Chapter 1 Introduction and Literature review

1.1 Introduction

Ceramics and glasses have been given a wide attention as bioceramics for implants as they have shown highly attractive characteristics for biomedical applications. Bioactive glasses and glass-ceramics have been widely investigated for repair and regeneration of bone defects due to their excellent bioactivities [1]. It has been established that bioactive glasses in the SiO_2–Na_2O–CaO–P_2O_5 system have higher bioactivity in comparison to hydroxyapatite (HA) [2,3]. Hench and Clerk [4] had first demonstrated in 1970s that bioactive glasses are able to bind with bone and to promote bone formation. When bioactive glasses are soaked in physiological fluids or implanted in vivo, they can bind to living bone through the formation of hydroxy carbonate apatite (HCA) layer on their surfaces which is a mineral phase of the bone. The sequence of the reaction steps was reported by Clark and Hench [4]. The main characteristics of the bioactive glasses are their highly reactive nature when it is soaked in simulated body fluid (SBF) or an analogous solution [5][6]. Many bioactive glasses have been developed and most of the bioactive glasses contain both CaO and P_2O_5 as main components which are very essential during the formation of HCA layer [4][6][5]. Moreover, the dissolution of silica simultaneously in the body fluids results in the formation of silanol groups on their surface which are necessary for nucleation and amorphous HA formation [1]. However, the bioactive glasses have critical drawbacks due to the low mechanical properties and this causes limitations to use it in the load bearing areas. For this reason, bioactive glasses are often doped with other ions which possess tough nature and provide excellent surface properties without a great loss in the bioactivity. An addition of oxides, like MgO, Al_2O_3, ZrO_2, CoO and TiO_2 etc., may be

used to control some physical and chemical properties without affecting its bioactivity [7] [8][9]. Moreover, the special thought has been given to the development of multifunctional bioactive glasses which can promote osteogenesis, angiogenesis and antibacterial properties. This is due to the release of therapeutic ions or drug ions into the biological environment. Hence, the bioactive glasses can be substituted with different therapeutic ions such as Sr, Cu, Zn, Ag, Ga and Co, etc., to provide a better controlled release of ions *in situ* and to show the multifunctional behavior [10][11][12].

1.1.1 Regions of bioactive glass composition

Many glasses have been developed and studied in the three components Na_2O-CaO-SiO_2 system containing a constant content of P_2O_5 of 6.0 wt% as shown in **Table 1.1**. The compositional range for bonding of bone to bioactive glasses and glass-ceramic has been shown in **Figure 1.1**. The glasses with the highest level of bioactivity lie in the middle (region A) of the Na_2O-CaO-SiO_2 diagram (assuming a constant 6 wt% of P_2O_5) [1]. The boundaries marked in the diagram are kinetic boundaries not phase equilibrium boundaries. Compositions which exhibit slower rates of bonding lie between 52 to 60% by weight of SiO_2 in the glass. Glass compositions with greater than 60% SiO_2 (region B) do not possess bioactivity and also do not bond with bone. They lie in the composition range of traditional silicate glasses and hence they are called as bio-inert glasses. The scientific basis for the compositional regions is associated with the rate of the surface reactions of bioactive glasses. However, the rate of surface reactions depends also on the presence of the multivalent ions in the glass network.

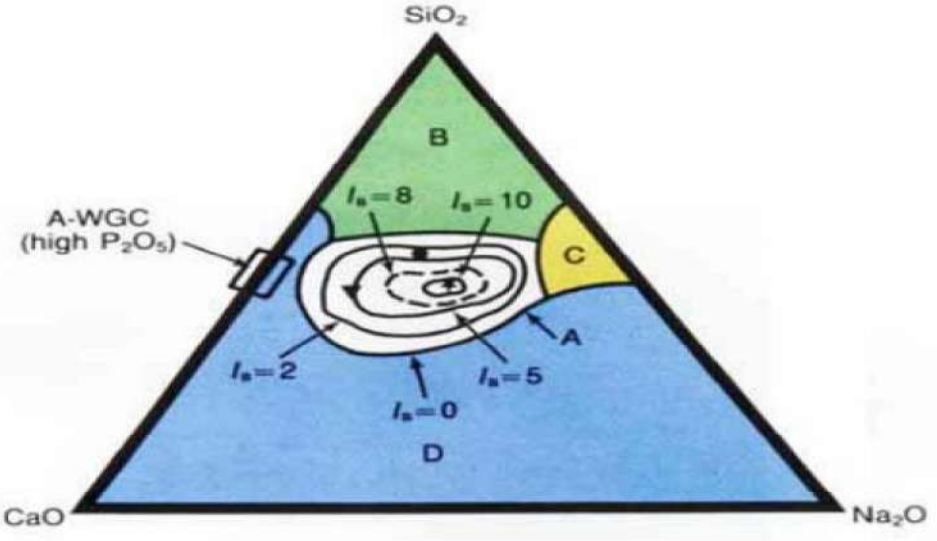


Figure 1.1 Ternary phase diagram of SiO_2-CaO-Na_2O containing constant P_2O_5 [1].

Table 1.1 Some bioactive glass compositions (wt %) containing constant P_2O_5 [1].

Bioglass	SiO_2	CaO	Na_2O	P_2O_5	B_2O_3	F_2Ca	K_2O
45S5	45	24.5	24.5	6.0			
45S5F	43	12	23	6.0		16	
45B15S5	30	24.5	24.5	6.0	15		
45B5S5	40	24.5	24.5	6.0	5		
KCP1	45	24.5		6.0			24.5
45S5-N	50	24.5	19.5	6.0			
45S5-C	50	19.5	24.5	6.0			

The lines indicated in the ternary diagram represent the bioactivity of the glasses. Further, the level of bioactivity is defined as $I_b = 100/t_{0.5bb}$, where $t_{0.5bb}$ is the necessary time period of an implant to bind with the bone as more than 50% of the surface. The bioactive glasses in zone 'A' create an attachment with the bone. In the diagram for the line ($I_b > 8$), the glasses can also bond with the soft tissues. The I_b decreases on moving from the centre and hence the rate of the reaction also decreases. In the zone 'B', the glass compositions behave like almost inert bioceramics whereas

the glasses in the zone 'C' are reabsorbed with time which include between 10 - 30 days. The compounds in the region 'D' did not result any technical interest and therefore they were not found suitable for implant. Therefore, the optimal composition can be chosen based on the functional zones and also essential for rapid bone formation as an implant material.

1.1.2 Types of bioceramics according to tissue attachments

The mechanism of tissues attachment to an implant is directly related to the tissue response at the implant interface as shown in **Figure 1.2** [13]. There are four types of bioceramics given as follows according to types of tissue attachment.

1. Nearly inert bioceramics
2. Porous bioceramics
3. Bioactive bioceramics
4. Resorbable bioceramics

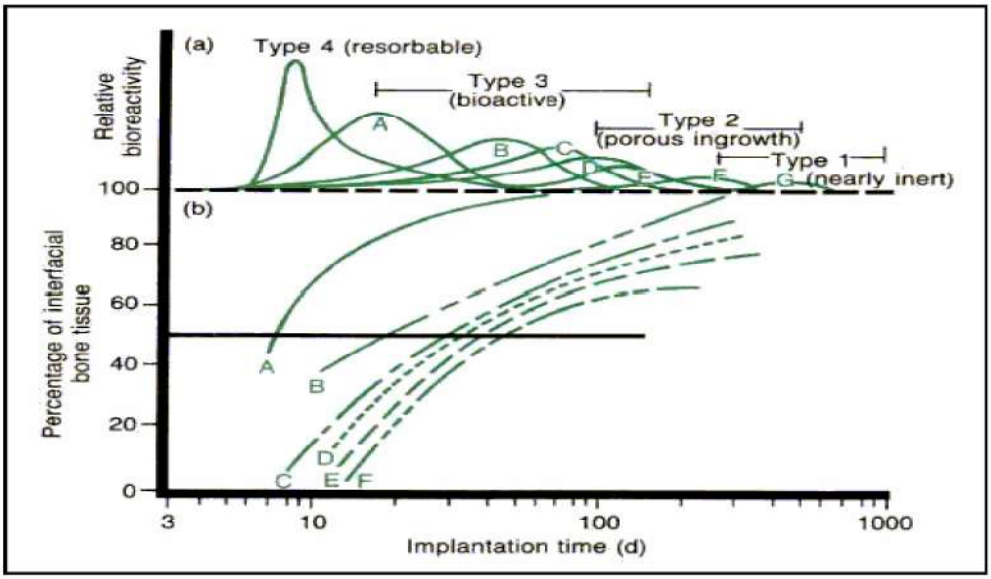


Figure 1.2 The spectrum of bioactivity for various bioceramic implants. (A) The reactivity take place between the implant and the tissue and (B) The time dependence of bone bonding at an implant interface for the different groups of biomaterials [13].

1.1.3 Nearly inert bioceramics:

Nearly inert bioceramics are those which do not form a bond with tissue and non-adherent fibrous tissues around the implant site. This method of attachment of such an implant with tissue is termed as morphological fixation and nearly inert bioceramics are alumina, zirconia and etc., A limitation of this type of implant is that when implanted in the body the interfacial movement occurs. The thicknesses of fibrous tissues around the implant are very less and the implant loosens very quickly [1]. Loosening of implant invariably leads to fracture of the implant material or the adjacent bone.

1.1.4 Porous bioceramics:

Porous bioceramics are which provide in-growth of tissues into its pores. This method of attachment is termed as biological fixation. Mainly, the Porous bioceramics are hydroxyapatite, HA coated metals. The porous ceramics has got its limitations that this type of implant should have a pores size at least 100 micrometers in diameter. Moreover, the larger size of pores is required for interfacial tissue growth but which decreases the strength of implant.

1.1.5 Bioactive bioceramics:

A bioactive is defined as: “a material that elicits a specific biological response at the interface of the material which results in the formation of an interfacial bond between the tissues and the material without any harmful effect to the living tissues” [13]. This method of attachment is termed as bioactive fixation. Bioactive ceramics are such as bioglass®, bioactive glasses, apatite/wollastonite (A/W) glass-ceramics, glass-ceramic, hydroxyapatite, polyethylene-hydroxyapatite composite and bioactive hydroxyapatite coating on porous titanium alloy.

A common characteristic of this type of bioactive implant is the formation of a hydroxyl-carbonate apatite (HCA) layer on their surfaces when implanted in human body. The HCA is an alike in composition and structure to the mineral phase of human bone. The HCA layer grows on the surface of the implant as polycrystalline agglomerates. The collagen fibrils are incorporated within the agglomerates thereby binding the inorganic surface to the organic constituents of tissues. Thus the interface between an implant and tissue is nearly identical to the naturally occurring interfaces between tissue and tendons as well as ligaments.

The following are the four types of bioactive bioceramics:

- Bioactive Glasses.
- A/W glass-ceramics and Phosphates glasses
- Dense Hydroxyapatite
- Prous Hydroxyapatite

1.1.6 Resorbable bioceramics:

Resorbable bioceramic degrades gradually with respect to time and can be restored with natural tissues of the implanted site. Bioresorbable bioceramics are calcium silicate, tricalcium phosphate and calcium phosphate salts. An essential condition for this type of implant material is that the resorption rates of implants must be matched with the repair rates of tissues. They greatly differ in the sense that some implant materials degrade too rapidly and some too slowly.

1.2 Application of Bioceramics

Generally bioceramics are used in the repair or reconstruction of the musculoskeletal system in the human body. Therefore, bioceramic materials are in contact with either hard tissues such as bone or soft tissues such as tendons,

ligaments, muscle, and subcutaneous tissues [1]. In numerous applications a bioceramic may be in contact with hard tissues at one portion of the surface, such as the root of the tooth implant, and also be in contact with soft tissues, like the gingival tissues, at the same implant site etc as shown in **Figure 1.3** [14]. More attention has been paid on bioceramics in the field of biomedical science as an implant in the body. The attachment of implant materials is also an important property of bioceramics. These are called total joint replacement and the attachment of the implant to the remaining bone stock must be stable for the implant to survive. Recently, novel compositions of bioactive glasses and bioactive glass-ceramics have been developed for therapeutic use in medicine. Examples include the use of radioactive bioactive glass beads for the treatment of tumors.

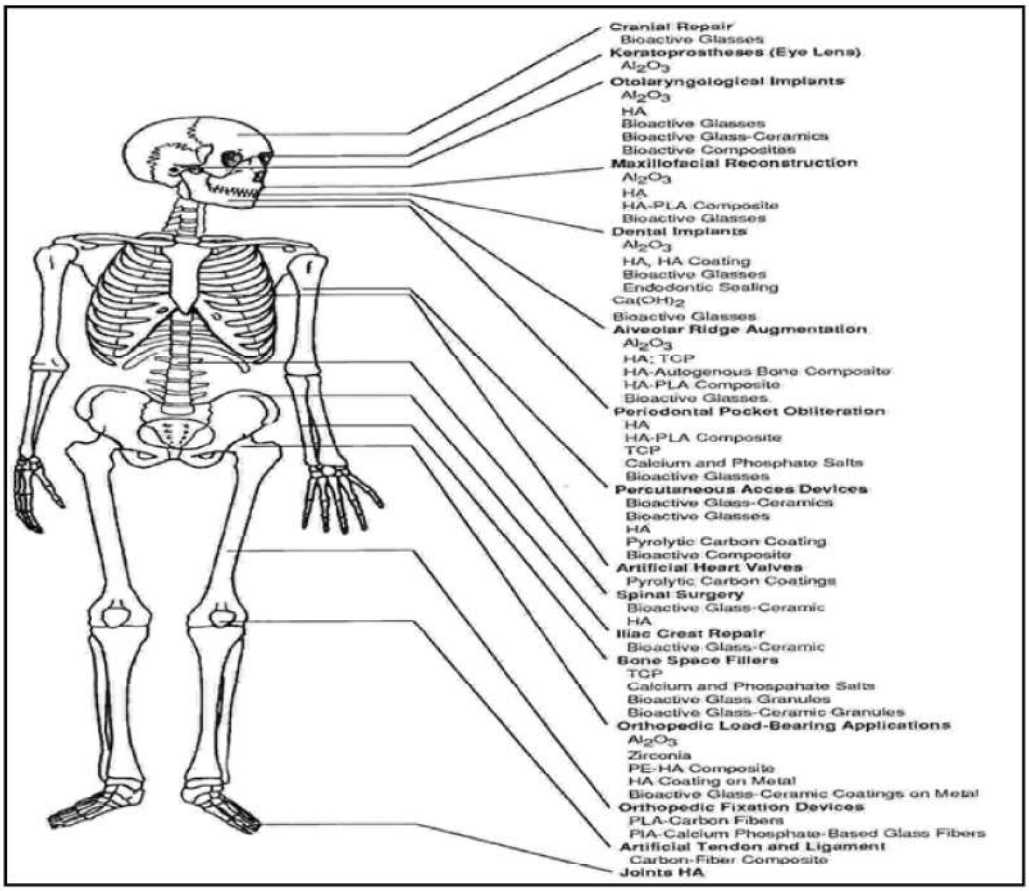


Figure 1.3 Wide range of clinical applications of bioceramics [14]

1.3 Hydroxyapatite:

Apatites are a structural type of compounds having the general formula $M_{10}(XO_4)_6Y_2$ rather than specific compounds where M represents as a cation (M = Ca, Sr, Ba, Zn, Mg, Na, K, etc), (XO_4) represents an anionic group (where the element X=P, Si, B, S etc consitituting phosphate, silicate, borate and sulphate groups etc) and Y represents an anion (Y= OH, CO_3, F, Cl, etc.). Generally, apatites are known to be capable of accommodating a wide variety of modifications and combinations due to substitutions of various ions and groups within the apatitic lattice [15]. Amongst all the apatites, hydroxyapatite (HA) is the most important in biological systems as it is the major component of the bones and teeth. HA is having similar structure and composition like major mineral component of the bone. Its formula is given as $Ca_5(PO_4)_3OH$ or more often $Ca_{10}(PO_4)_6(OH)_2$ and chemical structure is shown in **Figure 1.4**. The Ca/P molar ratio in HA is 1.667 commonly defines the stoichiometry of a hydroxyapatite [16].

In this, Ca^{2+} ions are commonly referred to as Ca (I) and it is coordinated to nine O atoms, with six shorter bonds which define an approximate trigonal prism and three longer bonds capping the prism faces. The Ca-O_9 polyhedra share the trigonal faces to form chains parallel to the c-axis. The remaining six Ca^{2+} ions (6h sites, referred to as Ca (II) or triangular Ca) form two triangular sets at z = 0.25 and Z=0.75 on the mirror planes. The Ca (II) ions are seven-coordinated, with six O atoms and one OH^- ion. The six PO_4^{3-} ions occupy 6h positions similar to that of the Ca (II) ions, in expanded triangular positions as shown in **Figure 1.4**. Adjacent Ca (I) and Ca (II) polyhedra are linked through O atoms of the PO_4^{3-} tetrahedra. Because of the crystallographic mirror symmetry imposed by the space group, each OH^- ion has to be considered at statistically disordered positions both above and below the mirror planes at z = 0.25

and 0.75. It has been shown by neutron diffraction studies that O atoms in hydroxide ions are situated at 0.34 Å away from the mirror plane with the OH^- direction pointing away from the mirror planes.

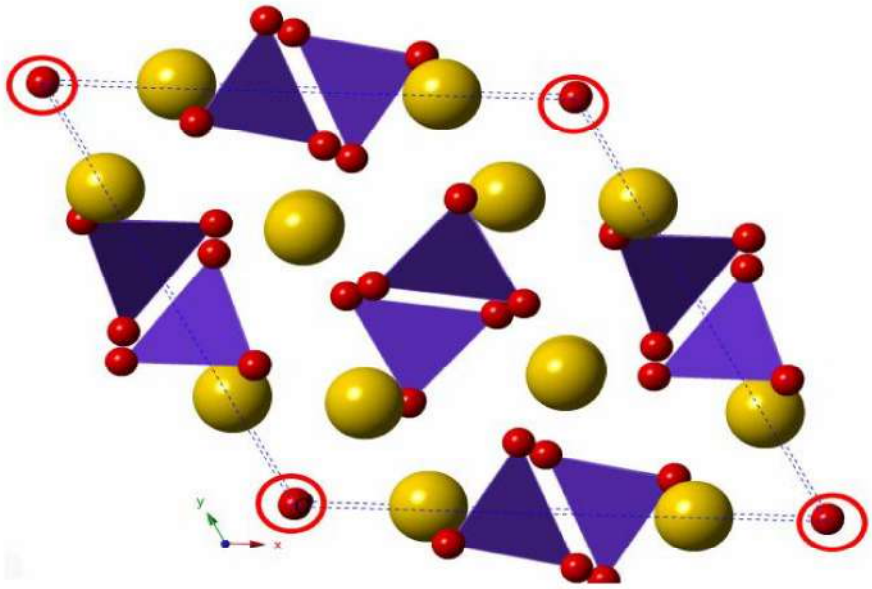


Figure 1.4 Structure of hydroxyapatite, Red: Oxygen, Yellow: Calcium, Purple: Phosphate PO_4 tetrahedra, and Channel OH sites circled

1.4 Bioactive glass

Bioactive glass was first discovered by Larry Hench and colleagues in 1969 at the University of Florida to which bone can bond chemically to certain glass compositions. This group of glasses has been known as bioactive glasses based upon the following definition[13]: *"A bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of an interfacial bond between the tissues and the material without any harmful effect to the living tissues"* Bioactive glasses have got their various applications in the repair and reconstruction of diseased and damaged tissues in the human body. One aspect that makes bioactive glasses different from other bioactive ceramics and glass-ceramics is

the possibility of controlling a range of chemical properties and rate of bonding to tissues. The mol% glass composition of the first bioactive glass is $46.1SiO_2$-$24.2Na_2O$-$26.9CaO$-$2.6P_2O_5$ and was termed 45S5 Bioglass®, which is now trademarked name [17]. Hench had chosen this composition as it provided a large amount of CaO with some P_2O_5 in a Na_2O-CaO-SiO_2 matrix. The composition is very close to a ternary eutectic, making it easy to melt. The first implant was done in a rat femur. After 6 weeks it was removed with great difficulty from the implant site. A quantitative evaluation of interfacial shear strength in rat and monkey models showed that the strength of the interfacial bond between bioglass and cortical bone was equal to or greater than the strength of the host bone [18]. Hench's discovery launched the field of bioactive ceramics and bioglass particulate which has been in clinical use since 1985 (US Biomaterials Corp., Florida). Later studies have also found that other compositions of bioactive glasses bond to soft tissues. The interest in bioactive glasses has focused now on the ability of a material to stimulate new bone growth. The regenerated mechanism has got its potential applications which can restore diseased or damaged part of the bone to its original condition and functions.

1.5 Glass composition and structure

1.5.1 Role of network former in glass

The network former is that which forms the network complex and overall it holds the structure together. They form multiple bonds which link to oxygen atoms. These are produced in most of the bioactive glasses with SiO_2, P_2O_5 and B_2O_3 etc. In a silica network, the bridging oxygens (Si-O-Si) form a SiO_4 tetrahedra and it is through the interconnecting corners of the disordered three dimensional structure of glass as illustrated in **Figure 1.5**. These oxygen atoms which connect the network formers are

referred as bridging oxygens (BOs) and it bonds to two silicon atoms (Si-O-Si) together. The earlier studies had shown the bond angles and bond lengths between the oxygen and silicon atoms in the tetrahedra [19].

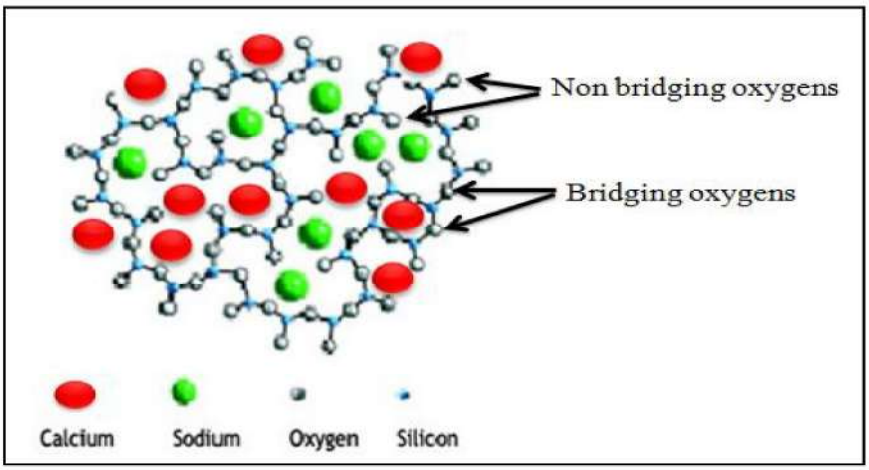


Figure 1.5 Schematic diagram for the glass structure with bridging and non bridging oxygens.

1.5.2 Role of network modifiers in glass

Network modifiers are alkali and alkaline earth oxides such as Li_2O, Na_2O, K_2O, MgO, CaO, SrO etc., which can alter the chemistry of bonding in the glass network. They interrupt the formation of bridging oxygens (SiO-Si) and produce non bridging oxygens ($Si\text{-}O^-$) as shown in **Figure 1.5**. The bonds formed between the modifier and the oxygen ions are ionic in nature. Typically, network modifiers are used to lower the melting temperature of glasses and consequently it saves the energy [19]. The modifiers are also being used in the bioactive glasses. In addition, they are highly beneficial during bioactivity and bone regeneration processes which mainly depend on the specific modifier used in the composition. Possibly the most important aspect is that on increasing the concentration of network modifiers resulted in an increase in the

dissolution rate of glass in the human body fluid [20]. However, release of certain modifiers like K_2O, CaO and SrO etc., can also stimulate cells in the human body.

1.5.3 Role of stabilizers in the glass

Stabilizers are those oxides which can possess the properties like the glass formers but do not form glasses alone rather they can improve the properties of glass. These intermediate oxides can participate in the glass network along with the network formers like MgO, CoO, NiO and ZnO etc., [21][22] . These ions can play the role of either a network former or a network modifier or both depending on the glass composition.

1.5.4 'Q^n' structure

In the Q^n structure, n depicts the number of bridging oxygens around a tetrahedral network former. For example the structure of Q^2 means that two bridging oxygens have surrounded the network former as shown in **Figure 1.6**. An increase in silica content in the glass increases the fraction of cross-linked Q^3 $[SiO_4]^{4-}$ chains at the cost of the more soluble Q^2 chains. Thus, the glass solubility would decrease significantly and the degradation would be minimized. The pure silica glass has a network connectivity of 4.0 theoretically as it is only composed of silicon and oxygen atoms. However, the bioactive glasses tend to have low network connectivity around the region of 2.0. This would be the suitable network structure of the glass as for as disrupted possibly and allowing the ions for fast dissolution while maintaining its stable mechanical structure

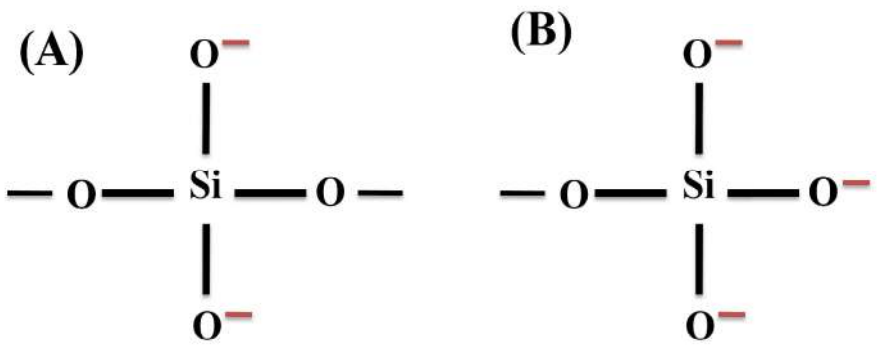


Figure 1.6 Schematic diagrams of a SiO_4 tetrahedra possessing (A) Q^2 structure and (B) Q^1 structure

1.5.5 Relation between network connectivity and bioactivity

The network former, silica can produce a maximum of four bridging oxygens. The mol% of silica in the glass is therefore multiplied by 4 to give the number of bridging oxygens which can contribute to the system. Each network modifier in the system, irrespective of its valency, such as Na^+ or Ca^{2+} , etc., would break a single bridging oxygen to produce two non-bridging oxygens. Most of the bioactive glasses contain phosphate as a component in the system which is due to its ability to assist in the formation of hydroxy carbonate apatite. Many research groups reported that the phosphate in bioactive glasses is present as orthophosphate. Generally, the phosphate is a network former in phosphate glasses (P-O-P bond) and hence, it was thought to be a part of the silica network structure (Si-O-P bond). However, O'Donnell [23] had demonstrated through MAS-NMR studies that phosphorus did not form any Si-O-P bonds in the silicate based glasses. Moreover, the phosphate forms an individual phase within the glass network and therefore, the release of phosphate ions from the glass were faster when immersed in simulated body fluids [24].

Network connectivity (NC) can be used to predict a number of properties of the bioactive glass, such as structural, physico-mechanical, chemical, bioactivity and biological [25–27]. NC represents the average number of bridging oxygen (BOs) atoms per unit glass forming elements in the glass structure. In general, the glass NC tends to decrease with increasing the concentration of modifiers in the glass system. Network Connectivity can be calculated on the basis of the following general equation (1.1) [25,28] assuming that SiO_2 forms the network structure in the glass whereas, P_2O_5 remains in orthophosphate phase as reported above. The lower is the glass NC value, the more is the disruption in the glass network and more would be the glass degradation and hence thereby increase in bioactivity [20]. Moreover, Fujibayashi et al. [29] had demonstrated that there was a decrease in bone formation as the network connectivity of a system increased at the implant site as shown in **Figure 1.7**.

$$\mathrm{NC} = \frac{[\text{No. of bridging oxyggens }] - [\text{No. of non} - \text{bridging oxygens}]}{[\text{Bridging species}]} \qquad -(1.1)$$

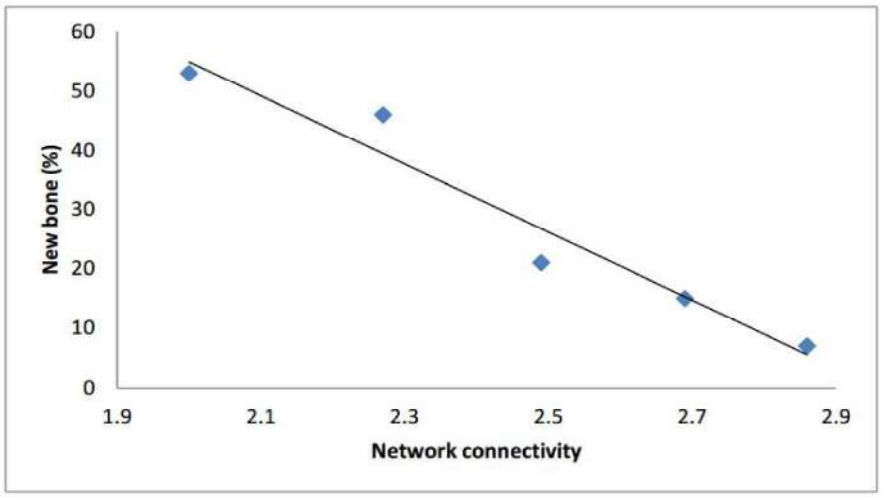


Figure 1.7 Relation between network connectivity of bioactive glasses and the amount of new bone formed in vivo [29].

1.5.6 Mechanism of bioactivity, HA formation and bone bonding

For *in vitro* bioactivity, simulated body fluid (SBF) test method is most suitable and it is widely accepted [30]. The SBF is comparable to that of human blood plasma in terms of its ion concentration so that one can assess the bioactivity of the material outside the human body. This involves immersion of the samples in SBF for different time periods. When a bioactive glass reacts with an aqueous solution, both biochemical and structural changes occur as a function of time within the bioactive glass surface. Accumulation of dissolution products causes a change in both the chemical composition and pH of solution. The formation of hydroxyl carbonate apatite (HCA) layer on bioactive glasses and the release of sodium, calcium ions and soluble silica to the surrounding fluids are key factors in the rapid bonding of these bioactive glasses with hard and soft tissues.

There are 12 stages in the process of complete bonding of bioactive glass to bone formation as shown in **Figure 1.8** [31]. Stages 1-5 are the chemical and stages 6-12 are the biological response. These stages are:

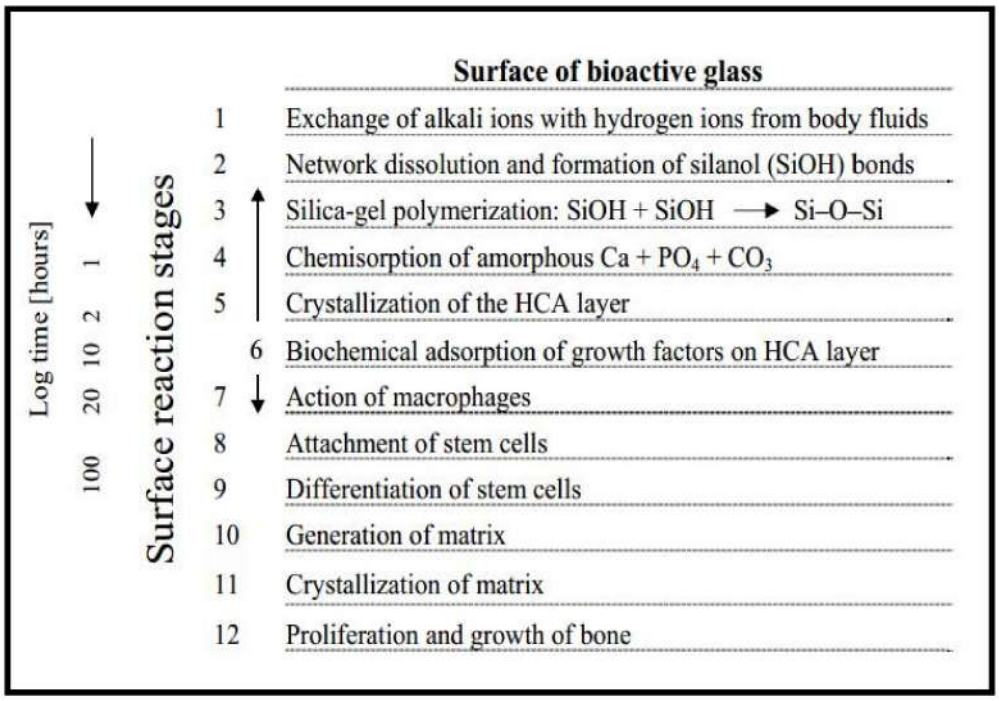


Figure 1.8 Order of interfacial reactions involved between bioactive glasses and bone during bone bonding mechanism [31].

Stage (1) the loss of sodium ions (Na^+) from the surface of the glass via ion exchange with hydrogen ion (H^+ or H_3O^+). This reaction occurs very rapidly, within minutes of material exposure to body fluids and causes a dealkalinization of the surface layer with a net negative surface charge. This stage is usually controlled by diffusion and it exhibits time dependence as $t^{-1/2}$.

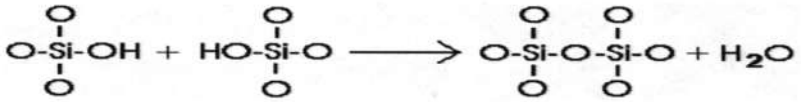
$$\mathbf{Si\text{-}O\text{-}Na^+ + H^+ + OH^- \rightarrow Si\text{-}OH^+ + Na^+(solution) + OH^-}$$

Stage (2) Loss of soluble silica in form of $Si(OH)_4$ to the solution resulting from breaking of Si-O-Si bonds and formation of Si-OH (silanols) at the glass solution interface.

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

This stage is usually controlled by interfacial reaction and it depends upon time as $t^{1.0}$. Hench had proposed that the loss of soluble silica from the surface of bioactive glasses might be at least partially responsible for stimulating the proliferation of bone-forming cells in the area of the glass surface.

Stage (3) Condensation and repolymerization of a SiO_2-rich layer on the surface depleted in alkalis and alkaline earth cations.



Stage (4) Migration of Ca^{2+} and PO_4^{3-} ions to the surface through the SiO_2- rich layer forming a CaO-P_2O_5-rich film on top of the silica-rich layer, followed by growth of the amorphous CaO-P_2O_5-rich layer due to incorporation of soluble calcium and phosphates from solution.

Stage (5) Crystallization of the amorphous CaO-P_2O_5 film by incorporation of OH^- or CO_3^{2-} anions from the solution to form a mixed hydroxyl carbonate apatite layer.

Tissue bonding stages**:**

Stage (6) Adsorption and desorption of biological growth factors in the hydroxyl carbonate apatite (HCA) layer (continues throughout the process), to activate differentiation of stem cells.

Stage (7) Action of macrophages to remove debris from the site allowing cells to occupy the space.

Stage (8) Attachment of stem cells on the bioactive surface.

Stage (9) Differentiation of stem cells to form tissue growing cells.

Stage(10) Generation of extracellular matrix by the tissue growing cells to form tissue.

Stage(11) Crystallization of inorganic calcium phosphate matrix to enclose cells in a living composite structure.

Stage(12) Cell proliferation and growth of new bone.

The reaction stages one and two are responsible for the dissolution of a bioactive glass, and therefore greatly influence the rate of HCA formation. The HCA is precipitated and crystallized on the collagen fiber and bioactive glass surfaces [1]. For hard tissue, interfacial bonding occurs because of the biological equivalence of the inorganic portion of hard tissue and the growing HCA layer on the bioactive glass surface. For soft tissues, the collagen fibrils are chemisorbed on the porous silica rich layer via electrostatic, ionic and/or hydrogen bonding. Several studies have shown that the leaching of silicon and sodium to solution is initially rapid, following a parabolic relationship with time for the first 6 hours of the reaction and then stabilizes followed by a linear dependence on time[5][32].

The adsorption of proteins and other biologic moieties take place simultaneously with the 4 stages of reaction and it was believed to contribute a favorable biological environment of the HCA layer within approximately 3 to 6 hours in vitro. Further, the calcium phosphate layer will crystallize into the HCA layer and this surface is chemically and structurally almost like natural bone mineral. Therefore, the tissues are able to attach directly to this mineral phase. The thickness of the HCA layer increases as the reaction takes place with time and forms a bonding zone mechanically which yields an interfacial bonding between bioactive implant and the natural tissue.

The bioactivity of glasses depends on their composition. Fujibayashi et al (2003) [29], Oonishi et al (1999) [2] and Wheeler et al.(2000) [33] had demonstrated a great difference between the rates of bone growth and degree of bone repair for different glasses *in vivo*. This result in two classes of bioactivity as one of the bioactive glasses stimulate both osteoconduction and osteoinduction and another bioactive glasses that show only osteoconduction in which bone migration takes place along an interface. The main reasons for different degrees of bioactivity have been linked to the composition of the glass and the stability of the glass network. The mechanism of bioactivity is mainly dependent on the rate of glass dissolution and as it increases the rate of formation of the HCA layer also increases and hence the bone bonding takes place more rapidly [31]. However, it would only show an enhanced osteoconductivity of bioactive glasses over a less bioactive composition, but not on the basis of osteoinduction. Therefore, the mechanism for osteoinduction is more difficult process. It is very important to understand the biological response with the bioactive glasses which involve the osteogenic cells which receive the signals from the implant materials. In general, bioactive glass degrades and releases ions such as Si^{4+}, Ca^{2+}, Na^{+} and PO_4^{3-} etc into the solution. The combination of some of these ions activates the cells to produce new bone during which the critical concentration of soluble silicon and calcium ions play mainly a significant role [34].

1.6 Literature review

1.6.1 Bioglass

Bakry et al. [35] had established that 45S5 bioglass® can also be used in dental paste, as it has been found to show good biocompatibility with pulp cells when it is compared to commercially available dental materials. The authors had suggested that

the use of 45S5 bioglass® in the dental paste is safe and helpful in dentine hypersensitivity treatment.

Lefebvre et al [36] had demonstrated that 45S5 bioactive glass crystallized mainly in the $Na_2CaSi_2O_6$ phase rather than $Na_2Ca_2Si_3O_9$ in the temperature range of 600 – 700 ^oC. The authors believed that the initial one leads to the major crystalline phase and the second one leads to phosphate phase. The 45S5 Bioglass® had shown the rapid crystallization tendency of the material to crystallize. Moreover, time–temperature-transformation (T-T-T) curves were developed which could be used to control the degree of crystallinity of porous blocks. It was also reported that these results cannot be extended to solid blocks.

Cerruti et al. [37] had demonstrated that the pH and ionic strength of triss buffered solution significantly effected on the reactivity of 45S5 Bioglass® in the formation of HCA layer at pH=8.0. Calcium phosphate precipitation occurred after immersion in the SBF at higher pH but the precipitate prevented the release of larger ions. Hence, calcium carbonate was deposited more than HCA in these conditions. Further, at lower pH, no HCA precipitation was observed during initial two days of the reaction but only total breakdown of silicate network took place.

Hench L. L. had reviewed [31] that bioceramics can be used for the repair and reconstruction of diseased or damaged parts of the musculoskeletal system which is termed bioceramics and it may be bioinert (e.g., alumina and zirconia), porous scaffold for tissue ingrowth (e.g., hydroxyapatite-coated metals), bioactive (e.g., hydroxyapatite, bioactive glasses, and glass-ceramics), resorbable (e.g., calcium sulphate, tricalcium phosphate and calcium phosphate salts), The applications include replacements for hips, knees, teeth, tendons, ligaments and repair for periodontal disease, maxillofacial reconstruction, augmentation and stabilization of the jaw bone,

spinal fusion, and bone repair after tumour surgery. The tissue bonding mechanisms with bioactive ceramics is due to the molecular design of bioceramics for interfacial bonding with hard and soft tissues. For high toughness and elastic modulus, the bioactive composites are being developed to match with bone. Therapeutic treatment of cancer has been accomplished by localized delivery of radioactive isotopes through the glass beads. Clinical success of bioceramics has led to a remarkable advance in the quality of life for many people.

It was proposed by Hench and Polak [38] that the third generation biomedical materials are those which possess a combination of bioactive and resorbable nature of biomaterials. While, the second generation biomaterials were designed and demonstrated to be either resorbable or bioactive. Moreover, the third generation biomaterials are proposed with the aim of developing the materials that should heal by itself in the body once implanted.

In vivo comparison study was performed by Vogel et al. [39] on the powders of bioactive glasses of 45S5, 52S ($52.0SiO_2$ -$21.0Na_2O$ -21.0CaO -$6.0P_2O_5$) and 55S ($55.0SiO_2$ -$19.5Na_2O$ -19.5CaO -$6.0P_2O_5$) particles in rabbits. The 45S5 bioglass® had shown the maximum bone bonding kinetics due to its higher degradation rates. The behavior of the multinuclear giant cell (MNGC) line seemed to react inversely to the bone.

Hench et al. [17] had prepared the parent bioactive glass (45S5) containing $45.0SiO_2$ -$24.5Na_2O$ -24.5CaO -$6.0P_2O_5$ (wt%) and it had been found to bond with bone through formation of biologically active hydroxyl carbonate apatite (HCA) layer on its surface in physiological conditions. Based upon *in vitro* and *in vivo* studies, the surface of implant has been found chemically and structurally equivalent to the mineral phase in the bone.

1.6.2 Barium oxide (BaO) as an additive

Kaur et al [40] had reviewed that the strontium and barium belong to alkaline earth metal oxide group like calcium and magnesium. Generally, the ionic field strength decreases with increasing the ionic radii of alkaline earth ions; Mg^{2+}, Ca^{2}+, Sr^{2+}, and Ba^{2+} (0.45, 0.33, 0.30, and 0.24), respectively. Hence, substitution of barium in the glass would increase the surface adherence by reducing surface tension.

Austin et al [41] had shown the barium distribution in human in teeth in early-life dietary transitions. They have reported that a direct correlation between Ba/Ca distributions in human baby teeth and breastfeeding data collected. It was reported that the Ba concentration in mother's milk is consistent with Ba/Ca in dental tissues. It was noticed that the Ba/Ca ratio decreased consistently from the onset of supplementation. The study demonstrated that distribution of Ba is due to intake of mother's milk.

Kaur et al. [42] had studied the bioactivity in the system of barium–zinc-borosilicate glass system containing 30 mol% of barium in their compositions. It was reported that glass contained $40SiO_2$–$30BaO$–$20ZnO$–$2.5B_2O_3$–$7.5Al_2O_3$ and it had shown the bioactivity after immersion in SBF for 30 days. They also mentioned that the band gap decreased drastically as compared to other glasses.

Moreover, Makita et al. [43] had studied the effect of barium concentration on the radiopacity and biomechanics of bone cement. They have demonstrated that the radiopacity of the cement has increased with increasing barium concentration. It was concluded that 30% or 40% barium concentration in the cement has got more advantages than 10% or 20% because of its better visibility.

Lewis et al. [44] had reported that the commercially available bone cement (poly- methylmethacrylate, or PMMA) contained 10% of barium concentration as $BaSO_4$ to increase the radiopacity of the cement. Therefore, the presence of barium in bone does not harm the host tissues.

Yamaguchi et al. [45] had demonstrated previously the barium entry in osteoblast like cells. The osteoblasts cell was loaded with Fura 2, the fluorescence measurement was taken in the presence of a voltage-sensitive Ca^{2+} and Ba^{2+} ions and the entry pathway was recognized as L-type depolarization-activated Ca^{2+} and Ba^{2+} channel in these cells.

Mathew et al [46] had reported that the larger Ba^{2+} ion can be substituted in the mineral fluoroapatite [$Ca_{10}(PO_4)_6F_2$] crystal structure. They demonstrated the formation of $Ba_{10}(PO_4)_6F_2$ crystal structure by single –crystal X-ray diffraction. They also mentioned that the coupled substitution of Ba^{2+} ion prevents the charge balance during filling of the atomic sites.

1.6.3 Strontium oxide (SrO) as an additive

It has been demonstrated Liu et al [47] that the substitution of Sr in the bioactive glass has resulted in the mixed Sr-HA ($Sr_5Ca_5(PO_4)_6(OH)_2$) layer formation on the surface of the glass. Santocildes-Romero et al. [48] substituted strontium (50% and 100% of CaO) for calcium in 45S5 bioglass. It has been shown in significant modifications in density and solubility of the glasses. The bioactive glass containing strontium (100%) showed the greatest inhibition of cellular metabolic activity. Further, the Sr-contained bioactive glasses had regulated the expression of *Alpl* and *Bglap* genes in standard and osteogenic cell culture media. This signifies that the strontium-substituted bioactive glasses have superior regenerative properties.

Massera et al [49] had substituted SrO (5 to 21.77 mol%) for CaO in $53.85SiO_2$–$22.66Na_2O$–$1.72P_2O_5$– 21.77CaO (S53P4) bioactive glass and no change was seen in the glass structure but a small change was noticed in the thermal properties of glasses. The glass dissolution increased initially with an increase in SrO concentration in the glass which led to increase in pH of the solution. Moreover, as the concentration of strontium increases in the glass the thickness of the reacted area also increases at the glass surface and hence a gradual decrease in silica rich layer was observed. They suggested that substituting SrO for CaO can lead to the formation of mixed Sr-HA layer on the glass surface.

Strobel et al. [50] had reported that the doping of SrO (5 wt%) for CaO in the system $53SiO_2$–$6Na_2O$–$4P_2O_5$–$12K_2O$–5MgO–20CaO significantly increased the osteocalcin, collagen type 1 and vascular endothelial growth factor (VEGF). This has indicated that the Sr-doped glass stimulates the osteogenic and angiogenic properties of human bone marrow stromal cells (hBMSCs).

Fujikura et al. [51] revealed that Sr^{2+} has influenced the silicate network when it replaced more than 50% of calcium in 45S5 Bioglass. It was suggested that glass structure preferentially changed from chains toward rings with increasing SrO substitution. Upon heat treatment of 45S5, amorphous phosphate-rich phases were formed along with a crystalline silicate, combeite. Further heat treatment at the temperature above 800 ^{o}C led to formation of crystalline phosphate phases in Sr-substituted glasses, whereas the 45S5 composition still contained a large amount of amorphous phosphate and meta-phosphate species. The findings from this study have got their important implications in understanding the effect of Sr^{2+} on dissolution and bioactivity of silicate glasses.

Isaac et al. [52] had substituted 2.9 mol% of SrO for CaO in SiO_2-CaO-SrO system. The osteoblast differentiation had improved in the presence of SrO (2.9%) as shown by better alkaline phosphate activity in comparison to Sr- free bioglass.

Gentleman et al [53] substituted SrO for CaO from 0, 10, 50 and 100 (mol%) in the bioglass 46.46 SiO_2 – 1.07 P_2O_5 – 26.38 Na_2O – 23.08 CaO composition. They concluded that strontium can be substituted for calcium and it has a significant increase in osteoblast proliferation and alkaline phosphate activity (ALP) activity. The presence of SrO in the glass inhibits tartrate-resistant acid phosphatase (TRAP) activity and osteoclast mediated resorption of apatite layer. It was highlighted that substitution of SrO for CaO in BG was an effective approach for early bone repair and regeneration therapies.

Hesaraki et al. [54] substituted SrO (7.9 wt%) for CaO in the bioglass SiO_2–P_2O_5–CaO system and concluded the density and glass crystallization temperature were found to increase with the addition of SrO. The rate of ionic dissolution from the glass also increased with increasing SrO substitution in the composition. The formation of apatite phase on the surface of Sr-contianed bioglass was slightly inhibited after soaking in SBF. However, the Sr-contained bioglasses had increased the proliferation and ALP activity of rat calvaria osteoblastic cells.

O'Donnell, M. D et al. [55] showed that the strontium had a beneficial effect on bone remodelling and osteoblast activity while suppressing the bone resorbing osteoclasts. Strontium can be added to bioactive glasses in place of calcium and also in combination of Ca with minimal alteration of glass structure and properties while exhibiting bioactivity (HA formation) as comparable to bioceramics such as 45S5 Bioglass®. Complete substitution of strontium for calcium did not result in decrease in cell proliferation or increase in toxicity as compared to all calcium base Bioglass

(45S5). Biomaterials which slowly released strontium at a controlled rate locally at the defect site could offer superior clinical performance as compared to current ceramic materials available as synthetic bone grafts and would also prove useful as new regenerative therapies for hard tissue repair.

Bonnelye et al. [56] had demonstrated that the strontium ranelate has dual effect in bone. They reported a positive effect on osteoblast differentiation and a negative effect on osteoclast formation and resorption. This is due to its capacity to interrupt the sealing zone formation. Strontium ranelate has shown a significant role in treatment of osteoporosis of postmenopausal which reduces the risk of vertebral and non-vertebral fractures, including hip fractures.

O'Donnell at al. [57] studied a series of SrO substitution for CaO in hydroxy apatite $(Sr_xCa_{1-x})_5(PO_4)_3OH$, where x = 0.00, 0.25, 0.50, 0.75 and 1.00.They reported the mixed Sr-HA formation and hence the lattice parameters, unit cell volume and density which increased linearly with increasing the strontium concentration.

Marie P. [58] reviewed the strontium, as therapy for osteoporosis. The available pharmacological and clinical data indicated that strontium ranelate was a unique anti-osteoporotic drug that had reduced the risk of vertebral and non-vertebral fractures in postmenopausal women with good tolerability in patients; it has also represented an important advance in the osteoporotic therapy. Unlike the available anti- osteoporotic drugs, the compound was found to induce anti-resorbing and bone-forming effects on bone remodelling which resulted in improvement of bone mass and its strength. Putative mechanisms of action of strontium ranelate on bone cells have been recently postulated. However, further studies are needed to establish the exact cellular and molecular mechanisms by which strontium ranelate exerts its anti- osteoporotic effects in bone.

1.6.4 Magnesium oxide (MgO) as an additive

Anand et al. [59] prepared the bioactive glass samples containing magnesium and zinc in 45S5 bioactive glass system xZnO-(22.4-x)Na_2O -46.1SiO_2-26.9CaO -2.6P_2O_5-2MgO. X-ray diffraction had shown the formation of hydroxyl apatite layer after immersion in SBF for 7 and 14 days. The growth of hydroxyl apatite phase (P-O bond) was observed by Raman spectra and also by field emission scanning electron microscopy. The Ca/P ratio and ion dissolution rate have been studied and confirmed the growth of apatite layer with increasing time during *in vitro* analysis. It was reported that the glass dissolution rate had decreased which might be due to the presence of MgO and the authors also claimed low drug delivery properties for longer duration. It was predicted that porosity, surface area and chemical composition of the glasses have played an important role in controlling the bioactive behavior of the glasses.

Al-Noaman et al. [60] had studied the effect of MgO substitution on bioactivity and structure of SiO_2-CaO-MgO-ZnO-Na_2O-K_2O-P_2O_5 bioactive glasses. They substituted MgO upto 20 mol% for CaO. The MgO substitution increased the compactness of silicate network connectivity significantly. Bioactivity assays indicated that the layer of apatite was seen after one month of immersion in SBF. These series of MgO substitution revealed the biocompatibility with fibroblast cells but no response was seen with osteoblast cells.

Ma et al. [61] studied that the degradability and bioactivity of MgO contained bioglasses where MgO was partially substituted for CaO in the ternary CaO-P_2O_5-SiO_2 glass system. The presence of MgO in the glass composition had decreased the dissolution of the glass in SBF and delayed the HA formation. This might be due to the higher ionic field strengh of Mg^{2+} over Ca^{2+} ion according to the authors (ionic field strength I ($I = Z/r^2$) where Z is cationic charge and r is its radius. For Mg^{2+} and Ca^{2+} , the

ionic radii, r is 0.65Å and 0.99 Å, and I is 4.73 $Å^{-2}$ and 2.04 $Å^{-2}$, respectively. They concluded that the ionic field strength of Mg^{2+} influenced on the glass degradation siginificantly.

Verne et al. [62] had studied the series of bioglasses containing SiO_2–MgO–CaO–P_2O_5–Na_2O–K_2O where MgO was present as 10-15 mol%. The cell adhension and proliferation results indicated that the glasses were non-toxic. The formation of HCA layer on the surface of these glasses was found to vary with composition and also it influenced on mechanism of bioactivity. The presence MgO in some of the systems had affected the glass stability, thermals properties as well as surface reactivity of both bioactive glass and glass-ceramics.

Saboori et al. [63] had concluded that the bioactive glass $64SiO_2$ -26CaO -5MgO -$5P_2O_5$ (mol%) contianing MgO had shown significant enhancement in bioactivity in 7 days of immersion in SBF. The HA layer formation was confirmed by XRD, FTIR and SEM analyses. Moreover, the *in vitro* cell culture studies indicated that the presence of Mg^{2+} ion in the glass stimulates bone cell production of alkaline phosphate activity.

Agathopoulos S et al. [32] had investigated CaO– MgO–SiO_2 based glass compositions, B_2O_3,P_2O_5,Na_2O, and CaF_2 with additives which possess the features of *in vitro* bioactivity. There were evidences of formation of both silica gel and HA at the surface of the glasses after immersion in SBF for 1 week. Glass surfaces were completely covered with HA layer after 2–3 weeks in SBF. The influence of the structural features of the glasses on bioactivity performance was evident after prolonged immersion for 120 days in SBF. They showed that increasing amount of phosphates had favoured the deposition of carbonated HA, while the trend was opposite on increasing CaO and SiO_2 contents.

1.6.5 Silver oxide (Ag_2O) as an additive

Goh et al. [64] had studied the effect of Ag_2O and CuO contained bioactive glasses on the bacterial migration. The Ag_2O and CuO 1, 5, and 10 mol% were substituted for CaO in the bioactive glass. It was noticed that CuO in BG was present in Cu^{2+} ion, while AgO was present in ionic and metallic states in glasses contained as 5Ag and 10Ag. It has been demonstrated that the addition of CuO (10 mol%) and Ag_2O (>5 mol%) prevented the bacterial migration effectively after 24 h of incubation. It was concluded that Ag-contained bioactive glasses had shown to be faster bacteria-killing agent than that of Cu-containing glasses within 24 h.

Jacquart et al. [65] had demonstrated that the chemical reactions were involved in silver-doped $CaCO_3$–Calcium phosphate cement. The formation of apatite layer was enhanced with Ag-contained cement in comparison with Ag-free reference cement. The Ag contained cements had shown the *in vitro* antibacterial resistance against *S. aureus and S. epidermidis* bacteria. The *in vitro* cytotoxicity analysis using human bone marrow stromal cells (HBMSC) has revealed that these samples are non toxic to the HBMS cells as well as exhibited the anti-bacterial properties such as anti-adhesion and anti-biofilm formation on the samples. Therefore, the silver-doped $CaCO_3$–CaP cement was regarded as a promising bone substitute material to reduce implant associated infections.

Gargiulo et al. [66] had demonstrated the effect of silver addition in mesoporous bioactive glass (MBG). The elemental silver was successfully incorporated into the matrix of MBG in order to provide antibacterial properties. The bioactivity of these samples was evaluated using a commercial DMEM solution revealing the formation of hydroxyl carbonated apatite layer on the surface. Furthermore, Ag-contained MBG had shown a very good antibacterial property against *S. aureus* strain. The authors had

reported the mechanism of Ag on bacterial viability which was due to the formation of intermediate crystalline AgCl.

El-kady et al [67] had prepared the bioactive glasses with substitution of a series of Ag_2O such as 1, 3, 5 and 10 wt% for CaO through a quick alkali-mediated sol– gel technique. The degradation study had shown that the incremental addition of silver in the glass not only decreased the weight loss (%) of glasses but also decreased the dissolution rates of silver ions from the glass. The microbiological investigation had revealed that the glasses were resistant to *S. aureus* and *E.coli* bacteria.

Blacker et al [68] had prepared the silver-doped bioactive glass powder (AgBG) which was used to coat on commercially available Mersilks (silk) and Vicryl® (polyglactin 910) sutures. The formation of crystalline HA and *in vitro* bioactive behaviour of the coated sutures was demonstrated after immersion in SBF for 3 days. The AgBG coated sutures had demonstrated both antibacterial and bioactive properties. The resorbable sutures are the coated bioactive AgBG and are also attractive reinforcement elements for resorbable bioceramics such as calcium phosphates and HA.

Bellantone et al. [69] had prepared silver free SiO_2-CaO-P_2O_5 (BG) and silver contained bioactive glass (AgBG) systems through sol-gel process. They have performed the bacterial study using E. coli bacteria against both the glasses. BG sample had not shown the antimicrobial activity over the quantity of the glass in the concentration range (0.1–40.0 mg/mL) studied. The AgBG had shown significant antibacterial effect on *E.coli* due to the release of Ag^+ ions into the media and also maintained its bioactivity.

Gristina et al. [70] had performed investigation on infected implants and confirmed the presence of bacterial like *Staphylococcus epidermidis* and *Staphylococcus aureus* as well as with *Escherichia coli* which are the most commonly isolated pathogens. It was reported that the frequent formation of biofilm and its integrity with tissue cells on the surface of implants had led to its failure. Moreover, the percentage of infected artificial joints, catheters and heart valves contaminated by *S. epidermidis* and *S. aureus* were reported to be about 50% and 23%, respectively.

1.6.6 Porous scaffold

Francesco et al. [71] had reviewed the bioceramics and scaffolds for bone tissue engineering. In the last few decades, a general increase of age related orthopedic and dental problems in the human body globally. Hence, there is the need for new biomaterials which can regenerate the diseased or damaged tissues of the body on its own and promote the healing. Porous templates referred to as "scaffolds" which are showing a three-dimensional tissue growth. Bioceramics, such as calcium phosphates, bioactive glasses, and glass–ceramics have shown to repair and reconstruction of diseased parts of the human body tissues. These bioactive glasses have potential applications as scaffold materials because of their high bioactive nature. The bulk bioceramics materials have been used for repair of hard tissues as a filling and restore material in bone and dental defects. Moreover, the special attention has been given to the development of multifunctional scaffolds which are showing the release of therapeutic ions or drug and they had shown promising applications beyond hard tissue repair. Hence, bioactive glasses can be doped with different therapeutic ions such as Sr, Cu, Zn, Ag, Ga and Co, etc., to provide a better controlled delivery of ions *in situ*. This would provide therapeutic effects upon the release of ions into the biological environment to promote osteogenesis, angiogenesis and antibacterial properties.

Wu et al [11] had successfully prepared copper-containing bioactive porous scaffolds using co-templates of non-ionic block polymer P123 and polyurethane sponges. P123 was used to produce mesoporous structures (mesopore size: several nanometres) and polyurethane sponges were used to create large pores (large pore size: several hundred micrometers). The large pores of 300 - 500 μm and mesopores of 5 nm were reported. The incorporation of therapeutic Cu^{2+} ions into MBG scaffolds had shown the multifunctional characteristics with improved angiogenesis capacity, osteostimulation, drug delivery and antibacterial properties. The multifunctional characteristics of the bioactive scaffolds were suggested to be of great potential for bone regeneration.

Eqtesadi Siamak et al [72] had demonstrated the preparation of highly stable solids loading colloidal suspensions line inks from 45S5 Bioglasss® using carboxymethyl cellulose (CMC) as a single multifunctional (dispersant, binder, gelation agent) processing additive. The 45S5 bioglass 3D scaffold was made using robocasting. This simple and versatile recipe would enable the fabrication of scaffolds with customized external geometry and optimized pore architecture. This method was proposed to show the way for the use of this bioactive material in a broader range of tissue engineering applications.

It was reviewed by Bose et al. [73] that scaffolds with porous structure exhibited high potential for bone tissue engineering due to their interconnected network structure which facilitate sufficient space for cell migration and ingrowth of new bone as well as soft tissues. Fabrication of bioresorbable scaffolds with tailored porosity and controlled pore sizes are possible now a day because of available advanced technologies. They pointed out the drawback of porous scaffolds which is independent of composition and also it has low mechanical properties. Porosity and pore size of the

scaffolds is uniformly distributed in most of the samples. It was highlighted that porosity of the scaffold need not to be uniform. The porosity of natural bone does not have uniform distribution as it has lesser porosity at outer surface and higher at inner core area.

It was reviewed by Fu et al [74] for the repair and regeneration of large bone defects caused due to the disease or trauma which had remained a significant clinical challenge. Bioactive glasses had been showing the characteristics as a scaffold material for bone tissue engineering, but the application of these scaffolds for the repair of load-bearing areas of bone defects was often limited because of their low mechanical strength and fracture toughness. It was highlighted on the mechanical behavior of the scaffolds for the repair of loaded bone defects. This review had shown the fact that mechanical strength was not a real limiting factor in the use of bioactive glass scaffolds for bone repair and it was not frequently documented by most researchers and clinicians. The present limitations of the bioactive glass scaffolds include their low fracture toughness (low resistance to fracture) and inadequate mechanical reliability, which have so far received little consideration. Future research directions must include the development of strong and tough bioactive glass scaffolds.

Qian et al. [75] had prepared a novel biomorphic 45S5 bioglass scaffold from a biotemplate sugarcane successfully by infiltrating 45S5 bioglass solution into the biotemplate and allowing the same in air followed by subsequent sintering at 1030 °C for 1 h. The inherent microstructure of the sugarcane was converted into 45S5 bioglass scaffold very well and there was a reduction in average pore size. The major crystal phase of the 45S5 bioglass was found as $Na_2Ca_2Si_3O_9$. A new secondary crystal phase, orthorhombic $NaCaPO_4$, was found in the sol–gel derived 45S5 bioglass. The 45S5 bioglass contains both nonbonding oxygen (Si–NBO–Na, –Ca or –H) and bridging

oxygen atoms (Si–BO–Si). The biotemplate method may be utilized to fabricate scaffold for bone tissue engineering.

Yan et al.[76] had prepared highly ordered mesoporous bioactive glasses (MBG) and demonstrated the higher bioactivity than the sol-gel bioactive glasses (BG). This is due to MBGs have higher specific surface area and more pore volume which have greatly enhanced the bioactivity and formation of HCA layer than bioactive glasses (BGs).

Jones and Hench [77] had demonstrated that porosity of the scaffold had played an important role. It must be interconnected with porous network having a wide variety of pore sizes like macropores (>400–500 μm) which allow tissue ingrowth and vascularization and the microporous pores (2-50nm) which promote protein adhesion and consequently cell attachment and growth.