definitive restoration. Indeed the manufacturer of this material recommends placement of the veneering restoration within a maximum period of 6 months after placing the initial Biodentine™ bulk restoration. However, an in-vitro study was conducted prior to the start of the clinical trial to investigate the potential for bonding the veneering resin composite restoration at the same visit as placing the Biodentine™ as this would be less time consuming, eliminating the need to bring the patient back for a second visit.

In the clinical trial, the integrity of the veneering resin composite restoration was evaluated using the USPHS criteria and the more recently introduced FDI criteria which claims to provide a more sensitive assessment method with enhanced discriminative power compared with the original Ryge (USPHS) criteria. Therefore, another aim of the clinical trial was to compare the efficacy of FDI criteria to the more traditional USPHS criteria.

In this clinical study, CBCT detected lesions in some of the teeth which were diagnosed clinically and using periapical radiography with reversible pulpitis. This indicates that in reality, these teeth have irreversible pulpitis although the signs/ symptoms and tests indicate reversible pulpitis. This is consistent with studies indicating no correlation between the signs/ symptoms and tests used to determine the condition of the pulp and the actual inflammatory status of the pulp (Mejare *et al.*, 2012). This is also consistent with the studies confirming that CBCT is more sensitive than conventional radiographs in detecting periapical radiolucencies (Estrela *et al.*, 2008; Patel *et al.*, 2009a; Paula-Silva *et al.*, 2009; Patel *et al.*, 2011). Indeed CBCT appears to provide a more accurate diagnosis of the condition of the pulp than the traditional methods however, the question remains as to whether CBCT should be used routinely to assist in the diagnosis of the pulp condition especially when considering the radiation dose. The radiation dose of a limited volume CBCT which captures a 30 mm high by

40 mm diameter cylindrical volume of data similar in overall height and width to a periapical radiograph equals to approximately 2–3 standard periapical radiograph exposures (Patel *et al.*, 2007b). At present, routine use of CBCT to support the decision to undertake vital pulp therapy or root canal treatment is not recommended, however CBCT is indicated to aid diagnosis of radiographic signs of periapical pathosis when there are contradictory (nonspecific) signs and/or symptoms (Patel *et al.*, 2014).

Furthermore, the in-vitro study on the ex-vivo model confirmed the possibility of reducing the CBCT radiation dose by using 180° (low resolution) rotation of the tube head instead of 360° (high resolution) while maintaining a diagnostic yield with detail sufficient to make a sound clinical judgment. However, a 360° rotation of the tube head was used in the clinical study as the trial had initiated before the in-vitro study was completed and consistency of the imaging technique was fundamental for the results to be comparable.

The clinical trial confirmed no difference between Biodentine™ and GIC when used as therapeutic indirect pulp capping agents in teeth clinically diagnosed with reversible pulpitis. However radiographically, the majority of teeth with healing lesions after one year viewed on CBCT had received Biodentine™ while the majority of teeth with lesions that did not heal had received GIC. Although sample numbers in this case were low, careful consideration must be given as there was a statistically significant difference between the two materials in this regard. This is in line with earlier clinical studies on GIC placed in non-exposed deep cavities in human teeth. No symptoms were recorded during the observation periods however when extracted, an increased inflammatory cell infiltrate in the odontoblast layer was found with more odontoblast aspiration and changes in the odontoblast layer which mostly resolved towards the end of the experiments (Tobias *et al.*, 1978; Cooper, 1980; Plant *et al.*, 1984). It is worthy to note that the teeth used in these experiments were caries-free which may have contributed to the resolution of the inflammation at the end of the experiments.

Biodentine™ induces an early form of reparative dentine synthesis through the modulation of pulp cell TGF-β1 secretion (Laurent *et al.*, 2012a). Furthermore, Biodentine™ induces cell proliferation and remineralisation by the increased uptake of calcium and silicon ions in addition to its caustic effect from the high alkalinity enhancing apatite formation and remineralisation (Tran *et al.*, 2012; Han and Okiji, 2013; Watson *et al.*, 2014). Glass ionomer cements on the other hand lack this "caustic" effect as they are acidic in nature. However, this acidity promotes self-etching adhesion and when placed on moist dentine, triggers an ionic exchange creating an intermediate ion-enriched layer derived from both substrates (Atmeh *et al.*, 2012). Their direct effect on pulp cells however has a cytotoxic effect. Although GIC was not placed directly on the pulp in the present study, it may have aggravated a pre-existing compromised histopathological situation in the pulp. A histology analysis would be appropriate before a definitive explanation could be given.

Before commencement of the clinical trial, the timing of placement of the overlying resin composite restoration, the quality of the interface between Biodentine™ and the resin composite restoration, and the bonding method was assessed using microshear testing. Clinical relevance of the results of this in-vitro study showed that placing the resin composite restoration immediately in the same visit as placing the Biodentine™ led to a weaker interfacial bond. This could be attributed to the polymerisation shrinkage of the resin composite during setting stressing the structurally weak Biodentine™ in this early sensitive phase resulting in greater cohesive failure within Biodentine™ itself. Indeed this is important clinically as microleakage may result in premature failure of the treatment in a tooth which is already compromised. Leaving Biodentine™ to set completely for at least two weeks is a clinical recommendation concluded from this study which also allows time to monitor the pulp before placing the definitive restoration.

In the clinical trial, Biodentine™ was left for one month before placement of the resin composite restoration. This was done to give sufficient time to monitor the

pulp and ensure the stability of the status of the pulp prior to veneering Biodentine™ (or GIC). The resin composite restoration was evaluated thereafter using the USPHS criteria introduced by Cvar and Ryge in 1971 and the FDI criteria introduced by Hickel et al. in 2007. Although FDI criteria claim to be a more sensitive assessment method, with an increased discriminative power compared to the USPHS criteria, no significant difference was found in the efficacy of the assessment of the restorations between the two criteria. However, from data analysis in the present study, due consideration must be given to modify some aspects in the FDI criteria for improvement. This would include the addition of sensitivity in the subcategory “patient’s view”, adding gingival hypersensitivity to the subcategory “Postoperative (hyper)sensitivity and tooth vitality”, and radiolucency beneath the restoration in the subcategory “radiographic examination”.

The clinical trial was conducted in compliance with the principles of the Declaration of Helsinki and the principles of GCP (Good Clinical Practice) and has been reported following the CONSORT statement (Consolidated Standards of Reporting Trials). The clinical trial is registered at ClinicalTrials.gov with registration number NCT02201641. All documents related to the clinical trial can be found in the appendix.

6.2 Suggestions for future work

1. Longer term follow up of the patients in the clinical trial. This involves:

- Clinical and radiographic assessment of the tooth vitality. This is important to assess the longer term success rate of Biodentine™ compared to GIC.
- Assessment of the integrity of the overlying resin composite restoration. One year follow up is insufficient to detect changes in the restorations. A

longer observation period is necessary to evaluate the long term performance of the resin composite restorations.

- Further CBCT scans taken perhaps at 3 or 5 years to monitor any changes to the periapical area of the related tooth. This is fundamental to confirm the evidence supporting the use of Biodentine™ rather than GIC as therapeutic indirect pulp capping materials in teeth with deep carious lesions.
- Further PA radiographs to monitor the radiolucency subjacent to the Biodentine™ restorations and compare this with the GIC restorations to find out whether the radiolucency decreases in subsequent PA radiographs.

This is currently being undertaken, while amendment or new ethical approval for further CBCT scans of the patients will be pursued.

2. In-vivo study which includes conventional radiography, CBCT, and histology analysis as a reference standard to establish the effect of Biodentine™ and GIC on the healing dynamics of the pulp and periapical area. This can be conducted on carious teeth planned for orthodontic extraction. Another way of investigating/confirming the effect of Biodentine™ and GIC on the pulp and periapical area is to recruit a sufficient number of patients with deep carious lesions and conventional signs of reversible pulpitis with baseline CBCT lesions. Biodentine™ and GIC may then be applied following randomisation and CBCT scans taken after one year to see whether the lesions heal or not.

3. Investigate whether a chemical union exists between Biodentine™ and the overlying veneering resin composite restoration. The functional monomer 10-MDP, present in the adhesive used in the in-vitro study, has been proven to bind to calcium in tooth structure. It would be useful to investigate if 10-MDP monomer binds chemically to the calcium in Biodentine™ hence promoting chemical adhesion in addition to micromechanical attachment.

Appendices

Appendix 1: Published papers

- Hashem, D., Brown, J. E., Patel, S., Mannocci, F., Donaldson, A. N., Watson, T. F. & Banerjee, A. 2013. An In Vitro Comparison of the Accuracy of Measurements Obtained from High- and Low-resolution Cone-beam Computed Tomography Scans. Journal of Endodontics, 39, 394-397.

- Hashem, D. Foxton, R., Manoharan, A., Watson, T. F. & Banerjee, A. 2014. The physical characteristics of resin composite–calcium silicate interface as part of a layered/laminate adhesive restoration. Dental Materials, 30, 343-349.

- Hashem, D., Mannocci, F., Patel, S., Manoharan, A., Brown, J., Watson, T. F. & Banerjee, A. 2015. Clinical and Radiographic Assessment of the Efficacy of Calcium Silicate Indirect Pulp Capping: A Randomized Controlled Clinical Trial. Journal of Dental Research, 94, 562-568.

Basic Research—Technology

An *In Vitro* Comparison of the Accuracy of Measurements Obtained from High- and Low-resolution Cone-beam Computed Tomography Scans

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Abstract

Introduction: This study aimed to investigate in an *ex vivo* model the reduction in patient radiation dose while maintaining accurate linear measurements by comparing cone-beam computed tomography (CBCT) scans taken at 360° versus 180° rotation, with porcine jaw specimens as a reference standard. **Methods:** CBCT scans of 12 sectioned porcine hemimandibles at 360° and 180° rotations were taken with standardized clinical exposure parameters. To assess interobserver variability, 6 assessors who were blinded to the degree of rotation took linear measurements of anatomic structures on each scan. The measurements were repeated after 2 weeks to assess intraobserver variability. Accuracy of measurement was judged against the corresponding measurements taken from the porcine jaw specimens. **Results:** Intraclass correlations signaled good-to-excellent intraobserver and interobserver agreement (0.65–0.98 and 0.79–0.98), respectively. Mixed regression analysis found no significant difference between the measurements from 180° or 360° rotations and no difference between the 2 rotations and porcine jaw specimens. **Conclusions:** A CBCT image sufficient to make accurate clinical measurements with a reduced radiation exposure may be obtained by using 180° rotation of the CBCT tube head. *(J Endod 2013;39:394–397)*

Key Words

Accuracy of measurements, cone-beam computed tomography (CBCT), diagnostic imaging, radiation dose

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Cone-beam computed tomography (CBCT) has been introduced in response to the high demand for an advanced imaging technique that allows a 3-dimensional view of the oral and maxillofacial region. This technique provides images at a high resolution with lower cost, lower radiation dose, rapid scanning time, ease of handling, and accessibility compared with medical CT scans for improved detection and diagnosis (1, 2). Since the introduction of dedicated dentomaxillofacial CBCT scanners, research has been accomplished, and clinical CBCT applications have become more commonplace in dental practice (3). For example, in endodontics, the use of CBCT is becoming more popular because it has been proposed that the technique allows detection of otherwise undetectable apical lesions (4). Studies have also revealed superior diagnostic accuracy in the detection of vertical root fractures (5, 6) and increased sensitivity in the detection of root perforations, which allows earlier diagnosis (7). The outcome of root canal treatment may also be improved by using this imaging modality (8).

CBCT scanners are variable in terms of essential exposure parameters including tube current/voltage, exposure time, field of view, and the extent of rotation of the gantry around the patient's head (9, 10). This variability results in diverse absorbed radiation doses for patients and is linked to the quality of the image obtained (11). The need to keep the radiation dose as low as reasonably achievable is of fundamental importance, and the balance between obtaining an optimized diagnostic image and a low radiation dose remains a challenge (12).

Currently, many CBCT scanners including the Morita Accuitomo (J Morita Corporation, Osaka, Japan) offer a choice of rotation of the gantry at 360° (full rotation of the x-ray tube around the patient's head) or at 180° (half rotation of the x-ray tube around the patient's head). According to the operating parameters used in this study, the absorbed dose for the 360° and 180° rotation is 0.7 and 0.3 mGy, respectively. Although there is an increased radiation dose by approximately more than half with the 360° rotation, higher-resolution images are obtained. However, the fundamental question to be answered is whether this high-resolution image actually helps improve the accuracy of clinical diagnosis or whether it merely exposes the patient to a higher radiation dose without diagnostic benefit.

The aim of this study was to investigate this possibility by comparing linear measurements of clinically relevant anatomic structures viewed on CBCT (Morita Accuitomo) scans taken of sectioned porcine mandibles at 360° (high resolution) and 180° (low resolution) rotation of the tube head with porcine jaw specimens of the same anatomic structures considered as a reference standard.

The null hypothesis was that there is no difference between the measurement accuracy obtained from the CBCT images taken at 360° and 180° rotation of the tube head and when compared with the measurements obtained from the porcine jaw specimens.

Materials and Methods

Specimen Preparation

Twelve fresh porcine hemimandibles were sectioned by using a handsaw in a vertical and horizontal direction to produce a block section including 2 teeth. This

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The physical characteristics of resin composite–calcium silicate interface as part of a layered/laminate adhesive restoration

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ABSTRACT

Objectives. To compare in-vitro micro-shear bond strengths (μSBS) of resin composite to calcium silicate cement (Biodentine™) vs. glass ionomer cement vs. resin modified glass ionomer cement (RM-GIC) using an adhesive in self-etch (SE)/total etch (TE) mode after aging three substrates and bond and characterizing their failure modes.

Methods. Resin composite was SE/TE bonded to 920 standardized disks of Biodentine™, GIC & RM-GIC. Dividing samples into two groups, the first underwent early (t = 0 min, 5 min, 20 min, 24 h) or delayed (t = 2 wk, 1 month, 3 months, 6 months) substrate aging before bonding and μSBS (t = 24 h) testing. In the second, adhesive was applied after either early (t = 5 min) or delayed (t = 2 wk) substrate aging and then tested after bond aging (t = 2 wk, 1 month, 3 months, 6 months). The failure modes were identified using stereomicroscope. SEM images of selected samples were analyzed.

Results. No significant differences were observed between (SE)/(TE) bonding modes ($P = 0.42$). With substrate aging, a significant reduction in μSBS occurred between early and delayed time intervals for Biodentine™ ($P = 0.001$), but none for the GIC/RM-GIC ($P = 0.465$, $P = 0.512$ respectively). With bond aging, there was no significant difference between time intervals for all groups, except at 6 months for the GIC ($P < 0.05$). Modes of failure were primarily cohesive within all the substrates (68.82%) followed by adhesive failure at the resin–substrate interface (21.71%).

Significance. Biodentine™ is a weak restorative material in its early setting phase. Placing the overlying resin composite as part of the laminate/layered definitive restoration is best delayed for >2 wk to allow sufficient intrinsic maturation to withstand contraction forces from the resin composite. A total-etch or self-etch adhesive may be used.

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Clinical and Radiographic Assessment of the Efficacy of Calcium Silicate Indirect Pulp Capping: A Randomized Controlled Clinical Trial

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Abstract

The aims of this study were to assess the effectiveness of calcium silicate cement (Biodentine) versus glass ionomer cement (GIC; control group) as indirect pulp capping materials in patients with reversible pulpitis and to compare the effectiveness of cone beam computed tomography (CBCT) versus periapical (PA) radiographs in detecting PA changes at baseline (T0) and at 12 mo (T12) postoperatively. Seventy-two restorations (36 Biodentine, 36 Fuji IX) were placed randomly in 53 patients. CBCT/PA radiographs were taken at T0 and T12. Two calibrated examiners assessed the presence/absence and increase/decrease in the size of existing PA radiolucencies under standardized conditions. The Kappa coefficient evaluated statistically the effectiveness of CBCT versus PA radiographs in detecting PA changes. Chi-square/Mann-Whitney tests were used to evaluate the association between PA changes in CBCT with various clinical measures. Significance was predetermined at $\alpha = 0.05$. Clinical success rates for Biodentine and Fuji IX GIC were 83.3%. CBCT was significantly more effective in detecting PA radiolucencies compared with radiographs ($P = 0.0069$). Of the teeth, 65.4% and 90.4% were deemed healthy using CBCT and PA radiographs, respectively, at T12. Healing/healed rates were 17.3%/0%, while new/progressed radiolucency were 30.8%/9.6% with CBCT/PA radiographs, respectively. Seventy-one percent of healed lesions had received Biodentine; 88% of new/progressed lesions received Fuji IX GIC. Teeth presenting with an initial CBCT PA lesion had a failure rate of 63%, whereas teeth with no initial lesion had a failure rate of 16%. Although no statistically significant difference was detected in the clinical efficacy of Biodentine/Fuji IX when used as indirect pulp capping materials in patients with reversible pulpitis, CBCT showed a significant difference in that most healed CBCT lesions had received Biodentine while most that did not heal received Fuji IX. Longer-term follow-up is needed to establish their effect on the healing dynamics of PA tissues (ClinicalTrials.gov NCT02201641).

Keywords: pulpitis, periapical disease, dental radiography, cone beam computed tomography, glass ionomer cements, dental caries

Introduction

There is no reliable objective method of evaluating clinically the extent of pulp inflammation and/or its pathological condition. Identifying reversible/irreversible pulpitis relies on patients' subjective descriptions of symptoms, pulp sensibility testing, and radiographic examination (Bjørndal 2002; Pitt Ford and Patel 2004). In addition, treating deep carious lesions can prove to be challenging especially when approaching the pulp as an increased risk of pulp exposure reduces the predictability of the treatment outcome (Barthel et al. 2000; Bjørndal et al. 2010; Dammaschke et al. 2010). Indirect pulp capping (IPC) is one treatment modality that maintains pulp vitality by facilitating healing/repair (Tziafas et al. 2000). Calcium silicate cements (Biodentine; Septodont, Saint Maur des Fosses, France) can be used both for pulp capping and provisional restoration. Biodentine encourages dentine bridge formation with no inflammatory pulp response through secretion of transforming growth factor (TGF)–b1 (Laurent et al. 2012; Zanini et al. 2012; Nowicka et al. 2013). Glass ionomer cements (GICs) are used as liners/sealers and dentine replacement materials in the sandwich technique with no pulp exposure (Sidhu 2011). They exhibit biocompatibility with minimal cytotoxic effect when used indirectly over the pulp (Hume and Mount 1988; Six et al. 2000). Fuji IX (GC Corporation, Tokyo, Japan) contributes to carious dentine remineralization by releasing fluoride and strontium ions (Ngo et al. 2006).

Cone beam computed tomography (CBCT) is more sensitive than intraoral periapical (PA) radiography in detecting PA radiolucencies in teeth subsequently requiring root canal

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Appendix 2: Modified USPHS criteria

Category	Rating and Characteristics	Score
Anatomical form	A: Restoration's contour is continuous with existing anatomical form and margins B: Restoration is slightly over contoured or under contoured C: Marginal overhang or tooth structure (dentin or enamel) is exposed D: Restoration is missing, traumatic occlusion or restoration causes pain in tooth or adjacent tissue	
Secondary caries	A: No visible caries C: Caries contiguous with the margin of the restoration	
Colour match	A: No mismatch in colour, shade or translucency between restoration and adjacent tooth structure B: Mismatch between restoration and tooth structure within the normal range of tooth C: Mismatch between restoration and tooth structure outside the normal range of tooth D: Aesthetically displeasing colour, shade and translucency	
Retention	A: Present B: Partial loss C: Absent	
Marginal adaptation	A: Excellent continuity at resin–enamel interface; no ledge formation, no discoloration B: Slight discoloration at resin–enamel interface; ledge at interface C: Moderate discolouration at resin–enamel interface measuring 1 mm or greater D: Recurrent decay at margin	
Polishability	A: Smooth and highly shiny, similar to enamel B: Smooth and satin, highly reflective C: Rough and shiny, satin, somewhat reflective D: Rough and dull or satin, not reflective	
Surface staining	A: Absent C: Present	
Sensitivity	Preoperative sensitivity (yes/no) Post-operative sensitivity (yes/no)	
Soft tissue health	A: Excellent response, no inflammation B: Slight inflammation of gingival tissue C: Moderate to severe gingival inflammation	
Proximal contact points	A: Present C: Absent	

A alpha, B bravo, C charlie, D delta

Modified USPHS criteria for clinical evaluation of restorations.

Appendix 3: FDI criteria

A. Aesthetic properties	**1. Surface luster**	**2. Staining** **a. surface** **b. margin**	**3. Color match and translucency**	**4. Esthetic anatomical form**
1. Clinically excellent/very good	1.1 Luster comparable to enamel.	2a.1 No surface staining. 2b.1 No marginal staining.	3.1 Good colour match, no difference in shade and/or translucency.	4.1 Ideal
2. Clinically good (after polishing probably very good)	1.2.1 Slightly dull, not noticeable from speaking distance. 1.2.2 Some isolated pores.	2a.2 Minor surface staining, easily removable by polishing. 2b.2 Minor marginal staining, easily removed by polishing.	3.2 Minor deviations in shade and/or translucency.	4.2 Form deviates only slightly from the norm.
3. Clinically sufficient/satisfactory (minor shortcomings, no unacceptable effects but not adjustable w/t damage to the tooth.)	1.3.1 Dull surface but acceptable if covered with film of saliva. 1.3.2 Multiple pores on more than one third of the surface.	2a.3 Moderate surface staining that may also be present on other teeth, not aesthetically unacceptable. 2b.3 Moderate marginal staining, not aesthetically unacceptable.	3.3 Distinct deviation but acceptable. Does not affect aesthetics: 3.3.1 more opaque 3.3.2 more translucent 3.3.3 darker 3.3.4 brighter	4.3 Form deviates from the norm but is aesthetically acceptable.

4. Clinically unsatisfactory (but reparable)	1.4.1 Rough surface, cannot be masked by saliva film, simple polishing is not sufficient. 1.4.2 Voids	2a.4 Unacceptable surface staining on the restoration and major intervention necessary for improvement. 2b.4 Pronounced marginal staining; major intervention necessary for improvement.	3.4 Localised clinical deviation that can be corrected by repair: 3.4.1 too opaque 3.4.2 too translucent 3.4.3 too dark 3.4.4 too bright	4.4 Form is affected and aesthetically unacceptable. Intervention/correction is necessary.
5. Clinically poor (replacement necessary)	1.5 Very rough. Unacceptable plaque retentive surface.	2a.5 Severe surface staining and/or subsurface staining, generalised or localised, not accessible for intervention. 2b.5 Deep marginal staining, not accessible for intervention.	3.5 Unacceptable. Replacement necessary.	4.5 Form is unsatisfactory and/or lost. Repair not feasible/reasonable, replacement needed.
Overall aesthetic score	Acceptable aesthetics (n and %)		Not acceptable(n,% and reasons):	

B. Functional properties	**5. Fracture of material and retention**	**6. Marginal adaptation**	**7. Occlusal contour and wear** **a. qualitatively** **b. quantitatively**	**8. Approximal anatomical form** **a. contact point** **b. contour**	**9. Radiographic examination (when applicable)**	**10. Patient's view**
1.Clinically excellent/very good	5.1 No fractures/cracks.	6.1 Harmonious outline, no gaps, no white or discoloured lines.	7a.1 Physiological wear equivalent to enamel. 7b.1 Wear corresponding to 80-120% of enamel.	8a.1 Normal contact point (floss or 25 µm metal blade can pass). 8b.1 Normal contour.	9.1 No pathology, harmonious transition between restoration and tooth.	10.1 Entirely satisfied with aesthetics and function.
2. Clinically good (after polishing probably very good)	5.2 Small hairline crack.	6.2.1 Marginal gap (<150µm), white lines. 6.2.2 Small marginal fracture removable by polishing. 6.2.3 Slight ditching,	7a.2 Normal wear only slightly different from that of enamel. 7b.2 50-80% or 120-150% wear compared to that of corresponding enamel.	8a.2 Contact slightly too strong but no disadvantage (floss or 25 µm metal blade can only pass with pressure). 8b.2 Slightly deficient	9.2.1 Acceptable material excess present 9.2.2 Positive/negative step present at margin <150 µm.	10.2 Satisfied 10.2.1 Esthetics. 10.2.2 Function, eg. Minor roughness.

		slight step/flashes, minor irregularities.		contour.		
3.Clinically sufficient/ satisfactory (minor shortcomings, no unacceptable effects but not adjustable w/t damage to the tooth.)	5.3 Two or more or larger hairline cracks and/or material chip fracture not affecting the marginal integrity or approximal contact.	6.3.1 Gap <250 µm not removable. 6.3.2 Several small marginal fractures 6.3.3 Major irregularities, ditching or flash, steps.	7a.3 Different wear rate than enamel but within the biological variation. 7b.3 <50% or 150-300% of corresponding enamel.	8a.3 Somewhat weak contact, no indication of damage to tooth, gingiva or periodontal structures; 50 µm metal blade can pass. 8b.3 Visibly deficient contour.	9.3.1 Marginal gap <250 µm. 9.3.2 Negative steps visible <250 µm. 9.3.3 Poor radiopacity of filling material. No adverse effects noticed.	10.3 Minor criticism but no adverse clinical effects. 10.3.1 aesthetic shortcomings. 10.3.2 Some lack of chewing comfort. 10.3.3 Unpleasant treatment procedure.
4.Clinically unsatisfactory (but reparable)	5.4.1 Material chip fractures which damage marginal quality or approximal contacts 5.4.2 Bulk fractures with partial loss (less than half of the restoration).	6.4.1 Gap>250 µm or dentine/base exposed. 6.4.2 Severe ditching or marginal fractures. 6.4.3 Larger irregularities or steps (repair necessary).	7a.4 Wear considerably exceeds normal enamel wear; or occlusal contact points are lost. 7b.4 Restoration >300% of enamel wear or antagonist >300%.	8a.4 Too weak and possible damage due to food impaction; 100 µm metal blade can pass. 8b.4 Inadequate contour. Repair possible.	9.4.1 Marginal gap >250 µm. 9.4.2 Material excess accessible but not removable. 9.4.3 Negative steps >250 µm and reparable.	10.4 Desire for improvement. 10.4.1 Esthetics. 10.4.2 Function, eg. Tongue irritation. Reshaping of anatomic form or refurbishing is possible.
5. Clinically poor (replacement necessary)	5.5 (Partial or complete) loss of restoration or multiple fractures.	6.5.1 Restoration (complete or partial) is loose but in situ. 6.5.2 Generalised major gaps or irregularities.	7a.5 Wear is excessive. 7b.5 Restoration or antagonist >500% of corresponding enamel	8a.5 Too weak and/or clear damage due to food impaction and/or pain/ gingivitis. 8b.4 Insufficient contour, requires replacement.	9.5.1 Secondary caries, large gaps, large overhangs. 9.5.2 Apical pathology. 9.5.3 Fracture/loss of restoration or tooth.	10.5 Completely dissatisfied and/or adverse effects, incl. pain.
Overall Function score	Acceptable function (n and %)		Not acceptable(n,% and reasons):			

C. Biological properties	**11. Postoperative (hyper)sensitivity And tooth vitality**	**12. Recurrence of caries (CAR), erosion, abfraction**	**13. Tooth integrity (enamel cracks, tooth fractures)**	**14. Periodontal response (always compared to a reference tooth)**	**15. Adjacent mucosa**	**16. Oral general health**
1.Clinically excellent/very good	11.1 No hypersensitivity, normal vitality.	12.1 No secondary or primary caries.	13.1 Complete integrity.	14.1 No plaque, no inflammation, no pockets.	15.1 Healthy mucosa adjacent to restoration.	16.1 No oral or general symptoms
2. Clinically good (after polishing probably very good)	11.2 Minor hypersensitivity for a limited period of time, normal vitality.	12.2 Small and localised. 1. Demineralisation 2. Erosion or 3. Abfraction	13.2.1 Small marginal enamel fracture (<150 µm). 13.2.2 Hairline crack in enamel (<150 µm).	14.2 Little plaque, no inflammation (gingivitis), no pocket development. 14.2.1 Without 14.2.2 With overhangs gaps or inadequate anatomic form.	15.2 Healthy after minor removal of mechanical irritations (plaque, calculus, sharp edges, etc.)	16.2 Minor transient symptoms of short duration; local or generalised.
3.Clinically sufficient/satisfactory (minor shortcomings, no unacceptable effects but not adjustable w/t damage to the tooth.)	11.3.1 Moderate hypersensitivity. 11.3.2 Delayed/ mild sensitivity; no subjective complaints, no treatment needed.	12.3 Larger areas of 1. Demineralisation 2. Erosion or 3. Abfraction/ dentine not exposed. Only preventive measures necessary.	13.3.1 Marginal enamel defect <250 µm. 13.3.2 Crack <250 µm. 13.3.3 Enamel chipping 13.3.4 Multiple cracks.	14.3 Difference up to one grade in severity of PBI compared to baseline and compared to control tooth. 14.3.1 Without 14.3.2 With overhangs gaps or inadequate anatomic form.	15.3 Alteration of mucosa but no suspicion of causal relationship with restorative material.	16.3 Transient symptoms, local and/or general.
4.Clinically unsatisfactory (but reparable)	11.4.1 Intense hypersensitivity. 11.4.2 Delayed with minor subjective symptoms. 11.4.3 No clinical detectable sensitivity. Intervention necessary, but not replacement.	12.4.1 Caries cavitation and suspected undermining caries. 12.4.2 Erosion in dentine. 12.4.3 Abrasion/ abfraction in dentine. Localised and accessible can be repaired.	13.4.1 Major marginal enamel defects; gap >250 µm or dentine or base exposed. 13.4.2 Large cracks >250 µm, probe penetrates. 13.4.3 Large enamel chipping or wall fracture.	14.4 Difference of more than one grade PBI in comparison to control tooth or increase in pocket depth>1 mm requiring intervention. 14.4.1 Without 14.4.2 With overhangs gaps or inadequate anatomic form	15.4 Suspected mild lichenoid or toxic reaction.	16.4 Persisting local or general symptoms of oral contact stomatitis or lichen planus or allergic reactions. Intervention necessary but

						no replacement.
5. Clinically poor (replacement necessary)	11.5 Intense, acute pulpitis or nonvital tooth. Endodontic treatment is necessary and restoration has to be replaced.	12.5 Deep caries or exposed dentine that is not accessible for repair of restoration.	13.5 Cusp or tooth fracture.	14.5 Severe/ acute gingivitis or periodontitis. 14.5.1 Without 14.5.2 With overhangs gaps or inadequate anatomic form	15.5 Suspected severe allergic, lichenoid or toxic reaction.	16.5 Acute/ severe local and/or general symptoms.
Overall Biological score	Acceptable biologically (n and %)		Not acceptable(n,% and reasons):			

Appendix 4: CONSORT checklist*

CONSORT 2010 checklist of information to include when reporting a randomised trial*

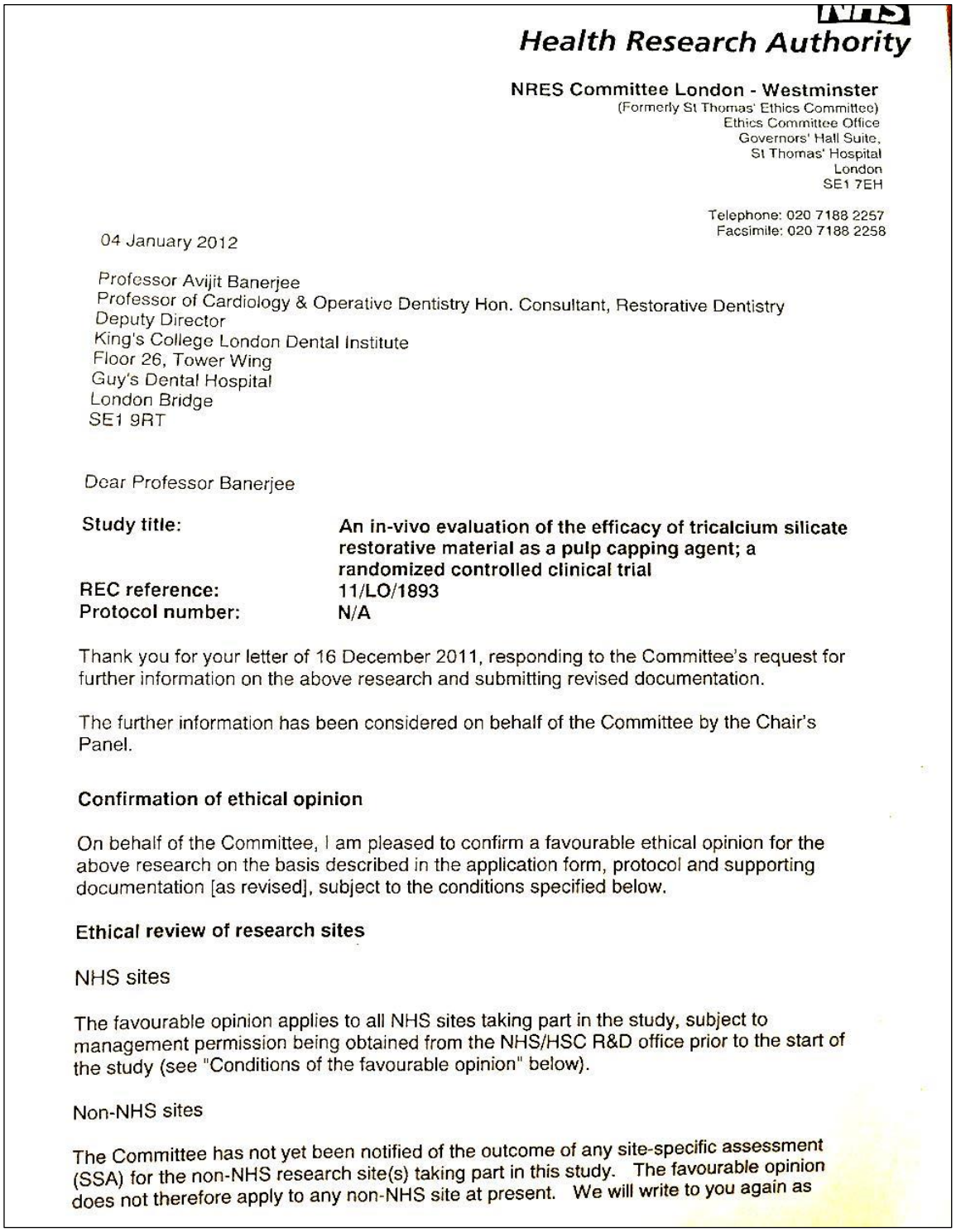
Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	2
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n/a
Participants	4a	Eligibility criteria for participants	Table 1
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6 +fig 1

Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7,8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n/a
Sample size	7a	How sample size was determined	5
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n/a
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	5,7
	11b	If relevant, description of the similarity of interventions	n/a
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	7,8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	7,8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Fig 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Fig 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Fig 1
	14b	Why the trial ended or was stopped	n/a
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	8, Table 1

Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	8,9
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	8,9, table 2
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	n/a
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	n/a
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	10,11,12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	10,11,12
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	10,11,12
Other information			
Registration	23	Registration number and name of trial registry	NCT02201641
Protocol	24	Where the full trial protocol can be accessed, if available	ClinicalTrials.gov
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	n/a

* The CONSORT checklist above contains the page numbers which applies to the paper published in the Journal of Dental Research. All checklist items are included in the thesis.

Appendix 5: Research Ethics Committee approval letter



NHS
Health Research Authority

NRES Committee London - Westminster
(Formerly St Thomas' Ethics Committee)
Ethics Committee Office
Governors' Hall Suite,
St Thomas' Hospital
London
SE1 7EH

Telephone: 020 7188 2257
Facsimile: 020 7188 2258

04 January 2012

Professor Avijit Banerjee
Professor of Cardiology & Operative Dentistry Hon. Consultant, Restorative Dentistry
Deputy Director
King's College London Dental Institute
Floor 26, Tower Wing
Guy's Dental Hospital
London Bridge
SE1 9RT

Dear Professor Banerjee

Study title: **An in-vivo evaluation of the efficacy of tricalcium silicate restorative material as a pulp capping agent; a randomized controlled clinical trial**
REC reference: 11/LO/1893
Protocol number: N/A

Thank you for your letter of 16 December 2011, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair's Panel.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation [as revised], subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as

soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		01 November 2011
GP/Consultant Information Sheets	1	01 November 2011
Investigator CV	Supervisor	01 November 2011
Investigator CV	Student	02 November 2011
Letter from Statistician		01 November 2011
Other: Medical Physics Expert report	1	12 October 2011
Other: Case Report Form	1	01 November 2011
Participant Consent Form	1	01 November 2011
Participant Information Sheet	2	12 December 2011
Protocol	1	01 November 2011
REC application		02 November 2011
Referees or other scientific critique report		
Response to Request for Further Information		16 December 2011
Summary/Synopsis	1	01 November 2011

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating

Appendix 6: Research and Development approval letter

Guy's and St Thomas' NHS
NHS Foundation Trust

Research & Development
16th Floor Tower Wing
Guy's Hospital
Great Maze Pond
London SE1 9RT
Tel: 020 7188 7188

Dr Avijit Banerjee
Reader in Cariology & Operative Dentistry, Hon Consultant
King's College London Dental Institute
Floor 26, Guy's Tower,
Guy's Hospital
Great Maze Pond
London SE1 9RT

9th July 2012

Dear Dr Banerjee

Title: An in-vivo evaluation of the efficacy of tricalcium silicate restorative material as a pulp capping agent; a randomized controlled clinical trial

In accordance with the Department of Health's Research Governance Framework for Health and Social Care, all research projects taking place within the Trust must receive a favourable opinion from an ethics committee and approval from the Department of Research and Development (R&D) prior to commencement.

- **Ethics Number:** **11/LO/1893**
- **Sponsor:** **KCL/GSTFT**
- **Funder:** **No Funding**
- **End Date:** **09/01/2014**
- **Protocol:** **Version 2.0 Dated 26/04/2012**
- **Site:** **Guy's and St Thomas' NHS Foundation Trust**
- **R&D Approval Date:** **09/07/2012**
- **Chief Investigator:** **Dr Avijit Banerjee**

NHS permission for the above research has been granted on the basis described in the application form, protocol and supporting documentation as listed in the ethics letter of favourable opinion letter dated 04/01/2012. I am pleased to inform you that we are approving the work to proceed within Guy's and St Thomas' NHS Foundation Trust and that the study has been allocated the Trust R&D registration number **RJ112/N200**. Please quote the R&D registration number in any communications with the R&D Department regarding your project.

Whilst the Trust takes on non funded research without charge for sponsorship, research management and governance or research costs we encourage all research to be funded and particularly encourage UKCRN portfolio eligible research. Prior to your next research proposal please contact the R&D department about portfolio eligibility and how to gain funding for research so as to ensure that the study can gain appropriate funding prior to your research application.

Conditions of Approval:

- The principal investigator must ensure that the recruitment figures are reported.
- The principal investigator must notify R&D of the actual end date of the project.
- R&D must be notified of any changes to the protocol prior to implementation.
- The project must follow the agreed protocol and be conducted in accordance with all Trust Policies and Procedures especially those relating to research and data management.
- Members of the research team must have appropriate substantive or honorary contracts with the Trust prior to the study commencing. Any additional researchers who join the study at a later stage must also hold a suitable contract.

Data Protection:
Please ensure that you are aware of your responsibilities in relation to The Data Protection Act 1998, NHS Confidentiality Code of Practice, NHS Caldicott Report and Caldicott Guardians, the Human Tissue Act 2004, Good Clinical Practice, the NHS Research Governance Framework for Health and Social Care, Second Edition April 2005 and any further legislation released during the time of this study.

The Principal Investigator is responsible for ensuring that Data Protection procedures are observed throughout the course of the project.

If the project is a clinical trial under the European Union Clinical Trials Directive the following must also be complied with:

1. The EU Directive on Clinical Trials (Directive 2001/20/EC) and UK's implementation of the Directive: The Medicines for Human Use (Clinical Trials) Regulations 2004;
2. The EU Directive on Principles and Guidelines for Good Clinical Practice (EU Commission Directive 2005/28/EC); and UK's implementation of the Directive: The Medicines for Human Use (Clinical Trials) Amendment Regulations 2006;
3. If a clinical trials team has to keep a subject in a department" out of hours" for whatever reason, the Senior Nurse for the Hospital should be informed of their presence – as should the Resuscitation Team.
4. For CtiMP studies hosted by GSTFT, the sponsor is responsible for reporting updates and providing updated documents related SMPC at this site

Amendments:
Please ensure that you submit a copy of any amendments made to this study to the R&D Department.

ISRCTN registration:
If appropriate it is recommended that you register with the Current Controlled Trials website http://isrctn.org/. Find out more about registering for an International Standard Randomised Controlled Trial Number (ISRCTN) as part of the Portfolio application process. Non-commercial studies with an interventional component that are eligible for NIHR CRN support can register for an ISRCTN for free via the Portfolio Database.

Annual Progress Report:
It is obligatory that an annual report is submitted by the Chief Investigator to the research ethics committee, and we ask that a copy is sent to the R&D Department. The yearly period commences from the date of receiving a favourable opinion from the ethics committee.

Please submit a copy of the progress report on the anniversary of the Ethics favourable opinion **(4th January).**

Should you require any further information please do not hesitate to contact us.

In line with the Research Governance Framework, your project may be randomly selected for monitoring for compliance against the standards set out in the Framework. For information, the Trust's process for the monitoring of projects and the associated guidance is available from the Trust's intranet or on request from the R&D Department. You will be notified by the R&D Department if and when your project has been selected as part of the monitoring process. No action is needed until that time.

Thank you for registering your research project.

Yours sincerely

Salma Kibalda
R&D Governance Specialist

Appendix 7: Clinical trial participant's information sheet

Guy's and St Thomas' NHS
NHS Foundation Trust

KING'S College LONDON

Guy's Hospital
Great Maze Pond
London SE1 9RT

PARTICIPANT INFORMATION SHEET

1. **Study title**

"An evaluation of the efficiency of a new filling material to preserve the tooth vitality".

2. **Invitation paragraph**

You are being invited to take part in a research study. Before you decide whether or not to do so, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information.
Take time to decide whether or not you wish to take part. *Thank you for reading this.*

3. **What is the purpose of the study?**

Tooth decay is one of the most widespread diseases in the world. Very often, a patient comes to the dental clinic when experiencing pain which commonly means the decay is deep and is very close to or has penetrated the nerve of the tooth. When the decay is very close to the pulp or nerve of the tooth, a procedure called pulp capping is done in an attempt to save the tooth and to prevent root canal treatment. A material has been introduced to the market for this purpose, called Biodentine. This study aims to investigate the nerve response to this material and to assess the quality of the overlying filling compared to another material (glass ionomer cement) used more commonly. Also, X-rays are a routine procedure used to assess how close the decay is to the nerve and to see if there are abnormalities around the tooth. However conventional X-rays may not be accurate. A new technique called Cone Beam Computed Tomography (CBCT) has been developed which shows a 3-D image of the tooth and may be more accurate. We aim to compare the images gained from conventional X-rays and CBCT to help improve diagnosis and care planning for patients. This study is being carried out as

the researcher's partial fulfilment of the requirements for the degree of Doctor of Philosophy (PhD).

4. **Why have I been invited?**

We are inviting you to take part in this study because you have deep caries and need the pulp capping procedure. This makes you suitable for this study. We hope to recruit 74 volunteers.

5. **Do I have to take part?**

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you do not take part in the study you will not be required to give a reason and there will be no detriment. This will not affect the standard of care you receive in any way. We will ask your consent for informing your general dental practitioner (GDP) about your participation in this study. If you don't consent to us telling your GDP, you can still stay in the study.

6. **What will happen to me if I take part?**

If you decide to take part in this research your dental care will proceed as normal. Every procedure done in this research study is a routine dental procedure with the exception of CBCT. The following flow chart will help you understand what will happen:

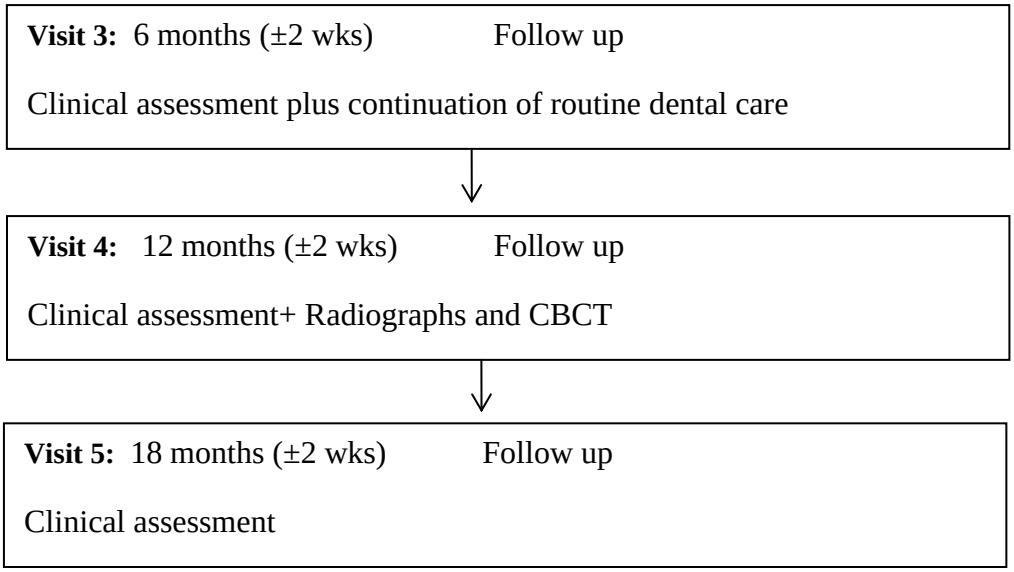
Visit 1: The study is explained and information sheet given. Informed consent is signed. Dental treatment begins. This involves a regular clinical assessment and radiographic assessment (Both conventional radiography and CBCT). Random placement of either test material (Biodentine) or control material (Glass ionomer cement).

↓

Visit 2: One month (±2 wks) Follow up

Clinical assessment+ Placement of the permanent restoration and continuation of your routine dental care

↓



7. **What do I have to do?**

Patients who agree to take part in this study will be required to attend 4 additional visits to Guy's Hospital for follow-up of the restorations during an 18 month period as shown in the flow chart. Should you require other dental treatment, this will be provided at your follow-up visits, free of charge, within the clinical acceptance criteria of Guy's and St. Thomas' Hospitals Foundation Trust (GSTFT).

8. **What is the drug or procedure that is being tested?**

We are not testing any new procedure or material under development. The material we are testing is already fully authorised and approved for routine clinical use and is available on the market. We are trying to find out the beneficial effect of this material (Biodentine) in comparison to another material (glass ionomer cement) commonly used for the procedure of pulp capping. Cone beam computed tomography has been proved by previous research studies to detect the presence of bony disease around the tooth roots earlier than conventional X-rays. This can help improve our diagnosis and care plan for future patients.

9. **What are the side effects of taking part?**

There are no side-effects of taking part in this study other than those expected from routine dental care.

10. **What are the possible disadvantages and risks of taking part?**

Every exposure to ionising radiation (x-rays) carries a risk. However, due to the low doses of radiation from dental x-rays including CBCT, this risk is negligible. Periapical x-rays are normally taken for routine dental treatment and the effective dose from this conventional x-ray is equal to 0.19% of annual background radiation. This is the same as cosmic radiation exposure on board an aircraft for a 3 hour flight. The additional dose comes from the CBCT scan which is equal to 2.43% annual background radiation and is the same as cosmic radiation on board an aircraft for a 20 hour long-haul flight, e.g from the UK to Japan or Australia.

11. **What are the possible benefits of taking part?**

There are no advantages to you personally from taking part. If you participate or not, you will still receive appropriate dental care as per the acceptance criteria of GSTFT. The information we get from this study may help us to treat future patients better.

12. **What if something goes wrong?**

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaint mechanisms may be available to you.

13. **Will my taking part in this study be kept confidential?**

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

14. **What will happen to the results of the research study?**

Results of this research will be published in appropriate dental and scientific journals. No personal information or other information that could be identified as relating to you will be published. You will be informed of the results of the study.

15. **Who is organising and funding the research?**

King's College London.

16. **Who has reviewed the study?**

This study was reviewed by the London-Westminster Research Ethics Committee.

17. **Summary**

You are invited to participate in this study because you have deep caries. This will be cleaned out and you will receive either a Biodentine filling or Glass ionomer cement filling. After one month, these fillings will be covered with a permanent filling (composite resin). During the next follow-up visits your tooth will be examined and the fillings evaluated. You will receive x-rays at your first visit and after one year.

18. **Contact for Further Information**

For further information please contact:
Danya Hashem
King's College London Dental Institute
Biomaterials research group
Floor 17 Guy's Tower
SE1 9RT
Tel: 02071881820 E-mail: danya.hashem@kcl.ac.uk

Appendix 8: Clinical trial consent form

Guy's and St Thomas' NHS
NHS Foundation Trust

KING'S College LONDON

Study Number:
Patient Identification Number for this trial:

CONSENT FORM FOR RESEARCH STUDY

Title of Project: An in-vivo evaluation of the efficacy of tricalcium silicate restorative material as a pulp capping agent; a randomized controlled clinical trial".

Name of Researcher: Danya Hashem

	Please tick to confirm
• I confirm that I have read and understood the information sheet dated **12-12-2011** (version **2.0**) for the above study.	☐
• I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
• I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
• I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from Guy's Hospital, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
• I agree to my GDP being informed of my participation in the study.	☐
• I agree to take part in the above research study.	☐

Name of Patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

When complete, 1 copy for patient: 1 copy for researcher site file: 1 (original) to be kept in medical notes.

Appendix 9: Clinical trial case report form

Guy's and St Thomas' NHS
NHS Foundation Trust

KING'S College LONDON

Case Report Form

Baseline Visit

Patient Identification Number/Code:

Date:

Demographics:

Gender	Male (0) ☐ Female (1) ☐
Date of Birth	☐☐ ☐☐ ☐☐☐☐ Day Month Year
Age	
Ethnicity	Caucasian ☐ Black ☐ Asian ☐ Hispanic ☐ Other ☐
Occupation	
Smoking status	Smoker ☐ Non-Smoker ☐
Pregnancy	Pregnant ☐ Not Pregnant ☐ N/A ☐
Alcohol Consumption	

Medical History*:

Are there any medical conditions to report? Yes* ☐ No ☐ *If "Yes" please describe below:
***Note:** A separate detailed medical history chart is included in the patient's notes.

Dental History:

Presenting Complaint	
History of presenting complaint	Commencement: Location: Type of pain: Incidence: Duration: Initiating/Relieving factors:
Past Dental History	Previous dental treatment: How regularly do you visit your dentist? Preventive protocols do you follow?
Social History	Availability for appointments:
Habits	OH procedures/frequency: Diet: Parafunctional habits:

Full Clinical Oral Examination

Extra-Oral Examination			
	Present	Absent	Comment
Normal Facial Symmetry	☐	☐	
Normal Temporomandibular Joint	☐	☐	
Normal Lips	☐	☐	
Normal Upper Cervical Lymph Nodes	☐	☐	
Normal Submandibular Triangle	☐	☐	
Normal Salivary Glands	☐	☐	
Other, (specify)	☐	☐	

Intra-Oral Examination*			
	Present	Absent	Comment
Normal Mucosa	☐	☐	
Normal Outer and Inner lips	☐	☐	
Normal Gingiva	☐	☐	
Normal Tongue	☐	☐	
Normal Floor of the Mouth	☐	☐	
Normal Hard Palate	☐	☐	
Normal Soft Palate	☐	☐	
Normal Oropharynx	☐	☐	

***Note:** A separate detailed examination chart is included in the patient's notes.

Clinical Examination of the involved tooth	
Involved tooth number (FDI tooth notation system)	
mICDAS score	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐
EPT	
Cold Test	1-Exaggerated response, disappears when stimulus is removed. ☐ 2-Exaggerated response, remains for a while after stimulus is removed. ☐ 3- Negative response ☐
Percussion Test	TTP ☐ Not TTP ☐
Palpation Test	Normal ☐ Abnormal ☐
Sinus Tract, fistula, swelling, abscess	Present ☐ Absent ☐
Mobility	0 ☐ 1 ☐ 2 ☐ 3 ☐
Probing Depth (mm)	Buccal ☐ Lingual ☐ Mesial ☐ Distal ☐

Radiographic Examination:

Periapical Date taken:	CBCT Date taken:
Report:	Report:

Inclusion Criteria:

Please mark the correct answers to the following questions:

	Yes	No*
Age Over the age of 16	☐	☐
General health Good general health with (in the opinion of the investigator) no clinically relevant abnormalities of medical history or oral soft tissue examination.	☐	☐
Carious lesion A minimum of one carious lesion (occlusal or proximal).	☐	☐
Pulp response A positive pulp response to electric pulp test or thermal stimulation of the tooth involved.	☐	☐
Compliance Understands and is willing, able and likely to comply with all the study procedures and restrictions.	☐	☐
Consent Demonstrates understanding of the study and willingness to participate as evidenced by voluntary written informed consent and has received a signed and dated copy of the informed consent form.	☐	☐

*Note: *If any of the above questions are answered "No", the subject should be discontinued from the study as a"screen failure"on the Study Conclusion page (p).*

Please mark the correct answers to the following questions:

	Yes*	No
1- Pregnancy Women who are known to be pregnant or who are intending to become pregnant over the duration of the study.	☐	☐
2- Condition of the tooth/teeth involved		
• Clinical symptoms of irreversible pulpitis requiring endodontic treatment.	☐	☐
• The presence of fistulas or swelling.	☐	☐
• Anterior tooth/teeth with aesthetic concerns.	☐	☐
• The presence of radiolucencies or widening of the apical periodontal ligament space.	☐	☐
• External or internal root resorption.	☐	☐
• Mobile tooth/teeth or tenderness to percussion.	☐	☐
3- Allergy/Intolerance Known or suspected intolerance or hypersensitivity to the study materials (or closely related compounds) or any of their stated ingredients.	☐	☐
4- Dental condition Evidence of gross intra oral neglect.	☐	☐

*Note: *If any of the above questions are answered "Yes", the subject should be discontinued from the study as a "screen failure"on the Study Conclusion page (p).*

Fitness and Eligibility to Participate in Study:

In the investigator's opinion, on the basis of the screening assessment and Inclusion and Exclusion criteria at this visit, is the subject eligible and fit to participate in the next part of the study? ☐ Yes ☐ No

Investigator's Signature_____ Date_____

Tooth Eligibility and Randomisation

Pulp exposure after caries affected dentine removal.	☐ Yes* ☐ No
If "Yes" please record size and time taken for haemostasis.	Size: Time taken for haemostasis:
Is the tooth still eligible for inclusion?	☐ Yes ☐ No*
	*If "No" is checked, the subject should be discontinued from the study due to "Protocol deviation" on the Study Conclusion page (p) unless another tooth is eligible for inclusion.
Was the tooth/teeth randomised?	☐ Yes* ☐ No
	*If "Yes" is checked, please complete the following
Date of randomisation	__ __ / __ __ / __ __ __ __ Day Month Year
Randomisation code	

Digital Imaging Procedure (Photography)

Has the subject had digital imaging?	☐ Yes ☐ No

Subject Eligibility:

Has there been any deviation from the protocol since the last visit? ☐ Yes* ☐ No If "Yes", please record the deviation details on the comments page. Did any untoward signs/symptoms appear or worsen since the last visit? ☐ Yes* ☐ No If "Yes", please describe on the comments page. Is the subject eligible to continue in the study? ☐ Yes ☐ No* If "No", please continue the Study Conclusion page.

Clinical Examination:

Clinical Examination of the involved tooth	
Involved tooth number (FDI tooth notation system)	
EPT	
Cold Test	1-Exaggerated response, disappears when stimulus is removed. ☐ 2-Exaggerated response, remains for a while after stimulus is removed. ☐ 3- Negative response ☐
Percussion Test	TTP ☐ Not TTP ☐
Palpation Test	Normal ☐ Abnormal ☐
Sinus Tract, fistula, swelling, abscess	Present ☐ Absent ☐
Mobility	0 ☐ 1 ☐ 2 ☐ 3 ☐
Probing Depth (mm)	Buccal ☐ Lingual ☐ Mesial ☐ Distal ☐

Assessment of the Test/Control Material:

Modified USPHS criteria

Category	Score
Anatomical form	
Secondary caries	
Colour match	
Retention	
Marginal adaptation	
Polishability	
Surface staining	
Sensitivity	
Soft tissue health	
Proximal contact points	

Hickel et al.'s criteria

Score

Study Conclusion:

Did the subject complete the entire study? ☐ Yes ☐ No*

*If "No" is marked, please indicate the primary reason below. Please mark only one.

Lost to follow-up ☐

Protocol deviation ☐

Withdrawal of Consent ☐ Please specify:

Adverse Event ☐ Please specify:

Other ☐ Please specify:

Was there contact with the subject after the final visit? ☐ Yes ☐ No

If there was contact in relation to this study, please complete the following.

Method of contact: ☐ Telephone ☐ Letter

☐ Other Please specify:

Date of last contact:

Investigator's Signature:

I confirm that I have reviewed all the data collected in this Case Report Form and take responsibility that the information is accurate and complete.

Principle Investigator's Signature:_____ Date:_____

Appendix 10: Raw data of the measurements of the CBCT scans

Sample ID	Location	Measurement	Rotation	Replication	Operator
1	1	2.241	360	1	1
1	2	13.108	360	1	1
1	3	8.907	360	1	1
1	4	0.64	360	1	1
1	5	1.054	360	1	1
1	6	0.959	360	1	1
1	1	1.904	180	1	1
1	2	13.33	180	1	1
1	3	8.758	180	1	1
1	4	0.652	180	1	1
1	5	0.611	180	1	1
1	6	0.713	180	1	1
1	1	2.027	360	2	1
1	2	13.637	360	2	1
1	3	9.251	360	2	1
1	4	0.754	360	2	1
1	5	0.426	360	2	1
1	6	0.965	360	2	1
1	1	1.86	180	2	1
1	2	13.523	180	2	1
1	3	9.122	180	2	1
1	4	0.548	180	2	1
1	5	0.611	180	2	1
1	6	0.611	180	2	1
2	1	1.941	360	1	1
2	2	20.195	360	1	1
2	3	15.984	360	1	1
2	4	0.415	360	1	1
2	5	0.504	360	1	1
2	6	0.613	360	1	1
2	1	1.816	180	1	1
2	2	19.875	180	1	1
2	3	15.649	180	1	1
2	4	0.535	180	1	1
2	5	0.697	180	1	1
2	6	0.398	180	1	1
2	1	1.727	360	2	1

2	2	19.984	360	2	1
2	3	16.285	360	2	1
2	4	0.612	360	2	1
2	5	0.504	360	2	1
2	6	0.706	360	2	1
2	1	1.894	180	2	1
2	2	19.884	180	2	1
2	3	16.052	180	2	1
2	4	0.535	180	2	1
2	5	0.598	180	2	1
2	6	0.696	180	2	1
3	1	2.219	360	1	1
3	2	5.548	360	1	1
3	3	5.605	360	1	1
3	4	0.403	360	1	1
3	5	0.402	360	1	1
3	6	0.504	360	1	1
3	1	2.017	180	1	1
3	2	5.355	180	1	1
3	3	5.606	180	1	1
3	4	0.415	180	1	1
3	5	0.403	180	1	1
3	6	0.504	180	1	1
3	1	2.625	360	2	1
3	2	5.65	360	2	1
3	3	6.175	360	2	1
3	4	0.303	360	2	1
3	5	0.403	360	2	1
3	6	0.605	360	2	1
3	1	2.128	180	2	1
3	2	5.347	180	2	1
3	3	5.793	180	2	1
3	4	0.604	180	2	1
3	5	0.403	180	2	1
3	6	0.613	180	2	1
4	1	2.079	360	1	1
4	2	8.184	360	1	1
4	3	12.119	360	1	1
4	4	0.407	360	1	1
4	5	0.576	360	1	1
4	6	0.6412	360	1	1
4	1	1.961	180	1	1

4	2	13.558	180	1	1
4	3	13.58	180	1	1
4	4	0.322	180	1	1
4	5	0.509	180	1	1
4	6	0.612	180	1	1
4	1	2.243	360	2	1
4	2	8.184	360	2	1
4	3	12.037	360	2	1
4	4	0.407	360	2	1
4	5	0.652	360	2	1
4	6	0.714	360	2	1
4	1	2.245	180	2	1
4	2	8.417	180	2	1
4	3	13.304	180	2	1
4	4	0.509	180	2	1
4	5	0.721	180	2	1
4	6	0.52	180	2	1
5	1	1.565	360	1	1
5	2	5.887	360	1	1
5	3	4.598	360	1	1
5	4	0.313	360	1	1
5	5	0.418	360	1	1
5	6	0.52	360	1	1
5	1	1.575	180	1	1
5	2	4.688	180	1	1
5	3	4.683	180	1	1
5	4	0.313	180	1	1
5	5	0.417	180	1	1
5	6	0.52	180	1	1
5	1	1.665	360	2	1
5	2	5.307	360	2	1
5	3	4.579	360	2	1
5	4	0.521	360	2	1
5	5	0.522	360	2	1
5	6	0.728	360	2	1
5	1	1.772	180	2	1
5	2	5.531	180	2	1
5	3	4.598	180	2	1
5	4	0.313	180	2	1
5	5	0.43	180	2	1
5	6	0.624	180	2	1
6	1	2.006	360	1	1

6	2	8.448	360	1	1
6	3	6.249	360	1	1
6	4	0.996	360	1	1
6	5	0.633	360	1	1
6	6	0.844	360	1	1
6	1	2.136	180	1	1
6	2	8.439	180	1	1
6	3	6.324	180	1	1
6	4	0.453	180	1	1
6	5	0.532	180	1	1
6	6	0.649	180	1	1
6	1	2.112	360	2	1
6	2	8.659	360	2	1
6	3	6.365	360	2	1
6	4	0.972	360	2	1
6	5	0.422	360	2	1
6	6	0.739	360	2	1
6	1	2.029	180	2	1
6	2	8.65	180	2	1
6	3	6.451	180	2	1
6	4	0.833	180	2	1
6	5	0.544	180	2	1
6	6	0.544	180	2	1
7	1	2.071	360	1	1
7	2	4.766	360	1	1
7	3	4.654	360	1	1
7	4	0.935	360	1	1
7	5	1.343	360	1	1
7	6	0.628	360	1	1
7	1	2.289	180	1	1
7	2	4.894	180	1	1
7	3	4.791	180	1	1
7	4	0.418	180	1	1
7	5	1.257	180	1	1
7	6	0.624	180	1	1
7	1	2.061	360	2	1
7	2	4.766	360	2	1
7	3	4.654	360	2	1
7	4	1.136	360	2	1
7	5	1.342	360	2	1
7	6	0.628	360	2	1
7	1	2.084	180	2	1

7	2	5.212	180	2	1
7	3	4.89	180	2	1
7	4	0.417	180	2	1
7	5	1.335	180	2	1
7	6	0.728	180	2	1
8	1	2.173	360	1	1
8	2	8.496	360	1	1
8	3	12.094	360	1	1
8	4	0.516	360	1	1
8	5	0.66	360	1	1
8	6	0.721	360	1	1
8	1	2.378	180	1	1
8	2	8.596	180	1	1
8	3	12.01	180	1	1
8	4	0.309	180	1	1
8	5	0.652	180	1	1
8	6	0.621	180	1	1
8	1	2.266	360	2	1
8	2	8.696	360	2	1
8	3	12.331	360	2	1
8	4	0.413	360	2	1
8	5	0.875	360	2	1
8	6	0.824	360	2	1
8	1	2.275	180	2	1
8	2	8.527	180	2	1
8	3	12.331	180	2	1
8	4	0.413	180	2	1
8	5	0.66	180	2	1
8	6	0.824	180	2	1
9	1	1.754	360	1	1
9	2	8.371	360	1	1
9	3	10.035	360	1	1
9	4	0.413	360	1	1
9	5	0.516	360	1	1
9	6	0.627	360	1	1
9	1	1.889	180	1	1
9	2	7.996	180	1	1
9	3	10.738	180	1	1
9	4	0.533	180	1	1
9	5	0.842	180	1	1
9	6	0.329	180	1	1
9	1	1.854	360	2	1

9	2	8.225	360	2	1
9	3	10.643	360	2	1
9	4	0.413	360	2	1
9	5	0.516	360	2	1
9	6	0.412	360	2	1
9	1	2.007	180	2	1
9	2	8.184	180	2	1
9	3	10.842	180	2	1
9	4	0.523	180	2	1
9	5	0.898	180	2	1
9	6	0.312	180	2	1
10	1	1.426	360	1	1
10	2	9.458	360	1	1
10	3	12.455	360	1	1
10	4	0.504	360	1	1
10	5	0.514	360	1	1
10	6	1.115	360	1	1
10	1	1.26	180	1	1
10	2	9.001	180	1	1
10	3	12.609	180	1	1
10	4	0.305	180	1	1
10	5	0.322	180	1	1
10	6	0.619	180	1	1
10	1	1.426	360	2	1
10	2	9.602	360	2	1
10	3	13.162	360	2	1
10	4	0.403	360	2	1
10	5	0.415	360	2	1
10	6	0.605	360	2	1
10	1	1.529	180	2	1
10	2	9.186	180	2	1
10	3	12.684	180	2	1
10	4	0.508	180	2	1
10	5	0.424	180	2	1
10	6	0.713	180	2	1
11	1	1.413	360	1	1
11	2	4.549	360	1	1
11	3	5.44	360	1	1
11	4	0.451	360	1	1
11	5	0.357	360	1	1
11	6	0.504	360	1	1
11	1	1.594	180	1	1

11	2	4.713	180	1	1
11	3	5.63	180	1	1
11	4	0.332	180	1	1
11	5	0.535	180	1	1
11	6	0.642	180	1	1
11	1	1.617	360	2	1
11	2	4.644	360	2	1
11	3	5.455	360	2	1
11	4	0.415	360	2	1
11	5	0.733	360	2	1
11	6	0.403	360	2	1
11	1	1.737	180	2	1
11	2	4.796	180	2	1
11	3	5.551	180	2	1
11	4	0.509	180	2	1
11	5	0.509	180	2	1
11	6	0.714	180	2	1
12	1	1.594	360	1	1
12	2	3.838	360	1	1
12	3	4.191	360	1	1
12	4	4.127	360	1	1
12	5	0.82	360	1	1
12	6	0.329	360	1	1
12	1	1.595	180	1	1
12	2	3.617	180	1	1
12	3	4.383	180	1	1
12	4	4.123	180	1	1
12	5	0.696	180	1	1
12	6	0.315	180	1	1
12	1	1.692	360	2	1
12	2	3.986	360	2	1
12	3	4.28	360	2	1
12	4	4.148	360	2	1
12	5	0.98	360	2	1
12	6	0.498	360	2	1
12	1	1.592	180	2	1
12	2	3.803	180	2	1
12	3	4.289	180	2	1
12	4	3.882	180	2	1
12	5	0.629	180	2	1
12	6	0.497	180	2	1
1	1	2.241	360	1	2

1	2	15.024	360	1	2
1	3	10.573	360	1	2
1	4	0.682	360	1	2
1	5	0.426	360	1	2
1	6	0.639	360	1	2
1	1	2.264	180	1	2
1	2	15.232	180	1	2
1	3	10.612	180	1	2
1	4	0.682	180	1	2
1	5	0.42	180	1	2
1	6	0.619	180	1	2
1	1	2.241	360	2	2
1	2	15.342	360	2	2
1	3	10.549	360	2	2
1	4	0.768	360	2	2
1	5	0.426	360	2	2
1	6	0.754	360	2	2
1	1	2.032	180	2	2
1	2	15.337	180	2	2
1	3	10.764	180	2	2
1	4	0.796	180	2	2
1	5	0.509	180	2	2
1	6	0.713	180	2	2
2	1	1.948	360	1	2
2	2	19.893	360	1	2
2	3	16.248	360	1	2
2	4	0.543	360	1	2
2	5	0.415	360	1	2
2	6	1.008	360	1	2
2	1	1.992	180	1	2
2	2	20.737	180	1	2
2	3	16.86	180	1	2
2	4	0.667	180	1	2
2	5	0.41	180	1	2
2	6	0.597	180	1	2
2	1	1.914	360	2	2
2	2	20.843	360	2	2
2	3	16.674	360	2	2
2	4	0.571	360	2	2
2	5	0.503	360	2	2
2	6	0.807	360	2	2
2	1	1.894	180	2	2

2	2	20.501	180	2	2
2	3	16.842	180	2	2
2	4	0.718	180	2	2
2	5	0.41	180	2	2
2	6	0.597	180	2	2
3	1	2.219	360	1	2
3	2	5.447	360	1	2
3	3	7.061	360	1	2
3	4	0.403	360	1	2
3	5	0.513	360	1	2
3	6	0.605	360	1	2
3	1	2.421	180	1	2
3	2	5.65	180	1	2
3	3	6.065	180	1	2
3	4	0.504	180	1	2
3	5	0.604	180	1	2
3	6	0.504	180	1	2
3	1	2.421	360	2	2
3	2	5.649	360	2	2
3	3	6.761	360	2	2
3	4	0.403	360	2	2
3	5	0.402	360	2	2
3	6	0.613	360	2	2
3	1	2.12	180	2	2
3	2	5.75	180	2	2
3	3	5.7	180	2	2
3	4	0.403	180	2	2
3	5	0.415	180	2	2
3	6	0.605	180	2	2
4	1	2.659	360	1	2
4	2	9.021	360	1	2
4	3	14.198	360	1	2
4	4	0.42	360	1	2
4	5	0.548	360	1	2
4	6	0.612	360	1	2
4	1	2.151	180	1	2
4	2	9.358	180	1	2
4	3	13.916	180	1	2
4	4	0.509	180	1	2
4	5	0.795	180	1	2
4	6	0.612	180	1	2
4	1	3.059	360	2	2

4	2	9.621	360	2	2
4	3	14.156	360	2	2
4	4	0.407	360	2	2
4	5	0.796	360	2	2
4	6	0.721	360	2	2
4	1	2.345	180	2	2
4	2	9.319	180	2	2
4	3	14.256	180	2	2
4	4	0.455	180	2	2
4	5	0.509	180	2	2
4	6	0.714	180	2	2
5	1	1.561	360	1	2
5	2	6.472	360	1	2
5	3	4.792	360	1	2
5	4	0.521	360	1	2
5	5	0.418	360	1	2
5	6	0.832	360	1	2
5	1	1.561	180	1	2
5	2	5.762	180	1	2
5	3	4.792	180	1	2
5	4	0.417	180	1	2
5	5	0.417	180	1	2
5	6	1.041	180	1	2
5	1	1.873	360	2	2
5	2	5.404	360	2	2
5	3	4.787	360	2	2
5	4	0.417	360	2	2
5	5	0.627	360	2	2
5	6	0.728	360	2	2
5	1	1.977	180	2	2
5	2	5.78	180	2	2
5	3	4.58	180	2	2
5	4	0.417	180	2	2
5	5	0.313	180	2	2
5	6	0.634	180	2	2
6	1	2.112	360	1	2
6	2	9.077	360	1	2
6	3	5.805	360	1	2
6	4	1.04	360	1	2
6	5	0.851	360	1	2
6	6	0.851	360	1	2
6	1	2.029	180	1	2

6	2	9.291	180	1	2
6	3	6.545	180	1	2
6	4	1.056	180	1	2
6	5	0.754	180	1	2
6	6	0.427	180	1	2
6	1	2.123	360	2	2
6	2	8.973	360	2	2
6	3	6.574	360	2	2
6	4	1.141	360	2	2
6	5	0.527	360	2	2
6	6	0.739	360	2	2
6	1	2.139	180	2	2
6	2	10.256	180	2	2
6	3	7.478	180	2	2
6	4	0.769	180	2	2
6	5	0.543	180	2	2
6	6	0.64	180	2	2
7	1	2.064	360	1	2
7	2	5.962	360	1	2
7	3	5.032	360	1	2
7	4	1.446	360	1	2
7	5	1.768	360	1	2
7	6	0.526	360	1	2
7	1	1.977	180	1	2
7	2	5.203	180	1	2
7	3	4.683	180	1	2
7	4	1.252	180	1	2
7	5	1.461	180	1	2
7	6	0.624	180	1	2
7	1	1.958	360	2	2
7	2	5.152	360	2	2
7	3	4.757	360	2	2
7	4	1.136	360	2	2
7	5	2.066	360	2	2
7	6	0.619	360	2	2
7	1	2.185	180	2	2
7	2	4.637	180	2	2
7	3	4.583	180	2	2
7	4	0.939	180	2	2
7	5	2.09	180	2	2
7	6	0.416	180	2	2
8	1	2.472	360	1	2

8	2	9.705	360	1	2
8	3	13.637	360	1	2
8	4	0.426	360	1	2
8	5	0.744	360	1	2
8	6	0.95	360	1	2
8	1	2.371	180	1	2
8	2	10.418	180	1	2
8	3	14.046	180	1	2
8	4	0.526	180	1	2
8	5	0.936	180	1	2
8	6	0.75	180	1	2
8	1	2.474	360	2	2
8	2	9.621	360	2	2
8	3	13.507	360	2	2
8	4	0.31	360	2	2
8	5	0.583	360	2	2
8	6	1.03	360	2	2
8	1	2.369	180	2	2
8	2	9.254	180	2	2
8	3	13.059	180	2	2
8	4	0.426	180	2	2
8	5	0.786	180	2	2
8	6	0.782	180	2	2
9	1	1.854	360	1	2
9	2	9.335	360	1	2
9	3	13.347	360	1	2
9	4	0.413	360	1	2
9	5	0.66	360	1	2
9	6	0.618	360	1	2
9	1	1.992	180	1	2
9	2	8.882	180	1	2
9	3	12.187	180	1	2
9	4	0.419	180	1	2
9	5	0.842	180	1	2
9	6	0.625	180	1	2
9	1	1.857	360	2	2
9	2	8.728	360	2	2
9	3	11.789	360	2	2
9	4	0.326	360	2	2
9	5	0.66	360	2	2
9	6	0.515	360	2	2
9	1	2.295	180	2	2

9	2	9.336	180	2	2
9	3	11.787	180	2	2
9	4	0.432	180	2	2
9	5	0.984	180	2	2
9	6	0.417	180	2	2
10	1	1.114	360	1	2
10	2	10.259	360	1	2
10	3	14.041	360	1	2
10	4	0.302	360	1	2
10	5	0.645	360	1	2
10	6	0.605	360	1	2
10	1	1.529	180	1	2
10	2	9.822	180	1	2
10	3	12.738	180	1	2
10	4	0.305	180	1	2
10	5	0.509	180	1	2
10	6	0.644	180	1	2
10	1	1.715	360	2	2
10	2	10.56	360	2	2
10	3	13.896	360	2	2
10	4	0.302	360	2	2
10	5	0.588	360	2	2
10	6	0.713	360	2	2
10	1	1.529	180	2	2
10	2	11.046	180	2	2
10	3	14.343	180	2	2
10	4	0.406	180	2	2
10	5	0.455	180	2	2
10	6	0.591	180	2	2
11	1	1.717	360	1	2
11	2	4.708	360	1	2
11	3	5.556	360	1	2
11	4	0.403	360	1	2
11	5	0.705	360	1	2
11	6	0.605	360	1	2
11	1	1.839	180	1	2
11	2	4.996	180	1	2
11	3	5.608	180	1	2
11	4	0.303	180	1	2
11	5	1.018	180	1	2
11	6	0.918	180	1	2
11	1	1.818	360	2	2

11	2	4.741	360	2	2
11	3	5.606	360	2	2
11	4	0.403	360	2	2
11	5	0.806	360	2	2
11	6	0.605	360	2	2
11	1	1.962	180	2	2
11	2	4.191	180	2	2
11	3	5.31	180	2	2
11	4	0.42	180	2	2
11	5	1.077	180	2	2
11	6	0.52	180	2	2
12	1	1.393	360	1	2
12	2	3.582	360	1	2
12	3	4.284	360	1	2
12	4	3.793	360	1	2
12	5	1.294	360	1	2
12	6	0.399	360	1	2
12	1	1.894	180	1	2
12	2	4.08	180	1	2
12	3	4.975	180	1	2
12	4	3.813	180	1	2
12	5	0.995	180	1	2
12	6	0.398	180	1	2
12	1	1.593	360	2	2
12	2	3.506	360	2	2
12	3	4.21	360	2	2
12	4	3.617	360	2	2
12	5	1.098	360	2	2
12	6	0.399	360	2	2
12	1	1.794	180	2	2
12	2	4.081	180	2	2
12	3	4.479	180	2	2
12	4	3.882	180	2	2
12	5	1.194	180	2	2
12	6	0.597	180	2	2
1	1	2.142	360	1	3
1	2	13.87	360	1	3
1	3	9.742	360	1	3
1	4	0.859	360	1	3
1	5	0.533	360	1	3
1	6	0.959	360	1	3
1	1	2.151	180	1	3

1	2	13.751	180	1	3
1	3	8.954	180	1	3
1	4	0.796	180	1	3
1	5	0.719	180	1	3
1	6	0.509	180	1	3
1	1	2.155	360	2	3
1	2	13.645	360	2	3
1	3	9.358	360	2	3
1	4	0.754	360	2	3
1	5	0.426	360	2	3
1	6	0.959	360	2	3
1	1	1.94	180	2	3
1	2	13.736	180	2	3
1	3	9.156	180	2	3
1	4	0.822	180	2	3
1	5	0.611	180	2	3
1	6	1.019	180	2	3
2	1	1.928	360	1	3
2	2	20.419	360	1	3
2	3	16.386	360	1	3
2	4	0.604	360	1	3
2	5	0.403	360	1	3
2	6	0.706	360	1	3
2	1	2.291	180	1	3
2	2	19.64	180	1	3
2	3	15.952	180	1	3
2	4	0.802	180	1	3
2	5	0.597	180	1	3
2	6	0.724	180	1	3
2	1	1.749	360	2	3
2	2	20.256	360	2	3
2	3	16.084	360	2	3
2	4	0.542	360	2	3
2	5	0.503	360	2	3
2	6	0.605	360	2	3
2	1	1.794	180	2	3
2	2	19.946	180	2	3
2	3	15.761	180	2	3
2	4	0.638	180	2	3
2	5	0.667	180	2	3
2	6	0.497	180	2	3
3	1	2.219	360	1	3

3	2	5.49	360	1	3
3	3	5.605	360	1	3
3	4	0.513	360	1	3
3	5	0.402	360	1	3
3	6	0.813	360	1	3
3	1	2.32	180	1	3
3	2	5.772	180	1	3
3	3	5.522	180	1	3
3	4	0.504	180	1	3
3	5	0.604	180	1	3
3	6	0.605	180	1	3
3	1	2.12	360	2	3
3	2	5.346	360	2	3
3	3	5.342	360	2	3
3	4	0.403	360	2	3
3	5	0.402	360	2	3
3	6	0.606	360	2	3
3	1	2.219	180	2	3
3	2	5.47	180	2	3
3	3	5.362	180	2	3
3	4	0.503	180	2	3
3	5	0.415	180	2	3
3	6	0.514	180	2	3
4	1	3.168	360	1	3
4	2	9.461	360	1	3
4	3	13.328	360	1	3
4	4	0.518	360	1	3
4	5	0.652	360	1	3
4	6	0.714	360	1	3
4	1	2.557	180	1	3
4	2	8.861	180	1	3
4	3	13.714	180	1	3
4	4	0.42	180	1	3
4	5	0.684	180	1	3
4	6	0.612	180	1	3
4	1	2.455	360	2	3
4	2	8.337	360	2	3
4	3	12.241	360	2	3
4	4	0.509	360	2	3
4	5	0.407	360	2	3
4	6	0.62	360	2	3
4	1	2.549	180	2	3

4	2	8.438	180	2	3
4	3	13.625	180	2	3
4	4	0.61	180	2	3
4	5	0.595	180	2	3
4	6	0.51	180	2	3
5	1	1.98	360	1	3
5	2	6.45	360	1	3
5	3	4.59	360	1	3
5	4	0.635	360	1	3
5	5	0.636	360	1	3
5	6	0.832	360	1	3
5	1	1.769	180	1	3
5	2	5.907	180	1	3
5	3	4.684	180	1	3
5	4	0.522	180	1	3
5	5	0.626	180	1	3
5	6	0.624	180	1	3
5	1	1.876	360	2	3
5	2	5.276	360	2	3
5	3	4.371	360	2	3
5	4	0.313	360	2	3
5	5	0.522	360	2	3
5	6	0.865	360	2	3
5	1	1.769	180	2	3
5	2	6.108	180	2	3
5	3	4.684	180	2	3
5	4	0.418	180	2	3
5	5	0.532	180	2	3
5	6	0.833	180	2	3
6	1	2.427	360	1	3
6	2	9.924	360	1	3
6	3	7.181	360	1	3
6	4	0.675	360	1	3
6	5	0.851	360	1	3
6	6	0.87	360	1	3
6	1	2.458	180	1	3
6	2	9.291	180	1	3
6	3	7.481	180	1	3
6	4	0.918	180	1	3
6	5	0.533	180	1	3
6	6	0.854	180	1	3
6	1	2.322	360	2	3

6	2	8.551	360	2	3
6	3	6.47	360	2	3
6	4	0.747	360	2	3
6	5	0.632	360	2	3
6	6	1.055	360	2	3
6	1	2.136	180	2	3
6	2	9.719	180	2	3
6	3	6.855	180	2	3
6	4	0.813	180	2	3
6	5	0.745	180	2	3
6	6	0.961	180	2	3
7	1	1.958	360	1	3
7	2	5.461	360	1	3
7	3	4.646	360	1	3
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7	5	1.55	360	1	3
7	6	0.619	360	1	3
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7	3	4.579	180	1	3
7	4	0.466	180	1	3
7	5	1.29	180	1	3
7	6	0.53	180	1	3
7	1	2.061	360	2	3
7	2	5.258	360	2	3
7	3	4.489	360	2	3
7	4	0.723	360	2	3
7	5	1.141	360	2	3
7	6	0.413	360	2	3
7	1	1.873	180	2	3
7	2	4.895	180	2	3
7	3	4.505	180	2	3
7	4	0.667	180	2	3
7	5	1.291	180	2	3
7	6	0.416	180	2	3
8	1	2.68	360	1	3
8	2	9.76	360	1	3
8	3	13.127	360	1	3
8	4	0.31	360	1	3
8	5	0.583	360	1	3
8	6	0.728	360	1	3
8	1	2.472	180	1	3

8	2	8.93	180	1	3
8	3	12.128	180	1	3
8	4	0.413	180	1	3
8	5	0.661	180	1	3
8	6	1.035	180	1	3
8	1	2.369	360	2	3
8	2	9.502	360	2	3
8	3	13.229	360	2	3
8	4	0.31	360	2	3
8	5	0.832	360	2	3
8	6	0.95	360	2	3
8	1	2.275	180	2	3
8	2	8.506	180	2	3
8	3	12.773	180	2	3
8	4	0.56	180	2	3
8	5	0.583	180	2	3
8	6	0.721	180	2	3
9	1	2.06	360	1	3
9	2	8.728	360	1	3
9	3	11.15	360	1	3
9	4	0.73	360	1	3
9	5	0.831	360	1	3
9	6	0.824	360	1	3
9	1	2.19	180	1	3
9	2	8.831	180	1	3
9	3	11.215	180	1	3
9	4	0.418	180	1	3
9	5	0.794	180	1	3
9	6	0.73	180	1	3
9	1	1.751	360	2	3
9	2	8.348	360	2	3
9	3	10.774	360	2	3
9	4	0.62	360	2	3
9	5	0.516	360	2	3
9	6	0.618	360	2	3
9	1	1.89	180	2	3
9	2	8.401	180	2	3
9	3	11.318	180	2	3
9	4	0.627	180	2	3
9	5	0.753	180	2	3
9	6	0.625	180	2	3
10	1	1.516	360	1	3

10	2	10.1	360	1	3
10	3	13.382	360	1	3
10	4	0.604	360	1	3
10	5	0.57	360	1	3
10	6	0.908	360	1	3
10	1	1.835	180	1	3
10	2	10.93	180	1	3
10	3	14.094	180	1	3
10	4	0.508	180	1	3
10	5	0.652	180	1	3
10	6	0.917	180	1	3
10	1	1.516	360	2	3
10	2	9.771	360	2	3
10	3	13.431	360	2	3
10	4	0.503	360	2	3
10	5	0.504	360	2	3
10	6	0.606	360	2	3
10	1	1.644	180	2	3
10	2	10.396	180	2	3
10	3	14.316	180	2	3
10	4	0.407	180	2	3
10	5	0.421	180	2	3
10	6	0.971	180	2	3
11	1	1.717	360	1	3
11	2	4.724	360	1	3
11	3	5.38	360	1	3
11	4	0.504	360	1	3
11	5	0.605	360	1	3
11	6	0.605	360	1	3
11	1	1.53	180	1	3
11	2	4.69	180	1	3
11	3	5.552	180	1	3
11	4	0.407	180	1	3
11	5	0.509	180	1	3
11	6	0.822	180	1	3
11	1	1.616	360	2	3
11	2	4.846	360	2	3
11	3	5.422	360	2	3
11	4	0.503	360	2	3
11	5	0.504	360	2	3
11	6	0.605	360	2	3
11	1	1.53	180	2	3

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11	3	5.767	180	2	3
11	4	0.305	180	2	3
11	5	0.611	180	2	3
11	6	0.621	180	2	3
12	1	1.792	360	1	3
12	2	3.782	360	1	3
12	3	4.284	360	1	3
12	4	3.793	360	1	3
12	5	0.995	360	1	3
12	6	0.589	360	1	3
12	1	1.493	180	1	3
12	2	3.782	180	1	3
12	3	4.28	180	1	3
12	4	3.901	180	1	3
12	5	1.094	180	1	3
12	6	0.597	180	1	3
12	1	1.506	360	2	3
12	2	3.786	360	2	3
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12	4	3.814	360	2	3
12	5	0.796	360	2	3
12	6	0.489	360	2	3
12	1	1.492	180	2	3
12	2	3.886	180	2	3
12	3	4.284	180	2	3
12	4	4.099	180	2	3
12	5	0.802	180	2	3
12	6	0.498	180	2	3
1	1	2.131	360	1	4
1	2	13.637	360	1	4
1	3	8.965	360	1	4
1	4	0.859	360	1	4
1	5	0.746	360	1	4
1	6	0.853	360	1	4
1	1	2.447	180	1	4
1	2	13.598	180	1	4
1	3	9.219	180	1	4
1	4	0.877	180	1	4
1	5	0.814	180	1	4
1	6	1.325	180	1	4
1	1	2.131	360	2	4

1	2	13.317	360	2	4
1	3	9.435	360	2	4
1	4	0.754	360	2	4
1	5	0.639	360	2	4
1	6	1.172	360	2	4
1	1	2.141	180	2	4
1	2	14.818	180	2	4
1	3	10.9	180	2	4
1	4	0.548	180	2	4
1	5	0.407	180	2	4
1	6	0.619	180	2	4
2	1	1.841	360	1	4
2	2	20.243	360	1	4
2	3	16.077	360	1	4
2	4	0.805	360	1	4
2	5	0.806	360	1	4
2	6	0.706	360	1	4
2	1	2.189	180	1	4
2	2	20.243	180	1	4
2	3	16.534	180	1	4
2	4	0.725	180	1	4
2	5	0.597	180	1	4
2	6	0.896	180	1	4
2	1	1.916	360	2	4
2	2	20.018	360	2	4
2	3	15.964	360	2	4
2	4	0.929	360	2	4
2	5	0.604	360	2	4
2	6	0.706	360	2	4
2	1	2.189	180	2	4
2	2	20.309	180	2	4
2	3	16.431	180	2	4
2	4	1.094	180	2	4
2	5	0.598	180	2	4
2	6	0.697	180	2	4
3	1	2.322	360	1	4
3	2	5.447	360	1	4
3	3	5.285	360	1	4
3	4	0.403	360	1	4
3	5	0.302	360	1	4
3	6	0.505	360	1	4
3	1	2.118	180	1	4

3	2	5.75	180	1	4
3	3	6.601	180	1	4
3	4	0.403	180	1	4
3	5	0.604	180	1	4
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3	1	2.12	360	2	4
3	2	5.346	360	2	4
3	3	5.881	360	2	4
3	4	0.504	360	2	4
3	5	0.302	360	2	4
3	6	0.404	360	2	4
3	1	2.221	180	2	4
3	2	5.16	180	2	4
3	3	6.093	180	2	4
3	4	0.605	180	2	4
3	5	0.403	180	2	4
3	6	0.514	180	2	4
4	1	2.755	360	1	4
4	2	8.184	360	1	4
4	3	12.076	360	1	4
4	4	0.611	360	1	4
4	5	0.961	360	1	4
4	6	0.918	360	1	4
4	1	2.651	180	1	4
4	2	8.918	180	1	4
4	3	13.817	180	1	4
4	4	0.519	180	1	4
4	5	0.795	180	1	4
4	6	0.816	180	1	4
4	1	2.243	360	2	4
4	2	8.16	360	2	4
4	3	12.297	360	2	4
4	4	0.518	360	2	4
4	5	0.652	360	2	4
4	6	0.822	360	2	4
4	1	2.549	180	2	4
4	2	8.48	180	2	4
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4	5	0.652	180	2	4
4	6	0.612	180	2	4
5	1	1.769	360	1	4

5	2	5.757	360	1	4
5	3	4.58	360	1	4
5	4	0.417	360	1	4
5	5	0.522	360	1	4
5	6	0.624	360	1	4
5	1	1.769	180	1	4
5	2	5.663	180	1	4
5	3	4.891	180	1	4
5	4	0.522	180	1	4
5	5	0.431	180	1	4
5	6	0.937	180	1	4
5	1	1.873	360	2	4
5	2	5.681	360	2	4
5	3	4.475	360	2	4
5	4	0.419	360	2	4
5	5	0.417	360	2	4
5	6	0.52	360	2	4
5	1	1.769	180	2	4
5	2	17.482	180	2	4
5	3	5.022	180	2	4
5	4	0.522	180	2	4
5	5	0.417	180	2	4
5	6	0.832	180	2	4
6	1	2.428	360	1	4
6	2	8.55	360	1	4
6	3	6.461	360	1	4
6	4	1.419	360	1	4
6	5	0.843	360	1	4
6	6	0.95	360	1	4
6	1	2.349	180	1	4
6	2	9.613	180	1	4
6	3	7.369	180	1	4
6	4	0.917	180	1	4
6	5	0.754	180	1	4
6	6	0.861	180	1	4
6	1	2.111	360	2	4
6	2	8.553	360	2	4
6	3	6.569	360	2	4
6	4	1.194	360	2	4
6	5	0.633	360	2	4
6	6	1.161	360	2	4
6	1	2.139	180	2	4

6	2	8.886	180	2	4
6	3	6.11	180	2	4
6	4	0.984	180	2	4
6	5	0.639	180	2	4
6	6	0.961	180	2	4
7	1	2.267	360	1	4
7	2	4.664	360	1	4
7	3	4.842	360	1	4
7	4	0.723	360	1	4
7	5	0.936	360	1	4
7	6	0.619	360	1	4
7	1	2.185	180	1	4
7	2	5.308	180	1	4
7	3	4.994	180	1	4
7	4	0.532	180	1	4
7	5	0.626	180	1	4
7	6	0.735	180	1	4
7	1	1.549	360	2	4
7	2	5.563	360	2	4
7	3	4.739	360	2	4
7	4	0.827	360	2	4
7	5	1.552	360	2	4
7	6	0.722	360	2	4
7	1	1.977	180	2	4
7	2	5.099	180	2	4
7	3	4.89	180	2	4
7	4	0.43	180	2	4
7	5	1.982	180	2	4
7	6	0.53	180	2	4
8	1	2.575	360	1	4
8	2	9.114	360	1	4
8	3	13.042	360	1	4
8	4	0.628	360	1	4
8	5	0.888	360	1	4
8	6	1.133	360	1	4
8	1	2.369	180	1	4
8	2	8.873	180	1	4
8	3	12.619	180	1	4
8	4	0.517	180	1	4
8	5	0.752	180	1	4
8	6	1.339	180	1	4
8	1	2.369	360	2	4

8	2	8.672	360	2	4
8	3	12.604	360	2	4
8	4	0.527	360	2	4
8	5	0.609	360	2	4
8	6	1.03	360	2	4
8	1	2.266	180	2	4
8	2	8.728	180	2	4
8	3	12.064	180	2	4
8	4	0.31	180	2	4
8	5	0.744	180	2	4
8	6	1.133	180	2	4
9	1	2.06	360	1	4
9	2	8.549	360	1	4
9	3	10.643	360	1	4
9	4	0.723	360	1	4
9	5	0.875	360	1	4
9	6	0.721	360	1	4
9	1	2.19	180	1	4
9	2	8.4	180	1	4
9	3	10.92	180	1	4
9	4	0.628	180	1	4
9	5	0.963	180	1	4
9	6	1.043	180	1	4
9	1	1.957	360	2	4
9	2	8.371	360	2	4
9	3	10.515	360	2	4
9	4	0.516	360	2	4
9	5	1.096	360	2	4
9	6	0.721	360	2	4
9	1	1.982	180	2	4
9	2	8.714	180	2	4
9	3	11.303	180	2	4
9	4	0.628	180	2	4
9	5	0.816	180	2	4
9	6	0.635	180	2	4
10	1	1.715	360	1	4
10	2	9.933	360	1	4
10	3	13.092	360	1	4
10	4	0.402	360	1	4
10	5	0.676	360	1	4
10	6	1.663	360	1	4
10	1	1.529	180	1	4

10	2	10.889	180	1	4
10	3	14.27	180	1	4
10	4	0.518	180	1	4
10	5	0.72	180	1	4
10	6	1.223	180	1	4
10	1	1.92	360	2	4
10	2	9.872	360	2	4
10	3	13.528	360	2	4
10	4	0.514	360	2	4
10	5	0.363	360	2	4
10	6	0.913	360	2	4
10	1	1.427	180	2	4
10	2	9.358	180	2	4
10	3	12.251	180	2	4
10	4	0.305	180	2	4
10	5	0.594	180	2	4
10	6	0.815	180	2	4
11	1	1.818	360	1	4
11	2	4.842	360	1	4
11	3	5.85	360	1	4
11	4	0.605	360	1	4
11	5	0.451	360	1	4
11	6	0.605	360	1	4
11	1	1.839	180	1	4
11	2	4.893	180	1	4
11	3	5.913	180	1	4
11	4	0.611	180	1	4
11	5	0.915	180	1	4
11	6	1.122	180	1	4
11	1	1.513	360	2	4
11	2	4.842	360	2	4
11	3	5.547	360	2	4
11	4	0.612	360	2	4
11	5	1.31	360	2	4
11	6	0.813	360	2	4
11	1	1.533	180	2	4
11	2	4.385	180	2	4
11	3	5.498	180	2	4
11	4	0.509	180	2	4
11	5	1.018	180	2	4
11	6	0.918	180	2	4
12	1	1.991	360	1	4

12	2	4.123	360	1	4
12	3	4.379	360	1	4
12	4	3.719	360	1	4
12	5	1.294	360	1	4
12	6	0.902	360	1	4
12	1	1.692	180	1	4
12	2	4.001	180	1	4
12	3	4.577	180	1	4
12	4	3.927	180	1	4
12	5	0.398	180	1	4
12	6	0.597	180	1	4
12	1	1.298	360	2	4
12	2	4.731	360	2	4
12	3	4.379	360	2	4
12	4	3.91	360	2	4
12	5	1.21	360	2	4
12	6	0.6	360	2	4
12	1	1.692	180	2	4
12	2	4.08	180	2	4
12	3	4.379	180	2	4
12	4	3.604	180	2	4
12	5	0.507	180	2	4
12	6	0.497	180	2	4
1	1	2.036	360	1	5
1	2	13.53	360	1	5
1	3	9.212	360	1	5
1	4	1.214	360	1	5
1	5	0.746	360	1	5
1	6	0.959	360	1	5
1	1	2.347	180	1	5
1	2	13.209	180	1	5
1	3	8.867	180	1	5
1	4	0.821	180	1	5
1	5	0.509	180	1	5
1	6	0.713	180	1	5
1	1	2.451	360	2	5
1	2	13.424	360	2	5
1	3	9.913	360	2	5
1	4	0.714	360	2	5
1	5	0.32	360	2	5
1	6	0.639	360	2	5
1	1	2.449	180	2	5

1	2	14.209	180	2	5
1	3	9.131	180	2	5
1	4	0.734	180	2	5
1	5	0.367	180	2	5
1	6	0.713	180	2	5
2	1	2.14	360	1	5
2	2	20.171	360	1	5
2	3	16.177	360	1	5
2	4	0.705	360	1	5
2	5	0.302	360	1	5
2	6	0.706	360	1	5
2	1	1.835	180	1	5
2	2	19.804	180	1	5
2	3	15.562	180	1	5
2	4	0.588	180	1	5
2	5	0.598	180	1	5
2	6	0.497	180	1	5
2	1	1.819	360	2	5
2	2	19.73	360	2	5
2	3	16.059	360	2	5
2	4	0.302	360	2	5
2	5	0.403	360	2	5
2	6	0.706	360	2	5
2	1	1.993	180	2	5
2	2	20.299	180	2	5
2	3	16.345	180	2	5
2	4	0.497	180	2	5
2	5	0.4498	180	2	5
2	6	0.769	180	2	5
3	1	2.522	360	1	5
3	2	5.548	360	1	5
3	3	5.681	360	1	5
3	4	0.604	360	1	5
3	5	0.402	360	1	5
3	6	0.614	360	1	5
3	1	2.726	180	1	5
3	2	5.851	180	1	5
3	3	5.504	180	1	5
3	4	0.403	180	1	5
3	5	0.514	180	1	5
3	6	0.807	180	1	5
3	1	2.221	360	2	5

3	2	5.149	360	2	5
3	3	6.283	360	2	5
3	4	0.303	360	2	5
3	5	0.414	360	2	5
3	6	0.404	360	2	5
3	1	2.221	180	2	5
3	2	5.447	180	2	5
3	3	6.16	180	2	5
3	4	0.302	180	2	5
3	5	0.225	180	2	5
3	6	0.706	180	2	5
4	1	2.466	360	1	5
4	2	8.011	360	1	5
4	3	11.647	360	1	5
4	4	0.305	360	1	5
4	5	0.652	360	1	5
4	6	0.714	360	1	5
4	1	2.345	180	1	5
4	2	8.283	180	1	5
4	3	13.55	180	1	5
4	4	0.508	180	1	5
4	5	0.795	180	1	5
4	6	0.816	180	1	5
4	1	2.354	360	2	5
4	2	8.184	360	2	5
4	3	11.671	360	2	5
4	4	0.407	360	2	5
4	5	0.433	360	2	5
4	6	0.51	360	2	5
4	1	2.449	180	2	5
4	2	8.163	180	2	5
4	3	12.388	180	2	5
4	4	0.407	180	2	5
4	5	0.742	180	2	5
4	6	0.816	180	2	5
5	1	2.601	360	1	5
5	2	6.235	360	1	5
5	3	4.878	360	1	5
5	4	0.522	360	1	5
5	5	0.233	360	1	5
5	6	1.045	360	1	5
5	1	2.609	180	1	5

5	2	4.571	180	1	5
5	3	4.519	180	1	5
5	4	0.208	180	1	5
5	5	0.522	180	1	5
5	6	0.52	180	1	5
5	1	1.873	360	2	5
5	2	5.389	360	2	5
5	3	4.683	360	2	5
5	4	0.233	360	2	5
5	5	0.209	360	2	5
5	6	0.624	360	2	5
5	1	1.781	180	2	5
5	2	5.184	180	2	5
5	3	4.828	180	2	5
5	4	0.313	180	2	5
5	5	0.418	180	2	5
5	6	0.633	180	2	5
6	1	2.217	360	1	5
6	2	8.339	360	1	5
6	3	6.442	360	1	5
6	4	0.824	360	1	5
6	5	0.972	360	1	5
6	6	0.739	360	1	5
6	1	2.243	180	1	5
6	2	8.65	180	1	5
6	3	6.557	180	1	5
6	4	0.453	180	1	5
6	5	0.533	180	1	5
6	6	0.748	180	1	5
6	1	2.325	360	2	5
6	2	8.659	360	2	5
6	3	6.25	360	2	5
6	4	0.675	360	2	5
6	5	0.527	360	2	5
6	6	0.528	360	2	5
6	1	1.925	180	2	5
6	2	9.292	180	2	5
6	3	6.848	180	2	5
6	4	0.533	180	2	5
6	5	0.32	180	2	5
6	6	0.641	180	2	5
7	1	2.267	360	1	5

7	2	5.086	360	1	5
7	3	4.749	360	1	5
7	4	0.93	360	1	5
7	5	1.343	360	1	5
7	6	0.619	360	1	5
7	1	2.393	180	1	5
7	2	5.519	180	1	5
7	3	4.692	180	1	5
7	4	1.043	180	1	5
7	5	1.878	180	1	5
7	6	0.728	180	1	5
7	1	2.166	360	2	5
7	2	5.057	360	2	5
7	3	4.654	360	2	5
7	4	1.343	360	2	5
7	5	1.965	360	2	5
7	6	0.516	360	2	5
7	1	1.977	180	2	5
7	2	4.821	180	2	5
7	3	4.683	180	2	5
7	4	0.945	180	2	5
7	5	1.043	180	2	5
7	6	0.429	180	2	5
8	1	2.472	360	1	5
8	2	8.622	360	1	5
8	3	12.552	360	1	5
8	4	0.309	360	1	5
8	5	0.786	360	1	5
8	6	0.927	360	1	5
8	1	2.371	180	1	5
8	2	8.621	180	1	5
8	3	11.882	180	1	5
8	4	0.206	180	1	5
8	5	0.692	180	1	5
8	6	0.728	180	1	5
8	1	2.481	360	2	5
8	2	8.704	360	2	5
8	3	12.348	360	2	5
8	4	0.103	360	2	5
8	5	0.516	360	2	5
8	6	0.515	360	2	5
8	1	2.378	180	2	5

8	2	8.672	180	2	5
8	3	11.948	180	2	5
8	4	0.206	180	2	5
8	5	0.527	180	2	5
8	6	0.618	180	2	5
9	1	1.559	360	1	5
9	2	8.296	360	1	5
9	3	10.056	360	1	5
9	4	0.723	360	1	5
9	5	0.602	360	1	5
9	6	0.824	360	1	5
9	1	2.505	180	1	5
9	2	8.711	180	1	5
9	3	10.445	180	1	5
9	4	0.894	180	1	5
9	5	0.886	180	1	5
9	6	0.939	180	1	5
9	1	1.677	360	2	5
9	2	8.047	360	2	5
9	3	10.429	360	2	5
9	4	0.516	360	2	5
9	5	0.462	360	2	5
9	6	0.627	360	2	5
9	1	1.697	180	2	5
9	2	7.996	180	2	5
9	3	10.255	180	2	5
9	4	0.418	180	2	5
9	5	0.636	180	2	5
9	6	0.737	180	2	5
10	1	1.614	360	1	5
10	2	9.759	360	1	5
10	3	11.962	360	1	5
10	4	0.302	360	1	5
10	5	0.443	360	1	5
10	6	0.707	360	1	5
10	1	1.529	180	1	5
10	2	10.442	180	1	5
10	3	14.118	180	1	5
10	4	0.407	180	1	5
10	5	0.619	180	1	5
10	6	1.019	180	1	5
10	1	1.614	360	2	5

10	2	9.647	360	2	5
10	3	13.26	360	2	5
10	4	0.302	360	2	5
10	5	0.403	360	2	5
10	6	0.606	360	2	5
10	1	1.325	180	2	5
10	2	10.118	180	2	5
10	3	13.895	180	2	5
10	4	0.306	180	2	5
10	5	0.322	180	2	5
10	6	0.714	180	2	5
11	1	1.714	360	1	5
11	2	4.64	360	1	5
11	3	5.46	360	1	5
11	4	0.612	360	1	5
11	5	0.504	360	1	5
11	6	0.706	360	1	5
11	1	1.632	180	1	5
11	2	4.796	180	1	5
11	3	5.336	180	1	5
11	4	0.407	180	1	5
11	5	0.814	180	1	5
11	6	0.62	180	1	5
11	1	1.614	360	2	5
11	2	4.943	360	2	5
11	3	5.851	360	2	5
11	4	0.403	360	2	5
11	5	0.806	360	2	5
11	6	0.406	360	2	5
11	1	1.948	180	2	5
11	2	4.182	180	2	5
11	3	5.224	180	2	5
11	4	0.407	180	2	5
11	5	0.814	180	2	5
11	6	0.62	180	2	5
12	1	1.891	360	1	5
12	2	3.782	360	1	5
12	3	4.678	360	1	5
12	4	3.882	360	1	5
12	5	0.802	360	1	5
12	6	0.598	360	1	5
12	1	2.189	180	1	5

12	2	3.992	180	1	5
12	3	4.407	180	1	5
12	4	3.782	180	1	5
12	5	1.194	180	1	5
12	6	0.597	180	1	5
12	1	1.496	360	2	5
12	2	3.436	360	2	5
12	3	4.778	360	2	5
12	4	3.682	360	2	5
12	5	0.796	360	2	5
12	6	0.498	360	2	5
12	1	1.393	180	2	5
12	2	3.604	180	2	5
12	3	4.582	180	2	5
12	4	3.781	180	2	5
12	5	0.896	180	2	5
12	6	0.703	180	2	5
1	1	2.77	360	1	6
1	2	13.125	360	1	6
1	3	9.554	360	1	6
1	4	0.917	360	1	6
1	5	0.649	360	1	6
1	6	1.385	360	1	6
1	1	2.243	180	1	6
1	2	14.75	180	1	6
1	3	10.079	180	1	6
1	4	0.804	180	1	6
1	5	0.61	180	1	6
1	6	0.815	180	1	6
1	1	2.024	360	2	6
1	2	13.638	360	2	6
1	3	9.29	360	2	6
1	4	0.716	360	2	6
1	5	0.64	360	2	6
1	6	0.746	360	2	6
1	1	2.252	180	2	6
1	2	13.624	180	2	6
1	3	9.055	180	2	6
1	4	0.368	180	2	6
1	5	0.611	180	2	6
1	6	0.917	180	2	6
2	1	2.119	360	1	6

2	2	6.267	360	1	6
2	3	16.177	360	1	6
2	4	0.806	360	1	6
2	5	0.504	360	1	6
2	6	1.009	360	1	6
2	1	1.891	180	1	6
2	2	20.423	180	1	6
2	3	15.847	180	1	6
2	4	0.597	180	1	6
2	5	0.498	180	1	6
2	6	0.696	180	1	6
2	1	1.788	360	2	6
2	2	20.206	360	2	6
2	3	16.574	360	2	6
2	4	0.705	360	2	6
2	5	0.302	360	2	6
2	6	0.807	360	2	6
2	1	1.932	180	2	6
2	2	19.837	180	2	6
2	3	16.335	180	2	6
2	4	0.597	180	2	6
2	5	0.498	180	2	6
2	6	0.796	180	2	6
3	1	2.725	360	1	6
3	2	5.447	360	1	6
3	3	6.561	360	1	6
3	4	0.645	360	1	6
3	5	0.302	360	1	6
3	6	0.807	360	1	6
3	1	2.421	180	1	6
3	2	5.657	180	1	6
3	3	6.456	180	1	6
3	4	0.403	180	1	6
3	5	0.605	180	1	6
3	6	0.605	180	1	6
3	1	2.118	360	2	6
3	2	5.447	360	2	6
3	3	6.556	360	2	6
3	4	0.403	360	2	6
3	5	0.503	360	2	6
3	6	0.605	360	2	6
3	1	2.017	180	2	6

3	2	5.448	180	2	6
3	3	6.255	180	2	6
3	4	0.303	180	2	6
3	5	0.403	180	2	6
3	6	0.606	180	2	6
4	1	2.365	360	1	6
4	2	7.917	360	1	6
4	3	11.837	360	1	6
4	4	0.306	360	1	6
4	5	0.796	360	1	6
4	6	0.62	360	1	6
4	1	2.447	180	1	6
4	2	8.958	180	1	6
4	3	14.056	180	1	6
4	4	0.102	180	1	6
4	5	0.51	180	1	6
4	6	0.612	180	1	6
4	1	2.447	360	2	6
4	2	8.261	360	2	6
4	3	11.998	360	2	6
4	4	0.407	360	2	6
4	5	0.432	360	2	6
4	6	0.51	360	2	6
4	1	2.039	180	2	6
4	2	9.137	180	2	6
4	3	13.476	180	2	6
4	4	0.61	180	2	6
4	5	0.652	180	2	6
4	6	0.918	180	2	6
5	1	1.98	360	1	6
5	2	6.174	360	1	6
5	3	5.208	360	1	6
5	4	0.522	360	1	6
5	5	0.627	360	1	6
5	6	1.25	360	1	6
5	1	1.885	180	1	6
5	2	5.511	180	1	6
5	3	5.005	180	1	6
5	4	0.313	180	1	6
5	5	0.209	180	1	6
5	6	0.737	180	1	6
5	1	1.668	360	2	6

5	2	6.45	360	2	6
5	3	4.909	360	2	6
5	4	0.313	360	2	6
5	5	0.43	360	2	6
5	6	0.728	360	2	6
5	1	1.694	180	2	6
5	2	5.806	180	2	6
5	3	4.919	180	2	6
5	4	0.313	180	2	6
5	5	0.522	180	2	6
5	6	0.839	180	2	6
6	1	2.217	360	1	6
6	2	9.08	360	1	6
6	3	6.565	360	1	6
6	4	1.136	360	1	6
6	5	0.538	360	1	6
6	6	0.844	360	1	6
6	1	2.032	180	1	6
6	2	8.976	180	1	6
6	3	6.315	180	1	6
6	4	0.754	180	1	6
6	5	0.639	180	1	6
6	6	0.545	180	1	6
6	1	2.122	360	2	6
6	2	8.972	360	2	6
6	3	6.341	360	2	6
6	4	0.85	360	2	6
6	5	0.422	360	2	6
6	6	0.746	360	2	6
6	1	1.925	180	2	6
6	2	8.973	180	2	6
6	3	6.209	180	2	6
6	4	0.533	180	2	6
6	5	0.648	180	2	6
6	6	0.534	180	2	6
7	1	2.061	360	1	6
7	2	5.358	360	1	6
7	3	4.43	360	1	6
7	4	0.826	360	1	6
7	5	1.136	360	1	6
7	6	0.628	360	1	6
7	1	2.395	180	1	6

7	2	4.901	180	1	6
7	3	4.682	180	1	6
7	4	0.233	180	1	6
7	5	1.068	180	1	6
7	6	0.728	180	1	6
7	1	2.061	360	2	6
7	2	4.841	360	2	6
7	3	4.739	360	2	6
7	4	0.62	360	2	6
7	5	1.55	360	2	6
7	6	0.207	360	2	6
7	1	1.977	180	2	6
7	2	5.308	180	2	6
7	3	4.787	180	2	6
7	4	0.43	180	2	6
7	5	1.565	180	2	6
7	6	0.624	180	2	6
8	1	2.266	360	1	6
8	2	8.708	360	1	6
8	3	8.551	360	1	6
8	4	0.206	360	1	6
8	5	1.033	360	1	6
8	6	0.95	360	1	6
8	1	2.268	180	1	6
8	2	8.506	180	1	6
8	3	12.518	180	1	6
8	4	0.413	180	1	6
8	5	0.517	180	1	6
8	6	0.927	180	1	6
8	1	2.474	360	2	6
8	2	9.558	360	2	6
8	3	12.778	360	2	6
8	4	0.516	360	2	6
8	5	0.66	360	2	6
8	6	1.133	360	2	6
8	1	2.165	180	2	6
8	2	8.649	180	2	6
8	3	12.552	180	2	6
8	4	0.413	180	2	6
8	5	0.66	180	2	6
8	6	0.824	180	2	6
9	1	1.957	360	1	6

9	2	8.468	360	1	6
9	3	10.231	360	1	6
9	4	0.62	360	1	6
9	5	0.786	360	1	6
9	6	0.721	360	1	6
9	1	1.569	180	1	6
9	2	8.427	180	1	6
9	3	11.23	180	1	6
9	4	0.314	180	1	6
9	5	0.732	180	1	6
9	6	0.626	180	1	6
9	1	1.651	360	2	6
9	2	7.901	360	2	6
9	3	11.105	360	2	6
9	4	0.31	360	2	6
9	5	0.516	360	2	6
9	6	0.618	360	2	6
9	1	2.089	180	2	6
9	2	8.528	180	2	6
9	3	11.698	180	2	6
9	4	0.418	180	2	6
9	5	0.669	180	2	6
9	6	0.635	180	2	6
10	1	1.617	360	1	6
10	2	9.836	360	1	6
10	3	13.039	360	1	6
10	4	0.504	360	1	6
10	5	0.451	360	1	6
10	6	0.713	360	1	6
10	1	1.325	180	1	6
10	2	9.372	180	1	6
10	3	12.959	180	1	6
10	4	0.204	180	1	6
10	5	0.42	180	1	6
10	6	0.611	180	1	6
10	1	1.416	360	2	6
10	2	9.885	360	2	6
10	3	13.015	360	2	6
10	4	0.302	360	2	6
10	5	0.637	360	2	6
10	6	0.807	360	2	6
10	1	1.227	180	2	6

10	2	9.992	180	2	6
10	3	12.661	180	2	6
10	4	0.305	180	2	6
10	5	0.367	180	2	6
10	6	0.612	180	2	6
11	1	1.714	360	1	6
11	2	5.153	360	1	6
11	3	6.052	360	1	6
11	4	0.415	360	1	6
11	5	1.151	360	1	6
11	6	0.706	360	1	6
11	1	1.432	180	1	6
11	2	4.691	180	1	6
11	3	5.812	180	1	6
11	4	0.42	180	1	6
11	5	1.06	180	1	6
11	6	0.918	180	1	6
11	1	1.726	360	2	6
11	2	4.439	360	2	6
11	3	5.753	360	2	6
11	4	0.318	360	2	6
11	5	0.604	360	2	6
11	6	0.706	360	2	6
11	1	1.432	180	2	6
11	2	4.588	180	2	6
11	3	5.507	180	2	6
11	4	0.305	180	2	6
11	5	0.618	180	2	6
11	6	0.51	180	2	6
12	1	1.298	360	1	6
12	2	3.981	360	1	6
12	3	4.877	360	1	6
12	4	3.787	360	1	6
12	5	0.796	360	1	6
12	6	0.498	360	1	6
12	1	1.791	180	1	6
12	2	3.881	180	1	6
12	3	4.479	180	1	6
12	4	3.981	180	1	6
12	5	0.896	180	1	6
12	6	0.597	180	1	6
12	1	1.493	360	2	6

12	2	3.886	360	2	6
12	3	4.877	360	2	6
12	4	3.682	360	2	6
12	5	0.995	360	2	6
12	6	0.598	360	2	6
12	1	1.393	180	2	6
12	2	3.687	180	2	6
12	3	4.578	180	2	6
12	4	3.787	180	2	6
12	5	0.597	180	2	6
12	6	0.497	180	2	6
1	1	2.118	gold	1	7
1	2	12.982	gold	1	7
1	3	8.291	gold	1	7
1	4	0.394	gold	1	7
1	5	0.15	gold	1	7
1	6	0.448	gold	1	7
1	1	2.323	gold	2	7
1	2	13.171	gold	2	7
1	3	8.911	gold	2	7
1	4	0.763	gold	2	7
1	5	0.412	gold	2	7
1	6	0.588	gold	2	7
2	1	2.173	gold	1	7
2	2	19.697	gold	1	7
2	3	15.304	gold	1	7
2	4	0.71	gold	1	7
2	5	0.262	gold	1	7
2	6	0.604	gold	1	7
2	1	1.985	gold	2	7
2	2	19.742	gold	2	7
2	3	15.284	gold	2	7
2	4	0.476	gold	2	7
2	5	0.456	gold	2	7
2	6	0.426	gold	2	7
3	1	2.492	gold	1	7
3	2	6.056	gold	1	7
3	3	5.612	gold	1	7
3	4	0.402	gold	1	7
3	5	0.095	gold	1	7
3	6	0.142	gold	1	7
3	1	2.586	gold	2	7

3	2	6.193	gold	2	7
3	3	5.707	gold	2	7
3	4	0.244	gold	2	7
3	5	0.427	gold	2	7
3	6	0.295	gold	2	7
4	1	2.209	gold	1	7
4	2	7.872	gold	1	7
4	3	11.632	gold	1	7
4	4	0.192	gold	1	7
4	5	0.719	gold	1	7
4	6	0.728	gold	1	7
4	1	2.24	gold	2	7
4	2	7.887	gold	2	7
4	3	11.701	gold	2	7
4	4	0.345	gold	2	7
4	5	0.751	gold	2	7
4	6	0.688	gold	2	7
5	1	1.787	gold	1	7
5	2	6.538	gold	1	7
5	3	5.277	gold	1	7
5	4	0.401	gold	1	7
5	5	0.533	gold	1	7
5	6	0.783	gold	1	7
5	1	1.828	gold	2	7
5	2	6.431	gold	2	7
5	3	5.159	gold	2	7
5	4	0.353	gold	2	7
5	5	0.518	gold	2	7
5	6	0.578	gold	2	7
6	1	2.277	gold	1	7
6	2	8.387	gold	1	7
6	3	5.717	gold	1	7
6	4	0.953	gold	1	7
6	5	0.488	gold	1	7
6	6	0.549	gold	1	7
6	1	1.99	gold	2	7
6	2	8.392	gold	2	7
6	3	6.238	gold	2	7
6	4	0.998	gold	2	7
6	5	0.561	gold	2	7
6	6	0.624	gold	2	7
7	1	2.119	gold	1	7

7	2	4.726	gold	1	7
7	3	5.14	gold	1	7
7	4	0.986	gold	1	7
7	5	1.185	gold	1	7
7	6	0.443	gold	1	7
7	1	1.881	gold	2	7
7	2	5.16	gold	2	7
7	3	5.206	gold	2	7
7	4	1.045	gold	2	7
7	5	1.648	gold	2	7
7	6	0.43	gold	2	7
8	1	2.246	gold	1	7
8	2	8.22	gold	1	7
8	3	11.711	gold	1	7
8	4	0.542	gold	1	7
8	5	0.748	gold	1	7
8	6	1.058	gold	1	7
8	1	2.327	gold	2	7
8	2	8.362	gold	2	7
8	3	11.729	gold	2	7
8	4	0.471	gold	2	7
8	5	1.055	gold	2	7
8	6	0.643	gold	2	7
9	1	2.032	gold	1	7
9	2	8.032	gold	1	7
9	3	10.072	gold	1	7
9	4	0.56	gold	1	7
9	5	0.814	gold	1	7
9	6	0.475	gold	1	7
9	1	2.033	gold	2	7
9	2	7.734	gold	2	7
9	3	10.003	gold	2	7
9	4	0.363	gold	2	7
9	5	0.728	gold	2	7
9	6	0.541	gold	2	7
10	1	1.483	gold	1	7
10	2	8.484	gold	1	7
10	3	11.622	gold	1	7
10	4	0.374	gold	1	7
10	5	0.526	gold	1	7
10	6	0.588	gold	1	7
10	1	1.531	gold	2	7

10	2	8.45	gold	2	7
10	3	11.775	gold	2	7
10	4	0.322	gold	2	7
10	5	0.415	gold	2	7
10	6	0.806	gold	2	7
11	1	1.693	gold	1	7
11	2	5.338	gold	1	7
11	3	6.379	gold	1	7
11	4	0.492	gold	1	7
11	5	1.19	gold	1	7
11	6	0.436	gold	1	7
11	1	1.754	gold	2	7
11	2	5.369	gold	2	7
11	3	5.767	gold	2	7
11	4	0.544	gold	2	7
11	5	1.208	gold	2	7
11	6	0.43	gold	2	7
12	1	1.833	gold	1	7
12	2	3.786	gold	1	7
12	3	4.201	gold	1	7
12	4	3.744	gold	1	7
12	5	1.248	gold	1	7
12	6	0.357	gold	1	7
12	1	1.734	gold	2	7
12	2	3.832	gold	2	7
12	3	4.081	gold	2	7
12	4	4.065	gold	2	7
12	5	1.329	gold	2	7
12	6	0.329	gold	2	7

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