General Introduction

1.1 Bioceramics

MANY millennia ago, the discovery that fire would irreversibly transform clay into ceramic pottery led to an agrarian society and an enormous improvement in the quality and length of life. Further, another revolution occurred in the use of ceramics during the past four decades to improve the quality of life. This revolution is the innovative use of specially designed ceramics for the repair, reconstruction and replacement of diseased or damaged parts of the human body. Ceramics used for this purpose are termed ‘‘bioceramics’’. The field of bioceramics encompasses single crystal and polycrystalline alumina, zirconia or partially stabilized zirconia (PSZ), hydroxyapatite, bioactive glasses, bioactive glass-ceramics, A/W glass ceramics as well as bioactive composite (polyethylene–hydroxyapatite). Several special ceramics and glasses have been designed and developed during this century for use in the health care such as eyeglasses, diagnostic instruments, chemical wares, thermometers, tissue culture flasks, fiber optics for endoscopy and carriers for enzymes & antibodies (Hench, 1982). Ceramics are also used widely in dentistry as restorative materials, gold porcelain crowns, glass-filled ionomer cements, dentures, etc. The materials used in these applications are called dental ceramics (Preston, 1988).

Bioceramics have been earlier produced in several forms and phases which serve different functions in the repair of the body, as given in Fig. 1.1 and Table 1.1. In different area of applications ceramics are used in the form of bulk materials of a specific shape as *implants*, *prostheses*, or *prosthetic devices* (Hench, 2013). Bioceramics are also used to fill space while the natural repair processes restore functions. In other situations

the ceramic is used as a coating on a substrate, or as a secondary phase in a composite which combine the characteristics of both into a new material with enhanced mechanical and biochemical properties.

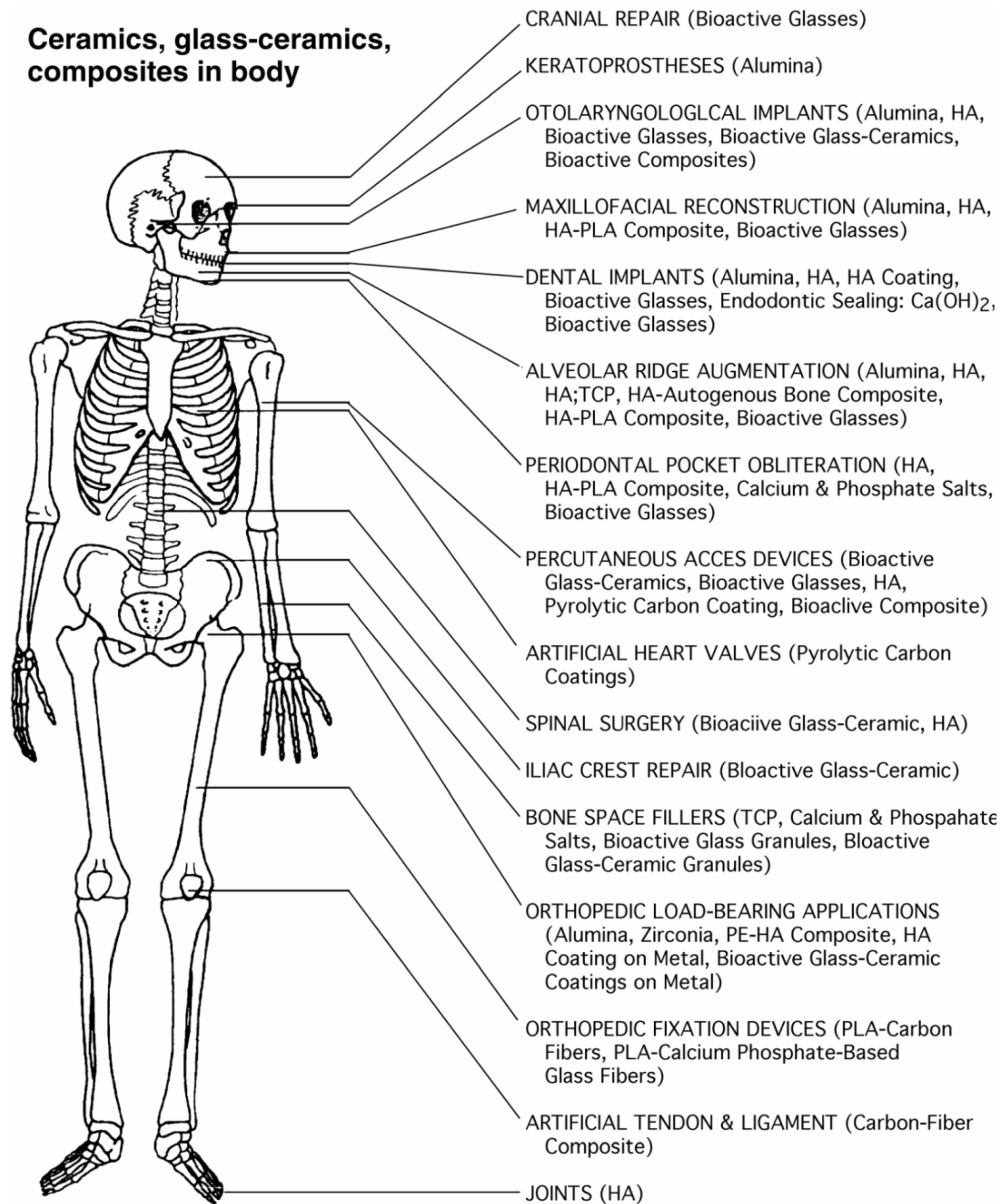

Fig. 1.1: Clinical uses of ceramics, glasses and composites in the human body (Hench, 2013).

Bioceramics are made in various different phases. They can be single crystals (sapphire), polycrystalline (alumina), hydroxyapatite, Bioglass®, bioglass ceramics, A/W glass-ceramics or composites (polyethylene-hydroxyapatite). The phases used depend on the properties and required functions. For example, single crystal sapphire is used as a dental implant because of its high strength. A/W glass-ceramic is used to replace vertebrae because it has high strength and also bonds to bone. Bioactive glasses have low strength but bond rapidly to bone, as they are used to augment the repair of boney defects.

Table 1.1 Form, phase and function of bioceramics.

Form	Phase	Function
Powder	Polycrystalline, Glass	Space-filling, therapeutic treatment, regeneration of tissues
Coating	Polycrystalline, Glass and Glass-Ceramic	Tissue bonding, thromboresistance, corrosion protection
Bulk	Single Crystal Polycrystalline, Glass and Glass-Ceramic, Composite (Multi-Phase)	Replacement and augmentation of tissue, replace functioning parts

The first total hip replacement was done with alumina days back to 1971 (Boutin, 1972). The earlier work done by Hench *et al* (Hench *et al*., 1971) concerning the mechanism of bonding of biomaterials to host tissue has opened the field of research on bioactive materials. Shortly after their report, Ceravital® was patented (Bromer *et al*., 1973) which

refers to a number of different glass compositions used for the replacement of the ossicular chain in the middle ear. In the mid seventies, three independent groups commercialized a synthetic form of HA for orthopaedic applications (Jarcho, 1976, de Groot, 1983, Denissen, 1979 and Aoki *et al*., 1977). HA can be used either as dense or as porous material. Tricalcium phosphate (TCP) has been also used to make resorbable implants (Koster *et al*., 1977). A/W glass ceramics in the $MgO\text{-}CaO\text{-}SiO_2\text{-}P_2O_5$ system, containing apatite and wollastonite phases, were introduced by Kokubo and others (Kokubo *et al*., 1982). The A/W glass ceramics have been used for vertebral replacement, iliac crest prostheses and bone defect fillers. Later on, composites made of a variety of bioceramics and other materials have been investigated. Such as stainless steel fiber reinforced Bioglass®, titanium fiber reinforced Bioglass®, TCP or HA reinforced polyethylene (Hench *et al*., 1993).

Bioceramics can be used in granular or bulk form, depending on the requirement as: bulk samples for tissue replacement or augmentation, whereas powders for space-filling, therapeutic treatment, tissue regeneration, or coatings (Hench *et al*., 1971).

1.2 Need for Bioceramics

Bioceramics are needed to alleviate pain and restore function to diseased or damaged parts of the human body. A major contributor to the need for ‘‘spare parts’’ for the body is the progressive deterioration of tissue with increasing age. Bone is especially vulnerable to fracture in older people due to a loss of bone density and strength with age (Hench *et al*., 1993). Fig. 1.2 shows the effect of time on the bone mass (bone strength and density). The effect is severe in women especially because of hormonal changes associated with menopause. Bone density decreases because bone-growing cells

(osteoblasts) become progressively less productive in regenerating new bone and repairing microfractures. The lower density greatly deteriorates the strength of the porous bone, called as trabecular or cancellous bone, in the ends of long bones and in vertebrae. As a consequence many old people suffer fracture unfortunately of their hips or they have collapsed vertebrae and spinal problems.

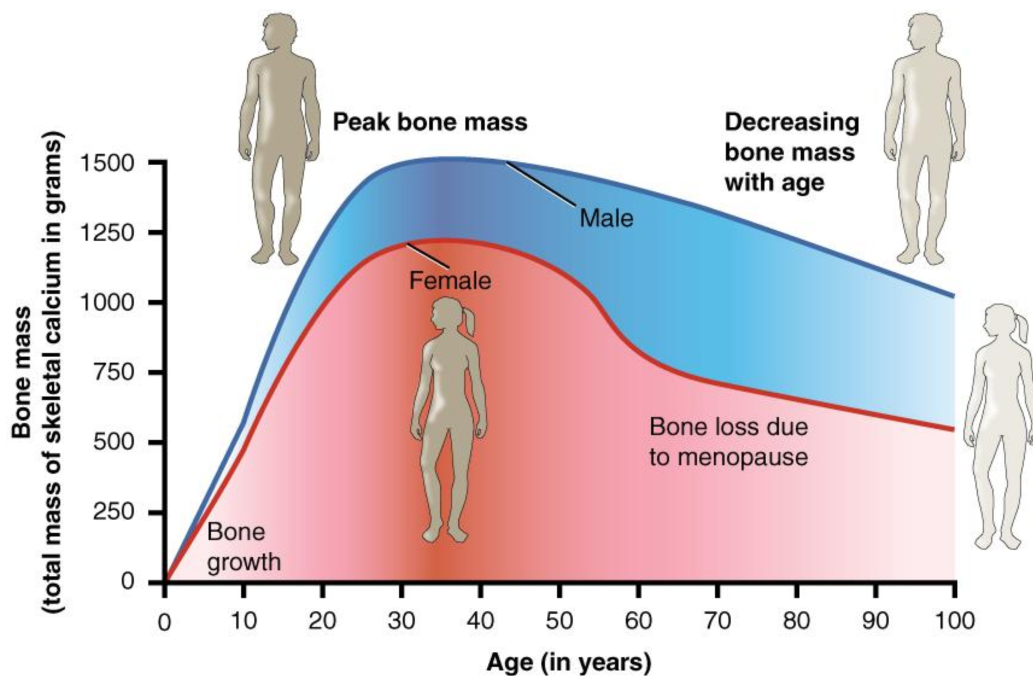

Fig. 1.2: Effect of age on the strength of bone or bone mass in male and female (Hench *et al*., 1993).

The great challenge before the use of ceramics in the body is to replace old, deteriorating bone with a material that can function for the remaining years of the patient's life. Because the average life span of human is now around 80 years and the need for spare parts begins at the age of about 60 years. Bioceramics need to last for around 20 years. This demanding requirement of survivability is under conditions of use which are especially harsh to ceramic materials such as corrosive saline solutions at 37°C under

variable, multiaxial, cyclical mechanical loads. The excellent performance of the specially designed bioceramics which have survived these clinical conditions represents one of the most remarkable accomplishments of ceramic research and development, production as well as quality assurance during this century (Hench, 1998).

1.3 Types of Bioceramic-Tissue Attachments

The mechanism of attachment of tissue to an implant is directly related to the tissue response at the implant interface (Hench *et al*., 1982, Hench *et al*., 1993 and U Gross *et al*., 1988). There are four types of bioceramics each having different type of tissue attachment as presented in Table 1.2 along with certain examples. The factors that influence the implant–tissue interfacial response are listed in Table 1.3 and they also affect the type and stability of tissue attachment as given in Table 1.2.

Table 1.2 Types of Tissue Attachment of Bioceramic Prostheses.

Type of Implant	Type of Attachment	Example
(1) Nearly inert	Mechanical interlock (Morphological Fixation)	Al_2O_3, ZrO_2
(2) Porous	In-growth of tissues into pores (Biological Fixation)	Hydroxyapatite (HA) HA-coated porous metals
(3) Bioactive	Interfacial bonding with tissues (Bioactive Fixation)	Bioactive glasses, Bioactive glass-ceramics, HA
(4) Resorbable	Replacement with tissues	Calcium sulphate, TCP, calcium phosphate salts & Bioactive glasses

Type 1is nearly inert and implant does not form a bond with bone. Type 2 is porous and implant forms a mechanical bond via in-growth of bone into the pores. Type 3 is bioactive and implant forms an interfacial bond with bone via chemical reactions at the interface. Type 4 is resorbable and implant is replaced by the bone through dissolution in human body fluid. The mechanisms of attachment were respectively termed as T_1, T_2, T_3 and T_4 type of mechanisms.

Table 1.3 Factors affecting interfacial response.

Sl. No.	Tissue Side	Implant Side
1.	Type of Tissue	Composition of Implant
2.	Health of Tissue	Phases in Implant
3.	Age of Tissue	Phase Boundaries
4.	Blood Circulation in Tissue	Surface Morphology
5.	Blood Circulation at Interface	Surface Porosity
6.	Motion at Interface	Chemical Reactions
7.	Closeness of Fit	Closeness of Fit
8.	Mechanical Load	Mechanical Load

For many years, it was thought that interactions between body and implants would cause only undesirable reactions, such as tissue irritation, damage and finally death. It was due to the observation that if a toxic material is put in contact with a tissue then it will cause the death of tissue. Due to this reason, the guiding principle used in biomaterials development at the beginning was that they should be as chemically inert as for as possible (Hench *et al*., 1982). Still, even the most inert materials (*bioinert*) elicit a reaction of the body when it is implanted (Hench, 1994 and Hench, 1991). A thin non-adherent fibrous capsule is developed on the bioinert materials after they remain in contact with body for some time under physiological condition. This prevents further

interactions with the tissues. The thickness of the protective fibrous layer developed depends on the type of bioinert material as well as on the motion and fit at the interface. This type of interface cannot last for a longer time. Eventually, deterioration occurs and surgical removal of the device becomes necessary. Owing to this reason, research on biomaterials switched over to the development of new materials that could interact with the body inducing a desirable response by the host tissue.

Porous materials can achieve *biological fixation* and in this case, a mechanical bond is obtained by in growth of bone into the pores if the pore diameter >100 μm (Hulbert, 1993). The increased interfacial area between the implant and the tissue results in an increase in the resistance for the movement of the device in the tissue. Still, also porous materials do not last for longer time when once implanted. Their mechanical resistance is not as high as bulk materials and the corrosion due to the exposure of a large surface area to body fluids further causes a decrease in their strength.

Resorbable bioceramics represent an alternative solution to the problem of the long-term implant failure. These materials are supposed to exploit and increase the human body capacity of self repairing. This happens as these materials degrade gradually over a period of time and are replaced by the natural host tissue. An important issue is the biocompatibility of the products of resorption. Moreover, resorption should occur at a rate similar to cellular metabolism. These requirements are very difficult to be fulfilled and due to this reason, many resorbable biomaterials are not yet clinically applied.

The valuable solution to the problem of achieving a stable implant-tissue interface is *bioactive fixation*. This can be obtained only when *bioactive materials* are used.

1.4 Bioactive materials

“**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**”. This definition was given by Hench, who initiated this subject of research with his colleagues in the early nineteen seventies (Hench *et al.*, 1971). They discovered that certain compositions of glasses in the soda-lime-phosphosilicate (SiO_2-CaO-Na_2O-P_2O_5) system were able to form a bond with bone when they are implanted. In fact, when these glasses were put in contact with biological fluids, a layer of hydroxyapatite (HA) analogous to the mineral phase of bones was deposited on their surface. Collagen molecules were incorporated into this layer and a biological bond could be formed. Later work by Wilson and Nolletti (Wilson *et al.*, 1990) showed that a bond with soft tissue could be achieved too, if the rate of apatite formation was high enough.

The rate of bonding of bioactive glasses depends on many factors. One is the bulk composition and the most rapid rates of bonding for bioactive glasses composed of SiO_2, CaO, Na_2O and P_2O_5 are obtained with SiO_2 contents of 45-52% by weight. In this compositional range, bonding of implant to soft and hard connective tissue occurs within 5 to 10 days. Bioactive glasses or glass ceramics containing 55-60% SiO_2 require a longer time to form an interfacial bond with bones and do not bond to soft tissues. Glass compositions with more than 60% SiO_2 do not bond either to bone or soft tissues and they elicit formation of a non adherent fibrous interfacial capsule (Hench, 1998). These concepts have been summarized in Fig.1.3 which shows the Na_2O-CaO-SiO_2 ternary phase diagram, referring to glasses with a constant 6 wt% of P_2O_5 (Hench, 1991).

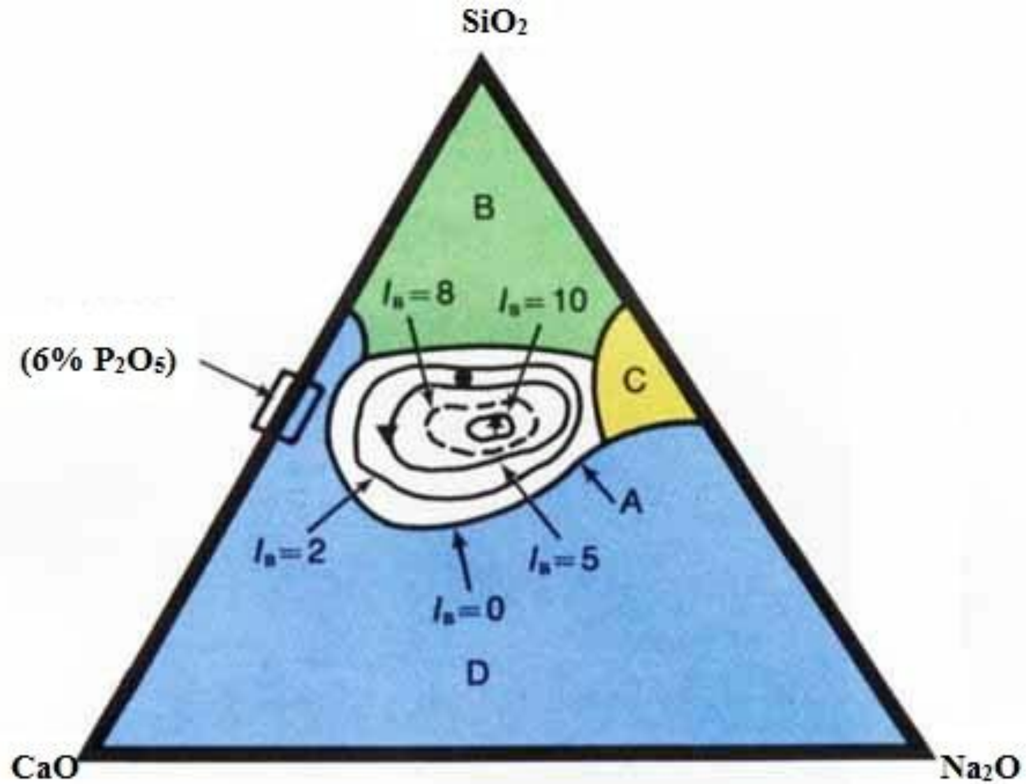

Fig.1.3: Compositional dependence (wt %) of bone bonding and soft tissue bonding of bioactive glasses and glass-ceramics. All compositions in region A have a constant 6 weight % of P_2O_5, A/W glass-ceramic has higher P_2O_5 content. Region A develops HA both *in vitro* and *in vivo*. Compositions inside the dashed line bind also to soft tissues. The materials in region B are inert and those in region C are resorbable. Region D is a non-glass forming and nonbonding region (Hench, 1993).

The values of the *index of bioactivity* (I_B) are given in Fig.1.3. I_B is a measure of the level of bioactivity of bioactive materials and it is defined as the inverse of the time required for more than 50% of the interface to be bonded as given in following equation no. (1).

$$(I_B = 100/t_{0.5\ bb}) \qquad \text{--------------------} \ (1)$$

In the SiO_2-CaO-Na_2O-P_2O_5 system the I_B changes from o to 10 and I_B=0 for bio-inert, I_B=5 for bioactive and I_B=10 for bio resorbable materials, respectively. The change of I_B is very large on comparing glasses in region A and B. If the ions other than Ca and Na are added into glass composition, large variations in I_B have been observed. Greenspan (Greenspan *et al*., 1972) showed that the addition of only 3% Al_2O_3 had destroyed the glass bone-bonding ability and Gross (Gross *et al*., 1980) proved the same for other multivalent cations. The thickness of the bonding zone is approximately proportional to the I_B value and the failure strength of a bioactive bond appears to be inversely proportional to the thickness of the zone. Thus, a very high I_B value gives a thick bonding zone and low shear strength. Depending on the preference of rapid bonding or high shear strength is preferred, different compositions are optimal. The most bioactive composition of glasses in the SiO_2-CaO-Na_2O-P_2O_5 system is called (45S5) Bioglass® which was the first bioactive material discovered in 1969 by Hench (Hench *et al*., 1971). This material also known as Hench glass has found many orthopedic and dental applications. Now days, it is one of the most clinically used bioceramics.

1.5 Solutions used to test *in vitro* bioactivity

The importance of assessing bioactivity *in vitro* prior to *in vivo* test is quite clear. *In vivo* studies require animal sacrifices which are more costly and less easily reproducible and also involve ethical issues. Due to these reasons, before testing bioactivity of the materials *in vivo*, it is necessary to carry out screenings in chemical or biological labs *in vitro*. The choice of the solution used to simulate *in vitro* for the reactions occurring on the surface of the biomaterial is very important. Simple solutions which mimic only the

inorganic composition of human body fluids can be used or more complex solutions that contain some biological moieties also such as proteins. Moreover, cell-containing solutions can be employed, thus increasing both the similarity to real body fluids and the complexity of the test.

The rate of release of ions and pH increase also depend on the dynamic or static methods used to simulate biomaterial reactivity. Many studies were done in 'static', which means that the solution used to dissolve the biomaterial *in vitro* is never changed in the course of the experiment. In other investigations instead, the solution in which the biomaterial is dissolved is periodically changed and refilled with some fresh one (Falaize *et al*., 1999). In some recent experiments, the solution was continuously ricirculated, so that the new solution may be in contact with the biomaterial for all the times (Izquierdo *et al*., 2000). It is difficult to prove which experimental method simulates the best *in vivo* situation. In fact, human body fluids circulate at the interface with the wounded area, but it is far to be define well the extent of this circulation. It has been shown experimentally that the static method quickly induces saturation of the solution and so the apatite precipitates faster and the pH increases more than in the dynamic method (Ramila *et al*., 2001).

Earlier studies concerning bioactivity of Bioglass® were carried out in simple TRIS-buffered solution (Clark *et al*., 1976, Clark *et al*., 1976 and Hench, 1981). TRIS base (tris-hydroxy methyl amino methane) has a pK_a=8.1, and can be used to buffer solutions in the range of pH~7.1-9.1. Since this solution does not contain any ions other than those which are dissolved by the materials immersed into it as such it can be very useful if one needs to analyze the basic steps involved in HA deposition on bioactive materials. Kokubo *et al*. (1990) introduced the use of simulated body fluid (SBF) to analyze

bioactivity of different materials (Kokubo *et al*., 1990). SBF is an aproteic and acellular solution containing different salts that simulate the concentration and pH of human plasma (Table 1.4).

Both SBF and plasma are saturated with respect to hydroxyapatite. For this reason, only a few nucleation sites are sufficient to observe HA nucleation on the surface of some material. This allowed the analysis of bioactivity also on some simple materials that did not contain calcium and phosphorous in their composition. Moreover, the rate of HA deposition in SBF is much higher than in TRIS-buffered solution, obviously because the degree of super saturation with respect to HA is reached more easily in SBF. As an alternative to the use of SBF is a solution containing TRIS and the electrolytes typical for plasma (Radin *et al*., 1997).

Table 1.4 Concentration (mM) and pH of simulated body fluid (SBF) and human plasma (Fujibayashi *et al*., 2003)

Sl. No.	Ions	SBF (mM Concentration)	Plasma (mM concentration)
1.	Na^{+}	142.0	142.0
2.	K^{+}	5.0	5.0
3.	Mg^{2+}	1.5	1.5
4.	Ca^{2+}	2.5	2.5
5.	Cl^{-}	147.8	103.0
6.	HCO_3^{-}	4.2	4.2
7.	HPO_4^{2-}	1.0	1.0
8.	SO_4^{2-}	0.5	0.5
9.	pH	7.25	7.20-7.40

Bovine and human serum is often used for *in vitro* studies when researchers wanted to analyze protein adsorption on biomaterials (Bosetti *et al*., 2001 and Rosengren *et al*., 2003). The reactions occurring at the surface of biomaterials in contact with protein containing solutions have also been studied with Dulbecco's Modified Eagle's minimum essential medium supplemented with 10% Nu-Serum™ (Effah Kaufmann *et al*., 2000), which contains growth factors, hormones and vitamins.

A step further to simulate *in vitro* the real condition of biomaterials immersed into body fluids is the immersion in cell-containing solutions. Osteoblast cells have often been used. In order to understand the influence of the presence of biomaterials on cells, different tests can be done. At first, usually cell morphology, adhesion and proliferation are examined and then cell activity can be tested by the amount of some specific enzymes produced. As for example, osteoblasts synthesizing bone matrix produce alkaline phosphatase. Another important protein that can be evaluated is osteocalcin. This is a non-collagenous extracellular matrix protein and its presence is indicative of the beginning of bone mineralization.

1.6 Bioactive glasses

It was discovered by Hench and his colleagues in 1969 that bone can bond chemically to certain glass compositions (Hench *et al*., 1972). This group of glasses was known as bioactive glasses, based upon definition of bioactive materials as given earlier: **"*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*"**

(Hench *et al*., 1991, 1991, 1998 and 2004). Bioactive glasses have got their numerous applications in the repair and reconstruction of diseased and damaged tissue, especially hard tissue (bone). 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 most reactive glass compositions develop a stable, bonded interface with soft tissues (Wilson *et al*., 1981). It is possible to design glasses with properties specific to a particular clinical application. This is also possible with some glass-ceramics but their heterogeneous microstructure restricts their versatility.

1.6.1 Compositions

The base components in most bioactive glasses are SiO_2, Na_2O, CaO and P_2O_5 (Table 1.5). The first and foremost well-studied composition in wt%, termed Bioglass® 45S5 (Registered trademark University of Florida, Gainesville, FL), contains 45% SiO_2, 24.5% Na_2O, 24.4% CaO and 6% P_2O_5 (Hench *et al*., 1972). The 45S5 bioglass® composition in mol% is also given in Table 1.5, along with several other compositions investigated for the kinetics of surface reaction. Hench and coworkers have studied a series of glasses in this four-component system (SiO_2-Na_2O-CaO-P_2O_5) with a constant 6.0 weight % P_2O_5 content. This work is summarized in the ternary SiO_2-Na_2O-CaO diagram as shown previously in Fig. 1.3.

Table 1.5 Composition (wt %), structure and index of bioactivity of different melt-derived glasses (Hench, 1991).

Constituents, structure and I_B	45S5	45S5.4F	52S4.6	KGC Ceravital®	A/W-GC
SiO_2	45	45	52	46.2	34.2
P_2O_5	6	6	6	-	16.3
CaO	24.5	14.7	21	20.2	44.9
$Ca(PO_3)_2$	-	-	-	25.5	-
CaF_2	-	9.8	-	-	0.5
MgO	-	-	-	2.9	4.6
Na_2O	24.5	25.5	21	4.8	-
K_2O	-	-	-	0.4	-
Structure	Glass	Glass	Glass	Glass-ceramic	Glass-ceramic
I_B	12.5	12.5	10.5	5.6	6.0

The figure establishes the bioactive-bonding-boundary of compositions (Hench *et al.*, 1991, 1991, 1998, 2004). In the region A the glasses are bioactive and bond to bone. In the middle of this area a smaller region is indicated with broken line, within which soft tissue bonding also occurs. Glasses in region B behave as nearly-inert materials and are encapsulated by non-adherent fibrous tissue when implanted. Compositions in region C are bioresorbable and resorbed within 10 to 30 days in tissue. In region D, the compositions are not technically practical and have not been implanted. The boundary between region A and C depends upon the ratio of surface area of the glass to the

effective solution volume of the tissue and the glass composition. Fine glass powders resorb more quickly than bulk implants.

In the early 1990s, Li and colleagues synthesized some bioactive glasses by sol-gel technique (Li, 1991 and Li *et al*., 1991). This new class of bioactive glasses has shown a higher compositional range of bioactivity. Glasses in the SiO_2-CaO-P_2O_5 system could form a layer of HA with silica content up to 90%. This is quite different from that of the melt-derived bioactive glasses which showed bioactivity only up to 60% SiO_2 content.

One of the main differences between melt-derived and sol-gel glasses lies in surface area which ranges for sol-gel glasses from ~200 to 650 m^2/g, whereas the melt-derived glasses show surface area less than $1m^2/g$ for rough particles and ~2 m^2/g for micron sized particles. This major difference is due to the temperature and synthesis conditions of the two types of materials. Sol-gel glasses are synthesized in an aqueous environment at lower temperature and then dried and stabilized at temperatures not exceeding 600ºC. Surface and structural properties such as surface area and porosity can be finely modulated depending on composition and synthesis conditions. At the end, controlled nanostructured materials can be obtained. Melt derived bioactive glasses are prepared at temperatures higher than 1000ºC with a procedure analoguous to that used to melt common window glasses. The finished product does not have any porosity at all and the surface area depends only on particle size obtained by grinding up the powders.

HA is deposited much faster on sol-gel bioactive glasses than on the traditional melt derived glasses and the materials can be resorbed in some cases. In fact, the porous structure of gel bioactive glasses allows the formation of a hydrated layer inside the

material, where biological moieties can enter maintaining their structural configuration and biological activity (Hench, 1998). This way, gel glasses can become an indistinguishable part of the host tissue. For example, it has been earlier shown that when trabecular rabbit bone was proliferated on 45S5 Bioglass® particles, a structure similar to normal bone was obtained, but some large particles of Bioglass® were still present. Instead, if gel-glasses were used, no residual particles could be observed (Oonishi *et al*., 1997 and Wheeler *et al*., 1997). The introduction of sol-gel technique for synthesizing glasses has opened the research for new types of biomaterials. Many possible dopants can be introduced in a material synthesized through sol-gel route. Recently some researchers have added Ag^+ ion to sol-gel bioactive glass composition (Bellantone *et al*., 2000), which gives antimicrobial properties to the material.

1.6.2 Interfacial Reaction Kinetics

The basis of the bone-bonding property of bioactive glasses is the chemical reactivity of the glass in the body fluids. The surface chemical reactions result in the formation of a hydroxy carbonate apatite (HCA) layer to which bone can bond. Bonding occurs due to a sequence of reactions. On immersion of a bioactive glass in an aqueous solution, three general processes occurs namely leaching, dissolution and precipitation. Leaching is usually characterized by release of alkali or alkaline earth elements by cation exchange with H^+ or H_3O^+ ions. Ion exchange is easy because these cations are not part of the glass network. They only modify the network by forming non-bridging oxygen bonds. The release of network-modifying ions is rapid for glasses in the bioactive compositional region (Region A in Fig. 1.3). This ion exchange process leads to an increase in interfacial pH values greater than 7.4 ($pH > 7.4$).

Network dissolution occurs concurrently, by the breaking of –Si–O–Si–O–Si– bonds through the action of hydroxyl (OH) ions. Breakdown of the network occurs locally which releases silica into solution in the form of silicic acid [$Si(OH)_4$]. The rate of dissolution of silica depends very much on glass composition. The dissolution rate decreases greatly for compositions containing greater than 60% SiO_2 because of the large number of bridging oxygen bonds in the glass structure. The hydrated silica (Si-OH) formed on the glass surface by these reactions undergoes rearrangement by polycondensation of neighboring silanols, resulting in a silica rich gel layer. In the precipitation reaction, calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions released from the glass, together with those from the solution, form a calcia-phosphate-rich (CaP) layer on the glass surface (Ohtsuki *et al*., 1991). The CaP layer formed *in vitro* is mainly located on the top of the silica gel, whereas it is formed *in vivo* within the gel layer. The calcium phosphate phase that accumulates on the gel surface is initially amorphous (a-CaP). It later crystallizes to a HCA structure by incorporating carbonate anions (CO_3^{2-}) from solution within the amorphous CaP phase. The mechanism of nucleation and growth of the HCA layer appears to be the same *in vitro* as well as *in vivo* and it is accelerated by the presence of hydrated silica. It is important to note that the mixed organic–inorganic bonding occurs within a region that has Si as well as Ca and P.

Thus, the stages of the reactions on the implant side of the interface with a bioactive glass are given as follows:

Stage 1: Leaching and formation of silanols (Si-OH)

Stage 2: Loss of soluble silica and formation of silanols

Stage 3: Polycondensation of silanols to form a hydrated silica gel

Stage 4: Formation of an amorphous calcium phosphate layer

Stage 5: Crystallization of a hydroxycarbonate apatite layer

Clark and Hench first proposed a detailed sequence of reactions occurring at the surface of silica-based bioactive glasses (Clark *et al*., 1976 and Hench, 1991). These involved the following steps:

Stage	
1.	Rapid exchange of Na^+ or K^+ with H^+ or H_3O^+ from solution: $Si - O - Na^+ + H^+ + OH^- \rightarrow Si\text{-}OH^+ + Na^+ (solution) + OH^-$ This stage is usually controlled by diffusion and exhibits a $t^{-1/2}$ dependence.
2.	Loss of soluble silica in the form of $Si(OH)_4$ to the solution, resulting from breaking of Si−O−Si bonds and formation of silanols (Si−OH) at the glass solution interface: $Si - O - Si + H_2O \rightarrow Si - OH + OH - Si$ This stage is usually controlled by interfacial reaction and exhibits time dependence ($t^{1.0}$).
3.	Condensation and repolymerization of a SiO_2-rich layer on the surface depleted in alkalis and alkaline-earth cations:
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 SiO_2-rich layer as followed by the growth of amorphous $CaO-P_2O_5$ rich film by incorporation of soluble calcium

and phosphates from solution.

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

The following equation (no. 2) describes the overall rate of change of glass surfaces and gives rise to the interfacial reaction profile (Ratner *et al.*, 1996). The reaction rate (R) for a single glass phase depends upon at least four terms of the equation (no. 2)

$$\mathbf{R = -k_1t^{0.5} - k_2t^{1.0} + k_3t^{1.0} + k_4t^y + k_nt^z} \quad \text{---------} \qquad (2)$$

Where k_1, k_2, k_3 and k_4 are the respective rate constant for first, second, third and fourth stage of reactions. The first term ($\mathbf{-k_1t^{0.5}}$) describes the rate of alkali extraction from the glass and it is known as first stage of the reaction. The second term ($\mathbf{- k_2t^{1.0}}$) describes the rate of interfacial network dissolution and it is called as second stage of the reaction. The surface of the glass is protected by third stage of reaction and its time dependent can be given by the third term ($\mathbf{k_3t^{1.0}}$) of the above equation (no. 2). The fourth term ($\mathbf{k_4t^y}$) in the equation describes the precipitation reaction which results in formation of multiple films as characteristics of glasses.

A layer of biologically active HCA must form for the occurrence of a bond with tissues. This appears to be the only common characteristic of all the known bioactive implants. The rate of tissue bonding appears to depend on the rate of HCA formation.

The five stages of the reaction that occur on the material side of the interface do not depend on the presence of tissues. They occur in distilled water, tris-buffer solutions or simulated body fluids. Bonding to tissues requires an additional series of reactions.

The sequence of events associated with formation of a bond with tissues is as follows

Stage 6: Adsorption of biological moieties in the SiO_2-HCA layer

Stage 7: Action of macrophages

Stage 8: Attachment of stem cells

Stage 9: Differentiation of stem cells

Stage 10: Generation of matrix

Stage 11: Mineralization of matrix

Stage 12: Proliferation and growth of bone

The time dependence of the reaction stages is depicted in Fig. 1.4. Compositions in

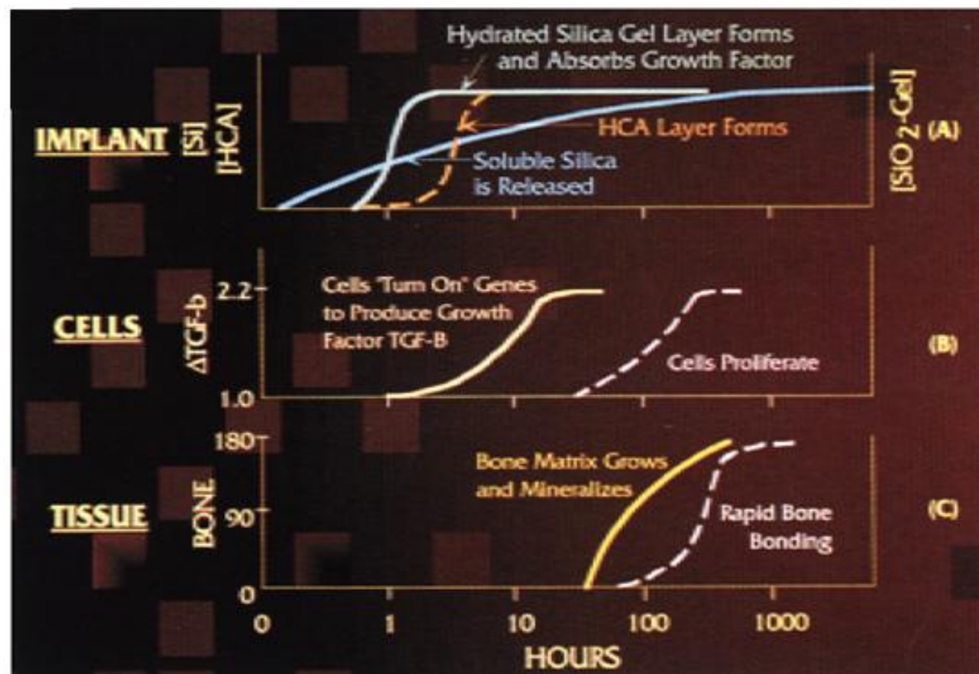

Fig.1.4: Time dependence of reactions occurring on a bioactive implant surface and the effects on cells leading to rapid bone bonding and bone proliferation.

the center of the field of bioactive bonding in Fig. 1.3 exhibit very rapid rates of stages 1–12. When the concentration of SiO_2 in the glass network exceeds more than 55%, the rates of reaction become much slower as indicated by the iso-bioactivity contours in Fig. 1.3, and bonding to bone becomes very slow also. At a concentration of 60% SiO_2, the rates of reaction are sufficiently slow due to that the material is biologically inert. Bioactive glass-ceramics, such as A/W glass-ceramic, are intermediate in reactions rates and bioactivity.

Several workers (Kokubo, 1990 and Yamamuro *et al.*, 1990) have previously shown that a calcium and phosphorus-rich layer is also present at the bonding interface between the polycrystalline apatite and wollastonite-based A/W glass-ceramic and bone. However, the SiO_2-rich layer was not present, even though a substantial concentration of soluble silicon was lost into the solution. CaO–SiO_2-based glasses without phosphate form an apatite layer on their surfaces (Yamamuro *et al.*, 1990 and Kokubo, 1990) when exposed for 2–30 days in simulated body fluid which contains only 1.0 $\mathrm{mMHPO_4^{2-}}$ concentration. The CaO–SiO_2 based glasses were found to bond to living bone by the surface apatite layer (Yamamuro *et al.*, 1990). Ogino *et al.* (1980) showed earlier that P_2O_5 free Na_2O–SiO_2 glasses form an apatite layer on their surfaces when exposed to an aqueous solution containing calcium and phosphate ions. Li *et al.*, (1991) demonstrated that highly porous sol–gel derived glasses containing primarily SiO_2, with only 10 mol% of CaO and P_2O_5 without Na_2O, form apatite layers in a *tris*-buffer solution. Earlier, Walker (1977) demonstrated that even highly pure SiO_2 eventually forms a bone bond if the surface has a very high surface area, greater than 400 m^2/g. Synthetic HA ceramic implants, which

contain no SiO_2 or alkali ions, bond to bone by forming a new epitaxial apatite phase at the interface(de Groot, 1983, Jarcho, 1981 and Williams, 1985).

Consequently, it is mentioned herewith that bioactivity occurs only within certain compositional limits and very specific ratios of oxides in the Na_2O–K_2O–CaO–MgO–P_2O_5–SiO_2 systems. ***A layer of biologically active HCA must form for a bond with tissues to occur. This is the common characteristic of all the known bioactive implant materials***. It is the rate of HCA formation (stage 4) and the time for its crystallization (stage 5) that varies greatly. When the rate becomes extremely slow, no bond is formed and the material is no longer bioactive.

The thickness of the bonding zone is approximately proportional to the I_b value and the failure strength of a bioactive bond appears to be inversely proportional to the thickness of the zone. Thus, a very high I_B value gives a thick bonding zone and low shear strength. Depending on the presence of rapid bonding or high shear strength is preferred, different compositions are optimal.

1.6.3 Interaction with cells

The presence of proteins does not influence only the type of calcium-phosphate containing layer formed on bioactive glasses immersed in body fluids, but also their further reactivity towards cells (Ducheyne *et al*., 1990).

Fibronectin is a protein found in plasma. It has been shown that a coating of this protein on bioactive material surface enhances fibroblasts attachment and proliferation (Seitz *et al*., 1982 and Cannas *et al*., 1988). Other workers observed that the configuration of

fibronectin adsorbed was different depending on the type of surface of the biomaterial exposed. A specific fibronectin conformation found on bioactive glasses treated in SBF in order to form a surface of CaP amorphous layer induced a very strong cell adhesion (Garcia *et al*., 1998).

Moreover, the type of surface exposed by the biomaterials influence cell functions. Ghannam *et al*. showed that when bioactive glasses were immersed in osteoblast-like solutions, cell proliferation on their surface was high during the first 7 days, but it slowed down as the bone matrix began to synthesize. On the contrary, cell were continued to proliferate on HA (Ghannam *et al*., 1997).

In order for a better understanding of these data, cell life cycle is presented herewith in the following Fig. 1.5.

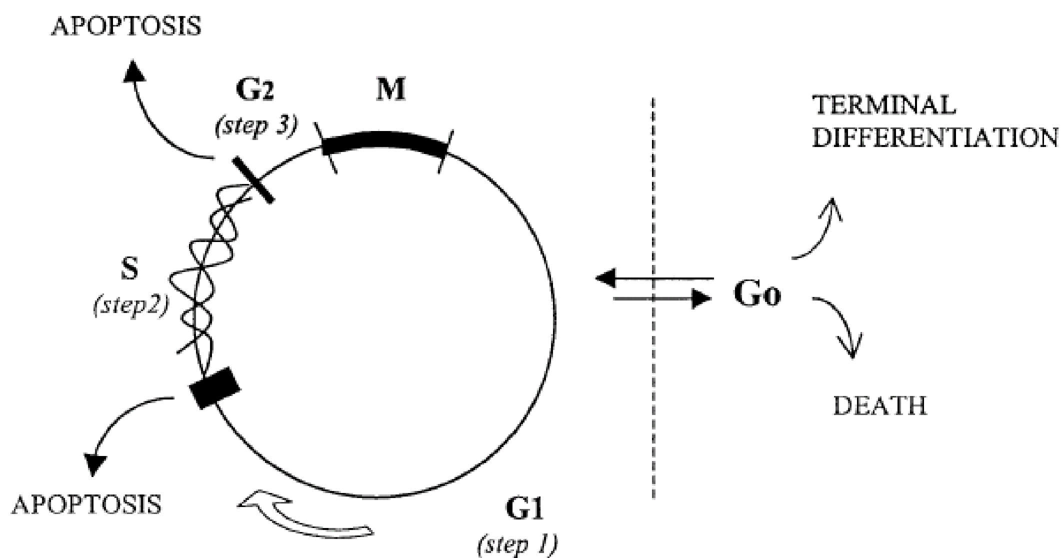

Fig.1.5: Cell cycle (Hench *et al*., 2003).

A resting cell is in the G0 state. In G1 phase, the cell grows and carries out its normal metabolism. For example, osteoblasts produce alkaline phosphate (ALP) and tropocollagen molecules, which can self-assemble into collagen. Later, cells enter in the S phase and begin to synthesize DNA. When all chromosomes have been duplicated, cells enter a secondary growth phase (G2) and finally divide M phase or mitosis. There are some feedback mechanisms in cells, controlling the state of the cell before switching from one phase to the next one. If the control fails, the cell enters a phase of programmed death, called apoptosis.

The difference in cell functions observed after contact with bioactive glasses and HA can be explained in terms of the cell cycle (Ghannam *et al*., 1997) and are paralleled with the results obtained by Xynos *et al*. (Xynos *et al*., 2000). After 6 days of reaction, the number of cells attached to a bioinert material is higher than on a bioactive material. Still, the number of cells that are in the S and G2-M phases is higher on the bioactive material. This means that on a bioactive material, cells that are not capable of differentiating into the osteoblast phenotype die according to the apoptosis process. After about 12 days, ALP production decreases in the cell attached to bioactive materials, and osteocalcin, characteristic of bone formation, increases.

It is not yet completely understood how these differences in cell function depend on the interaction with biomaterials. Surface morphology is definitely a relevant factor (Ducheyne *et al*., 1999), but also the ions released in body fluids, and the changes induced in pH should be taken into consideration. Alkalinization and increase in [Ca^{2+}] ion was observed both in body fluids and inside osteoblast cells in contact with Bioglass® (Silver *et al*., 2001). It was hypothesized that the higher glycolitic activity

shown by these osteoblasts should be specifically related to the changes in pH and Ca^{2+} content. It is well known, in fact, that bone formation is always connected to an increase in pH (Cuervo *et al*., 1971). The concentration of Ca^{2+} increases glycolysis for some systems (e.g., skeletal muscles), and in general, is a modulator of intracellular events. Also Si release, which is always observed when bioactive glasses are dissolved, is relevant for bone formation. It has been shown that chicken and rats fed with a diet poor in Si had problems with their skeletal structure (Carlisle, 1981), and vice versa, solutions rich in Si induced osteoblast proliferation (Keeting *et al*., 1992).

Recently, something very intriguing has been shown that ionic release from bioactive materials also influences the expression of some specific genes (Xynos *et al*., 2001). Osteoblasts were treated with the ionic product of Bioglass® dissolution in Dulbecco culture media for 24 hours, then RNA was removed and genes analyzed. A lot of different genes were stimulated by the contact with these ions, and in particular, some of those are strongly involved in bone formation.

The main goal of this work is to contribute gaining a better understanding of the process of bioactive glass reactivity. As previously shown, a lot of work has been carried out in the past few years to study bioactive glass dissolution and re-precipitation in simulated body fluid (SBF), mostly focusing on the characterization of the HCA layer deposited. Improvement in composition and synthesis procedure of bioactive glasses has been obtained mostly by trial-and-error, and by comparing *in vitro* and *in vivo* results. Still, the actual surface sites of HCA deposition are not completely known, and the role of the different elements that bioactive glasses are made of is not fully understood.

A reason for this lack of knowledge is that the most important interactions occur at the bioactive glass/solution interface, which is a nanometer-sized continuously changing region of space. A thorough study of this region should involve analysis of changes in surface morphology, cristallinity, composition, hydroxylation, acidity, potential and charge of the material as well as composition and pH of the solution. Definitely, such large and varied information cannot be obtained with only one analytical technique. Moreover, only a few techniques can analyze the changes of a material immersed in solution without being affected by the presence of the liquid.

In my work, I faced the problem from different sides, using many analytical techniques to study the changes occurring both on the material and in the solution, although the main focus of the work was the analysis of changes in the material surface. The importance of biomaterials surface analysis has been recently pointed out.

Nevertheless, this type of study is still quite limited, especially if compared with the years long research carried out on the surface properties of other materials. I hope that the following work may contribute extending the application of some of the typical surface chemistry analytical tools to the field of biomaterials science.

My research work consists of five chapters which include preparation and characterization of various bioactive glasses containing Li_2O, K_2O, SrO, CuO, and (TiO_2+ZrO_2), respectively.

Chapter 3 consists of preparation and characterization of Li_2O-CaO-Al_2O_3-P_2O_5-SiO_2 glasses as bioactive material. A comparative study on structural and physico-mechanical properties as well as bioactivity of glasses was reported. The structural properties of

glasses were investigated by XRD, FTIR spectrometry, SEM and the bioactivity of the glasses was evaluated by *in vitro* test in simulated body fluid. Density, compressive strength, Vickers hardness and ultrasonic wave velocity of glass samples were measured to investigate physical and mechanical properties. Results indicated that partial molar replacement of Li_2O by Al_2O_3 resulted in a significant increase in mechanical properties of glasses. *In vitro* studies of samples in SBF had shown that the pH of the solution increased after immersion of samples during the initial stage and then after reaching maxima it decreased with increasing immersion time. *In vitro* test in SBF indicated that addition of Al_2O_3 up to 1.5 mol% resulted in an increase in bioactivity where as further addition of Al_2O_3 caused a decrease in bioactivity of the samples. The biocompatibility of these bioactive glass samples was studied using human osteoblast (MG-63) cell lines. The results obtained suggested that Li_2O-CaO-Al_2O_3-P_2O_5-SiO_2 based bioactive glasses containing alumina would be potential materials for biomedical applications.

Chapter 4 consists of structural characterization and *in vitro* bioactivity assessment of SiO_2–CaO–P_2O_5–K_2O–Al_2O_3 glass as bioactive ceramic material. The potassium based bioactive glasses have shown a better biocompatibility than soda containing bioactive glasses. Therefore, we have prepared a bioactive glass system containing potassium oxide and substituted with Al_2O_3 for further enhancement of bioactivity, physico-chemical properties, mechanical strength as well as its behavior to human osteosarcoma cells. The prepared bioactive glasses have a general formula, $42SiO_2$-$34CaO$-$6P_2O_5$-(18-x) K_2O, where x=0, 0.5, 1, 1.5 and 2.5 mol% of Al_2O_3. The *in vitro* bioactivity of these samples was assessed by immersion in SBF solution for different time periods under physiological conditions. The formation of hydroxy carbonate apatite (HCA) layer on the surface of the

glass samples after SBF treatment was confirmed by FTIR, XRD and SEM. The partial substitution of Al_2O_3 for K_2O in glass demonstrated a significant increase in mechanical properties such as compressive strength and elastic modulus, respectively. The cytotoxicity, cell viability, proliferation, apoptosis and cell attachment were assessed using human osteosarcoma U2-OS cell lines. The cell culture studies demonstrated that the samples containing high concentration of Al_2O_3 showed a cytotoxic nature against cell lines. But the blood compatibility showed that all the samples were tolerant. Finally, this study clearly concludes that the optimization of Al_2O_3 in present potash based bioactive glasses would be potential biomaterials for biomedical applications.

Chapter 5 consists of structural characterization and *in vitro* bioactivity assessment of SiO_2–CaO–P_2O_5–SrO–Al_2O_3 glass as bioactive ceramic material. A comparative study on structural and physico-mechanical properties as well as bioactivity of glasses was reported. The structural properties of glasses were investigated by XRD, FTIR spectrometry, SEM and the bioactivity of the glasses was evaluated by *in vitro* test in simulated body fluid. Density, compressive strength, Vickers hardness and ultrasonic wave velocity of glass samples were measured to investigate physical and mechanical properties. The partial substitution of Al_2O_3 for SrO in glass demonstrated a significant increase in mechanical properties such as compressive strength and elastic modulus, respectively. The cytotoxicity and cell viability were assessed using human osteosarcoma U2-OS cell lines. The cell culture studies demonstrated that the samples containing high concentration of Al_2O_3 showed a cytotoxic nature against cell lines. But the blood compatibility showed that all the samples were tolerant. Finally, this study clearly

concludes that the optimization of Al_2O_3 in present strontium based bioactive glasses would be potential biomaterials for biomedical applications.

Chapter 6 consists of studies on preparation, characterization and antibacterial properties of CuO substituted 45S5 bioactive glass as bioactive ceramic material. The aim of the present investigation was to evaluate the role of CuO in the system of 45S5 bioactive glass for improving the bioactivity as well as other physical and mechanical properties of 45S5 bioactive glass. The partial substitution of 1, 2, 3, 4 mol% of CuO for CaO in 45S5 bioactive glass system was done by melting route at 1400^0C in globar rod furnace in air. A comparative study on structural and mechanical properties as well as bioactivity of the glasses was reported. The properties of glasses were determined by XRD, FTIR spectrometry, SEM and the bioactivity of the glass samples were investigated by in vitro test in simulated body fluid (SBF). Density and compressive strength of glass samples were measured. The results indicate that with partial substitution of CuO for CaO in 45S5 bioactive glass system, the mechanical properties of the glasses were found to increase significantly. The glass samples exhibited higher density and compressive strength as compared to their corresponding 45S5 bioactive glass. The in-vitro studies of glass samples in SBF had shown that the pH of the solution increased with increasing time period for immersion during initial stage of reaction. This indicated that bioactivity of the samples had increased with increasing duration of time. On later stages the decrease in pH of the solution with time had shown that the bioactivity of the samples had decreased. Antibacterial tests of these glasses had shown that after introducing CuO from 0.5-2.5 mol% these glasses develops an antibacterial property.

Chapter 7 consists of studies on preparation and characterization of 45S5 bioactive glass doped with (TiO_2 + ZrO_2) as bioactive ceramic material. The aim of the present investigation was to evaluate the role of (TiO_2 + ZrO_2) in the system of 45S5 bioactive glass for improving the bioactivity as well as other physical and mechanical properties of 45S5 bioactive glass. The partial substitution of 1, 2, 3, 4 mol% of mixed (TiO_2 + ZrO_2) (3:2) for SiO_2 in 45S5 bioactive glass system was done by melting route at 1400^0C in globar rod furnace in air. A comparative study on structural and mechanical properties as well as bioactivity of the glasses was reported. The properties of glasses were determined by XRD, FTIR spectrometry, SEM and the bioactivity of the glass samples were investigated by in vitro test in simulated body fluid (SBF). Density and compressive strength of glass samples were measured. The results indicate that with partial substitution of (TiO_2 + ZrO_2) for SiO_2 in 45S5 bioactive glass system, the mechanical properties of the glasses were found to increase significantly. The glass samples exhibited higher density and compressive strength as compared to their corresponding 45S5 bioactive glass. The in-vitro studies of glass samples in SBF had shown that the pH of the solution increased with increasing time period for immersion during initial stage of reaction. This indicated that bioactivity of the samples had increased with increasing duration of time. On later stages the decrease in pH of the solution with time had shown that the bioactivity of the samples had decreased.

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