SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

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FABRICATION AND CHARACTERIZATION OF BIOACTIVE GLASS/ALGINATE SCAFFOLDS

By

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitles "**Fabrication and Characterization of Bioactive Glass/Alginate Scaffolds**". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or university.

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LIST OF ABBREVIATIONS

Symbols	Description
BG	Bioactive glass
HCA	Hydroxyl carbonate apatite
CaP	Calcium phosphate
3D	Three dimensional
Ca	Calcium
P	Phosphorus
O	Oxygen
HA	Hydroxyapatite
SiO_2	Silicon dioxide
Na_2O	Sodium oxide
CaO	Calcium oxide
P_2O_5	Phosphorus pentoxide
SBF	Simulated body fluid
MgO	Magnesium oxide

LIST OF SYMBOLS

Symbol	Description
Wt.%	Weight percent
kV	kilo Volt
α	alpha
β	beta
δ	lambda
°C	Degree Celsius
MPa	Mega Pascal

FABRIKASI DAN PENCIRIAN PERANCAH KACA BIOAKTIF/ALGINAT

ABSTRAK

Kejuruteraan tisu telah berevolusi dari bidang perkembangan bahan bio dan berpandukan kepada praktik penggabungan perancah, sel dan molekul aktif secara biologi ke dalam tisu berfungsi. Matlamat kejuruteraan tisu adalah untuk memulihkan pemasangan pembinaan berfungsi, mempertahankan atau memperbaiki tisu yang rosak atau seluruh organ. Kaca bioaktif adalah salah satu jenis bahan bio di mana perkembangannya telah mempengaruhi kehidupan manusia secara besar-besaran oleh aplikasi perubatan serba boleh dan masa depan yang sangat menjanjikan. Dalam kajian ini, kaca bioaktif (BG) jenis 45S5 telah digunakan bersama-sama dengan mineral kordierit dan oksida kordierit untuk mereka bentuk perancah melalui kaedah pengeringan beku. Perancah dibuat melalui teknik pengeringan beku dengan pra-beku 20 gram sluri untuk setiap komposisi (15 gram 45S5 BG 5 gram mineral kordierit dan 15 gram 45S5 BG 5 gram oksida kordierite) dengan suhu pra-beku yang berbeza; -10^{o}C dan -40^{o}C. Kaedah ini telah disediakan untuk mengkaji kesan mineral dan oksida kordierit kepada 45S5 BG dan kesan suhu pra-pembekuan yang berbeza kepada perancah mineral kordierit-45S5 dan perancah oksida kordierit-45S5. Ujian mampatan dan keliangan telah dilakukan dan perancah dengan 15 gram 45S5 + 5 gram oksida kordierit dengan suhu pra-beku -40^{o}C memberikan peratusan tertinggi keliangan dan ujian mampatan adalah yang paling rendah. Perancah yang sama disalut dengan 5% kepekatan larutan natrium alginat untuk mengkaji kesan natrium alginat pada sifat mekanik perancah. Daripada ujian mampatan, kekuatan perancah yang disalut dengan natrium alginat adalah lebih tinggi berbanding dengan perancah yang tidak bersalut dengan nilai 0.037 MPa dan 0.01 MPa untuk perancah oksida kordierit-45S5 yang tidak bersalut dengan suhu pra-beku -40^{o}C.

FABRICATION AND CHARACTERIZATION OF BIOACTIVE GLASS/ALGINATE SCAFFOLDS

ABSTRACT

Tissue engineering evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells and biologically active molecules into functional tissues. The goal of tissue engineering is to assemble functional constructs that restore, maintain or improve damaged tissues or whole organs. Bioactive glass is one type of biomaterials where its development has influenced human lives to a large extent by its versatile medical applications and very promising future. In this present study, 45S5 type of bioactive glass (BG) was used together with mineral cordierite and oxide cordierite to fabricate scaffolds through freeze drying method. The scaffolds were fabricated through freeze-drying technique by pre-freezing 20 gram of slurry for each composition (15 gram 45S5 BG + 5 gram mineral cordierite and 15 gram 45S5 BG + 5 gram oxide cordierite) with subjected pre-freezing temperature; -10^{o}C and -40^{o}C. This fabrication was fabricated in order to study the effect of mineral and oxide cordierite to 45S5 BG and the effect of different pre-freezing temperature to the mineral cordierite-45S5 and oxide cordierite-45S5 scaffolds. Compression and porosity test were done and scaffold with 15 gram 45S5 + 5 gram oxide cordierite with -40^{o}C of pre-freezing temperature give the highest percentage of porosity hence the compression test is the lowest. The same scaffold was coated with 5 wt.% concentration of sodium alginate to study the effect of sodium alginate on mechanical properties of the scaffold. Result from the compression test shows that the strength of the scaffold coated with sodium alginate is higher compared to the uncoated scaffold with value of 0.037 MPa and 0.01 MPa for uncoated oxide cordierite-45S5 scaffold with pre-freezing temperature -40^{o}C.

CHAPTER 1

INTRODUCTION

1.1 Research Background

There are over 200 bones of different shapes, sizes and functions in the human body. Besides providing the weight-bearing structure for the body, they also play several important roles such as protection of the most vital organs, movement and locomotion of the body, production of blood cells, support and protection of soft tissues, calcium and phosphate storage and acting as a storehouse for growth factors and minerals (Clarke, 2008). Each bone continuously undergoes modelling or reshaping during lifetime to help it fit biochemical forces changes, as well as remodelling to remove micro-damaged bone, old bone and replace it with new, mechanically stronger bone to help retain bone strength. Hence, loss of this multifunctional tissue adversely affects the patient's quality of life and represents a burden for the healthcare system. This multifunctional tissue loss due to the bone tissue tends to lose more calcium than is replaced. Fortunately, bone exhibits unique regenerative capacity and can heal without structural or functional impairment (Noori et al., 2017).

Bone tissue is capable of self-repair, where from a fracture, the cell starts to migrate and differentiate followed by tissue synthesis and cytokine and growth factor release occur, which regulated by the mechanical environment. Self-repair of bone tissue results in the production of new bone exhibiting all the characteristics of normal bone. However, when the natural bone repair mechanisms fail, autologous bone grafting is the current standard of care. The osteogenic cells and bone matrix in the graft provide the osteoinductive and osteoconductive properties required for successful bone repair.

Osteoinduction describes that the graft can induce the basic, undifferentiated and pluripotent stem cells to develop into the bone-forming cell lineage, by which osteogenesis is induced. Osteoconduction refers to the ability to support the attachment of osteoblast and osteoprogenitor cells, and allow the migration and ingrowth of the cells within the three-dimensional architecture of the graft (Wang and Yeung, 2017). So, fracture or bone defect filling by an autologous cancellous bone graft results from interactions among osteogenic cells, cytokines, an osteoconductive matrix, and a mechanically stable environment with a good blood supply. This results in the production of new bone exhibiting all the characteristics of normal bone (Rosset et al., 2014). However, if the defect size is greater than the healing capacity of osteogenic tissues or bone tissue formation, the site will not regenerate spontaneously. Furthermore, diseased bones are incapable for complete healing. In this situation, orthopaedic surgeons are left with two options: autogenous bone grafting, or the use of synthetic biomaterials (Noori et al., 2017).

Nevertheless, the autogenous bone grafting has its own limitations include added operative time for graft harvest, moulding challenges, graft resorption, donor site morbidity and limited availability, especially in paediatric population. Since then, there are numerous alternatives to bone graft have become available to address these limitations, sadly, most of these products are expensive, have unpredictable biologic activity and do not osseointegrate (harmonious coexistence of implant, bone, and soft tissue) (Rogers and Greene, 2012). As mentioned, the serious shortage of natural bone graft and the little chance of supply meeting the demands in an ageing population has triggered the blossom of the bone grafts market. Hence, synthetic biomaterials is a better option. Calcium phosphate (CaP) ceramics, CaP cements, calcium sulfate, bioactive glass or combinations, therefore, are most frequently synthetic bone substitutes available at

present (Wang and Yeung, 2017). The synthetically derived material is classified in alloplastic bone graft materials. Some synthetic bone grafts are made of calcium carbonate such as bioactive glass, which start to decrease in usage because it is completely absorbable in short time and makes the breaking of the bone easier .

The field of tissue engineering in the last decade has advanced dramatically, offering the potential for regenerating, repairing or replace portions of or whole tissue of the human body (Fisher and Mauck, 2013). The advances involve researchers in a multitude of disciplines, including cell biology, biomaterials science, imaging, and characterization of surfaces and cell-material interactions. Tissue engineering aims to restore, maintain, or improve tissue functions that are defective or have been lost by different pathological conditions, either by developing biological substitutes or by reconstructing tissues. The general strategies adopted by tissue engineering can be classified into three groups: (i) implantation of isolated cells or cell substitutes into the organism, (ii) delivering of tissue-inducing substances (such as growth factors), and (iii) placing cells on or within different matrices (Vacanti and Langer, 1999).

This field relies extensively on the use of porous 3D scaffolds to provide the appropriate environment for the regeneration of tissues and organs. These scaffolds essentially act as a template for tissue formation and are typically seeded with cells and occasionally growth factors, or subjected to biophysical stimuli in the form of a bioreactor; a device or system, which applies different types of mechanical or chemical stimuli to cells (O'Brien, 2011) .

Scaffold plays a unique role in tissue regeneration. During the past two decades, many works have been done to develop potentially applicable scaffold materials for tissue engineering. Scaffold design and fabrication are major areas of biomaterial

research, and they are important subjects for tissue engineering and regenerative medicine research (Agrawal and Ray, 2001). Scaffold fabricated from inorganic materials such as calcium phosphate-based bioceramic and bioactive glass can provide higher mechanical strength such as compression and hardness than other biomaterial scaffolds (Witte et al., 2005). Scaffolds for bone tissue engineering are subject to many interlinked and often opposing biological and structural requirements. A major hurdle in the design of tissue engineering scaffolds is that host materials are not simultaneously mechanically competent and bioresorbable, i.e. mechanically strong materials are usually bioinert, while degradable materials tend to be mechanically weak. Hence, the fabrication of composites comprising biodegradable polymers and bioactive glass becomes a suitable option to fulfil the requirement of bioactivity, degradability and mechanical competence (Chen et al., 2008) .

A possibility to optimize the large-scale production of important pharmaceutical substances is the culture of cells of particular tissues and monocellular bacteria in continuous-flow, solid-bed reactors. Polymers, glasses and ceramics can be used to immobilize cells and microorganisms in the reactor. However, the higher mechanical and chemical resistance, better porous surface and lower production costs make ceramics a more suitable support, including bioactive glass than the other two types of materials. In particular, ceramics made from cordierite can be considered among the best support for cell growth for the possibility to identify suitable macro- and micro-porosity and to control the mineralogical phases in the bone tissue engineering (Orlandi et al., 1997).

Bone tissue engineering seeks to restore and maintain the function of human bone tissues using the combination of cell biology, materials science and engineering principles. The three main ingredient for tissue engineering are, therefore, harvested

cells, recombinant signalling molecules and three – dimensions matrices. Cells and signalling molecule such as growth factors are seeded into highly porous biodegradable scaffolds, cultured in vitro, and subsequently, the scaffolds are implanted into bone defects to induce and direct the growth of new bone. Signalling molecules can be coated onto the scaffolds or directly incorporated into them. Hence, the first and foremost function of a scaffold is its role as basement that allows cells to attach, proliferate, differentiate (i.e., transform from a non-specific or primitive state into cells exhibiting the bone-specific functions), and organize into normal, healthy bone as the scaffold degrades (Chen et al., 2008).

Since natural bone matrix is a composite of biological ceramic (natural apatite) and biological polymer (collagen), it is not surprising that synthetic or naturally occurring ceramics, polymers, and their composites have been extensively considered to construct scaffolds for bone tissue engineering. Since bone consists of large amounts of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) and related calcium phosphates (CaP) (e.g., β-tricalcium phosphate) have been considered to develop the mineral phase of bone and exhibit excellent biocompatibility. The close similarity of HA properties to the mineral component of bone properties give stability in the body if being implanted. However, it results in the lack of biodegradation of HA in the body, which is generally an undesirable feature for tissue engineering scaffold materials. For example, a recent clinical report on 6 – 7-year follow-up study has confirmed that implanted crystalline HA is not biodegradable, remaining in the body for extended periods with no visible signs of biomaterial resorptions (Marcacci et al., 2007).

However, bioactive glasses (BG) were first developed in 1969 by Hench, and represent a group of reactive materials that are able to bond to mineralized bone tissue in

physiological environment. Hench and co-workers discovered that 45S5 bioglass contains 45% SiO_2, 24.5% Na_2O, 24.4% CaO and 6% P_2O_5 in weight percent and the phase diagram as proposed by him is given in Figure 1.1. Bioactive glasses are widely used in the biomedical area. Early applications of bioactive glasses were in the form of solid pieces for small bone replacement in middle ear surgery. Later, several applications of bioactive glasses have been proposed, including the dental field (Subramani et al., 2013). Recently, bioactive glasses have been widely studied for potential application in tissue engineering and regenerative medicine. Bioactive glasses and glass-ceramics are a diverse group of materials possessing a unique set of physicochemical properties that make them useful for bone repair (Fiume et al., 2018).

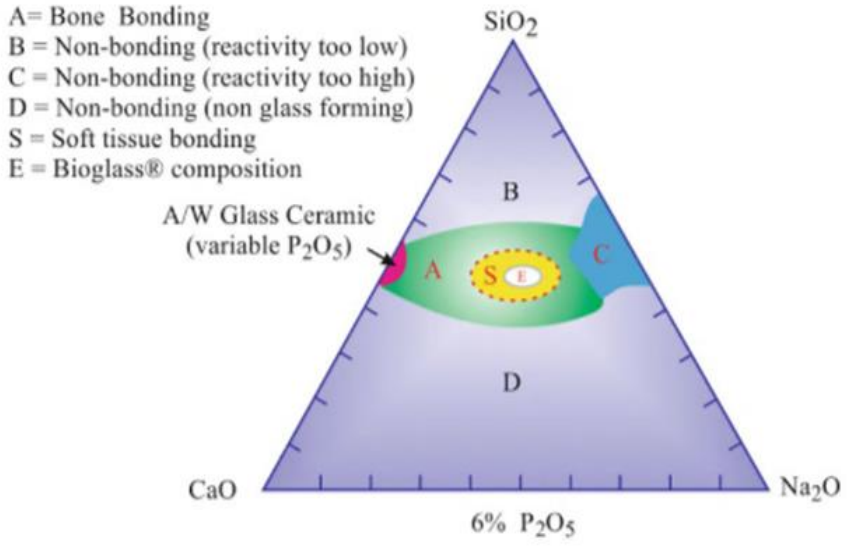

Figure 1.1: Phase diagram indicating the glass-forming region as proposed by Hench (Subramani et al., 2013)

BG is a highly bioactive material and expresses other properties of a similar kind as osteoinductive and osteoconductive, which allows them to be implanted in medical application, e.g. bone replacement and regeneration in dental and orthopaedic treatment and even in tissue engineering (Oryan et al., 2014). The ability of BG to precipitate hydroxyapatite (HAp), form a chemical bond with the host tissue, and ultimately resorb or become integrated with the bone make these as viable substitutes to restore and improve the function. The growth of hydroxycarbonate apatite (HCA) layer on the glass surface is initiated upon immersion in simulated body fluid (SBF) due to the ion exchange between the biological fluid and bioactive glass. Bioactivity indicates the capability of BG to interact with defective living cells and tissues, stimulate and regenerate new tissue upon glass dissolution. Attachment and spread of bone cell on the HCA layer induce tissue regeneration once collagen produced by bone cells bind to HCA layer (Stanic, 2017). Considering all the privileges of 45S5 BG, this type of bioglass is the main material use in this research.

In this research, cordierite is used together with 45S5 BG and acts as matrix to scaffold formation. Generally, cordierite has stoichiometric formula of $2MgO.2Al2O_3.5SiO_2$ and can exist under three polymorphic forms; α-cordierite, β-cordierite and μ-cordierite. However, α-cordierite is the most suitable cordierite glass-ceramic to be applied in engineering materials because of their good properties in dielectric properties (~5 at 1 MHz, with low tan δ), low Coefficient Thermal Expansion (CTE) (1-2 x 10-6 /°C), chemical stability and excellent thermal shock resistance (Mei et al., 2001). Due to these properties, cordierite has been widely used in various applications such as material in kiln furniture, carriers of purifying exhaust emission, filters for liquid at high temperature, glaze for tiles and partial electronic component (Ibrahim et al., 2018). These properties of cordierite may improve the properties of the

bioactive glass materials when use together in producing scaffold for bone tissue engineering. Together with that, cordierite was a chosen material to act as matrix in composite material with 45S5 bioglass.

1.2 Problem Statement

A variety of biomaterials and fabrication techniques have been used in the field of tissue engineering to produce numerous scaffolds in order to regenerate different tissues and organs in the body. Regardless of the tissue type, a number of key considerations are important when designing or determining the suitability of a scaffold for use in tissue engineering such as biocompatibility, biodegradability, mechanical properties, scaffold architecture and manufacturing technology (O'Brien, 2011).

Mechanically, bioceramics and glasses are stronger than polymer and play a critical role in providing mechanical stability to constructs prior to synthesis of new bone matrix by cells. However, ceramics and glasses are very fragile and prone to catastrophic failure due to their intrinsic brittleness and flaw sensitivity. Although brittle, scaffolds fabricated from inorganic materials such as calcium phosphate-based bioceramic and bioactive glass can provide higher mechanical strength than polymeric scaffolds. The formation of composites thus capitalises on the advantages of both material types and minimises their shortcomings (Mano and Reis, 2007). One major challenge to optimise the biological and mechanical performance of bioactive glass or ceramic composites is to balance between the porosity of the biomaterial on one hand and maintaining the mechanical strength. The strength tends to deteriorate as the increase of the porosity value according to the previous studies (Zohora et al., 2014). In the other hand, the important feature of bioactive glasses, which makes them suitable candidates for bone

tissue engineering, is their ability to enhance mechanical properties, increasing the initial compressive strength and its positive influence on the scaffolds bioactivity (Kaur, 2017).

In this research, 45S5 type of BG is chosen as main material due to the deployment of BG for clinical treatment received massive interest following its first introduction by Hench (Xiaoxia et al., 2004). Modification of BG composition influences the reactivity and bioactivity of the glass itself. Glass dissolution depends on glass composition and its textual features. Many BG compositions based on the SiO_2-CaO-Na_2O-P_2O_5 network system have been developed for improving the BG bioactivity, yet the golden 45S5 BG composition is still used commercially and remained as subject of interest in many researches (Jones, 2013). 45S5 BG as an implant is able to integrate with living tissue without fibrous encapsulation formation, which enables its use in clinical applications. The bond between BG and tissue is connected through the formation of established biologically active apatite layer, hydroxyl carbonate apatite (HCA).

Both mineral cordierite and oxide cordierite powder are fabricated through glass route method where both used as matrix with 45S5 BG to form scaffold. Generally, cordierite has stoichiometric formula of 2MgO.2Al2O3.5SiO2 and α-cordierite has good properties in dielectric properties, chemical stability and excellent thermal shock resistance. Hence, this cordierite specialty brought to improve and enhance the properties of 45S5 BG scaffold which act as matrix component.

Alginate is a biosynthesized material that available in large quantities and typically comprises from 30% to 60% of brown algae. The natural functionality of alginate is to give flexibility and mechanical strength to the seaweed. Additionally, it also serves as a water reservoir to prevent hydration of alginate to exposed to the air (Lee and Mooney, 2012a). Recently, alginate has been extensively evaluated as the most employed

biopolymers in food industry, agricultural fields and biomedical applications due to its outstanding gelling performance and viscosity. Previously, alginate were used as an adhesive binder. Nevertheless, recent developments in the field of alginate functionality have led to a renewed interest in the usage of alginate as thickening agent, emulsifiers and gels formation substances. Besides one of the most important role of alginate, it serves as a biomaterial in engineered scaffold applications as it demonstrates soft gelling properties and biochemical characteristics (Sun and Tan, 2013).

In a recent study, in biomedicine, alginate is commonly used in the form of a hydrogel including wound healing, drug delivery and tissue engineering application. Hydrogels are three-dimensionally crosslinked networks composed of hydrophilic polymers with high water content. Hydrogels are often biocompatible, as they are basically similar to the macromolecular-based components in the body, and can often be delivered into the body via minimally invasive control (Lee and Mooney, 2012b). In the other hand, a research confirmed that the highly porous scaffolds that fabricated by the foam replication technique and then coated with sodium alginate did not affect the interconnectivity of the scaffold pore structure. The resulting composite scaffold exhibited antibacterial effect and improved mechanical properties as well as high bioactivity (Viviana et al., 2010).

In order to fulfil the requirement of bone substitute with acceptable characteristic, in this current study, bioactive glass will be fabricated by melt-derived method and the bioactive glass with mineral and oxide cordierite scaffold fabricate by the freeze-drying method and coat with sodium alginate right after the scaffold requirements are obtained. The porosity, mechanical properties and morphology will be observed to investigate the effect of different composition and after coating with sodium alginate.

1.3 Objectives

The objectives of this research are:

i. To investigate the effect of mineral cordierite and oxide cordierite in 45S5 bioactive glass.

ii. To investigate the effect of pre freezing temperature on mineral cordierite-45S5 scaffold and oxide cordierite-45S5 scaffold.

iii. To evaluate the effect of sodium alginate on the mechanical properties of the scaffold.

1.4 Scope of Research

In this research, the bioactive glass 45S5 was fabricated by melt-derived method composed of 45 g SiO_2, 24.5 g CaO, 24.5 g Na_2O and 6 g P_2O_5. Both mineral cordierite and oxide cordierite also were fabricated by melt-derived method. Batch composition of mineral cordierite are 81.25 g kaolin, 21 g MgO and 12.38 g SiO_2, and the batch composition of oxide cordierite composed by 21 g MgO, 26 g Al_2O_3 and 53 g SiO_2. The powder obtained at this stage were analysed via XRD, XRF and SEM.

15 g of 45S5 BG and 5 g mineral or oxide cordierite were mixed together using mechanical stirrer at 300-350 rpm to form slurry before diluted gelatine, polyvinyl alcohol (PVA) and glutaraldehyde solution were added. Then the slurry was pour into a polypropylene mould and directly put into freezer under -10^oC and -40^oC for 24 hours for pre-freezing.

After 24 hours, the sample was put in the freeze-drying machine with temperature of -50^oC and pressure of 0.05 mBar. The samples were left for 24 hours in freeze-drying

machine. The obtained scaffolds were characterized using Instron Testing Machine for compression and Archimedes' principle for porosity test. Finally, the scaffolds were coated with 5 wt. % concentration of sodium alginate. Followed by characterization and mechanical evaluation.

CHAPTER 2

LITERATURE RIVIEW

2.1 Introduction

This chapter is focusing more on the materials and selected method used in this present work, which are 45S5 bioactive glass, cordierite, alginate and freeze-drying technique where all of this will be introduced and explained further. In this chapter also explained the general overview of scaffolds in bone tissue engineering applications and the basic requirements needed for ideal biomaterials in order to fabricate scaffolds. In the other hand, the choices of biomaterials also being discussed in this chapter and 45S5 bioactive glass will be highlighted.

The properties and advantageous of the cordierite material which was used together in 45S5 scaffolds in this research also been explored more. Lastly, the other scaffold fabrication techniques and the advantages of the chosen technique which is freeze drying, also been review in this chapter.

2.2 Overview of bone tissue engineering

Tissue engineering technology is based on the cells combination from the body with scaffolds that act as templates for tissue regeneration to facilitate and guide the growth of new tissue. The concept of tissue engineering was officially defined at a National Science Foundation in 1988 as the application of principles and methods of engineering and life sciences towards the crucial understanding of structure and function relationships in normal and pharmaceutical mammalian tissues and the development of biological substitutes to restore, preserve, or improve tissue function (María and

Eduardo, 2011). The study area of tissue engineering is a highly multidisciplinary field, with experts from materials science, medicine, mechanical engineering, physics, biology and chemistry, as schematically represented in Figure 2.1 below. The biology, cellular and molecular biology, and biochemistry knowledge is needed for the design of the new tissues. At the same time, materials science and engineering together with chemistry bring the required knowledge for designing and building the scaffolds, in which cells should attach and grow. Lastly, medicine's knowledge applies practical issues to necessities and real problems (Vallet-Regi et al., 2013).

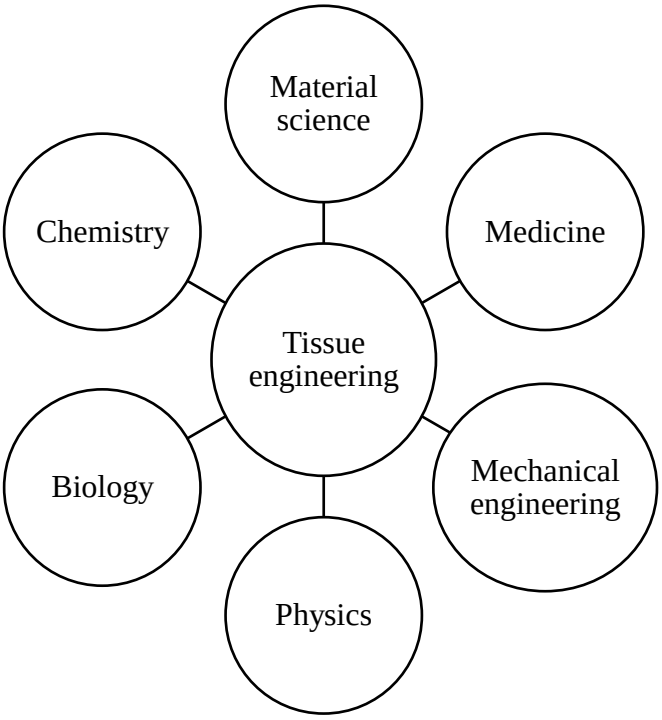

Figure 2.1: A highly multidisciplinary field for tissue engineering (María and Eduardo, 2011)

Considering the definition of tissue engineering published by Langer and Vacanti (Langer and Vacanti, 1993), bone tissue engineering can be defined as an emerging interdisciplinary field that seeks to address the needs by applying the principles of biology and engineering to the development of viable substitutes that restore and

maintain the function of human bone tissues. Bone tissue engineering is based on the understanding of structure of bone, bone mechanics and formation of the tissue as it aims to induce bone tissues with new functional (Amini et al., 2012). Thus, the real challenge in bone tissue engineering is to mimic nature's behavior (Place et al., 2009). It is for this reason that is necessary to understand the hierarchical structure of bone in Figure 2.2 before starting to design any scaffold for bone tissue engineering.

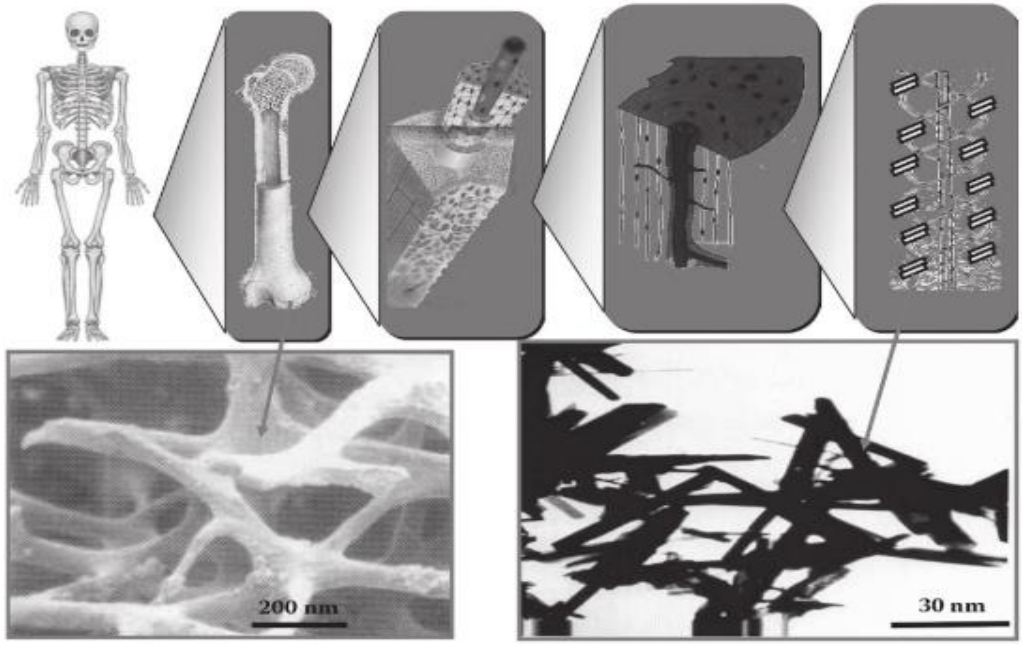

Figure 2.2: Representation of the hierarchical structure of bone (top); and micrographs of bone macro porosity (bottom left) and carbonate hydroxyapatite (bottom right) (Vallet-Regi et al., 2013).

Bone tissue is a natural composite material made of a combination of organic and inorganic components. For example, collagen and carbonate hydroxyapatite as organic biopolymer and inorganic ceramic respectively. A collagen is a triple helix of protein chains that presents high tensile and flexural strength. The character of this biopolymer is to provide a framework for bone tissue. Carbonate hydroxyapatite is a crystalline calcium phosphate that provides the stiffness and high compressive strength of bone

(María and Eduardo, 2011). Additionally, it has been observed that there are two types of bone in vertebrates. First, cortical bone (also called compact bone) is dense structure with high mechanical strength. Second, cancellous bone (also called trabecular or spongy bone) is less dense and weaker than cortical bone because of its porous nature. Because of its porous structure, it is highly vascularized and contains red bone marrow to produce blood cells. Bone plays very important parts in critical functions in human physiology, such as movement, protection, support of other critical organs, mineral storage and homeostasis, blood production, multiple progenitor cell housing, blood pH regulations and other (Porter et al., 2009).

Bone tissue engineering is a complicated and dynamic process that initiates with movement and enrolment of osteoprogenitor cells followed by their generation, differentiation, matrix formation along with remodelling of the bone. Major advances in bone tissue engineering with scaffolds are achieved through growth factors, drugs and gene deliveries (Bose et al., 2012). Figure 2.3 shows the bone cells that are arranged in cylindrical patterns throughout bone around thin tubes called Haversian canals, it is explained how the cells movement in the bone.

Osteoprogenitor cells are the stem cells of bone that undergo mitosis, producing daughter calls that differentiate into osteoblasts. It aid in repair of bone fractures. Osteoblasts, lining in the surface of bone and perform osteogenesis. It produces and releases proteins and other parts of matrix. Before calcium salts are deposited, the matrix is called osteoid which elevate the level of calcium salts in matrix and converting osteoid to bone. Next, the osteocytes is to maintain the protein and mineral content of the matrix. It secretes chemicals that dissolve old matrix and then stimulate the depositing of calcium crystals. Besides, it also assist in the repair of damaged bone where it can become

osteoblast and osteoprogenitor cells if released from lacunae (Lowe and Anderson, 2015).

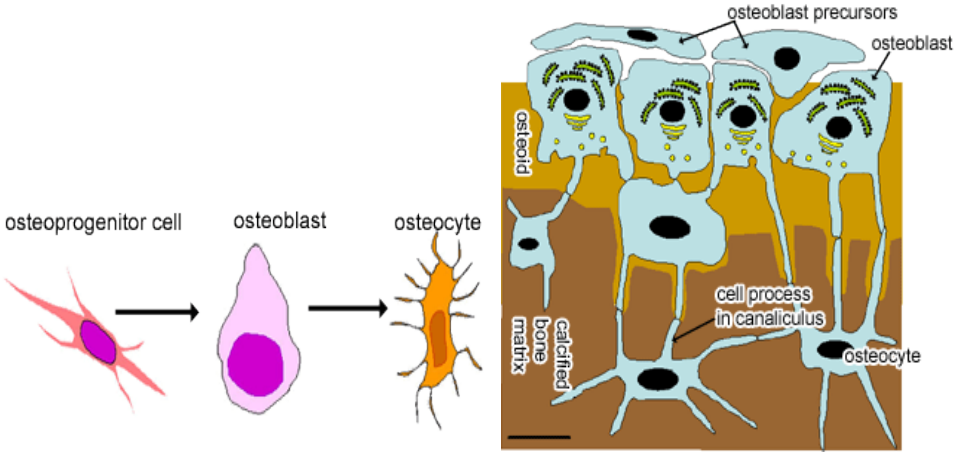

Figure 2.3: The bone cells that are arranged in cylindrical patterns throughout bone around thin tubes called Haversian canals (Lowe and Anderson, 2015)

Disease, injury and trauma that cause damage and degradation of tissues have powered the development of treatments to promote their repair, replacement or regeneration (O'Brien, 2011). Traditionally, the treatment has been based on: (1) transplanting healthy tissue from the same patient to the damaged area (autograft), and (2) transplanting healthy tissue from a donor to the patient (allograft). Although those approaches have been a great solution within the last few years, there are major drawbacks associated with both techniques. The use of autografts is painful, restricted to anatomical limitations, and, more importantly, related to donor-site morbidity because of possible infection and hematoma. On the other hand, allografts also present the limitation of accessing enough tissue for all the patients, the risk of rejection by the immune system of the patient, and the chance of infection together with the possibility of a disease contagion from the donor to the patient (Vallet-Regi et al., 2013).

According to Somaiya and Kaur (Somaiya and Kaur, 2015), because of the limitations of conventional treatments for bone fracture, such as limited quantity for autografts, there is a demand to investigate better alternatives for bone healing. Their research methods such as low-level laser therapy, mesenchymal stem cell-based therapy, nanomaterials, biodegradable hydrogels, extracellular matrix-mimetic materials, and controlled delivery of growth factors from polymer scaffolds look promising for bone healing. Meanwhile, in a research done by Bigham-Sadegh and Oryan (Bigham-Sadegh and Oryan, 2014), they found out many researchers have fabricated nano-hydroxyapatite/collagen by biomimetic strategy and it shows great promise in clinical applications because its composition and structure are similar to natural bone. In addition, to select an appropriate treatment strategy in achieving a successful and secure healing, more information concerning injuries of bones, their healing process and knowledge of the factors involved are required.

The developing field of tissue engineering targets the treatment and repair of damaged bone tissues with a different approach. Instead of replacing them, tissue engineering aims to regenerate damaged tissues by developing biological substitutes that restore, maintain or improve tissue function (Langer, 2000). From the materials perspective, and in the case of critical size defect scenarios, the implantation of a biomaterial scaffold is essential for filling the defect and stimulating the self-repairing processes of bone. In this sense, the scaffold should be able to deliver biological factors that will promote bone regeneration. Additionally, the scaffold should fulfil profoundly challenging biological and biochemical functions. In general, ideal scaffolds must fulfil many design requirement, which will explained in next section.

2.2.1 The basic biomaterial design criteria to fabricate scaffolds

Scaffold-based tissue engineering combines viable cells, biomolecules, and a structural scaffold into a tissue-engineering construct to promote the repair and/or regeneration of tissues. The construct is intended to support cell migration, growth, and differentiation, and guide tissue development and organization into a mature and healthy state. The science in the field is still developing and various approaches and strategies are under experimental investigation. It is by no means clear what defines ideal scaffold/cell or scaffold/neotissue constructs, even for a specific tissue type. The considerations are complex and include architecture, structural mechanics, surface properties, degradation products, composition of biological components, and the changes of these factors with time in vitro and/or in vivo (Hutmacher et al., 2014).

However, tissue-engineering scaffolds do have certain minimum requirements for biochemical as well as chemical and physical properties. A biomaterial is regarded as material intended to undergo a series of chemical surface reactions to replace damaged tissue/organ and hence retaining the normal body functions. For the normal functionality of the biomaterials, a number of factors such as physical/chemical/biological compatibility are required (Gurbinder, 2017). These prerequisites for any biomaterial dictate the desired shape, size and configurations of the implant for uninterrupted working. In general, the biomaterials should comply with the following basic criteria in Table 2.1.

Table 2.1: Basic biomaterial design criteria (Ruvinov et al., 2015)

Basic Criteria	**Explanation**
Biocompatibility	This term refers to the ability of a material to perform with an appropriate host response in a specific situation or ability to deliver cells (Williams, 1999). In tissue engineering, biocompatibility refers to the ability of a scaffold to perform as a substrate that will support the appropriate cellular activity, including the facilitation of molecular and mechanical signaling, in order to optimize tissue regeneration, without eliciting any undesirable effects in these cells, or including any undesirable local or systemic responses in the host.
Mechanical properties	The strength of a material is its ability to withstand an applied stress without failure. Scaffolds in tissue engineering should have the mechanical properties to contain and protect the seeded or recruited cells and maintain their structure under mechanical perturbation existing during cultivation and at implant site. At the same time, the scaffold mechanical properties should be compatible with the host tissue to allow its integration without interfering with the normal function of the organ. This is especially critical when a biomaterial is used as ECM replacement of damaged myocardium.
Biodegradable/bio resorption	Ideally, the scaffold should disappear from the host when tissue regeneration has been accomplished and normal function is restored. Biodegradable scaffolds can do so via polymer backbone degradation (e.g., hydrolysis, enzymatic cleavage) or by dissolution of the matrix. It is fundamental that the products of this process would be biocompatible and be resorbed by the body or removed from it via extraction from the urine.

Scaffold fabrication	Ideally, this process should use safe reagents, which do not affect material properties, such as its cell recognition motifs. For example, cross-linking between polymer chains is often used in hydrogel fabrication from natural materials such as alginate, collagen, hyaluronan, and others. Cross-linking can be physical, where the polymer chains self-assemble due to electrostatic interactions, response to temperature and irradiation, or chemical, where covalent bonds are introduced between the polymer chains. Chemical cross-linking often changes the material properties (degradability, mechanical strength, and cell recognition) due to lack of precise control over the position where the cross-link linkages are formed. In addition, chemical cross-linking often involves the use of harsh reagents, thus raising concern about the material biocompatibility.
Scaffold internal morphology	Ideal bone scaffolds should have interconnected pore networks to promote waste exchange, oxygen and nutrient. This structure plays a crucial role in the osteogenesis of seed cells. The microscopic structure of pore for the scaffold mainly refers to pore size, porosity, the uniformity of pore distribution, pore connectivity, the twist of connected channels, and the specific surface area of the scaffold. Scaffolds with high porosity and large specific surface area promote seed cell adhesion and growth, extracellular matrix deposition, nutrient and oxygen entry, metabolite discharge, and the ingrowth of blood vessels and nerves. A must have property for scaffolds is interconnected porosity >90%, where pore size should be at least 100 μm in diameter for successful diffusion of essential nutrients and oxygen for cell survivability. However, pore sizes in the range of 200 to 350 μm are found to be optimum for bone tissue in-growth and to enable cell-cell interactions and support vascularization after implantation.

2.2 Biomaterial classification

Biomaterial is non-vital material with unique properties such as cytocompatibility, biodegradation, bioactivity, adequate mechanical strength, and osteoinduction/osteogenesis/osteoconduction capability. The first generation biomaterials were developed in 1960s and 1970s, whose main aim was to accomplish desired mixture of physical and chemical properties to match as that of the host tissue (with minimum or no cytotoxic response). The main intention for designing this era biomaterials was inertness to avoid any biological rejection and foreign body reaction (Kaur, 2017).

In order to meet the ideal scaffold's requirement, the choices of the biomaterial need to be highlighted. In most cases, a biomaterial is any biocompatible material, natural or man-made, that is used to replace or assist part of an organ or its tissue, while in intimate contact with living tissue (Chen and Thous, 2015). Many types of biomaterial can be used to the generation of three-dimensional scaffolds for tissue engineering. Four categories of materials; metals, ceramics, polymers and composites are the most common materials used as biomaterials based on their classification and functions. Major characters of chemical bonds and representative properties of four materials types as following Table 2.2.

Table 2.2: Major characteristics of four materials types (Chen and Thous, 2015)

	Metallic	**Ceramic**	**Polymeric**	**Composite**
Character of chemical bonding	Metallic bonding	Ionic bonding	Covalent bonding within molecular chain	Physical mixture
Typical properties	Conductive, tough, ductile and strong	Nonconductive, inert (corrosion resistant), thermally stable, strong and hard	Nonconductive, inert, soft, flexible, plastic or elastic	Combination of component materials
Major problems	Corrosion	Brittle	Thermally unstable, oxidation (aging)	Expensive processing

2.2.1 45S5 bioactive glasses

The term of bioactive has been used with different definitions in different scientific fields. In the biochemistry field, for example, the bioactive component of an enzyme refers to its biochemically reactive part. In the field of biomaterials, bioactive often refers to a material, which upon being placed within the human body interacts with the surrounding tissue (Lanza et al., 2000). According to the mentioned definition, bioactive is restricted to surface bioactive materials, as opposed to bulk bioresorbable materials. Surface bioactive ceramics are virtually nonresorbable in the body but exhibit an ability to bond with the bone. Surface-erodible and surface-bioactive are often used interchangeably. There are three types of surface bioactive ceramics: (i) hydroxyapatite and related calcium phosphates, (ii) bioactive glasses and (iii) glass-ceramics. Most surface bioactive ceramics can, however turned to become bulk biodegradable via the

alteration of crystallinity and/or composition. In general, crystalline ceramics are more stables in aqueous environments than their amorphous counterparts of the same compositions, and many glass bioceramics (e.g., amorphous calcium phosphates) are biodegradable (Chen and Thous, 2015).

2.2.1.1 Composition and biodegradability of bioactive glasses

The constituent of bioactive glasses is similar to that of soda-lime glass. The most bioactive glasses are composed of SiO_2, Na_2O, CaO and P_2O_5. The well-known 45S5 Bioglass (first bioactive composition) contains 45% SiO_2, 24.5% Na_2O, 24.4% CaO and 6% P_2O_5, in wt.%. The bioreactivity of these materials is composition-dependent. In the 1960s, Larry Hench (Lanza et al., 2000) systematically studied a series of glasses in the four-component systems with a constant 6 wt.% P_2O_5 content. This work is graphically summarized in the ternary SiO_2-Na_2O-CaO diagram shown in Figure 2.4. the major features are listed as follows. In region A, the glasses are bioactive and bond to bone. In region B, glasses are nearly inert when implanted. In region C, the compositions are resorbed within 10-30 days in tissue and in region D, the compositions are not technically practical.

The key advantage that makes bioactive glasses attractive bioceramics is possibility of controlling the bone-bonding ability and biodegradation kinetics by modification of their chemical properties. The structure and chemistry of glasses can thus be tailored at the molecular level by varying either composition or thermal processing parameters. It is possible to design glasses with properties specific to a particular clinical application.