國立臺灣大學工學院暨醫學院醫學工程學研究所

碩士論文

Institute of Biomedical Engineering
College of Medicine and College of Engineering
National Taiwan University
Master Thesis

發展不含鐵之介穩水泥做為牙科逆向封填材料的研究

The Development of Iron Free Partially Stabilized Cement for Dental Retrograde Filling Material

董芳慈
Fatou Ndong

指導教授：林峯輝 博士
林俊彬 教授
Advisor: Feng-Huei Lin, Ph.D.

Chun-Pin Lin, DDS, MS, PhD

中華民國 100 年 01 月

January, 2011

Abstract

A Nobel one-step sol-gel process for adding zinc to PSC-system was developed. Different weight percentage of zinc concentrations (1%, 3%, and 5%) were added to PSC-system and analysis of the new final material was done. In this study, 5 wt% PSC-Zn significantly decreased the initial setting time of PSC Cement to 10 minutes and its final setting time to 30 minutes compared to MTA which has an initial setting time of 30minutes and a final setting time of 210 minutes. Zn is naturally abundant in the human body and is the key element for bone development and healing. PSC with different percentages of zinc added was not only non-toxic at cellular level but also has adequate mechanical properties that qualify it to be a good root-end filling material.

At a preparation temperature of 80^0C, PSC-Zn increased the phase contents of Ca_2SiO_5 (C_2S) and Ca_3SiO_6 (C_3S) but decreased CaO. As a result, the setting time was shortened. It was discovered that as more weight percentage of Zn concentration was added, more unstable state was effectively created and monoclinic structure of C_3S was favored to form, which increased the hydrated reaction of PSC-Zn and shortened the setting time as shown in the SEM and XRD data. The new PSC-Zn material has great sealing ability, good bio-compatibility and an ideal setting time which is believed to have magnificent potential in its application to perforation repair and retrograde filling in Apical surgery.

Key Words: Endodontic Surgery, Calcium Silicate Cement, Apicoectomy

Acknowledgement

To my late father who is the best man known to have born on earth and my family for always been supportive and encouraging. To Roderick who left the USA to support me in Taiwan and being a wonderful and understanding husband.

My deepest appreciation goes to professor Feng-Huei Lin of National Taiwan University, Department of Biomedical Engineering for believing in me from day one and advising as well as sponsoring this research. Professor Chun-Pin Lin of National Taiwan University, Department of Dentistry for his advice and providing MTA samples as well as allowing me to use some of the equipments in his lab. Professor Rock C.K Hsu of National Taipei University of Technology, Department of Materials and Mineral Resources for being supportive and allowing me to use some of the equipments in his lab. Wang Wen-Hsi my mentor for always being there despite his busy schedule.

I sincerely thank the following for helping in one way or the other to make this research a success. Kai-Chun, Chun-Jei, Dr Kuo, Luise, Ja-Jin, Goldfish and Yu-Ting Lin.

Chart List

Table of List

Content

Chapter 1

1-0 INTRODUCTION

1-1 Root-End Surgery

The food we eat is broken down into smaller pieces by the teeth for better enzyme activity. The scientific name for this process is Digestion. The process start in the mouth and ends in the smaller intestine, this is the process by which the teeth initially breaks down the food into manageable sizes for further enzyme activity in the small intestines. The process of breaking down food in the mouth results in the smaller particles of food to lodge between the teeth, causing decay, plague and subsequently tooth lo**ss.** Endodontic treatment is one of the most common dental procedures that can be performed to prevent tooth loss. To help understand endodontic treatment, a sectional photo of the tooth anatomy is shown in Figures 1and 2.

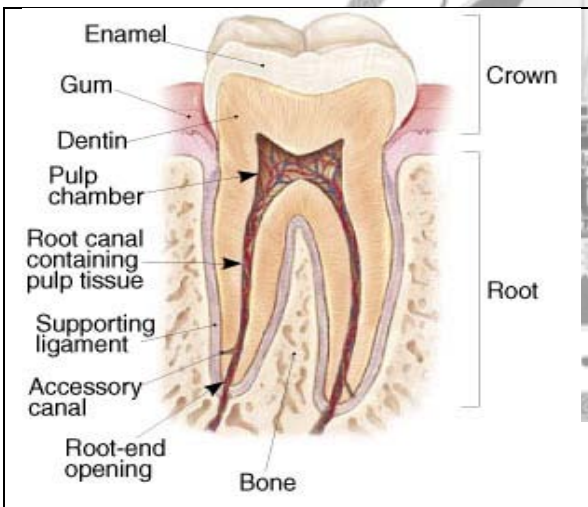


Figure 1: A healthy tooth Anatomy

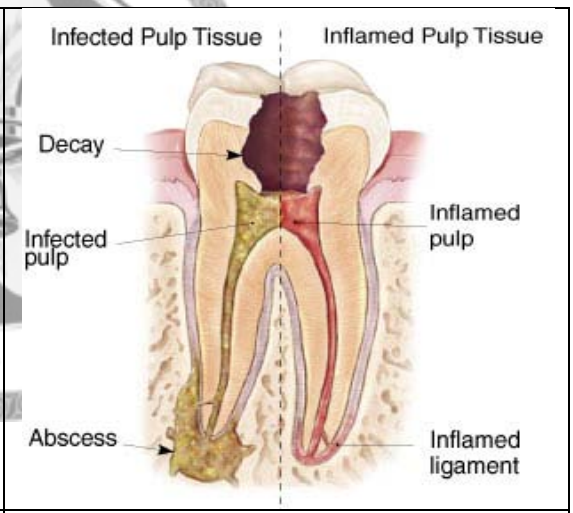


Figure 2: An infected tooth Anatomy

The pulp lies underneath the dentin inside the enamel; it is composed of soft connective tissues with an abundant supply of nerves, blood vessels, and lymphatics. The peripheral tissues including cementum, periodontal ligament, and alveolar bone are connected with the dental pulp via the apical foramen and lateral canals [89]. When the pulp is exposed to micro-organisms, the use of a material for sealing canal is necessary to avoid its propagation through the pathways of communication between the root canal system and the oral cavity. MTA is the best known

clinical material in the market but one of its major drawbacks is long setting time. Partial stabilized cement (PSC) is a novel innovated material prepared to address some of MTA's drawbacks if not all. If the pulp inside the root canal of a tooth becomes inflamed or infected, then a root canal treatment is necessary. There can be a variety of factors that cause the inflammation or infection such as: repeated dental procedures on the tooth, deep decay, cracks or chips in tooth. The endodontist removes the inflamed or infected pulp, carefully cleans and shapes the inside of the canal, a channel inside the root, then fills and seals the space with root-end filling material.

1-1-1 **Why would Patients need endodontic surgery?**

Endodontic surgery is a procedure where the endodontist opens the gum tissue around the tooth to inspect the bone and or to remove inflamed/ infected tissue. The end of the root can be removed and filled to seal the root canal. The gingival is sutured after surgery to ensure proper tissue healing which may take months. Other surgeries may include dividing a tooth in half, repairing an injured root, or removing one or more roots. In certain cases, a procedure called intentional replantation may be performed. Intentional replantation is a procedure, in which a tooth is extracted, treated with an endodontic procedure whiles it is out of the mouth, and then replaced in its socket.

Surgery can help save an infected tooth in a variety of situations:

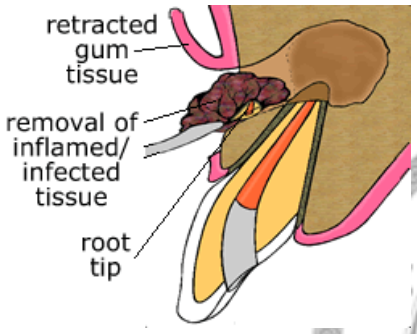
- It may be used as part of a diagnosis to intrusively investigate problems that are not detected by x- ray. Particularly in situations where the symptom persist, but eludes x-ray detection. E.g. a tiny fracture or canal that could not be detected during nonsurgical treatment. In such a case, surgery allows your endodontist to examine the entire root of your tooth, investigate the problem, and provide appropriate treatment.

- In situations where calcium deposit causes the canal to narrow down beyond the reach of instruments used in nonsurgical root canal treatment. If a tooth develops "calcification," then the endodontist may have to perform endodontic surgery to clean and seal the remainder of

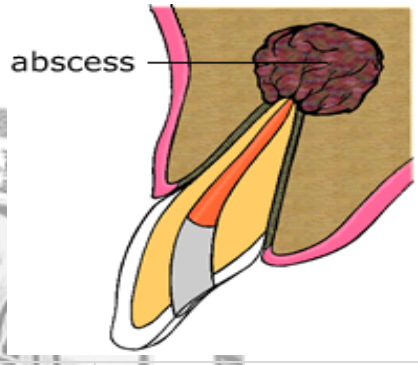
the canal.

- A tooth that has undergone a root canal treatment can last a life time and never need further endodontic treatment. However, in a few isolated cases, a tooth may not heal properly or may become re-infected after successful previous treatment. If such a situation arises then surgery may be the most viable option available.

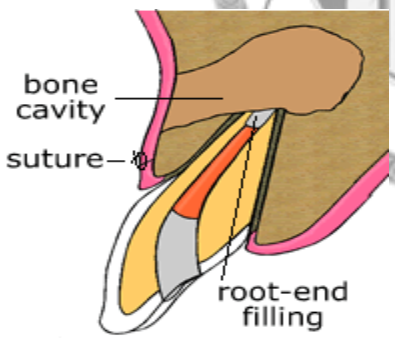
- Surgery may also be performed to treat damaged root surfaces or surrounding bone.



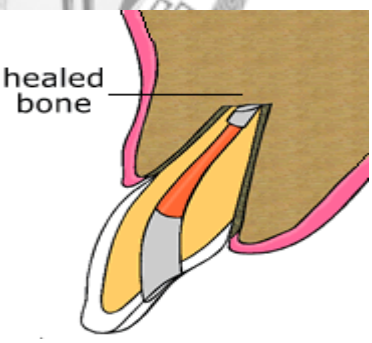
Step 1 **The gum is opened to remove the infected tissue**



Step 2 **The root canal is cleaned**



Step 3 The canal is filled and the gum sutured



Step 4 Healing takes place

Figure 3 Diagrams of the Steps involve in Apicoectomy [48]

1-2 Root-End Filling Materials

An ideal root-end filling material must be bio-active or bio-compatible, easy to handle, dimensionally stable, non-resobable and radiopaque [22, 23], have a good sealing ability, non-toxic, non-mutagenic and a great anti-bacterial ability. In addition, a novel root-end material should have a short setting time.

None of the commercially available root-end materials fulfills all or most of the criteria as an ideal material [23, 25]. Below is a list of currently used root-end filling materials with their drawbacks: In the previous study [46], a dental root-end filing material called Partial Stabilized Cement PSC was synthesized by traditional powder mixing method. The reaction efficiency of traditional powder mixing method was quite low, and this resulted in longer setting time, low initial strength of the material, and poor handling property [22]. In order to improve on the drawbacks of PSC, transition metal elements such as zinc, cobalt and chromium were added to the material by sol-gel method [43]. Zinc was specifically added in this study to cancel the concern of metal toxicity in PSC [47]. Cobalt and chromium added to PSC has the same effect but the cytotoxicity concern is raised [44]. The human body has and can tolerate traces of zinc which plays an important role in biochemistry activity such as the development of bone structures [48]. The addition of zinc to the PSC system has greatly improved the initial strength of PSC, its reaction efficiency and setting time. In this study, a one-step sol-gel process of PSC synthesis with the addition of zinc weight percentages was done. XRD and SEM were used for the material analysis, and the performance of PSC was evaluated by setting time test and hydration product. The purpose of this study was to develop a root-end filling material known as **Partial Stabilized Cement (PSC)** to improve on the drawbacks of the already existing materials if not totally cancel their disadvantages.

1-2-1 Amalgam

Amalgam is an alloy of silver and tin used in dental restoration with mercury as a combiner. It is the longest and most widely used dental material. Farrar was the first scientist to use Amalgam as a root-end filling material in 1884. It is easily manipulated with good radio opacity and it's

non-soluble in tissue fluid. Marginal sealing adaption improves as amalgam ages due to its corrosive characteristics. However, there are several limitations which are initial marginal leakage, mercury contamination, very sensitive to moisture, and cytotoxicity due to un-reacted mercury which is gradually released to the body.

1-2-2 Zinc Oxide Eugenol (ZOE) and reinforced ZOE cement

There isn't much information about ZOE used as conventional root-end sealing agent. However, there are newer modification of ZOE compound such as IRM and super EBA that provide a better apical seal. IRM is zinc oxide-eugenol cement reinforced by addition of 20% polymethacylate by weight to the powder [26]. Studies reveal that IRM seals better than non zinc amalgam [27]. Super EBA is zinc oxide-eugenol cement modified with ethoxybenzoic acid to alter the setting time and increase the strength of the mixture [26]. Super EBA shows better physical properties than conventional ZOE in high compressive strength, high tensile strength, neutral pH and low solubility. In a series of investigations performed recently [49], super EBA and IRM are found to be more bio-compatible than other formulations of ZOE [24].

When properly handled, IRM performs very well. However, IRM lacks cohesive property and should be inserted as a single mass with further condensation rather than incrementally placed [28]. Super EBA provides an optimum seal, minimal tissue toxicity and good handling property when properly mixed. It is difficult to mix, requiring more effort and practice than most other root-end filling materials [23].

1-2-3 Composite Resin

Composite resin another material (and technique) borrowed from restorative dentistry and adapted to endodontic surgery [23]. Composite resin is mostly used in combination with dentin bonding agent. Rut el al has demonstrated excellent long-term clinical success with the use of Retroplast composite resin and Gluma (Bayer AG) dentin bonding agent [30]. Its drawbacks are

sensitivity to moisture, Micro-leakage [29] and uncured oxygen-inhibited surface layer that may interfere with initial healing after surgery.

1-2-4 Mineral Trioxide Aggregate (MTA)

Mineral Trioxide Aggregate is a potential alternative conventional material developed at the Linda University in 1993 [31]. The composition of MTA includes Tricalcium Silicate, Tricalcium Aluminates, Tricalcium Oxide, Silicate Oxide and other mineral oxides [32]. Adamo et al.[33] found no statically significant difference in the rate of micro-leakage amongst MTA, Supper EBA, Composite and Amalgam. However, the studies of Torabinejad et al. [34, 35] and Fischer et al. [36] prove that MTA is superior, when compared to Super EBA and IRM. Several in-vitro and in-vivo studies have demonstrated that the sealing ability and bio-compatibility of MTA are superior to that of Amalgam, IRM, and Super EBA [37-39]. MTA has a high pH value of 12.2 at the beginning of hydration, and 3hours after mixing; the pH rises to 12.5 [24], which may contribute to its anti-bacterial ability [40]. It drew a lot of attention due to its role in apical surgery. However, it is not easy to handle, has a long setting time and obtaining consistent results in clinical applications can be difficult. In addition, Lee et al. [41] demonstrated that hydration behavior of MTA will be affected by an acidic environment during setting, which results in the weakening of the material's micro-structure and the decreasing of its physical strength. This study suggests that the long setting-time of MTA is a possible disadvantage as stated by Wang Wen-Hsi et al [29], because many environmental factors may affect the properties of MTA during setting period.

1-2-5 Calcium Silicate Cement

Calcium Silicate cement was first developed as a Portland cement and obtained as a patent by Joseph Aspdin, a British bricklayer. He is usually regarded as the inventor of modern Portland cement [46]. There are four major components of calcium silicate cement: Tricalcium silicate ($(CaO)_3.SiO_2$, C_3S), Di-calcium silicate ($(CaO)_2.SiO_2$, C_2S), Tricalcium aluminates ($(CaO)_3.Al_2O_3$, C_3A), and tetra-calcium aluminoferrite ($(CaO)_4.Al_2O_3.Fe_2O_3$, C_4AF). Phase's hydration

in materials gives calcium silicate its strength. Each phase has a specific reaction with water to produce a different kind of hydration product which gives a dense and strong solid. C_3A has the fastest reaction rate amongst all and generates the highest heat but contributes least in the ultimate strength of the cement, albeit it's early significant contribution to strength. C_3S is most reactive, giving early strength but C_2S has a better long term contribution. C-S-H is the principal binding phase in calcium silicate, and is quantitatively the most significant hydration product. The reaction rate of C_4AF is intermediate between C_3S and C_2S but it has an important long term contribution to strength and durability [46, 47].

Calcium silicate cement such as Portland cement and MTA has great potential as root-end filling material. The sealing ability of calcium silicate cement can efficiently close the channel between the oral environment and the root canal. It has anti-bacterial ability due to its rising pH value and is bio-compatible with bone tissue proved in the study of Saidon in 2003 [53]. However, the development of calcium silicate cement as a root-end filling material is limited by its long setting time, similar to the drawback of commercial MTA. We have previously reported the development of partial-stabilized cement PSC [43], which is a modified calcium silicate cement composing of Tricalcium silicate, Di-calcium silicate, Tricalcium aluminates and calcium aluminoferrite, with a specific ratio of each component. The composition of MTA and PSC is quite similar, except that MTA lacks calcium aluminoferrite and PSC lacks bismuth [43]. Since the base material for both MTA and PSC is calcium silicate cement, they share similar advantages and disadvantages. The drawback of long setting time is greatly improved by the addition of transition metal elements such as Co, Cr and Zn, in PSC [43]. However, with the addition of transition metal elements, the concern of toxicity is also raised.

In the study of Wang Wenhsi et al. [43], the addition of Co and Cr showed significant cytotoxicity and thus influence the cell function and metabolism. The effects of metal ions in medical devices and implants have been investigated for years [43-58]. Metals are unique among pollutants because of their naturally adverse health effects which are ubiquitous in their environment. Adequate level of exposure of metal ions in the human body is inevitable most times. Furthermore, many are biologically essential but become toxic with increasing dosage [60]. The toxicity of cobalt and chromium has been reported in many studies [43, 60-63]. These two metal ions have various influences on cell proliferation, gene expression and cytokine

secretion [63]. In the study of Anissian et al. [61], high cobalt ion concentration reduced proliferation and osteoblast activities. Moreover, the addition of Co and Cr will lead to the development of cytoplasmic vacuolation and extensive cell death [43]. Wang et al. [43] also indicated that chromium ion will enhance the production of IL- 1b, IL-6 and TNF-α and inhibit synthesis of osteocalcin and type 1 collagen, which suggests that chromium ions will cause defects in osteoblast bone formation. This result may be due to the oxidation state of chromium in the solution. According to the study of Rose et al. [66], there are two different oxidation states of chromium which are hexa-valent and tri-valent in the calcium silicate and hexa-valent chromium has been shown to be more toxic than the tri-valent chromium in in-vitro in an intact cell system [67, 68]. Hexa-valent chromium is recognized as an environmental and occupational carcinogen [63]. It is toxic because it is easily taken up into cells. At physiological pH, hexa-valent chromium exists mainly as the tetrahedral chromate anion, $CrO_4^{2-,}$ and there are also a number of physiological anions such as sulfate and phosphate, which are also tetrahedral. The hexa-valent chromium can enter cells through a relatively non-selective anion channel because of the chemical similarity [63]. Once inside the cells, unstable hexa-valent chromium is reduced by intracellular reductants through the formation of reactive intermediates, such as tetra-valent chromium and hexa-valent chromium to form more stable tri-valent chromium [69]. The formation of tri-valent chromium and the intermediate oxidation states at physiological pH plays an important role in the mechanism of hexa-valent chromium cytotoxicity. This intracellular reduction phenomenon is also quite critical in the expression of DNA damage and toxicity [70-72].

According to the above description, several cell functions are disordered and cell cycle is regulated toward apoptosis due to the addition of transition metal elements in PSC although the setting property is greatly improved [43, 73]. As a result, the addition of zinc was chosen in this study.

Hydration reaction of each component of calcium silicate cement [47]

Reaction of Principal Phases in calcium silicate cement

$$C_3S + H_2O \rightarrow C\text{-}S\text{-}H^* + Ca(OH)_2$$

$$C_2S + H_2O \rightarrow C\text{-}S\text{-}H^* + Ca(OH)_2$$

$$2C_3A + 18H_2O \rightarrow C_2AH_8 + C_4AH_{10}$$

$$2C_3A + 32H_2O + 3(Ca_{2+\,(aq)} + SO_4^{2-}{}_{(aq)}) \rightarrow C_6AS_3H_{32}$$

$$C_6AS_3H_{32} + 2C_3A \rightarrow 3C_4ASH_{12}$$

C_4AF has analogous reaction to C_3A, but produces $C_6(AF)S_3H_{32}$

*C-S-H is an amorphous hydrogen having variable

Composition in terms of Ca/Si ratio and H_2O/SiO_2 ratios

Chapter 2 THEORETICAL BASIS

2-1 Sol-Gel Process

There are many definitions about sol-gel process. For example Dislich [75] thinks that the sol-gel process only includes multi-components that are homogenous at atomic level. However, this definition does not include those colloidal co-precipitates of hydroxides and oxyhydrates since they become homogenous only by reaction at high temperature. Under this definition, the term “sol-gel” is restricted only to those gels that synthesized from alkoxides. Segal [76], on the other hand, considers sol-gel as the production of inorganic oxides, either from colloidal dispersion or metal alkoxides. Moreover, the term “sol-gel process” no longer includes solely oxides but also some other components such as nitrides and sulfides for the synthesis of hybrid organic-inorganic materials.

There are so many variations that can be brought to sol-gel synthesis of ceramics. As a matter of fact, sol-gel process does not only designate a unique technique, but a broad type of procedures [77]. The first step of sol-gel process is the selection of precursors for the wanted materials. Precursors react with each other spontaneously to form either colloidal particles or polymeric gels. The resultant gel can be fabricated into several different forms, such as coating, fiber, powder, and monoliths, depending on the fabrication process.

There are many advantages to sol-gel processing. For example, very pure product is produced by simply purifying the precursor either by distillation, crystallization, or electrolysis, and the first step of the chemical process is always carried out in low temperatures. By controlling the nucleation and growth of the primary colloidal particles, specific shape, size, and size distribution of the particles can be easily obtained. Moreover, sintering temperature of both amorphous and crystalline materials can be lowered of several hundred degrees Celsius than conventional process. The structure of sol-gel ceramics can also be easily controlled as the size of the particles. An amorphous or semi-vitreous state is for example, quite easy to obtain and many new types of glasses that are not feasible by conventional quench techniques can now be synthesized [77]. One of the most outstanding advantage of sol-gel process for mixed oxide systems is the chemical homogeneity of the various elements can be controlled down to atomic level. This condition is virtually impossible when solid powders are mechanically mixed in the conventional processes [77].

There are also limitations to the synthesis of ceramic by sol-gel process. One of the greatest limitations is the cost of the precursors, especially that of alkoxides. Most of these alkoxides are not quite easy to prepare, especially for those that do not tend to polymerize. Therefore, sol-gel synthesis of ceramics will never be able to compete for the mass production of some large scale materials such as window glass for which the conventional process can rely on much cheaper raw materials [77]. However, sol-gel process is much more superior to conventional process in the synthesis of highly advanced ceramics.

2-2 Precursors

There are two major groups of precursors which are metallic salts and alkoxides. The general formula of metallic salts is M_mX_n where M is the metal, X an anionic group, and m and n represent stoichiometric coefficients, such as $AlCl_3$. As for alkoxides, their general formula is $M(OR)_n$ which indicates that they are a combination of cation M with n alcohol groups ROH, such as $AL(OC_2H_5)_3$. The choice of the solvent could be either water or organic liquid, depend on the precursor because of the different solution chemistry in these two groups.

When metal salts are used in the sol-gel process, they are often dissolved in an aqueous medium. As the salt MX dissociates into ions, the anions' negative charge X^{z-} balances the positive charge of the metal atom M^{z+}. The absolute formal charge z is the same in both cation and anion. Anions are sometimes considered as impurities, even though they could be eliminated in order to produce pure oxide ceramics. However, they are also very important in channeling the chemical transformations within the solution. Ions will first solvate with water molecules because of the polar nature of the solvent.

Alkoxides which are also called alcoholates are the compounds with chemical formula $M(OR)_z$ that are the products of direct and indirect reactions between a metal M and an alcohol ROH. There is a great variety of alkoxides that can be produced for each metal because of the existence of many different alcohols.

2-2-1 Hydrolysis and Condensation Reactions of Metal Salt Precursors

Hydrolysis and condensation are two major reactions of sol-gel process. In order to understand hydrolytic equilibriums in aqueous solution of metal salts, the calculation of partial charges on the atoms is needed. There will be a partial electron transfer occurring until the electro-negativity χ_i of each atom is equal to the mean electro-negativity χ for the system. The atoms have acquired appropriate partial charge δ_i. The electro-negativity of an atom is related to its partial charge by the equation:

$$\chi_i = \chi_i^0 + \eta_i \delta_i$$

Where χ_i^0 is the electro-negativity of the neutral atom and η_i is the "hardness" defined as:

$$\eta i = k\sqrt{\chi i0}$$

Where k is a constant (1.36 for electro-negativities on the Pauling scale).The mean electro-negativity χ is given by

$$X = \frac{\sum_i Pi\sqrt{\chi i0 + kz}}{\sum i(Pi/\sqrt{\chi i0})}$$

(Phi is the stoichiometry of the i^{th} atom in the compound and z is the next charge on the ionic species). From these equations, the partial charge δ_i is given by

$$\delta_i = (\chi - \chi_i^0)/\sqrt{\chi i0}$$

and can thus be calculated since the electro-negativities of the neutral atoms, the stoichiometric composition and the ionic charge are all known. The nature of the species present in aqueous solutions of metal salts is determined by the position of the equilibrium:

$$[M\ldots OH_2]^{z+} \leftrightarrow [M-OH]^{(z-1)+} + H^+ \leftrightarrow [M=O]^{(z-2)+} + 2H^+$$

If the partial charge on OH is positive, the reaction

$$M^{\delta+} - O^{\delta-} - H^{\delta+} + H_2O \leftrightarrow M - O^- + H_3O^+$$

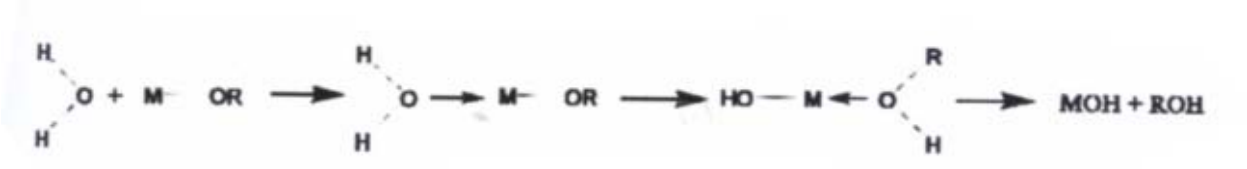
will proceed to the right. From the above equations, the proton removal is facilitated by high formal charge on the metal ion and by low coordination number, with a relatively minor effect of electro-negativity.

After the hydrolysis, condensation reaction will take place in the sol-gel process. One of the ligands acts as an attacking group for linking with a second metal species depending on whether or not this species already has its preferred coordination number, and the other existing ligand group may act as a leaving group in the process. For pure aquo ions, water of hydration is a very poor nucleophilic attack group and there is thus no tendency toward condensation. For oxo species, the oxygen atoms which are highly negative partial charges give very good nucleophilic attack properties, but are very poor leaving groups because of the strong electrostatic attraction to the highly positive metal centre. Condensation will only

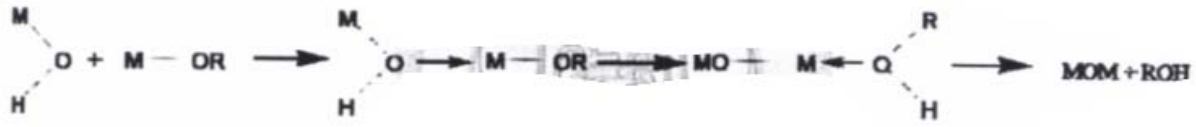
occur for oxo ions when the coordination shell can be expanded, permitting addition condensation.

2-2-2 Hydrolysis and Condensation of Metal Alkoxide Precursors

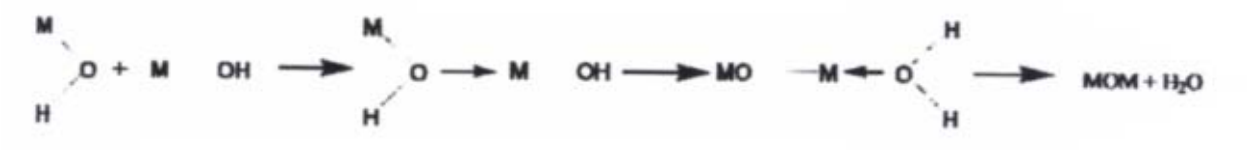
Where there is no acid or base catalyst in the system, metal alkoxides react first by hydrolysis involving nucleophilic addition of a water molecule followed by proton transfer from water to the alkoxy group which then leaves as alcohol:



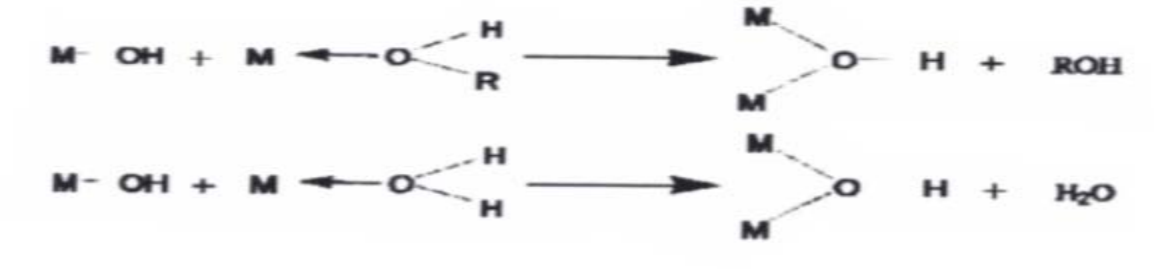
This is followed by reaction of the resulting MOH species with a further alkoxides (alcoxolation):



Or another MOH species (oxolation):



Or solvated metal species (olation):



The thermodynamics of these different process are determined by the partial negative charge of the incoming nucleophile in hydrolysis, the partial positive charge of the electrophilic metal and the partial charge and stability of the leaving group (with more positively charged groups leaving most readily) [78].

2-3 Objective/purpose of the Study

In this study, it was assumed that adding zinc as a transition metal element using a one-step sol-gel process to synthesize PSC will help obtain the unstable phase of PSC at room temperature and thus improves its setting time as a root-end filling material. To also improve the reddish-brown and gray color of PSC gel and powder respectively by removing iron from the PSC system, and the handling property by adding calcium sulfide powder which is used as a combiner in dentistry.

2-4 Reasons for Adding Zinc to PSC Cement

- Highly reactive
- Non-toxic to human body
- Ability to create more defects
- Creates defects by replacing either Ca^{+2} or Si^{+4} with Zn^{+2} in the PSC system as Shown in Figure4:

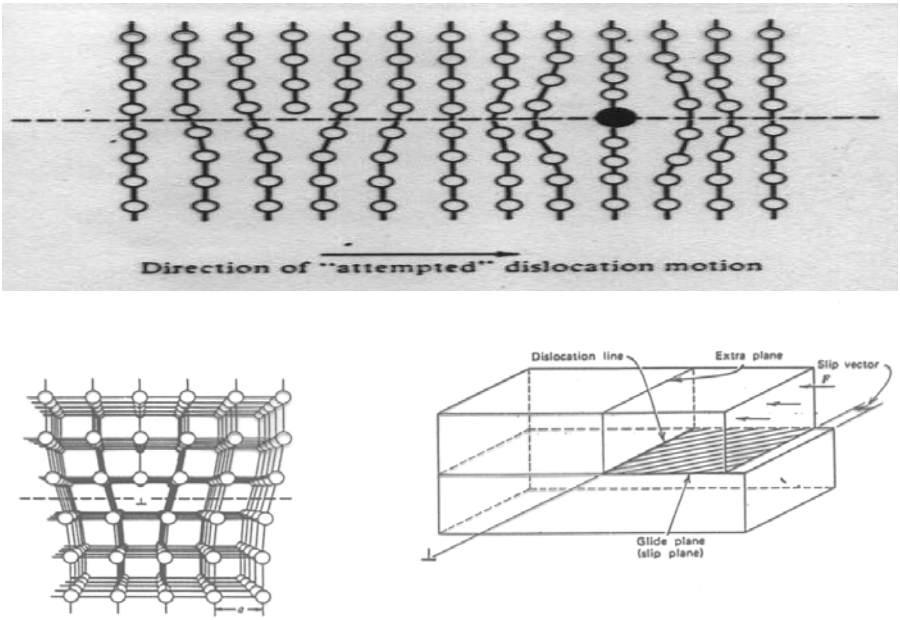


Figure 4: Diagram showing Ionic Dislocations

- Zinc ions (Zn^{+2}) are larger in size than calcium ions (Ca^{+2}). When zinc is added to PSC system, zinc ions (Zn^{+2}) partially replaces calcium ions (Ca^{+2}). A defect is formed due to the difference in ion size. According to Kroger-Vink, neutral atoms are electrically added or subtracted from crystals to avoid making judgments about bond types. In this system, we can assume substitution reaction for Zn and C_3S as:

$$\mathbf{ZnO \xrightarrow{3CaO.SiO2} Zn''_{Si} + O_0 + V_0}$$ Wang et al

From this equation, 1 mol of Zn''_{Si} substitution can create 1mol of oxygen vacancy. This creates an unstable phase of the PSC material at room temperature which enables it to react with water and body fluids. More defects in PSC increases the rate of nucleation of portlandite and finally the hydration reaction which shortens the setting time. Hence, the ability of PSC to be a good retrograde filling material.

Chapter 3 MATERIALS AND METHODS

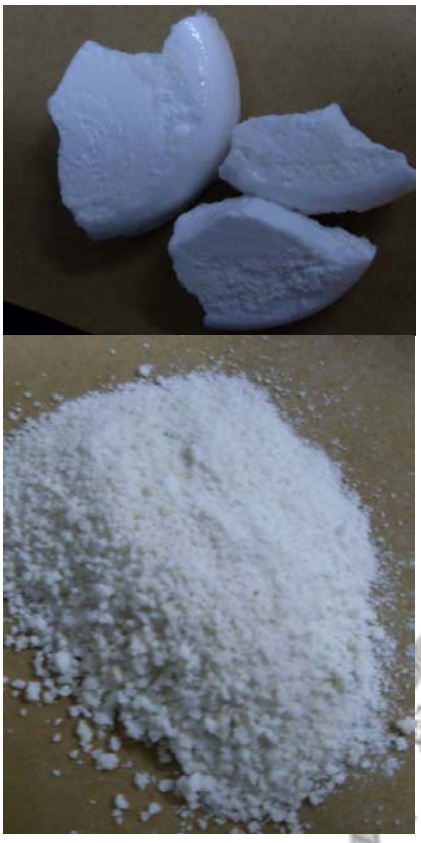
3-1 Selection of Alkoxide and Metal Salts

Tetraethyl orthosilicate ($Si(OEt)_4$), aluminum sec-butoxide (ASB, $Al(OBu^s)_3$), zinc nitrate ($Zn(NO_3)_2$), and calcium nitrate ($Ca(NO_3)_2$) were used as the source of Si, Al, Zn, and Ca, respectively in the sol-gel process. Acetyl acetone (acac) was used as the complex ligand for the modification of $Al(OBu^s)_3$. Ammonia water was used as a catalyst in the process in order to facilitate reaction between alkoxides.

3-2 Material Preparation

A schematic diagram of the preparation is presented in figure 5. First, $Al(OBu^s)_3$ (5.7g) reacted with acac for 4h in the complex ratio of (x) equaled to 1 and $Al(OBu^s)_3$/(acac) complex was formed before further sol-gel process took place. Complexion ratio (x) represents the molar ratio of acac and $Al(OBu^s)_3$, Al(OBus)3/acac). After the surface modification of $Al(OBu^s)_3$, TEOS was added into the solution and well mixed. The aqueous solution of $Ca(NO_3)_2$ and $Zn(NO_3)_2$ were added and 1.5mL of ammonia water were added as a catalyst. $Zn(NO_3)_2$ was added in concentrations of 1%, 3%, and 5% in weight ratio. Gel was formed after 24h of stirring and 72h of rest in air. The gel was heated for 1400 ^{o} C for 2h and quenched in air.

A one-step sol-gel process of PSC with iron added was used as a control group in this preparation. This has the same precursors and preparation procedure as the above except that Zinc nitrate was replaced by 3.2% iron nitrate. Note: Iron was removed from the system to improve the gray color of the final powder product where a white powder was obtained.



PSC Dry Gel and Calcined PSC powder at 1400^0C

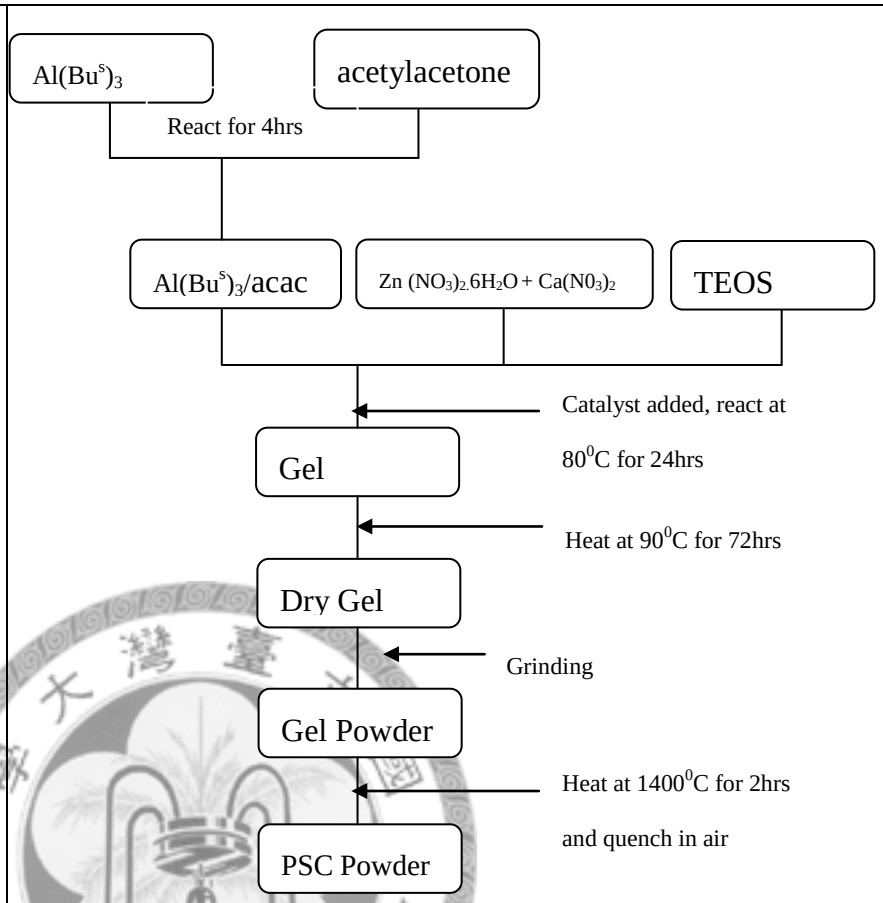


Figure 5 Schematic Diagram for the Preparation of PSC powder by sol-gel process.

Several ways of ceramic synthesis are known. One of the most common techniques is powder mixing method used in laboratories and industries. The starting powders need to be well blended and then ground or milled. The resulting powder is then calcined at the right temperatures. In most cases, high temperatures are required for better and proper reaction between components. This can result in the lost of volatile oxides and a complete reaction is not always guaranteed after milling. Hence the difficulty in reproducing uniform materials especially; when one component is present in a smaller fraction than the others. In-homogeneity and coarse particle size are some of the drawbacks that limit the traditional powder mixing method. Particle size, morphology, purity and chemical composition greatly affects the property of the ceramics produced [1]. A better method, sol-gel, which is a chemical technique, was confirmed to efficiently control the morphology and chemical composition of the prepared powder [2].

Sol-gel is an attractive chemical method of ceramics and glass preparation that has been used by many scientists [3]. Many researchers are drawn to this technique due to its outstanding characteristics such as: high chemical homogeneity, low processing temperature, and uniform phase distribution in multi-component systems, higher reactivity of the products and small particle size availability to name a few [4]. In-organic polymerization reaction is the basic mechanism of sol-gel process. The oxopolymers are formed during the process of hydrolysis and condensation, and will transform into oxide network upon calcination [1]. The chemical procedure strongly influences the structure and morphology of the resulting powders [5]. Albeit the wide use of sol-gel process for the synthesis of ceramics and glass [1, 6-16], there are only few cases regarding the synthesis of calcium silicate cements by sol-gel method [4, 17-21]. In most of these cases, researchers used sols of SiO_2 or Al_2O_3 as the source of Si or Al and Ca $(NO_3)_2$ was used as a source of CaO.

3-3 Material Analysis

3-3-1 Crystalline Phase Determination XRD

The crystalline phases of the specimens were determined by a Rigaku X-ray powder diffractometer (Rigaku Geigerflex, Japan), with CuKα radiation and Ni filter. The scanning range of the samples was from 10^o to 60^o with a scanning speed of 10^o/min. Crystallinity of the samples were obtained by auto matching, using a computer aided JADE5 software for the experimental data with standard JCPDs data. Origin 8 software was use to draw all graphs.

3-3-2 Hydration Product Evaluation

The addition of water to dry cement powder results in a thin cement slurry that can be easily manipulated and cast into different shapes. In time, the slurry sets and develops strength through a series of hydration reactions. Hydration of cement is not linear through time; it proceeds very slowly at first, allowing the thin mixture to be properly placed before hardening. The chemical reactions that cause the delay in hardening are not completely understood; however, they are critical to developing a rational methodology for the control of cement setting.

3-3-2-0 Tri- and di-calcium silicates

The tri- and di-calcium silicates (C_3S and C_2S, respectively) comprise over 80% by weight of most cement.

It is known that C_3S is the most important phase in cement for strength development during the first month, while C_2S reacts much more slowly, and contributes to the long-term strength of the cement. Both the silicate phases react with water as shown below to form calcium hydroxide and a rigid calcium-silicate hydrate gel, C-S-H, (1) and (2).

$$2\,(CaO)_3(SiO_2) + 7\,H_2O \rightarrow (CaO)_3(SiO_2)_2{\cdot}4(H_2O) + 3\,Ca(OH)_2 \quad (1)$$

$$2\,(CaO)_2(SiO_2) + 5\,H_2O \rightarrow (CaO)_3(SiO_2)_2{\cdot}4(H_2O) + Ca(OH)_2 \quad (2)$$

The detailed structure of C-S-H is not completely known, however it is generally agreed upon that it consists of condensed silicate tetrahedral sharing oxygen atoms with a central, calcium hydroxide-like CaO_2 layer. From a compositional point of view, C-S-H gel can be characterize according to its Ca/Si ratio which typically ranges from 0.7- 2.3. This variability is the major reason why many features of nano-structure of C-S-H gel remain unknown [85].

Calcium hydroxide consists of hexagonal layers of octahedrally coordinated calcium atoms and tetrahedrally coordinated oxygen atoms. Taylor has proposed that the structure is most similar to either Tobermorite or Jennite, both of which share a skeletal silicate chain as shown in Figure 6.

The hydration products of each group were determined using XRD. The PSC powdered samples were mixed with distilled water in the volume ratio of 3:1 to form a paste. The paste was compacted into molds of 5mm diameter and 4mm thickness and pressed with a spatula to obtain a smooth surface. The compacted molds were put in a beaker containing hydrated cotton with de-ionized water and SBF solution, and then incubated in a water bath at a temperature of 37^0C after sealing the top with a cling film. The samples were collected separately after 3hrs, 1, 3 days of hydration, rinsed with de-ionized water. They were then dried and ground into powder for XRD.

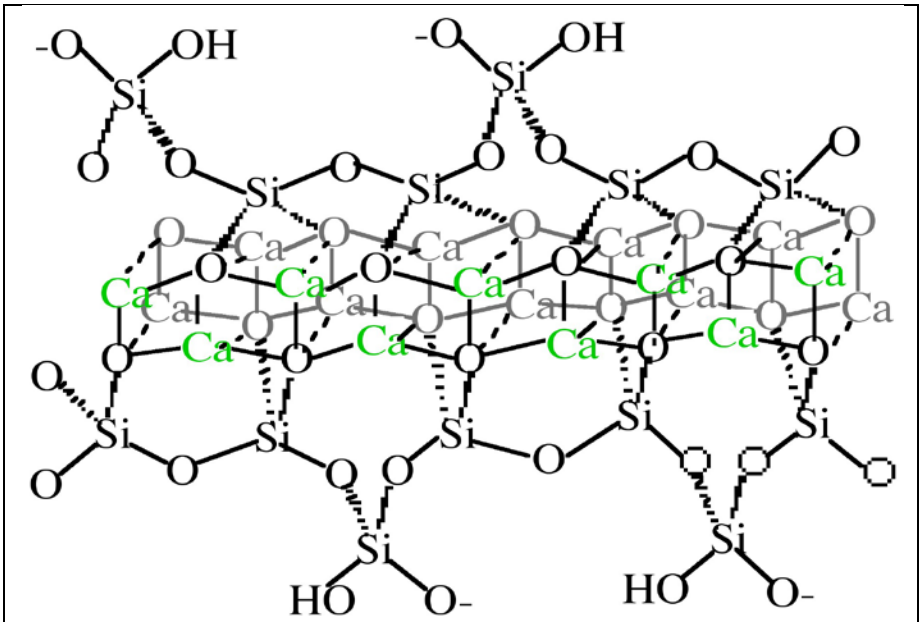


Figure 6(a): Schematic representation of Tobermorite, viewed along a polysilicate chain. Silicate ions either share oxygen atoms with a central CaO_2 core or bridge silicate tetrahedra. Interlayer calcium ions and water molecules are omitted for clarity. [82]

3-3-3 Setting Time Evaluation

Cement paste setting time is affected by a number of items including: cement fineness, water-cement ratio, chemical content (especially gypsum content) and admixtures. Setting tests are used to characterize how a particular cement paste sets. Additionally, setting times can give some indication of whether or not cement is undergoing normal hydration (PCA, 1988). Normally, two setting times are defined (Mindess and Young, 1981): [80]

1. Initial set. Occurs when the paste begins to stiffen considerably.
2. Final set. Occurs when the cement has hardened to the point at which it can sustain some load.

These particular times are just arbitrary points used to characterize cement; they do not have any fundamental chemical significance. Both common setting time tests, the Vicat needle and the Gilmore needle, define initial set and final set based on the time at which a needle of particular

size and weight either penetrates a cement paste sample to a given depth or fails to penetrate a cement paste sample. The Vicat needle test is more common and tends to give shorter times than the Gilmore needle test.

An ASTM Vicat needle testing machine with a needle of 1mm and a loading of 300g was used to evaluate the setting time of the different experimental groups. Depth of initial setting time was recorded to be less than 1mm and that of final setting time to be zero. PMMA Molds with 3, 3mm diameter holes were compacted with PSC paste of the different experimental groups. The molds were placed in a water bath at a temperature of 37^0C recording the time they take to set. The depths of initial setting times were taken after 20 minutes. The molds were placed on the Vicat glass plate and the needle with a load of 300g was brought in contact with the smooth surface of the already set material for about 30 seconds. The surface is then inspected for any indentation. The final setting time is recorded when no indentation was made.

3-3-4 *Scanning Electron Microscopy (SEM)*

Scanning Electron Microscopy (SEM) (Hitachi-810A) was used for the observation of hydration product, the microstructure and the element analysis of the developed materials. The sample powders were mixed with distilled water into a paste and compacted into PMMA molds with 3, 3mm diameter holes. The molds were immersed in SBF solution and de-ionized water, and then incubated in a temperature of 37^0C for 3hr, 1day and 3days. The incubated molds were carefully washed with de-ionized water and dried for two days at a temperature of 60^0C for SEM.

3.3.5 A recipe for preparing simulated body fluid (SBF) [79]

Reagents for SBF

The following powder reagent grade chemicals have to be stoked in desiccators. De-ionized and distilled water is used for the preparation of SBF:

1. Sodium Chloride (NaCl)
2. Sodium Hydrogen Carbonate ($NaHCO_3$)

3.Potassium Chloride (KCl)

4.Di-potassium Hydrogen Phosphate Trihydrate (K_2HPO_4. $3H_2O$)

5.Magnesium Chloride Hexahydrate ($MgCl_2$. $6H_2O$)

6.Calcium Chloride ($CaCl_2$)

7.Sodium Sulfate (Na_2SO_4)

8.Tris-hydroxymethyl Aminomethane ($(HOCH_2)_3CNH_2$) (Tris)

9.1 M (mol/l) Hydrochloric Acid, 1M HCl

10. pH standard solution, (pH 4, 7 and 9)

In order to prepare 1000ml of SBF, put 1000ml of de-ionized water and a stirring bar into a 1000ml plastic beaker. Set it in the water bath on the magnetic stirrer and cover it with a plastic wrap. Heat the water in the beaker to 36.5 ± 1.5^0C under stirring.

Dissolve only the reagents of 1^{st} to 8^{th} order into the solution at 36.5 ± 1.5^0C one by one in the order given in table 3, taking care of the indications of the following list. The reagents of 9th (Tris) and 10th order (small amount of HCl) are dissolved in the following process of pH adjustment.

(a) In preparation of SBF, glass containers should be avoided, but a plastic container with smooth surface and without any scratches is recommended, because apatite nucleation can be induced at the surface of a glass container or the edge of scratches. If the container has scratches, replace it by a new one.

(b) Never dissolve several reagents simultaneously. Dissolve a reagent only after the preceding one (if any) is completely dissolved.

(c) Since the reagent $CaCl_2$, which has great effect on precipitation of apatite, takes usually granular form and takes much time to dissolve on granule at a time, completely dissolve one before initiation of dissolution of the next.

(d) Measure the volume of 1M-HCl by cylinder after washing with 1M-HCl.

(e) Measure the hygroscopic reagents such as KCl,
K_2HPO_4. $3H_2O$, $MgCl_2$. $6H_2O$, $CaCl_2$, Na_2SO_4 in as short a period as possible.

Table 3: Order, amounts, weighting containers, purities and formula weights of reagents for preparing 1000ml of SBF

Order	Reagent	Amount	Container	Purity (%)	Formula weight
1	NaCl	8.035g	Weighing paper	95.5	58.4430
2	$NaHCO_3$	0.355g	Weighing paper	95.5	84.0068
3	KCl	0.225g	Weighing bottle	95.5	74.5515
4	$K_2HPO_4.3H_2O$	0.231g	Weighing bottle	99.0	228.2220
5	$MgCl_2.6H_2O$	0.311g	Weighing bottle	98.0	203.3034
6	1.0.M-HCl	39ml	Graduated cylinder	_	_
7	$CaCl_2$	0.292g	Weighing bottle	95.5	110.9848
8	Na_2SO_4	0.072g	Weighing bottle	99.0	142.0428
9	Tris	6.118g	Weighing paper	99.0	121.1356
10	1.0M-HCl	0-5ml	Syringe	_	_

Set the temperature of the solution at 36.5±1.5 ^{0}C.

Insert the electrode of the pH meter into the solution. Just before dissolving the Tris, the pH of the solution should be 2.0±1.0.

With the solution temperature between 35 and 38^{0}C, preferably to 36.5±0.5^{0}C, dissolve the reagent Tris into the solution little by little taking careful note of the pH change. After adding a small amount of Tris, stop adding it and wait until the reagent already introduced is dissolved completely and the pH has become constant; then add more Tris to raise the pH gradually. When the pH becomes 7.3±0.05, make sure that the temperature of the solution is maintained at 36.5±0.5 ^{0}C. With the solution at 36.5±0.5 ^{0}C, add more Tris to raise the pH to under 7.45.

Note 1: Do not add a large amount of Tris into the solution at a time, because the radical increase in local pH of the solution can lead to the precipitation of calcium phosphate. If the solution

temperature is not within 36.5±0.5 ^{0}C, add Tris to raise the pH to 7.30±0.05 stop adding it and wait for the solution temperature to reach 36.5±0.5 ^{0}C.

Note 2: The pH shall not increase over 7.45 at 36.5±0.5 ^{0}C; taking account of the pH decrease with increasing solution temperature (the pH falls about 0.05/^{0}C at 36.5±1.5 ^{0}C).

When the pH has risen to 7.45±0.01, stop dissolving Tris, then drop 1M-HCl by syringe to lower the pH to7.42±0.01, taking care that the pH does not decrease below 7.40. After the pH has fallen to 7.42±0.01, dissolve the remaining Tris little by little until the pH has risen to p7.45. If any Tris remains, add the 1M-HCl and Tris alternately into the solution. Repeat this process until the whole amount of Tris is dissolved keeping the pH within the range of 7.42–7.45. After dissolving the whole amount of Tris, adjust the temperature of the solution to 36.5±0.2 ^{0}C. Adjust the pH of the solution by dropping 1M-HCl little by little at a pH of 7.42±0.01 at 36.5±0.2 ^{0}C and then finally adjust it to 7.40 exactly at 36.5 ^{0}C on condition that the rate of solution temperature increase or decrease is less than 0.1 ^{0}C/min.
Remove the electrode of the pH meter form the solution, rinse it with distilled water and add the washings into the solution.
Pour the pH-adjusted solution from the beaker into 1000 ml volumetric flask. Rinse the beaker with de-ionized and distilled water, add the washings into the flask several times, fixing the stirring bar with a magnet as if to prevent it from falling into the volumetric flask. Put a lid on the flask and close it with plastic film.
After mixing the solution in the flask, keep it in the water to cool it down to 20 ^{0}C. SBF should be preserved in a plastic bottle with a lid put on tightly and kept at 5-10^{0}C in a refrigerator. The SBF shall be used within 30days after preparation.

3.4 In-Vitro Evaluation

3.4.1 Cell Culture and Extract Condition

All of the materials and equipments used in this experiment were autoclaved before in-vitro evaluation.PSC materials of each group were mixed with distilled water first to form a paste. The following procedure was adapted from Keiser, Johnson and Tipton et al. [90]. The materials were mixed according to the manufacturer's recommendations and placed into the bottom of a 6-well tissue culture plates to achieve a thickness of≤ 5mm. These samples were divided into two

groups. The first group included all materials in a freshly mixed state, whereas in the second group, the materials were allowed to set for 24 hr at 37^0C at 100% relative humidity. ISO 10993-5 (0.1g/mL) extract condition ratio was used and a serum-free Modified Eagle's Medium (MEM) added. The plates were incubated at 37^0C at 100% relative humidity for 24 hr. The medium was then drawn off and sterile-filtered at 0.22μm for cytotoxicity and cell viability tests. Cells with serum-free medium and commercial MTA were used as positive and negative control groups respectively in these tests.

The cranium of Wistar rat osteoblast primary cell was used as a target cell in this study. Modified Eagle's Medium (MEM) was used as a growth medium for the primary cells. The MEM medium contained 10% foetal bovine serum (FBS), 100μg/ml streptomycin, 100μg/ml penicillin, and 0.25μg/ml amphotericin B. Cells were grown in plastic culture dish, with no prior surface treatment. Confluent cells were trypsinized (1x trypsin), collected by centrifugation (5mins at 1600rpm), re-suspended in completed medium and then sub-cultured in Petri dish. Cells from 3^{th} generation were used for the experiment. In order to determine cell number, cells were detached from the dish by treatment with trypsin. Cells were then pelleted and re-suspended in 1 ml medium, and an aliquot of the cell suspension mixed with tripan blue was injected into a haemocytometer (Fisher). The number of cells in a grid of volume less than 100nl was counted using a light microscope, and the values for eight separate grids measured to give an average cell count. The cells were seeded into 96 well flat-bottom plates, $5x10^3$ cells per well as determined by haemocytometer counting in medium and incubated in an average humidified atmosphere of air and 5% CO_2 at 37^0C for 1 day. The culture medium was then replaced with 200μl of aliquots of the test extracts or control media, and the cells thus exposed were incubated for 24 and 72 hour at 37^0C under humidified air and 5% CO_2. Six wells were used for each single experimental group. Figure 6(b) shows the follow diagram of the primary cell culture.

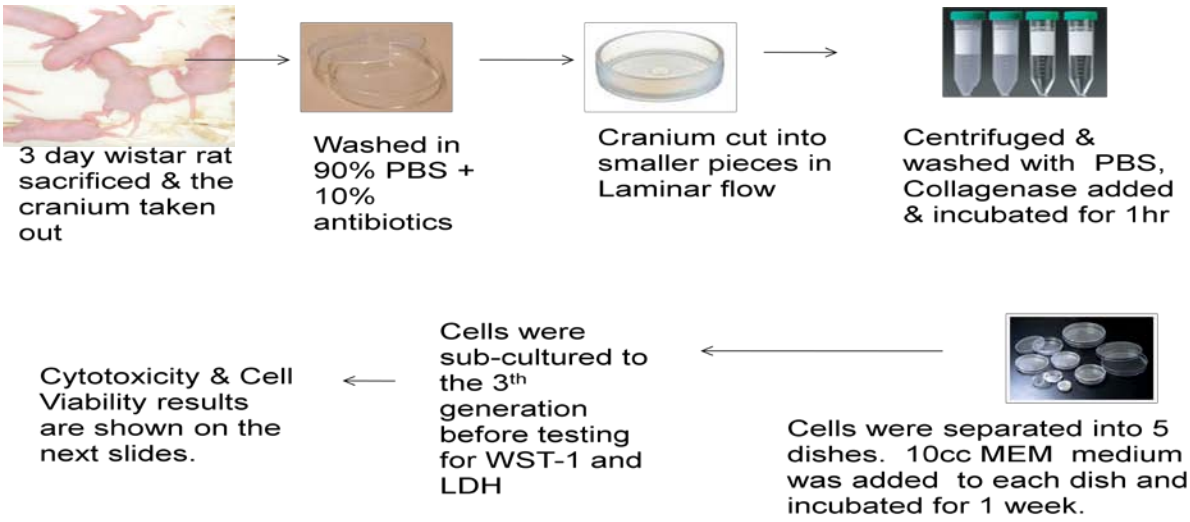


Figure 6(b) Follow chart of Primary Cell Culture

3.4.2 Lactate Dehydrogenase (LDH) Assay

CytoTox 96 ® Non-Radioactive Cytotoxicity Assay (Promega) was used to test for cell cytotoxicity. The CytoTox 96 ® Assay quantitatively measures lactate dehydrogenase LDH, a stable cytosolic enzyme that is released upon cell lysis. Released LDH in culture medium was measured with a 30-minute coupled enzymatic assay, which resulted in the conversion of a tetrazolim salt (INT) into a red formazan product. The amount of color formed is proportional to the number of lysed cells [81]. Visible wavelength absorbance data was collected by using a standard 96-well plate ELISA reader.

The general chemical reactions of the assay are as follows:

$$NAD+ + lactate \rightarrow pyruvate + NADH$$

$$NADH + INT \rightarrow NAD+ + formazan\ (red)$$

LDH assay has been commonly used in many fields as an index of cellular cytotoxicity in-vitro.

3.4.3 Cell Proliferation Reagent (WST1) Assay

The measurement of cell proliferation and cell viability has become a key technology in the life sciences. The need for sensitive, reliable, fast and easy methods has led to the development of

several standard assays. In this study, a colorimetric assay for the quantification of cell proliferation and cell viability, based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells was used for the evaluation of cell viability.

The general chemical reaction of WST1 is shown below:

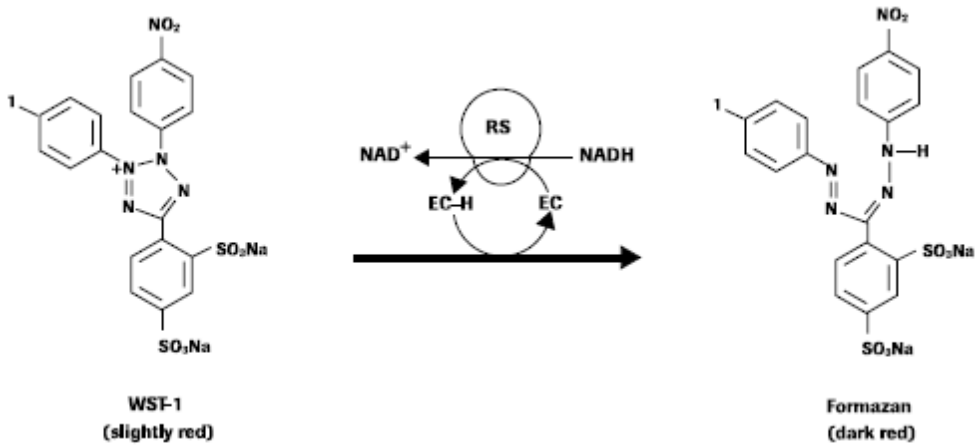


Figure 7: Chemical Reaction of WST-1

Cell Proliferation Reagent WST-1 has several advantages compared to other assays:

• In contrast to MTT which is cleaved to water insoluble formazan crystal and therefore has to be solubilized after cleavage, WST-1 yields water soluble cleavage products like XTT and MTS which can be measured without an additional solubilization step.
• In contrast to XTT and MTS, WST-1 is more stable. Therefore, WST-1 can be used as a ready-to-use solution and can be stored at 2-8°C for several weeks without significant degradation.
• WST-1 has a wider linear range and shows accelerated color development compared to XTT.

The Cell Proliferation Reagent WST-1 is designed to be used for the non-radioactive, spectrophotometric quantification of cell growth and viability in proliferation and chemo-sensitivity assays. It can be used e.g. for:

- The measurement of cell proliferation in response to growth factors, cytokines, mitogens and nutrients.
- The assessment of growth inhibitory antibodies and physiological mediators.

After 1, 3 days of incubation with extracted medium, the medium was removed with sterile pipette and washed with PBS. 100µl WST1 kit was added to each well and gently swirled for 1 minute. The cells were incubated for 3 hours at 37^0C in a 5% CO_2 atmosphere and protected from light. After 3 hours of incubation, measurement was taken using spectrophotometer.

Chapter 4 RESULTS

*4.*1 XRD Analysis of PSC Material

Figure 8 shows the XRD patterns of PSC powder with zinc added in different weight percent concentrations. Table 1 gives the phases found in each group. All the patterns are similar to the control group. However, there is a slight difference between the 1%, 3% groups which has a phase of CaO and 5%, control groups that has no CaO phase. This indicates low reaction efficiency in the process of 1% and 3% groups. C2S, C3S and C3A which are the characteristic components of calcium silicate cement were found in each group. The characteristic peaks of C2S and C3S where superimposed mostly. As a result, the peaks at the positions of 20.3^0, 29^0, $30^0$and 37.3^0 were used as the major peaks for the identification of C2S, C3S, C3A, and CaO respectively. All these peaks were identified at a heating temperature of 1400^0C. PSC synthesized by sol-gel process was observed to have shown diffraction pattern of monoclinic while C2S/C3S synthesized by conventional method remained triclinic at the same firing temperature (1400^0C) in the study of Wang et al. It was discovered that the content of C2S and CaO decreased at 1400^0C, while that of C3S increased [43]. The content of CaO was noted to be decreased as zinc weight percent concentration increases while C2S content remained the same, Wang et al. Hence the C3S/CaO ratio becomes higher. The higher C3S/CaO ratio was supposed to have higher activity of PSC in reaction with water [43]. This effect was well enhanced in the 5% group where no trace of CaO was found.

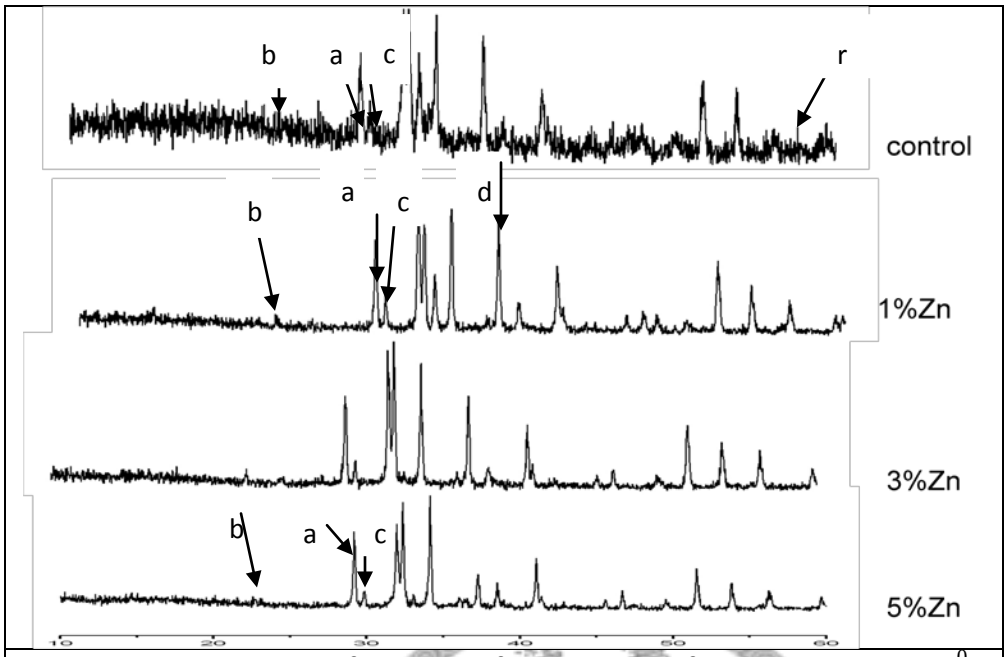


Figure 8: XRD pattern of product of each group after calcined at 1400^0C for 2 hours: a-C_3S, r-C_4AF, d-CaO, b-C_2S, and c-C_3A

Table 1 Experimental groups and their XRD phases: C2S-Ca_2SiO_4, C3S-Ca_3SiO_5, C3A-$Ca_3Al_2O_6$, C4AF-$Ca_4Al_2Fe_2O_{10}$, CaO

Groups	1% Zn	3% Zn	5% Zn	Control
Phases Found	C2S C3S C3A CaO	C2S C3S C3A CaO	C2S C3S C3A	C2S C3S C3A C4AF

4.2 Hydration Product Evaluation

Figure 9(a) shows the XRD pattern of the hydration product that reacted with water for 1, 3 and 7 days while Figure 9(b) shows that which reacted with water for 7 days. Portlandite with a

characteristic peak of 17.5 degrees is the major hydration product of calcium silicate cements. The content of portlandite was observed to have increased with increasing days of hydration [50-52] as shown in figure 9(a). The same scale was used for the different groups for a valid comparison.

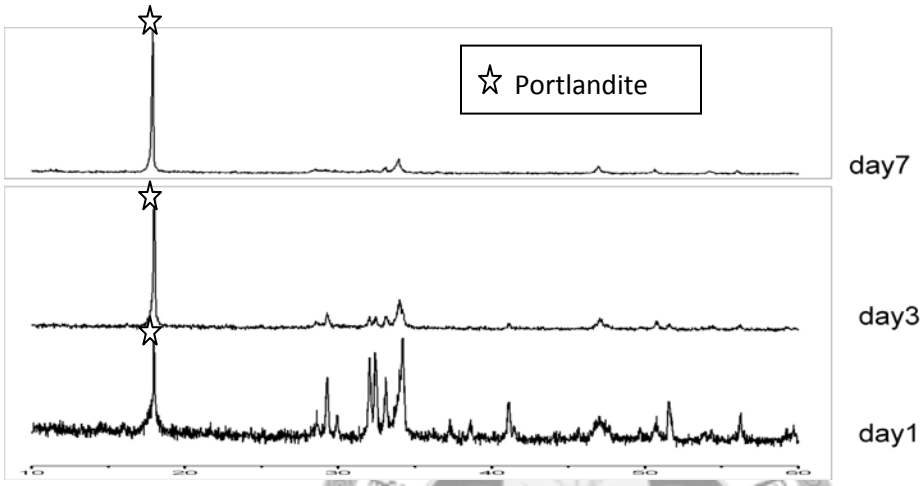
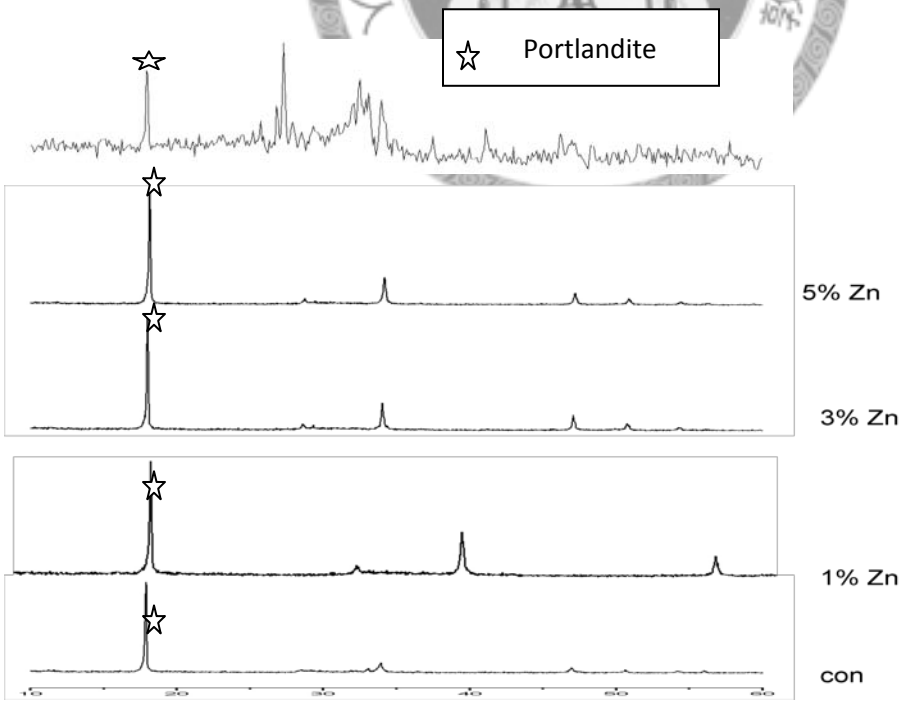


Figure 9(a): 1, 3 and7 Days Hydration



(b)Day 7 Hydration

Figure 9 XRD Pattern of the Hydration Product of (a): Representative of PSC groups and (b): Day 7 comparison of all groups of PSC.

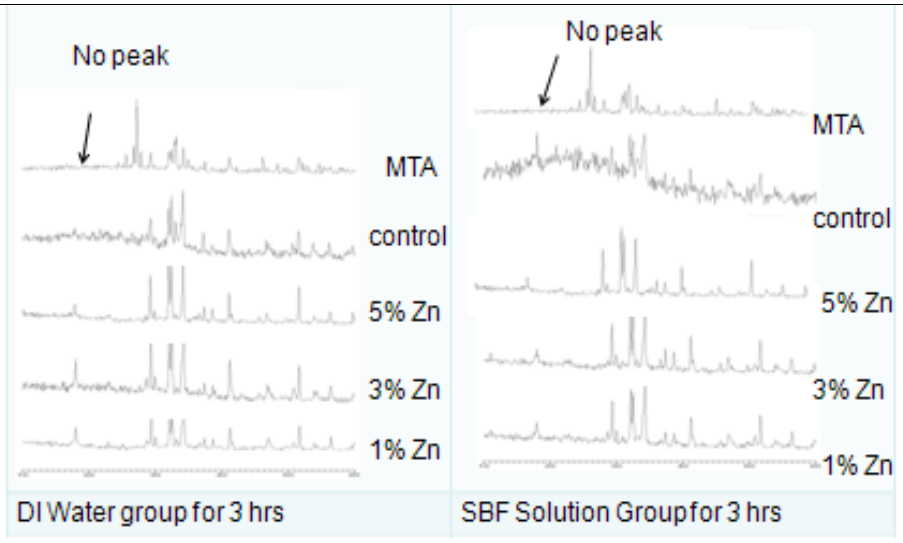


Figure 10(a) 3 hrs Hydration Test Results

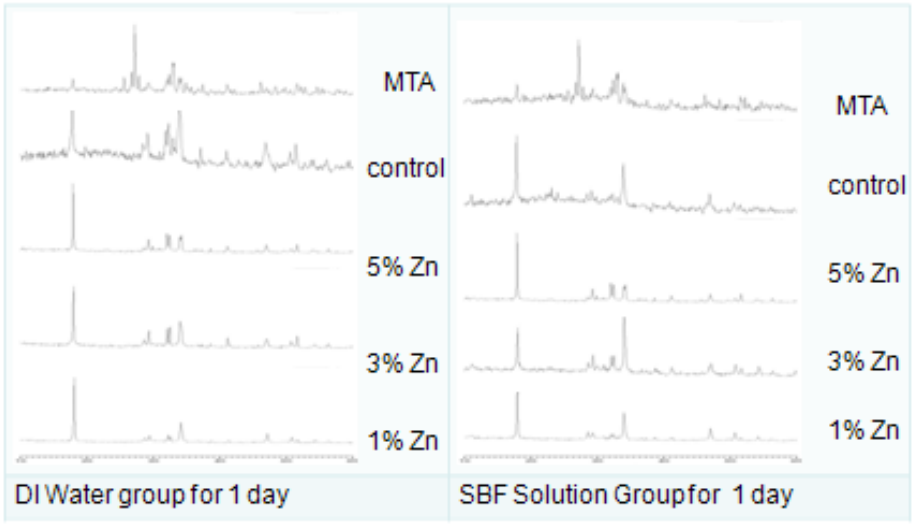


Figure 10(b) 1 day Hydration Test Result

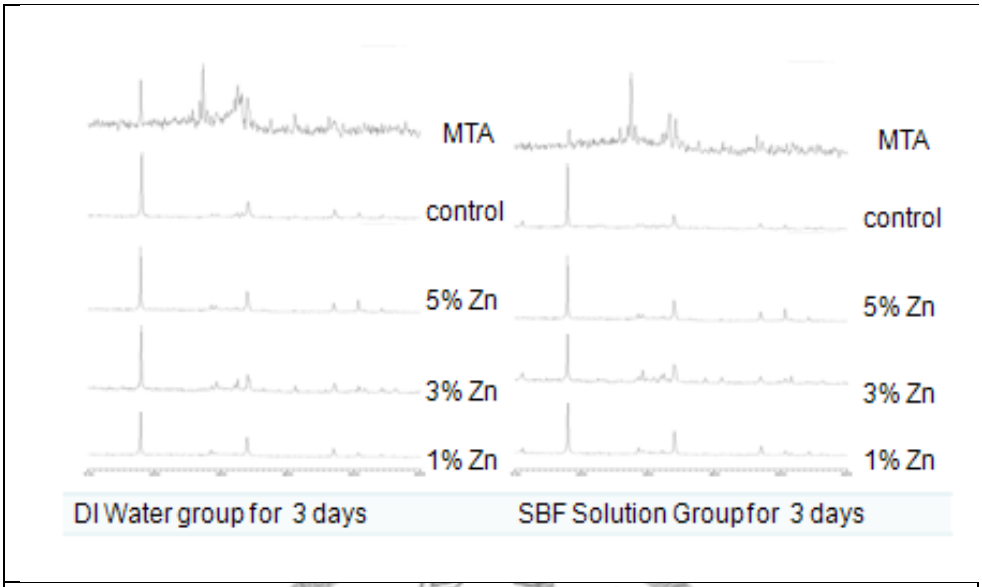


Figure 10(c) 3days Hydration Test Results

Figures 10(a), (b) and (c) show the XRD patterns of hydration product for 3 hours, 1 and 3 days in simulated body fluid (SBF) compared to de-ionized or distilled water for all five experimental groups. Experimental groups immersed in de-ionized water were used as control for this experiment. There was not much significant difference in the portlandite peak between the two solutions except for the 3 hours group where there was no portlandite peak observed on the MTA group. This was due to the fact that MTA takes more than 3 hours to set and hence there was merely any reaction. Portlandite peak increased as the days of immersion increased. A uniform scale was use for validity of this experiment. This shows that simulated body fluid can also be used as one of the liquid components of PSC material. Normal saline will be investigated in the future.

4.3 Micro-Structure of Hydrated PSC

Micro-structures and surface morphology of 1, and 3 weeks hydrated PSC for the five experimental groups was examined using Hitachi-model-810A scanning electron microscopy. PSC powder was mixed with water in the ratio of 3:1 and the paste compacted in a PMMA mold with 3, 3mm diameter holes. A spatula was used to flatten the surface. The molds were immersed in both de-ionized water and SBF solution. They were given 100% humility at 37^0C and incubated in a water bath for 1 and 3 weeks. A carbon film was used to mount the mold samples because carbon is electrically conductive and PSC powder is not electrically magnetic material compared to other cements. As a result, a thin gold film was used to cover the sample for a better and quality image. Figure 11 shows the images of the different groups.

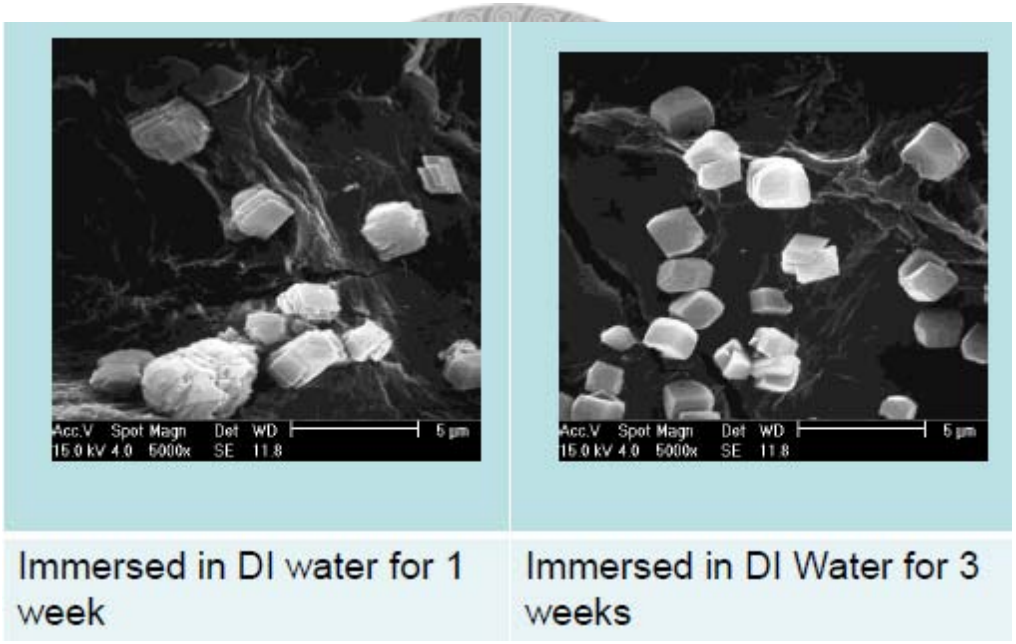


Figure 11(a): SEM Image of 1% Zn-PSC

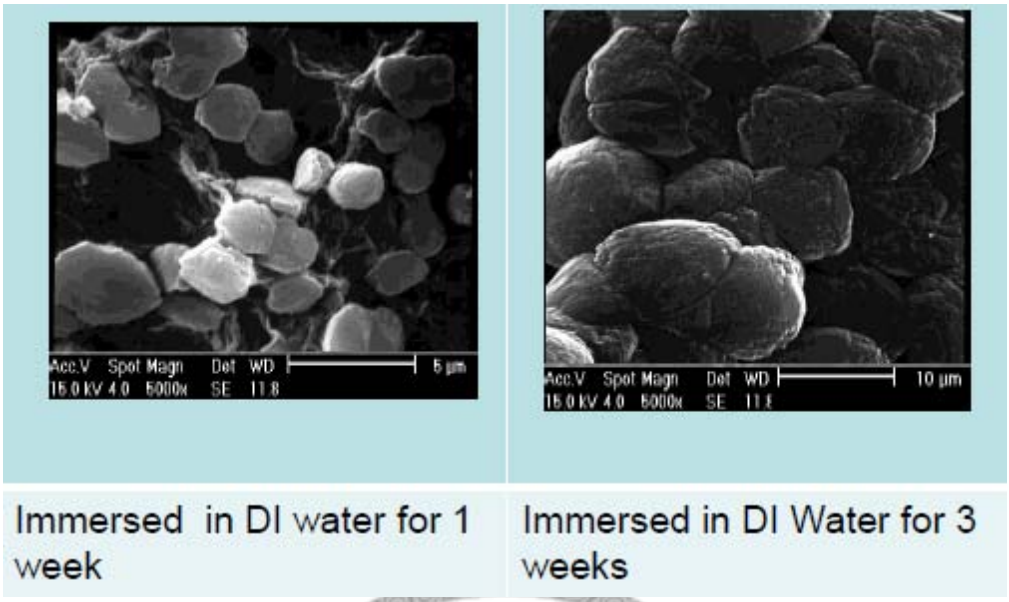


Figure 11(b): SEM Image of 3% Zn-PSC

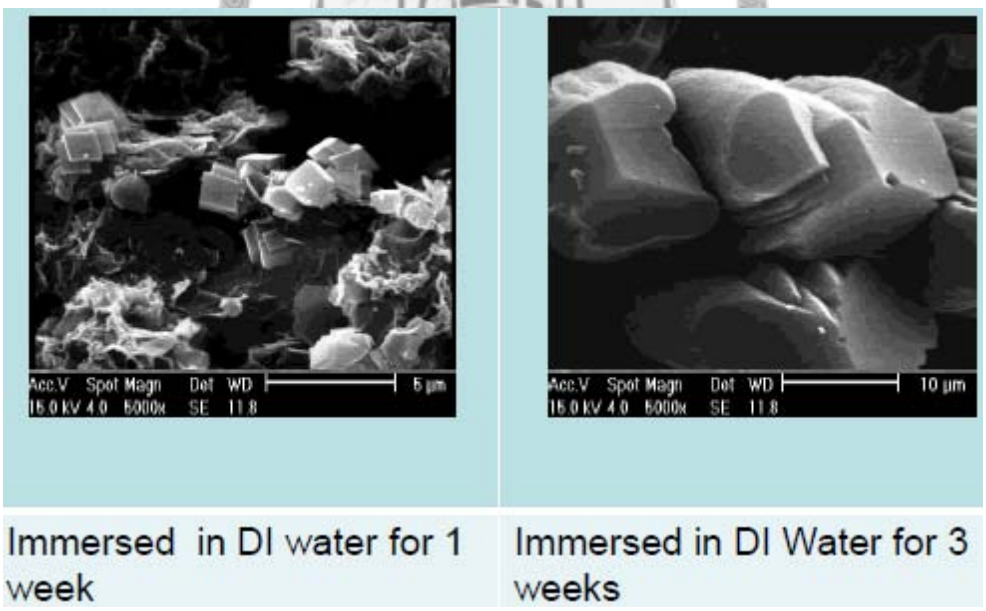


Figure 11(c): SEM Image of 5% Zn-PSC

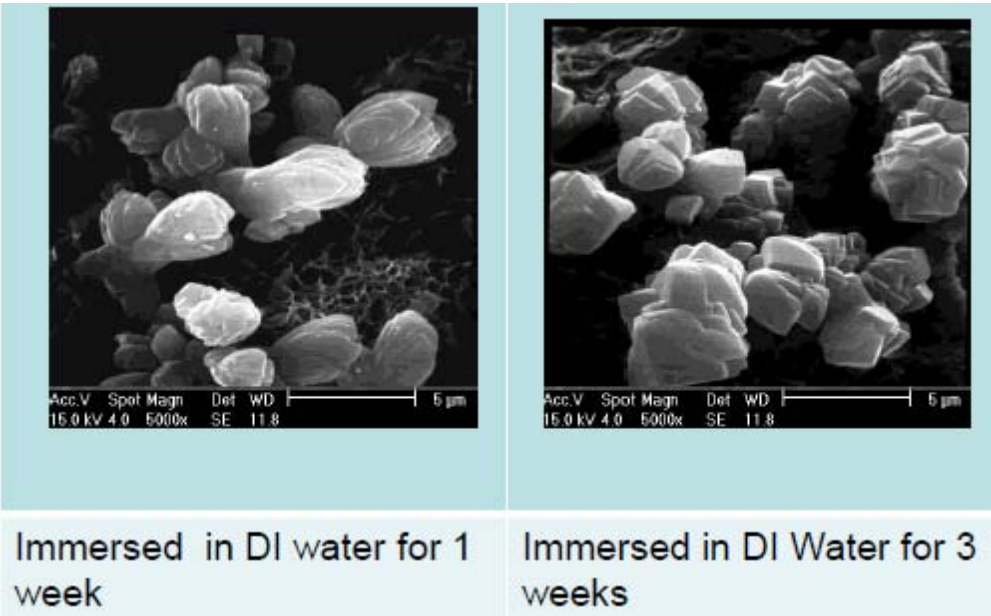


Figure 11(d): SEM Image of control (PSC with Fe)

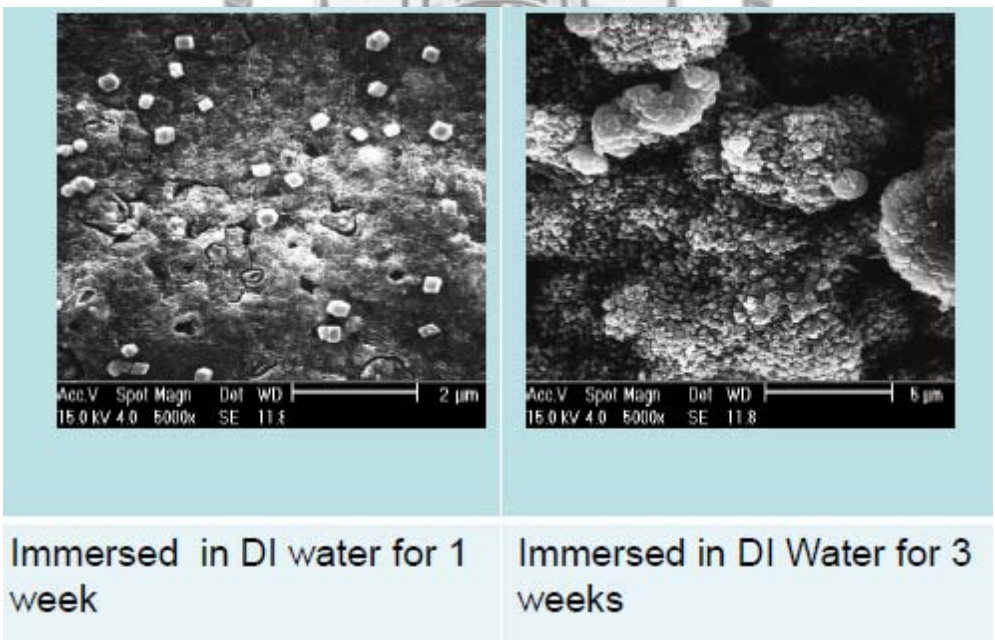


Figure 11(d): SEM Image of MTA

4.4 Setting Time Evaluation

PSC paste was prepared by mixing the powder components and distilled water in a volume ratio of 3:1. The paste was then filled upon a PMMA mold with 3, 3-mm diameter holes. The extra paste was scooped out and pressed by a spatula to obtain a compacted flat surface for indentation. The PMMA molds with the compacted PSC paste was incubated in a water bath at a temperature of 37^{o} C for a period of time. The PMMA molds were placed on the Apparatus Vicat needle test machine to evaluate the initial and final setting times of the material. Commercial MTA was used as a negative control group in this experiment.

ASTM C187-04

Needle: 1mm

Loading: 300g

Depth: ≤ 1mm (initial setting)

Depth: 0 (final setting)

Figure 12: Vicat Apparatus Machine

Table 2: Setting time of PSC groups in Minutes

Experimental Groups	Initial setting time	Final Setting Time	Initial depth
MTA	≤ 30mins	210 ± 0.5 mins	≤ 1mm
Control	≤ 20mins	90 ± 0.5 mins	≤ 1mm
1% Zn	≤ 15mins	80 ± 0.5 mins	≤ 1mm
3% Zn	≤ 10mins	45 ± 0.5 mins	≤ 1mm
5% Zn	≤ 10mins	35 ± 0.5 mins	≤ 1mm

4.5 Cytotoxicity Test

Figures 13 (a) and (b) show results of LDH of rat osteoblast primary (ROB) cells respectively. Overall, there was no significant difference in cytotoxicity.

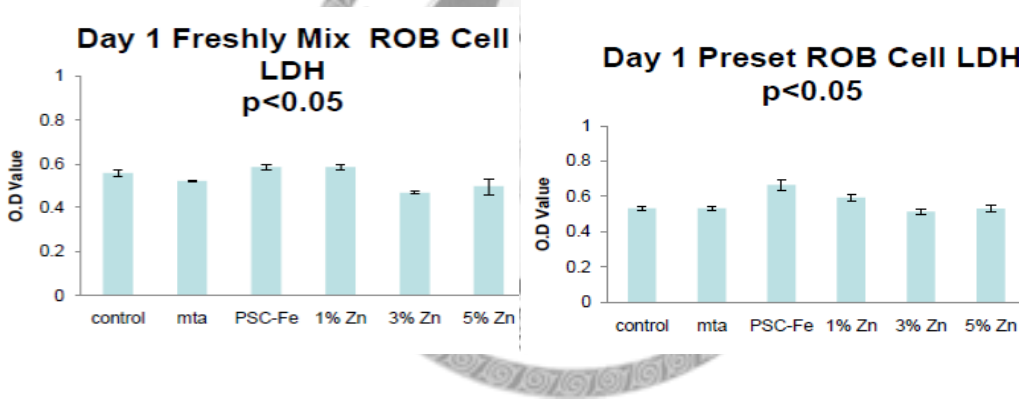


Figure 13(a): Day 1 LDH test results

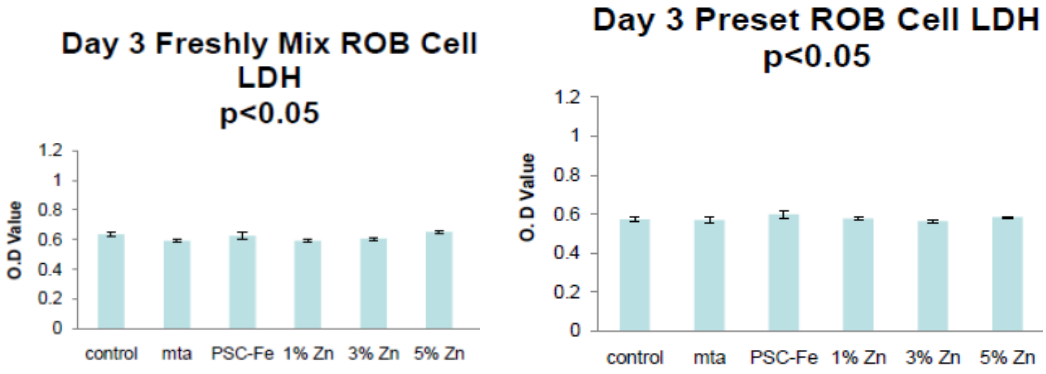


Figure 13 (b): Day 3 LDH test results

4.6 Cell Viability Test

Figures 14 (a) and (b) show results of WST-1 of rat osteoblast primary (ROB) cells respectively. An increase in cell viability was observed for both groups. However, freshly mixed group out-performs the preset group in day 3.

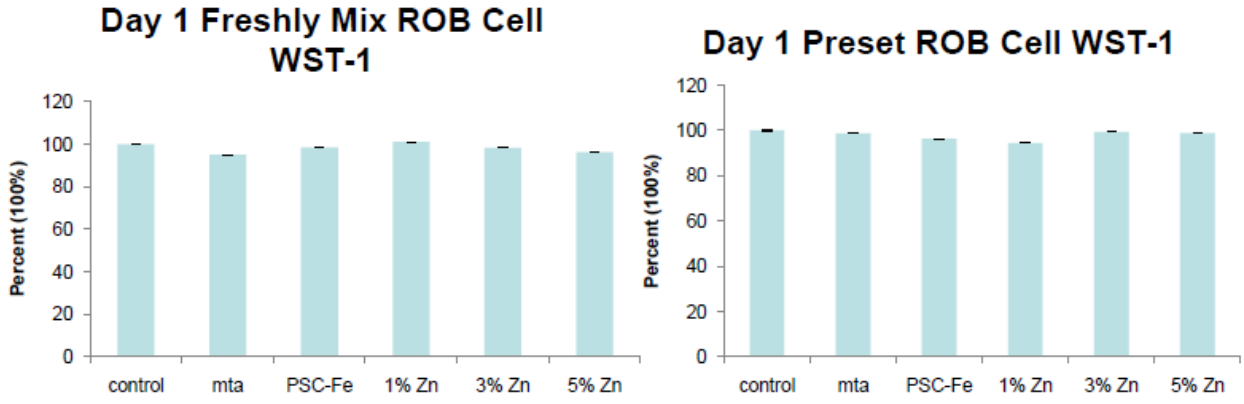


Figure 14 (a) Day 1 WST-1 test result

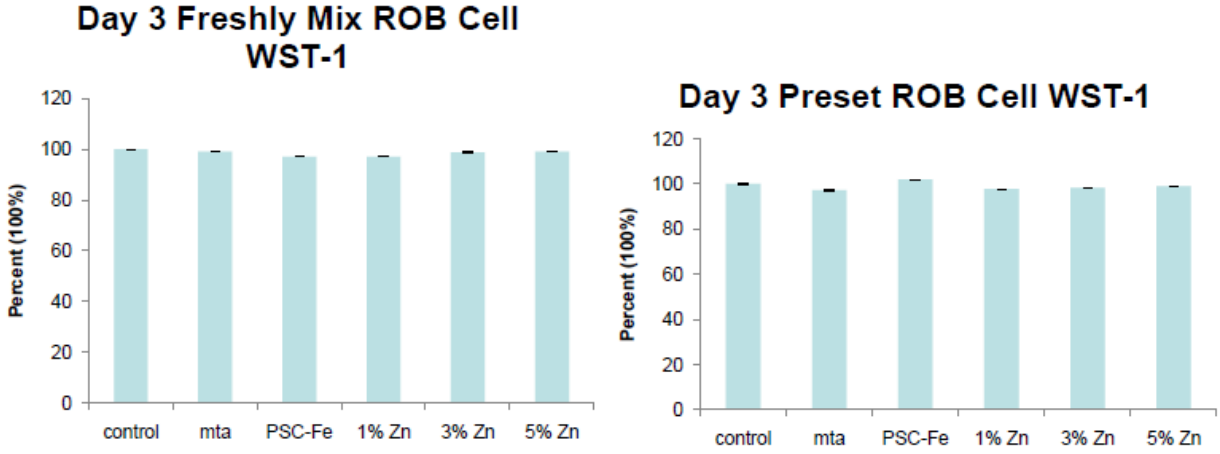


Figure 14 (b): Day 3 WST-1 test result

Chapter 5 DISCUSSIONS

Discussion of Synthesis of PSC by sol-gel Process

According to the XRD results, no CaO was found in 5% Zn and control groups calcined at 1400^0C. This indicates that calcium atoms completely react with other atoms (Si, Al, Zn, and Fe) in the system to form C_3S, C_2S, C3a, and C_4AF. On the other hand, CaO was found in the 1% and 3% Zn groups. The formation of calcium oxide is due to insufficient energy for calcium atom to react with Si, Al and Zn atoms to form C_3S, C_2S, and C_3A, this result in calcium atoms reacting with oxygen atoms to form calcium oxide which requires lower energy. Thus it means that there is much higher reaction efficiency in the 5% Zn and control groups than the other two groups. In a single component sol-gel system, there is no significant difference in homogeneity of the product between network structures and long chain structures. As a result, a molecular level homogeneity is attained and there should be higher reaction efficiency between atoms, Wang et al. One step sol-gel process is less time consuming, requires lower energy, lower temperature, has better particle oxide homogeneity and a greater reproductive efficiency than traditional powder mixing process. The size of raw materials in a traditional mixing process is around several micro-meters, and reaction occurs through diffusion between surfaces of particles. It is therefore difficult to achieve homogeneity and this leads to low reaction efficiency.

On the other hand, starting materials of sol-gel process are prepared by dissolving desired metal salts and alkoxides in a common solvent. The whole process takes place in the liquid phase giving possible greater specific surface area and higher molecular level reaction leading to lower energy requirement and hence lower temperature. One of sol-gel process major characteristics is the ability to synthesize all components of a system in a single batch, this makes it time efficient. The product of modified sol-gel process is more active in the monoclinic phase compared to the traditional method which is most active in the triclinic phase [83, 84]. The result of triclinic-monoclinic transformation is in agreement with the hydration product result as shown in Figure11.

Discussion of Hydration Product Evaluation

At the early stage of hydration of calcium silicate cement, initial hydration product is amorphous due to low Ca/Si ratio in the solution [86]. Crystalline hydration product such as portlandite is

formed at the later stage of hydration because of the high Ca/Si ratio in the solution [87, 88]. The high intensity in diffraction and prominent portlandite peaks in both days 3 and 7 of the different experimental groups on Figures 9(a) and 10(c) confirms this phenomenon.

Discussion of Setting Time

It was observed that the setting time of PSC material increases as the zinc weight percent added increases. This is shown in table 2 of the setting time evaluation using Apparatus Vicat machine. However, there is a limit to which zinc is added. The optimum percent of zinc that can be added without inhibiting hydration reaction is wt 5%. The initial setting time was improved from 20mins to 10mins where as final setting time was improved from 4hrs to 30mins.

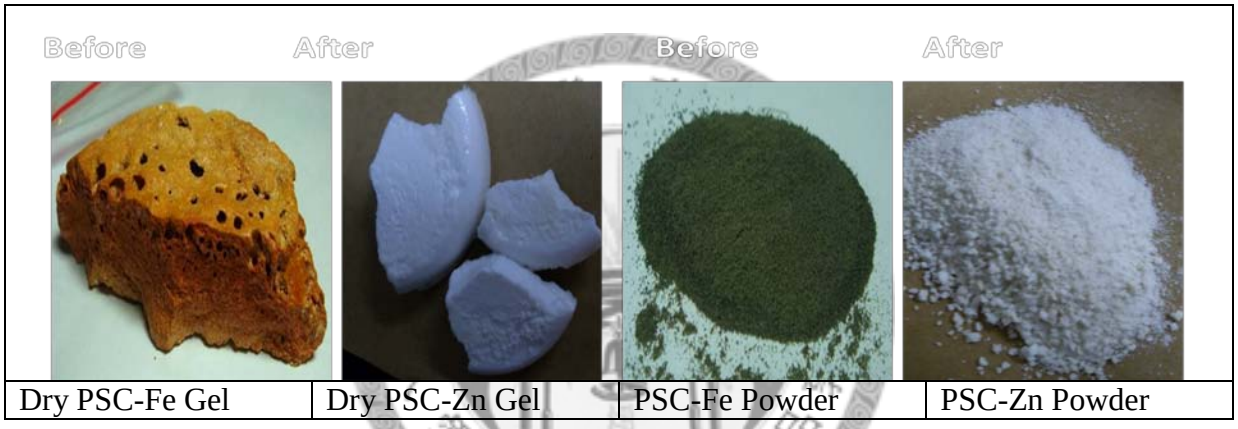
Discussion of In-Vitro Evaluation

Toxicity of root canal sealer or root-end filling material is generally determined in three-stage approach. First stage is to screen a candidate material by using series of in-vitro cytotoxicity tests. If the cytotoxicity test results of the material turn out to be non-toxic, it then can be implanted in subcutaneous tissue or muscle for the evaluation of local tissue reaction. Finally, the target tissue response with the material must be evaluated in human or animal subjects [38]. This section involves the first stage of toxicity evaluation. In order to determine the cytotoxicity of the materials, proper test system is needed for evaluation. There are many test systems to evaluate the cytotoxicity of dental materials in mammalian cell culture [80]. The decision made on each particular test system should take into consideration the chemical and physical characteristics and natures of the materials being tested. Eluates (extracts), complying with ISO10993-5 of test materials were used in this study. This method offers the advantage of the extract medium being easily sterilized by filtration, and the ability to examine the effect of materials on cells that are both distant to and in contact with them. Sterilization of test material may however alter their properties during the process in direct contact method. Possible contamination may be avoided by using extraction medium. The use of extract medium also stimulates the immediate post-surgical root-end environment in which toxic elements of the retrograde-filling material leach into the surrounding fluids in the bony crypt [90]. PSC varies enormously during the paste

preparation if the material is not handled properly. The use of extraction medium instead of PSC paste can address this problem.

Conclusion

- It was observed that adding Zn to the PSC system caused defect in the lattice structure of the PSC-Zn Cement to create the desired unstable state at room temperature which enhances the hydration reaction and significantly decreased the setting time.
- The “grey” color of PSC was successfully changed to “white” by removing Iron from the PSC system as shown in Figure 15 below:



Dry PSC-Fe Gel	Dry PSC-Zn Gel	PSC-Fe Powder	PSC-Zn Powder

Figure 15: Dry PSC-Zn/Fe gels and powder

References

Adamo HL, Buruiana R, Schertzer L, Boylan RJ. A comparison of MTA, Super-EBA, composite and amalgam as root-end filling materials using a bacteria microleakage model. International Endodontic Journal 1999;32(3):197-203.

Allen MJ, Myer BJ, Millet PJ, Rushton N. The effects of particulate cobalt, chromium and cobalt-chromium alloy on human osteoblast-like cells in vitro. Jounal of bone and Joint Surgery-British Volume 1997 May;79-B(3): 475-482.

Andrew R. Barron. Hydration of Portland Cement. Connexions Module: m16447.

Angela P. Noguera, DDS, MS and Paula L. Russi, DDS, MS Diplomats, American Board of Endodontics.

Brinker CJ, Scherer GW. Sol-Gel Science. San Diego: Academic Press, 1990.

Bye GC Portland Cement-Composition, Production and Properties: Pergamon Press, 1983.

Cohen S, Burns RC. Pathways of the Pulp. 8 ed. New Delhi, India: Harcourt(India) Private Limited, 2001.

Cotton FA, Wilkinson G. Advanced inorganic Chemistry. Ney York: John Wiley and Sons, 1980.

D.B. Jaroch, D.C Clupper, Modulation of Zinc Release from bioactive sol-gel derived SiO_2-CaO-ZnO glasses and ceramics DOI: 10. 1002/jbm.a.31180

Da Silva MGF, Valente MA. The iron behavior in aluminosilicate gel-derived materials. J Sol-Gel Sci Technol 2000; 17:47-53.

Dalado JS, Griebel M, Hamaekers J. A Molecular Dyanamics study of cementitious calcium silicate hydrate C-S-H gels: Institute of numerical simulation; 2007.

Deshpande VV, Patil MM, Navale SC, Ravi V.A Coprecipitation Technique to prepare ZnNb2O6 Powders. Bull Mater Sci 2005; 28:205-207.

Dovgan JS. Root canal surgery, Endodontic surgery, Apicoectomy (Apico) and Retrofill (retro) or Surgical Endodontic Therapy. When is the procedure done? . 2004

Fischer EJ, Arens DE, Miller CH. 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 1998;24:176-179.

Foulkes E. Metal Toxicology: Academic Press Inc., 1995.

Garner EM. A proposed mechanism for growth of C-S-H during the hydration of Tricalcium silicate. Cement and concrete research 1997; 27 (5): 665-672.

Gartner A, Dorn SO. Advances in endodontic surgery. Dental Clinics of North America 1992; 36:364-373.

Goktas AA, Weinberg MC. Preparation and crystallization of sol-gel calcia-alumina compositions. J Am Ceram Soc 1991; 74:1066-1070.

Hallab NJ, Vermes C, Messina C, Roebuck KA, Glant TT, Jacobs JJ. Concentration and composition-dependent effects of metal ions on human MG-63 osteoblasts. Journal of Biomedical Material Research 2002;60(3):420-433.

Harty FJ, Parkins BJ, Wengraf AM. Success rate in root canal therapy. A retrospective study of conventional cases. British Dental Journal 1970;128(2):65-70.

Hawkins P, Hadimli HH, Ataoglu H, Orstavik D. Anti-bacterial effect of selected root-end filling materials. Journal of endodontics 2006; 32 (4): 345-349.

Hawkins P, Tennis PD, Detwiler R. The use of lime stone in Portland cement: A State- of- the art report. Skokie, 2003. 44p.

Hong S-H, Young JF. Hydration Kinetics and phase stability of dicalcium silicates synthesized by the Pechini process. J Am Ceram Soc 1999; 82: 1681-1686.

Irma Araceli Belio-Reyes, Lauro Bucio, Esther Cruz-Chavez. Phase composition of ProRoot Mineral Trioxide Aggregate by X-ray powder diffraction. JOE- Volume 35, Number 6, June 2009.

Ishikawaa K, Miyamotob Y, Itoc A, Nagayamab M, Suzuki K. Fabrication of Zn containing apatite cement and its initial evaluation using human osteoblastic cells. Biomaterials 2002;23:423-428.

Ito A, Ojima K, Naito H, Ichinose N, Tateishi T. Preparation, Solubility, and cytocompatibility of zinc-releasing calcium phosphate ceramics. Journal of Biomedical Materials Research 2000 May;50(2):178-183.

Johnson BR. Considerations in the selection of a root-end filling material. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 1999;87(4):398-404.

Jou YT, Pertl C. Is there a best retrograde filling material? Den Clin North Am 1997;41:555-561.

Keiser K, Johnson CC, Tipton DA. Cytotoxicity of Mineral Trioxide Aggregate using human periodontal ligament fibroblast. Journal of Endodontics 2000 May;26(5):25-29.

Kim S. Endodontic Microsurgery. In: Cohen S, Burns R, editors. Pathways of the pulp. 8th ed: Mosby, 2002. p. 718-721.

Koh ET, McDonald F, Pitt FTR, Torabinejad M. Cellular response to Mineral Trioxide Aggregate. Journal or Endodontics 1998;24:543-547.

Kortenkamp A, Casadevall M, Faux S, Jenner A, Shayer ROJ, Woodbridge N, et al. A role of molecular oxygen in the formation of DNA damage during reduction of the carcinogen chromium(VI) by glutathione. Archives of Biochemistry and Biophysics 1996;329:199-207.

Laudisio G, Branda F. Sol-gel synthesis and crystallisation of 3CaO.2SiO2 glassy powder. Thermochim Acta 2001; 370:119-124.

Laudisio G, Catauro M, Costantini A, Branda F. Sol-gel preparation and crystallization of $2.5CaO{\cdot}2SiO_2$ glassy powders. Thermochim Acta 1998; 322:17-23.

Lea FM. The chemistry of cement and concrete. London: Edward Arnold publishers, 1976.

Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. Journal of Endodontics 1993;19:541-544.

Lee YL, Lee BS, Lin FH, Yun Lin A, Lan WH, Lin CP. Effects of physiological environments on the hydration behavior mineral trioxide aggregate. Biomaterials 2004;25:787-793.

Lin FH, Wang WH, Lin CP. Transition element contained partial-stabilized cement (PSC) as a dental retrograde-filling material. Biomaterials 2003;24:219-233.

Livage J, Babonneau F, Chatry M, Coury L. Sol-gel synthesis and NMR characterization of ceramics. Ceram Int 1997; 23:13-18.

Lopatin CM, Pizziconi VB, Alford TL. Crystallization kinetics of sol-gel derived hydroxyapatite thin films. J Mater Sci: Mater Med 2001; 12:767-773.

Lucas Anissian AS, Henrik Dahlstrand, Barbro Granberg, Victoria Good, Elisabet bucht. Cobalt ions influence proliferation and function of human osteoblast-like cells. Acta Orthopaedica Scandinavica 2002;73(3): 369-374.

Macphee DE Cement Chemistry. 2005

Mckay GC, Macnair R, Macdonald C, Grant MH. Interaction of othopaedic metal with an immobilized rat osteoblast cell line Biomaterials 1996;17(13):1339-1344.

Medevescek S, Gabrovsek R, Kaucic V, Meden A. Hydration Product in Water Suspension of Portland Cement containing Carbonates of various Solubility. Acta Chim Slov 200;53:172-179

Mezinskis G, Frischat GH, Radlein E. Micro- and nanostructures of Fe_2O_3—SiO_2 sol-gel derived thick coatings. J Sol-Gel Sci Technol 1997; 8:489-492.

Nachlas MM. The determination of lactic dehydrogenase with a tetrazolium salt. Analytical Biochemistry 1960;1:317-326.

NING J, GRANT MH. Chromium (VI)- induced cytotoxicity to osteoblast-drived cells. Toxicology in vitro 1999;13:879-887.

Oki A, Parveen B, Hossain S, Adeniji S, Donahue H, Preparation and in vitro bioactivity of zinc containing sol-gel –derived bioglass materials. Journal of Biomedical Material Research Part A 2004 Mar;69A(2):216-221.

Orefice RL, Hench LL, Clark AE, Brennan AB. Novel sol-gel bioactive fibers. J Biomed Mater Res 2001; 55:460-467.

Osorio RM, Hefti A, F.J. V, Shawley AL. Cytotoxicity of endodontic materials. Journal of Endodontics 1998;24:91-96.

Pierre AC. Introduction to sol-gel Processing. Norwell: Kluwer Academic Publishers, 1998.

Pitt Fort TR, Anderson JO, Dorn SO, Kariyawasam SP. Effect of IRM Root-End Fillings on healing after Replantation. Journal of Endodontics 1995;21:13-15.

Popper HH, Grygar E, Ingolic E, Wawaschinek O. Cytotoxicity of chromium III and VI compounds. I. In vitro studies using different cell culture systems inhalation Toxicology 1993;5:345-369.

Prasadarao AV, Selvaraj U, Komarneni S, Bhalla AS, Roy R. Enhanced densification by seeding of sol-gel-derived aluminum titanate. J Am Ceram Soc 1992; 75:1529-1533.

Rodney PF, Robert JJ, Lay PA, Dixon NE, Raker RSU, Bonin AM. Chromium (V)-induced cleavage of DNA: are chromium (V) complexes the active carcinogen in chromium(VI)-induced cancer? Chemical research in Toxicology 1989;2:227-229.

Rose J, Benard A, Susini J, Borschneck D, Hazemann JL, Chelan P, et al. First insights of Cr Specation in Leached Portland Cement Using X-ray Spectromicroscopy. Environmental Science and Technology 2003 November 1, 2003;37(21):4864-4870.

Roy DM, Oyefesobi SO. Preparation of very active Ca_2SiO_4 powder. J Am Ceram Soc 1977; 60:178-180.

Rud J, Rud V, Munksgaard EC. Long-term evaluation of root filling with dentin-bonded resin composite. Journal of Endodontics 1996;22:90-93.

Saidon J, He J, Zhu Q, Safavi K, Spangberg LSW, Conn F. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 2003;95:483-489.

Saravanapavan P, Hench LL. Mesoporos calcium silicate glasses. I. Synthesis. J Non-Cryst Solids 2003; 318:1-13.

Schweikl H, Schmalz G. Toxicity parameters for cytotoxicity testing of dental materials in two different mammalian cell lines. European Journal of oral sciences 1996 August; 104(4p1): 292-299.

Segal DL. Chemical Synthesis of Advanced Ceramic Materials. Cambridge University Press, 1989. P19-20

Segal DL. Chemical Synthesis of advanced ceramic materials: Cambridge University Press, 1989.

Smee G, Bolanos OR, Morse DR, Furst ML, Yesilsoy C. A Comparative leakage study of P-30 resin bonded ceramic, Teflon, amalgam, and IRM as retrofilling seals. Journal of Endodontics 1987;13:117-121.

Soe KKK, Maeda M, Suzuki I. Sol-gel processing of $Pb_{1-x}Ca_xTiO_3$ thin films. Mater Lett 1996; 27:373-379.

Stephan D. Wilhelm P. Synthesis of pure cementitious phases by sol-gel process as precursor. Z Anorg Allg Chem 2004; 630:1477-1483.

Storrie H, Stupp SI. Cellular response to zinc-containing organoapatite: An in-vitro study of proliferation, Alkaline phosphatase activity and biomineralization. Biomaterials 2005;26:5492-5499.

Sugiyama M, Tsuzuki K, Ogura R. Effect of ascorbic acid on DNA damage, cytotoxicity, glutathione reductase, and formation of paramagnetic chromium in Chinese hamster V-79 cells treated with sodium chromate(VI). Journal of cell Biology 1991;266: 3383-3386.

Sugiyama M. Role of physiological antioxidants in chromium(VI)-induced cellular injury. Free radical Biology and Medicine 1992;12:397-407.

Tadashi Kokubo, Hiroaki Takadama. How useful is SBF in predicting in vivo bone bioactivity? Chubu University, Japan: Biomaterials 27 (2006) 2907-2915

Thomas J. Webster CERHDRB. Hydroxylapatite with substituted magnesium, zinc,cadmium, and yttrium. 11. Mechanisms of osteoblast adhesion. Journal of Biomedical Material Research 2002;59(2):312-317.

Tkalcec E, Sauer M, Nonninger R, Schmidt H. Sol-gel-derived hydroxyapatite powders and coatings. J Mater Sci 2001; 36:5253-5263.

Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. Journal of Endodontics 1999;25:197-205.

Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR. Dye leakage of four root end filling material: effects of blood contamination. Journal of Endodontics 1994;20:159-163.

Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. Journal of Endodontics 1995;21:603-608.

Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root-end-filling materials. Journal of Endodontics 1995;21:403-406.

Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of mineral trioxide aggregate when used as a root-end filling material. Journal of Endodontics 1993;19:591-595.

Vasudev SK, Goel BR, Yyagi S. Root end filling materials –A Review. Endodontology 2003;15:12-18.

Verdenelli M, Parola S, Hubert-Pfalzgraf LG Lecocq S. Tin dioxide thin films from Sn(IV) modified alkoxides—Synthesis and structural characterization of $Sn(OEt)_2(n^2\text{-acac})_2$ and $Sn_4(u^3\text{-O})_2(u^2\text{-OEt})_4(OEt)_6(n^2\text{-acac})_2$. Polyhedron 2000; 19:2069-2075.

Wang JY, Wickhnd BH, Gustilo RB, Tsukayama DT. Prothestic metals interfere with the functions of human osteoblast cells in vitro. Clinical Orthopasedics and related research 1997;339:216-226.

Wang W-H, Lin F-H, Lee Y-L, Lin C-P. Cytotoxicity of partial-stabilized cement. J. Biomed Meter Res A 2007;81A:195-204.

Wang W-H, Lin F-H, Lee Y-L, Lin C-P. Synthesis of partial-stabilized cement (PSC) via sol-gel-process DOI:10. 1002/jbm-a-31532.

Warren CJ, Reardon EJ. The solubility of Ettringite at 250C. Cement Concrete Res 1994;24:1515-1524.

Warren CJ, Reardon EJ. The solubility of ettringite at 25^0C. Cement and concrete research 1994; 24 (8): 1515-1524.

Wright JD, Sommerdijk NAM. Sol-el Materials : Chemitry nd Applications CRC, 2001.

Wu A, Vilarinho PM, Salvado IMM, Baptiista JL. Sol-gel preparation of lead zirconate titanate powders and ceramics: Effect of alkoxide stabilizers and lead precursors. J Am Ceram Soc 2000; 83:1379-1385.

Yamaguchi G, Shirasuka K, Ota T. Highway research board. Symposium on Portland cement paste and concrete. 1966; 1966.p.263-268.

Yoshizawa J, Matsushita H, Katsui A. Preparation of $CaSiO_3$ and Ca_2SiO_4 by sol-gel method. J Adv Sci 2002; 13:50-52.

Zhi Lin Sun JCWTCH. Effects of metal ions on osteoblast-like cells metabolism and differentiation. Journal of Biomedical Material Research 1997;34(1):29-37.