SCHOOL OF MATERIAL AND MINERAL RESOURCES ENGINEERING
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EFFECT OF SILVER ON SOL-GEL DERIVED BIOGLASS SYSTEM

By

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled **"Effect of Silver on Sol-gel Derived Bioglass System"**. I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or University.

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LIST OF ABBREVIATIONS

BG	Bioglass
Ag	Silver
SiO_2	Silica dioxide
$P_2\ O_5$	Phosphate
$Na_2\ O$	Sodium Oxide
CaO	Calcium oxide
TEOS	tetraethoxysilane
HNO_3	nitric acid
TEP	Triethyl phosphate
$Ca(NO_3)_2.4H_2O$	Calcium nitrate tetrahydrate
$NaNO_3$	Sodium nitrate
$AgNO_3$	silver nitrate
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
HA	Hydroxyapatite
SBF	Simulated Body Fluid
XRD	X-ray Diffraction
DTA-TGA	Thermogravimetry/Differential Scanning Calorimetre

LIST OF SYMBOL

˚C	Degree Celsius
%	Percentage
mL	millilitre
g	gram
M	Molarity
Mm	milimetre

KESAN PERAK KEPADA BIOKACA BERASASKAN SOL-GEL

ABSTRAK

kaca bioaktif (BG) yang berasaskan sol-gel dengan komposisi 55% SiO_2 -4% P_2O_5 22%Na_2O 19%CaO (% berat) telah disintesis menggunakan Tetraetoksisilan (TEOS), 0.1m asid nitrik (HNO_3) , Trietil fosfat (TEP), Kalsium nitrat tetrahdrat (Ca (NO3) 2.4H2O), Natrium nitrat ($NaNO_3$). Argentum nitrat ($AgNO_3$) dengan komposisi yang berbeza (2%, 5% dan 8%) telah ditambah ke kacabio menggunakan kaedah yang sama. Ketiga-tiga sampel dibandingkan dengan kacabio yang tiada kandungan Argentum. Proses pengilangan berlaku untuk mendapatkan serbuk halus dan diayak sebelum ditekan ke dalam bentuk pelet. Proses pengkalsinan kemudian dilakukan keatas sampel pada suhu 700° C selama 5 jam. Selepas pengkalsinan, ia boleh diperhatikan bahawa pelet BG yang mengandungi 5% dan 8% perak pecah di bahagian tengah di mana bentuk seperti buih-buih boleh dilihat dengan jelas. Sampel yang sama telak dikalsin dalam bentuk serbuk dibawah suhu yang sama. Keputusan yang sama telah didapati. Kaca bio sampel dengan 8% perak juga mempunyai peratusan keliangan tertinggi. Untuk ujian bioaktiviti, pembentukan lapisan apatit yang paling banyak berlaku keatas permukaan sampel dengan kandungan perak tertinggi selepas direndam dalam larutan SBF untuk 3, 7 dan 14 days berbanding sampel lain. Oleh itu, pertambahan argentum ke dalam struktur biokaca yang berjaya meningkatkan sifat biokaca.

EFFECT OF SILVER ON SOL-GEL BIOGLASS SYSTEM

ABSTRACT

A sol-gel derived bioactive glass (BG) with the composition of 55% SiO_2 - 4%P_2 O_5 -22% Na_2 O-19% CaO (wt %) was synthesized using tetraethoxysilane (TEOS), 0.1M nitric acid (HNO_3), Triethyl phosphate (TEP), Calcium nitrate tetrahydrate(Ca(NO3)2.4H2O), Sodium nitrate($NaNO_3$). Silver nitrate(AgNO3) with different composition (2%, 5% and 8%) was added into the bioglass using the same method. All three samples were compared with silver free bioglass. Calcination process then performed on the samples at 700°C for 5 hours. After calcined, it can be observed that BG pellets that contained 5% and 8% silver burst in the middle part where bubbles-like shaped can clearly be seen. The same samples have been calcined in powder form under the same temperature. But resultant with the same result. Bioglass sample with 8% silver also have the highest porosity percentage. For bioactivity test, the formation of apatite layer occurred the most on the surface of samples with highest silver content after soaking in SBF solution for 3, 7 and 14days compared to the other samples. Thus, additioning silver into the bioglass structure successfully enhanced the properties of bioglass.

CHAPTER 1

INTRODUCTION

1.1 Background

Bone applies important functions in the human body such as assist in movement activity, ensure skeletons have sufficient bearing capacity and protection of internal organs (Florencio-Silva et al. 2015). Nowadays, accidents, injuries or diseases are a common phenomenon that makes bone susceptible to fracture. Fracture existences in bone are related to the quality of bone which influenced by several biological factors, the mechanical behaviour and the microstructure (Kataruka et al. 2017).

Worldwide, the incidence of bone fractures is increasing dramatically and thus increase the demand for bone grafting due to the increase of aging population. Base from the data collected from hospitals treating hip fractures, more than 300,000 hip and knee replacement surgeries have been performed on senior citizens of age above 50. It has been estimated that by the year 2050, the frequency of bone fractures will reach 3.25 million in Asia due to an increase in the elderly population. In the United States, approximately 1.5 million fractures are attributed to osteoporosis (Lee & Khir 2007).

In Malaysia, the statistics have shown that the overall incidence of hip fractures about 90 per 100 000 individuals. Race-specific incidence data showed that the fracture rates and the highest patients come from the Chinese (160 per 100 000) followed by Indians (150 per 100 000) and Malays (30 per 100 000) (Lee & Khir 2007). Therefore, the understanding of biological and mechanical properties of bone will help in developing better orthopaedic treatments. Technological research has looked forward for synthesis of new substituting biomaterials that mimic biological bone tissue (Kisailus 2016).

Bioactive glasses were special glass systems which were generally composed of silicon dioxide (SiO_2) , calcium oxide (CaO), phosphorus pentoxide ($P_2 O_5$) and sodium oxide (Na_2 O) (Bellantone *et al.*, 2002). The first bioactive glass was made in 1960 by Prof. L. L. Hench of University of Florida. Prof L.L.Hench found that bioactive glass was the first material that formed an interfacial bond with host tissue that did not surrounded by fibrous tissue after implantation (Hench, 2006). It was also stated that, once the bioactive was implanted in the body, it can react to the physiological fluids and form a strong chemical bond with bones. Its bioactivity was associated with the formation of the hydroxyl carbonated apatite (HCA) layer on its surface which was similar to the bone mineral. The HCA layer forms a rapid sequence of chemical reactions on the surface of the implant when in contact with body fluid (Mahmood *et al.*, 2015).

Bioactive glasses were initially obtained via melting at higher temperatures. There were two common processes for the formation of bioactive glasses which were melt derived and sol-gel process. The formation of bioactive glasses with a composition of SiO_2 - CaO-$P_2 O_5$ -Na_2 O by sol-gel processing was observed and it was found that glasses made from the sol-gel process required lower temperatures as compared to conventional melting method (Pirayesh and Nychka, 2013).

There were a lot of advantages of sol-gel method compared to traditional melting process. Some of them were lower fabrication temperature which can reduce the cost while improving the quality of the product at the same time, better control on composition and homogeneity, and has higher surface area. Higher surface area can increase the dissolution rate in body fluid which can raise the bioactivity of the resultant material. This in turn, increases the rate of hydroxyapatite (HA) or hydroxycarbonate apatite (HCA) formation, which is essential for preparing a suitable environment for attachment and differentiation of stem cells (Pirayesh and Nychka, 2013).

The high demand of bioactive glass and glass-ceramics in clinical use due to the fact that they offer the possibility of improving the long-term survivability of implants while improving and replacing the diseased or damaged bone (Grove, 2010). Biomedical implants were usually used in body to substitute not well functioning organs. However, the risk of microbial infections often occurred especially in metallic implants because of electrochemical corrosion when an implant was introducing into the body since metallic implants usually used for the fixation of open-fractured bones (Balamurugan *et al.*, 2008).

The infections come from the result of bacteria adhesion and following biofilm on the implants surface which can lead to the development of serious infections and surgery revision. If these situations occur, the body will resist to antibiotics and system in the body will immune to any kind of treatments. As already mention before, bioactive glasses show bioactivity that bond well with living bond, but a group of bacteria on the surface of the implant can lead to failure of the treatment. The result of implant infections were so serious and sometimes it can lead to second surgery with lots of suffering and the patient usually need to be hospitalize longer than the intended time (Mahmood *et al.*, 2015).

It is well known that silver (Ag_2O) and its compounds show an antibacterial behaviour. silver-doped glass and ceramics are expected to be candidates for antibacterial materials, since they showed high chemical durability and antibacterial activity (Pouraghaei *et al.*, 2016). Due to the of the antimicrobial properties of silver, recent researchers focuses on development of silver-doped implants are increasing (Nandi and Mahato, 2016). The introduction of Ag_2O into the bioactive glass composition is aimed to minimized the risk of microbial contamination through the potential antimicrobial activity (Bellantone *et al.*, 2002).

1.2 Problem statement

Bioglasses can be formed by the traditional method, which is regarded as simple and suitable for mass production. However, this method was limited by the evaporation of the volatile component P_2O_5 during high-temperature processing. Thus, the sol–gel method was an alternative approach to fabricate bioglasses. The advantages of the sol–gel process are well known where by the process takes place at lower temperatures and gives homogeneous mixtures in the final glass composition. Introduction of silver (Ag_2O) into the bioactive glass compositions aimed to minimize the risk of microbial contamination through the potential antimicrobial activity of the leaching Ag^+ ions. It has been shown that a bioactive glass composition doped with Ag_2O can prevent infections by inhibiting the growth or action of microorganisms.

Bioactive glass consisted of the main component SiO_2 and three other basic components. Na_2O, CaO, and P_2O_5. By varying all of these components different forms of bioactive glasses can be made bioactive glasses and have shown good results in bone regeneration. The process of bone-graft bonding of bioactive glasses starts with the release of soluble ions after a silica gel layer is formed on the bioactive glass surface.

After the formation of the silica gel layer, amorphous calcium phosphates precipitate on this layer where they form a natural hydroxyapatite (HA) layer due to crystallization. The HA layer activates the osteoblastic cells to start the formation of new bone (Kumar, 2009). Bioactive glasses and glass-ceramics are studied because of their surface chemical reactivity where the precipitation of bone-like apatite from the solution provides a strong chemical bonding with tissues when in contact with body fluids by a complex mechanism of ions leaching and partial dissolution of the glass surface. Since bioactive glasses and glassceramic are brittle materials, they are especially used in the field of small bone defects reconstruction, or as coatings on inert substrates for load-

bearing prostheses. Bonding between bioactive glass or glass ceramic and the surrounding tissues takes place through the formation of a HA layer, which is very similar to the mineral phase of bone.

When the bioactive glass is placed in contact with physiological fluids, this layer is formed through a complex ion-exchange mechanism with the surrounding fluids, known as bioactivity. This biologically-active layer of HA can form on the surface of glasses that having a wide compositional range. Its presence is widely recognized to be a sufficient requirement for the implant to chemically bond with the living bone. Kokubo et al. proposed the Tris-buffered simulated body fluid (SBF) for the in vitro study of bioactive glass and glass–ceramic, since its ion concentration is almost equal to that of human blood plasma.

In the past decade the popularity of bioactive glasses in the treatment of infections increased. Their versatility in composition and physiological properties enables us to use bioactive glasses in treatment of infection based on different antibacterial mechanisms. By adding antimicrobial element such as silver to the bioactive glass during the manufacturing process. The sol–gel-derived glasses have low release rates of antibiotics and have a low strength what makes them less applicable in treatment of bone infections. However, by adding silver to the bioglass enhance the bioative activity on the surface of the glass. Higher concentration of silver gave higher surface porosity. which accelerate the formation of HA layer.

1.3 Objectives

The main objectives of this projects are:

I. To study the effect of sol-gel method on bioative glass system.

II. To investigate the effect of silver concentrations on bioglass system.

1.4 Thesis overview

This thesis is organized in five chapters consequently. Chapter 1 describes a brief introduction, problem statement and objectives of the research. Chapter 2 a comprehensive review on the silver antibacterial behaviour, fundamental concepts and applications of silver and bioactive glass are discussed. Chapter 3 details the experiment procedures that are used in this study. This includes the experimental design, synthesis of silver doped bioglass and characterization techniques. This covers a brief explanation on the characterization equipment, their operation principles and sample preparation. Chapter 4 presents the experimental results and comprehensive discussion on the effect of silver on bioglass system. Finally, Chapter 5 is devoted to the conclusions of this research work and suggestions for future work.

Figure 1.1 showed the experiment procedure for the entire research. The experiment started with the synthesis of bioglass with different weight percentage of $AgNO_3$ which are 2%, 5% and 8%. After that the samples are undergo twice heating process to get rid the water. However prior to that, the samples are kept in room temperature for 5 days for aging process. After heating proess, milling and seiving process take place to get fine and unform powder particles. Then the samples are calcined at high temperature to get rid residue nitrate before sent for characterization.

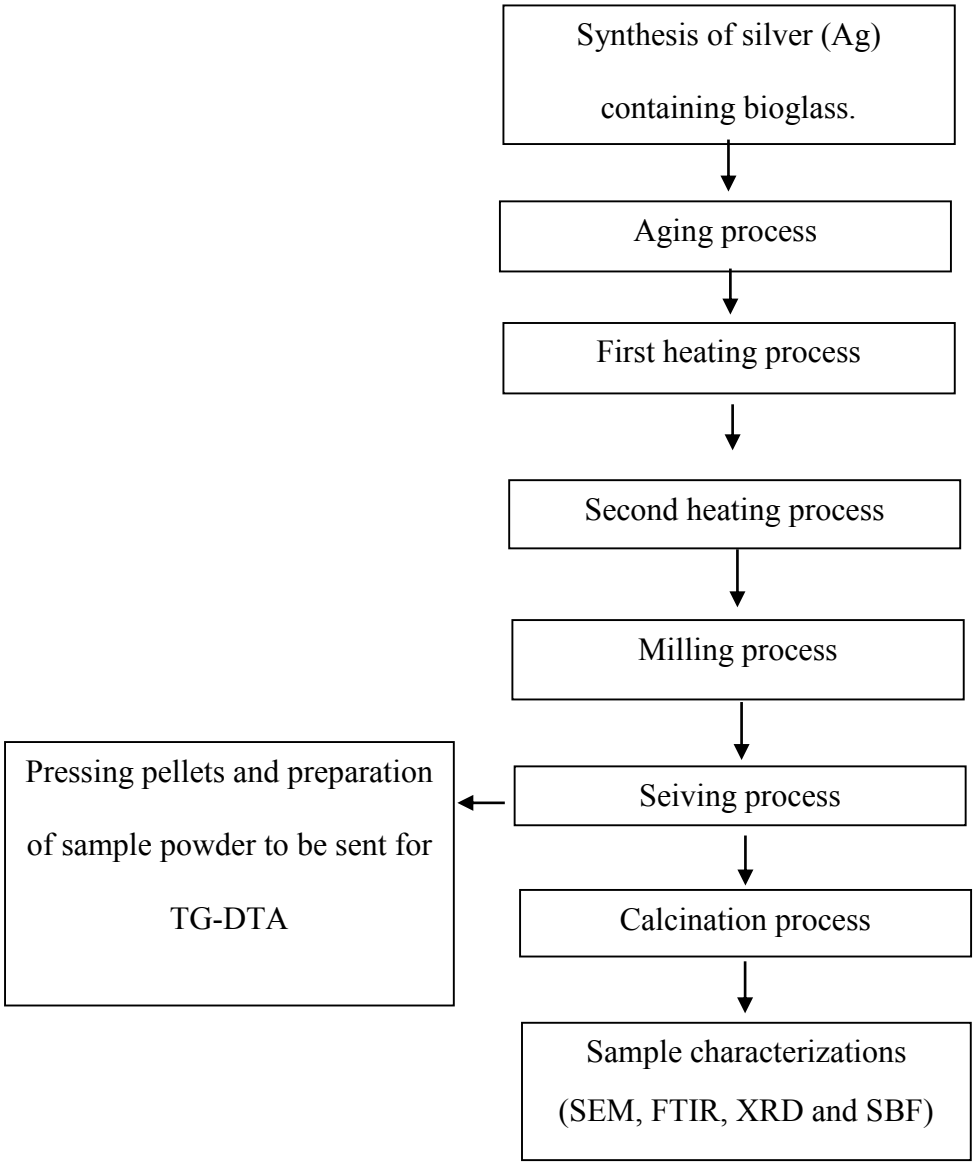

Figure 1.1 : Experiment procedure

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter describes the basic concept of sol-gel method and the necessary of understanding the effect of silver on bioglass system. This effect could provide benefit advancement in the engineering technology and other industry applications.

2.2 Bioactive glasses

Worldwide, bone injury or fracture is considered as a major health issue. Therefore, there have been extensive investigations into the fracture behaviour of human bone. Bone fracture is among of the expensive surgery for medical applications. In nature, bone has some characteristics such as good structure, lightweight and yet tough (Kataruka et al. 2017). However, the fracture properties of human bone beneath fall-like loading conditions remains poorly documented (Gauthier et al. 2017). Fractures can happen in variety ways such as trauma, cancerous, total hip revisions and metabolism (Umadevi & Geethalakshmi 2011). Thus, bone substitutes were becoming important in bone surgery due to high demands to overcome bone fracture issues over the last few decades.

Bone grafting is a surgical procedure which replacement of bone fracture with the material from either patient's own body (autograft), donor (allograft) or artificial bone (Teresa Mao 2013). However, both of bone graft techniques have drawback that needs to be considered. Generally, autograft is the standard technique that used for the surgical procedure. However, it has some limitations such as pain, donor site morbidity and long period time of operation (Kheirallah & Almeshaly 2016). For allograft technique, its look to overcome issues that related to autograft due to allograft shows better results which allow a faster recovery and without involved donor site morbidity (Rodríguez et al. 2015).

Unfortunately, allograft does not provid osteogenic properties which it will interact with host tissue. Another type of bone graft is the use of substitute materials that could not suffer both mechanical, osteoinductive and osteogenic properties.

The first bioactive glass as an alternative implant materials was invented by Prof. Larry Hench at the University of Florida in 1969. One day a US army colonel who just retured from Vietnam war asked him if a new material can be developed that could sustain the enviroment of human body because he already witnessed numerous amputations during the war that most of the time, the situations cannot be avoided because all available materials at that time such as metal and polymer cannot be used because the body rejected them (Hench, 2006).

So together with a friend who is a research assistance at a medical school and two orthopaedic faculty members, Prof L.L.Hench started the research. The research funded by US Army Medical R and D Command (Hench, 2006). Bioactive glasses are special glass system which are generally composed of silicon dioxide (SiO_2), calcium oxide (CaO), phosphorus pentoxide ($P_2\ O_5$) and sodium oxide (Na_2 O) (Bellantone *et al.*, 2002)(Nandi, Kundu and Datta, 2011).

These glasses possesses bioactive behaviour which is the ability to bond to soft and hard tissues by chemical and biochemical reactions that can produces strong bond between the glass and the tissues (Bellantone *et al.*, 2002). This ability is related to both ion release (Ca^{2+},Na+ PO^{3-}) from the glass and the subsequent precipitation of a calcium and phosphate surface layer to the negatively charged Si-OH groups that present on the glass surface like in Figure 2.1. This reaction is considered as a precursor to bone bonding in vivo (Haas *et al.*, 2015).

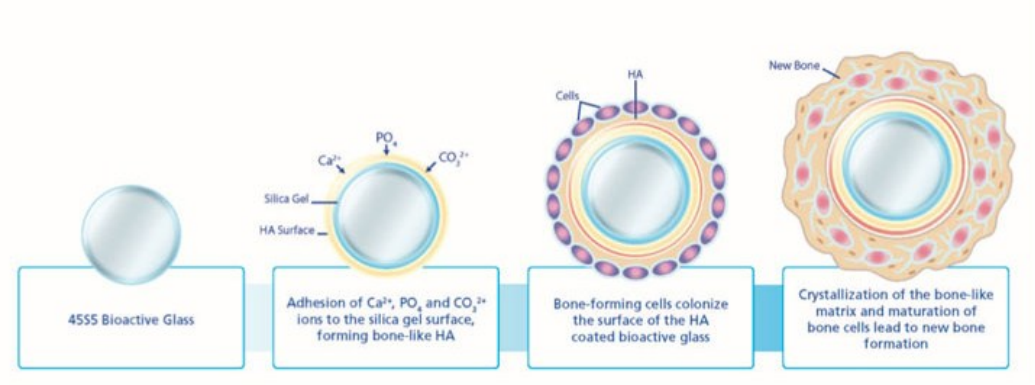

Figure 2.1: bioactive glass surface reaction

Bioactive silicate glasses are amorphous solids. The basic building unit of silicate glasses is the SiO_4 tetrahedron in a network, which is interconnected in a network through Si–O–Si bonds, commonly referred to as bridging oxygen atoms. Referring to Figure 2.1, these tetrahedra are commonly referred to as Qn units, where "n" represents the number of bridging oxygen per tetrahedron.

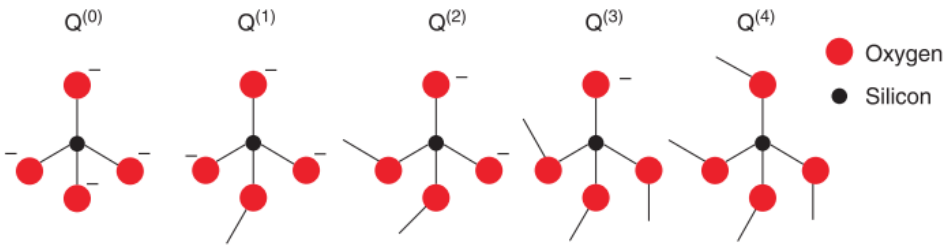

Figure 2.2: Silica tetrahedral sites of silicate glasses Bridging

During the synthesis process, the network modifiers such as Na^+, K^+ and Ca^{2+} disturbed the continuity of the glassy network, breaking some of the Si–O–Si bonds leading to the formation of non-bridging oxygen groups like in Figure 2.2. The properties of bioactive silicate glasses are influenced by a portion of non-bridging oxygen atoms. Network modifiers are often necessary to modify the properties of the glass.

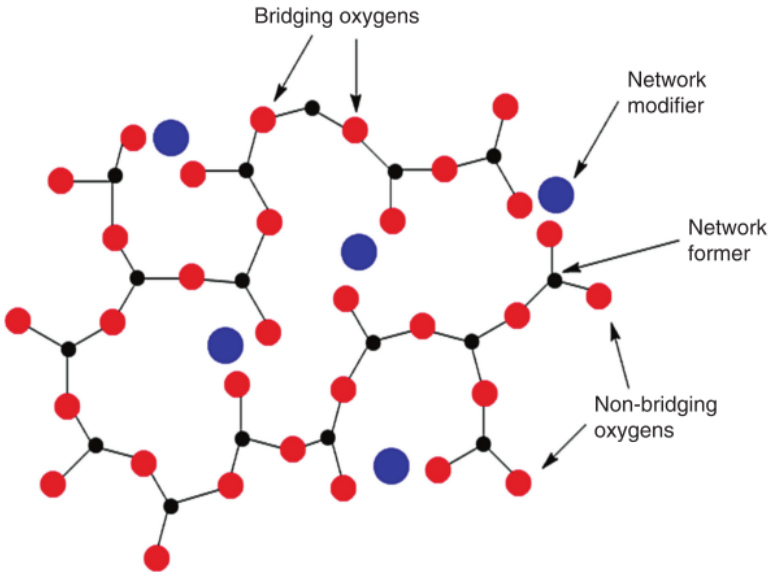

Figure 2.3: 2D presentation of random glass network modifiers and network formers

Bioactive materials gave a specific biological response at the interface of the material leading to the formation of a natural bond and development of new mineralized bone tissue. Bioactive glasses have been extensively studied for more than thirty years since Hench first invented Bioglass. Because of the good bioactivity osteoconductivity and biodegradability, bioglasses as bone repair materials or fillers owing to their ability to form a bond to living bone, have been used in clinic for more than ten years.

Also, Hench has described a sequence of five reactions that resulted in the formation of hydroxy apatite (HA) layer on the surface of these bioactive glasses. A bioactive glass is one that undergoes surface dissolution in a physiological environment in order to form a hydroxycarbonate apatite (HCA) layer. The larger the solubility of a bioactive glass, the more pronounced is the effect on bone tissue growth

2.3 Application of bioactives glass

Bioactive glasses have been used clinically in a variety of situations due to their high level of tissue integration and regeneration (Bellantone et al., 2002). They can form an interfacial bond with host tissue and will not surrounded by fibrous tissue after implantation process. Once they are implanted in the body, the bioactive glass will react to the physiological fluid and strong chemical bond with bones will form (Mahmood et al., 2015).

Nowadays, many medical materials are being developed from bioactive glasses as different compositions and structures can be designed for specific surgical applications. These include bone void filler composite materials with polymers to improve bioactivity, composite bone cements, glass–ceramic scaffolds to facilitate cellular and tissue ingrowth, and yttrium containing glass microspheres to treat tumor growths with radiotherapy (Haas *et al.*, 2015).

Furthermore, due to their high level of tissue integration and regeneration, bioactive glasses have been used clinically in a variety of situations. Bioactive glass devices are now available to treat conductive deafness and alveolar ridge resorption and bone loss due to periodontal disease and to fill cystic and surgically created defects, particularly in craniomaxillofacial sites (Bellantone *et al.*, 2002).

Metallic is known to have better mechanical properties. However, they have gained concerns over the years as bad host responses due to wear or electrochemical corrosion. Electrochemical corrosion can produce degradation products thus they may result in clinical failure of the implant (Pouraghaeia, Moztarzadeha and , N. Nezafatib, 2016). Because of that, there is an increasing clinical use of bioactive glass and glass ceramic. “Bioactive” glass can be defined as one that acquire a specific biological response at the

interface of the material that result in the formation of a bond between the tissue and the material. While “glass” often related as solid that possesses a noncrystalline structure at the atomic scale that show a glass transition when heated towards the liquid state. To put it briefly, bioactive glass has been designed to obtain a particular biological reaction at the interface of the material to stimulate cell growth, gene response and the formation of bond between living tissues and the material (Nandi and Mahato, 2016).

2.4 Silver Doped Bioglass

Bacterial infection after implant placement is a significant rising complication. Although infection is not a common reason for implant failure, it causes for a lot of medical cost and a decrease in patient satisfaction. So in order to reduce the incidence of infections after implant placement antimicrobial agents has recently been used in a variety of medical applications (Chen *et al.*, 2006). In particular, specific ions like Ag^{+}, Cu^{2+} and Zn^{2+} when eluted from medical materials can act as microbial agents. Ion release from these materials is powerful in terms of safety, durability and heat resistance than conventional organic compounds such as antibiotics (Balamurugan *et al.*, 2008).

There are some serious concerns involving antibiotic use which are including allergic reactions, microbial flora depletion and bacterial resistance. In particular, the evolution of antibiotic resistant strains of bacteria such as Methicillinresistant S. aureus (MRSA), Vancomycinresistant S. aureus and Methicillinresistant S. epidermidis (MRSE) are known to pose significant concerns to elderly or immune-compromised patients in hospitals. Because of that, alternative antibiotic-free methods have been investigated to import antibacterial properties to implantable materials (Haas *et al.*, 2015).

Ag^{+} in particular is known to have a wide antibacterial spectrum and to be relatively safe to humans. Silver-based antimicrobial agents receive much attention

because of the low toxicity of the active Ag+ ion to human cells, as well as it being a long-lasting biocide with high thermal stability and low volatility. In addition, microbes such as S. epidermidis can form a thick multi-layered biofilm which is composed of an extracellular polysaccharide known as polysaccharide intercellular adhesion (PIA) which can be very resilient to antimicrobial compounds, hence it is highly desirable to synthesize antimicrobial surfaces on medical materials that can reduce the possibility of biofilm colonization.

In addition, silver can help in skin wound healing. It reduces the inflammatory and granulation tissue phases of healing, and encouraged epidermal repair (Haas *et al.*, 2015). It has been reported that Ag can cause bacterial inactivation in vitro by binding both to microbial DNA, preventing bacterial replication (Chen *et al.*, 2006).

Bioactive glasses doped with small amounts of silver ions showed a broad spectrum of antimicrobial activity. Low concentrations of silver ions in BG are not toxic, but high concentrations can cause cytotoxicity. Silver-containing bioactive glasses are mostly obtained by a sol-gel method because of the homogenous product. The melt-quenching method is not suitable for the synthesis of Ag-doped BG, because homogeneity and reproducible of the product cannot be provided (Kaur, 2017).

Addition of silver ions into the BG structure induced lower bioactivity as a result of lower solubility and surface area. The release of silver ions from glasses in SBF is slow compared with the dissolution of other constituents. There were several important factors which limit the dissolution of AgBG and release of silver ions. Replacing calcium with silver ions in the bioactive glass structure increases glass network connectivity as a result of reducing the number of non-bridging oxygen groups, which are essential for the solubility.

Silver helped in the formation of hydroxyapatite layer(HA). The formation of the HA layer on the glass surface can be limited or even stop the dissolution and release of silver ions. Released Ag ions in the AgBG surface layer can interact with phosphate and chloride ions, building a silver phosphate compound and difficult soluble AgCl. Apatite materials can incorporate silver ions into the structure during its formation, or they can be absorbed from the solution. Silver ions may have a strong stimulatory effect on the formation of carbonate apatite.

The increase in the amount of silver in a BG leads to the formation of secondary phases: quartz and metallic silver, which reduce the BG transformation into HA. The textural characteristics of AgBG also play an important role; a higher surface area is favorable for obtaining a higher dissolution rate of glasses and therefore a higher bioactivity. Some studies have reported that with the increase silver content in a BG, there occurs a progressive decrease of the surface area and pore volume and the progressive broadening of the pores distribution

However, the previous researched done by a few researchers have been proved that bacteria showed a low tendency to develop resistance to silver-based products, and therefore both metallic and ionic silver have been incorporated into several biomaterials such as polyurethane, hydroxyapatite (HA) and bioactive glasses. Silver-containing products materials helped for wound repaired. When silver reacts with moisture on the skin surface or with wound fluids, silver ions are released, damaging bacterial RNA and DNA, thus inhibiting replication.

Sustained silver-release products have a bactericidal action and manage wound exudates and odor. Silver treatment appeared to reduce the inflammatory and granuation tissue phases of healing and induced epidermal repair (Balamurugan *et al.*, 2008). It has

been proved that BG dissolution products could stimulate cell proliferation and differentiation that eventually can promote new bone formation and, according to silicon oxide quantity, bone formation is faster with a BG implant than synthetic hydroxyapatite (Quintero and Escobar, 2017).

2.5 Processing Methods

Mechanical and structural properties of the bioactive glasses were highly dependent upon synthesis techniques, composition, particle size and crystallization. Thus many techniques were being used to make bioactive glasses (Kaur *et al.*, 2014). The most common methods for the production of bioactive glass materials are melt-quenching and the traditional melting process or more modern sol-gel process (Balamurugan et al., 2008).

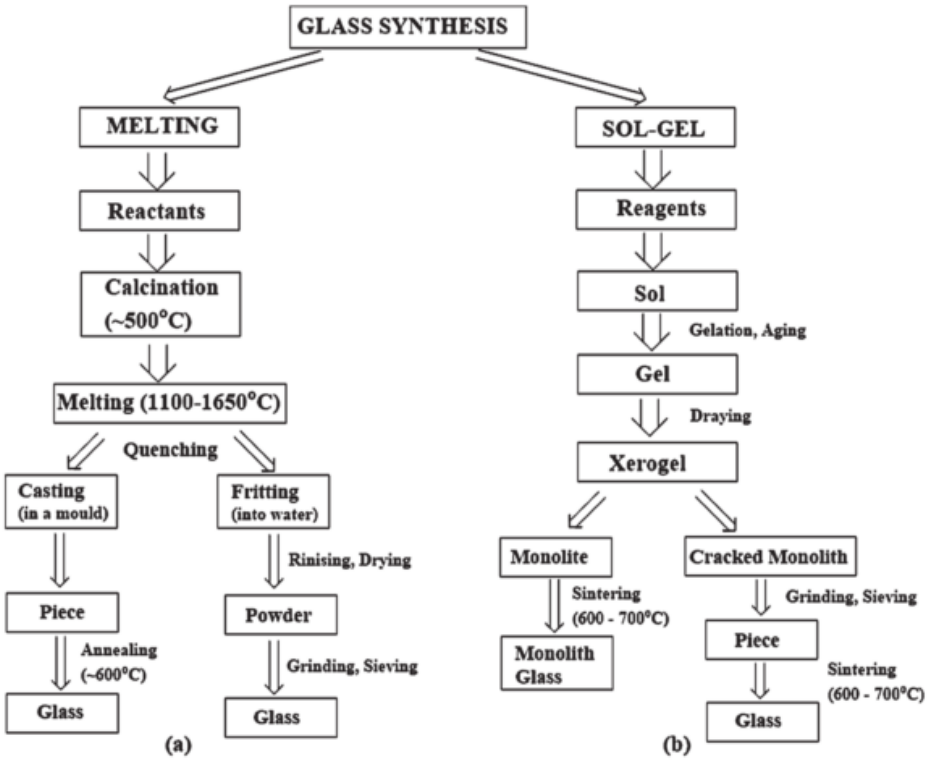

Figure 2.4: Schematic view of the preparation route for (a) melt quench and (b) sol-gel bioactive glasses

2.5.1 Sol-gel Method

Sol-gel method have been intensely studied as an alternative method for preparing ceramics and glasses for a wide variety of applications including bioceramics.The process involves the transition of the colloidal solution (sol) into a solid phase (gel). Gel can be described as a three-dimensional solid skeleton surrounded by a liquid phase, where both phases are continuous and nanometre dimensions (Kaur, 2017).

The gelation process is achieved by reactions of hydrolysis and condensation. These reactions can occur very slowly at room temperatures. So acidic or basic catalysts are added to help accelerate the process. The gelation phase is followed by a drying process, in which the solvent is removed from the gel and forms a solid, porous matrix called xerogel. The resulting xerogel is heat-treated in order to obtain the final product. Annealing process frequently leads to agglomeration and coarsening of nanoparticles .

The first step of the sol–gel process typically involves mixing the precursor, silicon alkoxide, solvent, and an acid catalyst. This step can dramatically affect the homogeneity of a multicomponent gel, which at the same time influenced by the nature and reactivity of the precursors, the nature and solubility of the reactants in the selected solvent, the concentration of the selected solvent, the sequence of addition, the pH and the time and temperature of the reaction. After mixing, the alkoxide precursor is hydrolysed to silicic acid, which then condenses to yield the silica gel.

The sol-gel–derived powders contained mainly mesopores within the range of 6.5–9.5 nm. The porous nature of these materials originates from the manner by which the gel is formed. The alkoxide precursors react readily with water, and the hydrolysed species link together in a condensation reaction that can form a three-dimensional polymeric network, because the monomer is tetrafunctional [$Si(OH)_4$]. The liquid solvents that

participate in the process are retained in the capillaries of the structure and, after drying, a solid network with continuous porosity results. The pores may vary in size and may be interconnected or completely closed, depending on the processing conditions and stabilization temperature. The properties of the obtained material are affected by many factors that influence the rate of hydrolysis and condensation, such as pH value, temperature, reaction time, concentration of reagents, the type and concentration of the catalyst, temperature, and time of aging and drying.

The advantages of the sol-gel method are the low-temperature processing, the purity and homogeneous distribution of the components, higher porosity and specific surface area values, and the possibility of particle size control. Increasing the specific surface area and pore volume of bioactive glasses greatly accelerates its dissolution and HA formation on the surface and therefore enhances the bioactive behaviour. The sol-gel–glass texture is highly porous; thus it promotes a higher degree of hydroxylation of the surface forming SiOH than melt-derived glasses (Lenza and Vasconcelos, 2001).

For sol-gel–derived materials the silica-rich gel layer provides more sites for calciumphosphate layer nucleation. Different textures can be promoted in sol-gel–derived material by simply altering specific processing parameters, such as pH, reagent concentrations, and drying and stabilization conditions. Furthermore, the large porosity of sol-gel–derived powders decreases their ability to be compacted into a fixed volume, which implies that a small mass of material is necessary to fill a void. This favors applications where faster resorption in vivo is needed. Because of its mesoporous texture and high surface area, which can adsorb a range of substances such as proteins and cells, the sol-gel method has become an alternative for preparing glasses for a number of applications in the biomedical field.

2.5.2 Melt Quenching Method

The melt-quenching method is based on melting a heterogeneous reactant mixture in a specified molar ratio. The reaction mixture calcined at about 500 °C to remove moisture, which is adsorbed onto the materials or may be formed by dehydration of hydroxides. In addition, there comes to the release of gases caused by decomposition of the possibly present precursors: carbonate, nitrate, and sulfate. Usually for this method oxides are mainly used as precursors.

The melting temperature (1,100–1,650 °C) is above the glass transition temperature (Tg) of the target bioactive glasses, to afford a viscous state. The Tg of the BG is lower than its crystallization temperature (Tc) which leads to the formation of glass-ceramics. The glasses are often melted twice in order to increase homogeneity. Melting process of individual components like alkalis, boron, phosphorus, and fluorides is carried out in covered crucibles to prevent evaporation from happening.

The molten glass is then cast into a preheated graphite or steel molds to make bulk implants or is immersed in water which is used for quenching. Bioglass have been used clinically in various medical and dental applications. Some of the bioglass products used to repair damaged bones, teeth and skin where the bacterial infections present (Grove, 2010).

Melt-derived powders, contained very little porosity and almost negligible adsorption levels. Although the pore-size measurements (1.6–2.1 nm) are not very precise at such low adsorption levels, they lie within the size scale of glass surface roughness. Surface roughness of particles produced from melt-derived glasses has been associated

with surface tension of the melt (melt-formed surfaces) and with intrinsic heterogeneities in glass structure. Meltderived glass particles exhibit mainly nonporous surfaces with low

intrinsic roughness and low surface area, dependent on the degree of grinding. Because of the lack of textural variation produced through melting, the melt-derived glasses have shown a dissolution behavior that can be directly correlated to the chemical composition.Therefore, for melt-derived powders, the change in particle-size range caused by different grinding periods could provide an efficient way to control dissolution rates. Finer powders exhibit higher specific surface areas; therefore, they provide more exposed surface for dissolution.

The comparative studies of gel-derived and melt-quenched glasses showed that the synthesis technique causes differences in the texture and the glass structure. The sol-gel-derived glasses showed more polymerized structure and higher porosity and specific surface area values, enhancing the solubility. The rate of HA formation is higher for the sol-gel-prepared glasses, and they exhibit bioactivity with a content of higher than 90% of SiO_2 while bioactivity at melt-derived glasses is present with a content only up to 60% of SiO_2.

2.6 Hydroxyapatite (HA) layer

For many years, HA has been considered as an important inorganic biomaterial which has attracted the attention of researchers related to biomaterials field due to its potential to stimulate optimal bone tissue regeneration (Wilcock et al. 2017). However, researchers have then realized that human biological bone is not solely HA with respect to its chemical composition, percentage crystallinity and crystal structure (Kulanthaivel et al. 2015).

Besides that, when exposed in direct contact with biological system, HA resulted in extremely slow in vivo degradation and bioresorption rate to regenerate new bone tissue. This has limits its applications for orthopaedic implants clinically (Kamitakahara et al. 2015). In addition, HA has also shown low mechanical properties particularly in terms of its tensile strength and fracture toughness (González Ocampo et al. 2016). For the aforementioned limitations, currently, HA can only use for non-loadbearing bone substitutes applications.

Furthermore, HA properties can be modified to make it suitable for broader biomedical applications by substitutions new element. The variety of substitutions can be introduced into the HA lattice by anions, cations, and functional groups. Among the ions that have been recommended to improve the HA properties are like carbon, cobalt, strontium, zinc, copper and silver. These substitutions can give big impact toward properties of HA and also act as a tailor to modify physical, chemical, mechanical, and biological properties of HA (Kramer et al. 2014). These elements are could play the important role towards improving cell-material interactions of HA (Mardziah et al. 2009).

As have been mention before, bioactive glasses are a group of inorganic materials based on silicon oxide which have been used widely due to their known excellent

bioactivity which can increases apatite layer formation allowing a suitable and safe chemical bond between the material and living bone (Quintero and Escobar, 2017).The surfaces of bioactive glasses represent the site of interaction with the surrounding living tissues. Therefore, it is crucial to enhance their biological performance.

The bioactivity of a glass is usually evaluated by its ability to form a hydroxyapatite (HA) layer on its surface upon immersion in SBF (Kaur *et al.*, 2014). Bioglass in presence of SBF solution can form a bone like apatite layer, especially those obtained by sol-gel techniques due to OH^- groups on their surface, which are able to induce HA nucleation (Quintero and Escobar, 2017).

A shown in Figure 2.5, the mechanism of bonding silicate bioactive glasses to the bone has been attributed to the formation of a HA layer on the glass surface in contact with the body fluid. The mechanism of HA layer formation on bioactive glasses has been widely studied *in vitro*. This process is complex and can be simplified to be shown through sequence of various stages. Some of these stages are played out partly in parallel, such as 6 and 7 with stages 3–5. The initial stages (1 and 2) involve the partial dissolution of the bioactive glass after contact with the body fluid (SBF), with substitutions of Na^+ and Ca^{2+} with H^+ ions and the pH increase of solution. As shown in equation 2.1.

$$Si - ONa + H^+ \longrightarrow Si - OH + Na^+ \qquad (2.1)$$

As a result, this leads to network degradation by breaking the Si–O–Si bonds, formation of Si–OH groups, and release of $Si(OH)_4$ and larger silicate fragments as shown in equation 2.2.

$$Si - O - Si + H_2O \longrightarrow Si - OH - OH \qquad `(2.2)$$

The continuous formation of silanol groups results (three stages) in their poly-condensation and formation of a porous silica-rich layer. Fourth, creating an amor- phous