EFFECT OF SINGLE AND CO-DOPING OF STRONTIUM AND SILVER ON PROPERTIES OF SOL-GEL DERIVED QUATERNARY BIOACTIVE GLASS

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LIST OF SYMBOLS

Å	Angstrom
cm	centimeter
°C	Degree Celsius
θ	Bragg Angle
λ	Wavelength
d	Distance between Planes
kV	Kilovolt
mA	Milliampere
KBr	Potassium Bromide
V_s	Volume of HBSS
g	Gram
h	Hour
t	Thickness
M	Molecular Weight
mol	Mole
rpm	Rotation Per Minute
<	Less than
m	Meter
mL	millilitre
n	Number of Samples
p	Significance Level
r	radius
h	height
min	Minute

>	Greater than
M	Molarity
mm	Millimeter
μm	Micrometer
cm^{-1}	Per centimeter
ppm	Part per million

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
α-MEM	Alpha modified minimum essential medium
BG	Bioactive Glass
FESEM	Field Emission Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
HA	Hydroxylapatite
HBSS	Hank's balanced salt solution
HCA	Hydroxylcarbonate apatite
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ISO	International Organization for Standardization
MC3T3-E1	Mouse calvaria osteoblastic cell line
MPa	Mega Pascal = 1 N/mm^2
OD	Optical density
PBS	Phosphate-buffered saline
PDF	Powder diffraction file
PET	Polyethylene terephthalate
PMMA	Poly-methyl methacrylate
SBF	Simulated body fluid
SD	Standard Deviation
TG-DTA	Thermogravimetric-differential thermal analysis
XRD	X-ray diffraction
S50P4	$50SiO_2$-21.5CaO-24.5Na_2O-4P_2O_5 (wt. %)
S53P4	$53SiO_2$-20CaO-23Na_2O-4P_2O_5 (wt. %)
S55P4	$55SiO_2$-19CaO-22Na_2O-4P_2O_5 (wt. %)

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KESAN PENGEDOPAN TUNGGAL DAN GABUNGAN STRONTIUM DAN PERAK TERHADAP SIFAT-SIFAT KACA BIOAKTIF KUARTENARI BERASASKAN SOL-GEL

ABSTRAK

Kaca bioaktif (BG) telah dianggap sebagai salah satu bahan yang paling sesuai bagi pertumbuhan semula tulang disebabkan sifatnya yang dapat terikat dengan kedua-dua tisu lembut dan keras serta keupayaannya untuk membebaskan ion berkadar dengan masa yang secara *in-vivo* dan *in-vitro* mempunyai kesan positif pada percambahan dan pembezaan sel tulang. Namun, pertumbuhan semula tulang pada pesakit yang menderita penyakit osteoporosis dan berlakunya jangkitan semula bakteria menghadkan penggunaannya dalam aplikasi bioperubatan. Bagaimanapun, had ini dapat diatasi dengan mengdopkan ion t ke dalam sistem BG. Oleh itu, tujuan kajian ini adalah untuk mengdopkan strontium (Sr^{2+}) dan perak (Ag^{+}) ke dalam sistem kuaterner (quaternary) silikat BG untuk meningkatkan bioaktiviti secara *in-vitro* (terutamanya pembentukan tulang untuk penyakit osteoporotik) dan sifat antibakteria (jangkitan semula bakteria). Hal Ini dilakukan dengan kaedah sol-gel yang merangkumi proses pencampuran, pengejelan, penuaan, pengeringan dan pengkalsinan. Komposisi S53P4 BG digunakan sebagai komposisi kawalan dan dua komposisi baru (S50P4 dan S55P4) telah dibangunkan dari sistem kuataneri (quaternary) SiO_2-CaO-Na_2O-P_2O_5. Strontium (Sr^{2+}) merupakan unsur tuju-tulang (bone seeker element) yang mempunyai kesan positif terhadap pembentukan tulang (osteogenesis). Oleh itu, kepekatan Sr^{2+} sebanyak 1, 3 dan 5 % mol dipilih dan dalam kajian ini komposisi tersebut disebut sebagai BG-1S, BG-3S dan BG-5S. Sebaliknya, kepekatan Ag^{+} yang rendah mempunyai kesan terhadap jangkitan bakteria dan

kepekatan sebanyak 1, 2 dan 3 % mol dipilih untuk pengdopan perak dan dinamakan sebagai BG-1A, BG-2A dan BG-3A. Pembelauan sinar-X (XRD) dan spektroskopi inframerah jelmaan Fourier (FTIR) menunjukkan bahawa pengdopan Sr^{2+} dan Ag^{+} tidak mengubah struktur silikat dan juga fasa combeite. Ujian bioaktiviti *in-vitro* dilakukan dengan merendam spesimen ke dalam larutan garam-seimbang Hank (HBSS) selama 3, 7 dan 14 hari dan mengesahkan pembentukan hidroksiapatit-berkarbonat (HCA) di permukaan spesimen dengan munculnya puncak HAp di dalam analisis XRD dan kumpulan terkarbonat (ikatan CO) serta fosfat (ikatan PO) dalam spektrum FTIR. Walaubagaimanapun, pembentukan apatit yang kurang ketara diperhatikan pada BG terdop Ag berbanding BG terdop Sr, sementara Sr-BG menunjukkan peningkatan pembentukan apatit setelah 14 hari terendam di dalam HBSS. Ujian bioserasi *in-vitro* dengan sel seperti osteoblas (MC3T3-E1_ membuktikan bahawa 3 % mol Sr^{2+} dan 1 %mol Ag^{+} menunjukkan aktiviti percambahan sel (daya maju sel) dan alkali fosfatas (ALP) yang paling tinggi. Tambahan pula, pewarnaan mineralisasi (mineralization staining) bagi pembentukan seakan-akan tulang dapatditingkatkan setelah Sr^{2+} dan Ag^{+} didopkan ke dalam formulasi kaca-bio berbanding spesimen yang tidak didop (BG-0). Menariknya, spesimen kaca-bio pelbagai dop (Sr dan Ag) (BG-3S1A) menunjukkan kebolehserasian-cyto yang menunjukkan kesan gabungan ion Sr^{2+} dan Ag^{+} yang positif terhadap percambahan sel osteoblas MC3T3-E1 serta kawasan pewarnaan untuk BG -3S1A yang jauh lebih tinggi daripada spesimen lain. Tambahan lagi, tindak balas bakteria bagi komposisi pelbagai dop (BG-3S1A) menunjukkan zon perencatan Ag-BG yang setanding melawan bakteria E.coli dan S.aureus. Ringkasnya, kombinasi pelbagai dop ion Sr^{2+} dan Ag^{+} ke dalam kaca bioaktif telah berjaya dibangunkan

melalui kaedah sol-gel dan menunjukkan peningkatan sifat *in-vitro* keserasian-bio dan sifat antibakteria.

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ABSTRACT

Bioactive glasses (BG) have been considered as one of the most promising materials for bone regeneration due to its property to bond with both soft and hard tissues and the capability to release ions overtime that have a positive effect on the bone cell proliferation and differentiation *in-vitro* and *in-vivo*. However, the bone regeneration in patient who suffer osteoporosis disease and the re-occurrence of bacterial infection limit its biomedical applications. BG can bond to hard tissue and degrades over time but the bone regeneration capacity of osteoporotic bones is generally slower than that of normal bones. Therefore it limits the application of BG. Also, bacterial infection is serious sometimes it needs to second surgery with a lot of suffering and there is still re-occurrence of bacterial infection after surgery. To overcome this limitation, the implant BG should possess long-term antibacterial property. However, these limitations could be overcome by doping of therapeutic ions into BG system. Hence, the purpose of this study is to dope strontium (Sr^{2+}) and silver (Ag^{+}) into quaternary silicate BG to enhance the *in-vitro* bioactivity (particularly bone formation for osteoporotic disease) and antibacterial properties (bacterial infection re-occurrence). This is performed by sol-gel method which include mixing, gelation, aging, drying and calcination. BG with S53P4 composition was used as a control and two new compositions (S50P4 and S55P4) was developed from SiO_2-CaO-Na_2O-P_2O_5 quaternary system. Strontium (Sr^{2+}) is bone seeker trace elements which possess positive effects on bone formation (osteogenesis). Thus, the Sr^{2+} concentration of 1, 3

and 5 mol% was chosen and the compositions were categorized as BG-1S, BG-3S and BG-5S in this study. On the other hand, low concentration of Ag^{+} has an effect on bacterial infection and the concentration of 1, 2 and 3 mol% was chosen for silver doping and denoted as BG-1A, BG-2A and BG-3A accordingly. X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) revealed that the doping of Sr^{2+} and Ag^{+} did not change the silicate structure as well as combeite phase. The *in-vitro* bioactivity test soaking in Hank's balanced salt solution (HBSS) for 3, 7 and 14 days confirmed on the hydroxyl-carbonated (HCA) formation on the specimens' surface with emerging of HA peaks in XRD and the carbonated (C-O bond) and phosphate groups (P-O bond) in FTIR spectra. However, less intense apatite formation was observed in Ag doped BGs compared to Sr-doped BG while Sr-BG showed the enhanced in apatite formation after 14 days soaking in HBSS. The *in-vitro* biocompatibility test with MC3T3-E1 osteoblast like cells proved that 3 mol% of Sr^{2+} and 1 mol% of Ag^{+} observed the highest in both cells proliferation (cells viability) and Alkaline phosphatase (ALP) activity. Moreover, the mineralization staining for bone-like formation was improved after Sr^{2+} and Ag^{+} doped into glass formulation compared with undoped specimen (BG-0). Interestingly, the multi-doped (Sr and Ag) bioglass specimen (BG-3S1A) showed cytocompatibility which demonstrated the positive effect of the combination of $Sr^{2+}$and Ag+ ions on MC3T3-E1 osteoblast cell proliferation as well as the staining areas for BG-3S1A are significantly higher than those of other specimens. Moreover, the bacterial response of multi-doped composition (BG-3S1A) had the comparable inhibition zone of Ag-BG against *E.coli* and *S.aureus* bacteria. Summarizing, the multi-doped combination of Sr^{2+} and Ag^{+} ion into bioactive glass was successfully developed by sol-gel method and exhibiting improved *in-vitro* biocompatibility and antibacterial properties.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bone tissue is a dynamic and vascularized tissue by which it continuously remodels throughout the lifetime. However, bones are prone to defects, resulting from either trauma, infection or tumour (Porter *et al.*, 2009). Therefore, there is an increasing demand to replace bone defects due to disease or injury remains as a serious health problem challenge for researchers in the biomedical field (Chen *et al.*, 2020). Millions of bone grafting procedures are performed in order to repair bone tissue damages. Among them, autograft has been known as the clinical gold standard for bone treatment due to its ability to meet the requirement for bone regeneration such as growth factors, cells and a biocompatible matrix (Laurencin *et al.*, 2006).

However, autograft bone grafting possesses some challenges including donor site morbidity after the operation as well as the mechanical loss of the donor site regardless of gender and age (Hung, 2012) as well as high cost for harvesting. According to the above-mentioned drawbacks of bone grafting, the development of synthetic bone substitutes were demanded for bone regeneration and repair. To obtain a satisfactory outcome and have an adequate response at the implantation site, appropriate implanted biomaterials should have certain required characteristics such as biocompatible with living tissue (Hu *et al.*, 2013).

Biomaterial is defined as any substance or combination of substances that can be used for measuring, restoring and improving physiological function and quality of life for any period, as a whole or as part of a human body system. Biomaterials should be compatible with living tissue to show their function potentially. Otherwise, they can

lead to unfavourable immune reactions, unwanted interactions between blood and body fluid (Jones, 2015).Bioactive glass (BG) is one of the biomaterials, which widely used to repair bone defect due to the ability to bond and integrate with bone in living body through rapid formation of apatite layer on the material surface upon exposure to biological environment (Mosbahi *et al.*, 2016). Bioactive glass is composed with four oxide system such as silicon dioxide (SiO_2), calcium oxide (CaO), sodium oxide (Na_2O) and phosphorus pentoxide (P_2O_5). In general, BGs show a rapid initial dissolution of the alkaline elements from the surface which is followed by a precipitation of a Ca- and P-rich layer on top of the alkali-depleted SiO_2 layer, forming hydroxycarbonate apatite (HCA) which is the mineral phase of bone (Moghanian *et al.*, 2020).

The used of BG has received a great attention for bone and dental treatment since its first invention by Professor Larry Hench in 1969. After the invention of Hench, many new compositions have been proposed for the specific clinical applications (Rahaman *et al.*, 2011). S53P4 BG was developed by Andersson with a nominal oxide composition of (wt.%) 53% SiO_2, 20% CaO, 23% Na_2O and 4% P_2O_5 (Andersson, 1990). S53P4 BG has been shown to possess better bacterial growth inhibitory effect by comparing bactericidal effects of different compositions BGs (Leppäranta *et al.*, 2008;Munukka *et al.*, 2008). As mentioned earlier, this S53P4 bioactive glass does not contain any kind of antibiotics (contain only four oxides), but the antimicrobial properties of this glass are based on the increased pH level and osmotic pressure caused by the exchange of alkali ions and the release of salt ions. Thus, it is used as a local antimicrobial biomaterial and as a bone defect filling biomaterial especially in the treatment of chronic osteomyelitis (Ferrando *et al.*, 2017).

Bioactive glass can be made using different method by varying of oxide components (Giannoudis *et al.*, 2005). Generally, there are two popular methods to fabricate BG: (i) conventional melt-derived method (Massera *et al.*, 2014) and (ii) sol-gel method (Sharifianjazi *et al.*, 2020). However, it had been reported that the BG consisting more than 60% of SiO_2 prepared by the melt-derived is not able to induce apatite layer even after several weeks immersion in simulated body fluid (SBF) solution and it failure to bond to either bone or soft tissue (Bejarano *et al.*, 2015). It is because the dissolution rate of silica depends very much on glass composition. The rate of dissolution decreases greatly for compositions containing of >60% SiO_2 because of the larger number of bridging oxygen bonds in the glass structure. Network dissolution is occurred by the breaking of –Si–O–Si–O–Si– bonds (bridging oxygen) through the action of hydroxyl (OH) ions from the solution. The larger the bridging oxygen, the slower the dissolution. It affects the crystallization of hydroxyapatite layer. On the other hand, Fiume *et al.* (2020) reported that sol-gel derived glasses show bioactivity within a much wider compositional (approximately ≤90% of SiO_2) range than melt-derived glasses. Additionally, Wang *et al.* (2014c) found that the surface area of sol-gel BGs increase by two times compared to melt-derived BG of a similar composition since high porosity of sol-gel derived BG leads to increase surface area. Since then, sol-gel derived bioactive glasses have been studied intensively. Moreover, the required processing temperatures of sol-gel method (600°C-700°C) are lower than the melting method (1250°C-1450°C) thus sol-gel method is energy efficient and environmentally friendly (Sharifianjazi *et al.*, 2020).

1.2 Problem Statements

In bone implantation surgeries, there are two main factors that should be considered: (i) bonding with a living bone; (ii) prevent bacterial infection. The consequences of implant infections are serious and sometimes it leads to second surgery with a lot of suffering (such as high cost and long-stay in hospital). Bioactive glass (BG) shows well bioactivity, bond with hard and soft tissues and also possess antibacterial properties without local antibiotics (Nandi *et al.*, 2016a;Skallevold *et al.*, 2019). Regarding of antibacterial property of BGs, after they are implanted into the defects, the leaching of Na^{2+} ions cause an increase of pH values of surrounding medium and osmotic pressure rise caused by the releasing of P and Ca salts makes the environment hostile for bacterial adhesion and proliferation, reducing the possibility of infection (Drago *et al.*, 2014;Lindfors *et al.*, 2016a).

However, the bacterial infection still occurs after surgery although BGs possess antibacterial property and it becomes its main drawback. Lindfors *et al.* (2016a) reported that the persistence of infection occurred up to 6 months after implanting S53P4 bioglass in the tibia and femur of patients with osteomyelitis. Besides, Ferrando *et al.* (2017) also found that after implanting S53P4 bioglass in patients who suffer from chronic osteomyelitis, the recurrence of infection persisted and the healing of wound was delayed. Moreover, regarding of bone regeneration, there is the limitation for the patients who suffer osteoporosis disease because the regeneration capacity of osteoporotic bones is generally slower than that of normal bones (Wang *et al.*, 2018;Gómez-Cerezo *et al.*, 2019).

Such limitations forced the researchers to find ways to enhance the biological efficiency of BGs to increase cell-material interactions to ensure rapid regulated bone

regeneration along with long-term antibacterial property. In fact, this limitations have been further improved by metal ions doping that has greatly strengthened new bone formation as well as antibacterial response. There are many metal ions such as magnesium (Mg), strontium (Sr), manganese (Mn), iron (Fe), zinc (Zn), silver (Ag) and other rare earths that have been effectively doped into bioactive glass to improve their mechanical and biological properties (Nandi *et al.*, 2016b). Therefore, the incorporation of metallic ions into the bioactive glass structure (to produce a composite) is one method to improve those required biological properties. Among the metallic ions, strontium (Sr^{2+}) is bone seeker trace elements that possess positive effects on bone formation. Although the Sr positively affects bone metabolism to promote bone formation and osteoblast replication while inhibiting bone resorption by osteoclasts. However, too much Sr may increase the number of osteoclast cells which can inhibit bone regeneration and remodeling, leading to osteonecrosis. Thus, strontium has very good effects up to an optimum level. Hesaraki et al. (2010) and Sharifianjazi et al. (2017) reported that bioactivity of sol-gel derived bioglass improved after 5 mol% of SrO was substituted in CaO. Furthermore, Moghanian *et al.* (2017a) proved that non-cytotoxicity and high activity of osteoblast cells were obtained after incorporating similar concentration of SrO dopants in bioglass composition system. The previous studies of different doping amount of SrO (>5, 10,…100 mol%) have proven that 5 mol% of SrO produced good osteoconductivity and improved cell response. However, the *in-vitro* bioactivity of Sr doped BG was decreased when SrO contents was higher than 5 mol% (Solgi *et al.*, 2015;Massera *et al.*, 2014). Thus, the lower concentration of SrO in bioglass system is essential to understand its effect on cells' activity, since no study so far has reported to date in

incorporating a low amount (< 5 mol%) of SrO. Therefore, in this study, the amount of SrO 1, 3, and 5 mol% were chosen to be incorporated in bioglass system.

Moreover, doping of metal-ions into BG became one method to improve antibacterial property of BGs. It is because they could be more reliable in the long-term, with the growing prevalence of antibiotic-resistant bacteria (Bassetti *et al.*, 2013;Lindfors *et al.*, 2016b). Among the many metal-ions, silver (Ag^{+}) ions have been widely used as antimicrobial agent because of its highly promising antibacterial and anti-inflammatory properties on gram-positive and gram-negative bacteria (Nandi *et al.*, 2016b). The key benefit of integrating silver ions into the sol-gel derived bioglass system is that the porous glass matrix enables to control in order to be sustained delivery of the antibacterial agent. Shahrbabak *et al.* (2019) reported that after incorporating 2-4 mol% of Ag_2O in bioglass formulation, apatite formation was stimulated with good antibacterial properties. Contradictorily, Luo *et al.* (2010) found that by adding more than 1 wt.% of Ag_2O in the bioglass formulation, a high cytotoxic effect was produced. Hence, the amount of Ag_2O (1, 2, and 3 mol%) were chosen to be incorporated in bioglass system to determine the optimum amount of Ag_2O as dopant in this study.

Therefore, based on the advantages shows by Sr and Ag after incorporation in the bioglass system, in this research, two new compositions of bioactive glass were fabricated by fixing the Na_2O: CaO ratio (1.00:0.87) and contain similar amount of P_2O_5 (4 wt%) with the S53P4 bioactive glass based using sol-gel method and these two new compositions are denoted as S50P4 and S55P4. Presently, there was no report in the literature on the preparation and characterization of multi-doped Sr and Ag S53P4 bioactive glass by sol-gel method. Hence, strontium (Sr^{2+}) will introduce as

bone formation enhancement (osteogenesis) whereas silver (Ag^{+}) for antibacterial property (microbial). Both metal ions were doped into the glass system to induce faster bone formation with good antibacterial performance that is required for clinical application. Thus, if these two biological properties could be achieved simultaneously, the quality of life could be improved for patients who suffer from bacteria-infected bone defect diseases.

1.3 Research Objectives

This study focused on the preparation of single and multi-doped Sr and Ag sol-gel derived quaternary bioactive glass and to evaluate their effectiveness on the biological properties of the bioglass. More specifically, the objectives of the thesis are as follows.

i. To prepare an optimized composition of bioglass using the sol-gel method which exhibits excellent bioactivity.

ii. To evaluate the effectiveness of Sr and Ag single doping on the antibacterial properties and cell response of BG

iii. To investigate the effect of (Sr+Ag) multi-doping on the properties and biocompatibility of the synthesized BG composites.

1.4 Scope of Research

This research work is divided into three parts, the first part is synthesis and characterization of quaternary bioglass using sol-gel method, the second part is the *in-vitro* biocompatibility of single doped Sr and Ag doped quaternary bioglass and the final part consists the antibacterial property of the multi-doped (Sr+Ag) bioglass. The

properties of each BG composition was characterized using thermal analysis, X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope (SEM). The *in-vitro* apatite formation on each BG composition were studied by immersion in Hank's balanced salt solution (HBSS) (pH=7.78) for different period of immersion time (3, 7, and 14 days). The releasing of ions of each immersion time for each composition was characterized by inductively coupled plasma optical emission (ICP-OES) and the apatite formation on BG was examined by SEM. Then, *in-vitro* cell-study test such as proliferation, alkaline phosphatase activity (ALP), was performed by using MC3T3-E1 osteoblast-like cells.

Among three BGs, only one composition with good biological response against MC3T3-E1 cells was optimized for further study on doped BGs. Similar material characterizations and cell studies were conducted for single and multiple doped Sr and Ag, respectively. Among different doping amounts of Sr and Ag, only one doping amount was optimized based on the bioactivity and cell-response results. Then, the final part is the simultaneously doped Sr and Ag BG to produce high bioactivity with good antibacterial results. In summary, the scope of research is presented in Figure 1.1.

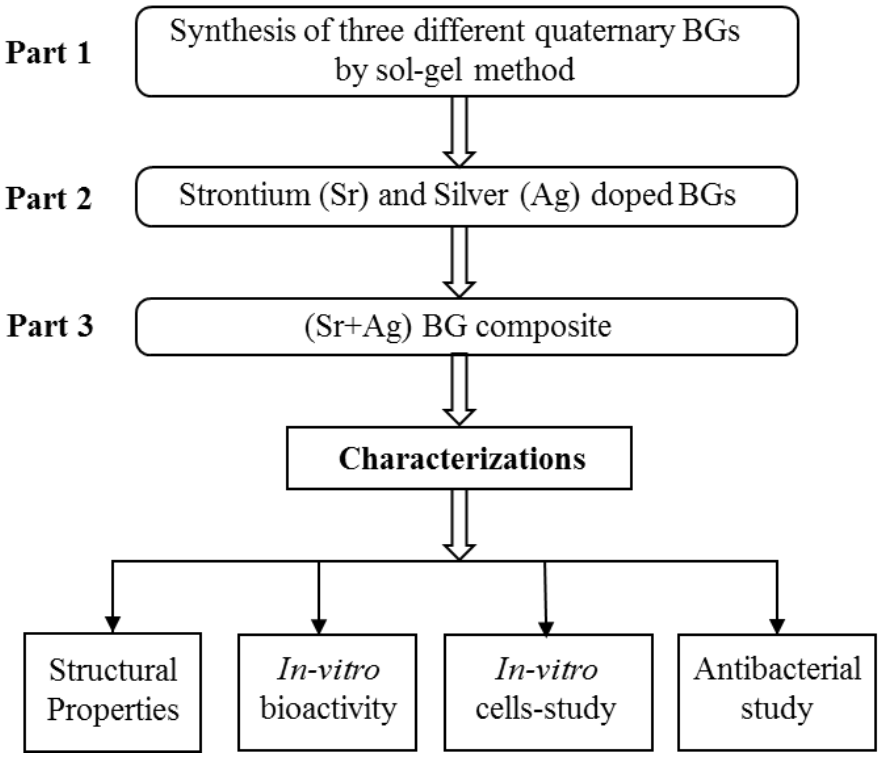


Figure 1.1 Overall flow chart of research

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Bone grafting can be identified as bone tissue being transplanted from another human body or animal. The bone graft should have strong compatibility with the local and systemic as well as the potential to replace the bone and fill the bony defects fully (Palacios *et al.*, 2018). Three forms of grafting are carried out by bone grafting, including autograft, allograft, and xenograft.

Autograft implants are known to be the gold standard for bone implants for a variety of reasons, the primary one being that this type of implant is obtained from a patient needing bone graft. This form of graft prevents the issue of the body refusing the implant but, as with all transplantations, there is still a chance of infection (Gual-Vaqués *et al.*, 2018). Autograft implants are typically obtained from the patient's iliac crest. While this method of implant is the implant of choice, it has drawbacks, including that there is only a very small amount of bone that can be collected to be used as a graft and that the harvesting process is not only difficult, but has its own collection of complications (Bates *et al.*, 2007).

Allograft implants are obtained from a dead human donor. This type of implant has the advantage over autograft implants that it does not require a secondary surgical site since the implant originates from another source (Wang *et al.*, 2014a). However, there is a problem that the host body may reject the implant because this implant originates from another human. Furthermore, the incompatible blood group in bone transplantation is also another issue and can develop antibody reactions within the human body system (Markel, 2019). This type of implant also has a risk of infection

due to the implantation of a foreign material in the patient; this also results in an inflammatory reaction from the tissue around the implant (Wang *et al.*, 2014a).

The other bone grafting replacement is xenograft where live cells, tissues or organs are transplanted from other mammals to humans. These tissues, organs or cells are called xenografts or xeno-transplants. The bone is typically taken from cows or pigs as a source of xenograft. Comparable to allograft, there is a high risk for disease spread, which contributes to frequent alternation of the animal genetic code (Vagaská *et al.*, 2010). Since, pig lifespan is shorter than humans meaning that pig tissue age at a different rate (Fillingham *et al.*, 2016).

Another sensitive problem with xenograft after their first trial was that of animal support organisations that were actively opposed to destroying animals to extract their organs for transplantation (Markel, 2019). According to the aforementioned facts, there are several disadvantages to available bone grafting that restrict their biomedical applications. There is therefore a great demand for other methods for bone regeneration and repair to be established.

2.2 Biomaterials

Due to the above-mentioned serious shortcomings related to bone graft substitutes, it has been required to produce synthetic materials. There have been different definitions related to the term "Biomaterial" (Ratner *et al.*, 2006). The biomaterials is defined as "A substance that has been engineered to take a form which alone or as a part of a complex system, is used to direct course of any therapeutic or diagnostic procedure in human or veterinary medicine through controlling the interactions with the components of living systems". By contrast, biological materials are the one that is produced by the biological systems such as tooth enamel and the

bone matrix. It should be noted that biomaterials are different from artificial material. The artificial material is in contact with skins such as hearing aid and wearable artificial limbs. They are not considered as biomaterials because the skin acts as a barrier with the external world, while biomaterials are used in the body system and are in contact with the internal tissues (Woodard *et al.*, 2007). The fundamental functions of biomaterials are to contribute to healing defects, correct abnormalities and thus improve the function (Vagaská *et al.*, 2010). The examples of biomaterials and their function in the human organ are summarized in Table 2.1.

Table 2.1 Example and function of biomaterials used in the body system, in contact with the internal tissues(Woodard *et al.*, 2007).

Function	**Example**	**Organ**
Replacement of damaged part	The artificial hip joint, dialysis machine	Bone, kidney
Assist in healing	Bone plates and screws	Bone
Improve function	Contact lens, cardiac pacemaker	Eye, heart
Aid to diagnosis	Probes, catheter	Bladder

After biomaterial implantation, it acts as a medium for the interaction of bone-implant with the surrounding tissue. Therefore, bone biomaterial should be carefully selected in order to prepare an ideal bone implant (Kocabey *et al.*, 2013). In general, bone biomaterials are selected based on their inherent biocompatibility, mechanical properties, and cellular behaviour. Their physicochemical properties, molecular weight, hydrophilicity/hydrophobicity are of great importance (Floren *et al.*, 2016). There are two other criteria of great importance for bone biomaterials: biodegradability and bioactivity. Some bioceramics such as alumina and zirconia, polymers such as polyurethane and silicone rubber, and some biomedical metals such as stainless steel and titanium (Ti) alloys are bioinert materials and non-biodegradable. Although they possess good biocompatibility and/or excellent mechanical strength, they cannot

biodegrade and thus, they remain permanent implants *in-vivo*. In addition, these biomaterials do not have bioactivity and can only bond to the bone tissue through mechanical interlocking which leads to wear and loosening after long-term implantation (Mohanapriya *et al.*, 2016).

Many factors contribute to the successful biomaterial implant including material properties, material biocompatibility, and material design. If the human immune system rejects the implant and has an undesirable effect on the body, the biomaterial fails, and this may lead to toxicity such as inflammation and cancer. The material should meet the following requirements to be considered as a biomaterial: (1) non-toxic, (2) non-carcinogenic, (3) non-allergic, (4) non-inflammatory, (5) biocompatible, (6) biodegradability and (7) biofunctional for a lifetime (Desai *et al.*, 2008). The common types of bone biomaterials can be classified as bioceramics, biopolymers, and biomedical metals (Webber *et al.*, 2016). Simply, a biomaterial is a non-living material such as polymers, ceramics, metals, glasses, carbons, and composites which is used in a medical device to interact with biological systems(Ducheyne, 2015).

Biomaterial may be pursued exclusively in the living body or used for cell delivery or growth factors as a scaffold. Implant product does not induce adverse reactions and harmful effects on implantation in the living body and should be degraded at a controlled rate without causing toxicity (Segers *et al.*, 2011). The use of metallic material and elements in the human body, such as copper (Cu), will cause harmful results if the use of the material reaches a minimal dosage (Letelier *et al.*, 2010;Jin *et al.*, 2016). The substance can also have minimal and restricted external reactions and inflammatory reactions (Cui *et al.*, 2016). The use of polymeric content

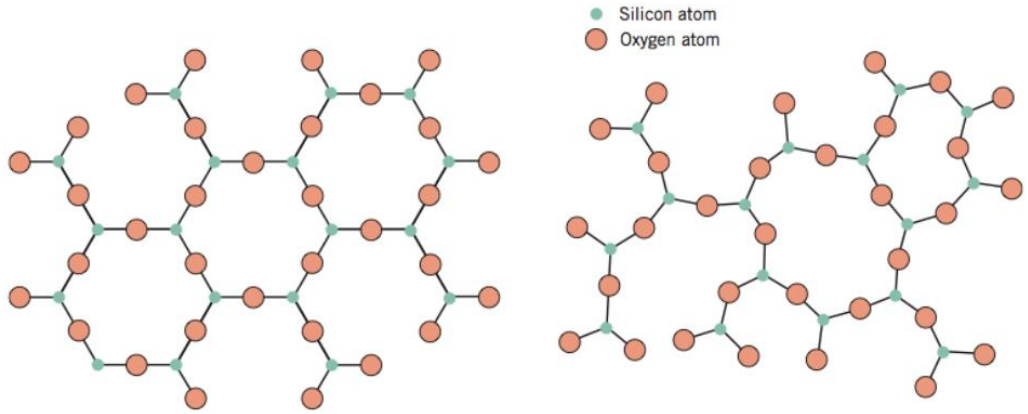
such as polyethylene glycol (PEG) has been shown to produce foreign body reactions with giant cells (Dobner *et al.*, 2009;Segers *et al.*, 2011).

Adequate shelf life or lifespan of the implant material is also a significant aspect that needs to be identified. This is to ensure that the time of degradation of the implant material correlates to the period of regeneration of the new tissue. In addition, the degradation composition should be free of toxic compounds and released from the body by metabolism (Nair *et al.*, 2007). Inert materials such as biomass, steel, silicones and polymers such as poly (methyl-methacrylate) show strong biocompatibility responses but are restricted due to non-degradation properties (Wang, 2016). In comparison, the use of glass as bone fillers and replacements, for example, indicates a strong biological reaction without interrupting bone remodelling. Based on clinical observation, BG has steadily experienced dissolution, surface reactions and osteoplastic activity within one to four years, depending on the cavity and volume of the defect (Lindfors *et al.*, 2010;Ylänen, 2017).

2.3 Glass as a biomaterial

Glass wording originated from Latin which refers to a transparent or translucent body. Since the index of glass reflection is comparable to the air, the glass reflects very little when light waves pass through. Non-reflected photon will interact with the atoms in glass molecules giving a transparent appearance (Fakhruddin *et al.*, 2018). Glass substances are also called vitreous. Glass can be produced either from organic (carbon) or inorganic (non-carbon) base (Angelini *et al.*, 2019). According to the hypothesis suggested by Rosenhain and Zachariasen, glass is a structure that is built in a random array of atoms connected by directional bonding. However, details on the random network term were first introduced by Wright *et al.* (2004).

Random network in glass structure can be defined as a non-crystalline solid that lacks the systematic and regular atom arrangement over relative atomic distance. Such behaviour, lead to glass being also called as amorphous, supercooled liquid (atomic structure resembles liquid). An overview of amorphous structure can be explained by understanding the illustration on comparison between crystalline and non-crystalline of ceramic compound, silicon dioxide. Figure 2.1 presents schematic diagram on two dimensional structure of both (crystalline and non-crystalline) state of SiO_2. Atom in disordered and irregular arrangement is observed for non-crystalline SiO_2 structure. The transformation into crystalline or amorphous structure depends on the conversion of random atomic structure in the liquid into ordered state during solidification (Callister Jr *et al.*, 2020).



(a) (b)

Figure 2.1 Illustration on two dimensional structure of SiO_2; (a) crystalline SiO_2 and (b) non-crystalline SiO_2 (Callister Jr *et al.*, 2020)

Glass is a common group of ceramic that has been typically used in wide range of applications such as insulating materials, structural flat glass, packaging, electrical devices or even as bioactive materials (Yadav *et al.*, 2015). Glass is also known as non-crystalline silicate normally contain of oxides such as lime, potassium oxide, sodium oxide and alumina which will influence the end product properties of glass

such as the mechanical properties (Erdem *et al.*, 2017), physical properties such as viscosity, electrical conductivity and thermal expansion (Yadav *et al.*, 2015) as well as biological properties (Bellucci *et al.*, 2017).

The most well-known type of glass is soda-lime glass. Typically, soda-lime glass contains 75% of silicon dioxide, sodium oxide, lime and other minor additives. Sodium oxide is added in the glass structure in order to reduce the melting points of glass while oxide such as CaO is added as glass stabilizer in the structure (Harper, 2001). Traditionally, glass is formed by cooling from melt to solid state. The melt will be poured into stainless steel compartment and then annealed for several hours to obtain bulk glass samples (Wang *et al.*, 2014b).

However, other techniques have been adopted over the years in glass synthesize for wide range of applications such as through physical vapour deposition for high mechanical strength glass application (Bokas *et al.*, 2017), neutron irradiation of material for high level waste immobilization application in nuclear reactor (Tang *et al.*, 2014) and sol-gel technique for optical semiconductor application (El Hamzaoui *et al.*, 2017). However, prominent techniques in glass synthesize for biomaterial application is either through melt-derived route or sol-gel method (Mezahi *et al.*, 2013).

2.3.1 Bioactive glass

Bioactive glasses (BGs) are synthetic bone graft replacements that have been widely researched over the last decades. Bioactive glasses are bioceramic solid, non-porous and strong materials composed of the main ingredient of silicon dioxide (or silicate) and three other essential components: sodium dioxide, calcium oxide and phosphorus oxide. Different types of bioactive glasses may be produced by varying all

of these elements. BGs can be developed as microspheres, fibres and porous implants as well as in granular form (Jones, 2013).

Bioactive glass was first invented in 1969 by Professor Larry Hench. It is a melt-derived bioactive glass designed (using phase diagrams, Figure 2.2) to have a significant amount of CaO with some P_2O_5 in the Na_2O-SiO_2 matrix (Hench, 2006). In 1972, Hench et al. recorded that 45S5 (45 mol% SiO_2, 24.5 mol% Na_2O, 24.5 mol% CaO and 6 mol% P_2O_5) Bioglass® formed a tight interfacial attachment to the bone, equivalent to or greater than the host bone strength (LeGeros, 1988).

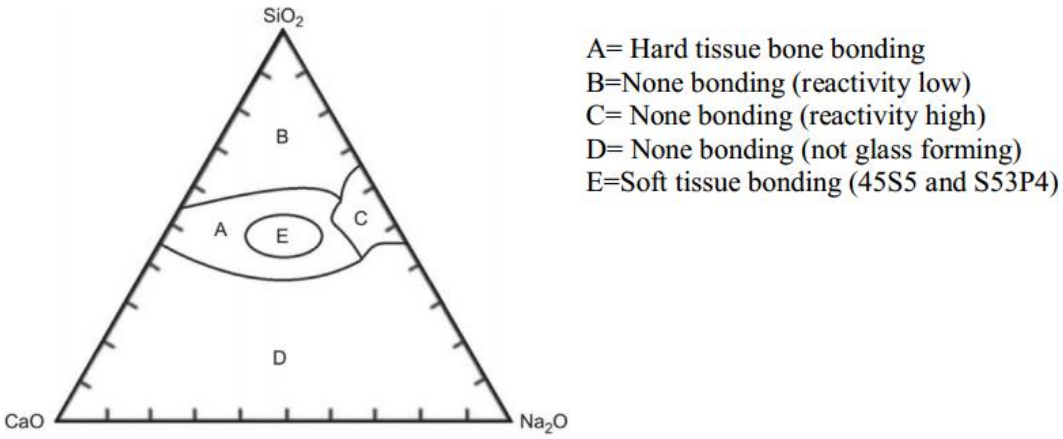


Figure 2.2 Compositional diagram for bone-bonding bioactive glasses. (Hench, 2006)

The first successful therapeutic use of bioactive glass was as a substitute for middle ear bones to restore conductive hearing loss (Merwin, 1986). The selected composition (45S5) has been shown to produce a hydroxyapatite (HA) layer in an *in-vitro* investigation. Subsequently, this substance was tested *in-vivo* and found to be closely bound to the bone, thereby confirming the theory. BGs have been found to be non-toxic to tissue and have formed a bond through their apatite surface layers to the host bone. The first in vivo study was completed for Bioglass monoliths on the rat femurs that has been documented after 6 weeks the interfacial shear strength of the bond between the glass and the cortical bone was equal or greater than the strength of

the host bone (Hench *et al.*, 1977;Hench, 1994). Right after Professor Hench's first results, other research centres began to produce related materials.

In the 1980s, numerous BGs with a wide variety of compositions were crafted by Åbo Akademi at the University of Turku, Finland. Andersson *et al.* (1992) studied these various compositions on their *in-vivo* and *in-vitro* activity, surface reactions and bone acceptance. Andersson has developed a statistical approach to the definition of glass bioactivity in terms of glass composition (Andersson *et al.*, 1990). Based on the findings of these trials, S53P4 was chosen as the most appropriate and most interesting glass for clinical use. S53P4 BG has a minimal oxide composition (wt% / mol%) of 23.0/22.7 Na_2O, 20.0/21.8 CaO, 4.0/1.7 P_2O_5, and 53.0/53.9 SiO_2. Clinically used granules of this glass are formed by the melting of oxides and the dissolving of silica into the melt. The popularity of bioactive glasses in the treatment of infections has grown over the last decade. In the treatment of bone infections with bioactive glasses, only the S53P4 silicate bioactive glass has demonstrated an antimicrobial activity of its own and has been extensively tested relative to other bioactive antibacterial glasses (van Vugt *et al.*, 2017).

2.3.2 Ions doped bioactive glass

The clinical demand of bioactive glass is increasing rapidly day by day due to its versatile properties such as bioactivity, resorbability, ostoeoinductive and osteoproductive. The bioactivity of a glass depends mainly on its surface reactivity and structure, and it may be possible to enhance the mechanism by modifying it. Modification is often required to address the drawbacks of conventional bioactive glasses, such as high solubility and low fracture toughness. Over the last two decades, researchers have shown that the sites in implantation of different parts of our body need different chemical and physical properties and have indicated that ion dissolving

products from inorganic materials are crucial to recognising and assuming the actions of *in-vitro* and *in-vivo* bioactive glasses (Hoppe *et al.*, 2011).

Since there are some trace elements present in the human body; such as strontium, copper, zinc, magnesium or cobalt; which are noted for their anabolic effects on bone metabolism. New bioactivity-enhancing approaches are being implemented to mimic the natural system, beneficial and appropriate ions are being introduced (Cacciotti, 2017). The incorporation of these therapeutic ions is assumed to improve the bioactivity of the implant. The release of these ions following exposure to the physiological environment helps to enhance the bioactive function of the implant relevant to both osteogenesis and angiogenesis. As a result, the latest trend is to introduce various ions into the formulations of bioactive glasses in order to improve their functional properties and therapeutic benefit (Baino *et al.*, 2018).

Doping means the incorporation of foreign atoms or ions of a trace element into the host lattice structure to yield materials with desirable functionality and properties (Yang *et al.*, 2012). The dopant is usually found at low concentration compared to the main constituents of the material in the range of few parts per million (ppm) to a few percent (Nedelec *et al.*, 2008). In order to enhance bioactivity, stimulating effects on osteogenesis, angiogenesis and antibacterial effects of bioactive glasses in a particular physiological environment, a number of methods have been tested to incorporate different metal ions into the silicate network (Hoppe *et al.*, 2013). Some of the ions including including silver, magnesium, strontium, zinc, aluminium, fluoride and zirconia on the characteristics of bioactive glass as summarized in Table 2.2.

Table 2.2 Effects of some ions on bioactive glass properties

Ion	Biological Activity	Reference
Ag	Comprising antibacterial activity to bioactive glass	(Bellantone *et al.*, 2002;Ranga *et al.*, 2019)
	Influencing on the toxicity of bioactive glass	(Luo *et al.*, 2010;Sharifianjazi *et al.*, 2017a)
	Increasing the bridging oxygen	(El-Kady *et al.*, 2012b)
	Declining the dissolution of the bioactive glass	(El-Kady *et al.*, 2012b)
Mg	Decreasing the glass transition	(Watts *et al.*, 2010)
	Increasing the expansion coefficient	(Watts *et al.*, 2010)
	Altering the bioactivity	(Ma *et al.*, 2012)
	Acting either as a network modifier or as a network former	(Watts *et al.*, 2010)
Sr	Enhancing the metabolic activity in osteoblasts	(Gentleman *et al.*, 2010)
	Inhabiting osteoclast activity	(Gentleman *et al.*, 2010)
	Altering the bioactivity and rate of HA formation	(Moghanian *et al.*, 2017a)
Zn	Decreasing the glass transition [87]	(Shahrabi *et al.*, 2011)
	Shows anti-inflammatory effect	(Yamaguchi, 1998)
	Bone formation in-vitro by activation of protein synthesis in osteoblasts	(Kwun *et al.*, 2010)
	Acting both as network modifier and as intermediate oxide	(El-Kady *et al.*, 2012a)
	Decreasing the bioactivity	(Shahrabi *et al.*, 2011)
Al	Decreasing the bioactivity	(Karakuzu-Ikizler *et al.*, 2020)
	Stabilizing the glass structure	(El-Kheshen *et al.*, 2008)
	Decreasing the expansion coefficient	(El-Kheshen *et al.*, 2008)
F	Decreasing the glass transition and glass crystallization	(Brauer *et al.*, 2010)
	No effect on the bioactivity	(Brauer *et al.*, 2010)
	Decreasing the chemical reactivity	(Lusvardi *et al.*, 2009)
	Reducing the tendency to crystallization	(Ben-Arfa *et al.*, 2016)
	No effect on the thermal expansion coefficient	(Ben-Arfa *et al.*, 2016)
Zr	Decreasing the bioactivity [127]	(Kasuga *et al.*, 1992)
	Altering the coating morphology [129]	(Rabiee *et al.*, 2013)

Gentleman et al. (2010) studies have stated that substitution of strontium (Sr^{2+}) ions for calcium Ca^{2+} in the bioactive glass system would increase the dissolution rate of ions, which has a major effect on bone cells *in-vitro* and *in-vivo* (Liu *et al.*, 2016b). Gentleman *et al.* (2010) have demonstrated that ions released from strontium-doped bioactive glass improve metabolic activity in osteoblasts. Moreover, osteoclast production is limited by both declining tartrate-resistant acid phosphatase activity and inhibiting calcium phosphate film resorption. Increased proliferation and alkaline phosphatase activity have also been found in osteoblasts grown in contact with strontium-substituted bioactive glass. Sharifianjazi *et al.* (2020) reported that the better apatite formation on Sr-doped bioactive glass than undoped composition after being immersed in SBF solution by increasing the soaking time, the amount of apatite crystals increased.

Since strontium is a biologically beneficial element and is abundant in human tissues. In addition, it is an element of group 2A of the periodic table and its biological characteristics are related to its chemical correspondence with Ca^{2+} (O'donnell *et al.*, 2010;Sharifianjazi *et al.*, 2017a). Due to this similarity to Ca, a high concentration of Sr can accumulate in bone and displace Ca in hard tissue metabolic processes (Salman *et al.*, 2012). In addition, strontium can be used as a medicine to cure and prevent osteoporosis by stimulating the formation of new bones and avoiding osteoclast-mediated resorption (Sharifianjazi *et al.*, 2020). Inclusion of strontium in the surface of the biomaterial provides the possibility of gradual release of this ion at the site of the defect, which is therapeutically useful (O'donnell *et al.*, 2010).

In addition, the invasion of bacteria on the surface of the implant may lead to a failure of treatment. The effects of implant infections are severe and often lead to