Chapter 2
Variation in Properties of Bioactive Glasses After Surface Modification

Vojislav Stanić

Abstract Surface modification is one of the most effective ways to improve properties of biomaterials for specific applications in medicine, dentistry, pharmacology, and biotechnology. The surface properties of biomaterials play a significant role in the interaction with the surrounding tissues. This chapter is mainly focused on bioactive silicate glasses, in the following three aspects: (1) ion doping glass, (2) covalent modification of a bioactive glass's surfaces by silanes, and (3) biological surface functionalization of bioactive glass. The incorporation of various ions in the structure of bioactive glasses can improve their bioactivity, stimulating effects on osteogenesis, angiogenesis, and antibacterial activity. The goal of covalent modification by silanes is to improve the interaction with the surrounding bone tissue, to enhance dispersion stability of inorganic particles in various liquids, or to act as anchors for the immobilization of drugs. Biological functionalization of bioactive glasses can improve their bone integration.

Keywords Bioactive glasses • Biomaterials • Surface modification • Silinization • Bone

2.1 Introduction

Bioactive glasses have been widely investigated as biomaterials in medicine and stomatology for hard tissue substitution. Hench and co-workers first made bioactive silicate glasses by melt quenching of chemical composition: 45% SiO_2, 24.5% CaO, 24.5% Na_2O, and 6% P_2O_5 in weight percent, denoted Bioglass® 45S5 [1]. Bioactive materials are surface active and form a stable bond with round hard and soft tissues: muscle and tendons (Class A) or to hard tissues only (Class B) [2, 3]. Class A biomaterials such as bioactive glasses showed rapid bonding to the bone and enhanced bone proliferation. Most calcium-phosphate biomaterials such as synthetic hydroxyapatite are an example of a Class B material; the bonding rate to the bone is slow

V. Stanić (✉)
University of Belgrade, Vinca Institute of Nuclear Sciences,
P.O. Box 522, 11001 Belgrade, Serbia
e-mail: voyo@vin.bg.ac.rs

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with no enhancement of bone proliferation. Surfaces of bioactive glasses represent the site of interaction with the surrounding living tissues and are therefore crucial to enhance their biological performance. The bioactivity of a glass is usually evaluated by its ability to form a hydroxycarbonate apatite (HCA) layer on its surface upon immersion in SBF. HCA is very similar to the mineral component of the bone; its presence on the glass surface promotes further attachment of biomolecules, cells, and tissue growth factors, which then favor the development of bonds with surrounding tissues and the creation of new tissue. The bioactivity of glasses depends on its network structure, chemical composition, particle size, surface area, and textural properties (pore size, pore volume, pore structure) and various organic compounds present in their composites. The surface modification of bioactive glasses should be viewed in several aspects: improving bioactivity; binding of biomolecules; binding, proliferation, and differentiation of cells; delivery of drugs, cytotoxicity; antimicrobial properties, and diagnosis, monitoring, and control of disease.

The mechanism of bonding silicate bioactive glasses to the bone has been attributed to the formation of a carbonate-substituted hydroxy apatite (HCA)-like layer on the glass surface in contact with the body fluid. The mechanism of HCA layer formation on bioactive glasses has been widely studied in vitro [4–6]. This process is complex and can be simplified to be shown through sequence of various stages (Table 2.1).

Some of these stages are played out partly in parallel, such as 6 and 7 with stages 3–5 [7]. The initial stages (1 and 2) involve the partial dissolution of the bioactive glass after contact with the body fluid or simulated body fluids (SBF), with substitutions of Na + and Ca2+ with H+ ions and the pH increase of solution (Eq. 2.1) [4, 7].

$$\mathrm{Si-ONa + H^{+}_{(aq)} \rightarrow Si-OH + Na^{+}_{(aq)}} \tag{2.1}$$

Table 2.1 Stages of interfacial reactions between bioactive glass and surrounding bone tissue

LogT (hour)	Surface reaction stages	
	1 and 2	Exchange of Na^+ ions with H^+ ions leads to formation of silanol groups (Si–OH)
		Network dissolution: formation of Si–OH groups and release of $Si(OH)_4$
	3	Polycondensation of silanol groups
1	4	Formation of amorphous $CaO–P_2O_5$
2	5	Crystallization of hydroxycarbonate apatite (HCA)
10	6	Adsorption of biological moieties in HCA layer
	7	Action of macrophages
20	8	Attachment of osteoblast stem cells
	9	Differentiation and proliferation of osteoblasts
	10	Generation of matrix
	11	Crystallization of matrix and growth of the bone
100	12	Proliferation of the bone

As a result, this leads to network degradation by breaking the Si–O–Si bonds, formation of Si–OH groups, and release of $Si(OH)_4$ and larger silicate fragments (stage 2, Eq. 2.2).

$$Si-O-Si+H_2O \rightarrow Si-OH+Si-OH \tag{2.2}$$

The continuous formation of silanol groups results (three stages) in their polycondensation and formation of a porous silica-rich layer. Fourth, creating an amorphous $CaO–P_2O_5$-rich film with a low Ca/P atomic ratio on the surface of the silica-rich layer results from the liberated Ca^{2+} and PO_4^{3-} ions from the glass and from a solution. The silica-rich layer has high density of surface silanol (Si–OH) groups, which are essential as nucleation centers for the precipitation of calcium phosphate. The formation of an amorphous calcium silicate and an amorphous calcium phosphate is the result of electrostatic interactions between the Si–OH groups on the glass surface and the calcium and phosphate ions in a solution [4]. The bioactivity of Na-free and P-free silicate glasses comes from the hydrated silica-rich layer [6, 8, 9]. Fifth, the amorphous calcium phosphate further increased its Ca/P atomic ratio, incorporating OH− and CO_3^{2-} anions from the solution and crystallized into hydroxycarbonate apatite (HCA). The crystallization of amorphous calcium phosphate into crystalline HCA can be explained by the increase in its stability. Apatite minerals are the most thermodynamically stable and have a lower solubility in water than any other calcium phosphates [10, 11]. In parallel with chemical reactions in the HCA layer, cellular stages 6–12 occur, such as action of macrophages, adsorption, and desorption of proteins, growth factors, and collagen which triggers proliferation and differentiation of cells and the creation of osteoblasts, thus encouraging bone growth on the surface glass [12]. Osteoblast cells create an extracellular matrix, which mineralizes to form a nanocrystalline mineral and collagen on the surface of the glass implant, while the degradation and conversion of the glass continue over time [12, 13]. These stages are very complex and not fully clarified.

2.2 Glass Structure

Bioactive glasses (BG) are built from glass formers, network modifiers, and intermediate oxides. The primary glass formers (network formers) in bioactive glasses are silica (SiO_2), boric acid (B_2O_3), and phosphoric oxide (P_2O_5), which can form single-component glasses. The generic name of glass is generally derived from its network former. Bioactive silicate glasses are amorphous solids. The basic building unit of silicate glasses is the SiO_4 tetrahedron in a network, which is interconnected in a network through Si–O–Si bonds, commonly referred to as bridging oxygen atoms [14]. These tetrahedra are commonly referred to as Q^n units, where "n" represents the number of bridging oxygens per tetrahedron (Fig. 2.1).

The network modifiers (Na^+, K^+, Ca^{2+}, etc.) provoke, during the synthesis, the disruption of the continuity of the glassy network, due to the breaking of some of

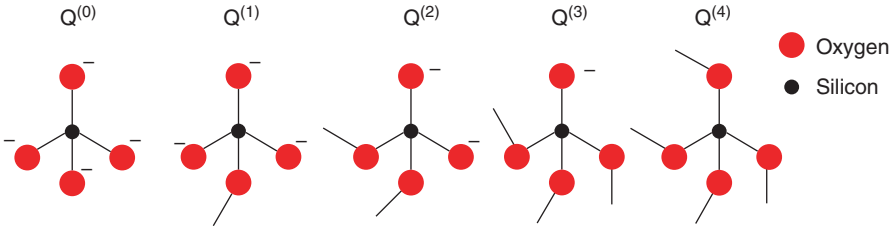

Fig. 2.1 Silica tetrahedral sites of silicate glasses

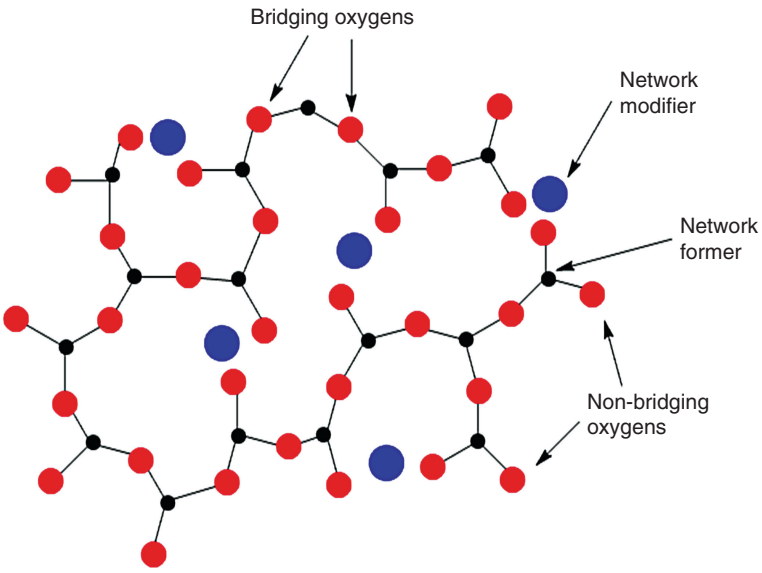

Fig. 2.2 2D presentation of random glass network modifiers and network formers

the Si–O–Si bonds leading to the formation of non-bridging oxygen groups (Fig. 2.2). The properties of bioactive silicate glasses are, to a large extent, influenced by a portion of non-bridging oxygen atoms. Network modifiers are often necessary to modify the properties of the glass. The intermediate oxides (ZnO and MgO) can act as typical network formers and modifiers [14].

Borate Bioactive Glasses

The major glass former in bioactive borate glasses is B_2O_3 and possesses a more complex structure due to a greater number of building blocks [15]. Some structural elements of borate glasses are shown in Fig. 2.3 [15, 16]. Borate glass structure can be built of trigonal planar BO_3 and/or tetrahedral BO_4 units. Adding metal oxides to borate glass comes to the crossing of planar into tetrahedral units, resulting in a higher degree of network connectivity. Non-bridging oxygen atoms are formed when the content of metal-doped ions is high.

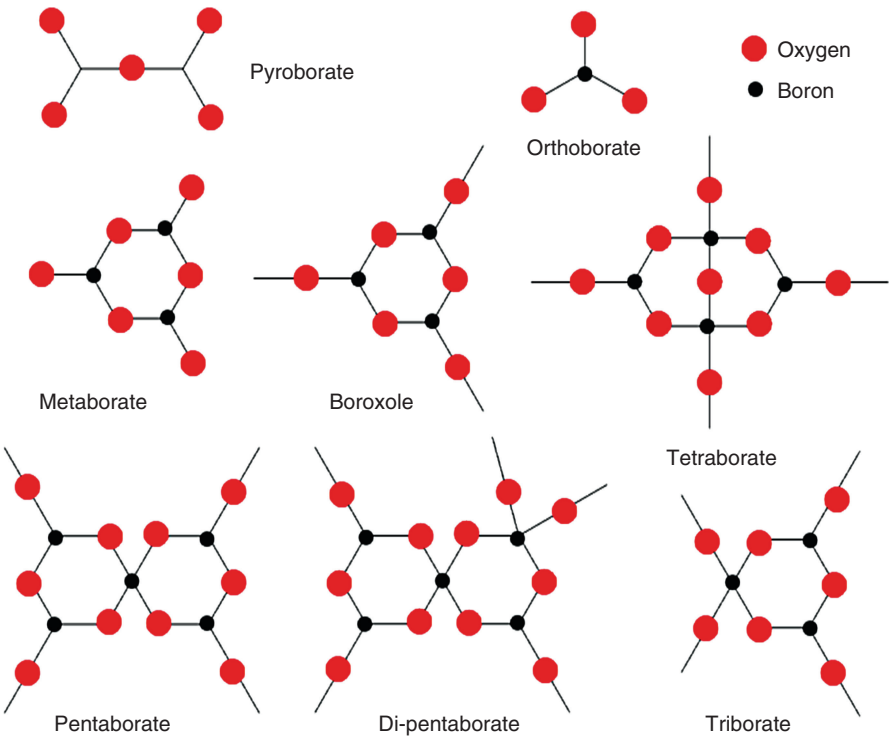

Fig. 2.3 The network units of borate glasses

Borate glasses are very reactive and have lower chemical durability; hence, they can convert faster and more completely to hydroxyapatite (HAp) in an aqueous phosphate solution, when compared to silica counterparts. Huang et al. [17] studied the formation of HAp, in a dilute phosphate solution, during the conversion of partial and full replacement of SiO_2 content in 45S5 glass with B_2O_3. The glasses with higher B_2O_3 content produced a more rapid conversion to HAp and a lower pH value of the phosphate solution. The borate glass was fully converted to HAp in less than 4 days, while silicate and borosilicate compositions were partially converted after 70 days and contained residual SiO_2 in a Na-depleted core. The borate glasses, unlike silicate glasses, form HCA directly on the surface without forming a borate-rich layer. For the borosilicate glasses, a conversion mechanism is similar to that of silicate 45S5 glass. A similar study was performed subsequently by Fu et al. [18] for 13–93 bioactive glass. This study showed the conversion rate of the scaffolds to HAp in the SBF increased with the B_2O_3 content of the glass. In vitro studies showed that on the surface of some borate glasses comes to attachment, proliferation, and differentiation of cells, while in vivo they are reported to enhance tissue infiltration [19–24]. Brown et al. [25] reported that glasses with higher B_2O_3 content showed an increase conversion rate to HAp, but also resulted in a greater inhibition of cell proliferation under static culture conditions. Boron compounds such as borax and

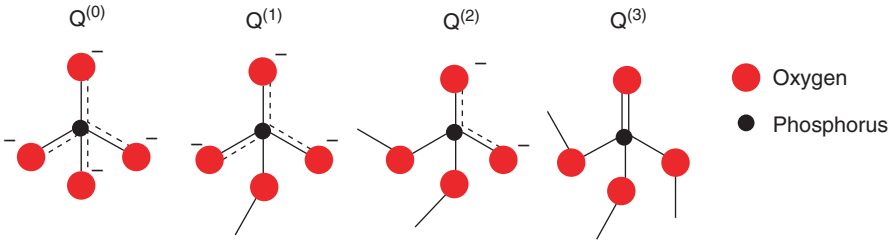

Fig. 2.4 Phosphate tetrahedral sites that can exist in phosphate glasses

boric acid in high concentrations are toxic [26]. The borosilicate scaffolds containing 12.5 wt% B_2O_3 showed cytocompatibility to a stromal cell line (ST2) [27].

Phosphate Bioactive Glasses

Phosphate glasses consist of P_2O_5 as the network former oxide and CaO and Na_2O as modifiers. The chemical composition of phosphate glasses is similar to the inorganic phase of the bone. These glasses have great potential as biomaterials because they are completely biodegradable and nontoxic [28]. Compared to silicate glasses, phosphate glasses have relatively poor chemical durability which durability limits their use in tissue engineering [29]. The solubility of phosphate glasses decreases with the increase of CaO content [30]. The basic building blocks of glasses are the P-tetrahedra, similar to those in silicate glasses [31]. The tetrahedra in the glass structure are interconnected through covalent bridging to form various phosphate anions (Fig. 2.4).

2.3 Ion-Doped Bioactive Silicate Glasses

In recent years, bioactive glasses are modified with a variety of trace elements such as Cu, Zn, Sr, and others. Many of these ions are essential or nonessential. Many nonessential metal ions are used for therapeutic purposes or are subject to various biological examination. These ions in bioactive glasses can cause changes in the crystal structure, specific surface, thermal stability, morphology, solubility, and chemical and biological properties. These trace elements have been found to play absolutely vital roles in the formation, growth, and repair of the bone.

Various studies have also demonstrated that the addition of trace elements to bioactive glasses materials can lead to controlled degradation and increase in mechanical strength of the materials and positively influence the biological response. Incorporation of various metallic dopants into the structure of bioactive glass (BG) can be made predominantly by methods of direct synthesis and sorption of ions from the solution. Doping of metal ions into the structure of BG by direct synthesis provides a greater amount and a more uniform distribution of ions over the entire volume.

The most common methods for the production of bioactive glass materials are melt-quenching routes and the sol-gel technique. The schematic illustration of melt-quenching and of sol-gel processes is shown in Fig. 2.5.

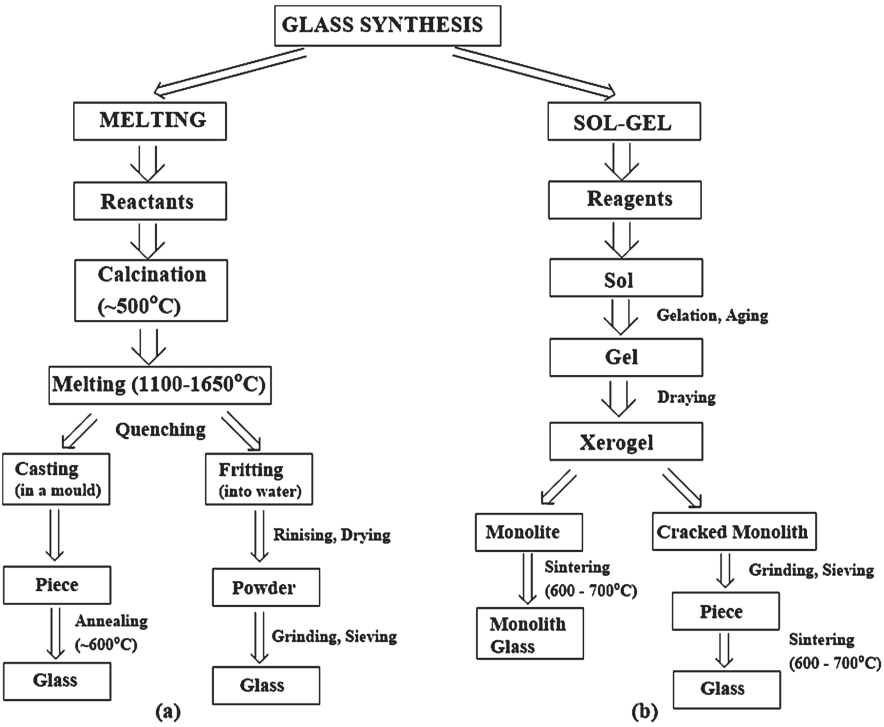

Fig. 2.5 Schematic view of the preparation route for (**a**) melt quench and (**b**) sol-gel bioactive glasses

The melt-quenching method is based on melting a heterogeneous reactant mixture in a specified molar ratio. The reaction mixture calcined at about 500 °C to remove moisture, which is adsorbed onto the precursor materials or may be formed by dehydration of hydroxides [15, 32]. Additionally, there comes to the release of gases caused by decomposition of the possibly present precursors: carbonate, nitrate, and sulfate. Oxides are mainly used as precursors. The melting temperature (1,100–1,650 °C) is above the glass transition temperature (Tg) of the target bioactive glasses, to afford a viscous state. The Tg of the BG is lower than its crystallization temperature (Tc) at which that leads to the formation of glass-ceramics. The glasses are often melted twice in order to increase homogeneity. In order to prevent evaporation of individual components: alkalis, boron, phosphorus, and fluorides, melting is carried out in covered crucibles [2]. The molten glass is then cast into a preheated graphite or steel molds to make bulk implants or is immersed in water which is used for quenching. The sol-gel method is most applied in the synthesis of bioactive glasses. This method may prepare materials in various forms: nanoparticles, thin film coatings, microporous, monoliths, and aerogel materials. Sol-gel process involves the transition of the colloidal solution (sol) into a solid phase (gel). Gel can be described as a three-dimensional solid skeleton surrounded by a liquid phase,

where both phases are continuous and nanometer dimensions. The gelation process is achieved by reactions of hydrolysis (Eq. 2.3) and condensation (Eqs. 2.4 and 2.5).

$$M(OR)n + xH_2O = M(OR)n - x(OH)x + xROH \tag{2.3}$$

$$-M-OH + HO-M- = -M-O-M- + H_2O \tag{2.4}$$

$$-M-OH + R-O-M- = -M-O-M- + R-OH \tag{2.5}$$

These reactions can occur very slowly at ambient temperatures so that they are added to acidic or basic catalysts for their acceleration. The gelation phase is followed by a drying process, in which the solvent is removed from the gel and forms a solid, porous matrix called xerogel. The resulting xerogel is heat-treated in order to obtain the final product. Annealing process frequently leads to agglomeration and coarsening of nanoparticles. The properties of the obtained material are affected by many factors that influence the rate of hydrolysis and condensation, such as pH value, temperature, reaction time, concentration of reagents, the type and concentration of the catalyst, temperature, and time of aging and drying. The advantages of the sol-gel method are the low-temperature processing, the purity and homogeneous distribution of the components, higher porosity and specific surface area values, and the possibility of particle size control [33]. Increasing the specific surface area and pore volume of bioactive glasses greatly accelerates its dissolution and HCA formation on the surface and therefore enhances the bioactive behavior. The porosity of ∼90% and pore size of >100 μm are desirable, as well as high pore interconnectivity being important for the formation of bone tissue, enabling the migration and proliferation of osteoblasts and mesenchymal cells, vascularization, and nutrient delivery to the newly formed tissue [34, 35]. In addition, the porous surface promotes mechanical coupling between the implanted biomaterial and the surrounding natural bone, providing greater mechanical stability in critical areas. The comparative studies of gel-derived and melt-quenched glasses showed that the synthesis technique causes differences in the texture and the glass structure [36, 37]. The sol-gel-derived glasses showed more polymerized structure and higher porosity and specific surface area values, enhancing the solubility. The rate of HCA formation is higher for the sol-gel-prepared glasses, and they exhibit bioactivity with a content of higher than 90% of SiO_2 [38]. Bioactivity at melt-derived glasses is present with a content of up to 60% of SiO_2.

2.3.1 Flourine-Doped Bioactive Glass

Fluoride ions are not natural constituents of bones, but in vivo it is mainly associated with calcified tissue, the bone, and teeth, replacing the hydroxyl groups in hydroxyapatite phase producing its partial conversion into fluorapatite [39, 40]. Compared to pure hydroxyapatite, fluorapatite has a much higher physic-chemical stability, such as an increased resistance to dissolution by acid [41]. Dental caries is one of the most

widespread bone diseases. Acidogenic bacteria are the main cause of dental caries, where the fermentation of sugars and starches in food accumulated on the surface of teeth can lead to the formation of organic acids, which then cause demineralization, and can lead to complete destruction of teeth [42]. The ability of fluorine ions to stabilize the apatitic structure against demineralization by acid is a useful way in preventing tooth degradation. Fluoride-doped biomaterials in an acidic environment, upon dissolution, lead to the release of fluorine ions, which act as an antimicrobial agent [43, 44]. Liu et al. [45] reported that the F-doped BG significantly inhibited the growth of periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis*, the antibacterial activity being dependent on the F^- content of the BG. Low concentrations of fluoride ions are not toxic to humans, but high concentrations are toxic and can lead to enamel fluorosis [46]. The fluoride glasses have good biological compatibility because they do not cause a hemolysis reaction and have no toxicity to cells and living animals [47]. Liu et al. reported that alkaline phosphatase activity, cell number, collagen formation, bone-like mineral nodules, and osteogenic gene expression of MC3T3-E1 cells were significantly promoted in low fluoride – BG-conditioned medium [45]. Currently, fluoride is one of the most common anticaries agents present as primary components in toothpaste and mouthwash [47, 48].

Fluoride ions in a bioactive glass increase the polymerization of the silicate network binding for modifiers (CaO and Na_2O) from the siliceous matrix and reduce its reactivity and bioactivity [49, 50]. The high fluoride-content glasses in melt-derived glass SiO_2–P_2O_5–CaO–Na_2O mainly form calcium fluorite (CaF_2) in SBF, while the formation of apatite is reduced compared to the fluoride-free composition [51]. With the increase in P_2O_5 content in fluoride-containing glasses comes the increase in glass degradation and ion release and favors the formation of fluorapatite (FAP) rather than CaF_2. FAP formation occurred more rapidly (within 6 h) with increased phosphate content in the glass, compared to 3 days for low phosphate-content glasses.

2.3.2 Magnesium-Doped Bioactive Glass

Magnesium is an essential element that is needed for a broad variety of physiological functions.

It is a cofactor for many enzymes, stabilizes the structures of DNA and RNA, and is important for the metabolism of Ca, K, P, Zn, Cu Fe, Na, Pb, and Cd [52–55]. Magnesium ions have a significant role in bone formation, enhance osteoblast cell activity, and inhibit osteoclasts [56]. Several investigations showed that the effect of long-term magnesium-deficient diets cause osteopenia and the inhibition of growth of the bone [54, 57, 58]. The effect of magnesium ions on the structure of a bioactive glass is questionable; they can act as modifiers [59] or as an intermediate oxide, partially entering the silicate network as MgO_4^{2-} tetrahedral units [60, 61]. Zhao et al. [60] have illustrated when MgO content in the bioactive glass surpasses 10 mol%; then a part of Mg ions enter the silicate structure as a network former. Watts et al. [61] suggested that magnesium oxide acts more as an intermediate oxide than as a

modifier, with a proportion of 86% of MgO acting as a network-modifying cation while up to 14% entering the silicate network as tetrahedral MgO_4 species. The presence of magnesium in the glasses increases the surface area and porosity [62, 63]. In contrast, Ma et al. [64] reported that the presence of MgO (0–20 mol%) in glass composition (SiO_2–CaO–P_2O_5–MgO) has little influence on its textural properties.

Some in vitro results indicate that magnesium ions in bioactive glasses delay apatite formation [62, 64–66], while others suggest that it does not effect on mineralization [24]. Ma et al. investigated the effects of the substitution of MgO (0–20 mol%) for CaO on sol-gel-derived glass degradation and bioactivity. The studies of in vitro showed that the rate of glass degradation gradually decreases with the increase of MgO, and the formation of an apatite layer on glass surface is retarded. The retardation in the formation of the layer on the surface of glass could be attributed with the decrease of the solubility of the glass and influence of the Mg^{2+} leached to the solution. Leached Mg^{2+} ions from a glass into the solution are considered as an inhibitor of calcium-phosphate crystallization and are marked as a delay to the transformation of amorphous calcium phosphates to more stable apatite phases [65, 66]. Moya et al. [67] reported that a glass of nominal composition (wt%) 54.5 SiO_2, 12.0 Na_2O, 4.0 K_2O, 15.0 CaO, 8.5 MgO, and 6.0 P_2O_5 found that the role of Mg^{2+} in the formation of Ca–P-rich layer was insignificant.

Numerous in vitro studies showed that Mg-doped bioactive glasses have better results in terms of cell adhesion, proliferation, and differentiation of osteoblasts cells than controlled samples [68–71]. Bioactive glass containing 5 mol% of MgO (SiO_2–CaO–P_2O_5–MgO) has been shown to enhance differentiation of human fetal osteoblastic cells (hFOB 1.19) [68]. This bioactive glass did not induce any signs of toxicity after 48 h with L929 mouse fibroblast cells. Bioactive glass scaffolds (SiO_2–CaO–P_2O_5) doped with MgO at different concentrations of up to 2.25 mol.% were demonstrated a higher proliferation and ALP activity of mesenchymal stem cells (MSCs) than controls: scaffold without doping and hydroxyapatite after 14 days of culture [69]. The MSCs on the scaffolds with 2.25 mol.% Mg show the highest MSC proliferation and ALP activity among those of the Mg-doped scaffolds. Balamurugan et al. [70] reported that SiO_2–CaO–MgO–P_2O_5 bioactive glass with 13 mol% MgO has the ability to support the growth of human osteoblast-like cells (MG63) and to promote osteoblast differentiation by stimulating the expression of alkaline phosphatase activity. Bioactive glasses SiO_2–CaO–P_2O_5–MgO doped with MgO (0, 10, and 20 mol%) did not show cytotoxicity to human gastric adenocarcinoma cells and antibacterial activity [71]. The presence of MgO in the glass composition increases the formation of the apatite layer, whereas when compared with base glass, the formation of HAp layer decreases when the concentration of MgO increases above 10%. The bioactive glass with 10% MgO had the highest specific surface area and solubility.

There are a few investigations focusing on the in vivo behavior of Mg-containing bioactive glasses [72–74]. Bioactive glass based on the SiO_2–P_2O_5–CaO–Na_2O–K_2O–Al_2O_3 system with the addition of 1–3 mol% of MgO has been prepared by melt technique as implants are embedded in the muscle and bone of white rabbits [72]. This bioactive glass elicits a favorable response both in the muscle and bone; a gradual degradation process leads to disruption and partial resorption of the

material, and a tight apposition is promoted with the newly formed bone. Implants did not produce any adverse inflammatory response in the muscle at any time.

Tamura et al. [73] reported that histological examination of rat tibiae showed that two types of bioactive bone cement containing either $MgO–CaO–SiO_2–P_2O_5–CaF_2$ (4.6 mol% MgO) or glass-ceramic powder, incorporated in bone defects, formed direct contact with the bone.

A bioactive study of 26 glasses in system $Na_2O–K_2O–MgO–CaO–B_2O_3–P_2O_5–SiO_2$ in vivo showed that glasses that contained 4–30 mol% alkali oxides, 14–30 mol% alkaline earth oxides, and <59 mol% SiO_2 can create links with bone tissue [74]. Glasses which contain potassium and magnesium (0–7.8 mol% MgO) bind to the bone in a similar way as other glasses that bind to bone.

2.3.3 Strontium-Doped Bioactive Glass

Strontium (Sr) is an important trace element in the human body and has a significant impact on bone metabolism. Its compounds strontium ranelate and strontium chloride are currently used to treat osteoporosis [75–78]. In vitro and in vivo studies showed that a low dose of strontium ions promotes bone formation and osteoblast replication while inhibiting bone resorption by osteoclasts. In contrast, high doses of strontium may induce skeletal abnormalities [76]. Sr ions exhibit cariostatic properties depending on their concentration, predominantly in the presence of fluoride [79]. Liu et al. reported that Sr-doped BG showed antimicrobial activity against subgingival bacteria, *A. actinomycetemcomitans* and *P. gingivalis* and that it depends on the amount on the percentage of strontium in the glasses [46]. Incorporation of strontium ions in a bioactive glass may be an effective way to deliver a steady supply of strontium ions to a bone defect site and in this way speed up the recovery of the patient.

The effect of strontium ions on the structure of several bioactive glasses has been reported. Substitution of strontium for calcium does not lead to significant structural changes, but there is a small expansion of the glass network. The density of the glasses increased with strontium substitution, while the oxygen density decreased [80, 81]. Expansion of the glass network was associated with the characteristics of metal ions. Strontium has a higher ionic radius and lower ionic field strength (r = 0.127 nm; I = 0.24) compared to the calcium ion (r = 0.106 nm; I = 0.35). Calcium and strontium ions were found to preferentially distribute in glass around phosphorus ions [46, 81, 82]. Glasses with a high content of silica showed a slight decrease in solubility, building Sr-substituted apatite layers, and bioactivity with increasing concentrations of strontium ions [80, 83–85]. The bioactive glasses with a higher content of phosphates exhibit greater solubility and bioactivity with increasing strontium content [83, 86].

Several studies have reported the enhancing effects of strontium-doped BG on osteogenesis in vitro using different cell sources, demonstrating their potential for bone tissue regeneration.

Strontium-doped BG promotes osteoblast proliferation and activity and decreases osteoclast activity and resorption [87, 88]. The concentration of Sr is a critical parameter for its increasing effect on cell proliferation. Bioactive glass containing little amounts of SrO (<5 mol%) has higher proliferation and alkaline phosphatase activity of the rat osteoblastic cells than samples without Sr and with its high dose [84]. Zhang et al. [89] showed that 5 mol% Sr significantly increased the proliferation and osteogenic differentiation of bone marrow stromal cells in a concentration-dependent manner. Sr-doped BG 64S with 5% Sr accelerates the differentiation of mesenchymal stem cells but not proliferation [90].

The available studies in vivo show that strontium-doped BG scaffolds can successfully regenerate bone defects [88, 89, 91–94]. Gorustovich et al. [91] reported that new lamellar bone had formed along the surface of both 45S5 and 45S5.6Sr BG particles within 4 weeks. Studies that were performed by Zhang et al. [89] have shown that the incorporation of Sr into mesoporous bioactive glass (MBG) scaffolds significantly stimulated new bone formation in osteoporotic bone defects when compared to MBG scaffolds alone. Recently, Zhao et al. [92] reported that Sr–MBG scaffolds had good osteogenic capability and stimulated new blood vessel formation in critical-sized rat calvarial defects within 8 weeks. Zhang et al. [88] have done a study on the immune response affected by Sr–BG. The results showed that Sr−BG in vivo initiated a less severe immune response and had an improved effect on bone regeneration than BG, which corresponded with the in vitro evaluation.

2.3.4 Silver-Doped Bioactive Glass

Orthopedic implant infections are significant because of their morbidity and usually require the removal or replacement of installed materials [95]. Incorporation of antimicrobial agents such as antibiotics, fluorine, and biocide metal ions in the implant biomaterial alone proved to be very successful in the prophylaxis [96]. Silver ions have expressed an oligodynamic effect with a minimal development of microorganism's resistance [97–99]. Bioactive glasses doped with small amounts of silver ions showed a broad spectrum of antimicrobial activity [100, 101]. Low concentrations of silver ions in BG are not toxic, but high concentrations can cause cytotoxicity. The Ag-doped borate bioactive glasses containing 0.75 and 1 wt% Ag were not toxic to the mouse MC3T3 osteoblasts and L929 fibroblast cells, whereas the glass containing 2 wt% Ag was toxic [101, 102]. Phetnin and Rattanachan reported that Ag-doped silicium glasses exhibited anticancer properties against human liver cancer HepG2 cells [103]. Silver-containing bioactive glasses are mostly obtained by a sol-gel technique because of the homogenous product. The melt-quenching technique is not suitable for the synthesis of Ag-doped BG, because homogeneity and reproducible of the product cannot be provided [104]. Modification of the surface of the bioactive glass with silver using techniques of ion exchange may be done in two different ways: in molten salts and in an aqueous solution [105]. The amount of Ag within the glass was reported to be very low (up to 0.66 wt.% Ag/glass), but its concentration

within the glass surface layer was high. Addition of silver ions into the BG structure induced lower bioactivity as a result of lower solubility and surface area [106]. The release of silver ions from glasses in SBF is slow compared with the dissolution of other constituents. There are several important factors which limit the dissolution of AgBG and release of silver ions. Replacing calcium with silver ions in the bioactive glass structure increases glass network connectivity as a result of reducing the number of non-bridging oxygen groups, which are essential for the solubility [107].

The formation of the HCA or HCA/AgCl layer on the glass surface can be limited or even stop the dissolution and release of silver ions. Released Ag ions in the AgBG surface layer can interact with phosphate and chloride ions (SBF), building a silver phosphate compound and difficult soluble AgCl (Ksp = 1.8 × 10–10 at 25 °C) [105]. Apatite materials can incorporate silver ions into the structure during its formation, or they can be absorbed from the solution. Silver ions may have a strong stimulatory effect on the formation of carbonate apatite [99]. The increase in the amount of silver (3%) in a BG leads to the formation of secondary phases: quartz and metallic silver, which reduce the BG transformation into HCA [107]. The textural characteristics of AgBG also play an important role; a higher surface area is favorable for obtaining a higher dissolution rate of glasses and therefore a higher bioactivity. Some studies have reported that with the increase silver content in a BG, there occurs a progressive decrease of the surface area and pore volume and the progressive broadening of the pores distribution [108, 109].

2.3.5 Copper-Doped Bioactive Glass

Copper is an essential trace metal found in all living organisms and is necessary for a lot of biological processes. It is an angiogenic agent because it increases the expression of pro-angiogenic and growth factors (VEGF, bFGF, TNF-α, and IL-1β), enhances the in vivo angiogenesis, and stimulates the human endothelial cell proliferation [110–112]. Insufficient amounts of copper in a diet can cause a reduction of bone mineral density [113]. Copper ions in vitro diminished the proliferation rate of mesenchymal stem cells but increase their ability to differentiate into osteogenic lineage [114]. Previous studies suggested that Cu^{2+} ions could enhance cell activity and proliferation of osteoblastic cells and inhibit osteoclast activity [115, 116]. Copper and its compounds are highly significant as antimicrobial agents in the prevention of postoperative infections [117, 118]. Incorporation of Cu into BG may offer an alternative route for sustained delivery of Cu ions. The in vitro bioactivity of Cu-doped glasses was dependent on the concentration of Cu^{2+} ion incorporated which decreased the formation of apatite at higher concentrations [119, 120]. Cu^{2+} ions acted as network modifiers and disrupted the silicate network of BG [121]. Its effect on the network is inferior to Mg^{2+} and Zn^{2+} ions (Cu < Mg < Zn) [124]. The effect of Cu on the textural properties and microstructure of the doped glass matrices depended on their compositions. Bejarano et al. [120] reported that the incorporation of CuO increased the surface area and pore volume of 58S BG

($60SiO_2$–$36CaO$–$4P_2O_5$), whereas an opposite effect was observed in NaBG ($60SiO_2$–$25CaO$–$11Na_2O$–$4P_2O_5$).

In vitro and in vivo studies reported that Cu–BG scaffolds release Cu^{2+} ion and stimulate processes such as angiogenesis as well as osteogenesis. Li et al. [122] reported that the composite of Cu–BG nanocoatings on a natural eggshell membrane can stimulate angiogenesis and neoepidermis formation during wound healing process. The composite containing 5 mol% Cu stimulated proangiogenesis by improving the vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1a protein secretion. In a previous study, Wu et al. [123] also found that Cu–BG scaffolds (1, 2, and 5% Cu) significantly enhance hypoxia-like tissue reaction leading to the coupling of angiogenesis and osteogenesis. Furthermore, Cu–MBG scaffolds showed a sustained release of ibuprofen. Studies by Lin et al. [124] have demonstrated that Cu–BG (13–93) scaffolds with 0.4 and 0.8 wt.% CuO did not have a significant effect on the response of pre-osteoblastic MC3T3–E1 cells in vitro and on angiogenesis and osteogenesis in rat calvarial defects at 6 weeks post-implantation. The scaffold with the highest dopant concentration of 2.0 wt.% CuO significantly enhanced angiogenesis in the fibrous tissue that infiltrated the scaffolds and significantly reduced osteogenesis as a result of cytotoxic effects of high concentrations of copper.

Copper-doped BG materials showed antibacterial activity in suppressing some bacterial pathogens involved in postsurgical infections, such as *S. aureus*, *S. mutans*, *E. coli,* and *P. aeruginosa* [123, 125–128].

2.3.6 Zinc-Doped Bioactive Glass

Zinc is an essential trace element to the structure of biomolecules and function of metabolism. It plays a physiologically important role in bone metabolism, formation, and resorption [129, 130]. Zinc deficiency results in a retardation of bone growth, indicating that the element is required for the growth, development, and maintenance of healthy bone [131]. Excess zinc may have adverse and serious effects on health such as reduced bone formation, anemia, hypertension at rats, as well as systemic cytotoxicity [132–135].

The possibility of incorporating Zn^{2+} ions in bioactive glasses has received special interest lately, and several formulations of bioactive glasses doped with ZnO have been recently obtained, both by melting and sol-gel techniques [136–141]. ZnO in the structure of bioactive glass might act as divalent network modifier and/or network former depending on the composition and its content. Several studies based on experimental and computational approaches have shown that Zn^{2+} ions in BG adopt a tetrahedral coordination (ZnO_4^{2-}) and so act as a weak tetrahedral network former and participate in the copolymerization with the Si tetrahedra units [136, 137]. Zinc ions in the presence of sufficient amounts of alkali ions act as a network former. Conversely, if there are insufficient alkaline ions, the zinc ion will be a network modifier [138]. Haimi et al. [139] reported that ZnO in BG (Na_2O,

K_2O, MgO, CaO, B_2O_3, TiO_2, ZnO, P_2O_5, and SiO_2) acts both as network former and network modifier [142]. The addition of Zn^{2+} ions to silicate and phosphosilicate glasses enhances its chemical durability and improves the thermal and mechanical strength of BG [138, 140]. The textural properties such as the surface area, pore volume, and pore size diameter of the scaffolds progressively decreased with the increasing concentration of Zn^{2+} ions in BG [136, 141]. These changes can be associated with the structural role played by Zn^{2+} species in the glass network. Zinc has been found to have a great influence on the growth kinetics of HCA in SBF [140, 142–145]. The increasing Zn content in BG leads to a decrease in the solubility of glasses. Srivastava et al. [140] reported that there is no effect on the formation of HCA layer by addition of 1% of ZnO by weight in 45S5 bioactive glass, but increasing the ZnO content more than 1% decreases the formation of HCA layer. Zinc ions potently inhibit the growth of hydroxyapatite crystals [118, 146]. It has been recognized that ZnO retards the crystal nucleation of HCA during the initial periods of in vitro bioactivity studies in SBF, but apatite growth still takes place within a few hours to a few days of immersion. The bioactivity and biocompatibility of Zn-doped BG materials were not only strongly associated with the apatite forming ability but also related with the release of zinc ions, which have a stimulatory effect on bone cells' proliferation and differentiation. Zinc ions must be released slowly from the BG because its elevated concentrations can have harmful effects. Uncontrolled fast release of Zn^{2+} ions from BG can create negative effects on the growth of new bone tissue and have a cytotoxic effect. Aina et al. [143] reported that 45S5 glasses with a zinc content of 5 wt% showed reduced solubility and bioactivity (monitored by HCA formation) in relation to the parent glass, while the endothelial cell adhesion on the surface thereof was the best. The sample with 20 wt% Zn has completely inhibited the growth of HCA. Balamurugan et al. [147] reported that BG with 5 wt% ZnO showed proliferation and differentiation of osteoblast rat's cells. In contrast, another study reported that BG scaffolds with 0–5% ZnO had no effect on proliferation and osteogenesis of human adipose stem cells (hASCs) [139].

Several studies have reported that Zn-doped BG exhibits antimicrobial activity as an important feature in the prevention of postoperative infections [141, 148, 149].

2.3.7 Cobalt-Doped Bioactive Glass

Cobalt is an essential trace element and is a constituent of several enzymes and vitamin cyanocobalamin (vitamin B12) [150, 151]. The investigation of cobalt materials in bone tissue engineering implants and as anticancer and antimicrobial agents is a broad area attracting increasing attention [152–156]. Highly vascularized bone tissue is essential for successful clinical application of engineered implants. Cobalt ions can stimulate angiogenesis via inducing hypoxic conditions and activate the hypoxia-inducible factor-1 (HIF-1) in mesenchymal stem cells and subsequently activate HIF-α target genes including VEGF, EPO, and p21 [157–159]. Hypoxia can also create a potentially lethal environment and limit cellular respiration and growth

[160]. High doses of cobalt may be cytotoxic and genotoxic and can cause cancer [161]. Hence, for applications in bone tissue engineering, a BG matrix is needed for the controlled release of Co^{2+} ions into a physiological environment. In this context, BG matrix has been shown to be suitable carriers for therapeutic ions [162]. Cobalt doped BG is bioactive and, in SBF, develops a hydroxycarbonate apatite layer on the surfaces [163–165]. Cobalt ions ware present in both the silicate and phosphate phases of the BG and formed Si–O–Co and P–O–Co linkages [165]. It plays a concentration-dependent role in the glass network, acting as network modifier at 1 wt% and a network former at ≥5 wt% [163]. The results indicated that the doping of CoO in 45S5 bioactive glass and glass-ceramics enhanced its density, compressive, bending strength, and elastic properties [163, 165]. Several studies have shown positive effects of the addition of Co^{2+} ions to BG scaffolds in angiogenesis and osteogenesis. Mesoporous bioactive glass (MBG) scaffolds showed that low amounts of Co (<5%) incorporated into MBG scaffolds had no significant cytotoxicity and that their incorporation significantly enhanced VEGF protein secretion, hypoxia-inducible factor HIF-1α expression, and bone-related gene expression in BMSCs, and also that the Co–MBG scaffolds support BMSC attachment and proliferation [166]. Another study showed that 1393 BG with 1 wt.% of CoO was biocompatible with osteoblast-like cells and endothelial cells which showed slightly stimulating effects on osteoblast-like cells, while the addition of 5 wt.% of CoO was cytotoxic to both cell types [167]. A recent study has shown that incorporation of CoO (0.5 mol%) in the BG significantly promotes osteogenic activity of human osteosarcoma cells without any cytotoxicity effect [164].

2.4 Silanization: Covalent Modification of a Bioactive Glass's Surfaces by Silanes

Silanization is an effective covalent coating method to modify material surfaces that are rich in hydroxyl groups, such as bioactive glasses, hydroxyapatite, titania, and many other metal oxide surfaces [168, 169]. The goal of silanization is to form bonds across the interface between the inorganic components and organic molecules or biomolecules in order to improve the interaction with the surrounding bone tissue and to enhance dispersion stability of inorganic particles in various liquids or as anchors for the immobilization of drugs. The mechanism of the silanization of inorganic materials is well studied [170, 171]. The reaction conditions such as nature and concentration of the alkoxysilane, solvent type, temperature, and reaction time must be carefully controlled to prevent the forming of a thick polymerized silane network on the surface. The resulting chemical bonds between alkoxysilane and the surface of a material can be hydrolyzed in some conditions. Silanol groups from hydrolyzed silicon alkoxides are able to condense with the hydroxyl groups present on the material surface, while the alkyl chain bears the functional group such as amino, chloro, carboxyl, epoxide, thiol, vinyl, cyanide, or phenyl that can be exploited for further functionalization [172–174]. The amino ($-NH_2$) groups are

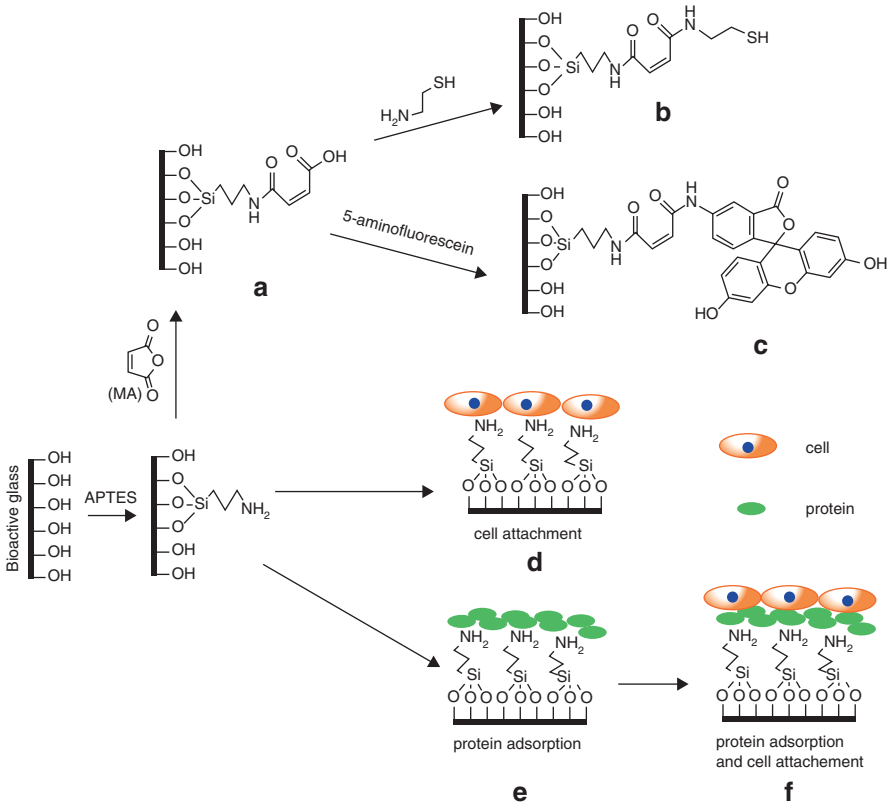

Fig. 2.6 Synthetic procedure for the preparation of APTS-BG-MA (**a**); synthesis of the APTS-BG-MA and cysteamine conjugate (**b**); synthesis of the APTS-BG-MA and 5-aminofluorescein conjugate (**c**); models for cell binding (**d**, **f**) and protein adsorption (**e**, **f**)

responsible for the covalent bonding and electrostatic interactions with negatively charged groups present on a variety of molecules such as DNA and proteins. The capability of biomaterials to adsorb proteins on their surface can affect cell adhesion and their growth. The 3-aminopropyltriethoxysilane (APTES) is one of the most frequently used silanes for the modification of different materials in many in vitro and in vivo biological studies (Fig. 2.6). Surface modification with APTES can be done during the synthesis of BG [175] or adsorption from solution [176, 177]. The highest calcination temperature of 150 °C is used in order to avoid the decomposition of the $-CH_2-CH_2-CH_2-NH_2$ chains of APTS molecules inserted during the synthesis [175]. In vitro tests indicated that APTES on a bioactive glass surface does not reduce its bioactivity [175, 178, 179]. Chen et al. [12] reported that the APTES layers themselves do not influence the kinetics of structural and chemical changes of the 45S5 Bioglass®-derived glass-ceramic material in SBF, while the aqueous treatment involved during the surface modification plays a key role in speeding up these changes. Zhang et al. [180] described the synthesis of mesoporous bioactive

glass (MBG) and its functionalization with APTES (N-MBG) and triethoxysilylpropyl succinic anhydride (TESPSA) (C-MBG). In vitro studies showed that all samples could significantly promote the proliferation and osteogenic differentiation of rabbit bone marrow stromal cells; the effect was greatest with N-MBG. In vivo results demonstrated that N-MBG could promote higher levels of bone regeneration compared with MBG and C-MBG. Amino groups present on the surface are likely to have a significant role in improving cell proliferation and differentiation. The type of charge and hydrophilicity of functional groups can influence protein and cell adhesion by changing the hydrophilicity-hydrophobicity of surfaces [181]. The amino groups are less hydrophilic than carboxyl groups present on the surface of the C-MBG sample [180]. Consequently, surfaces containing amino groups exhibit hydrophilic-hydrophobic balance, which is beneficial for cell adhesion. Composites consisting of polymers such as polylactide (PLA) and bioactive glasses have been developed as bone-repairing devices because of their bioactivity and biodegradability [182, 183]. The APTES proved successful for surface modification of BG as a coupling agent for improving the interface between PLLA and BG particles [184]. The APTES-treated glass particles without agglomeration are uniformly dispersed into the polymer phase compared to non-treated glass. The bending strength, bending modulus, and shearing strength of PLLA/BG-APS composites were all higher than those of unmodified composites. The acid anhydride reagents and glutaraldehyde (GA) are the most common used heterobifunctional cross-linker molecules for derivatization of the $-NH_2$ in the $-COOH$ groups (Fig. 2.6). Aina et al. [175] described derivatization of APTES-functionalized 25SG423 glass with maleic or cis-aconitic anhydrides and then conjugation with cysteamine and 5-aminofluorescein, used as model molecules to simulate a drug (Fig. 2.6a–c).

Degradation studies have shown that total release of conjugates from composites occurs only in an acid solution (pH 4.5), whereas at physiological pH (7.4) in conditions close to neutrality, a slow release of these organic molecules has been observed. The use of GA as a protein coupling agent allows the control of protein release kinetics and almost completely maintains the native protein structure [185–187]. The surface functionalization of the BG substrate with APTES and GA does not induce significant conformational changes in the methemoglobin and 5-methylaminomethyl-uridine forming enzyme structure [186, 187]. The GA also improved the stability of hemoglobin attachment and induces its polymerization on the surface of Ag-doped BG [188].

2.5 Biological Surface Functionalization of Bioactive Glass

Biological functionalization of bioactive glasses can be described as the attachment (immobilization) of biological species such as proteins, cells, and other biomolecules to material surfaces. Biomolecules can be bound to the surface of the materials by the weak physical interaction (electrostatically and Van der Waals forces) and/or chemical bonds (covalent and ionic). Physical and chemical immobilization may occur on

the surface at the same time; a primary layer of molecules may be physically adsorbed on top of an underlying chemisorbed layer. Chemical immobilization is highly selective and occurs only between certain adsorptive and adsorbent species, for example, salinization (Sect. 2.3). Many physicochemical characteristics of materials may affect the binding biomolecules, for example, chemical composition, dissolution behavior or pH, degree of crystallization, microstructure, hydrophobicity, z-potential, surface roughness, surface reactivity, particle sizes, etc. [189]. Surface functionalization of bioactive glasses with the proteins can improve their bone integration. The interactions of cells and tissues with biomaterials are the main condition for its survival and function in the human body. Biomaterials applied in most cases remain a long-term contact with local cells and tissues at the site of installation by entering into contact with them. The interaction of cells with biomaterials starts the moment when tissue comes into contact with biomaterial elements, first, through the adsorption of proteins on the surface of biomaterials in a very short time (<1 s). The formation of a protein monolayer on almost the entire surface of the implant is played for several seconds to minutes [190]. The type, amount, and conformation of adsorbed proteins on the surface are important for adhesion, proliferation, and differentiation of cells, and they can be an important factor in controlling the next bioprocess on the implant [189, 191]. Chemical composition of biomaterial surfaces can greatly influence the absorption of proteins. A calcium-phosphate surface on bioactive glass plays an important role in enhancing protein attachment. El-Ghannam et al. [192] reported that the amount of serum proteins adsorbed to the calcium-phosphate surface-modified porous 45S5 bioactive glass was significantly higher than that to the unmodified porous bioactive glass. Porous stoichiometric hydroxyapatite bound significantly higher amount of total proteins than the amount adsorbed to the bioactive glass substrates. The surface-modified porous bioactive glass selectively adsorbed higher amounts of fibronectin from serum than the hydroxyapatite or unmodified bioactive glass. BG in vivo showed a more intense bioactive effect than HAp [193]. The greater bioactive effect (i.e., bone bonding) of BG compared to hydroxyapatite was due to its ability to concentrate active proteins on its surface [192, 194]. Fibronectin is one of the most abundant extracellular matrix glycoproteins that adsorbs to biomaterials, mediating cell adhesion. In vitro, other proteins such as vitronectin, laminin, and collagen have been shown to be involved in cell adhesion [195]. On the contrary, albumin from the plasma can be used to "passivate" surfaces preventing cell adhesion and greatly reducing the acute inflammatory response to the material [196, 197]. Metal ions in the structure of BG can increase or decrease adsorption of proteins on its surface. High content of 8 mol% Ag_2O in bioactive glasses ($CaO–SiO_2–P_2O_5$) contributes to the improvement of its protein binding capability [198]. Silver ions in the particle surface of biomaterial particles can form bonds with proteins primarily through thiol-containing amino acids [199]. Rosengrena et al. [200] reported that two bioactive glass-ceramics, AP40 and RKKP, exhibit good absorption capacity to apolipoprotein J, fibrinogen, and fibronectin from human plasma. The presence of La or Ta in bioactive glass-ceramics decreased the adsorption of proteins. The proteins adsorbed on the surface of the glass act as promoters or inhibitors of the formation of apatite. The fibrinogen adsorbed on the BG surfaces induces a growing of the

apatite-like layer [201]. The presence of serum proteins delayed apatite precipitation for fluoride-containing glasses, while Bioglass 45S5, despite a considerably higher phosphate content, formed only amorphous calcium phosphate [202]. The cells are primarily associated with proteins as the main coating than to the actual surface of biomaterials [203, 204]. Cells adhered to the adsorbed proteins on biomaterial surface through integrins, a family of heterodimeric calcium-dependent membrane receptor proteins [191]. The role of fibronectin for in vitro cell adhesion on BG surfaces has been highlighted by several authors [190, 192]. Adherent cells on the surface in the absence of fibronectin are only spread 5%, but the expansion of the increased is close to 100% if the fibronectin adsorbed to the surface of the previously [190]. Osteogenic cell (MC3T3-El) adhesion to porous 45S5 BG glass treated to form a dual layer of calcium-phosphate and serum protein was significantly higher than adhesion to porous hydroxyapatite with adsorbed serum protein [192]. Hydroxyapatite adsorbed the greatest amount of total protein, while BG demonstrated selectivity. The calcium-phosphate surface on BG plays an important role in the selective concentration of fibronectin required to promote the accumulation of cells [192, 204].

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