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REVIEW ARTICLE

Progress of three-dimensional macroporous bioactive glass for bone regeneration

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Abstract Bioactive glasses (BGs) are ideal materials for macroporous scaffolds due to their excellent osteoconductive, osteoinductive, biocompatible and biodegradable properties, and their high bone bonding rates. Macroporous scaffolds made from BGs are in high demand for bone regeneration because they can stimulate vascularized bone ingrowth and they enhance bonding between scaffolds and surrounding tissues. Engineering BG/biopolymers (BP) composites or hybrids may be a good way to prepare macroporous scaffolds with excellent properties. This paper summarizes the progress in the past few years in preparing three-dimensional macroporous BG and BG/BP scaffolds for bone regeneration. Since the brittleness of BGs is a major problem in developing macroporous scaffolds and this limits their use in load bearing applications, the mechanical properties of macroporous scaffolds are particularly emphasized in this review.

Keywords bioactive glass, biopolymer, bone regeneration, macroporous scaffolds, tissue engineering

1 Introduction

Repairing diseased or damaged tissues is the ultimate goal for studying bioactive materials. Since the 1960s, two generations of biomaterials, bioinert and bioactive implants, have been developed. Millions of clinical trials and applications have proved that prostheses implants based on biomaterials can effectively relieve pain. However, both first and second generation biomaterials have drawbacks, such as high failure rates after long-term prostheses implantation and the lack of response to changing physiologic environments, which are driving scientists to continue the search for a third generation of biomaterials which can stimulate cellular response at the molecular level and thus promote tissue regeneration [1]. Silica based-bioactive glasses (BGs) have excellent osteoconductive, osteoinductive, biocompatible and biodegradable properties, and have high bone bonding rates and these properties have been reviewed in other papers [2–6]. Research on BG-based materials has produced an optimistic outlook of third generation biomaterials.

The bioactivity of BGs depends on their components and structures. The main components of BGs are SiO_2, P_2O_5, CaO and other metal oxides. SiO_2 and P_2O_5 are network formers, whereas Ca^{2+} and other ions, such as Na^+, Mg^{2+}, Zn^{2+}, Sr^{2+} and Ag^+, are network modifiers or have dual roles. The bonding mechanism of BGs to bone tissue has been well explained [1,7]. Briefly, network modifiers disrupt the SiO_2 network by forming ionic interactions with O atoms which make it unstable in body fluids. In the ion exchange process between the BG and the body fluid, Ca^{2+} is released to the surface of the material and forms Si–OH on the glass surface. The released Ca^{2+} ions react with the PO_4^{3-} in the surrounding fluid and precipitate on the Si–OH surface, forming a hydroxyl carbonate apatite (HCA) layer which has a composition that is similar to inorganic natural bones. Thus the HCA layer allows the proliferation and differentiation of osteoblast stem cells to form new bone tissue which enables the scaffold to bond firmly with the bone tissue.

The most promising application of BG is in bone tissue engineering, where three-dimensional (3D) macroporous scaffolds are required. Macroporous scaffolds with interconnected pores bigger than 100 μm can provide a 3D extracellular matrix which enables cell growth on the surface of the pores, forms 3D tissues, vascularizes the inside of the implant and improves the surface interlocks between the scaffold and the surrounding natural tissues

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[8,9]. These scaffolds have higher surface areas and ion exchange rates compared to bulk solid materials and thus they have better bioactivity and biodegradability [10–13]. Macroporous scaffolds can provide 3D structural support for new tissues that are being formed. Additionally they can also stimulate tissue regeneration, deliver drugs locally for therapy and be made to be anti-inflammatories by incorporating specific growth factors, bone morphogenetic proteins, drugs or antibiotics into them. Research on these special functions has been extensively reviewed [13–20]. Many 3D macroporous BG scaffolds have been prepared, but there are still problems that limit their clinical applications. BGs are brittle materials, which causes poor mechanical properties in 3D BG macroporous scaffolds. Improving the mechanical properties of 3D macroporous BG scaffolds is still a major challenge.

Natural bone is a type of reinforced inorganic/organic polymer composite. The subtle macro- and micro-structures endow the bones with excellent mechanical properties and biologic functions [21]. Thus, it is reasonable to design bioactive glass/biopolymer (BG/BP) 3D macroporous scaffolds with structures and mechanical properties that are similar to natural bones. Although great strides have been made in the past, it still remains a major challenge to prepare 3D macroporous scaffolds with good mechanical properties. Research in this field before 2006 has been reviewed by Rezwan et al. [22]. In this review, the progress over the last few years in preparing 3D macroporous bioactive glasses with good mechanical properties is reviewed with the hope of elucidating methodologies that will produce scaffolds that can fulfill the requirements needed for clinical applications. The focus is not only on 3D macroporous scaffolds composed of pure BG, but more importantly, those composed of BG/BP composites.

2 Species of bioactive glass

Many kinds of BGs have been developed since Hench et al. reported this material in 1971 [7]. BGs with different chemical and mechanical properties can be obtained by changing their components or the preparation method. Thus the species of BGs must be taken into account to prepare macroporous BG scaffolds with the desired chemical and mechanical properties.

2.1 Melt-quenching and sol-gel derived BGs

BG can be prepared by a melt-quenching or a sol-gel process. In a melt-quenching process, powders of inorganic species such as SiO_2, CaO, and P_2O_5 are mixed together, and then are melted above the glass transition temperature (T_g) of the target bioactive glasses so that they become a viscous flow. Inorganic ions in the viscous flow enter into the silica network at high temperature. Bioactive glasses form when the viscous flows are suddenly poured into a casting mold or cold water. Because the T_g of the glass is very close to its crystallization temperature (T_c), crystallization occurs and glass-ceramics are formed. Since the first melt-quenching derived bioactive glass (45S5 Bioglass®) was developed in 1971, many other kinds of BGs have been prepared by the melt-quenching method. However, the required high temperature in the melting process limits the strategies that can be used for preparing BG scaffolds with complicated 3D structures.

The first sol-gel derived BG was investigated in 1991 [23]. In a sol-gel process, a solution of silica precursor containing Ca, P, and all other species is prepared and then after hydrolysis and polycondensation reactions, the BG gels which are composed of particles are formed. These gels are usually sintered at 600°C–800°C to cause further condensation, ions to enter into the SiO_2 network and the removal of organic and toxic species [24]. Compared with a melt-quenching method, a sol-gel process has many merits. The sol-gel precursors are in a liquid solution at room temperature and the sintering temperature in a sol-gel process is much lower than the temperature needed to melt inorganic powders which is usually above 1450°C in a melt-quenching method. The liquid precursor at room temperature and the lower sintering temperature supports the preparation of scaffolds with complicated structures, and helps avoid the formation of crystals and the contamination caused by ions diffusing from the mold to the glass at high temperature. A sol-gel process also makes it possible to prepare BG/BP composites or hybrids at low temperature without destroying organic species (this will be discussed in a later part of this review).

Melt-quenching and sol-gel derived materials with similar compositions can have very different structures and properties [25]. This is because sol-gel derived BGs have lots of mesopores due to the incomplete condensation process at low temperature [2,26], which has been confirmed by our group (Fig. 1). All the reactants are homogenously mixed at the molecular level, which leads to good quality glasses with easily controlled morphologies. The composition of the glass formed also dramatically affects the glass. For example, binary phosphosilicate glasses have been prepared with a high phosphate content (P : Si atomic ratio 4 : 35), which changes the silicon coordination from tetrahedral to octahedral after heat treatment (Fig. 2) [27].

Recently a new phytic acid derived sol-gel glass, which provides a much broader bioactive compositional range especially at high phosphate content (Fig. 3), was reported [28]. The phytic acid helped calcium enter into the gel network without further calcination and also provided better control over the overall sol-gel BG dissolution rate which is a requirement for various terminal biomedical applications [28]. These samples are chemically homogeneous on the micron scale (Fig. 4) [26].

Compared with sol-gel derived BGs, melt-quenching derived BGs have denser SiO_2 networks because the dense pure SiO_2 glass powder is added to the mixture before melting at high temperature. Thus they have better mechanical properties than sol-gel derived BGs with the same compositions. However, because the microporous or mesoporous structures produce bioactivity, the sol-gel derived BGs are bioactive with up to 90 mol-% SiO_2 content, which is much higher than the 60 mol-% SiO_2 content limit for melt-quenching derived BGs [23]. Thus the mechanical properties of sol-gel derived BGs can be improved by increasing the content of the SiO_2 network with little loss of bioactivity. Sol-gel derived BGs without P_2O_5 provide another choice for bioactive materials with good mechanical properties because of their strong SiO_2 network [26].

2.2 BGs containing different components

Introducing ions into a BG can optimize its properties or create new functions in the BG. Besides Ca^{2+} and Na^{+}, the most important ions being introduced into BGs are K^{+}, Mg^{2+}, Sr^{2+}, Zn^{2+} and Ag^{+}. These network modifiers form metal oxides inside the BGs and significantly affect their properties. Some typical research on adding these network modifiers to improve the BGs' properties are listed in Table 1. Adding alkali oxides like Na_2O and K_2O to reduce the amount of SiO_2 decreases the melting temperature and the viscosity of the BGs, however the BGs devitrify if the SiO_2 content is lower than 54 mol-% [29]. Replacing some of the Na_2O with K_2O, increases the amount of alkali earth metal oxide and replacing some of the CaO with MgO affects the BG's crystallization behavior and changes the devitrified glass to one with a large working range which produces the properties needed for manufacturing. Thus SiO_2-P_2O_5-CaO-MgO-Na_2O-K_2O [30,31] and SiO_2-CaO-Na_2O-MgO [32,33] are very popular systems for making BG scaffolds. Incorporating MgO into BGs has also been found to induce the formation of whitelockite-like phases in the biomimetic layer that is formed on the BGs; this in turn affects the cell behavior on the scaffold surface and the bonding to natural tissues [34–36]. Introducing silver into a BG can give the scaffold an antibacterial property, which is very important for minimizing local bacterial infections [37,38]. BGs containing ZnO have higher specific surface areas which favor the precipitation of HCA [39,40]. CaO-SiO_2 BGs with SrO incorporated into them showed better in vitro bioactivity [41]. BGs containing different amounts and species of ions have different mechanical properties. However, this difference is not significant enough to change the mechanical properties of the macroporous scaffolds.

2.3 Apatite-wallastonite bioactive glass-ceramic (AW GC)

Because of its excellent mechanical properties, AW GC which is composed of MgO-CaO-SiO_2-P_2O_5-CaF_2 has attracted great interest since it first reported by Nakamura et al. [42]. AW GC had a bending strength of 157 MPa and a compressive strength of 1060 MPa, but its fracture toughness of 10 MPa is lower than that of natural cortical bones. A mixture of AW GC and fibrin has better osteoconductivity as a bone defect filler than a mixture of HA and fibrin. When the ratio of AW GC to fibrin was about 1 : 4 by weight, the formed bone tissues after 24 weeks of implantation had similar compressive strength, compressive stiffness and fracture toughness as those of natural cancellous bones [43]. These excellent mechanical properties make AW GC a good candidate for implants used under high load conditions [44]. AW GC can be prepared by both melting and sol-gel methods [45,46], which supports the preparation of AW GC with complex structures. Making AW GC/biopolymer composites is a good way to obtain 3D macroporous scaffold [45,47,48].

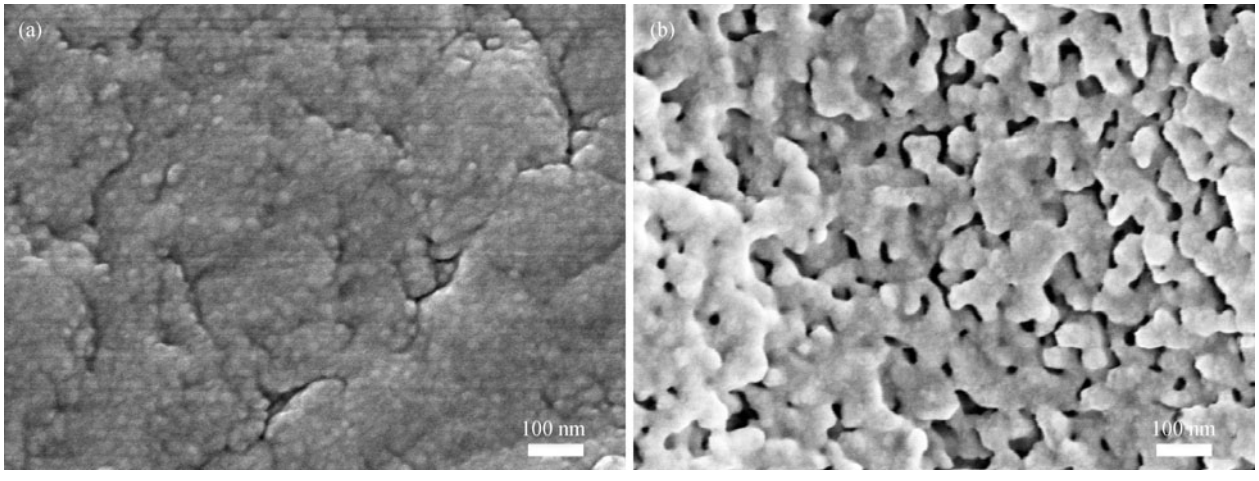

Fig. 1 Scanning electron microscopy (SEM) images of two typical BGs synthesized by a melt-derived method and a sol-gel process respectively: a) melt-derived BG 45S5; b) sol-gel derived BG 70S30C

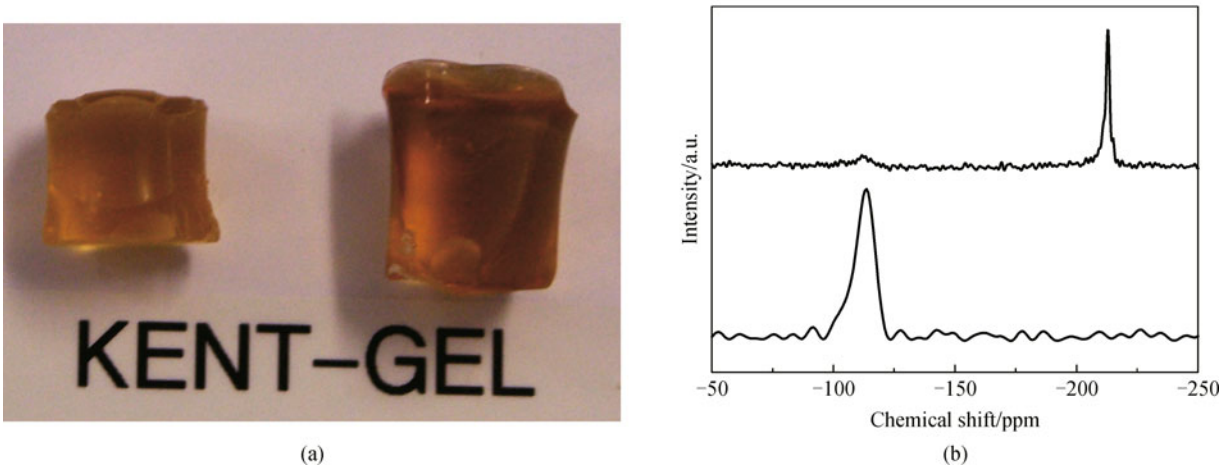

Fig. 2 (a) Picture of gel dried at 120°C (b) ^{29}Si magic angle spinning (MAS) NMR spectra of gel dried at 120°C (lower) and 450°C (upper) [27]

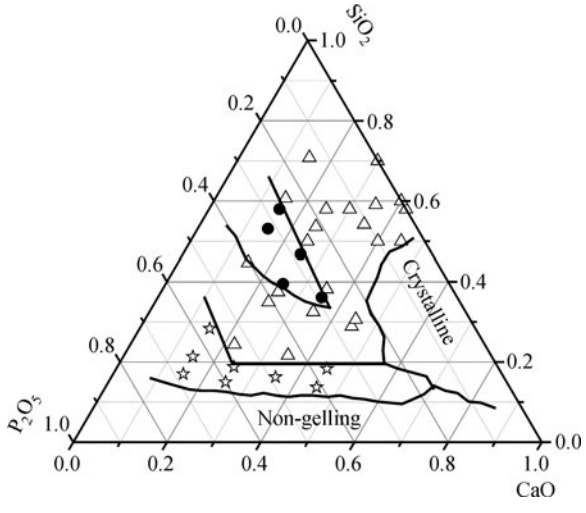

Fig. 3 Composition dependence of bioactive CaO-SiO_2-P_2O_5 gel-glasses. Triangle HA formation, filled black circle no HA formation, star dissolution [28]

3 Natural biodegradable polymers used for bioactive glass composites

Although BGs have a higher compressive strength, they are very brittle and have much lower fracture toughness than natural bones. This inherent drawback limits their application as load-bearing scaffolds. BG/BP composites that mimic the structure of bone could be a good strategy for obtaining scaffolds with good mechanical properties. Many BPs, including both natural BPs and synthetic BPs, have been used to prepare BG/BP composites. The mechanical properties of synthetic BPs, such as saturated aliphatic polyesters, polypropylene fumarates, polyhydroxyalkanoates, poly(anhydrides), poly(ortho-ester) and polyphosphazene, have been reviewed in other papers [22,49]. Saturated aliphatic polyesters including poly (lactic acid) (PLA), poly(glycolic acid) (PGA), poly (lactic-co-glycolide) (PLGA), and poly(ε-caprolactone) (PCL), and polyhydroxyalkanoates including poly(hydroxybutyrate) (PHB), poly(hydroxybutyrate-co-hydroxyva-

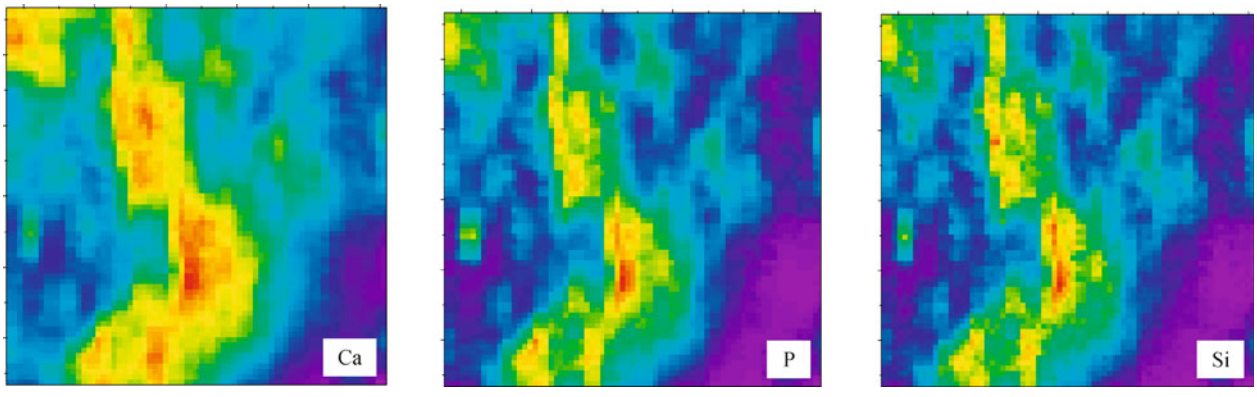

Fig. 4 μ-X-ray fluoroscopy (XRF) element mapping of a 210 × 210 μm^2 zone of the BG after heat treatment at 120°C [26]

lerate) (PHBV), and poly(vinyl alcohol) (PVA) are very popular. Natural BPs that are commonly used in BG/BP composite preparation and bone tissue engineering, and their mechanical properties are emphasized in this review.

The physical and chemical properties of natural BPs are not as reproducible as those of synthetic BPs because of their animal origins and natural BPs have a potential for the transfer of diseases. However many researchers are engineering BG/natural BP composites that mimic natural bone structure and which contain about 35% (by mass) type I collagen, because natural BPs possess excellent biocompatibility, bioactivity, and biodegradability. The mechanical and integrated properties of natural BPs can be improved significantly by three basic methods: crosslinking, compounding with other polymers, and plasticization.

3.1 Chitosan

Chitosan is a cationic polysaccharide prepared by the deacetylation of chitin, which comes from many natural organisms including mushrooms, the exoskeletons of crustaceans, and the cell walls of fungi. Because chitosan is most often prepared as films for applications, chitosan films have been extensively studied. Table 2 shows the properties of chitosan materials.

Chitosan is often plasticized, or incorporated with other polymers such as collagen, gelatin [51], saturated aliphatic polyesters, or starch by chemical grafting or blending [50,52] which improves the mechanical properties and the range of applications for the materials in fields like bone tissue engineering and cartilage, nerve, blood vessel, and skin replacement [53]. Plasticization can decrease the tensile strength of chitosan films but it increases their elongation at break [50]. Incorporating chitosan films into other polymers usually imparts the excellent properties of the other material to the chitosan film. Blending chitosan with PLA can improve its tensile strength and water vapor barrier [54]. Dry commercial chitosan films have break stresses an order of magnitude higher than those of wet chitosan films, but after blending with PCL and solvent annealing, a significant improvement in the mechanical properties of the chitosan films in wet conditions was observed [55]. By soaking chitosan films made from prawn shell waste in 2-ethyl-2-hydroxymethyl-1,3-propandiol trimethacrylate (EHMPTMA), a trifunctional monomer and 2-ethylhexylacrylate (EHA), a monofunctional monomer, Khan et al. improved the tensile strength of their chitosan film from 7 to 29 Mpa [58].

3.2 Collagen

Bones composed of collagen and hydroxyapatite nanocrystals have excellent mechanical properties, but how to prepare collagen composites with mechanical properties that are comparable to natural bone is still not clear. Thus, research on the preparation of bone scaffolds with collagen is very important for clinical application. Table 3 shows the mechanical properties of some types of collagens.

Crosslinking is an effective way to improve the mechanical properties of collagen films, fibrils and hydrogels. The crosslinkers used to crosslink collagens include malic acid derivatives, 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide, *N*-hydroxysuccinimide, diepoxypropylether, glutaraldehyde [61–67], and 4-butyrothiolactone [68]. It has been reported that the break modulus of a collagen film increased when the dry crosslinking network was allowed to swell in a fibroblast culture medium [62]. In another report, collagen fibrils crosslinked with carbodiimide showed bending moduli ranging from 1.0 to 3.9 GPa, but a corresponding fibril that was allowed to swell in a phosphate-buffered saline

Table 1 Species of bioactive glasses for the preparation of scaffolds

Composition	Preparation method	Improved property or added function	Ref.
MgO, K_2O, Na_2O, CaO, B_2O_3, P_2O_5, SiO_2	Melting	Large working range	[29]
AgO, MgO, K2O, Na_2O, CaO, SiO_2, P_2O_5, CaF_2	Melting	Antibacterial	[38]
SiO_2, CaO, ZnO	Sol-gel	Enhanced specific surface area	[39]
SiO_2, CaO, P_2O_5, ZnO	Sol-gel	Enhanced specific surface area	[40]
MgO, CaO, SiO_2, P_2O_5, CaF_2	Melting	Mechanical properties	[42,43]

Table 2 Mechanical properties of chitosan materials

Biopolymer	Method of treatment	Tensile strength /MPa	Elastic modulus /MPa	Ref.
Chitosan film	–	1.4–89	$2.7–4.1 \times 10^3$	[50,52,54–57]
Chitosan film	Plasticization	10–74	60–364	[50,52]
Chitosan/PVA/PCL/PLA films	Blending	0.5–73	2–470	[52,54,55,57]
Chitosan/EHMPTMA/EHA	IPN compounding	29	–	[58]

solution showed a decrease in bending moduli to 0.07–0.17 Gpa [63].

3.3 Gelatin

Due to different origins and different degrees of crosslinking, the reported tensile strengths and elastic moduli of gelatins vary greatly (Table 4) [69–79]. In addition, as the number of triple-helixes increases, the gelatin films become stronger [80].

Similar to the preparation of chitosan and collagen composites, gelatin is also often crosslinked, plasticized or combined with polymers to integrate the material properties [81]. The most popular plasticizers are water and polyols including hydroxypropyl starch, glycerol, sorbitol, and sucrose. After gelatin films were plasticized with water or polyols, their tensile strength decreased significantly and the elongation at break increased [82–84]. Synthesizing poly(2-hydroxyethylmethacrylate) (PHEMA)-gelatin interpenetrating polymer networks improved the elastic moduli of the gelatin films [74]. Some processing technologies can also improve the mechanical properties of gelatin films. Gelatin films that were plasticized by glycerol and sorbitol and processed by extruding and heat-pressing had a tensile strength of 20.4 Mpa [84]. By using uniaxial stretching, the elastic modulus and stress at break of gelatin films increased linearly with the increase of draw ratio and was five times as high as those of undrawn samples [76]. The polymerization of nordihydroguaiaretic acid (NDGA) inside gelatin hydrogels effectively improved the mechanical and thermal properties of the hydrogels [85]. The fracture stress and compressive stiffness of the hydrogels achieved maximum values of 0.5 ± 0.1 MPa and 5.1 ± 1.2 MPa for 10% gelatin gels, respectively.

4 Synthesis methods, structure and mechanical properties of three-dimensional macroporous bioactive glass

The properties of 3D macroporous scaffolds primarily depend on their chemical composition, their preparation method, and the structures of the BG, the bioceramic and the composite which include crystal, amorphous and macroporous structures. The porosity and pore size of the scaffolds is determined by the preparation methods and has a big effect on their mechanical properties [86]. Lots of 3D macroporous scaffolds made of pure BGs, bioceramics or their biopolymer composites have been prepared in an effort to make implants with perfect performance. The pore size in 3D macroporous scaffolds, including the interconnected pore size, must exceed 100 μm in order to meet the requirement of tissue ingrowth [24]. The composition, preparation method, pore size and mechanical properties of various BG 3D macroporous scaffolds are listed in Tables 5 and 6.

4.1 Foam synthesis

Foam synthesis is a convenient way to make macroporous materials. Here a surfactant is added to the precursor solution to form an emulsion and macroporous structures can be obtained after gelation and sintering at high temperature. Using this method and a sol-gel process, Jones et al. prepared macroporous 70S30C (70 mol-% SiO_2, 30 mol-% CaO) with mesopores of 10–20 nm [87]. After sintering at 800°C, this material had a compressive strength similar to that of trabecular bone. An online small-angle X-ray scattering (SAXS) study showed that the average pore size of the 70S30C foam decreased from 40.5 to 30.7 nm after being immersed in simulated body fluid

Table 3 Mechanical properties of collagen materials

Biopolymer	Method of treatment	Tensile strength/MPa	Elastic modulus /MPa	Ref.
Collagen matrices and films	–	20–92.5	1.16×10^3–2.0×10^3	[59,60,62,65,68]
Collagen gel	–	0.13	0.4 – 0.6	[66,67]
Collagen matrices and films	Crosslinking	10.1–77.9	$1.12 \times 10^3 - 2.5 \times 10^3$	[62,65,68]
Collagen gel	Crosslinking	–	2.1–8.0	[66,67]

Table 4 Mechanical properties of gelatin materials

Biopolymer	Method of treatment	Tensile strength/MPa	Elastic modulus/MPa	Ref.
Gelatin film	–	1.7–15	4.6×10^{-2}–3.5×10^3	[71,73–75,78]
Gelatin/ PHEMA	Compounding	–	8.0×10^{-2}	[74]
Gellan/gelatin	Compounding	10–60	–	[79]
Gelatin film	Crosslinking	80–96	3.3×10^3–3.7×10^3	[72]
Gelatin film	Uniaxial stretching	–	0.5×10^2–2.5×10^2	[76]
Gelatin film	Plasticization	20.4–130	2.5×10^2–2.1×10^3	[82–84]

Table 5 Preparation methods, pore size and mechanical properties of typical macroporous BG scaffolds

Bioactive glass	Preparation method	Pore size/μm	Compressive strength/MPa	Elastic modulus /MPa	Ref.
SiO_2, P_2O_5, CaO, MgO, Na_2O, K_2O	PU sponge template	100–500	1		[30]
SiO_2, P_2O_5, CaO, MgO, Na_2O, K_2O, CaF, Ag		> 100	2		[38]
SiO_2, P_2O_5, CaO, Na_2O		510–720	0.3–0.4		[90]
Na_2O, K_2O, MgO, CaO, SiO_2, P_2O_5, B_2O_3		200–500	10		[91]
SiO_2, P_2O_5, CaO, MgO, Na_2O, K_2O	Phase separation	6–120	16–180	4×10^3–25×10^3	[31]
SiO_2, CaO	Foam synthesis	500; 100	2.26		[87]
SiO_2, CaO, Na_2O, K_2O, P_2O_5, MgO, CaF_2	Polymer particle template	> 100	20		[94]
SiO_2, P_2O_5, CaO, MgO, Na_2O, K_2O	Freeze extrusion	300	140	5×10^3–6×10^3	[106]

Table 6 Preparation methods, pore size and mechanical properties of typical macroporous BG/BP scaffolds

Bioactive glass	Biopolymer	Preparation method	Pore size/μm	Compressive strength/MPa	Elastic modulus/ MPa	Ref.
MgO, CaO, SiO_2, P_2O_5, CaF_2 (AW GC)	Chitosan	PU sponge template	100–500	3.11		[93]
SiO_2, CaO, P_2O_5	PHB	Salt particle	250–300			[99]
SiO_2, NaO, P_2O_5, CaO (45S5)	PU	Salt particle	100–400			[100]
SiO_2, CaO, P_2O_5	Gelatin	Phase separation	200–500		50–80	[101]
SiO_2, CaO, P_2O_5	PLLA*		10–400	0.35	8	[102]
SiO_2, CaO, P_2O_5, MgO	PLLA		113, 149	0.75, 0.65	11,7	[103]
SiO_2, NaO, P_2O_5, CaO (45S5)	PCL		100–300	0.2	0.251	[104]

* Poly(lactic acid)

(SBF) for 6 h (Fig. 5) [88]. Gel-cast foaming is another foam synthesis method. In this method, instead of a sol-gel precursor, a melt-quenching derived BG powder is mixed with the surfactant, monomer, and initiator. After a series of steps, including an initiating reaction, casting the gel into a mold and thermal decomposition of the organic species, 3D macroporous scaffolds can be obtained. A 3D macroporous scaffold composed of melt-quenching derived SiO_2-P_2O_5-CaO-Na_2O-K_2O BG was prepared using this method [89].

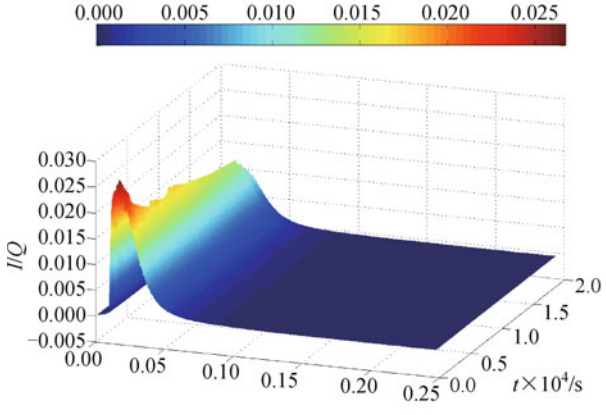

Fig. 5 Graphical representation of SAXS data for a foam immersed in SBF as a function of time [84]

4.2 Template synthesis

Good control of the porous structure of 3D macroporous scaffolds is important to fulfill the requirements of tissue ingrowth. Template synthesis may be the easiest way to control the porous structure of synthesized scaffolds, because the pore size, pore size distribution and interconnectivity of the pores of the scaffolds can be easily replicated from templates which are obtained by traditional methods. Many kinds of templates have been chosen to prepare 3D macroporous scaffolds, such as starch, polyurethane (PU) sponges, polymer beads and particles. A macroporous GC scaffold of SiO_2-CaO-Na_2O-MgO was prepared by thermally treating a mixture of glass powders and three different starch templates [32].

PU is a popular template for preparing macroporous GC scaffolds. Template synthesis with a PU sponge is also called sponge impregnation or organic foam impregnation. In this method, a slurry containing BG particles, a polymer binder and water is impregnated into a PU sponge. After thermal treatment to remove the PU template and the binder, the BG particles are sintered and a replica of the PU

template is obtained. A macroporous structure with a high porosity of 90% was prepared with a typical bioactive glass 45S5 using PU template synthesis [90]. The bending and compressive strengths of these materials were tested and reached 0.4–0.5 MPa. A 3D macroporous bioactive borosilicate scaffolds prepared using a PU template had pore sizes of 200–500 μm and a porosity of about 70% [91]. Its compressive strength was close to that of human cancellous bone. A BG scaffold containing silver ions has also been prepared with a PU template; it had a compressive strength of 2 MPa and demonstrated antibacterial activity [38].

Macroporous SiO_2-P_2O_5-CaO-MgO-Na_2O-K_2O and AW GC scaffolds have also been prepared using this same method [30,92]. After coating the pore surface of the macroporous AW GC scaffold with chitosan (CS), the macroporous composite scaffold had pore sizes of 100–500 μm and a compressive strength of 3.11 MPa [93], which is eight times higher than that of the scaffold before coating and is in the range of the compressive strength of trabecular bone (2–12 MPa) [87].

A cubic macroporous scaffold with excellent compressive strength and a porosity of up to 50% was prepared by uniaxial pressing and sintering a mixture of polyethylene particles and GC powders [94]. A macroporous scaffold of 45S5 bioglass® prepared via a similar pressing and burning out method and with a similar polyethylene particle template had a compressive strength of 123 MPa with a porosity of 60% and a pore size greater than 100 μm [95].

Colloidal crystals are ideal templates for preparing 3D ordered macroporous materials because they have perfect monodispersed microspheres with a close-packed structure. Yan et al. prepared BG with 3D ordered macroporous structures by a sol-gel process and by replicating from a polymer colloidal crystal template [96]. They confirmed hydroxycarbonate apatite mineralization on the BG surface in vitro.

BG with 3D ordered mesoporous-macroporous structures have also been prepared by combining self-assembled polystyrene beads, polyester fibers and surfactants [97,98]. Mesostructures can promote immobilization of proteins and the adhesion of cells, and thus are useful for tissue ingrowth [19]. Salt leaching can also be classified as a template synthesis method, where the template is salt particles with a chosen size. In this method, BG and BP are dispersed and dissolved in a solvent, and then the solution is poured into a bed of salt particles. After rinsing the mixture with water, the salt particles are leached out, leaving a BG/BP macroporous scaffold. For example, a macroporous BG/PHB (polyhydroxybutyrate) scaffold with a porosity of 84% and pore sizes of 250–300 μm has been prepared [99]. Similarly, biodegradable polyurethane (PU) and bioactive glass 45S5 were used to prepare macroporous materials through salt leaching [100]. The porosity exceeded 70% and the pore sizes were 100–400 μm. It was also found that the storage modulus of the macroporous materials increased as the BG content increased.

4.3 Phase separation method

Phase separation combined with freeze-drying is a popular way to prepare 3D macroporous BG/BP composite scaffolds. In this method, suspensions of BG particles or BG particle/BP mixtures are mixed with water or a small organic molecule solvent that can sublimate or be exchanged by another solvent at low temperature. After casting the mixture into a mold at low temperature and then freeze-drying, water or the volatile organic solvent are removed, leaving macropores inside the materials. In this way, BG/Gelatin macroporous nanocomposites with porosities of 72%–86% have been prepared [101].

In another work, PLLA containing different amounts of GC were mixed with dioxane. After phase separation at low temperature and freeze-drying, macroporous GC/PLLA structures with porosity and compressive strength that depended on the amounts of GC were prepared [102]. The porosity was in the range of 88%–92% and a composite containing 20 wt-% GC had the best mineralization property in simulated body fluid. A SiO_2-P_2O_5-CaO-MgO/PLLA macroporous structure that was prepared in the same way, showed similar mechanical properties with pore sizes between 110 μm and 150 μm [103], whereas a BG/PCL macroporous structure with porosities that varied from 88%–92% showed a 60% deformation but its compressive strength and elastic modulus were a little weaker [104].

Unidirectional freezing suspension is a novel freeze-drying method to prepare scaffolds with oriented porous structures. Liu et al. prepared a macroporous scaffold from melt-quenching derived SiO_2-P_2O_5-CaO-MgO-Na_2O-K_2O BG [31]. The scaffold had a very high compressive strength which varied from 180 to 16 MPa, but the average pore diameter was 6–120 μm, which is a little smaller than the requirement for tissue ingrowth. Recently, macroporous scaffolds from sol-gel derived BG, instead of melt-quenching derived BG, were prepared by coupling sol-gel with freeze drying; however the mechanical properties of the scaffold have not been reported [105].

4.4 Freeze extrusion fabrication

Doiphode et al. reported a scaffold prepared from a melt-quenching derived 13–93 BG by freeze extrusion fabrication [106]. The 13–93 BG slurry was extruded through a nozzle and deposited layer by layer in a cold environment with the aid of a computer to form a grid-like 3D network structure. After sintering at 700°C, the pore width of this macroporous structure was about 300 μm. The preparation process is very easy and reproducible. The scaffold had a porosity of 50% and the compressive strength and elastic

modulus were comparable to those of cortical bone. The uniform pore width and grid diameter of the dense glass may be the reason for the good mechanical properties.

Hierarchical porous BG/PCL composite scaffolds with meso-macro-giant pores have recently been prepared by combining nonionic triblock copolymer templates for creating mesopores and polymer templates or salt-leaching methods for creating macropores with freeze extrusion fabrication [107,108]. However, the compressive modulus in these materials decreased to 2–4 MPa due to the existence of meso-macro pores [108].

5 Structures of BG/BP composites

5.1 BG/BP blends

BG/BP composites can be incorporated or blended at the molecular, nanoscale or macroscale level. The macroporous materials discussed above are BG and BP composites that were synthesized independently and then blended together. Thus they have separated phases from the nano to the macroscale level. From the viewpoint of traditional polymer composite materials, there are three kinds of blended BG/BP composite structures which are classified by the ratio of BG to BP. When the BP in the composite is dominant, BP forms a continuous phase and BG forms a dispersed phase (Fig. 6(a)). In this case, BP has a greater effect on the integrated properties of the composite. When the amount of BG is dominant, BG forms the continuous phase and BP the dispersed phase (Fig. 6(b)). Here, BG has more effect on the integrated properties of the composite. When BG and BP both form continuous phases, the composite has properties of both materials (Fig. 6(c)). Figure 6(d) shows BG/BP hybrid without phase separation.

The brittle macroporous BG scaffold can be softened and strengthened by combining it with BP. However, there are still some problems that need to be resolved. The different properties of the components and the weak interactions at the interfaces between the different phases can lead to nonuniform properties throughout the scaffold. For example, when the thickness of a pore wall is comparable to the size of the BG particles, the pore wall can have a much higher BG content than the average BG content throughout the whole macroporous composite since big BG particles can be dominant in a small area. This causes a material that is as brittle as a pure BG. In addition, the mechanical properties can decrease in vivo because BP degrades much more quickly than BG and leaves brittle and porous BG particles behind After the degradation of BP, the BG particles may be exposed and released, which may cause inflammation in vivo. A

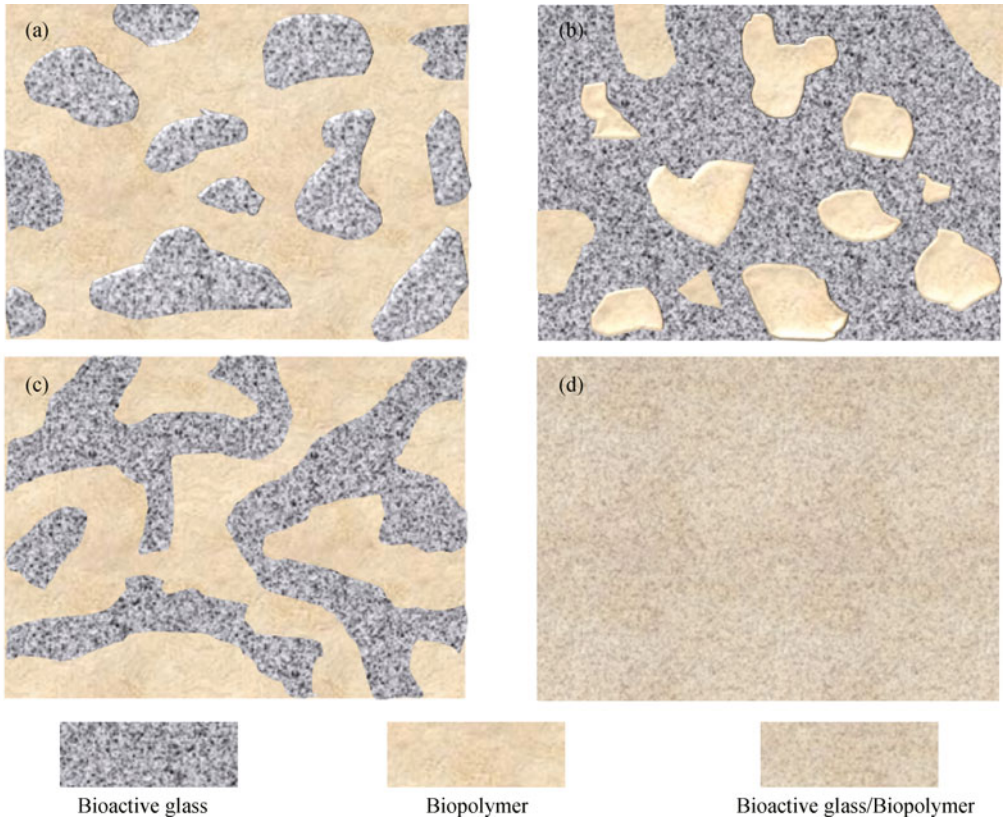

Fig. 6 Types of BG/BP blend and hybrid structures: (a) BP is dominant and forms a continuous phase in the blend; (b) BG is dominant and forms a continuous phase in the blend; (c) BG and BP both form continuous phases; (d) BG/BP hybrid without phase separation

possible way to resolve these problems may be to combine BG/BP at the molecular level.

5.2 BG/BP hybrid

BG/BP hybrids combined at the molecular level have shown great potential in clinic applications due to their uniform structures and properties, but there are still some problems that need to be resolved in the synthesis of these hybrid materials [109]. BG/BP hybrids have strong interactions like covalent bonding, van der Waals forces, hydrogen bonding and molecular entanglements between the inorganic BG molecules and the BPs. They have no phase separation and show uniform surface bioactivity, biocompatibility, and mechanical strength throughout all parts of the scaffold. No BG particles are released when the materials degrade which prevents problems with inflammation. Their components degrade as one material at the same time and rate and thus the hybrid scaffolds maintains uniform properties throughout the whole degradation process. It has been shown that macroporous scaffolds made of silica/gelatin hybrids have covalent interactions between the silica and gelatin constituents and these are essential to enable the precise control of mechanical and dissolution properties [110]. This hybrid was stronger and not as brittle as BG due to the incorporation of BP at the molecular level.

Molecular design is very important to synthesize BG/BP hybrids with excellent integrated properties because BG and BP molecules must be grafted with functional groups that can interact strongly or react with each other in order to bond BG and BP molecules together and enhance their compatibility. BPs and inorganic ions which are necessary for bone formation must be mixed together without phase separation or sedimentation, and need to form uniform composites without toxic residues after reaction. However, BG composites can only be prepared by melting and sol-gel processes. BPs inevitably discompose at high temperatures if they are added to melted BG and the low solubility of BPs in the BG sol-gel precursors also limits their applications in sol-gel systems. Thus designing new BP molecules that are soluble in sol-gel systems is important for the preparation of BG/BP hybrids.

PVA has been successfully dissolved into BG sol-gel precursors to form BG/PVA hybrids [111–115], and the macroporous scaffolds of the BG/PVA hybrids showed mechanical properties comparable to those of trabecular bone (2–12 MPa). Another problem is that ions, such as calcium, can only diffuse into silica networks at temperatures above 400°C [87], and nitrates which are popular ion sources have to be heated to above 600°C to remove toxic nitrate by-products [116]. Thus it is necessary to find new ion sources that can diffuse into the silica network and new methods that can help to form BG/BP hybrids at low temperatures. Li and Qiu have shown that with the help of non-toxic phytic acid, calcium ions can be incorporated into networks at temperatures as low as 120°C [28], which may prove to be a suitable strategy.

6 Conclusions and outlook

Natural bones have complicated and subtle organic/inorganic composite structures. It continues to be a major challenge to synthesize biomaterials that mimic the structure and function of natural bones. An ideal artificial scaffold should have several features which include bioactive and biodegradable compositions; nontoxic degradation products; a uniform degradation rate throughout the whole scaffold; suitable 3D macroporous structures with an interconnected pore size greater than 100 μm and excellent mechanical properties. As reviewed above, many 3D macroporous BG and BG/BP scaffolds with excellent integrated properties have been synthesized in the past few years by novel methods. Most of these scaffolds have outstanding bioactive and biodegradable properties, have nontoxic degradation products and have suitable interconnected pore sizes. However, to the authors' knowledge, no BG/BP composites with uniform degradation rates have been prepared by mixing BG particles with BPs. Synthesizing BG particles with degradation rate similar to BPs is an important area of research that still needs to be pursued. Neither BGs nor BPs by themselves have mechanical properties that are good enough to meet the requirements needed for strong load-bearing scaffolds. Improving the interactions between BGs and BPs by chemical modification, copolymerization or by blending BPs with polymers with good mechanical properties could be good ways for enhancing the mechanical properties of BG/BP macroporous scaffolds. BG/BP hybrids which have enhanced fracture toughness and uniform bioactive properties may be another good choice for 3D macroporous scaffolds.

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