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Bioactive Glasses

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Abstract: Bioactive glasses were discovered in 1969 and provided for the first time an alternative to nearly inert implant materials. They formed a rapid, strong, and stable bond with host tissues. This article examines the frontiers of research crossed to achieve clinical use of bioactive glasses and glass–ceramics. In the 1980s, it was discovered that bioactive glasses could be used in particulate form to stimulate osteogenesis, which thereby led to the concept of regeneration of tissues. Later, it was found that the dissolution ions from the glasses behaved like growth factors, providing signals to the cells. Hereby, the frontiers of knowledge crossed during four eras of development of bioactive glasses led from concept of bioactivity to widespread clinical and commercial use, with emphasis on the first composition, 45S5 Bioglases® were mentioned. The four eras are (a) discovery, (b) clinical application, (c) tissue regeneration, and (d) innovation. Questions still to be answered for the fourth era are included to stimulate innovation in the field and exploration of new frontiers that can be the basis for a general theory of bioactive stimulation of regeneration of tissues and application to numerous clinical needs.

Keywords: Bioactive glass, History, Development, Types, Production, Properties, Application

Biyoaktif Camlar

Özet: Biyoaktif camlar 1969 yılında keşfedilmiş ve ilk kez neredeyse tamamen inert malzemelere bir alternatif olmuştur. Dokularla hızlı, sağlam ve kararlı bağ oluştururlar. Bu makalede konuyla ilgili yapılan çalışmalardan ve biyoaktif camlarla cam seramiklerin klinik uygulamalarından bahsedilecektir. 1980'lerde biyoaktif camların özellikle dokuların yeniden şekil alması anlamında kullanılabilecekleri bulunmuştur. Daha sonra, camdan çözünen iyonların hücrelere sinyal vererek büyütücü etken gibi davrandıkları belirlenmiştir. Mevcut çalışmada, biyoaktif camların gelişiminde (a) keşif, (b) klinik uygulama, (c) doku oluşturma (d) yenilik olmak üzere dört evrenin ortaya çıkışına ve bu grup malzemelerin biyoaktiflikleri sayesinde yaygın klinik ve ticari kullanımlarına yol açan ilk bileşimden (45S5 Bioglases®) bahsedilerek devam edilecektir.

Anahtar kelimeler: Biyoaktif cam, Tarihçe, Gelişim, Tür, Üretim, Özellikler, Uygulama

1. History

In 1969, the discovery of bioactive glasses bonding to living bone was made. The most significant frontier was the discovery by Hench, Splinter, Allen, and Greenlee that certain compositions of $Na_2O–CaO–P_2O_5–SiO_2$ glasses formed a strong, adherent bond to bone [1].

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Figure 1. Larry Hench joined Imperial in 1995 from the University of Florida, having made the seminal discovery in 1969 of bioglases−the first reported synthetic material to form a bond with living tissues [2].

These biomaterials have become known as "bioactive," reacting in the physiological environment to form a bond between an artificial material and living tissue. Studies showed stable and strong bonding between bone and soft tissues in a wide range of mammals: mice, rats, guinea pigs, rabbits, dogs, sheep, pigs, monkeys, and baboons. A stable bone−bonded implant in the anterior region of the mandible of a baboon after 4 years of functional use was reported, one of the longest in vivo studies of biomaterials in primates ever published [3].

Until 1973, the second frontier was development of in vitro and in vivo tests that established the mechanisms and limits of bonding of bioactive glasses and glass–ceramics to bone. The in vitro tests indicated that the 45S5 Bioglass composition (Table 1) developed a HA layer in test solutions. This hydroxyapatite (HA) phase developed on the surface of the implants in vitro was equivalent to the interfacial HA crystals observed in vivo by Dr. Greenlee's transmission electron micrographs of the bonded interface. The HA crystals in vivo were bonded to layers of collagen fibrils produced at the interface by osteoblasts. The chemical bonding of the HA layer to collagen created the strongly bonded interface [1, 4].

Table 1. Composition and properties of bioactive glasses and glass–ceramics used clinically for medical and dental applications [1]

Composition (wt. %)	**45S5 Bioglass (NovaBone, Perioglass, NovaMin, Biogram)**	**S53P4 (AbminDent1, BonAlive)**
Na_2O	24.5	23
CaO	24.5	20
CaF_2	0	0
MgO	0	0
P_2O_5	6	4
SiO_2	45	53

From 1973 to 1979, two important aspects of the frontiers were explored in the era of discovery. First, the methodology for investigating the reactive glass surface and bonded interfaces of bioactive implants with living tissues had to be developed. There was no precedent for such analyses. Examples are instrumental techniques such as infrared reflection spectroscopy, developed by Sanders and Hench (1973), and applied to bioactive glasses and cryogenic Auger electron spectroscopy (AES), developed by Ohuchi, Pantano, Ogino, and Hench [5–6]. At this stage, tests were conducted primarily on bulk samples or as bioactive coatings on metal, e.g., Co–Cr alloys, or ceramic (e.g., alumina) implants. It was assumed that the eventual applications of bioactive bonding would be to replace a diseased or damaged bone.

In 1980, the Era of Clinical Applications of bioglasses has started. An important frontier to cross was clinical translation. The discovery by Wilson et al. that bioglass could bond to soft tissue paved

the way for development of the first bioactive glass clinical applications that required both stable bone and soft tissue interfaces: the MEP (middle ear replacement prostheses) [7].

In 1993–1997, alternative Class B bioactive implants made of synthetic HA were lost by extrusion or exfoliation from the jaw after only a few years post implantation. In contrast, 45S5 Bioglass implants maintained stable bonding in alveolar bone and a stable gingival interface for long term and maintained thickness of the bone without resorption generally experienced by denture wearers [8–9].

In 1985–2005, studies on tissue regeneration frontiers have begun. The discovery of osteoproduction (osteostimulation) and the concept of using bioglass particulate for regeneration of bone was the key frontier crossed that led to the Era of Tissue Regeneration. Wilson et al. described the effect of various sizes of bioglass particulate on regeneration of bone in periodontal defects created in a monkey model [10].

Figure 2. Bioglass is a bioactive material which causes a proper biological response from the tissue through the formation of a bond with the hard bone tissue. These compounds are mainly used in repairing and modifying damaged bone tissues. The unique property that makes a difference between bio glasses and other bioactive ceramics is the chemical control of these compounds. Different oxides of calcium (CaO), silicon (SiO_2), and phosphorus (P_2O_5) form different types of bioactive glasses [11].

In 2006, the first non–45S5 composition to reach the market was S53P4 (Table 1), now known as BonAlive® (BonAlive Biomaterials, Turku, Finland), which received European approval for orthopaedic use as bone graft substitute. It has higher silica content; so bioactivity is expected to be lower than 45S5. The next frontier was identifying what was really stimulating bone regeneration. While the in vivo data exhibited differences between implants, they were not answering the question why there were differences. Initially, the dissolution of the 45S5 Bioglass particles were thought to cause more bone formation by the HCA layer forming more rapidly and by the glass degrading, making more space for bone ingrowth. This was not the complete story though. Dissolution is important, but mainly because the dissolution ions act as signals to the cells. This was revealed through in vitro experiments that indicated critical concentrations of Si and Ca ions released from the glasses stimulated cells at the genetic level [12].

After 2005, the fourth era which is called as Innovation (2005–2025) Frontiers and Unmet Challenges has begun. There are many challenges still ahead for the clinical use of bioactive glasses that require advances in a fourth era, an era of innovation. Significant scientific and technological issues remain unanswered. As a result, the bioactive glass eras can be classified as four separate periods. Respectively;

(a) Era of Discovery (1969–1979);
(b) Era of Clinical Application (1980–1995);
(c) Era of Tissue Regeneration (1995–2005);
(d) Era of Innovation (2005–2025).

2. The Developments

The US Army Medical R and D Command funded the proposal for a one year test of the hypothesis. The glass composition of 45 % SiO_2–24.5 % Na_2O–24.5 % CaO–6 % P_2O_5 was selected to provide a large amount of CaO with some P_2O_5 in a Na_2O−SiO_2 matrix. The composition is very close to a ternary eutectic, making it easy to melt. The glass was melted, cast and made into small rectangular implants for testing in a rat femoral implant model designed by Dr. Ted Greenlee. The implants were made in the Department of Materials and inserted into the rats at the Gainesville, Florida Veterans Administration Hospital. The first tests were for six weeks. Dr. Greenlee reported at the end of the six weeks, *"These ceramic implants will not come out of the bone. They are bonded in place. I can push on them, I can shove them, I can hit them and they do not move. The controls easily slide out."*

This finding was the basis for the first paper published in 1971 in the Journal of Biomedical Materials Research that summarized the in vivo results and the in vitro tests that provided an explanation for the interfacial bonding of the implant to bone [13]. The in vitro tests showed that the 45S5 Bioglass® composition developed a hydroxyapatite layer in test solutions that did not contain calcium or phosphate ions. This rapid formation of HA in vitro was equivalent to the interfacial HA crystals observed in vivo by Dr. Greenlee's transmission electron micrographs of the bonded interface [14]. The HA crystals were bonded to layers of collagen fibrils produced at the interface by osteoblasts. The chemical bonding of the HA layer to collagen created the strongly bonded interface [13, 15].

Table 2. Chronology of science and clinical product development of Bioglass®

1969 Discovery of bone bonding to 45S5 Bioglass at University of Florida (Report to US Army Medical RD Command).
1971 First peer reviewed publications of bonding of bone to bioactive glasses and glass–ceramics [1, 16].
1972 Bonding of bioglass bone segments and coated femoral stems in monkeys [17–18].
1975 Bioglass coated alumina bone bonding to sheep hip implants (in Germany) [19].
1977 Bonding of bioglass implant in guinea pig middle ear. Patent applications filed for Bioglass coatings on metals and alumina ceramics [20].
1981 Discovery of soft connective tissue bonding to 45S5 Bioglass. Toxicology, biocompatibility studies (20 in vitro and in vivo) to establish safety for FDA clearance of bioglass products [21].
1985 First medical product (Bioglass Ossicular Reconstruction Prosthesis) (MEP) cleared by FDA via the 510 (k) process [22–24].
1987 Discovery of osteoproduction (osteostimulation) in use of bioglass particulate in repair of periodontal defects [20, 25–26].
1988 Bioglass Endosseous Ridge Maintenance Implant (ERMI) cleared by FDA via the 510 (k) processes [20, 27–30].
1991 Development of sol-gel process method to make bioactive gel−glasses extending bioactive compositional range of bioactivity [31–32].
1993 Bioglass particulate for use in bone grafting to restore bone loss from periodontal disease in infrabony defects (Perioglas) cleared by FDA via the 510 (k) process [20].
1995 Perioglas obtained CE Mark in Europe [20].
1996 Use of perioglas for bone grafts in tooth extraction sites, alveolar ridge augmentation cleared by FDA via the 510 (k) process [20, 24–25].
1999 European use of 45S5 particulate for orthopaedic bone grafting (Nova Bone) [20, 33–34].

2000 FDA clearance for use of Nova Bone in general orthopaedic bone grafting in non–load bearing sites [20, 33–34].
2000 Quantitative comparison of rate of trabecular bone formation in presence of bioglass granules versus synthetic HA and A/W glass–ceramic [35].
2000 Analysis of use of 45S5 Bioglass ionic dissolution products to control osteoblast cell cycles [36–37].
2001 Gene expression profiling of 45S5 Bioglass ionic dissolution products to enhance osteogenesis [38–39].
2004 FDA clearance of 45S5 particulate for use in dentinal hypersensitivity treatment (NovaMin) [20, 40].

3. General Composition of Bioglass

Bioglass is a commercially available family of bioactive glasses, composed of SiO_2, Na_2O, CaO and P_2O_5 in specific proportions. The proportions differ from the traditional soda−lime glasses in low amount of silica (less than 60 mol. %), high amount of sodium and calcium, and high calcium/phosphorus ratio [41]. High ratio of calcium to phosphorus promotes formation of apatite crystals; calcium and silica ions can act as crystallization nuclei [42]. Bioglasses have different formulations. Some bind to soft tissues and bone (e.g. 45S5), some only to bone (e.g. 5S4.3 or Ceravital), some do not form a bond at all and after implantation get encapsulated with non−adhering fibrous tissue, and others are completely resorbed within few weeks. Fine powders resorb faster than bulk materials. A thin layer of apatite forms on the glass−tissue interface, facilitating strong bond to the bone. Some formulations can facilitate growth of osteoblasts through the material [1]. Generally, there are four classes of bioglasses [42]:

1. 35–60 mol. % SiO_2, 10–50 mol. % CaO, 5–40 mol. % Na_2O: bioactive, bonds to bone, some formulations bond to soft tissues
2. < 35 mol. % SiO_2: non glass−forming
3. > 50 mol. % SiO_2, < 10 mol. % CaO, < 35 mol. % Na_2O: bioactive, resorption within 10–30 days
4. > 65 mol. % SiO_2: non−bioactive, nearly inert, gets encapsulated with fibrous tissue

Some CaO can be replaced with MgO and some Na_2O with K_2O without much effect to bone bonding. Some CaO can be replaced with CaF_2 without altering bone bonding; this, however, modifies the dissolution rate of the glass. B_2O_3 or Al_2O_3 may be added for easier material processing, however these influence the bone bonding; alumina inhibits bonding and its content is therefore restricted to small levels of about 1–1.5 %.
Phosphate−free glasses also exhibit bioactivity. The role of the phosphate is only in aiding of nucleation of apatite on the surface; phosphate ions adsorbed from the organism itself can play the same role [42].
Bioglasses are divided into two categories [43]:

- **Class A** bioglasses are osteoproductive. They bind with both soft tissues and bone. The HCA layer forms within several hours.
- **Class B** bioglasses are osteoconductive. Bond to soft tissues is not facilitated. The HCA layer takes one to several days to form.

When the proportions of these minerals are altered, the properties of the bioglass change, which can be suited to be used in various body parts accordingly. As depicted in the triangle (Figure 3), varying proportions of the components cause the bioglass to be bioinert, bioresorbable, or bioregenerative.

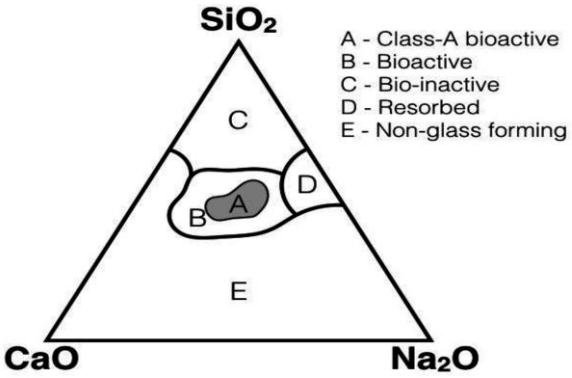


Figure 3. Property changes of bioglass materials [44].

Bioglass is available in multiple forms: Particulate, pellets, powder, mesh, and cones. Interestingly it can be moulded into any desired form.

Table 3. Composition of bioglass and glass−ceramics [45–46]

Glass	SiO_2	P_2O_5	CaO	$Ca(PO_3)_2$	CaF_2	Na_2O	Others	Properties
Bioglass 42S5.6	42.1	2.6	29.0			26.3		Mol%
Bioglass 46S5.2	46.1	2.6	26.9			24.4		Mol%; best tissue bonding of bioglass formulas
Bioglass 49S4.9	49.1	2.6	25.3			23.8		Mol%
Bioglass 52S4.6	52.1	2.6	23.8			21.5		Mol %
Bioglass 55S4.3	55.1	2.6	22.2			20.1		Mol %
Bioglass 60S3.8	60.1	2.6	19.6			17.7		Mol%, no phosphate film formed
Bioglass 45S5	45	6	24.5			24.5		The original Bioglass formulation; binds with bone and soft tissues
Bioglass 45S5F	45	6	12.25		12.25	24.5		
Bioglass 45S5.4F	45	6	14.7		9.8	24.5		
Bioglass 40S5B5	40	6	24.5			24.5	5 B_2O_3	
Bioglass 52S4.6	52	6	21			21		
Bioglass 55S4.3	55	6	19.5			19.5		
Bioglass 8625	?	?	?			?	Fe_2O_3	Highly biocompatible, does not bind with tissues, fibrous encapsulation; absorbs infrared radiation, can be laser sealed, used for RFID tag encapsulation

4. The Preparation and Synthesis

Up to now, various methods have been developed for the synthesis of bioglass and its composites including conventional melt quench, sol–gel, flame synthesis and microwave irradiation. Bioglass synthesis has been reviewed by various groups. Amongst all, the sol–gel synthesis of bioglass composites is the highly efficient technique for bioglass composites for tissue engineering applications.

4.1. Melt Quench Synthesis

The first bioactive glass itself made by Professor Larry Hench in the 1970's was made through melt–quenched method. The idea behind the invention was to make an implant material which can form a hydroxyapatite (HA) layer on its surface when implanted, which can develop a living bond with the host [47]. As the main aim was to mimic bone and bone contains hydroxyapatite [$Ca_5(PO_4)_3OH$], Ca^+ and PO_4 were taken as a component of glass. The other main components of glass Si^{4+} and Na^+ can also be found in human body. Among the compositions Hench and co–workers made, 45S5 were found to bond with rat femur. The selection of the components of this glass, named as Bioglass®, was ideal. The low silica content compared to the previous soda–lime–silicate glasses forms a layer of silica and amorphous calcium phosphate on the surface of the implant. Since then the research on bioactive glass somehow concentrated mostly compositions similar to 45S5 bioactive glass. As summarized in Table 4, a variety of methods can be used to form the particles of a melt–derived bioactive glass into a porous construct, producing different pore architectures (Figure 4). For a given bioactive glass scaffold, the porosity, pore size and pore inter−connectivity are critical parameters. In general, interconnected pores with a mean diameter (or width) between neighbouring pores of 100 μm or greater, and open porosity of > 50 % are generally considered to be the minimum requirements to permit tissue ingrowth and function in porous scaffolds [48–49]. Most of those bioactive glasses were produced by melting raw materials at an elevated temperature because it is a simple, low–cost technique and does not take much time to complete. It typically involves raw materials selection, weighing, mixing of components in appropriate proportion and removal of impurities to get a homogeneous melt.

Table 4. Methods used to create bioactive glass scaffolds, and characteristics of the fabricated scaffolds [48–49]

Method	Glass	Porosity(%)	Pore size(μm)	Strength(MPa)
Thermal Bonding of				
Particles	13-93	40-45	100-300	22 ±1
Short fibers	13-93	45-50	>100	5
Polymer foam replication	45S5	89-92	510-720	0.4 ±0.1
	13-93	75-85	100-500	11 ±1
	13-93B3	80-85	100-500	5 ±0.5
Sol-gel foam	70S30C	82	500(100)*	2.4
Undirectional freezing of suspensions	13-93	53-57	90-110	25 ±3
	13-93	50-55	60-120	27±8
	13-93	50	50-150	47 ±5
Solid freeform fabrication				
Selective laser sintering	13-93	58-60	700-1000	15 ±1
Freeze extrusion fabrication	13-93	50	300	140 ±70
Robocasting	6P53B	60	500	136 ±22

The reactivity of a glass in aqueous solutions is strongly dependent on the composition of the glass and thus the choice of composition is very important. Because the limited range of glass

composition shows bioactivity, the glass composition should be chosen in a way so that it can be melted and formed into required shapes with available methods. The raw materials can be divided into five different categories according to their role: glass former, flux, modifier, colorant and fining agent. Glass formers are the most important components of glass as they form the matrix of the glass structure. Silica (SiO_2), boric acid (B_2O_3) and phosphoric acid (P_2O_5) are the most common types of glass former normally present in oxide glass. In between these silica is widely used; however, the melting temperature of silica is too high (1600–1725 °C) and so different types of flux, such as Na_2O and PbO, can be used to decrease the melting temperature of the mixture. The addition of flux sometime degrades the properties of glass, which can be overcome by introducing different property modifier or intermediates such as boron, sodium, magnesium, titanium and calcium. Colorants are used to control the colour in the final product. Finally, fining agents such as arsenic, antimony oxides, potassium and sodium nitrates are added to raw materials to remove bubbles from the melt.

During melting of the raw materials inside the furnace, they react with each other and carbon dioxide and water–vapour emission takes place, which causes the formation of bubbles. To raise the bubbles up to the upper surface of the melt, low viscosity is maintained. Batch particle size and their mixing in proper proportion are other factors that provide homogeneity in glass structure. Glass forming is an intermediate stage in between glass melting and annealing. The stages of glass synthesis are illustrated schematically in Figure 5.

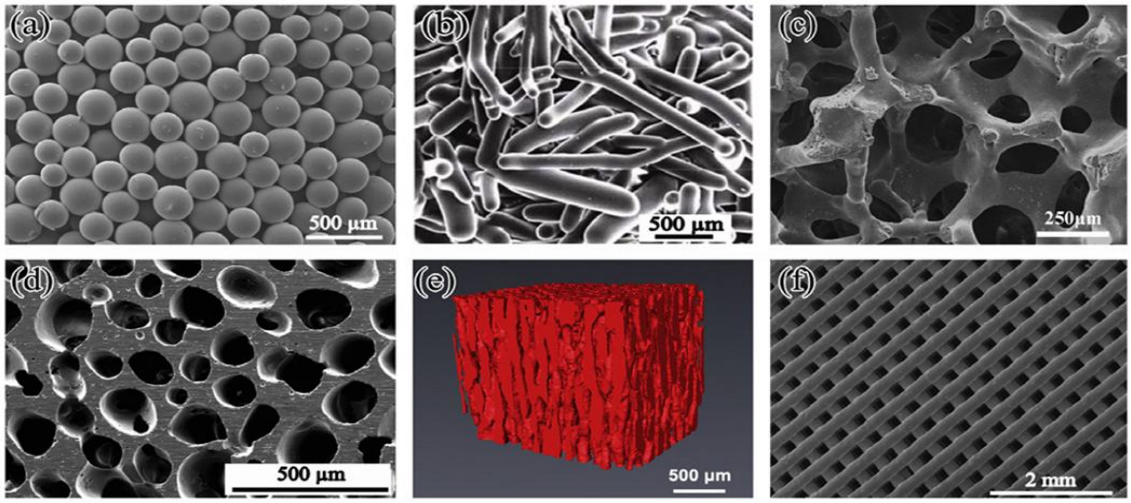


Figure 4. Microstructures of bioactive glass scaffolds created by a variety of processing methods: (a) thermal bonding (sintering) of particles (microspheres); (b) thermal bonding of short fibres; (c) "trabecular" microstructure prepared by a polymer foam replication technique; (d) oriented microstructure prepared by unidirectional freezing of suspensions (plane perpendicular to the orientation direction); (e) X–ray micro CT image of the oriented scaffold shown in (d); (f) grid–like microstructure prepared by robocasting. Glass composition: (a) $16CaO{-}21Li_2O{-}63B_2O_3$; (b–e) 13–93; (f) 6P53B [48–49].

Practically appropriate amount (mole/weight fraction) of initial ingredients is mixed, followed by grinding, to break agglomerated particles. In order to obtain more uniform powder, the mixture of ingredients is ground in ball mill using acetone (water can also be used unless some ingredient is hygroscopic). After drying the mixture in air, the powder can be transferred in platinum crucible and melted in a high–temperature furnace. Generally, around 500 ^oC, the gaseous substances (moisture and gas) come out of the composition. Hence, it is better to calcine the mixture at 500 ^oC for at least 2 h. Before taking out the melt, it must be confirmed that the glass mixture is held at the melting temperature for at least an hour to achieve homogeneous, bubble–free molten materials.

Then, the molten glass can be quenched in liquid such as water, liquid nitrogen, etc. Granules of different sizes formed collectively known as frits can be collected and milled to get glass powder. Desirable size and shapes can be made by pouring the molten mixture into moulds of particular shapes. In the case of preparation of glass with particular shape, the poured glass is annealed slightly below the glass transition temperature of the corresponding glass for 12 h in air in pit furnace.

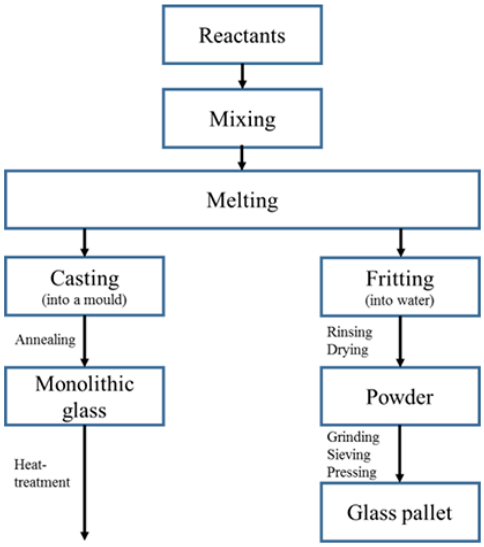


Figure 5. Schematic representation of melt–derived glass synthesis [50].

4.1.1. Important factors of melt quench synthesis

Important factors to remember while melting a glass are viscosity, thermal expansion and crystallization characteristics. Low viscosity helps the melt to be bubble free and homogeneous and also facilitates easy elimination from the platinum pot. It is a crucial factor in determining the best possible procedure for a particular composition. Viscosity values at high temperatures can be linked with melt–forming processes and low–temperature values indicate the suitability of the glass, whether for sintering into porous bodies or coating on metal implants. The approximate viscosity values for a bioactive–glass–forming process are given in Table 5.

Table 5. Approximate viscosity values (dPas) for bioactive–glass–forming process [50]

Processing	Viscosity (η) (dPa s)
Melting	10-10^2
Pressing	10^4-10^6
Drawing of continuous fibres	10^2.5-10^3.5
Sinter glass powder to porous body	10^8-10^9
Annealing	10^12-10^13

Bioactive glass coating provides better bone−implant connection when coated on metal prostheses [51–56]. According to the implantation area, lower surface reactivity may be preferred and in such cases glass composition with less bioactivity are favoured. Whatever be the case the thermal expansion of the glass must be compatible with the metal otherwise cracks may appear on the

coating leading to peeling off of the coating. Another important factor is that the melting temperature should be higher than liquidus temperature of the compositions. Recent development of bioactive glasses focuses on the change of chemical composition and different heat treatment condition [57–58]. Aboud et al. analysed the effect of increasing temperature on the crystallisation behaviour and the phase formation order of different crystals of SiO_2–P_2O_5–Al_2O_3–MgO–Na_2O glasses [59]. The changes in microstructure, mechanical and chemical properties of this glass with different heat treatment conditions result in an important application in dental restoration [60]. Also, thermal treatments of bioactive glass tend to enable the glass to attain different elastic properties and a range of bioactivity, which could be helpful for making patient−specific implant [61].

4.2. Sol-Gel Synthesis

Sol-gel glasses are made by a chemical−based process at much lower temperatures than the traditional processing methods [62–66]. The method has been recently accepted by a number of research groups to make a new generation of bioactive glass and offers assurance for tailoring the composition to match the specific requirements. Recently, scientists have preferred the sol–gel method in order to increase the specific surface area, and thus, the surface reactivity and degradability of the material [67]. It also provides better control over homogeneity and purity [31]. A sol is a colloidal suspension of solid particles (with a diameter of 1–100 nm) in a liquid, where the colloids exhibit Brownian motion, a random walk driven by momentum imparted by collisions with molecules of the suspending medium. Gel can be described as a rigid network of covalently bonded silica comprised of interconnected pores [68–69]. Three methods can be used to make sol–gel materials: gelation of colloidal particles, hypercritical drying or controlled hydrolysis and condensation of metal alkoxide precursors followed by drying at ambient pressure. All the three methods create a three–dimensional, interconnected network. Gels can be categorized into three types, such as alcogels, xerogels and aerogels [31]. Alcogels are generally alcohol based, whereas xerogels are formed from thermal removal of pore liquid. Gels with low density (80 kg m^{-3}) and large pore volumes (up to 98 %) are called aerogels, which are the result of removal of pore liquid from the rigid network without collapsing it.

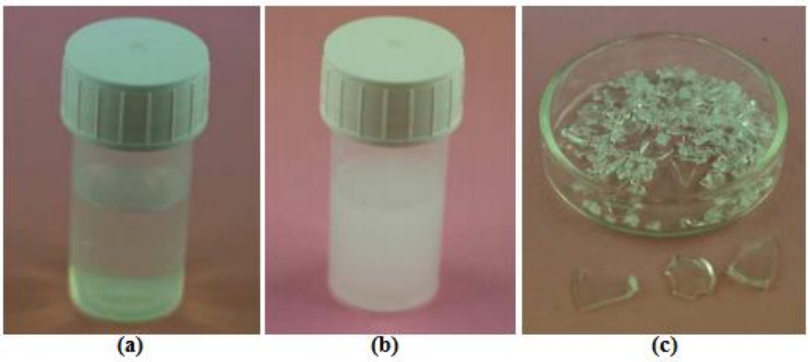


Figure 6. (a) Sol (b) Gel (c) Glass (Laboratoire des Matériaux Céramiques et Procédés Associés (LMCPA) Université de Valenciennes–France).

Preparation of gel glasses by a sol–gel method composed of seven steps. First, the alkoxide or organometallic precursors are mixed to form the low−viscosity sol, followed by hydrolysis of liquid alkoxide precursors with de–ionised water [70–71]. Hydrolysis of silicon alkoxide forms silanol groups [$Si(OH)_4$], eventually interact with each other to make the Si–O–Si bond and increase the viscosity of the sol (Figure 7). This is the time where the sol can be applied as a coating, be pulled into fibre, electro spun, impregnated into a composite or formed into powders. During the process of gelation, the viscosity of the solution sharply increases [72]. The gelation time depends upon the concentration of the solvent, nature of the oxide group and the amount of water used for the

hydrolysis [73–74]. While aging of a gel for several hours at 25–80 °C, decrease in porosity and increase in the strength can be observed due to polycondensation and reprecipitation of the gel network [75–77]. Aging process also affects the pore volume, surface area and density of the gel. The removal of pore liquid has different effect on arising stress for colloidal gels (pore size > 100 nm) and alkoxide−based gels with pore size 1–10 nm. Colloidal gels can be dried easily; however, in the case of alkoxide−based gels, large capillary stress may arise during drying. Hypercritical drying at elevated temperature and pressure, above the pore–liquid–solid critical point, avoids the solid−liquid interface and eliminates drying stress [78]. In order to control the stability of the material, chemical stabilization of the dried gel is required. Sintering of the gel at 500-900 °C desorbs silanol groups from the surface and eliminates 3–Si rings from the gel. It also increases the density, strength and hardness of the gel. The sintering temperature of alkoxide–based gels is in the range of 900–1150 °C depending upon composition. The schematic diagram of the sol–gel process is provided in Figure 8. The physical differences between the two synthesis routes are that sol–gel glasses tend to have inherent nano porosity whereas melt–derived glasses are dense in nature [79]. The surface area of sol–gel glasses is also higher than melt–quenched glass, which results in greater dissolution rate, and hence higher cellular response. The hierarchical pore structure consisting of interconnected macro pores (> 100 micrometre) and nano pores is beneficial for interaction and stimulation with cells as it mimics the hierarchical structure of natural tissues. Also bioactive glasses in the form of nano porous powders or monoliths or as nanoparticles can be made by changing the pH of the sol–gel process [80]. However, the sol–gel made scaffolds have lower strengths than melt–quenched glasses, and thus inappropriate to use in hard tissue engineering (Figures 9–10).

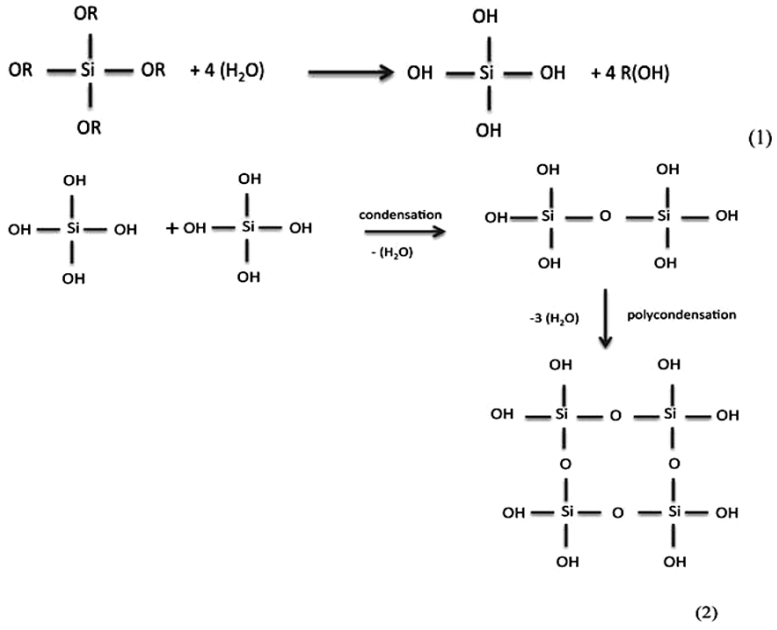


Figure 7. (1) Hydrolysis of $Si(OH)_4$; (2) formation of Si–O–Si bond [50].

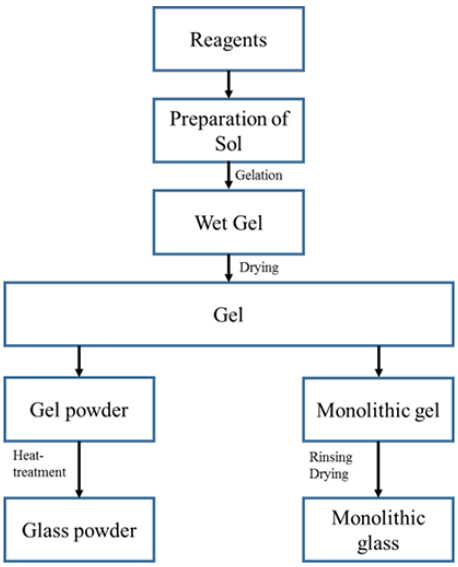


Figure 8. Schematic representation of sol–gel glass synthesis [50].

4.2.1. Important factors of sol-gel synthesis

The physical and chemical properties of sol–gel bioactive glass mainly depend upon silica and so the hydrolysis and condensation of silica plays an important role. The kinetics of hydrolysis and condensation of silica depend upon several factors such as pH, composition, temperature, precursor, catalysis and concentration of ions and the ratio of moles of water/moles of tetraethyl orthosilicate (TEOS). The polymerization of silica can be divided in between three pH ranges: < pH 2, pH 2–7 and > pH 7. pH 2 and pH 7 appear to be boundaries because at pH 2 the surface charge (PZC) and the electrical mobility of silica (isoelectric point, IEP) are zero, whereas above pH 7 the solubility and dissolution rates of silica are maximized leading to particle growth without gelation [80].

4.3. Microwave Synthesis

Recently ultrasonic assisted synthesis and microwave assisted synthesis are gaining attention as they can help to reaction in a short time and can modify the reaction environment to produce nano phase powders. It is a rapid and low cost powder synthesis method for powders. For synthesis, the precursors were dissolved in de–ionized water and transferred to the ultrasonic bath. The irradiation time was varied to obtain the optimum synthesis condition. Microwave operation was performed in a second batch of powders after the ultrasonic irradiation. The obtained amorphous powder was washed in de–ionized water and filtered. After drying for 24 hours in oven at 80 °C the powders were calcined at 700 °C for the development of bioglass [81].

5. Composition and Types of Bioactive Glass and Their Effects on Bioactivity

Since the report of bone–bonding properties of bioactive glass, silica has been used as the major component of glass composition and also most widely researched with changing its amount. Silicate glasses comprise an amorphous network structure based on SiO_4 tetrahedron, which are linked to each other at the oxygen centres. Silicate glasses have open structure of silica due to the presence of non–bridging oxygen ions attached with silicon. Addition of network modifiers such as Na^+, K^+, Ca^{2+} also causes the opening of silica network structures. These ions replace bridging oxygens of the network with non–bridging oxygens, hence opening of the glass structure. The number of modifier ion–oxygen bonds and non–bridging oxygen bonds determines several properties of the corresponding glass [82]. Detailed structural features of silicate glasses and their effect on different

physical and chemical properties have been reported by various research groups [83–85]. In the case of bioactive silicate (SiO_2 less than 60 wt. %) glasses, each silica tetrahedron contains more than 2.6 number of non-bridging oxygen ions, which is necessary in order to be bioactive [86].

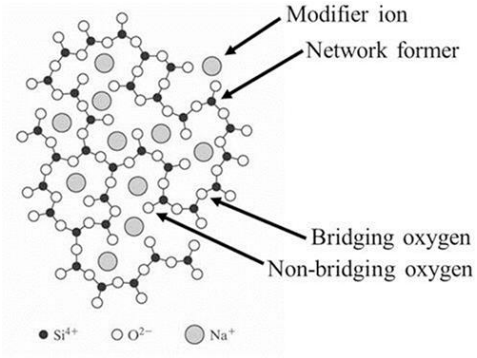


Figure 9. 2D presentation of random glass network modifiers and network formers [86].

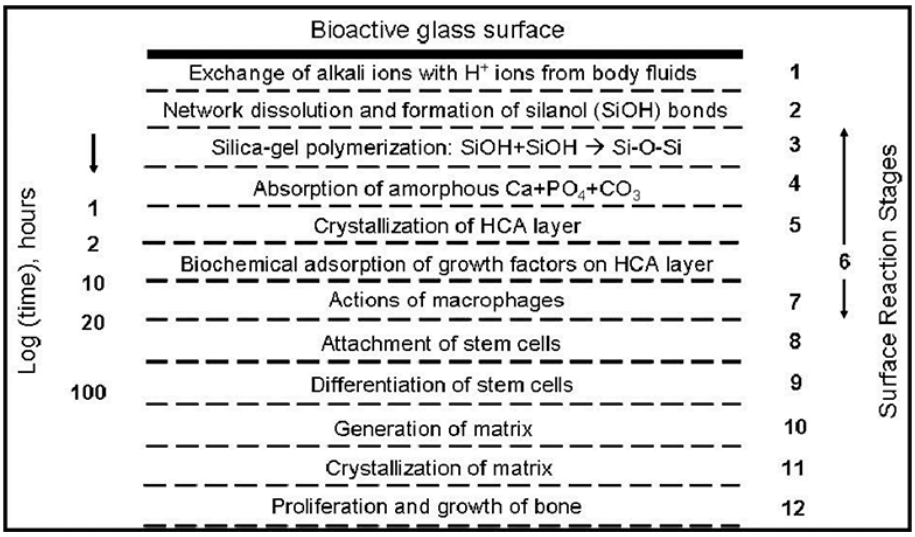


Figure 10. Sequence of interfacial reactions kinetics involved in forming a bond between bone and a bioactive glass [87].

The composition of bioactive glass is different from the traditional soda−lime−silica gasses that consist of more than 65 wt. % of silica. Basic components required for a glass to obtain bioactivity are SiO_2, Na_2O, CaO and P_2O_5, which can be distinguished in three main features according to Hench and Anderson [87]; the amount of SiO_2 should be in between 45 and 60 wt. %, Na_2O and CaO content must be high and a high CaO/P_2O_5 ratio. Higher content of SiO_2 decreases the dissolution rate of the glass ions from the surface, leading to decrease of bioactivity. Very low content of silica also leads to totally dissolvable monomeric SiO_4 units. Silica content also plays an important role to form hydroxyapatite carbonate (HCA) upon contact with physiological fluids, thus leading to the chemical attachment to soft/hard tissues. As a result, the interfacial bonding strength with bone increases, and a stable bond with strength equivalent to or greater then bone forms. High CaO/P_2O_5 ratio tends to enable the release of ions from the surface of the material when soaked in body fluid, forming a surface layer of HCA in a very short time span. It also supports cell proliferation on the surface of the implant by maintaining the ion concentration [47]. Previously, Hench and co–workers assumed that a typical range (2–6 wt. %) of P_2O_5 is required for a glass to be bioactive as it aids the formation of calcium phosphate phase on the surface, but later Hench and Anderson observed that bioactivity can be independent of P_2O_5 as phosphate ion is also available in physiological fluids.

In the last two decades, a number of different oxide systems have been studied to understand the effect on glass bioactivity and to increase its mechanical strength, still a complete understanding of the correlation between composition and bioactivity is insufficient but mechanical improvement can be possible. Different partial substitutions in the already approved glass compositions have been made, as CaO by 12.5 wt. % CaF_2, SiO_2 by 5–15 wt. % B_2O_3, but no significant effects were found. Even fluoride substitution reduced the bone bonding capability of the glass [78]. The substitution of MgO for CaO or K_2O for Na_2O showed slight increase in bioactivity. During 1990's glasses with alumina and boron oxide gained enormous interest. Sadly, the addition of small 3 wt. % Al_2O_3 to the 45S5 formula was found to prevent bonding with bone. Anderson proved that substitution by Al_2O_3 (1–1.5 wt. %) can reduce the bioactivity of glass because of its carcinogenicity [88]. Osaka et al. and Saranti et al. studied glasses with B_2O_3 content and found that the presence of boron has a positive impact on the bioactivity of the glass [89–90]. In the case of only B_2O_3–substituted glass, the ratio between B_2O_3 and SiO_2 plays an important role in the rate of formation of calcium phosphate layer on the surface of the implant [91]. Later, de Arenes proposed to control the B_2O_3/Al_2O_3 ratio in B_2O_3 and Al_2O_3 containing glasses in order to exhibit bioactivity [92]. In recent years, researchers tend to play with the composition of glass incorporating the ions that are abundant in human bone, such as Mg, Zn, Cu etc. [90–99]. Xia Li et al. found that by incorporating Mg, Zn or Cu in different amounts in place of Ca^{2+} can affect the bioactivity of the glass to different extent in a sequence of Cu < Mg < Zn [100]. Potassium substitution in place of Na^+ reduces the viscosity of silicate glasses and their susceptibility of crystallization [101]. Even now, a lot of research is going on to find a relation between the composition of the glasses, which have more than four components and tissue connectivity through phase diagram, but relation between these two factors is yet to come. Some researchers such as Anderson et al. and Brink et al. predicted the in vivo reactivity of glasses with six or seven oxides as a function of their composition with phenomenological models suggested by regression analysis [88, 102].

5.1. Silicate Bioactive Glass

Since the report of its bone−bonding properties nearly 40 years ago [1], the bioactive glass designated 45S5, sometimes referred to by its commercial name Bioglass®, has been the most widely researched glass for biomedical applications [103]. This glass is a silicate glass based on the three−dimensional (3–D) glass–forming SiO_2 network in which Si is fourfold coordinated to O. The key compositional features that are responsible for the bioactivity of 45S5 glass are its low SiO_2 content (when compared to more chemically durable silicate glasses), high Na_2O and CaO (glass network modifiers) content, and high CaO/P_2O_5 ratio (Table 6).

Table 6. Compositions of various bioactive glasses [103]

Composition (wt%)	45S5	13-93	6P53B	58S	70S30C	13-93B1	13-93B3	$P_{50}C_{35}N_{15}$
N_2O	24.5	6.0	10.3	0	0	5.8	5.5	9.3
K_2O	0	12.0	2.8	0	0	11.7	11.1	0
MgO	0	5.0	10.0	0	0	4.9	4.6	0
CaO	24.5	20.0	18.0	32.6	28.6	19.5	18.5	19.7
SiO_2	45.0	53	52.7	58.2	71.4	34.4	0	0
P_2O_5	6.0	4.0	6.0	9.2	0	3.8	3.7	71.0
B_2O_3	0	0	0	0	0	19.9	56.6	0

5.1.1. Modified silicate glasses: general structural features

Silicate glasses are amorphous solids, characterized by a network of covalent SiO_4 tetrahedral building blocks, linked together by bridging oxygen (BO) atoms, each BO shared by two Si. While the short–range order within the tetrahedra is similar to their crystalline counterparts, no long range order is present; the high flexibility in the angle between linked tetrahedra and in their relative orientation determines a high degree of structural disorder beyond the short range. Amorphous SiO_2 is characterized by a continuous network, fully interconnected in three dimensions, with every tetrahedron linked by BOs to four adjacent tetrahedra. The addition of alkali or alkaline−earth metal cations ('modifier' cations) breaks the silicate network by replacing Si–BO–Si bonds with Si–NBO, where NBO is a non–BO. Ionic bonds between NBOs and the modifier cations ensure the local charge balance and the overall charge neutrality; while weaker than the covalent Si–O bonds, the ionic interaction between NBOs and modifiers is extremely important to stabilize 'invert' glasses containing low silica amounts, such as the bioactive glasses (Figure 12). For instance, the 45S5 composition contains 45 % SiO_2, 6 % P_2O_5 and 24.5 % of both Na_2O and CaO (wt. %), with less than one–third of the oxygen atoms as BOs; owing to the low silica amount, the modifier–NBO interaction is crucial for the formation of a stable glass dominated by chain–like fragments, occasionally interconnected to each other (Figures 11–12) [83].

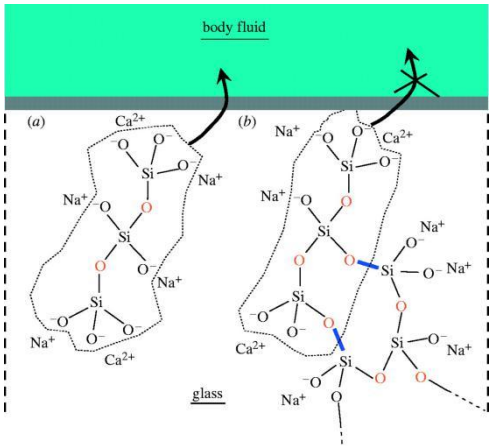


Figure 11. General scheme of the chemical structure of bioactive glasses; BOs are marked in red. The fragment in *(a)* is a three–membered silicate chain, with no covalent links to the rest of the structure, whose dissolution will be relatively fast, compared with the fragment in *(b)*: the latter is part of a five–membered ring and covalently cross–linked through the additional Si–O bonds coloured in blue [104].

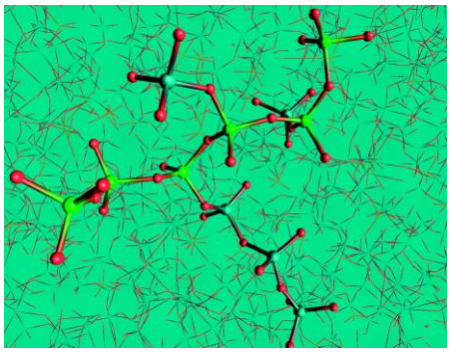


Figure 12. The silicate network of 45S5 Bioglass as obtained from SM MD; Na and Ca ions are not shown for clarity. Ball–and–stick visualization is used to highlight an individual silicate chain fragment, with Si atoms coloured in green, and its interconnections to other fragments, with Si atoms coloured in light blue [104].

5.2. Borate Bioactive Glass

More recent work has depicted that certain compositions in other glass−forming systems, such as borate glass [105–109], are also bioactive (Table 6). Because of their lower chemical durability, some borate bioactive glasses degrade faster and convert more completely to an HA–like material, when compared to silicate 45S5 or 13–93 glass [91, 110–112]. The conversion of borate bioactive glass to HA appear to follow a process similar to that described for 45S5 glass, but without the formation of a SiO_2–rich layer [110–111]. Borate bioactive glasses have been shown to support cell proliferation and differentiation in vitro [113–114], as well as tissue infiltration in vivo [115]. Borate bioactive glasses have also been shown to serve as a substrate for drug release in the treatment of bone infection [116–118]. A concern associated with borate bioactive glass is the toxicity of boron released into the solution as borate ions, (BO_3). In conventional "static" in vitro culture conditions, some borate glasses were observed to be toxic to cells, but the toxicity was diminished in "dynamic" culture conditions [119]. Scaffolds of a borate bioactive glass, designated 13–93B3, with a composition obtained by replacing all the SiO_2 in 13–93 glass with B_2O_3 (Table 6), were found to be toxic to murine MLO−A5 osteogenic cells in vitro [115]. However, the same scaffolds did not show toxicity to cells in vivo and supported new tissue infiltration when implanted subcutaneously in rats [115, 120]. Borate glass pellets implanted in rabbit tibiae produced boron concentrations in the blood far below the toxic level [121]. Recent work has shown the ability to control the degradation rate of bioactive glass by manipulating its composition. For example, by partially replacing the SiO_2 in silicate 45S5 or 13–93 glass with B_2O_3 (yielding a borosilicate bioactive glass), or fully replacing the SiO_2 with B_2O_3 (producing a borate bioactive glass), the degradation rate can be varied over a wide range [91, 111–112]. The ease of manufacture and the ability to control the degradation rate of these borate−based glasses make them particularly useful for promoting the regeneration of bone. By controlling the glass composition, it should be possible to match the degradation rate of borate−based bioactive glass with the bone regeneration rate. Another possibility is to exploit the compositional flexibility of glass so that it also can serve as a source of many of the minor elements known to favour bone growth, such as Zn, Cu, F, Mn, Sr and B. As the glass degrades in vivo, these elements are released at a biologically acceptable rate.

5.3. Phosphate Bioactive Glass

Phosphate glasses, based on the P_2O_5 glass–forming network and CaO and Na_2O as modifiers (Table 6), have also been developed for biomedical use [122–126]. As their constituent ions are present in the organic mineral phase of bone, these glasses have a chemical affinity with bone. Their solubility can be controlled by modifying their composition; therefore they may have additional clinical potential as resorbable materials.

6. The Structure

In order to achieve a deeper understanding of the biological activity of Hench's glasses, we need to focus on those properties of the glass which may impact:

1. The partial dissolution of the silicate network and
2. The reactivity of the glass surface

The basic information needed to begin any rational study of these effects is the *bulk structure* of the glass; despite its obvious importance, and the relatively long history of successful applications of bioactive glasses, investigations on the atomic structure of bioactive glasses have only started to appear very recently. This is undoubtedly related to the highly disordered and multicomponent nature of bioglass systems, which represents a serious challenge to standard experimental and

computational techniques to unveil their atomistic structure. However, prompted by recent advances in experimental and computational methods and available resources, in the last few years, several groups have started to focus their investigations on the structure of these complex systems [127–129]. These studies have produced interesting new insight on the medium-range arrangement and other structural features.

7. The Mechanical Performance

A key property, particularly for scaffolds intended for the repair of loaded bone, is the mechanical response. As previously discussed, scaffolds should have mechanical properties comparable to those of the tissue to be replaced. Bone is generally classified into two types: cortical bone also referred to as compact bone, and trabecular bone, also referred to as cancellous or spongy bone. Cortical bone, found primarily in the shaft of long bones and as the outer shell around trabecular bone, is much denser, with a porosity of 5–10 % [130]. Trabecular bone, found at the end of long bones, in vertebrae and in flat bones such as the pelvis, is much more porous, with porosity in the range 50–90 % [131]. The mechanical properties of bone vary between subjects, from one bone to another and within different regions of the same bone. The mechanical properties are also highly anisotropic, as a result of the oriented microstructure. However, based on the testing of large specimens, the compressive strength and elastic modulus of cortical bone have been reported in the range 100–150 MPa and 5–15 GPa, respectively, in the direction parallel to the orientation axis (long axis) [132–134]. The strength and modulus in the direction perpendicular to the long axis are typically 1.5–2 times lower. A wide range has been reported for the elastic modulus (0.1–5 GPa) and compressive strength (2–12 MPa) of trabecular bone [110–111]. The mechanical properties of porous scaffolds depend on the type of biomaterial, the microstructure and the fabrication method. Table 4 indicates the compressive strength of bioactive glass scaffolds prepared by a variety of methods. This summary is not meant to be exhaustive but, rather, to indicate representative examples. Bioactive glass scaffolds prepared by methods such as polymer foam replication, gel–casting and sintering of particles or short fibres typically have strengths comparable to that of human trabecular bone. Methods such as rapid prototyping and unidirectional freezing of suspensions have resulted in the creation of porous bioactive glass scaffolds with compressive strength and elastic modulus which are comparable to, or approach the values for, human cortical bone. These scaffolds have potential application in the regeneration of load–bearing bones. Figure 13 compares the mechanical response of 13–93 bioactive glass scaffolds formed by a polymer foam replication technique [135], unidirectional freezing [136] and by freeze extrusion fabrication (FEF), a rapid prototyping method. Scaffolds prepared by the polymer foam replication technique (porosity= 85 %; pore size= 100–400 μm) initially show an elastic response, followed by several peaks and valleys in the stress–strain curve. These peaks and valleys may be related to progressive breaking of the solid glass struts in the “trabecular” structure and compaction of the sample. Conversely, the constructs prepared by uniaxial freezing (porosity= 50 %, pore size= 60–80 μm) or by rapid prototyping (FEF) (porosity= 50 %, pore size= 100–500 μm) show a typical brittle response, consisting of an elastic response followed by fracture.

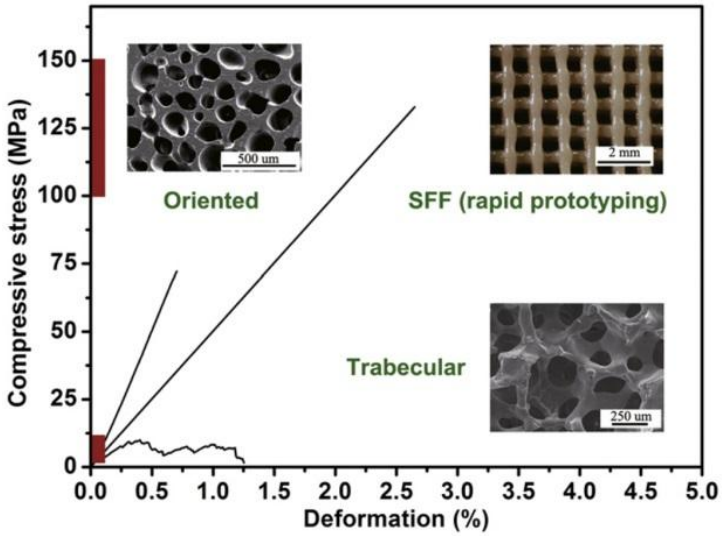


Figure 13. Mechanical response (compressive stress vs. deformation) of bioactive glass (13–93) scaffolds with: a trabecular microstructure prepared by a polymer foam replication technique; an oriented microstructure prepared by unidirectional freezing of suspensions; a grid–like microstructure prepared by freeze extrusion fabrication (a solid freeform fabrication technique). The ranges of compressive strength values for trabecular and cortical bone are given in red [133].

8. Surface Reaction Kinetics

Chemical reactivity of a glass in contact with body fluid holds the key of the bone bonding properties of the glass. Due to the chemical reactions, a layer of hydroxycarbonate apatite forms on the surface to which bone can connect. When immersed in an aqueous solution, such as SBF (simulated body fluid) or PBS (phosphate−buffer solution), three general processes occur: leaching, dissolution and precipitation. Leaching can be characterized as release of ions, generally by exchange of alkali or alkaline earth metals ions with H^+ or H_3O^+ ions of the solution. Glass modifier ions leach very easily from the surface of the glass when immersed in an aqueous solution, as they are not part of the glass network. The ion exchange process leads to increase in the hydroxide ion concentration, i.e., the basicity of the solution increases to pH > 7. Network dissolution occurs simultaneously by breaking of the network forming silica bonds (–Si–O–Si–O–Si–) by the attack of hydroxyl ions (OH^-). It releases silica into the solution in the form of silicic acid [$Si(OH)_4$]. In this step, glass composition plays an important role as the rate of silica dissolution depends very much on glass composition. Silica dissolution rate rapidly decreases if the weight percentage of SiO_2 goes beyond 60 % because of the increase of bridging oxygen, which can hold the network very strongly. Hydrated silica then undergoes polycondensation with neighbouring silanols to form silica–rich layer. In the precipitation part, calcium and phosphate ions released from the glass together with those from solution to form a calcium–phosphate–rich layer on the glass surface. Slowly, it crystallizes to form HCA by incorporating carbonate ions from solution. Generally, there are five reaction stages on the implant side of the interface with a bioactive glass [78].

- Stage 1: Leaching and formation of silanols (SiOH)
- Stage 2: Loss of soluble silica and formation of silanols
- Stage 3: Polycondensation of silanols to form a hydrated silica gel
- Stage 4: Formation of an amorphous calcium phosphate layer
- Stage 5: Crystallisation of a hydroxycarbonate apatite layer

Hench et al. have been extensively described the reaction processes [78, 121, 137–139].

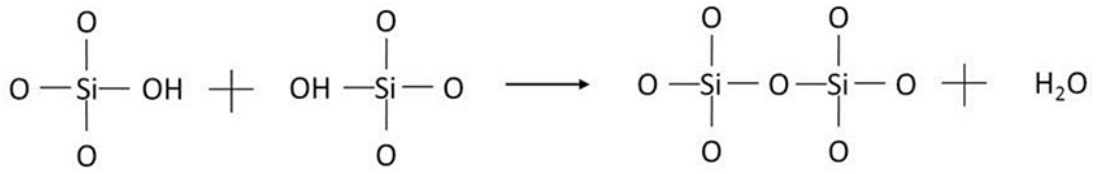
1. Rapid exchange of alkali or alkaline earth metal ions Na^+ or K^+ with H^+ or H_3O^+ from solution

$$Si–O–Na^+ + OH \rightarrow Si–OH^+ + Na^+ (solution) + OH^-$$

2. –Si–O–Si–O–Si– bonds break through the action of hydroxyl ions and form Si–OH (silanols)

$$Si–O–Si + H_2O \rightarrow Si–OH + OH–Si$$

3. Condensation of Si–OH groups near the glass surface: re–polymerisation of the silica rich layer



4. Migration of Ca^{2+} and PO_4 groups to the surface through the SiO_2–rich layer forming a $CaO–P_2O_5$–rich film on top of the SiO_2–rich layer, followed by growth of the amorphous $CaO–P_2O_5$–rich film by incorporation of soluble calcium and phosphate ions from solution.
5. Incorporation of hydrolysis and carbonate from solution and crystallization of the $CaO–P_2O_5$ film to HCA.

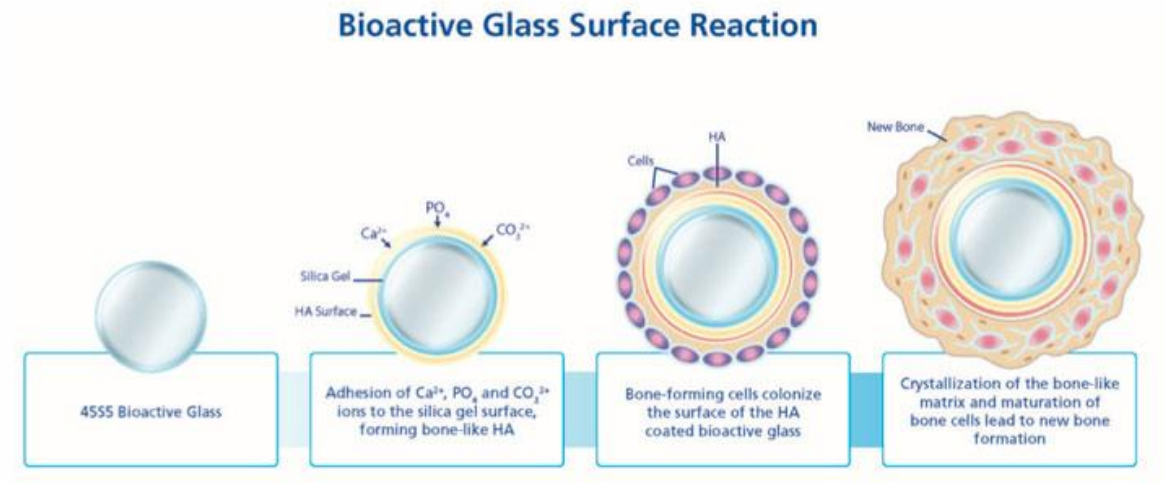


Figure 14: Surface reaction of bioactive glass [140].

As these stages were proposed many years ago, they are proved through time by various types of characterization techniques. 17O nuclear magnetic resonance (NMR) confirmed the increase of bridging oxygen bonds during leaching, which indicates the repolymerisation of Si–OH groups in the silica–rich layer. The formation of crystallise HCA layer on the surface was confirmed by surface–sensitive–small–angle X–ray diffraction (XRD) [82 141]. Calcium phosphate nucleate on the Si–OH groups as they have negative charge in solution and the separation of the Si–OH groups is thought to dictate the orientation of the apatite crystals, which grow with a preferred orientation in the 001 plane on Bioglass 45S5 [122–126].

9. The Properties of Bioactive Glass in Vivo

The bioactivity of glasses can only be investigated and confirmed after testing with living tissues. If a calcium phosphate layer can be found on a silica gel layer at the surface of the implants, the glass can be called bioactive. The extent of bioactivity of the glass is directly dependent on the ability of the glass to form calcium apatite layer. The above–mentioned five stages on the surface of bioactive glass do not depend on the presence of tissues. The sequence of in–vivo reactivity of bioactivity glass with tissues has been investigated by Hench and Anderson [52, 104, 135].

- Stage 6: Adsorption of biological moieties in the SiO_2–hydroxycarbonate apatite layer
- Stage 7: Action of macro phases
- Stage 8: Attachment of stem cells
- Stage 9: Differentiation of stem cells
- Stage 10: Generation of matrix
- Stage 11: Mineralisation of matrix

Through the 11 stages, a bioactive glass bonds with the bone. Gradually, the bioactive glass will be absorbed with increasing bone ingrowth.

45S5 Bioglass® was the first bioactive glass successfully investigated in vivo by many researchers [78]. After that another bioactive glass S53P4 was developed by Anderson and Karlson and has been successfully used in clinical applications [141–143]. Later, glass 13–93 and glass 1–98 also presented good bioactivity in vivo [102, 144–146].

10. The Efficiency of Bioactive Glass in Bone and Soft Tissue

Bioactive glass is very reactive and provides unique chemical environments in the tissue it's placed in causing the body to generate similar tissue to that surrounding it. It's this unique response by the body to these chemical signals that give bioactive glass such a wide range of applications. Bioactive glass is effective in bone regeneration due to its ability to interact with the body to form new bone tissue. The chemical reaction from glass to hydroxyapatite and the biological incorporation of this material into new and living bone is what makes the glass so unique.

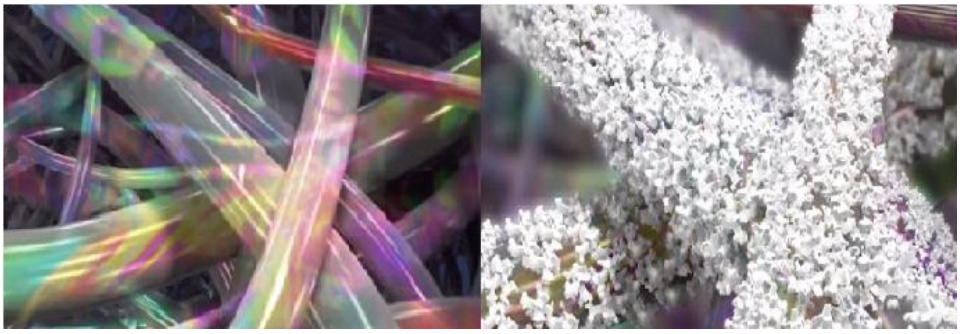


Figure 15. Appearance of bioactive glass in tissue [147].

During the course of conversion from glass into hydroxyapatite and then incorporation by the body's bone forming cells, the grafting material is completely consumed by the process leaving the site indistinguishable from its native form. Not all mechanisms of action have been identified in soft tissue applications, but it is known that when the glass is placed in a wound site changes in the protein production change rapidly. This is due to the up regulation of certain genes that produce these proteins. The ionic change in the local environment has also been shown to attract a specific type of white blood cell called neutrophils that help with the body's immune system responded helping fight infections [147].

10.1. Bioglass in Bone Tissue Engineering

One of the biggest hurdles in tissue engineering was to mimic the extracellular matrix. Scaffolds built using bio composite nanofibers and nano hydroxyapatites were naturally very porous, which in turn facilitated good cell occupancy, vascularity, movement of nutrients, and metabolic waste products. Studies comparing bio inert with bioactive glass ceramic templates produced increased osteoblast proliferation and differentiation. This system helped the human fatal osteoblasts to

adhere, migrate, proliferate, and mineralize into bone, which was a tremendous step ahead in the bone defect filling [148–149].

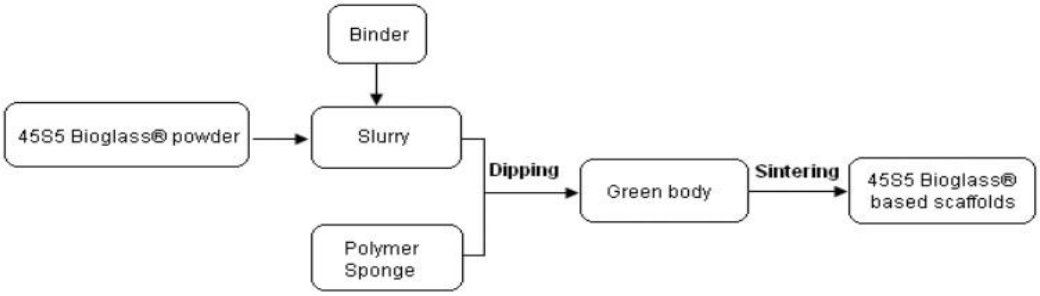


Figure 16. Schematic diagram presenting the foam replica method to fabricate bioglass tissue engineering scaffold [150].

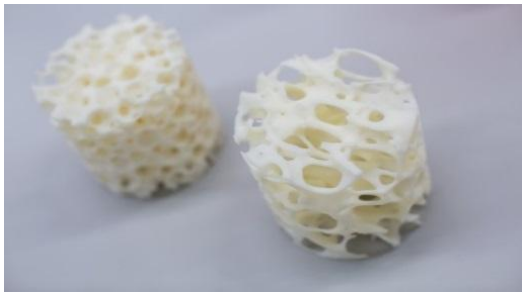


Figure 17. The graphitic nano carbon created by the PlasCarb technology has been tested by Abalonyx as reinforcement to bioglass. This material is used for the generation of transplantable bone tissue scaffolds [151].

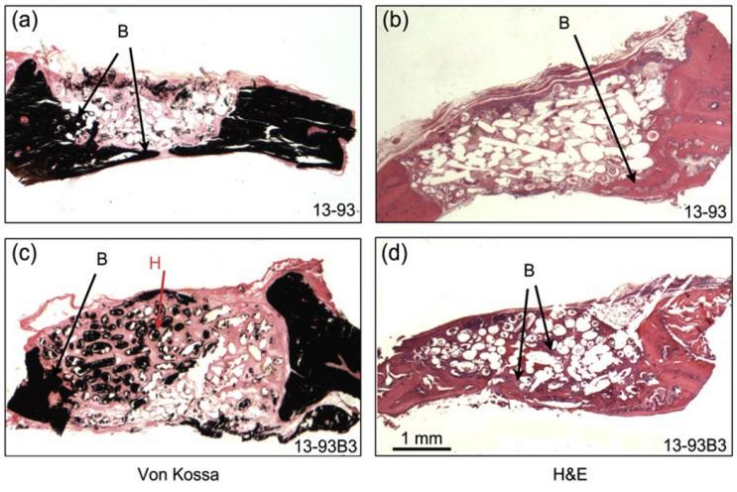


Figure 18. (Left) Von Kossa−and (right) H&E–stained sections of silicate 13–93 bioactive glass scaffolds (a and b) and borate 13–93B3 bioactive glass scaffolds (c and d), after implantation for 12 weeks in rat calvaria defects. B, bone; H, hydroxyapatite within scaffold [152].

11. Applications

Bioactive glasses are available in a wide variety of different forms, ranging from cast shapes, quenched frit, rods, fibres, disks, spheres, porous scaffolds, and millimetre to submicron sized powders. These can be surface–treated or modified as needed for particular applications. Depending on the composition, thin plates similar to microscope slides may be available as a custom item through R&D [153].

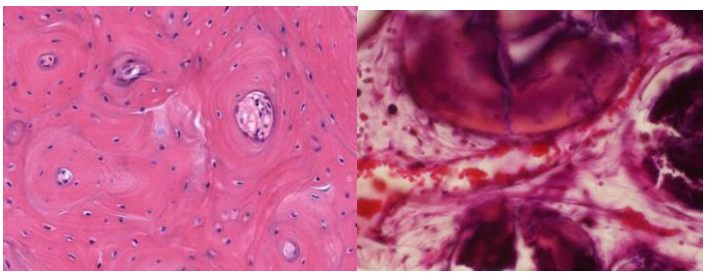


Figure 19. Appearance of bioactive glass in tissue and bone [153].

Uses include:

- Implant components for bone grafting biomaterials such as granules, putty, strips, or porous scaffolds
- Repair of periodontal defects
- Cranial and maxillofacial repair
- Dental applications such as a desensitization component for toothpaste
- Bioactive coatings for metal, ceramics, or plastics
- Fibre reinforcement for composites
- Wound care for traumatic or no healing wounds
- Haemostatic devices for blood loss control
- Stimulation of vascular regeneration
- Nerve repair

11.1. Bioglass as Endosseous Implant

After dental extraction, resorption of alveolar bone affects majority of patients [154–156]. This resorption leads to ill–fitting dentures resulting in compromised masticatory efficiency, oral and systemic health problems, and aesthetics. Alveolar bone height is maintained on stimulation by the periodontal membrane and teeth or roots being present [157–158]. After extraction, stimulation is lost to the alveolar bone and the pressure from dentures cause bone resorption [159–160]. The resorption rate varies with from individual to individual and at varying levels in the same individual [157–158, 161].

Many treatment modalities have been suggested for augmentation of the atrophic ridge [162]. Although autogenous bone grafting can be a recommended treatment modality and also with reduced antigenicity of freeze dried bone rejection, infections and transmission of disease limit its usage. Ankyloses, resorption, and pocket formation make replantation of natural roots a failure. Thus, maintaining the residual alveolar ridge is better than trying to augment it. While many materials such as carbon, calcium phosphate ceramics, tricalcium phosphate, hydroxyapatite, coraline hydroxyapatite, and bioglass have been used in augmentation of alveolar ridge, dehiscence of these materials, mostly within 12 months, made implantation difficult.

Considering these obstacles, bioglass was the most promising implant material, as proved by the study carried out by Stanley et al., using cone−shaped bioglass [163–164]. The study was done on baboons for 2 years. Bioglass implants were placed in the extracted sockets of incisors, splinted to adjacent natural teeth for 3 months and then desplinted for another 3 months. Bioglass caused ankyloses, usually by direct deposition of bone on the implant surface, [2] with the added advantage of gradation of mineralization within the bioglass gel layer reducing from outward to inward providing mechanical compliance like the periodontal membrane in the natural tooth. Another study [155, 165–166] had 242 cone implants placed in 29 patients. The patients were observed from 12 to 32 months. The implants were found to be surrounded with new bone on postoperative evaluation of surgical exposure. Dehiscence was not encountered even at 12 months, compared with

dehiscence at 10 months with other materials. Inflectionless normal tissue healing with new bone formation as sighted in radiographs made bioglass a highly biocompatible innovation.

Figure 20. Conical and cylindrical prosthetic connections for dental implants [167].

11.2. Bioglass as A Graft Material

Materials chosen for grafting need to be biocompatible, bioresorbable, and osteogenic. Treatment for the elimination of osseous defects due to periodontal diseases, pathologies, and surgeries include autogenous bone grafts, alloplast, guided tissue regeneration, combination of guided tissue regeneration and decalcified freeze–dried bone. Limitations of autogenous bone grafts are additional surgical trauma and not enough tissue material to fill the defect. To overcome these restrictions, alloplastic materials were used. But again adverse immune response and disease transmission have restricted its widespread acceptance. The membrane exposure and the local infection that follows in guided tissue regeneration obstruct bone formation. The last three decades saw the trials of many glass and glass–ceramic compositions. The glass–silicate composition developed by Hench showed bonding to bone. The bioactive glass has been observed to bond with certain connective tissue through collagen formation with the glass surface. Bioactive glass with its interconnected porosity has added advantages in hard–tissue prosthesis.

Figure 21. Engineered tissue graft for a knee articular cartilage lesion [168].

The porous structure supports tissue in/on growth and improves implant stability by biologic fixation. But its low fracture resistance makes it more useful in load−free areas. Trials have been conducted to compare repair response of bioactive glass synthetic bone graft particles and open debridement in treatment of human periodontal osseous defects. Fifty–nine defects in 16 healthy adults were chosen. Clinical parameters of probing depths, clinical attachment levels, and gingival recession were recorded. Radiographs and soft tissue presurgical measurements were repeated at 6, 9, and 12 months. There was significantly less gingival recession in bioactive sites compared with control sites. More defect fill in bioactive glass sites. Bioactive glass sites showed significant improvement in clinical parameters compared with open flap debridement [169]. Bioglass was used in particle form to fill periodontal osseous defects [10, 44, 169–170]. Bone was seen to be surrounding individual particles from many sites [171]. Twenty patients age 23–55 years (44 sites)

with intrabony defects completed the 1–year study. Follow–up was carried out weekly, at 3 months, 6 months, 9 months, and 1 year post surgery. Results exhibited a significant increase in radiographic density and volume between the defects treated with bioactive glass when compared with those treated with surgical debridement only. Thus, bioactive glass was found to be effective in the treatment of intrabony defects [172]. Another study [173] was conducted with bioglass particulates in periodontal osseous defects of 12 patients. Data was collected initially and at 3, 6, 24 months post−treatment intervals. Considerable improvements of all clinical parameters of mean probing depth reduction, mean attachment gain, and mean radiographic bone fill were noted. Follow–up of over 24 months showed stable results. The material elicited extraordinary tissue response and hassle–free handling.

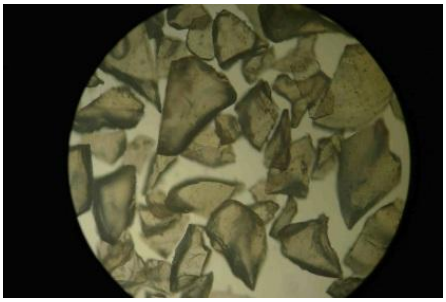


Figure 22. Bone graft materials in veterinary dentistry. Structure of a typical bioglass, showing the smooth surface [174].

11.3. Bioglass in Drug Delivery

The basic criteria for selection of any drug delivery system should be that it is inert; biologically compatible; has good mechanical strength; is good from the aspect of patient comfort; has the ability to carry high doses of the drug, with no risk of accidental release; and is in easy administering, removal, fabrication, and sterilization. There are three basic mechanisms through which active agents can be delivered: By diffusion, activation of solvent or swelling, and degradation. Controlled drug delivery means pre–planned delivery of a drug. The aim was to be more effective without possibilities of increased or decreased dosages, and also greater patient acceptance, maximal usage of the drug, with least administrations. The importance is more so ever when this accuracy is limited while using conventional drugs or injections. For example, when water soluble drugs should be slowly released, low soluble drugs should be released fast, specific–site delivery, nano particulate drug delivery systems, and where carriers should be quickly removed. Studies have proved that bioglass in such cases can be a successful carrier in drug delivery. A study used Fick's diffusion law to treat osteomyelitis with teicoplanin [173]. Teicoplanin was the liquid and borate bioactive glass the solid carrier along with chitosan, citric acid, and glucose. The results of the study showed bioactivity of hydroxyapatite forming from the bioglass when the drug was being released. This system cured the osteomyelitis in tibial bone of rabbits in vivo, and also promoted formation of the tibial bone.

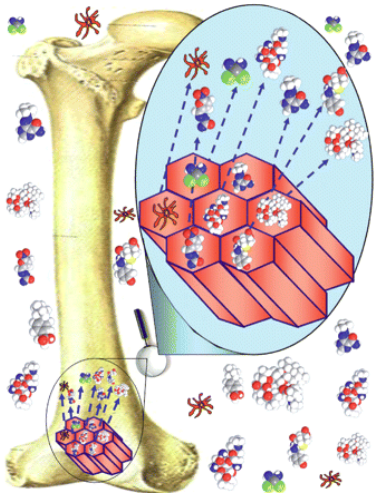


Figure 23. The concept of MBG for drug delivery and bone regeneration [175].

Bioglass has been tried as a vehicle for drug delivery. Vancomycin on bioglass carrier has been tested for treating osteomyelitis with success [176]. Indomethacin was tried with self–setting bioactive cement based on $CaO–SiO_2–P_2O_5$ glass. This mixture hardened and formed hydroxyapatite in about 5 minutes with volume shrinkage of 5 % in simulated body fluid [177]. The fast−acting anti−inflammatory drug ibuprofen was released in the first 8 hours when immersed in simulated body fluid [178–179].

12. The Latest Studies

Baiona et al. published a review paper focusing on research that demonstrates the suitability of bioactive glasses in contact with tissues outside the skeletal system, including muscle and nerve tissue regeneration, treatment of diseases affecting sense organs (eye and ear), embolization of neoplastic tissues, cancer radiotherapy via injectable microspheres, and wound dressing [180]. Ben–Arfa et al. studied the effects of three functional ions (yttrium Y^{3+}, fluorine F^-, titanium Ti^{4+}) on the glass forming ability, sintering, crystallization, and thermo–physical properties of glasses and glass–ceramics in a diopside–calcium pyrophosphate (90 % $CaMgSi_2O_6$–10 % $Ca_2P_2O_7$) system [181]. Liu et al. made an investigation on manufacturing and assessing bioactivity of low fluoride/high phosphate (low F^-/high P_2O_5) bioglases (BGs). Then the effects of BG–conditioned medium on osteoblast–like cell behaviour and BG particles on bactericidal activity [182]. The effect of SrO substitution for CaO into sol–gel glasses with different chemical compositions (mol %) A2Sr:(54–x)$CaO–xSrO–6P_2O_5–40SiO_2$ and S2Sr:(16–x)$CaO–xSrO–4P_2O_5–80SiO_2$ (x= 0, 1, 3 and 5) stabilized at 700 ^oC on their structure (XRD, FTIR) and bioactive properties (SBF test) was searched by Diadek et al. [183]. ElBatal et al. searched for the bone–bonding ability or bioactivity of some prepared borate glasses and their glass–ceramic derivatives from the two systems ($Na_2O–CaO–B_2O_3$) and ($NaF–CaF_2–B_2O_3$) [184]. Siyu et al. reported that the sol−gel derived bioactive $CaO–SiO_2–Ag_2O$ materials were successfully decorated onto and into PAA nano−pores by a sol dipping method and subsequent calcination of gel–glasses at 500 °C. The $CaO–SiO_2–Ag_2O$ decorated porous anodic alümina (PAA) significantly enhanced PAA's apatite–forming ability in SBF. An in vitro antimicrobial activity test demonstrated that the $CaO–SiO_2–Ag_2O$/PAA system was highly effective in inhibiting the growth of both E. coli and S. aureus bacteria [185]. Strontium contained biomaterials have been reported as a potential bioactive material for bone regeneration, as it reduces bone resorption and stimulates bone formation. Therefore, Areplli et al. designed the bioactive glasses to partially substitute SrO for SiO_2 in $Na_2O–CaO–SrO–P_2O_5–SiO_2$ system. This work demonstrates that the substitution of SrO for SiO_2 has got significant benefit than substitution for CaO in the bioactive glass [186]. Abdelghany et al. examined borate glasses containing SrO substituting both CaO and NaO and characterized them for their bioactivity or bone bonding ability [187]. Orgaz et al. developed novel bioactive amorphous glass–glass composite scaffolds with

interconnected porosity [188]. With a continuously increasing aging population and the improvement of living standards, large demands of biomaterials are expected for a long time to come. Further development of novel biomaterials, that are much safer and of much higher quality, in terms of both biomedical and mechanical properties, are therefore of great interest for both the research scientists and clinical surgeons. Compared with the conventional crystalline metallic counterparts, bulk metallic glasses have unique amorphous structures, and thus exhibit higher strength, lower Young's modulus, improved wear resistance, good fatigue endurance, and excellent corrosion resistance. For this purpose, bulk metallic glasses (BMGs) have recently attracted much attention for biomedical applications. Consequently, Lie and Zeng discussed and summarized the recent developments and advances of bulk metallic glasses, including Ti–based, Zr–based, Fe–based, Mg–based, Zn–based, Ca–based and Sr–based alloying systems for biomedical applications [189]. Karasu et al. also gave a review on metallic glasses and their uses [190]. A promising strategy in regenerative endodontics is the combination of human dental pulp stem cells (hDPSCs) with an appropriate biomaterial substrate. Huang et al. studied the effects of zinc and zinc containing bioactive glasses (ZnBGs) on hDPSCs [191]. Szesz and Lepienski published a report on the use of the anodic bonding technique to bond titanium alloy and two different bioactive glasses aiming the medical application of these biomaterials [192]. Tantalum is a bioactive and biocompatible transition metal that has been used as an orthopaedic medical device. It has a range of biological and physical properties that make its incorporation into ionic form into bioactive glass systems promising for various clinical applications. Alhalawani and Towler mentioned about the characterization and properties of novel tantalum–containing glasses of their work [193]. Lizzi et al. have given a systematic review aiming to identify the relationship between the composition of bioactive glasses used in medical applications and their influence on the mechanical and biological properties [194]. The realization of surfaces with antibacterial properties due to silver nanoparticles loaded through a green approach is a promising research challenge of the biomaterial field. Ferraris et al. in their research, have doubly surface functionalized two bioactive glasses with polyphenols (gallic acid or natural polyphenols extracted from red grape skins and green tea leaves) and silver nanoparticles deposited by in situ reduction from a silver nitrate aqueous solution [195]. Kumari et al. synthesized calcium oxy fluoro boro phosphate glasses with fixed concentration of CuO and mixed with different modifier oxides (viz., BaO, SrO, ZnO and MgO) that play a vital role in collagen deposition, cellular activity, proliferation of osteoblasts and in blood vessel maturation, producing enzymes etc., that are necessary for normal functioning of human body [196]. Melli et al. conducted a study on the evaluation of the microstructure and the effect of crystallization on the dissolution mechanism of a Bioglass®–based glass−ceramic scaffold, produced with a powder metallurgy inspired technology [197]. Satyanarayana et al. synthesized glasses of a particular composition (60–x) P_2O_5–20CaO–17Na_2O–3K_2O: xSrO ($0.5 \leq x \leq 1.5$) mol. % using conventional melt quenching technique [198]. Samudrala et al. aimed to elucidate the applications of titania (TiO_2) doped calcium borosilicate glass as a biocompatible material in regenerative orthopaedic applications. In this context, they examined the bioactivity of various concentrations of TiO_2 doped glasses with the help of simulated body fluid (SBF). Cytocompatibility, cell proliferation, and protein expression studies revealed the potential candidature of TiO_2 doped glasses on osteoblast cell lines (MG–63) [199]. Bellucci et al. tested a set of novel materials for bone tissue regeneration in vivo in an animal model. In fact, despite many studies have been devoted to amorphous 45S5 Bioglass®, there is lack in the literature of works aimed to study the in vivo performance of heat–treated–and thus partially crystallized–45S5. As widely reported crystallization limits the bioactivity of 45S5 and is the main reason that prevents a broader use of this material [200].

13. Conclusions

During the past decades, there has been a major breakthrough in development of biomedical materials including various ceramic materials for bone and dental repair as well as implantable drug

delivery systems. Both increases in life expectancy and the social obligations to provide a better quality of life appeared to be the vital factors to this development. Significant attention has been paid towards the use of synthetic graft materials in bone tissue and dental repair and development of new implant technologies has led to the design concept of novel bioactive materials. Bioactive glass inducing active bio mineralization in vivo has been a high demand in the development of clinical regenerative medicine. The replacement of tissues demands very high importance in this technological era. As highlighted in the present article, bioglass is a versatile replacement material, as it is available in multiple forms and also can be moulded into desired forms as per the need of the user. Thus, its scope for use also increases manifold. After two decades of being in use, the most telling is that bioglass has not reported any adverse responses when used in the body. As the use of these compositions increases, in varying clinical fields, it will bring into sight, better applications in repair as well as regeneration of natural tissues. Bioactive glass may be explored by the scientists/researchers/clinicians in a better way and dimension for wellbeing of human kind.

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