SOL-GEL PREPARATION OF 58S AND 77S BIOACTIVE GLASSES BASED ON DECON® SURFACTANT

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Bioactive glasses are biocompatible materials which have been extensively investigated for development and advancement of tissue engineering and medical implantation. There are various kinds of bioactive glasses and many different pathways for the production. Sol-gel preparation of 58S and 77S bioactive glasses had been chosen for this study. These two 58S and 77S bioactive glasses consist of different percentage of compositions in SiO$_2$-CaO-P$_2$O$_5$ system. Besides investigated the preparation of bioactive glasses, this study was focused on the effect of different temperature used in thermal treatment on the bioactivity of bioactive glasses. After accurate amount of the precursors were added, Decon$^\text{®}$ surfactant was added to foam the sol obtained. The foamed gel is then aged, dried and calcinated under suitable temperature and period. Simulated body fluid was used to represent the body fluid for the in-vitro study of bioactivity of produced bioactive glasses. The formation of hydroxycarbonate apatite clusters or layer on the surface of bioactive glasses indicated that the produced bioactive glasses was eligible for the tissue growth. From this study, it was found that after immersion of simulated body fluid solution, the intensity of hydroxycarbonate apatite clusters formed on the 600°C calcinated bioactive glasses was higher as compared to the other calcinated bioactive glasses. Hence, the most optimal calcination temperature for 58S and 77S bioactive glasses was 600°C.
Penyediaan sol-gel untuk 58s dan 77s kaca bioaktif berdasarkan Decon® surfactant.

ABSTRAK

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<td>Bioactive glass</td>
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<td>HCA</td>
<td>Hydroxycarbonate apatite</td>
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<td>SBF</td>
<td>Simulated body fluid</td>
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<td>c-SBF</td>
<td>Corrected SBF</td>
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<tr>
<td>n-SBF</td>
<td>Newly improved SBF</td>
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<tr>
<td>R&amp;D</td>
<td>Research and development</td>
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<td>TEOS</td>
<td>Tetraethyl orthosilicate</td>
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<td>TMOS</td>
<td>Tetramethyl orthosilicate</td>
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<td>MAS-NMR</td>
<td>Magic-angle spinning-nuclear magnetic resonance</td>
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<td>BET</td>
<td>Brunauer-Emmett-Teller</td>
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<td>MEP</td>
<td>Middle ear prosthesis</td>
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<td>ERMI</td>
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XRD  X-Ray Diffraction
TGA  Thermogravimetric Analysis
DTA  Differential Thermal Analysis
FTIR  Fourier Transform Infrared Spectroscopy
IR  Infrared
SEM  Scanning Electron Microscope
FEG  Field Emission Gun
\( \rho \)  Density
PE  Polyethylene
PS  Polystyrene
N  Normality
M  Molarity
ACP  Amorphous Calcium Phosphate
CHAPTER 1

INTRODUCTION

1.1 Background of Study

Various kinds of traumas, diseases and cancers cause significant loss of tissue. Graft materials from other body parts of patients (autografts) and donors (homografts) or even from other living things (heterografts) and non-living things (xenografts) can be used to replace and heal the injured areas (Sepulveda et al., 2002). Recently, autografts are favoured by many surgeons. However, there are some drawbacks in autografts which are the shortage of available natural graft materials and most patients bear severe pain at the donor places. Thus, usage of synthetic grafting materials has become an alternative way to overcome these limitations of autografts (Jones, 2013).

Bioactive materials are defined as the synthetic materials that are capable of releasing ions which stimulate the beneficial cellular responses at the interface of the materials (Bosetti & Cannas, 2005; Jones, 2013). These materials play an important role in cell regeneration. They particularly influenced the attachment, proliferation, differentiation and mineralization of the cells after forming mechanically strong bonds between the material and the living host tissues, such as the bone tissues. The surface reactivity of bioactive materials contributes to its ability of bonding and tissue formation (Ducheyne & Qiu, 1999).

Bioactive materials comprise of bioactive glasses, bioactive glass-ceramics, bioactive calcium phosphate ceramics, and synthetic hydroxyapatite (HA) ceramics (Cao & Hench, 1995). They have been mainly used as bone or dental fillers.
and coating for implantation in medical fields over the last decade (Gwendolen et al., 2007). The results for the application of bioactive materials are far more superior to bioinert materials as they could bond spontaneously to the bone without evoking any fibrous encapsulation after implantation (Hench, 1991). Additionally, bioactive materials are characterized as osteogenic, osteoconductive, osteoinductive, or in combination thereof. They are also very useful for bone grafting in the reconstructive orthopaedic surgery (Bauer & Smith, 2002).

According to Hench & Fielder (2004), there are two classes of bioactivity, i.e. class A and B, which can be distinguished by the rate and mechanism of implant-tissue interactions. According to Hench, Class A is the osteoproduction and Class B is osteoconduction where Class A shows bones and tissues bonding while class B only bonds with bones (Peitl et al., 2001). There are two pathways to optimize the biochemical and biomechanical compatibility of the bioactive materials. The optimization can be achieved either structural tailoring of bioactive composites or molecular tailoring of the surface chemistry (Cao & Hench, 1995).

Besides that, the bioactive glasses (BAGs) are found to have the most capability to stimulate the bone regeneration among the bioactive materials. BAGs are multi-component systems that composed of CaO-P2O5-SiO2 (Jones, 2013) and mainly confined to powder, granules, or small monoliths (Sepulveda et al., 2001). They have been denoted as a latent osteogenic porous bioactive glass template for tissue engineering (Gwendolen et al., 2007) and transplantation (Sepulveda et al., 2001). Tissue engineering can be expounded as the formation of new tissues is promoted after the introducing of certain new material that involves the interactions with the surrounding cells (Hardouin et al., 2000).

Based on the in-vivo studies, BAGs bond with bone more rapidly than bioactive ceramics. The in-vitro studies show that the osteogenic properties of BAG are due to the stimulation of osteoprogenitor cells at genetic level by the dissolution products of BAG. However, calcium phosphate and synthetic HA bioactive ceramics are more widely applied in the clinic. This is because BAGs are lag behind of other bioactive materials in the context of commercial success but mainly due to the scientific limitations. For example, the first invented BAG, 45S5 Bioglass® found to be
crystallizes during sintering and thus it is difficult to fabricate porous bioactive glass scaffolds for bone regeneration (Jones, 2013).

Basically, the bioactive glasses are synthesized via two major processes: melt-casting and sol-gel route (Ungureanu, 2011). All 45S5 Bioglass® are prepared via traditional melt-quenching methods. Recently, a new sol-gel route has been proposed to overcome the sintering problem faced by melt-derived 45S5 Bioglass® (Jones, 2013). The new sol-gel glasses based on SiO₂-CaO-P₂O₅ system are characterized by a low propensity to devitrify. They have higher crystallization temperature up to 900 °C and the calcination occurs at 850 °C (Bellucci et al., 2014). This enables them to preserve their amorphous nature (Bellucci et al., 2014) and the silica network is fabricated at room temperature (Jones, 2013). Moreover, the sol-gel glass powders have been successfully applied to produce scaffolds that have higher potential application in bone tissue engineering than those melt-derived counterparts (Bellucci et al., 2014).

Furthermore, bioactive sol-gel glasses are belongs to class A bioactivity (Hench & Fielder, 2004). The most commonly used bioactive sol-gel glasses are in ternary system, e.g. 58S and 77S with compositions of (60 mol. % SiO₂, 36 mol. % CaO, 4 mol. % P₂O₅) and (80 mol. % SiO₂, 16 mol. % CaO, 4 mol. % P₂O₅) respectively (Li, 1991). A significant advantage of bioactive sol-gel glasses is that the composition and texture of the glass controls the rate of dissolution and resorption during bone repair and tissue engineering (Hench & Fielder, 2004).

However, on account of the progress of the foaming process, fabrication and nanofibre spinning technologies, porous BAG scaffolds can be produced through both synthesis routes. The ability to share loads together with bone will be an important key point for an ideal scaffold. Bioceramics are outmoded in application for bone regeneration because they are brittle. Nowadays, new nanoscale BAG glass polymer hybrids are invented. They are tough and require the control of the chemistry of sol-gel route (Jones, 2013).
1.2 Objectives of Study

The objectives of this study are:

a) to synthesize two different types of porous bioactive glasses, i.e. 58S and 77S,

b) to determine the most suitable calcination temperature among the parameters for thermal stabilization, and

c) to study the morphology and bio-mineralization properties of the bioactive glasses before and after immersion in simulated body fluid (SBF).

1.3 Scope of Study

This study focuses on the preparation of two different composition of bioactive glasses in ternary system, i.e. 58S (60 mol.% SiO₂, 36 mol.% CaO, 4 mol.% P₂O₅) and 77S (80 mol.% SiO₂, 16 mol.% CaO, 4 mol.% P₂O₅) by sol-gel route. Each type of BAG was calcinated with three different thermal stabilization temperature, i.e. 400 °C, 600 °C, and 800 °C. These temperatures were denoted as the parameters of this study which were determined through the analysis of TGA. The basic precursors for this study were tetramethyl orthosilicate (TMOS), phosphorus pentoxide, and calcium nitrate. First, the precursors were hydrolysed in the presence of the catalyst, nitric acid to produce aliquots of sol that contains nanoparticles. Secondly, Decon® detergent was added into the aliquots of sol as foaming agent. It is a type of detergent that contains low concentration mixture of anionic and non-ionic surfactants. Vigorous agitation of the mixture produced porous scaffolds. As the gelling point approached, which means when the sol viscosity rise more steeply, the foamed sols are transferred or casted into moulds. Finally, the samples are allowed to undergo ageing at 60 °C, drying at 130 °C and calcinated at different temperatures. Lastly, the porous sol-gel glasses were soaked in SBF solution to study and compare the bioactivity of these glasses. In this study, the physical and structural characteristics of the BAGs were characterized using TGA, and SEM. TGA was used to determine the suitable parameters for this study while SEM was applied to study the morphology and the growth of hydroxycarbonate apatite (HCA) layer on the bioactive glasses before and after SBF immersion respectively.
2.1 Historical background

Before the discovery of bioactive materials, various kinds of products that are readily available at that time were used for implantation. However, due to the rapid advancement of the science and technology in medical fields, the knowledge about the human immune system has been fully understood. Scientists have found out and proven that most of the materials used are toxic and pathogenic to human body. This leads to the development of the first-generation of biomedical material during 1960s and 1970s. More than 50 prostheses were fabricated from 40 different newly-discovered materials for the clinical usage by 1980. Unfortunately, these materials are most likely to trigger the formation of fibrous encapsulation around them due to their biological "inertness" property. Hence, second-generation of biomedical materials were invented to improve the first-generation of biomedical material and overcome the previous problems (Hench & Polak, 2002).

The research and development (R&D) of the second-generation biomaterials are mainly focussed on generating bioactive components which can evoke a controlled mechanism in the biological system. The living tissues growth on the BAGs through the interaction with hydroxycarbonate apatite (HCA) layer. The whole bonding mechanism comprises 11 steps which are 5 surface reaction and 6 cellular reaction steps. In the mid-1980s, bioactive materials had been widely used in orthopaedic and dental applications (Hench & Polak, 2002). In addition, resorbable biomaterials were under intensive research and development as they can control chemical breakdown and resorption. By 1984, resorbable biomaterials were applied
in clinical use for sutures. All these great discoveries have boosted the aging population (Hench & Polak, 2002). However, the survival analyses shown that one third to half of the skeletal prostheses (Wroblewski et al., 2009) and artificial heart valves (Schoen et al., 1992) implantation failed within 10 to 25 years. Afterwards, revision surgery is needed (Hench & Polak, 2002). The biomaterials at this stage are still exhibits some limitations. They are unable to respond to the biochemical stimuli and changes of physiological loads. Thus, the lifetime of artificial biomaterials is limited (Hench & Polak, 2002).

Those limitations present in the second-generation of biomaterials have induced a change in direction of development in third-generation biomaterials towards a more biologically based processing route. The newly invented third-generation biomaterials are able to activate genes and cells to stimulate the cellular responses of the specific tissues at molecular level. Moreover, these bioactive materials will liberate chemicals known as ionic dissolution products and growth factors which induce the in-situ self-assemble of genes and cells into desire tissues. Nowadays, these biomaterials have been commonly applied in cell transplants of many body parts and also often use in treatment of neurological disorders (Hench & Polak, 2002). In the past few decades, a lot of works have been done to study BAGs in SBF. The synthesis routes and compositions of BAGs are improved by trial-and-error method and also through the comparison of the in vivo and in vitro experiments and studies (Cerruti, 2014).

The first BAG was invented by Professor Larry L. Hench from University of Florida, United States of America in 1969. He gained the idea of invention after sharing a bus ride conversion with Colonel Klinker, a US Army colonel who had just returned from Vietnam War on the way to an US Army Materials Research Conference in the summer of 1967. The colonel had witnessed numerous amputations failed during the war as the body always rejected the implant materials e.g. bioinert plastics and metals which are available at that time. Those materials do not bone with body tissues but triggered fibrous encapsulation around the implant materials. Thus, he asked Professor Hench for a favor to find a new material that can overcome all these medical problems (Hench, 2006; Jones, 2013).
Finally, Bioglass® is the achievement of this quest (Hench, 2006). The 45S5 Bioglass® was the first manmade material that is able to bond with bone. This leads to the new era of bioactive material and launch the development of bioactive ceramics (Jones, 2013).

2.2 Synthetic routes of bioactive glass

The two common processing pathways to fabricate porous bioactive glass scaffolds are melt-quenching route and sol-gel route. Both methods produce high bioactivity BAGs that are very useful in tissue regeneration. The melt-quenching route is traditional fabrication method that used in production of the first invented bioglass®, i.e. BAG with 45S5 composition. On the other hand, the later modified bioactive glasses with binary system, e.g. 70S30C and ternary system, e.g. 58S and 77S are normally synthesized via sol-gel route (Jones, 2013). The bio-mineralization properties of both melt-derived and sol-gel derived BAGs has been studied and found that the morphologies and compositions of the bio-mineralized products on the surfaces of the three BAGs were different (Chen et al., 2008).

2.2.1 Melt-quenching route

This is the traditional fabrication method that involves the heating and melting of precursor mixture in a platinum crucible at temperature higher than 1300°C. The molten product formed is then quenched in a graphite mould or in water to form the glass (Bellucci et al., 2014; Jones, 2013). It has been reported that the melt-derived BAGs with more than 60 mol% SiO₂ in Na₂O-CaO-P₂O₅-SiO₂ system are biologically inactive (Hench & West, 1996).

This method has some significant disadvantages. Firstly, the content of SiO₂ is low and percentage of alkali or alkaline earth metal is high in the BAGs produce through this traditional method. This gives rise to highly reactive BAGs compositions. These compositions tend to dissolve of the crucible produced by refractory material and cause a series of impurities appear in the melt derived BAGs. Hence, highly contaminated final product is produced and it is very difficult to synthesize high purity of melt-casted BAGs (Li et al., 1991). Besides that, this method results in high
production cost as high temperatures are required during the process (Ungureanu et al., 2011).

2.2.2 Sol-gel route

Sol-gel route is an inorganic polymerization process which had been discovered by Ebelmann in 1846 (Corriu & Anh, 2009). It is a "wet" chemistry-based synthesis route that involves the formation of gel in the solution under mild conditions. Room temperature is enough for the precursors to undergo polymerization (Corriu & Anh, 2009; Hench & West, 1990).

Besides that, this synthesis route possesses a degree to govern the composition and structure of BAGs at molecular level which cannot be achieved via the conventional melt-quenching method (McCormick, 1994). The production of ultrafine powders with controlled compositions becomes possible through this method. The BAGs synthesized via this route have low devitrify tendency, higher purity and homogeneity as compared to the traditional melt-quenching method (Bellucci et al., 2014).

Due to all these advantages, the sol-gel technology has drawn lots of attention in various fields recent years. During the last two decades, this inorganic polymerization route has been revived and became the general oxides preparation method. Various thermolabile entities in chemistry are allowed to be incorporated via this process (Corriu & Anh, 2009). Figure 2.1 depicts the synthesis of BAG via acid-catalysed sol-gel route in a flow chart.