PHYSICAL PROPERTIES OF MODIFIED CALCIUM SILICATE-BASED CEMENT (CSC) AS ROOT CANAL MEDICAMENT

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ABSTRACT

Title: Physical properties of modified Calcium Silicate-Based Cement (CSC) As Root Canal Medicament.

Objectives: To evaluate the physical properties of Calcium Silicate-Based Cement (CSC) by retarding its setting time after mixing with 2% Chlorhexidine gel (CHX) to be used as a root canal medicament.

Methods: Three types of CSC were used in this study: white Portland cement (Aalborg, Malaysia), white ProRoot MTA (Tulsa, USA), and Biodentine (Septodont, France). CSCs were mixed with 2% CHX (n=8) with ratio of 2g powder:1g CHX. Calcium Hydroxide paste (Master-Dent, UAS) was used as a control in this study. The setting time, calcium ion release, pH of the water surrounding the specimens, film thickness and flowability of the CSC/CHX mixture (experimental cements) were assessed and measured following the standards of the ISO specification. Differences between materials and changes over time were compared with ANOVA and Tukey's post-hoc tests at a significance level of 0.05.

Results: CSC/CHX mixture did not set until 84 days. Calcium ion release of the experimental cements were significantly higher compared to control over the period of 14 days ($P<0.001$). The mean pH value were above 11.45 for all materials tested in this study over the period of 14 days, however, there was a statistical significant difference between them ($P<0.001$). The results also showed that there were no significant difference in film thickness of the experimental cements compared to control ($P>0.05$), however, the results of the flowability of the experimental cements was significantly higher than the control ($P<0.05$).

Conclusions: The addition of 2% CHX to CSCs is retarding or inhibiting its setting reaction over a period of 84 days. The calcium ion release and flowability of this

CSC/CHX mixture cement were found to be better than Calcium Hydroxide. Therefore, this experimental cement has the potential to be used as an enhanced root canal medicament. Further research on this formulation (particularly biocompatibility and antibacterial properties) is needed.

ABSTRAK

Title: Sifat Fizikal Simen Berasaskan kalsium Silikat (CSC) Sebagai Ubatan Rawatan Akar.

Objektif: Untuk menilai sifat-sifat fizikal simen berasaskan silikat kalsium (CSC) dengan memperlahankan masa terbenamnya selepas bergaul dengan 2% Chlorhexidine gel (CHX) untuk digunakan sebagai ubatan rawatan akar.

Kaedah: Tiga jenis CSC telah digunakan dalam kajian ini: putih simen Portland (Aalborg, Malaysia), putih ProRoot MTA (Tulsa, Amerika Syarikat), dan Biodentine (Septodont, Perancis). CSC dicampurkan dengan 2% CHX (n = 8) dengan nisbah (serbuk 2g: 1g CHX). Kalsium pes Hidroksida (Master-Dent, UAS) telah digunakan sebagai kawalan dalam kajian ini. Masa penyediaan, melepaskan ion kalsium, pH air yang mengelilingi spesimen, ketebalan filem dan kebolehaliran CSC / campuran CHX (simen eksperimen) telah dinilai dan diukur mengikut standard spesifikasi ISO. Perbezaan dalam bahan-bahan dan perubahan dari masa ke masa dibandingkan dengan ANOVA Tukey dan ini ujian post-hoc di tahap kepentingan sebanyak 0.05.

Keputusan: Masa penyediaan campuran / CHX CSC tidak ditetapkan teupohnya. pelepasan ion kalsium daripada simen eksperimen adalah jauh lebih tinggi berbanding kawalan tempoh 14 hari ($P < 0.001$). Nilai min pH adalah 11.45 ke atas untuk semua bahan-bahan yang diuji dalam kajian ini tempoh 14 hari, walau bagaimanapun, terdapat perbezaan yang signifikan di antara mereka ($P < 0.001$). Keputusan juga menunjukkan bahawa tidak terdapat perbezaan yang signifikan dalam ketebalan filem daripada simen eksperimen berbanding dgngan kawalan ($P > 0.05$), walau bagaimanapun, keputusan kebolehaliran daripada simen eksperimen adalah lebih tinggi daripada kawalan ($P < 0.05$).

Kesimpulan: Penambahan 2% CHX untuk CSC adalah untuk memperlahankan atau menghalang reaksi terbenamnya dalam tempoh 84 hari. Pembebasan kalsium ion dan kebolehaliran ini CSC / CHX campuran simen didapati lebih baik daripada Kalsium

Hidroksida. Oleh itu, simen eksperimen ini mempunyai potensi untuk menbaiki ubat rawatan akar. Benyelidikan lanjut mengenai penggubalan ini (terutamanya biocompatibility dan ciri-ciri antibakteria) diperlukan.

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LIST OF SYMBOLS AND ABBREVIATIONS

$Ca(OH)_2$	:	Calcium Hydroxide
CSC	:	Calcium Silicate-based Cement
NaOCl	:	Sodium Hypochlorite
CHX	:	Chlorhexidine Gel
MTA	:	Mineral Trioxide Aggregate
PC	:	Portland Cement
WPC	:	White Portland Cement
Bio	:	Biodentine
EDTA	:	Ethylenediaminetetraacetic acid
MTFS	:	Microtensile fracture strength
PDL	:	Periodontal ligament
CEM	:	Calcium Enriched Mixture
CER	:	Cimento Endodntico Rapido

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background

An intracanal medicament is a "temporary placement of medicament with good biocompatibility into root canals for the purpose of inhibiting coronal invasion of bacteria from the oral cavity" (Tronstad et al., 2000). It is an antimicrobial agent placed in the root canal among appointments to demolish remaining bacteria and stop reinfection (Weine 2004). It is used after shaping and cleaning the root canal to reduce periapical tissue inflammation and to achieve a root canal free of bacteria after instrumentation (Chong & Ford, 1992). It helps to control inflammatory root resorption, remove apical exudate and prevent contamination among treatment visits (Farhad & Mohammadi, 2005). Several commonly used intracanal medicaments include calcium hydroxide, phenolics, aldehydes, halides, steroids, antibiotics (Torabinejad & Walton, 2009) and chlorhexidine (Sinha et al., 2013).

The use of EDTA, NaOCl, triple antibiotic paste (TAP), or $Ca(OH)_2$ was found to significantly reduce dentin microhardness, flexure strength and root resistance to fracture (Yassen & Platt, 2013),(Zhang et al., 2010),(Yassen et al., 2013).

Chlorhexidine is used as a gluconate salt and is a cationic bis-guanide molecular structure. It has been broadly utilized in dentistry as a root canal medicament owing to its broad spectrum antimicrobial activity, low toxicity and its solubility in water (Kumar, 2013; Oliveira et al., 2007) thus, being an effective therapy of infected root systems. It was reported by various studies that chlorhexidine is highly efficient in removal of *E. faecalis* within dentinal tubules. It has been demonstrated to attain 78% negative cultures after a week of application. However, the disadvantage is that it does not have any detoxifying ability against endotoxins and it does not act as a physical

barrier against microbial recolonization. Therefore, it stays in the canal for a shorter time (Sinha et al., 2013). Moreover, chlorhexidine with calcium hydroxide and chlorhexidine alone exhibited more antibacterial efficiency against *E faecalis* than calcium hydroxide alone (Sinha et al., 2013).

Calcium hydroxide $Ca(OH)_2$ has been successfully utilized in endodontic treatment for decades since it has the potential to stimulate calcified tissue formation (Freeman et al., 1994) and induce antimicrobial properties (Kontakiotis et al., 1995, Lima et al., 2012) that are extensivly attributed to its high pH. Ca(OH)2 has been recommended for use in different endodontic procedures such as an indirect and direct pulp capping (Willershausen et al., 2011, Leye Benoist et al., 2012) inter appointment intracanal dressing (Sjögren et al., 1991), treatment of internal and external root resorption (Benenati 2001, Cunha et al., 2011) apexification (Yas-sen et al., 2012), treatment of horizontal and vertical root fractures (Maki et al., 2005, Misra & Toumba, 2008), perforation repair (ElDeeb et al., 1982), apexogenesis (Cvek 1978) and pulp regeneration (Cehreli et al., 2011, Chen et al., 2012).

Calcium silicate based cements such as ProRoot MTA (Dentsply, USA), MTA Angelus (Angelus, Brazil) and many others have been recommended for many clinical uses including retrograde filling, perforation repair, apexification, pulp-capping (Nair et al., 2008; Pace et al., 2008; Torabinejad & Chivian, 1999). These cements are contemporary biologically active materials (Gandolfi et al., 2010; Taddei et al., 2009) and have been suggested to biomimic the dentine remineralization (Gandolfi et al., 2008; Gandolfi et al., 2011; Gandolfi et al., 2010; Tay et al., 2007). CSC contains tricalcium silicate, dicalcium silicate, and tricalcium aluminate (Chen et al., 2009) whereas MTA powder is basically a mixture of bismuth oxide and Portland cement (PC) and has been used successfully in dental applications for the previous decade (Chen et al., 2009;

Torabinejad et al., 1995). Since the early 1990s, MTA was investigated for endodontic applications and then given approval for endodontic use by the U.S. Food and Drug Administration in 1998 (Robertset al., 2008). However, bioceramic materials with a uniform consistency and shorter setting time during placement might provide a useful substitute to MTA with enhanced handling characteristics (Islam et al., 2006). Recently, a new bioactive cement (CSC), Biodentine (Septodont, UK) was newly launched in the dental market in 2010 as a dentine substitute. Biodentine is stated to be utilized as a dentine restorative material in addition to endodontic indications similar to those of MTA (Han & Okiji, 2011). The consistency of Biodentin is similar to that of phosphate cement (Dammaschke, 2010). Although MTA has many favourable properties that support its clinical uses, there are several shortcomings. The setting time of MTA has been reported to be about 3 hours which would result in better marginal adaptation and less shrinkage (Torabinejad et al., 1995).

However, some clinical situations warrant the use of an additional barrier with a fast set time to protect the integrity of MTA during the setting period. Moreover, when MTA is used as a root-end filling material, it may wash out of the preparation if special care is not taken. The manufacturer (ProRoot MTAs and MTAs Angelus) recommends mixing MTA with sterile water. This produces a grainy, sandy mixture which is typically difficult to deliver to the required site and hard to compact adequately. However, CSC including MTA have not been used as root canal medicament since the removal of MTA from the root canal after being fully set and cured is almost impossible and extremely difficult. Several commonly used additives to MTA have the potential to enhance the working properties of the cement such as saline, 2% lidocaine, 3.0% NaOCl gel, chlorhexidine gluconate gel, K-Y Jelly, 3% and 5% $CaCl_2$. Several of these additives such as NaOCl gel may reduce the setting time and improve the handling characteristics of the mixture; they may also provide antimicrobial action. On the other hand, chlorhexidine gluconate

gel may retard and impaired the setting time of the cement (Kogan et al., 2006). Furthermore, It was reported by several researchers that MTA perhaps coud be a superior material when compared to calcium hydroxide. According to Mente et al., (2010) MTA appeared to favour long-term pulp vitality after direct pulp capping when compared with calcium hydroxide (Mente et al., 2010) Calcium hydroxide has been shown to be less effective against Enterococcus faecalis and Candida albicans (Mohammadi & Dummer, 2011). Therefore, the prime concern was the assessment of the Calcium Silicate–based Cement (CSC) and its characteristics as an intracanal medicament.

1.2 Aim

To use Calcium Silicate–based Cement (CSC) as an intracanal medicament by retarding its setting time and exploring its physical properties.

1.3 Objectives

- To evaluate the setting time of different types of CSC after mixing with different vehicles.
- To determine and compare the physical properties of different types of CSC, namely (calcium ions release, pH, film thickness and flowability and radiopacity after mixing with vehicle- 2% Chlorhexidine gel.

1.4 Null Hypothesis

There is no difference in the physical and mechanical properties (setting time, pH, calcium ions release, film thickness, and flow ability) between the different types of CSC when mixed with 2% Chlorhexidine gel.

CHAPTER 2: LITERATURE REVIEW

2.1 Intracanal medicaments- Historical origin and development

The historical background of intracanal medicaments belongs to a predate era where Beeechwood creosote had been initially used to sterilize and desensitize a tooth ache (Flagg, 1860). Later, the use of orangewood with phenol was recommended by Richmond to deaden the pulp (Milas, 1976). Medicaments such as eugenol, phenols like camphorated monoparacholorophenol, cresatin, cresol, thymol and creosote have been used extensively in the former days that dates back to 1800s. Several combinations of these drugs were manufactured which played a significant role in root canal therapy during the 19-20th century. However, Formocresol had come into being that played a vital role and was found to be efficacious in root canal therapy (Buckley, 1904).

2.2 Ideal properties of Intracanal medicaments

1. It should be a potential germicidal and bactericacidal in action.
2. It should be non-irritant.
3. It should not dissolve in solution.
4. It should have a long term bactericidal effect.
5. It should readily respond in field of blood, serum and protein cognate of tissue.
6. It should penetrate the tissue adequately.
7. It should not impede in the repair mechanism of periapical tissues.
8. It should be instilled in the root canal with ease.
9. It should be abe to get inactivated and be neutralized within the culture vehicle.
10. It should not counteract the coronal microleakage and disseminate through the interim restoration.

2.3 Classification of intracanal medicaments

According to Grossman intracanal medicament can be classified as

1. Essential oils

• Eugenol

2. Phenolic compounds

• Phenol

• Parachlorophenol

• Camphorated parachlorophenol

• Cresol

• Formocresol

• Creosote

• Cresatin

• Cresanol

3. N2

4. Salt of heavy metals

• Metaphen

• Merthiolate

• Mercurophen

5. Halogens

• Sodium hypochlorite

• Iodides

• Chlorexidine

6. Quaternary ammonium compounds

• 9-aminoacidine

7. Fatty acids

Propionic acid

• Caproic acid

• Cuprylic acid

8. Sulphonamides

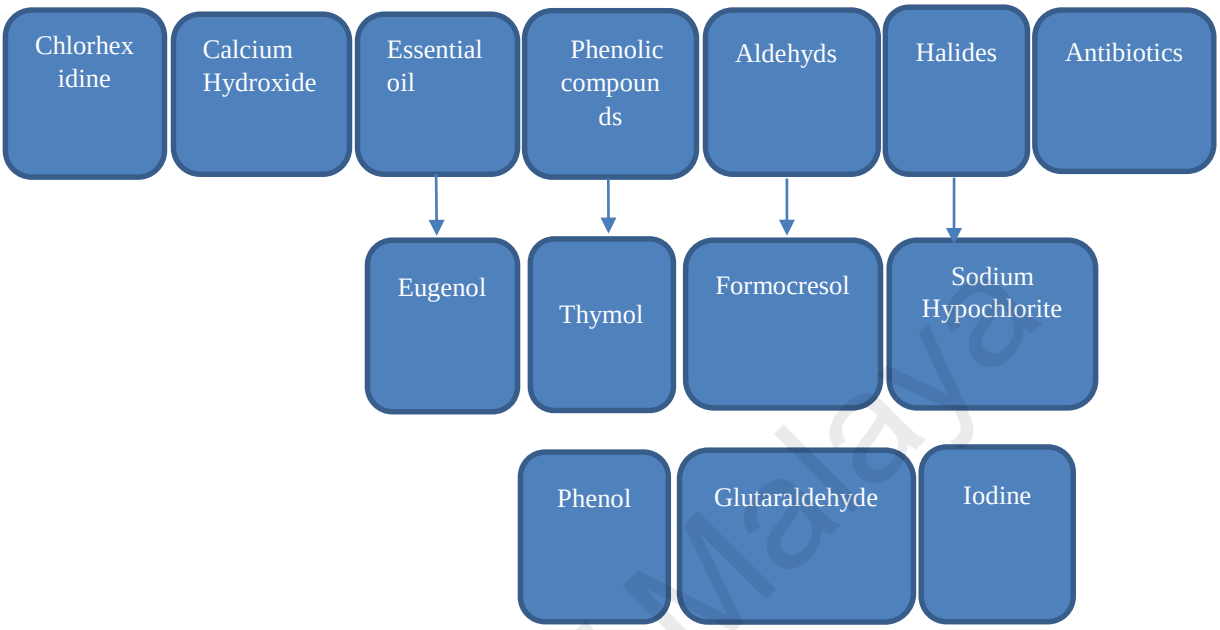

Figure 2.1: Commonly used Intracanal medicaments.

2.4 Calcium Hydroxide – Gold Standard Intracanal Medicament

Calcium hydroxide was introduced to endodontics as a direct pulp-capping agent (Hermann, 1920).

2.4.1 Characteristics of calcium hydroxide

2.4.1.1 Chemical composition

$Ca(OH)_2$ is a white neutral powder with a molecular weight of 74.08 (Farhad & Mohammadi, 2005). It has low solubility in water (around 1.2 g L)1 at 25 C) which decreases with a rise in temperature (Siqueira et al., 1999).

The pure powder is insoluble in alcohol and has a high pH (approximately 12.5–12.8) (Farhad & Mohammadi, 2005). It acts as a strong base via the ionic detachment of OH

and Ca_2+ ions and their effect on vital tissues thus, results in hard-tissue deposition and owes to its antibacterial activity (Siqueira et al., 1999).

2.4.1.2 $Ca(OH)_2$ and Chlorhexidine

Chlorhexidine reaches its optimal antibactierial activity within a range of 5.5-7.0 pH (Athanassiadis et al., 2007) and by the addition of $Ca(OH)_2$ to CHX results in the deposition of CHX particles thus, reducing its efficiency (Mohammadi & Abbott, 2009). It has been reported that the pH of $Ca(OH)_2$ when mixed with CHX remains unchanged because it has low solubility in water (Haenni et al., 2003). Hence, the efficacy of adding $Ca(OH)_2$ to CHX remains uncertain (Athanassiadis et al., 2007), in order to obtain a wide-spectrum antimicrobial preparation with a long-lasting effect (Waltimo et al. 1999). However, the effects of these irrigation solutions on $Ca(OH)_2$ and vice-versa have not been studied in detail. When used as a root canal medicament, CHX was found to be highly effective than $Ca(OH)_2$ in abolishing *E. faecalis* within dentinal tubules (Athanassiadis et al., 2007).

It was reported by another study that when 1%CHX gel and $Ca(OH)_2$ were mixed in a preparation of 50 : 50 ratio, it was found to be more effective in removing *E. faecalis* from dentine tubules (Almyroudi et al., 2002). These findings were also supported by Almeida et al. (2006). Moreover, 2% CHX gel was reported to be more effective against *E. faecalis* found in human dentine followed by $Ca(OH)_2$ and CHX/$Ca(OH)_2$ alone (Schäfer & Bössmann, 2005). Chlorhexidine had revealed to increase the calcium ion release of $Ca(OH)_2$ medication (Signoretti et al., 2011).

2.4.1.3 Effect of $Ca(OH)_2$ on dentine

Following 7–84 days of $Ca(OH)_2$ application, the microtensile fracture strength (MTFS) of teeth was reduced by almost 50% (Rosenberg et al., 2007). $Ca(OH)_2$ after a 5

week application in the bovine dentine reduced fracture strength by 32% and found to be maintained in petri dishes (White et al., 2002). Moreover, after 1 year of $Ca(OH)_2$ applcation in sheep dentine, the fracture strength was reduced by 50% (Andreasen et al., 1989). It has been reported that the mean elastic modulus of dentine significantly increased due to exposure to $Ca(OH)_2$ paste resulting in more susceptible to fracture (Kawamoto et al., 2008). The flexural strength of dentine was also found to be reduced when treated with $Ca(OH)_2$ (Grigoratos et al., 2001).

However, significant decrease in the fracture resistance of dentine was observed when $Ca(OH)_2$ was utilized as an intracanal medication in human root dentine (Doyon et al., 2005). Furthermore, dentine exposed to $Ca(OH)_2$ for a prolonged period (6 months to 1 year) results in lower fracture resistance and reduced flexural strength. Therefore, other treatment approaches such as the apical barrier method utilizing mineral trioxide aggregate (MTA) should be used to treat teeth with open apices and non-vital pulps following a short period of $Ca(OH)_2$ medication should be indicated.

2.4.1.4 Antimicrobial activity

The antimicrobial activity of $Ca(OH)_2$ is associated with the release of (OH) ions in an aqueous atmosphere (Siqueira 2001). Hydroxyl ions are extremely oxidant free radicals that show highly reactivity with numerous biomolecules. This reactivity is high and the free radical rarely diffuses away from places of generation (Siqueira et al., 1999).The lethal influences of hydroxyl ions on bacterial cells are possibly due to the protein denaturation, damage to the bacterial cytoplasmic membrane and damage to the DNA(Siqueira et al., 1999).

2.4.1.5 $Ca(OH)_2$ removal from root canals

$Ca(OH)_2$ as a root canal medicament when placed into the canal has to be removed from it before obturation. Laboratory researches have discovered that fragments of

$Ca(OH)_2$ can obstruct the diffusion of root sealers inside dentinal tubules (Çalt & Serper, 1999), obstruct the adhesion of sealers to dentine, increase the leakage of root canal fillings (Kim & Kim, 2002) and possibly act together with zinc oxide eugenol sealers making them granular and brittle (Margelos et al., 1997). Hence, $Ca(OH)_2$ has been recommended to be completely eliminated before root canal obturation (Mohammadi & Dummer, 2011). However, it was reported by several studies that despite effectvely removing $Ca(OH)_2$ from the root canal utilizing various vehicles like normal saline, 3% NaOCl + 17% EDTA, 3% sodium hypochlorite (NaOCl) as irrigants along with hand filing, 45% of the canal surface remained coated by $Ca(OH)_2$.The study concluded that the quantity of $Ca(OH)_2$ powder in the paste did not affect elimination, however, the vehicle did (Lambrianidis et al., 1999). Using NaOCl or 15% EDTA alone as irrigants did not affect the removal of $Ca(OH)_2$ from the root canal, but was improved when used in combination with hand instrumentation (Margelos et al., 1997).

Oil-based $Ca(OH)_2$ paste is much harder to eliminate than $Ca(OH)_2$ powder mixed with sterilized water. Both 10% citric acid and 17% EDTA are able to eliminate $Ca(OH)_2$ powder mixed with sterilized water whereas 10% citric acid was found to be better than EDTA in removing an oil-based $Ca(OH)_2$ paste (Nandini et al., 2006). In another study where comparing the removal efficiency of $Ca(OH)_2$/CHX solution, $Ca(OH)_2$/CHX gel and $Ca(OH)_2$/saline pastes by instrumentation with or without a patency file and irrigation with EDTA and NaOCl solutions, remnants were found in all canals regardless of the trial material or use of filing. When examining the root canal as a whole, $Ca(OH)_2$/CHX gel paste had significantly more amount of residue in comparison to $Ca(OH)_2$/CHX solution paste that had less residue than the other two groups with or without the use of filing. They also observed that the use of filing assisted removal of extra medicament in the apical third of straight canals (Lambrianidis et al., 2006). Ultrasonic devices is another source of removing remnants of $Ca(OH)_2$ from the root canal. Another study used various

techniques such as hand filing, ultrasonics and rotary instrumentation alongwith vehicles like combinations of EDTA with NaOCl irrigation to assess the amount of $Ca(OH)_2$ remaining in root canals after removal. None of the procedures were capable to eliminate the $Ca(OH)_2$ entirely. However. ultrasonic and rotary methods eliminated $Ca(OH)_2$ more signiicantly than irrigants (Kenee et al., 2006).

2.4.1.6 Clinical applications of Calcium Hydroxide

$Ca(OH)_2$ was initially recommended to be used in teeth suffering from horizontal root fractures (Cvek, 1974). Cvek et al, (1974) reported that the canal at the level of fracture line could be compared to apical foramen of an immature tooth. Hence, it was assumed by him that the repair mechanism could be homologous to the apexification opted for an open apex tooth (Cvek, 1974). Root canal therapy using $Ca(OH)_2$ is advantageous perhaps because of its antibacterial activity and its potential to stimulate the calcification at the open apex of the coronal segment, thereby promoting filling with guttapercha (Cvek et al., 2008) as root canal therapy can result in failure due to perforations/ furcations leading to teeth loss (Bramante & Berbert, 1994).

$Ca(OH)_2$ has several advantages in this field of therapy due to easy handling, quick resorption when ejected into the periodontium, facilitating the transformation of periodontal tissues and inducing calcified deposits material (Bramante & Berbert, 1994). $Ca(OH)_2$ when comes in close nearness to biological fluid perhaps could be displaced as a result of which good seal could not be achieved (Schuurs et al., 2000) and in that case a more reliable restorative material like MTA could be used. Pitt Ford et al. (1995) reported that cementum was formed underneath MTA in most of the teeth with perforations in comparison to those teeth with perforation/furcation locations sites were sealed with amalgam $Ca(OH)_2$ has been recommended as an established agent to repair

perforations and is still being used for infection control. It stops bleeding and could be used temporarily when there is not enough time for a permanent repair.

2.5 Chlorhexidine (CHX)

CHX is most routinely used as disinfectant which is bacteriostatic at lower concentrations and bactericidal at higher concentrations. It is positively charged with a pH of 5-8. It is hydrophobic and lipophilic molecule that functions via interacting with lipopolysaccharides and phospholipids on the cell membrane of bacteria cell membrane and infilterates the cell via passive or active transport mechanism. It is efficient owing to its interaction of the positive charge of the molecule with the phosphate groups on cellwall of microrganism hence, altering the cells' osmotic equilibrium (Kumar, 2013). CHX has been used widely and available commercially as oral rinses & subgingival irrigants with a concentration of 2%, exhibiting no obvious side effects (Spratt et al., 2001). Even though CHX possess a diverse range of antimicrobial property and substantivity but it should not be used as a root canal irrigant because of the absence of tissue solubility, sensitivity to organic content and inactivation via dentine, its components & root canal debris. Nevertheless, it should be recommended as a final rinse or intracanal medicament (Kanisavaran, 2008).

Moreover, chlorhexidine with calcium hydroxide and chlorhexidine alone exhibited more antibacterial efficiency against *E faecalis* than calcium hydroxide alone (Sinha et al., 2013). It has been reported through experimental studies that CHX is found to be very cytotoxic to human periodontal ligament (PDL) cells and fibroblasts through the process of protein synthesis inhibition (Chang et al., 2001). The clinical importance of these findings are yet to be proved (CHX apart from being an antimicrobial agent have also known to increase the release of calcium of $Ca(OH)_2$ (Jacinto et al., 2015)).

The addition of 2% CHX will prohibit setting of the material. The adding of CHX to E-MTA increased its calcium ion release and pH (Jacinto et al., 2015). The bacterial attachment to the oral medium is a significant step in the pathological process and CHX has proven to effectively inhibit the pathogenic adherence (Grenier, 1996).

CHX is much more active against Gram positive than the Gram-negative bacteria, the least vulnerable of which includes the Proteus strains, Pseudomonas, Enterobacter, Actinobacter and Kleibsiella (Emilson, 1977) The evidence of CHX being resistant in Gram-negative mico-organism and to be plasmid-borne or transferable is not yet reported (Russell & Path, 1986) which perhaps explains the reason why an application of CHX for long duration in dogs led to a prominence in plaque sample of Gram negative rods. The effectiveness of CHX against Gram positive bacteria perhaps could result in an over-estimation of the clinical efficacy of this agent (Zehnder, 2006).

2.6 Calcium Silicate Based Cement (CSCS)

Recently available products of CSCs are:

- Portland Cement (PC)
- MTA Angelus (Grey and White)
- ProRoot MTA (Grey and White)
- DiaRoot BioAggregate
- IBC BioAggregate
- Biodentine
- iRoot
- Calcium Enriched Mixture (CEM) Cement
- MTA FILLAPEX (MTA based root canal seller)
- Endo-CPM
- Cimento Endodontico Rapido (CER)

- ProRoot Endo Sealer
- MTA Plus
- Ortho MTA
- MTA Bio
- MTA Sealer (MTAS)
- Capasio
- Fluoride-Doped MTA Cement
- Generex A
- EndoSequence BC Sealer
- Nano-Modified MTA (NMTA)
- Endosequence Root Repair Material (Putty and Paste)
- Ceramicrete-D
- Light-Cured MTA
- Calcium Silicate (CS)
- Endocem

2.6.1 Clinical application of CSC

2.6.1.1 Root canal repair material

ProRoot MTA (Grey and White), MTA Angelus (Grey and White) (Carvalho et al., 2013; Kvinnsland et al., 2010; Parirokh & Torabinejad, 2010b), DiaRoot BioAggregate(De-Deus, Canabarro, et al., 2009; Hashem & Amin, 2012), Biodentine (dentine substitute) (Laurent et al., 2012), Calcium Enriched Mixture (CEM) Cement (Asgary et al., 2011), Cimento Endodontico Rapido (CER) (Santos et al., 2008), Endosequence Root Repair Material (Putty and Paste) (Damas et al., 2011), I Root BP, Generex A, CERAMICRETE-D.

2.6.1.2 Root canal filling

Portland cement PC (Sakai et al., 2009), MTA Plus, I Root SP, Ortho MTA, MTA Bio, CAPASIO, Nano-Modified MTA (NMTA), Light-Cured MTA, Endoce.

2.6.1.3 Root canal sealing material

MTA FILLAPEX (MTA based root canal seller)(Nagas et al., 2012), ENDO-CPM (Parirokh & Torabinejad, 2010a), EndoSequence BC Sealer (de Miranda Candeiro et al., 2012), ProRoot Endo Sealer (Huffman et al., 2009), MTA Sealer (MTAS), Fluoride-Doped MTA Cement, I Root BP plus, Calcium Silicate (CS).

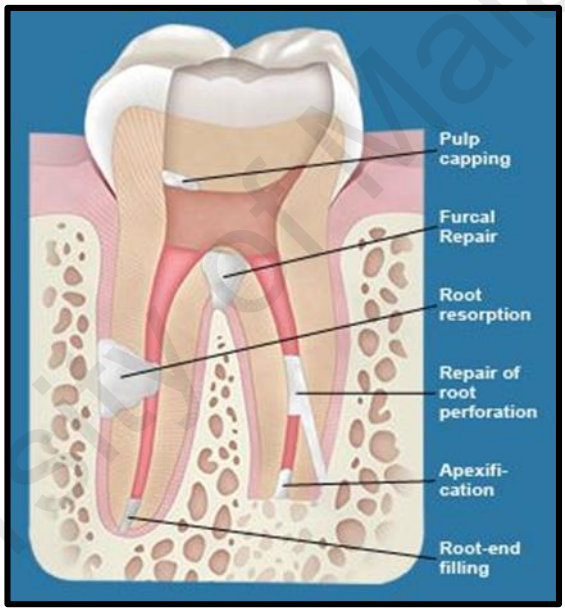

Figure 2.2: Clinical applications of CSC (Figure from PRO Root MTA).

2.6.2 Mineral Trioxide Aggregate (MTA)

MTA was primarily developed as material for pulpotomy, pulp capping or as material for the formation of apical barrier in teeth with immature root and later for retrograde root canal obturation (Torabinejad & Ford, 1996). MTA was marketed as a gray-colored powder, but for esthetic reasons, it was replaced by white MTA (ProRoot MTA; Dentsply, Johnson City, TN, and MTA Branco, Angelus, Londrina, Brazil). Eventually after commercialization of white MTA, the company Angelus Dental Solutions produced

MTA BIO cement (an experimental material) and which has the same indications of white MTA (De-Deus, de Souza, et al., 2009).

Currently, it is used as root-end filling during endodontic surgery and in the threapy of a variety of pulpotomy, canal perforations and treatment of vital pulp. In addition, it has the ability to produce an apical barrier in teeth with necrotic pulp, and open apex (Jokanović et al., 2011; Parirokh & Torabinejad, 2010b). MTA appeared to favour long-term pulp vitality after direct pulp capping in comparison to the calcium hydroxide (Mente et al., 2010) Hence, MTA, replacing calcium hydroxide as the material of choice (Revathi & Sharath, 2014). MTA is used in the treatment of a variety of root canal perforations, pulpotomy and treatment of vital pulp. In addition, it has the ability to produce an apical barrier in teeth with necrotic pulp and open apex.

In contact with synthetic tissue fluids, MTA after its application forms hydroxyapatite crystals which acts as a base for the formation of calcified structures (Parirokh & Torabinejad, 2010b). A successful outcome clinically and radiographically of pulpotomised teeth using MTA Angelus and PC was reported with follow up to 24 months (Sakai et al., 2009) where calcium hydroxide was used as a dressing material to stimulate apexification of a root canal and MTA as a plug to permit root canal filling. There was no evidence of apical periodontitis and thus was considered as the treatment of choice (Moore et al., 2011; Paula-Silva et al., 2011).

2.6.2.1 Composition of MTA

MTA materials are a mixture of refined Portland cement –tricalcium silicate (3CaO.SiO2), tricalcium oxide, tricalcium aluminate (3CaO.Al2O3), silicate oxide (SiO2) and bismuth oxide (Bi2O3). The bismuth oxide is added to improve properties and the radioopacity (Torabinejad et al., 1995). MTA shows smaller and uniform particle size (Dammaschke et al., 2005).

MTA is available in two forms namely gray (GMTA) and white (WMTA) and the difference between them is the presence of Al_2O_3, MgO and especially FeO in the former material.

2.6.2.2 Setting reaction of MTA

MTA is a type of hydraulic cement (harden under water) that requires water to set. Hydraulic cements are finely ground materials (powder) which when mixed with water gradually or instantly set or harden in air or in water leading to a reaction resulting in the formation of hydrated compounds whose strength increases with time. MTA consists of fine hydrophilic particles which when comes in contact with water, sets to a hard composition via the formation of colloidal gel (Camilleri et al., 2005). Chemical analysis of MTA in water revealed the presence of calcium. The pH becomes high as a result of this hydration process and calcium hydroxide is released (Fridland & Rosado, 2003). During MTA hydration, calcium disilicate and tri-silicate react to form calcium hydroxide and hydrated calcium silicate gel (Belío-Reyes et al., 2009). Calcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalciumaluminoferrite are the main component of all types of MTA (Islam et al., 2006). The tricalcium silicate phase also provides the long-term mechanical strength of the cement.

2.6.2.3 Setting Time

MTA takes a long time to set in contrast to other cements. The exact setting time needed to set varies in different studies. The setting time of GMTA is about 2 hours and 45 minutes (Torabinejad et al., 1995). Another study reported 2 hours;55 minutes for GMTA and 2 hours;20 minutes for WMTA (Islam et al., 2006). By the addition of different solutions such as dibasic sodium phosphate which are setting accelerators to WMTA, non phosphate solutions had an average setting time of 151 minutes in contrast to the added phosphate solutions which reduced the setting time to 26 minutes (Huang et

al., 2008). The addition of $CaCl_2$ provided a significant reduction (50%) in the initial setting time of cements. The final setting time of WMTA was reduced o 35.5% and the final setting time of WPC to 68.5%. The WMTA with $CaCl_2$ absorbed water and gained weight with time (Bortoluzzi et al., 2009). In a study to evaluate the setting time of experimental accelerated calcium-silicate cements and ProRoot MTA in deionized water, phosphate-buffered saline (PBS), 20% fetal bovine serum (FBS)/80% PBS or hexadecane oil, all experimental cements showed initial setting times between 28 and 45 minutes and final setting times between 52 and 80 minutes. MTA showed a final setting time of 170 minutes. Final setting time of all experimental cements was faster than MTA (Gandolfi et al., 2009).

It has also shown that the effect of various chemicals on dissolution of (WMTA) Carbonic acid can effectively be used as an adjunct to dissolve set WMTA even after 21 days, whereas 2% chlorhexidine gluconate showed significant surface dissolution only within 24 hours of WMTA placement. Thus, usage of chlorhexidine gluconate as a root canal irrigant in which WMTA is used during endodontic procedure should be avoided for 24 hours (Nandini et al., 2010). The addition of water soluble polymer to MTA reduced its setting time but PC displayed the shortest setting time ($P < 0.05$). PC exhibited a much higher degree of shrinkage than MTA (Camilleri & Mallia, 2011). By the addition of several hydration accelerators such as calcium chloride, low-dose citric acid, calcium lactate gluconate solution resulted in an improved setting time of MTA. The setting time of MTA mixed with hydration accelerators was significantly shorter than that of MTA mixed with water (Lee et al., 2011). Moreover, by the addition of radiopacifying agents: bismuth oxide (BO), calcium tungstate (CT), and zirconium oxide (ZO) to MTA and PC, MTA showed shorter setting time than the other materials (Duarte et al., 2012; Tanomaru-Filho et al., 2012).

2.6.2.4 The pH and calcium ion release

Due to the high alkalinity of MTA and the ability to release calcium which is believed to be in its hydroxide state, gives the MTA a major advantage in the capability to induce mineralization (Fridland & Rosado, 2003). MTA has a pH of 10.2 initially which rises to 12.5 after three hours of the mixing (Torabinejad et al., 1995). In another study, the pH of colored MTA was 13.0 after 1 hour of mixing (Islam et al., 2006). It was reported by several studies based on evaluating the pH of calcium release of two commercially available mineral trioxide aggregate (MTA) cements (white MTA Angelus and MTA Bio) and of three experimental cements (light-cured MTA, Portland cement with 20% bismuth oxide and 5% calcium sulfate and an epoxy resin–based cement). A higher pH and calcium ion release was observed with white MTA Angelus and MTA Bio. However, the epoxy resin–based cement and light-cured MTA presented lower cacium ion release and pH values (vivan et al., 2010).

2.6.3 Portland cement

Portland cement was discovered in 1824 by a British bricklayer. It was then named after the inventor named it 'PORTLAND CEMENT' due to its similarity with the stone to that found in the British coast (Rayn, 1929).

Portland cement (PC) is a fine powder that is created by grinding cement clinker and is composed of 20% silica, 65% lime, 10% alumina and ferric oxide and 5% other compounds. Lime contains calcium and magnesium oxides. PC is created by a process called calcinations which results in chemical and physical changes by grinding clay and lime-bearing minerals in accurate percentage and then is later heated to 1,400°C. The resulting powder and small amounts of gypsum is added to retard the setting process (Steffen & Van Waes, 2009).

2.6.3.1 Composition of PC

Lately, portland cement is produced by crushing, grinding and blending raw materials (limestone and clay) and by heating the produced powder to a high temperature and which eventually produces clinker nodules (Taylor, 1997). The clinker nodules are then cooled and grounded 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). The resulting″clinker" is grounded to a fine powder and a small amount of gypsum is added to retard the setting process (Steffen & Van Waes, 2009). PC is classified as a hydraulic cement which normally is composed of 65% lime, 20% silica, 10% alumina and ferric oxide and 5% of other compounds.

Lime is composed of calcium and magnesium oxides. It contains four main phase fractions including tricalcium silicate ($3CaO.SiO_2$), dicalcium silicate ($2CaO.SiO_2$), tricalciumalminate ($3CaO.Al_2O_3$) and tetracalciumaluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$)(Ghosh, 2003). PC and MTA have similar components except for the bismuth oxide and is mainly composed of dicalcium and tricalcium silicate which produces calcium hydroxide and calcium silicate hydrate gel during hydration. MTA showed absence of potassium and a reduced amount of calcium sulphate unhydrated and calcium dialuminate compared to PC (Parirokh & Torabinejad, 2010a). PC also has higher concentrations of lead, arsenic and chromium compared to MTA Angelus (Camilleri et al. 2012). There are two types of Portland cement: - (gray and white). The chemical compositions are the same for the exception of iron in the gray Portland cement (Dhanirachna et al., 2012).

2.6.3.2 Setting reaction of PC

The addition of water to PC results in a complicated hydration reaction as PC sets in a series of stages. Firstly, there is a dispersion of clinker grain in water. Secondly, hydration

products eat into and grow out from surface of each grain. Thirdly, setting occurs when a different clinker grains join together. Finally, hardening occurs with further development of the gel and ventually crystalline particles are disseminated throughout (Steffen & Van Waes, 2009). Chemical expression is called alite and belite phase reaction. The simplified reaction of alite with water may be expressed as:

$$2Ca_3OSiO_4+6H_2O \Longrightarrow 3CaO_2SiO_2.3H2O+ 3Ca(OH)_2$$

It is a fast reaction and causes setting and strength development in the first few weeks.

The simplified reaction of belite is:

$$2Ca_2SiO_4+4H_2O \Longrightarrow 3CaO.2SiO_2.3H2O+Ca(OH)_2$$

This is a relatively slowly reaction responsible for gaining strength after one week (Taylor, 1997).

2.6.4 Biodentine

Recently, new calcium silicate based cements (CSBC) were produced such as Biodentine™ and was supplied by dental materials' manufacturer Septodont in September 2010 and came into being commercially available in January 2011. It is biologically active cement which has dentine-like mechanical properties. It is a biocompatible and biologically functions like dentin. It also can be used as a dentine replacement coronal and radicular portion of tooth. Lately, EndoSequence Root Repair Material (ERRM) came into existence which is a bioceramic material composed of calcium silicates, zirconium oxide, titanium oxide, calcium phosphate monobasic, thickening agents, and proprietary fillers. EndoSequence has been manufactured to overcome some of the difficult handling characteristics of MTA. EndoSequence materials are ready-to-use as packaged. Depending on the consistency desired, ERRM is

manufactured in a syringeable form which is flowable and a putty form which is firm and moldable.

2.6.4.1 Composition of Biodentine

Biodentine consists of tricalcium silicate, dicalcium silicate, calcium carbonate and oxides such as iron oxide, and zirconium oxide as its powder components and calcium chloride and a water – soluble polymer as its liquid component (Han & Okiji, 2011). It has been shown in an investigation that biodentine has the higher amount of lead leached than MTA Angelus, PC, Tricalcium silicate and Bioagregate into an acidic environment. However, the amount of arsenic released from Biodentine was the same as that from Bioagregate and PC in the same environment. Regardless of the presence of high lead content some investigators recommended Biodentine safe for use in dentistry (Grech et al., 2013).

Biodentine powder is mixed with the liquid in a capsule in the triturator for 30 seconds. Once mixed, Biodentine was found to set in approximately 12 minutes (Septodont, UK).

2.7 Mixing different vehicles with CSC

2.7.1 To retard its setting time and improve working properties

Normal saline was able to extend the setting time to 90 minutes, while 2% lidocaine mixed with MTA raised the setting time to double compared to when mixed with water. Furthermore, mixing 2%CHX gel with MTA did not set until the end of the experimental period of 4 hours (Kogan et al., 2006).The addition of $CaCl_2$ provided a significant reduction (50%) in the initial setting time of cements. The final setting time of WMTA was reduced in 35.5% and the final setting time of WPC in 68.5% (Bortoluzzi et al., 2009).

The addition of deionized water, Phosphate-Buffered Saline (PBS), 20% Fetal Bovine Serum (FBS)/80% PBS or hexadecane oil to experimental cements exhibited initial setting times between 28 to 45 minutes and final setting times between 52 to 80 minutes. MTA showed a final setting time of 170 minutes (Gandolfi et al. 2009). The addition of a water-soluble polymer to MTA reduced its setting time but pulp canal sealer (PCS) displayed the shortest setting time ($P < 0.05$). PCS exhibited a much higher degree of shrinkage than MTA (Camilleri & Mallia, 2011).

The addition of KY liquid and NaOCl to ProRoot Gray MTA improved the handling properties and decreased the setting time(AlAnezi et al., 2011). The addition of Propylene Glycol (PG) to MTA-Angelus increased its setting time (Duarte et al. 2012).The addition of Carbonic Acid can be effectively used as an adjunct to dissolve set White MTA even after 21 days, whereas 2% Chlorhexidine Gluconate showed significant surface dissolution only within 24 hours of White MTA placement (Nandini et al., 2010).

The addition of sodium hypochlorite (5.25%), chlorhexidine (2%), and Glyde File Prep on MTA-dentin showed that the bond strengths were significantly lower in Glyde File Prep group ($p < 0.05$) and there was no significant difference in the chlorhexidine group or in the NaOCl group ($p > 0.05$). The addition of Sodium Fluoride accelerated apatite formation on calcium silicate cements. Fluoride-doped calcium silicate cements had higher bioactivity and earlier formation of fluorapatite. Sodium fluoride may be introduced in the formulation of mineral trioxide aggregate cements to enhance their biological behaviour (Gandolfi et al., 2011).

2.7.2 To improve the antibacterial effect

The addition of chlorhexidine gluconate (0.12%) to WMTA enhanced its antimicrobial effect (Stowe et al., 2004). Mixing 2%CHX liquid with MTA showed larger zone of inhibition against Enterococcus faecalis than sterile water (Holt et al., 2007). It was

reported by another study that the substitution of CHX (0.12%) for sterile water in MTA increases its cytotoxicity (Hernandez et al., 2005). MTA mixed with CHX (0.12%) was surrounded by fibrous connective tissue indicating that it was well tolerated by the tissues. Although, MTA/CHX seemed to be biocompatible (Sumer et al., 2006). The amount of calcium hydroxide produced in MTA has been found to be approximately 10–15% of the hydrated material (Camilleri, 2008; Chedella & Berzins, 2010).

CHAPTER 3: MATERIALS AND METHODS

3.1 PART ONE

3.1.1 Retarding the Setting Time of CSC Materials

The materials were utilized in this work according to the manufacturer's instructions which are mentioned in Table 3.1 and Table 3.2. The materials used in this study are presented in Figure 3.1 and 3.2.

Table 3.1: Different types of CSC used for investigations.

No.	Materials	Compositions	Manufacturer	Batch No.
1	White Portland Cement (WPC)	Dicalcium silicate ($2CaO.SiO_2$), Tricalcium silicate ($3CaO.SiO_2$), Tetracalciumaluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$) and Tricalciumalminate ($3CaO.Al_2O_3$)	Aalborg, Malaysia	EN 197-1 CEM I 52.5N
2	White ProRoot MTA (MTA)	Tricalcium aluminate ($3CaO.Al_2O_3$), Tricalcium silicate ($3CaO.SiO_2$), Silicate oxide (SiO_2) and Bismuth oxide (Bi_2O_3)	Tulsa Dental products, Tulsa, ok, USA	13102906
3	Biodentine® (Bio)	Dicalcium silicate, Tricalcium silicate, Calcium carbonate, Oxides, Zirconium oxide as its powder components, Iron oxide, calcium chloride and a water –soluble polymer as its liquid component	Septodont, Saint-Maur-des-Fossés, France	B15371

Figure 3.1: CSC used in this study.

Figure 3.2: Different vehicles used to mix with CSC.

Table 3.2: Vehicles and its compositions used to mix with CSC.

No.	Materials	Compositions	Manufacturer	Batch No.
1	2% Chlorhexidine gluconate gel (CHX)	Sorbitol, Aqua, PEG-40 Hydrogenated Castor Oil, Hydroxyethycellulose, panthenol, Aroma (flavor) Cinnamal, Allantoin, Chlorhexidine Digluconate, Sodium Methylparaben, Sodium Saccharin, PVM/MA Copolymer, Ctiric Acid.	Foramen, Cantabria, Spain.	1301-0483
2	Fluoride gel (FL)		Medicom (Asia)	Lot 24525
3	Eugenol	Eugenol	Roth eugenol liquid USP, (Auckland, New Zealand)	Il 60610
4	Local anesthesia with/ epinephrine	Mepivacaine Hydrochloride, Adernaline, Sodium Chloride, Potassium Metabisulfite, Disodium Edetate, water.	Septodont, Saint-Maur-des-Fossés, France	B15170AA
5	Sodium hypochlorite 5% (NaClO)	Sodium hypochlorite 5%	ACROS ReAgent (USA)	Lot 219250025
6	Phosphate-buffered saline (PBS)	Potassium Chloride, Sodium Chlorid.	Medlin (USA)	Lot 107743
7	Calcium chloride ($CaCl_2$)	Calcium Chloride 96% extra.	ACROS organics (USA)	Lot A0320147

3.1.2 Preliminary Study

A volume of 0.3g of each experimental cement and its corresponding vehicle were measured using weighing scale (Acculab, Sartorius group) and then mixed. The results were based on assessing the working properties and manipulation of the cement.

The three main CSCs were mixed with 7 different vehicles: 2%Chlorhexidine gel, fluoride gel, Eugenol, Local anesthesia with and without epinephrine, Sodium hypochlorite 5% (NAClO), Phosphate-buffered saline (PBS), and Calcium chloride ($CaCl_2$).

3.1.3 Main Experiment

3.1.3.1 Specimen preparation

Disc shaped samples were prepared from each material using a custom perspex mould (Zecttron® Snd Bhd, Kuala Lumpur, Malaysia) measuring 2 mm in height and 10 mm in diameter (Figure 3.3). Experimental medicaments were prepared by adding the cements to the corresponding vehicles in the determined powder/vehicle ratio 2:1 (at the room temperature and placed inside the mould as shown in (Figure 3.4). Air entrapment inside the mixed cement was avoided by using mechanical vibration for 10 seconds to obtain even distribution of the mixture (Figure 3.5). The samples were then kept inside an incubator (Memmert GmbH, Schwabach, Germany) at 37°C of 100% humidity (Figure 3.6).

Samples (n=105) were fabricated using the perspex moulds and samples were divided into 3 groups of experimental cements (CSC) (n=35). The samples from each group were divided into 7 subgroups corresponding to seven different vehicles which are: 2% Chlorhixeden Gel , Fluoride Gel, Eugenol , Local Anesthesia with Epinephrine, 5% Sodium Hypochlorite (NACLO), Phosphate -Buffered Saline (PBS), and Calcium Chloride ($CaCl_2$) (Figure 3.5). Each subgroup was allocated with 5 samples (n=5) and mixed with the vehicles in the powder/vehicle ratio of 2:1 as seen in (Figure 3.7).

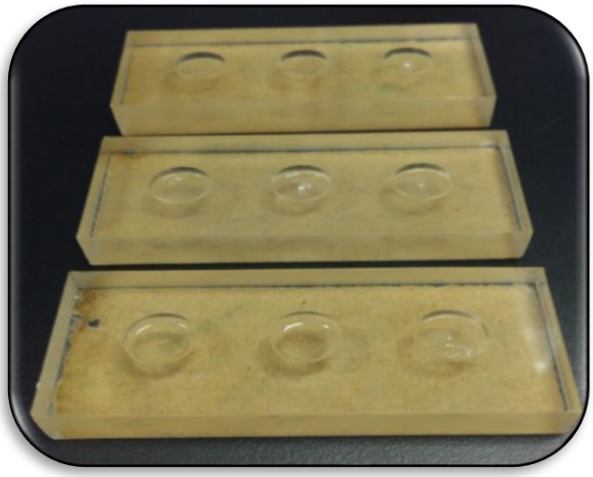

Figure 3.3: Customized perspex mould.

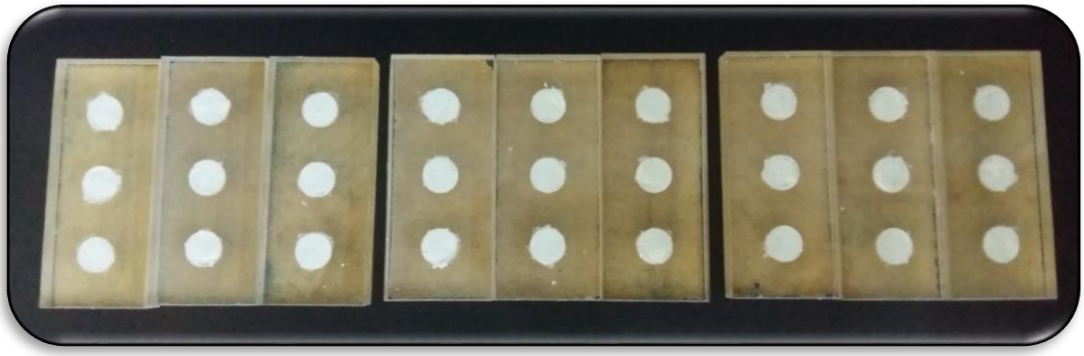

Figure 3.4: Customized perspex mould with experimental cements.

Figure 3.5: Ultrasonic scaler hand piece.

Figure 3.6: Incubator used.

3.1.3.2 Procedure

The setting of the material was analyzed in a similar way used for setting time test that was performed as demonstrated by the ADA specification 57 and ASTM specification C266-03. The final setting of the materials were determined utilizing the modified Gilmore needles weighing 456 g according to the methodology described by Bortoluzzi as shown in (Figure 3.8). (Bortoluzzi et al., 2009). Setting of the materials was recorded at 24h, 2, 3, 4, 5, 6, 7, 14, 28, 60 and 84 days until the needle failed to penetrate the cement surface as shown in (Figure 3.9). Descriptive analysis was used to describe the setting of the material.

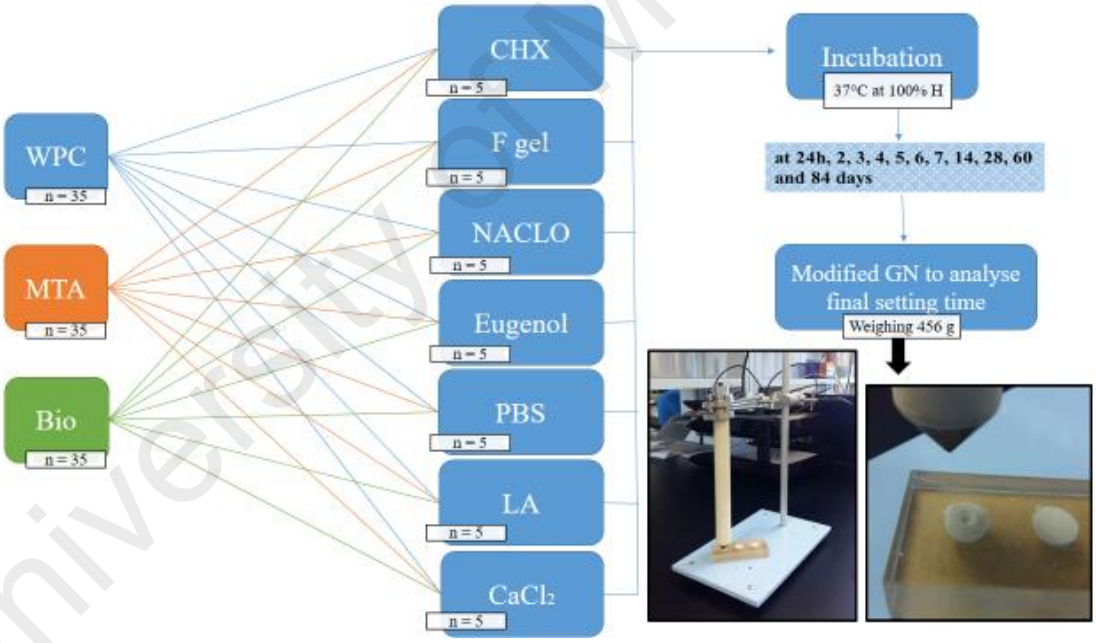

Figure 3.7: Flowchart describing the steps used in retarding the setting time of CSC.

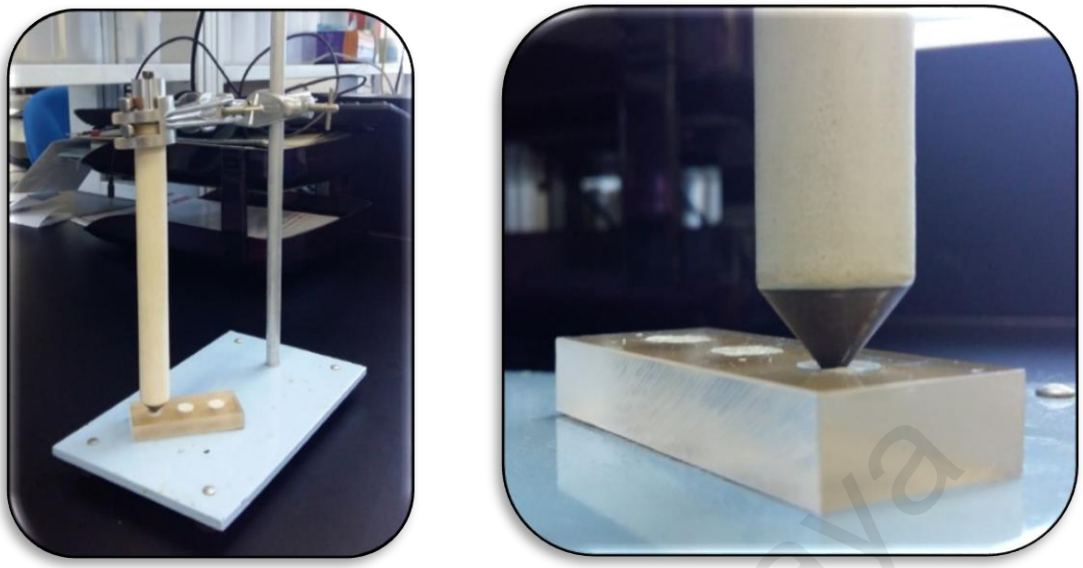

Figure 3.8: Gilmore needles weighing 456g.

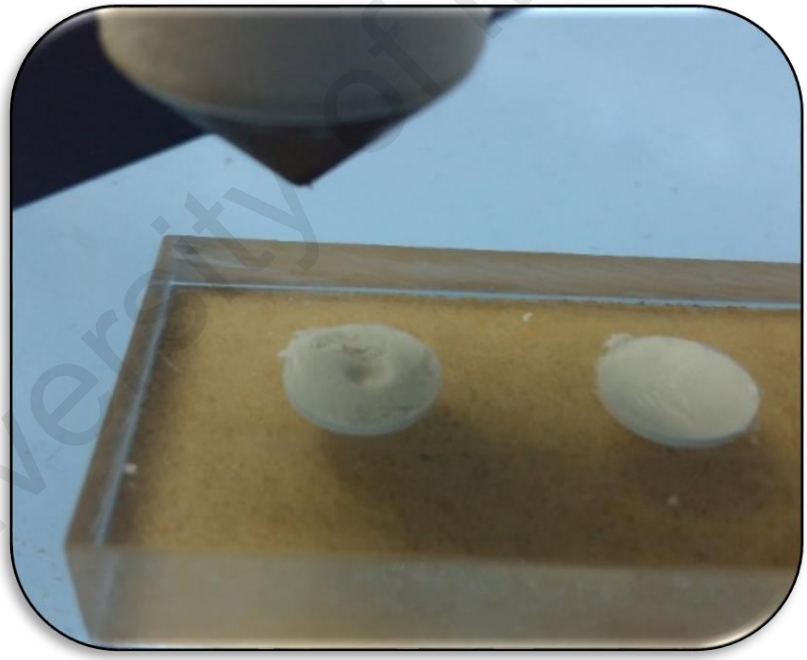

Figure 3.9: Visible indentation in the cement.

3.2 PART TWO

3.2.1 Physical properties of CSC after mixing with CHX gel

3.2.1.1 Materials

Table 3.3: Material used and its specifications.

No.	Materials	Compositions	Manufacturer	Batch No.
1	Calcium Hydroxide Paste($Ca(OH)_2$	Calcium Hydroxide with Barium Sulfate	USA	V115AJ
2	White Portland Cement (WPC)	Dicalcium silicate ($2CaO.SiO_2$), Tricalcium silicate ($3CaO.SiO_2$), Tetracalciumaluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$) and Tricalciumalminate ($3CaO.Al_2O_3$)	Aalborg, Malaysia	
3	White ProRoot MTA (MTA)	Tricalcium aluminate ($3CaO.Al_2O_3$), Tricalcium silicate ($3CaO.SiO_2$), Silicate oxide (SiO_2) and Bismuth oxide (Bi_2O_3)	Tulsa Dental products, Tulsa, ok, USA	13102906
4	Biodentine® (BIO)	Dicalcium silicate, Tricalcium silicate, Calcium carbonate, Oxides, Zirconium oxide as its powder components, Iron oxide, calcium chloride and a water –soluble polymer as its liquid component	Septodont, Saint-Maur-des-Fossés, France	B15371
5	2%Chlorhexidine gluconate gel (CHX)	Sorbitol, Aqua, PEG-40 Hydrogenated Castor Oil, Hydroxyethycellulose, panthenol, Aroma (flavor) Cinnamal, Allantoin, Chlorhexidine Digluconate, Sodium Methylparaben, Sodium Saccharin, PVM/MA Copolymer, Ctiric Acid.	Foramen, Cantabria, Spain.	1301-0483

3.2.2 Ca^{+2} ion release

3.2.2.1 Specimens preparation

White Portland Cement (Aalborg, Malaysia), Biodentine (Septodont, Saint-Maur-des-Fossés, France) and White ProRoot MTA (Tulsa Dental products, Tulsa, ok, USA) was mixed with 2% chlorhexiden gel with a volume of 0.03g in the powder/vehicle ratio of 2:1 using a weighing scale (Figure 3.10). The specimens were then stored in vials at 37°C ±1 in incubator with 100% humidity for 24 hours. Each Specimen was then fully immersed in a separate 5ml aliquot of fresh distilled water (pH~ 5.0) and stored for the following periods of 1st, 7th, 14th, 21th, and 28th day. For the control group (calcium hydroxide) was mixed and prepared according to the manufacturer's recommendations. The volume of 0.3grams was used for the control group and experimental medicamen. The process of calcium ion analysis is summarized in Figure 3.10.

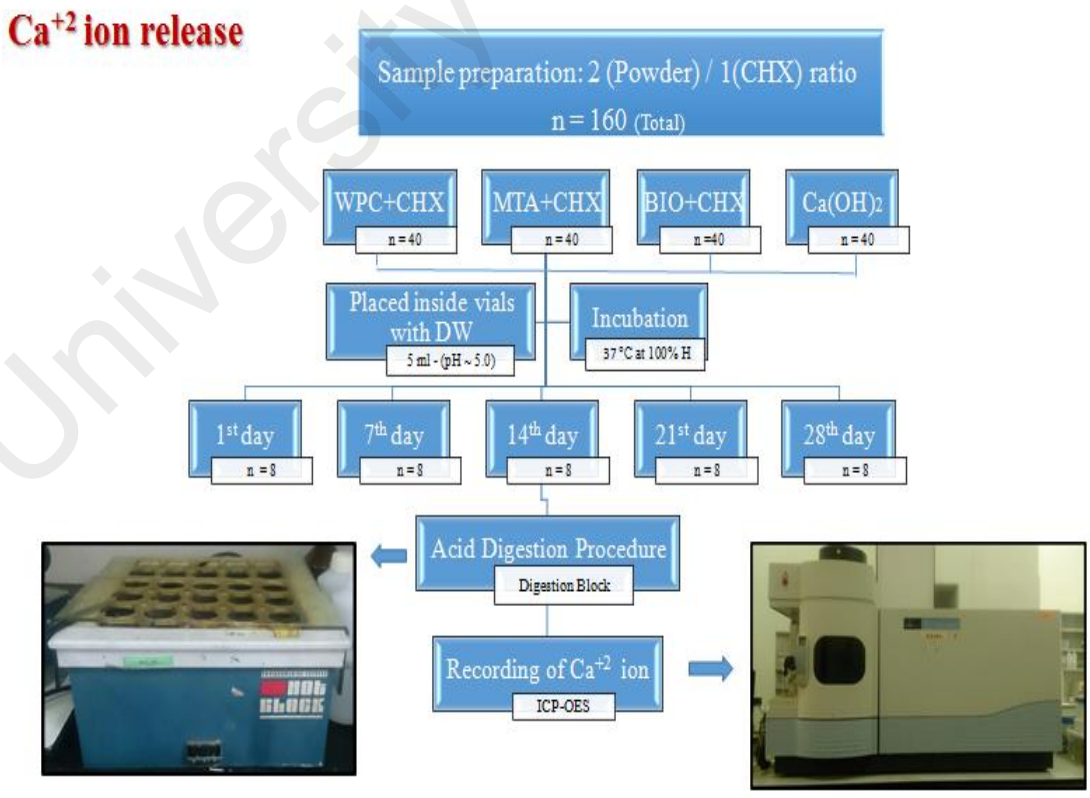

Figure 3.10: Flow chart describing the procedute to analyse calcium ion release.

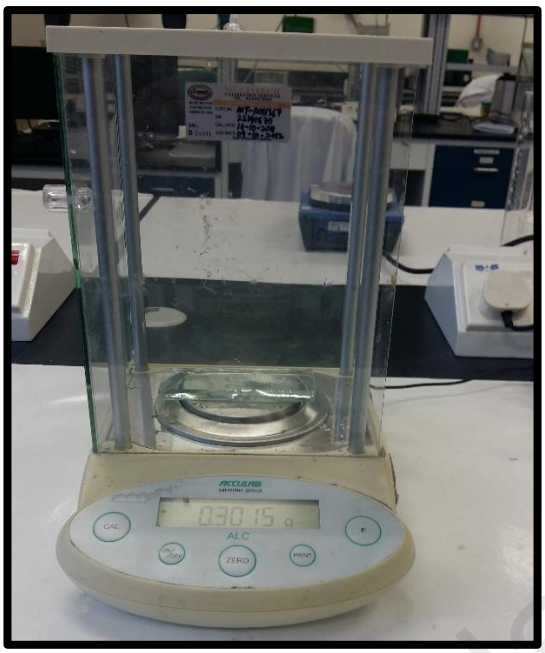

Figure 3.11: Weight of the mixed cements being measured on a weighing scale.

3.2.2.2 Procedure Analysis for Ca+2 ion release using inductively coupled plasma optical emission spectrometry (ICP-OES)

Acid Digestion of Sediments, Sludge and Soils by ICP-OES (OPTIMA® 7000 DV, PerkinElmer, Inc., Waltham, USA) were used to analyse the Ca^{+2} ion release from experimental and control cements (Figure 3.12, 3.13 & 3.14). At the first day of testing 0.1g of each sample was weighed and carefully placed in a beaker. 1 mL of 1:1. HNO_3 was taken using micropipette (Figure 3.15) and was added into the beaker containing the sample and eventually the mixture was gently swirled. The specimen was then heated at 95 °C for 10 min. Subsequently, 5 mL of concentrated Nitric acid (HNO_3) was added and then reheated for 30 minutes. Deionized water (2 mL) and 3 mL of 30% H_2O_2 (Hydrogen peroxide) were added after the samples were cooled. Then the sample was heated again at 95°C for 2 hours. Later, 7 mL of 30% H_2O2 and 10 mL of concentrated Hydrochloric acid (HCl) were added to the specimen. It was then heated at 95°C for 15 minutes. The sample was later cooled, filtered and marked up with deionized water until 100 mL. The digested sample was eventually analyzed for the following period's 1^{st}, 7^{th}, 14^{th}, 21^{th}, and 28^{th} day using ICP-OES.

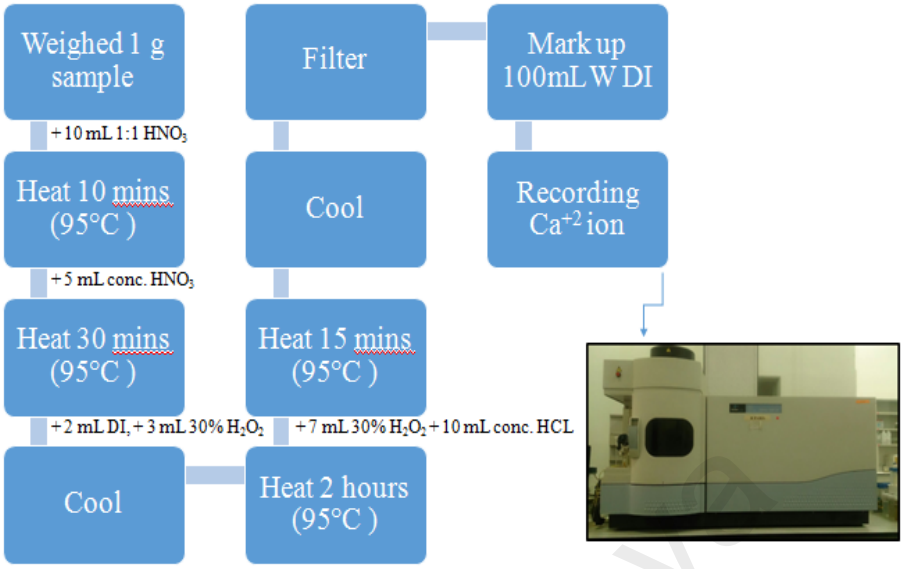

Figure 3.12: Flow chart describing the acid digestion of sediments, sludge and soils method.

Figure 3.13: Digestion Block – for acid digestion (metal analysis).

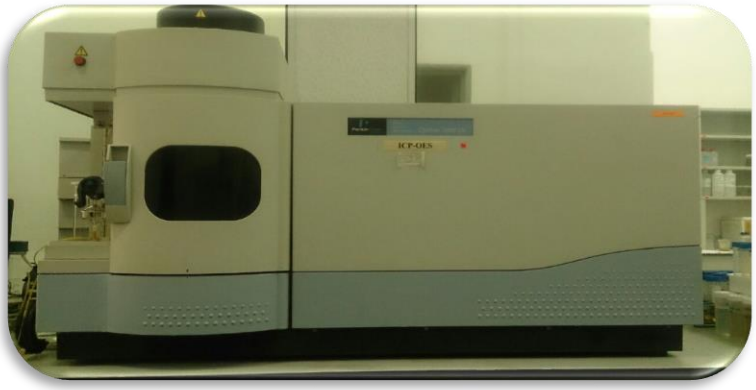

Figure 3.14: ICP-OES – for metal analysis.

Figure 3.15: Micropipette 100 µL.

3.2.3 Analysis of the pH

The pH for all the experimental and control cements were analyzed using digital pH meter (Eutech, pH 700) (Figure 3.16). The device was calibrated with buffer solutions with pH 4, 7, and 10 before each readings. The manipulated specimens were transferred into vials containing 5 ml fresh distilled water and allowed to equilibrate for 24 hours in the incubator to avoid liquid saturation before the test. Subsequently, the pH was measured by inserting the electrode of the device into the vials containing the samples. After recording the readings, the sample was left inside the water in the vial and restored until the next test. Replenishing of water into the vial was done 1 day prior to each testing periods (1^{st}, 7^{th}, 14^{th}, 21^{th}, and 28^{th} day). The flow chart illustates pH analysis is shown in Figure 3.17.

Figure 3.16: Digital Eutech pH meter.

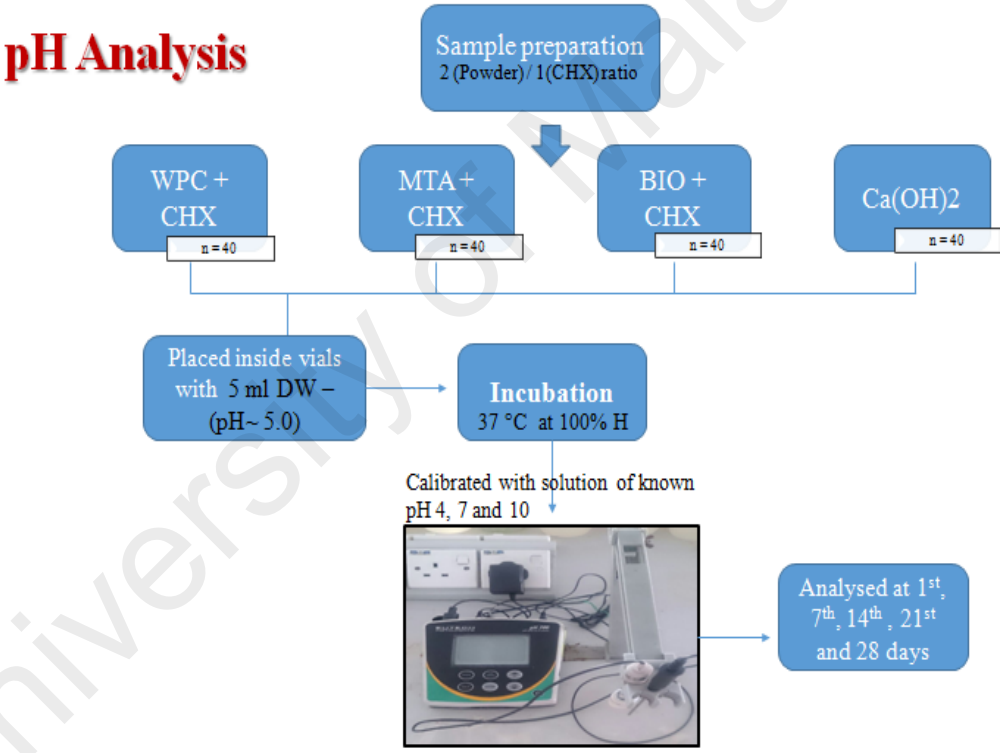

Figure 3.17: Flow chart describing the pH analysis.

3.2.4 Film Thickness Measurements

Film thickness was analysed using two 5mm thick optically flat glass plates with a contact surface area of 200 ±25 mm² was positioned together and their combined thickness was taken with a digital caliper (Absolute Digimatic 500-197, Mitutoyo Corp, Kawasaki, Japan) to an accuracy of ±0.01 µm. The manipulated experimental and control cements were placed between the plates as shown in Figure 3.22 and after 2 minutes of the mixing, a load of 150 N was applied on the top surface of the glass plate for 10 minutes (Figure 3.18) according to the method described in ISO Standard 6876/2001. The difference in thickness of the two glass plates with and without mixture was recorded as the film thickness of the material (Figure 3.19 and Figure 3.20). Samples (n=5) from each experimental and control cements were measured. The process of film thickness analysis is summarized in (Figure 3.21).

Figure 3.18: Uni axial hydraulic used to apply 150N.

Figure 3.19: Two glass without mixture. **Figure 3.20:** Two glass with mixture.

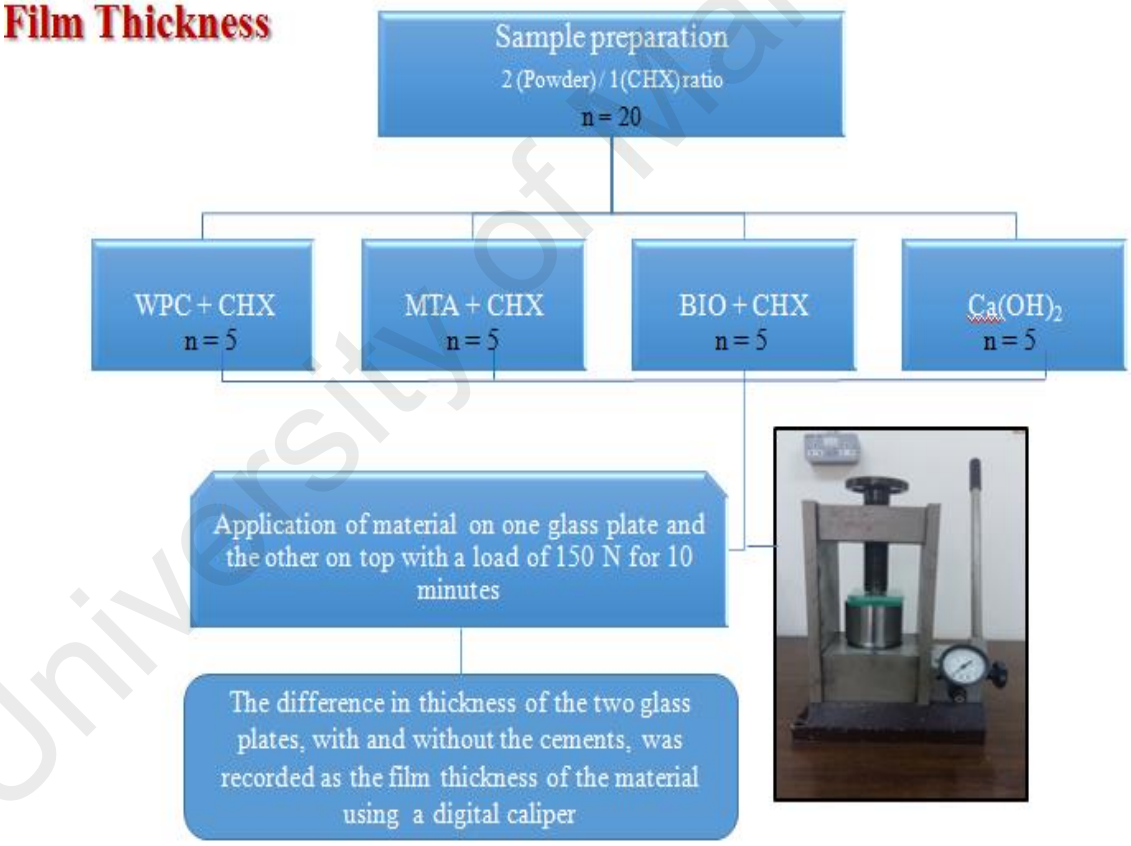

Figure 3.21: Flowchart describing the steps taken for analysis of film thickness.

3.2.5 Flowability

Following the standards of the ISO specification 6876/2001, two optically flat glass plate with a mass of 20 ±2 g were used. An amount of 0.05 ml of each experimental and control cements using a (1 ml) syringe was placed on the center of a glass plate. At 180 ±5 seconds after mixing, another plate was placed carefully on top of the mixed cement followed by a load of 100g that was carefully applied onto the top of the cements (Figure 3.22). Ten minutes after mixing the cement, the load was removed and the major and minor diameters of the compressed discs were measured using a digital caliper with a resolution of ±0.01 µm (Absolute Digimatic 500-197, Mitutoyo Corp, Kawasaki, Japan). The mean of three such determinations for each cement (n=5) was taken as the flow of the material. If the major and minor diameter discs were not uniformly circular or did not match within 1mm as shown in Figure 3.23, the test was repeated. The processs of flowablitlty is summarized in (Figure 3.24).

Figure 3.22: Application of 100g of load.

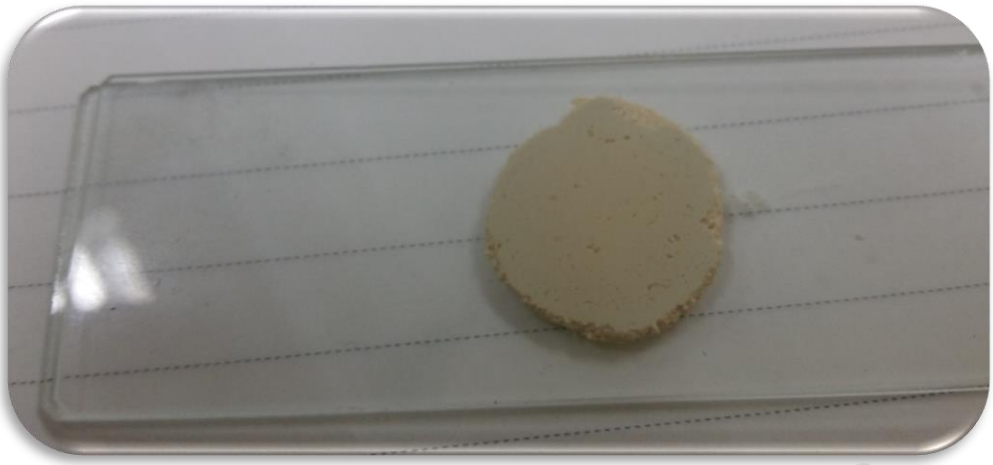

Figure 3.23: A uniformly circular shaped disc achieved.

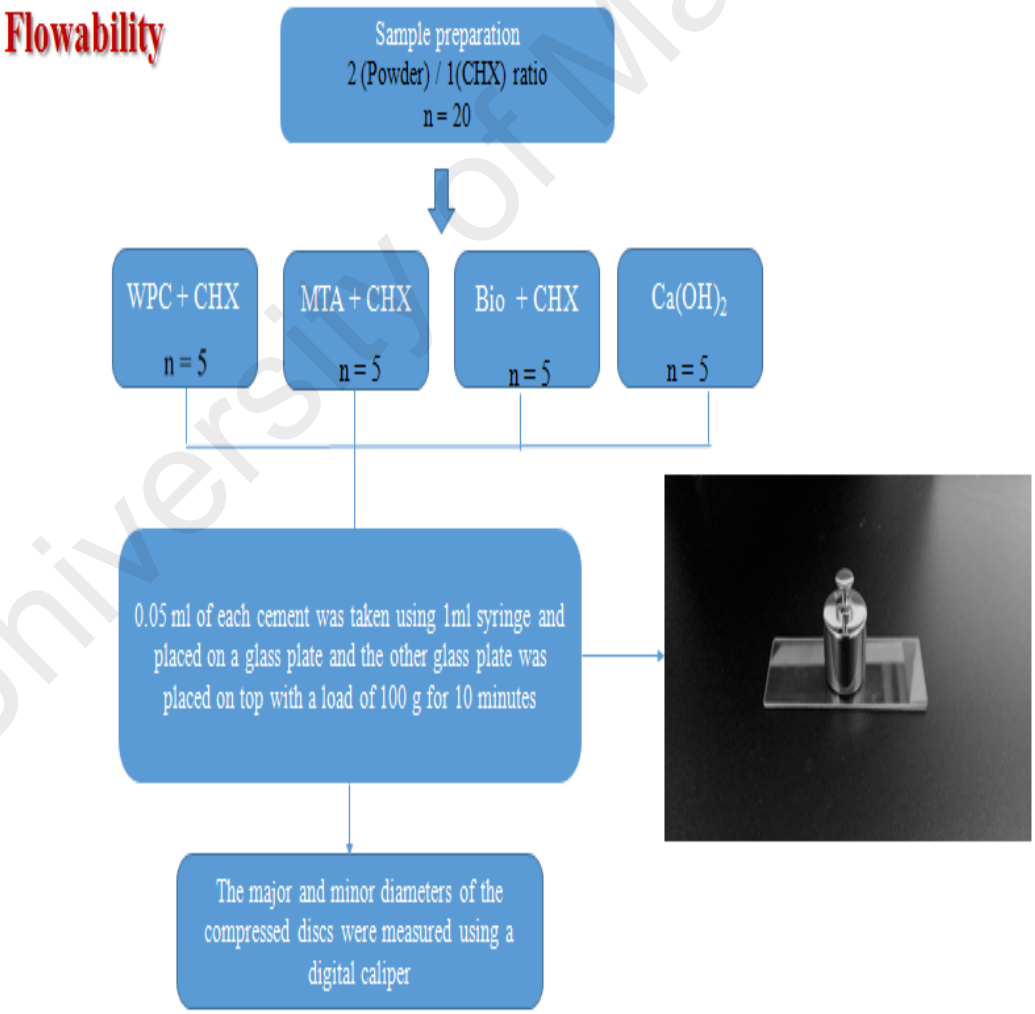

Figure 3.24: Flow chart describing the analysis for flowablity.

3.3 Statistical Analysis

All data were statistically analyzed by using SPSS 20.0. The normality distribution of data and homogeneity of variances were checked using levene test. Analysis of variance (ANOVA) was utilized to compare significant differences amongst CSC+CHX experimental and control $Ca(OH)_2$ cements and Tukey post hoc test were used to perform multiple comparison for Ca^{+2} ion release, pH value, film thickness and flowability. The significant level was set as $p<0.05$.

CHAPTER 4: RESUTS

4.1 PART ONE

4.1.1 Retarding the setting time of CSC materials

4.1.1.1 Results of Preliminary Study

Preliminary study showed that the best powder/vehicle ratio for CSCs after mixing with different vehicles is 2g powder: 1g vehicle. In fact the more liquid added, the longer is the delay of setting time. The results were based on assessing the working properties and manipulation of the cement.

4.1.1.2 Analysis of the setting time

The descriptive data for the setting time of the material are seen in Table 4.1. It was observed that all the experimental cements mixed with the different vehicles set after 24 hours except for the 2% Chlorhexidine gel which did not set even after 24 hours until 84 days.

Table 4.1: Setting time of the different experimental medicaments that mixed with different vehicles.

Experimental medicaments	2%,CHX Gel (n=5)		Fluoride gel	Eugenol	LA with epin	5%NaOCl	Phospha te-Buffered Saline	$CaCl_2$
	24h	2-84 Days	24h	24h	24h	24h	24h	24h
White Portland Cement	Not Set	Not Set	Set	Set	Set	Set	Set	Set
White ProRoot MTA	Not Set	Not Set	Set	Set	Set	Set	Set	Set
Biodentine	Not Set	Not Set	Set	Set	Set	Set	Set	Set

4.2 PART TWO

4.2.1 Physical properties of CSCs after mixing with CHX gel

4.2.1.1 Results of Ca^{+2} Ion Release

Table 4.2 and Figure 4.1 shows the means and standard deviations (mg/L) for the experimental medicament and control $Ca(OH)_2$ at different periods of time. All experimental medicaments showed significantly higher Ca^{+2} ion release compared to control $Ca(OH)_2$ ($P< 0.001$) for the 1st, 7th, 14th, and 21st day (Table 4.3-4.7). Except for 21st day, there were no significant differences between MTA+CHX and the control $Ca(OH)_2$ cements ($P>0.001$) (Table 4.6). For the 28th day, Bio+CHX and MTA+CHX cements showed significant lower Ca^{+2} ion release compared to the control $Ca(OH)_2$ ($p< 0.05$) (Table 4.7). However, WPC+CHX cement showed significantly higher Ca^{+2} ion release compared to other experimental medicaments and the control ($P< 0.001$).

The release of Ca^{+2} ion on 1st, 7th, 14th and 21st day for experimental medicaments and control cements in decreasing order were Bio+CHX > WPC+CHX > MTA+CHX > $Ca(OH)_2$. However, on 14th and 21st day the order were MTA+CHX > WPC+CHX and $Ca(OH)_2$ > MTA+CHX respectively. On 28th day the Ca^{+2} ion release in decreasing order were WPC+CHX > $Ca(OH)_2$ > Bio+CHX > MTA+CHX. (see Appendix B, Table 1. 2, 3, 4).

Table 4.2: Means and standard deviations of Ca^{+2} ion release of different experimental medicaments and control with different times expressed in mg/L.

Times	Groups			
	WPC+CHX	Bio+CHX	MTA+CHX	Ca (OH)2
1st Day	1979.75±6.32[b]	2460.25±33.96[a]	1884.00±52.48[c]	485.63±3.15[d]
7th Day	1926.25±27.11[b]	2443.75±35.38[a]	1845.00±64.18[c]	494.63±2.61[d]
14th Day	1058.38±25.90[c]	1898.62±43.68[a]	1486.12±33.430[b]	476.62±2.82[d]
21st Day	1050.00±26.75[b]	1238.25±7.166[a]	509.62±46.356[c]	529.62±3.37[c]
28th Day	745.88±28.68[a]	494.38±21.567[c]	397.00±28.005[d]	532.62±3.37[b]

(ANOVA/Tukey tests, $p>0.05$; n= 8)

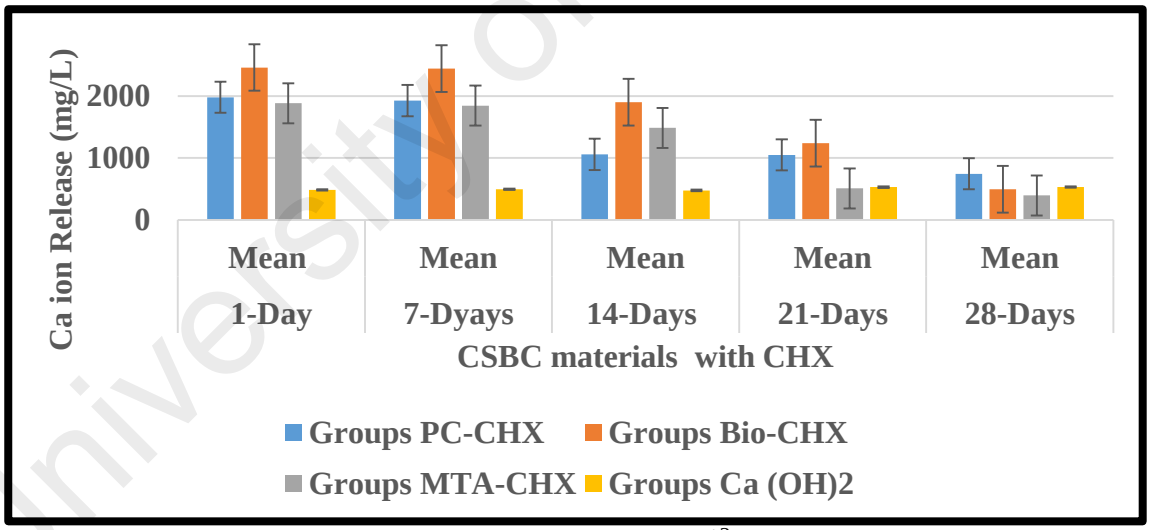

Figure 4.1: Means and standard deviations of Ca^{+2} ion release of different experimental medicaments and control.

An analysis of variance showed that the effect of Ca^{+2} ion release on 1st day was significant, F (3, 28) = 5.83, P = 0.000.

4.2.1.2 pH value

Table 4.11 and Figure 4.2 shows the means and standard deviations of pH value for experimental medicaments and control at different periods of time. Until 28 days, the mean pH value of the experimental medicaments was significantly lesser than control in general with significant differences ($p<0.05$). Amongst the experimental medicaments, Bio+CHX showed significantly higher pH values ($p<0.05$) compared to others during all periods except for 1st day. It was also noted that mean pH values of all experimental medicaments after 1st day was varying between 10.55 – 12.18. The highest pH values for the experimental medicaments were found to be on 7th day (Figure 4.2).

The pH value at 1st day for experimental medicaments and control in decreasing order were $Ca(OH)_2$> WPC+CHX > Bio+CHX > MTA+CHX. However, on 7th, 14th, 21st, and 28th day the order were $Ca(OH)_2$> Bio+CHX > WPC+CHX > MTA+CHX (see Appendix C, Table 1. 2, 3, 4).

Table 4.3: Means and Standard Deviations of pH value of different experimental medicaments and control with different times.

Times	Groups			
	WPC+CHX	Bio-+CHX	MTA+CHX	Ca (OH)2
1st Day	11.84±.027[b]	11.66±.028[c]	11.34±.029[d]	12.56±.029[a]
7th Day	12.09±.025[c]	12.18±.028[b]	11.75±.035[d]	12.56±.027[a]
14th Day	11.74±.026[c]	12.02±.027[b]	11.45±.029[d]	12.22±.039[a]
21st Day	10.95±.029[c]	11.64±.028[b]	10.55±.037[d]	12.25±.029[a]
28th Day	11.71±.027[c]	11.97±.037[b]	11.56±.202[d]	12.16±.035[a]

Values followed by the same letter in the same row are not statistically different (ANNOVA/ Tukey tests, $p > 0.05$; n= 8).

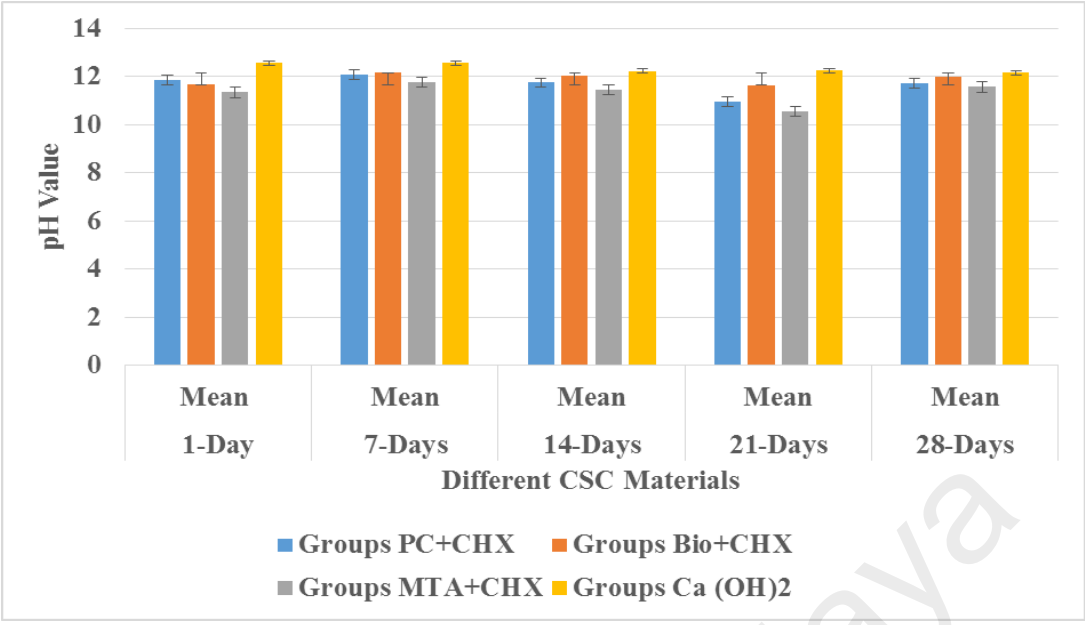

Figure 4.2: Means and standard deviations of pH value level for different experimental medicaments and control.

4.2.2 Film thickness

Table 4.14 and Figure 4.3 shows means and standard deviations of film thickness (mm) for experimental medicaments and control. The results showed that there is no statistically significant difference in film thickness of the experimental medicaments compared with the control ($p>0.05$) as shown in (see Appendix D, Table 1.).However the ranking order of the film thickness was WPC+CHX = Bio+CHX = MTA+CHX < $Ca(OH)_2$.

Table 4.4: Means and Standard Deviations of the Film thickness for experimental medicaments and control.

Groups	Film thickness in (mm) (mean ± sd)
WPC+CHX	0.10±0.016[a]
Bio+CHX	0.10±0.031[a]
MTA+CHX	0.10±0.019[a]
$Ca(OH)_2$	0.11±0.016[a]

Values followed by the same letter in the Colum are not statistically different (ANOVA/ Tukey tests, $p>0.05$; n= 5).

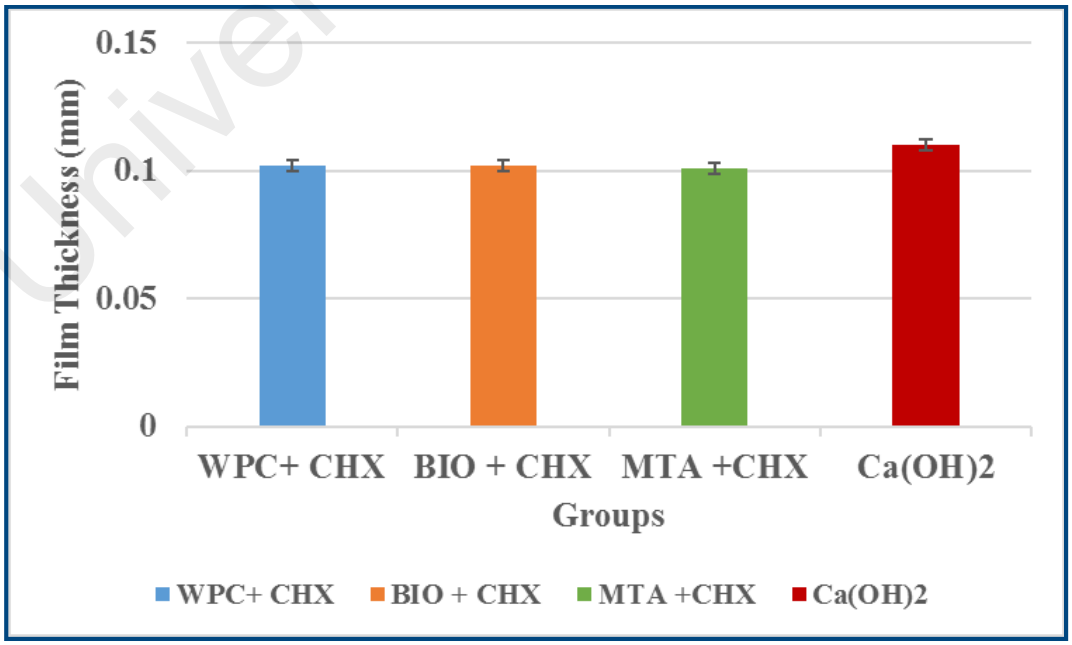

Figure 4.3: Means and and standard deviations of the film thickness for different experimental medicaments and control.

4.2.4 Flowability

Table 4.27 and Figure 4.5 shows means and standard deviations of flowability for experimental medicaments and control. The results showed that the flowability for the experimental medicaments was significantly higher compared to the control ($p<0.05$). It was also noted that within the experimental medicaments, MTA+CHX cement showed significantly higher flowability than the others ($p<0.05$). However, there were no difference in flowability between WPC+CHX and BIO+CHX cements ($p>0.05$) (see Appendix E, Table 1).

Table 4.5: Means and Standard Deviations of the Film thickness for experimental medicaments and control.

Groups	Flowability in (mm) (mean± sd)
WPC+CHX	18.27 ± 1.79^{b}
Bio+CHX	17.32 ± 1.80^{b}
MTA+CHX	22.80 ± 1.00^{a}
$Ca(OH)_2$ (Control)	14.15 ± 0.52^{c}

Values followed by the same letter in the Colum are not statistically different (ANOVA/ Tukey tests, $p<0.05$; n= 5).

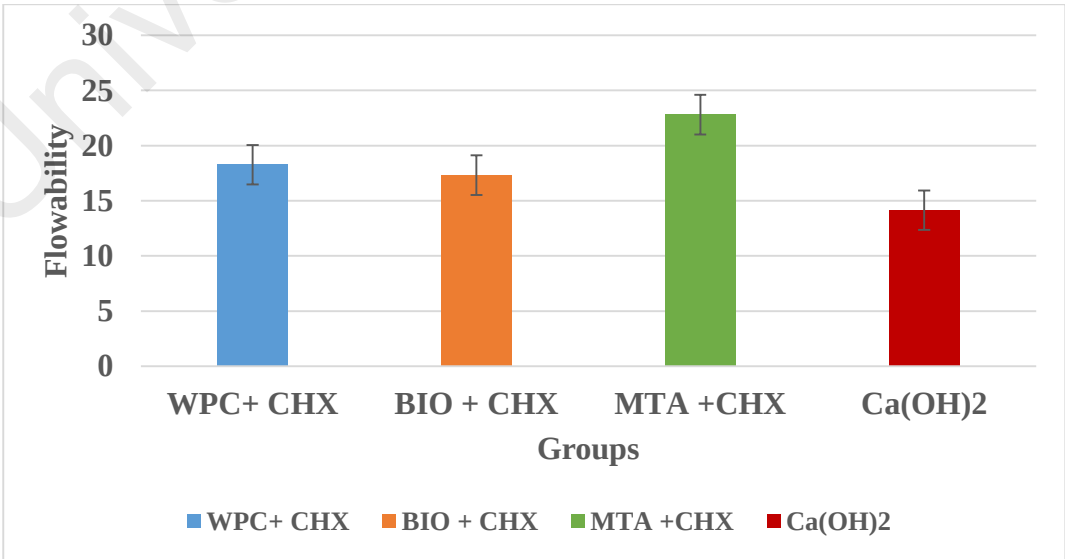

Figure 4.4: Means and standard deviations for the flowability of different experimental medicaments and control.

CHAPTER 5: DISCUSSION

Several studies have been conducted to evaluate and improve the handling characteristics of the MTA mixture. They had improved antimicrobial action and setting time of MTA by the addition of different additives in an attempt to decrease its setting time (Kogan et al., 2006; Stowe et al., 2004). The present in-vitro study had used calcium silicate based cements combined with different vehicles to retard its setting time to be used as intra canal medicament.

MTA has also been shown to be well tolerated by pulpal and periradicular tissues (Baek et al., 2005). MTA has shown significantly better frequency in dentin bridge formation, less pulp inflammation, less and thicker porous dentin, in comparison with calcium hydroxide (Kulan et al., 2016; Parirokh & Torabinejad, 2010). MTA which is one type of calcium silicate based cement are the only alternative agent which are comparable to calcium hydroxide cements.

A case report had investigated the outcome of various root perforation repair done with the aid of MTA and showed that there were no significant clinical and radiological changes in the periraducular area after the follow up period which ranged from 12 – 45 months (Main et al., 2004). A cohort study by Mente et al, (2014) examined 64 teeth which underwent treatment for root perforation using MTA for the period between 12 – 104 months had presented with 86% success rate. A recent long term prospective study for a period of 13years, which reported primary healing after repairing the iatrogenic perforations with MTA had revealed the outcome of 110 patients who had single perforation repair. The study results had showed that in 89% and 3% of cases, the repaired site were healed during first and second year review period respectively (Gorni et al., 2016).

Some investigators had proposed to use calcium silicate cement based agents as a root canal sealer. They suggested this type of sealer can decrease the healing time of periapical radiolucencies and improve the success rate of root canal treatment. They also recommend that this material could be used during obturating a canal with wide open apices where the material is in direct contact with peripaical tissues (Prati et al., 2014). In this situation conventional sealers such as zinc-oxide, calcium-oxide and resin based sealers has the limitation of insufficient healing due to higher cytotoxicity and less biocompatibility (Huang et al., 2002, Bouillaguet et al., 2006). Chang et al. (2014) had showed in their *in-vitro* study that a calcium silicate based sealer (MTA Fillapex) has superior osteogenic potential and lower expression of proinflammatory mediators compared to calcium hydroxide based sealer (Sealapex). However, they had concluded in their study that both the sealers were not cytotoxic (Chang et al., 2014). Additionally, these sealers have poor sealing qualities compared to calcium silicate based sealers in the area where moisture control is suboptimal (Prati et al., 2014).

An unpublished data by Prati et al. (2014) had demonstrated that the inclusion of chlorhexidine in calcium silicate based root sealer revealed that the combination has effective antibacterial property with pronounced effects on dentine remineralization.

Most recently a systematic review of *in-vitro* study comparing the premixed calcium silicate based endodontic sealers with conventional root canal sealers had concluded that calcium silicate based sealers has good physicochemical and biological properties. The properties were similar or better than conventional root canal sealers. (Almeida et al., 2017). However, until now there was no single clinical study describing the outcome of calcium silicate based endodontic sealers.

These studies justify that calcium silicate cements are biocompatible and safer within the root canals. Therefore, it could be used as intra canal medicaments by retarding its setting time.

Therefore, these are the reasons to choose MTA and this is the first study which explores the use of MTA as an intracranial medicament.

Biodentine is believed to have superior biologic and physical properties, which can be used in a variety of clinical treatments as an alternative to MTA, which could be used in restorative dentistry endodontics, pediatric dentistry, and dental traumatology (Rajasekharan et al., 2014).

The white Portland cement (WPC) has similar composition and physical properties to MTA (Funteas et al., 2003). Due to high cost of CSC such as MTA and Biodentine in this current study, WPC was chosen because it is less expensive and readily available in the market.

Chlorhexidine is a broad spectrum antimicrobial agent that has been used in root canal treatment (Signoretti et al., 2011). Studies showed that Chlorhexidine is more effective in elimination of *E. faecalis* within dentinal tubules. The disadvantage is that it does not act as a physical barrier against microbial recolonization and does not have any detoxifying ability against endotoxins (Sinha et al., 2013). However the addition of CHX to MTA showed significantly better antimicrobial activity (Jacinto et al., 2015).

In this present study, three CSC namely White Portland cement (WPC), Biodentine (Bio) and Pro Root MTA (MTA) were mixed with different vehicles in an attempt to retard its setting time and to improve the working properties of the mixture to be used as an intracanal medicament. Physical properties such as Ca^{+2} ion release, pH value, film thickness and flowability were evaluated for the experiment medicament (CSC+CHX).

CSC were mixed with Fluoride Gel, Eugenol, Local Anesthesia with Epinephrine, 5% Sodium Hypochlorite (NACLO), Phosphate-Buffered Saline (PBS) and Calcium Chloride (CACL2) all mixtures were set at the first 24 hours. While CSC mixed with 2% chloxexidine gel (CHX) delayed its setting time to more than 60 days. The results of this study were in agreement to a previous study by Kogan et al. (2006). They had reported that, there is an inconsistency in setting time for the MTA/CHX gel where the mixture did not set even after 7 days. Also in this study, the powder/liquid ratio of the mixture was defined. The materials were prepared with different ratios. The proper ratios assigning adequate texture for easy insertion in root canals were selected. Thus, powder/liquid ratio of 2/1 was used. The ratio used might explain one of the reasons that CSC did not set even after 60 days. According to (Nandini et al., 2010), rinsing of CHX on MTA showed significant surface dissolution, which can explain the impairment of the mixture to set which has been found in this study.

Calcium ion release and alkaline pH stimulate tissue repair and provide antimicrobial environment. It is also suggested that the material should have alkaline pH and calcium release to stimulate mineralization (Estrela et al., 1995). In this study, the release of Ca^{+2} ions were assessed to make sure the vehicle used should not interfere with its release and also not to decrease the pH values. The methodology used in this study to analyse the Ca^{+2} ion release was similar to work done in the literature (Carvalho et al., 2016; Fan et al., 2016; Natale et al., 2015). All materials analysed presented suitable release of calcium in the ICP-OES analysis. The results of this study had demonstrated a reduction in values over time and presenting higher values at the initial period. All CSC mixed with CHX experimental cements had an overall reduction in calcium release after 21 days for the exception of MTA+ CHX showed lower Ca^{+2} ion release than $Ca(OH)_2$ at 21st day. While $Ca(OH)_2$ was likely to be stable during all periods. Among the group of CSC, Biodentine showed the highest release of Ca^{+2} ion until 21st day.

A recent study by Natale et al., (2015) revealed that all cements promote a strong alkalinization of the medium. ICP-OES analysis for two different pH had revealed that calcium released from Dycal was lower than MTA Angelus (pH 5.5) and than Biodentine (pH 7.0). However, several previous studies found that a lower Ca^{+2} ion release from Dycal compared to calcium silicate materials at neutral pH (Gandolfi et al., 2012; Takita et al., 2006). In the present study, the results revealed that higher Ca^{+2} ion release of CSC+CHX at pH 5.0 than the control group and in agreement with results of previous studies (Gandolfi et al., 2012, Takita et al., 2006).

MTA biocompatibility could be related to the high pH. A previous study determined that MTA was able to maintain a high pH in the range of 11 to 12 for 78 days (Fridland & Rosado, 2005). The pH of the CSC mixed with CHX experimental cements had an overall significantly high pH value of 11.97 up to 28th day and a maximum value of 12.18 for Bio+ CHX at 7th day despite the fact that $Ca(OH)_2$ had higher pH value of 12.57 at 7th day and 12.16 at 28th day.

The high pH value of CSC in this study were similar to the findings of Torabinejad et al., (1995). The addition of CHX to CSC increased the Ca^{+2} ion release and the pH and prevented the setting of the material. The results of this work were similar to the findings of a previous study by Jacinto et al., (2015). Elimination of bacteria such as *Enterococcus faecalis* needs an alkaline media greater than pH 11 (McHugh et al., 2004).

Since an inverse relationship between the flow rate and the film thickness was observed (Bagheri, 2013). In this study, the film thickness and flowability were tested because of the importance of the intracanal medicament adequate distribution and penetration into the fine dentine tubules to ensure a complete removal of the bacteria (bactericidal). Also, the results observed that there was no statistically different in film thickness between the CSC+ CHX experimental cements and the control $Ca(OH)_2$. The

results were in agreement with the ISO Standard 6876/2001 film thickness not more than 50 µm. It could be the addition of CHX gel which affected the CSC and still unclear. Therefore, further studies should be conducted to explain the mechanism and the relation between the setting, film thickness and flowability.

However, the new material exhibited reasonable film thickness and flow, which was statistically different from MTA. The slight expansion and reasonable flow and film thickness of MTA can ensure an effective seal after setting and reduction in the subsequent leakage. It has been reported by a recent study whose findings are in agreement with the present study that is Mesoporous calcium-silicate nanoparticles combined with chlorhexidine resulted in the release of ions and CHX, less cytotoxicity, excellent antibacterial potential and in vitro mineralization. Hence, this material could be used as an effective intra-canal medicament or a latest bone defect filling material for infected bony defects (Fan et al., 2016).

These physical properties may account for the superior sealing ability of MTA over other root-end–filling materials (Asgary et al., 2008; Maltezos et al., 2006; Torabinejad et al., 1993). It has been reported by a recent study that 2% CHX gel was known to be vehicle which brought some improvements to antimicrobial activity of MTA (De andrade et al., 2015). However, Biodentine was found to exhibit much better antibacterial property against E. faecalis than MTA did and therefore, could be used as a better alternative to MTA being a root end filling and root repair material (Jose et al., 2016).

ISO specification 6876/2001 establishes as 20 mm is the acceptable minimum value for the diameter of the disc formed by the sealer. In this study, the highest flow was presented in MTA+ CHX (22.80 mm), followed by WPC+CHX (18.27 mm), followed by Bio+ CHX (17.32 mm) then $Ca(OH)_2$ (14.15mm). Thus, only MTA+ CHX was in agreement with ISO specification 6876/2001. However, in comparison to the control CSC

+ CHX had better flow. In this study, it has been observed that the increasing liquid to powder ratios leads to improve flowability of the cement. High liquid-to powder ratios might be beneficial for releasing calcium hydroxide (Fridland & Rosado, 2003).

More recently study by Marciano et al., (2016) observed that the zirconium oxide has been tested as a substitute to bismuth oxide due to its adequate radiopacity (Duarte et al., 2009; (Camilleri et al., 2014) and no interference with hydration of WPC (Camilleri et al., 2013). Calcium tungstate has also been investigated as alternative radio- opacifier to bismuth oxide, and it shows adequate physical and chemical properties when associated with WPC (Duarte et al., 2009).

However, it was not possible to make a real comparison between the results of the present study and literature because of the absence of studies assessing the effect of 2% CHX gel added to CSC in the ratio of 2/1 powder/liquid. The addition of CHX improved the flowability and film thickness of the MTA.

In this study, it has been observed that the increasing liquid to powder ratios leads to improve flowability of the cement. High liquid-to powder ratios might be beneficial for releasing calcium hydroxide (Fridland & Rosado, 2003).

CHAPTER 6: CONCLUSIONS AND FUTURE STUDIES

6.1 Conclusions

1. The addition of 2% CHX to CSC had inhibited its setting reaction over a period of 84 days.
2. The calcium ion release and flowability of this CSC/CHX mixture cement were found to be better than non-setting Calcium Hydroxide.
3. The pH value of $Ca(OH)_2$ was higher at all times compared to CSC+CHX experimental cements, However it still had high pH.
4. There were no significant difference in Film thickness.
5. Among CSC experimental cements BIO+CHX showed superior physical properties compared to others.

Clinical Significant: because the failure of some endodontic cases due to microbial regeneration we mixed cholrhexidine which is known as a bactericidal agent with MTA which has similar or better properties than calcium hydroxide and hoping to deliver a better intercanal medicament with no drawbacks.

6.2 Recommendations for Future Studies

1. This material need more investigations for its cytotoxiety.
2. This material should be investigated for its antibactiral effect.
3. This material should be investigated for clinical trial for short and long period.
4. Chemical investigations should be conducted to show the effect of ingredients for long clinical trails.

5. The removal of the root canal medicament from the root canal system should be investigated.
6. Radio opacity of the material should be investigated.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

1. Poster presentation at IADR 2014 in Malysia.
2. Patent for developing new intracanal medicament in 2014.
3. Oral presentation at IADR 2017 in USA.
4. One paper (article) under- review.