BIOACTIVITY IMPROVEMENT OF 45S5 BIOGLASS THROUGH CALCIUM COATING

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By

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DECLARATION

I hereby declare that I have conducted, completed the research, and written the dissertation entitled **"Bioactivity Improvement of 45S5 Bioglass Through Calcium Coating**". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar titles of this for any other examining body or University.

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Witnessed by

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Date:

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LIST OF ABBREVIATIONS

BG	Bioactive glass
BO	Bridging oxygen
FTIR	Fourier transform infrared spectroscopy
HA	Hydroxyapatite
HCA	Hydroxyl carbonate apatite
NBO	Non-bridging oxygen
SBF	Simulated body fluid
T_g	Glass transition temperature
T_r	Room temperature
XRD	X-ray diffraction
XRF	X-ray fluorescence

PENAMBAHBAIKAN BIOAKTIVITI BIOGLASS 45S5 MELALUI SALUTAN KALSIUM

ABSTRAK

Kaca bioaktif (BG) 4555 telah digunakan dalam aplikasi klinikal, terutamanya dalam penjanaan semula tulang. Walau bagaimanapun, BG mempunyai bioaktiviti terhad yang menyekat penggunaannya dalam tisu hidup. Rawatan kalsium digunakan untuk menggalakkan pembentukan lapisan HA untuk meningkatkan bioaktiviti BG. Bioaktiviti dikaji dengan mempelbagaikan jenis garam kalsium iaitu kalsium asetat, nitrat dan klorida. BG 45S5, yang mempunyai peratusan mol (mol%) sebanyak 45% SiO_2, 24.5% CaO, 24.5% Na_2O, dan 6% P_2O_5, telah disintesis melalui kaedah terbit-lebur. Mengumpul, mencampurkan, mencairkan pada 1400 °C, pelindapkejutan air, mengisar, menapis, menekan dan mensinter semuanya merupakan sebahagian daripada persediaan BG. BG telah dirawat dengan garam kalsium kemudian diletakkan di dalam inkubasi cecair badan simulasi untuk menentukan bioaktiviti untuk pembangunan lapisan hidroksil karbonat apatit (HCA), yang diikuti oleh pencirian. Kalsium asetat yang dirawat BG 45S5 mempunyai kumpulan berfungsi P-O dan C-O, yang menyumbang kepada pembangunan lapisan HCA. Nilai pH ditentukan dengan menganalisis SBF selepas setiap tempoh rendaman. Kalsium asetat yang dirawat menunjukkan pertumbuhan lapisan HCA pada permukaan kerana nilai pH hampir malar selama 7 hari rendaman berbanding garam kalsium lain. Pengukuran kehilangan berat juga menunjukkan bahawa BG yang dirawat dengan kalsium asetat mempunyai kehilangan berat yang ketara yang menunjukkan pelarutan bioglass diikuti oleh kalsium klorida dan kalsium nitrat. Ia menunjukkan keberkesanan pemisahan ion larut ke dalam larutan SBF.

BIOACTIVITY IMPROVEMENT OF 45S5 BIOGLASS THROUGH CALCIUM COATING

ABSTRACT

4555 bioactive glass (BG) has been utilized in clinical applications, especially in bone regeneration. However, BG has limited bioactivity that restricts its use in live tissue. Calcium treatment is used to promote the formation of the HA layer hence boost the bioactivity of the BG. The bioactivity was studied by varying the type of calcium salt which is calcium acetate, nitrate and chloride. BG 45S5, having mole percentages (mol%) of 45% SiO_2, 24.5% CaO, 24.5% Na_2O, and 6% P_2O_5, was synthesized via a melt-derived method. Batching, mixing, melting at 1400 °C, water quenching, grinding, sieving, pressing, and sintering were all part of the BG preparations. The BG were treated with calcium salt then subjected to simulated body fluid incubation to determine bioactivity for hydroxyl carbonate apatite (HCA) layer development, which was followed by characterisations. Calcium acetate treated BG 45S5 had functional groups of P-O and C-O bonds, which contributed to the development of HCA layers. The pH value was determined by analyzing SBF after each immersion period. Calcium acetate treated showed the growth of the HCA layer on the surface as the pH value almost constant for 7 days of immersion among other calcium salts. The weight loss measurement also indicate that BG that treated with calcium acetate has the significant weight loss that shows bioglass dissolution followed by calcium chloride and calcium nitrate. It shows the effectiveness of soluble ion dissociation into the SBF solution.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bioceramics are ceramic materials used in medical and dental applications such as repairing and reconstructing sick or damaged body parts. Bioceramics are a type of biomaterial that comes in a range of forms and phases and is used in several applications within the human body. A biocompatible ceramic is made of calcium and phosphate, for example, hydroxyapatite (HA) (Ramakrishna et al., 2001). It is either a permanent replacement, such as coating gliding surfaces to minimize wear in prosthetic joints, or a temporary construction, such as bioresorbable pins, plates, and screws. To be employed in medical applications, a material must have a few specialized qualities, the most important of which are linked to biocompatibility. Because of their biocompatibility, suitable materials are termed biomaterials. Biocompatibility is a descriptive phrase that denotes a material's capacity to execute the necessary host reaction in a certain application (Seeram Ramakrishna, 2004).

The fundamental components of bioglass are SiO_2, Na_2O, CaO, and P_2O_5. Glass is an inorganic material formed by melting numerous minerals together at high temperatures and cooling the molten state to its solid state without crystallization through its glass transition point. Bioglass differs from glass ceramic in that it can manage a variety of chemical characteristics as well as the pace of attaching with tissues. Bioactive materials are materials that may induce a biological response at their contact by creating links between tissues and the substance itself. Bioactive materials induce a biological reaction from the body, such as tissue bonding. Bioactive materials are classified into two types which is osteoconductive and osteoproductive. Osteoconductive materials, such as synthetic

hydroxyapatite and tricalcium phosphate ceramics, link to hard tissue (bone) and encourage bone development along the surface of the bioactive substance. Osteoproductive materials, such as bioactive glasses, which may also attach to soft tissue such as gingival (gum) and cartilage, induce the formation of new bone on the material distant from the bone/implant contact (Hench & Jones, 2005).

Bioactive glass (BG) is a biomaterial capable of forming connections with both hard and soft tissues, including bone and teeth. Professor Hench invented 45S5 Bioglass® in 1969, with a mole percentage composition of 46.1 wt. % of SiO_2, 26.9 wt. % CaO, 24.4 wt. % Na_2O, and 2.5 wt. % P_2O_5 (Hench et al., 1971). This glass has high bioactivity and biocompatibility, as well as osteoconductivity and osteoinductivity. The exceptional performance of bioactive glasses in bone repair is attributed primarily to the reactivity of their surface in body fluids, which ends within the release of critical concentrations of soluble Si^{4+}, Ca^{2+}, P^{5+}, and Na^{+} ions, which might cause favourable intracellular and extracellular responses promoting rapid bone formation (Zheng et al., 2014).

Following Professor Hench's first discoveries, other research institutions began developing comparable materials. Various conceivable bioglasses with a broad range of compositions were devised in the 1980s at Abo Akademi and the University of Turku, Finland and has been studied the in vivo and in vitro behaviour, surface responses, and bone acceptance of these various formulations (Hench, 2006). BG may be manufactured in a variety of forms suited for a variety of applications, including powders, rods, discs, and nanoparticles. The melt-derived technique and the sol-gel route are two ways typically utilized to create BG (Ibrahim et al., 2017). The melt-derived approach necessitates high temperatures, often above 1000 °C, whereas the sol-gel technique necessitates temperatures

below 1000 °C. When BG is dissolved in simulated physiological fluids, it forms hydroxyapatite (HA) and hydroxyl carbonate apatite (HCA) layers on its surfaces, indicating its bioactivity (Kokubo & Takadama, 2006).

Bioactivity is an interface-driven phenomena that is heavily influenced by the material's composition, structure, surface charge, particle size, and shape, since these characteristics dictate the material's capacity to induce cellular proliferation and differentiation (Malafaya et al., 2007). Bioactivity of glasses is defined as their capacity to cause HA production when they come into contact with a physiological environment such as simulated body fluid (SBF) (Hench, 2005). In other words, the formation of a HA layer on the surface of bioglasses in vitro serves as a preliminary test to assess whether or not the glass is bioactive. HA is the most abundant bone mineral (with minor amounts of intermediate calcium phosphates and calcium carbonate), with a needle-like structure that changes to cauliflower-like clusters on the surface of the glasses (Hench, 2006).

The SBF has the same pH and ionic content as human blood plasma, and the BGs will exchange ions with it, resulting in the production of silanol groups on their surface and the nucleation of a HA layer (Baino & Yamaguchi, 2020). Sol–gel glasses often generate the HA surface layer faster than melt-derived glasses because to their quick reaction kinetics and large surface area for ion exchange with biological fluids such as SBF (Lu & Ducheyne, 2000). Many researchers are interested in bioglass because of its unusual properties, such as its comparatively low softening temperature, which may be employed as a sintering aid during sintering to connect ceramic particles and fill micropores (Boccaccini et al., 2017). Aside from that, bioglass has the situation of compositional design based on attributes specific to a certain therapeutic use. It also has a broad range of

controllability over its chemical characteristics and rate of bonding with tissues, as well as a fast rate of surface reactivity, which leads to direct chemical bonding with bone (Balamurugan et al. 2007).

1.2 Problem Statement

Bioactive glass is primarily used as a synthetic bone graft in orthopaedic and periodontal applications (Skallevold et al., 2019). The glass is utilized in both of these applications to restore and cure bone defects (holes in bone) caused by trauma or tumour/cyst excision. Periodontal abnormalities are often found in the jawbone around the tooth root. Autografting, which is the transplantation of bone from another area of the body (a donor site) to the defect location, is being used by orthopaedic surgeons. Bioceramics such as BG are appropriate for bone and tooth restoration owing to the released ionic dissolution products, which may encourage cells to regenerate and self-repair (Ibrahim et al., 2018).

The problem of BG is its limited bioactivity, which restricts its use in live tissue. HA is related to bone minerals, and because of its similarities to bone and tooth minerals, it is commonly employed in dental implants, orthopedic coatings, and other biological substances. As a result, HA is necessary to promote osteoconduction qualities for the formation of new bone. Calcium treatment on 45S5 BG is used to increase the bioactivity of BG. Additionally, due to the released ionic dissolution products that might encourage the cells toward a route of regeneration and also self-healing, biomaterials like BG are suitable for bone and dental repair (El-Bassyouni et al., 2016).

According to Diba & Boccaccini (2014), melt- derived method provide easier synthesis technique compared to the sol-gel method. It is also commercially

accessible materials and gives superior mechanical and flexural characteristics. Fabrication of BG requires longer period for sol-gel route compared to melt derived route. Bioactivity in melt-derived silicate glasses is only viable in a narrow compositional range, since SiO_2 concentrations greater than 60 mol% make the material chemically inert in interaction with body fluids (Hench, 2006). However, melt-derived glass has a broad workability window, making it an ideal option for pellet manufacturing that it is feasible to sinter highly densified structures at a wide temperature range without altering the material's reactivity (Fiume et al., 2018). Furthermore, the primary crystalline phase can be observed in 45S5 BG pellet which is combeite-type, $Na_2Ca_2Si_3O_9$ that numerous researchers discovered in melt-derived 45S5 Bioglass® after sintering at 650 °C ((Baino et al., 2015, Lefebvre et al., 2008).

One of the novel ways is calcium treatment, in which bioceramic materials are treated with calcium salt to promote the formation of the HA layer and hence boost the bioactivity of the bioceramic materials (Filho et al., 1996). Calcium salt functions as a molecule that releases Ca^{2+} ions, increasing the degree of supersaturation in relation to HA (Ibrahim et al., 2018). When BG-treated material interacts with simulated body fluid (SBF), a calcium phosphate layer forms on the surfaces and transforms into a hydroxyapatite layer (Kaur et al., 2019). As a result, the presence of calcium ion in cordierite will aid in the synthesis of hydroxyapatite while having no effect on its mechanical qualities. Thus, the influence of calcium salt (calcium acetate, nitrate and choride) on the bioactivity characteristics of BG in this work is studied.

The calcium phosphate hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) has a remarkable biological affinity for live bone. Synthetic hydroxyapatite is a popular substance for

filling bone deficiencies. Furthermore, hydroxyapatite possesses various unique features, including protein and virus adsorption, ionic species exchange in its crystal lattice, and the elimination of volatile chemical molecules (Kawai et al., 2006). According to research on hydroxyapatite formation on SiO_2-based systems (Kim et al., 2008; Kokubo & Takadama, 2006) the creation of a silanol (Si-OH) group leads to heterogeneous hydroxyapatite nucleation on a material's surface. The generated nuclei develop by consuming calcium and phosphate ions from their surroundings. Calcium treatment, on the other hand, is beneficial on hydroxyapatite coating because the release of calcium ions from the material increases the degree of supersaturation with regard to hydroxyapatite. A few types of calcium salt can be used to study the HA coating. Studied have done that the calcium salt coating is very potential for HA formation that will improve bioactivity of BGs. As a result, hydroxyapatite development on BG involves the formation of Si-OH by hydration and/or treatment with a calcium salt solutions (Mori et al., 2003).

1.3 Research Objectives

The objectives of this project are:

i. To fabricate 45S5 bioactive glass by melt-derive method.

ii. To determine the effect of different types of calcium salt immersion on 45S5 bioglass on bioactivity and mechanical properties.

1.4 Thesis Outline

This thesis is divided into five chapters. The first chapter provides a quick overview of the general research approach, problem statement, objectives, and scope of research activities.

The second chapter of this thesis contains a thorough evaluation of the literature. The basic principles of bioactive glass are discussed. The standard melting approach and the sol-gel procedure are discussed in further detail. The applicability and significance of bioactive glass for clinical applications are also thoroughly discussed. Finally, the effects of calcium salt on melt-derived bioactive glass are discussed. Chapter three outlines the study's general technique. Details such as material specifications and chemicals used, experimental procedures, and the procedure involved in bioactive glasses synthesis are described. The characterization apparatus and its functioning are briefly detailed. Chapter four explained the influence of different calcium salt and simulated body fluid (SBF) on the bioactivity of bioactive glasses with the support of characterization methods such as X-Ray Diffraction (XRD), X-Ray Fluorescence (XRF), Fourier Transform Infrared Spectroscopy (FTIR), diametral tensile strength (DTS) test, pH evaluation, density and porosity test. Chapter five, the last chapter, deduces the full thesis. An overview of the obtained results is provided, as well as suggestions and recommendations for future studies related with bioactive glass. The general research work is illustrated as shown in Figure 1.1.

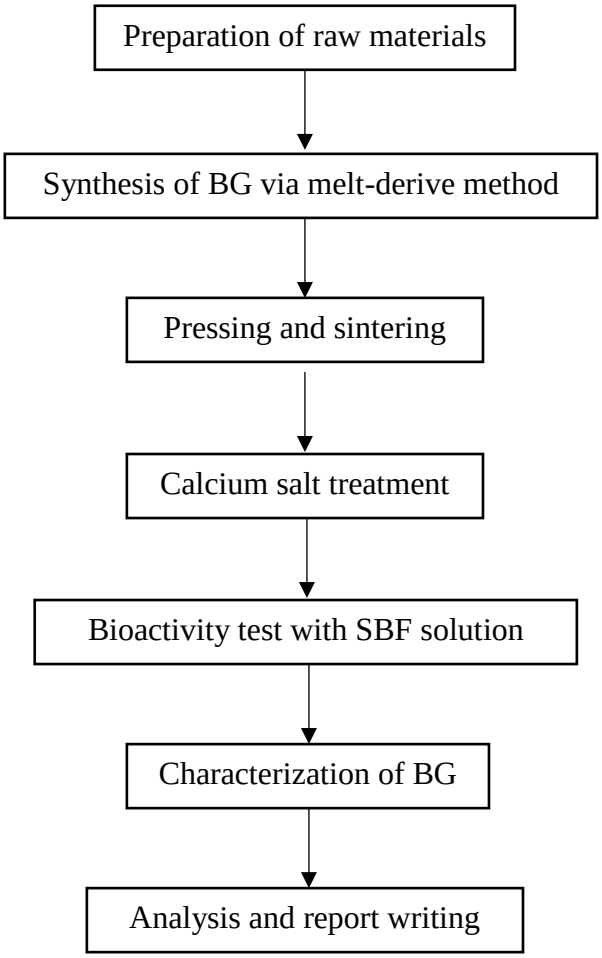

Figure 1.1: General research work flow chart.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioactive Materials

Hench et al. (1998) were the first to report on bioactive glasses' capacity to connect to bone via the creation of a hydroxyapatite surface layer in late 1970s. Since the discovery of bioactive glasses as bone implant materials in the 1970s, there has been a great deal of interest in producing bioactive glass-ceramics. Bioactive glass-ceramics, such as sintered Bioglass®, have the ability to spontaneously connect and integrate with live bone, resulting in the formation of a biologically active bone-like apatite layer on their surfaces. However, because of their weak mechanical qualities, such as low flexural strength and fracture toughness, these materials are not suitable for replacing bones when subjected to load. A biomaterial's primary role is to replace damaged or diseased tissues. Bioactivity indicates the properties of a material's ability to form bonds with host tissues. Bioactive materials are materials that may generate unique biological activity at the material's interface owing to the connection established between live tissues and the material itself (Hench, 2005).

Bioactive materials are those that have the capacity to generate particular biological reactions that result in bone bonding between tissues and implant material. There are two types of bioactive materials: group A and group B. Both groups show that the rate and mechanism of interaction between implant material and faulty tissues varies. Group A is considered an osteoproductive material, while Group B is considered an osteoconductive substance. When the implant material elicits intracellular and extracellular reactions at the implant interface, group A bioactivity is detected. The phrases intracellular and extracellular refer to the

position of the cell inside or outside the cell (Leonor et al., 2002). This group's bioactive substance can create bonds with bone and soft tissues. Meanwhile, group B material only exhibits extracellular reactions at the implant interface. Because of its capacity to encourage new bone formation with or without contact with host bone, bioactive glass such as 4SS5, also known as Bioglass®, falls into categories A and B. (Jones et al., 2007). Synthetic hydroxyapatite (HA) is an example of a bioactive substance in category B. (El-Ghannam and Ducheyne, 2017).

Bioactive glass, bioactive glass ceramic, bioactive calcium phosphate, HA, bioactive coatings, and composites are examples of bioactive materials (El-Bassyouni et al., 2016). Bioactive materials have the properties of both bioinert and biodegradable materials. When bioactive materials have a high surface reactivity, they make strong bonds with faulty tissue when implanted in the live organism (Magri et al., 2017). The bond contact between surfaces is produced by a physiologically active hydroxycarbonate apatite (HCA) layer. The HCA layer is responsible for the union of implant material and faulty tissues by chemically and physically mimicking the mineral phase of bone (Caruta, 2006).

A bioactive material has been broadly described as a material that has been developed to induce specific biological activity. A bioactive substance has been described more narrowly as a material that, when transplanted into the body, undergoes specific surface reactions that result in the creation of a HA-like layer that is responsible for the establishment of a firm bond with hard and soft tissues. The capability of a substance to generate a HA-like surface layer in vitro when submerged in a simulated body fluid (SBF) is frequently used to indicate its bioactivity (Kokubo and Takadama, 2006).

At first glance, glass' inherent brittleness does not appear to make it a viable material for implanted medical devices. Glass, on the other hand, has a number of advantages that make it a good candidate for biomaterial applications. Most biomaterials made of glass or ceramics are intended to improve human health and quality of life by recreating the function of live tissue and organs. The single most crucial characteristic of a biomaterial is that it may come into contact with human body tissues without inflicting unacceptable harm, that is, the material is biocompatible. Understanding the nature and characteristics of glass lays a strong foundation for evaluating its biological potential. In general, a material's appropriateness for a certain application is determined by its performance, characteristics, fabricability, and manufacturing costs (Williams, 2008).

2.2 Bioactive Glass

Hench et al. (1998) invented bioactive glasses in 1969, and they are a class of reactive materials that can bond to mineralized bone tissue in a physiological environment. Bioactive glass, initially synthesized by Hench, is the general term for a kind of amorphous material with a wide range of bioactivity due to chemical composition differences (El-Ghannam and Ducheyne, 2017). The majority of bioactive glasses on the market today are based on Hench et al formula (Hench et al., 1971). Silica is a key component of bioactive glass because it acts as a nucleation site for calcium and phosphate ions to precipitate during the formation of HA (Carvalho et al., 2013).

Bioactive glasses are a type of inorganic bioactive ceramic that may react with physiological fluids to build strong linkages with bone through the production of bone-like HA layers and the biological interaction of collagen with the material

surface (Fernandes et al., 2018). There were several BG compositions to choose from. Different types of bioactive glasses may be created by altering all of these components. Several forms of bioactive glasses have been created over the years, including traditional silicate glass (45S5 bioactive glass or Bioglass®), glass ceramics (S53P4 bioactive glass or BonAlive®), and borate-based glasses (19-93B3 bioactive glass). The biocompatibility of bioactive glasses is typically determined by the silicate content of the glass, with 45-52% silicate achieving the best graft-bone bonding (Arts and Geurts, 2017).

Because of its remarkable bioactivity and biocompatibility, 24.5 wt. % of Na_2O, 24.5 wt. % of CaO, 45 wt. % of SiO_2, and 6 wt. % of P_2O_5 bioglass (45S5) is the most well-known of these systems and has been extensively investigated in recent decades. Because of their high bioactivity index and ability to attach to both soft and hard connective tissues, they provide exceptional benefits as inorganic components of composite scaffolds. These bioactive glasses are osteogenetic and osteoconductive materials, whereas other bioactive materials (such as HA) simply display osteoconductivity (Fiume et al., 2018). Furthermore, 45S5 Bioglass has been shown to increase vascular endothelial growth factor secretion in vitro and to improve vascularization in vivo, implying that scaffolds containing controlled concentrations of Bioglass may stimulate neovascularization, which is advantageous to large tissue engineered constructs (Conrado, 2016).

As 45S5 glass has a low silica content (24.5 wt. % of Na_2O, 24.5 wt. % of CaO, 45 wt. % of SiO_2, and 6 wt. % of P_2O_5), a high calcium-phosphorus ratio, and sodium ions, it exchanges ions quickly with H^+ and H_3O^+ in simulated body fluid. After being implanted in the human body, ion exchange between bioglass, soft tissue, and bone can occur smoothly, resulting in faster bone repair and

regeneration. By activating genes involved in bone formation and vascularisation, bioglass ionic dissolution may induce osteogenesis and angiogenesis (Li et al., 2013).

When used, a bioactive glass degrades gradually. Ions are released as a result of the breakdown, prompting the creation of a carbonated HA layer on the surface of the bioactive glass. It was discovered that high concentrations of Na_2O, CaO, and CaO/P_2O_5, referred to as modifiers, might stimulate glass reactivity in the physiological environment. When bioactive glass is implanted in the body, one of its properties is the kinetics of surface alteration as a function of time. The quantity of bridging oxygen atoms determines the network activity of bioactive glass, which may be used to analyze bioactivity, surface reactivity, and solubility. The capacity of bioactive glass to generate carbonated HA in vitro is investigated using simulated body fluid (SBF), which may be created using ionic compositions comparable to blood plasma (Khurshid et al., 2019).

BG varies from other bioactive materials in that they have unique features and characteristics (Khalid et al., 2017). Bond formation with bone was also seen when hydroxyapatite (HA) and hydroxyl carbonate apatite (HCA) layers were found following BG 45S5 immersion in simulated body fluid (SBF). When a bioactive glass is submerged in simulated body fluid, its capacity to create a hydroxyapatite (HA) layer is generally viewed as a sign of its bioactivity (SBF). In a short amount of time, BG may regenerate human bone, disintegrate in solution, release ionic dissolution products from the bioactive glass, and form an apatite layer on the bioactive glass's surface. Furthermore, the BG ionic dissolution products may be regulated and adjusted for tissue regeneration (Houreh et al., 2017).

Figure 2.1 illustrates the compositional phase diagram for BG, as well as the mixture-levels at which certain biomaterial features emerge (Hench, 2006). For numerous decades, BG has been used in clinical settings for orthopaedic surgery. When BG is implanted in a defect area near bone, reactions on the BG surface cause the release of critical concentrations of soluble Si^{4+}, Ca^{2+}, P^{5+}, and Na^{+} ions, which induce favourable intracellular and extracellular responses, resulting in rapid bone formation (Xynos et al., 2001), this bone formation is then followed by the formation of silica-rich gel on its surface. When silica-rich gel combines with ions in body fluids, hydroxyapatite (HAp)-like crystals grow on the surface of the BG. Furthermore, osteoblasts generate new bone in the silica-rich gel, enabling BG to link with bone through the creation of bone-like hydroxyapatite layers as well as biological interactions with collagen (Figure 2.2) (Höland et al., 1985).

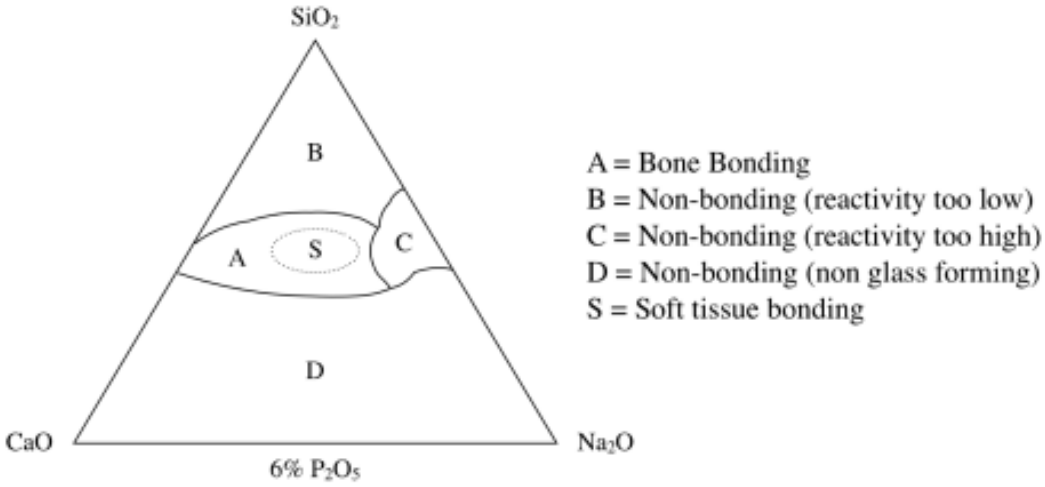

Figure 2.1: A compositional phase diagram of bioactive glasses with an emphasis on bone-bonding is shown. Zone S is the Class A bioactivity region where bioactive glasses connect to both bone and soft tissues and exhibit gene activating properties (Hench, 2006).

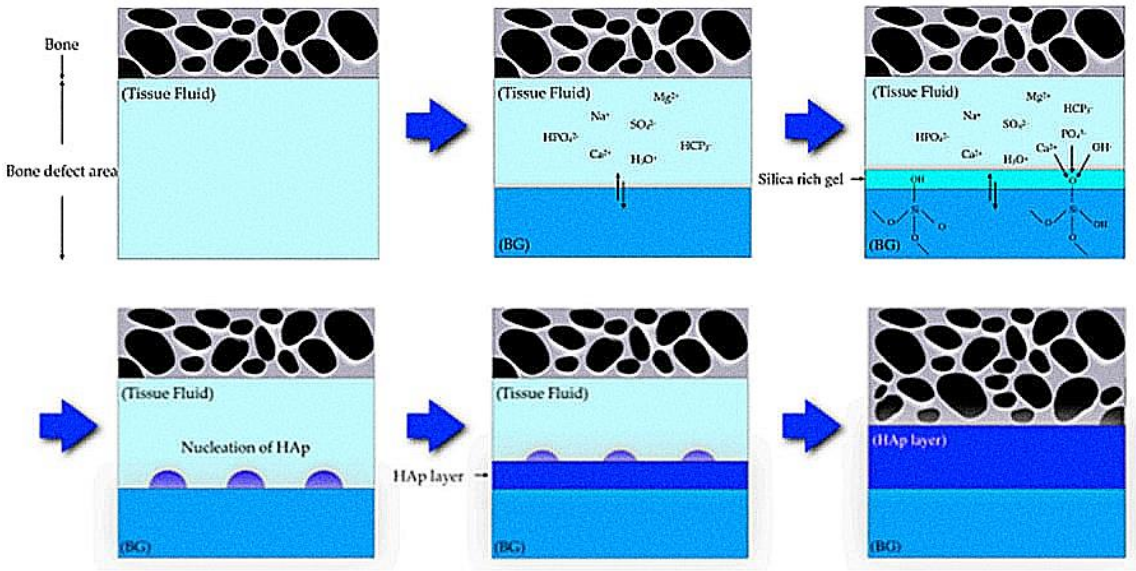

Figure 2.2: A diagram showing a proposed bonding process of bioactive glass with bone (Höland et al., 1985).

Furthermore, BG may encourage bone cells to renew and mend themselves, dramatically speeding tissue healing kinetics (Xynos et al., 2001). These are known as osteoconductivity and osteoinductivity (Hench et al., 1971). BG has mostly been employed in situations where it would come into touch with bone tissue, but it has lately showed potential in inducing soft tissue healing as well (Baino and Yamaguchi, 2020). Many researchers are interested in BG because the ionic dissolution products of BG have been reported to induce angiogenesis. Furthermore, alternative BG-based medicines are also available for use in wound healing and peripheral nerve regeneration (Gilchrist et al., 1998). These uses indicate that BG is suitable and biocompatible as a biomaterial capable of being applied to both hard tissues like dentin or cementum (which are akin to bone) and soft tissues like dental pulp and periapical tissue (Oguntebi et al., 1993).

To begin, the word bioactive refers to the material's ability to create a connection with live tissue. When BG is exposed to extracellular fluids, mechanisms on its surface initiate this connection. Furthermore, BG offers a surface

for cells to connect, grow, and deposit matrix on, and it even stimulates osteoblast recruitment and differentiation. In BG-filled defects, bone develops and proliferates, and the glass eventually degrades over time (Van Gestel et al., 2015).

Bacterial infections have long been a source of concern in orthopaedic and trauma surgery. Despite breakthroughs in both antibiotic medicines and surgical procedures, an ever-increasing number of interventions and an increase in antibiotic-resistant bacteria result in an increase in absolute numbers of infections. The capacity of BG to combat bacterial infections in vivo is a unique trait, and possibly the most critical benefit over other graft materials. This means that the material can act as both a local antibiotic-like therapy and a permanent graft layer at the same time. Extensive in vitro research on the antibacterial effects of BG has been conducted. Powdered BGs have been proven to have a high antibacterial impact on a wide range of therapeutically relevant pathogens (Arts and Geurts, 2017).

45S5 Bioglass® is a type of Class A bioactive material. Due to the high surface area and nanometer-scale porosity of the hydrated silica gel and rapidly forming hydroxycarbonate apatite layers, the release of soluble silica and other ionic species from this glass has intracellular effects on bone tissue proliferation, but it also has extracellular effects (Oonishi et al., 2000). Oonishi et al. discovered a relationship between a material's bioactivity class and the rate of bone formation: class A bioactive materials, such as 45S5 Bioglass®, encourage tissue formation more than class B bioactive materials, which dissolve slowly (Arts and Geurts, 2017).

2.2.1 Properties of Bioglass

Bioactive implants for clinical use should, ideally, mirror the mechanical characteristics of the host tissue and form strong interfacial interactions with both hard and soft tissues. Because of the inorganic origin and mechanical qualities of bioactive glasses, which have physical properties that are similar to 'hard' bone tissue, there has been a lot of interest in utilizing these biomaterials in contact with bone and teeth. Table 2.1 summarizes the key mechanical characteristics of several commercial bioactive glasses and glass-ceramics, hydroxyapatite, and human bones. Table 2.1 shows that all of the synthetic materials are much less tough than natural load-bearing cortical bone (Kaur et al., 2019).

Bioactive glasses are fragile and have a poor fracture toughness by nature. When bioactive glasses come into contact with (body) fluid, surface reactions occur, resulting in bone bonding. As a result, mechanical properties will change over time in an in vivo scenario. To simulate the in vivo environment, simulated body fluid (SBF) is used in in vitro experimental settings (Ylänen, 2018).

Bioactive glass has an amorphous structure, whereas glass-ceramics are crystalline glasses. Glass ceramics are made by heating glass in a controlled environment at a specific temperature for a specific amount of time. After controlled heat treatment of the glass, a glass-ceramic is formed with improved mechanical properties over its parent glass, such as viscous behavior, toughness, and hardness. However, crystallization reduces the mechanical strength of low-strength glass-ceramic scaffolds in the case of 45S5 glass (Kaur et al., 2013).

Table 2.1: Bioactive glasses, ceramics, and human bones mechanical characteristics (HA = hydroxyapatite; AW = apatite wollastonite) (Kaur et al., 2019).

Material	Compressive Modulus (GPa)	Compressive Strength (MPa)	Fracture Toughness (MPa/$m^{\frac{1}{2}}$)	Vickers Hardness (MPa)	Structure
HA	35-120	100-150	0.8-1.2	90-140	Ceramic
Bioglass® 45S5	60	-	0.6	-	Glass
Bioglass® 52S4.6	60	-	-	-	Glass
Cerabone® AW	120	1080	2	680	Glass-ceramic
Ceravital®	100-160	500	-	-	Glass-ceramic
Bioverit® I	70-90	500	1.2-1.8	-	Glass-ceramic
Bioverit® II	70	450	1.2-1.8	-	Glass-ceramic
Bioverit® III	45	-	0.6	-	Glass-ceramic
Trabecular bone	0.05-0.6	1.5-7.5	0.1-0.8	40-60	-
Cortical bone	7-30	100-135	2-12	60-75	-

Crystallization increases the mechanical and flexural strength of glass, resulting in its high fracture strength. In glass-ceramics, crystalline phases are embedded in an amorphous glassy matrix. The crystallization of glasses has been shown to affect their bioactivity in numerous studies. Crystallization in bioactive glass reduces bioactivity, making it an inert material, according to Filho and Li's findings (Filho et al., 1996). While glass-ceramic is mechanically stronger than amorphous glass, it has significantly less bioactivity (Kaur et al., 2013).

Table 2.2 displays Bioglass's thermal, physical, mechanical, and chemical characteristics. Bioglass and other bioactive glasses have inferior mechanical qualities, with lower tensile strength and greater modulus compared to cortical bone (50–150 Mpa and 7–30 Gpa, respectively), which implies they cannot be implanted alone into load-bearing bone defects. As a result, they could not be employed to regenerate a full-thickness (segmental) bone defect on their own metallic attachment is necessary in those applications to withstand cyclic strain. As a result, bioactive glasses and other bioactive ceramics are increasingly being employed to heal lesions surrounded by host bone (Jones and Clare, 2012).

Table 2.2: Properties of bioglass (Jones and Clare, 2012).

Property	Value
Density	2.7 g/cm^3
Glass transition temperature	538 °C
Crystallization onset temperature	677 °C
Melting temperature	1224 and 1264 °C
Thermal expansion coefficient	15.1×10^{-6} °C^{-1}
Refractive index	1.59
Tensile strength	42 MPa
Young's modulus	35 MPa
Shear modulus	30.7 GPa
Fracture toughness	0.6 MPa m$^{1/2}$
Vickers hardness	5.75 GPa

2.2.2 Bioactivity of bioglass

The same oxides are present in bioactive glasses as in regular soda-lime glasses, but in different amounts, resulting in major changes in several properties. The chemical stability of bioactive glasses is the most important factor in determining bioactivity; in watery environments, bioactive glasses dissolve at significantly faster rates than soda-lime glasses used in containers or windows, for example. Bioactive glasses have been shown to have quicker rates of HCA production and bone-bonding formation (Jones et al., 2007).

Bioactive glasses, for example, have greater osteoconductivity than bioactive ceramics, since osteoconductivity is connected with the creation of HCA, and the rate of surface HCA generation in bioactive glasses is higher than in

ceramics. When these materials come into touch with body fluids, they degrade over time and are eventually replaced by new bone growth and tissue regeneration. In order for the dissolving glass to promote and increase tissue regeneration and development, its dissolving rate must be compatible with cellular processes, whereas regular glasses are assumed to be inert throughout routine use (Miguez-Pacheco et al., 2015).

As a consequence, bioactive glasses can help with tissue repair and regeneration for a limited time. Brittleness is one of the greatest barriers to the use of bioactive glasses, particularly in load-bearing applications. Brittleness problems could be addressed in the future by incorporating bioactive glass into composites with polymers, or by creating tissue engineering scaffolds with unique designs tailored to the needs of loaded bone (Jones, 2013).

The first bioactive glasses investigated as prosthetic materials were melt-derived compositions within the system 24.5 wt. % Na_2O, 24.5 wt. % CaO, 6 wt. % P_2O_5, and 45 wt. % SiO_2. It was a unique and wise choice to utilize glass as a material in contact with human bones. Professor Hench, the inventor of bioactive glasses, had the brilliant idea of creating a material composed entirely of human body parts. In addition, the oxide ratios in the compositions studied were chosen to promote rapid alkali dissolution from the glass surface in aqueous solutions, followed by the precipitation of an outer layer rich in calcium and phosphorus at the inner alkali-depleted silica layer (Fagerlund and Hupa, 2017).

Almost all glasses dissolve gradually in aqueous solutions, but the rate of ion leaching and the ability to form surface layers vary significantly depending on the overall composition. It is believed that if the calcium phosphate surface layer's composition is comparable to that of hydroxyapatite (HCA), a hydrated calcium

phosphate component found in bone tissue, the glass will not be rejected by the body (Miguez-Pacheco et al., 2015).

As a result, bioactive glasses have been shown to be useful in bone rehabilitation. Novel bioactive glass compositions for bone and soft tissue engineering are undergoing extensive research. Researchers have been adjusting and tailoring bioactive glass compositions far beyond the initial bone tissue use to develop areas in soft tissue regeneration, thanks to a growing understanding of the effect of different ions released from glasses on tissue regenerating capabilities. Despite the fact that bioactive glasses have a long history, the majority of commercial products today are used to treat bone tissue damage, illness, or injury (Jones, 2013).

2.2.3 Bone bonding Mechanism

Bioactive glasses are designed to break down and respond in a controlled manner in the human body. Surface-active materials capable of making a mechanically strong chemical contact with live tissue, mainly bone, have historically been thought of as bioactive glasses. The foundation for the glass to form chemical bonding with tissue is the glass's time-dependent dissolution and precipitation interactions with its surrounding solution. Variations in the ion concentrations of the solution around the reacting glass promote the formation of the carbonated hydroxyapatite (HCA) interfacial layer (Jones, 2013). The HCA layer resembles the inorganic mineral apatite found in bone, providing a chemical attachment of the implant to the surrounding tissue rather than the mechanical fixation provided by fibrous capsules around biostable implants. Not only do biomimetic crystals have a comparable composition to biological bone apatite, but

their crystallite size is also compatible with nano-sized bone or dentine apatite crystals (Rahaman et al., 2011).

The glass is also osteoconductive due to the HCA layer's surface. The bioactive glasses can be categorized as resorbable materials because they dissolve over time while enabling apatite precipitation at the dissolving surface and adjacent tissue. The bioactive glasses' ion release products are known to activate various gene families, including those that govern osteogenesis and the synthesis of growth factors, affecting protein adsorption and cell attachment at the surface of the responding glass (Valerio et al., 2004).

As a result, bioactive glasses that promote new bone regeneration via ionic dissolution products are both osteoconductive and osteostimulative. In vitro and in vivo, dissolution and precipitation processes of bioactive glasses produce comparable surface template structures that facilitate hydroxyapatite precipitation. As a result, the production of HCA on the glass surface in vitro is commonly regarded as a sign of bioactivity. The glass surface partially degrades before acting as a substrate for HCA precipitation. The precipitate is initially amorphous, but with time it transforms into carbonated hydroxyapatite (Boccaccini et al., 2017).

Surprisingly, the use of bioactive glasses is not limited to bone tissue engineering or other applications that require the bone-bonding properties of HCA crystals. The effect of the released ions on the activation of genes and cells involved in hard and soft tissue regeneration, wound healing, and angiogenesis has been studied in several studies. Bioactive glasses, among other things, have the ability to regenerate heart, lung, nerve, and gastrointestinal tissue. The HCA layer that forms on the surface of the bioactive glass also serves as a soft tissue bonding interface.

On the other hand, the mechanisms by which the bioactive glass interacts with the cells that make up the various soft tissues are unknown (Rahaman et al., 2011).

2.2.4 Apatite growth on bioglass surface

After the production of the hydroxycarbonate apatite (HCA) layer, the biological processes involved in bone growth and bone bonding on the glass surface may be separated into phases. In vitro and in vivo, the formation of silica and apatite layers is identical. Nonetheless, when exposed in vitro, a calcium phosphate (Ca-P) layer forms on the glass surface, but in vivo, a calcium phosphate (Ca-P) layer forms inside the surface of the Si rich layer. In vivo, adhering collagen and protein generated by bone cells are seen on the implant glass surface (Zadpoor, 2014).

In general, in vitro immersion using physiological fluids is a process used to assess the capacity of implant materials to generate apatite. For apatite formation assessment, the bioactive material is exposed to biological solutions such as simulated body fluid (SBF) with pH 7.4 for many days at a constant temperature of 37 °C. HCA layer may be seen, although it grows more easily in SBF, particularly when the glass composition includes less calcium (Bellucci et al., 2011). Bioactive material exposure to cells is also classified as an in vitro test, in which implant material is exposed to cell cultures such as fibroblast cells and human bone cells in order to examine the cells' reaction, durability, and cytocompatibility. Cells are also incubated at a steady temperature of 37 °C for many days.

Meanwhile, in vivo testing involves implanting bioactive material in tissues or organs of a live person or animal (Liu et al., 2010). Urine and blood are often used to assess ion exposure and release caused by biological reactions between implant material and faulty tissue or organ. The essential observation that must be