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Review Article

A review of bioactive glasses: Their structure, properties, fabrication, and apatite formation

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Abstract: Bioactive glass and glass–ceramics are used in bone repair applications and are being developed for tissue engineering applications. Bioactive glasses/bioglass are very attractive materials for producing scaffolds devoted to bone regeneration due to their versatile properties, which can be properly designed depending on their composition. An important feature of bioactive glasses, which enables them to work for applications in bone tissue engineering, is their ability to enhance revascularization, osteoblast adhesion, enzyme activity and differentiation of mesenchymal stem cells as well as osteoprogenitor cells. An extensive amount of research work has been carried out to develop silicate, borate/borosilicate bioactive glasses and phosphate glasses. Along with this, some metallic glasses have also been investigated for biomedical and technological applications in tissue engineering. Many trace elements have also been incorporated in the glass network to obtain the desired properties, which have beneficial effects on bone remodeling and/or associated angiogenesis. The motivation of this review is to provide an overview of the general requirements, composition, structure-property relationship with hydroxyapatite formation and future perspectives of bioglasses. Attention has also been given to developments of metallic glasses and doped bioglasses along with the techniques used for their fabrication. J Biomed Mater Res Part A: 102A: 254–274, 2014.

Key Words: glass, glass ceramics, bioactivity, biodegradable, hydroxyapatite

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INTRODUCTION

The natural or man-made materials, which are used to replace or supplement the functions of living tissues, are known as biomaterials.[1] The imperative terminology for the study of biological performance of materials includes: biocompatibility and biomaterials. The biomaterials should be biocompatible that is *in vivo* harmony of biomaterial and *vice versa*.[2] In addition to this, biomaterials must possess features like bioinert behavior, bioactivity, biostability, and biodegradibilty.[3] Biomaterials are generally categorized as (a) natural or synthetic polymers, (b) metals, (c) composites, and (d) ceramics (bioglasses) as shown in Figure 1.

Although the composition flexibility for polymers provides them with their unique characteristics, but their low mechanical strength cannot withstand the stresses required in many applications. Metals have high wear resistance, strength and ductility. However, their high corrosion rate and low biocompatibility are undesirable for living tissues, and the high diffusion of metal ions may lead to allergic reactions.[4] Some composites have cross-linked elastomers which give them high elastic moduli which in turn are beneficial for biocompatibility. Ceramics generally posses good biocompatibility along with resistance to corrosion and compression. Unfortunately these materials are brittle and maintain small resilience, high density and low fracture strength. The use of biodegradable polymer scaffolds for the regeneration of bones is limited and challenging. These polymers lack a mechanically biocompatible hydroxyapatite (HA) inorganic phase.[5-8] The scaffolds which are fabricated from calcium phosphate-based inorganic materials or bioceramics such as bioactive glass usually provide a higher mechanical strength.

Bioactive glass and glass–ceramics are also used in bone repair applications and are being developed for tissue engineering applications.[9] Bioactive glasses/bioglasses are very attractive materials for producing scaffolds devoted to bone regeneration due to their versatile properties, which can be properly designed depending on their composition.[10] The

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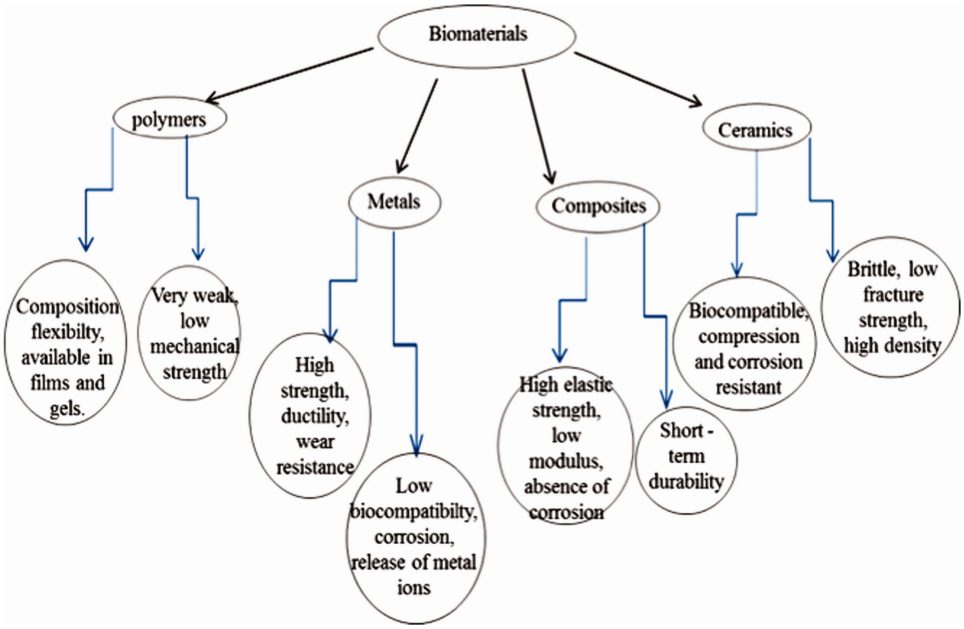

FIGURE 1. Schematic of classification of biomaterials. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

first bioactive glass was synthesized 40 years ago by Hench et al.[10] and belonged to the SiO_2-Na_2O-CaO-P_2O_5 system (Bioglass VR). In 1969, Hench et al. discovered that certain glass compositions had excellent biocompatibility as well as the ability to bond bone.[10-12] The bioactivity of this glass system can vary from surface bioactive to bulk degradable that is, resorbed within 10 to 30 days in tissue.[11] The 45 S5 Bioglass W contains 45% SiO_2, 24.5% Na_2O, 24.4% CaO, and 6% P_2O_5, in weight percent.[3] The phase diagram as proposed by Hench is given in Figure 2. Hench published a very broad review describing the various stages involved during Bioglass synthesis along with biochemical/biological/structural testing ultimately leading to its commercialization.[13]

This invention led to a revolution in the development of biomaterials and doped system of bioglasses for the human body. Progressively this perspective explored inert materials for implantation and to promote natural tissue regeneration.[14,15] Ancient civilizations like Egyptians, Chinese, and Indian used biomaterials for reconstructing the defective parts of the body, but Bioglass has been in clinical use since 1985 in the form of a fine particulate for dental application (Perioglas, NovaBone) to the present.[16]

Broadly a bioactive material represents a material that is designed for inducing target specific biological activity.[17] More specifically, a bioactive material represents a material, which follows a two-step process upon its implantation inside the body. In the first step it undergoes specific surface reactions especially with simulated body fluid (SBF) and during the second step it forms a HA-like layer, which is responsible for the interactions within hard and soft tissues.[18] The formation of a HA-like surface interaction layer when immersed in a simulated body fluid (SBF) *in vitro* is an imperative criteria to decide the biological activity of a biomaterial *in vivo*.[19-21] However, some materials like dicalcium phosphate dehydrate show *in vitro* formation of an HA-like surface layer when immersed in a SBF but no direct bone bonding *in vivo*.[22,23] In contrast to this, β-TCP does not always lead to the formation of an HA-like material in a SBF in spite of the fact that it shows extensive bonding to bone.[24]

Recently, for bone replacement, the attention has been inclined toward materials exhibiting chemical and crystallographic similarity to natural bone mineral hydroxyapatite (HA), fluorapatite, and other calcium phosphates in addition to their biodegradability.[25] Calcium phosphate-based bioceramics, such as HA, $Ca_{10}(PO_4)_6(OH)_2$, β-tricalcium phosphate (β-TCP), $Ca_3(PO_4)_2$, and biphasic calcium phosphate (BCP), and a mixture of HA and β-TCP are inorganic materials

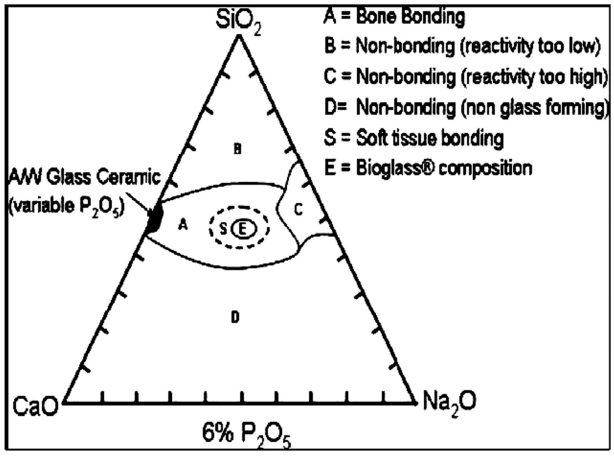

FIGURE 2. Ternary compositional diagram given for 45% SiO_2-24.5% Na_2O-24.5% CaO-6%P_2O_5 glass by Hench for bone-bonding.[13]

composed of the same ions as bone and have received most attention for bone repair applications.[26,27] HA resorbs slowly and undergoes little conversion to a bone-like material after implantation, but possess a higher mechanical strength in comparison to other calcium phosphates.[28] The use of BCP with different HA to β-TCP ratios allows control over the degradation rate, in addition to other properties.[29,30]

Bioglasses should be designed in such a way so that they provide appropriate structural compatibility without any detrimental effects on living tissues.[31] By tailoring the initial composition of bioglass and upon changing the processing conditions that is melt quenching or sol-gel, we can design target and application specific bioglass.[18,32,33] An important feature of bioactive glasses, which enables them to work for applications in bone tissue engineering, is their ability to enhance revascularization, osteoblast adhesion, enzyme activity, and differentiation of mesenchymal stem cells.[18,21,32,34] In addition to this, they can also act as promising filler materials/coatings for polymer structures.[35-45] However, while using bioglasses for producing porous scaffolds, dental materials, or filler materials, the properties of bioglasses due to the form of their powders/particles of various shapes and sizes and granulates of different sizes should be considered. The risk of toxicity must also be taken into consideration while designing the compositions of bioactive biomaterials so that the release of elements is lower than their biologically safe levels and hence exhibit no or negligible cytotoxicity.

A significant amount of research work has been carried out to develop silicate, borate/borosilicate bioactive glasses, and phosphate glasses. Some metallic glasses have also been investigated for biomedical and technological applications in tissue engineering. To this extent, many trace elements have also been incorporated in the glass network to obtain the desired properties, which have beneficial effects on bone remodeling and/or associated angiogenesis. This has amplified the interest in the field of biomedical application of bioactive glasses over the last four decades. This is a field of intense research, which is clearly manifested in the increasing number of publications in the field of bioglasses, their properties and applications. This paper is presented mainly to provide an overview of requirements, composition, the structure-property relationship with hydroxyapatite formation and future perspectives of bioglasses. Attention has also been given to developments of metallic glasses and doped bioglasses along with the techniques used for their fabrication. The authors do not intend to imply that bioglasses are the only suitable material for biomedical applications.

DESIRED PARAMETERS FOR BIOGLASSES/ GLASS-CERAMICS

Bioactive glass exhibits an amorphous structure, whereas glass–ceramics are crystallized glasses. Glass ceramics are obtained through a process in which the glass is heated at a fixed temperature and duration in controlled atmosphere. Upon controlled heat treatment of the glass, a glass–ceramic is formed which exhibits superior mechanical properties with respect to its parent glass like viscous behavior, toughness and hardness. However, in the case of 45S5 glass, the crystallization leads to a decrease in the mechanical strength of glass–ceramic scaffolds with low strength ($<$1 MPa).[5] In contrast, silicate 13–93 glass and borate 13–93B3 bioactive glass scaffolds have a higher compressive strength and elastic modulus values.

Crystallization enhances the mechanical and flexural strength of glass leading to its high fracture strength. Glass ceramics consist of crystalline phases embedded inside an amorphous glassy matrix. The crystallization of glasses affects the bioactivity of glass as shown by many researchers.[46-48] According to the reports by Filho et al.[46] and Li et al.,[47] the crystallization in bioactive glass leads to decreased level of bioactivity, which probably makes it an inert material. This indicates that while the glass ceramic is mechanically stronger than amorphous glass, at the same time the bioactivity is greatly reduced. This competition between strength and bioactive behavior has been addressed after the discovery of the Na-containing glass, 45 S5BioglassW. This glass has been sintered to obtain a mechanically strong crystalline phase inside a residual glassy matrix. At body temperature this crystalline phase transforms into an amorphous calcium phosphate while in a biological environment and remains biodegradable as well as bioactive.[49,50] It has also been observed that the healing profile of bone matches the biodegradation profile of the 45 S5BioglassW. This desirable property is a unique feature of this 45 S5 Bioglass W and has not previously been found in any other material like hydroxyapatites, alloys, polymers or calcium phosphates. Lefebvre et al. and Huang et al. investigated and modeled the sintering behavior of Bioglass.[51,52]

Following are listed some of desired parameters for bioactive glasses/glass–ceramics to function as a suitable biomaterial:[53-57]

1. Biocompatibility of bioglasses is an indispensable property. They should be non-toxic and hence promote cell proliferation in addition to cell adhesion.
2. For making scaffolds, almost every bioglass requires a thermal heat treatment, which leads to nucleation and growth of crystalline phases embedded in a matrix of glass. These crystallized phases must not induce cytotoxic effect or hamper any bioactive process inside the cell/tissue.
3. When these glasses are in contact with SBF, there must be a formation of a hydroxyapatite layer.
4. It should not exhibit any inflammatory response, demonstrate cytotoxicity or immunogenicity. Tissue scaffolds provide a temporary structure for cells to synthesize new tissue and they must exhibit neogenesis, however they must degrade into nontoxic products, which can be easily resorbed or be excreted by the body. Moreover, both the surface and the bulk material must be sterile.
5. The bioglass must posses required mechanical properties to withstand any kind of pressure or strain in order to prevent any structural failure during handling of the

TABLE I. Role of Elements in Human Body

Name of the Element	Role
Calcium	Constituent of bones and teeth, regulation of nerves, enzyme activation, neuromuscular excitability
Phosphorous	Constituent of teeth, bones, adenosine triphosphate and nucleic acids
Sodium	Principal cation of extracellular fluid, Regulates plasma volume, maintains osmotic pressure, transmission of nerve impulses, absorptive processes for bile salts and amino acids
Potassium	Principal cation in extracellular fluid, regulation of osmotic pressure, glycogenesis, muscle contraction of cardiac muscles
Chlorine	Fluid and electrolyte balance, principal anion in extracellular fluid and gastric juice
Magnesium	Component of enzyme system with thymine pyrophosphate cofactor. Constituent of bones and teeth, activator for phosphate transferring enzymes
Chromium	Maintains the configuration of RNA molecule, active ingredient in glucose tolerant factor
Cobalt	Constituent of vitamin B_{12}, cofactor of enzymes involved in DNA biosynthesis
Copper	Essential for hematologic and neurologic systems, formation of myelin sheaths in nervous systems, constituent of many enzymes, helps in iron absorption
Iodine	Component of thyroid hormones
Iron	Required for hemoglobin, component of enzymes for cellular respiration, myelination of spinal cord, synthesis and packaging of neurotransmitters
Manganese	Cofactor of hydrolase, decarboxylase, involved in glycoprotein, part of enzymes required for urea formation and pyruvate metabolism
Molybdenum	Part of metalloenzymes, helps in cellular metabolism
Selenium	Constituent of glutathione peroxidase, part of defense system protecting organisms from harmful free radicals, oxidant with vitamin E
Silicon	Calcification of bone, component of mucopolysaccharides, component of connective tissues, cross linking agent, helps in resiliency of connective tissues
Zinc	Cofactor for many enzymes, cell replication, metabolism of vitamin A and E, tissue repair and wound healing
Fluorine	Increases hardness of bones, increases enamel remineralization, prevents dental caries
Sulfur	Required for amino acid, connective tissue, skin, nails and hair
Strontium	Helpful in calcification of bones and teeth, bone healing, bone resorption
Nickel	Maintenance of membrane structure, control of prolactin
Boron	Helps in bone formation
Barium	Bone opacifier

material and during the patient's normal routine activities. The bioglass scaffolds must exhibit, mechanical properties that are comparable to those of the tissue to be replaced for better compatibility.

6. For bone engineering, bioglass should posses controllable interconnected porosity to support vascularization so as to direct cells to grow into the required physical structure. A typical porosity of 90% along with a pore diameter of at least 100 μm is required for proper vascularization of the tissue.
7. The architecture aspect of a bioglass scaffold should have a porous three-dimensional (3D) structure for cell proliferation, vascularization and diffusion of nutrients. This provides a regulated microenvironment for new tissue synthesis.
8. For commercialization, the bioglass should be cost-effective while still maintaining the desired features.

Hence, all these criteria are desirable to obtain suitable bioglasses for the biomedical and technological applications.

ELEMENTS REQUIRED BY HUMAN BODY

The human body requires some elements as they are vital constituents of organs or body parts. In addition, they are required for maintenance of acid-base balance and regulation of body fluids. For understanding the biological significances of these elements inside the human body, it is necessary to obtain an insight of their relative abundance in biological cells/tissues. Human body tissue consists of up to 90% water.[58] Carbon, hydrogen, nitrogen and oxygen are the major constituents of proteins, amino acids, deoxyribonucleic acids (DNA) and ribonucleic acids (RNA). Hence these elements are regarded as the basic building blocks. The remainder of the elements required for the body can be divided into macroelements and microelements. Macroelements include calcium, phosphorus, sodium, chlorine, potassium and so forth are required $>$100 mg/dL and microelements like copper, iron, zinc, sulfur, magnesium, chromium, strontium and so forth are required up to $<$100 mg/dL.[59-68] The deficiencies of these elements can cause major public health problems especially in developing nations, whereas their high concentrations can be toxic. All the elements required by human tissues are listed in Table I along with their significance. These elements are also important components of bioglasses. Hence for uninterrupted working of these bioactive glasses (scaffolds), it is necessary for glass to degrade *in vivo* so that the trace elements in scaffolds must be released below the toxic level.

Calcium and phosphorus function as major constituents while magnesium as a minor constituent for bone, teeth and adenosine triphosphate (ATP). Calcium is required for membrane permeability, muscle contraction and neuromuscular

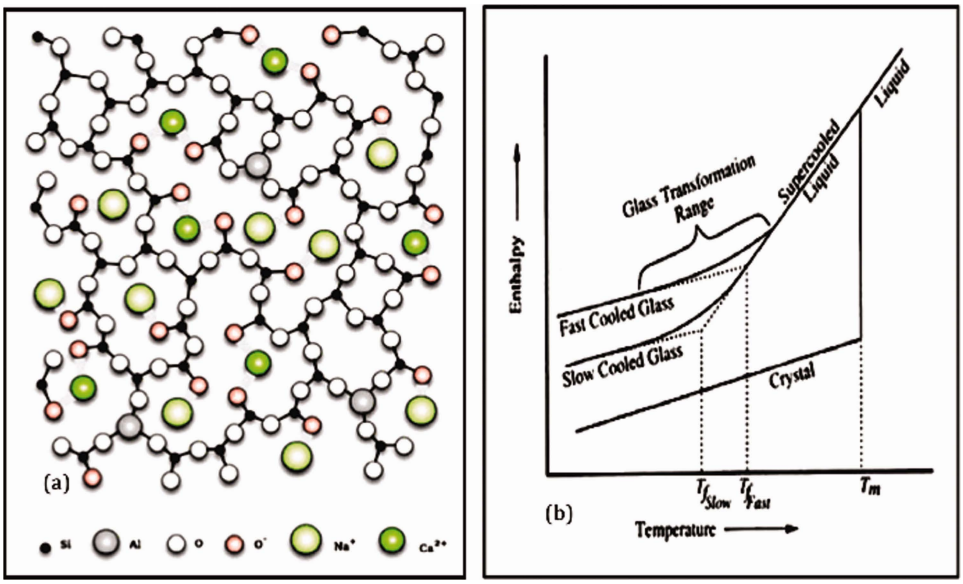

FIGURE 3. (a) Structure of glass and (b) enthalpy–temperature diagram.[90] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

excitability. Phosphorus is also involved in the synthesis of phospholipids and phosphoproteins. Its deficiency may cause De-Toni Fanconi syndrome or osteomalacia whereas its increase content can lead to chronic nephritis and hypoparathyroidism. Hypothyroidism includes low Ca/P ratio.[69] Magnesium is also an active component of several enzymes having thymine pyrophosphate as a cofactor and its deficiency can lead to deeply depressed tendon reflexes. Strontium and barium also belong to the same alkaline earth metal oxide group of calcium and magnesium. The field strengths of Mg^{+2}, Ca^{2+}, Sr^{2+}, and Ba^{2+} are 0.45, 0.33, 0.30, and 0.24, respectively. Oxides of barium increase the surface adherence by reducing surface tension.[70] Basically, it is a very strong modifier and is associated with providing nonbridging oxygen (NBOs) to the glass structure, which may enhance the formation of the apatite layer.[71] Barium crystals are also used as an opacifier in bone cements and radiopaque bioactive RSA glasses.[72-74] Strontium has shown positive effects during treatment of osteoporosis.[62-65]

Other elements like silicon, zinc, copper, iron, and so forth also play a vital role for the normal functioning of the human body. Silicon is a biological cross-linking agent contributing to the structure and resilience of connective tissues.[75,76] Silicic acid is the physiological form of silica, which interacts with aqueous aluminum to form hydroxyl aluminosilicates having low toxicity.[77] Zinc acts as a cofactor and constituent of many dehydrogenase enzymes, which are necessary for macronutrient metabolism as well as cell replication.[60] Metabolism of vitamin A and E are dependent on zinc. Along with playing a vital role in plasma component and insulin, it also helps in developing taste buds, anti-inflammation, healing wounds and tissue repair.[66-68] It binds specific DNA regions to monitor genetic control of cell proliferation.[78] Copper is also a constituent of many oxidase enzymes and helps in iron absorption.[79] It is imperative for hematologic and neurologic systems as it forms myelin sheaths. Copper containing proteins are known to be erythrocuperin in red blood cells, hepatocuperin in liver and cerebrocuperin in brain.[69] Though its deficiency is associated with anemia due to reduced ferroxidase function, cardiac hypertrophy and cardiac failure but its excess causes Wilson disease and liver poisoning. Copper is reported to help in formation of bones but Zhang et al. reported that Cu^{2+} at a concentration of $10^{-6}M$ inhibits osteoclast activity.[80-82] Copper is reported to trigger endothelial cells towards angiogenesis[83] and hence acts as an angiogenic agent. Copper and angiogenesis growth factor FGF-2 exhibited synergistic stimulatory effects on angiogenesis *in vitro*.[84] Cu promotes the differentiation of mesenchymal stem cells towards the osteogenic lineage.[85,86] Iron (Fe) is an important constituent of succinate dehydrogenase and heme of hemoglobin (Hb), myoglobin, and cytochrome.[79] It is required for myelination of white matter of cerebellar folds in brain. Along with this, it is a cofactor for many enzymes of neurotransmitter system.[87] Fe is transferred as transferrin, stored as ferritin/hemosiderin and lost in sloughed cells.[69] Iron deficiency causes anemia whereas its excessive accumulation causes hemosiderosis, neurologic disorders like Alzheimer's disease, Parkinson, and neurodegeneration.[88] In summary, the level of all these elements must be optimized inside the human body for healthy functioning.

TYPES OF BIOGLASSES

Glass is an amorphous solid, without long-range order.[89,90] Glasses are typically brittle, and often optically transparent. Glasses and crystals have the same building blocks (cation polyhedra) arranged in a different pattern; for example, glasses have broader distributions of bond angles. The atoms in glass are arranged in a random manner more similar to that of a liquid because glass is essentially a superstiff liquid as shown in Figure 3(a). Every glass exhibits

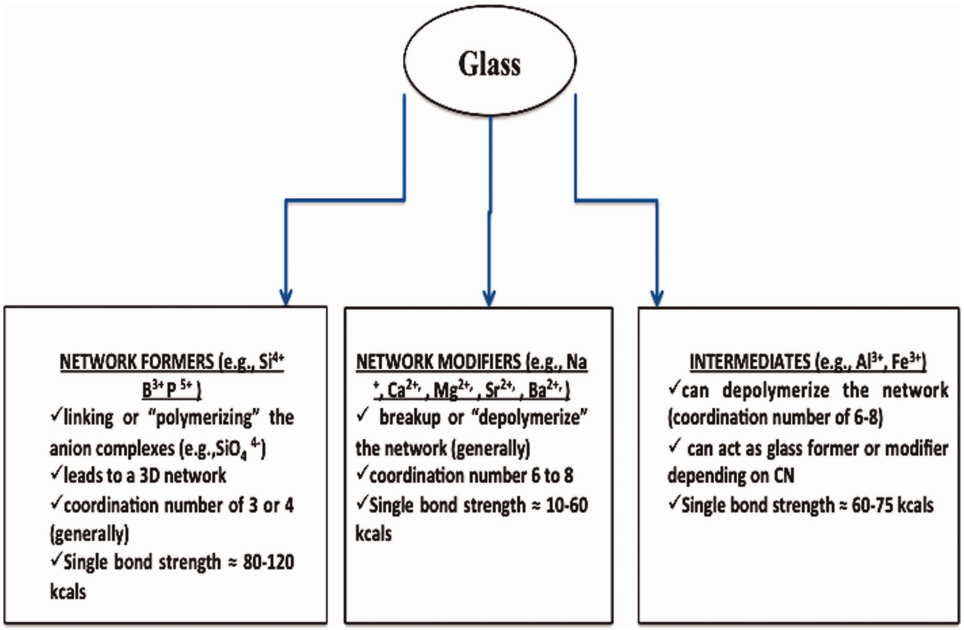

FIGURE 4. Glass and its components. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

time-dependent glass transformation behavior. This behavior occurs over a temperature range known as the glass transformation region. As indicated by the enthalpy temperature diagram [Fig. 3(b)], the glass transformation occurs over a range of temperatures and cannot be characterized by any single temperature. According to Zachariasen's criterion of glass formation:[89,90]

1. No oxygen atom may be linked to more than two cations (A).
2. The number of oxygen atoms surrounding the cations must be small (e.g., less than six)
3. The oxygen polyhedra shares corners with each other, but do not share edges or faces.
4. The polyhedra are linked in a three-dimensional network.
5. Cooling rate $\approx 10^6$ K/s is required.

Figure 4 indicates the components of glasses and their role. The generic name of the glass is generally derived from its network former. One of the major challenges of tissue engineering has been concerned with the design and development of materials and their bioresorability after performing their function so that the tissue can be remodeled to its natural form again. Hence, it is important to get insight into the glass and its structural design. From the compositional aspect of glasses that is the main oxide present in glass and various other oxides as dopants, some of the reported bioactive glasses and their structural components are described as follows:

Silicate glasses

Although the basic tetrahedra $(SiO_4)^{4-}$ are present in most silica structures, the connectivity varies widely into 1-, 2-, and 3-dimensional arrangements. Both ionic and covalent natures of the Si-O bond contribute to the preference for $(SiO_4)^{4-}$ tetrahedron formation in both crystalline and glassy silicas. In addition, each O anion is coordinated by two Si cations, corresponding to corner sharing of the oxide tetrahedra, preventing the close-packing of anion layers and resulting in relatively open structures.[89,90] Silicon plays a significant role in bone mineralization along with gene activation. Consequently, substitution of silicon for calcium into synthetic hydroxyapatites is the current area of investigation for many research groups.[91-93] The intracellular and extracellular response of bioactive glass depends upon the release of soluble ionic forms of Si, Ca, P, and Na, from glass surface. The 45 S5 Bioglass W is a silica-based composition, which has shown increased secretion of vascular endothelial growth factor *in vitro*.[36] SCK is Na_2O-free silica-based bioactive glass used for making scaffolds in which the bioactivity phenomena involves H^+/K^+ exchange process.[94] The 13-93 glass proposed by Fu et al.[95] remained amorphous even after heat-treatment confirmed from X-ray analysis. The 13-93 glass has more facile viscous flow behavior than bioglass, and fewer tendencies to crystallize.[96] The 13-93 glass scaffolds fabricated are shown in Figure 5(a,b) shows the needle like structure of hydroxyapatite formed on the surface of 13-93 glass after dipping in SBF solution for 6 days.

Silicate bioactive glasses (45S5 or 13-93) are well known to support the proliferation and differentiated function of osteoblastic cells such as murine MC3T3-E1 cells and MLO-A5 cells, during conventional *in vitro* cell culture.[95] Goel et al. synthesized alkali free system diopside.[97] The system showed considerably smaller weight loss in comparison to 45 S5 composition. In addition, during the 12 hr of immersion in SBF, highest level of bioactivity was also observed. The sintered glass was found to be amorphous. Silica spheres along with organic ligands find suitable applications in immunoarrays and detection of biological

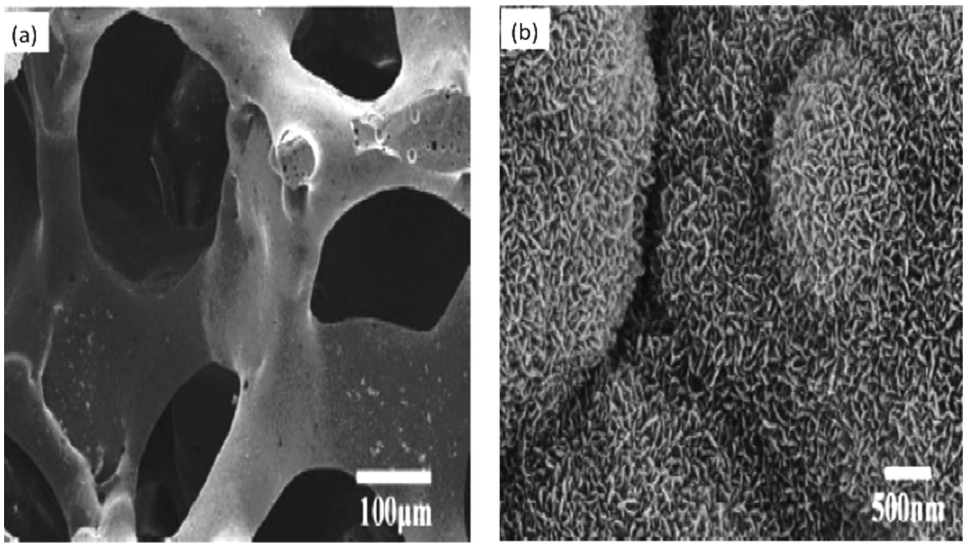

FIGURE 5. (a) 13-93 glass scaffold (b) formation of hydroxyapatite needles on the surface of 13-93 glass after 6 days in SBF.[95]

molecules.[98] Silica is also attracting attention of researchers in the field of nanomedicine and drug delivery.[99] Kokubo and Takadama found these materials to produce good prosthetic devices.[18,19] Silica-based star gels were developed by Du-Pont Corporation in 1995. These are organic-inorganic hybrids with unique structure of organic core surrounded by flexible arms. These arms terminate into alkylosilane groups.[100]

Borate/borosilicate glasses

Brink[96] proposed the first borosilicate glasses for biomedical applications in 1990. In order to get the desirable bioactive properties, the relative proportion of B_2O_3 was tailored. Borate glasses are very reactive and have lower chemical durability, hence they convert more completely and rapidly to HA than their silica counterparts.[101,102] Huang et al.[102] replaced SiO_2 with B_2O_3 in steps and found substantial increase in the conversion of the glass to HA in aqueous phosphate solutions. The conversion mechanism of bioactive glass to apatite is similar to that of silicate 45 S5 glass, with the formation of a borate-rich layer, similar to the silicate-rich layer of the former.[11] The complete degradation rate of the glasses can be controlled within a wide range of time periods by replacing silica with boron. In addition to this, the sintering behavior of borate/borosilicate glass is more controlled than silicate glasses.[103,104] The 45 S5 silicate compositions have been widely investigated over the course of many years but borate- and borosilicate-based compositions have recently been explored.[5,95,105] Boron is a trace element, which is required for bone health.[106-108] Borate glass leads to higher pH value of the culture medium. *In vitro*, borate glasses support cell proliferation along with differentiation whereas *in vivo* they are reported to enhance tissue infiltration.[109,110] However, $(BO_3)^{3-}$ ions are associated with the toxicity. Some of the reports indicate that certain compositions of borate glasses exhibited cytotoxicity under static conditions during *in vitro* culture testing whereas no considerable toxicity was detected under more dynamic culture conditions.[101-102,111] Before tissue culture, borate-based glass can be partially converted to hydroxyapatite so as to reduce the toxic effects. Other alternatives include dynamic cell culture or dilution of the phosphate solution[112] for reducing toxicity of the medium. Borate glasses rapidly release high concentrations of boron resulting in high level of local concentrations of boron near the vicinity of the glass. However, the boron concentrations detected in the blood around borate glass pellets implantation in rabbit tibiae were found to be at an acceptable level.[111] Hence borate-based glasses offer opportunities to regulate and tailor the degradation rate of synthetic biomaterials. For the first time in 2005, Scaffolds were derived from borate glasses using soft pressing and sintering treatment.[113,114] The 13-93B2 glass is of prime interest these days as it is one of the most promising materials for making foam-like scaffolds.[57] Polymer foam replication was used to successfully produce 13-93B2 glass scaffolds.[95] These scaffolds possessed microstructure nearly identical to human trabecular bone. The peculiar bioactive properties of sponge-derived borate glass scaffolds, as well as their mechanical behavior and structural similarity to trabecular bone, make them very promising candidates for clinical applications as bone grafts.[115-117] Below a threshold concentration (0.65 m*M*), borate ions released into the culture media due to conversion of the glass to HA did not hinder the proliferation of bone marrow stromal cells. Moreover, extracts of the scaffold dissolution products supported the proliferation and function of murine MLO-A5 cells. The *in vitro* bioactivity of the glass was confirmed from the hydroxyapatite (HA) layer on the glass surface after immersion of the scaffolds in a dilute phosphate solution (0.02*M* K_2HPO_4) for 7 days. Though conversion of the scaffolds in the aqueous phosphate solution resulted in a weight loss of 13.0%, an increase in the pH of the solution from 7.0 to 8.7 after 30 days was observed. The as-prepared scaffolds had a

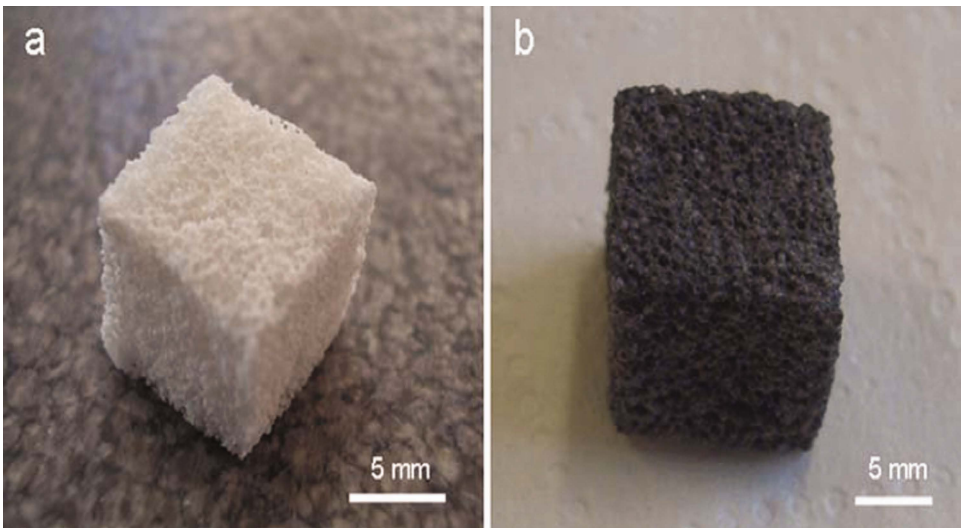

FIGURE 6. (a) GC-CEL2 scaffold and (b) GC-ICEL2 scaffold.[130] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

compressive strength of 6.4 ± 1.0 MPa, which decreased to 1.5–2.0 MPa after 15 to 30 days immersion in the phosphate solution. The mechanism of conversion of 13-93B2 scaffolds in HA after soaking in dilute phosphate solutions has been recently investigated in detail by Liu et al.[115-117] However, as demonstrated by Liu et al., the progressive material degradation carries a significant drop in the 13-93B2 scaffold strength (from 6.2 to 2.8 MPa after soaking for 15 days in phosphate solution). Silica free 13-93B3 borate glass scaffolds were reported to be toxic for murine MLO-A5 osteogeneic cells *in vitro*[118] but the same scaffolds were harmless to cells *in vivo* and supported new tissue infiltration upon subcutaneous implantation in rats.

Phosphate glasses

Phosphate-based glasses were proposed in 1980 in which P_2O_5 acts as network former oxide. These glasses contain a phosphate [PO_4] tetrahedron structural unit, which is highly asymmetric in nature. This asymmetry is the origin of their low durability, along with the ease of P-O-P bonds hydration.[119,120] Phosphate glasses have great potential as regenerative medicine because their solubility is strongly composition dependent. Hence, their dissolution rate is tailored by adding appropriate metal oxides, such as TiO_2, CuO, NiO, MnO, and Fe_2O_3 to the glass composition.[121-125] Phosphate glasses have been widely investigated as controlled release vehicles of antibacterial ions such as silver, copper, zinc, and gallium along with 3D construction of muscular tissues.[126] Phosphate glasses can also be spun to fabricate glass fibers. This special feature of phosphate based glasses provides the ability to be used in soft-tissue engineering as guides for muscle or nerve repair. Therefore, some developments and *in vivo* tests have been performed on phosphate glass nerve guides, such as tubes or meshes.[127,128] These tests yielded positive results and these glasses have been regarded by some as "smart materials" for soft-tissue engineering. For hard tissue engineering, the phosphate glasses are regarded as bone tissue regenerative materials in the form of bulk or powders in conjunction with polymers in composite materials. Vitale-Brovarone et al. manufactured phosphate glass-ceramic scaffolds[129,130] using ICEL2 powders as glassy inorganic phase as shown in Figure 6(a,b). GC-ICEL2 scaffolds were found to be resorbable as they underwent a process of continuous dissolution after soaking in water, Tris-HCl, and SBF. Moreover, GC-ICEL2 scaffolds were found to be bioactive because HA layer was observed to form on their trabeculae after soaking in SBF. In addition to this, bone marrow stromal cells which were cultured on the scaffold materials exhibited regulated metabolic activity and proliferation as well as differentiation. Abou Neel et al. studied Na_2O-CaO-SrO-P_2O_5 system and presented its physical and structural characterization as a bone regenerative material.[131] Figure 7 presents degradation rate and release of ions as a function of composition of glass used by Abou Neel et al. Substitution of Na_2O with SrO from 0 to 5 mol % produced a significant increase in density, glass transition temperature, and degradation rate of these glasses. This increase in degradation rate was further supported by the levels of cations and anions released from these glasses, which in turn changes the pH of the surrounding medium. Sr^{2+} was found to be directly related to the amount of SrO in the glass and not to the degradation rate. Successful fabrication of 3D trabecular scaffolds from phosphate glass using H_2O_2 foaming is also reported.[132] Changing thermal treatment conditions and H_2O_2 concentration could vary the percentage of crystallinity, pore content, and size. The phosphate glass as reinforcing phase in b-TCP-based scaffolds (b-TCP/PG1) composite scaffolds exhibited superior mechanical properties (up to 6 MPa) with respect to pure b-TCP scaffolds (up to 2.3 MPa). This may be attributed to the fact that glass must have acted as a viscous binder during the sintering process, hence strengthening the final scaffold structure.[133]

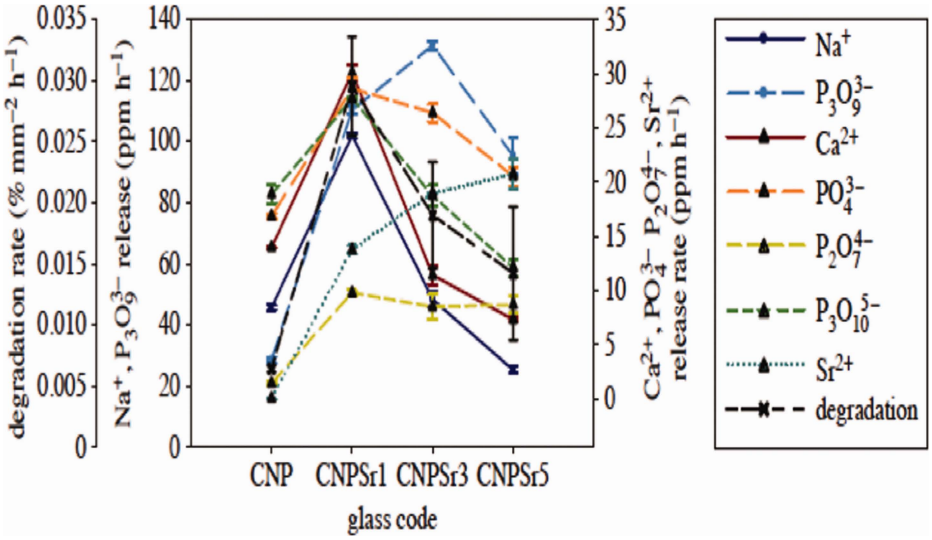

FIGURE 7. Degradation rate and ion release as a function of composition by Abou Neel et al.[131] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Doped glasses

The composition of glass is modified using "dopants" or additional additives in the glass composition to make it bioactive, bioresorable, and/or biodegradable.[132,133] The properties of bioactive glasses have been modified by doping with elements such as Cu, Zn, In, Ba, La, Y, Fe, Cr, and Sr.[134-138] In addition, bioactive glass compositions doped with silver have been shown to elicit antibacterial properties while maintaining their bioactive function.[139] In addition to SiO_2, B_2O_3, and P_2O_5, various amounts of other oxides may be incorporated in the glass composition to endow particular properties to the glass for example, CaO, K_2O, Na_2O, and MgO are useful to adjust the pH of surroundings, ZnO, CuO, AgO, and TiO_2 allow the release of proper ions that can impart antibacterial properties to the material. Alumina has high bioinertness, high abrasion resistance and high hardness, which makes it suitable material for dental and bone implants.[140] Al_2O_3 is helpful to strengthen the mechanical properties of glasses. Zinc[66-68] and magnesium[141] are known to exert a stimulatory effect on osteoblast proliferation, differentiation, and bone mineralization ability. Strontium incorporation in the bioactive glasses can stimulate osteogenesis, accelerate bone-healing processes and reduce bone resorption.[62,63] The drug strontium ranelate has been reported to increase the fracture-healing ability of rat bones in terms of callus resistance. The group treated with only strontium ranelate showed a significant increase in callus resistance compared with the untreated control group. On adding dopants, the X-ray imaging contrast increases. Iron is considered useful for the cancer treatment because of its magnetic properties. Singh and Bahadur[136] doped a borosilicate glass composition with iron and found that only the samples having 10-15% Fe_2O_3 showed the formation of an apatite layer on glass. Compositions having less than 5% Fe_2O_3 did not yield any apatite. For treatment of cancer, Luderer et al.,[142] also incorporated Fe_2O_3 in aluminoborosilicate glasses. Recently Singh et al.[135] also studied the effect on Al, Y, La, and Cr on the bioactive behavior of calcium borosilicate glasses. Yttria and chromium based glasses showed apatite formation after soaking in SBF solution. Though chromium is known to increase load resistance, at the same time yttria increases the devitrification resistance of glass. Recently, our research group has also elucidated the study on barium zinc alumino-borosilicate glasses.[71] It was observed that for Al_2O_3 >5%, the formation of brushite and whitlockite was apparent, though no hydroxyapatite formation could be observed on the glass surface.

Vitale-Brovarone et al.[143] developed the SiO_2-Na_2O-CaO-MgO (SNCM) glass system using three different organic starches (corn, potatoes, and rice) to make the scaffolds by their starch consolidation. The glass–ceramic possessed residual amorphous phase enriched with Mg^{2+} ions and $Na_2Ca_2(SiO_3)_3$ as the major crystalline phase. Excellent *in vitro* bioactivity was observed due to Mg^{2+} enriched amorphous phase and good bioactivity index of $Na_2Ca_2(SiO_3)_3$. In addition to this, the scaffolds showed interesting mechanical properties as well as a certain degree of resorption. The 13-93/13-93B1/13-93B2 and 6P53B compositions are doped with MgO.[5,144] The sintered 6P53B glass scaffolds show a porosity (60%) in the range of trabecular bone, whereas compressive strength of (136 $\pm$ 22 MPa) is obtained which is comparable with human cortical bone. Titania doped glass compositions based on the P_2O_5–CaO–Na_2O–TiO_2 system have shown controlled solubility.[145,146] In addition to this, the chemical composition is close to the bone mineral phase. It further demonstrates that this glass has an advantage over polymeric scaffolds due to the fact that the glass can positively affect the material–cell interaction. According to reports by Branda et al.,[134] the dopants like La, In, Ga, and so forth decreased the bioactive behavior of glasses though indium doped glasses exhibit higher HA formation on their surface as compared with other glasses as indicated in Figure 8.

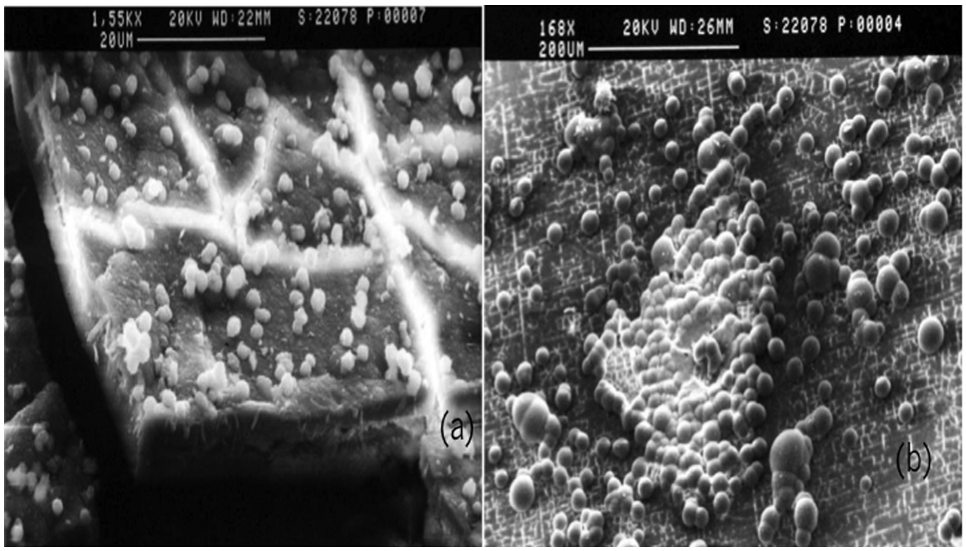

FIGURE 8. SEM micrograph of (a) gallium doped (b) Indium doped glass sample soaked in SBF for 7 days.[134]

Metallic glasses

The bulk metallic glasses (BMG) possess unique properties of superior strength, high elastic strain limit, high fracture toughness, and low young's modulus.[147-149] These glasses are biodegradable *in vivo* without hydrogen evolution. Zirconium based metallic glasses has found applications in biomedical engineering as zirconium has high mechanical strength and fracture toughness.[150-152] Hiromoto et al.[152,153] have re-passivated $Zr_{65}Cu_{17.5}Ni_{10}A_{l7.5}$ amorphous alloy in Hanks' solution and found lower metal dissolution during the re-passivation process. Morrison conducted cyclic polarization studies of $Zr_{41.2}Ti_{13.8}Ni_{10}Cu_{12.5}Be_{22.5}$ in phosphate buffered saline solution and compared the corrosion resistance with conventional biomaterials like 316L steel and Ti-6Al-4V alloy.[154,155] It was observed that BMG possess superior properties than conventional biomaterials. Horton and Parsell confirmed the biocompatibility of Zr-10Al-5Ti-17.9Cu-14.6Ni by viability of cells on the cell surface.[156] The main drawback of BMG is the inclusion of nickel. Nickel causes an allergic response and is possibly carcinogenic.[157,158] Hence many researchers have been trying to develop Ni-free metallic glasses. Jin and Löffler[159] developed $(Zr_xCu_{100-x})_{80}(Fe_{40}Al_{60})_{20}$ ($x = 62$–81) glasses, which have shown remarkably good biocompatibility with the cell. Zirconium oxide formed on the surface of the bioglass presumably helps in controlling the dissolution of toxic ions. A series of MgZnCa glasses[160,161] have been developed by Loffler SWISS group. These glasses exhibit high tensile strength. Huazhong group of China is also focusing on the studies of nickel free BMG glasses.[162,163] The hydroxyapatite formation on $Zr_{60.5}Cu_{19.5}Fe_5A_{l9.5}Ti_{5.5}$ BMG after immersion in SBF for 5 and 10 days are shown in Figure 9(a,b), respectively. In addition, when the immersion time exceeded 5 days, the apatite phase became porous as shown in Figure 9(b). Sometimes zirconium contains traces of radioelements.

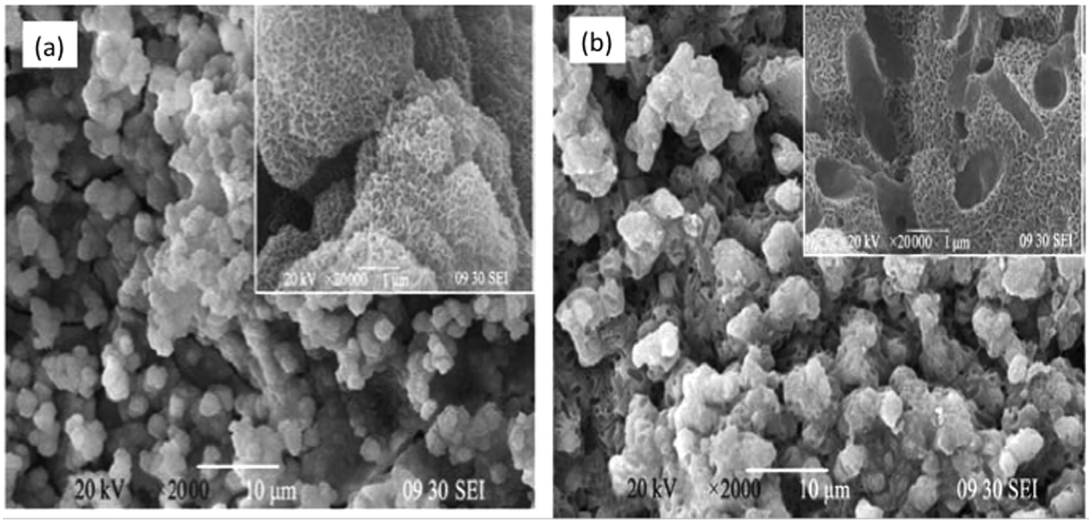

FIGURE 9. SEM images of MAO-treated Zr60.5Cu19.5Fe5Al9.5Ti5.5 BMG after immersion in SBF for (a) 5 and (b)10 days.[163]

These effects of radioelements and its cytotoxicity were investigated using gamma rays on zirconium head and no cytotoxicity was reported.[164,165]

45S5 Bioglass–titanium bulk and scaffold composites

For biomedical applications, 45S5 bioglass is the most widely used glass for over 40 years. The reactions on the glass surface induce release as well as exchange of critical concentrations of silicon, calcium, sodium, and phosphorus ions. These further lead to favorable extracellular and intracellular responses enhancing the bone formation process.[3,10-13] The 45S5 bioglass has shown increased secretion of vascular endothelial growth factor (VEGF) and its gene expression *in vitro*.[35,36,166] The 45S5 granules have been implanted in the tibiae and muscles of rabbits to determine the pathway of silicon released during the degradation of glass *in vivo*.[167] After 7 months of postimplantation, histopathological analyses of tissues indicated the excretion of silica through urine in soluble form. Though 45S5 is the standard bioactive material but it has certain limitations like its slow degradation rate and conversion to hydroxyapatite like material.[101,102] Hence the rate at which new tissue formation takes place is not in equilibrium with the rate at which scaffold derades. Hence, unconverted glass containing SiO_2 remains in scaffold, which can have effects for, long term. A new glass 13-93 has been formulated based on 45S5 composition except its higher silica content as well as K_2O and MgO as additional modifier atoms.[96,168] The 13-93 composition has better processing characteristics, yet its degradation is slower than 45S5 along with no marked difference in their bioactivity. However, some titania based composites have allowed the development of mesoporous scaffold with high bioactivity, as well as enhanced biomechanical behavior.[169,170] Titanium composites show high compressive strength, improved densification and enhanced biocompatibility. Novak et al. fabricated[171] TiO_2 foam-like scaffolds with pore size of the order of 300 nm with 95% porosity using the foam-replication method. PDLLA or PDLLA/Bioglass_coatings were developed to improve the structural integrity. A few micron PDLLA coating improved the compressive strength of scaffold seven times. In addition to this, composite coating involving Bioglass particles enhanced the bioactivity as is clear from the formation of dense hydroxyapatite layer on the surface of the foams upon immersion in SBF for 1 week. This makes rutile TiO_2 scaffold suitable for the applications in bone tissue engineering.

Pazo et al.[172] developed a method for producing bioactive coatings on Ti-Titanium based prosthetic implants to improve their adhesion with bone. The presence of small amount of TiO_2 in parent glass composition yielded reduced reactivity as well as broader firing range. This group used simple enamling technique to develop durable silicate glass coatings on Ti6Al4V substrates.[173] In order to increase the bioactivity of these coatings, hydroxyapatite and/or bioglass particles were incorporated into them. Saiz et al.[174] further found that higher silica coating on Ti6Al4V did not form apatite but were more resistant to corrosion, lower coefficient of thermal expansion and slow crack growth.

TECHNIQUES FOR FABRICATION OF BIOGLASSES

The most common techniques for the production of bioactive glasses are traditional melt quenching routes and the sol–gel technique.[71,72,89-90] In melt quenching technique, glass is prepared by taking required stoichiometric amounts of different constituent oxides or carbonates of high purity (99.9%). These constituents are first mixed together by ball-mill in an acetone medium. The powder obtained after ball-milling is melted at high temperatures in a high resistance furnace depending upon the composition chosen. The melt is then poured into molds to produce rods/cylinders or any other desired shape of interest. The melt can also be quenched in air using copper plates to obtain frits. The quenched glass is then annealed at 500°C to remove the internal stresses from the glasses.[70,71] Sometimes, the glass-forming batch is heated before the melting process in order to release the combined water of hydration or hydroxyl groups. Glasses containing less than 10% alkali oxide are difficult to melt due to their high viscosities. Generally, the silica content should be less than 60 mol % to allow the glass to bond with bone if it is obtained by melt quenching. Whereas, by using the sol-gel method for glass making, HA layer formation and bone bonding can be obtained with glasses having up to 90 mol % silica.[48] Metallic glasses are also prepared by melt-spinning, atomic evaporation and RF sputtering, among other methods, to obtain dimensions up to 100 μm. in order to obtain bulk dimensions, conventional mold-casting is used. The set-up used by the HUST group is shown in Figure 10.[159]

A sol–gel process has been used to prepare porous scaffolds of a few bioactive glasses, such as the glass-designated 58S, with the composition (mol %): 60% SiO_2, 36% CaO, 4% P_2O_5.[48] Though the prepared scaffold possessed a similar microstructure as that of dry human trabecular bone, the pore structure consisted of interconnected macropores (>100 μm) resulting from the foaming process and nano pores (less than several tens of nanometers). This may be attributed to the fact that these pores are inherent to the sol–gel process.[175] The process involves hydrolysis, polymerization, gelation, drying, and a dehydration process. Sol (or solution) evolves towards the formation of a gel-like diphasic system (with the aid of surfactant) containing both a liquid phase and solid phase. Its morphology can range from discrete particles to continuous polymer networks. Nano pores present in the glass prepared from a sol–gel-method yield a high surface area. Consequently, this leads to degradation and a faster conversion of these glasses to HA than scaffolds of melt-derived glass with the same composition. In addition, the sol-gel method provides high purity glasses with more homogeneity. Moreover, a lower processing temperature is required. However, these sol–gel-derived scaffolds have low strength (2–3 MPa) and consequently they are suitable for substituting defects in low-load sites only.

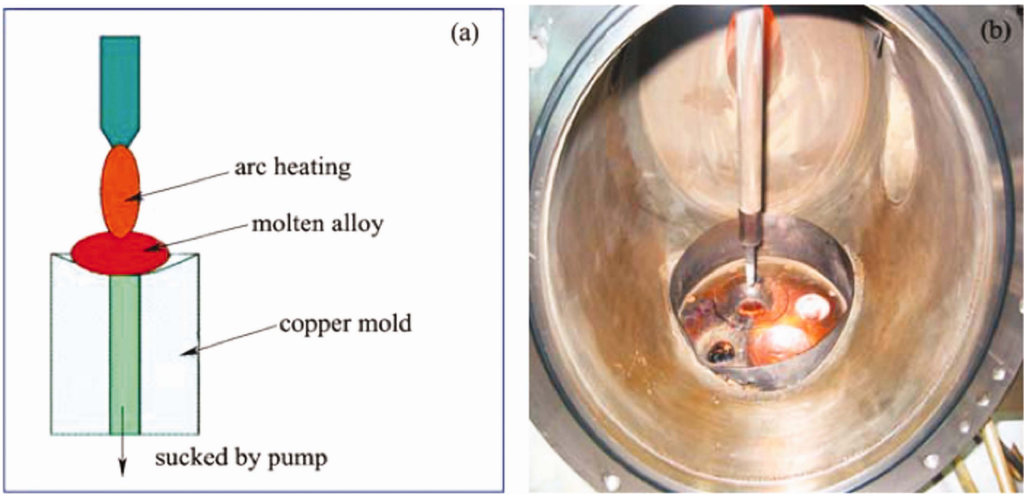

FIGURE 10. (a) schematic of casting and (b) set-up of soak mold casting.[163] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The fabrication method has a great impact on determining the structural properties of the biomaterial. The mechanical strength, pore size and pore inter-connectivity are critical parameters for bioactive glass scaffolds. Interconnected pores with a mean diameter (or width) between neighboring pores of 100 μm or greater, and open porosity of >50% are the criteria for tissue growth.[176] One method for forming a scaffold is to thermally bond a random packing of loose particles in a mold of the desired geometry.[103,118] But this method does not provide the desired porosity range and connectivity. Mixing the bioactive glass particles with some organic material and then removing it before the sintering process is also a useful method, but does not fully resolve the pore size issues. However, silicate, borosilicate and borate bioactive glass have been prepared with porosities in the range 60–90% using polymer foam replication method.[96,115,118] This method usually produces scaffolds similar to human trabecular bones. Disordered macro-porous structures of polymers and bioceramics can be produced by freezing of aqueous solutions and suspensions.[177] Porous scaffolds with an oriented microstructure have been prepared by optimized and controlled freezing technique as it leads to preferred direction of ice-growth.[95,118] An oriented microstructure is more beneficial than the random microstructure as it can provide higher strength in the direction of orientation.[178] Sponge replication is a very advantageous processing technique for making scaffolds, as it is relatively inexpensive and quick. The main drawback of using this technique is the lower mechanical strength of the scaffolds. Scaffolds produced by the polymer burning-out method show higher mechanical strength than that obtained through sponge replication.[113,114] An electrospinning method is also used to produce nano-fibrous bioactive glass scaffolds.[95] These glasses have high surface area, even more than sol-gel derived glasses. In addition to this, the composition of silica can be varied over a larger composition range.

HYDROXYAPATITE FORMATION

Hydroxyapatite is a calcium-deficient, carbonated phosphate surface layer developed on the surface of bioactive glass when in contact with SBF through interfacial and cell-mediated reactions. This layer mimics the chemical and crystallographic characteristics of bone, which allows it to chemically bond to host bone.[3,13-16] In fact almost two-thirds of a bone is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Hydroxyapatite formation is highly bioactive glass composition dependent.[5-10] The proposed structure for HA is shown in Figure 11 indicating its hexagonal symmetry with lattice parameters $a = 9.5$ and $c = 6.8$ Å.[179] For a material to be regarded as bioactive, biologically active carbonated hydroxyapatite (HCA) must form on its surface.[3] Hydroxyapatite is the most stable phase among various calcium phosphates. It is stable in body fluid and when fired up to 1200°C and does not show decomposition.[140] HA is osteoconductive as it supports bone regeneration along the implant at the bone–implant interface. However 45S5 bioglass is considered to

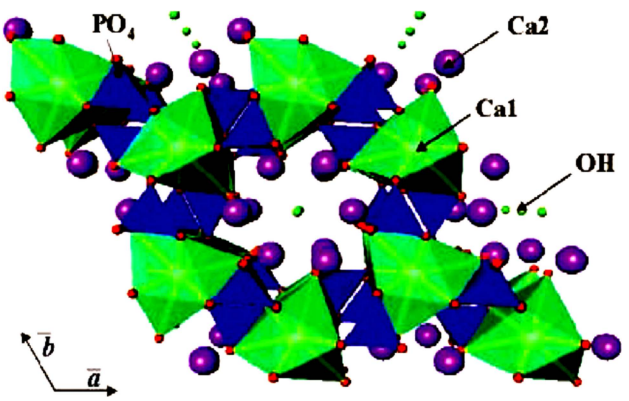

FIGURE 11. Proposed structure of hydroxyapatite.[179] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II. Calcium Phosphates along with their Ca/P Ratios[179]

Calcium Phosphate	Chemical Formula	Ca/P
Calcium metaphosphate	$Ca(PO_3)_2$	0.5
Calcium phosphate monohydrate	$Ca(H_2PO_4)_2{\cdot}H_2O$	0.5
Tetracalcium phosphate diacid	$Ca_4H_2P_6O_{20}$	0.67
Heptacalcium phosphate	$Ca_7\ (P_5O_{16})_2$	0.7
Calcium pyrophosphate dehydrate	$Ca_7\ P_2O_7{\cdot}2H_2O$	1
Calcium phosphate	$Ca_7\ P_2O_7$	1
Dicalcium phosphate	$CaHPO_4$	1
Dicalcium phosphate dihydrate	$CaHPO_4{\cdot}2H_2O$	1
Octacalcium phosphate	$Ca_8\ H_2\ (PO_4)_6{\cdot}5H_2O$	1.33
Tricalcium phosphate	$Ca_3(PO_4)_2$	1.5
Calcium phosphate	$Ca_{10-x}H_{2x}(PO_4)_6(OH)_2$	–
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Tetracalcium phosphate	$Ca_{10}\ O(PO_4)_2$	2.0

be osteoconductive as well as osteoinductive as it not only supports the regeneration at the interface but also away from the interface.[95-98] In addition to bioactive glasses, hydroxyapatite and some other calcium phosphates also show an excellent ability to bond to bone, although the compositional properties of biological apatite that is material to be substituted should be evaluated. Biological apatites like enamel, bone and dentine and so forth exhibit a wide compositional range in their sublattices as compared with stoichiometric hydroxyapatite. First of all, the size of biological apatite crystals is smaller than 500 Å. Biological apatites are endowed with carbonate in their structure and calcium deficiency leading to the non-stoichiometric phases. They also possess low crystallinity and large amounts of lattice defects. The presence of carbon in apatite is the cause of lattice distortion leading to crystalline defects and microstresses in the network. These stresses and defects play a vital role in the solubility of apatite. Hence, the synthetic apatites should exhibit small particle sizes along with the presence of CO^{3-} ions. At high temperatures, the carbonate ion occupies OH^- (mainly in synthetic aqueous systems) and they are known as A type apatites whereas in biological apatites the carbonates occupy ${PO_4}^3$ as a result of dissolved CO_2 in the aqueous phosphate solution and are known as B type apatites.[179] There is one more fundamental criteria, which is very helpful in determining the stoichiometry, acidity, and solubility of apatites that is Ca/P ratio. Higher Ca/P ratio yields lower acidity and solubility and *vice versa*. The Ca/P ratio of the converted material generally varies from the surface of the reacted glass to the interior. Ca/P ratios for some of the calcium phosphates is listed in Table II. Clinical investigations indicated that implanted hydroxyapatites and calcium phosphates are virtually inert and remain within the body for 6 to 7 years post-implantation.[180] Though the degradation rates of amorphous HA are high, it does not possess enough mechanical strength to build a 3D porous network. Hench proposed the following set of interactions and reactions to explain hydroxyapatite formation.[11]

Ion exchange reactions

In the first step, exchange of ions between the H^+ from the solution and the glass network modifiers (Na^+ and Ca^{2+}) leads to formation of silanol groups as a consequence of hydrolysis of the silica groups. This process results in a net increase of pH of the solution due to the increasing OH^- ions. The reaction mechanism follows the equation:

$$\mathrm{Si---O---Ca^{2+} + H^+ \rightarrow Si---O---H + Ca^{2+}} \quad (1)$$

Dissolution of silica

The silica network is attacked due to an increase in pH. The dissolution of silica occurs resulting in the formation of silicic acid leads that is $Si(OH)_4$ on the surface of glass as follows:

$$\mathrm{Si---O---Si + H_2O \rightarrow 2Si---OH} \quad (2)$$

These silanol groups play a vital role as the nucleation centers of the apatite formation. At this stage, network dissolution leads to the formation of insoluble form of silica. The dissolution process is controlled by an interface reaction with a linear t^1 dependence as follows:[181,182]

$$dC/dt = kS(C_e - C) \quad (3)$$

where C is concentration of silica in solution, C_e is equilibrium concentration of silicon, S is surface of solid phase, k is rate constant, and t is time. This reaction also dominates at high pH range during static solution conditions.

Formation of silica rich/calcium phosphate layer

A silica gel layer of 1 to 2 μm thickness is formed on the surface of the glass due to a polymerization process.[3] The precipitation and migration of calcium ions from the supersaturated solution onto the surface of ceramics occurs followed by the incorporation of $OH^-/{PO_4}^{2-}$ anions from the solution to form a mixed hydroxyl amorphous calcium phosphate (ACP) layer follows the silica polymerization. Hence, the concentrations of Ca^{2+} and $Si(OH)_4$ are critical parameters to determine hydroxypatite formation.

Formation of hydroxyapatite

The incorporation of $(OH)^-$ and $(CO_3)^{2-}$ from the solution continues, which ultimately converts the ACP layer to an HA layer. After the HA layer is formed the adsorption of growth factors, attachment, proliferation and differentiation of osteoprogenitor cells are the biological mechanisms of bonding to bone. In addition to this, adsorption of adhesion proteins (e.g., fibronectin, vitronectin, etc.) is necessary condition for cellular attachment. The formation of HA is pseudomorphic as it starts at the surface of the glass and moves inward.[118] Figure 12 shows how the hydroxyapatite layer increases with soaking time duration[183] on glass scaffolds. The spheres grow with the soaking time and after 7 and 14

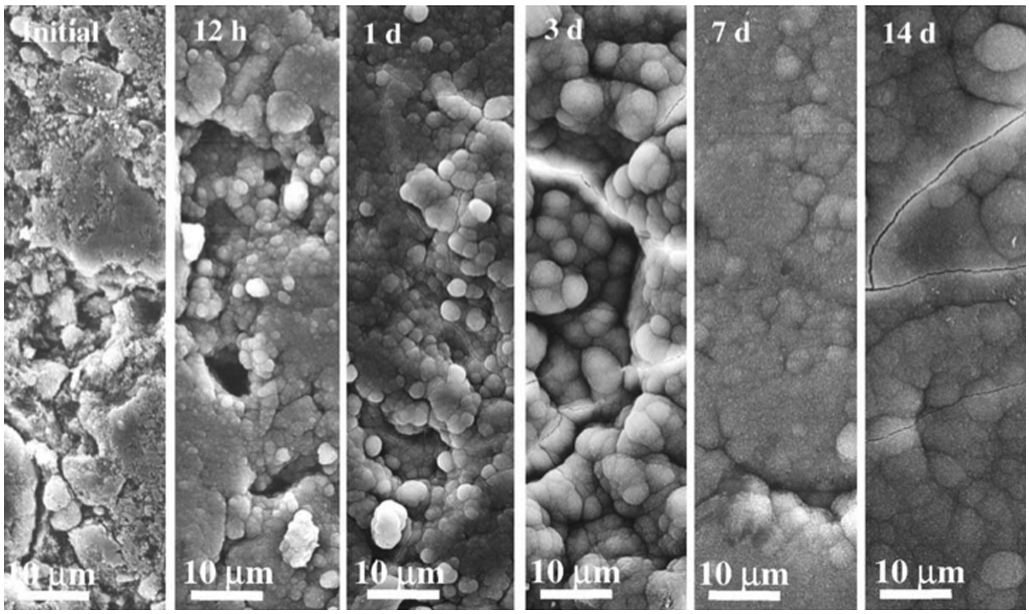

FIGURE 12. Scanning micrographs of the G–OHAp/700°C surface before and after soaking in SBF for different time periods.[183]

days they aggregate, causing densification and difficulty of separation.

The release of Si from 45S5 granules implanted in the muscle and tibiae of rabbits has confirmed the pathway of silicon released during the degradation of the glass *in vivo*.[111] In addition, the chemical and histopathological analyses of silicon released in urine and blood samples for up to 7 months postimplantation was conducted from 45S5 degradation and it was found that it was harmlessly excreted in soluble form through the urine. In contrast, some studies showed no evidence of Si release from Si substituted calcium phosphates.[184] The degradation kinetics for glass and conversion to HA *in vitro* has been evaluated by immersing the glass in an aqueous phosphate solution such as SBF at 37°C and measuring the weight loss of the glass as a function of time.[71,135,136] The degradation and dissolution of ions and soluble species like silanol groups into the solution result in change of the pH as well as concentration of the solution as a function of time. The variation in pH with time for some borosilicate glass compositions dipped in SBF has been shown in Figure 13. Along with pH variation, there is also a variation observed in resonance peaks of Fourier Transform Infra-red spectroscopy (FT-IR), because the infrared absorption spectra of the glasses gives information about the possible changes of vibration spectra due to the process of structural rearrangement during degradation of the glass composition after soaking as shown in Figure 14(a,b).[135] The broad band centered at 3438 cm^{-1} is assigned to the hydroxyl group (–OH) or the silanol group (Si-O–H). The glass sample GY after dipping in simulated

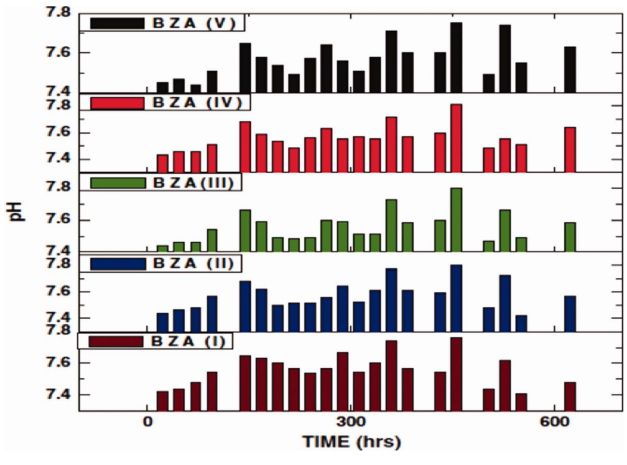

FIGURE 13. pH variation of glass dipped in SBF with time.[71] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

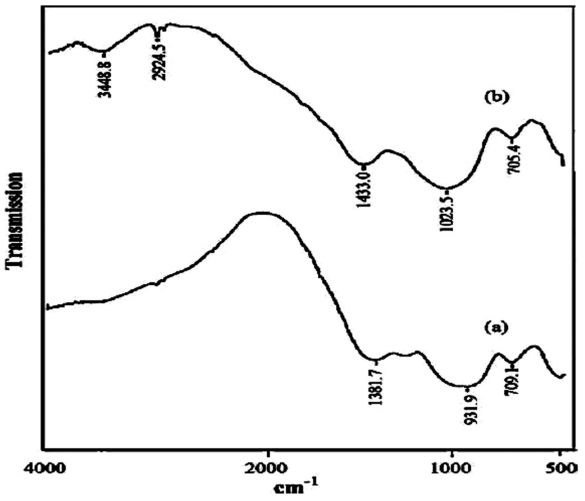

FIGURE 14. FT-IR spectra showing changes in GY glass after (a) 0 hr dipping and (b) 600 hr dipping in SBF solution.[135]

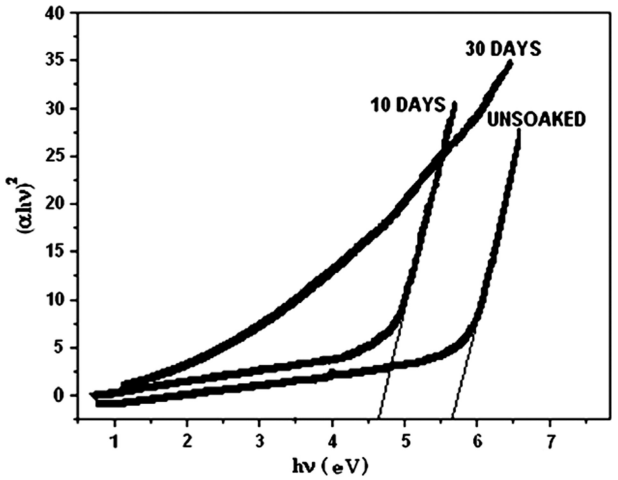

FIGURE 15. Change in band-gap of BZA glass after and before dipping in SBF solution.[71]

body fluid (SBF) solution shows the broad hump at 3500 cm^{-1} due to the OH^- group. On the other hand, there is no peak in that region in sample GY before dipping in simulated body fluid (SBF) solution. There is another peak at 700 cm^{-1}, which again shows the presence of OH^- ions as shown in Figure 14. As the layer formation involves a set of reactions like dissolution, precipitation and ion exchange between glass and SBF, some compositional changes in the glasses must have taken place during the ion exchange process leading to change in their optical properties. Our group has conducted the band gap measurements and found appreciable change in the band gap of samples before and after soaking in SBF solution as shown in Figure 15. After dipping, BZA glasses show sharp decrement in the band gap. Basically, the change in the optical band gap is attributed to the structural changes due to the different site occupancies by cations. For the formation of an apatite layer in borate glasses a similar mechanism is followed as followed by silica glasses except no silica rich layer is formed. Borate bioactive glasses have been reported to support cell proliferation and differentiation *in vitro* and *in vivo*. Even certain compositions of silica free borate bioactive glass enhance new bone formation to a greater extent than 45S5 glass.[95] Phosphate glasses have also shown chemical affinity with bone as the constituent ions are present in the organic mineral phase of bone.[95] Similar to silicate glasses, the ion exchange reaction takes place with Na-H ion exchange subsequently followed by phosphate network breakage due to the cleavage of P-O-P bonds. Current research is focused on the resorption ability of phosphate glasses as the bioactive behavior of these glasses has been low in comparison to that of silicate and borate glasses. Hence the solubility of these glasses needs to be optimized in order to implement them as bioresorable bioactive materials.

CYTOTOXICITY OF DISSOLUTION PRODUCTS

Bioglasses should be tested for their biocompatibility with the physiological environment upon their implantation.[3,185] Critical levels of the ions released by bioactive glasses regulate genes in osteogenic cells, which initiate a self-repair mechanism for tissue regeneration.[91-93,186] In other words, dissolution products of bioactive glasses should not be detrimental to the tissues of the host. Bioglasses have been implanted in many mammalian species including dogs, monkey, mice, and baboons and no evidence of toxic effects have been found.[187-189] Xynos et al.[93] investigated the effect of ionic products of 4S5S dissolution on the gene –expression profile of human osteoblasts for 1176 genes. Murphy et al.[186,190] investigated the ion release profiles for Novabone, BT111 and BT112 glass compositions. For Novabone, Si^{4+} ion released from 1 to 48 ppm with respect to time, which indicates their potential for bone-graft applications. Studies have shown that silica has stimulatory effects on osteoblasts if its release level is between 0.1 and 100 ppm.[93,191] A cellular receptor of silicon and its role in bone homeostasis still needs to be addressed.[92,93] Reports indicate a decrease in trabecular bone loss in ovariectomised mice and increase in eggshell thickness of hen.[192] Calcium also increases osteoblast activity in the range 13.1 to 90 ppm.[93] Sodium in plasma is 3200 ppm, hence the released amount does not show much significant physiological effects except for imparting degradability and control over release of other constituent ions.[18,185] For bone grafting purpose, strontium concentrations ranging from 8.7 to 87.6 ppm have shown stimulatory effects whereas zinc concentration above 6.5 ppm has revealed cytotoxic responses.[190,193] The cytotoxicity tests conducted for zinc have shown an increased concentration of zinc ions.[193] Human MG-63 osteoblast was incubated with bioglasses having a variation in zinc content. The ions increased the release of LDH in extracellular medium, which is regarded as an index of cytotoxicity as well as increased PPP activity. Several studies also indicate mitochondrial dysfunction in zinc cytotoxicity.[194] NADPH oxidase gets activated in cortical neurons, which further generates ROS generation in these cells.[195] Ito et al.[196] found that the growth rate in mouse osteoblastic MC3T3-E1 cells decreased considerably when zinc exceeded 1.2%. Bioglass containing zinc more than 5% also exhibited cytotoxic effects on human osteoblasts. Endothelial, retinal and peripheral blood lymphocytes have undergone oxidative damage triggered by zinc.[197-199] For practical biomedical applications, glass compositions needs to be developed which exhibit slower release of zinc from glass matrix.

CELL MICROENCAPSULATION

A substantial amount of research has focused on immune-isolation technologies in order to reduce transplant immunogenicity. These technologies reduce the chance of graft rejection upon transplantation of biologically active molecules.[200,201] Cell entrapment devices, aggregation systems, and cell microcapsules are the common immunoisolating devices, which have been improved over several years.[200] Cell microcapsulation technology is the preferred system for transplantation as it can treat multiple diseases in the absence of immunosuppression.[202] Microencapsules consist of biologically active materials within a polymeric matrix,

which are further surrounded by semi-permeable membrane.[200-203] The membrane provides bidirectional diffusion of oxygen, nutrients and waste. Microcapsules posses higher surface/volume ratio, which is helpful for nutrients and oxygen permeability.[204] In addition to this, the membrane protects the biologic cell from the immune system of host as well as mechanical stress. Overall, this contributes to reduction in chronic administration of immunosuppressants, which is mandatory for organ transplants.[200] The prime requirement for clinical implementation of cell microencapsulation technology is biocompatibility of matrix material so that it should not interfere with cell function or trigger the immune system of patient. Alginates are the most potential candidates for encapsulation technology. Alginates form three-dimensional structure upon their reaction with multivalent ions. Alginates are anionic polysaccharides composed of guluronic (G block) and mannuronic (M block) units intermixed with each other. Some barium alginate gels have been found to improve the mechanical stability whereas silicon capsules have resulted in control of diffusion properties.[205,206] Some polycations like PLL, PLO, and PMCG have also been employed to coat the microcapsule.[207] Leoni and Desai microfabricated PTFE based on the immune-isolation biocapsule concept where PTFE is used to support the vascularization for encapsulated cells.[208] Recently, enzymatically tailored alginate with increased resistance toward osmotic swelling and improved biocompatibility has been reported.[209] Clinically, cell microencapsulation has been used to develop bioartificial organs, treat mendelian disorders caused due to gene product deficiency, cancer treatment, and various other disorders. Scientists have introduced islet immobilization in macrocapsules to develop artificial pancreas.[204] Furthermore, it will decrease the insulin dose required by diabetic patients and subsequently reduce the complications associated with hypoglycemia. Hasse et al.[210] have developed barium chloride hardened alginate capsules enclosing allogenic parathyroid tissue to treat hypoparathyroidism. There was 50% reduction in patient's calcium and vitamin D replacement therapy. Xu et al.[211] have focused on the treatment of cancer by developing microencapsulated cells capable of expressing inducible nitric oxide synthase (iNOS). It triggered the Fas ligand and other similar proteins, which activated apoptotic pathways ultimately causing tumor suppression in mice. Also, there have been attempts by many research groups to treat degenerative diseases of central nervous system (CNS) like armyotrophic lateral sclerosis, chronic pain, Parkinson's disorder and Hutington's disease.[212] Hence, cell encapsulation is a non-viral approach in which cells and tissues can be immobilized and multiple diseases can be cured, but biosafety and toxicity needs to be addressed for achieving long-term performance goals.

CHALLENGES AND FUTURE SCOPE

The major challenge is to develop bioglasses that are both mechanically strong and biocompatible. Unfortunately, there is competition between mechanical strength and bioactive behavior specially biodegradability. Some mechanically strong materials like crystalline hydroxyapatite, polymer composites and Ti alloys are virtually inert, while biodegradable materials amorphous hydroxyapatite and glasses tend to be mechanically fragile. According to the reports of Filho et al.[46] and Li et al.,[47] the crystallization in bioactive glass leads to decreased level of bioactivity, which probably makes it an inert material. For bone engineering at load-bearing sites, annealing has been done during processing of 45 S5 BioglassW in order to obtain crystalline phases embedded in a residual glassy phase.[50] But in the case of 45S5 glass, the crystallization leads to a decrease in mechanical strength of the glass–ceramic scaffolds with low strength ($<$1 MPa). Hence, the optimization of mechanical strength is also an issue, which needs to be addressed. Though devitrification does not hinder the ability of 45S5 glass to form HA-like surface layer, it reduces the rate of conversion to HA. Ideally, bioglass needs to be degradable because biodegradation would avoid the harmful effects of a foreign entity and its gradual replacement with the new bone (in scaffolds). The degradation rate of 45S5 glass is very slow and hence a large amount of glass would remain unconverted to an HA layer. The rate at which glass degrades is slower than the rate at which new tissue is formed. This unconverted glass remains inside the scaffold and therefore *in vivo* stability remains an issue. This point implies a deep insight into all of the detrimental effects that can be potentially induced in the human body due to the ions released by bioglass in SBF. Ions like silicon, zinc, magnesium, strontium and calcium released by bioactive glasses can exert a gene control regulation along with osteoblastic cell proliferation, differentiation, and bone mineralization. The proper design of bioactive glasses is an imperative criterion as the hydroxyapatite formation and tissue engineering is composition dependent. Hence, while designing a glass, it is important to dope it with elements, which provide a target and application specific approach. In addition, the bioglass should not hamper the biological functioning of other organs.

Many issues such as testing, sterilization, packaging and international standards for the production of bioglasses for clinical use are required to make the bioglass commercially viable. Fabrication process for making 3D scaffolds from bioglass 45S5 also involves some difficulty. Porous bioactive glass scaffolds are commonly prepared by sintering of glass particles of desired 3D geometry and then bonding these particles into a strong glass phase with interpenetrating network of pores. The glass stability region for 45S5 glass is very narrow due to the small temperature interval difference between glass transition temperature and onset of the crystallization temperature. Hence the viscous flow of the glass has to overcome the high density and cannot be sintered properly leading to a decreased strength of the scaffold. In contrast, 13-93 glass products are widely used *in vivo* in Europe due to its large sintering interval, which enables the glass phase in porous 3-D scaffolds to be sintered to high density without crystallization. However, 13-93 glass degrades (and converts to an HA-like material) even more slowly than 45S5 glass. The pH of solution changes rapidly

during the initial stage of bioglass degradation as it releases ions into the solution leading to a change in the concentration as well as the local environment. The effect of this pH concentration on the human body along with the biological roles of these soluble species and their toxicity are a matter of concern. Even the process of their removal is not clearly understood as the biological effects of these changes are difficult to predict only from *in vitro* experiments. Sterilization is an issue regarding scaffold commercialization and safe clinical use as the retention of the original properties after sterilization might be a problem for polymer/glass composite scaffolds. In fact, although bioglass was demonstrated to be an excellent bioactive material for promoting bone tissue regeneration, all porous bodies produced from it exhibited relevant brittleness and poor mechanical strength. The toxicity of the borate glass system due to release of borate ions has been an issue, but the toxicity of borate glasses to cells and tissues have been addressed showing that borate glasses are non-toxic in small animals. In addition to the osteogenesis capacity of bioactive glasses, some glasses have revealed proangiogenic potential which provides benefits to soft-tissue repair.

Despite its brittleness, bioactive glasses have a unique set of properties, such as the ability to degrade at a controllable rate and convert to an HA-like material, to bond firmly to hard and soft tissues, and to release ions during the degradation process which can promote bone cell growth. These ions elevate osteogenesis and angiogenesis, as well as chondrogenesis. Future research is focused on limiting the detrimental effects of its brittleness through innovative scaffold design and processing, particularly for repair of load-bearing bones, keeping in mind the beneficial properties of the bioactive glass. Advanced studies on the interactions between the host cell and the biomaterial, as well as cell gene expression, and their response to biomaterials should be undertaken in order to understand surface topology, activity of cells and adhesion dynamics at the nanoscale. Complete *in vitro* biological, biochemical, morphological characterizations should be conducted in order to investigate advanced functionalities when used in drug delivery systems.

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REFERENCES

1. Ramakrishna S, Meyer J, Wintermantel E, Leong KW. Biomedical applications of polymer-composite materials: A review. Comp Sci Tech 2001;61:1189–1224.
2. Williams DF. Consensus and Definitions in Biomaterials, Advances in Biomaterials. Amsterdam: Elsevier Science; 1988. p 11–16.
3. Hench LL. Bioceramics: from concept to clinic. J Am Ceram Soc 1991;74:1487–1510.
4. Speidel MO, Uggowitzer PJ. Biocompatible Nickel-Free Stainless Steels to Avoid Nickel Allergy, Material in Medicine. Seitzerlansd: vdf Hochschulverlag AG ander ETH zurich; 1998. p 191–208.
5. Rehman MN, Ray DE, Bal BS, Fu Q, Jung SB, Bonewald LF, Tomsia AP. Bioactive glass in tissue engineering. Acta Biomater 2011;7: 2355–2373.
6. Zhang R, Ma PX. Poly(a-hydroxyl acids)/hydroxyapatite porous composites for bone tissue engineering I. Preparation and morphology. J Biomed Mater Res 1999;44:446–455.
7. Lu HH, El-Amin SF, Scott KD, Laurencin CT. Three-dimensional, bioactive, biodegradable, polymer-bioactive glass composite scaffolds with improved mechanical properties support collagen synthesis and mineralization of human osteoblast-like cells in vitro. J Biomed Mater Res 2003;64:465–474.
8. Kim S-S, Ahn KM, Park MS, Lee J-H, Choi CY, Kim B-S. A poly(lactide coglycolide)/hydroxyapatite composite scaffold with enhanced osteoconductivity. J Biomed Mater Res 2007;80: 206–215.
9. Ducheyne P, Qiu Q. Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function. Biomaterials 1999;20:2287–2303.
10. Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomed Mater Res 1972;2:117–141.
11. Hench LL. Biomaterials: A forecast for the future. Biomaterials 1998;19:1419–1423.
12. Hench LL, Wilson J. Introduction to Bioceramics. Singapore: World Scientific; 1993.
13. Hench LL. The story of BioglassVR. J Mater Sci Mater Med 2006; 17:967–978.
14. Rouquerol J, Avnir D, Fairbridge CW, Everett DH, Haynes JM, Pernicone N, Ramsay JDF, Sing KSW, Unger KK. Recommendations for the characterization of porous solids. Pure Appl Chem 1994;66:1739–1758.
15. Chevalier J, Gremillard L. Ceramics for medical applications: A picture for the next 20 years. J Eur Ceram Soc 2009;29: 1245–1255.
16. Williams DF, J. Cunningham. Materials in Clinical Dentistry. Oxford, UK: Oxford University Press; 1979.
17. Williams DF, editor. Definitions in Biomaterials. New York: Elsevier; 1987.
18. Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 2006;27:2907–2915.
19. Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T. Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W. J Biomed Mater Res 1990;24:721–734.
20. Ducheyne P. Bioceramics: Materials characteristics versus in vivo behavior. J Biomed Mater Res 1987;21:219–236.
21. Bohner M, LemaŸtre J. Can bioactivity be tested in vitro with SBF solution? Biomaterials 2009;30:2175–2179.
22. Apelt D, Theiss F, El-Warrak AO, Zlinszky K, Bettschart-Wolfisberger R, Bohner M, et al. In vivo behavior of three different injectable hydraulic calcium phosphate cements. Biomaterials 2004;25:1439–1451.
23. Theiss F, Apelt D, Brand B, Kutter A, Zlinszky K, Bohner M, et al. Biocompatibility and resorption of a brushite calcium phosphate cement. Biomaterials 2005;26:4383–4394.
24. LeGeros RZ, LeGeros JP. Phosphate minerals in human tissues. In: Nriagu JO, Moore PB, editors. Phosphate Minerals. Berlin: Springer-Verlag; 1984. p 351–385.
25. Witte F, Kaese V, Haferkamp H, Switzer E, Meyer-Lindenberg A, Wirth CJ, et al. In vivo corrosion of four magnesium alloys and the associated bone response. Biomaterials 2005;26:3557–3563.
26. Bandyopadhyay A, Bernard S, Xue W, Bose S. Calcium phosphate-based resorbable ceramics: Influence of MgO, ZnO, and SiO2 dopants. J Am Ceram Soc 2006;89:2675–2688.
27. Ohura K, Bohner M, Hardouin P, LemaŸtre J, Pasquier G, Flautre B. Resorption of and bone formation from, new β-tricalcium phosphate–monocalcium phosphate cements: An in vivo study. J Biomed Mater Res 1996;30:1993–2000.
28. Daculsi G, Laboux O, Malard O, Weiss P. Current state of the art of biphasic calcium phosphate bioceramics. J Mater Sci Mater Med 2003;14:195–200.
29. Rahaman MN, Brown RF, Bal BS, Day DE. Bioactive glasses for non-bearing applications in total joint replacement. Semin Arthroplasty 2007;17:102–112.
30. Wagoner Johnson AJ, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. Acta Biomater 2011;7:16–30.

31. Chen Q, Zhu C, Thouas GA. Progr Biomater, doi: 10: 1186/2194-0517-2.
32. Lobel KD, Hench LL. In-vitro protein interactions with a bioactive gel-glass. J Sol Gel Sci Technol 1996;7:69–76.
33. Ohgushi H, Dohi Y, Yoshikawa T, Tamai S, Tabata S, Okunaga K, Shibuya T. Osteogenic differentiation of cultured marrow stromal stem cells on the surface of bioactive glass ceramics. J Biomed Mater Res 1996;32:341–348.
34. Kotani S, Fujita Y, Kitsugi T, Nakamura T, Yamamuro T, Ohtsuki C, et al. Bone bonding mechanisms of β-tricalcium phosphate. J Biomed Mater Res 1991;25:1303–1315.
35. Keshaw H, Forbes A, Day RM. Release of angiogenic growth factors from cells encapsulated in alginate beads with bioactive glass. Biomaterials 2005;26:4171–4179.
36. Day RM, Boccaccini AR, Shurey S, Roether JA, Forbes A, Hench LL, Gabe S. Assessment of polyglycolic acid mesh and bioactive glass for soft tissue engineering scaffolds. Biomaterials 2004;25: 5857–5866.
37. Gatti AM, Valdre G, Andersson OH. Analysis of the in vivo reactions of a bioactive glass in soft and hard tissue. Biomaterials 1994;15:208–212.
38. Roether JA, Gough JE, Boccaccini AR, Hench LL, Maquet V, Jerome R. Novel bioresorbable and bioactive composites based on bioactive glass and polylactide foams for bone tissue engineering. J Mater Sci Mater Med 2002;13:1207–1214.
39. Schepers E, de Clercq M, Ducheyne P, Kempeneers R., Bioactive glass particulate material as a filler for bone lesions. J Oral Rehabil 1991;18:439–452.
40. Griffith LG. Emerging design principles in biomaterials and scaffolds for tissue engineering. Ann NY Acad Sci 2002;961:83–95.
41. Chen QZ, Harding SE, Ali NN, Lyon AR, Boccaccini A. Biomaterials in cardiac tissue engineering: ten years of research survey. Mater Sci Eng R Rep 2008;59:1–37.
42. Chen QZ, Rezwan K, Armitage D, Nazhat SN, Boccaccini AR. The surface functionalization of 45S5 Bioglass (R)-based glass-ceramic scaffolds and its impact on bioactivity. J Mater Sci Mater Med 2006;17:979–987.
43. Boccaccini AR, Maquet V. Bioresorbable and bioactive polymer/BioglassW composites with tailored pore structure for tissue engineering applications. Compos Sci Technol 2003;63:2417–2429.
44. Boccaccini AR, Blaker JJ, Maquet V, Day RM, Jéróme R. Preparation and characterisation of poly(lactide-*co*-grycolide) (PLGA) and PLGA/Bioglass W composite tubular foam scaffolds for tissue engineering applications. Mater Sci Eng C 2005;25:23–31.
45. Boccaccini AR, Notingher I, Maquet V, Jérôme R. Bioresorbable and bioactive composite materials based on polylactide foams filled with and coated by BioglassW particles for tissue engineering applications. J Mater Sci Mater Med 2003;14:443–450.
46. Filho OP, Latorre GP, Hench LL. Effect of crystallization on apatite-layer formation of bioactive glass 45 S5. J Biomed Mater Res 1996;30:509–514.
47. Li P, Yang Q, Zhang F, Kokubo T. The effect of residual glassy phase in a bioactive glass-ceramic on the formation of its surface apatite layer in vitro. J Mater Sci Mater Med 1992;3: 452–456.
48. Sepulveda P, Jones JR, Hench LL. Bioactive sol-gel foams for tissue repair. J Biomed Res A 2002;49:340–348.
49. Chen QZ, Liang SL, Wang J, Simon GP. Manipulation of mechanical compliance of elastomeric PGS by incorporation of halloysite nanotubes for soft tissue engineering applications. J Mech Behav Biomed Mater 2011;4:1805–1818.
50. Chen QZ, Boccaccini AR. Coupling mechanical competence and bioresorbability in BioglassW-derived tissue engineering scaffolds. Adv Eng Mater 2006;8:285–289.
51. Lefebvre L, Gremillard L, Chevalier J, Zenati R, Bernache-Assolat D. Sintering behaviour of 45S5 bioactive glass. Acta Biomater 2008;4:1894–1903.
52. Huang R, Pan J, Boccaccini AR, Chen QZ. A two-scale model for simultaneous sintering and crystallization of glass-ceramic scaffolds for tissue engineering. Acta Biomater 2008;4:1095–1103.
53. Chaikof EL, Matthew H, Kohn J, Mikos AG, Prestwich GD, Yip CM. Biomaterials and scaffolds in reparative medicine. Ann NY Acad Sci 2002;961:96–105.
54. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26:5474–5491.
55. Levenberg S, Langer R. Advances in tissue engineering. Curr Top Dev Biol 2004;61:113–134.
56. Mikos AG, Temenoff JS. Formation of highly porous biodegradable scaffolds for tissue engineering. Electron J Biotechnol 2000; 3:114–119.
57. Hutmacher DW. Scaffold design and fabrication technologies for engineering tissues—State of the art and future perspectives. J Biomater Sci: Polym Ed 2001;12:107–24.
58. Darby WJ. Trace elements in human health and disease. In: Prasad AS, Oberleas D, editors. Zinc and copper, Vol. 1. New York: Academic Press; 1976. p 17.
59. Seeley RR, Stephens TD, Rate P. Anatomy and physiology, 8th ed. New York: McGrew Hill; 2006.
60. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: A review. Afr J Food Sci 2010;4:200–222.
61. Whitney EN, Rolfes SR. Understanding Nutrition. Belmon: Wadsworth Publishing; 2010.
62. Marie PJ, Ammann P, Boivin G, Rey C. Mechanisms of action and therapeutic potential of strontium in bone. Calcif Tissue Int 2001;69:121–129.
63. Wong CT, Chen QZ, Lu WW, Leong JCY, Chan WK, Cheung KMC, Luk KDK. Ultrastructural study of mineralization of a strontium-containing hydroxyapatite (Sr-HA) cement in vivo. J Biomed Mater Res A 2004;70:428–435.
64. Marie PJ, The calcium-sensing receptor in bone cells: A potential therapeutic target in osteoporosis. Bone 2010;46:571–576.
65. Meunier PJ, Slosman DO, Delmas PD, Sebert JL, Brandi ML, Albanese C, Lorenc R, Pors-Nielsen S, de Vernejoul MC, Roces A, Reginster JY. Strontium ranelate: Dose-dependent effects in established postmenopausal vertebral osteoporosis—A 2-year randomized placebo controlled trial. J Clin Endocrinol Metab 2002;87:2060–2066.
66. Yamaguchi M. Role of zinc in bone formation and bone resorption. J Trace Elem Exp Med 1998;11:119–135.
67. Lang C, Murgia C, Leong M, Tan L-W, Perozzi G, Knight D, Ruffin R, Zalewski P. Anti-inflammatory effects of zinc and alterations in zinc transporter mRNA in mouse models of allergic inflammation. Am J Physiol Lung Cell Mol Physiol 2007;292:L577–L584.
68. Cousins RJ, A role of zinc in the regulation of gene expression. Proc Nutr Soc 1998;57:307–311.
69. Murray RK, Granner DK, Mayer PA, Rodwell VW. Harper's Biochemistry, 25th ed. Health Profession Division: Mc-Graw Hill; 2000.
70. Kaur G, Pandey OP, Singh K. Interfacial study between high temperature SiO_2-B_2O_3-AO-La_2O_3 (A= Sr, Ba) glass seals and Crofer 22 APU for solid oxide fuel cell applications. Int J Hydrogen Energy 2012;37:6862–6874.
71. Kaur G, Sharma P, Kumar V, Singh K. Assessment of in-vitro bioactivity of SiO_2-BaO-ZnO-B_2O_3-Al_2O_3 glasses: An optico-analytical approach. Mater Sci Eng C 2012;32:1941–1947.
72. Madanat R, Moritz N, Vedel E, Svedstro E, Aro HT. Radio-opaque bioactive glass markers for radiostereometric analysis. Acta Biomater 2009;5:3497–3505.
73. Lewis G, Van Hooy-Corstjens CS, Bhattaram A, Koole LH. Influence of the radiopacifier in an acrylic bone cement on its mechanical, thermal, and physical properties: barium sulfate-containing cement versus iodine-containing cement. J Biomed Mater Res B 2005;73:77–87.
74. Pierce DT, Spicer WE. Electronic structure of amorphous silicon from photoemission and optical studies. Phys Rev B 1972;5: 3017–3029.
75. Schwarz K. A bound form of silicon in glycosaminoglycans and polyuronides. Proc Natl Acad Sci USA 1973;70:1608–1612.
76. Barrett AJ. Cartilage. In: Florkin M, Stotz EH, editors. Comprehensive Biochemistry, Vol. 26B. New York: Elsevier; 1968. p 438–442.
77. Birchall JD, Bellia JP, Roberts NB. On the mechanisms underlying the essentiality of silicon interactions with aluminium and copper. Coord Chem Rev 1996;49:231–240.

78. Kwun IS, Cho YE, Lomeda AR, Shin HI, Choi JY, Kang YH, Beattie JH. Zinc deficiency suppresses matrix mineralization and retards osteogenesis transiently with catch-up possibly through Runx 2 modulation. Bone 2010;46:732–741.
79. Chandra RK, Micronutrients and immune functions: An overview. Ann NY Acad Sci 1990;587:9–16.
80. Zhang JC, Huang JA, Xu SJ, Wang K, Yu SF. Effects of Cu^{2+} and pH on osteoclastic bone resorption in vitro. Prog Nat Sci 2003; 13:266.
81. Smith BJ, King JB, Lucas EA, Akhter MP, Arjmandi BH, Stoecker BJ. Skeletal unloading and dietary copper depletion are detrimental to bone quality of mature rats. J Nutr 2002;132:190–196.
82. Cashman KD, Baker A, Ginty F, Flynn A, Strain JJ, Bonham MP, O'Connor JM, Bugel S, Sandstrom B. No effect of copper supplementation on biochemical markers of bone metabolism in healthy young adult female despite apparently improved copper status. Eur J Clin Nutr 2001;55:525–531.
83. Finney L, Vogt S, Fukai T, Glesne D. Copper and angiogenesis: Unravelling a relationship key to cancer progression. Clin Exp Pharmacol Physiol 2009;36:88–94.
84. Gerard C, Bordeleau LJ, Barralet J, Doillon CJ. The stimulation of angiogenesis and collagen deposition by copper. Biomaterials 2010;31:824–831.
85. Hu GF. Copper stimulates proliferation of human endothelial cells under culture. J Cell Biochem 1998;69:326–335.
86. Rodriguez JP, Rios S, Gonzalez M. Modulation of the proliferation and differentiation of human mesenchymal stem cells by copper. J Cell Biochem 2002;85:92–100.
87. Beard JL. Iron biology in immune function, muscle metabolism and neural functioning. J Nutr 2001;131:5685–5695.
88. Sadarzadeh SM, Saffari Y. Iron and brain disorder. Am J Clin Pathol 2004;121:64–70.
89. Kingery WD, Bowen HK, Uhlmann DR. Introduction to Ceramics, 2nd ed. New York: John Wiley and Sons; 1976.
90. Shelby JE. Introduction to Glass Science and Technology, 2nd ed. Cambridge: The Royal Society of Chemistry; 2005.
91. Xynos ID, Edgar AJ, Buttery LDK, Hench LL, Polak JM. Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis. Biochem Biophys Res Commun 2000;276:461–465.
92. Xynos ID, Hukkanen MVJ, Batten JJ, Buttery LD, Hench LL, Polak JM. Bioglass W45 S5 stimulates osteoblast turnover and enhances bone formation in vitro: Implications and applications for bone tissue engineering. Calcif Tissue Int 2000;67:321–329.
93. Xynos ID, Edgar AJ, Buttery LDK, Hench LL, Polak JM. Gene-expression profiling of human osteoblasts following treatment with the ionic products of BioglassW 45 S5 dissolution. J Biomed Mater Res 2001;55:151–157.
94. Vitale-Brovarone C, Verne E, Appendino P. Macroporous bioactive glass-ceramic scaffolds for tissue engineering. J Mater Sci Mater Med 2006;17:1069–1078.
95. Fu Q, Rahaman MN, Bal BS, Brown RF, Day DE. Mechanical and in vitro performance of 13–93 bioactive glass scaffolds prepared by a polymer foam replication technique. Acta Biomater 2008;4: 1854–1864.
96. Brink M. The influence of alkali and alkali earths on the working range for bioactive glasses. J Biomed Mater Res 1997;36: 109–117.
97. Goel A, Kapoor S, Rajagopal RR, Pascual MJ, Kim HW, Ferreira JMF. Alkali-free bioactive glasses for bone tissue engineering: A preliminary investigation. Acta Biomater 2012;8:361–372.
98. Martins TB, Burlingams R, Van Muhlen CA, Jaskowski TD, Litwin CM, Hill HR. Evaluation of multiplexed fluorescent microsphere immunoassay for reflection of antibodies to nuclear antigens. Clin Diagn Lab Immunol 2004;11;1054–1059.
99. Regi MV, Bala F. Silica material for biomedical applications. Open Biomed Eng J 2008;2:1–9.
100. Michalczyk MJ, Sharp KG. Single Component Inorganic/Organic Network Materials and Precursors There of. US Patent5,378,790, 1995.
101. Yao A, Wang D, Huang W, Fu Q, Rahaman MN, Day DE. In vitro bioactive characteristics of borate-based glasses with controllable degradation behavior. J Am Ceram Soc 2007;90:303–306.
102. Huang WH, Day DE, Kittiratanapiboon K, Rahaman MN. Kinetics and mechanisms of the conversion of silicate (45 S5), borate, and borosilicate glasses to hydroxyapatite in dilute phosphate solutions. J Mater Sci Mater Med 2006;17:583–596.
103. Liang W, Rahaman MN, Day DE, Marion NW, Riley GC, Mao JJ. Bioactive borate glass scaffold for bone tissue engineering. J Non-Cryst Solids 2008;354:1690–1696.
104. Lahl N, Singh K, Singheiser L, Hilpert K, Bahadur D. Crystallisation kinetics AO- Al_2O_3-SiO_2-B_2O_3 glasses (A = Ba, Ca, Mg). J Mater Sci 2000;35:3089–3096.
105. Yang X, Zhang L, Chen X, Sun X, Yang G, Guo X, Yang H, Gao C, Gou Z. Incorporation of B2O3 in CaO-SiO2-P2O5 bioactive glass system for improving strength of low-temperature co-fired porous glass ceramics. J Non-Cryst Solids 2012;358:1171–1179.
106. Uysal T, Ustdal A, Sonmez MF, Ozturk F. Stimulation of bone formation by dietary boron in an orthopedically expanded suture in rabbits. Angle Orthod 2009;79:984–990.
107. Nielsen FH. Is boron nutritionally relevant? Nutr Rev 2008;66: 183–191.
108. Chapin RE, Ku WW, Kenney MA, McCoy H, Gladen B, Wine RN, Wilson R, Elwell MR. The effects of dietary boron on bone strength in rats. Fundam Appl Toxicol 1997;35:205–215.
109. Vitale-Brovarone C, Miola M, Balagna C, Verne E. 3D-glass-ceramic scaffolds with antibacterial properties for bone grafting. Chem Eng J 2008;137:129–136.
110. Marion NW, Liang W, Reilly GC, Day DE, Rahaman MN, Mao JJ. Borate glass supports the in vitro osteogen differentiation of human mesenchymal stem cells. Mech Adv Mater Struct 2005; 12:239–246.
111. Zhang X, Jia W, Gua Y, Wei X, Liu X, Wang D, Zhang C, Huang W, Rahaman MN, Day DE, Zhou N. Teicoplanin-loaded borate bioactive glass implants for treating chronic bone infection in a rabbit tibia osteomyelitis model. Biomaterials 2010;31: 5865–5874.
112. Ning J, Yao A, Wang D, Huang W, Fu H, Liu X, Jiang X, Zhang X. Synthesis and in vitro bioactivity of a borate-based bioglass. Mater Lett 2007;61:5223–5226.
113. Rahaman MN, Liang W, Day DE, Marion NW, Reilly GC, Mao JJ. Preparation and bioactive characteristics of porous borate glass substrates. Ceram Eng Sci Proc 2005;26:3–10.
114. Marion NW, Liang W, Liang W, Reilly GC, Day DE, Rahaman MN, Mao JJ. Borate glass supports the in vitro osteogenic differentiation of human mesenchymal stem cells. Mech Adv Mater Struct 2005;12:239–246.
115. Liu X, Huang W, Fu H, Yao A, Wang D, Pan H, Lu WW. Bioactive borosilicate glass scaffolds: improvement on the strength of glass-based scaffolds for tissue engineering. J Mater Sci Mater Med 2009;20:375–362.
116. Liu X, Pan H, Fu H, Fu Q, Rahaman MN, Huang W. Conversion of borate-based glass scaffold to hydroxyapatite in a dilute phosphate solution. Biomed Mater 2010;5:15005.
117. Liu X, Huang W, Fu H, Yao A, Wang D, Pan H, Lu WW, Jiang X, Zhang X. Bioactive borosilicate glass scaffolds: In vitro degradation and bioactivity behaviours. J Mater Sci Mater Med 2009;20: 1237–1243.
118. Fu Q, Rahaman MN, Bal BS, Bonewald LF, Kuroki K, Brown RF. Silicate borosilicate, and borate bioactive glass scaffolds with controllable degradation rate for bone tissue engineering applications. II. In vitro and in vivo biological evaluation. J Biomed Mater Res A 2010;95:172–179.
119. Bunker BC, Arnold GW, Wilder JA. Phosphate glass dissolution in aqueous solutions. J Non-Cryst Solids 1984;64:291–316.
120. Gao H, Tan T, Wang D. Dissolution mechanism and release kinetics of phosphate controlled release glasses in aqueous medium. J Controlled Release 2004;96:29–36.
121. Abou Neel EA, Mizoguchi T, Ito M, Bitar M, Salih V, Knowles JC. In vitro bioactivity and gene expression by cells cultured on titanium dioxide doped phosphate-based glasses. Biomaterials 2007;28:2967–2977.

122. Abou Neel EA, Knowles JC. Physical and biocompatibility studies of novel titanium dioxide doped phosphate-based glasses for bone tissue engineering applications. J Mater Sci Mater Med 2008;19:377–386.
123. Abou Neel EA, Ahmed I, Pratten J, Nazhat SN, Knowles JC. Characterisation of antibacterial copper releasing degradable phosphate glass fibres. Biomaterials 2005;26:2247–2254.
124. Ahmed I, Collins CA, Lewis MP, Olsen I, Knowles JC. Processing, characterisation and biocompatibility of iron-phosphate glass fibres for tissue engineering. Biomaterials 2004;25:3223–3232.
125. Abou Neel EA, Ahmed I, Blaker JJ, Bismarck A, Boccaccini AR, Lewis MP, Nazhat SN, Knowles JC. Effect of iron on the surface, degradation and ion release properties of phosphate-based glass fibres. Acta Biomater 2005;1:553–563.
126. Valappil SP, Pickup DM, Carroll DL, Hope CK, Pratten J, Newport RJ, Smith ME, Wilson M, Knowles JC. Effect of silver content on the structure and antibacterial activity of silver-doped phosphatebased glasses. Antimicrob Agents Chemother 2007;51: 4453–4461.
127. Jeans LA, Gilchrist T, Healy D. Peripheral nerve repair by means of flexible biodegradable glass fibre wrap: A comparison with microsurgical epineuril repair. J Plast Reconstr Aesthet Surg 2007;60:1302–1308.
128. Shah R, Sinanan ACM, Knowles JC, Hunt NP, Lewis MP. Craniofacial muscle engineering using a 3-dimensional phosphate glass fibre construct. Biomaterials 2005;26:1497–1505.
129. Vitale-Brovarone C, Verne E, Baino F, Ciapetti G, Leonardi E, Baldini N. Bioresorbable phosphate scaffolds for bone regeneration. Key Eng Mater 2008;361:241–244.
130. Vitale-Brovarone C, Baino F, Bretcanu O, Verne E. Foam-like scaffolds for bone tissue engineering based on a novel couple of silicate-phosphate specular glasses: Synthesis and properties. J Mater Sci Mater Med 2009;20:2197–2205.
131. Abou Neel EA, Chrzanowski W, Pickup DM, O'Dell LA, Mordan NJ, Newport RJ, Smith ME, Knowles JC. Structure and properties of strontium-doped phosphate-based glasses. J R Soc Interf 2009;6:435–446.
132. Navarro M, Del Valle S, Marti'nez S, Zeppetelli S, Ambrosio L, Planell JA, Ginebra MP. New macroporous calcium phosphate glass ceramic for guided bone regeneration. Biomaterials 2004; 25:4233–4241.
133. Cai S, Xu GH, Yu XZ, Zhang WJ, Xiao ZY, Yao KD. Fabrication and biological characteristics of b-tricalcium phosphate porous ceramic scaffolds reinforced with calcium phosphate glass. J Mater Sci Mater Med 2009;20:351–358.
134. Branda F, Arcobello-Varlese F, Costantini A, Luciani G. Effect of the substitution of M2O3 (M= La, Y, In, Ga, Al) for CaO on the bioactivity of 2.5 CaO.2SiO_2 glass Biomaterials 2002;23:711–716.
135. Singh K, Bala I, Kumar V. Structural, optical and bioactive properties of calcium borosilicate glasses Ceram Int 2009;35:3401–3406.
136. Singh K, Bahadur D. Characterization of SiO2±Na2O±Fe2O3± CaO±P2O5±B2O3 glass ceramics J Mater Sci Mater Med 1999; 10:481–484.
137. Hoppe A, Guldal NS, Boccaccini AR. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials 2011;32:2757–2774.
138. Wang XP, Li X, Ito A, Sogo Y. Synthesis and characterization of hierarchically macroporous and mesoporous CaO-MO-SiO(2)-P(2)O(5) (M = Mg, Zn, Sr) bioactive glass scaffolds. Acta Biomater 2011;7:3638–3644.
139. Bellantone M, Williams HD, Hench LL. Broad-spectrum bactericidal activity of Ag2O-doped bioactive glass. Antimicrob Agents Chemother 2002;46:1940–1945.
140. Thamaraiselvi TV, Rajeswari S. Biological evaluation of bioceramic materials—A review. Trends Biomater Artif Organs 2004;18:9–17.
141. Saboori A, Sheikhi M, Moztarzadeh F, Rabiee M, Hesaraki S, Tahriri M. Sol–gel preparation, characterisation and in vitro bioactivity of Mg containing bioactive glass. Adv Appl Ceram 2009; 108:155–161.
142. Luderer AA, Borrelli NF, Panzarina JN, Mansfield GR, Hess DM, Brown JL, Barnett EH, Hawn EW. Glass-ceramic-mediated, magnetic-field-induced localized hyperthermia: response of a murine mammary carcinoma. Radiat Res 1983;94:190–198.
143. Vitale-Brovarone C, Verne E, Bosetti M, Appendino P, Cannas M. Microstructural and in vitro characterization of SiO2-Na2O-CaO-MgO glass-ceramic bioactive scaffolds for bone substitutes, J Mater Sci: Mater Med 2005;16:909–917.
144. Fu Q, Saiz E, Tomsia AP. Direct ink writing of highly porous and strong glass scaffolds for load-bearing bone defects repair and regeneration. Acta Biomater 2011;7:3547–3554.
145. Navarro M, Ginebra MP, Clement J, Martinez S, Avila G, Planell JA. Physico-chemical degradation of soluble phosphate glasses stabilized with TiO2 for medical applications. J Am Ceram Soc 2003;86:1345–1352.
146. Navarro M, Clement J, Ginebra MP, Martinez S, Avila G, Planell JA. Improvement of the stability and mechanical properties of resorbable phosphate glasses by the addition of TiO_2. In: Proceedings of the 14th International Symposium on Ceramics in Medicine, Bioceramics 14, November 14–17, Palms Springs; 2002. p 275–278.
147. Johnson WL. Bulk glass-forming metallic alloys: science and technology. MRS Bull 1999;24:42–56.
148. Wang WH, Dong C, Shek CH. Bulk metallic glasses. Mater Sci Eng R Rep 2004;44:45–89.
149. Kawamura Y, Shibata T, Inoue A, Masumoto T. Workability of the supercooled liquid in the Zr65Al10Ni10Cu15 bulk metallic glass. Acta Mater 1997;46:253–263.
150. Hiromoto S, Tsai A P, Sumita M. Effect of chloride ion on the anodic polarization behavior of the Zr65Al7.5Ni10Cu7.5 amorphous alloy in phosphate buffered solution. Corros Sci 2000;42:1651–1660.
151. Hiromoto S, Tsai AP, Sumita M. Effect of pH on the polarization behavior of Zr65Al7.5Ni10Cu17.5 amorphous alloy in a phosphate-buffered solution. Corros Sci 2000;42:2193–2200.
152. Hiromoto S, Tsai AP, Sumita M. Effects of surface finishing and dissolved oxygen on the polarization behavior of Zr65Al7.5Ni10-Cu17.5amorphous alloy in phosphate buffered solution. Corros Sci 2000;42:2167–2185.
153. Hiromoto S, Hanawa T. Re-passivation current of amorphous Zr65Al7.5Ni10Cu17.5 alloy in a Hanks' balanced solution. Electrochim Acta 2002;47:1343–1349.
154. Morrison ML, Buchanan RA, Peker A. Cyclic-anodicpolarization studies of a Zr41.2Ti13.8Ni10Cu12.5Be22.5 bulk metallic glass. Intermetallics 2004;12:1177–1181.
155. Morrison M L, Buchanan R A, Leon RV. The electrochemical evaluation of a Zr-based bulk metallic glass in a phosphate-buffered saline electrolyte. J Biomed Mater Res A 2005;74:430–438.
156. Horton JA, Parsell DE. Biomedical potential of a zirconium-based bulk metallic glass. Mater Res Soc Symp Proc 2003;754:CC1.5.1.
157. Maruyama N, Hiromoto S, Ohnuma M. Fretting fatigue properties of Zr-based bulk amorphous alloy in phosphate buffered saline solution. J Jpn Inst Metals 2005;69:481–487.
158. McGregor DB, Baan RA, Partensky C. Evaluation of the carcinogenic risks to humans associated with surgical implants and other foreign bodies—A report of an IARC Monographs Programme Meeting. Eur J Cancer 2000;36:307–313.
159. Jin KF, Löffler JF. Bulk metallic glass formation in Zr-Cu-Fe-Al alloys. Appl Phys Lett 2005;86:241909.
160. Zberg B, Arata ER, Uggowiter PJ. Tensile properties of glassy MgZnCa wires and reliability analysis using Weibull statistics. Acta Mater 2009;57:3223–3231.
161. Zberg B, Uggowiter PJ, Löffler JF. MgZnCa glasses without clinically observable hydrogen evolution for biodegradable implants. Nat Mater 2009;8:887–891.
162. Liu L, Yu Y, Chan KC. Bio-activation of Ni-free Zr-based bulk metallic glass by surface modification. Intermetallics 2009;18:1978–1982.
163. Chen Q, Liu L, Zhang SM. The potential of Zr-based bulk metallic glasses as biomaterials. Front Mater Sci China 2010;4:34–44.
164. Kanematsu N, Shibata K, Yamagami A, Kotera S, Yoshihara Y. Cytotoxicity of anodized titanium and polycrystalline zirconia in cultured mammalian cells. Jpn J Oral Biol 1985;27:382–384.
165. Yamada K, Nakamura S, Tsuchiya T, Yamashita K. Electroceramics in Japan IV. Key Eng Mater 2002;216:149–152.
166. Day RM, Maquet V, Boccaccini AR, Jerome R, Forbes A. In vitro and in vivo analysis of macroporous biodegradable poly(D,L-lactide-co-glycolide) scaffolds containing bioactive glass, J Biomed Mater Res A 2005;75:778–787.

167. Lai W, Garino J, Ducheyne P. Silicon excretion from bioactive glass implanted in rabbit bone. Biomaterials 2002;23:213–217.
168. Brink M, Turunen T, Happonen R, Yli-Urppo A. Compositional dependence of bioactivity of glasses in the system Na2O-K2O-MgO-CaO-B2O3-P2O5-SiO2. J Biomed Mater Res 1997;37: 114–121.
169. Cunha C, Sprio S, Panseri S, Dapporto M, Marcacci M, Tampieri A. High biocompatibility and improved osteogenic potential of novel Ca-P/titania composite scaffolds designed for regeneration of load-bearing segmental bone-defects. J Biomed Mater Res A. DOI: 10:1002/Jbm,a.34479.
170. Seeley Z, Bandyopadhay A, Bose S. Influence of TiO_2 and Ag_2O addition on tricalcium phosphate ceramics. J Biomed Mater Res A 2007;82:113–121.
171. Novak S, Druce J, Chen QZ, Boccaccini AR. TiO_2 foams with poly-(D,L-lactic acid) (PDLLA) and PDLLA/Bioglass® coatings for bone tissue engineering scaffolds. J Mater Sci 2009;44: 1442–1448.
172. Pazo A, Saiz E, Tomsia AP. Silicate glass coatings on Ti-based implants, Acta Mater 1998;46:2551–2558.
173. Gomez-Vega JM, Saiz E, Tomsia AP, Marshall GW, Marshall SJ. Bioactive glass coatings with hydroxyapatite and Bioglass particles on Ti-based implants. 1. Processing. Biomaterials 2000;21: 105–111.
174. Saiz E, Goldman M, Gomez-Vega JM, Tomsia AP, Marshall GW, Marshall SJ. In vitro behavior of silicate glass coatings on Ti6Al4V. Biomaterials 2002;23:3749–3756.
175. Zones JR, Ehrenfried LM, Hench LL. Optimising bioactive glass scaffolds for bone tissue engineering. Biomaterials 2007;27:964–973.
176. Hollinger JO, Brekke J, Groskin E, Lee D. Role of bone substitutes. Clin Orthop Relat Res 1999;324:55–65.
177. Fukasawa T, Ando M, Ohji T, Kanzaki S. Synthesis of porous ceramics with complex pore structure by freeze drying processing. J Am Ceram Soc 2001;84:230–232.
178. Deville S., Saiz E, Tomsia A, Freeze coating of hydroxyapatite scaffolds for bone tissue engineering, Biomaterials 2006;27: 5480–5489.
179. Regi MV. Ceramics for medical applications. J Chem Soc Dalton Trans 2001;2:97–108.
180. Marcacci M, Kon E, Moukhachev V, Lavroukov A, Kutepov S, Quarto R, Mastrogiacomo M, Cancedda R. Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue Eng 2007;13:947–955.
181. O'Connor TL, Greenberg SA. The kinetics for the solution of silica in autoes solutions. J Phys Chem 1958;62:1195–1198.
182. Hench LL, Clark DE. Physical chemistry of glass surfaces. J Non--Cryst Solids 1978;28:83–105.
183. Regi CV, Vallet-Regi M, Rodriguez Lorenzo LM. Preparation and in vitro of hydroxyapatite/solgel glass biphasic material, Biomaterial 2002;23:1865–1872.
184. Bohner M. Silicon substituted calcium phosphates: A critical review. Biomaterials 2009;30:6403–6406.
185. Wilson J, Pigott GH, Schoen FJ, Hench LL. Toxicology and biocompatibility of bioglasses. J Biomed Mater Res 1981;15:805–817.
186. Murphy S, Wren AW, Towler MR, Boyd D. The effect of ionic dissolution products of Ca-Sr-Na-Zn-Si bioactive glass on in vitro cytocompatibilty. J Mater Sci: Mater Med 2010;21:2827–2834.
187. Weinstein AM, Klawitter, Cook SD. Implant-bone interface characteristics of bioglass dental implants. J Biomed Mater Res 1980;14:23–29.
188. Smith JR. Bone dynamics associated with the controlled loading of bioglass-coated aluminium oxide endosteal implants. Am J Orthod 1979;76:618–636.
189. Stanley H, Hench LL, Going R, Benett C, Chellemi SJ, King C, Ingersoll N, Ethridge E, Kreutziger K. The implantation of natural of natural tooth form bioglasses in baboons. Oral Surg Oral Med Oral Pathol 1976;42:459–469.
190. Murphy S, Boyd D, Moane S, Bennett M. The effect of composition on ion release from Ca–Sr–Na–Zn–Si glass bone grafts. J Mater Sci: Mater Med 2009;20:2028–2035.
191. Keeting P, Oursler MJ, Wiegand KE, Bonde SK, Spelsberg TC, Riggs BL. Zeolite A increases proliferation, differentiation and TGF-beta production in normal adult human osteoblast-like cells in vitro. J Biomed Mater Res 1992;7:1281–1289.
192. Hott M, de Pollak C, Modrowski D, Marie PJ. Short-term effects of organic silicon on trabecular bone in mature ovariectomized rats. Calcif Tissue Int 1993;53:174–179.
193. Aina V, Perardi A, Bergandi L, Malavasi G, Menabue L, Morterra C, Ghigo D. Cytotoxicity of zinc containing bioactive glasses in contact with human osteoblasts. Chem-Biol Interat 2007;167: 207–218.
194. Pal S, He K, Aizenman E. Nitrosative stress and potassium channel-mediated neuronal apoptosis: is zinc the link? Pflugers Arch 2004;448:296–303.
195. Noh KM, Koh JH. Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes. J Neurosci 2000;20:RC111.
196. Ito A, Ojima K, Naito H, Ichinose N, Tateishi T. Preparation, solubility, and cytocompatibility of zinc-releasing calcium phosphate ceramics. J Biomed Mater Res 2000;50:178–183.
197. Wood JPM, Osborne NN. Zinc and energy requirements in induction of oxidative stress to retinal pigmented epithelial cells. Neurochem Res 2003;10:1525–1533.
198. Del Rio MJ, V'elez-Pardo C. Transition metal-induced apoptosis in lymphocytes via hydroxyl radical generation, mitochondria dysfunction, and caspase-3 activation: An in vitro model for neurodegeneration. Arch Med Res 2004;35:185–193.
199. Tang ZL, Wasserloos K, Croix CMS, Pitt BR. Role of zinc in pulmonary endothelial cell response to oxidative stress, Am J Physiol (Lung) 2001;281:243–249.
200. Orive G, Gascon AR, Hernandez RM, Igartua M, Pedraz JL. Cell microencapsulation technology for biomedical purposes: novel insights and challenges, Trends Pharmacol Sci 2003;24:207–210.
201. Hasse C, Zielke A, Klock G, Schlosser A, Barth P, Zimmermann U, Sitter H, Lorenz W, Rothmund M. Amitogenic alginates: Key to first clinical application of microencapsulation technology. World J Surg 1998;22:659–665.
202. Scharp DW Swanson CJ, Olack BJ, Latta PP, Hegre OD, Doherty EJ, Gentile FT, Flavin KS, Ansara MF, Lacy PE. Protection of encapsulated human islets implanted without immunosuppression in patients with type I or type II diabetes and in nondiabetic control subjects. Diabetes 1994;43:1167–1170.
203. Murua A, Portero A, Orive G, Hernandez RM, de Castro M, Pedraz JL. Cell microencapsulation technology: towards clinical application. J Controlled Release 2008;132:76–83.
204. De Vos P, Andersson A, Tam SK, Faas MM, Halle JP. Advances and barriers in mammalian cell encapsulation for treatment of diabetes. Immunol Endocr Metabol Agents Med Chem 2006;6:139–153.
205. Zimmermann U, Mimietz S, Zimmermann H, Hillgärtner M, Schneider H, Ludwig J, Hasse C, Haase A, Rothmund M, Fuhr G. Hydrogel-based non-autologous cell and tissue therapy. Biotechniques 1998;29:564–581.
206. Sakai S, Ono T, Ijima H, Kawakami K. Synthesis and transport characterization of alginate/aminopropyl-silicate/alginate microcapsule: Application to bioartificial pancreas. Biomaterials 2001; 22:2827–2834.
207. Calafior R, Basta G, Luca G, Boselli C, Bufalari A, Bufalari A, Cassarani MP, Giustozzi GM, Brunetti P. Transplantation of minimal volume microcapsules in diabetic high mammalians. Ann NY Acad Sci 1999;875:219–232.
208. Leoni L, Desai TA. Nanoporous biocapsules for the encapsulation of insulinoma cells: Biotransport and biocompatibility. IEEE Trans Biomed Eng 2001;48:1335–1341.
209. Strand BL, Morch YA, Syvertsen KR, Espevik T, Skjak-Braek G. Microcapsules made by enzymatically tailored alginate. J Biomed Mater Res A 2003;64:540–550.
210. Hasse C, Klock G, Schlosser A, Zimmermann U, Rothmund M. Parathyroid allotransplantation without immunosuppression. Lancet 1997;350:1296–1297.
211. Xu W, Liu L, Charles IG. Microencapsulated iNOS-expressing cells cause tumor suppression in mice. FASEB J 2002;16:213–215.
212. Visted T, Bjerkvig R, Enger PO. Cell encapsulation technology as a therapeutic strategy for CNS malignancies. Neurol Oncol 2001; 3:201–210.