CHAPTER-1

Introduction & Literature review

INTRODUCTION

1.1 Biomaterials:

A biomaterial refers to any substance, whether natural or manufactured, that is introduced into a living organism body tissue as a part of an implanted medical device or used to replace an organ, bodily function etc. According to Williams in ‘Definitions in Biomaterials’ (Williams, 1986) and ‘Dictionary of Biomaterials’ (Williams, 1999), biomaterials are inert materials used in medical devices that interface with biological structures. Prof. L. L. Hench classified materials broadly into four categories according to their material tissue interactions and biocompatibility, i.e.

- **(i)** Toxic: Not suitable for bioactive purposes.
- **(ii)** Bioactive (e.g. bioglass): enhanced adherence by stimulating bone regrowth.
- **(iii)** Bio-inert: (Al_2O_3, glassy carbon) Avoid eliciting or experiencing responses from the medium that comes into touch with the body's normal functioning. and
- **(iv)** Resorbable: (hydroxyapatite) Filler materials that enable the breakdown and subsequent reformation of tissues. (Hench et al.,1971).

Williams defines a biomaterial as a biocompatible natural or synthetic something that is made to work with a biological system to fix, enhance, or replace any body tissue, organ, or physical activity while eliciting a minimum biological reaction (Ratner et al., 1996). A biomaterial represents a biocompatible substance which may be utilized to fabricate synthetic organs, psychological rehabilitation devices, prostheses, and tissue replacements. An implant's tissue attachment mechanism is closely linked to the tissue reaction at the implant interface (Hench 1991). All materials placed in a live organism provoke a reaction taken by the tissue of the host, indicating that none are inert. However, according to Prof. Hench, there are no such materials as ‘absolute inert’: these stimulations provoke a reaction from the host tissue. upon implantation (Hench et al., 1970). Due to the high mechanical strengths and nearly bioinert nature, some metallic implants were initially considered natural bone grafts substitute (Schalock and Thyssen, 2013), yet the materialtissue incompatibility related complications like hypersensitivity, allergy, and inflammation (Teo and Schalock, 2017, Basko-Plluska et al., 2011, Rostoker et al., 1987, Merle et al., 1992, Munro-Ashman and Miller, 1976) restricted them from conventional (i.e., ceramic and polymeric) biomaterials (Wang et al., 1978, Puleo and Nanci, 1999). Even the so-called biocompatible titanium implants have caused unwanted immunity reactions in metals on metals (MOM) joint prostheses and total hip arthroplasty (THA) are a few of the examples of the drawbacks of metallic implants (Kazantzis, 1978; Black et al., 1990). Nevertheless, polymeric biomaterials have enormous contributions to tissue engineering (TE) applications, but repairing or replacing hard tissues with polymeric materials is very challenging since they always lack mechanical strength due to their high degradable tendency.

Biomaterials can be divided into different types, as shown in Figure 1.1. Firstly, they were classified as inorganic and organic materials; afterwards, it was classified according to their application. They are classified as ceramics, metals, bio-composites and polymers. The wide range of biomaterials accessible expands the selection of materials for particular medical purposes; for example, chemically inert metals may be chosen for their high electro-conductivity for electrodes in artificial organs with long-term restoration of lost body function. However, biodegradable materials, like stitches, can be utilized as a temporary structure for patients whose function or missing tissues may be recovered.

In Figure 1.2, we see Mr. L. L. Hench carrying 45S5 glass. He is the renowned scientist who spearheaded the production of the very promising 45S5 bioactive glass, a revolutionary bioglass. The invention of the 45S5 glass and other composites formed with the substitution of this glass led to a wide range of applications in the medical field.

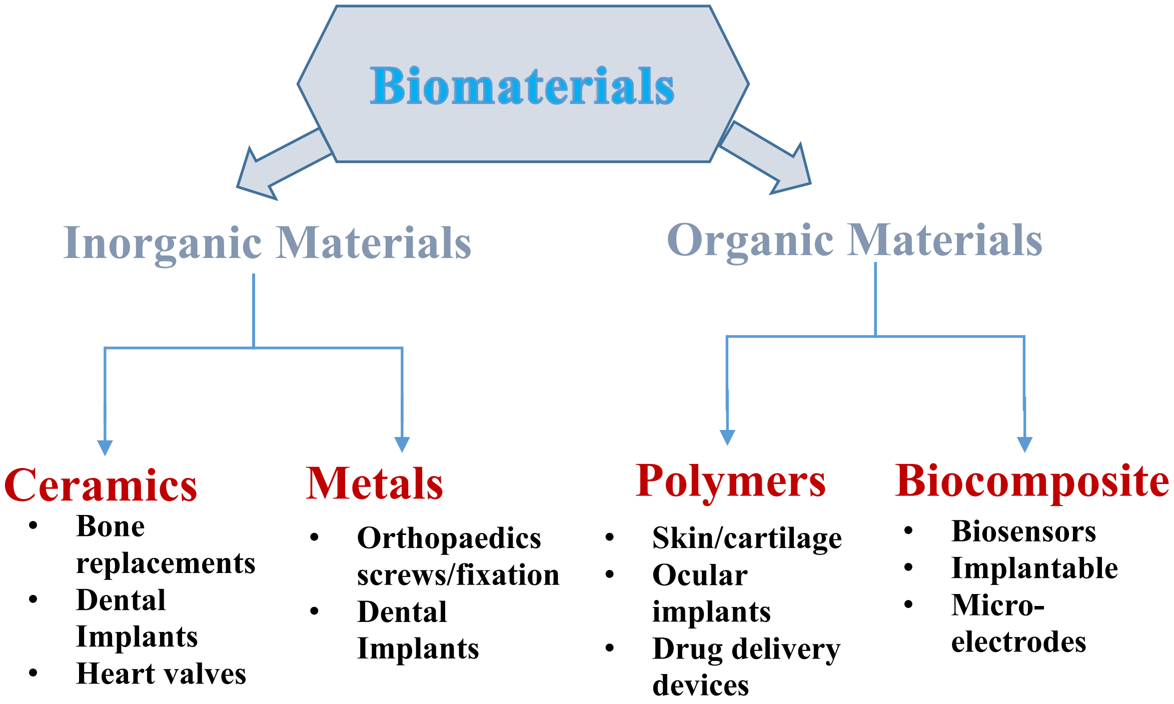

Figure 1.1 Types of Biomaterial showing their application.

Figure 1.2 L.L. Hench with 45S5 Bioglass.

Nevertheless, ceramic biomaterials, particularly bioactive glasses apart from Autografts and Allograft (the gold standard for bone grafts) (Bi et al., 2013), are so far the best synthetic grafting material because they are biologically compatible and can be converted to hydroxyapatites, the mineralogical component of ‘bone and teeth’ and also can be tailored to the architecture of the physiological organ to mimic natural tissues (bone, skin, etc.) (Liu et al., 2013). A revolution has occurred during the last 60 years to improve human life quality using ceramic-based biomaterials. Since then, ceramics have continuously been contributing to repairing diseased and damaged parts of the body. Such ceramics, which have gained significant trust in the betterment of human lives by their considerable contribution to tissue engineering applications, are known as bioceramics. Some clinical achievements of bioceramics include successful repair and augmentation of the skeletal system, bone joints, damaged teeth, and other hard and soft tissues. Ceramics were also used to replace parts of the cardiovascular system, especially heart valves. Special formulations of bioglasses were also used therapeutically for the treatment of tumours. When implanted, bioceramics show material-tissue interactions followed by the formation of a strong, compliant interface between the host tissue and the material. The quality of the interface indicates the biocompatibility of the bioceramics. The soft and hard tissue attachment to the materials is governed by the quality of the interface formed due to material-tissue interactions (Hench, 2013). However, bioactive glasses are the most important bioceramics for tissue engineering applications. The discovery of bioactive glass, was a breakthrough for the ceramic-driven biomaterials. The need for bioactive glasses was perceived when a Vietnam war returnee and Prof. Hench had a conversation about the necessity of such a material that acts like normal bones (Hench, 2006) to avoid the complexity of polymeric and metal implants and their substantial rejection. After almost two years of research, Prof. Lary L. Hench and the team discovered 45S5® bioactive glass, also known as Hench glass at the University of Florida, US, in 1969. Since then, bioactive glasses/ scaffolds have triggered a revolution in tissue engineering applications. However, bioactive glasses are known for their ability to elicit a specific response from the host tissue and form a strong, compliant bond at the material-tissue interface. The bioactivity of such biomaterials is ascribed by the formation of biocompatible HCA (hydroxy carbonate apatite) layer on the surface by a series of reactions while remain in contact with the physiological fluid. Introducing a biologically inactive and almost chemically unreactive implant triggers the development of a fibrous capsule that does not attach to the surrounding tissue. The name of this connection is "morphological fixation". The fibrous layer thickness is contingent upon several aspects, including the implant's conditions, the host tissue's circumstances, the mobility and fit conditions at the surface interface and mechanical stress. Alumina, which is chemically stable, forms a thin capsule when it is mechanically appropriately fitted. The increased chemical reactivity of metallic implants leads to a thicker layer of fibrous tissue at the interface. Micro-motion may arise due to the absence of chemical or biological bonding at the contact. This motion causes the gradual formation of the fibrous capsule that does not stick to the surface, ultimately leading to a decline in the performance of the implant, the tissue it is attached to, or both. Porous biomaterials facilitate interface attachment by allowing tissue to grow into surface pores or throughout the implant’s structure. The name of this attachment is "Biological fixation". It can endure more intricate stress than thick, almost inactive implants that simply accomplish "morphological fixation". Resorbable implants are engineered to undergo slow degradation over time and then be substituted by the body's natural tissues. For instance, metabolic processes break down biodegradable structures made of poly (lactic acid) - poly (glycolic acid) into carbon dioxide and water. Tricalcium phosphate ceramics undergo degradation, resulting in the formation of calcium and phosphate ions. Cells need the ability to manage high amounts of materials. Hence, the components of a resorbable implant must be metabolically compatible. A resorbable implant must have a resorption rate that aligns with the rates at which tissues heal themselves. Bioactive implants provide an alternative method for achieving interfacial attachments. Upon implantation of a bioactive substance in the body, a sequence of biophysical and biochemical processes occurs at the interface between the implant and the surrounding tissue. These processes ultimately produce a chemically robust interfacial bond with high mechanical strength. The name of this attachment is "Bioactive Fixation" as shown in Figure 1.3. Here the schematic diagram of long bone and its upper and centre part being explained.

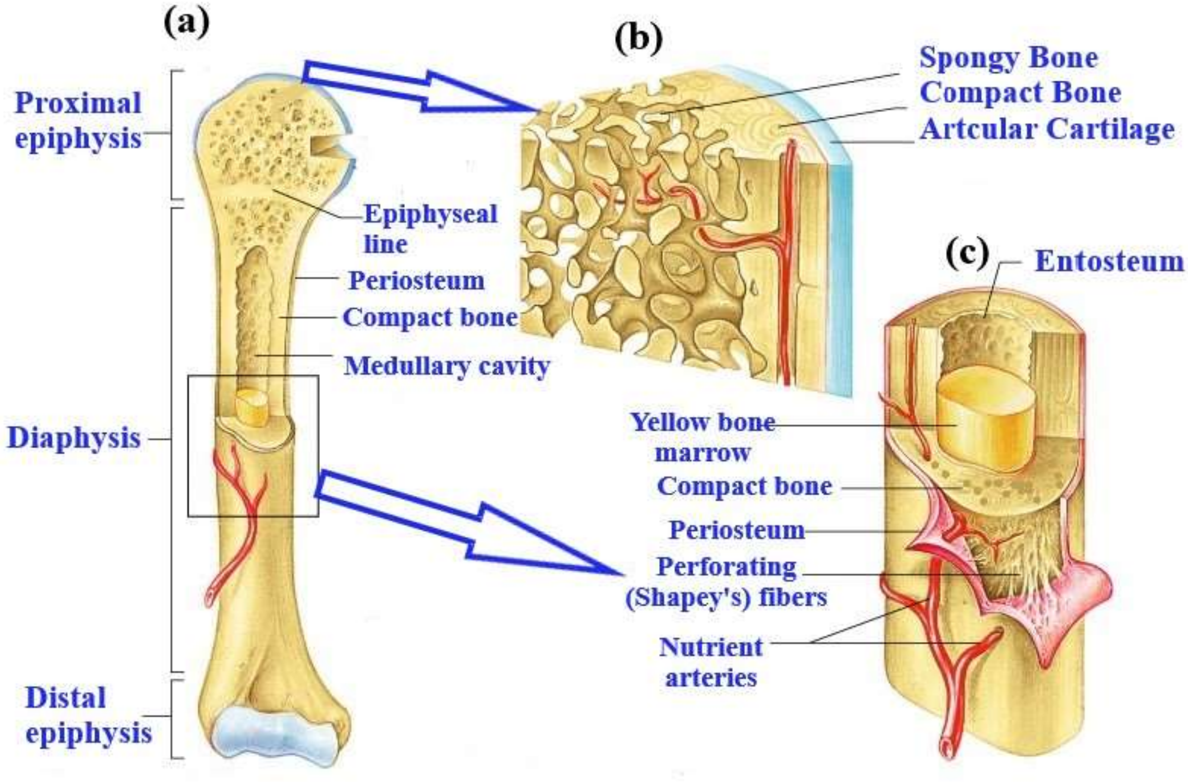

Figure 1.3 Shows the in vitro and in vivo performance of Bone **(a)** long bone **(b)** upper part **(c)** centre part of bone.

Glass products are also used in medicine departments in different fields. Some of them are shown in Figure 1.4. In the years since their invention, biomaterials have undergone a broad spectrum of transformations. Figure 1.5 explains the progression of applications of biomaterials and their job in the medical field as the year passes. Figure 1.6 explains the chronological analysis of the applications of bioglass, and the changes in their worldwide market trends are explained in this figure according to the data collected throughout the world. They are widely used in North America.

Medpor@PlusTM (Porex Surgical USA)

PerioGlass (NovaBone Products, USA)

RediHeal (Avalon Medical,

Butler@NuCare (Florida, USA)

Figure 1.4 Commercially produced glass available in market.

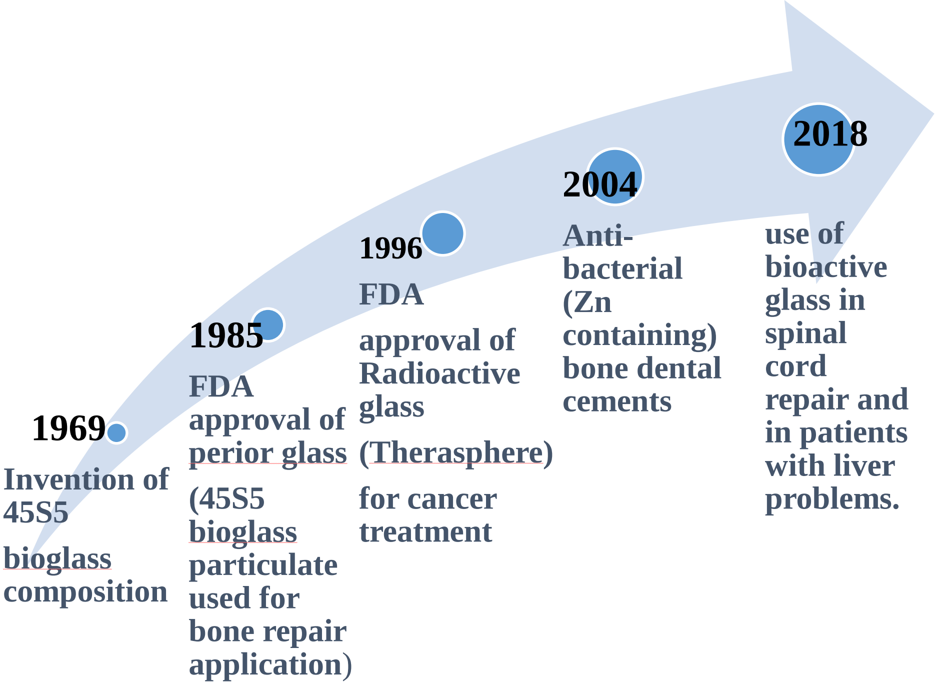

Figure 1.5 Progression of applications of biomaterials with the corresponding years.

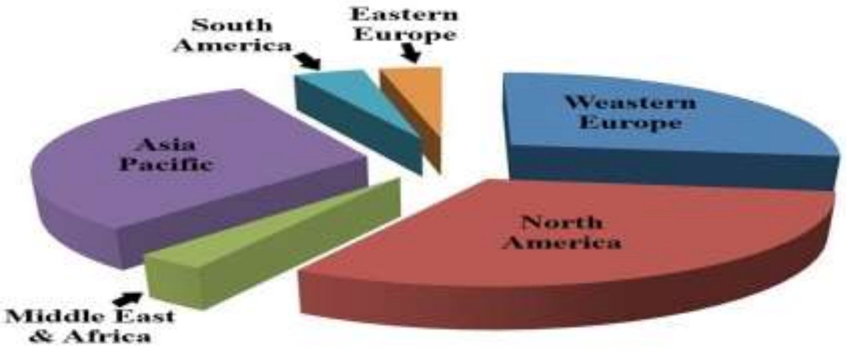

Figure 1.6 Chronological analysis of the applications of bioglass in worldwide.

1.2 What factors led to using Transition Metal substitution for various bioglass?

Transition metals, known for their ability to exist in different oxidation states and their coordination chemistry, have long fascinated researchers in the study of bioinorganic chemistry because of their essential functions in biological systems. The study of the role of transition metals in biological processes began with the ground breaking research of biochemists Hugo Theorell and Fritz Lipmann in the mid-20th century. In recent decades, there has been a significant and rapidly growing importance of transition metals in many industrial, agricultural, and medicinal technologies (Bernot et al., 2006; Hayes, 2008; Ayanda and Adekola, 2011; Rehder, 2016).

Transition metals are a group of elements with distinct chemical characteristics essential for biological systems. Their capacity to engage in redox reactions, interact with biomolecules, and facilitate various biochemical activities highlights their importance in vital biological processes. The complex interaction between transition metals and biological molecules provides the basis for many essential processes in living beings. Recent research has extensively investigated the functions of transition metals in biological systems, revealing their vital roles in physiological processes such as oxygen transport, cellular respiration, and DNA synthesis.

Transition metal ions function as cofactors in several enzymes, altering their catalytic activity and aiding in substrate binding. In addition, metallic proteins, which use the distinct characteristics of transition metals, have significant functions in signal transmission, gene regulation, and immunological response. Understanding the basic principles that regulate the interactions between transition metals and biological molecules has great potential for several scientific fields. The study of transition metal biology is a source of inspiration for ground significant advances, ranging from understanding the molecular underpinnings of illnesses to creating new medicinal medicines and biomimetic catalysts. Laxmi et. al. (2019) explore the coordination chemistry of transition metals, their functions as cofactors in enzyme processes, the structural characteristics of metal proteins, and the significance of transition metal biology in relation to health and illness. In this investigation, we want to elucidate the intricate relationship between transition metals and biological systems, which will facilitate future advancements and breakthroughs in both basic scientific knowledge and practical research. All these properties led us to select transition metals for substitution in the following bioactive glasses to increase their bioactivity as well as biocompatibility to the human body tissues. Here in present work we used the transition metal oxides as doping are like V_2O_5, TiO_2, ZrO_2 and ZnO due to their better strength and lower thermal coefficient propert.

LITERATURE REVIEW:

1.3 Bioactive Materials:

A bioactive material is a substance that triggers a targeted biological reaction at the point where it interacts with the material. This reaction creates a connection between the surrounding tissues and the material (Hench et. al. 1994). More specifically, a bioactive material is a substance that, when inserted into the body, triggers specific surface reactions, creating a hydroxyl-carbonate apatite (HCA) layer. This layer establishes a strong connection with hard and soft tissues (Kokubo et al., 2006).

The bioactivity level of a certain bioactive substance may be correlated with the duration required for over 50% of the contact to become bound. The bioactivity index (Ib), as established by Hench (Hench et al. 1993), is defined as in equation (i):

$$\mathbf{Ib} = \frac{\mathbf{100}}{\mathbf{t0.5bb}} \ldots\ldots\ldots\ldots\ldots\ldots (\mathbf{i})$$

Where $t_{0.5bb}$ is the time required for more than 50% of the implant interface to be bonded to tissues.

The bonding strength and bonding duration depend upon the bioactive material's nature, the bonding process, and the thickness of the bonding zone. The key attribute of a bioactive substance is its capacity to form chemical or biological bonds at the interface. According to the specific kind of biochemical interaction that occurs at the interface, bioactive materials may be categorized into two groups: Class A, which refers to materials that promote bone formation (osteoproductive materials), and Class B, which refers to materials that support bone growth (osteoconductive materials) (Hench et al., 1994). Osteoproduction, as described by Wilson et al. in 1994, refers to the process in which osteogenic stem cells, present in the defect environment, colonize a bioactive surface after surgical intervention. Class A bioactivity refers to the ability of a substance to induce both intracellular and extracellular responses at its contact. Class B materials, known as osteoconductive materials, only trigger an extracellular response at their contact (Cao et al., 1996).

1.3.1 Material selection criteria for biomedical applications:

When selecting a biomaterial, it is common practice to consider two aspects: the first is a functional need, and the second is the possibility of interaction with the tissue next to the biomaterial. The functional requirements consist of the physiologic role that will be replaced by the implant, as well as the amount of time that will be necessary to attain that performance. The link between the body and the implanted material has to be explored from two different points of view: the impact of the human environment on the characteristics of the material, the effect of the samples, and any deterioration of the body's internal and systemic physiology (Kutz, 2002).

In Table 1.1 we can observe various factors regarding the selection of biomaterials for different medical applications. Other aspects beyond these include the medical/surgical treatment, the duration of application, and the cost.

Table 1.1 Numerous factors to consider while selecting materials for biomedical applications **(Ramakrishna et al.)**.

Factors	**Description**		
	Chemical/biological characteristics	**Physical Characteristics**	**Mechanical/Structural characteristics**
1st Level material properties	Chemical composition (bulk and interface)	Density	Elastic Modulus Poisson's ratio Yield strength Tensile Strength Compressive Strength
2nd Level material properties	Adhesion	Surface topology (texture and roughness)	Hardness Shear Modulus Shear Strength Flexural Modulus Flexural Strength
Specific Functional requirements **(based on application)**	Biofunctionality (non-thombogenic, cell adhesion etc. Bioinert (non-toxic, non-irritant, non-allergic, non-carcinogenic, etc.) Bioactive Biostability (resistant to corrosion, hydrolysis, oxidation etc.) Biogradation	Form (solid, porous, coating, film, fibre, mesh, powder) Geometry Coefficient of thermal expansion Electrical conductivity Colour, aesthetics Refractive index Opacity or translucency	Stiffness or rigidity Fracture toughness Fatigue strength Creep resistance Friction and wear resistance Adhesion strength Impact strength Proof stress Abrasion resistance
Processing & Fabrication	Reproducibility, quality, sterilizability, packaging, secondary process ability		
Characteristics of host	tissue, organ, species, age, sex, race, health condition, activity, systemic response		

1.3.2 Solution employed to assess in vitro bioactivity (SBF):

The significance of evaluating biological activity in vitro before conducting in vivo tests is known. In vivo studies need creature penances, which are more exorbitant and difficult to replicate, and furthermore include moral problems. Because of such problems, it is important to do screening in synthetic or organic laboratories in vitro is necessary before assessing the bioactivity of the samples in vivo. The arrangement employed to recreate in vitro reactions occurring on the biomaterial's surface is crucial. Bioactivity in vitro before conducting in vivo tests is known. In vivo studies in 1991, Kokubo suggested that for an artificial material to adhere to living tissues, it must generate hydroxyl carbonate apatite (HCA) on its surface when implanted in the body. Furthermore, Kokubo proposed that this formation of HCA can be replicated in a simulated body fluid (SBF) with ion concentrations that closely resemble those found in human blood plasma (Table 1.2). The SBF produced by Kokubo exhibits variations in some ions compared to human blood plasma (Kokubo et al., 2006). Other studies have attempted to rectify this disparity by producing simulated body fluid (SBF) using different compositions. Oyane et al. developed a modified simulated body fluid (r-SBF) by adjusting the concentrations of CI^- and HCO_3^- ions to match those found in human blood plasma. Nevertheless, calcium carbonate exhibited a pronounced inclination to precipitate from r-SBF. Takadama and Kokubo et al. 2008 suggested a modified simulated body fluid (n - SBF) where only the concentration of Cr ions was raised. The stability and repeatability of this n-SBF are comparable to Kokubo's SBF.

Table 1.2 Ion Concentration (mM/L) of simulated body fluid (SBF) and human blood plasma.

Ion	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**HCO_3^-**	**Cl^-**	**HPO_4^{2-}**	**SO_4^{2-}**
Simulated Body Fluid	142	5.0	2.5	1.5	4.2	148	1.0	0.5
Human Blood plasma	142	5.0	2.5	1.5	27.0	103	1.0	0.5

1.3.3 Hydroxyapatite:

It is a crystalline calcium phosphate compound that closely resembles the mineral found in bone. The compound in question has a well-defined crystallographic structure and a precise composition, denoted as $Ca_{10}(PO_4)_6(OH)_2$.

Hydroxyapatite is the commonly used term for the mineral component of human bone, known as biological apatite. There is a distinction between biological apatite and pure hydroxyapatite, and it is more accurate to label it as carbonate apatite (Hench et al. 1993). Therefore, the optimal molar ratio of calcium to phosphorus in pure hydroxyapatite (1.67) is somewhat different from that seen in biological apatite (1.72-1.80) (Ylanen 2000). Researchers have been motivated to actively replace ions in apatite to affect its characteristics and behaviour based on the potential for enhancing apatite by altering its composition (Osaka et al. 2007). The primary objective of these investigations has been to improve osseo-integration between the implant and surrounding tissue.

Hydroxyapatite may be synthesized in either compact or porous structures. The surface chemistry of porous sintered and cemented hydroxyapatite is identical to that of the dense forms (Hench et al. 1993). Nevertheless, the reaction of tissue to porous hydroxyapatite implants is fundamentally distinct from its response to dense hydroxyapatite due to the potential for development. Consequently, porous hydroxyapatite has superseded the thick form of hydroxyapatite. Upon insertion of porous hydroxyapatite into bone defects, the extent of bone infiltration into the pores has varied between 18% and 74% (Holmes et al., 1988; Martin et al., 1993). The implant's porosity gap is unlikely bone-filled, as shown by Hulshoff et al. (1997) and Rosen et al. (1991). Porous hydroxyapatite has osteoconductive properties, is biocompatible, and exhibits high inertness. Over time, it undergoes resorption, but the rate of disintegration is relatively modest (Ikeda et al., 1999). Porous hydroxyapatite is limited to non-loading areas because of its inherent brittleness. Synthetic hydroxyapatite is often used in dental, craniofacial, and orthopaedic surgery, primarily as granules and as a bioactive coating on load-bearing implants, among other applications.

Materials used as medicine were reported to be recognized after the first successful aseptic surgery by Dr. Joseph Lister in the 1860s (Kuhn, 2014; Worboys, 2013; Cartwright, 1965; Stanton, 2012). However, the biomaterials used in biomedical applications in recent times were not known to people about 80 years ago (Ratner, 2004), yet some materials in the form of crude biomaterials with poor results have been reported to be used (Ratner, 2004).

Table 1.3 The application of silica-based bioactive glasses in medical fields **(Arcos et al. 2010)**.

Material form	**Clinical Applications**
Particulates	- Bone tissue replacement in periodontal diseases - Soft tissue augmentation in paralysis of vocal cords.
Particulates by injection	- Urological tissue augmentation
Particulates and autologous bone	- Maxillofacial reconstructions - Spine
Solid Shapes	- Ossicle replacement in the middle ear - Curved plates for restoring eye orbit floor - Cone shaped devices for jaw defects filling. - Soft tissue sealing for transdermal implants.

1.3.4 Bioactive Glasses:

Most of the available literature on bioactive glasses focuses on materials mainly composed of silica. Over the last several decades, silica-based bioactive glasses have effectively addressed various bone abnormalities and soft tissue therapies (Hench et al., 1993). Due to their excellent biocompatibility and the beneficial biological effects of their reaction products, whether leached or generated at the surface, silica-based bioactive glasses have been highly regarded as bioactive materials during the last four decades (Arcos et al. 2010).

However, the inadequate mechanical qualities of these bioactive glasses have significantly restricted their potential for use in many therapeutic applications. Diverse clinical applications need various kinds of bioactive glasses.

Table 1.3 provides examples of clinical applications using silica-based bioactive glasses. In addition to their ability to attach to live tissues, studies have shown that fine powders of silica-based bioactive glasses possess antibacterial properties when exposed to bacteria (Allan et al., 2001; Stoor et al., 1998). These bioactive glasses provide extra advantages in tissue replacement due to this characteristic.

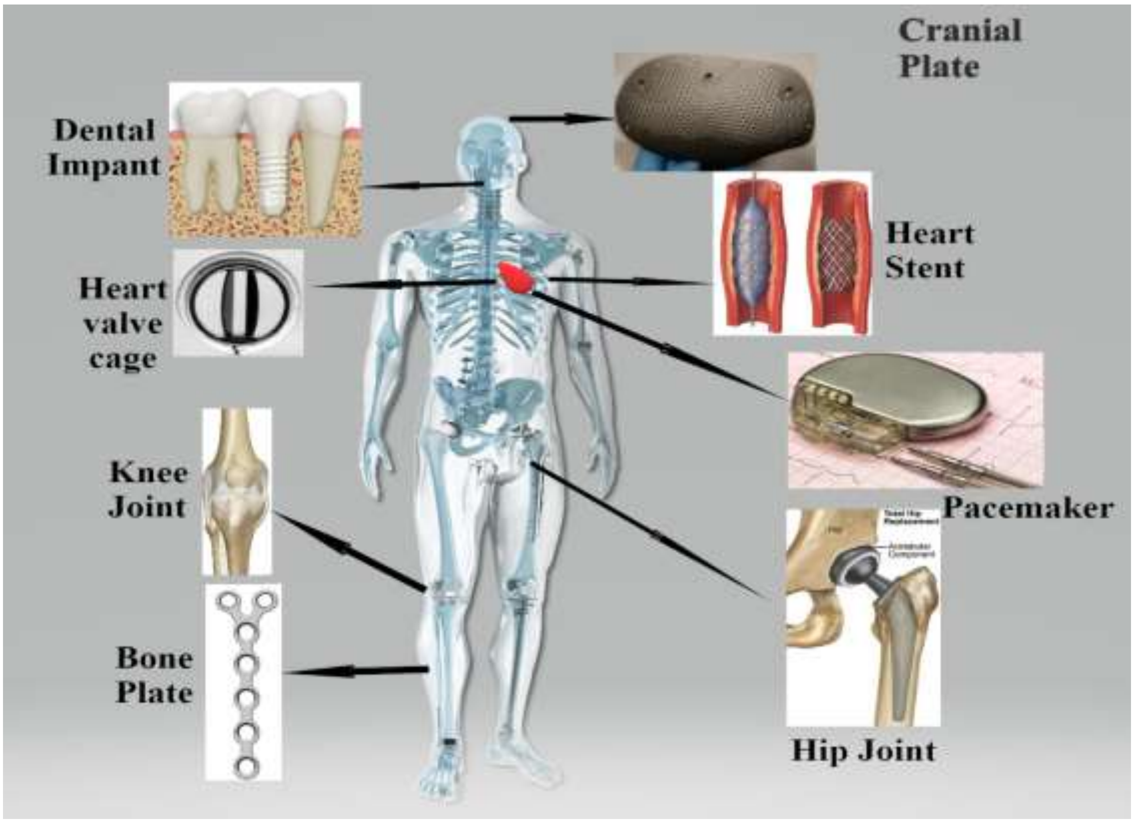

Figure 1.7 Pictorial representation of bioactive materials surgical implant uses in different human body parts.

Figure 1.7 depicts the application of bioactive materials in various bodily parts. The image of the instruments and gadgets is presented in the figure, showing the part where they are being used. These are utilized as surgical implants in different human body parts to treat a number of diseases.

Hench et al. (1991) identified three distinct compositional characteristics that differentiate bioactive glasses from conventional SiO_2 - Na_2O - CaO glasses:

1. The weight percentage of SiO_2 is between 40% and 60%.
2. There is a high concentration of Na_2O and CaO.
3. The CaO/P_2O_5 ratio is high.

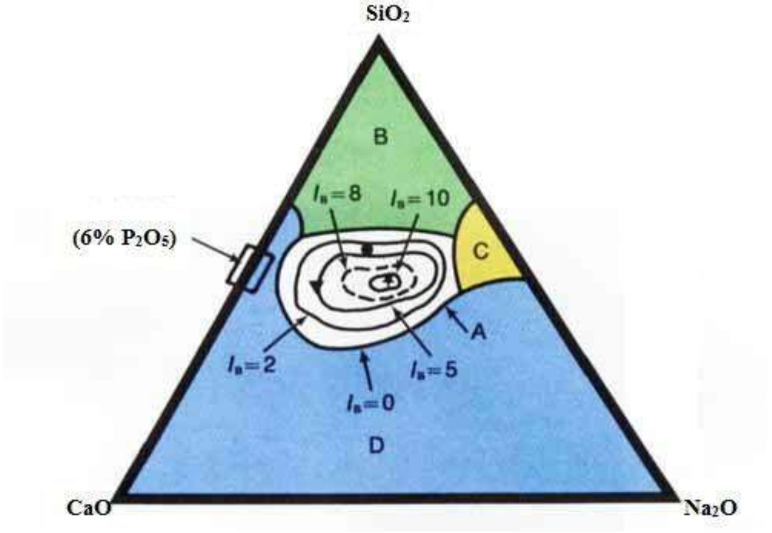

Figure 1.8 Bioactive regions in the CaO-SiO_2-Na_2O system.

Figure 1.8 shows the bioactive regions in case of CaO-SiO_2-Na_2O system. If the weight percentage of SiO_2 exceeds 60%, the amount of bridging oxygen ions significantly diminishes the pace at which the glass network dissolves, resulting in a decline in bioactivity. Nevertheless, a SiO_2 level below 40% by weight will completely dissolve monomeric SiO_2-4 units. The feasibility of achieving a glass phase for this combination is uncertain (Ducheyne 1987). Hence, to demonstrate bioactivity, the weight percentage of SiO_2 in the glass should range from 40% to 60%. The primary constituents in most silica-based bioactive glasses are SiO_2, Na_2O, CaO, and P_2O_5. Previously, it was postulated that P_2O_5 was necessary for a glass to exhibit bioactivity. However, it was subsequently shown that phosphate in the glass facilitates the formation of the calcium phosphate phase on the surface. Phosphate is not essential since the surface can attract and hold phosphate ions from the solution (Hench et al. 1993).

The tissue bonding of silica-based bioactive glass is believed to occur by creating a hydroxy carbonate apatite (HCA) layer on the glass surface upon contact with bodily fluids. Although some specifics of the chemical and structural alterations are not exact, it is widely accepted that the formation of the HCA layer occurs due to a series of reactions taking place on the surface of the bioactive glass implant, as Hench explained (1998).

Gradual crystallization of ACP turns to crystalline HCA (hydroxy carbonate apatite). The reactions are as follows:

(i) Ion exchange: $Si\ \text{-}O\text{-}Na^+ + H^+ + OH^- \rightarrow Si\text{-}OH + Na^+$ (solution) $+ OH^-$

(ii) Formation of silanols: $Si\text{-}O\text{-}Si + H_2O \rightarrow Si\text{-}OH + OH - Si$

(iii) Condensation and polymerization:

$OH\text{-}Si\text{-}OH + OH\text{-}Si\text{-}OH \rightarrow OH - Si - O - Si - OH + H_2O$

(iv) Accumulation of species (i.e., Ca^{2+} and PO_4^{3-}) on SiO_2 rich layer:

$Ca^{2+} + PO_4^{3-} \rightarrow Ca_3(PO_4)^2$

(v) Crystallization of ACP by incorporation of OH^-, CO_3^{2-} anions from the solution to form a crystalline HCA layer:

$Ca_3(PO_4)^2 + OH^- + CO_3^{2-} \rightarrow Ca_{10}(PO_4)_6\text{-}x(OH)_2(CO_3)x$

The HCA layer present on the surface of silica-based bioactive glass closely resembles biological apatite, with a chemical composition as shown below (Hench et al. 1993):

The creation of an HCA layer initiates a series of actions that seem to be linked to the bonding process with tissues (Hench 1998).

1. The adhering of living things to the porous SiO_2-rich and HCA layer.
2. Function of macrophages.
3. Stem cell attachments.
4. Stem cell differentiation.

5. Matrix generation.
6. Matrix mineralization.

The compatibility of silica-based bioactive glass with living organisms has been well-established significantly (Wilson 1985). Following implantation, silica-based bioactive glass experiences breakdown, releasing alkali ions such as Na^+ and Ca^{2+}. Silicon, most likely in the form of $Si(OH)_4$, is also liberated by dissolving processes throughout the deterioration process. A study by Lai et al. 2002 investigated the release of Si from silica-based bioactive glass implanted in a live organism to discover the specific mechanism of Si release. Through the analysis of urine and blood samples taken seven months after the implantation and by conducting chemical and histopathological examinations of bone and various tissues, it was discovered that the silicon (Si) released from the degradation of the 45S5 bioactive glass was safely excreted in a soluble form through urine. Phagocytes ingested and expelled the SiO_2-rich layer, which had an amorphous structure

1.3.5 45S5, 1393 and 1393B3 bioactive glass:

The very first bioactive glass, known as 45S5 bioactive glass, was created by Hench et al. in 1993. It comprises 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5 (wt%). This silica-based bioactive glass has exceptional bioactivity and can quickly bond with soft tissues. The bioactive glass known as 13-93 is composed of 53% SiO_2, 6% Na_2O, 12% K_2O, 20% CaO, 5% MgO, and 4% P_2O_5 (wt%). It is derived from the composition of 45S5 bioactive glass but has a higher concentration of SiO_2 and includes additional network modifiers like K_2O and MgO. This glass is also utilized in clinical applications (Rahaman et al., 2011). Recent studies have shown that specific borate-based glasses, such as those with a composition of 53% B_2O_3, 10.3% Na_2O, 28% K_2O, 18% CaO, 10.2% MgO, and 6% P_2O_5, as well as those with a composition of 56.6% B, 0% Na_2O, 5.5% K_2O, 11.1% CaO, 4.6% MgO, and 3.7% P_2O_5, exhibit bioactive properties (Rahaman et al., 2011). Borate-based bioactive glasses show higher degradation rates and undergo complete conversion into a substance resembling hydroxyapatite (HA) than silica-based bioactive glasses due to their lower chemical endurance. In vitro studies have shown that bioactive glasses containing borate promote the growth and specialization of cells, while in vivo studies have shown that these glasses facilitate the infiltration of tissues. Borate-based bioactive glasses have demonstrated their ability to function as a surface for medication delivery in managing bone infection. An issue related to borate-based bioactive glass is the potential toxicity of boron that is released into the solution in the form of borate ions (BO_4^-).

Manipulating the composition of silica-based bioactive glasses has been shown to provide control over their disintegration rate, as evidenced by recent research. As an illustration, one may create a borosilicate bioactive glass by substituting a portion of the SiO_2 in silica-based bioactive glasses with B_2O_3. An example of such a glass is the 45S5 B15 bioactive glass, which has a composition of 30% SiO_2, 15% B_2O_3, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5 (wt%). Another example is the 13-93B1 glass. The composition comprises 34.4% SiO_2, 19.9% B_2O_3, 5.8% Na_2O, 11.7% K_2O, 19.5% CaO, 4.9% MgO, and 3.8% P_2O_5 (wt%). By adjusting the composition, the degradation rate may be controlled within a broad range. The manufacturability and controllable degradation rate of silica-based bioactive glasses render them highly advantageous for facilitating tissue regeneration. By manipulating the composition of the glass, it is feasible to align the degradation rate of silica-based bioactive glass with the rate of tissue regeneration.

Based on the production process, bioactive glass may be categorized into two primary classes: sol-gel bioactive glasses and melt-derived bioactive glasses. Nevertheless, melting is a straightforward and cost-effective method that requires less time than sol-gel processing. The melting procedure is very suited and dependable for producing a substantial quantity of bioactive glasses. Due to these advantages, the melting technique is the primary method for manufacturing bioactive glasses.

Even so, the HCA may exist in other configurations when calcium is mixed with other elements, such as $[(Ca, M)_{10} (PO_4, CO_3, Y)_6 (OH, F, Cl)_2]$ (where M represents metals like Sr, Fe, Zn, etc.). Aside from Hench glass (45S5), the 1393 and 1393B3 bioactive glasses are often used to reconstruct and regenerate soft and hard tissues. While 45S5® and 1393 are silicate-based bioactive glasses, the 1393 bioglass has a greater silica concentration, two more network modifiers, and specific unique programmed characteristics. The composition of 1393 glass is generally 53% SiO_2, 20% CaO, 6% Na_2O, 4% P_2O_5, 12% K_2O, and 5% MgO by weight compared to the 45S5 material. The 1393 bioglass does not readily undergo crystallization despite its glassy condition since it is less inclined to crystallize during the sintering process. The preservation of the glassy state in 1393 is attributed to the inclusion of MgO inside the glass structure. However, 1393 may be readily transformed into particles or fibres of various lengths without undergoing devitrification, and it exhibits superior bioactivity compared to 45S5® (Fu et al., 2008). According to the literature, 1393 bioactive glass is often used in clinical studies and is considered one of the most extensively used bioglasses (Baino et al., 2018). The bioglasses known as 1393 and 45S5® have received approval for in vivo usage in Europe and the US, as stated by Rahaman et al. in 2011. The 1393B3, sometimes called cotton candy and commercialized under the names DermaFuse™/ Mirragen™, has remarkable wound healing capabilities (Baino et al., 2018). The term "cotton candy" was coined after a certified nurse named Peggy Taylor, who specialized in wound care, mistakenly applied 1393B3 to a persistent lesion because it resembled cotton (Wray, 2011). Remarkably, the persistent wound was successfully cured by using the 1393B3 bioglass. B_2O_3 serves as the primary component for the glass structure in borate-based 1393B3 glass, similar to how SiO_2 functions in silicate glasses. Although both substances act as network formers, both vary in terms of their viscosity in a molten state and their inclination to crystallize.

Borate glasses exhibit more easily flowing viscous behaviour compared to silicate glasses at their liquidus temperature. As a result, they have a greater propensity to undergo devitrification compared to glasses based on silicate (Schmelzer et al., 2015). Both glasses have distinct compositions and characteristics, resulting in diverse areas of use. The chemical composition of 1393B3 is as follows: B_2O_3-56.6%, CaO-18.5%, Na_2O-5.5%, MgO-4.6%, K_2O-11.1%, P_2O_5-3.7% (by weight) (Rahaman et al., 2011). The 1393B3 glass exhibits reaction kinetics similar to those of 45S5 and undergoes conversion to glass ceramics upon sintering (Fu, 2009). Contrary to the silicate-based 1393, the borate 1393B3 can only be readily transformed into glass fibres and scaffolds if it undergoes controlled heat treatment to turn it into a crypto-crystalline form (Jung and Day, 2009). 1393B3-a borate-based material is regarded as a critical bioceramic for tissue engineering applications due to its quicker breakdown and conversion to bone minerals (HA; $Ca_{10}(PO_4)_6(OH)_2$) (Huang et al., 2007). In addition, the 1393B3 glasses do not produce any residual layer rich in silica (similar to silicate glasses) when transformed into bone minerals (Bi et al., 2013; Yao et al., 2007). Boron in sealing glasses reduces viscosity and improves wettability on steel surfaces by delaying crystallization (Borhan, Gromada, et al. 2016). Adding substantial levels of B_2O_3 to silica reduces thermal characteristics due to weaker B-O bonds compared to Si-O bonds (Coillot, Méar et al. 2012). This also reduces the inclination for crystallization. Thermal characteristics such as TEC have been shown to be reliant on the B_2O_3/SiO_2 ratio in the composition (Sohn, Choi et al. 2004, Tulyaganov, Reddy et al. 2013). The relationship between TEC and the B_2O_3/SiO_2 ratio is complex due to the existence of additional oxides in multi-component oxide glasses or compositions, making it difficult to predict. Henao et al., 2019 discussed the SBF immersion dissolving behaviour effect with silicate as well as borate glasses, as shown in Figure 1.9. He later explained the ion exchange phenomena in SBF and alkali ions during the immersion process as shown in Figure 1.10.

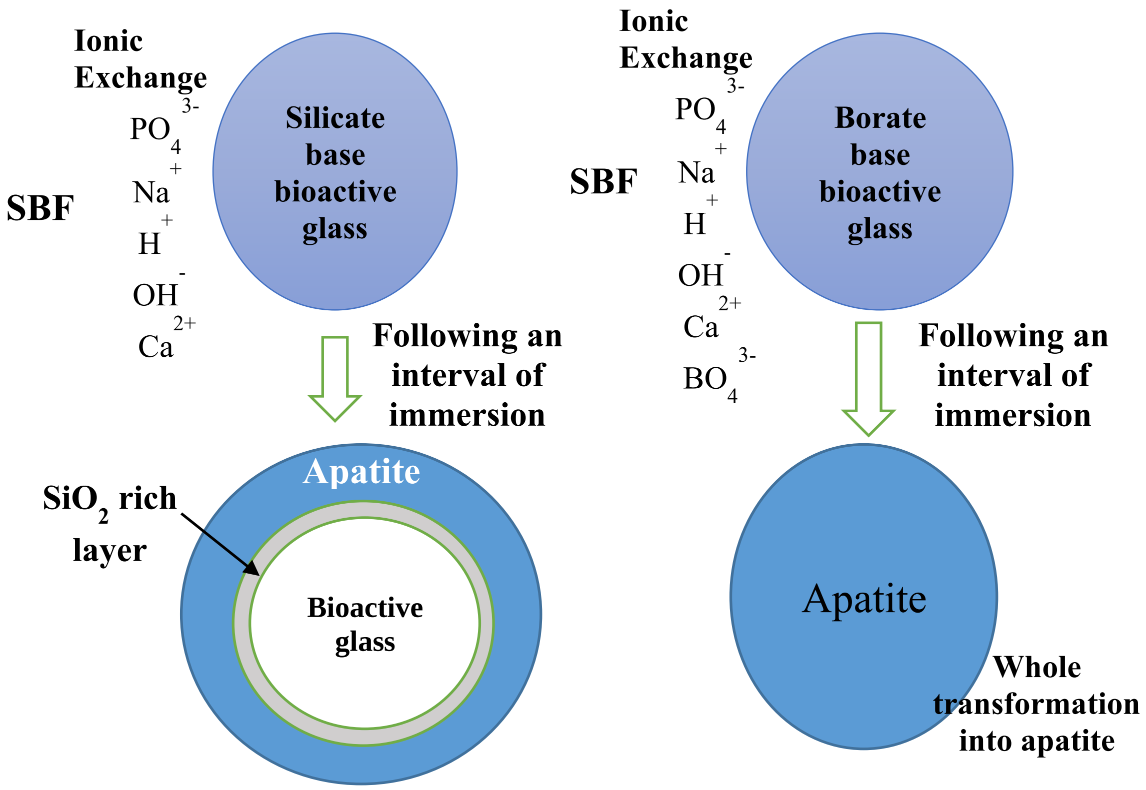

Figure 1.9 Schematic illustration of the dissolving behaviour of silicate and borate bioactive glasses in simulated bodily fluid (Henao et al. 2019).

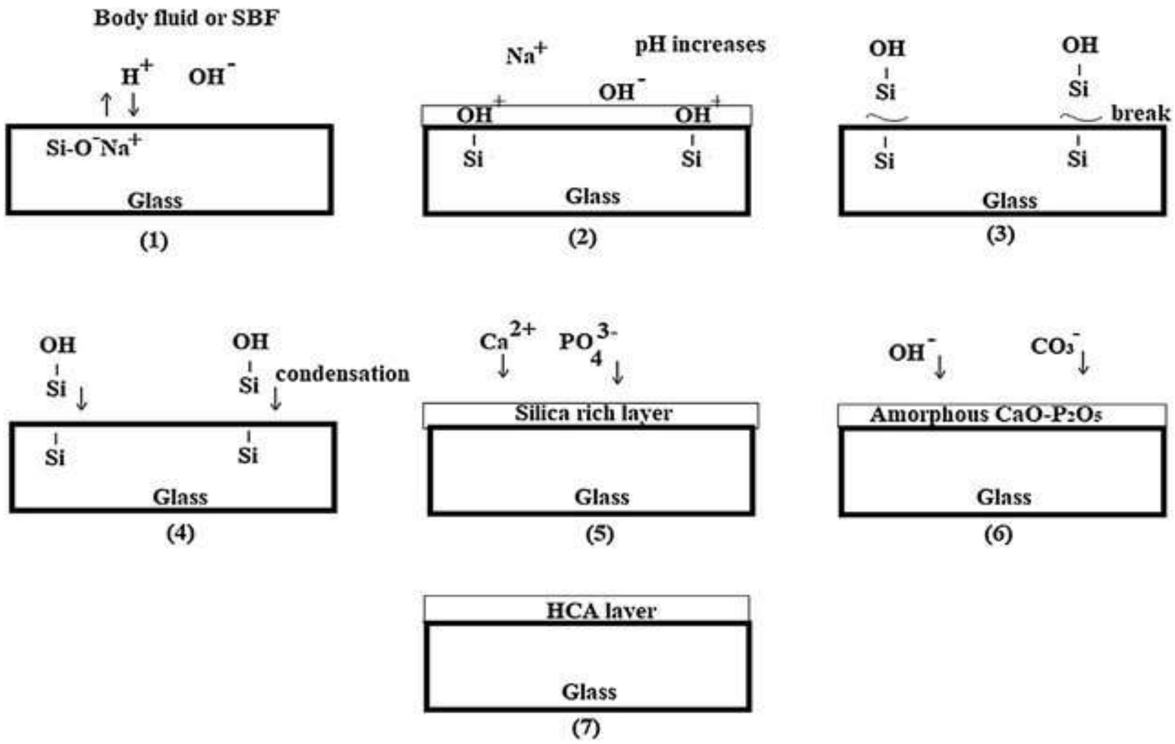

Figure 1.10 Schematic representation of the formation of hydroxy carbonate apatite (HCA) on the surface of bioactive glasses.

The in-vitro study conducted on 1393-B3 glass samples, which included transition metals, revealed that the material did not hinder the development and survival of human osteosarcoma cells (MG-63) when used at low concentrations. The components were well-tolerated by human red blood cells, as determined by a hemolytic experiment. This study assessed the characteristics of transition metal therapeutic ions (titanium, zirconium, and vanadium) when integrated into 1393 or 1393B3 glass-based samples. This evaluation aims to determine their potential as biomaterials in the future.

Here, in my thesis study, we have used three different types of glasses for doing transition metals. 45S5 glass,1393-B3 glass and Borosilicate glass. Till now, we discussed about 45S5 glass and 1393B3 glass and the relationship between them in detail. Here are some aspects of Borosilicate Glasses discussed in detail including the silica-based glasses.

1.3.6 Borosilicate Glass:

Borosilicate glass comprises silica and boric oxide, with trace quantities of alkali and aluminium oxide (Figure 1.12). This glass is chemically durable and thermally resistant, so it will not shatter under fast temperature changes. It is primarily employed in the pharmaceutical and chemical-related sectors, as well as in high-intensity lighting applications. Glasses made from Borosilicate are of technical importance because of their numerous and varied applications. Borosilicate glasses are employed in a variety of weight compositions for various applications. We emphasize multi-component sodium-borosilicate glasses with Si, B, and Na as network formers. Firstly, we should analyze their underlying silicate structure to determine structural modifications in silica-based glasses, such as the ones made from alumina and boron oxide. Following that, we will talk about network formers and modifiers that are incorporated with silicate glasses.

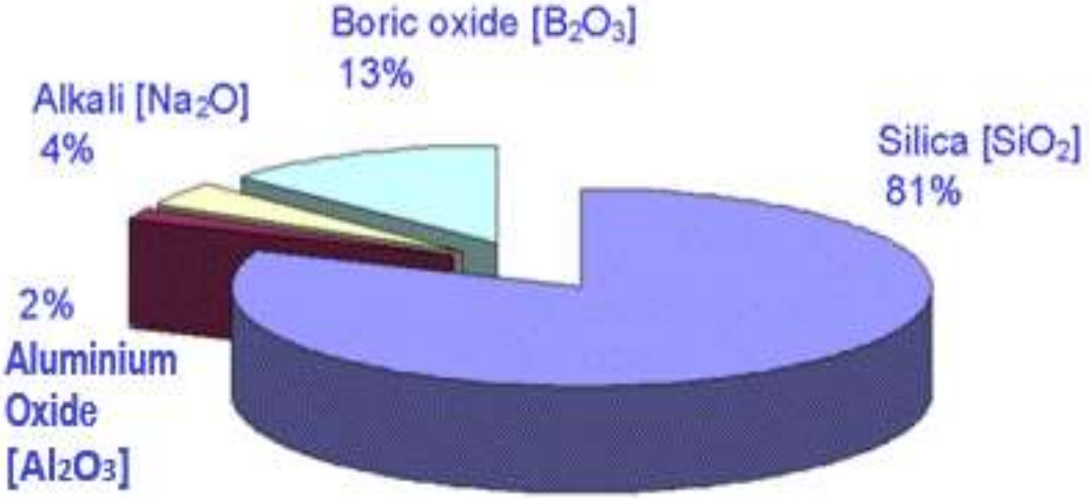

Figure 1.11 Chemical composition of Borosilicate Glass.

1.3.7 Structure of silicate glasses:

The structure of borosilicate glasses may be either amorphous or crystalline. Both of these structures are structures. Figure 1.13 illustrates the distinction between the two kinds of structures that may be found within the category of glasses. In contrast to the crystalline structure, the amorphous structure is deformed compared to the crystalline structure. This is something that we can see since the crystalline structure is found to have a standard structure across the atom.

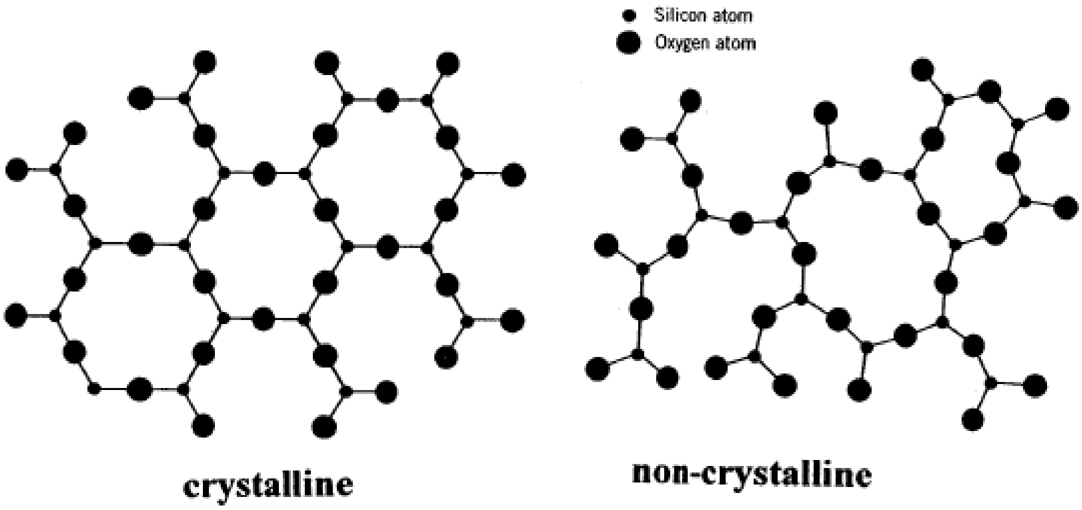

Figure 1.12 Comparison between crystalline and non-crystalline (amorphous) silica structure.

The simplest basic silicate glass structure uses SiO_2 as the network former. Common modifiers include alkaline earth oxides like CaO or BaO and alkali oxides like Na_2O. Silicon produces SiO_4 tetrahedral, which has four oxygen molecules covalently bonded to the core Si (Figure:1.13a). The tetrahedral forms a network by connecting oxygens (BOs) (Si-O-Si). A silicate melt will break one Si-O-Si bond per unit charge when an alkali or alkaline earth modifier cation is introduced. This will result in four coordinated silica and an NBO ion at the SiO_4 tetrahedral, as shown in Figure 1.13(b) (Shelby 2005). Nevertheless, a glass made of silica exhibits a more porous arrangement as a result of the presence of non-bridging oxygen ions. The open structure of the silica-based glass is created by the disruption of the network structure caused by the presence of network modifiers, such as Na^+, K^+, and Ca^{2+} (Figure:1.13b).

Strand (1996) proposed that the average quantity of non-bridging oxygen ions present in the silica tetrahedron determines the bioactivity of glass. The oxygen ion in the corner is balanced by a network modifier anion, such as Na^+, K^+, or Ca^{2+}, rather than sharing a corner with another tetrahedron. This is seen in this figure that each silicon atom in silica-based glass forms four chemical bonds with oxygen atoms. Hence, the tetrahedron may contain any number of non-bridging oxygen ions ranging from 0 to 4. The number 0 denotes a crystalline structure of SiO_2, often known as quartz glass. On the other hand, the numeral 4 signifies the presence of a dissolved SiO_4^{4-} ion. In order for a silica-based glass to exhibit bioactivity, the quantity of non-bridging oxygen ions per tetrahedron must exceed 2.6, as stated by Ylanen in 2000. Conventional silica-based glasses typically include over 65% SiO_2 by weight, less than 15% Na_2O by weight, and around 10% CaO by weight. The composition of silica-based bioactive glasses differs from that of ordinary silica-based glasses, yet bioactive glasses have a resemblance to them. Bioactive glasses often have a SiO_2 content of less than 60% by weight and a significant proportion of alkali or alkaline earth oxides.

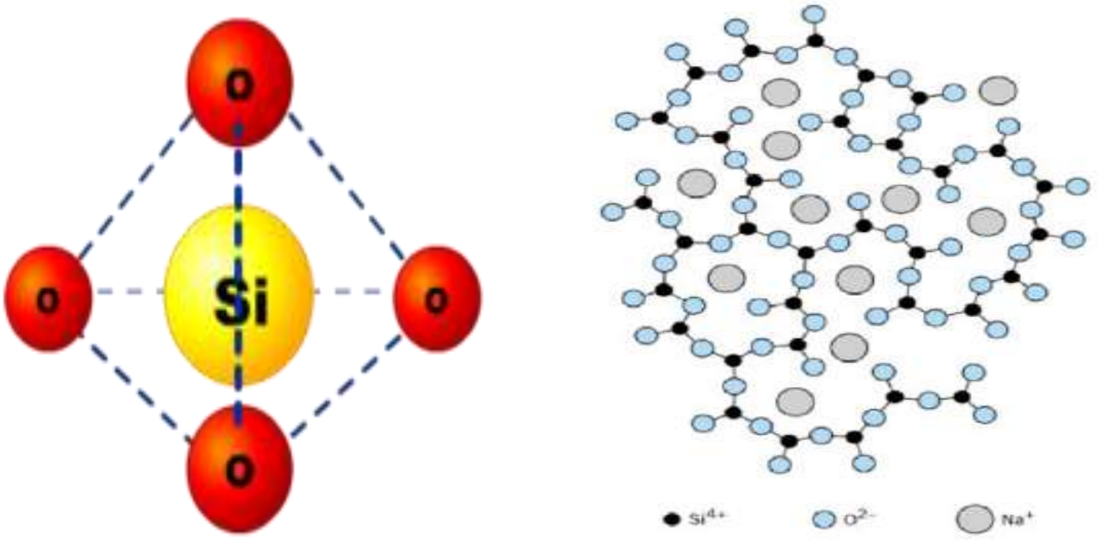

Figure 1.13 (a) Tetrahedral structural unit of silica (SiO_2) **(b)** Effect of introduction of Na+ cations in a silica network.

The main difference between borosilicate glass and silicate glass is that the former develops a layer that is rich in borate, while the latter generates a layer that is rich in silicate. Borosilicate glass transforms into an apatite layer in the same manner as silicate glass. The incorporation of boron into silicate glass results in a reduction in the melting point, a quickening of the transformation into HCA, and an increase in the rate of biodegradation.

Innovative bioactive glasses formulated with borate and borosilicate compositions have shown a superior capacity to stimulate the growth of new bone compared to silicate bioactive glass. Borate-based bioactive glasses have adjustable degradation rates, allowing for a more precise alignment between the bioactive glass implant's breakdown and the new bone growth rate.

1.4 Objective of this work:

This study aimed to formulate and evaluate a group of ceramic-based bioactive glass materials by substituting them with various transition metals, intending to explore their possible use in biological applications. To ensure the continuation of the present work, the following measures were implemented:

(i) The process of generating and evaluating a combination of transition metal substituted bioactive glasses. The composition of the prepared bioactive glasses is following:

(74-X) SiO_2,16Na_2O,10B_2O_3,(X) V_2O_5 (X=0 to 4 wt%)

(53-X) B_2O_3,6 Na_2O,5MgO,20CaO,12 K_2O,4 P_2O_5, X TiO_2 (X=0 to 2.5 wt%)

(53-X) B_2O_3,6 Na_2O,5MgO,20CaO,12 K_2O,4 P_2O_5, X V_2O_5 (X=0 to 2.5 wt%)

(53-X) B_2O_3,6 Na_2O,5MgO,20CaO,12 K_2O,4 P_2O_5, X ZrO_2 (X=0 to 2.5 wt%)

46.1 SiO_2,26.9 CaO,2.6 P_2O_5,(24.4-X) Na_2O, X ZnO (X = 0 to 3 mole %)

(ii) Fabrication and investigation of transition metals like titanium, zirconium and vanadium, substituted borosilicate, 1393-B3 and 45S5 bioactive glasses samples studied.

The solid-state approach was used to synthesize these bioactive glasses, and several characterization techniques were employed to acquire their distinct characteristics.

(iii) Strengthening of these transition metal-substituted bioactive glass systems:

A bioactive glass was strengthened by substituting additional transition metals in varying concentrations. Subsequently, ball milling was used to get a uniform blend of the components.

(iv) Physical and thermal properties measurement:

Density and thermal properties like DSC-TGA are to be determined. DSC-TGA shows the heat flow and weight loss in the corresponding samples.

(v) Study of mechanical characteristics:

Mechanical properties like the compressive strength, flexural strength and elastic properties of glass samples are to be determined. To check its better mechanical properties for bone implant conditions.

(vi) Evaluation of the in-vitro performance of the composites in terms of their bioactivity, pH measurement, and absorption of simulated body fluid (SBF). The capacity for apatite formation, absorption of water, and pH level of bioactive glasses are to be evaluated and compared with a reference material during immersion in SBF.

(vii) The *in-vitro* characteristics are to be evaluated by using X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) along with energy-dispersive X-ray spectroscopy (EDS) techniques.

(viii) Hemolysis and *in-vitro* cell culture characterization, such as cellular proliferation and viability, to be carried out on prepared powdered bioactive glass samples to determine the hemocompatibility and in-vitro cell culture analysis using the MG-63 cell line.

(ix) Polarization process and electrical property evaluation: The samples are being palatalized and separated as ‘N-surface’ and ‘P-surface’. Then, the dielectric property and the bioactivity of the bioactive glass samples are to be studied.

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