Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Biomaterials normally described as a combination of substances originating from natural, inorganic or organic materials that is biologically compatible when they exactly or partially came in contact with the physiological fluid over healing period. They involve as part of a living being or biomedical devices which enhances or repair any damaged or diseased natural physiological condition. Biomaterial is a nonviable substance used in medical devices for the intention of the interaction with biological systems [J.WBoretoset al. 1984]. Their usage within the physiological medium must need the characteristic features such as efficiency and reliability. These characteristic features have provided with a suitable combination of chemical, mechanical, physical and biological properties [D.F.Williamset al. 1987]. Recently, biomaterials are widely used in various medical devices and systems; drug delivery systems; tissue engineering; screws, plates, wires and pins for bone treatments; total artificial joint implants; partial or total hip replacement, skull repair or reconstruction; dental and maxillofacial applications [Binnazet al. 2012].In other words, a biomaterial is a non-toxic material that can be used to construct artificial organs, rehabilitation and augmentation of medical devices or prostheses, and to replace bodily tissues. Hench classified the application of biomaterials in tissue engineering into three time frames [L.L. Hench 1998]:

The past: removal of tissues;

The present: replacement of tissues;

The future: regeneration of tissues;

In the past years, the aim of developing biomaterial was to create much strongand chemically inert biomaterials for the augmentation of mechanical strength of the bones or others physiological parts. The first skeletal repairing materials used were metals, which were considered purely asbio-inert. However, a lot of fruitful applications in orthopedics attempted with metallic embeds in past. However, no material implanted in living tissues is completely inert: all materials elicit a response from the host tissue. Although inert metal implants can provide high strength and corrosion resistance, relative movement, called micro motion, can occur due to a lack of chemical or biological bonding at the interface [W.P. Cao et al. 1996]. Also, the inert metal implants have the risk of releasing some ions from the surface texture to cause immunological effects in the body. These led to a search for materials that can repair and regenerate tissues rather than replace them. These types of materials are called bioactive materials.

In a general sense, a bioactive material has been defined as a material that has been designed to induce specific biological activity. In a more narrow sense, a bioactive material has been defined as a material that undergoes specific surface reactions, when implanted into the body, leading to the formation of an hydroxyl - carbonate apatite (HCA) layer which is responsible for the formation of a firm bond with tissues [T. Kokuboet al. 1991]. The ability of a bioactive material to form a HCA layer when immersed in body fluid is often taken as an indication of its bioactivity.

1.2 Aim and objective of the work

The present study is to improve in-vitro bioactivity and mechanical properties of 45S5 bioactive glass substituted with rare earth oxide (CeO_2, $La_2O_3$and Sm_2O_3)

and Al_2O_3up to 2 wt %. The motivation for the selection of CeO_2, La_2O_3, Sm_2O_3 and Al_2O_3 are due to their good biological and mechanical property with low toxicity.

- Preparation of bioactive glass by melting route.
- Preparation of bioactive glass-ceramics by controlled crystallization.
- Structural analysis of bioactive glass by DTA, FTIR and XRD
- Physical properties of bioactive glass and glass-ceramics
- Assessment of in-vitro bioactivity in SBF
- Biocompatibility with human blood cell
- Identification of HCA formation by pH, FTIR and SEM
- Mechanical properties of bioactive glass and glass-ceramic (Destructive test)
- Elastic moduli of bioactive glass and glass-ceramic (Non-Destructive test)

Bioactive glasses were invented invented by Dr. L.L. Hench which which helped ininterfacial bonding with the surrounding or the damaged tissue regarded as the second generation bio-materials. Since then, various kinds of bioactive materials have been developed over the last three decades. Among these, the main bioactive materials used clinically are: silica based bioactive glasses [L.L. Hench et al.1971], hydroxyapatite (HA) [$Ca_{10}(PO_4)6(OH)_2$] [M. Jarcho et al. 1977], β - tricalcium phosphate (TCP) [$Ca_3(PO_4)_2$] [Rejda et al. 1977], HA/TCP bi - phase ceramic and bioactive glass - ceramic A-W containing crystalline oxyfluoroapatite [$Ca_{10}(PO_4)_6(O, F)_2$] and β - wollastonite [$CaO.SiO_2$] in a MgO - CaO - SiO_2 glassy matrix [T. Kokubo et al. 1982]. Figure 1.1shows the in vitro and in vivo performance.

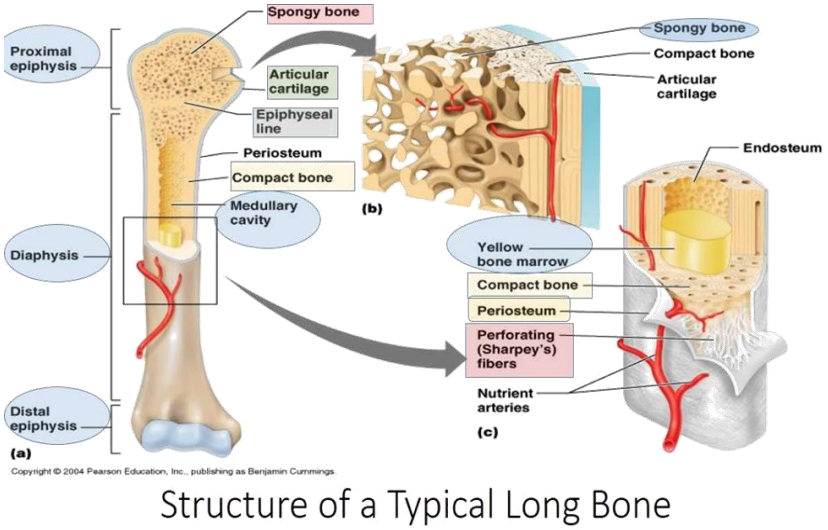

Figure 1.1: In vitro and In vivo performance [Antonio et al. 2013]

In 1991 Kokubo developed simulated body fluid (SBF) [T. Kokubo et al. 1992], SBF has become the most widely used solution for in vitro investigation of material bioactivity by providing conditions very close to those found in vivo, which is carried out in living body. A fast, economical and reliable bioactivity test of any material can thus be carried out in SBF solution.

By adjusting the oxide composition of a glass, its properties and rate of bonding to tissues can be controlled. These aspects make bioactive glasses different from other bioactive materials [L.L. Hench et al. 1998]. The 45S5 bioactive glass and glass-ceramic have been used widely because of bonding capability with hard & soft tissues. One of the major applications of bioactive glass and glass-ceramic is as an artificial bone graft. Therefore, it is a promising material in the field of biomedical application. It has inferior mechanical properties in comparison to cortical bone. Generally, the purpose of biomaterials is to substitute for replacing a damaged or diseased part of human body bones. In the 45S5 bioactive glass [$45SiO_2$-$24.5Na_2O$-$24.5CaO$-$6P_2O_5$ (wt %)] S denotes the network former SiO_2 followed by a specific Ca/P molar ratio of 5:1 [J.A.Juhasz etal.2008]. The key compositional features that are responsible for the bioactivity of 45S5 bioactive

glass are its low SiO_2, high Na_2O and CaO contents as well as high CaO/P_2O_5 ratio [A. Srivastava et al. 2012]. However, it suffers from a mechanical weakness and low fracture toughness due to an amorphous nature of glass and it may not be suitable for load-bearing applications.The network former in the bioactive glass holds the three dimensional non-periodic glass structure during selective dissolution of cations (Na^+,Ca^{2+}, etc.,) by suppressing the detachment of some other ions [L.L. Hench et al. 1971]. The presence of SiO_2 also helps in the precipitation or surface reconstruction of the loose silica-rich layer and hence enhances the formation of hydroxyl apatite layer [Hench et al. 1991, Paschallet al.1974 and L.L. Hench et al. 1997]. The interactions between the bone tissues and the bioactive implants, in particular the interfacial reaction kinetics and the sequence of reactions have been critically reviewed by earlier workers [L.L. Hench et al. 1991,L.L. Hench et al. 1998 and Lim et al.2005].

The aim of present investigation is the studies on In Vitro Bioactivity and Mechanical Behavior of bioactive glasses and their ceramic derivatives containing REEs (CeO_2, La_2O_3, and Sm_2O_3) oxides. In view of literature survey Ce^{4+}, La^{3+}, Sm^{3+}, and Al^{3+} ions are being considered as a possible alternative to growth factors and genetic approaches in tissue engineering because of their biocompatibility, easy processing, high temperature stability and tunable release kinetics. The motivation for the selection of REEs (CeO_2, La_2O_3, and Sm_2O_3) oxides is due to their good biological properties. The cerium and lanthanum are the components which did not affect the bone formation.

Therefore, the substitution of CeO_2, La_2O_3, and Sm_2O_3 in 45S5 bioactive glass would be highly beneficial in improving its biological and mechanical properties.I have prepared for first time the Sm_2O_3 contained bioactive glasses and its

ceramic derivatives. In order to assess their physical, mechanical and bioactivity, further studies need to be carried out.

Rear earth oxides such as CeO_2, La_2O_3, and Sm_2O_3 have been shown to increase the transcription factor. Bioactive glass offers an exciting route for potential delivery system of REEs within tissue regeneration scaffolds due to their ability to incorporate a large variety of elements and their largely controllable dissolution properties within physiological fluid [Jones et al.2001].

The role of La, Ce and Sm in the glass network will determine the network connectivity (NC) and macroscopic properties, such as ion release rate and Hydroxyapatite formation. Lanthanum oxide, Cerium oxide and Samarium oxide are cytotoxic in higher concentrations.[A. El-Adawy et al. 2006] have shown that bioactive scaffolds containing less than 4weight % CeO_2, La_2O_3, and Sm_2O_3 are not cytotoxic. The low concentration of REEs incorporated in these glasses means it is not possible to extract structural information from a single total (i.e. predictable) diffraction pattern.

1.3 Role of REEs (CeO_2, La_2O_3, and Sm_2O_3)

A redox-active rare earth oxide like cerium oxide nanoparticles attracted interest of various researchers in the field of orthopedic tissue engineering due totheir therapeutic applications potential such as oxygen buffering capacity [Das et al. 2012]. Moreover, it was reported that cerium oxide based nanoparticles play vital role as neuroprotective agents through limiting the amount of oxygen required to kill the cells [Schubert et al.2006]. The radical-scavenging potential of cerium nanoparticles are relied on reversibility of the Ce^{3+}/Ce^{4+} redox couple with the subsequent generation of surface defects. Particularly, partially reduced cerium

oxides possess oxygen vacancies in the lattice structure as a result of the loss of oxygen in the reaction [Eq. (1)]. Although, the non-stoichiometric cerium oxide ($CeO_{2-\delta}$) is readily re-oxidized back to CeO_2 and partially O_2 will produced. The oxidized CeO_2 nanoparticles are readily reduced when placed in water or coated by PEG.

$$\mathbf{CeO_2} \longleftrightarrow \mathbf{CeO_{2-\delta} + \delta/2\ O_2} \text{ ----------Eq. (1)}$$

Also, it confirms the encouraging effect of cerium nanoparticle in bioactive glass over the proliferation, differentiation and mineralization of primary osteoblasts [Zhang et al.2010]. Furthermore, materials such as bioglass doped or incorporated with cerium have shown promising antibacterial activities [Goh et al. 2014]. However, the introduction of cerium based bioglass or its oxide into the glass network may affect bioactivity and thus need to be evaluated. Previouly it was shows that bioactivity decreases with increasing Ce content in bioglass [D.W. Wheeler; Leonelli et al.2003; Cacaina et al.2006]. Thereby, it has been observed that deposition of anapatite layer was delayed in these glasses forming after 7–14 days incubation in SBF.

1.4 Uses of rare earth element

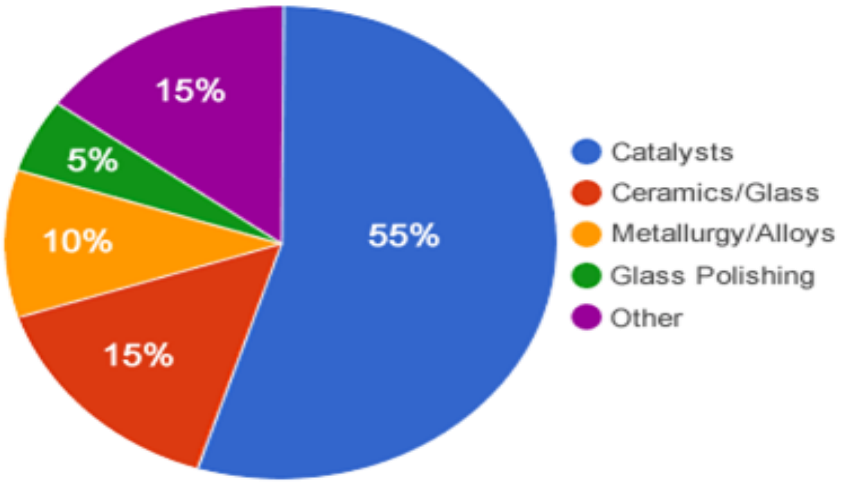

Figure 1.2: Uses in the United States geological survey mineral commodity, 2017.

Application of rare earth elements including all lanthanide elements (such as lanthanum, cerium, and samarium) in orthopaedicbioimplants have been increased drastically in the last few years [Fujimori et al. 1999]. Specifically, La has been widely used to substitute Ca in hydroxyapatite or other bioceramic such as bioglass in order to enhance the physicochemical and biological properties of substituted bioceramics [E. Willbold et al. 2015]. Also, it has been observed that the incorporation of La^{3+} in HA stabilized its apatite structure [G. Fei et al. 2007] and thus improves tensile and bending strengths of HA. Moreover, La incorporated hydroxyapatite was observed to facilitate formation of fibrous tissue around the matrices without causing significant inflammation after implantation in the bones of rats [J.L. Rygel et al 2009]. Furthermore, it was reported that the lanthanum substitution for Ca in apatite enhances the resistance of hard tissues to acid dissolution. Thus, a previous observation shows promising application potential of lanthanum to improve the performance of orthopedic implants in biomedical applications.

Al_2O_3 has been widely investigated as an additive in melt-quenched based glasses in order to enhance their mechanical properties as early as the 1990s. Alumina (Al_2O_3) and $Sm_2O_3$is a good example of network intermediate, by adding alumina to an alkali glass or glaze gives strength, chemical resistance and higher devitrification resistivity. On the other hand, the addition of alumina also rises the softening and melting point of the glass.Also, aluminum incorporation was observed to accelerate the generation of bioactive hydroxyapatite layers over the surface of phosphate-rich bioglasses, [El-Kheshen et al. 2008]. Incorporation of Al_2O_3 in bioactive glass was done previously with expectation to improve the bone tissue defect regeneration potential and to control the biodegradation of

bioglass based implants for long-term stability. It has been reported that the incorporation of Al^{3+} in bioglass at appropriate concentration leads to enhanced mechanical resistance of the bioglasses for successful application as orthopedic implants [M. A. K. Elfayoumi]. Incorporation of Al_2O_3 leads to the breaking of P = O bonds and the Si–O–P linkages are replaced by Al–O–P linkages in the glass network and thus enhances resistance of the bioglass towards biodegradation via enhancing its stability under physiological conditions.

1.5 Biomaterials

Any natural or synthetic material that is engineered to interact with the tissues in a living body is defined as biomaterial. According to Williams "a biomaterial is a non-toxic material of natural or man-made origin, which is intended to interface with a biological system to treat, augment or replace any tissue, organ, or function of the body and that evokes a minimal biological response" [Ratner et al. 1996]. In simple words, a biomaterial is a non - toxic material that can be used to construct artificial organs, rehabilitation devices or prostheses, and to replace natural tissues. The mechanism of tissue attachment of an implant is directly related to the tissue response at the implant interface [L.L. Hench et al, 1998]. No material implanted in living body is inert: all materials elicit a response from the host tissue. According to the different types of implant-tissue attachment, biomaterials are classified into four types, which are summarized in Table 1.1.

Table 1.1: ***Types of tissue attachment of biomaterials [L. L. Hench et al. 1996]***

Type of implant	Type of attachment	Example
Nearly inert	Mechanical interlock (morphological fixation)	Metals, Alumina, Zirconia, Polyethylene
Porous	In growth of tissues into pores (biological fixation)	Hydroxyapatite , Hydroxyapatite coated porous metals
Bioactive	Interfacial bonding with tissues (bioactive fixation)	Bioactive glasses, Bioactive glass - ceramics, Hydroxyapatite
Reorbabale	Replacement with tissues	Tricalcium phosphate, Polylactic acid

All compositions in region A have a constant 6 weight % of P_2O_5, A/W glass-ceramic has higher P_2O_5 content shown in Figure 1.3. Region A develops HA both in vitro and in vivo. Compositions inside the dashed line bind also to soft tissues. The materials in region B are inert and those in regions C are restorable. Region D is a non-glass forming and nonbonding region [L.L. Hench et al, 1991].

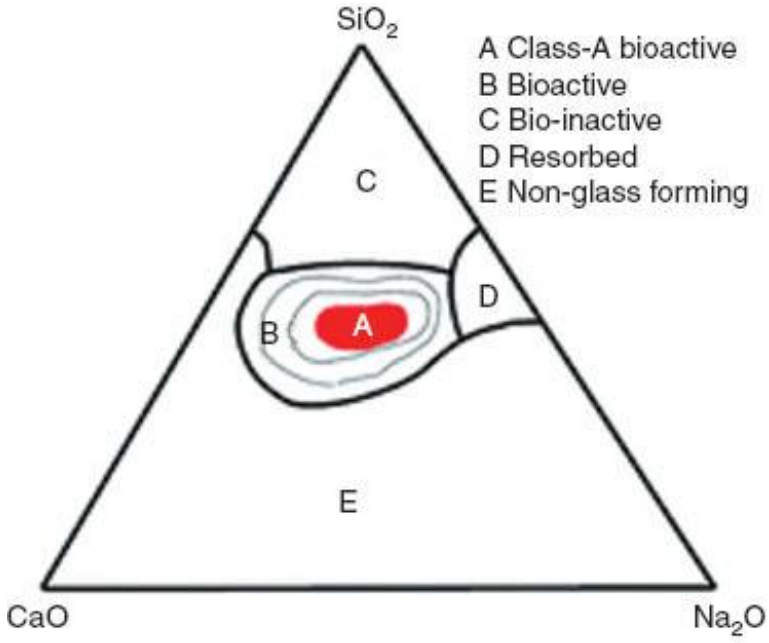

Figure 1.3: Kineticdiagram of bioactivity, compositional dependence (wt %) of bone bonding and soft tissue bonding of SiO_2-Na_2O-CaO bioactive glasses and glass-ceramics [L.L. Hench et al., 1993].

The tissue response to a biologically inactive, nearly inert implant is formation of a non-adherent fibrous capsule. This attachment is called "morphological fixation". The thickness of the fibrous layer depends on many factors, such as the conditions of the implant, the conditions of the host tissue, the conditions of motion and fit at the interface and the mechanical load. A chemically stable material like alumina elicits a very thin capsule under an optimal mechanical fit. More chemically reactive metallic implants elicit thicker interfacial fibrous layer. Because the interface is not chemically or biologically bonded, relative movement can occur, called micro motion. This movement results in progressive development of the non-adherent fibrous capsule and eventually leads to deterioration in function of the implant or the host tissue at the interface or both. Porous biomaterials provide interfacial fixation by ingrowths of tissue into pores on the surface or throughout the implant. This attachment is called "Biological Fixation". It is capable of withstanding more complex stress than dense nearly inert implants which achieve only "morphological fixation". Resorbable implants are designed to degrade gradually with time and be replaced with natural host tissues. For example, resorbable sutures composed of poly (lactic acid) - poly (glycolic acid) are metabolized to carbon dioxide and water. Tricalcium phosphate ceramics degrade to calcium and phosphate salts. Because large quantities of materials must be handled by cells, the constituents of a resorbable implant must be metabolically acceptable. Another requirement for a resorbable implant is that the resorption rate must be matched to the repair rates of tissues. Bioactive implants offer another approach to achieve interfacial attachment. When a bioactive material is implanted in the body, a series of biophysical and biochemical reactions occur at the implant-tissue interface. These reactions

eventually results in a mechanically strong chemical interfacial bonding. This attachment is called "Bioactive Fixation".

1.6 Bioactive Materials

In a general sense, a bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material [L.L. Hench et al. 1994]. In a more narrow sense, a bioactive material has been defined as a material that undergoes specific surface reactions, when implanted into the body, leading to the formation of a hydroxyl - carbonate apatite (HCAp) like layer that is responsible for the formation of a firm bond with hard and soft tissues [T. Kokubo et al. 2006)].

The level of bioactivity of a specific bioactive material can be related to the time for more than 50% of the interface to be bonded. An index of bioactivity (I_b) introduced by Hench [L.L. Hench et al. 1993] as:

$$I_b=100/t_{0.5bb}$$

Where $t_{0.5bb}$ is the time for more than 50% of the implant interface to be bonded to tissues bond strength and the time needed for bonding depend on the type of bioactive material and its bonding mechanism, as well as the thickness of the bonding zone. However, the critical character of a bioactive material is its ability to undergo chemical/biological bonding in the interface. Based on the type of biochemical bonding at the interface, bioactive materials have been classified into two types: Class A (osteoproductive materials) and Class B (osteoconductive materials) [L.L. Hench et al. 1994]. Osteoproduction has been defined as the where by bioactive surface is colonized by osteogenic stem cells free in the defect environment as a result of surgical intervention. Class A bioactivity occurs

when a material elicits both an intracellular and an extracellular response at its interface. However, the materials of Class B, the osteoconductive materials, elicit only an extracellular response at their interface [W. Cao et al. 1996]. Bioactive material includes a wide range of materials such as: bioactive glasses, bioactive glass - ceramics, hydroxyapatite**,** bioactive composites and bioactive coatings.

1.7 Simulated Body Fluid

In 1991, Kokubo proposed that the essential requirement for an artificial material to bond to living tissues is the formation of hydroxyl-carbonate apatite (HCAp) on its surface when implanted in the living body, and that this in vivo HCAp formation can be reproduced in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma. SBF developed by Kokubodiffers in some ions compared to human blood plasma. Some other researchers have tried to correct this difference by preparing SBF with alternative compositions. It was made a revised SBF (r - SBF) in which the concentrations of Cl^- and HCO_3^- ions were adjusted to the levels in human blood plasma. However, calcium carbonate showed a strong tendency to precipitate from r - SBF. Takadama et al. [Takadama et al. 2005, T. Kokubo et al.2006]. It was also a modified SBF (n - SBF) in which only the Cl^-ion concentration was increased. This n - SBF does not differ from the SBF by Kokubo in stability and reproducibility.

1.8 Bioactive Glasses

Most of the published works on bioactive glasses are concentrated on silica based materials. Silica - based bioactive glasses have supplied successful solutions to

different bone defects and soft tissue treatments during the last decades [L.L. Hench et al.1993]. The high biocompatibility and the positive biological effects of their reaction products (both leached or formed at the surface) after implantation, have made silica-based bioactive glasses one of the most interesting bioactive materials during the last 40 years. In contrast, the poor mechanical properties of these bioactive glasses have seriously limited the range of clinical applications.

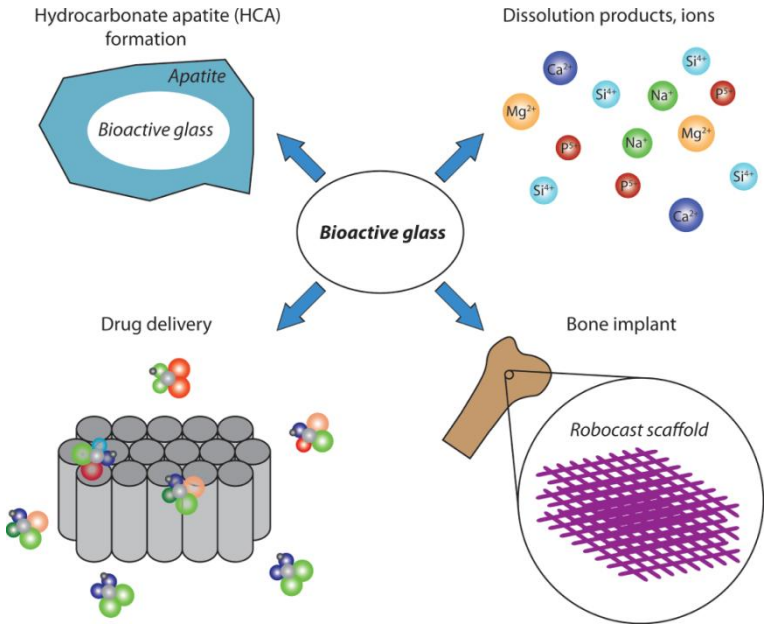

Figure 1.4: Different aspects of bioactive glass (BG) materials can be exploited to induce specific biological activities [A. Polini et al. 2013].

These bioactive glasses in different forms are needed for different clinical applications. Some clinical applications of silica based bioactive glasses are given in Table 1.2.

Table 1.2: ***Clinical applications of silica - based bioactive glasses***

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

Silica based glasses have an amorphous network structure based on the SiO_4^{4-} tetrahedron as the structural unit. The tetrahedra are linked to each other only at the oxygen ions at the corners. In crystalline silica, the tetrahedra are regularly arranged as shown in Figure 1.2.However, a silica based glass has a more open structure due to the existence of non-bridging oxygen ions.The open structure of the silica based glass is formed by the disruption of the network structure by the presence of network modifiers, e.g. Na^+, K^+, Ca^{2+}, Figure 1.3suggested that the bioactivity of a glass is based on the mean number of non-bridging oxygen ions in the silica tetrahedron. Instead of sharing a corner with another tetrahedron, the charge of the oxygen ion in the corner is balanced by a network modifier anion, e.g. Na^+, K^+, Ca^{2+}, and Figure 1.3.In silica based glass, each silicon is bonded to four oxygen atoms and thus the number of non - bridging oxygen ions in the tetrahedron can take any value between 0-4. The number 0 represents a crystalline SiO_2 structure or quartz glass; the number 4 means a dissolved SiO_4^{4-} ion. To be

bioactive for a silica based glass the number of non-bridging oxygen ions per tetrahedron must be greater than 2.6 [H. Ylanen et al. 2000]. Traditional silica based glasses consist of more than 65% SiO_2 by weight, less than 15% Na_2O by weight and about 10% CaO by weight. The composition of silica based bioactive glasses is different from traditional silica based glasses, though bioactive glasses resemble them. Bioactive glasses typically contain less than 60% SiO_2 by weight and large amounts of alkali and/or alkaline earth oxides.

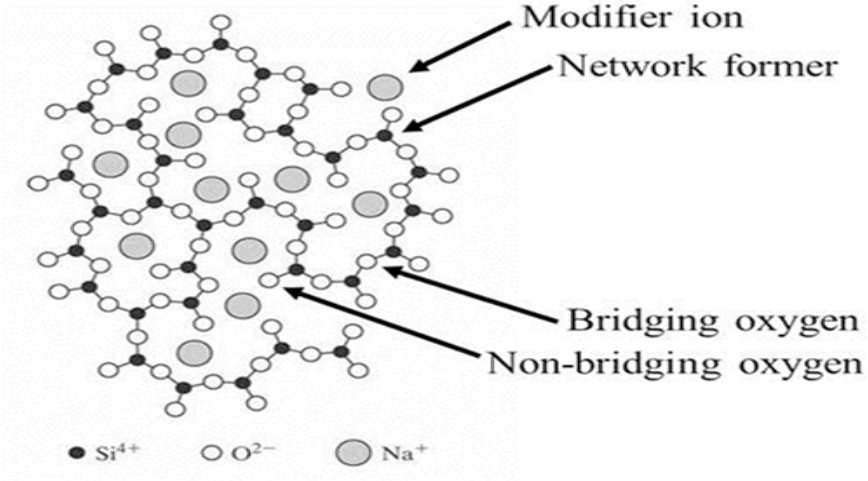

Figure 1.5: Two – dimensional presentation of a random glass network composed of network modifiers and network formers (SiO_4^{4-} units) [Starnd et al. 1992].

According to Hench et al. [L.L. Hench et al. 1991], three key compositional features distinguish bioactive glasses from traditional SiO_2 - Na_2O - CaO glasses:

- Amount of SiO_2 is 40 - 60% by weight;
- High Na_2O and high CaO content;
- High CaO/P_2O_5 ratio.

If the content of SiO_2> 60% by weight, the number of bridging oxygen ions is so large that it will dramatically reduce the network dissolution rate of the glass, thus leading to loss of bioactivity.However, a content of SiO_2< 40% by weight, will give totally dissolved monomeric SiO_4^{4-} units. It is questionable whether obtaining

a glass phase of this composition is possible [K.E. Healy et al. 1987]. Thus, in order to show bioactivity the SiO_2 content of the glass should be between 40 and 60% by weight. The base components in most silica based bioactive glasses are SiO_2, Na_2O, CaO and P_2O_5. Previously, it was found that P_2O_5 was very much necessary for the development of bioactive glasses. However, phosphate in the glass was later found only to aid in nucleation of the calcium phosphate phase on the surface. Phosphate is not a critical constituent because the surface can adsorb phosphate ions from solution [L.L. Hench et al. 1993].

The mechanisms of tissue bonding of silica based bioactive glass have been attributed to the formation of a hydroxyl - carbonate apatite (HCAp) layer on the glass surface when it is in contact with the body fluid.While some details of the chemical and structural changes are not clear, the HCAp layer is generally believed to form as a result of a sequence of reactions on the surface of the bioactive glass implant, as described by Hench [Hench 1998b]:

1. Rapid exchange of cations such as Na^+ or Ca^{2+} with H^+ or H_3O^+ ions from the solution, leads to creation of silanol (Si - OH) groups on the glass surface: $Si - O - Na^+ + H^+ + OH^- \rightarrow Si - OH + Na^+$ (solution) + OH^-

2. Loss of soluble silica in the form of silicic acid, $Si(OH)_4$, to the solution, resulting from breaking of Si - O - Si and the continued formation of Si - OH groups on the glass surface: $Si - O - Si + H_2O \rightarrow Si - OH + OH - Si$

3. Condensation and polymerization of amorphous silica (SiO_2) - rich layer on the surface of the glass depleted in Na^+ and Ca^{2+}: $OH - Si - OH + OH - Si - OH \rightarrow OH - Si - O - Si - OH + H_2O$

4. Migration of Ca^{2+} and PO_4^{3-} ions to the surface through the amorphous

SiO_2 - rich layer, leading to the formation of an amorphous calcium phosphate ($CaO-P_2O_5$) layer on the surface of the amorphous SiO_2 - rich layer, followed by growth of the amorphous $CaO-P_2O_5$ by incorporation of soluble Ca^{2+} and PO_4^{3-}ions from solution.

5. Crystallization of amorphous $CaO-P_2O_5$ layer by incorporation of OH^-, CO_3^{2-} anions from the solution to form crystallineHCAp layer.

The HCAp layer on the silica based bioactive glass surface is similar to biological apatite having the chemical formula given below [L.L. Hench et al.1993]:

$[(Ca, M)_{10} (PO_4, CO_3, Y)_6 (OH, F, Cl)_2]$

WhereM = Na^+, K^+, Mg^{2+}, Sr^{2+}, Pb^{2+}, Ba^{2+} etc. and Y = HPO_4^{2-}, SO_4^{2-}, BO_3^{2-} etc.

With the initial formation of an HCAp layer a sequence of events that appear to be associated with the formation of a bond with tissues are [L.L. Hench 1998]:

- Adsorption of biological moieties in the amorphous SiO_2 - rich and HCAp layer.
- Action of macrophages.
- Attachments of stem cells.
- Differentiation of stem cells.
- Generation of matrix.
- Mineralization of matrix.

The biocompatibility of silica based bioactive glass has long been established [J.Wilson et al. 1981]. After implantation, silica based bioactive glass undergoes degradation, releasingalkali ions such as Na^+ and Ca^{2+}. Si, presumably in the form of Si $(OH)_4$, is also releasedduring the degradation by dissolution

mechanisms. The release of Si from silica based bioactive glass implanted in living body has been studied to determine the pathway of released Si [Lai et al. 2002]. By measuring the Si released in urine and blood samples for up to 7 months post implantation, and using chemical and histopathological analyses of bone and several tissues, it was found that the Si resulting from the 45S5 bioactive glass degradation was harmlessly excreted in soluble form through the urine.

The first bioactive glass developed by Hench et al. [L.L. Hench et al. 1993] and named 45S5 bioactive glass [Composition (weight %) 45 SiO_2 - 24.5 Na_2O - 24.5 CaO - 6 P_2O_5], which exhibits a high bioactivity and can join readily even to soft tissues, is a silica based bioactive glass. A silica based bioactive glass designated 13 - 93 [Composition (weight %) 53 SiO_2 - 6 Na_2O - 12 K_2O - 20 CaO - 5 MgO - 4 P_2O_5] is based on the 45S5 bioactive glass composition, but it has a comparatively higher SiO_2 content and additional network modifiers, such as K_2O and MgO, when compared to 45S5 bioactive glass, is also used clinically [M.N. Rahaman et al. 2011]. However, 13-93 bioactive glass degrades and converts to an HCAp material more slowly than 45S5 bioactive glass. Other invented silica based bioactive glasses are: 45S5.4F bioactive glass [Composition (weight %) 45 SiO_2 - 24.5 Na_2O - 14.7 CaO - 9.8 CaF_2 - 6 P_2O_5], 52S4.6 bioactive glass [Composition (weight %) 52 SiO_2 - 21 Na_2O - 21 CaO - 6 P_2O_5], 55S4.3 bioactive glass [Composition (weight %) 55 SiO_2 - 19.5 Na_2O - 19.5 CaO - 6 P_2O_5], 55S4.3 bioactive glass [Composition (weight %) 55 SiO_2 - 19.5 Na_2O - 19.5 CaO - 6 P_2O_5], 6P53S bioactive glass [composition (weight %) 52.7 SiO_2 - 10.3 Na_2O - 2.8 K_2O - 18.0 CaO -10.2 MgO - 6 P_2O_5] and 58S bioactive glass [Composition (weight %) 58.2 SiO_2 - 32.6 CaO - 9.2 P_2O_5], [Andersson et al. 1990; Deaza et al. 2007; Hench et al. 1993; Uchino et al. 2009].More recent

works have shown that certain borate based glasses such as [composition (weight % 53) B_2O_3 - 10.3 Na_2O - 2.8 K_2O - 18 CaO - 10.2 MgO - 6 P_2O_5] and [Composition (weight %) 56.6 B_2O_3 - 5.5 Na_2O - 11.1 K_2O - 18.5 CaO - 4.6 MgO - 3.7 P_2O_5] are also bioactive [M.N. Rahaman et al. 2011]. Because of their lower chemical durability, borate based bioactive glasses degrade faster and convert more completely to an HCAp like material, when compared to silica based bioactive glasses. Borate based bioactive glasses have been shown to support cell proliferation and differentiation in vitro as well as tissue infiltration in vivo. Borate based bioactive glasses have also been shown to serve as a substrate for drug release in the treatment of bone infection. A concern associated with borate based bioactive glass is the toxicity ofboron released into the solution as borate ions $(BO_3)^{3-}$. It concludes that the ability to control the degradation rate of silica-based bioactive glasses by manipulating its composition by recent work. For example, by partially replacing the SiO_2 in silica based bioactive glasses with B_2O_3 (yielding a borosilicate bioactive glass), such as: 45B15S5 bioactive glass [Composition (weight %) 30 SiO_2 - 15 B_2O_3-24.5 Na_2O-24.5 CaO-6 P_2O_5] and 13 - 93B1 [Composition (weight %) 34.4 SiO_2 - 19.9 B_2O_3 - 5.8 Na_2O - 11.7 K_2O - 19.5 CaO - 4.9 MgO - 3.8 P_2O_5], the degradation rate can be varied over a wide range. The ease of manufacture and the ability to control the degradation rate of silica based bioactive glasses make them particularly useful for promoting the regeneration of tissue. By controlling the glass composition, it should be possible to match the degradation rate of silica based bioactive glass with the tissue regeneration rate.Some phosphate based glasses such as composition (weight %) 9.3 Na_2O - 19.7 CaO 71 P_2O_5 are also bioactive. As their constituent ions are present in the organic mineral phase of bone, these glasses have a chemical

affinity with bone. The solubility of these glasses can be controlled by modifying their composition; therefore these glasses may have additional clinical potential as resorbable materials.Depending on manufacturing process, bioactive glass can be divided mainly into two groups: sol - gel bioactive glasses and melt - derived bioactive glasses. Sol - gel bioactive glasses are made by a chemical based process at much lower temperatures than the traditional processing methods. Sol - gel bioactive glasses have been investigated by many research groups [Balamurugan et al. 2007; Li et al. 2005; Liu et al. 2004; Xia et al. 2006]. Li and co - workers in 1991 have shown that sol - gel bioactive glasses in the system of SiO_2 - Na_2O - CaO are bioactive even up to 85 mol % SiO2. The wide range of bioactive oxide compositions makes it possible to tailor the reactivity of the glasses to various applications. Also, sol - gel processing offer the potential advantages of ease of powder production, high purity of the material and better control of bioactivity through changes in processing parameters [Li et al. 1991]. Compared with the sol - gel process, melting requires much higher working temperatures. However, melting is a simple and low cost technique and is much less time consuming than sol-gel processing. For production of a large amount of bioactive glasses the melting process is very suitable and reliable. Because of these benefits, melting is the dominate process for producing bioactive glasses. There are some challenges also present in the bioactive glass.

1.9 Hydroxyapatite

Hydroxyapatite is a crystalline form of calcium phosphate similar to the mineral present in bone. It is a compound with a definite crystallographic structure and of a definite composition, $Ca_{10}(PO_4)_6(OH)_2$.The mineral component in the living bone is also a hydroxyapatite, the so - called biological apatite. The amount of the

biological apatite in bone is approximately 70% by weight. It was believed that hydroxyapatite used for bone replacement would be entirely compatible with the body. When exposed to body fluids, hydroxyapatite will bond to bone by forming indistinguishable unions. The bonding starts by formation of hydroxyl - carbonate apatite (HCAp) crystals on the bone, thus promoting the adhesion of matrix - producing cells and organic molecules as a result of surface chemistry and surface charges [L.L. Hench et al. 1993]. Biological apatite, which comprises the mineral phase of human bone, is usually referred to as hydroxyapatite. Actually, biological apatite differs from pure hydroxyapatite, and it is more appropriate to refer to as carbonate apatite [L.L. Hench et al. 1993]. Biological apatite contains ions such as Na^{+}, K^{+}, Mg^{2+}, F^{-} andCl^{-} in solid solution. Some of the PO_4^{3-} may also be replaced by CO_3^{2-}. Consequently, the ideal Ca/P molar ratio of pure hydroxyapatite (1.67) differs slightly from that of biological apatite (1.72-1.80) [H. Ylanen et al. 2000].. The possibility of improving apatite by changing its composition has inspired researchers to deliberately substitute ions in order to modify its properties and behavior [Hayakawa et al. 2008; Osaka et al. 2007]. The main goal in these studies has been to enhance bone bonding between the implant and tissue.

Hydroxyapatite can be prepared in either dense or macroporous forms. The surface chemistry of porous sintered and cemented hydroxyapatite is the same as that of the dense forms [L.L. Hench et al. 1993]. However, tissue response to porous hydroxyapatite implants is inherently different from its response to dense hydroxyapatite because of the opportunity for in growth. Thus, porous hydroxyapatite has now replaced dense hydroxyapatite form. When porous hydroxyapatite has been placed into bone defects, bone growth into the pores has ranged from 18% to 74% [Holmes et al. 1988; Martin et al. 1993]. The entire

porous space of the implant is probably never completely filled with bone [Hulshoff et al. 1997; Rosen et al. 1990]. Porous hydroxyapatite is osteoconductive, biocompatible and practically inert; it resorbs with time but the degradation rate is slow. Due to the brittle nature of porous hydroxyapatite, it can be used only in non - loading sites. In general, synthetic hydroxyapatite is widely used in dental, craniofacial and orthopedic surgery, mainly as granules, and as a bioactive coating on load-bearing implants, etc.

1.10 Bioactive Composites

Although bioactive materials can form a strong biochemical bond with the bone and soft tissues, the mechanical properties of the materials themselves are usually unsuited for load-bearing applications. One approach to solve this problem is to combine them with a fracture tough material to produce a composite. The bioactive composites are divided into two groups based on the goal of the implant [W. Cao et al. 1996]. The first group consists of the compositions in which a fracture resistant phase (metal fibers or tough ceramic particles) is used to reinforce the bioactive material [W. Cao et al. 1996]. In other words, the matrix of the composite is a bioactive material and the reinforcing phase is a tough material (matrix/reinforce bioactive material/tough material). In 1993, Ducheyne et al. introduced the first bioceramic composites of metal fibers and glasses, i.e. stainless steel fiber/bioactive glass and titanium fiber/bioactive glass composites. These discontinuous metal fiber/ceramic composites were shown to maintain the bioactivity of the ceramics, but to have enhanced fracture resistance and strength compared to the ceramic alone [Gheysen et al. 1983]. One year later, the metal fiber reinforced bioactive glass composites were patented by Ducheyne [L.L. Hench et al. 1993]. Other types of composites also have been developed. For

example, B. Cabal et al. [B. Cabal et al. 1997] described a processing route for the fabrication of metallic fiber mat reinforced bioactive glass matrix composites. However, the disadvantage of these composites is that they have elastic moduli greater than bone and thus give rise to stress shielding of bone.

The second group of composites uses powders, particles or fibers of bioactive materials to reinforce an elastically compliant and biocompatible polymer matrix, for example, poly(D,LLactice)/45S5 bioactive glass composite [Helen et al. 2006, Roether 2002], Poly(etheretherketone)/hydroxyapatite composite and poly(DL - Lactide - coglycolide)/ bioactive glass composite foams. Polymer - biomaterial composites solve the problem of stress shielding of bone. Bonfielddemonstrated that an increase in the volume fraction of particulate hydroxyapatite from 0 to 0.5 (50% volume) produced an increase in the Young's Modulus of the hydroxyapatite reinforced polyethylene composite, thus approaching the lower range of values associated with bone itself. Rich et al. studied in vitro bioactivity of poly (ε-caprolactone-co-DL-Lactide) reinforced by different amounts (40, 60 and 70 % by weight) of the bioactive glass S53P4 [Rich et al. 2002]. They found that the in vitro bioactivity was dependent on the weight fraction and granule size range of the bioactive glass used. The in vivo studies of the glass-fiber-reinforced composite have been done [Tuusa et al. 2005]. They also developed glass-fiber-reinforced composite with bioactive glass granule coating, and the in vivo tests showed that the coated composite implant provided an alternative for bone defect reconstruction, especially in head and neck area [Tuusa et al. 2007]. The bioactive part of the composite can also be a mixture of two biomaterials [Juhasz et al. 2004]. For applications without load-bearing requirements, composites derived from two bioactive materials have also been developed. Bioactive glass -

reinforced hydroxyapatite composites have higher fracture resistance and greater bone bonding ability than that of commercial hydroxyapatite [Goller et al. 2003]. Miao et al. [Miao et al. 2007] recently developed a porous calcium phosphate ceramic modified with poly (lactic-co-glycolic acid) bioactive glass. This composite showed enhanced strength due to infiltration of the PLGA (poly (lactic-co-glycolic acid)) into the inner pores of the porous ceramic. Bioactivity was achieved by an additional coating with the bioactive glass PLGA.

1.11 Bioactive Coatings

To solve the problems of the mechanical limitations of bioactive materials in load-bearing applications it is to apply the material as a coating on a mechanically tough substrate. Bioactive coatings can modify the surface of implants and create an entirely new surface, thus giving the implant bioactive properties which are quite different from those of the uncoated implant. The bioactive coating materials successfully combine the bioactivity of bioactive materials and the good mechanical properties of tough materials. The bone-bonding capacity of these coatings may help to provide cement less fixation of orthopedic prostheses, especially for short term stabilization of the implants [Cao et al. 1996]. But in long term implantation, the bioactive coated materials suffer from a lack of stability of the coating/implant interface. Because of its similarity to the inorganic component of bone and tooth, hydroxyapatite was one of the first materials considered for coating metallic implants.

A research group from Japan developed a carbonate hydroxyapatite coating to titanium, for use in bone bonding implants. The coating significantly increased the bone bonding strength by providing a bioactive surface. Similar research was carried out by [S. Kumar et al. 2002] by producing coating materials with

different ratios of TiO_2/hydroxyapatite. Hydroxyapatite a coating on the other hand is clinically important implants such as porous zirconia have also been studied [Miao et al. 2007]. Osaka et al. [Osaka et al. 2007] developed a bioactive composite coating consisting of one layer of titania and one layer of apatite on a titanium substrate. Thermal spraying, in particular plasma spraying, is the most common method for applying hydroxyapatite coatings. Other techniques have also been investigated for commercial applications, including electrophoretic deposition processes, hot isostatic pressing, ion beam sputtering, radio frequency sputtering, and thermal spray techniques other than plasma spraying, such as the high velocity oxy-fuel technique.

1.12 Area of Biomaterials

✓ Bioactive glass ✓ Tissue culture vessels ✓ Diagnostic devices ✓ Thermometers	✓ Dental sector
✓ Filling materials ✓ Gold porcelain coating ✓ Prosthetic parts	✓ Dental sector