The effect of contamination on selected physical and chemical characteristics of Mineral Trioxide Aggregate

Thesis submitted in fulfilment of the degree of

Doctor of Philosophy

Mohammad Hossein Nekoofar

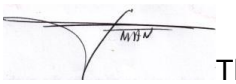
School of Dentistry

Cardiff University

(April 2006-August 2011)

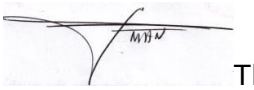
DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

 Thursday, 23 June 2011

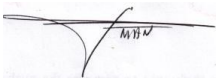
STATEMENT 1

This thesis is being submitted in partial fulfillment of the requirements for the degree of **PhD**

 Thursday, 23 June 2011

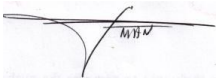
STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated.
Other sources are acknowledged by explicit references.

 Thursday, 23 June 2011

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

 Thursday, 23 June 2011

STATEMENT 4: PREVIOUSLY APPROVED BAR ON ACCESS

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans **after expiry of a bar on access previously approved by the Graduate Development Committee.**

Signed …… (candidate) Thursday, 23 June 2011

Oh God, grant me the serenity to accept the things I cannot change and courage to change the things I can and the wisdom to know the difference [(i)].

Acknowledgments

Above all I wish to give my thanks to God, the creator, who has endowed us with the ability to understand some of the complexities of our surroundings and to recognise the fascinating order of the universe, which is only a reflection of God's infinite intelligence and beauty. This comprehension indeed can make possible to man a definite appreciation of life [(ii)].

I would like to dedicate this thesis to my Mother, **"*Mrs Safa Akhavan*",** for her endless love and to the memory of my beloved Father, **"*Dr Abbas Nekoofar*"**, my main role model. I also dedicate the thesis to my wife, **"*Dr Fatemeh Rokni Yazdy*",** whom I would be nothing without and to my lovely children and son in law "***Hosniyeh, Zeynab, Zoha"*** and "***Mohammad"*** for allowing me to embark on this PhD programme that took me away from them.

This thesis is also dedicated to my forever wonderful supervisor, **"*Professor Paul Michael Howell Dummer*",** without whose inspiration this doctorate would not have been possible.

Professor Paul M H Dummer has taught me how to think professionally, design and manage research projects and how to write and publish academically. Words are not able to express my appreciation to you ***Paul***. It is an honour to be your student.

I am also heartily thankful to *"Professor Robert Newcombe"* for his ongoing support and valuable advice during the statistical analysis of the data.

I also offer my regards and blessings to *"Professor Jeremy Rees"*, my second supervisor, for his support and encouragement and to all of the collaborators and those who supported me during the completion of the project, including:

Dr M S Sheykhrezae, Dr DF Stone, Dr MS Namazikhah, Dr N Shokouhinejad, Dr T E Davies, Dr FB Basturk, Mrs S Bryant, Dr MB Kayahan, Mr SJ Hayes, Mr Brian Western, Mrs W Rowe, Dr G Adusei, Dr Z Aseeley, Dr K Oloomi, Mr D C Stone, Mrs S Oliver, Mr C Fenton, Dr DC Haddad, J Dr Nolde, Dr R Tabor, Dr A Carle, and Dr MM Mohammadi,

i) Justin Kaplan, ed., Bartlett's Familiar Quotations 735 (17th ed. 2002) (attributing the prayer to Niebuhr in 1943).

ii) Ghandi Mahmoud Energy Spectrum of Electrons in Non-Crystalline Materials. Thesis (PhD) University of California, Davis 1971

Summary

Aim: To evaluate the effect of various environmental (clinical) conditions on the physical and chemical characteristics of Mineral Trioxide Aggregate (MTA).

Methodology: Initially preparation of specimens was standardised. Moreover, a novel mixing technique, trituration of encapsulated MTA, was developed. The effects of acid and blood contamination on various characteristics of MTA including compressive strength, surface microhardness, push-out bond strength and total porosity were then evaluated. Furthermore, by using X-ray diffraction analysis the hydration process of blood contaminated MTA was studied. In addition, the microstructure of contaminated MTA specimens was compared with control groups.

Results: Methods of mixing and placing MTA significantly affected the hydration process and consequently the physical properties of the material. The lowest and greatest compressive strength, Vickers surface microhardness, and push-out strength values of MTA were found after exposure to pH levels of 4.4 and 7.4, respectively. In addition, scanning electron microscopy revealed a lack of needle-like crystals when the material was in contact with more acidic solutions. The hydration state of specimens partially mixed with blood was more complete than those mixed entirely with blood and less than specimens that were hydrated only with water.

Conclusion: In experimental investigations, use of controlled mixing and placement techniques when using MTA is essential in order to standardise specimen preparation. Delaying the placement of the final coronal restoration in clinical situations when MTA is contaminated is recommended so that the material can acquire sufficient physical properties to withstand the acid-etch procedure and the condensation pressures that occur during the placement of a restoration and/or produced through indirect masticatory forces.

CHAPTER 1

INTRODUCTION

1. Introduction

The ultimate goal of endodontic treatment is to conserve the integrity of the masticatory system by saving teeth at risk of developing pulp inflammation and those with established pulp and periradicular disease. Prevention of pulp inflammation through elimination of caries and restoration of teeth is the primary aim of Operative Dentistry. However, several treatment modalities within the scope of Endodontics, such as direct pulp capping and pulpotomy are used to eliminate infected dentine and pulp tissue in order to preserve the integrity of the remaining uninfected tissues. Such treatments are generally known as "vital pulp treatments". On the other hand, when pulp inflammation is irreversible and the pulp is non-savable treatments are categorised as "non-vital pulp treatments". "Root canal treatment" is the preferred option when attempting to retain teeth, the aim being to eliminate infection from the pulpodentinal complex, which is achieved by debridement and disinfection of the root canal system. Filling the root canal system and restoring the tooth prevents re-infection by re-establishing the surface integrity of the body to prevent microbial ingress.

1.1. **Vital pulp treatments**

Management of an exposed vital pulp in an adult tooth, particularly when root formation is incomplete, is a controversial topic in Endodontics and a broad range of treatments including direct pulp capping and pulpotomy are available (Pitt Ford *et al.* 1996, Ward 2002). The materials used in direct pulp capping and pulpotomy are placed adjacent to pulp tissue. Therefore, they must be non-toxic (Saidon *et al.* 2003) and of low solubility (El-Araby & Al-Jabab 2005). A pulp capping material should also have sufficient compressive strength to tolerate pressures resulting from the condensation of restorative materials (Shazad &

Kennedy 1994). The ability of a pulp capping material to set in a wet environment is also important (Karabucak *et al.* 2005) as well as its ability to control bleeding during treatment (Tunca *et al.* 2007). Such a material should also prevent bacterial leakage, which is known to be the main aetiological factor in post-treatment disease (Fuks 2002).

1.2. **Root canal treatment**

As a consequence of extensive caries and/or a traumatic injury the dental pulp may become irreversibly inflamed and/or infected with the result that conserving the pulp tissue is not feasible. Root canal treatment includes removal of the infected pulp and the micro-organisms that inhabit the canal system followed by the placement of a root filling. Such treatments are not always straightforward and a number of complications can impede thorough shaping, cleaning and filling of the root canal system. In some instances, because of these complications, periapical disease may persist or emerge following treatment (Wu *et al.* 2006).

1.2.1. **Post-treatment disease**

Ideally post-treatment disease caused by intra-canal infection should be treated by revision of the primary root canal treatment. However, for pragmatic reason such cases are often treated using a surgical approach, which is the appropriate approach for extra-radicular infection, foreign body reactions and cysts (Wu *et al.* 2006). The outcome of endodontic surgery is largely dependent on the elimination of the antigenic source through the removal of infection or on the ability to trap micro-organisms within the tooth. It is generally accepted that the root-end cavity in a resected root should be prepared and then sealed with an appropriate root-end filling material (Theodosopoulou & Niederman 2005) to prevent the passage of the antigenic source from the contaminated root canal into the periapical tissue (Kim & Kratchman 2006). In addition, an ideal root-end filling material should be non-toxic, of low

solubility and be dimensionally stable (Andreasen & Pitt Ford 1994). Numerous root-end filling materials have been used, such as reinforced zinc oxide-eugenol cements, amalgam, composite resins, and glass-ionomer cement (Pereira *et al.* 2004). None of them have been considered to fulfill the requirements of an ideal root-end filling material (Harrison 1992).

1.2.2. **Incomplete root formation**

In some instances, incomplete development of the root apex may result in a wide root canal with thin and delicate dentine walls and an open apex that creates a major challenge during conventional root canal treatment. Pulp necrosis in a tooth with incomplete root formation can result from trauma at a young age. Conventional root canal treatment including cleaning, shaping and filling of such root canals can be problematic.

1.2.2.1.**Apexification**

Various methods and different materials have been suggested to deal with teeth having immature apices; such treatments are generally called "apexification". Apexification has been defined by the American Association of Endodontists (2003) as:

> *"a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incomplete root in teeth with necrotic pulp".*

The most essential requirements of a material in this situation are to be non-toxic osteoconductive and antibacterial (Shabahang & Torabinejad 2000),. Another important necessity is the capacity of the material to seal the apical part of root canal system (Morse *et al.* 1990). In addition, because of the lack of root formation control of bleeding and effective drying of the root canal system is a challenge with the result that a material that can set in an aqueous environment will be more efficacious (Simon *et al.* 2007).

1.2.2.2. **Regenerative endodontics**

The aim of regenerative endodontics in treatment of teeth with incomplete root formation and necrotic pulp is to replace the irreversibly inflamed pulp tissue with newly regenerated tissue in an attempt to stimulate root maturation (Murray *et al.* 2007). This novel procedure of revitalization, introduced by Banchs & Trope (2004), involves chemical disinfection of the root canal system through profuse irrigation using sodium hypochlorite and the placement of a tri-antibiotic paste inside the root canal system without mechanical instrumentation. The tri-antibiotic paste was described by Hoshino *et al.* (1996) as an effective medicament against the multi-bacterial root canal infection. At the second appointment a blood clot is induced inside the canal to act as a matrix for regeneration and then the coronal access cavity is sealed to prevent bacterial penetration and to allow regeneration in a bacteria free environment (Banchs & Trope 2004). More recently, the use of platelet-rich plasma rather than blood clot has been suggested (Hiremath *et al.* 2008).

The material used to provide the bacteria tight seal in this context is important (Bose *et al.* 2009, Torabinejad & Turman 2011) as it should ideally have the ability to up-regulate signaling molecules and provoke regeneration (Huang 2008, Thomson & Kahler 2010). Moreover, since it is not practical to avoid blood contamination the sealing ability and basic physical properties of the material should not be jeopardized by moisture and/or blood exposure.

1.2.3. **Root perforation**

To prevent infection and/or re-infection of periodontal tissues, root canal treatment procedures should remain within the root canal system. One of the iatrogenic accidents that results in an unintended communication between the root canal system and periodontium is a perforation, which can be categorized according to its anatomical location (Fuss & Trope

1996). Perforations can result in an inflammatory reaction within the periodontal tissues through infection that can then lead to bone loss. Adequate illumination and magnification (de Carvalho & Zuolo 2000), choosing a non end-cutting bur during access cavity preparation (Riitano 2005) and use of ultrasonic instruments to remove dentine interferences to obtain clear and unhindered access to each orifice (Plotino *et al.* 2007) can reduce the possibility of perforating the pulp chamber floor during access cavity preparation. Moreover, various root canal length measurement methods (Nekoofar *et al.* 2006) and preparation techniques (Abou-Rass *et al.* 1980, Torabinejad 1994, Dummer *et al.* 1998) have been described to reduce the possibility of apical, lateral and strip root perforations. Despite the ability of these techniques to prevent iatrogenic perforation, it has been reported as the second greatest cause of post-treatment disease (Ingle *et al.* 2007).

Dealing with perforations is challenging. Ideally, a perforation should be repaired immediately following its creation (Fuss & Trope 1996). Various materials have been suggested to repair these defects with the outcome dependent on the biocompatibility of the chosen material and its sealing ability (Bryan *et al.* 1999). Ideally, the material should be osteoconductive and be able to provide an environment for cementum re-growth (Alhadainy 1994). In addition, since in some instances control of bleeding is difficult, the use of a material that can set in an aqueous environment is advantageous (Alhadainy 1994, Gulsahi *et al.* 2007).

1.2.4. **Root resorption**

Root resorption is a pathological process that involves specific clastic cell activity resulting in destruction of the mineral and organic structures of cementum and dentine (Heithersay 1994). The aetiology of this entity is not known but it is a destructive process that can develop gradually or more rapidly, with some types of resorption being self-limiting (Benenati 1997).

Various treatment approaches have been suggested for resorption based on its anatomical position, morphology, histopathological findings and severity, as well as its extent and speed of progression. As a common goal of these treatment modalities clastic cells should be eliminated and the defect sealed by an appropriate material (Bakland 1992) against future antigenic leakage (Panzarini *et al.* 2007). Since thorough eradication of clastic cells is not practical, progression of the disease may also be controlled by release of calcium ions from such materials (Ozdemir *et al.* 2008). This ionic release, by changing the pH, may block clastic activity and prevent progression and/or recurrence of resorptive defects (Rehman *et al.* 1996, Sari & Sonmez 2006). In cases of pathological root perforation as a consequence of extensive resorption the repair material should also be non-toxic (Hsien *et al.* 2003) and be able to seal the defect effectively.

1.3. **Mineral Trioxide Aggregate**

1.3.1. **Advantages**

In all of the procedures mentioned above, the selected material is placed in contact with connective tissues. Therefore, its biocompatibility is a most critical requirement. In addition, the materials should ideally be non-toxic, set in a wet environment, be unaffected by blood contamination, provide a good seal against bacteria and fluids, release calcium hydroxide, induce or conduct bone deposition, have antibacterial properties and have reasonable compressive strength and hardness in order to withstand functional loads and the compaction forces that might apply when restorative materials are used subsequently.

Mineral trioxide aggregate (MTA), which was developed by Torabinejad and co-workers at Loma Linda University, CA, USA (Lee *et al.* 1993), has been shown to cause low levels of inflammation (Torabinejad *et al.* 1995c), be less cytotoxic than conventional materials

(Keiser *et al.* 2000, Ribeiro *et al.* 2006) and can be used safely in contact with pulp and periodontal tissues (Abedi & Ingle 1995, Torabinejad & Chivian 1999). In addition, Tunca *et al.* (2007) reported that MTA induced vasoconstriction that may facilitate haemorrhage control.

Thus, MTA appears to be a most promising material for use in all of the aforementioned challenging endodontic modalities (Mente *et al.* 2010a, Mente *et al.* 2010b, Parirokh & Torabinejad 2010b, Tang *et al.* 2010). Initially MTA was available commercially as ProRoot? MTA (Dentsply Tulsa Dental, Tulsa, OK, USA); however, another MTA-based cement has been launched commercially as MTA Angelus (Angelus Dental Industries Ltd, Londrina, Brazil). Both manufacturers produce two types of MTA (grey and tooth coloured); the initial grey formulation was thought to stain teeth (Antunes Bortoluzzi *et al.* 2007), hence the development of the tooth coloured variety. More recently, a range of other MTA-based and related materials have been developed and marketed, e.g. Biodentine? (Septodont, Saint-Maur-des-) RVps, France), CEM-cement? (BioniqueDent, Tehran, Iran) and Biosealer? (Isasan, Rovello Porro, Italy).

According to the US patent 5,415,547, the principle component of MTA is Portland cement (Torabinejad & Dean 1995). Numerous authors have examined similarities between several types of MTA and Portland cement (Estrela *et al.* 2000, Asgary *et al.* 2004, Camilleri *et al.* 2005b, Dammaschke *et al.* 2005) and demonstrated that both tooth coloured and grey MTA have a similar chemical constitution to Portland cement except for the addition of bismuth oxide to make them radiopaque (Hwang *et al.* 2009). Saidon *et al.* (2003) reported that MTA and Portland cement were both biologically tolerated and had similar biocompatibility; several further studies have confirmed these findings (Ribeiro *et al.* 2005, de Morais *et al.* 2006, Ribeiro *et al.* 2006).

MTA was introduced initially for the repair of root perforations (Lee *et al.* 1993, Mente *et al.* 2010b) and was subsequently recommended as a root-end filling material following root-end resection (Torabinejad *et al.* 1993, Torabinejad *et al.* 1995a). It has also been suggested for use in vital pulp treatments (Pitt Ford *et al.* 1996, Mente *et al.* 2010a). Since hard tissue induction is one of its exceptional properties it has been recommended for use as an apical barrier in treatment of immature teeth with non-vital pulps and open apices (Bakland 2000, Mente *et al.* 2009). Schwartz (1999) reported the use of MTA for repair of resorptive root defects.

Compared to other materials that have been used in similar clinical applications, MTA has been shown to have a high degree of biocompatibility (Geurtsen 2001, Ribeiro *et al.* 2005), low degree of cytotoxicity (Keiser *et al.* 2000, Ribeiro *et al.* 2006), good marginal adaptation (Xavier *et al.* 2005), antibacterial effects (Al-Hezaimi *et al.* 2006) and good sealing ability (De Bruyne *et al.* 2006). However, to date the number of clinical trials undertaken using MTA is limited (Theodosopoulou & Niederman 2005, Nair *et al.* 2008, Christiansen *et al.* 2009).

1.3.2. **Disadvantages**

Despite its unique combination of properties and great potential, the prolonged setting time of the original MTA material is a major disadvantage (Antunes Bortoluzzi *et al.* 2006) as are its poor handling characteristics (Levenstein 2002). After mixing MTA with water by hand the resulting material is a "grainy" and "sandy" mixture, which is difficult to manipulate (Kogan *et al.* 2006). On occasions, even when MTA powder is mixed with the recommended amount of water it can become too dry and have poor handling characteristics; unfortunately, adding more water may reduce its resistance to movement and result in even more difficult handling (Kogan *et al.* 2006, Felekoglu *et al.* 2007, Jafarnia *et al.* 2009).

In addition, since MTA requires water to initiate and complete the setting reaction, placing a wet cotton pellet next to MTA has been suggested following its use (Torabinejad 2004). Therefore, it is recommended that other filling materials are not placed adjacent to it at the same appointment, a problem that obviously increases the number of appointments required to complete treatment.

In surgical endodontics after placement of MTA in a root-end cavity, it may be lost from the preparation when the surgical site is irrigated (Kogan *et al.* 2006). In addition, MTA may sometimes remain unset at subsequent appointments to suggest incomplete hydration of the material (Torabinejad *et al.* 1995b). Different physiological conditions may also interfere with the hydration of MTA and its microhardness (Lee *et al.* 2004). Low compressive strength (Harrington 2005), low viscosity (Clark 2007), shrinkage (Kogan *et al.* 2006) and poor chemical bonding to dentine (Yan *et al.* 2006) are other potential disadvantages of the material.

1.4. **Gaps in knowledge**

Following the development of MTA by Torabinejad and co-workers (Lee *et al.* 1993, Torabinejad *et al.* 1993) research tended to focus on its advantageous biological properties, clinical applications of MTA and sealing ability rather than on basic physical and chemical properties. Indeed, few early independent studies reported on the physical and chemical properties of the material. Furthermore, the hydration process of MTA was not evaluated fully. Over time, investigators began to investigate these important properties (Lee *et al.* 2004, Camilleri *et al.* 2005a, Dammaschke *et al.* 2005, Danesh *et al.* 2006, Islam *et al.* 2006a, Camilleri 2007, Camilleri 2008b, Gandolfi *et al.* 2009, Gandolfi *et al.* 2010b, AlAnezi *et al.* 2011, Cutajar *et al.* 2011). In such studies, to achieve valid and consistent results, variables that may affect the properties and performance of the materials tested should be

controlled. However, various confounding variables, such as water content, mixing methodology and spatulation pressure were uncontrolled and not reported in these previous studies with the result that their conclusions could be compromised.

When testing materials it is also imperative to adhere to standards that govern their evaluation. Unfortunately, MTA is not a typical dental, cement-like material and testing its physical and chemical properties requires a wider consideration of the appropriate standards. Some studies have focused on testing MTA using standards based on dental cements (Torabinejad *et al.* 1995b, Fridland & Rosado 2005), e.g. specification for dental root canal filling materials (ISO 6876) and for dental zinc oxide eugenol cements and zinc oxide non-eugenol cement (ANSI/ADA No.30). However, since MTA is a Portland-cement-like material Camilleri *et al.* (2006) and Danesh *et al.* (2006) used Portland cement standards, which are applicable in the construction industry, e.g. composition, specification and conformity criteria for common cements (EN 197-1) and specification for physical testing of Portland cement (BS 4550 Section 3).

Obviously, clinical applications of MTA are not similar to industrial applications of Portland cement. Moreover, MTA is neither a restorative material nor an endodontic sealer; rather it is a unique material and does not easily meet the criteria of the available standards. Thus, it could be argued that MTA should have its own "standard".

Characterization of MTA under standardized and controlled conditions should lead to an improved understanding of its behaviour and the optimisation of its use in clinical practice. However, although MTA has been suggested for use in a variety of clinical applications there is limited information available on the effect of various environmental factors on the physical properties of MTA. In particular, little is known about the effect of mixing technique, including spatulation pressure, ultrasonic agitation, cement to water ratio and acid or blood

contamination on its chemical and physical characteristics. In addition, the effects of other restorative materials that may be placed in direct contact with MTA, such as glass ionomer, have not been studied comprehensively (Parirokh & Torabinejad 2010a).

In order to overcome its various disadvantages, to improve its physical and chemical properties and propose a specific standard for MTA, it is essential that knowledge of the physical properties and chemical characteristics of MTA is enhanced. In addition, since in most of its clinical applications it would be exposed to blood and/or tissue fluids, the effects of various clinical situations on the characteristics of MTA should be studied. Hydration, setting and hardening process should also be investigated to enhance understanding and provide base-line data to develop the material further.

1.5. **Overall aim**

The overall aim of this work is to evaluate several physical and chemical characteristics of ProRoot? MTA (Dentsply Tulsa Dental,) while exposed to acidic preparations or blood, the type of contamination that is likely to occur in the clinical environment. However, prior to the main studies a substantial body of work was undertaken to standardise the methodology relating to specimen preparation in order to ensure the control and/or elimination of confounding variables such as mixing and placement techniques as well as powder to water ratios.

1.6. **Structure of thesis**

In Chapter 1, the "Introduction", the background to the development and use of MTA are described as well as deficiencies in knowledge. By highlighting the disadvantages of MTA the reasons for undertaking this series of studies and their importance are justified.

Chapter 2, the "Review of the literature", contains a comprehensive review and critical appraisal of more than 300 articles that describe various aspects of MTA, including its biological, physical and chemical properties. Where possible these properties have been related to its clinical applications.

In Chapter 3 the aims of the studies are described, whilst the experimental studies undertaken in fulfillment of this PhD are reported in Chapters 4 to 5. Chapter 4 contains four separate fundamental studies undertaken to improve the background knowledge of how MTA (ProRoot? MTA, Dentsply Tulsa Dental) should be mixed and how specimens should be prepared. These preliminary studies allowed a consistent methodology (standard) for the main investigations that followed. In addition, the lack of consistency in the water content of ProRoot? MTA (Dentsply Tulsa Dental) ampoules is reported, which led to the development of a novel mixing technique by encapsulating MTA. Moreover, the effect of mixing and placement methodology and various water-to-cement ratios on selected properties of MTA were evaluated.

In Chapter 5, the effect of contamination on selected properties of MTA is reported. In these studies, 'standard' specimens were subjected to various experimental conditions that included contamination with two types of acid or blood in an attempt to simulate several challenges that occur in the clinical situation and their effect on the physical and chemical properties of MTA.

Chapter 6, the final chapter, contains conclusions and areas for further research.

CHAPTER 2

REVIEW OF THE LITERATURE

2. Mineral Trioxide Aggregate – a review of the literature

2.1. **Introduction**

Mineral Trioxide Aggregate (MTA) was developed by Torabinejad and co-workers at Loma Linda University, CA, USA in an attempt to fulfill the ideal criteria of a root perforation repair material (Lee *et al.* 1993). The material was recommended subsequently as a "root-end filling material" (Torabinejad *et al.* 1993). MTA was initially approved for use in vital pulp treatments by the United States Food and Drug Administration in 1997 (USFDA 1997). Its use in other clinical applications such as repair of root perforations and/or as an apical plug during apexification was approved later (USFDA 1998a). Subsequently, the United States Food and Drug Administration supported the safety and effectiveness of MTA for use as a root-end filling material (USFDA 1998b).

MTA is a type of hydraulic cement that requires water to set. In simple terms, hydraulic cements are finely ground materials (powders) that when mixed with water gradually or instantly set and harden in air or in water; the reaction resulting in the formation of hydrated compounds whose strength increases with time. MTA consists of fine hydrophilic particles that on contact with water sets to a hard composition through the creation of a colloidal gel (Pitt Ford *et al.* 1995, Lee *et al.* 2004, Camilleri *et al.* 2005a).

2.2. **Clinical applications**

Mineral trioxide aggregate (MTA) has shown potential as an endodontic material in several *in vivo*, *in vitro* and *ex vivo* studies (Mitchell *et al.* 1999, Torabinejad & Chivian 1999, Moretton *et al.* 2000, Schmitt *et al.* 2001) and because of its many potential advantages, it is being used increasingly in a wide range of clinical treatments. It was first developed and

introduced in endodontics for the repair of root perforations (Lee *et al.* 1993). Subsequently, it has been widely used in surgical endodontics as a root-end filling material (Torabinejad *et al.* 1993, Aqrabawi 2000). It has also been used in vital pulp treatments, including direct pulp capping and pulpotomy of pulps in immature teeth (apexogenesis) (Abedi & Ingle 1995, Torabinejad & Chivian 1999). In addition, as hard tissue induction is one of its exceptional properties, it has been suggested as an apical barrier in treatment of teeth with open apices and necrotic pulps (apexification) (Shabahang & Torabinejad 2000, Witherspoon & Ham 2001).

MTA also provides an effective seal against penetration of bacteria and their by-products (Tselnik *et al.* 2004) and thus has been recommended as a temporary filling material (Schmitt *et al.* 2001) and as a coronal plug after filling of the root canal system (Mah *et al.* 2003, Tselnik *et al.* 2004). Moreover, it is recommended for the non-surgical repair of invasive cervical root resorption (Schwartz *et al.* 1999). Yildirim & Gencoglu (2009) reported new hard tissue formation in two horizontal root fracture lines after a 5-year follow-up and suggested the use of MTA in the treatment of such cases. In addition, Gomes-Filho *et al.* (2009) reported that a sealer based on MTA stimulated mineralization and thus advocated its use as a root canal sealer. The use of MTA has also been suggested in regenerative endodontics for treatment of immature permanent teeth with periapical disease (Banchs & Trope 2004, Thibodeau & Trope 2007, Petrino *et al.* 2010, Thomson & Kahler 2010, Torabinejad & Turman 2011).

2.3. **Commercially available products**

The first commercial MTA product was launched as ProRoot? MTA (Dentsply Tulsa Dental). The initial product was a grey-coloured material but as this initial formulation was thought to stain teeth (Antunes Bortoluzzi *et al.* 2007) a tooth coloured version was

subsequently developed. A second commercially available cement was later launched as MTA Angelus? (Angelus Dental Industries Ltd, Londrina, Brazil) with both grey and tooth coloured versions being available.

Since the commencement of this PhD a number of other MTA-like materials such as Biodentine? (Septodont, Saint-Maur-des-) RVps, France), CEM? cement (BioniqueDent, Tehran, Iran), Biosealer? (Isasan, Rovello Porro, Italy) and DiaRoot? BioAggregate (Innovative BioCeramix, Vancouver, Canada) have been marketed. The manufacturers of these new products claim that the handling and biological properties of their products are better than those of MTA. This thesis, however, will focus on ProRoot? MTA (Dentsply Tulsa Dental), which was available at the outset of the project.

2.4. **Chemical constituents**

According to the US patent number 5,415,547, the principle component of MTA is Portland cement (Torabinejad & Dean 1995). Portland cement was invented in 1824 by a British bricklayer, Joseph Aspdin who burned crushed limestone and clay in his kitchen stove and produced a hydraulic cement that hardened following mixing with water (Bates 1926). The inventor named it 'Portland cement' because it was similar to a stone extracted from the Isle of Portland off the British coast (Ryan 1929).

Today Portland cement is produced by crushing, grinding and blending raw materials (limestone and clay or shale) followed by heating the resulted powder in a rotary kiln up to a temperature of approximately 1400-1500^{o} C to partially fuse them (Taylor 1997) and thus produce clinker nodules. To modify the properties of the final product, clinker nodules are then cooled and ground with 3-6% calcium sulphate ($CaSO_4$). The clinker has a composition of calcium oxide (CaO) 50–75%, silicon dioxide (SiO_2) 15–25%, aluminum oxide (Al_2O_3) 1-5% and iron oxide (Fe_2O_3) 1-3% (Taylor 1997) and contains four main phase fractions

including “alite” or ticalcium silicate ($3CaO.SiO_2$), “belite” or dicalcium silicate ($2CaO\_SiO_2$), “aluminate” or tricalcium aluminate ($3CaO.Al_2O_3$) and “ferrite” or tetracalcium aluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$) (Aranda 2001, Bensted 2002).

Camilleri (2007) chemically analysed Portland cement and MTA powders and reported low levels of the aluminate phase in the latter and concluded that MTA powder was very likely manufactured in a laboratory rather than in an industrial rotary kiln.

For simplification in cement chemistry a single letter can be used to express the name of common oxides, such as: C=CaO, $S=SiO_2$, $A=Al_2O_3$, $F=Fe_2O_3$, $H=H_2O$, $\bar{S}= SO_3$ and $\bar{C}=CO_2$. In addition, chemical formulae may be abbreviated in the same way, for example: $3CaO.SiO_2$ or tricalcium silicate can be abbreviated to C_3S (Table 1, page 26). This system can be also used in a chemical equation, for example:

$3CaO + SiO_2 \rightarrow 3CaO.SiO_2$

or

$3C + S \rightarrow C_3S$

Several authors have examined similarities between different types of MTA and Portland cement. Using fluorescence spectrometry Estrela *et al.* (2000) compared the chemical constituents of grey ProRoot? MTA (Dentsply Tulsa Dental) and Portland cement. The results of their study revealed that with the exception of the presence of bismuth in ProRoot? MTA (Dentsply Tulsa Dental), the other chemical ingredients of these two materials were similar. As a result of a comparative chemical study using inductively coupled plasma emission spectrometry (ICP-ES) Funteas *et al.* (2003) reported that the absence of bismuth in Portland cement was the only dissimilarity between the chemical elements of grey ProRoot? MTA (Dentsply Tulsa Dental) and Portland cement. Asgary *et al.* (2004) compared tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) and two types of white Portland cement

using SEM and X-ray microanalysis. In their study a smaller range of particle sizes was observed in samples of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) compared to white Portland cements. As a result a finer texture has also been observed in tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) products. According to Estrela *et al.* (2000), Funteas *et al.* (2003) and Asgary *et al.* (2004) both tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental) have a similar chemical constituents to their Portland cement counterparts, except for the presence of bismuth that is intentionally added to make MTA radiopaque; this information was confirmed subsequently by Camilleri *et al.* (2005b).

The chemical composition of powder and set forms of grey MTA Angelus? (Angelus) has been compared to grey ProRoot? MTA (Dentsply Tulsa Dental), tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) and white and grey Portland cement (Song *et al.* 2006) using X-ray diffraction analysis. In agreement with other studies it was revealed that Portland cement varied from both commercially available MTA products by the lack of bismuth ions. Hwang *et al.* (2009) compared chemical and certain physical properties of Portland cement and tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) using energy dispersive X-ray analysis. In accordance with previous studies the chemical resemblance of Portland cement and MTA, apart from the absent of bismuth oxide, was confirmed. Moreover, the presence of large jagged particles that were dispersed irregularly between small particles was illustrated in both MTA and Portland cement, although the number of large particles was more prominent in the latter (Dammaschke *et al.* 2005). The multifaceted chemistry of Portland cement and its dental version, MTA, has been the subject of many studies but is not yet determined fully, although an accepted simplification is that they generally consists of four major hydraulic phases: tricalcium silicate ($3CaO.SiO_2$), dicalcium silicate ($2CaO.SiO_2$), tricalcium aluminate ($3CaO.Al_2O_3$) and tetracalcium aluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$)

(Table 1, page 26). A very low level of aluminum and magnesium and absence of iron in tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) compared to grey ProRoot? MTA (Dentsply Tulsa Dental) has been reported by Camilleri *et al.* (2005b) and confirmed by Asgary *et al.* (2006). The absence of iron compound in tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) was described as the reason of its colour difference to the original (grey) ProRoot? MTA (Dentsply Tulsa Dental) (Asgary *et al.* 2006, Camilleri 2008c).

Common name	Chemical Name	Composition	Symbol
Alite	Tricalcium silicate	$3CaO.SiO_2$	C_3S
Belite	Dicalcium silicate	$2CaO.SiO_2$	C_2S
Aluminate	Tricalcium aluminate	$3CaO.Al_2O_3$	C_3A
Ferrite	Tetracalcium aluminoferrite	$4CaO.Al_2O_3.Fe_2O_3$	C_4AF
Gypsum	Calcium sulphate	$CaSO_4$	
Bismite	Bismuth Oxide	Bi_2O_3	

Table 1: Major components of Mineral Trioxide Aggregate (MTA)

These constituent elements hydrate at different rates to influence the setting time, hardening, workability, strength and other physical properties of the material. Calcium sulphate ($CaSO_4$) and bismuth oxide (Bi_2O_3) are also added to control flash setting and make it radiopaque, respectively. In other words, the physical and chemical properties of these cements are governed by the way in which the phases react with water, which can be modified by altering the composition of each phase or by adding other elements/materials. Camilleri (2008b) demonstrated that the aluminate phase and sulphate levels of un-hydrated tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) compared with the white Portland cement were low and therefore suggested that it was manufactured in a laboratory rather than in an industrial rotary kiln. The aluminate phase in Portland cement is essential for sufficient clinkering (fusing by burning) of the raw materials in a kiln (Taylor 1997).

2.4.1. **Hydration**

The chemical reaction between water molecules and cement particles is called hydration. During the hydration process, water molecules are absorbed by the anhydrous cement particles to produce a gel matrix that encompass the cement grains and results in the formation of a plastic paste followed by the release of hydrates into solution (Taylor 1997). The density of ionic hydrates gradually increases and following saturation, the ionic constituents precipitate out as a solid phase and the material begins to harden (Boumiz *et al.* 1996). With progress of the hydration process over time, stiffening (loss of workability) of the cement occurs, which is referred to as the 'initial set' of the cement (Walker *et al.* 2006). The cement then acquires discernible resistance to fracture from either compressive or tensile stresses (Boumiz *et al.* 1996). A certain degree of hardness, sufficient to bear a given light weight without indentation, is referred to as the 'final set' (solidification) (Camilleri 2007). With continued hydration of the cement over time, its compressive strength increases and it becomes harder (Lee *et al*. 2004).

2.4.2. **Calcium silicates (C_3S & C_2S)**

Most of the binding power, early strength and biological reactions of Portland cement and MTA are attributed to the hydration of its major constituent, the calcium silicates, including tricalcium silicate and dicalcium silicate (Bensted 2002). As a result of the hydration process, calcium hydroxide is formed and released (Fridland & Rosado 2003). In addition, the main binding agent of the cement, a gel of calcium silicate hydrates (C-S-H), is formed simultaneously (Taylor 1997, Camilleri 2007).

2.4.2.1. **Tricalcium silicate ($3CaO.SiO_2$ or C_3S)**

Tricalcium silicate or Alite (C3S) is the main component of all normal Portland cements (Camilleri *et al.* 2005b) and all types of MTA (Islam *et al.* 2006b). Hydration of Alite begins in the early stages of the reaction and continues for approximately one month when hydration is nearly complete (Bensted 2002). Since it reacts quickly with water; Alite is considered as the most important constituent for initial strength development. The tricalcium silicate phase also provides the long-term mechanical strength of the cement. The main products of the hydration reaction of the Alite phase are a C-S-H gel and a soluble calcium hydroxide that precipitate after saturation.

2.4.2.2. **Dicalcium silicate ($2CaO.SiO_2$ or C_2S)**

Dicalcium silicate or Belite (C_2S) reacts slowly with water, thus, its contribution to early strength development is less than Alite (Bensted 2002); however, delayed strength development is attributable to this phase (Eglinton 1987). The hydration of this phase also results in the formation of calcium hydroxide that is released in an aqueous environment (Fridland & Rosado 2005). The proportion of dicalcium silicate in MTA is higher than in Portland cement (Camilleri 2008b), which may result in a longer hydration process and a greater release of calcium hydroxide (Camilleri 2010).

2.4.3. **Tricalcium aluminate ($3CaO.Al_2O_3$ or C_3A)**

Pure tricalcium aluminate or aluminate reacts rapidly with water and exhibits flash setting (Taylor 1997). Therefore, the hydration of tricalcium aluminate is more rapid than that of the other phases with the result it influences the early setting process of the cement (Gemelli *et al.* 2004) and contributes to its early strength (Bensted 2002). By increasing the aluminate content rapid-hardening or high early strength can be produced (Stutzman 2004). Following

the initiation of the hydration process and in the presence of calcium hydroxide, asymmetrical flakes of calcium aluminate hydrate (C-A-H) with poor crystalline formation develop on the surface of the cement particles (Hewlett & Lea 1997). Consequently, as a result of a reaction between calcium sulphate dihydrate (gypsum) and C-A-H the compound ettringite (hydrated calcium sulphoaluminate) is formed that results in a network of needle-like crystals that contribute to early stiffening and strength. These ettringite crystals absorb more water to provide an effective barrier against rapid hydration of the cement by delaying formation of more C-A-H and thus regulate the hydration process (Camilleri 2007). The formation of ettringite from aluminate and the sulphate phases requires calcium ions that are provided by the calcium hydroxide produced during the hydration of the calcium silicate phases (Camilleri 2008a). Incomplete hydration of the cement restricts the formation of calcium hydroxide and consequently in a failure to produce ettringite (Camilleri 2008b). The absence of the aluminate phase has been reported in several recently developed MTA-like materials, which is claimed to be associated with improvements in their biological properties (De-Deus *et al.* 2009a).

2.4.4. **Calcium sulphate dihydrate ($CaSO_4$-$2H_2O$)**

Calcium sulphate dihydrate (gypsum) is an essential constituent of Portland cement and MTA. Gypsum regulates the rate of hydration and retards the initial stiffening of the cement, thereby, increasing the initial setting time of the cement, in contrast to the aluminate phase (Black *et al.* 2006, Camilleri 2008d). In the initial phases of the hydration process, sulphur ions are gradually absorbed on the surface of the C-S-H gel and results in hardening of the cement (Dammaschke *et al.* 2005). Formation of ettringite crystals also depends on the presence of gypsum (Gandolfi *et al.* 2010b). Dammaschke *et al.* (2005) reported that the

amount of sulphur in ettringite crystals originating from the $(SO_4)^{2-}$ groups of gypsum increases as a result of the setting reaction.

2.4.5. **Tetracalcium aluminoferrite (Ca_2AlFeO_5)**

Tetracalcium aluminoferrite (Ca_2AlFeO_5) or ferrite reacts rapidly with water in the initial stages of hydration; however, this reaction reduces with time (Bensted 2002). The rate of its hydration is related to the aluminum/ferric composition ratio and is regulated by Gypsum. As a result of the reaction between hydrated ferrite and Gypsum, close to the aluminate surface, prismatic crystals of ettringite may form (Taylor 1997). The dark colour of grey Portland cement and grey MTA is a result of ferrite (Bensted 2002, Asgary *et al.* 2005). White Portland cement and tooth coloured MTA are made by increasing the ratio of AL_2O_3 to Fe_2O_3 (Taylor 1997, Dammaschke *et al.* 2005). A fluxing agent is used to remove the ferrite phase during the clinkering process. The lack of this phase in tooth coloured MTA has been confirmed by Song *et al.* (2006).

2.4.6. **Bismuth oxide (Bi_2O_3)**

An important property of dental materials is radiopacity, by which they can be distinguished from surrounding tooth tissues and bone on radiographs (Torabinejad *et al.* 1995b, Tagger & Katz 2004). In the first prototype of MTA, bismuth oxide powder (wt 25%) was added to Portland cement (wt 75%) to make it radiopaque (Torabinejad *et al.* 1995b). It has been shown that bismuth oxide may interact with the hydration process of MTA by producing calcium silicate bismuth hydrate (Camilleri 2007, Camilleri 2010). Coomaraswamy *et al.* (2007) evaluated the effect of the ratio of bismuth oxide on the physical properties of Portland cement. They demonstrated that a higher content of bismuth oxide resulted in a cement with greater porosity and lower compressive strength. The use of other radiopacifiers

to overcome the suggested detrimental effects of bismuth oxide has been suggested (Coomaraswamy *et al.* 2008 , Camilleri 2010, Camilleri & Gandolfi 2010).

2.4.7. **Presence of arsenic**

A particular concentration of arsenic is harmful for human health and may cause cancer (Chen *et al.* 2010). Therefore, the presence of arsenic in various types of Portland cement and MTA has been of concern. However, Duarte *et al.* (2005) demonstrated that the concentration of arsenic in both Portland cement and MTA was less than the Food & Agriculture Organisation and World Health Organisaton (FAO/WHO) recommended harmful dosage and concluded that Portland cement could be used clinically. This conclusion was strongly criticized by Primus (2006) who emphasised the unhygienic manufacturing process used to produce Portland cement compared to the more controlled conditions used to produce ProRoot? MTA (Dentsply Tulsa Dental). Bramante *et al.* (2008) and De-Deus *et al.* (2009b) confirmed that the levels of arsenic in various types of MTA and Portland cement were lower than the harmful concentration. Primus (2009) has raised doubts about the validity of the methodology used by Duarte *et al.* (2005) and Bramante *et al.* (2008) to trace arsenic. Further controlled studies are needed to determine the precise amount of the various types of arsenic in the hydraulic cements that have been suggested for use in endodontic treatments. .

2.4.8. **Presence of phosphorus**

The presence of phosphorus that can explain the bioactivity of MTA is a matter of debate (Gandolfi *et al.* 2010b). In a preliminary laboratory study using X-ray energy dispersive spectrometry in conjunction with scanning electron microscopy Torabinejad *et al.* (1995b) indicated that the main elements present in MTA were calcium and phosphorus. However, in other studies phosphorus in grey and tooth coloured ProRoot? MTA (Dentsply Tulsa

Dental) has not been detected (Asgary *et al.* 2004, Camilleri *et al.* 2005a, Asgary *et al.* 2006, Gandolfi *et al.* 2010a). This significant inconsistency might have occurred because of a modification to the chemical composition of the initial prototype of MTA, which was used in preliminary studies but not in the product that was eventually marketed (Asgary *et al.* 2005). On the other hand, Camilleri *et al.* (2005a) suggested that unintentional exposure of MTA to phosphate solutions during laboratory testing was the reason of this discrepancy. Sarkar *et al.* (2005) demonstrated that MTA in contact with a synthetic tissue fluid containing phosphate buffered saline produced precipitates similar to hydroxyapatite. Accordingly, Gandolfi *et al.* (2010a, 2010b) demonstrated the absence of phosphorus in MTA and Portland cement powder and indicated that the source of phosphorus in hydroxyapatite crystals was not from the MTA powder itself.

2.5. **Biocompatibility**

Biocompatibility is a characteristic of a material that indicates its ability to produce appropriate inflammatory and/or immunological responses, whilst in contact with host tissue in a specific application (Peppas & Langer 1994). In addition to a non-significant immunological reaction, a biocompatible material does not cause any genotoxic, mutagenic or cytotoxic effects (Schmalz 1998).

Various methodologies have been used to evaluate the biocompatibility of dental materials including:

- *In vitro* mutagenicity tests;
- Genotoxicity examinations;
- *In vitro* cytotoxicity assays;
- Evaluation of bioactivity by measurement of cytokines and biological markers as a result of exposure to the extracts of materials;

- Assessment of local toxicity reactions and healing processes following subcutaneous and/or intraosseous implantation of materials in laboratory animals.

Subjected to various basic and advanced biocompatibility tests, Portland cement and its derivative, Mineral Trioxide Aggregate (MTA), have been reported to be highly biocompatibility; indeed, MTA has been described as a bioactive endodontic material (Torabinejad *et al.* 1995d, Keiser *et al.* 2000, Asrari & Lobner 2003, Gandolfi *et al.* 2008, Silva *et al.* 2008, Jafarnia *et al.* 2009, Dammaschke *et al.* 2010a, Hasheminia *et al.* 2010, Ko *et al.* 2010, Zeferino *et al.* 2010).

2.5.1. **Mutagenicity and Genotoxicity**

Potential mutagenicity of MTA, IRM (Dentsply Caulk, Milford, DE, USA) and Super EBA (Harry J. Bosworth Co., Skokie, IL, USA) was studied by employing the standard "Ames mutagenicity assay" with the conclusion that IRM, Super EBA, and MTA were not mutagenic (Kettering & Torabinejad 1995).

Causing genetic damage, such as breakage of chromosomes, gene mutation and cellular transformation, are some of the indicators of the carcinogenicity of a material (Shelby 1988). Therefore, before application of a new material in humans, *in vitro*, *ex vivo* and *in vivo* genotoxicity tests are needed to evaluate its potential carcinogenic effects (Barlow *et al.* 2006). Genotoxic effects of various concentrations of Portland cement and MTA Angelus were evaluated by the single-cell gel (comet) assay and trypan blue exclusion test in mouse lymphoma cells by Ribeiro *et al.* (2005) who concluded that Portland cement and MTA did not cause genotoxic effects. Barz *et al* (2006) evaluated the concentration-related genotoxic effect of MTA Angelus and two types of Portland cement on peripheral lymphocytes using an alkaline single cell gel (comet) assay. They concluded that exposure of cultured human peripheral lymphocytes to various concentrations of MTA or Portland cement (dissolved in

phosphate buffered serum) ranging from 1 to 1000 ?g mL^{-1} for 1 h at 37^{o}C did not cause significant DNA defects (Braz *et al.* 2006). The potential genotoxic effects of various types of MTA have also been evaluated on cell cultures of V79 Chinese hamster fibroblasts (Camargo *et al.* 2009), MG63 human osteosarcoma cell line (Ding *et al.* 2010) and murine fibroblasts (Zeferino *et al.* 2010) and in accordance with previous findings it was confirmed that MTA did not cause DNA damage.

2.5.2. **Cytotoxicity**

In a pioneer study Torabinejad *et al.* (1995c) compared the cytotoxicity of freshly mixed and set experimental materials including amalgam, Super EBA, IRM, and MTA on mouse L929 fibroblasts using the agar overlay and radiochromium methodologies. According to the results of the agar overlay technique, cytotoxicity of fresh and set MTA, ranked second after amalgam since the average zone of lysis for fresh and set experimental materials in a downward order was IRM, Super EBA, MTA and amalgam. Additionally, the percentage of radioactive chromium release, as an indicator of cellular lysis, following 24 h incubation with radiochromium-labeled mouse L929 fibroblasts in descending order was IRM, Super EBA, amalgam and MTA. This means that the degree of cytotoxicity of fresh and set MTA was the least followed by amalgam, Super EBA and IRM (Torabinejad *et al.* 1995c).

Osorio *et al.* (1998) assessed the cytotoxic effects of original Mineral Trioxide Aggregate (ProRoot? MTA), amalgam, Ketac Silver, Gallium GF2 and Super-EBA on cell culture of human gingival fibroblasts and L-929 mouse fibroblast using the MTT assay as an indicator of mitochondrial succinate dehydrogenase activity (cell metabolism) and the crystal violet assay for calculation of cell numbers. The results of their study revealed that among all tested materials MTA was not cytotoxic as it did not affect mitochondrial enzyme activity and did not impair cell proliferation in the L-929 mouse fibroblasts culture. However, in cultures of

human gingival fibroblasts the material caused a small but significant reduction in cell proliferation (Osorio *et al.* 1998).

In another study, using human periodontal ligament cell cultures, Keiser *et al.* (2000) compared the cytotoxicity of freshly mixed amalgam, Super EBA and MTA. In addition, to evaluating the cytotoxicity of set materials, they incubated the experimental materials for 24 h at 37^{o} C and fully saturated humidity. Then, various concentrations of the extracts of each experimental material were exposed to cell cultures. The results indicated that the toxicity of freshly mixed MTA was lower than Super EBA and amalgam. In addition, compared to Super EBA and amalgam, high concentrations of set MTA were the least cytotoxic (Keiser *et al.* 2000).

The effect of Portland cement and two various types of MTA, ProRoot? MTA (Dentsply Tulsa Dental) and MTA Angelus, on mitochondrial enzyme activity of human ECV endothelial cell lines were evaluated using an MTT assay (De-Deus *et al.* 2005) with no significant difference being reported. Indeed, the cytotoxic effect of all tested materials declined with time (De-Deus *et al.* 2005).

Souza *et al.* (2006) compared the cytotoxic effect of gutta-percha and set specimens of SuperEBA, N-Rickert, amalgam, glass–ionomer and MTA and concluded that all the materials were cytotoxic, however, MTA was ranked as the least cytotoxic.

The cytotoxicity of ProRoot? MTA (Dentsply Tulsa Dental) and MTA Angelus was compared with SuperEBA and Vitrebond using rat pulp cells (RPC-C2A) and human lung fibroblasts (MRC-5) with the conclusion that both MTA materials caused the least cytotoxic effect and could be regarded as biologically inert materials (Koulaouzidou *et al.* 2008).

Badr (2010) compared the cytotoxicity of MTA, amalgam and bone cement using human periodontal ligament fibroblast tissue culture and reported that the cytotoxicity of amalgam

was greater than bone cement and MTA. Cytotoxicity of MTA was similar to bone cement, the latter being used clinically in orthopaedic surgery in which biocompatibility is crucial to achieve successful outcomes (Badr 2010).

2.5.3. **Bioactivity**

In general, materials that have been suggested for the repair of hard tissue defects (bone, dentine and cementum) are categorised into two main groups: bioinert or bioactive. Bioinert materials do not encourage the formation of hard tissues and do not bond biologically to them. Bioactive materials stimulate the formation of hard tissues and therefore facilitate a biological link with bone and form a mechanically strong biomaterial-tissue interface.

Bioactive materials that are used for repair of hard tissues are also divided into two main categories: inductive or conductive. The hard tissue-conductive materials are able to support the formation of new hard tissues on their surfaces following application into and/or close to existing hard tissues. Bioactive materials that contain hydroxyapatite are usually considered as hard tissue-conductive biomaterials since it can provide a matrix for the formation of hard tissues.

Inductive biomaterials are able to provoke formation of new hard tissue (LeGeros 2008) regardless of the anatomical location of the application. This phenomenon was reported by Urist (1965) who demonstrated the induction of bone formation following implantation of demineralized bone matrix (DBM) in non-osseous tissues (Reddi 2003, LeGeros 2008). The osteoinductive property of demineralized bone matrix is due to bone osteogenic and morphogenetic proteins (BMPs) (Vandersteenhoven & Spector 1983, Urist *et al.* 1984, Lee *et al.* 2010).

Mineral Trioxide Aggregate is considered as a bioactive material with possible osteoinductive properties since it has been shown that MTA up-regulates bone

morphogenetic protein-2 (BMP-2) expression (Maeda *et al.* 2010). In addition, in several *in vitro* studies up-regulation of various cytokines and biologic markers such as osteopontin (Nakayama *et al.* 2005, Kuratate *et al.* 2008), osteocalcin (Koh *et al.* 1997, Thomson *et al.* 2003), bone sialoprotein (Min *et al.* 2008, Yang *et al.* 2010), BMP-2 (Ham *et al.* 2005, Maeda *et al.* 2010) and alkaline phosphatase (Bonson *et al.* 2004, Paranjpe *et al.* 2010) as a result of the presence of MTA in various cell cultures has been reported. Bonson *et al.* (2004) exposed cell cultures of gingival and periodontal ligament fibroblasts to various root-end filling materials including ProRoot? MTA (Dentsply Tulsa Dental) and indicated that only MTA was capable of modifying differentiation of both fibroblast populations, resulting in significantly increased levels of alkaline phosphatase activity. Activity of alkaline phosphatase is regarded as an indicator of bone formation. Moreover, the potential property of MTA to promote differentiation of dentinoblasts from clonogenic cells of the dental pulp has been demonstrated by Zhao *et al*. (2011).

2.5.4. **Animal studies**

Torabinejad *et al.* (1995c) compared bone tissue reaction to implanted MTA and Super EBA in guinea pigs. Two Teflon cups, filled with each material, were implanted surgically in the mandibles of the animals and the tissue reactions to each material were evaluated by recording the presence of inflammation and the main types of inflammatory cells as well as the thickness of fibrous connective tissue adjacent to each implanted material. Within the parameters of that study both experimental materials were considered as biocompatible materials; however, because of the limitations of the study no statistical analysis was reported. In another study, the periradicular tissue response in dogs to MTA and amalgam when used as a root-end filling material were evaluated by Torabinejad *et al.* (1995a). Compared to amalgam, more fibrous capsule formation and less inflammatory response were

reported adjacent to MTA. In addition, deposition of cementum, as an indicator of proper healing, on the surface of MTA was a common finding (Torabinejad *et al.* 1995a). Favorable inflammatory reactions to implanted MTA in tibia and mandible of guinea pigs was also reported (Torabinejad *et al.* 1998) suggesting the biocompatibility of MTA. The root-end induction capability of MTA in dogs was demonstrated by Shabahang *et al.* (1999) suggesting its use as an apical plug in teeth with open apices and necrotic pulps. Dentine bridge formation following pulpotomy by MTA and Portland cement was reported in dogs (Holland *et al.* 2001) confirming the tissue compatibility of MTA.

The potential healing effect and formation of dentinal bridges following direct pulp capping with MTA in cats were also demonstrated by Hasheminia *et al.* (2010). They reported that treatment of exposed pulp tissue by lasers before direct pulp capping with MTA had no significant effect on the healing process or on the formation of dentine bridges (Hasheminia *et al.* 2010). The superior healing process following direct pulp capping with MTA has also been reported in rats (Dammaschke *et al.* 2010b).

2.5.5. **Human studies**

The number of high quality, well-designed and large scale randomized controlled clinical trials with long term follow-up to confirm the outcome of MTA in its clinical applications are very limited (Steffen & van Waes 2009, Tang *et al.* 2010).

Following approval of MTA by U.S. Food and Drug Administration (FDA) in 1988, Schwartz *et al.* (1999) reported elimination of clinical symptoms and conduction of bone healing in five cases that MTA were used in various applications including repair of a root perforation. Torabinejad & Chivian (1999) reported several cases with favourable outcome while MTA were used as the root repair material. In a preliminary clinical trial Eidelman *et al.* (2001) compared the outcome of dressing the exposed pulp of forty five pulpotomised

primary molars with formocresol or MTA and reported that at the 17 months postoperative follow-up none of the MTA-treated teeth had signs of pathosis. The authors concluded that MTA was a suitable replacement for formocresol in pulpotomy treatment of primary teeth. Favourable outcomes of pulpotomy in primary teeth by MTA was also reported by Agamy *et al.* (2004), Farsi *et al.* (2005) and Holan *et al.* (2005). In a multicentre, multioperator, prospective, randomized, controlled clinical trial (Zealand *et al.* 2010) a 100% success rate for pulpotomies in primary teeth by grey MTA was reported at the 6 months follow-up. In a prospective clinical study by Barrieshi-Nusair & Qudeimat (2006) thirty-one first permanent molars with cariously exposed pulp tissue were subjected to partial pulpotomy and then capped by grey MTA. The outcome of the intervention was evaluated clinically and radiographically at 24 months and a success rate of 64% was reported (Barrieshi-Nusair & Qudeimat 2006), however, since no control group was employed it was not possible to determine the superiority of MTA over other methods. In another study by Nair *et al.* (2008) the response of healthy human pulp tissue to direct pulp capping with MTA at various time intervals was evaluated histologically and compared to a control group where the healthy pulp tissue was directly capped by Dycal (Dentsply Caulk, Milford, DE, USA) a hard setting calcium hydroxide liner. The absence of inflammation and formation of a hard tissue barrier were significant differences found in the MTA group compared to control group in which the presence of inflammation and less consistent formation of the hard tissue barrier was a common finding (Nair *et al.* 2008).

The application of MTA as a root-end filling material was compared to IRM in a randomised controlled trial and a high success rate of both materials was reported following a 24 month follow up (Chong *et al.* 2003). In another randomised clinical trial with a shorter follow-up time, the success rate of MTA versus gutta-percha when applied as the root-end filling

material was compared and a significantly higher success rate for MTA was reported at 12 months (Christiansen *et al.* 2009).

2.6. **Physical properties**

2.6.1. **Particle size**

MTA powder is composed of fine particles that during the hydration process absorb water molecules and produced a stiff gel composed mainly of calcium silicate hydrate (CSH). Consequently, following formation and precipitation of several crystalline structures, the MTA slurry becomes harder and a solid but porous material forms (Fridland & Rosado 2003). In general, the kinetics of the hydration process and the rheology of this hydraulic cement are influenced mainly by the size of the particles (Dammaschke *et al.* 2005, . RP DED\ DVKI6 SnQJ EHJ . Therefore, to predict the rheological properties, setting reaction and hydration behaviour of various types of MTA, assessment of their particle size distribution, as a fundamental characteristic, is important.

There are various methods for evaluating the particle size distribution of materials including sieving, laser diffraction, flow particle image analysis, electrical zone sensing, X-ray gravitational sedimentation (XRS) and scanning electron microscopy (SEM). Dammaschke *et al.* (2005) compared the particle size of tooth coloured MTA with two various types of Portland cement using SEM. They demonstrated that ProRoot? MTA (Dentsply Tulsa Dental) had a homogeneous morphology with an equal particle size, whereas a wide range of particle size distribution was observed in both types of the Portland cements tested (Dammaschke *et al.* 2005). In another study using SEM, a wide range of particle sizes was observed in Portland cement compared to tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) where homogenous particle sizes were observed (Hwang *et al.* 2009). For better

understanding of the particle size and shape of various types of MTA, Komabayashi & SSl QJ EHJ (2008) employed a flow particle image analyzer and reported that MTA Angelus particles had a broad size distribution and were less homogeneous than ProRoot? MTA (Dentsply Tulsa Dental).

2.6.2. **Setting time**

MTA requires water to initiate and complete the setting reaction. The speed of setting and hardening are independent; the latter refers to "increase of compressive strength" which, characteristically, is a time-consuming process (Hewlett 2004). Setting has been defined as stiffening without considerable increases in compressive strength (Hewlett 2004). Both processes are consequences of cement hydration and are indicative of this process (Eglinton 1987). The setting times of hydraulic cements depend on their composition, particle size, pH, water/cement ratio, presence of various admixtures and the mixing technique (Hewlett 2004). Various methods have been used to record cement setting times (Hewlett 2004), which are based on recommendations from various organisations dealing with standards. The method that is recommended by the International Organization for Standardization (ISO) to measure setting time of root canal sealing materials (ISO 6876:2001) is similar to the recommendation of the American Society for Testing and Materials (ASTM C266-03). The latter describes the "standards techniques" necessary to determine both initial and final setting times of hydraulic-cement pastes using Gillmore needles (ASTM C266-03).

Torabinejad *et al.* (1995b) examined the setting time of the initial prototype of MTA using one needle from the Gillmore apparatus and reported a setting time of 165 minutes. However, it was not made clear whether this time was the initial or final set and which needle of the Gillmore apparatus was used.

The initial and final setting times of tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental) were determined according to ASTM C266-03 by Chng *et al.* (2005). They reported that the initial and final setting times of tooth coloured MTA were 45 and 140 minutes respectively, which were significantly quicker than the initial and final setting times of grey MTA that were 70 and 175 minutes respectively (Chng *et al.* 2005).

Huang *et al.* (2008) used the Gillmore apparatus and reported the final setting time of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) was 151 minutes. Islam *et al.* (2006a) used the Gillmore apparatus and reported the initial setting times of tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental) to be 40 and 70 minutes, respectively and the final setting times to be 140 and 175 minutes, respectively; they concluded that tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) set more quickly than grey MTA.

Kogan *et al.* (2006) measured the setting time of grey MTA while mixed with sterile water using the Vicat apparatus and reported that it was 50 minutes. In another study using the Vicat apparatus, the setting time of grey MTA was reported as 202 minutes (Ber *et al.* 2007). An obvious discrepancy in the reported values is seen in the results of studies that used the Vicat apparatus to determine time of setting compared with those using the Gillmore apparatus. As a consequence the results from these two different techniques cannot be compared.

The prolonged setting time of MTA is considered to be a significant disadvantage in clinical situations (Abdullah *et al.* 2002, Ber *et al.* 2007). Indeed, it is generally recommended that other filling materials should not be placed adjacent to MTA at the same appointment, which increases the number of appointments and the clinical time required (Simon *et al.* 2007). In addition, when used as a root-end filling material, care must be taken to prevent MTA from being displaced due to its extended setting time (Huffman *et al.* 2009, Shokouhinejad *et al.*

2010) and to decrease the possibility of the MTA slurry being rinsed out during irrigation of the surgical site. The manufacturer of MTA Angelus has suggested that the reduction in particle size in their product RP DED DVKL6SnQJEHJ and the reduced amount of gypsum (Bortoluzzi *et al.* 2006) results in a more rapid setting time.

In Portland cement chemistry it has been shown that admixtures of calcium chloride ($CaCl_2$) result in a reduced setting time. Not surprisingly, the effect of various additives has been investigated to determine their effect on the setting properties of MTA (Ber *et al.* 2007, Wiltbank *et al.* 2007, Bortoluzzi *et al.* 2009, Reyes-Carmona *et al.* 2009, Reyes-Carmona *et al.* 2010a). The addition of 10% calcium chloride ($CaCl_2$) significantly reduced the initial and final setting time of tooth coloured MTA (Bortoluzzi *et al.* 2009). It has been also shown that an admixture of 1% methylcellulose and 2% calcium chloride equal to 2% of the sample weight resulted in one third faster setting time of the MTA slurry (Ber *et al.* 2007). Abdullah *et al.* (2002) demonstrated that accelerating the setting time of Portland cement as a result of admixing with calcium chloride did not interfere with its biocompatibility and may have potential to promote bone healing.

2.6.3. **Setting expansion**

One of the main advantages of MTA is its sealing ability that can be explained by its expansion during the setting process (Gandolfi *et al.* 2009, Hawley *et al.* 2010). However, at the margins of a cavity being filled with an MTA-like material, the setting expansion may produce micro-cracks in the tooth structure (Shipper *et al.* 2004). In a laboratory study, Storm *et al.* (2008) monitored the linear expansion of grey and tooth coloured MTA as a function of time while the MTA material was covered by Hanks balance salt solution (HBSS) or water and reported that the linear setting expansion of grey MTA was significantly greater than that of tooth coloured MTA. In another laboratory study, Hawley *et al.* (2010) evaluated the effect

of various water to powder ratios on setting expansion and confirmed that the setting expansion of grey MTA was significantly more than tooth coloured MTA, although they did not find any correlation between various water to powder ratios and the setting expansion of MTA. The effect of various soaking media on the setting expansion of MTA was evaluated by (Gandolfi *et al.* 2009) who demonstrated that in the presence of foetal bovine serum and phosphate buffered serum the expansion of MTA was reduced significantly. The importance of expansion during the setting of MTA is unclear and requires further evaluation.

2.6.4. **Sealing ability**

The leakage of the MTA-tooth interface while used in various clinical applications has been investigated comprehensively (Bates *et al.* 1996, Tang *et al.* 2002, Al-Hezaimi *et al.* 2005, De Bruyne *et al.* 2006, Martin *et al.* 2007, Ferk Luketic *et al.* 2008, Brito-Junior *et al.* 2009, Torabinejad & Parirokh 2010, Yildirim *et al.* 2010). Leakage of MTA has been compared with other various materials using dye leakage (Torabinejad *et al.* 1993, Torabinejad *et al.* 1994a, Fischer *et al.* 1998, Aqrabawi 2000, Daoudi & Saunders 2002, Islam *et al.* 2005, Pichardo *et al.* 2006, Hashem & Hassanien 2008, Orosco *et al.* 2008, Lolayekar *et al.* 2009), bacterial penetration (Torabinejad *et al.* 1995e, Fischer *et al.* 1998, Hachmeister *et al.* 2002, Maltezos *et al.* 2006), fluid filtration (Bates *et al.* 1996), endotoxin leakage (Tang *et al.* 2002), dentine penetration (Vogt *et al.* 2006) and/or electrochemical analysis (Martell & Chandler 2002). In addition, in several studies the influence of a variety of factors on the microleakage of MTA was evaluated including blood contamination (Torabinejad *et al.* 1994a), thickness of MTA (Valois & Costa 2004), various admixtures (Shahi *et al.* 2007), environmental pH (Camilleri & Pitt Ford 2008), size of the cavity and thickness of the dentinal wall (Yildirim *et al.* 2010). The results of most of these leakage investigations revealed that in comparison to other materials MTA produced a good seal. However, there is

insufficient evidence to correlate the sealing ability of a material to clinical outcomes (Pitt Ford 1983, Torabinejad *et al.* 1994b, Oliver & Abbott 2001, de Chevigny *et al.* 2008, Ng *et al.* 2008a, Ng *et al.* 2008b, Ng *et al.* 2011).

2.6.5. **Marginal adaptation**

A highly adapted interface between tooth structure and biomaterials is one of the basic requirements of endodontic materials (Safavi *et al.* 1988, Costa *et al.* 2009, Bidar *et al.* 2010); however, no correlation has been found between sealing ability and marginal adaptation of various root-repair and root-end filling materials (Stabholz *et al.* 1985, Xavier *et al.* 2005, Costa *et al.* 2008). Moreover, the correlation between marginal adaptation and sealing ability and a desirable treatment outcome has not been established (Torabinejad *et al.* 1994b, Ng *et al.* 2008a, Ng *et al.* 2008b). In addition, the experimental laboratory model used may influence the results and must be controlled precisely. For example, to avoid the possible formation of artifacts and dentine microfractures during SEM investigations, the use of replicas has been recommended (Stabholz *et al.* 1985, Gondim *et al.* 2002, Gondim *et al.* 2003). Furthermore, Shipper *et al.* (2004) demonstrated that when using a low vacuum SEM technique and humid samples fewer gaps were observed. Several studies have demonstrated good marginal adaptation for MTA compared to other suggested root-repair and/or root-end filling materials such as IRM, Super EBA, glass ionomer and amalgam (Torabinejad *et al.* 1995f, Shipper *et al.* 2004, Camilleri & Pitt Ford 2008, Costa *et al.* 2009, Badr 2010). The effect of pretreatment with calcium hydroxide (Bidar *et al.* 2010), finishing (Gondim *et al.* 2005), methods of cavity preparation (Gondim *et al.* 2003) and occlusal loading (Peters & Peters 2002) on the marginal adaptation of MTA have been investigated and its superior adaptation confirmed.

2.6.6. **Push-out force**

It has been suggested that the two exceptional properties of MTA, biocompatibility and sealing ability, have originated from the physicochemical reactions between MTA and dentine (Sarkar *et al.* 2005), that results in an adhesion reaction between them (Reyes-Carmona *et al.* 2010a). The bond strength can be evaluated by employing push-out test methods (Reyes-Carmona *et al.* 2010b) that provide a value for this adhesion (Gancedo-Caravia & Garcia-Barbero 2006). In a laboratory study, Sluyk *et al.* (1998) evaluated the push-out force of MTA and showed that the bond strength of MTA increased gradually over time, suggesting that the placement of the permanent restoration over MTA should be delayed. In another study, it was shown that humidity significantly improved the bond strength between MTA and dentine (Gancedo-Caravia & Garcia-Barbero 2006). Loxley *et al.* (2003) evaluated the effect of various intracanal oxidizing agents on the push-out force of MTA, Super EBA and IRM and demonstrated that MTA was significantly more resistant to displacement than Super EBA or IRM. They concluded that this confirmed the suitability of MTA for the repair of perforations on the floor of the pulp chamber and as a direct pulp capping material.

2.6.7. **Microhardness**

One of the universal and non-destructive methods for investigating the quality of materials is the microhardness test (Cross *et al.* 2000, Munack *et al.* 2001, Lee *et al.* 2004, Ramp *et al.* 2006, Saghiri *et al.* 2009, Moshaverinia *et al.* 2010). According to the British Standard Institution (BS EN 843-4 2005) the microhardness test is based on static micro-indentation into the surface of a test material by loads of less than 1 kgf followed by the measurement of the dimensions of the indentation. Variations of the test use differently shaped indenters such

as the Knoop elongated diamond pyramid and the Vickers diamond pyramid (Quinn & Quinn 1997).

As the hydration process of hydraulic cements progresses the crystalline microstructures that form and mature result in a gradual increase in the microhardness of the resultant cement (Torabinejad *et al.* 1995b, Igarashi *et al.* 1996, Camilleri 2007, Camilleri 2008c, Camilleri 2008b). Therefore, for assessment of the progress and quality of the hydration process, as well as evaluation of the microstructural gradient of MTA materials, microhardness tests can be used (Lee *et al.* 2004, Saghiri *et al.* 2009). These tests are also powerful for investigation of the mechanical response of MTA in both optimal and compromised environments (Igarashi *et al.* 1996), e.g. acid, blood.

The effect of various storage environments on the Knoop microhardness of MTA was evaluated by Lee *et al.* (2004) and the detrimental effect of acidic pH was revealed. Danesh *et al.* (2006) compared the Vickers microhardness of MTA and two different types of Portland cement and reported that the mean microhardness value of ProRoot? MTA (Dentsply Tulsa Dental) was significantly greater than both types of Portland cements. The effect of various solvents including carbonic acid, EDTA and chlorhexidine on the Vickers surface microhardness of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) was evaluated at different time intervals and demonstrated that exposure to carbonic acid significantly reduced its Vickers microhardness; however, exposure to EDTA had no significant effect on surface microhardness (Nandini *et al.* 2010). The latter result was not in accordance with findings of Lee *et al.* (2007) who demonstrated the detrimental effect of EDTA on several properties of MTA including Knoop microhardness. The storage of MTA powder at 4^{o}C, compared to 25^{o}C and 40^{o}C, was reported to significantly decrease the Vickers surface microhardness of

the resultant cement suggesting that storage temperature influenced surface hardness (Saghiri *et al.* 2010a).

2.6.8. **Flexural strength**

Walker *et al.* (2006) evaluated the effect of the hydration process on the flexural strength of MTA using the three-point bend test and demonstrated that compared to other impaired hydrated specimens, double-sided hydration of MTA resulted in a significantly higher flexural strength value. The mean flexural strength value for the control group that were hydrated double-sided and incubated for 24 hours at 37° C [illegible] (Walker *et al.* 2006). In another study the effect of storage in various endodontic solutions such as EDTA, chlorhexidine, sodium hypochlorite and BioPure MTAD on certain physical properties of ProRoot® MTA (Dentsply Tulsa Dental) including the flexural strength was evaluated and revealed that its flexural strength when stored under distilled water was significantly higher than other tested solutions (Aggarwal *et al.* 2009). In the latter study the mean value of the flexural strength of control specimens that were stored in distilled water for 7 days at 37° [illegible] (Aggarwal *et al.* 2009).

From a practical point of view, performing a three-point bend test for measurement of flexural strength of a material with a low value of flexural strength such as MTA is complicated as the specimens may easily break during casting, handling and removal from the split moulds (Trost 2005).

2.6.9. **Compressive strength**

Compressive strength is the highest vertical compressive load that a material can tolerate before failure and is calculated by dividing the load at fracture by the area of the cross section of the test specimen; it is reported in SI units of stress - Pa (1 Pa=1 N/m^2) (van der Varst *et*

al. 1993). Thus, compressive strength is regarded as one of the main physical characteristics of hydraulic cements that is correlated to its stage of hydration (Boumiz *et al.* 1996, Kogan *et al.* 2006).

In the laboratory environment the compressive strength of dental materials is measured using a universal testing machine that applies a compressive load vertical to the long axis of the test specimen and records the load at rupture. In a pioneer study, Torabinejad *et al.* (1995b) compared the compressive strength of the initial prototype of MTA, super-EBA and IRM at 24 h and 21 days after mixing and demonstrated that the compressive strength of all cements increased after 3 weeks. The strength of Super-EBA was significantly higher than that of IRM and MTA. In another study, the compressive strength of MTA and Portland cement was compared at 3 and 28 days following hydration when it was shown that the compressive strength of both cements increased over time and that the compressive strength of MTA was greater than that of the Portland cement at 28 days (Islam *et al.* 2006a).

In an attempt to decrease the setting time of MTA, Kogan *et al.* (2006) evaluated the effect of various admixtures on the setting time of MTA and demonstrated that the addition of 5% calcium chloride decreased the setting time as well as its compressive strength. They concluded that the compressive strength of MTA could be affected by the nature of the liquid mixed with the powder (Kogan *et al.* 2006).

2.6.10. **Solubility**

In all of the suggested clinical applications of MTA (see section 2.2) resistance to solubility is a prerequisite characteristic of the ideal material. Torabinejad *et al.* (1995b) compared the solubility of MTA, IRM, Super-EBA and amalgam at various time intervals. The changes in mean weight loss at 1, 7 and 28 days for MTA, Super-EBA and amalgam were not significantly different (Torabinejad *et al.* 1995b). In another study it was found that by

decreasing the powder to water ratio the solubility of MTA increased (Fridland & Rosado 2003). Danesh *et al.* (2006) compared the solubility of MTA and two types of Portland cement and determined that ProRoot? MTA (Dentsply Tulsa Dental) was of lower solubility. In contrast, Bodanezi *et al.* (2008) indicated that MTA was more soluble than Portland cement while stored in an aqueous environment.

2.6.11. **pH**

Some of the major advantages of MTA, such as antibacterial activity and conduction of hard tissue, can be best rationalised as a result of its alkalinity (Fridland & Rosado 2003). In a laboratory study Torabinejad *et al.* (1995b) measured the pH value of the initial prototype of MTA and reported that its pH when freshly mixed MTA was 10.2, which rose to 12.5 after 3 h. Chng *et al.* (2005) and Islam *et al* (2006a) demonstrated that the pH value of tooth coloured MTA rose to 13.0 at 60 minutes after mixing, which was attributed to the continuous formation of calcium hydroxide during the hydration process (Fridland & Rosado 2003, Fridland & Rosado 2005). The pH value of tooth coloured MTA was reported to be higher than grey MTA (Chng *et al*. 2005, Islam *et al.* 2006a). The pH of tooth coloured and ordinary Portland cement was shown to be more alkaline than their corresponding MTA and also reached the peak pH values more rapidly than corresponding MTA materials.

2.6.12. **Porosity**

In general, porosity is defined as the volume fraction of a material that can contain gas and/or liquid and is calculated by dividing the total volume of the pores by the total volume of a specimen (Hu & Stroeven 2005).

Following mixing of MTA powder and water a porous semi-solid gel of calcium silicate forms that by precipitation of ettringite crystals gradually becomes hard. The resultant solid

MTA, that resembles hardened Portland cement, contains microscopic air bubbles, unbound trapped water, and an interconnected network of micropores and channels (Fridland & Rosado 2003, Islam *et al.* 2006a). The formation of this intricate network of microchannels and micropores then facilitates further hydration of the cement. The gradual and long-term release of calcium hydroxide, the bioactive byproduct of MTA hydration, as a result of the ongoing hydration process has been demonstrated by Fridland & Rosado (2003, 2005). Therefore, the formation of this interconnected network of micropores is necessary for the continuous hydration of MTA. With further crystalisation of MTA the initial connectivity of the microchannels decreases and results in the formation of closed pores that may hold trapped water. Therefore, hardened cement contains a large number of closed pores that are not connected (Camilleri 2007, Camilleri 2011a) resulting in the cement having low permeability (Feldman 1990, Lu *et al.* 2006). Evaluation of the porosity of MTA is important as it can provide information on the physical properties of the material (Fridland & Rosado 2003).

2.6.13. **Radiopacity**

One of the essential requirements of a dental material is radioapcity (Devito *et al.* 2004) in order to distinguish it from the adjacent hard tissues such as dentine, cementum and alveolar bone (Torabinejad *et al.* 1995b, Tagger & Katz 2004). According to ANSI/ADA Specification No. 57 (2006) an endodontic (root canal) sealing material should be at least the equivalent of 2 mm aluminium more radiopaque than adjacent hard tissue to allow monitoring of its potential dissolution, and to identify the presence of voids (Coutinho-Filho *et al.* 2008, Tanomaru-Filho *et al.* 2008). Shah *et al.* (1996) suggested a minimum radiopacity of the equivalent of 3 mm aluminium for root-end filling materials, which is greater than the suggested radiopacity of root canal sealers.

One of the main differences in the chemical constituents of MTA and Portland cement is the presence of bismuth oxide, which is added to make MTA radiopaque (Torabinejad & Dean 1995). Pure Portland cement would not be sufficiently radiopaque to be distinguished from adjacent anatomical hard tissues (Bortoluzzi *et al.* 2006). The equivalent thickness of DOXP LQXP IRUKXP DQGHQVH DVUHSRUWHGV? P P FRP SDUHGWK0 7$ WKLVDV reported as beiQJ HTXLYDOHQWWKLFNQHVVRI ? (Laghios *et al.* 2000). Amalgam ZLWKDQHTXLYDOHQWXP IQXP WKLFNQHVVRI ? KDGWKHJUHDWHVWRSDFLW DP RQJ the 13 root-end filling materials evaluated by Laghios *et al.* (2000). The equivalent aluminiXP WKLFNQHVVRI 0 7$ ZDVUHSRUWHGWEH P P E\ ' DQHMK *et al.* (2006), a significantly greater radiopacity than specimens of Portland cement.

CHAPTER 3

AIMS OF THE STUDY

3. Aims & objectives of the study

The main aim of this work was to evaluate the effect of various environmental (clinical) conditions on the physical and chemical characteristics of MTA in the hope that such information would inform those clinicians who used the material whilst also gaining new information about the physical properties of the existing materials. The key research questions were:

What was the effect of acid solutions used during restorative techniques on the physical properties of MTA?

The null hypothesis being that acid contamination of MTA had no detrimental effect on its physical properties.

What was the effect of blood contamination on the physical and chemical properties of MTA?

The null hypothesis being that blood contamination of MTA had no detrimental effect on its physical or chemical properties.

During the planning stage and before the main studies a series of laboratory studies were undertaken to standardise specimen preparation. This extensive preliminary work was essential in developing a standard methodology for use in the main studies and to gain an understanding of the material and how it reacted to various mixing and placement techniques. These studies were essential because few, if any, previous studies mentioned this critical information and because pilot studies had revealed inconsistency in the material when mixed and placed using 'normal' clinical techniques.

Preliminary studies: In Chapter 4 (page 58) the background to this preliminary work, the methodologies used together with the results and conclusions are described. Initially, to eliminate any potential effect of confounding variables, the amounts of powder and water

supplied in ProRoot? MTA (Dentsply Tulsa Dental) packages were measured precisely. In the second preliminary study, in an attempt to standardise potential confounding variables during mixing of MTA, the effect of passive water absorption and pressure on selected properties of MTA was evaluated. In the next phase of the preliminary studies, a novel mixing technique, mixing of encapsulated MTA, was developed to standardize further the mixing methodology. In addition, the potential effect of this innovative mixing technique on surface hardness and compressive strength of MTA was evaluated.

Next, in an attempt to achieve consistent slurries of MTA following placement the application of ultrasonic energy was investigated and its effects on certain physical properties of MTA evaluated. Finally, the effect of the powder to water ratio on selected physical properties of MTA was examined when specimens were mixed with the 'standard' mixing and placement technique.

By employing the findings of these preliminary studies in the main studies (Chapter 5, page 121) the effects of acid and blood contamination on various characteristics of MTA could be evaluated using standard mixing and placement techniques that eliminated potential confounding variables that had likely influenced the results of work undertaken by many previous authors.

Main studies: In most of the clinical applications using MTA the material is in contact with inflamed tissue that might have an acidic pH. Thus, the effect of various forms of acid on the physical properties of MTA including compressive strength, surface microhardness, push-out force and microstructure were evaluated. Initially, butyric acid, a by-product of anaerobic bacterial metabolism (Zeikus 1980, Tonetti *et al.* 1991) was used to simulate a potential clinical situation associated with periradicular infections. Another route for acid contamination of MTA is when a clinician restores a tooth using acid gel to etch the enamel

and dentine. During the use of such gels it is impossible to protect the MTA surface from exposure to the phosphoric acid they contain. Therefore, the effect of phosphoric acid gel on the selected physical properties of MTA was also evaluated.

During many of the application of MTA the material comes into contact with tissue fluid and blood. Therefore, in the final part of the study the effect of blood contamination on selected physical and chemical properties of MTA were evaluated.

CHAPTER 4

STANDARDISATION OF SPECIMENS

4. Standardisation of sample preparation - the preliminary studies

4.1. **Background**

In an experimental study, an *independent variable* is deliberately manipulated by the researcher and the effect of this intervention on the *dependent variables* is studied (Avis 1994). Any other *confounding variables* that could affect this experience must be controlled precisely and eliminated (Wunsch 2008). Lack of consistency in *controlled variables* may critically compromise the validity of the results (Fowkes & Fulton 1991). In the present work, to standardise the methodology of the laboratory tests on MTA, a series of studies was required to understand better the confounding variables that would likely have an impact on the several dependent variables studied.

4.1.1. **Study 1 - Weight of water in ampoules**

In each package (box) of grey ProRoot? MTA Original (Dentsply Tulsa Dental) and tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) there are five sachets (pouches) containing MTA powder and six plastic ampoules containing water. The manufacturer claims that each sachet contains 1 g of MTA powder and that the amount of water inside each ampoule is equal to 0.35 g. This information is written on the sides of each package (Figure 1, page 58).

Figure 1: Wording on a box of ProRoot? MTA
The volume of water in each ampoule is stated clearly (see arrow).

The first preliminary study undertaken was initiated as a result of a noticeable variation in the volume of water in the ampoules supplied within the ProRoot? MTA (Dentsply Tulsa Dental)

packages. Since the powder to water ratio is a key factor affecting the ultimate physical and chemical properties of the material (Fridland & Rosado 2003) it was essential to determine whether specimens of MTA for the main studies could be prepared when mixing the powder with the water from the ampoules or whether individual measures of water had to be used. Thus, the amount of water and MTA powder supplied in the packages of both original and tooth coloured ProRoot® MTA (Dentsply Tulsa Dental) were measured precisely.

4.1.2. **Study 2 – Manual mixing technique: saturation followed by application of pressure**

The physical and chemical properties of several dental materials, including MTA, can be affected by mixing technique. Many dental materials are available as two components that are mixed before use, including "powder and liquid", "two paste" and "paste and liquid" systems. When one component in the system is a liquid, the achievement of a homogenous mixture becomes less predictable (Powers & Wataha 2008). According to the manufacturer's recommendations predetermined amounts of powder and water should be spatulated on a glass slab to produce slurries. In the laboratory situation standardisation of spatulation pressure and technique when making hundreds of specimens is not feasible. Therefore, in the second preliminary study, in an attempt to eliminate this inconsistency in spatulation pressure and mixing technique, premeasured weights of distilled water were added to the appropriate amount of powder and left until it was absorbed fully – without spatulation. This powder saturation hydration methodology has the potential to produce a more consistent and repeatable method of water incorporation. In tandem with this saturation mixing technique the standardisation of pressure on the resultant slurry was also considered as another confounding variable and addressed in the same study; the pressure following saturation mixing was an attempt to simulate the pressure normally exerted during spatulation.

In most previous studies (Torabinejad *et al.* 1993, Hachmeister *et al.* 2002, Fridland & Rosado 2003, Lawley *et al.* 2004, Lee *et al.* 2004, Fridland & Rosado 2005, Walker *et al.* 2006) spatulation pressure was an uncontrolled variable that could have affected the properties and performance of the resultant MTA cement.

Torabinejad *et al.* (1993) reported that the characteristics of hardened MTA were related to the powder to water ratio, the humidity around the material and the amount of air trapped in the mixture. To allow MTA to harden sufficiently moisture must be present during the hydration process (Torabinejad *et al.* 1995b). Based on various clinical applications, moisture may be available from adjacent periodontal or pulpal tissues and/or from a damp cotton or sponge pellet that should be placed near the unset material (Walker *et al.* 2006). It is probable that the degree of spatulation pressure may change the molecular distance between water molecules and the particles of MTA powder and influence the space available for the hydration reaction. This in turn may influence the optimum water to powder ratio (Bordallo *et al.* 2006) and may also have an effect on entrapped air and consequently on the number of air inclusions in the hydraulic cement (Bentz 1997). In summary, it is possible that if the mixing of MTA is not controlled then the physical and biological properties of the hardened material will vary. Therefore, the aim of the second preliminary study was to evaluate the effect of application of various amounts of pressure on MTA slurries following the powder saturation hydration method on several physical properties of MTA: surface microhardness, microstructure and compressive strength.

These initial and early preliminary studies informed the first main study on contamination of MTA with acid (section 5.1, page 121). However, during the course of the acid contamination study parallel work on the mixing of MTA was ongoing. This resulted in a further refinement

in specimen preparation that was then used in the remainder of the studies on contamination with blood (section 5.1.6, page 155).

4.1.3. **Study 3 – Mechanical mixing technique – mixing of encapsulated MTA**

One of the most popular methods of mixing materials such as amalgam or glass ionomer is mechanical encapsulated mixing. For amalgam, the individual components of the material were at first placed manually into a mixing device (Curry 1945). Then, in an attempt to enhance consistency further, premeasured amounts of amalgam powder and mercury were placed inside a capsule containing two compartments that were then mechanically mixed (Lazarus 1951, Harcourt & Lautenschlager 1970). In due course, various dental materials including, glass ionomer (Mitchell *et al.* 1998), self-cured composite resin (Tani & Ida 1978), mixtures of gutta-percha and sealer (Nawal *et al.* 2011), eucoapercha (Campbell & Thorpe 1990), zinc phosphate (Branco & Hegdahl 1983) and calcium hydroxide cements (Schmid 1998) became available in capsules containing pre-set proportions of their components that were then mechanically mixed prior to use. The capsule often contained a small rod-like pestle, which improved the mechanical mixing (Darvell 1981). This system of encapsulation and mechanically mixed has the potential to produce a consistently uniform, void or pore-free mixture (Powers & Wataha 2008).

In all reported studies to date MTA was mixed manually according to the manufacturer's instructions (Parirokh & Torabinejad 2010b). Such a technique results in an uncontrolled mixing time and spatulation pressure with potential to produce a set material that has an inconsistent range of physical properties. In addition to the saturation method, which was described above, and in order to achieve even more consistency when mixing MTA it could be hypothesised that encapsulated pre-set proportions of MTA could confer advantages to the practitioner and the material.

As amalgamators are available in most dental clinics it was thought that the use of such a mixing technique would be effective and rapid, since it is a familiar and efficient mixing technique and can be used in both laboratory and clinical environments. Therefore, in the next preliminary study a novel technique was developed whereby mixing of MTA was standardised by encapsulated mixing 1 g of MTA powder with 0.33 g of liquid in a plastic mixing capsule containing a plastic pestle, at 4500 revolutions/min for 30 s using an amalgamator (GB patent No 0919270.9).

4.1.4. **Study 4 – Placement technique - application of ultrasonic agitation**

Following the development of a standard mixing technique for MTA using either the saturation or encapsulated methods further work was undertaken on a standard placement technique that was aimed at producing a consistent sample of MTA during specimen preparation. This work looked specifically at the application of ultrasonic energy to samples following placement of MTA slurries into moulds. Vibration has a dispersion effect on the particles of cement materials, which frequently cluster together (Bensted 2002). Indeed, ultrasonic energy has been used to enhance the mechanical properties of various restorative materials, including compressive strength (Kleverlaan *et al.* 2004, Barata *et al.* 2008), tensile bond strength (Algera *et al.* 2005, Fagundes *et al.* 2006), hardness (Towler *et al.* 2001) and fill density (Yeung *et al.* 2006). Ultrasonic energy may also increase the total reactive surface area, improve particle interaction and decrease setting time (Kleverlaan *et al.* 2004, Algera *et al.* 2005). In addition, by changing rheological properties it can improve the placement and handling characteristics of materials (Witherspoon & Ham 2001, Lawley *et al.* 2004, Schmidlin *et al.* 2005), as well as marginal adaptation to the cavity wall (Schmidlin *et al.* 2005).

Aminoshariae *et al.* (2003) compared two placement techniques for MTA, concluding that hand compaction of MTA provided less porosity, better adaptation and fewer voids than ultrasonic vibration. Hachmeister *et al.* (2002), in an attempt to improve the sealing ability of MTA in an immature root canal model, emphasised the importance of the delivery system rather than the material itself. Roberts *et al.* (2008) highlighted the use of ultrasonic vibration for MTA placement.

Since previous work appeared to be inconclusive, in this preliminary study, the effect of ultrasonic energy following two different mixing techniques on several physical properties of MTA was evaluated.

4.1.5. **Study 5 - Powder to water ratio**

The physical and chemical characteristic of hydraulic cements including MTA may be affected by variation in the powder to water ratio (Groves 1981, Hanehara *et al.* 2001, Fridland & Rosado 2003, Fridland & Rosado 2005, Islam *et al.* 2006a). Adhering to a consistent powder to water ratio is important as deviation may produce inconsistent setting and reduced physical properties (Hawley *et al.* 2010). Fridland & Rosado (2003) investigated the effect of various powder to water ratios on certain characteristic of MTA and showed that decreasing the powder to water ratio resulted in decreased solubility and porosity of the resultant hardened MTA. Given the development of the new encapsulated mixing method and the ultrasonic agitation of the resultant slurries during specimen placement it was thought essential to investigate further the effect of powder to water ratio. This was particularly relevant given that no previous work had been published on these parameters when linked to the novel methods of specimen production outlined above.

Thus, in this section of the preliminary studies the effect of various powder to water ratios on compressive strength, surface hardness and setting time of MTA were investigated.

In addition, for a better understanding of the hydration process of MTA material when mixed with various amount of water, the crystalline phase structure of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) while mixed with various amount of water was studied using X-ray diffraction (XRD) analysis. Moreover, for comparison reason and to identify and evaluate the by-products of the hydration process of MTA, the phase composition of the un-hydrated MTA (powder) was also evaluated.

"X-ray diffraction analysis" (XRD) is one of the powerful methods for the characterization of crystalline structure of materials (Figure 2, page 65). The key to this technique is that the diffraction pattern (angle and intensity) of each particular crystalline structure as a result of X-ray exposure is a unique pattern. Therefore, by comparing the diffraction pattern with reference standards it is possible to identify the crystalline structure of a material as the same substance always gives the same diffraction pattern (that is like a fingerprint of a substance). In polycrystalline materials, each crystalline structure produces its characteristic diffraction pattern, which is independent of the other crystalline formations.

Figure 2: An X-ray diffractometer (Panalytical X'pert pro, Almelo, Netherlands).

In the following section the aims and methodologies of each preliminary study along with the results are presented separately. The section ends with a holistic discussion and conclusion that summarises the findings and how they influenced specimen preparation during the main experimental work on acid and blood contamination (Chapter 5).

4.2. **Powder and water content of ProRoot? MTA packages**

4.2.1. **Aim**

To measure the weight of powder and water in packages of ProRoot? MTA (Dentsply Tulsa Dental).

4.2.2. **Materials & Methods**

4.2.2.1.**Specimens**

The total of 50 sachets of MTA powder and 58 ampoules of water were collected from five sealed packages of ProRoot? MTA Original (Dentsply Tulsa Dental) with LOT number of 05003087 (grey) and five sealed packages of ProRoot? MTA (Dentsply Tulsa Dental) with LOT number 09001920 (tooth coloured) respectively. The date of each package was checked to ensure they were within their expiry date. The samples were kept at a controlled laboratory temperature (20^{o}C) for 24 h before measurement.

4.2.2.2.**Weight measurements**

An analytical laboratory balance (Precisa 80A-200M, Zurich, Switzerland) was used. The accuracy of the laboratory digital scale was confirmed by using predetermined standard weights.

For measurement of the water content of each ampoule, the analytical balance was stabilized at zero. Then the weight of each ampoule with the water inside was measured and recorded to an accuracy of three decimal places at 20^{o}C. Subsequently, the tip of each ampoule was removed using a surgical scalpel and water was released into a glass container, which had also been kept at 20^{o}C for 24 h. Then, the weight of water was measured to an accuracy of three decimal places at 20^{o}C. Subsequently the weights of each empty plastic container and the removed tip were also determined. To calculate the definitive amount of water inside the

ampoules, the weight of each empty plastic container and its tip were subtracted from the weight of the ampoules before opening, i.e. with water inside. The value obtained was considered as the definitive amount of water inside each ampoule. The difference between the definitive water and measured water was calculated as the amount of water that could not be released from the plastic ampoule.

The MTA powder content of each sachet was then measured using the same principle. The analytical balance was stabilized at zero before the weight of each sachet with the powder inside was measured and recorded to an accuracy of three decimal places at room temperature (20^{o}C). Then, the border of each sachet was removed using a scissors and then MTA powder was poured into a pre-weighed glass container. Then the net weight of the powder within each sachet was measured to an accuracy of three decimal places at 20^{o}C. Subsequently the weight of each empty sachet and its cut edge were also determined. To calculate the definitive amount of powder inside the each sachet, the weight of each empty sachet and the cut edge were subtracted from the total weight of sachet before opening. The value obtained was considered as the definitive amount of powder inside each sachet. The difference between the definitive powder weight and measured powder weight was calculated as the amount of powder that could not be released from the sachet.

4.2.3. **Results**

The results of measurement of weight of water in ampoules are summarized in Table 2 (page 68). The average measured and definitive weights of water in the ampoules were 0.211 g (range: '0.140 g, 0.270 g'; SD: 0.40) and 0.223 g (range: '0.149 g, 0.290 g'; SD: 0.40) respectively. These values are 0.139 g (range: '0.210 g, 0.800 g') and 0.198 g (range: '0.201 g, 0.060 g') less than the amount of water claimed (Figure 1, page 58) to be inside the ampoules.

Weight of water	Average	Range	Standard deviation
Measured	0.211 g	0.140 g - 0.270 g	0.40
Definitive	0.223 g	0.149 g - 0.290 g	0.40

Table 2: The measured and definitive weights (g) of water inside the ampoules supplied in the ProRoot® MTA packages.

The average measured and definitive weights of powder in the sachets were 1.004 g (range: '1.001, 1.009 g'; SD: 0.003) and 1.006 g (range: '1.004g, 1.010 g'; SD: 0.005) respectively. By rounding these values to two decimal places, they are identical to the amount of powder claimed to be inside the sachets.

4.2.1. **Conclusions**

The results identified a major problem with the weight of water in the ampoules supplied in the MTA packages. Therefore, ampoules supplied by the manufacturer (Dentsply Tulsa Dental) should not be used to dispense the water used in research studies involving MTA. Rather the water component should be weighed accurately prior to mixing. Thus, in all studies of this thesis, the water used to produce MTA slurries was weighed individually; the ampoules were not used.

4.3. **Effect of application of various amounts of pressure on MTA slurry following the powder saturation hydration method on selected physical properties of MTA**

4.3.1. **Aim**

To evaluate the effect of application of various amounts of pressure on MTA slurries following the powder saturation hydration method on several physical properties of MTA.

4.3.2. **Materials and Methods**

The material investigated was tooth coloured ProRoot® MTA (Dentsply Tulsa Dental) with LOT number 03081235.

4.3.2.1.**Compressive strength**

Each sachet containing 1 g of MTA powder was mixed with a 0.33 g aliquot of distilled water. The distilled water was added to the powder and left until it was absorbed (saturation technique). The material was then placed with minimal pressure using the tip of a dental spatula into customized open-ended polycarbonate cylindrical moulds having an internal diameter of 4 mm and height of 6 mm (in accordance with ISO 4049:2000 & ISO 9917-1:2003), which were lying upright on a glass slab. Five groups of 10 specimens were prepared and the material within each mould was then subjected to pressures of 0.06, 0.44, 1.68, 3.22 or 4.46 MPa respectively by varying the weight applied to the open surface. The pressure on each specimen was applied for 1 minute using a custom-made device containing a stainless steel piston with an internal diameter that was similar to the polycarbonate cylindrical moulds (Figure 3, page 70). The samples were thus subjected to a vertical force that was translated into a transverse and equally distributed pressure that pressed the MTA evenly into the cylindrical mould. A wet cotton pellet was then placed onto the tube

containing the MTA and sealed in container with fully saturated humidity for 4 days at room

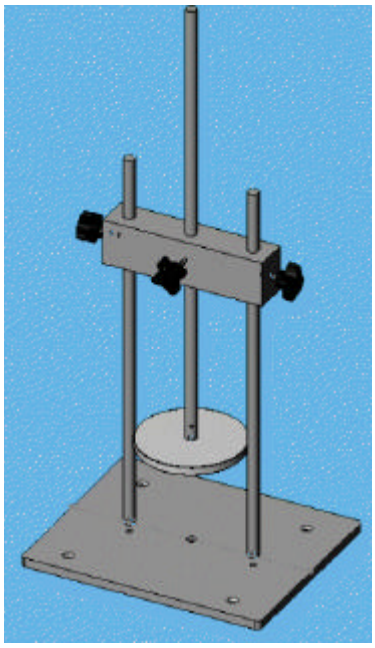


Figure 3: Custom-made device for application of controlled pressure on test specimens.

The compressive strength test was conducted using a universal testing machine (Lloyd LR MK1 machine; Lloyd Instruments, Fareham, UK). A flat steel rod was used at a crosshead speed of 1 mm min^{-1} whilst specimens were mounted vertically so that the compressive load was applied along the long axis of each specimen (Figure 4, page 70).

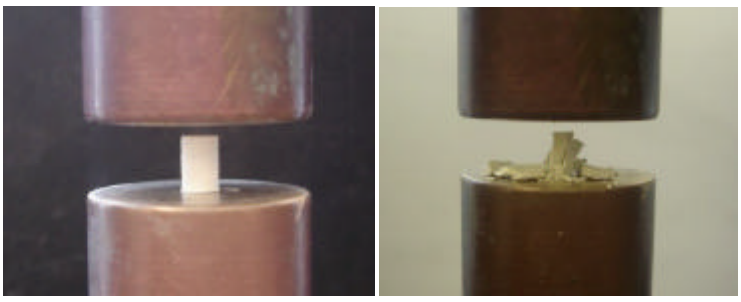


Figure 4: A tooth coloured ProRoot® MTA specimen during the compressive test using the universal testing machine.

The failure load was recorded and the compressive strength calculated using Equation 1 (page 71). The compressive strength of all specimens was recorded in MPa. The data obtained from the compressive strength tests were subjected to statistical analysis using one-way ANOVA for overall comparison and by Tukey's post hoc test for individual comparisons.

$$\mathrm{CS}(\sigma) = \frac{4P}{\pi d^2}$$

Equation 1: CS is compressive strength (MPa), P is the force (N) applied and d (mm) is the diameter of the specimen.

4.3.2.2. **Surface microhardness**

The same specimen preparation procedure was employed for testing surface microhardness (see section 4.3.2.1, page 69). Cylindrical specimens of 6 mm in diameter and 12 mm in height were prepared in polycarbonate cylindrical moulds. The same custom-made device (Figure 3, page 70) was used to apply pressure to the samples. Six groups of 10 specimens were prepared using pressures of 0.06, 0.44, 1.68, 3.22, 4.46 or 8.88 MPa. A wet cotton pellet placed onto each specimen, which was then sealed in a container with fully saturated humidity for 4 GD\V DW URRP WHP SHUDWXUH&

After 4 days, both surfaces of the specimens were wet polished at room temperature using minimum hand pressure and silicon carbide-based sandpapers of varying particle size (‘Wetordry™’ 600-grit, 737 SF ‘Tri-M-ite™’ and ‘Wetordry™’ 1200-grit; 3M, St Paul, MN, USA) to provide smooth surfaces for ease of indentation testing. The polished specimens were cleaned gently under light pressure using distilled water to remove surface debris. To prevent dissolution or water sorption the surfaces were dried gently by air spray. The Vickers

hardness test of each specimen was performed using a microhardness tester (MVK G_1, Mitutoyo Corp., Tokyo, Japan) and a square-based pyramid-shaped diamond indenter with a full load of 50 g for 5 s at room temperature that produced a quadrangular depression with two equal orthogonal diagonals in the polished surface of the cement. The angle between the RSSRVLWH GLDP RQG LQ HQWHU DV) LYH LQGHQWDWLRQV HUH PDGH RQ WKH polished surface of each specimen at discrete locations no closer than 1 mm to adjacent indentations or the specimen periphery. The diagonal of the resulting indention was measured immediately under the microscope and the Vickers hardness value displayed on the digital readout of the microhardness tester. Based on the Equation 2 (page 72), the Vickers hardness (H_V) is calculated.

$$H_V = \frac{2F \sin(136^\circ/2)}{d^2}, \quad H_V = 1.854\frac{F}{d^2} \text{ approximately}$$

Equation 2: Hv is Vickers microhardness, F is load (kg−1) and d (mm) is the mean of the two diagonals of the impression made by the indenter.

The mean of the hardness values obtained was calculated to determine the hardness value for each specimen. Differences between the experimental groups were analysed by one-way ANOVA for overall comparison and by Tukey's post hoc test for individual comparisons.

4.3.2.3. **Internal surface microstructure**

For the morphological evaluations, new specimens were prepared using the same pressures of 0.06, 0.44, 1.68, 3.22 or 4.46 MPa and the same storage conditions. To analyse the microstructure of the inner surfaces, the specimens were sectioned in two using a disposable surgical scalpel blade No. 15. The surfaces were sputter-coated with gold using a Polaron Sputter Coater (Quorum Technologies, Newhaven, UK) and specimens were visualised with

an EBT1 (Electron Beam Technology) Scanning Electron Microscope (S.E.M. Tech Ltd, Woodbridge, UK). The micrograph images from the SEM analysis showing the qualitative internal microstructure of the set MTA prepared with different pressures were evaluated in terms of the presence of microchannels and type of crystal formation.

4.3.3. **Results**

4.3.3.1. **Compressive strength**

The results of the compressive strength testing are shown in Figure 5 (page 73). Maximum compressive strengths occurred at a pressure of 0.06 and 1.68 MPa. The lowest compressive strength was associated with a pressure of 0.44 MPa. Despite the obvious variations there was no significant difference between the groups.

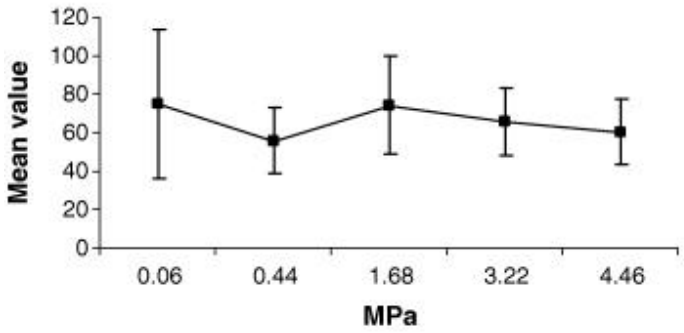


Figure 5: Mean compressive strength values (MPa) of specimens subjected to various pressures. No significant differences were apparent between the groups.

4.3.3.2. **Surface microhardness**

The results of the microhardness testing are shown in Figure 6 (page 74). Little difference occurred in mean surface hardness values up to a pressure of 3.22 MPa. However, a pressure of 8.88 MPa produced specimens with significantly lower values in terms of surface hardness than the other groups ($p<0.001$). A pressure of 3.22 MPa conferred the maximum hardness

value; however, it was not significantly different from the other groups except the 8.88 MPa group ($p<0.001$).

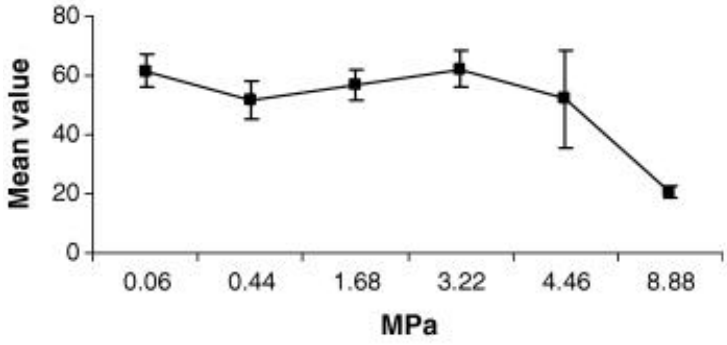


Figure 6: Mean Vickers surface microhardness (HV) values of specimens subjected to various amounts of pressure. The 8.88 MPa pressure produced specimens with significantly lower Vickers surface microhardness (HV) values than the other groups (P < 0.001).

4.3.3.3. Internal surface microstructure

The internal microstructure of all specimens that were prepared with various pressures revealed microchannels, depressions caused by air bubbles, pores, asymmetrical crystalline formation in the form of laminated cross-stratified structures, and bundles of jagged needle-like formations in a homogeneous matrix that resembled an epitaxial growth pattern (Figure 7-Figure 11, pages 75-77). It was not possible to score each characteristic and thus compare them quantitatively between groups. However, the SEM images demonstrated that the application of higher pressures resulted in fewer voids created by entrapped air. In addition, fewer microchannels could be seen in specimens prepared with application of higher pressures (Figure 12, page 77). More typical crystalline structures were observed in specimens prepared with application of lower amounts of pressure that tended to appear around microchannels (Figure 8 & Figure 9, page 75,76).

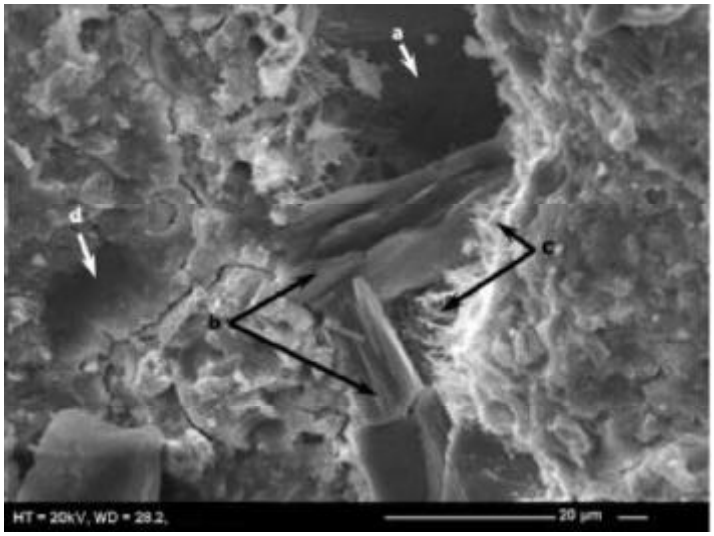


Figure 7: Scanning electron microscope image of specimen subjected to 3.22 MPa. A cross-section of a microchannel can be seen (a), together with laminated (b) and needle-like (c) crystalline formations and a depression from an air bubble (d).

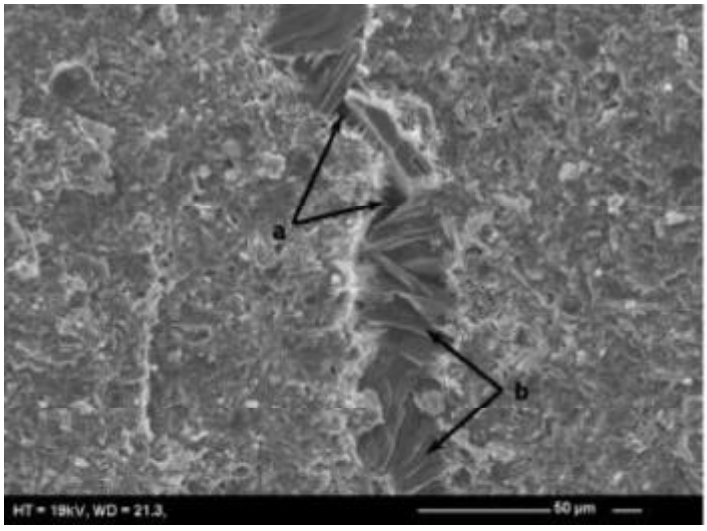


Figure 8: Scanning electron microscope image of a specimen subjected to a pressure of 0.06 MPa showing a cross-section of a microchannel (a), and a crystalline formation in the form of a laminated cross-stratified structure (b).

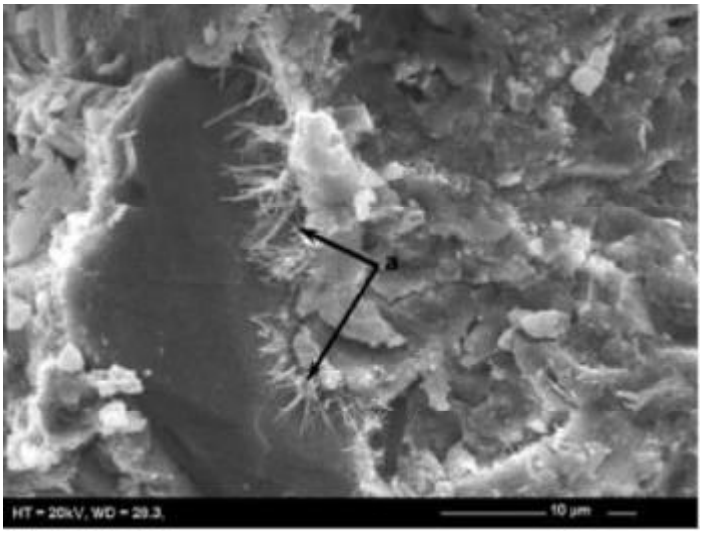


Figure 9: Scanning electron microscope image of a specimen subjected to a 0.06 MPa pressure. Bundles of jagged needle-like formations can be seen (a).

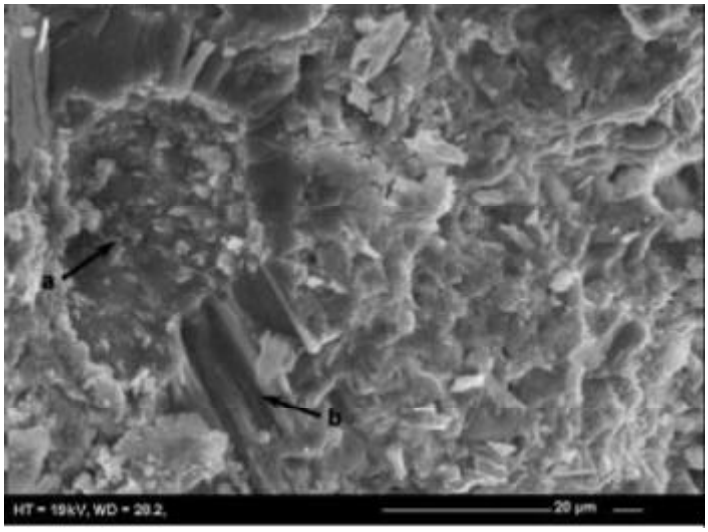


Figure 10: Scanning electron microscope image of a specimen subjected to a 1.67 MPa pressure. Cross-sections of bundles of jagged needle like formations (a) can be seen together with a laminated crystalline structure (b). This structure resembles an epitaxially growth pattern.

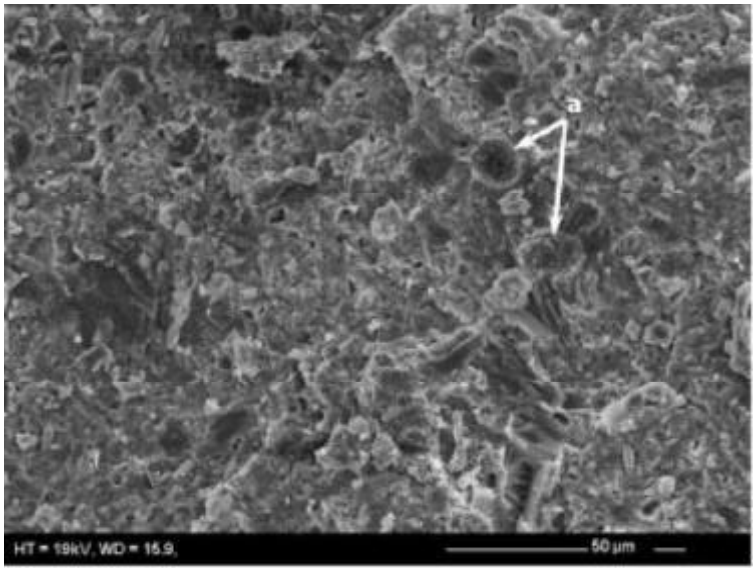


Figure 11: Scanning electron microscope image of a specimen subjected to a 0.06 MPa pressure. Depressions from an air bubble (a) can be seen.

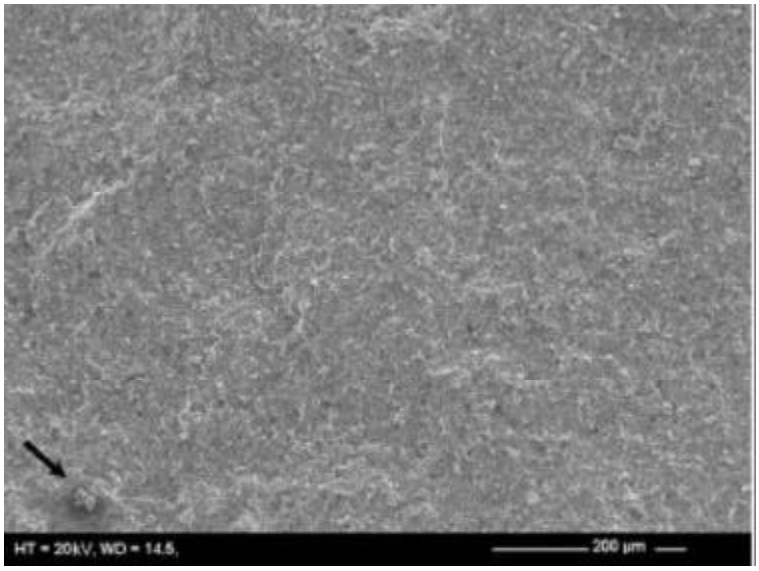


Figure 12: Scanning electron microscope image of a specimen subjected to a 4.46 MPa pressure. Greater pressure resulted in fewer voids and microchannels. Some unidentified debris can be seen (arrow).

4.3.4. **Conclusions**

Application of pressure during the mixing of MTA may affect the strength and hardness of the material. Based on the findings of this study, when greater pressures were applied to

MTA following saturation of the powder its surface hardness reduced significantly. Conversely, its maximum compressive strength occurred with the minimum pressure. This may occur because a greater pressure may reduce the intermolecular distance that results in less space for the ingress of water to hydrate the material adequately. Therefore, in experimental investigations, use of controlled pressure when mixing ProRoot? MTA (Dentsply Tulsa Dental) is essential in order to standardise specimen preparation.

4.4. **Effect of placement technique on certain physical properties of MTA**

4.4.1. **Aim**

To evaluate the effect ultrasonic agitation (during the placement of MTA slurries) following two different mixing techniques on surface microhardness, compressive strength and setting time of MTA. The mixing methods were the novel technique of encapsulated mixing MTA and the saturation of MTA powder with predetermined amount of water followed by application of 3.22 MPa pressure.

4.4.2. **Materials & Methods**

The materials investigated were:

- ProRoot? MTA Original (Dentsply Tulsa Dental) with LOT number of 05003087 (grey)
- ProRoot? MTA Tooth coloured (Dentsply Tulsa Dental) with LOT number of 083006

4.4.2.1. **Compressive strength**

Eighty custom-made polytetrafluoroethylene (PTFE) cylindrical moulds (internal dimensions P P KHLJKWDQG? P P GLDP HWHU ZHUH SUHSDUHG 7KH\ ZHUH LQLWLDOO\ GLYLGHG LQWR two groups of 40 samples each. Moulds in each group were then randomly divided into four groups of 10, with each subgroup to be filled with one of the two types of grey or tooth coloured MTA slurries prepared by the following four methods:

- Group 1: an aliquot of 0.33 g distilled water was gradually added to one gram of each type of MTA powder in the PTFE cylindrical container on a glass slab and left until it had absorbed (saturation method). It was then subjected to a constant pressure of 3.22 MPa as described in section 4.3.2.1 (page 73) to provide consistency in the MTA

slurry preparation. Resultant slurries were then transferred to the silicon experimental moulds with minimal pressure.

- Group 2: MTA materials were mixed using the novel technique of "mechanically mixing of encapsulated MTA" as described in section 4.1.3 (page 61). One g of each type of MTA powder (grey and/or tooth coloured) and 0.33 g of double distilled water mechanically mixing in a plastic capsule containing a plastic pestle, at 4500 revolutions/min for 30 s using an amalgamator (PromixTM, Dentsply Caulk, York, PA, USA). The resultant slurries were then transferred to the PTFE experimental moulds with minimal pressure.
- Groups 3 and 4: samples were prepared as described in Groups 1 and 2 respectively; however, following placement of the MTA slurries in the PTFE experimental moulds with minimal pressure, MTA slurries were subjected to ultrasonic energy using a BUC-1 Spartan tip (Obtura Spartan, Fenton, MO, USA) attached to a Suprasson? P5 Booster (Satelec, Acteon Group, 0 pUIJQF, France) (Figure 13, page 80).

Figure 13: MTA slurry being treated with ultrasonic vibration.

Excess MTA slurry was removed from each specimen by gently wiping the mould over the glass slab, this aided in keeping the samples parallel (Figure 14, page 81); they were then incubated in fully saturated humidity at 37^{o}C for 4 days.

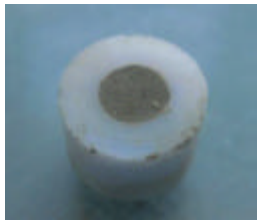


Figure 14: MTA specimen after surface wiping, thus ensuring parallelism of surfaces.

The hydrated MTA samples were then removed by cutting vertically through the wall of the moulds using a disposable surgical scalpel blade No.15, whilst taking care not to damage the MTA samples (Figure 15, page 81).

Figure 15: MTA sample being removed from the casing of the PTFE mould.

Following removal from the moulds, all samples of MTA were visually inspected to ensure they had no voids or flaws before being subjected to the compressive strength test. Then the end surfaces were polished with 1200-grit fine-grain sandpaper (3M, St Paul, MN, USA) to GLP HQVLRQVRI ? P P height DQG? P P GLDP HWHUDFFRUGDQFH ZLWK,62 - 1:2003 standards (Figure 16, page 81).

Figure 16: The dimensions of specimens were rechecked prior to the compressive test.

The compressive strength of each specimen was then determined using a universal testing machine (Lloyd LR MK1 machine; Lloyd Instruments, Fareham, UK) as described in section 4.3.2.1 (page 69) (Figure 4, page 70).

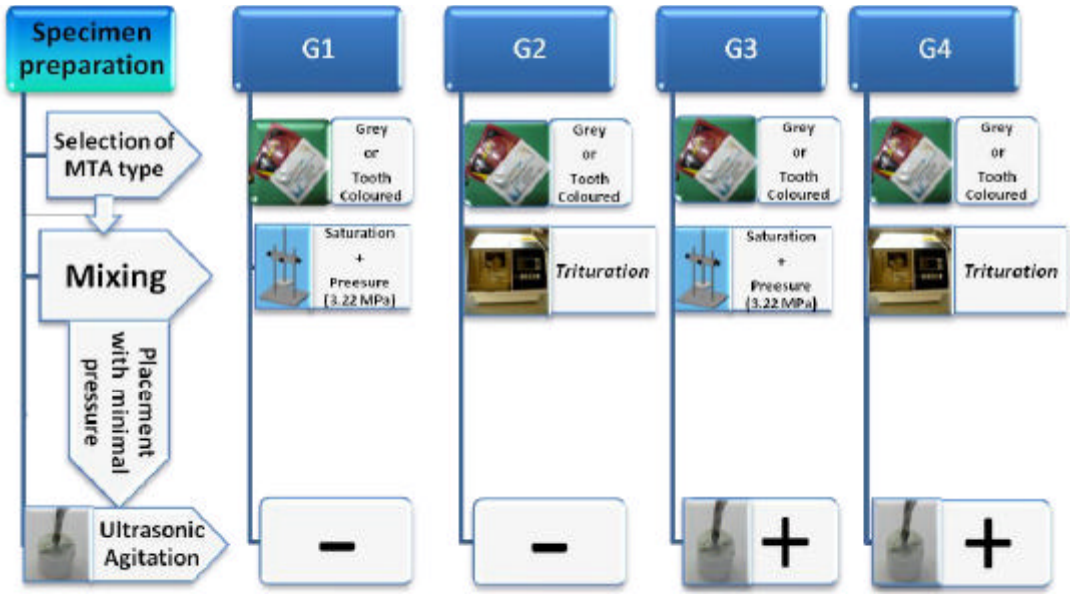


Figure 17: Allocation of the experimental groups for each type of MTA (grey or tooth coloured) according to specimen preparation technique (mixing and placement).

4.4.2.2. Surface microhardness

The same specimen preparation procedure was employed for testing the surface microhardness. After 4 days all samples were removed from the incubator and subjected to the Vickers surface microhardness test as described in section 4.3.2.2 (page 71). After microhardness testing, all samples were replaced immediately into the incubator. After a further 28 days samples were removed from the incubator and subjected to the surface microhardness test using the same methodology. The mean Vickers surface microhardness value and standard errors were calculated for each group and subjected to a two way analysis

of variance. All analysis was performed using the Statistical Package of Social Science version 16 (SPSS Inc., Chicago, IL, USA).

4.4.2.3.**Setting time**

Eighty silicon experimental moulds (Dentsply DeTrey, Konstanz, Germany) were prepared with an inner diameter of 10 mm and height of 2 mm. They were initially divided into four groups of 40 samples each. Moulds in each group were then randomly divided into four groups of 10, with each subgroup being filled with one of the two types of MTA slurry prepared by four different methods (Figure 17, page 82) that were employed for testing the compressive strength (see section 4.4.2.1, page 79).

The initial and final setting times of all samples were measured (in accordance with ASTM international standard C 266 – 07) at 37^{o}C inside an incubator using a Gillmore apparatus CT-5 (ELE International Inc. Loveland, CO, USA). The apparatus consisted of two needles, one to evaluate the initial set, the other the final set. The initial-set needle weight was 113.4 g and had a 2.12 mm diameter tip. The final-set needle weighed 453.6 g and had a 1.06 mm tip. The initial-set needle was applied lightly to the surface of each sample. This procedure was repeated each 60 seconds until the needle did not create a complete circular depression. This was verified by attempting two further unsuccessful indentations. The elapsed time, in minutes, between the start of contact between MTA and water and unsuccessful indentation, was recorded as the initial setting time. To measure the final setting time, the same procedure was followed every 10 minutes by applying the final-set Gillmore needle to the specimen surface. The final setting time was recorded when the needle did not mark the specimen surface with a complete circular depression. This was verified by two additional unsuccessful attempts and the final setting time recorded. The data were then analysed statistically using

two-way ANOVA for overall comparison followed by Tukey's post hoc test for individual comparisons. All analysis was performed using the SPSS version 16 (SPSS Inc.)

4.4.3. **Results**

4.4.3.1. **Compressive strength**

The results are summarised in Table 3 (page 84).

Table 3: Mean value, standard errors and 95% confidence interval of compressive strength values (MPa) for each experimental group categorised according to the mixing and placement techniques used.

MTA type	Mixing	Placement	Groups	Mean	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Tooth coloured	Saturation & Pressure	No Ultrasonic	G1	88.773	6.803	75.199	102.347
		Ultrasonic	G2	91.776	6.803	78.202	105.350
	Encapsulated mixing	No Ultrasonic	G3	89.743	7.171	75.435	104.052
		Ultrasonic	G4	**101.708**	7.171	87.399	116.016
Grey	Saturation & Pressure	No Ultrasonic	G1	**65.195**	7.171	50.887	79.504
		Ultrasonic	G2	74.533	6.803	60.959	88.108
	Encapsulated mixing	No Ultrasonic	G3	83.619	6.803	70.045	97.193
		Ultrasonic	G4	85.206	7.171	70.897	99.515

No significant differences were found between the various experimental groups except between specimens of tooth coloured MTA mechanically mixed and agitated by ultrasonics and specimens of grey MTA that were mixed by the saturation and pressure methodology without application of ultrasonic agitation ($p < 0.001$). The highest mean compressive strength value was observed in the specimens of group 4, tooth coloured MTA, which were mechanically mixed followed by application of ultrasonic agitation (Figure 18 page 85).

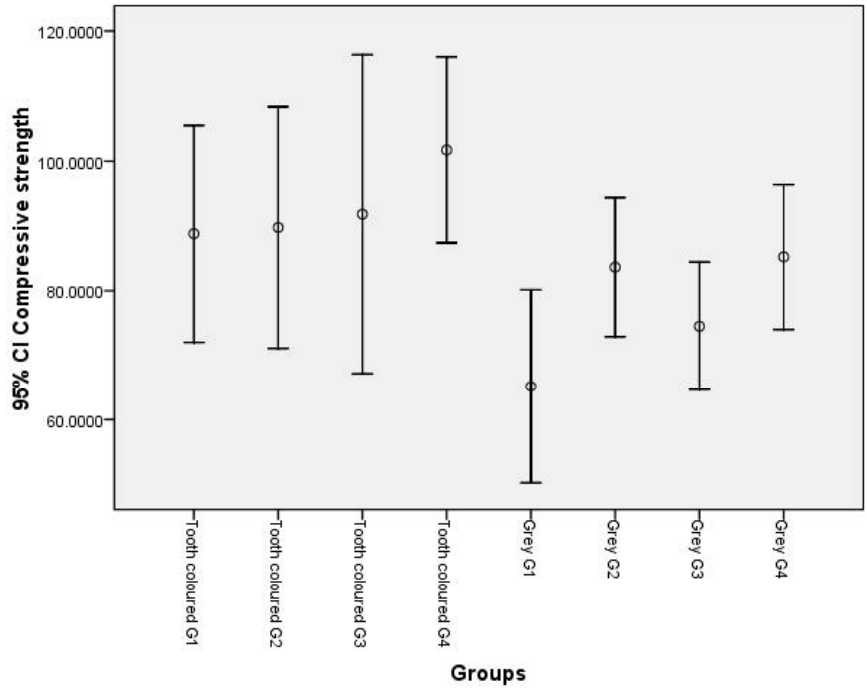


Figure 18: Error bar graph representing the effect of ultrasonic application on compressive strength values (MPa). A significant difference was observed between mean values of the compressive strength (MPa) of specimens of tooth coloured MTA group 4 and grey MTA group 1 (p <0.001).

4.4.3.2. **Surface microhardness**

The results are summarised in Table 4 (page 89). At 28 days of incubation, regardless of the type of MTA and/or techniques that were used for preparation of specimens, the surface microhardness values were significantly greater for all experimental groups compared to the 4 day values (p <0.00001) (Figure 19, page 86).

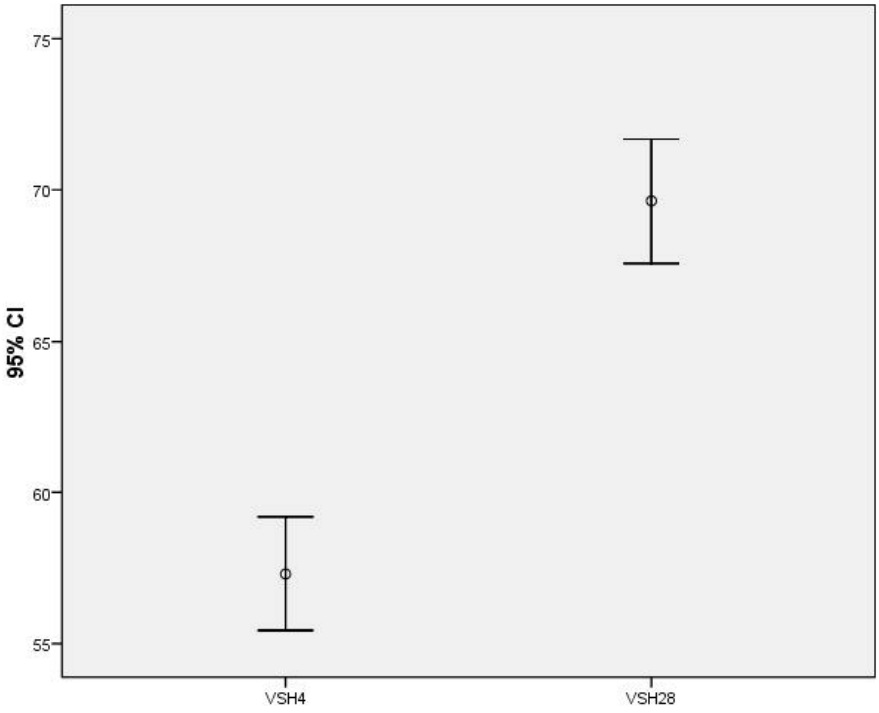


Figure 19: Error bar graph representing Vickers surface microhardness (HV) mean values and 95% confidence intervals at different incubation time intervals regardless of the type of MTA and/or techniques that were used for preparation of specimens. The surface microhardness (HV) values were significantly greater for all experimental groups at 28 days compared to 4 days incubation time (p <0.00001). (VSH4=Vickers surface microhardness (HV) at 4 days/ VSH28= Vickers surface microhardness (VH) at 28 days).

For tooth coloured ProRoot? MTA (Dentsply Tulsa Dental), the application of ultrasonic energy produced significantly higher surface microhardness values irrespective of the incubation time and/or the mixing techniques (saturation & pressure and/or encapsulated mixing) ($p < 0.0001$) (Table 4, page 89). For grey ProRoot? MTA (Dentsply Tulsa Dental) the application of ultrasonic vibration resulted in significantly higher values for surface microhardness at 4 days ($p < 0.0001$), however, at 28 days no significant difference existed between the specimens of the experimental groups (Figure 20, page 87 and

Table 4, page 89).

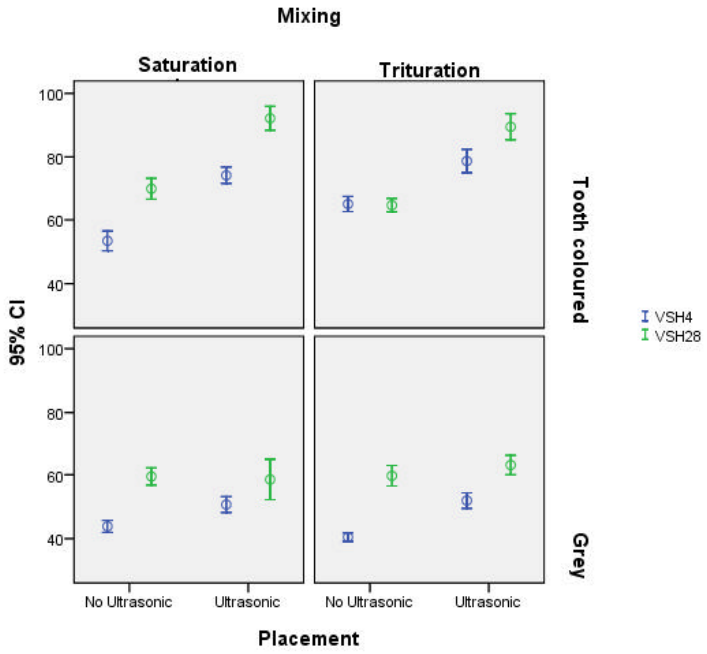


Figure 20: Error bar graph representing the effect of ultrasonic application and mixing techniques on the Vickers surface microhardness (VH) values of tooth coloured and grey MTA (VSH4=Vickers surface microhardness (VH) at 4 days/ VSH28=Vickers surface microhardness (VH) at 28 days).

Comparing the two types of MTA, irrespective of the mixing and placement techniques, a significant difference ($p<0.0001$) was observed in surface microhardness at both 4 and 28 days. Tooth coloured ProRoot[?] MTA (Dentsply Tulsa Dental) produced the highest Vickers surface microhardness value (Figure 21, page 88).

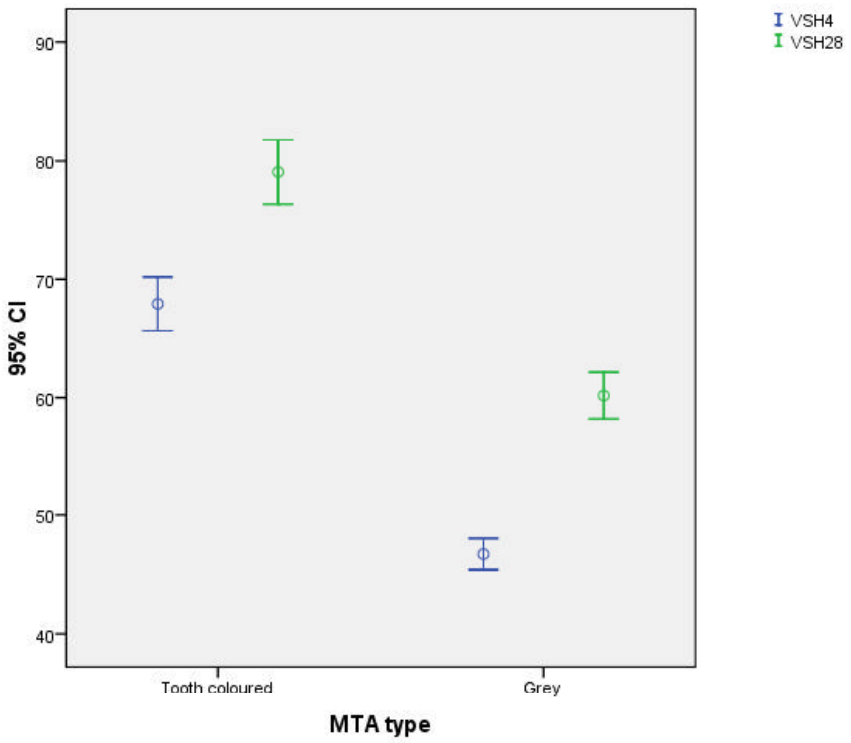


Figure 21: Error bar graph comparing the Vickers surface microhardness values (VH) of two types of ProRoot? MTA (Dentsply Tulsa), grey and tooth coloured, regardless of the mixing and placement techniques at 4 days and 28 days. (VSH4=Vickers surface microhardness (VH) at 4 days/ VSH28=Vickers surface microhardness (VH) at 28 days).

Mixing techniques regardless of the type of MTA and/or placement technique did not produce any significant difference in Vickers surface hardness values at any of incubation times (Figure 22, page 89).

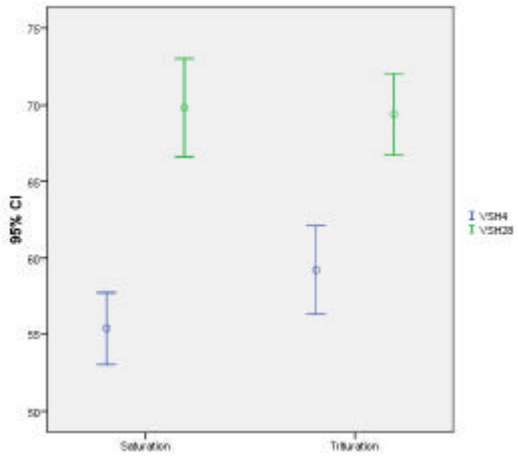


Figure 22: Error bar graph of mixing technique regardless of the types of MTA and/or placement techniques revealing no significant difference in Vickers surface hardness values (VH). (VSH4=Vickers surface microhardness (VH) at 4 days/ VSH28=Vickers surface microhardness (VH) at 28 days).

	MTA type	Mixing	Placement	Group	Mean	Std. Error	95% Confidence Interval	
							Lower Bound	Upper Bound
VSMH after 4 Days	Tooth coloured	Saturation & Pressure	No Ultrasonic	G1	53.423	1.264	50.933	55.914
			Ultrasonic	G2	74.203	1.286	71.670	76.737
		Encapsulated mixing	No Ultrasonic	G3	65.180	1.264	62.689	67.671
			Ultrasonic	G4	78.703	1.244	76.253	81.153
	Grey	Saturation & Pressure	No Ultrasonic	G1	43.813	1.264	41.323	46.304
			Ultrasonic	G2	50.650	1.264	48.159	53.141
		Encapsulated mixing	No Ultrasonic	G3	40.460	1.264	37.969	42.951
			Ultrasonic	G4	51.917	1.264	49.426	54.407
VSMH after 28 Days	Tooth coloured	Saturation & Pressure	No Ultrasonic	G1	69.977	1.841	66.349	73.604
			Ultrasonic	G2	92.155	1.873	88.466	95.845
		Encapsulated mixing	No Ultrasonic	G3	64.780	1.841	61.153	68.407
			Ultrasonic	G4	89.469	1.811	85.901	93.038
	Grey	Saturation & Pressure	No Ultrasonic	G1	59.467	1.841	55.839	63.094
			Ultrasonic	G2	58.510	1.841	54.883	62.137
		Encapsulated mixing	No Ultrasonic	G3	59.680	1.841	56.053	63.307
			Ultrasonic	G4	63.063	1.841	59.436	66.691

Table 4: Mean value, standard errors and 95% confidence intervals of Vickers surface microhardness (VH) values for each experimental group following 4 and 28 days of incubation. The groups were categorized according to the specimen preparation and placement techniques used, (for more details see Figure 17, page 82).

4.4.3.3.**Setting time**

The mean, standard error and 95% confidence interval values are shown in Table 5. In general, within each group, the mean values of the initial setting times were significantly shorter (P<0.00001) than their corresponding values for final setting times (Figure 23, page 92).

4.4.3.3.1. Initial setting time

The mean initial setting times for the different types of MTA revealed that tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) had a significantly shorter setting time (P<0.00001) than grey ProRoot? MTA (Dentsply Tulsa Dental) regardless of the mixing and placement techniques that were used for specimens preparation (Figure 23, page 92). The use of ultrasonic agitation following mixing by saturation and pressure led to a significantly more rapid setting time (P<0.00001) for both types of ProRoot? MTA (Dentsply Tulsa Dental). Ultrasonic application following slurry preparation by encapsulated mixing with tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) led to a significant decrease in setting time (P<0.00001), which was not noted for grey ProRoot? MTA (Dentsply Tulsa Dental).

	MTA type	Mixing	Placement	Mean	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Initial Setting Time	Tooth coloured	Saturation & Pressure	No Ultrasonic	94.00	3.090	87.840	100.160
			Ultrasonic	61.00	3.090	54.840	67.160
		Encapsulated mixing	No Ultrasonic	128.50	3.090	122.340	134.660
			Ultrasonic	89.00	3.090	82.840	95.160
	Grey	Saturation & Pressure	No Ultrasonic	130.0	3.090	123.840	136.160
			Ultrasonic	66.00	3.090	59.840	72.160
		Encapsulated mixing	No Ultrasonic	235.50	3.090	229.340	241.660
			Ultrasonic	224.00	3.090	217.840	230.160
Final Setting Time	Tooth coloured	Saturation & Pressure	No Ultrasonic	292.00	6.033	279.974	304.026
			Ultrasonic	240.00	6.033	227.974	252.026
		Encapsulated mixing	No Ultrasonic	319.00	6.033	306.974	331.026
			Ultrasonic	306.50	6.033	294.474	318.526
	Grey	Saturation & Pressure	No Ultrasonic	444.00	6.033	431.974	456.026
			Ultrasonic	392.00	6.033	379.974	404.026
		Encapsulated mixing	No Ultrasonic	428.00	6.033	415.974	440.026
			Ultrasonic	441.00	6.033	428.974	453.026

Table 5: The mean initial and final setting time (min) values, standard error and 95% confidence intervals of the various experimental groups (for more details of group allocation see, page 82).

Comparing tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental), within each group, except for slurries prepared by ultrasonic activation following saturation and pressure, toothcoloured ProRoot? MTA (Dentsply Tulsa Dental) had significantly shorter mean initial setting times ($P<0.00001$) when compared to grey ProRoot? MTA (Dentsply Tulsa Dental) (Table 5, page 91).

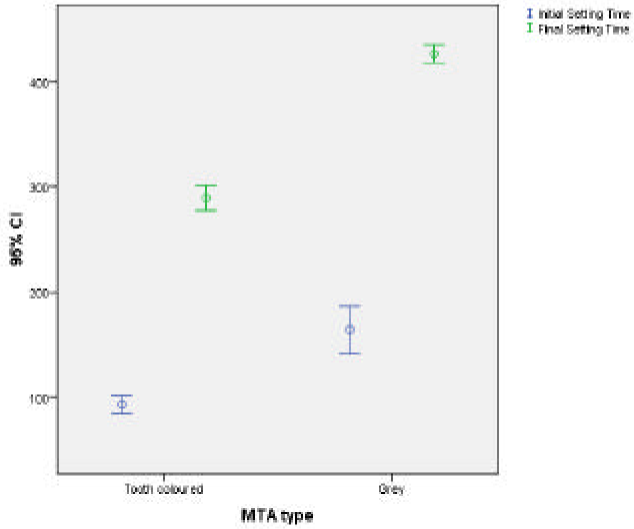


Figure 23: Error bar graph comparing initial and final setting times (min) of two types of ProRoot? MTA (Dentsply Tulsa Dental), grey and tooth coloured, regardless of the mixing and placement techniques.

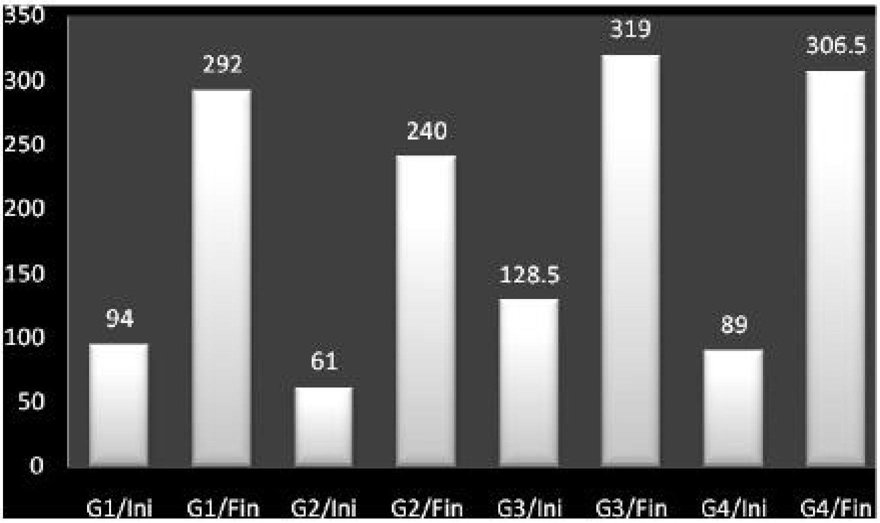


Figure 24: Mean values of the initial (Ini) and final (Fin) setting time (min) RI WRRWKFRORXUHG3 UR5 RRW MTA specimens (Dentsply Tulsa Dental) (for more details of groups allocation see Figure 17, page 82).

4.4.3.3.2. Final setting time

Comparing tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental), regardless of the mixing and placement techniques, tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) had a significantly (P<0.00001) shorter mean final setting time than grey ProRoot? MTA (Dentsply Tulsa Dental) (Figure 23, page 92).

The use of ultrasonic agitation following mixing by saturation and pressure led to a significantly more rapid final setting time (P<0.00001) for both types of ProRoot? MTA (Dentsply Tulsa Dental).

Ultrasonic application following slurry preparation by encapsulated mixing did not produce any significant change in the final setting time of both tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental).

Comparing tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental), within each group, tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) had significantly shorter mean initial setting times (P<0.00001) when compared to the corresponding grey ProRoot? MTA groups (Dentsply Tulsa Dental) (Table 5, page 91).

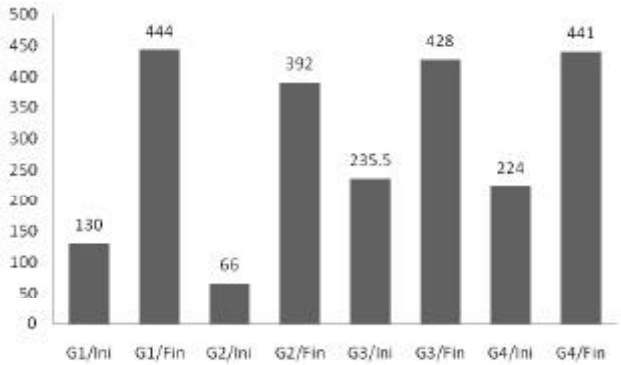


Figure 25: Mean values of the initial (Ini) and final (Fin) setting times (min) **of grey ProRoot? MTA (Dentsply Tulsa Dental) specimens in the various experimental groups (for more details of group allocation see Figure 17 (page 82).**

4.4.4. **Conclusions**

Methods of mixing and placement of MTA significantly affected the hydration process and consequently the physical properties of the material. Therefore, a consistent mixing and placement methodology should be employed. Overall, application of ultrasonic vibration following either the manual mixing technique (saturation and pressure) and/or mechanical mixing technique (mechanically mixing of encapsulated MTA) resulted in the most favourable physical properties of the MTA specimens.

4.5. **Effect of various powder to water ratios on selected physical properties of MTA**

4.5.1. **Aim**

To evaluate the effect of various powder to water ratios on compressive strength, surface hardness and setting time of various types of MTA.

4.5.2. **Materials & Methods**

The materials investigated were

- ProRoot® MTA Original (Dentsply Tulsa Dental) with LOT number of 05003087 (grey)
- ProRoot® MTA Tooth coloured (Dentsply Tulsa Dental) with LOT number of 083006; (tooth coloured)

4.5.2.1. **Compressive strength**

One hundred and twenty **custom-made** polytetrafluoroethylene (PTFE) cylindrical moulds (internal dimensIRQV? P P KHJKWQG? P P GLDPHWHU HPEHGGHG (ISO 9917-1:2003).

They were randomly divided into six groups of 20 with each group being filled with either grey or tooth coloured MTA slurries prepared using the following three different water to powder ratios.

Groups 1 and 4- MTA mix had a powder to water to ratio of 3.5

Groups 2 and 5- MTA mix had a powder to water ratio of 3.0

Groups 3 and 6- MTA mix had a powder to water ratio of 2.5

In groups 1-3 grey MTA and in groups 4-6 tooth coloured MTA were used respectively. Mixing was standardized by encapsulating one gram of corresponding type of MTA and 0.29g, 0.33g and 0.40g (in accordance with the group being tested) of distilled water (Table

6, page 96) in an empty plastic capsule with a plastic pestle to facilitate mechanically mixing. These were sealed and loaded into a Promix TM amalgamator (Dentsply Caulk) and mechanically mixed at 4500 revolutions/min for 30 s as described in section 4.1.3 (page 61).

Groups	Powder (g)	Water (g)	Powder to water ratio
1	1.00	0.40	2.5
2	1.00	0.33	3.0
3	1.00	0.29	3.5

Table 6: The amount of cement and water required for the different cement to water ratios.

The PTFE cylindrical moulds were filled with the resultant MTA slurry using a spatula with minimal pressure and then to standardise the specimens, all samples were agitated with ultrasonic energy using a BUC-1 Spartan tip (Obtura Spartan) attached to a SupUDWRQ? 3 Booster (Satelec, Acteon Group) as described in section 4.4.2.1 (page 79). The specimens were then incubated at 37^{o}C and fully saturated humidity for 4 or 28 days respectively. After their allocated time the moulds were removed and the MTA specimens extracted by cutting vertically through the wall of the moulds using a No.15 disposable surgical scalpel blade, whilst taking great care not to touch the MTA samples (Figure 15, page 81). The final height was measured and their parallelism checked with a micrometer (Figure 16, page 81).

A universal testing machine, (Lloyd LR MK1 machine; Lloyd Instruments) was used to perform the compressive strength testing as described in section 4.3.2.1 (page 69) (Figure 4, page 70). As the data was normally distributed statistical analysis was carried out using two-way ANOVA for overall comparisons and Tukey's post hoc test for individual comparisons. All analyses were performed using the Statistical Package for the Social Sciences version 16 (SPSS Inc.)

4.5.2.2.**Surface microhardness**

Three groups of 10 samples for each type of MTA (60 specimens in total) were prepared with various cement to water ratios as described in section Compressive strength 4.5.2.1 (page 95) and incubated for 4 days at 37^{o}C and fully saturated humidity. After 4 days all samples were removed from the incubator and subjected to the Vickers surface microhardness test in accordance with the European and British Standard (BS EN 843-4:2005) using a microhardness tester (MVK G1, Mitutoyo Corp.) as described in section 4.3.2.2 (page 71). After microhardness testing, all samples were covered immediately and then incubated at 37^{o}C in a fully saturated humidity. After 28 days the samples were again removed from the incubator and subjected to the surface microhardness test using the same methodology. The mean Vickers surface microhardness values were calculated for each group. All the results were subjected to a two way analysis of variance test. All analysis was performed using the Statistical Package for the Social Sciences version 16 (SPSS Inc.).

4.5.2.3.**Setting time**

One hundred and twenty silicon moulds (Dentsply DeTrey, Konstanz, Germany) were prepared with an inner diameter of 10.00 mm and height of 2.00 mm. They were initially divided into three groups as follows:

Group 1: Powder/Water = 3.5/1

Group 2: Powder /Water = 3.0/1

Group 3: Powder /Water = 2.5/1

Moulds in each group were divided into two groups of 10, each subgroup being filled with one of the types of ProRoot? MTA that were mixed and placed using the methodology described in section 4.5.2.1 (page 95). The initial and final setting times of all samples were measured (in accordance with ASTM international standard C 266 – 07) at 37^{o}C as described

in section 4.4.2.3 (page 83) using a Gillmore apparatus CT-5 (ELE International Inc.). All the results were subjected to a two way analysis of variance using the Statistical Package for the Social Sciences version 16 (SPSS Inc.).

4.5.2.4. **Phase composition**

A sample from each group of the hydrated tooth coloured ProRoot? MTA that were produced by mechanical mixing of encapsulated MTA with various amounts of water as described in section 4.5.2.1 (Table 6, page 26) were subjected to X-ray diffraction analysis. Three aluminum sample holders (Panalytical, Almelo, Netherlands) with a round depression in the centre (20 mm diameter & 2 mm depth) were randomly selected (Figure 26(A), page 98). The circular depression of each XRD sample holder (Panalytical, Netherlands) was filled by the corresponding MTA slurry with minimal pressure (Figure 26(B), page 98).

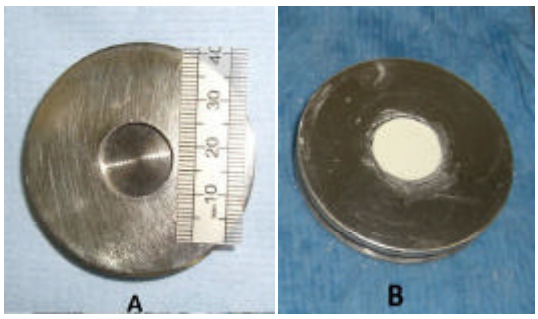


Figure 26: (A) An aluminium XRD sample holder (Panalytical, Almelo, Netherlands) with a round depression in the centre (20 mm diameter & 2 mm depth). (B) The depression of one sample holder was filled with MTA slurry using minimal pressure followed by the application of ultrasonic agitation.

To standardise the placement technique, the MTA slurry was then subjected to ultrasonic energy using a BUC-1 Spartan tip (Obtura Spartan, Fenton, MO, USA) attached to a 6XSUDVRQ? 3 %RRVWHU Satelec, France). The ultrasonic tip was moved throughout the MTA slurry without touching either the wall or floor of the sample holder, whilst being activated for 30 s at power scale 5. The assemblies were then incubated at 37°C in fully saturated humidity for 4 days. The specimen surface was then polished with 1200-grit fine-grain

sandpaper (3M, St Paul, MN, USA) to ensure the surface of the sample was level with the holder surface.

Phase compositions of each sample were then determined using an X-ray diffractometer (XRD, Panalytical X'pert pro, Almelo, Netherlands) (Figure 2, page 65). X-ray diffraction patterns were recorded using Ni filtered CuK_α radiation (40Kv and 40mA). Scans were undertaken in the range 10-80^o 2θ. All patterns were matched using the ICDD database (International Centre for Diffraction Data, Pennsylvania, USA). For comparison, the powder of tooth coloured ProRoot? MTA was also subjected to XRD analysis. For this, an extra sample holder (Panalytical, Netherlands) was filled with unhydrated powder of tooth coloured ProRoot? MTA and then a flat spatula was used to press and level the powder into sample holders to provide a smooth flat surface; then it was placed inside the X-ray diffractometer (XRD, Panalytical X'pert pro, Almelo, Netherlands) and subjected to XRD analysis.

4.5.3. **Results**

4.5.3.1. **Compressive strength**

The results are summarized in (page 102). Irrespective of the other variables, the mean compressive strength values at 30 days were significantly greater than at 4 days ($p < 0.00001$) (Figure 27, page 100). The only exception was observed in group 1 (grey MTA) in which the powder to water ratios was 2.5. In this group the mean compressive strength values calculated at 4 days and 30 days incubation time were not significantly different (Figure 28, page 101).

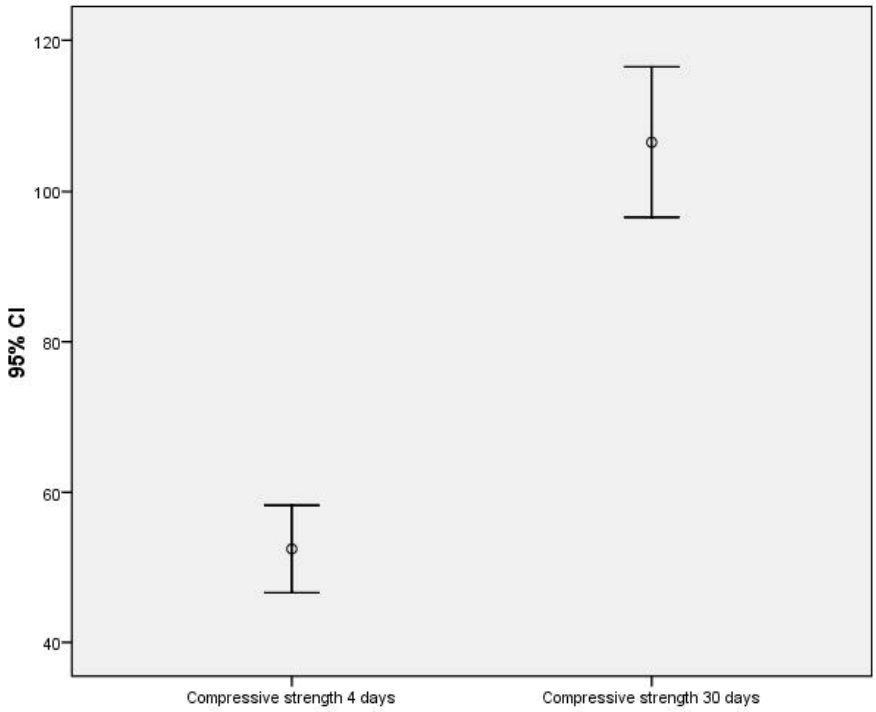


Figure 27: Error bar graph representing mean compressive strength (MPa) values and 95% confidence intervals at different incubation time intervals regardless of the type of MTA and/or the powder to water ratio used for preparation of specimens.

There was no significant difference between the mean compressive strength values of the various powder to water ratios at 4 days for either types of MTA. However, at 30 days, the mean values for compressive strength of the specimens of grey MTA (Group 1) was significantly lower than the other groups ($p < 0.00001$). In addition, for tooth coloured MTA at 30 days the mean compressive strength values of group three (powder to water ratio of 3.5) were significantly greater than the other two groups ($p < 0.00001$) (Figure 28, page 101).

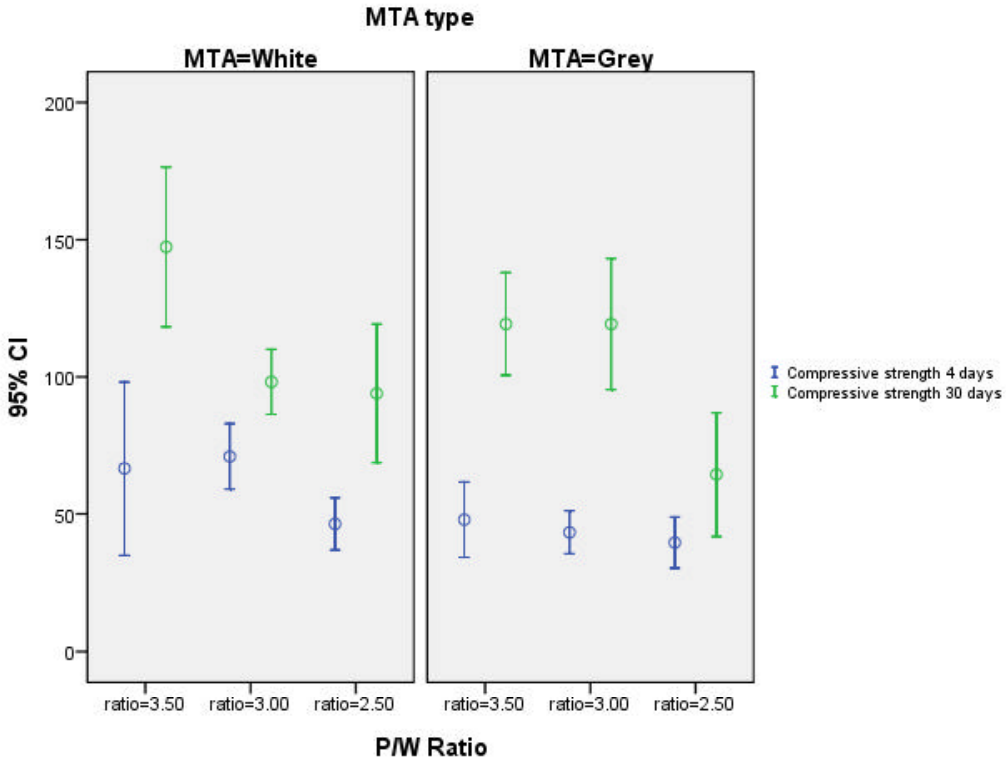


Figure 28: Error bar graph representing the effect of various powder to water ratios on compressive strength (MPa). The mean compressive strength (MPa) values calculated at 4 days and 30 days were not significantly different. At 4 days for both types of MTA there was no significant difference between mean compressive strength (MPa) values. At 30 days the compressive strength (MPa) of group one (P/W ratio: 2.5) of grey MTA was significantly lower than the other groups (p <0.00001). For tooth coloured MTA at 30 days the mean compressive strength (MPa) values of group three (P/W ratio: 3.5) were significantly greater than other two groups (p <0.00001).

Comparing mean compressive strength value of each group (in relation to various powder to water ratios), no significant difference was observed between the types of MTA, at each incubation time interval (Figure 28, page 101) except between the mean compressive strength values of group 2 (powder to water ratio of 3.0) at 4 days ($p < 0.0001$).

	MTA type	P/W ratio	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Compressive strength 4 days	Tooth coloured	3.5	66.504	6.854	52.716	80.293
		3.0	70.956	5.734	59.420	82.492
		2.5	46.361	5.468	35.362	57.361
	Grey	3.5	47.914	6.045	35.754	60.074
		3.0	43.310	6.045	31.150	55.470
		2.5	39.585	6.854	25.796	53.373
Compressive strength 30 days	Tooth coloured	3.5	147.391	10.842	125.580	169.203
		3.0	98.187	9.071	79.938	116.435
		2.5	93.965	8.649	76.566	111.364
	Grey	3.5	119.299	9.562	100.063	138.535
		3.0	119.296	9.562	100.061	138.532
		2.5	64.332	10.842	42.521	86.143

Table 7: Mean value, standard errors and 95% confidence interval of compressive strength (MPa) values of each experimental group categorised according to incubation time, MTA type and powder to water ratios used for specimen preparation.

4.5.3.2. **Surface microhardness**

The results are summarised in Table 8 (page 104). At 28 days, regardless of the powder to water ratio, the Vickers surface microhardness values were significantly greater for all experimental groups compared to 4 days ($p < 0.00001$), except for group 2 that used grey MTA in which the powder to water ratio was 3.0 (Figure 29, page 103).

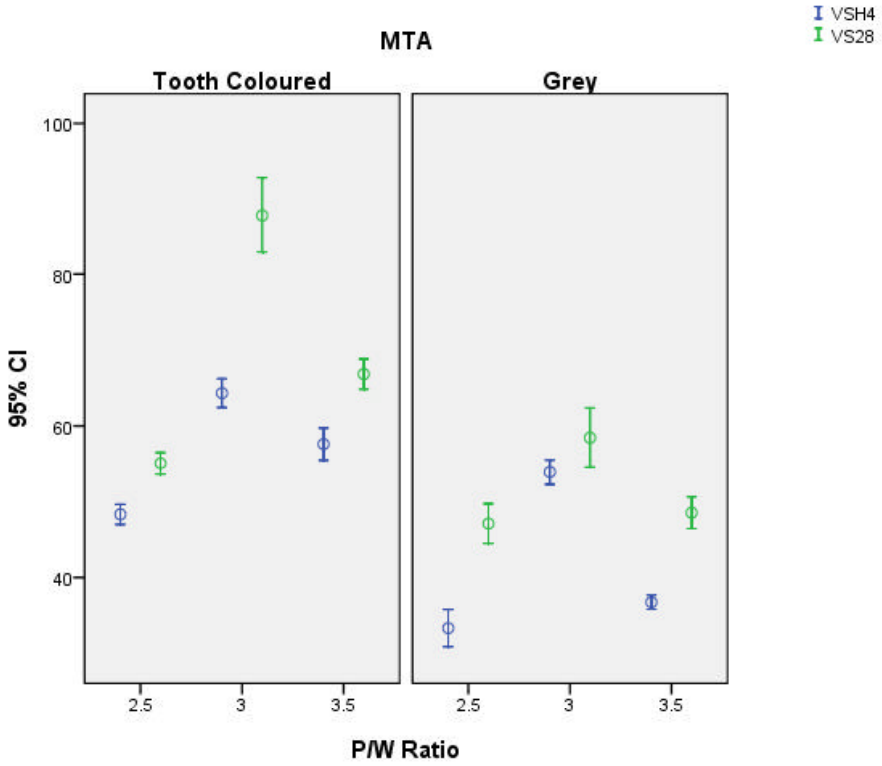


Figure 29: Error bar graph representing the effect of various water to powder ratios on the Vickers surface microhardness values (VH) of grey and tooth coloured ProRoot? MTA at two different incubation time intervals: mean values (VH) and 95% confidence intervals are presented. The surface microhardness (VH) values were significantly greater for all experimental groups at 28 days compared to 4 days (p <0.00001) except for group 2 (P/W ratio: 3.0) of grey MTA. The mean Vickers surface microhardness (VH) values of the group 2 (P/W ratio: 3.0) of both types of MTA at both intervals was significantly greater than group 1 (P/W ratio: 2.5) and 3 (P/W ratio: 3.5) (p <0.00001) (VSH4=Vickers surface microhardness (VH) at 4 days/ VSH28=Vickers surface microhardness (VH) at 28 days).

The mean Vickers surface microhardness (VH) values of group 2 (powder to water ratio - 3.0) of both types of MTA at both incubation time intervals was significantly greater than group 1 and 3 (p <0.00001) in which the powder to water ratios were 2.5 and 3.5 respectively (Figure 29, page 103).

	MTA	P/W Ratio	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
VSH4	Tooth Coloured	2.5	48.32	.893	46.56	50.08
		3	64.31	.893	62.56	66.07
		3.5	57.60	.893	55.85	59.36
	Grey	2.5	33.35	.893	31.61	35.11
		3	53.89	.893	52.13	55.65
		3.5	36.75	.893	34.99	38.51
VS28	Tooth Coloured	2.5	55.06	1.542	52.02	58.09
		3	87.88	1.542	84.84	90.91
		3.5	66.83	1.542	63.79	69.86
	Grey	2.5	47.11	1.542	44.07	50.14
		3	58.45	1.542	55.41	61.48
		3.5	48.53	1.542	45.50	51.56

Table 8: Mean value, standard errors and 95% confidence intervals of the Vickers surface microhardness (VH) values of each experimental group following 4 and 28 days. The groups were allocated according to various powder to water ratio (P/W Ratio) and types of MTA.

4.5.3.3.**Setting time**

The results are summarised in Table 9 (page 106). In general, within each group, the mean values of the initial setting time were significantly shorter than the mean values of final setting time (Figure 30, page 105) regardless of the powder to water ratios and the type of MTA that were used ($p<0.000001$).

4.5.3.3.1. Initial final setting time

Concerning the effect of powder to water ratio on the initial setting time of grey ProRoot? MTA, the mean initial setting time value of the specimens of group 1 (P/W ratio: 2.5) was significantly longer than the other two groups ($p<0.000001$). The same pattern was observed for the tooth coloured ProRoot? MTA in which the mean initial setting time value of the specimens of group 1 (P/W ratio: 2.5) was significantly longer than the other two groups

(p<0.000001). Comparing each corresponding group of grey and tooth coloured ProRoot® MTA, within each group, there was no significant difference between the mean initial setting time values (Figure 30, page 105) (p=0.412).

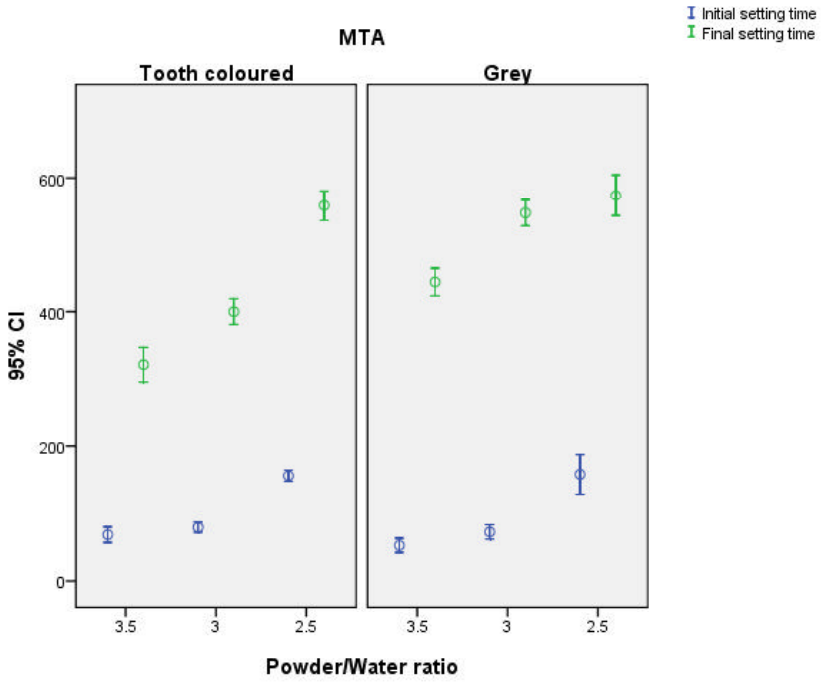


Figure 30: Error bar graph representing the effect of various water to powder ratios on the initial and final setting times (min) of grey and tooth coloured ProRoot® MTA. The mean value and 95% confidence intervals are presented.

4.5.3.3.2. Final setting time

Concerning the effect of powder to water ratio on the final setting time of grey ProRoot® MTA, the final setting time of the specimens of group 3 (P/W ratio: 3.5) was significantly shorter than the other two groups (p<0.000001). There was no significant difference between the final setting time of specimens of group 1 and 2 in which the powder to water ratio was 2.5 and 3.0 respectively (p=0.2058).

For tooth coloured 3URRW MTA a significant difference was observed between the mean final setting time values of all three groups (p<0.000001). The final setting time of specimens

of group 3 (P/W ratio: 3.5) was significantly shorter than other groups, whilst the final setting time of specimens in group 1 (P/W ratio: 2.5) was significantly longer than the specimens of other two groups ($p<0.000001$).

Dependent Variable	MTA	P/W Ratio	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Initial setting time	Tooth coloured	3.5	68.90	6.66	55.54	82.26
		3	80.00	6.66	66.64	93.36
		2.5	**156.00**	6.66	142.62	169.36
	Grey	3.5	53.00	6.66	39.64	66.36
		3	73.00	6.66	59.64	86.36
		2.5	**158.00**	6.66	144.64	171.36
Final setting time	Tooth coloured	3.5	**321.40**	10.30	300.73	342.07
		3	**400.00**	10.30	379.33	420.67
		2.5	**558.00**	10.30	537.3	578.67
	Grey	3.5	**444.00**	10.30	423.33	464.67
		3	547.00	10.30	526.33	567.67
		2.5	573.50	10.30	552.83	594.17

Table 9: Mean value, standard errors and 95% confidence interval of initial and final setting times (min) of each experimental group. The groups were allocated according to various powder to water ratios (P/W Ratio) and types of MTA.

4.5.3.4. **Phase composition**

X-ray diffraction analysis of the unhydrated tooth coloured ProRoot? MTA revealed the major phases to be bismuth oxide (α-Bi_2O_3, ICDD 00-027-0053), and tri-calcium silicate (Ca_3SiO_5, ICDD 00-055-0738). Di-calcium silicate (Ca_2SiO_4 ICDD 00-024-0037) was also determined but due to peak overlap was difficult to identify. The addition of water resulted in the formation of a calcium hydroxide phase ($Ca(OH)_2$ ICDD ICDD 00-044-1481) and calcium carbonate (ICDD 01-072-1652) with the corresponding decrease in the reflections associated with tricalcium silicate.

Analysis of the un-hydrated ProRoot? MTA demonstrated that the main phase present was α-Bi_2O_3 indicated by the strong reflections at 26.98, 33.31, and 27.42o 2θ. Other reflections were contributed by Ca_3SiO_5, indicated by the peaks at 32.26, 34.42 and 32.78. Addition of water to the samples resulted in the formation of $Ca(OH)_2$ and a corresponding decrease in the Ca_3SiO_4 reflection, which is consistent with the reaction:

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

Comparison of the three different water to cement ratios (Figure 31, page 107) revealed no difference between the three samples. The phases present and their relative concentrations were the same with the addition of water resulting in the formation of calcium hydroxide and calcium carbonate in all cases.

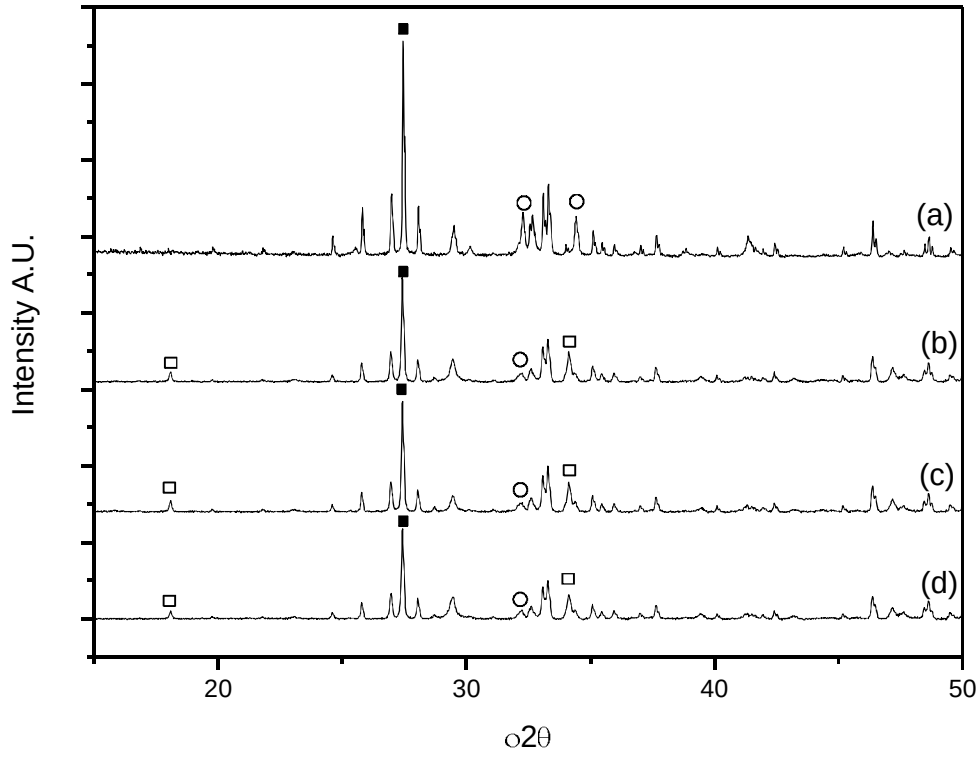


Figure 31: X-ray powder diffraction patterns of tooth coloured ProRoot? MTA showing the effect of water to cement ratio: unhydrated ProRoot? tooth coloured MTA (a), w/c ratio 2.0 (b), w/c ratio 3.0 (c), w/c ratio 3.5 (d). Main phases present highlighted with symbols: (■) α-Bi_2O_3, (○) Ca_3SiO_5 and (□) $Ca(OH)_2$.

4.5.4. **Conclusion**

Using various powder to water ratios can affect the physical properties of MTA. However, no significant change was observed in XRD results in terms of the phase composition of MTA. To achieve the optimum physical properties of MTA specimens employing the powder to water ratio of 3.0, which is recommended by the manufacturer, was confirmed.

4.6. **Overall discussion – preparation of standardised specimens**

Elimination of confounding variables by standardisation of controlled variables is one of the most important requirements of a valid experimental study. Therefore, the water and powder contents of ProRoot? MTA (Dentsply Tulsa Dental) packages were measured precisely in order to determine whether the weight of the water and powder were accurate and consistent and whether the product could be used directly from the packaging or not.

The results revealed that the amount of MTA powder in both grey and tooth coloured types of ProRoot? MTA (Dentsply Tulsa Dental) was consistent and as expected, however, an inconsistency was observed in the amount of water in the ampoules of both the tooth coloured and grey products (Table 2, page 68). This lack of consistency may have occurred as a result of poor quality control by the manufacturer or because of the migration of water molecules through the low-density polyethylene material of the ampoules (McCall *et al.* 1984). The storage conditions of the packages may influence the rate of water loss from the low-density polyethylene material 0 (OH *et al.* 2001). For example, storage of the ampoules in dry conditions would be expected to increase water loss. Indeed, it is recommended by the manufacturer to store the packages in dry conditions to provide better conditions for the sachets of powder, which must not become contaminated by moisture.

In the manufacturer's instructions for use, found inside the package, it is stated clearly that:

'Note: 1: Adding too much, or too little liquid will reduce the ultimate strength of the material.'

The results of Section 4.5.3.2 (page 102) is in accordance with this recommendation (Figure 29, page 103).

The setting of Portland cement and MTA takes place in two stages. After mixing with water the hydration reaction of calcium silicates begins and results in the formation of a gel consisting of calcium silicate hydrates, which liberates calcium hydroxide (Taylor 1997, Camilleri 2008b). The calcium hydroxide then gradually reacts with the minerals to form other hydrated compounds. The calcium silicates contribute most to the binding power and strength of the material. It is also the main binding agent of crystalline calcium hydroxide that leaches most readily from the gel (Eglinton 1987). The bioactive hydration product of MTA is calcium hydroxide (Camilleri 2007, Camilleri 2008b), which is released during and after completion of the hydration process. The characteristic of the resultant set material is likely to be dependent on various factors including water to powder ratio, temperature, environmental humidity and pH, entrapped air and water and the rate of packing (Roy & Gouda 1975, Ishikawa *et al.* 1994, Torabinejad *et al.* 1995b, Fridland & Rosado 2003, Lawley *et al.* 2004, Felekoglu *et al.* 2007).

The compressive strength of Portland cement is affected directly by the powder to water ratio (Papadakis *et al.* 2002). Walker *et al.* (2006) evaluated the effect of setting conditions (time and hydration) on flexural strength of MTA and showed that sufficient water was essential to optimize the flexural strength of the hardened material. Fridland & Rosado (2003) studied the effect of various water-to-powder ratios of MTA on its solubility and porosity. They reported that an increased water-to-powder ratio resulted in higher solubility, porosity and release of calcium hydroxide. However, the presence of excess water in the mixture might cause

difficulty in handling and placement of the material. According to the results of Section 4.4.2.14.5.3.1 (page 99) of the present study adding more water produced lower compressive strength values in the longer term; however, in the short term no significant difference was observed between the specimens that were prepared using various powder to water ratios (Figure 28, page 101). The results of Section 4.5.3.2 (page 102) revealed that a powder to water ratio of 3.0 resulted in the greatest surface microhardness compared to the other groups that were prepared with a lower (2.5) or higher (3.5) powder to water ratio respectively. Mixture of one gram of powder with 0.33 gram of powder, which is recommended by manufacturer, is in accordance with the powder to water ratio of 3.0. The results of Section 4.4.2.34.5.3.3 (page 104) confirmed that the admixture of more water to MTA powder resulted in longer initial and final setting times. A significantly shorter setting time was observed in the specimens of group 3 in which the powder to water ratio was 3.5 ($P<0.00001$), although from the clinical point of view this difference may not be relevant. According to the results of Section 4.5.3.4 (page 106) the hydration of all three groups that were mixed with different powder to water ratios (Table 6, page 96) resulted in the formation of the main phases of MTA, including calcium hydroxide (Figure 31, page 107). Therefore, considering the findings of Section 4.5.3 (page 99) within the range of powder to water ratio that were used, the ratio recommended by the manufacturer (3.0) can be supported and used for specimen preparation in the remainder of the study. Certainly mixing the inconsistent and underweight amount of water that is supplied in the ProRoot? MTA (Dentsply Tulsa Dental) packages with 1 g of MTA powder would result in an inconsistent powder to water ratio and in unpredictable and uncontrolled mechanical and chemical properties of the material. It may also be one of the reasons that the material does not set or solidify occasionally after its placement, which is the indicator of an incomplete hydration process (Torabinejad & Chivian

1999). In addition, in most of the laboratory and clinical research studies on MTA reported to date, specimens were prepared by mixing all the water in an ampoule with 1 g of MTA. As a result, the water to powder ratio, that is one of the most significant variables, might be unintentionally inconsistent. Obviously, supplying the precise and accurate amount of water is essential for a product such as ProRoot? MTA (Dentsply Tulsa Dental). According to the optimised powder to water ratio, which is 3.0, it is recommended that users calculate the amount of water themselves rather than relying on the water supplied in an ampoule, which is unreliable (Table 6, page 96). In the short term, the manufacturer should evaluate the batch weighing system in order to ensure the correct amount of water is supplied. In the longer term, the development of a different delivery system, such as encapsulated MTA is suggested. Encapsulation of pre-set proportions of MTA powder and water appears advantageous as it enables the powder to liquid ratio and the mixing technique to be regularised by the manufacturer. In turn, this reduces the variability that might occur when the material is dispensed and mixed conventionally.

MTA has been recommended for a wide range of clinical applications, although many properties of the material have not been investigated. For example, the effect of pressure during placement on its physical properties is unknown. Hydraulic cements are finely ground materials that when mixed with water gradually or instantaneously set and harden either in air or in water. The reaction results in the formation of hydrated compounds whose strength increases with time. The characteristics of the resultant set material are likely to be dependent on various factors including water to powder ratio, temperature, environmental humidity and pH, entrapped air and water and the rate of packing (Torabinejad *et al.* 1995b, Lawley *et al.* 2004, Lee *et al.* 2004, Fridland & Rosado 2005).

Because of the various clinical applications of MTA, such as direct pulp capping, sufficient strength is necessary to withstand compaction pressures applied to restorative materials. Compressive strength and surface hardness are indicators of the setting process and strength of the hydraulic cements (Bentz 1997) and formed the basis of some of the present preliminary investigations. In addition, in an attempt to evaluate the effect of pressure on the microstructure of MTA, a SEM evaluation was carried out.

Compressive strength, surface microhardness and setting time of hydraulic cements are not just a measure of a solitary property. They are influenced substantially by other fundamental properties of the material such as crystal structure stability (Taylor 1997, Hewlett 2004). Thus, they can be used as indicators of the hydration and setting process of the hydraulic cements (Lee *et al.* 2004, Camilleri 2007). They can also indicate the effect of various setting conditions on the overall strength of the cement (Danesh *et al.* 2006, Saghiri *et al.* 2010a). Evaluation of the ultrastructural morphology can also provide valuable information about the hydration process of MTA under various conditions (Camilleri 2011b). The effect of pressure during mixing on the physical properties of MTA has not been reported previously. However, one of the operator variable factors when mixing the powder of hydraulic cements with water is the pressure that is applied during spatulation.

Because of the lack of standardisation for MTA in the field of dentistry, some studies followed the standards of endodontic sealers (Chng *et al.* 2005) with samples being prepared on the basis of the standards developed for restorative materials (Torabinejad *et al.* 1995b, Fridland & Rosado 2003, Danesh *et al.* 2006). In fact, MTA is not a restorative material nor is it an endodontic sealer. It would seem to be essential that MTA, being a new and unique material with various clinical applications, should have its own standard. The standard testing

techniques for Portland cement are not always applicable for MTA, even though they have been used occasionally (Chng *et al.* 2005, Dammaschke *et al.* 2005, Danesh *et al.* 2006).

In most of the laboratory and clinical studies on MTA, it was stated that specimens "*were prepared in accordance with the manufacturer's instruction*". However, in the manufacturer's instruction there is no information about the optimum pressure or the spatulation time of MTA. Therefore, spatulation pressure was an uncontrolled variable in most experimental studies reported to date (Torabinejad *et al.* 1995b, Eidelman *et al.* 2001, Aminoshariae *et al.* 2003, Dammaschke *et al.* 2005, Walker *et al.* 2006, Yeung *et al.* 2006). Because of this lack of standardisation and use of uncontrolled hand placement methods, the results obtained in these studies may be inconsistent. Conversely, temperature, gradual incorporation of water, methods of drying, humidity, water to powder ratio, size of samples, time, humidity and other environmental conditions have been considered (Camilleri *et al.* 2005a, Dammaschke *et al.* 2005, Danesh *et al.* 2006, Walker *et al.* 2006).

In the present study to eliminate the effect of time taken during mixing as a confounding variable, an application time of 1 min was adopted based on preliminary studies. In order to apply even and equally distributed pressure on the specimens, a custom device was designed and constructed (Figure 3, page 70) so that the diameter of the piston matched the internal diameter of cylindrical polycarbonate moulds. In this way the entire MTA surface was under pressure and upward seeping of material prevented.

Based on the findings of Section 4.3.3 (page 73), when greater pressures were applied to MTA its surface hardness reduced significantly (Figure 6, page 74). In addition, maximum compressive strength occurred with minimum pressure (Figure 5, page 73). Therefore, in laboratory studies standardisation of mixing methodology is essential; saturation of MTA

powder with predetermined amounts of water followed by application of minimal pressure is likely result in a relatively strong material.

In a laboratory study on ProRoot[?] MTA (Dentsply Tulsa Dental) and two Portland cements (Danesh *et al.* 2006) an ultrasonic vibration intensity of 6000 min^{-1} was used, to avoid air entrapment and to control confounding variables when samples were prepared for measurement of Vickers microhardness. But in the same study samples that were prepared for radiopacity and solubility were neither vibrated nor condensed with a controlled pressure even though the molecular distance might affect solubility and radiopacity. In an attempt to improve the placement and seal of MTA in immature root canals, Lawley *et al.* (2004) used ultrasonics. They compared this method with conventional hand compaction, but failed to control the mixing methodology and the pressure applied. Clearly, use of standard mixing methodology and controlled pressure for all future studies is suggested.

In the present study it was anticipated that application of a greater pressure would result in a harder material, although the result showed that when the amount of pressure was more than 3.22 MPa surface hardness was reduced (Figure 5, page 73). This may occur because of insufficient intermolecular space for the ingress of water to hydrate the material adequately. In addition, SEM images demonstrated that application of higher pressures were associated with fewer voids that could result in a less than optimal volume of intermolecular space with a negative effect on the hydration process. Thus, applying a greater pressure in an attempt to achieve a harder material appears futile. The results also revealed that application of various amounts of pressure following the saturation of the MTA powder with predetermined amount of water during the preparation of samples did not have a significant impact on compressive strength. Compressive strength is the capacity of a material to withstand axially directed pressure generating compressive stress as a result of compression force. It could be

hypothesized that with the application of higher pressures, the formation of microchannels was limited as a result of the material being more compact. This may also reduce the amount and the rate of water diffusion through the material that is likely to impair the setting reaction and result in reduced compressive strength and surface hardness. The role of water molecules during the setting reaction is crucial. It does not only mix with powder, but it also chemically binds with various phases of the cement and has a direct effect on the setting process (Camilleri *et al*. 2005a, Santos *et al.* 2005, Walker *et al.* 2006). In other words, MTA hardens and gains strength as it hydrates; this process occurs rapidly at first and then slows down with time. When MTA powder is mixed with water, a special network of microchannels is created (Figure 7 & Figure 8 page 75 & 75). The continuity of microchannels is disrupted during the setting process (Fridland & Rosado 2005). Therefore, the hardened cement has pores and broken microchannels. The role of microchannels and pores during the hydration reaction is important; they provide pathways for the water to diffuse into the material and thus take part in the slow hydration process of the cement, when water becomes bound into the structure (Fridland & Rosado 2003, Fridland & Rosado 2005). In this study, specimens mixed with lower pressures had more typical crystalline structures around microchannels (Figure 8, page 75). This might be related to better water diffusion and therefore a greater degree of hydration leading to well developed crystalline structures in the form of laminated cross-stratified and bundles of jagged needle-like formations (Figure 9, page 76). In other words, application of a higher pressure may compress the powder molecules closer together to produce a drop in surface hardness and a reduction in crystalline formation due to lack of sufficient space for water molecules. Future analytical studies are suggested to correlate crystalline morphology and phase composition of the hydrated cement. Thus, in summary, according to the result of

this preliminary study samples of MTA should be prepared with low and controlled (consistent) pressure.

To achieve optimum properties, the hydraulic cement particles should be thoroughly mixed with water. The method chosen for mixing a material is fundamental to produce effective contact between powder particles and liquid and a set material with optimum physical, chemical and biological properties (Nomoto & McCabe 2001). The hydration process is a complex phenomenon that if modified might influence the biological, chemical and physical properties of the resulting product (Camilleri 2007).

To determine the most consistent mixing and placement technique for MTA specimen preparation; the effect of two different mixing methods followed by the application of ultrasonic agitation during the placement of MTA slurry on certain physical properties of MTA including surface microhardness, compressive strength and setting time were evaluated (section 4.4, page 79). The employed mixing methodologies were the novel technique of mechanically mixing encapsulated MTA and the saturation of MTA powder with predetermined amount of water followed by application of 3.22 MPa pressure. This amount of pressure was demonstrated as the optimum pressure that resulted in the most favorable physical properties of the resultant hard MTA material (section 4.3.3, page 73). In terms of the effect of the various mixing and placement techniques used (Figure 17, page 82) on the physical properties of MTA; the results of section 4.4.3.1 (page 84) demonstrated that the highest mean compressive strength value was observed in the specimens of group 4 (tooth coloured MTA) that were mixed mechanically using encapsulated MTA followed by the application of ultrasonic agitation (=101.70 MPa); the lowest mean compressive strength value was observed in specimens of group 1 (grey MTA) prepared by saturation and pressure (=65.195 MPa) ($p < 0.0001$). The results of Section 4.4.3.2 (page 85) revealed that for tooth

coloured ProRoot? MTA (Dentsply Tulsa Dental), the application of ultrasonic energy resulted in significantly higher surface microhardness values in all experimental groups ($p < 0.0001$). The same effect of ultrasonic agitation was observed for grey ProRoot? MTA at 4 days. The results of Section 4.4.3.3.2 (page 93) illustrated that the use of ultrasonic agitation following mixing by saturation and pressure led to a significantly more rapid final setting time ($P<0.00001$) for both types of ProRoot? MTA (Dentsply Tulsa Dental). Ultrasonic application following encapsulated mixing did not produce any significant change in the final setting time of both tooth coloured and grey ProRoot? MTA (Dentsply Tulsa Dental). These findings can be explained by the increased interaction of water and powder particles of MTA that were produced by the application of ultrasonic agitation. To achieve optimum properties, the hydraulic cement particles should be thoroughly mixed with water (Tymkowicz & Steffes 1997). The method chosen for mixing and treatment of cement is fundamental to produce effective contact between powder particles and liquid and a set material with optimum physical and chemical properties (Nomoto & McCabe 2001, Ilie & Hickel 2007). Aminoshariae *et al.* (2003) compared the effect of ultrasonic and hand compaction on the adaptation of MTA to experimental polypropylene containers as well as the occurrence of voids within the material. They concluded that ultrasonic techniques resulted in more voids than hand compaction. The presence of voids might not be a disadvantage for the MTA hydration process as they might provide pathways for the water to diffuse into the material (Fridland & Rosado 2003, Fridland & Rosado 2005). The results of Section 4.3.3.3 (page 74) illustrated that more typical crystalline structures were observed in specimens prepared with lower pressures that tended to appear around microchannels (Figure 8, page 75). However, higher pressures resulted in fewer voids and reduced compressive strength and surface

hardness values. These findings confirm the concept that more porosity may result in a better hydration and improved physical properties.

The results of Section 4.4.3 (page 84) demonstrated that the application of ultrasonic agitation produced better physical properties for MTA. This could also be explained by the dispersion effect of the ultrasonic energy on the material particles that might provide sufficient space for water molecules and better water diffusion producing a greater degree of hydration and subsequently a higher compressive strength, enhanced surface microhardness and shorter setting time. Total reactive surface area and particle interaction are increased by ultrasonic energy and might decrease setting time (Kleverlaan *et al.* 2004, Algera *et al.* 2005). In addition, ultrasonic vibration, by changing the rheological properties of a material, might also improve their handling characteristics (Witherspoon & Ham 2001, Lawley *et al.* 2004, Schmidlin *et al.* 2005). The same phenomenon may occur as a result of encapsulated mixing of MTA powder and water to enhance the hydration process and ease of handling. In terms of consistency, the materials mechanically mixed using the encapsulated mixing methodology were subjectively found to be consistently creamier and of a less grainy quality that made handling more controllable. To quantify these handling characteristics, further rheological investigations are recommended.

Yeung *et al*. (2006) in their *ex-vivo* study compared the fill density of MTA in simulated straight and curved canals using hand compaction and indirect ultrasonic vibration. They reported a heavier and denser filling in the latter group, suggesting the beneficial effects of ultrasonic vibration on MTA that is in accordance with the results of Section 4.4.3 (page 84) of the present study. In addition, the advantages of the application of ultrasonic vibration were reported by Lawley *et al.* (2004). They evaluated the effect of ultrasonic energy on MTA in relation to bacterial penetration in an apexification model and found that it improved

the seal after 45 days significantly. In the studies of Aminoshariae *et al.* (2003) and Yeung *et al.* (2006), the amount of pressure applied during the compaction of the material was an uncontrolled variable. The results of Section 4.3.3 (page 73) demonstrated that application of higher pressures during mixing of MTA produced lower surface microhardness values, suggesting that application of the higher pressures affects the hydration process, strength and surface microhardness of the material. Optimum physical properties were reported at a pressure of 3.22 MPa (Section 4.3.3, page 73), which was the selected pressure used in the present study for the saturation and pressure technique.

4.7. **Conclusion**

In an attempt to eliminate confounding variables and achieve standard MTA specimens during the main experimental studies a consistent powder to water ratio of 3.00 and a coherent mixing and placement methodology should be employed. In terms of the consistency and control of the variables evaluated there was no significant advantage for any particular mixing and placement methodology. When saturation and pressure was used the best physical properties were observed at 3.22 MPa. Therefore, this amount of pressure was employed in the main studies when the manual technique for mixing was used. In addition, application of ultrasonic energy following either the manual mixing (saturation and pressure) and/or mechanical mixing (encapsulated MTA) resulted in the optimum physical and chemical properties of MTA specimens. Therefore, in section 5.1.6 (page 155) of the main study (contamination with blood), ultrasonic agitation was used for the standard placement of MTA slurries. However, since during the course of the acid contamination study (section 5.1 page 121) parallel work on the mixing of MTA was ongoing, application of ultrasonic agitation was not used.

Chapter 5

EFFECT OF CONTAMINATION ON SELECTED PROPERTIES OF MTA

5. Effect of contamination on selected properties of MTA

In this chapter the effects of contamination with two types of acid and whole, fresh human blood "(WFH) blood" on selected physical and chemical properties of MTA were evaluated. Ethical approval was granted by a panel from the School of Dentistry, Cardiff University Ethical Committee and the ethical board of the local research review committee in the Faculty of Dentistry, Tehran University of Medical Sciences, Iran.

5.1. **Acid contamination**

5.1.1. **Introduction**

MTA appears to be a most promising material for use in a variety of complicated clinical applications, in which it is placed adjacent to the connective tissues in order to conduct hard tissue formation and/or act as a barrier against bacterial microleakage (section 2.2, page 21). In the most of these applications, MTA could be placed in an environment where inflammation is present and where a low pH is possible (Eidelman *et al.* 2001). Thus, MTA could be affected by the presence of tissue fluid (Torabinejad *et al.* 1995b, Camilleri *et al.* 2005b) that has been shown, in an infected area, to be acidic (Malmed 2004, Nekoofar *et al.* 2009). Therefore, it is hypothesized that variations in the pH value of host tissues because of pre-existing pathosis at the time of MTA placement could change its physical and chemical properties. For example, an acid pH in the environment may impede MTA setting (Walker *et al.* 2006), and reduce its strength and hardness (Yeung *et al.* 2006). Lee *et al.* (2004) immersed MTA specimens in various pH solutions and stored them for 7 days and reported the mean Knoop microhardness values of MTA specimens. They indicated that specimens stored at pH 5 were weaker than those stored at higher pH. However, although in clinical

situations MTA might be exposed to an acidic environment, extended immersion in acid does not simulate clinical conditions. Thus, to investigate further the response of MTA to acid under more relevant conditions, a study was designed to evaluate the push-out force between MTA and intraradicular dentine, as well as the compressive strength and surface microhardness of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) following exposure to a range of acidic environments during hydration. Furthermore, the morphological microstructural features of samples were studied by SEM.

The exposure of MTA to acidic pH does not only occur in altered biological conditions but also during restorative procedures. However, there is limited information about the effect of various restorative treatments on the physical properties of MTA. In particular, there is no information about the effect of tooth conditioning processes, including exposure to phosphoric acid, on the physical properties and crystalline structure of MTA, which are reflections of the hydration process (Taylor 1997, Wei *et al.* 2003). The effect of phosphoric acid, which is used to increase the retention and sealability of composite resin restorations (Gorucu *et al.* 2011), on the properties of pulp capping and root repair materials is a fundamental problem and one that could affect their durability and effectiveness. Fuss *et al.* (1990) evaluated the effect of the acid-etch technique on glass ionomer cements when used as a lining material and reported that a 15s etch had no detrimental effect. In a similar study, the surface structure of glass ionomer was evaluated under scanning electron microscopy (SEM) and no significant loss of cement was reported after acid-etching procedures (Smith & Martin 1990). However, no information is available on MTA. The purpose of this part of study was to evaluate the influence of acid-etch procedures 4, 24 or 96 h after mixing on surface microhardness, compressive strength and the surface crystalline structure of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental).

5.1.2. **Aim**

To evaluate the effect of two types of acid, butyric acid (to simulate exposure to inflammation) and phosphoric acid gel (to simulate etching prior to placement of resin restorations), on certain properties of MTA including compressive strength, surface microhardness, push-out force and microstructure.

5.1.3. **Materials & Methods**

5.1.3.1.**Materials**

The material investigated was the tooth coloured formula of ProRoot? MTA (Dentsply Tulsa Dental) with LOT number of 083006.

Two types of acid were used; butyric acid (Sigma-Aldrich, Gillingham, UK) and phosphoric acid 37% (3M ESPE Co., St Paul, MN, USA). The butyric acid was buffered at either pH 4.4, 5.4, 6.4 or 7.4 respectively, using sodium bicarbonate (Sigma-Aldrich).

5.1.3.2.**Methods**

5.1.3.2.1. Compressive strength (effect of butyric acid)

Forty customised polycarbonate cylindrical moulds having an internal diameter of 4 mm and height of 6 mm were filled with a slurry of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental). Mixing and placement of the MTA slurry was standardised by mixing 1 g of MTA powder with 0.33 g of distilled water under a standard pressure of 3.22 MPa as described in section 4.3 (page 69). The filled moulds were then randomly allocated to four groups each of ten specimens and placed within glass vials. The bottom of each vial contained a piece of gauze that had been soaked in butyric acid buffered at either pH 4.4, 5.4, 6.4 or 7.4, respectively. The latter group acted as the control group. Based on pilot experimentation, the acid-soaked pieces of gauze were replaced with fresh acid-soaked gauze every 24 h to ensure a consistent pH during the experimental period. The openings of the glass vials were then

covered by moist gauze and sealed to ensure the presence of sufficient humidity inside the vials. After 4 days, the MTA specimens were removed from the moulds and subjected to the compressive strength test using the methodology described in section 4.3.2.1 (page 69). The compressive strength of all specimens was recorded in MPa. The data obtained from the compressive strength tests were subjected to statistical analysis using one-way ANOVA for overall comparison and by Tukey's post hoc test for individual comparisons.

5.1.3.2.2. Compressive strength (effect of phosphoric acid gel)

Ninety customised stainless steel cylindrical moulds having an internal diameter of 4 mm and height of 6 mm were filled incrementally with tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) slurry using the methodology described in section 4.3.2 (page 69) and incubated at 37^{o}C in a fully saturated humidity. After 4 h, 30 samples were randomly selected and divided into two groups of 15. In the first group, the surface of one end of each specimen was exposed to 37% phosphoric acid (3M ESPE Co., St Paul, MN, USA) for 15 s then rinsed using tap water for 15 s and dried gently using a stream of air for 15 s. The second group of 15 specimens was used as the control group and was not exposed to acid. This procedure was repeated 24 and 96 h after mixing on a further 30 samples at each time period respectively. The compressive strength test was conducted on each specimen just after acid exposure and on the corresponding control specimens using a universal testing machine (Instron, 3345, Norwood, MA, USA) as described in section 4.3.2.1 (page 69). The compressive strength of all specimens was recorded in mega Pascal (MPa). The data obtained for compressive strength of all six groups were found to be non-parametrically distributed. Therefore, differences between groups were analysed using the Kruskall–Wallis test.

5.1.3.2.3. Surface microhardness (effect of butyric acid)

Forty customised polycarbonate cylindrical moulds having an internal diameter of 6 mm and height of 12 mm were filled with tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) slurry. Mixing and placement of MTA slurry was standardised by mixing 1 g of MTA powder with 0.33 g of distilled water under a standard pressure of 3.22 MPa as described in section 4.3.2 (page 69). The filled moulds were then randomly allocated to four groups each of ten specimens and placed within glass vials. The bottom of each vial contained a piece of gauze that had been soaked in butyric acid buffered at either pH 4.4, 5.4, 6.4 or 7.4, respectively. The latter group acted as the control group. Based on pilot experimentation, the acid-soaked pieces of gauze were replaced with fresh acid-soaked gauze every 24 h to ensure a consistent pH during the experimental period. The openings of the glass vials were then covered by moist gauze and sealed to ensure the presence of sufficient humidity inside the vials. After 4 days, the MTA specimens were removed from the moulds and the surfaces of each specimen, which were exposed to acid, were subjected to the Vickers surface microhardness test as described in section 4.3.2.2 (page 71). The mean Vickers surface microhardness value, standard deviations and standard errors were calculated for each group and subjected to a one way analysis of variance and post hoc Tukey's test.

5.1.3.2.4. Surface microhardness (effect of phosphoric acid gel)

Forty five customised polycarbonate cylindrical moulds having an internal diameter of 6 mm and height of 12 mm were filled with tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) slurry as described in section 4.3.2 (page 69) and incubated at 37^{o}C and fully saturated humidity. After 4 h, fifteen samples were randomly selected and the surface of one end of each sample was covered with 37% phosphoric acid (3M ESPE) for 15 s then rinsed using tap water for 15 s and dried gently using a stream of air for 15 s. The opposite surface of each sample was used as the control and was not subjected to acid exposure. Both surfaces of the

specimens were then subjected to the Vickers surface microhardness test using a Mitutoyo microhardness tester MVK G_1 (Mitutoyo Corp., Tokyo, Japan) as described in section 4.3.2.2 (page 71) and the mean Vickers surface microhardness values calculated. The same acid exposure procedures and surface microhardness tests were repeated on specimens 24 and 96 h following mixing on a further 15 samples at each time interval. Differences between the Vickers surface microhardness values obtained from the surfaces that were exposed to the acid-etch procedure at different time periods and control surfaces was compared statistically using one-way ANOVA for overall comparison followed by a post hoc Tukey's test for individual comparisons.

5.1.3.2.5. Push-out force (effect of butyric acid)

Freshly extracted human teeth including mandibular single-rooted premolars or maxillary anterior incisors that were either intact or contained only small carious lesions were selected and stored in 0.5% chloramine-T at 4 ^{o}C for up to 1 month before use. Mid-root dentine was sectioned horizontally into slices with a thickness of 1.0 mm. A diamond saw microtome 6 P IFURWP H/ HFD1 X?0RFK* HLP DQ\ ZDXVHGWRREWDQURRWGHQWLQ e slices. The lumen of the root dentine disks were instrumented with Gates Glidden burs (Dentsply Tulsa Dental), sizes 2 to 5, to achieve a standardised diameter of 1.3 mm. Eighty standard root dentine slices were then filled with tooth coloured ProRoot? MTA slurry (Dentsply Tulsa Dental) using the methodology described in section 4.4.2 (page 79). Mixing and placement of the MTA slurries were standardised by mixing 1 g of MTA powder with 0.33 g of distilled water (saturation method) followed by the application of the ultrasonic energy using a BUC-1 tip (Obtura Spartan) attached to a 6XSUDWRQ? 3 %RRWWLQ6DWDF The specimens were then divided randomly into four groups (n = 20). In group A, the specimens were wrapped in pieces of gauze soaked in phosphate buffered saline solution (pH = 7.4). In

groups B, C, and D, specimens were wrapped in pieces of gauze soaked in butyric acid buffered at pH values of 6.4, 5.4, or 4.4, respectively. Following 4 days of incubation at 37^{o}C in fully saturated humidity all test and control specimens were subjected to the push-out force test. The push-out force were measured using a universal testing machine (Z050; Zwick/Roell Group, Ulm, Germany). The samples were placed on a metal slab with a central hole to allow the free motion of the plunger. The compressive load was applied by exerting a downward pressure on the surface of the MTA using a 1.00 mm diameter cylindrical stainless steel plunger at a speed of 1 mm/min (Figure 32, page 127). The plunger had a clearance of approximately 0.2 mm from the margin of the dentinal wall to insure contact with MTA only. The maximum load applied to MTA at the time of dislodgement was recorded in Newtons. In order to express the bond strength in MPa, the values were divided by the adhesion area of the root filling calculated by the following equation:

$$A=2\pi rhK$$

where A is the area of the root canal, r is the root canal radius and h is the thickness of the root-dentine slice in millimetres. The data were analysed using one-way analysis of variance followed by the Tamhane post hoc test.

Figure 32: A cylindrical stainless steel plunger attached to the load cell of the universal testing machine loading on MTA inside a root section.

The slices were then examined under a light microscope at X40 magnification to determine the nature of the bond failure. Each sample was categorised into one of three failure modes (Figure 33, page 128): adhesive failure at the MTA and dentine interface, cohesive failure within MTA, or mixed failure.

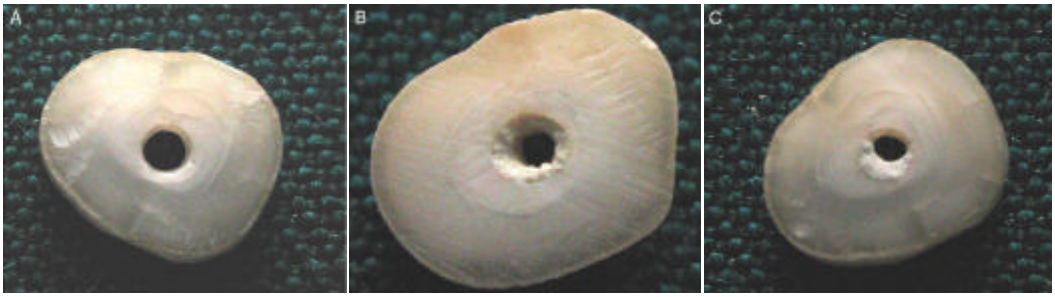


Figure 33: Various failure modes. (A) Adhesive failure; note the clean canal wall. (B) Cohesive failure within MTA. (C) Mixed failure; note the MTA residual inside the canal.

5.1.3.2.6. Push-out force (effect of phosphoric acid gel)

Forty standard root dentine slices with 1.00 mm thickness and 1.3 mm internal diameter were prepared and then filled with a slurry of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) as described previously (5.1.3.2.5, page 126). The assemblies were then incubated at 37^{o}C in a fully saturated humidity. After 24 h, 20 specimens were randomly selected and divided into two groups of 10. In the first group, one surface of each specimen was randomly selected and the dentine surface was subjected to the acid etch procedure using 37% phosphoric acid (3M ESPE) for 15 s. During the acid-etch procedure the margins of the MTA material were also deliberately exposed to acid gel, however, the whole surface of the MTA material did not cover. The dentine disk assembly, containing MTA material, was then rinsed using tab water for 15 s and dried gently using a stream of air for 15 s. The second group of 10 specimens was used as the control group and was not exposed to acid. The push-out force test was then conducted on each assembly just after acid etch procedure and on the control

specimens respectively using a universal testing machine (Z050) as described in section 5.1.3.2.5 (page 126). The same acid etch procedure, rinsing, drying and push-out force measurement, were repeated after 96 h incubation period on a further 20 samples.

The push-out force of each specimen was recorded in mega Pascal (MPa) and the data were then subjected to two way ANOVA analysis using SPSS to examine the difference between the mean push-out force values of each experimental group. The nature of the failure of each specimen was also examined under a light microscope at X40 magnification and the failure mode recorded accordingly.

5.1.3.2.7. Microstructure (effect of butyric acid)

For the microstructural morphological evaluations by SEM, eight specimens (two for each group) were prepared. Mixing and placement of MTA slurry was standardised by mixing 1 g of MTA powder with 0.33 g of distilled water under a standard pressure of 3.22 MPa as described in section 5.1.3.2.3 (page 124). To analyse the internal microstructure, the specimens were sectioned into two halves using a No. 15 disposable surgical scalpel blade to initiate a crack. The surfaces were sputter-coated with gold using a Polaron Sputter Coater (Quorum Technologies, Newhaven, UK) and specimens were analysed with an EBT1 (Electron Beam Technology) scanning electron microscope (S.E.M Tech Ltd, Woodbridge, UK). The micrograph images from the SEM analysis showing the qualitative internal microstructure of the set MTA were evaluated at the same depth within the specimens in terms of the presence of microchannels and type of crystal formation.

5.1.3.2.8. Microstructure (effect of phosphoric acid gel)

For the morphological evaluation, new specimens were prepared as described in section 5.1.3.2.1 (page 123) and stored under the same conditions. After 4, 24 and 96 h, samples were divided randomly into two groups of five. The surfaces of specimens in the test groups

were exposed to 37% phosphoric acid (3M ESPE Co.) for 15 s then rinsed using tap water for 15 s and then dried gently using a stream of air for 15 s. Specimens in the control group were not exposed to acid. The surfaces were sputter-coated with gold and analysed using an EBT1 Scanning Electron Microscope as described in section 5.1.3.2.7 (page 129). Gold sputter coating in the vacuum chamber was not possible on specimens removed after 4 h due to the amount of moisture in the samples; therefore, morphological evaluation by SEM after 4 h was impossible. The micrograph images from the SEM analysis of the two acid-exposed and control groups after 24 and 96 h were compared in terms of the surface morphology and type of crystal formation.

5.1.4. **Results**

5.1.4.1. **Effect of butyric acid on compressive strength**

The results of the compressive strength testing are shown in Table 10 (page 131). There was a significant difference between the mean compressive strength values of all experimental groups ($p<0.000001$) except between the specimens of the experimental groups that were exposed to pH 6.4 and 7.4. The lowest and the greatest mean compressive strength values were observed in the specimens exposed to pH 4.4 (29.66 ?) and 7.4 ( ) respectively.

pH	Mean	Standard Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
4.4	29.6680	6.52542	25.0000	34.3360	18.45	38.45
5.4	43.4780	6.12015	39.0999	47.8561	32.07	52.46
6.4	58.8265	7.37706	53.5493	64.1037	44.62	71.42
7.4	62.9940	10.15211	55.7316	70.2564	52.68	82.24

Table 10: Means, standard deviations, 95% confidence intervals, minimum and maximum values of the compressive strength (MPa) of experimental groups that were exposed to butyric acid with various pH values.

Mean compressive strength values of 43.47 ? DQG 58.82 ? ZHUHREVHUYHG following exposure to pH 6.4 and 5.4, respectively. In general, lower the compressive strength values of the material were associated with a more acidic environment (Figure 34, page 131).

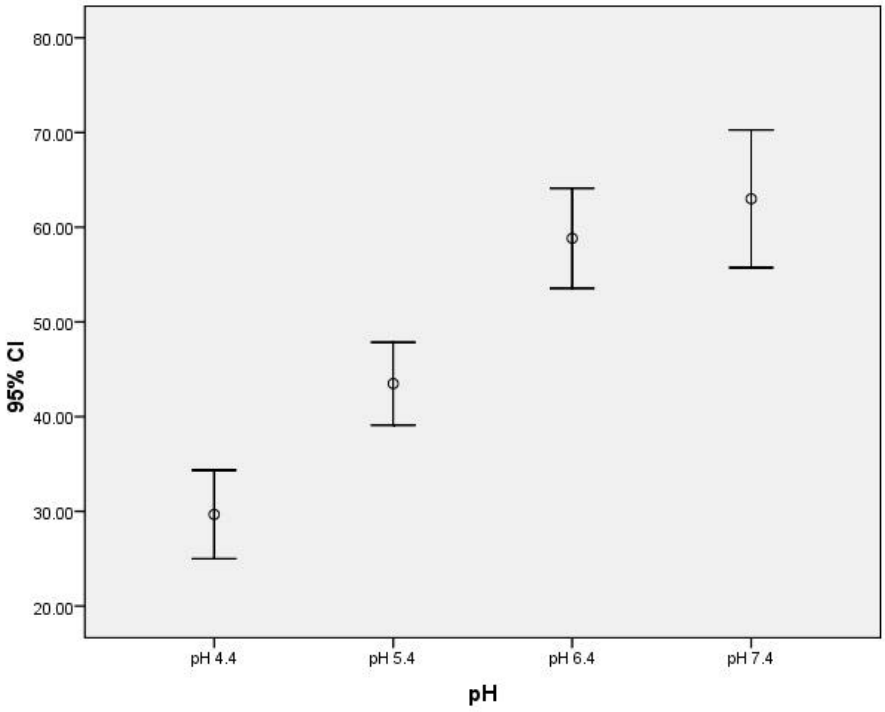


Figure 34: Error bar graph representing the mean compressive strength (MPa) values ? 95 % confidence interval of tooth coloured ProRoot? MTA exposed to butyric acid buffered at various pH values. Significantly lower compressive strength (MPa) values were associated with lower pH (p <0.00001).

5.1.4.2. **Effect of phosphoric acid on compressive strength**

The results of the compressive strength testing are shown in (Figure 35, page 132). The lowest compressive strength (mean=6.438 MPa, SD=2.360) was observed in specimens exposed to acid after 4 h. There was a statistically significant difference between compressive strength values of experimental specimens after 4 h compared with those groups exposed to acid for 24 and 96 h ($P < 0.0001$). However, there was no significant difference between specimens exposed to acid for 24 or 96 h respectively. Significant differences in compressive strength values between test and control groups occurred only after 4 h ($P < 0.0001$).

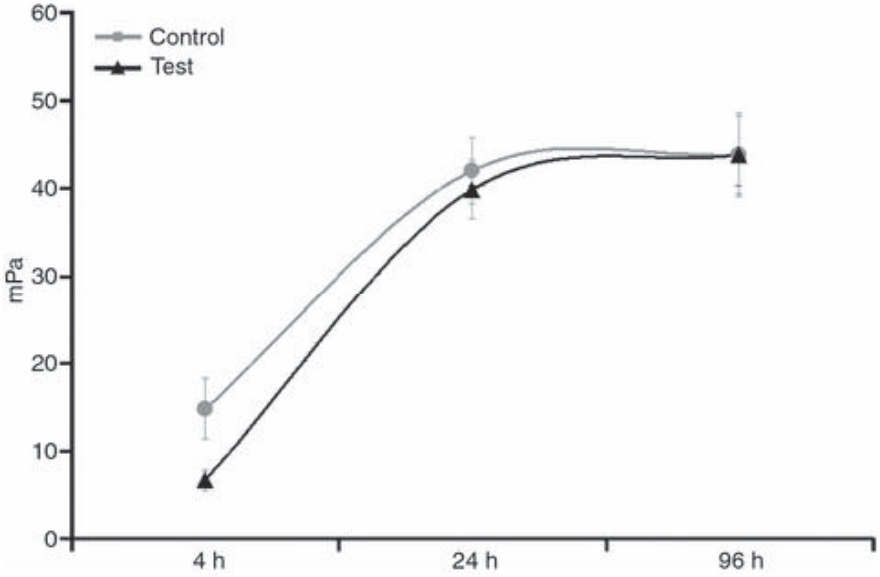


Figure 35: Mean compressive strength (MPa) of intact and acid-exposed specimens after 4, 24 and 96 h.

5.1.4.3. **Effect of butyric acid on surface microhardness**

The results of the microhardness testing are shown in (Figure 36, page 133). The greatest

P HDQVXUIDFHKDUGQHVVYDOXHV? ZHUHREVHUYHGIRUDZ LQ H¦ SRVXUHVRS+

ZLWKWHYDOXHVGHFUHDVLQ VW? IRUDZ LQ H¦ SRVXUHVRS+ 7KHGLIIHUHQFH

between these values at the 95% CI (33.39–44.30) was significant (P < 0.0001). Mean VXUIDFHP IFURKDUGQHVVYDOXHVRI ? DQG? ZHUHREVHUYHGIRORZLQJ

exposure to pH 6.4 and 5.4, respectively. Tukey's post hoc tests revealed that the difference between the values of specimens exposed to pH 6.4 and pH 5.4 at the 95% CI (-2.78 to 8.75) was not significant. However, the difference between the Vickers microhardness values of other groups was statistically significant (P < 0.001).

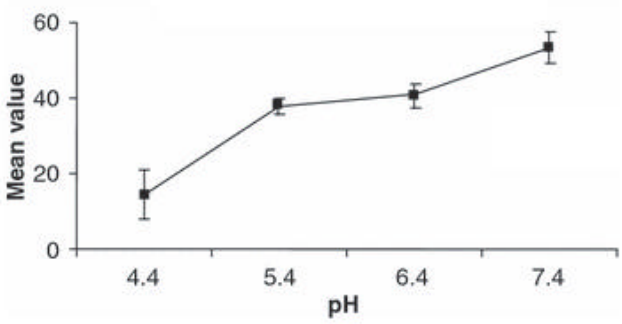


Figure 36: Effect of butyric acid on surface microhardness. The greatest mean surface microhardness 9 YDOXHV? DQGWKHORZHVWPHDQPLFURKDUGQHVV9 + YDOXHV? ZHUHREVHUYHGDIWHU exposure to pH 7.4 and pH 4.4, respectively (P < 0.0001).

5.1.4.4. **Effect of phosphoric acid on surface microhardness**

The results of the surface microhardness test are shown in (Figure 37, page 134). In the group exposed to acid, the lowest Vickers surface microhardness value (mean = 17.269, SD = 6.382) was observed after 4 h. There was a significant difference between specimens etched after 4, 24 and 96 h respectively (P < 0.0001). There was a statistically significant difference in Vickers surface microhardness values between test and control groups after 4, 24 and 96 h

respectively (P < 0.0001). The surface microhardness values of the test group were lower than the control group at all time intervals (Figure 37, page 134). In the control group, there was a significant difference in Vickers surface microhardness values between specimens after 4 h compared with the other control groups (P < 0.0001). However, there was no significant difference between control specimens after 24 and 96 h respectively.

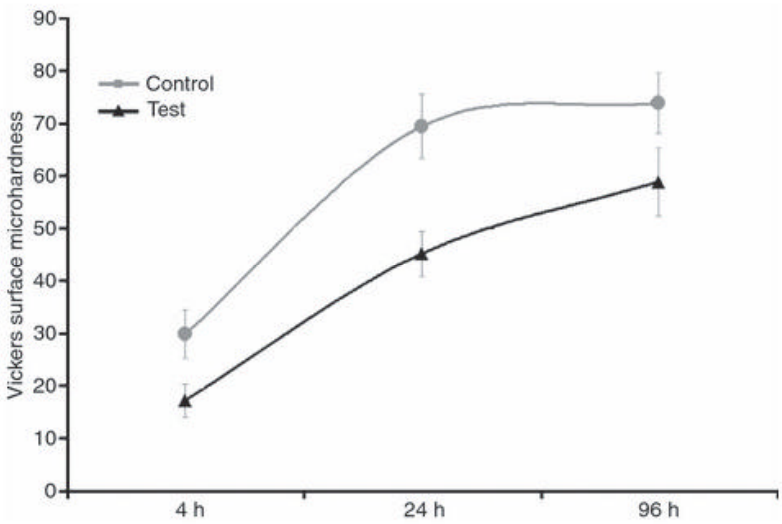


Figure 37: Mean Vickers surface microhardness (VH) of intact and phosphoric acid gel-exposed surfaces after 4, 24 and 96 h.

5.1.4.5. **Effect of butyric acid on push-out force**

The results are summarised in Figure 38 (page 135). The greatest mean push-out force (7.28 ? 0 3DZ DvREVHUYHGLWMDF[SRVXUHvRDS+ RI 7KHYDOXHvGHFUHDVHGvR?

MPa after exposure to a pH of 4.4. There were significant differences between the groups (p < 0.001). The Tamhane post hoc test revealed that the mean push-out force values of specimens exposed to pH 4.4 and 5.4 were significantly lower than the others (p<0.001). No significant difference was found between the values for specimens exposed to pH levels of

6.4 and 7.4. Inspection of the samples revealed the bond failure to be predominantly adhesive for all groups.

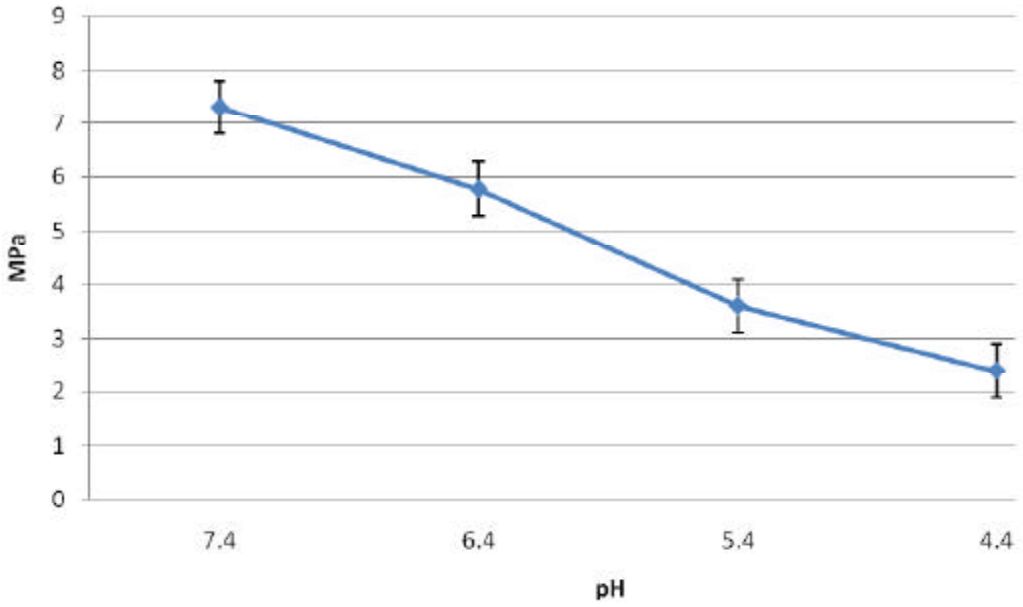


Figure 38: The effect of butyric acid at various pH values on the push-out force (MPa) between MTA and dentine.

5.1.4.6. Effect of phosphoric acid on push-out force

The results are summarised in Table 11 (page 135). Regardless of the exposure to the phosphoric acid gel, the mean push-out force values calculated following 96 h incubation were significantly greater than the push-out force values calculated at 24 h ($p < 0.0001$).

Incubation Time	Acid gel	Mean	95% Confidence Interval	
			Lower Bound	Upper Bound
24 hours	No etch	2.668	2.047	3.289
	Etch	1.444	.823	2.065
96 hours	No etch	7.082	6.461	7.703
	Etch	6.693	6.072	7.314

Table 11: Mean values and 95% confidence intervals of the push-out force (MPa) of tooth coloured ProRoot? MTA subsequent to acid etch with phosphoric acid performed following various incubation periods.

The acid etch procedure did not significantly affect the push-out force values of tooth coloured ProRoot? MTA measured following 24 h and/or 96 h incubation time. No significant difference was observed between the mean push-out force values of the etched and un-etched specimens at either incubation period.

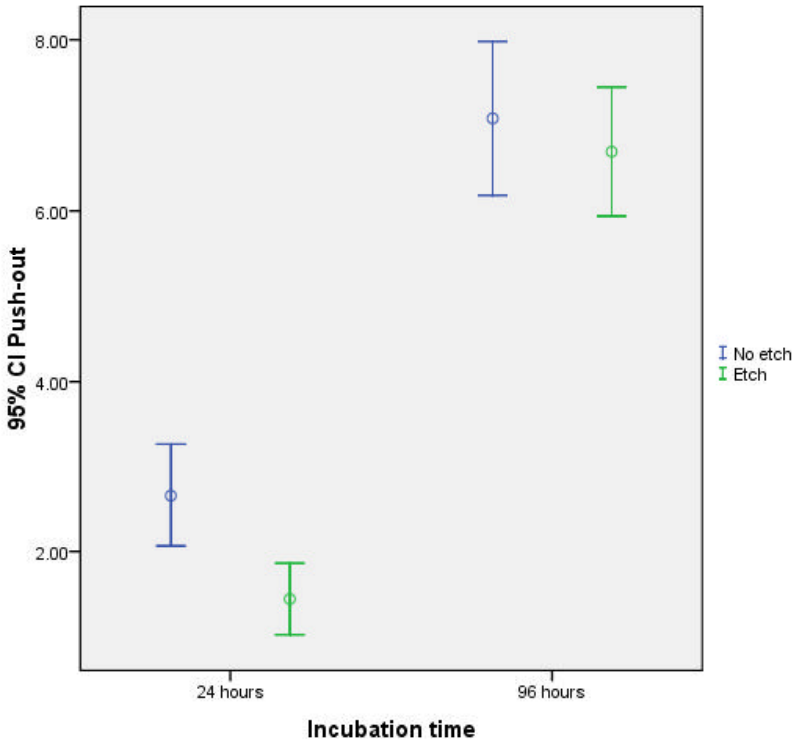


Figure 39: Error bar graph representing the mean push-out force (MPa) values of etched and un-etched tooth coloured ProRoot? MTA specimens at 24 h and 96 h with 95% confidence interval. No significant difference was observed between etched and control groups. Regardless of acid etching the push-out force (MPa) of specimens was significantly greater at 96 h ($p < 0.0001$).

In terms of failure mode, inspection of the samples revealed the bond failure to be predominantly adhesive for both etched and control groups at both incubation times.

5.1.4.7. Effect of butyric acid on surface microstructure

The internal microstructure of specimens exposed to pH 7.4 environments revealed distinctive structures such as asymmetrical crystalline formations in the form of laminated

cross-stratified structures (Figure 40, page 137), bundles of jagged needle like formations (Figure 40, page 137), microchannels (Figure 41, page 138), depressions caused by air bubbles (Figure 42, page 138) and pores. Development of these crystalline structures except the needle like crystals were observed in all specimens exposed to various acidic environments and in general it was not possible to score each characteristic and thus compare them quantitatively between groups. However, specimens kept in contact with butyric acid at pH 7.4 had distinctive needle like crystalline structures embedded within a more uniform matrix partially covered by colloidal gel that may have been involved in the bonding of the various phases of the cement (Figure 40, page 137). Specimens exposed to more acidic pH had extensive porosity (Figure 43, page 139). In addition, lack of needle like formations in specimens exposed to more acidic pH was noticeable (Figure 44, page 139).

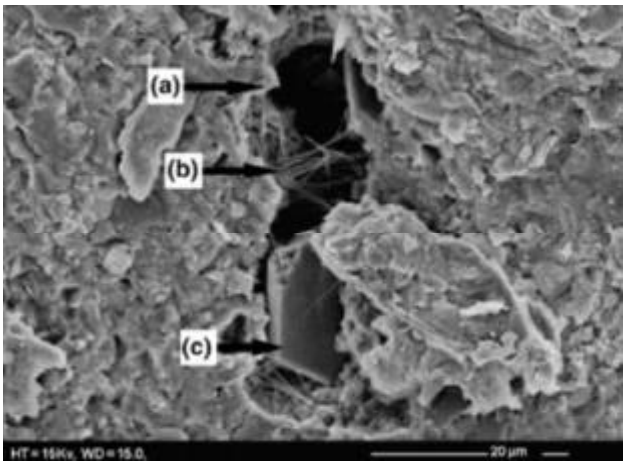


Figure 40: Scanning electron microscopy image of a specimen exposed to pH 7.4. A cross section of a microchannel (a), needle like (b) and laminated (c) crystalline formation can be seen.

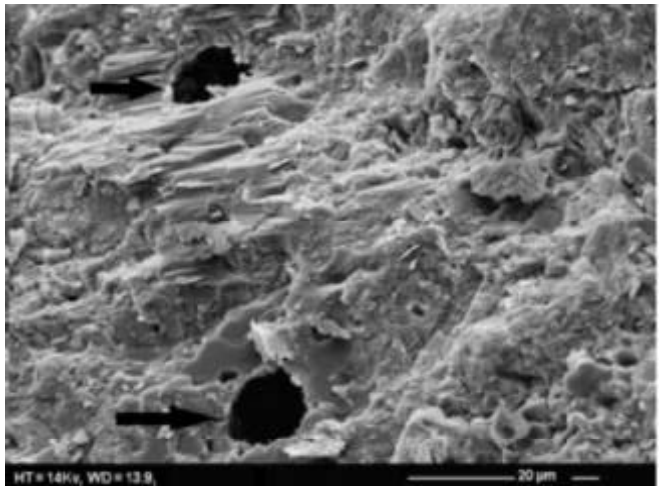


Figure 41: Scanning electron microscopy image of a specimen exposed to pH 7.4. Cross sections of two microchannels can be seen.

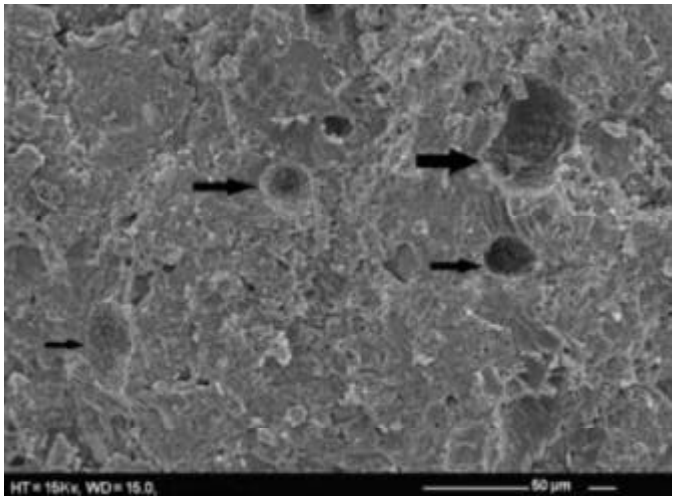


Figure 42: Scanning electron microscopy image of a specimen exposed to pH 6.4. Depressions caused by air bubbles can be seen.

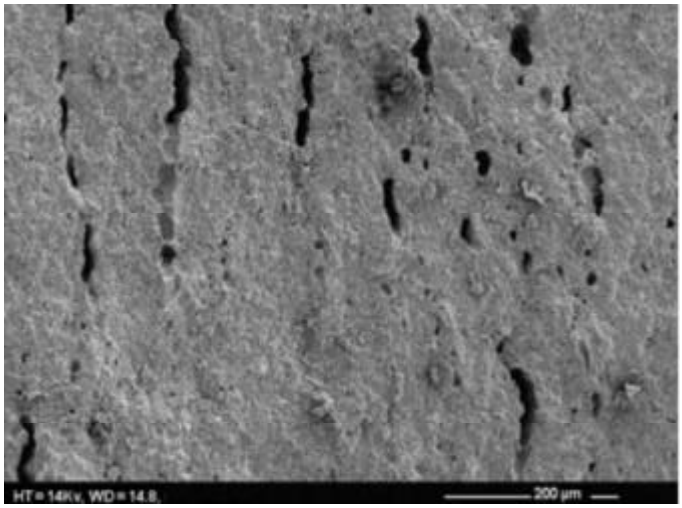


Figure 43: Scanning electron microscopy image of a specimen exposed to pH 4.4. Extensive porosity can be seen.

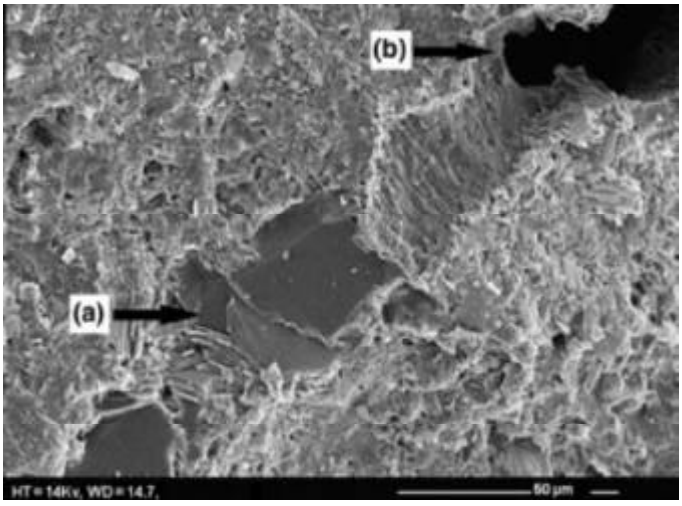


Figure 44: Scanning electron microscopy image of a specimen exposed to pH 5.4. Asymmetrical crystalline formations in the form of laminated cross-stratified structures (a) near the cross section of a microchannel can be seen (b). Lack of needle like formations is noticeable.

5.1.4.8. **Effect of phosphoric acid gel on surface microstructure**

The SEM examinations revealed distinct morphological differences between the intact (Figure 45, Figure 47 & Figure 48, pages 141, 142 & 142) and acid-exposed MTA surfaces

(Figure 49 to Figure 53, pages 143-145). No morphologically dissimilarity was observed within the test and control groups at 24 and 96 h after mixing (Figure 47 & Figure 48, pages 142, 142). The surface microstructure of the non acid-exposed MTA (control groups) after both time periods were similar and revealed an amorphous poorly crystallized superficial gel structure at x36 magnification. The presence of needle-like crystals was a common finding in the control groups at higher magnification after 24 and 96 h (Figure 47 & Figure 48, pages 142, 142). A plain poorly crystallized superficial gel structure containing globular aggregate particles was observed in the control group. After acid exposure at 24 or 96 h, a selective loss of matrix from around the crystalline structures were observed at various magnifications that produced a relatively uniform 'honeycomb' etched pattern without penetrating deeply or removing substantial amounts of the cement (Figure 49 to Figure 53, pages 143-145). No needle-like crystals were observed over the surfaces exposed to acid. Loss of the needle-like crystals was a significant morphological difference between acid gel exposed and intact (control) surfaces. In addition, acid gel exposure after 24 and 96 h created notable crystalline structures such as plate-shaped and laminated crystals on the MTA surface (Figure 53, page 145).

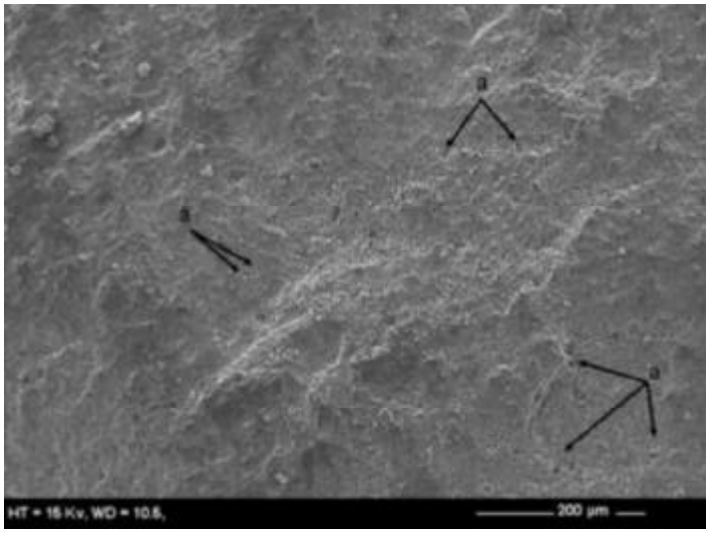


Figure 45: Intact surface of mineral trioxide aggregate in the control groups after 24 h. An amorphous poorly crystallized superficial gel structure and the cross-section of some microchannels (a) can be seen.

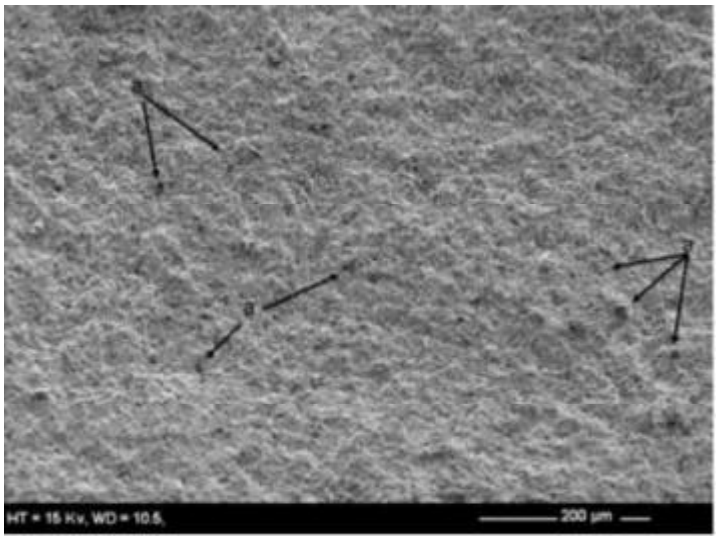


Figure 46: Surface of acid-exposed mineral trioxide aggregate after 96 h. An amorphous poorly crystallized superficial gel structure and the cross-section of several microchannels (a) can be seen.

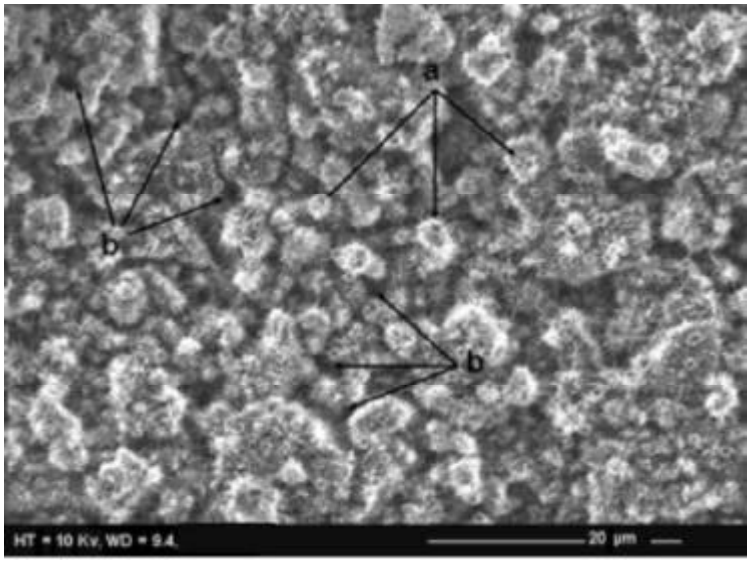


Figure 47: Intact surface of mineral trioxide aggregate from the control groups after 24 h. Irregular needle-like crystals that cover globular formations (a) and cross-sections of several microchannels (b) can be seen.

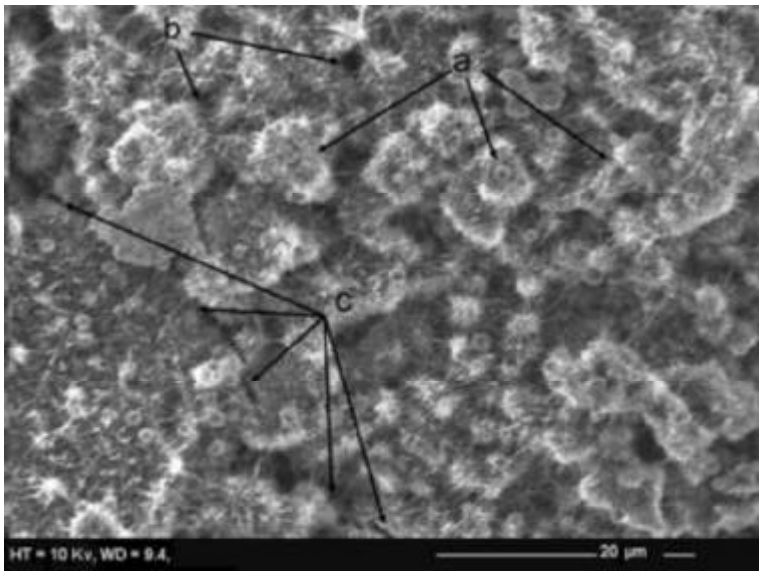


Figure 48: Intact surface of mineral trioxide aggregate from the control group after 96 h. Irregular needle-like crystals that cover globular formations (a), cross-sections of some microchannels (b) and a microchannel running transversely (c) can be seen.

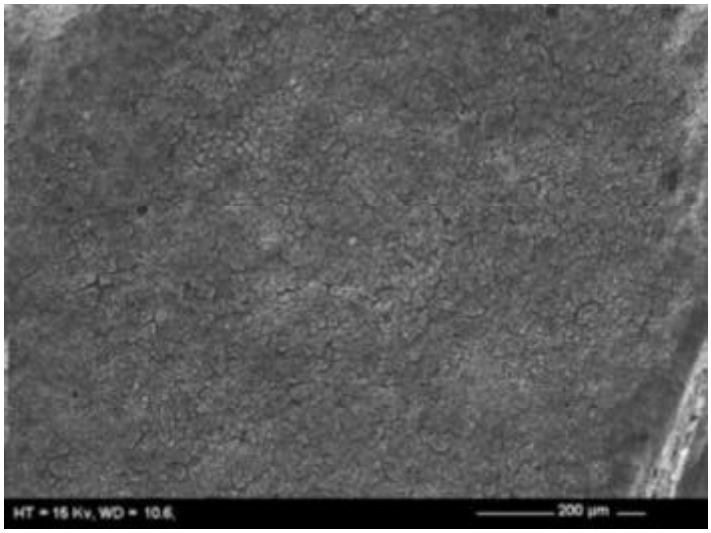


Figure 49: Acid exposed surface of mineral trioxide aggregate after 24 h. A relatively uniform 'honeycomb' etched pattern can be seen.

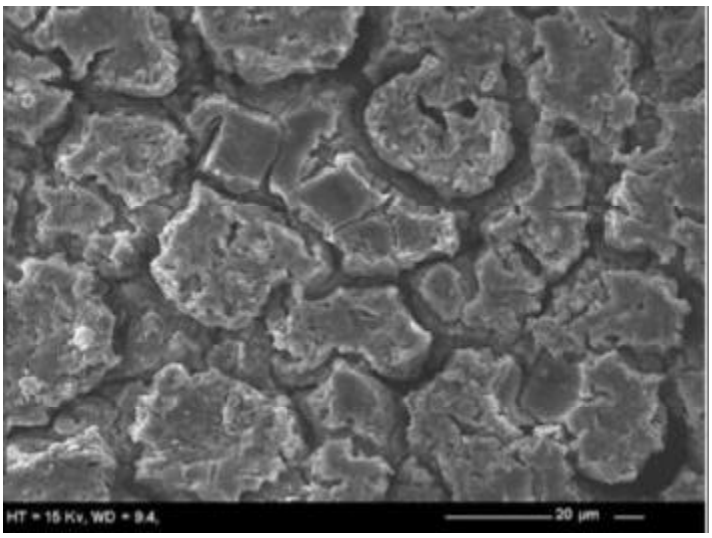


Figure 50: Acid exposed surface of mineral trioxide aggregate after 24 h. Selective loss of matrix from around the crystalline structures and relatively uniform 'honeycomb' etched pattern with selective loss of the cement can be seen. No needle-like crystals were observed.

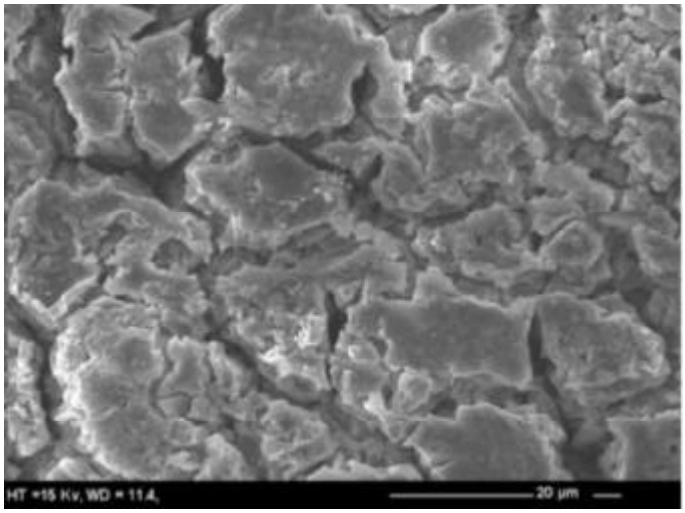


Figure 51: Acid exposed surface of mineral trioxide aggregate after 96 h. Selective loss of matrix from around the crystalline structures and relatively uniform 'honeycomb' etched pattern with selective loss of the cement can be seen. No needle-like crystals were observed.

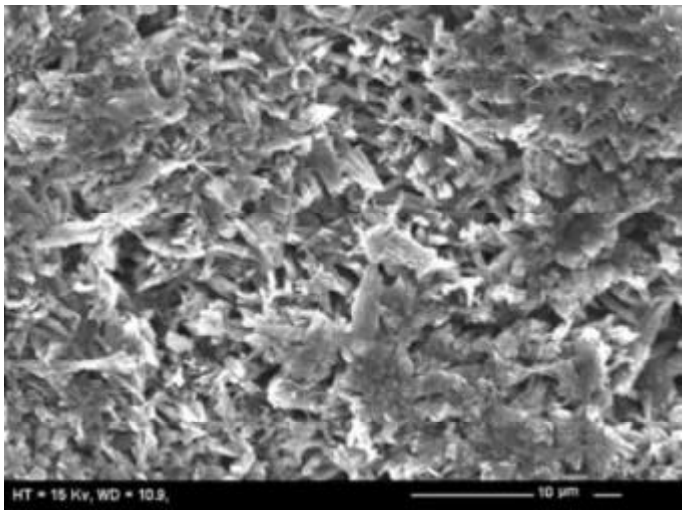


Figure 52: Acid exposed surface of mineral trioxide aggregate (MTA) after 96 h. Selective elimination of matrix can be seen. Laminated and plate-shaped crystals are notable and visible on the MTA surface. No needle-like crystals were observed.

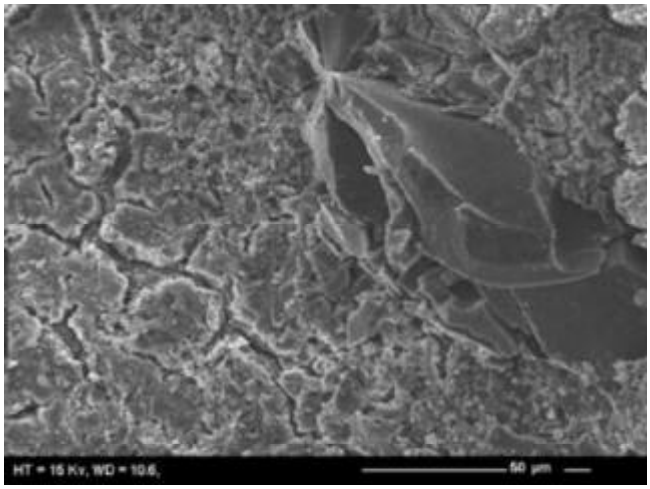


Figure 53: Acid exposed surface of mineral trioxide aggregate (MTA) after 96 h. Selective elimination of matrix with minimal loss of the cement can be seen. Laminated and plate-shaped crystals were a notable feature on the MTA surface. No needle-like crystals were observed.

5.1.5. **Discussion**

Mineral trioxide aggregate has been shown to release soluble fractions (mainly calcium hydroxide) in both the short and long-term (Lee *et al.* 2004, Camilleri 2007) sufficient to maintain the pH of the surrounding environment at a high level (pH 11–12) (Saghiri *et al.* 2009). Duarte *et al.* (2003) confirmed that MTA released calcium ions as a result of hydration of calcium oxide, the main component of MTA and Portland cement. Torabinejad *et al.* (1995b) reported the pH value of MTA to be between 10.5 and 12.9. The biological properties of MTA, e.g. the ability to induce changes in cellular activity of osteoblasts, have been attributed to its alkalinity (Fridland & Rosado 2003).

Santos *et al.* (2005) noted that the pH of MTA increased to a peak of 10.39 within the first 24 h after mixing followed by a decrease to 7.72 within 360 h. However, since different methodologies were used for pH measurement in these studies it is not possible to compare their results directly.

It is recommended that MTA be allowed to set untouched for 72 h or longer to decrease the chance of MTA displacement (Sluyk *et al.* 1998). In the present study, the samples were kept in a humid environment for 96 h to allow optimum setting.

Within the human body under normal physiologic conditions, any minor change in pH is controlled by the carbonic acid-bicarbonate buffer system and the other pH regulatory systems active in connective tissue (Wray 1988); periodontal tissue is no exception (Azuma 2006). However, in certain clinical applications, MTA is placed in an environment where inflammation may be present and the surface of the unset material will be exposed to a low pH environment (Nekoofar *et al.* 2009), e.g. when used as a root-end filling material, as an apical barrier in teeth with open apices or for repair of root perforations (Torabinejad & Chivian 1999). Placement of MTA in an inflamed low pH environment may influence its physical and chemical properties. Lee *et al.* (2004) studied the effect of pH on the hydration process of MTA. They immersed and stored MTA samples in solutions of pH 5, 7 and 7.4 for 7 days and reported that microhardness at low pH was reduced. However, immersion of the material in acid does not simulate clinical conditions as most often only one surface of the MTA will be exposed to an acidic environment. Therefore, to simulate clinical condition in a more relevant way, in the present study only one surface of the specimens were exposed to acid. Furthermore, in situations where the initiating and perpetuating factors of an inflammatory process are removed by appropriate treatment, it is possible that the pH of the environment returns to normal in a shorter time period than the 7 days used by Lee *et al.* (2004). In the present study a shorter incubation period of four days was used. Various types of acid have dissimilar effects on the physical and chemical characteristics of Portland cement (Taylor 1997) and might also have different effects on MTA. The type of acid used by Lee *et al.* (2004) was not stated. In the present study butyric acid, a by-product of

anaerobic bacterial metabolism (Zeikus 1980, Barker 1981, Tonetti *et al.* 1991, Tsuda *et al.* 2010) was used to simulate the clinical conditions within periradicular infections.

Lota *et al.* (2000) demonstrated that considerable changes in the microstructure of hydrated cements occurred in the presence of polyacrylic acid when compared with a control paste. Rai *et al.* (2004) reported that hydration of Portland cement was considerably retarded when malic acid was added. In the presence of tartaric acid, the silicate hydration-phase of Portland cement was retarded strongly (Rai *et al.* 2006). In contrast, Singh *et al*. (1986a) revealed that lactic acid accelerated the hydration of Portland cement by increasing the crystalline character of calcium hydroxide resulting in advanced growth of the hydration products. Different concentrations of citric acid have been shown to have dissimilar effects on Portland cement (Singh *et al.* 1986b). Singh *et al*. (1986b) indicated that 0.1% citric acid accelerated the hydration process of Portland cement whereas concentrations >0.1% retarded hydration.

The compressive strength and microhardness of a material are not the measures of two single properties. They are influenced substantially by other fundamental properties of the material such as yield strength, tensile strength, modulus of elasticity (Bentz 1997) and crystal structure stability (Gilman 1997) .Thus, they can be used as indicators of the setting process and the overall strength or resistance to deformation when compared with baseline information. They can also indicate the effect of various setting conditions on the overall strength of a material (Blake 1985).

There are two universal types of microhardness test, Vickers and Knoop. The main difference is attributed to the shape of the diamond indenter. The shape of the Vickers diamond indenter is a square pyramid whereas the shape of the Knoop diamond indenter is an elongated pyramid shape. Gong *et al.* (2002), when measuring silicon nitride ceramic samples, showed that Knoop hardness values were generally lower than the corresponding values for Vickers

hardness. However, there is a strong correlation between these two values that may be related to elastic recovery occurring at the indentation. Measurement of the Vickers microhardness formed the basis of some parts of the present investigation. Danesh *et al.* (2006) reported that the Vickers microhardness of MTA was 39.99. Lee *et al.* (2004) noted that the microhardness of MTA using the Knoop scale was 51.20. The results of the present study indicated that the Vickers microhardness of MTA was affected significantly by low pH environments. At pH 7.4, the surface microhardness of MTA was 53.19 with the Vickers scale. This value decreased significantly following exposure to pH 6.4, 5.4 and 4.4. This finding is in accordance with Lee *et al.* (2004) who reported that weaker specimens resulted from immersion and storage in a low pH environment.

It has been reported that on occasion MTA fails to set, requiring replacement at a further appointment (Torabinejad & Chivian 1999, Shabahang & Torabinejad 2000). One reason for this lack of hydration might be the acidic pH of inflamed tissues in contact with the material, including the presence of various acids secreted by bacteria in an infected site (Seltzer & Naidorf 1985, Lardner 2001, Costa *et al.* 2003, Nekoofar *et al.* 2009). The results of section 5.1.4.1 (page 130) revealed that the more acidic environment the MTA specimens were exposed to the lower were the compressive strength values (Figure 34, page 131). These findings are in accordance with the results reported by Lee *et al.* (2004) and support the observation that MTA does not harden as well in a low pH environment. Moreover, in the SEM analysis, a greater degree of porosity was seen in samples that were exposed to low pH environments, although it was not possible to grade precisely and objectively the degree of porosity within the context of the SEM examination.

Roy *et al.* (2001) compared the sealing ability of different root-end filling materials whilst exposed to acidic pH. In their study, MTA was placed on a matrix of Calcium Phosphate

Cement (CPC) that was claimed to release water and thus have the potential to enhance the hydration of MTA. They reported that the sealing ability of Super EBA, MTA and MTA with CPC matrix was not affected by low pH.

It has been suggested that the two exceptional properties of MTA, biocompatibility and sealing ability, originate from the physicochemical reactions between MTA and dentine (Sarkar *et al.* 2005). Investigation of the push-out force of MTA will reveal the value of bonding between them. There are various methods for evaluating the adhesion of a dental material to dentine including tensile, shear, and push-out force tests. Loxley *et al* (2003) evaluated the effect of various intra-canal oxidizing agents on the push-out force of MTA, Super EBA (Harry J. Bosworth Co., Skokie, IL, USA), and IRM (Dentsply Caulk, Milford, DE, USA). MTA was significantly less resistant to displacement than Super EBA or IRM. In the present study, the push-out test method was used to test the bond strength between MTA and dentine while exposed to solutions of butyric acid with several pH values.

In the presence of tissue fluid, hydration of MTA powder results in the development of hydroxyapatite crystals and formation of a hybrid layer between dentine and MTA (Sarkar *et al.* 2005). This reaction can be simulated by mixing MTA powder with disodium hydrogen phosphate, a phosphate-containing solution (Lotfi *et al.* 2009). The composition and morphology of the hydroxyapatite crystals is related to various factors, including the environmental pH (Qu & Wei 2008). The ideal pH for this reaction is 7.00 (Sarkar *et al.* 2005). The ensuing hydroxyapatite crystals cover the MTA, fill the microscopic gap between MTA and dentine, and create a chemical bondi; subsequently, because of the precipitation of calcium phosphate, the environmental pH rises to 11.00 (Sarkar *et al.* 2005). Torabinejad *et al.* (1995b) reported the pH value of MTA itself to be between 10.5 and 12.9. On hydration, MTA can release calcium hydroxide (Fridland & Rosado 2003). The formation of calcium

hydroxide and the precipitation of calcium phosphate can explain the ability of MTA to maintain the pH of the surrounding environment at a high level (Fridland & Rosado 2003, Fridland & Rosado 2005). It may also explain some of its biological properties such as the ability to increase osteoblast activity and the induction of hard-tissue formation (Camilleri 2008b).

In some clinical situations, MTA might be exposed to an inflamed environment with a low pH (Nekoofar *et al.* 2009). The application of MTA in a low pH situation may influence its physical and chemical properties (Lee *et al.* 2004). The results of the present study (section 5.1.4.3, page 130, section 5.1.4.3, page 132 5.1.4.1and section 5.1.4.5, page 134) revealed that the lowest and greatest compressive strength (MPa), surface hardness (VH), and push-out force (MPa) values of MTA were found after exposure to pH levels of 4.4 and 7.4, respectively. Scanning electron microscopy evidence also suggests the development of a porous surface and lack of needle-like crystals when the material is in contact with more acidic solutions. Furthermore, Saghiri *et al.* (2008) reported that the time needed for leakage to occur was significantly shorter in samples stored at lower pH values. Watts *et al* (2007) reported that the compressive strength of both tooth coloured and grey MTA decreased significantly when mixed with local anaesthetic solution and exposed to an environment of pH of 5.0. However, there was no significant difference in compressive strength of both tooth coloured and grey MTA when mixed with water and exposed to a pH of 5.0 or 7.4. They suggested the use of sterile water as the mixing liquid rather than local anaesthetic solution.

In addition, various types of acid may have different effects on the physical and chemical properties of MTA. The type of acid was not stated by Lee *et al* (2004) and Watts *et al* (2007), and this lack of information may be one of the reasons for the different findings. The results (5.1.4.5, page 134) showed that the mean push-out force of MTA to intra-radicular

dentine decreased significantly after exposure to pH levels of 4.4 and 5.4 compared with pH levels of 6.4 and 7.4. These results could be caused by alterations in the physical and chemical properties of MTA in such a low pH environment. Moreover, the formation of hydroxyapatite crystals and subsequently the formation of a hybrid layer at the MTA-dentine interfacial gap are likely to be disrupted in an acidic environment. In the present study, the bond failures observed in all experimental groups were predominantly at the MTA-dentine gap (adhesive type). This result is in accordance with Vanderweele *et al*. (2006) who reported that MTA-dentine bond failures were usually adhesive. The adhesive mode of failure may have occurred as a result of the short storage time before evaluation of the push-out force, which was 4 days in the present study and 7 days in the study by Vanderweele *et al*. (2006). Hachmeister *et al*. (2002) suggested that the formation of chemical bonding leads to enhanced attachment of dentine to MTA over time. Sarkar *et al.* (2005) showed that teeth filled with MTA and stored in synthetic tissue fluid for 2 months produced an adherent interfacial layer at the dentine wall that resembled hydroxyapatite in composition. They also reported that in the presence of humidity the tensile strength of the bond between dentine and MTA substantially increased at 3 days with a further moderate increase at 21 days (Sarkar *et al.* 2005). Further long-term studies are suggested to evaluate the effect of aging on the MTA-dentine bond strength.

Hachmeister *et al*. (2002) evaluated the retention characteristics of MTA when placed as an apical barrier with and without prior use of non-setting calcium hydroxide. They revealed that by increasing the thickness of the MTA plug and thus increasing the contact area between MTA and dentine, resistance to dislodgement regardless of the use of calcium hydroxide increased significantly. Therefore, according to the findings of the present study and Hachmeister *et al.* (2002) when exposure to an acidic environment is unavoidable, an

application of a thicker layer of MTA may be beneficial. In addition, before the placement of MTA in an infected and/or inflamed low pH environment, the application of non-setting calcium hydroxide to neutralize the pH is suggested. Under the conditions of this study, the force needed for the displacement of MTA from root dentine to occur was significantly lower in samples stored at lower pH values.

In the present study, the effects of an acid-etch procedure on the surface microhardness, compressive strength, push-out force and surface morphology of tooth coloured ProRoot? MTA (Dentsply Tulsa Dental) was investigated. The result of section 5.1.4.3 (page 132), section 5.1.4.1 (page 130) 5.1.4.7 (page 136) showed that exposure of MTA to a low pH environment may influence its physical properties. Because of the prolonged hydration and setting process of MTA and lack of knowledge about the effect of restoration procedures during this time, the effect of phosphoric acid on MTA was investigated 4, 24 and 96 h after mixing. There is anecdotal evidence that some operators tend to place the final coronal restoration in the same appointment as the MTA. However, the effect of various restoration procedures on the chemical and mechanical characteristics of MTA and the appropriate time of restoration after mixing of MTA are important issues that have not been evaluated adequately. Yan ***et al.*** (2006) evaluated the bond strength of MTA to dentine in different environments and demonstrated that there was no statistically significant difference between the strength of the bond even when the dentine had been exposed previously to sodium hypochlorite and chlorhexidine. Tunc *et al.* (2008) evaluated the adhesive properties of MTA and restorative materials by investigating the shear bond strength of two resin composites used with two different bonding systems to tooth coloured ProRoot? MTA (Dentsply Tulsa Dental). They recommended that composite resins used with a total-etch, one bottle adhesive system was an appropriate final restoration in contact with MTA.

The results of the section 5.1.4.2 (page 132) demonstrated that acid etch applied 4 h after mixing MTA with water, significantly reduced its resultant compressive strength compared with the controls. However, after 24 and 96 h, these differences were not significant. Therefore, to reduce the potential adverse effects of the acid on the compressive strength of MTA, it could be suggested that it is only necessary to postpone the acid-etch procedure and the restoration of a tooth for 24 h. This finding is not in accordance with the results of section 5.1.4.1 (page 130) that showed the adverse effect of butyric acid on the compressive strength of MTA. This inconsistency can be explained by the duration of the acid exposure. In section 5.1.4.1 (page 130) the specimens were exposed to butyric acid for 96 h, however in section 5.1.4.2 (page 132) the specimens were exposed to phosphoric acid for just 15 s, the normal time for an etching purpose.

On the other hand, the acid-etch procedure reduced significantly the surface microhardness of MTA when applied either 4, 24 and/or 96 h after mixing 5.1.4.4, page 133), which is in accordance with the findings of section 5.1.4.3 (page 132) that revealed that MTA did not harden as well in a low pH environment. In addition, according to the results of section 5.1.3.2.6 (page 128) the push-out force of MTA increased significantly following increased incubation periods in both etched and control groups. However, at 24 h and 96 h the acid etch procedure did not affect the push-out force of MTA. Therefore, in clinical applications such as repair of furcation perforations, direct pulp capping and/or pulpotomy MTA might dislocate under indirect masticatory forces. Thus, postponing the final restoration for a period longer than 24 h would appear to be sensible. At 24 and 96 h following mixing, the superficial gel-like amorphous structure and needle-like crystals were missing in the etched samples when they were observed under SEM. This selective loss of matrix from around the

crystalline structures with minimal loss of the cement resulted in a relatively uniform 'honeycomb' etched pattern and exposure of crystalline structures that could provide a satisfactory surface for bonding resin materials. Due to the humidity of the specimens, it was not possible to evaluate the specimens after 4 h of mixing. To overcome this limitation, the use of an environmental SEM that does not require a vacuum (Bergmans *et al*. 2005) may be advantageous.

Taken together the results would suggest that delaying tooth restoration for at least 96 h or longer is desirable. There was also a trend for increased compressive strength, surface microhardness and push-out force over time in both test and control groups; therefore by delaying the placement of the final coronal restoration the material can acquire sufficient compressive strength and push-out force to withstand acid-etch procedures and/or condensation pressures used during the placement of a restoration. Bodanezi *et al.* (2008) evaluated the short- and long-term solubility of MTA-Angelus (Angelus; Londrina, PR, Brazil) and suggested that at least 72 h was necessary to achieve the desirable sealability. Their conclusion was based on the finding that during the first 72 h after mixing, the degree of solubility of the material was high. Vanderweele *et al.* (2006) recommended that MTA should be allowed to set untouched for 72 h or longer to decrease the chance of material displacement. Sluyk *et al.* (1998) also reported that for achieving the desirable sealability, MTA should be untouched for 3 days when used to repair root perforations.

According to the findings of the SEM evaluation, the surface morphology of MTA after acid-etch procedures created a selective loss of matrix from around the crystalline structures that resulted in a relatively uniform 'honeycomb' etched pattern without penetrating deeply or removing substantial amounts of the cement (Figure 49-Figure 53, pages 143-145). This differential etching pattern is an essential characteristic for achieving a satisfactory bond to

resin restorations and is one of the requirements when selecting a material for the composite resin combined or 'sandwich' technique (Sheth *et al.* 1989, Fuss *et al.* 1990). Accordingly, it would appear that the combined MTA-composite restoration could provide a reliable chemical bond to dentine, as well as have the potential for micromechanical bonding of the composite to MTA surfaces (Sarkar *et al.* 2005, Yan *et al.* 2006, Tunc *et al.* 2008). Despite the fact that the type and duration of acid exposure was not the same as in this study, the 'honeycomb' pattern of the etched surfaces was reported previously by Lee *et al.* (2004) as well as the exclusive removal of the needle-like crystals. In addition, in the present study, the acid-etch procedures after 24 and 96 h created notable structures such as plate-shaped and laminated crystals on the MTA surface (Figure 49-Figure 53, pages 143-145). This finding is in accordance with the results of section 5.1.4.7 (page 136) although in that section MTA samples were exposed to butyric acid for 4 days and not phosphoric acid. The significance of these morphological changes is unclear. Lack of sufficient information about the hydration of MTA makes the interpretation of the SEM findings difficult. However, removal of the superficial gel-like amorphous structure, lack of needle-like crystals throughout the etched samples and exposure of remarkable crystalline structures were common findings in the etched samples. Further studies are suggested to determine the significance of these changes in terms of bonding to composite resins.

5.1.6. **Conclusion**

The lowest and greatest compressive strength, Vickers surface microhardness, and push-out force values of MTA were found after exposure to pH levels of 4.4 and 7.4, respectively. In addition, scanning electron microscopy revealed a lack of needle-like crystals when the material was in contact with more acidic solutions, which can explain the adverse physical properties that result as a consequence of acid exposure. Therefore, when exposure to an

acidic environment is unavoidable, an application of a thicker layer of MTA may be beneficial. Moreover, before the placement of MTA in an infected and/or inflamed low pH environment, the application of non-setting calcium hydroxide to neutralize the acidity is suggested. Additionally, since there was a trend for increased compressive strength, surface microhardness and push-out force over time in both etched and control groups; it can recommended that by delaying the placement of the final coronal restoration the material can acquire sufficient compressive strength and push-out force to withstand the acid-etch procedure, condensation pressures used during the placement of a restoration and/or indirect masticatory forces.

5.2. **Blood contamination**

5.2.1. **Introduction**

Blood may contaminate or even be incorporated into MTA during placement and have a detrimental effect on its physical properties. An ideal root repair and root-end filling material should not be affected by contamination of physiological solutions such as blood and/or saliva (Dorn & Gartner 1990, Gartner & Dorn 1992). Torabinejad *et al*. (1994a) evaluated the effect of blood contamination on MTA in an *ex-vivo* study by comparing the leakage of amalgam, Super EBA, IRM and the primary experimental prototype of MTA when applied to root-end cavities that were contaminated by blood immediately after root resection. They reported that there was no significant difference between dye leakage in contaminated and uncontaminated groups and that MTA leaked significantly less than the other materials. In another laboratory study, Martell & Chandler (2002) compared electrochemical and dye leakage of Super-EBA, IRM and MTA in root-end cavities after immersion for 24 hours in defibrinated horse blood. The authors concluded that MTA was associated with less leakage

than the other materials. In an animal study the same materials along with a zinc oxide and eugenol base material were passively exposed to blood in root-end cavities of mandibular premolar teeth in dogs (Bernabe *et al.* 2005). The materials used in their study, including MTA, all had similar effects on the healing process, except for ZOE that resulted in a significantly worse outcome.

To investigate MTA crystal formation in a simulated clinical situation; Tingey *et al.* (2008) analysed the surface characteristics of MTA when set in the presence of bovine serum. They demonstrated that bovine serum affected the dynamics of MTA crystal nucleation and lattice growth suggesting potential effects on the cellular response to the material. Due to biosafety issues and difficulties in obtaining fresh human blood, Tingey *et al.* (2008) emulated the exposure of MTA to human tissue fluid by exposing samples to foetal bovine serum, however, bovine serum could be considered to provide a poor alternative to human serum and/or whole human blood and it may not entirely replicate clinical conditions in humans.

In a laboratory bacterial leakage study Montellano *et al.* (2006) evaluated the effect of blood and/or saliva contamination on bacterial penetration of root-end cavities that were filled by tooth coloured MTA after root-end resection. Saliva contaminated specimens demonstrated significantly more bacterial penetration than the uncontaminated group. However, contamination or absence of blood had no significant effect on bacterial penetration of root-end cavities that were filled with MTA. Conversely, Vanderweele *et al.* (2006), when evaluating the retention characteristics of MTA in simulated furcation perforations, reported that in the blood contaminated group MTA had significantly less resistance to displacement compared to the uncontaminated group at 7 days. Therefore, they recommended that blood should be removed before the placement of MTA. In contrast, Arens & Torabinejad (1996) recommended that perforation sites should not be dried before the placement of MTA. In

addition, Sluyk *et al.* (1998) reported that the presence of moisture in a perforation site resulted in good adaptation of MTA to the perforation walls. Furthermore, they recommended a moistened matrix be positioned in the perforation defect before placement of MTA for ease of MTA condensation and to prevent over-extrusion of the material. However, Al-Daafas & Al-Nazhan (2007) found the use of an internal matrix beneath MTA, preventing its direct contact with the tissues, produced an adverse healing response and reduced connective tissue attachment and bone formation in the site of the perforation.

Porosity is one of the physical properties of MTA that has not been evaluated comprehensively. In a laboratory study, using the Archimedes principle, Fridland & Rosado (2003) evaluated the effect of various water to powder ratios on the initial porosity of MTA and demonstrated that by increasing the water ratio, porosity increased. By using SEM, in section 5.1.4.7 (page 136) a correlation between increased porosity and detrimental physical properties of MTA at various pHs has been suggested, however, SEM is a subjective method for porosity evaluation.

In general, porosity is defined as the volume fraction of a material that can contain gas and/or liquid and it is calculated by dividing the total volume of the pores by the total volume of a specimen. During the hydration process, MTA particles absorb water and amorphous calcium silicate gel is produced (Camilleri 2008b, Camilleri 2010). By progression of the hydration process, calcium hydroxide and calcium silicate crystals precipitate and produce an interconnected network of pores and microchannels (Dammaschke *et al.* 2005, Danesh *et al.* 2006). Formation of this network is critical for diffusion of water and development of crystalline structures (Kogan *et al.* 2006). Due to continued deposition of crystal precipitates, the initial connectivity of the microchannels decreases and form closed pores that may hold trapped water. Therefore, hardened MTA contains a high number of closed pores that are not

connected (Fridland & Rosado 2003). Pores in a material can be classified as closed/isolated or through/connected (Malcolm *et al.* 2007). Evaluation of porosity of hydraulic cements is important as it can provide information on the physical properties of the material (Feldman 1990).

There are several methodologies for evaluation of porosity, such as SEM, the "Archimedes"-method, gas or liquid perfusion and microcomputed tomography (micro-CT). The values calculated by each method are dependent on the methodology and do not yield similar values. Information can be obtained by utilizing a particular technique, however, none of them individually can provide comprehensive information about the porosity of a material and each has their own limitations. Micro-CT is a useful tool to distinguish between closed and connected pores of hydraulic cements compared to perfusion techniques in which closed pores could be compressed and broken (Ghasemi Mobarakeh *et al.* 2007, Malcolm *et al.* 2007). Micro-CT is a non intrusive methodology and therefore, the topography and connectivity of the pores and microchannel network can be evaluated without destruction of specimens. Zakizadeh *et al.* (2008) compared porosity of MTA, Fuji-Plus and Geristore using micro-CT and concluded that MTA was less porous than other materials, although no quantification analysis was employed. By quantification of MTA porosity it should be possible to evaluate the effect of blood contamination on MTA, a factor that has not been reported previously.

The aims of the studies within this section of the thesis were to evaluate the effect of whole fresh human (WFH) blood contamination on the certain physical and chemical properties of tooth coloured ProRoot® MTA (Dentsply Tulsa Dental): including compressive strength, surface microhardness, push-out force, porosity using computed microtomography and quantification analysis, surface microstructure, phase composition and elemental analysis.

WFH blood was used to contaminate MTA samples, rather than the various human blood substitutes that have been used in similar studies. In addition, the materials were also mixed solely with whole blood, in place of water, to determine if incorporation of this potential contaminant would affect their properties.

5.2.2. **Materials & Methods**

5.2.2.1.**Materials**

Fresh whole blood was collected from a healthy consented volunteer by a trained individual (Figure 54, page 160) in accordance with Helsinki ethical principles for medical research involving human subjects (2001) and approved by a panel from the School of Dentistry, Cardiff University Ethical Committee and the ethical board of the local research review committee in the Faculty of Dentistry, Tehran University of Medical Sciences, Iran. The material investigated was the tooth coloured formula of ProRoot? MTA (Dentsply Tulsa Dental) with LOT number of 083006.

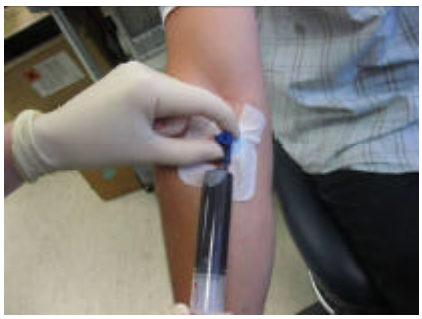


Figure 54: Fresh whole blood was collected from a healthy consented volunteer by a trained individual in accordance with Helsinki ethical principles for medical research involving human subjects (2001).

5.2.2.2.**Methods**

5.2.2.2.1. Compressive strength

Eighty custom-made polytetrafluoroethylene (PTFE) cylindrical moulds (internal dimensions P P height DQG? P P GLDPHWHU ZHUH UDQGRPO\ DOORFDWHG WR IRXU JURXSV SULRU WR filling with MTA slurries.

The groups consisted of:

Group 1: MTA mixed with distilled water and exposed to distilled water (control group);

Group 2: MTA mixed with distilled water and exposed to WFH blood;

Group 3: MTA mixed with WFH blood diluted with distilled water (50% v/v) and exposed to WFH blood;

Group 4: MTA mixed entirely with WFH blood and exposed to WFH blood.

Mixing of MTA was standardised by encapsulated mixing 1 g of MTA powder with 0.33 g of the appropriate liquid in the plastic mixing capsule as described in section 4.3.2.1 (page 69). The PTFE cylindrical moulds were then filled with the resultant MTA slurry using a spatula with minimal pressure and then subjected to ultrasonic energy as described in section 4.3.2.1 (page 69). In the test groups (2, 3 and 4), before placement of the MTA slurry, inside each mould was filled by WFH blood that was aspirated following 20 s. As a result, the inner walls of moulds were coated with WFH blood prior to placement of the MTA slurry (Figure 55, page 162). Each specimen was then placed in a 1.5 mL Eppendorf tube, which contained the appropriate liquid medium used to expose the lower surface of MTA specimen. A moist cotton pellet was then placed above the specimen before sealing the Eppendorf tube to provide a fully saturated humid environment prior to being incubated at 37^{o}C for a short (4 days) and long (30 days) period of time.

After 4 and 30 days of incubation, ten specimens of each four groups were randomly selected and subjected to the compressive strength test, using a universal testing machine (Lloyd LR MK1, UK), as described in section 4.3.2.1 (page 69).

Figure 55: In the test groups, before placement of the MTA slurry, the inner walls of moulds were coated with WFH blood.

The mean compressive strength values, confidence intervals and standard deviations were calculated for each group and, as the data was normally distributed, analysed using two-way ANOVA for overall comparisons and Tukey's post hoc test for individual comparisons. All analyses were performed using the statistical package of social science version 16 (SPSS Inc.).

5.2.2.2.2. Surface microhardness

Thirty custom-made borosilicate glass cylindrical moulds (internal dimensions 12 ? P P height and 6 ? P P GLDP HWZ HUHDQGRP \ DORFDWHG three groups, prior to filling with MTA slurry.

The groups consisted of:

Group 1: MTA mixed with distilled water and exposed to distilled water (control group);

Group 2: MTA mixed with distilled water and exposed to WFH blood;

Group 3: MTA mixed entirely with WFH blood and exposed to WFH blood.

Mixing of MTA was standardized by encapsulated mixing 1 g of MTA powder with 0.33 g of appropriate liquid in the plastic mixing capsule as described previously (section 4.3.2.2, page 71). The borosilicate cylindrical moulds were then filled with the resultant MTA slurry using a spatula with minimal pressure and then subjected to ultrasonic energy as described previously (section 4.3.2.2, page 71). In the test groups (2 and 3), before placement of the MTA slurry, the inner walls of moulds were coated with WFH blood. Each specimen was then placed in a 1.5 mL Eppendorf tube, which contained the appropriate liquid medium used to expose the lower surface of MTA specimen. A moist cotton pellet was then placed above

Eighty standard root dentine slices with 1.00 mm thickness and 1.3 mm internal diameter were prepared using the methodology described in section 5.1.3.2.5 (page 126). The specimens were then allocated randomly into four groups as described in section 5.2.2.2.1 (page 160) to be filled with the corresponding MTA slurry.

The groups consisted of:

Group 1: MTA mixed with distilled water and exposed to distilled water (control group);

Group 2: MTA mixed with distilled water and exposed to WFH blood;

Group 3: MTA mixed with WFH blood diluted with distilled water (50% v/v) and exposed to WFH blood;

Group 4: MTA mixed entirely with WFH blood and exposed to WFH blood.

Mixing of MTA was standardised by encapsulated mixing 1 g of MTA powder with 0.33 g of corresponding liquid in the plastic mixing capsule as described in section 4.4.2 (page 79). The MTA slurries were then introduced incrementally with no pressure into the lumens of the root-dentine slices. Placement of MTA slurries were standardised by the application of the ultrasonic energy using a BUC-1 Spartan tip (Obtura Spartan) attached to a 6XSUDWRQ? 3

Booster (Satelec) as described in section 4.4.2 (page 79). Following 4 and 30 days of incubation D[illegible] in a fully saturated humid atmosphere; 10 specimens of each group were randomly selected and subjected to the push-out force test using a universal testing machine as described in section 5.1.3.2.5 (page 126).

The push-out forces were measured using a universal testing machine (Z050; Zwick/Roell Group, Ulm, Germany) and recorded in mega Pascal (MPa). The data were then subjected to one way ANOVA analysis using the Statistical Package of Social Science version 16 (SPSS Inc.) to examine the difference between the mean push-out force values of each experimental group followed by the Tamhane post hoc test. The nature of the bond failure of each specimen was also examined under a light microscope at X40 magnification and the failure mode recorded accordingly.

5.2.2.2.3. Porosity

Thirty two samples were prepared and allocated into four groups by the methodology described in section 5.2.2.2.1 (page 160).

After 4 and 30 days, four specimens of each four groups were randomly selected and scanned using a desktop x-ray microtomograph (SkyScan 1072, SkyScan, Aartselaar, Belgium). The side of the specimen that was closest to the wet cotton pellet was fixed on a specimen holder using Super Glue™ (Henkel Loctite Adhesives Ltd, Gillingham, UK) and loaded centrally on the sampling plate within the machine. Specimens were scanned using the rotation range of H[illegible] times magnification. The beam-hardening artefact was reduced by using a 1-mm aluminium filter. Median filtration, geometrical correction, and flat field correction were applied during acquisition to minimize

noise. An average frame of 1 was chosen as a space for the acquisition of the serial cross-sections DQGD? URWDWLRQ VWHS To reduce amplify noise the gain was adjusted to 1. A typical cycle of data collection for reconstruction contained image acquisition from 200 to 280 views. After the acquisition of the projection images the reconstruction was performed using NRecon volumetric reconstruction software (Version 1.5.1.4, SkyScan). The reconstructed cross-sections had a 1024 x 1024 pixels format, 16-bits and the image pixel size of the specimen was 12.

The 3D data sets obtained were then analyzed using CT-Analyzer image analysis software (Version 1.10.1.0, SkyScan). The region of interest (ROI) was defined as a circle surrounding the specimen and the images were interpolated. The ROI was vertically limited by the radius and the cross-sections with a volume outside of this range were excluded. In addition, corrupted images from the surface which were in contact with Super Glue™ were also excluded. After selection of the ROI, the half tone images were transformed to binary images with two-brightness gradations only: black and white (Figure 56, page 165).

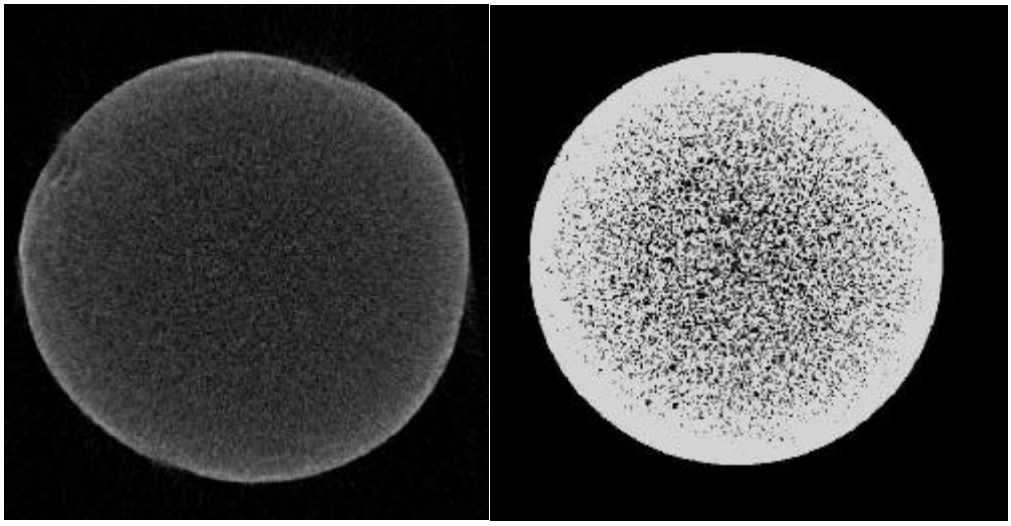


Figure 56: A half tone image (left) of a section of one of the specimens of group 1. The transformed binary image (right) of the same section.

A pixel intensity histogram was then created from the binary images (Figure 57, page 166) representing the intensity value that optimally distinguished pores from the solid phase.

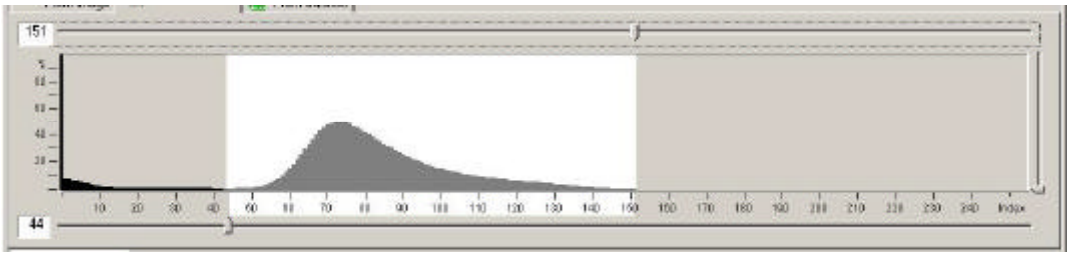


Figure 57: A pixel intensity histogram. The selected area in white represents the solid phase. The values between 0-44 represent pores.

In this situation, each 3D pixel (voxel) acquires a value between 0 and 255 corresponding to pores and solid material, respectively (Figure 57, page 166). For quantitative analysis the threshold was set at the minimum between two peaks of the histogram using the auto-threshold option of the software (Figure 57, page 166). Smoothing, despeckle, and ROI shrink-wrap, were then performed and the 3D total porosity percentage was measured.

5.2.2.2.4. SEM/EDX

Sixteen additional specimens were prepared and allocated to four groups using the same methodology described in section 5.2.2.2.1 (page 160) and incubated at 37^{o}C in fully saturated humidity for 4 days.

For the morphological evaluations and to analyse the microstructure of the inner surfaces, two specimens of each group were selected randomly and sectioned in two using a disposable surgical scalpel blade No. 15. The surfaces were sputter-coated with gold using a Polaron Sputter Coater (Quorum Technologies) and specimens were visualised with an EBT1 (Electron Beam Technology) Scanning Electron Microscope (S.E.M. Tech). The micrograph images from the SEM analysis showing the qualitative internal microstructure of the specimens of each group were evaluated in terms of the presence of various types of crystal

formations. The locations of the images were selected at random from the internal surface of the broken specimen and are considered to be representative of the material.

In addition, the surface characteristics of two additional specimens of each group were examined and subjected to elemental analysis. The two randomly selected specimen of each group were also sectioned using a disposable surgical scalpel blade No. 15 and mounted on aluminium stubs using adhesive carbon discs and analysed uncoated using a scanning electron microscope (SEM, Carl Zeiss EVO 40, Oberkochen, Germany) fitted with an energy dispersive X-ray detector (EDX, Oxford Instruments, Oxford, UK). The locations of the images were selected at random from the internal surface of the broken specimen and are considered to be representative of the material.

5.2.2.2.5. Phase composition (XRD)

MTA slurries were prepared according to the following three groups.

Group 1: MTA mixed solely with distilled water

Group 2: MTA mixed with WFH blood diluted with distilled water (50% v/v)

Group 3: MTA mixed solely with WFH blood

Mixing of MTA was standardized by encapsulated mixing 1 g of MTA powder with 0.33 g of appropriate liquid in the plastic mixing capsule as described in section 4.3.2 (page 69). The resulting MTA slurries were placed with minimal pressure in the circular depression of an XRD sample holder (Panalytical, Almelo, the Netherlands). To standardise the placement technique MTA slurries were then subjected to ultrasonic energy as described in section 4.3.2 (page 69). The ultrasonic tip was moved throughout the MTA slurry without touching either the wall or floor of the sample holder. Specimens were then incubated at 37^{o}C in fully saturated humidity for 4 days. Phase compositions of MTA specimens from each group were

determined using an x-ray diffractometer (XRD, Panalytical X'pert pro, Almelo, Netherlands). The specimen surfaces were polished with 1200-grit fine-grain sandpaper (3M, St Paul, MN, USA) to ensure the surface of the sample was level with the holder surface. X-ray diffraction patterns were then recorded using Ni filtered CuK_α radiation (40Kv and 40mA). Scans were undertaken in the range 10-80^o 2θ. All patterns were matched using the ICDD database (International Centre for Diffraction Data, Pennsylvania, USA). For comparison a specimen of unhydrated MTA powder was also subjected to X-ray diffraction analysis as group 4.

5.2.3. **Results**

5.2.3.1. **Compressive strength**

A summary of the results of the compressive strength tests are shown in Figure 58 & Figure 59 (pages 169 & 170).

After 4 days: A trend was observed between the degree of blood contamination and compressive strength of MTA (Figure 59, page 170). The more that blood was incorporated into the MTA slurries, the lower the mean compressive strength values were recorded. There was a statistically significant difference between the mean compressive strength of all groups ($p<0.00001$). The lowest mean compressive strength value was recorded for MTA specimens RI JURXS Z KLFKZ HUHQRWHV PL[HGZLWKDQH[SRVHGWR:) + EORRG? 0 3D

while the highest mean compressive strength value was recorded for the control specimens of group 1 which were only mixed and exposed to distilled wateU? 0 3D

After 30 days: A trend between the degree of blood contamination and reduction in mean compressive strength of MTA specimens was seen for all groups except for those of group 3 (Figure 58, page 169). There was a statistically significant difference between the mean compressive strength values of specimens mixed only with distilled water (groups 1 and 2)

and those mixed with either diluted (group 3) or WFH blood (group 4) (p<0.00001). The lowest mean compressive strength value was recorded for MTA specimens of group 4 which were entirely mixed with and e[SRVHGWR:) + EORRG? 0 3DZ KLQMKHKJKHW mean compressive strength value was recorded IRUWKHVSHFLP HQVRI JURXS ? 20.016 MPa). There was no significant difference between specimens of groups 1 and 2 (both mixed with water) and between specimens of groups 3 and 4 (mixed with diluted or WFH blood, respectively), irrespective of their exposure fluids. In general, except for group 3, the longer incubation time resulted in a greater compressive strength. There was a statistically significant difference between the mean compressive strength values of groups 1, 2 and 4 over the two experimental incubation time periods (p<0.00001).

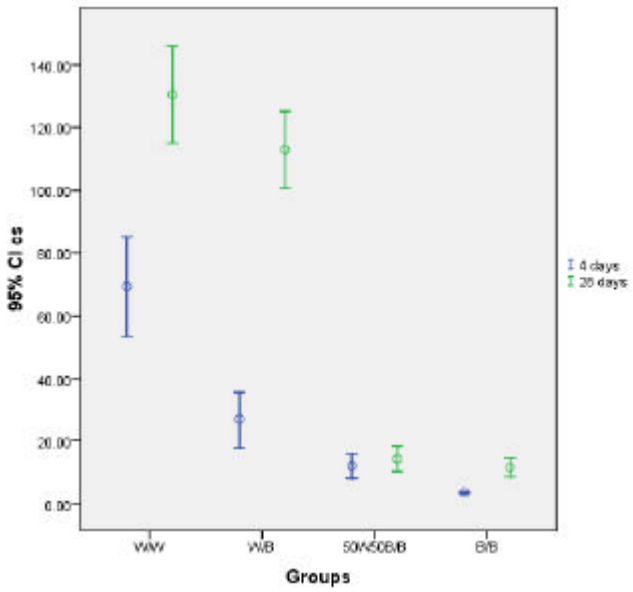


Figure 58: Error bar graph representing the mean compressive strength (MPa) values ? 95 % confidence interval of various tooth coloured ProRoot? MTA groups:

W/W= group 1: Mixed with water and exposed to water
W/B= group 2: Mixed with water and exposed to blood;
50W50B/B= group 3: Mixed with diluted blood and exposed to blood;
B/B= group 4: Mixed entirely with blood and exposed to blood.

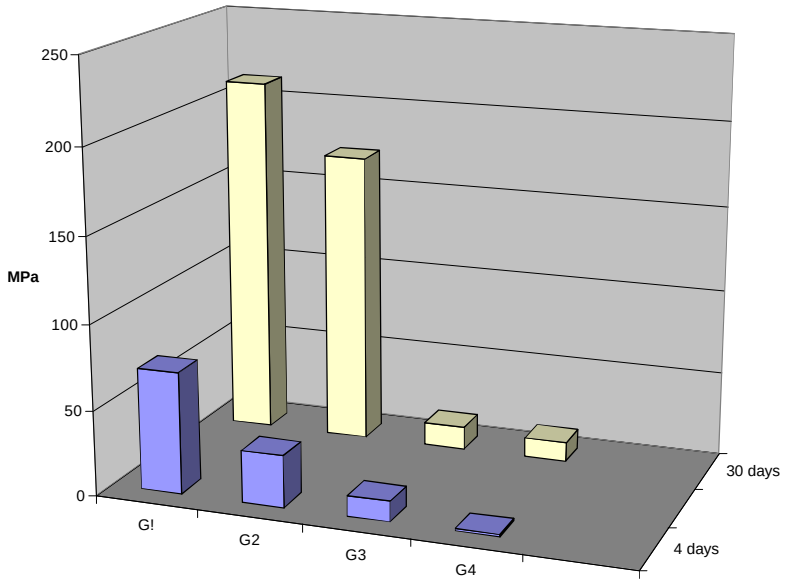


Figure 59: Compressive strength (MPa) of various tooth coloured ProRoot? MTA groups obtained after 4 and 30 days incubation period. For the description of each group see the legend of Figure 58.

5.2.3.2. **Surface microhardness**

The results are summarised in Figure 60 (page 171).

After 4 days: The mean Vickers surface microhardness value of the specimens of control JURXS Z DV? This was significantly greater than the mean surface microhardness value of the specimens of other experimental groups ($p < 0.00001$). No significant difference was observed between mean surface microhardness value of group 2 and 3 in which MTA slurries were exposed to WFH blood following mixing of MTA powder with water or blood respectively ($p < 0.00001$).

After 6 months: The microhardness values of the samples after 6 months had a similar pattern to those values obtained after four days. The control group, in which tooth coloured MTA powder was mixed with water and exposed to water, had significantly greater microhardness values than the experimental groups ($p < 0.00001$).

In general there was no significant difference between the microhardness values after 4 days and 6 months within the experimental groups. In accordance with the results obtained after 4

days, significant difference was observed between the mean surface microhardness values of the specimens of the control group in which MTA powder mixed and exposed to water and specimens of group 2 and 3 in which MTA slurries were exposed to WFH blood following mixing of MTA powder with water or blood respectively ($p < 0.00001$).

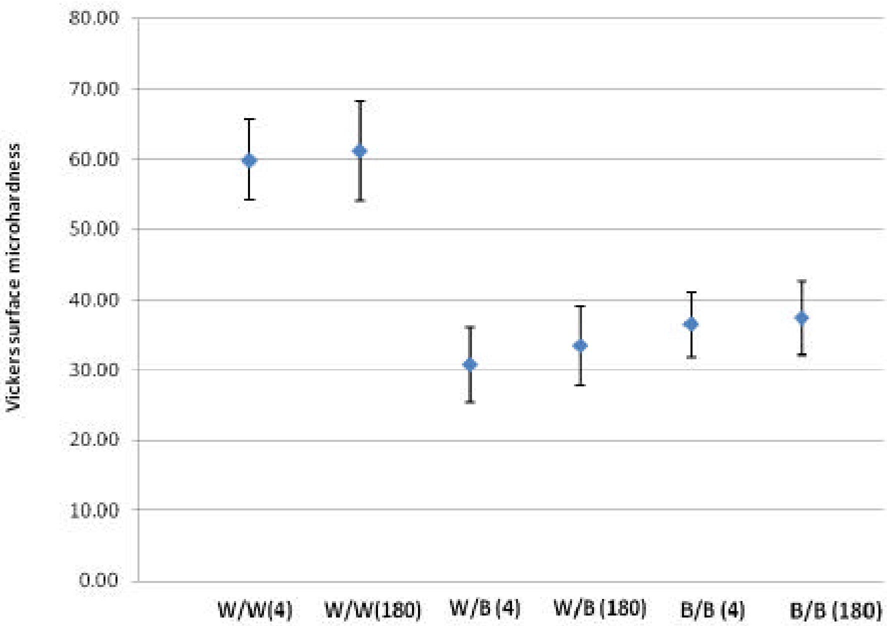


Figure 60: Error bar graph representing the mean Vickers surface hardness (VH) values ? 95 % confidence interval of various tooth coloured ProRoot? MTA groups:

W/W= group 1: Mixed with water and exposed to water
W/B= group 2: Mixed with water and exposed to blood;
B/B= group 3: Mixed entirely with blood and exposed to blood.
The numbers in bracket correspond the incubation period (days).

5.2.3.3. **Push-out force**

The results are summarised in Figure 61 (page 172).

After 4 days: There was a significant difference between the mean push-out force values of the control group and the experimental groups ($p<0.00001$). The greatest mean push-out force

ZDVVHHQLQVSHFLPHQVRIWKHFRQWUROJURXS JURXS LQZKLFK0 7$ SRZGHUZDV

mixed with water and exposed to water. No difference was observed between the other experimental groups.

After 30 days: Compared to groups 3 & 4, the significantly greatest mean push-out force YDOXHZ DVREVHUYHGIQJ URXS ? DQGJ URXS ? UHVSHFWWHO S + RZ HYHUQR

significant difference was observed between group 1 and 2 or between groups 3 and 4.

In general, the mean push-out force values of the specimens of group 1 & 2 significantly increased over time ($p<0.00001$). However, specimens in groups 3 and 4 did not gain more push-out force after 30 days of incubation. No difference was observed in the mean push-out force values of the specimens of groups 3 and 4.

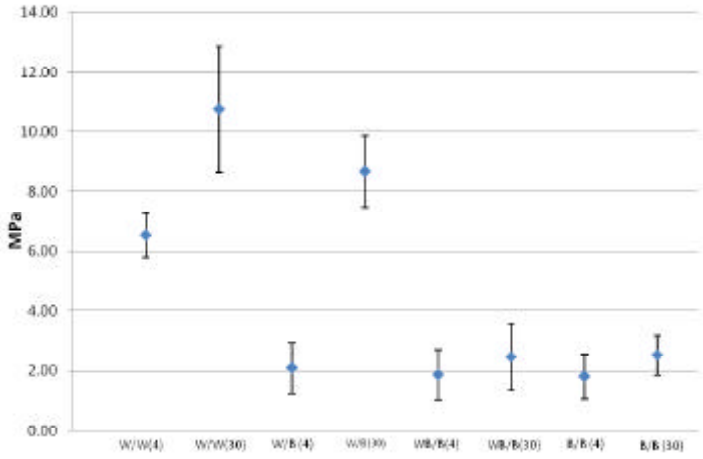


Figure 61: Error bar graph representing the mean push-out force (MPa) ? 95 % confidence interval of various tooth coloured ProRoot? MTA groups:

Group 1 (W/W): Mixed with water and exposed to water
Group 2 (W/B): Mixed with water and exposed to blood;
Group 3 (WB/B): Mixed with diluted blood and exposed to blood;
Group 4 (B/B): Mixed entirely with blood and exposed to blood.
The numbers in bracket correspond the incubation period (days).

5.2.3.4. **Porosity**

The results are summarised in Figure 62 (page 173).

Effect of blood after 4 days: Significantly higher percentages of total porosity were observed

LQJ URXSV? DQG? FRP SDUHGWRJ URXSV 2.894 0.713)

and 2 (3. S 1 RVLJ QLILFDQW LIHUHQFHZ HUHREVHUYHGEHZHHQWKH

percentages of total porosity of groups 1 and 2 nor between groups 3 and 4.

After 30 days: Significantly higher percentages of total porosity were observed in groups 3

? S + RZ HYHQR GLIIHUHQFHZ DREVHUYHG

between the percentages of total porosity between the specimens of groups 3 and 4. 6LJQLILFDQW GRZ HSHUFHQWDJHV RI WRWDO SRURVLW ? ZHUHREVHUYHGLQWKH

specimens of group 1 in which MTA powder was mixed with water and exposed to water ($p<0.0001$).

Effect of time: A significant reduction was seen in the specimens of group one ($p<0.00001$) when comparing the percentage of total porosity at 4 and 30 days. However, in the other experimental groups this reduction did not occur and no difference was observed between the percentages of total porosity after 4 days and 30 days respectively. In the group 2 the SHUFHQWDJHVRWRWDO SRURVLW DIWHUGD V? Z DVORZ HUWKDQDIWHU 4 days

DQGRXJKWHGLIIHUHQFHZ DVQRW QLILFDQW

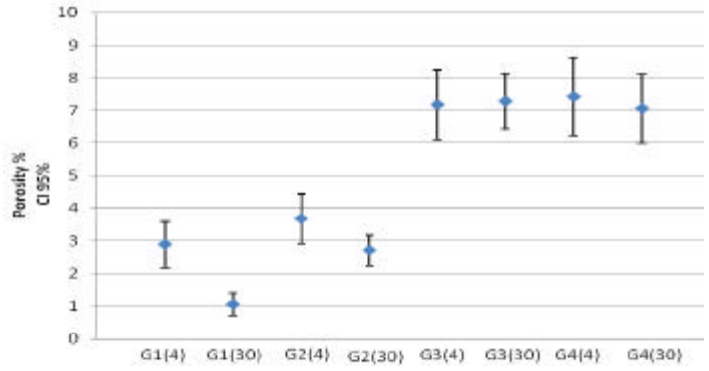


Figure 62: Error bar graph representing the percentages of total porosity ? 95 % confidence interval of the various tooth coloured ProRoot² MTA groups:

Group 1 (W/W): Mixed with water and exposed to water;
Group 2 (W/B): Mixed with water and exposed to blood;
Group 3 (WB/B): Mixed with diluted blood and exposed to blood;
Group 4 (B/B): Mixed entirely with blood and exposed to blood.
The numbers in bracket correspond the incubation period (days).

5.2.3.5.**SEM & EDX**

In the control groups, samples revealed notable crystalline characteristics when compared to experimental groups, including a wide variety of distinctive crystalline formations around cross-sections of micro-channels (Figure 63, page 175). Such formations included plate-like crystals with well-defined edges, which were embedded in a porous and rough crystalline matrix. In addition, angular and laminar crystals were present along with two main forms of acicular crystals that are characteristic of hydrated calcium sulphoaluminate (ettringite) (Gemelli *et al.* 2004). These characteristic formations include jagged or spiky ball-like clusters and bundles of longer spanning structures that interlinked with other crystals (Figure 63 & Figure 65, pages 175 & 177). All experimental blood contaminated groups had more globular formations rather than the angular crystals seen in the control groups. The surfaces of all experimental samples had a noticeably different appearance than control specimens (Figure 64, page 176). In the experimental groups there was a clear lack of angular crystal formations and an absence of both the jagged clusters and longer forms of the interlinking acicular ettringite crystals (Figure 64, page 176). However, in samples mixed with water and exposed to blood it was possible to see a more angular matrix than the other experimental groups. SEM & EDX analysis of group 1 (control group) revealed a homogeneous amorphous layer containing small microchannels (1-30μm) and dispersed with distinct 0-10μm structures shown to have high levels of bismuth by EDX analysis (Figure 65, page 177). At higher magnification, clusters of fine acicular (needle-like) crystals were seen. EDX analysis revealed higher sulphur and aluminium concentration in acicular clusters relative to the background matrix, which demonstrated a higher level of calcium, oxygen, silicon and magnesium in addition to trace quantities of iron.

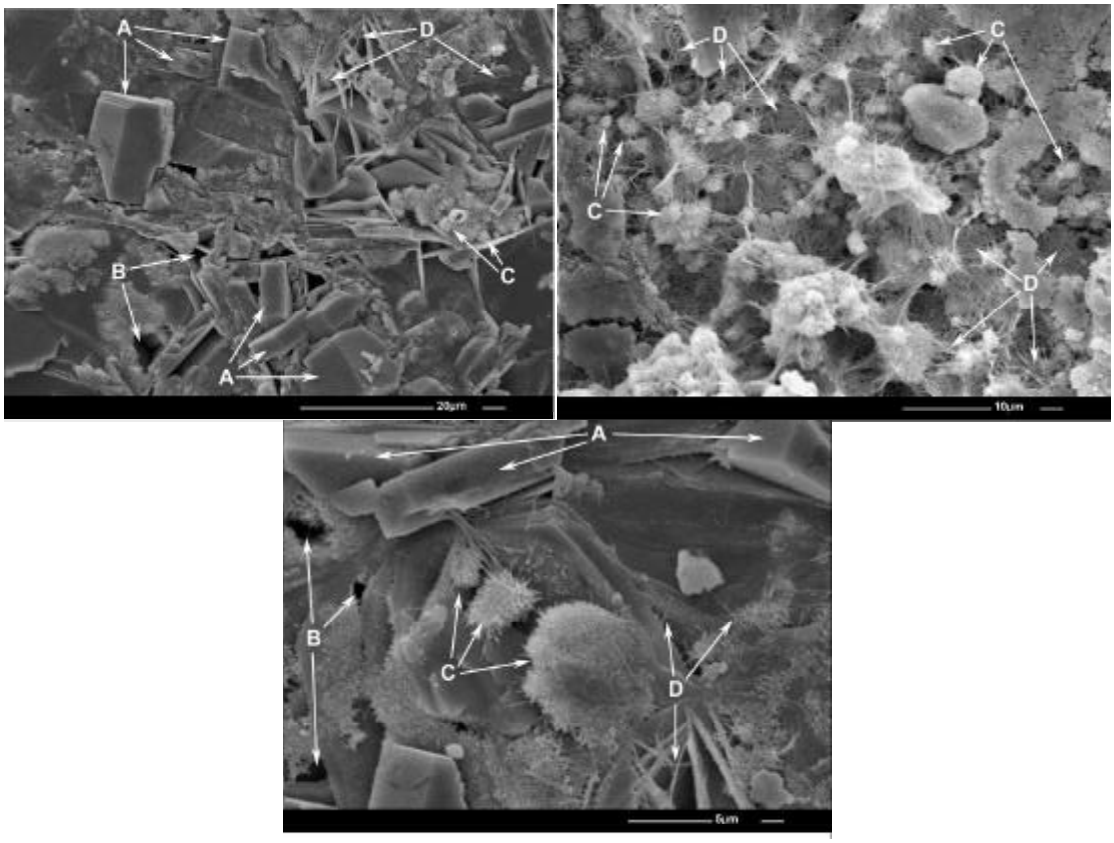


Figure 63: Scanning electron microscope image of a control sample of MTA mixed with and exposed to distilled water. Large laminated plate-like crystals with well-defined edges (A) were embedded in a rough crystalline matrix containing micro-channels (B). Acicular crystals were seen in clusters of spiky ball formations (C) as well as longer spanning forms (D) radiating from the rough matrix. Three levels of magnification are shown with the highest at the bottom.

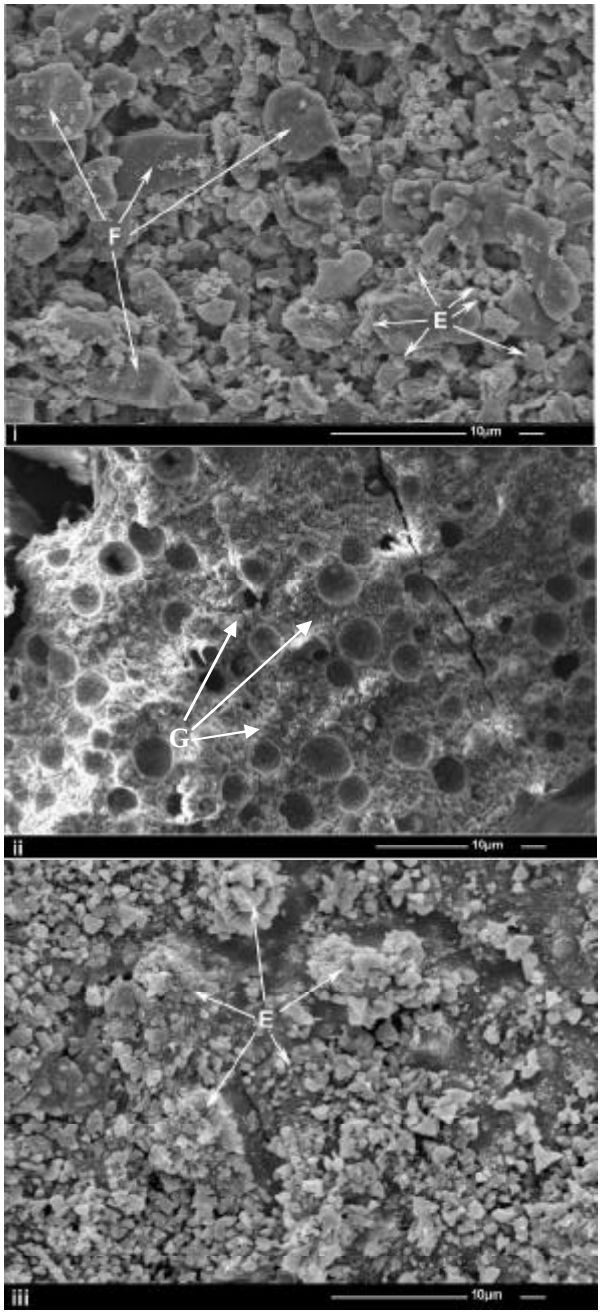


Figure 64: Scanning electron microscope images demonstrating MTA surfaces from experimental groups. Absence of both of acicular crystal formations, jagged clusters and long spanning shapes were observed in all experimental groups (i, ii & iii). A globular matrix (E) with less angular crystal formations (F) was seen in experimental groups (ii & iii). Large crystals with rounded edges embedded in globular matrix (F). The large number of depressions from air bubbles present throughout the bulk (G).

Group 1 (i) MTA mixed with water and exposed to WFH blood;
Group 2 (ii) MTA mixed with WFH blood diluted with distilled water (50% v/v) and exposed to blood;
Group 3 (iii) MTA mixed with WFH blood and exposed to WFH blood.

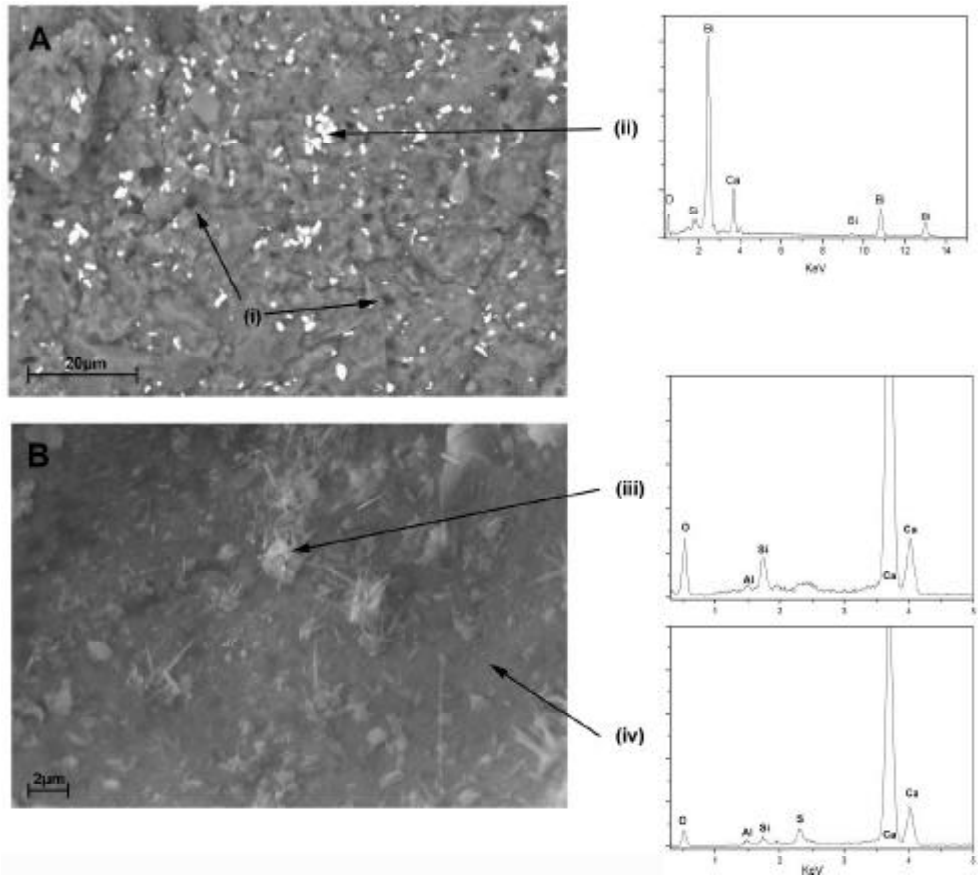


Figure 65: Scanning electron microscopy image of uncontaminated hydrated tooth coloured ProRoot® MTA (a) showing small microchannels (i) amongst distinct structures shown to have high levels of bismuth oxide by energy-dispersive X-ray (EDX) analysis (ii). Higher magnification SEM of uncontaminated hydrated WMTA (b) demonstrating the presence of acicular crystals (iii) on an amorphous background matrix (iv). EDX analysis showed the acicular crystals (iii) to have a higher level of sulphur and aluminium than the background matrix (iv), characteristic of ettringite (hexacalcium aluminate trisulphate hydrate).

5.2.3.6. **Phase composition (XRD)**

The results are illustrated in the Figure 66 (page 178).

All specimens analysed by XRD comprised bismuth oxide (α-Bi_2O_3, ICDD 00-027-0053) indicated by the main peaks at 27.38, 33.07 and 33.23° 2θ and tricalcium silicate (Ca_3SiO_5, ICDD 00-055-0738) indicated by the peaks at 32.13, 32.56 and 34.30° 2θ. Levels of tricalcium silicate and bismuth oxide were lower in groups 1 and 2 than in groups 3 and 4. Groups 1 and 2 showed reflections at 18.10, 28.69 and 34.10° 2θ indicative of calcium

hydroxide ($Ca(OH)_2$, ICDD 00-044-1481). The calcium hydroxide phase is absent in group 3 and in the unhydrated MTA powder.

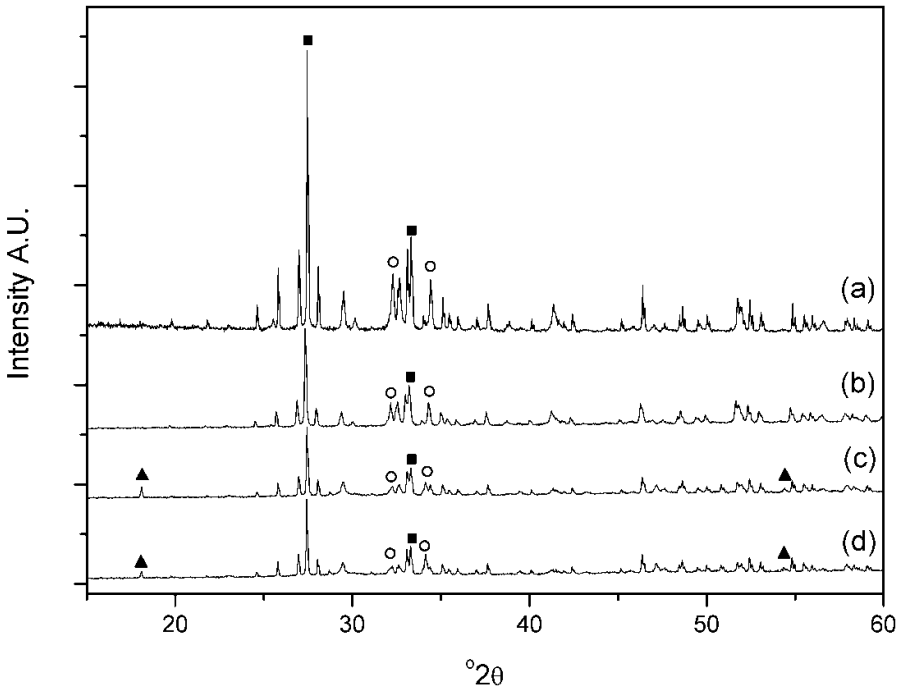


Figure 66: X-ray diffraction patterns showing the main compound present: unhydrated MTA powder (a), group 3 (b), group 2 (c), group 4 (a). Main phases present highlighted with symbols: (■) α-$Bi2O_3$, (○) Ca_3SiO_5 and (▲) $Ca(OH)_2$. The calcium hydroxide phase is absent in group 3 (b) and the unhydrated MTA powder (a).

5.2.4. **Discussion**

In the course of use, MTA is frequently in close contact with blood and this fluid may even mix with the body of the cement due to the specific clinical conditions. Therefore, it is imperative to investigate the setting reaction of the material in an environment where blood is present and explore any potential changes to the crystalline microstructure and surface microhardness because of this contamination.

In section 5.2.2.2.1 (page 160), the compressive strength of MTA was used as a measure of the hydration process. According to ISO 9917-1 (2003) standards, the use of a split-mould design, made of stainless steel or a material that will not be affected by the cement has been

advised. There have been a variety of methods used to form cylindrical MTA specimens for compression testing. In the present study, PTFE plastic cylindrical moulds were used to form 6x4 mm diameter MTA samples. In other investigations of MTA compressive strength, one-piece plastic cylindrical moulds (Kogan *et al.* 2006) or plastic split moulds (Holt *et al.* 2007) have been used. Due to the expansion of MTA during its hydration Holt *et al.* (2007) reported difficulties during the removal of specimens from moulds without exerting excessive force on the material prior to testing. Holt *et al.* (2007) used a two-part split-mould design to create MTA samples for compression testing and reported that samples required moderate force to allow removal, which resulted in fracture failure of some samples prior to testing. In the present work two types of moulds have been used including one-piece polycarbonate cylindrical moulds (section 4.3.2.1, page 69) and stainless-steel split-moulds (Torabinejad *et al.* 1995b). In addition, in a pilot study prior to the present study, single-piece borosilicate glass moulds were used to form MTA samples, which required a high push-out force to remove them for testing and resulted in multiple sample fractures. Therefore, in section 5.2.2.2.1 (page 160) a new method of MTA sample formation was developed that involved careful removal of two opposing sections of the cylindrical mould walls to reduce retention of the MTA samples. This novel method minimised the forces on MTA samples prior to compression testing that may otherwise have introduced confounding variables.

In the majority of endodontic applications, MTA slurry comes into contact with blood and in the extreme may become mixed with blood during placement. The results of the present study revealed that both these events adversely alter the compressive strength and surface microhardness of MTA. Moreover, it decreased the push-out force of MTA that could result in dislocation of MTA prior to its hardening. Furthermore, the porosity of MTA was high when contaminated with blood and did not reduce after 30 days to reflect its incomplete

hydration. XRD analysis confirmed this hypothesis since calcium hydroxide one of the main by-products of the hydration process did not form in contaminated groups. In addition, in the blood contaminated groups the absence of acicular crystals, characteristic of hydrated calcium sulphoaluminate (ettringite) (Gemelli *et al.* 2004, Stutzman 2004), which have a potential role in forming inter-crystal bonds (Ismail *et al.* 2002) was demonstrated by SEM (section 5.2.3.5, page 174). Accordingly, it can be suggested that blood contamination is a likely cause for encountering unset MTA at a subsequent evaluation appointment.

According to the manufacturer's instructions:

"if unhardened MTA is encountered at the second appointment the MTA material should be rinsed out and replaced."

However, in surgical applications MTA cannot be examined for setting, which could potentially result in unfavourable clinical outcomes. For better understanding of the clinical behaviour of the material, particularly when it cannot be examined at a later appointment, investigations into the effect of blood contamination on the physical properties of MTA are required.

In this studies, in an attempt to replicate the clinical situation in which blood becomes incorporated into MTA, the effects of WFH blood contamination on certain physical properties of tooth coloured Pro Root MTA were investigated. WFH blood was chosen to contaminate MTA rather than substitutes such as defibrinated horse blood (Martell & Chandler 2002), simulated human plasma fluid (Coleman *et al.* 2007), phosphate buffered saline (PBS) (Bozeman *et al.* 2006, Gandolfi *et al.* 2009) or foetal bovine serum (FBS) (Tingey *et al.* 2008). The advantage of using fresh, human blood is that it more closely replicates the human clinical situation. However, experiments involving WFH blood present

difficulties such as ethical considerations, biohazard issues and obtaining sufficient volumes of blood over a prolonged period of time without the addition of anticoagulant agents.

A pilot experiment was initially carried out to confirm the feasibility of the chosen method before commencing the study on the relatively large scale planned. For this pilot investigation, human blood was sourced from the National Blood Transfusion Service, a choice made on the basis of low cross-infection risk and ease of availability. This initial experiment was unsuccessful as the mixing of MTA powder with the supplied blood resulted in slurries that did not set to the usual consistency and the surface was too soft to be investigated. It was also possible to penetrate the entire depth of the samples with a dental probe. Therefore, in this situation performing compressive strength, surface microhardness and other physical tests were considered unpractical. This unexpected outcome was investigated by conducting the same experiment successfully with fresh blood with the method described for the main research experiments. It was, therefore, deduced that the incomplete hydration of MTA resulted from the addition of a citrate anticoagulant by the Blood Transfusion Service to the blood sample that was centrifuged to obtain the serum used. This addition is a standard practice by the Blood Transfusion Service (James 2005). This speculation is in accordance with the results of section 5.1.4 (page 130) that demonstrated the detrimental effects of low pH on the physical properties of MTA.

In the experimental groups of the present study, to exaggerate blood contamination of MTA, as occurs in some clinical applications, tooth coloured MTA powders were first mixed with, then exposed to WFH blood. In another experimental group, MTA powder was mixed with WFH blood diluted with distilled water (50% v/v) and then exposed to WFH blood. The latter situation most probably might happen while MTA is used for the repair of perforation, apexification and/or as a root-end filling following apical root resection. In the other

experimental groups the powder of tooth coloured ProRoot® MTA was mixed with distilled water and exposed to WFH blood to most closely simulate the clinical conditions where just the surface is exposed to blood, such as in direct pulp capping and/or pulpotomy. In the control groups MTA powder was mixed with and exposed to distilled water. According to the results the more blood becomes incorporated into MTA, the more the compressive strength, surface microhardness and push-out force of the material are reduced.

Haemoglobin or whole animal blood has been used in Portland cement as an air entrainment admixture to increase porosity (Remadnia *et al.* 2009). Jasiczak & Zielinski (2006) mixed powdered red blood cells taken from pigs and cows with Portland cement and demonstrated that even small amounts of the red blood cell powder resulted in reduced compressive strength and prolonged setting time of the cement. These findings have been explained by the air entrainment properties of blood proteins that affected the porous microstructure of cements (Remadnia *et al.* 2009). The air entrainment effects of blood on cement (Jasiczak & Zielinski 2006) and the resultant increased porosity (Remadnia *et al.* 2009) most likely explains the results of the present study, which demonstrated a decreased compressive strength of blood contaminated MTA and increased porosity as a result of blood contamination. These findings are in accordance with Hesaraki *et al.* (2006) who showed an increased porosity of calcium phosphate cement when mixed with an air-entrainment admixture.

The results of section 5.2.3.4 (page 172) revealed that the porosity of blood contaminated MTA was greater than the porosity of the control group most likely as a result of air entrainment of the blood. Greater blood incorporation into MTA resulted in greater porosity and lower compressive strength, surface microhardness and less push-out force. In the longer term, the physical characteristics of specimens that were mixed solely with blood did not

improve in comparison to the control group where compressive strength, surface microhardness and push-out force did increase over time. Those specimens that their surface were contaminated by WFH blood but not mixed with blood did also gain in terms of their physical properties over time.

At the microstructural level, the blood-contaminated groups exhibited a different morphology of crystals. Evaluation of the SEM images revealed a distinct lack of acicular crystals in all groups exposed to or mixed with WFH blood, when compared to control samples (5.2.3.5, page 174). Ismail *et al.* (2002) suggested that the bonds between particles of hydrated Portland cement are created by a dense meshwork of acicular crystals that radiate from the cement particles. Stutzman (2004) evaluated the microstructure of hydraulic cement using SEM and X-ray microanalysis and concluded that the interlinking crystal phase was composed of tricalcium aluminate and/or tetracalcium aluminoferrite. Therefore, in the present study, the reduced physical properties of MTA in the groups contaminated with blood is most likely explained by the lack of interlinking acicular crystals. The characteristic lack of interlinking acicular crystals has also been observed following MTA exposure to acidic conditions (section 5.1.4.7, page 136), which may replicate the clinical environment of infected tissues that have a lower pH than normal (Nekoofar *et al.* 2009). In section 5.2.3.5 (page 174), a similar lack of acicular crystals was observed despite the fact that the pH of healthy blood is slightly alkaline (pH 7.4). Future studies should attempt to determine the importance of the tricalcium aluminate and/or tetracalcium aluminoferrite crystal phases.

In addition to microstructural changes, uneven hemispherical expansion of MTA samples out of the ends of the cylindrical moulds was noted in all blood-contaminated groups (Figure 67, page 184). The most notable expansions were seen in the specimens mixed solely with and exposed to WFH blood. Unfortunately, due to the uneven hemispherical expansion of MTA,

precise dimensional measurements were unpractical. Storm *et al.* (2008) have also described the expansion of MTA when allowed to hydrate in a salt solution, which was used to simulate the *in vivo* environment.

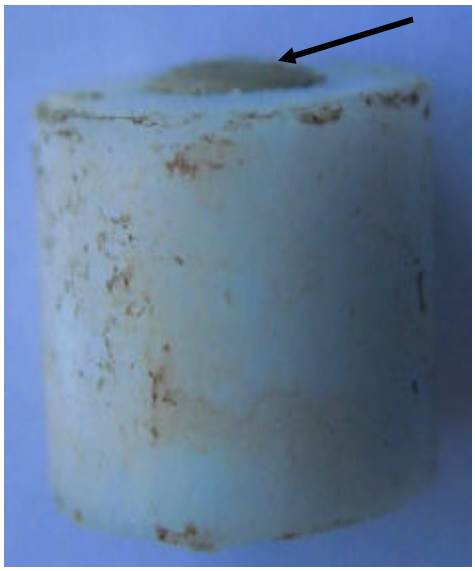


Figure 67: Uneven hemispherical expansion of MTA out of the end of a cylindrical mould that was exposed to WFH blood is shown by the arrow.

Gandolfi *et al.* (2009) examined the expansion of MTA when exposed to water, PBS and a mixture of PBS and FBS to replicate the tissue fluids encountered clinically. They found expansion of MTA exposed to the PBS and FBS mixture was less than that of PBS or the water control. They speculated that the proteins in tissue fluids adsorb onto the surface of MTA and block porosities, thus retarding the hydration processes and resulting in increased expansion. The effect of blood contamination on dye leakage of an initial prototype of MTA was investigated in an *ex vivo* endodontic surgery model, which concluded that blood contamination had no significant effect on dye leakage (Torabinejad *et al.* 1994b). The beneficial reduction in dye leakage (Torabinejad *et al.* 1994b) and bacterial penetration (Montellano *et al.* 2006) of blood contaminated samples of MTA may possibly be explained by the expansion of samples when exposed to blood proteins. In addition, Reyes-Carmona *et*

al. (2009) described the formation of an interfacial hydroxyapatite layer with tag-like structures at the junction of MTA and dentine following immersion of MTA samples in PBS, suggesting better adaptation. In sections 5.1.4.5 & 5.1.4.6 (pages 134 & 135) MTA samples were exposed to solutions at various pHs and a significantly lower push-out force was observed in specimens subjected to an acidic environment. In the present study on blood contamination, the same effect on push-out force was observed. This is in accordance with finding of VanderWeele *et al.* (2006) who reported that blood contaminated MTA decreased push-out force compared to controls. Therefore, it can be confirmed that the expansion observed in specimens of blood contaminated MTA was not associated with an increase in push-out force values. This may seem counter intuitive, but presumably the reduction of push-out force is a complex issue that is related to the inherent strength of the material as well as the nature of the interface between the material and dentine.

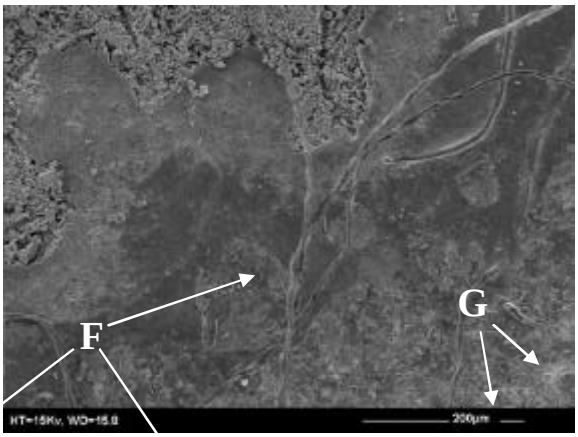


Figure 68: Scanning electron microscope image showing cotton fibres (F) on the surface of, and being incorporated into (G) MTA.

As an incidental SEM finding, incorporation of cotton fibres into the surface of uncontaminated MTA from the moist cotton pellet used to provide the humid environment

was observed (Figure 68, page 185). Therefore, for future studies and clinical applications, the use of an absorbable and non-fibrous material to maintain humidity during MTA setting is recommended.

In section 5.2.3.2 (page 170) the effect of blood contamination on the surface microhardness of MTA was studied. The surface microhardness of the material may provide an indication of the degree to which the material has undergone hydration during the initial setting reaction (Lee *et al.* 2004) and over time since the test is non-destructive (Igarashi *et al.* 1996). In this study, the surface microhardness of MTA was evaluated after four days and six months. There was no significant difference between the microhardness values after 4 days and 6 months within the specimens of each experimental group. However, at both incubation times the mean surface microhardness values of the specimens of the control group were significantly higher than other experimental groups in which MTA slurries were exposed to WFH blood following mixing of MTA powder with water or blood respectively ($p < 0.00001$). Thus, the results demonstrate that blood contamination has a detrimental effect on the surface microhardness of MTA.

In the study by Tingey *et al.* (2008) the bovine serum used was first frozen by the manufacturer and then thawed for use. According to the manufacturer there is the potential problem of flocculence on thawing due to denaturation of serum lipoproteins (http://tools.invitrogen.com/content/sfs/brochures/B-066802-Sera_Bro.pdf). Regular mixing during thawing is recommended and the possible need for centrifugation and refiltration is emphasised. Therefore, it is possible to hypothesise that the use of frozen and thawed blood products may have an effect on its biochemistry with the potential for differences between such products and fresh blood in terms of their interaction with MTA. Tingey *et al.* (2008) allowed 24 hours incubation of the MTA samples before the examination of the surface

microstructure. It is likely that the hydration of MTA was not complete at this time. In the present studies, the samples were incubated for four days before the initial testing. This time period is in accordance with Vanderweele *et al.* (2006) and Song *et al.* (2006) who suggested that MTA should be left for at least 72-96 hours to decrease the likelihood of displacement and increase surface microhardness and compressive strength. Bodanezi *et al.* (2008) also reported that the solubility of MTA decreased after 72 hours. In addition, Sluyk *et al.* (1998) concluded that for achieving the desirable sealability MTA should be untouched for at least 3 days when used to repair root perforations.

All of the experimental groups in contact with blood had a reduced hardness in comparison to the control groups. Therefore, it is possible to make a supported recommendation that clinicians should attempt to control bleeding when placing MTA in any clinical situation. In support of this finding, Tingey *et al.* (2008) reported that the presence of serum affected the setting reaction of MTA after examining the surface microstructure of MTA samples.

Other studies have reported that the setting of MTA is adversely affected by a number of environmental factors. These include an acidic pH (Lee *et al.* 2004, Saghiri *et al.* 2008) and an alkaline environment (Saghiri *et al.* 2010b). However, the most important issue to acknowledge is whether the adverse effect on the properties of MTA demonstrated by this study and others has a detrimental consequence for the material after placement and its subsequent longevity. Clearly, further research is necessary in this area.

In this study X-ray diffraction analysis was also used to determine the effect of blood contamination on the early stages of the hydration process of MTA. During the initial stage of the hydration process Ca^{2+} and OH^- ions are released from tricalcium silicate (C_3S) into the surrounding environment which, at supersaturation levels, forms calcium hydroxide (portlandite) precipitate and amorphous CSH (calcium silicate hydrate) gel (Camilleri 2007).

In the presence of sulphur and aluminium ions crystals of ettringite (hexacalcium aluminate trisulphate hydrate) are also formed (Camilleri 2008b). The sulphur and aluminium ions originate from the dissolution of gypsum and aluminate, respectively (Lee *et al.* 2007, Gandolfi *et al.* 2010b). The setting and strength of hydraulic cements have been attributed to the formation of CSH and ettringite on nucleation sites of calcium hydroxide (CH) crystals (Lee *et al.* 2007). Therefore, the formation of CH in the early stage is crucial for the progression of the hydration process (Banfill 1986). The antibacterial properties of MTA have been explained by the alkaline environment formed by the release of hydroxide ions (Zhang *et al.* 2009). Additionally, induction of hard tissue has been suggested to be a result of the presence of calcium ions (Shabahang *et al.* 1999).

MTA hydration in the presence of solutions containing phosphate ions, such as tissue fluid or blood, resulted in the precipitation of hydroxyapatite (HA) crystals (Sarkar *et al.* 2005), which has also been accredited for the bioactivity of MTA (Reyes-Carmona *et al.* 2009). In the present study, XRD analysis of MTA specimens exposed to blood did not demonstrate the presence of HA crystals, which may be due to the relatively short exposure period used when compared to longer term exposures described in other studies (Bozeman *et al.* 2006, Parirokh *et al.* 2009, Reyes-Carmona *et al.* 2009).

Analysis of specimens mixed with distilled water (group 1) revealed the presence of bismuth oxide, tricalcium silicate and calcium hydroxide. These results are consistent with those found by Camilleri (2008b) even though the incubation time and preparation technique were not identical.

Accordingly, the proposed hydration mechanism is:

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

expressed in cement nomenclature as:

$$2C_3S + 6H \rightarrow 3CSH + 3CH$$

Diffractograms of unhydrated MTA powder (group 4) and specimens mixed wholly with WHF blood (group 3) demonstrated a lack of reflections at 18.10, 28.69 and 34.10 $^{o}2\theta$ (▲ symbols in Figure 66, page 178), corresponding to CH, indicating that the hydration process had been impeded. In addition, the intensity of reflections corresponding to C_3S (○ symbols in Figure 66, page 178) were relatively higher than group 1 and 2, indicating that the transition of C_3S to crystalline CH was incomplete in group 3. However, the formation of amorphous CSH should not be discounted as in XRD analysis only crystalline formations are detectable. Additionally, the absence of this amorphous content could reflect the short hydration time (Camilleri 2008b). Therefore, for the future studies evaluation of the specimens after longer incubation times is suggested.

The CH phase was also seen in the diffractogram of specimens mixed with WFH blood diluted with distilled water (group 2). Camilleri (2007) showed that CSH takes up bismuth oxide during the hydration process. Accordingly, in the present study the lower intensity of bismuth oxide (■ symbols in Figure 66, page 178) shown in group 3, compared to the unhydrated specimens, suggests that CSH gel is being formed and taking up bismuth oxide. However, the lower intensity of bismuth oxide in groups 1 and 2, when compared to that of group 3, assumes the impeded hydration of the specimens in the latter group that were mixed entirely with WFH blood.

For elemental analysis of acicular crystals that were absent in blood contaminated specimens and for better understanding of the effect of WFH blood contamination on the hydration process of MTA slurries, EDX analysis was also employed. SEM analysis revealed several morphological differences between groups. Clusters of fine acicular crystals were only seen

in specimens mixed with distilled water (group 1) and not in specimens partially or wholly mixed with blood (groups 2 and 3, respectively). Spot analysis of the acicular crystals by EDX indicated that they were rich in sulphur and aluminium as compared to the background matrix (Fig. 2), suggestive of ettringite crystals.

Despite the similar appearance of the diffractograms of groups 1 and 2, the lack of acicular crystals in specimens that were partially or entirely mixed with blood (groups 2 and 3, respectively) can be explained by the inhibited hydration process due to the lower water concentration in these groups. Therefore, the SEM and XRD findings demonstrated the hydration state of specimens partially mixed with blood (group 2) to be more complete than those mixed entirely with blood (group 3) and less than that of the fully hydrated specimens (group 1), which is in agreement with the results for the compressive strength, surface microhardness and push-out force of blood contaminated MTA. The lower hydration reaction of blood contaminated specimens are likely to be the result of blood protein adhesion to crystal nucleation sites resulting in hydration inhibition (Gandolfi *et al.* 2009).

The specimens partially mixed with blood were created to best simulate the clinical applications of MTA, such as in direct pulp capping or repair of root perforations, in which MTA slurries often become partially mixed with blood during and after its placement. In addition, group 3 was included to represent the severe blood contamination that may also be experienced clinically when acute inflammation is present. Coleman *et al.* (2007) investigated the effect of simulated body fluid (SBF), the ionic composition of which approximates to that of human plasma (Kokubo & Takadama 2006), on set white Portland cement (WPC) and reported the absence of CH and ettringite as a result of WPC being in contact with SBF for 7 days at 37^{o}C. They attributed the absence of calcium hydroxide to the dissolution of the hydroxyl ions associated with hydroxyapatite formation (Coleman *et al.*

2007). In the present study, to better replicate the clinical situation, whole fresh human blood was used to contaminate MTA rather than SBF, despite the technical and ethical difficulties involved. Due to the differing methodologies used, direct comparison of the present findings with those of Coleman *et al* (2007) are not possible.

5.2.5. **Conclusion**

The hydration state of specimens partially mixed with blood was more complete than those mixed entirely with blood and less than specimens that were hydrated only with water. At the microstructure level, lack of formation of the crystalline calcium hydroxide in the early stage of the hydration process and the absent of acicular crystals, characteristic of ettringite crystals, in blood-contaminated specimens was a common finding. This can explain the reduction in compressive strength and surface microhardness. The further blood becomes incorporated into MTA, the more the compressive strength, surface microhardness, push-out force of the material are reduced. In addition, its porosity is increased. Therefore, in clinical situations in which blood becomes incorporated into MTA, its physical properties are likely to be compromised. Therefore, it might be suggested that when using MTA, attempts should be made to control bleeding. When only the surface of MTA is exposed to blood, its physical properties may improve over the time. However, when it is partially or solely mixed with blood it cannot hydrate properly and its physical properties were compromised substantially.

CHAPTER 6

CONCLUSIONS

6. Conclusions

- The hydration state of specimens partially mixed with blood was more complete than those mixed entirely with blood and less than specimens hydrated only with water;
- The scanning electron microscopy analysis revealed a lack of needle-like crystals when the material was in contact with more acidic solutions;
- The force needed for the displacement of MTA from root dentine to occur was significantly lower in samples stored at lower pH values;
- There was a trend for increased compressive strength, surface microhardness and push-out force over time in both etched and control groups;
- At the microstructural level, lack of formation of crystalline calcium hydroxide in the early stages of the hydration process and the absence of acicular crystals, characteristic of ettringite crystals, in blood-contaminated specimens was a common finding;
- Incomplete hydration due to blood contamination can explain the reduction in compressive strength and surface microhardness;
- The further blood becomes incorporated into MTA, the more the compressive strength, surface microhardness, push-out force of the material are reduced. In addition, its porosity is increased.
- A major problem with the weight of water in the ampoules supplied in the MTA packages was identified. The amount of water in the ampoules was inconsistent and less than the 0.35 g claimed by the manufacturer;
- Methods of mixing and placement of MTA significantly affected the hydration process and consequently the physical properties of the material;

- Application of pressure during the mixing of MTA affects the strength and hardness of the material. When saturation and pressure was used for the mixing of MTA powder and water, the best physical properties were observed when a pressure of 3.22 MPa was applied following the saturation of the powder;
- When greater pressures were applied to MTA following saturation of the powder its surface hardness reduced significantly; conversely, its maximum compressive strength occurred with minimum pressure;
- Application of ultrasonic vibration following either the manual mixing technique (saturation and pressure) and/or mechanical mixing technique (encapsulated MTA) resulted in the most favourable physical properties of the MTA specimens;
- To achieve the optimum physical properties of ProRoot? MTA (Dentsply Tulsa Dental) specimens prepared with a powder to water ratio of 3.0, as recommended by the manufacturer, was confirmed;
- The lowest and greatest compressive strength, Vickers surface microhardness, and push-out force values of MTA were found after exposure to pH levels of 4.4 and 7.4, respectively;

Recommendations

- In clinical situations in which blood becomes incorporated into MTA, its physical properties are likely to be compromised. Thus, when using MTA, attempts should be made to control bleeding.
- Delaying the placement of the final coronal restoration is recommended so that the material can acquire sufficient compressive strength and push-out force to withstand

the acid-etch procedure, the condensation pressures used during the placement of a restoration and/or indirect masticatory forces;

- In the clinical situation, when exposure to an acidic environment is unavoidable, an application of a thicker layer of MTA may be beneficial;
- In an infected and/or inflamed low pH environment, non-setting calcium hydroxide should be used initially to neutralize the acidity of the tissues;
- The ampoules supplied by the manufacturer of ProRoot? MTA (Dentsply Tulsa) should not be used to dispense the water used in research studies involving MTA. Rather, the water used to produce MTA specimens in future research studies should be weighed individually prior to mixing;
- The manufacturers are advised to change the batch weighing system in order to supply the correct amount of water;
- Development of different delivery systems for MTA packages are suggested;
- In future experimental investigations, in order to achieve consistency and standardise specimen preparation the use of controlled pressure when mixing MTA is essential. In addition, the use of the encapsulated pre-set proportions of MTA (GB patent No 0919270.9) offer an additional advantage;
- In an attempt to eliminate confounding variables and achieve standard MTA specimens during future experimental studies a consistent powder to water ratio of 3.00 and a coherent mixing and placement methodology should be employed;

Suggestions for further studies

- Further research into the significance of blood contamination on the outcome of MTA applications in the form of a clinical trial would be beneficial;
- Further study is needed to evaluate the correlation between the porosity and other physical properties of MTA including compressive strength and surface microhardness;
- Further study is required to evaluate the effect of blood contamination on the expansion of MTA and its correlation with the sealing ability of the material;
- Further study on the effect of blood contamination on setting time is also suggested;
- Further studies are suggested to evaluate the effect of various encapsulated mixing times on the physical and chemical properties of MTA while using the novel technique of encapsulated MTA;
- Further study is suggested to evaluate the effect of pH on the total porosity of MTA.

CHAPTER 7

REFERENCES

7. References

Abdullah D, Ford TR, Papaioannou S, Nicholson J, McDonald F (2002) An evaluation of accelerated Portland cement as a restorative material. *Biomaterials* **23**, 4001-10.

Abedi HR, Ingle JI (1995) Mineral trioxide aggregate: a review of a new cement. *Journal of the California Dental Association* **23**, 36-9.

Abou-Rass M, Frank AL, Glick DH (1980) The anticurvature filing method to prepare the curved root canal. *The Journal of the American Dental Association* **101**, 792-4.

Agamy HA, Bakry NS, Mounir MM, Avery DR (2004) Comparison of mineral trioxide aggregate and formocresol as pulp-capping agents in pulpotomized primary teeth. *Pediatric Dentistry* **26**, 302-9.

Aggarwal V, Jain A, Kabi D (2009) In vitro evaluation of effect of various endodontic solutions on selected physical properties of white mineral trioxide aggregate. *Australian Endodontic Journal*, Article published online : 23 OCT 2009, DOI: 10.1111/j.747-4477.2009.00211.x.

Al-Daafas A, Al-Nazhan S (2007) Histological evaluation of contaminated furcal perforation in dogs' teeth repaired by MTA with or without internal matrix. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **103**, e92-9.

Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, Oglesby S, Simon JH, Rotstein I (2006) Antibacterial effect of two mineral trioxide aggregate (MTA) preparations against Enterococcus faecalis and Streptococcus sanguis in vitro. *Journal of Endodontics* **32**, 1053-6.

Al-Hezaimi K, Naghshbandi J, Oglesby S, Simon JH, Rotstein I (2005) Human saliva penetration of root canals obturated with two types of mineral trioxide aggregate cements. *Journal of Endodontics* **31**, 453-6.

AlAnezi AZ, Zhu Q, Wang YH, Safavi KE, Jiang J (2011) Effect of selected accelerants on setting time and biocompatibility of mineral trioxide aggregate (MTA). *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **111**, 122-7.

Algera T, Kleverlaan C, De Gee A, Prahl-Andersen B, Feilzer A (2005) The influence of accelerating the setting rate by ultrasound or heat on the bond strength of glass ionomers used as orthodontic bracket cements. *The European Journal of Orthodontics* **27**, 472.

Alhadainy HA (1994) Root perforations. A review of literature. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **78**, 368-74.

American Association of Endodontists (2003) *Glossary of endodontic terms.* 7 edn. Chigago: American Association of Endodontists.

American National Standard, American Dental Association (2006) Endodontic Sealing Materials. *ANSI/ADA Specification No. 57*.

Aminoshariae A, Hartwell GR, Moon PC (2003) Placement of mineral trioxide aggregate using two different techniques. *Journal of Endodontics* **29**, 679-82.

Andreasen JO, Pitt Ford TR (1994) A radiographic study of the effect of various retrograde fillings on periapical healing after replantation. *Endodontics and Dental Traumatology* **10**, 276-81.

Antunes Bortoluzzi E, Juarez Broon N, Antonio Hungaro Duarte M, de Oliveira Demarchi AC, Monteiro Bramante C (2006) The use of a setting accelerator and its effect on pH and

calcium ion release of mineral trioxide aggregate and white Portland cement. *Journal of Endodontics* **32**, 1194-7.

Antunes Bortoluzzi E, Sivieri Araujo G, Maria Guerreiro Tanomaru J, Tanomaru-Filho M (2007) Marginal Gingiva Discoloration by Gray MTA: A Case Report. *Journal of Endodontics* **33**, 325-7.

Aqrabawi J (2000) Sealing ability of amalgam, super EBA cement, and MTA when used as retrograde filling materials. *British Dental Journal* **188**, 266-8.

Aranda M (2001) Full phase analysis of Portland clinker by penetrating synchrotron powder diffraction. *Analytical Chemistry* **73**, 151-6.

Arens DE, Torabinejad M (1996) Repair of furcal perforations with mineral trioxide aggregate: two case reports. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **82**, 84-8.

Asgary S, Parirokh M, Eghbal MJ, Brink F (2004) A comparative study of white mineral trioxide aggregate and white Portland cements using X-ray microanalysis. *Australian Endodontic Journal* **30**, 89-92.

Asgary S, Parirokh M, Eghbal MJ, Brink F (2005) Chemical differences between white and gray mineral trioxide aggregate. *Journal of Endodontics* **31**, 101-3.

Asgary S, Parirokh M, Eghbal MJ, Stowe S, Brink F (2006) A qualitative X-ray analysis of white and grey mineral trioxide aggregate using compositional imaging. *Journal of Materials Science. Materials in Medicine* **17**, 187-91.

Asrari M, Lobner D (2003) In vitro neurotoxic evaluation of root-end-filling materials. *Journal of Endodontics* **29**, 743-6.

Avis M (1994) Reading research critically. I. An introduction to appraisal: designs and objectives. *Journal of Clinical Nursing* **3**, 227-34.

Azuma M (2006) Fundamental mechanisms of host immune responses to infection. *Journal of Periodontal Research* **41**, 361-73.

Badr AE (2010) Marginal adaptation and cytotoxicity of bone cement compared with amalgam and mineral trioxide aggregate as root-end filling materials. *Journal of Endodontics* **36**, 1056-60.

Bakland LK (1992) Root resorption. *Dental Clinics of North America* **36**, 491-507.

Bakland LK (2000) Management of traumatically injured pulps in immature teeth using MTA. *Journal of the California Dental Association* **28**, 855-8.

Banchs F, Trope M (2004) Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *Journal of Endodontics* **30**, 196-200.

Banfill PFG (1986) Precipitation of calcium hydroxide in the presence of organic compounds. *Journal of Materials Science Letters* **5**, 33-4.

Barata TJE, Bresciani E, Adachi A, Fagundes TC, Carvalho CAR, Navarro MFL (2008) Influence of ultrasonic setting on compressive and diametral tensile strengths of glass ionomer cements. *Materials Research* **11**, 57.

Barker HA (1981) Amino acid degradation by anaerobic bacteria. *Annual Review of Biochemistry* **50**, 23-40.

Barlow S, Renwick AG, Kleiner J *et al.* (2006) Risk assessment of substances that are both genotoxic and carcinogenic: Report of an International Conference organized by EFSA and WHO with support of ILSI Europe. *Food and Chemical Toxicology* **44**, 1636-50.

Barrieshi-Nusair KM, Qudeimat MA (2006) A prospective clinical study of mineral trioxide aggregate for partial pulpotomy in cariously exposed permanent teeth. *Journal of Endodontics* **32**, 731-5.

Bates CF, Carnes DL, del Rio CE (1996) Longitudinal sealing ability of mineral trioxide aggregate as a root-end filling material. *Journal of Endodontics* **22**, 575-8.

Bates PH (1926) Portland cement research. *Journal of Chemical Education* **3**, 519.

Benenati FW (1997) Root resorption: types and treatment. *General Dentistry* **45**, 42-5.

Bensted J (2002) Hyrdation of Portland cement. In SN Ghosh ed. *Advances in cement technology: chemistry, manufacture and testing*; pp. 31-86. New Delhi, India: Tech Books International.

Bentz DP (1997) Three-dimensional computer simulation of Portland cement hydration and microstructure development. *Journal of the American Ceramic Society* **80**, 3-21.

Ber BS, Hatton JF, Stewart GP (2007) Chemical modification of proroot mta to improve handling characteristics and decrease setting time. *Journal of Endodontics* **33**, 1231-4.

Bergmans L, Moisiadis P, Van Meerbeek B, Quirynen M, Lambrechts P (2005) Microscopic observation of bacteria: review highlighting the use of environmental SEM. *International Endodontic Journal* **38**, 775-88.

Bernabe PF, Holland R, Morandi R *et al.* (2005) Comparative study of MTA and other materials in retrofilling of pulpless dogs' teeth. *Brazilian Dental Journal* **16**, 149-55.

Bidar M, Disfani R, Gharagozloo S, Khoynezhad S, Rouhani A (2010) Medication with calcium hydroxide improved marginal adaptation of mineral trioxide aggregate apical barrier. *Journal of Endodontics* **36**, 1679-82.

Black L, Breen C, Yarwood J, Deng CS, Phipps J, Maitland G (2006) Hydration of tricalcium aluminate (C 3 A) in the presence and absence of gypsum—studied by Raman spectroscopy and X-ray diffraction. *Journal of Materials Chemistry* **16**, 1263-72.

Blake A (1985) *Handbook of mechanics, materials, and structures* 1 edn. New York: Wiley-Interscience.

Bodanezi A, Carvalho N, Silva D *et al.* (2008) Immediate and delayed solubility of mineral trioxide aggregate and Portland cement. *Journal of Applied Oral Science* **16**, 127-31.

Bonson S, Jeansonne BG, Lallier TE (2004) Root-end filling materials alter fibroblast differentiation. *Journal of Dental Research* **83**, 408-13.

Bordallo HN, Aldridge LP, Desmedt A (2006) Water dynamics in hardened ordinary portland cement paste or concrete: from quasielastic neutron scattering. *The Journal of Physical Chemistry B* **110**, 17966-76.

Bortoluzzi EA, Broon NJ, Bramante CM, Felippe WT, Tanomaru Filho M, Esberard RM (2009) The influence of calcium chloride on the setting time, solubility, disintegration, and pH of mineral trioxide aggregate and white Portland cement with a radiopacifier. *Journal of Endodontics* **35**, 550-4.

Bortoluzzi EA, Broon NJ, Bramante CM, Garcia RB, de Moraes IG, Bernardineli N (2006) Sealing ability of MTA and radiopaque Portland cement with or without calcium chloride for root-end filling. *Journal of Endodontics* **32**, 897-900.

Bose R, Nummikoski P, Hargreaves K (2009) A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. *Journal of Endodontics* **35**, 1343-9.

Boumiz A, Vernet C, Tenoudji FC (1996) Mechanical properties of cement pastes and mortars at early ages : Evolution with time and degree of hydration. *Advanced Cement Based Materials* **3**, 94-106.

Bozeman TB, Lemon RR, Eleazer PD (2006) Elemental analysis of crystal precipitate from gray and white MTA. *Journal of Endodontics* **32**, 425-8.

Branco R, Hegdahl T (1983) Physical properties of some zinc phosphate and polycarboxylate cements. *Acta Odontologica Scandinavica* **41**, 349-53.

Braz MG, Camargo EA, Salvadori DM, Marques ME, Ribeiro DA (2006) Evaluation of genetic damage in human peripheral lymphocytes exposed to mineral trioxide aggregate and Portland cements. *Journal of Oral Rehabilitation* **33**, 234-9.

Brito-Junior M, Faria-e-Silva AL, Fonseca B, Camilo CC (2009) Sealing ability of MTA used as cervical barrier in intracoronal bleaching. *Acta odontologica latinoamericana : AOL* **22**, 118-22.

Bryan EB, Woollard G, Mitchell WC (1999) Nonsurgical repair of furcal perforations: a literature review. *General Dentistry* **47**, 274-8; quiz 9-80.

BS EN 843-4 (2005) Advanced technical ceramics — Mechanical properties of monolithic ceramics at room temperature — Part 4: Vickers, Knoop and Rockwell superficial hardness *London, UK: British Standards Organisation.*

Camargo SE, Camargo CH, Hiller KA, Rode SM, Schweikl H, Schmalz G (2009) Cytotoxicity and genotoxicity of pulp capping materials in two cell lines. *International Endodontic Journal* **42**, 227-37.

Camilleri J (2007) Hydration mechanisms of mineral trioxide aggregate. *International Endodontic Journal* **40**, 462-70.

Camilleri J (2008a) Characterization and chemical activity of Portland cement and two experimental cements with potential for use in dentistry. *International Endodontic Journal* **41**, 791-9.

Camilleri J (2008b) Characterization of hydration products of mineral trioxide aggregate. *International Endodontic Journal* **41**, 408-17.

Camilleri J (2008c) The chemical composition of mineral trioxide aggregate. *Journal of conservative dentistry : JCD* **11**, 141-3.

Camilleri J (2008d) The physical properties of accelerated Portland cement for endodontic use. *International Endodontic Journal* **41**, 151-7.

Camilleri J (2010) Hydration characteristics of calcium silicate cements with alternative radiopacifiers used as root-end filling materials. *Journal of Endodontics* **36**, 502-8.

Camilleri J (2011a) Evaluation of the effect of intrinsic material properties and ambient conditions on the dimensional stability of white mineral trioxide aggregate and Portland cement. *Journal of Endodontics* **37**, 239-45.

Camilleri J (2011b) Scanning electron microscopic evaluation of the material interface of adjacent layers of dental materials. *Dental Materials*, doi:10.1016/j.dental.2011.04.013

Camilleri J, Gandolfi MG (2010) Evaluation of the radiopacity of calcium silicate cements containing different radiopacifiers. *International Endodontic Journal* **43**, 21-30.

Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TR (2005a) The constitution of mineral trioxide aggregate. *Dental Materials* **21**, 297-303.

Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR (2005b) The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. *International Endodontic Journal* **38**, 834-42.

Camilleri J, Pitt Ford TR (2008) Evaluation of the effect of tracer pH on the sealing ability of glass ionomer cement and mineral trioxide aggregate. *Journal of Materials Science: Materials in Medicine* **19**, 2941-8.

Campbell JP, Thorpe JR (1990) Safe and easy eucapercha paste preparation. *Journal of Endodontics* **16**, 505-6.

Chen BC, Chou WC, Chen WY, Liao CM (2010) Assessing the cancer risk associated with arsenic-contaminated seafood. *Journal of Hazardous Materials* **181**, 161-9.

Chng HK, Islam I, Yap AU, Tong YW, Koh ET (2005) Properties of a new root-end filling material. *Journal of Endodontics* **31**, 665-8.

Chong BS, Pitt Ford TR, Hudson MB (2003) A prospective clinical study of Mineral Trioxide Aggregate and IRM when used as root-end filling materials in endodontic surgery. *International Endodontic Journal* **36**, 520-6.

Christiansen R, Kirkevang LL, Horsted-Bindslev P, Wenzel A (2009) Randomized clinical trial of root-end resection followed by root-end filling with mineral trioxide aggregate or smoothing of the orthograde gutta-percha root filling--1-year follow-up. *International Endodontic Journal* **42**, 105-14.

Clark PD (2007) An InVitro Leakage and Viscosity Analysis of Root End Filling Materials. Richmond: Virginia Commonwealth University.

Coleman NJ, Nicholson JW, Awosanya K (2007) A preliminary investigation of the in vitro bioactivity of white Portland cement. *Cement and Concrete Research* **37**, 1518-23.

Coomaraswamy KS, Lumley PJ, Hofmann MP (2007) Effect of bismuth oxide radioopacifier content on the material properties of an endodontic Portland cement-based (MTA-like) system. *Journal of Endodontics* **33**, 295-8.

Coomaraswamy KS, Lumley PJ, Shelton RM, Hofmann MP (2008) Evaluation of Different Radiopacifiers for an MTA-like Dental Cement. *Key Engineering Materials* **361-363**, 885-8.

Costa AT, Konrath F, Dedavid B, Weber JB, de Oliveira MG (2009) Marginal adaptation of root-end filling materials: an in vitro study with teeth and replicas. *The Journal of Contemporary Dental Practice* **10**, 75-82.

Costa AT, Post LK, Xavier CB, Weber JB, Gerhardt-Oliveira M (2008) Marginal adaptation and microleakage of five root-end filling materials: an in vitro study. *Minerva Stomatologica* **57**, 295-300.

Costa ED, de Souza-Filho FJ, Barbosa SV (2003) Tissue reactions to a component of root canal system bacteria: lipoteichoic acid. *Brazilian Dental Journal* **14**, 95-8.

Coutinho-Filho T, De-Deus G, Klein L, Manera G, Peixoto C, Gurgel-Filho ED (2008) Radiopacity and histological assessment of Portland cement plus bismuth oxide. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **106**, e69-77.

Cross WM, Sabnis KH, Kjerengtroen L, Kellar JJ (2000) Microhardness Testing of Fiber-Reinforced Cement Paste. *ACI Materials Journal* **97**.

Curry JJ (1945) Capsule. US Pat. 2382978.

Cutajar A, Mallia B, Abela S, Camilleri J (2011) Replacement of radiopacifier in mineral trioxide aggregate; characterization and determination of physical properties. *Dental Materials*, doi:10.1016/j.dental.2011.04.012.

Dammaschke T, Gerth HU, Zuchner H, Schafer E (2005) Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. *Dental Materials* **21**, 731-8.

Dammaschke T, Stratmann U, Wolff P, Sagheri D, Schafer E (2010a) Direct pulp capping with mineral trioxide aggregate: an immunohistologic comparison with calcium hydroxide in rodents. *Journal of Endodontics* **36**, 814-9.

Dammaschke T, Wolff P, Sagheri D, Stratmann U, Schafer E (2010b) Mineral trioxide aggregate for direct pulp capping: a histologic comparison with calcium hydroxide in rat molars. *Quintessence International* **41**, e20-30.

Danesh G, Dammaschke T, Gerth HU, Zandbiglari T, Schafer E (2006) A comparative study of selected properties of ProRoot mineral trioxide aggregate and two Portland cements. *International Endodontic Journal* **39**, 213-9.

Daoudi MF, Saunders WP (2002) In vitro evaluation of furcal perforation repair using mineral trioxide aggregate or resin modified glass lonomer cement with and without the use of the operating microscope. *Journal of Endodontics* **28**, 512-5.

Darvell BW (1981) Efficiency of mechanical trituration of amalgam. III. Practical comparisons. *Australian Dental Journal* **26**, 236-43.

De-Deus G, Canabarro A, Alves G, Linhares A, Senne MI, Granjeiro JM (2009a) Optimal cytocompatibility of a bioceramic nanoparticulate cement in primary human mesenchymal cells. *Journal of Endodontics* **35**, 1387-90.

De-Deus G, de Souza MC, Sergio Fidel RA, Fidel SR, de Campos RC, Luna AS (2009b) Negligible expression of arsenic in some commercially available brands of portland cement and mineral trioxide aggregate. *Journal of Endodontics* **35**, 887-90.

De-Deus G, Ximenes R, Gurgel-Filho ED, Plotkowski MC, Coutinho-Filho T (2005) Cytotoxicity of MTA and Portland cement on human ECV 304 endothelial cells. *International Endodontic Journal* **38**, 604-9.

De Bruyne MA, De Bruyne RJ, De Moor RJ (2006) Long-term assessment of the seal provided by root-end filling materials in large cavities through capillary flow porometry. *International Endodontic Journal* **39**, 493-501.

de Carvalho MC, Zuolo ML (2000) Orifice locating with a microscope. *Journal of Endodontics* **26**, 532-4.

de Chevigny C, Dao TT, Basrani BR *et al.* (2008) Treatment outcome in endodontics: the Toronto study--phases 3 and 4: orthograde retreatment. *Journal of Endodontics* **34**, 131-7.

de Morais CA, Bernardineli N, Garcia RB, Duarte MA, Guerisoli DM (2006) Evaluation of tissue response to MTA and Portland cement with iodoform. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **102**, 417-21.

Devito KL, Ortega AI, Haiter-Neto F (2004) Radiopacity of calcium hydroxide cement compared with human tooth structure. *Journal of Applied Oral Science* **12**, 290-3.

Ding SJ, Kao CT, Chen CL, Shie MY, Huang TH (2010) Evaluation of human osteosarcoma cell line genotoxicity effects of mineral trixoide aggregate and calcium silicate cements. *Journal of Endodontics* **36**, 1158-62.

Dorn SO, Gartner AH (1990) Retrograde filling materials: a retrospective success-failure study of amalgam, EBA, and IRM. *Journal of Endodontics* **16**, 391-3.

Duarte MA, De Oliveira Demarchi AC, Yamashita JC, Kuga MC, De Campos Fraga S (2005) Arsenic release provided by MTA and Portland cement. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **99**, 648-50.

Duarte MA, Demarchi AC, Yamashita JC, Kuga MC, Fraga Sde C (2003) pH and calcium ion release of 2 root-end filling materials. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **95**, 345-7.

Dummer PM, al-Omari MA, Bryant S (1998) Comparison of the performance of four files with rounded tips during shaping of simulated root canals. *Journal of Endodontics* **24**, 364-71.

Eglinton MS (1987) *Concrete and Its Chemical Behavior, 1st edn. London: Thomas Telford.*

Eidelman E, Holan G, Fuks AB (2001) Mineral trioxide aggregate vs. formocresol in pulpotomized primary molars: a preliminary report. *Pediatric Dentistry* **23**, 15-8.

El-Araby A, Al-Jabab A (2005) The influence of some dentin primers on calcium hydroxide lining cement. *Journal of Contemporary Dental Practice* **6**, 1-9.

Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD (2000) Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. *Brazilian Dental Journal* **11**, 3-9.

Fagundes TC, Barata T, Bresciani E, Cefaly D, Carvalho C, Navarro M (2006) Influence of ultrasonic setting on tensile bond strength of glass-ionomer cements to dentin. *The journal of adhesive dentistry* **8**, 401.

Farsi N, Alamoudi N, Balto K, Mushayt A (2005) Success of mineral trioxide aggregate in pulpotomized primary molars. *The Journal of clinical pediatric dentistry* **29**, 307-11.

Feldman R (1990) The porosity and pore structure of hydrated portland cement paste. *Materials Research Society Symposium Proceedings* **137**, 59-73.

Felekoglu B, Türkel S, Baradan B Effect of water cement ratio on the fresh and hardened properties of self-compacting concrete. *Building and Environment* **42**, 1795-802.

Ferk Luketic S, Malcic A, Jukic S, Anic I, Segovic S, Kalenic S (2008) Coronal Microleakage of Two Root-end Filling Materials Using a Polymicrobial Marker. *Journal of Endodontics* **34**, 201-3.

Fischer EJ, Arens DE, Miller CH (1998) Bacterial leakage of mineral trioxide aggregate as compared with zinc-free amalgam, intermediate restorative material, and Super-EBA as a root-end filling material. *Journal of Endodontics* **24**, 176-9.

Fowkes FG, Fulton PM (1991) Critical appraisal of published research: introductory guidelines. *British Medical Journal* **302**, 1136.

Fridland M, Rosado R (2003) Mineral trioxide aggregate (MTA) solubility and porosity with different water-to-powder ratios. *Journal of Endodontics* **29**, 814-7.

Fridland M, Rosado R (2005) MTA solubility: a long term study. *Journal of Endodontics* **31**, 376-9.

Fuks AB (2002) Current concepts in vital primary pulp therapy. *European Journal of Paediatric Dentistry* **3**, 115-20.

Funteas UR, Wallace JA, Fochtman EW (2003) A comparative analysis of Mineral Trioxide Aggregate and Portland cement. *Australian Endodontic Journal* **29**, 43-4.

Fuss J, Mount GJ, Makinson OF (1990) The effect of etching on a number of glass ionomer cements. *Australian Dental Journal* **35**, 338-44.

Fuss Z, Trope M (1996) Root perforations: classification and treatment choices based on prognostic factors. *Dental Traumatology* **12**, 255-64.

Gancedo-Caravia L, Garcia-Barbero E (2006) Influence of humidity and setting time on the push-out strength of mineral trioxide aggregate obturations. *Journal of Endodontics* **32**, 894-6.

Gandolfi MG, Iacono F, Agee K *et al.* (2009) Setting time and expansion in different soaking media of experimental accelerated calcium-silicate cements and ProRoot MTA. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **108**, e39-45.

Gandolfi MG, Pagani S, Perut F *et al.* (2008) Innovative silicate-based cements for endodontics: A study of osteoblast-like cell response. *Journal of biomedical materials research. Part A.*

Gandolfi MG, Taddei P, Tinti A, De Stefano Dorigo E, Rossi PL, Prati C (2010a) Kinetics of apatite formation on a calcium-silicate cement for root-end filling during ageing in physiological-like phosphate solutions. *Clinical Oral Investigations* **14**, 659-68.

Gandolfi MG, Van Landuyt K, Taddei P, Modena E, Van Meerbeek B, Prati C (2010b) Environmental scanning electron microscopy connected with energy dispersive x-ray analysis and Raman techniques to study ProRoot mineral trioxide aggregate and calcium silicate cements in wet conditions and in real time. *Journal of Endodontics* **36**, 851-7.

Gartner AH, Dorn SO (1992) Advances in endodontic surgery. *Dental Clinics of North America* **36**, 357-78.

Gemelli E, Lourenci S, Folgueras MV, Camargo NHA (2004) Assessment of industrial wastes in mortar layers deposited on stainless steel sheets of sinks. *&HUkPIFD***50**, 336-44.

Geurtsen W (2001) Biocompatibility of root canal filling materials. *Australian Endodontic Journal* **27**, 12-21.

Ghasemi Mobarakeh L, Semnani D, Morshed M (2007) A novel method for porosity measurement of various surface layers of nanofibers mat using image analysis for tissue engineering applications. *Journal of Applied Polymer Science* **106**, 2536-42.

Gilman JJ (1997) Chemical and physical" hardness". *Materials Research Innovations* **1**, 71-6.

Gomes-Filho JE, Watanabe S, Bernabe PF, de Moraes Costa MT (2009) A mineral trioxide aggregate sealer stimulated mineralization. *Journal of Endodontics* **35**, 256-60.

Gondim E, Jr., Gomes BP, Ferraz CC, Teixeira FB, Souza-Filho FJ (2002) Effect of sonic and ultrasonic retrograde cavity preparation on the integrity of root apices of freshly extracted human teeth: scanning electron microscopy analysis. *Journal of Endodontics* **28**, 646-50.

Gondim E, Jr., Kim S, de Souza-Filho FJ (2005) An investigation of microleakage from root-end fillings in ultrasonic retrograde cavities with or without finishing: a quantitative analysis. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **99**, 755-60.

Gondim E, Zaia AA, Gomes BP, Ferraz CC, Teixeira FB, Souza-Filho FJ (2003) Investigation of the marginal adaptation of root-end filling materials in root-end cavities prepared with ultrasonic tips. *International Endodontic Journal* **36**, 491-9.

Gong J, Wang J, Guan Z (2002) A comparison between Knoop and Vickers hardness of silicon nitride ceramics. *Materials Letters* **56**, 941-4.

Gorucu J, Gurgan S, Cakir FY, Bicer CO, Gorucu H (2011) The effect of different preparation and etching procedures on the microleakage of direct composite veneer restorations. *Photomed Laser Surg* **29**, 205-11.

Groves GW (1981) Microcrystalline calcium hydroxide in Portland cement pastes of low water/cement ratio. *Cement and Concrete Research* **11**, 713-8.

Gulsahi A, Gulsahi K, Ungor M (2007) Invasive cervical resorption: clinical and radiological diagnosis and treatment of 3 cases. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **103**, e65-72.

Hachmeister DR, Schindler WG, Walker WA, 3rd, Thomas DD (2002) The sealing ability and retention characteristics of mineral trioxide aggregate in a model of apexification. *Journal of Endodontics* **28**, 386-90.

Ham KA, Witherspoon DE, Gutmann JL, Ravindranath S, Gait TC, Opperman LA (2005) Preliminary evaluation of BMP-2 expression and histological characteristics during apexification with calcium hydroxide and mineral trioxide aggregate. *Journal of Endodontics* **31**, 275-9.

Hanehara S, Tomosawa F, Kobayakawa M, Hwang KR (2001) Effects of water/powder ratio, mixing ratio of fly ash, and curing temperature on pozzolanic reaction of fly ash in cement paste. *Cement and Concrete Research* **31**, 31-9.

Harcourt JK, Lautenschlager EP (1970) Trituration of amalgam. *Journal of Dental Research* **49**, 408-14.

Harrington PP (2005) Post retention with Mineral Trioxide Aggregate and accelerated Portland cement. Master of Science in Endodontics. Virginia, USA: West Virginia University.

Harrison JW (1992) Surgical management of endodontically treated teeth. *Current Opinion in Dentistry* **2**, 115-21.

Hashem AA, Hassanien EE (2008) ProRoot MTA, MTA-Angelus and IRM used to repair large furcation perforations: sealability study. *Journal of Endodontics* **34**, 59-61.

Hasheminia SM, Feizi G, Razavi SM, Feizianfard M, Gutknecht N, Mir M (2010) A comparative study of three treatment methods of direct pulp capping in canine teeth of cats: a histologic evaluation. *Lasers in Medical Science* **25**, 9-15.

Hawley M, Webb TD, Goodell GG (2010) Effect of varying water-to-powder ratios on the setting expansion of white and gray mineral trioxide aggregate. *Journal of Endodontics* **36**, 1377-9.

Heithersay GS (1994) External root resorption. *Annals of the Royal Australasian College of Dental Surgeons* **12**, 46-59.

Hesaraki S, Moztarzadeh F, Solati-Hashjin M (2006) Phase evaluation of an effervescent-added apatitic calcium phosphate bone cement. *Journal of Biomedical Materials Research Part B* **79**, 203.

Hewlett P (2004) *Lea's Chemistry of Cement and Concrete* 4th edn. Oxford: Butterworth-Heinemann.

Hewlett PC, Lea FM (1997) *Lea's chemistry of cement and concrete*: Arnold London.

Hiremath H, Gada N, Kini Y, Kulkarni S, Yakub SS, Metgud S (2008) Single-step apical barrier placement in immature teeth using mineral trioxide aggregate and management of periapical inflammatory lesion using platelet-rich plasma and hydroxyapatite. *Journal of Endodontics* **34**, 1020-4.

Holan G, Eidelman E, Fuks AB (2005) Long-term evaluation of pulpotomy in primary molars using mineral trioxide aggregate or formocresol. *Pediatric Dentistry* **27**, 129-36.

Holland R, de Souza V, Murata SS *et al.* (2001) Healing process of dog dental pulp after pulpotomy and pulp covering with mineral trioxide aggregate or Portland cement. *Brazilian Dental Journal* **12**, 109-13.

Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, Rutledge RE (2007) The anti-microbial effect against enterococcus faecalis and the compressive strength of two types of mineral trioxide aggregate mixed with sterile water or 2% chlorhexidine liquid. *Journal of Endodontics* **33**, 844-7.

Hoshino E, Kurihara-Ando N, Sato I *et al.* (1996) In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. *International Endodontic Journal* **29**, 125-30.

Hsien HC, Cheng YA, Lee YL, Lan WH, Lin CP (2003) Repair of perforating internal resorption with mineral trioxide aggregate: a case report. *Journal of Endodontics* **29**, 538-9.

Hu J, Stroeven P (2005) Local porosity analysis of pore structure in cement paste. *Cement and Concrete Research* **35**, 233-42.

Huang GT (2008) A paradigm shift in endodontic management of immature teeth: conservation of stem cells for regeneration. *Journal of Dentistry* **36**, 379-86.

Huang TH, Shie MY, Kao CT, Ding SJ (2008) The effect of setting accelerator on properties of mineral trioxide aggregate. *Journal of Endodontics* **34**, 590-3.

Huffman BP, Mai S, Pinna L *et al.* (2009) Dislocation resistance of ProRoot Endo Sealer, a calcium silicate-based root canal sealer, from radicular dentine. *International Endodontic Journal* **42**, 34-46.

Hwang YC, Lee SH, Hwang IN *et al.* (2009) Chemical composition, radiopacity, and biocompatibility of Portland cement with bismuth oxide. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*.

Igarashi S, Bentur A, Mindess S (1996) Microhardness testing of cementitious materials. *Advanced Cement Based Materials* **4**, 48-57.

Ilie N, Hickel R (2007) Mechanical behavior of glass ionomer cements as a function of loading condition and mixing procedure. *Dental Materials Journal* **26**, 526-33.

Ingle JI, Bakland LK, Baumgartner JC (2007) *Endodontics* 6th edn. Philadelphia: Lea & Febiger.

Ishikawa K, Takagi S, Chow LC, Ishikawa Y, Eanes ED, Asaoka K (1994) Behavior of a calcium phosphate cement in simulated blood plasma in vitro. *Dental Materials* **10**, 26-32.

Islam I, Chng HK, Yap AU (2005) Comparison of the root-end sealing ability of MTA and Portland cement. *Australian Endodontic Journal* **31**, 59-62.

Islam I, Chng HK, Yap AU (2006a) Comparison of the physical and mechanical properties of MTA and portland cement. *Journal of Endodontics* **32**, 193-7.

Islam I, Chng HK, Yap AU (2006b) X-ray diffraction analysis of mineral trioxide aggregate and Portland cement. *International Endodontic Journal* **39**, 220-5.

Ismail MA, Joer HA, Sim WH, Randolph MF (2002) Effect of cement type on shear behavior of cemented calcareous soil. *Journal of Geotechnical and Geoenvironmental Engineering* **128**, 520.

Jafarnia B, Jiang J, He J, Wang YH, Safavi KE, Zhu Q (2009) Evaluation of cytotoxicity of MTA employing various additives. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **107**, 739-44.

James V (2005) *Guidelines for the Blood Transfusion Services in the UK* 7th edn. London: Stationery Office Books.

Jasiczak J, Zielinski K (2006) Effect of protein additive on properties of mortar. *Cement and Concrete Composites* **28**, 451-7.

Karabucak B, Li D, Lim J, Iqbal M (2005) Vital pulp therapy with mineral trioxide aggregate. *Dental Traumatology* **21**, 240-3.

Keiser K, Johnson CC, Tipton DA (2000) Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. *Journal of Endodontics* **26**, 288-91.

Kettering JD, Torabinejad M (1995) Investigation of mutagenicity of mineral trioxide aggregate and other commonly used root-end filling materials. *Journal of Endodontics* **21**, 537-42.

Kim S, Kratchman S (2006) Modern endodontic surgery concepts and practice: a review. *Journal of Endodontics* **32**, 601-23.

Kleverlaan CJ, van Duinen RNB, Feilzer AJ (2004) Mechanical properties of glass ionomer cements affected by curing methods. *Dental Materials* **20**, 45-50.

Ko H, Yang W, Park K, Kim M (2010) Cytotoxicity of mineral trioxide aggregate (MTA) and bone morphogenetic protein 2 (BMP-2) and response of rat pulp to MTA and BMP-2. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **109**, e103-8.

Kogan P, He J, Glickman GN, Watanabe I (2006) The effects of various additives on setting properties of MTA. *Journal of Endodontics* **32**, 569-72.

Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F (1997) Mineral trioxide aggregate stimulates a biological response in human osteoblasts. *Journal of Biomedical Materials Research* **37**, 432-9.

Kokubo T, Takadama H (2006) How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials* **27**, 2907-15.

. RP DED\ DWKL7 6SnQJEHU / 6 &RP SDUDWLYH DQDO\VLV RI WKH SDUWLFOH PRUSKRORJ\ RI commercially available mineral trioxide aggregates and Portland cement: a study with a flow particle image analyzer. *Journal of Endodontics* **34**, 94-8.

Koulaouzidou EA, Economides N, Beltes P, Geromichalos G, Papazisis K (2008) In vitro evaluation of the cytotoxicity of ProRoot MTA and MTA Angelus. *Journal of Oral Science* **50**, 397-402.

Kuratate M, Yoshiba K, Shigetani Y, Yoshiba N, Ohshima H, Okiji T (2008) Immunohistochemical analysis of nestin, osteopontin, and proliferating cells in the reparative process of exposed dental pulp capped with mineral trioxide aggregate. *Journal of Endodontics* **34**, 970-4.

Laghios CD, Benson BW, Gutmann JL, Cutler CW (2000) Comparative radiopacity of tetracalcium phosphate and other root-end filling materials. *International Endodontic Journal* **33**, 311-5.

Lardner A (2001) The effects of extracellular pH on immune function. *Journal of Leukocyte Biology* **69**, 522-30.

Lawley GR, Schindler WG, Walker WA, 3rd, Kolodrubetz D (2004) Evaluation of ultrasonically placed MTA and fracture resistance with intracanal composite resin in a model of apexification. *Journal of Endodontics* **30**, 167-72.

Lazarus CH (1951) Amalgam. *Military Surgeon* **109**, 39-43.

Lee JH, Kim CS, Choi KH *et al.* (2010) The induction of bone formation in rat calvarial defects and subcutaneous tissues by recombinant human BMP-2, produced in Escherichia coli. *Biomaterials* **31**, 3512-9.

Lee SJ, Monsef M, Torabinejad M (1993) Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *Journal of Endodontics* **19**, 541-4.

Lee YL, Lee BS, Lin FH, Yun Lin A, Lan WH, Lin CP (2004) Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. *Biomaterials* **25**, 787-93.

Lee YL, Lin FH, Wang WH, Ritchie HH, Lan WH, Lin CP (2007) Effects of EDTA on the hydration mechanism of mineral trioxide aggregate. *Journal of Dental Research* **86**, 534-8.

LeGeros RZ (2008) Calcium phosphate-based osteoinductive materials. *Chemical Reviews* **108**, 4742-53.

Levenstein H (2002) Obturating teeth with wide open apices using mineral trioxide aggregate: a case report. *Journal of the South African Dental Association* **57**, 270-3.

Lolayekar N, Bhat SS, Hegde S (2009) Sealing ability of ProRoot MTA and MTA-Angelus simulating a one-step apical barrier technique--an in vitro study. *Journal of Clinical Pediatric Dentistry* **33**, 305-10.

Lota JS, Kendall K, Bensted J (2000) Mechanism for the modification of Portland cement hydration using polyacrylic acid. *Advances in Cement Research* **12**, 45-56.

Lotfi M, Vosoughhosseini S, Saghiri MA, Mesgariabbasi M, Ranjkesh B (2009) Effect of white mineral trioxide aggregate mixed with disodium hydrogen phosphate on inflammatory cells. *Journal of Endodontics* **35**, 703-5.

Loxley EC, Liewehr FR, Buxton TB, McPherson JC, 3rd (2003) The effect of various intracanal oxidizing agents on the push-out strength of various perforation repair materials. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **95**, 490-4.

Lu S, Landis EN, Keane DT (2006) X-ray microtomographic studies of pore structure and permeability in Portland cement concrete. *Materials and Structures* **39**, 611-20.

Maeda H, Nakano T, Tomokiyo A *et al.* (2010) Mineral trioxide aggregate induces bone morphogenetic protein-2 expression and calcification in human periodontal ligament cells. *Journal of Endodontics* **36**, 647-52.

Mah T, Basrani B, Santos JM *et al.* (2003) Periapical inflammation affecting coronally-inoculated dog teeth with root fillings augmented by white MTA orifice plugs. *Journal of Endodontics* **29**, 442-6.

Malcolm A, Leong H, Spowage A, Shacklock A (2007) Image segmentation and analysis for porosity measurement. *Journal of materials processing technology* **192**, 391-6.

Malmed SF (2004) *Handbook of Local Anesthesia, 5th edn. St Louis Mosby: Elsevier.*

Maltezos C, Glickman GN, Ezzo P, He J (2006) Comparison of the sealing of Resilon, Pro Root MTA, and Super-EBA as root-end filling materials: a bacterial leakage study. *Journal of Endodontics* **32**, 324-7.

Martell B, Chandler NP (2002) Electrical and dye leakage comparison of three root-end restorative materials. *Quintessence International* **33**, 30-4.

Martin RL, Monticelli F, Brackett WW *et al.* (2007) Sealing properties of mineral trioxide aggregate orthograde apical plugs and root fillings in an in vitro apexification model. *Journal of Endodontics* **33**, 272-5.

McCall DW, Douglass DC, Blyler Jr LL, Johnson GE, Jelinski LW, Bair HE (1984) Solubility and diffusion of water in low-density polyethylene. *Macromolecules* **17**, 1644-9.

Mente J, Geletneky B, Ohle M *et al.* (2010a) Mineral trioxide aggregate or calcium hydroxide direct pulp capping: an analysis of the clinical treatment outcome. *Journal of Endodontics* **36**, 806-13.

Mente J, Hage N, Pfefferle T *et al.* (2009) Mineral trioxide aggregate apical plugs in teeth with open apical foramina: a retrospective analysis of treatment outcome. *Journal of Endodontics* **35**, 1354-8.

Mente J, Hage N, Pfefferle T *et al.* (2010b) Treatment outcome of mineral trioxide aggregate: repair of root perforations. *Journal of Endodontics* **36**, 208-13.

Min KS, Park HJ, Lee SK *et al.* (2008) Effect of mineral trioxide aggregate on dentin bridge formation and expression of dentin sialoprotein and heme oxygenase-1 in human dental pulp. *Journal of Endodontics* **34**, 666-70.

Mitchell CA, Orr JF, Russell MD (1998) Capsulated versus hand-mixed glass-ionomer luting cements for post retention. *Journal of Dentistry* **26**, 47-51.

Mitchell PJ, Pitt Ford TR, Torabinejad M, McDonald F (1999) Osteoblast biocompatibility of mineral trioxide aggregate. *Biomaterials* **20**, 167-73.

0|OOHU *HYHUDW+ROPVWUyP $ ([DPLQDWLRQRI DORZ GHQVLW\ SRO\HWK\OHQH '3(
film after 15 years of service as an air and water vapour barrier. *Polymer Degradation and Stability* **73**, 69-74.

Monteiro Bramante C, Demarchi AC, de Moraes IG *et al.* (2008) Presence of arsenic in different types of MTA and white and gray Portland cement. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **106**, 909-13.

Montellano AM, Schwartz SA, Beeson TJ (2006) Contamination of tooth-colored mineral trioxide aggregate used as a root-end filling material: a bacterial leakage study. *Journal of Endodontics* **32**, 452-5.

Moretton TR, Brown CE, Jr., Legan JJ, Kafrawy AH (2000) Tissue reactions after subcutaneous and intraosseous implantation of mineral trioxide aggregate and ethoxybenzoic acid cement. *Journal of Biomedical Materials Research* **52**, 528-33.

Morse DR, O'Larnic J, Yesilsoy C (1990) Apexification: review of the literature. *Quintessence International* **21**, 589-98.

Moshaverinia A, Brantley WA, Chee WW *et al.* (2010) Measure of microhardness, fracture toughness and flexural strength of N-vinylcaprolactam (NVC)-containing glass-ionomer dental cements. *Dental Materials* **26**, 1137-43.

Munack J, Haubert H, Dogan S, Geurtsen W (2001) Effects of various storage media on surface hardness and structure of four polyacid-modified composite resins ("compomers"). *Clinical Oral Investigations* **5**, 254-9.

Murray PE, Garcia-Godoy F, Hargreaves KM (2007) Regenerative endodontics: a review of current status and a call for action. *Journal of Endodontics* **33**, 377-90.

Nair PN, Duncan HF, Pitt Ford TR, Luder HU (2008) Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial. *International Endodontic Journal* **41**, 128-50.

Nakayama A, Ogiso B, Tanabe N, Takeichi O, Matsuzaka K, Inoue T (2005) Behaviour of bone marrow osteoblast-like cells on mineral trioxide aggregate: morphology and expression of type I collagen and bone-related protein mRNAs. *International Endodontic Journal* **38**, 203-10.

Nandini S, Natanasabapathy V, Shivanna S (2010) Effect of various chemicals as solvents on the dissolution of set white mineral trioxide aggregate: an in vitro study. *Journal of Endodontics* **36**, 135-8.

Nawal RR, Parande M, Sehgal R, Naik A, Rao NR (2011) A comparative evaluation of antimicrobial efficacy and flow properties for Epiphany, Guttaflow and AH-Plus sealer. *International Endodontic Journal* **44**, 307-13.

Nekoofar MH, Ghandi MM, Hayes SJ, Dummer PM (2006) The fundamental operating principles of electronic root canal length measurement devices. *International Endodontic Journal* **39**, 595-609.

Nekoofar MH, Namazikhah MS, Sheykhrezae MS *et al.* (2009) pH of pus collected from periapical abscesses. *International Endodontic Journal* **42**, 534-8.

Ng YL, Mann V, Gulabivala K (2008a) Outcome of secondary root canal treatment: a systematic review of the literature. *International Endodontic Journal* **41**, 1026-46.

Ng YL, Mann V, Gulabivala K (2011) A prospective study of the factors affecting outcomes of non-surgical root canal treatment: part 2: tooth survival. *International Endodontic Journal.*

Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K (2008b) Outcome of primary root canal treatment: systematic review of the literature -- Part 2. Influence of clinical factors. *International Endodontic Journal* **41**, 6-31.

Nomoto R, McCabe JF (2001) Effect of mixing methods on the compressive strength of glass ionomer cements. *Journal of Dentistry* **29**, 205-10.

Oliver CM, Abbott PV (2001) Correlation between clinical success and apical dye penetration. *International Endodontic Journal* **34**, 637-44.

Orosco FA, Bramante CM, Garcia RB, Bernadineli N, Moraes IG (2008) Sealing ability of grar MTA AngelusTM, CPM TM and MBPc used as apical plugs. *Journal of Applied Oral Science* **16**, 50-4.

Osorio RM, Hefti A, Vertucci FJ, Shawley AL (1998) Cytotoxicity of endodontic materials. *Journal of Endodontics* **24**, 91-6.

Ozdemir HO, Ozcelik B, Karabucak B, Cehreli ZC (2008) Calcium ion diffusion from mineral trioxide aggregate through simulated root resorption defects. *Dental Traumatology* **24**, 70-3.

Panzarini SR, Holland R, de Souza V, Poi WR, Sonoda CK, Pedrini D (2007) Mineral trioxide aggregate as a root canal filling material in reimplanted teeth. Microscopic analysis in monkeys. *Dental Traumatology* **23**, 265-72.

Papadakis VG, Antiohos S, Tsimas S (2002) Supplementary cementing materials in concrete Part II: A fundamental estimation of the efficiency factor. *Cement and Concrete Research* **32**, 1533-8.

Paranjpe A, Zhang H, Johnson JD (2010) Effects of mineral trioxide aggregate on human dental pulp cells after pulp-capping procedures. *Journal of Endodontics* **36**, 1042-7.

Parirokh M, Askarifard S, Mansouri S, Haghdoost AA, Raoof M, Torabinejad M (2009) Effect of phosphate buffer saline on coronal leakage of mineral trioxide aggregate. *Journal of Oral Science* **51**, 187-91.

Parirokh M, Torabinejad M (2010a) Mineral trioxide aggregate: a comprehensive literature review--Part I: chemical, physical, and antibacterial properties. *Journal of Endodontics* **36**, 16-27.

Parirokh M, Torabinejad M (2010b) Mineral trioxide aggregate: a comprehensive literature review--Part III: Clinical applications, drawbacks, and mechanism of action. *Journal of Endodontics* **36**, 400-13.

Peppas NA, Langer R (1994) New challenges in biomaterials. *Science* **263**, 1715.

Pereira CL, Cenci MS, Demarco FF (2004) Sealing ability of MTA, Super EBA, Vitremer and amalgam as root-end filling materials. *Pesquisa odontologica brasileira = Brazilian oral research* **18**, 317-21.

Peters CI, Peters OA (2002) Occlusal loading of EBA and MTA root-end fillings in a computer-controlled masticator: a scanning electron microscopic study. *International Endodontic Journal* **35**, 22-9.

Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB (2010) Challenges in regenerative endodontics: a case series. *Journal of Endodontics* **36**, 536-41.

Pichardo MR, George SW, Bergeron BE, Jeansonne BG, Rutledge R (2006) Apical leakage of root-end placed SuperEBA, MTA, and Geristore restorations in human teeth previously stored in 10% formalin. *Journal of Endodontics* **32**, 956-9.

Pitt Ford TR (1983) Relation between seal of root fillings and tissue response. *Oral Surgery, Oral Medicine, Oral Pathology* **55**, 291-4.

Pitt Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP (1996) Using mineral trioxide aggregate as a pulp-capping material. *Journal of the American Dental Association* **127**, 1491-4.

Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP (1995) Use of mineral trioxide aggregate for repair of furcal perforations. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **79**, 756-63.

Plotino G, Pameijer CH, Grande NM, Somma F (2007) The Use of a Setting Accelerator and Its Effect on pH. *Journal of Endodontics* **33**, 81-95.

Powers JM, Wataha JC (2008) *Dental Materials: Properties and Manipulation* 9th edn. St. Louis: Mosby.

Primus CM (2006) Comments on arsenic release provided by MTA and Portland cement. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **101**, 416-7; author reply 7-8.

Primus CM (2009) Comments on testing for the presence of arsenic in MTA and portland cement. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **108**, 479-80; author reply 80-2.

Qu H, Wei M (2008) The effect of temperature and initial pH on biomimetic apatite coating. *Journal of Biomedical Materials Research Part B, Applied Biomaterial* **87**, 204-12.

Quinn J, Quinn G (1997) Indentation brittleness of ceramics: a fresh approach. *Journal of Materials Science* **32**, 4331-46.

Rai S, Chaturvedi S, Singh NB (2004) Examination of Portland cement paste hydrated in the presence of malic acid. *Cement and Concrete Research* **34**, 455-62.

Rai S, Singh NB, Singh NP (2006) Interaction of tartaric acid during hydration of Portland cement. *Indian Journal of Chemical Technology* **13**, 255-61.

Ramp LC, Broome JC, Ramp MH (2006) Hardness and wear resistance of two resin composites cured with equivalent radiant exposure from a low irradiance LED and QTH light-curing units. *American Journal of Dentistry* **19**, 31-6.

Reddi AH (2003) Marshall R. Urist: a renaissance scientist and orthopaedic surgeon. *The Journal of bone and joint surgery. American volume* **85-A Suppl 3**, 3-7.

Rehman K, Saunders WP, Foye RH, Sharkey SW (1996) Calcium ion diffusion from calcium hydroxide-containing materials in endodontically-treated teeth: an in vitro study. *International Endodontic Journal* **29**, 271-9.

5HPDGQLD$ 'KHLOO 50 /DLGRXGL%4XpQHXGHF0 8VHRIDQLPDOSURWHLQV foaming agent in cementitious concrete composites manufactured with recycled PET aggregates. *Construction and Building Materials* **23**, 3118-23.

Reyes-Carmona JF, Felippe MS, Felippe WT (2009) Biomineralization ability and interaction of mineral trioxide aggregate and white portland cement with dentin in a phosphate-containing fluid. *Journal of Endodontics* **35**, 731-6.

Reyes-Carmona JF, Felippe MS, Felippe WT (2010a) The biomineralization ability of mineral trioxide aggregate and Portland cement on dentin enhances the push-out strength. *Journal of Endodontics* **36**, 286-91.

Reyes-Carmona JF, Felippe MS, Felippe WT (2010b) A phosphate-buffered saline intracanal dressing improves the biomineralization ability of mineral trioxide aggregate apical plugs. *Journal of Endodontics* **36**, 1648-52.

Ribeiro DA, Duarte MA, Matsumoto MA, Marques ME, Salvadori DM (2005) Biocompatibility in vitro tests of mineral trioxide aggregate and regular and white Portland cements. *Journal of Endodontics* **31**, 605-7.

Ribeiro DA, Sugui MM, Matsumoto MA, Duarte MA, Marques ME, Salvadori DM (2006) Genotoxicity and cytotoxicity of mineral trioxide aggregate and regular and white Portland cements on Chinese hamster ovary (CHO) cells in vitro. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **101**, 258-61.

Riitano F (2005) Anatomic Endodontic Technology (AET)--a crown-down root canal preparation technique: basic concepts, operative procedure and instruments. *International Endodontic Journal* **38**, 575-87.

Roberts HW, Toth JM, Berzins DW, Charlton DG (2008) Mineral trioxide aggregate material use in endodontic treatment: A review of the literature. *Dental Materials* **24**, 149-64.

Roy CO, Jeansonne BG, Gerrets TF (2001) Effect of an acid environment on leakage of root-end filling materials. *Journal of Endodontics* **27**, 7-8.

Roy DM, Gouda GR (1975) Optimization of strength in cement pastes. *Cement and Concrete Research* **5**, 153-62.

Ryan JF (1929) The story of Portland cement. *Journal of Chemical Education* **6**, 1854.

6DDML (6SnQJEHJ / 6DSRXQDV* 0 DF$ 0DWM7 - ,QYHVWLJDWLRQRI
biocompatibility and marginal adaptation of root retrofilling materials. *Journal of Endodontics* **14**, 538-42.

Saghiri MA, Lotfi M, Joupari MD, Aeinehchi M, Saghiri AM (2010a) Effects of storage temperature on surface hardness, microstructure, and phase formation of white mineral trioxide aggregate. *Journal of Endodontics* **36**, 1414-8.

Saghiri MA, Lotfi M, Saghiri AM, Vosoughhosseini S, Aeinehchi M, Ranjkesh B (2009) Scanning electron micrograph and surface hardness of mineral trioxide aggregate in the presence of alkaline pH. *Journal of Endodontics* **35**, 706-10.

Saghiri MA, Lotfi M, Saghiri AM *et al.* (2008) Effect of pH on sealing ability of white mineral trioxide aggregate as a root-end filling material. *Journal of Endodontics* **34**, 1226-9.

Saghiri MA, Shokouhinejad N, Lotfi M, Aminsobhani M, Saghiri AM (2010b) Push-out bond strength of mineral trioxide aggregate in the presence of alkaline pH. *Journal of Endodontics* **36**, 1856-9.

6DLGRQ- +H- =KX4 6DIDYL. 6SnQJEHUJ /6 &HOODQGWLVVXHUHDFWLRQVWRPLQHUDO trioxide aggregate and Portland cement. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* **95**, 483-9.

Santos AD, Moraes JC, Araujo EB, Yukimitu K, Valerio Filho WV (2005) Physico-chemical properties of MTA and a novel experimental cement. *International Endodontic Journal* **38**, 443-7.

Sari S, Sonmez D (2006) Internal resorption treated with mineral trioxide aggregate in a primary molar tooth: 18-month follow-up. *Journal of Endodontics* **32**, 69-71.

Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I (2005) Physicochemical basis of the biologic properties of mineral trioxide aggregate. *Journal of Endodontics* **31**, 97-100.

Schmalz G (1998) Concepts in biocompatibility testing of dental restorative materials. *Clinical Oral Investigations* **1**, 154-62.

Schmid CE (1998) Calcium hydroxide package and method of forming same. United State Patent: 5819921.

Schmidlin PR, Zehnder M, Schlup-0DWWNR&*|KULQJ71 ,QWHUIDFHHYDOXDWLRQDIWHU manual and ultrasonic insertion of standardized class I inlays using composite resin materials of different viscosity. *Acta Odontologica* **63**, 205-12.

Schmitt D, Lee J, Bogen G (2001) Multifaceted use of ProRoot MTA root canal repair material. *Pediatric Dentistry* **23**, 326-30.

Schwartz RS, Mauger M, Clement DJ, Walker WA, 3rd (1999) Mineral trioxide aggregate: a new material for endodontics. *Journal of the American Dental Association* **130**, 967-75.

Seltzer S, Naidorf IJ (1985) Flare-ups in endodontics: I. Etiological factors. *Journal of Endodontics* **11**, 472-8.

Shabahang S, Torabinejad M (2000) Treatment of teeth with open apices using mineral trioxide aggregate. *Practical periodontics and aesthetic dentistry : PPAD* **12**, 315-20; quiz 22.

Shabahang S, Torabinejad M, Boyne PP, Abedi H, McMillan P (1999) A comparative study of root-end induction using osteogenic protein-1, calcium hydroxide, and mineral trioxide aggregate in dogs. *Journal of Endodontics* **25**, 1-5.

Shah PM, Chong BS, Sidhu SK, Pitt Ford TR (1996) Radiopacity of potential root-end filling materials. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **81**, 476-9.

Shahi S, Rahimi S, Yavari HR, Shakouie S, Nezafati S, Abdolrahimi M (2007) Sealing ability of white and gray mineral trioxide aggregate mixed with distilled water and 0.12% chlorhexidine gluconate when used as root-end filling materials. *Journal of Endodontics* **33**, 1429-32.

Shazad N, Kennedy JG (1994) A comparison of the compressive strength of calcium hydroxide based lining materials. *The European journal of prosthodontics and restorative dentistry* **3**, 33-40.

Shelby MD (1988) The genetic toxicity of human carcinogens and its implications. *Mutation Research/Genetic Toxicology* **204**, 3-15.

Sheth JJ, Jensen ME, Sheth PJ, Versteeg J (1989) Effect of etching glass-ionomer cements on bond strength to composite resin. *Journal of Dental Research* **68**, 1082-7.

Shipper G, Grossman ES, Botha AJ, Cleaton-Jones PE (2004) Marginal adaptation of mineral trioxide aggregate (MTA) compared with amalgam as a root-end filling material: a low-vacuum (LV) versus high-vacuum (HV) SEM study. *International Endodontic Journal* **37**, 325-36.

Shokouhinejad N, Nekoofar MH, Iravani A, Kharrazifard MJ, Dummer PM (2010) Effect of acidic environment on the push-out bond strength of mineral trioxide aggregate. *Journal of Endodontics* **36**, 871-4.

Silva MJ, Vieira LQ, Sobrinho AP (2008) The effects of mineral trioxide aggregates on cytokine production by mouse pulp tissue. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **105**, e70-6.

Simon S, Rilliard F, Berdal A, Machtou P (2007) The use of mineral trioxide aggregate in one-visit apexification treatment: a prospective study. *International Endodontic Journal* **40**, 186-97.

Singh NB, Prabha S, Singh AK (1986a) Effect of lactic acid on the hydration of Portland cement. *Cement and Concrete Research* **16**, 545-53.

Singh NB, Singh AK, Prabha Singh S (1986b) Effect of citric acid on the hydration of Portland cement. *Cement and Concrete Research* **16**, 911-20.

Sluyk SR, Moon PC, Hartwell GR (1998) Evaluation of setting properties and retention characteristics of mineral trioxide aggregate when used as a furcation perforation repair material. *Journal of Endodontics* **24**, 768-71.

Smith ED, Martin FE (1990) Acid etching of a glass ionomer cement base: SEM study. *Australian Dental Journal* **35**, 236-40.

Song JS, Mante FK, Romanow WJ, Kim S (2006) Chemical analysis of powder and set forms of Portland cement, gray ProRoot MTA, white ProRoot MTA, and gray MTA-Angelus. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **102**, 809-15.

Souza NJ, Justo GZ, Oliveira CR, Haun M, Bincoletto C (2006) Cytotoxicity of materials used in perforation repair tested using the V79 fibroblast cell line and the granulocyte-macrophage progenitor cells. *International Endodontic Journal* **39**, 40-7.

Stabholz A, Friedman S, Abed J (1985) Marginal adaptation of retrograde fillings and its correlation with sealability. *Journal of Endodontics* **11**, 218-23.

Steffen R, van Waes H (2009) Understanding mineral trioxide aggregate/Portland-cement: A review of literature and background factors. *European Archives of Paediatric Dentistry* **10**, 93-7.

Storm B, Eichmiller FC, Tordik PA, Goodell GG (2008) Setting expansion of gray and white mineral trioxide aggregate and Portland cement. *Journal of Endodontics* **34**, 80-2.

Stutzman P (2004) Scanning electron microscopy imaging of hydraulic cement microstructure. *Cement and Concrete Composites* **26**, 957-66.

Tagger M, Katz A (2004) A standard for radiopacity of root-end (retrograde) filling materials is urgently needed. *International Endodontic Journal* **37**, 260-4.

Tang HM, Torabinejad M, Kettering JD (2002) Leakage evaluation of root end filling materials using endotoxin. *Journal of Endodontics* **28**, 5-7.

Tang Y, Li X, Yin S (2010) Outcomes of MTA as root-end filling in endodontic surgery: a systematic review. *Quintessence international (Berlin, Germany : 1985)* **41**, 557-66.

Tani Y, Ida K (1978) Adhesiveness of the encapsulated composite restorative materials to human enamel. *Kyoto Daigaku Kokukagaku Kiyo. Bulletin of Stomatology, Kyoto University* **18**, 40-6.

Tanomaru-Filho M, da Silva GF, Duarte MA, Goncalves M, Tanomaru JM (2008) Radiopacity evaluation of root-end filling materials by digitization of images. *Journal of Applied Oral Science* **16**, 376-9.

Taylor HFW (1997) *Cement Chemistry*, 2nd edn. London: Thomas Telford.

Theodosopoulou JN, Niederman R (2005) A systematic review of in vitro retrograde obturation materials. *Journal of Endodontics* **31**, 341-9.

Thibodeau B, Trope M (2007) Pulp revascularization of a necrotic infected immature permanent tooth: case report and review of the literature. *Pediatric Dentistry* **29**, 47-50.

Thomson A, Kahler B (2010) Regenerative endodontics--biologically-based treatment for immature permanent teeth: a case report and review of the literature. *Australian Dental Journal* **55**, 446-52.

Thomson TS, Berry JE, Somerman MJ, Kirkwood KL (2003) Cementoblasts maintain expression of osteocalcin in the presence of mineral trioxide aggregate. *Journal of Endodontics* **29**, 407-12.

Tingey MC, Bush P, Levine MS (2008) Analysis of mineral trioxide aggregate surface when set in the presence of fetal bovine serum. *Journal of Endodontics* **34**, 45-9.

Tonetti M, Cavallero A, Botta GA, Niederman R, Eftimiadi C (1991) Intracellular pH regulates the production of different oxygen metabolites in neutrophils: effects of organic acids produced by anaerobic bacteria. *Journal of Leukocyte Biology* **49**, 180-8.

Torabinejad M (1994) Passive step-back technique. *Oral Surgery, Oral Medicine, Oral Pathology* **77**, 398-401.

Torabinejad M (2004) Clinical applications of mineral trioxide aggregate. *Alpha Omegan* **97**, 23-31.

Torabinejad M, Chivian N (1999) Clinical applications of mineral trioxide aggregate. *Journal of Endodontics* **25**, 197-205.

Torabinejad M, Dean J (1995) Tooth filling material and method of use. *United State Patent No. 5,415,547*. USA. Loma Linda University.

Torabinejad M, Ford TR, Abedi HR, Kariyawasam SP, Tang HM (1998) Tissue reaction to implanted root-end filling materials in the tibia and mandible of guinea pigs. *Journal of Endodontics* **24**, 468-71.

Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR (1994a) Dye leakage of four root end filling materials: effects of blood contamination. *Journal of Endodontics* **20**, 159-63.

Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR (1995a) Investigation of mineral trioxide aggregate for root-end filling in dogs. *Journal of Endodontics* **21**, 603-8.

Torabinejad M, Hong CU, McDonald F, Pitt Ford TR (1995b) Physical and chemical properties of a new root-end filling material. *Journal of Endodontics* **21**, 349-53.

Torabinejad M, Hong CU, Pitt Ford TR, Kaiyawasam SP (1995c) Tissue reaction to implanted super-EBA and mineral trioxide aggregate in the mandible of guinea pigs: a preliminary report. *Journal of Endodontics* **21**, 569-71.

Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD (1995d) Cytotoxicity of four root end filling materials. *Journal of Endodontics* **21**, 489-92.

Torabinejad M, Lee SJ, Hong CU (1994b) Apical marginal adaptation of orthograde and retrograde root end fillings: a dye leakage and scanning electron microscopic study. *Journal of Endodontics* **20**, 402-7.

Torabinejad M, Parirokh M (2010) Mineral trioxide aggregate: a comprehensive literature review--part II: leakage and biocompatibility investigations. *Journal of Endodontics* **36**, 190-202.

Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR (1995e) Bacterial leakage of mineral trioxide aggregate as a root-end filling material. *Journal of Endodontics* **21**, 109-12.

Torabinejad M, Smith PW, Kettering JD, Pitt Ford TR (1995f) Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. *Journal of Endodontics* **21**, 295-9.

Torabinejad M, Turman M (2011) Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: a case report. *Journal of Endodontics* **37**, 265-8.

Torabinejad M, Watson TF, Pitt Ford TR (1993) Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *Journal of Endodontics* **19**, 591-5.

Towler M, Bushby A, Billington R, Hill R (2001) A preliminary comparison of the mechanical properties of chemically cured and ultrasonically cured glass ionomer cements, using nano-indentation techniques. *Biomaterials* **22**, 1401-6.

Trost SM (2005) Method for verifying quality. US Patent App. 20,050/150,306.

Tselnik M, Baumgartner JC, Marshall JG (2004) Bacterial leakage with mineral trioxide aggregate or a resin-modified glass ionomer used as a coronal barrier. *Journal of Endodontics* **30**, 782-4.

Tsuda H, Ochiai K, Suzuki N, Otsuka K (2010) Butyrate, a bacterial metabolite, induces apoptosis and autophagic cell death in gingival epithelial cells. *Journal of Periodontal Research* **45**, 626-34.

Tunc ES, Sonmez IS, Bayrak S, Egilmez T (2008) The evaluation of bond strength of a composite and a compomer to white mineral trioxide aggregate with two different bonding systems. *Journal of Endodontics* **34**, 603-5.

Tunca YM, Aydin C, Ozen T, Seyrek M, Ulusoy HB, Yildiz O (2007) The effect of mineral trioxide aggregate on the contractility of the rat thoracic aorta. *Journal of Endodontics* **33**, 823-6.

Tymkowicz S, Steffes RF (1997) Vibration study for consolidation of Portland cement concrete. *Journal of the Transportation Research Board* **1574**, 109-15.

Urist MR (1965) Bone: formation by autoinduction. *Science (New York, N.Y.)* **150**, 893-9.

Urist MR, Lietze A, Dawson E (1984) Beta-tricalcium phosphate delivery system for bone morphogenetic protein. *Clinical Orthopaedics and Related Research*, 277-80.

USFDA (1997) Mineral Trioxide Aggregate K964174. *Food and Drug Administration United States*.

USFDA (1998a) Mineral Trioxide Aggregate K980332. *Food and Drug Administration United States*.

USFDA (1998b) Mineral Trioxide Aggregate K981620. *Food and Drug Administration United States*.

Valois CR, Costa ED, Jr. (2004) Influence of the thickness of mineral trioxide aggregate on sealing ability of root-end fillings in vitro. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **97**, 108-11.

van der Varst PG, Brekelmans WA, de Vree JH, de Groot R (1993) Mechanical performance of a dental composite: probabilistic failure prediction. *Journal of Dental Research* **72**, 1249-56.

Vandersteenhoven JJ, Spector M (1983) Histological investigation of bone induction by demineralized allogeneic bone matrix: a natural biomaterial for osseous reconstruction. *Journal of Biomedical Materials Research* **17**, 1003-14.

Vanderweele RA, Schwartz SA, Beeson TJ (2006) Effect of blood contamination on retention characteristics of MTA when mixed with different liquids. *Journal of Endodontics* **32**, 421-4.

Vogt BF, Xavier CB, Demarco FF, Padilha MS (2006) Dentin penetrability evaluation of three different dyes in root-end cavities filled with mineral trioxide aggregate (MTA). *Braz Oral Res* **20**, 132-6.

Walker MP, Diliberto A, Lee C (2006) Effect of setting conditions on mineral trioxide aggregate flexural strength. *Journal of Endodontics* **32**, 334-6.

Ward J (2002) Vital pulp therapy in cariously exposed permanent teeth and its limitations. *Australian Endodontic Journal* **28**, 29-37.

Watts JD, Holt DM, Beeson TJ, Kirkpatrick TC, Rutledge RE (2007) Effects of pH and mixing agents on the temporal setting of tooth-colored and gray mineral trioxide aggregate. *Journal of Endodontics* **33**, 970-3.

Wei YM, Tomita B, Hiramatsu Y, Miyatake A, Fujii T, Yoshinaga S (2003) Hydration behavior and compressive strength of cement mixed with exploded wood fiber strand obtained by the water-vapor explosion process. *Journal of Wood Science* **49**, 317-26.

Wiltbank KB, Schwartz SA, Schindler WG (2007) Effect of selected accelerants on the physical properties of mineral trioxide aggregate and Portland cement. *Journal of Endodontics* **33**, 1235-8.

Witherspoon DE, Ham K (2001) One-visit apexification: technique for inducing root-end barrier formation in apical closures. *Practical Procedures & Aesthetic Dentistry* **13**, 455-60; quiz 62.

Wray S (1988) Smooth muscle intracellular pH: measurement, regulation, and function. *American Journal of Physiology* **254**, C213-25.

Wu MK, Dummer PM, Wesselink PR (2006) Consequences of and strategies to deal with residual post-treatment root canal infection. *International Endodontic Journal* **39**, 343-56.

Wunsch G (2008) Confounding and control. *Demographic Research* **16**, 97.

Xavier CB, Weismann R, de Oliveira MG, Demarco FF, Pozza DH (2005) Root-End Filling Materials: Apical Microleakage and Marginal Adaptation. *Journal of Endodontics* **31**, 539-42.

Yan P, Peng B, Fan B, Fan M, Bian Z (2006) The effects of sodium hypochlorite (5.25%), Chlorhexidine (2%), and Glyde File Prep on the bond strength of MTA-dentin. *Journal of Endodontics* **32**, 58-60.

Yang IS, Lee DS, Park JT, Kim HJ, Son HH, Park JC (2010) Tertiary dentin formation after direct pulp capping with odontogenic ameloblast-associated protein in rat teeth. *Journal of Endodontics* **36**, 1956-62.

Yeung P, Liewehr FR, Moon PC (2006) A quantitative comparison of the fill density of MTA produced by two placement techniques. *Journal of Endodontics* **32**, 456-9.

Yildirim T, Er K, Tasdemir T, Tahan E, Buruk K, Serper A (2010) Effect of smear layer and root-end cavity thickness on apical sealing ability of MTA as a root-end filling material: a bacterial leakage study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **109**, e67-72.

Yildirim T, Gencoglu N (2009) Use of mineral trioxide aggregate in the treatment of horizontal root fractures with a 5-year follow-up: report of a case. *Journal of Endodontics* **35**, 292-5.

Zakizadeh P, Marshall SJ, Hoover CI *et al.* (2008) A novel approach in assessment of coronal leakage of intraorifice barriers: a saliva leakage and micro-computed tomographic evaluation. *Journal of Endodontics* **34**, 871-5.

Zealand CM, Briskie DM, Botero TM, Boynton JR, Hu JC (2010) Comparing gray mineral trioxide aggregate and diluted formocresol in pulpotomized human primary molars. *Pediatric Dentistry* **32**, 393-9.

Zeferino EG, Bueno CE, Oyama LM, Ribeiro DA (2010) Ex vivo assessment of genotoxicity and cytotoxicity in murine fibroblasts exposed to white MTA or white Portland cement with 15% bismuth oxide. *International Endodontic Journal.*

Zeikus JG (1980) Chemical and fuel production by anaerobic bacteria. *Annual Review of Microbiology* **34**, 423-64.

Zhang H, Pappen FG, Haapasalo M (2009) Dentin enhances the antibacterial effect of mineral trioxide aggregate and bioaggregate. *Journal of Endodontics* **35**, 221-4.

Zhao X, He W, Song Z, Tong Z, Li S, Ni L (2011) Mineral trioxide aggregate promotes odontoblastic differentiation via mitogen-activated protein kinase pathway in human dental pulp stem cells. *Molecular Biology Reports*, DOI 10.1007/s11033-011-0728-z.