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Advances in bioactive glass-containing injectable hydrogel biomaterials for tissue regeneration

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Abstract

Successful tissue regeneration requires a scaffold with tailorable biodegradability, tissue-like mechanical properties, structural similarity to extracellular matrix (ECM), relevant bioactivity, and cytocompatibility. In recent years, injectable hydrogels have spurred increasing attention in translational medicine as a result of their tunable physicochemical properties in response to the surrounding environment. Furthermore, they have the potential to be implanted via minimally invasive procedures while enabling deep penetration, which is considered a feasible alternative to traditional open surgical procedures. However, polymeric hydrogels may lack sufficient stability and bioactivity in physiological environments. Composite hydrogels containing bioactive glass (BG) particulates, synergistically combining the advantages of their constituents, have emerged as multifunctional biomaterials with tailored mechanical properties and biological functionalities. This review paper highlights the recent advances in injectable composite hydrogel systems based on biodegradable polymers and BGs. The influence of BG particle geometry, composition, and concentration on gel formation, rheological and mechanical behavior as well as hydration and biodegradation of injectable hydrogels have been discussed. The applications of these composite hydrogels in tissue engineering are additionally described, with particular attention to bone and skin. Finally, the prospects and current challenges in the development of desirable injectable bioactive hydrogels for tissue regeneration are discussed to outline a roadmap for future research.

Keywords: Injectable hydrogels; Bioactive glasses, Bone regeneration, Wound healing; Composite gels, Biomaterials

1. Introduction

Rapidly developing biomaterial technologies are revolutionizing the field of tissue engineering. Hydrogel materials have emerged as suitable candidates, among many biomaterials recently developed, for diverse biomedical applications as they provide a highly hydrated microenvironment that emulates, to a large extent, the dynamic nature of native extracellular matrix (ECM).[1, 2] Hydrogels are three-dimensional (3D) networks of hydrophilic polymer chains that are able to maintain a large amount of water or biological fluids (often $\geq$ 90%) within their structure while remaining structurally undamaged and insoluble, which render them particularly suitable for cell culture and tissue engineering applications. Hydrogels can be classified on various bases such as composition, appearance, type of crosslinking, configuration, and network electrical charge.[3] Hydrogels can be synthesized from a variety of naturally-derived and/or synthetic polymers, with pros and cons for each type of base material, as well as hybrid combinations, and through a plethora of chemical (e.g. Schiff-base reaction, Michael-type addition reaction, click chemistry, disulfide bond formation, and radical polymerization) or physical (e.g. hydrogen bonding, ionic interactions, hydrophobic interactions) crosslinking mechanisms, which should be precisely selected to match the specific application of interest.[4, 5] The molecular structure of hydrogels can be tailored in order to respond to the changes in the surrounding environment intelligently.[6] There are a range of comprehensive reviews available that discuss the hydrogel chemistry.[7-9] Injectable hydrogels offer significant advantages over the conventional treatment techniques. Needless to say, invasive surgical procedures are required for the implantation of conventional preformed hydrogel scaffolds, and sufficient knowledge of the size and shape of defects or cavities to be filled prior to the implantation of these hydrogel scaffolds is essential, which renders filling defects with irregular margins problematic. On the contrary, injectable hydrogels are in solution, suspension, or gel states prior to injection and solidification or self-healing will occur *in vivo*.[10] From a clinical point of view, injectable hydrogels not only can be minimally invasively delivered through a cannulated needle or catheter to the defect site, but can also fill out the defects of any shapes, enable a more uniform distribution of bioactive molecules within the scaffold matrices and allow co-injection of cell-suspension along with the hydrogel precursors. Consequently, these hydrogels can lead to a smaller operative site dead space, reduced patient discomfort, decreased risk of postoperative infection, shorter hospital stay, and lower cost of treatment. [11-13] As such, injectable hydrogels are promising biomaterials not only for engineering both hard and soft tissues, but also to serve as pro-healing tissue adhesives and controlled-release devices for local and sustained delivery of therapeutics.[14-17] To obtain desirable responses *in vivo*, a promising strategy is to prepare multicomponent composite hydrogels to gain distinct functionalities within a hydrogel network.[18] A number of organic and inorganic fillers, such as ceramics, carbon-based, metallic and polymeric materials, have been incorporated within hydrogel networks to attain a composite with superior mechanical and biological properties as well as tailored functionalities.[19] Composite hydrogels incorporated with bioactive glasses (BGs) have received significant interest in recent years due to their combined bioactivity, biocompatibility, pro-angiogenic properties, and osteogenic effects.

BGs belong to bioceramics and were first invented by professor Hench in 1969, commercialized under the tradename of 45S5 Bioglass® (Novabone, FL).[20] The Bioglass® a quaternary system with composition of 45 wt.% SiO_2-24.5 wt.% CaO-24.5 wt.% Na_2O-6.0 wt.% P_2O_5. Since then, numerous studies have been conducted to dope this parent glass system with different therapeutic elements or reformulate it towards more specific, tailored properties. This resorbable synthetic bioactive material was initially manufactured to repair bone defects

via stimulating bone regeneration. Upon implantation, the dissolution products of BGs facilitate bone bonding stemmed from the formation of surface hydroxycarbonate apatite (HCA), which has a similar composition and structure to the bone mineral.[21] Formation of HCA on the surface of BG-containing materials can modulate protein adsorption, which in turn, can promote fibronectin-mediated osteoblast-like cell adhesion and differentiation.[22, 23] It is also well-known that ionic dissolution products of BGs can stimulate osteogenesis and angiogenesis both *in vitro* and *in vivo*.[24, 25] More products of BGs have been commercialized in recent years for bone regeneration (e.g., PerioGlas®, NovaBone Products LLC., Alachua, Florida, USA, Biogran®, BIOMET 3i, Palm Beach Gardens, FL, USA, and BonAlive®, BonAlive Biomaterials, Turku, Finland) and tooth hypersensitivity (e.g., NovaMin®, GlaxoSmithKline, UK) benefiting from their outstanding bioactivity and biocompatibility.[26] In addition to the prominent osteoconductivity and osteostimulatory properties of BGs, myriads of studies also showed that the controlled release of certain therapeutic ions, such as silver (Ag^{+}), gallium (Ga^{3+}), zinc (Zn^{2+}), copper (Cu^{2+}), and cerium (Ce^{3+}), incorporated into the glass network can induce antibacterial effect.[27] The appealing properties of BGs resulted from the biological role of released ions have recently contributed to the extension of their application in soft tissue repair including skin wounds (e.g., Mirragen™, Avalon Medical, USA).[28, 29] Interestingly, ionic dissolution products of BGs were shown to enhance vascularization through stimulating the secretion of angiogenic growth factors from fibroblasts.[30]

Despite the exceptional advantages, the inherent brittleness of BGs has limited their applications where high and/or cyclic loads are involved.[26] Blending micro- or nano-sized BGs with polymeric hydrogels is a viable solution to address this challenge and to modulate the inflammatory response associated with their sharp morphology.[31] The properties of resultant material can be sum of functions of the BG particles and the hydrogels, or the arising characteristics can result from synergistic interactions. The properties of such composites can be highly influenced by the structural and compositional properties of BG particles.

In this review, after providing a short overview of the most common polymers utilized for the development of BG-reinforced composite hydrogels, we focus on the recent advances of BG-reinforced injectable hydrogels in biomedical applications (Figure 1). To this end, we first discuss the influence of BG particle size, composition, and concentration on the gel formation kinetics, rheological and mechanical properties as well as the swelling and degradation characteristics of such injectable composite hydrogels. Then, we highlight the recent reports on bone tissue engineering and wound healing applications of these composite hydrogels and conclude with a summary and future prospective.

Figure 1

2. Common polymers in hydrogel formulations

The selection of polymers for developing a hydrogel is highly dependent on the intended application and the expected outcomes. A variety of natural and synthetic polymers have been combined with BG nano- or microparticles to prepare injectable composite hydrogels for tissue regeneration. Hydrogels derived from natural polymers are considered to possess good degradability, biocompatibility and structural similarity to tissues. However, they may have suboptimal mechanical stability and could induce an inflammatory or immunogenic response.

Common natural polymers in hydrogel formulations include alginate (ALG), chitosan (CS), gellan gum, pectin, collagen and gelatin (GEL).

CS is a linear biodegradable polysaccharide derived from abundantly available chitin. CS is chemically analogous to glycosaminoglycan and possesses good biocompatibility and minimal toxicity to tissues, and thus has found numerous applications in biomedical fields including tissue regeneration, drug delivery systems and wound healing. In the human body, it can be biodegraded by lysozyme and colonic bacterial enzymes and its degradation kinetics can be modulated by the degree of deacetylation.[32] Indeed, the degradation rate decreases with an increase in the degree of deacetylation.[33] CS has been widely used for preparation of injectable hydrogels as it can form electrostatic interaction with anionic materials due to the presence of cationic amine in its structure. The main drawback of CS is its low solubility in neutral aqueous solutions and organic solvents.

ALG is a naturally occurring, linear, unbranched polysaccharide, typically extracted from brown seaweed. ALG-based biomaterials have been widely explored for biomedical applications due to their biocompatibility, inexpensiveness and the ability to undergo gel formation at mild conditions using divalent cations like Ca^{2+}, Sr^{2}, Cu^{2+}, Mg^{2+} and Ba^{2+}, which is an appealing feature required for cell encapsulation.[34] However, ALG does not mediate adhesion of mammalian cells, as it promotes very minimal protein absorption.[35, 36] ALG is intrinsically non-degradable in mammalian body fluids due to the scarcity of the alginase enzyme, which can cleave the chains of ALG.[37] While ionically crosslinked ALG hydrogels can be dissolved through the release of divalent ions into the physiological media, ALG with high molecular weight will likely remain in the body. Many research works have also been conducted to mitigate the biodegradation problem associated with pristine ALG by its oxidization and/or combination with other degradable polymers. Oxidation of ALG has been found to be a practical approach to enhance its biodegradability, as a result of a reduction in the molecular weight of ALG during oxidation.[38]

Gellan gum is a naturally-occurring anionic extracellular polysaccharide produced via fermentation of Sphingomonas elodea, and can form an injectable and thermoreversible hydrogel in the presence of divalent cations. It has been greatly used in biomedical applications due to its inherent biocompatibility and biodegradability.[39, 40] As with other polysaccharides, human enzymes such as lysozyme, amylase and trypsin can degrade its backbone. These hydrogels; however, are susceptible to hydrolytic degradation and physically crosslinked gellan gum hydrogels are mechanically weak and tend to lose their stability in physiological conditions.[41, 42] Notably, the mechanical properties of gellan gum hydrogels highly depend on its type (low-acyl or high-acyl gellan gums) and concentration.[43]

Pectin is a water soluble, anionic polysaccharide containing linear chains of α-(1-4)-linked-D-galacturonic acid residues. According to the degree of methylation, pectins are classified as low methoxyl (DM < 50%) and high methoxyl (DM > 50%) pectins. It is a heteropolymer abundantly available in the primary cell wall of plants and can form a gel in the presence of divalent cations like Ca^{2+}.[44] Pectins are promising for tissue engineering applications due to their good biocompatibility, biodegradability and low cost, as well as antioxidant and anti-inflammatory effects.[45, 46]

As the most abundant fibrous protein of ECM, collagen has been widely exploited for the engineering of bioscaffolds for tissue engineering applications. Collagen possesses the advantageous feature of low antigenicity, and the amino acid sequences of collagen

(particularly, arginine–glycine–aspartic acid, RGD) are recognizable by the cells as well as it can be degraded by the collagenase enzymes expressed by the cells.[47] However, the main drawbacks associated with using collagen in hydrogels are suboptimal mechanical strength at low biopolymer concentrations, high cost of manufacturing, some immunogenicity and high swelling ratio, which may give rise to its rapid absorption *in vivo*.[48]

GEL is a naturally-derived protein, typically produced by the denaturation of collagen via hydrolysis. While it retains the bioactive sequences present in collagen, such as RGD, which facilitate cell attachment and matrix remodelling, it benefits from less antigenicity and better solubility.[49-51] In recent years, growing attention has been paid to the development of GEL-based injectable hydrogels due to its cost-effectiveness, non-toxic nature, biodegradability and ease of modification.[52] Nevertheless, hydrogels purely made of GEL suffer from poor mechanical properties, uncontrolled degradability and inadequate osteogenesis and angiogenesis.[53] Unmodified GEL-based biomaterials can easily undergo gel-sol transition at physiological temperature. To counteract this issue, an appropriate method of crosslinking can be employed to improve their thermal and mechanical stability so as to render these formulations suitability for tissue engineering applications.[54] One possible solution is to modify GEL molecules with various functional groups, such as acrylamide or methacryloyl, which provides a greater control over the crosslinking process and, in turn, the ultimate characteristics of the hydrogel.[55] Injectable gelatin methacryloyl (GelMA) hydrogels have drawn a great deal of interests for use in biomedical applications due to their tunable physical properties and excellent biological response. GelMA prepolymer solution can undergo photocrosslinking upon exposure to UV and form a stable gel at body temperature.[56] As no reactions occur between the RGD sequences of GEL and methacrylic anhydride during the modification process, the good cell-adhesive properties of GelMA are retained.[57, 58] Meanwhile, owing to the retention of matrix metalloproteinase-sensitive degradation motifs, GelMA hydrogels can undergo accelerated enzymatic degradation.[59]

Synthetic polymers are attractive materials for the development of tissue-engineering hydrogels because of their lack of immune responses or possessing chemical inertness, ease of control over synthetic process and modification as well as robust mechanical properties as compared to the natural systems. A main disadvantage of synthetic polymer-based hydrogels is; however, the lack of cell recognition signals for engineering tissues. This category includes several biocompatible polymers, such as poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), Pluronic® F127, poly-(N-isopropylacrylamide) (PNIPAAm), and peptides, which have been widely used in tissue engineering applications.[60-62]

PEG and its derivatives such as polyethylene glycol diacrylate (PEGDA) are among the most widely studied synthetic polymers.[61, 63] They are broadly used for the development of injectable hydrogels for tissue engineering applications due to their favorable features such as high hydrophilicity, good biocompatibility, non-toxicity and non-immunogenicity. However, unmodified PEG hydrogels are not naturally degradable and are intrinsically protein resistant, which render them biologically inert as cells cannot effectively attach and spread on these hydrogels in the absence of proteins.[64]

PVA is generally synthesized through the hydrolysis of polyvinyl acetate. PVA is a biodegradable water-soluble polymer whose hydrophilicity and solubility are highly relied on the degree of hydrolysis and molecular weight.[65] The hydrogels generated from PVA have found broad applications in biomedical fields owing to their chemical stability, cell-compatibility and absence of toxicity.[66] Most notably, PVA hydrogels resist protein

adsorption and cell adhesion.[67] The abundant hydroxyl groups on PVA can be easily modified with a variety of bioactive molecules to promote cell adhesion.

Pluronics® are synthetic water-soluble amphiphilic block copolymers made up of polar (polyethylene oxide) and non-polar (polypropylene oxide) blocks. The well-known Pluronic F127 has drawn significant attention as its aqueous solution undergoes a reversible sol-gel transition close to the body temperature similar to PNIPAAm, and benefits of good biocompatibility, bioadhesiveness, lack of inherent myotoxicity and immunotoxicity.[62, 68] Thus, it has been widely investigated for various biomedical applications including drug delivery, tissue engineering and wound healing. However, its poor mechanical properties, high permeability and quick dissolution in body fluid necessitate its modification through, for example, copolymerization or reinforcement with organic or inorganic fillers.[69]

Peptides are another important class of synthetic materials including well-known elastin-like polypeptides (ELP) and polylysine. Under specific aqueous conditions (e.g., temperature, pH or ionic strength) and with the proper sequence in place, peptides could simultaneously self-assemble to form a hydrogel. The polypeptides have the ability to adopt secondary structures such as α-helices and β-sheets through the intramolecular hydrogen bonding within peptide chains rendering them conformation-specific self-assembly behaviors and bioactivity.[70] Hydrogels based on polypeptides have garnered exceptional attention in diverse biomedical applications, thanks to their remarkable biocompatibility, cell adhesive properties, enzyme biodegradability and structural similarity to ECM.[71] These hydrogels also possess good bioactivity enabling cells to effectively adhere, proliferate and differentiate. Importantly, the physical and biological properties of these hydrogels can be simply altered by changing the length of peptide sequences or replacing the amino acid residues.[71]

3. Properties of injectable hydrogels

Hydrogels are viscoelastic materials with highly tailorable rheological properties; therefore, they have been utilized in various biomedical applications, such as drug delivery, tissue engineering, hemostasis, and wound healing. The physical behavior of hydrogels is highly dependent upon polymer content as well as crosslinking nature and density, which can be manipulated to achieve desired hydrogels for different applications.[6] The gel formation kinetics as well as gel viscosity and injectability are some of the key characteristics of injectable hydrogels, which should be considered in developing new biomedical systems. There are a variety of parameters influencing the gel formation kinetics of hydrogels. Besides the precursor solution parameters, such as polymer and crosslinker concentrations, molecular weight of polymers, and concentrations of reactive groups of polymers, internal microenvironment factors, such as temperature, pH, biocatalysts and confinement, can noticeably influence the gel formation kinetics of hydrogels.[5, 72, 73] By optimizing the compositional proportions of mixtures, one can speculate to modulate the crosslinking degree and, consequently, the gel formation kinetics of hydrogels. Additionally, the viscosity of the hydrogel solution should be sufficiently low to allow easy injection, which is very helpful for minimally invasive surgical procedures. By engineering the gel parameters, it would be possible to access the potential performance of hydrogels *in vivo*. Rheological measurements are an appropriate approach to quantitatively measure these parameters, and rheological characteristics are regarded as physicochemical factors that can also regulate the printability of hydrogels.[74]

In addition to gelation and rheological behavior, mechanical properties of hydrogels play a critical role in selecting a hydrogel for a particular application. Indeed, the compatibility of hydrogel mechanical properties with host tissues should be precisely considered in designing systems for tissue regeneration since it can fundamentally affect the host inflammatory response and eventually the fate and success of implanted materials.[75] Matrix stiffness plays a key role in directing the cellular responses.[76] The stiffness mismatch between the hydrogel and native tissue is often found to lead to concentration of local stress at the interfaces and, if the gel is not appropriately adhered to the adjacent tissue, relative movement of the hydrogel implants. Shear stress can induce production of pro-inflammatory signals that recruit immune cells and can result in fibrosis.[77] However, since hydrogels are generally less stiff than the neighboring tissue, they may lead to minimal stress concentration, and thus fibrous capsule formation. Biomaterials that adapt their stiffness with tissue microenvironments *in vivo* are highly appealing in the fields of tissue engineering and regenerative medicine. Fracture toughness is another important mechanical property which characterize the ability of a material to resist the propagation of cracks and it should be considered in design of injectable hydrogels. Indeed, hydrogels are inherently brittle because of the reduced areal density of polymer chains resulted from their large water content.

Hydrogels can be engineered to form gels *in situ* upon injection through various physical or chemical crosslinking mechanisms that could occur during or after injection of the precursors. The precursors can be delivered and mixed *in situ* using a dual-barrel syringe or other mixing system, alternatively, an external stimulus, such as UV or visible light, is required to induce gelation.[5, 78-80] Stimuli-responsive units can be included in the hydrogel backbone to enable triggered and/or reversible sol-gel transitions.[81] Stimuli-responsive hydrogels, in particular, have garnered substantial attention due to their capability to alter their physicochemical characteristics in response to external and internal stimuli, enabling the regulation of cell microenvironment.[7] The key feature of *in situ* forming hydrogels for biomedical applications is related to their ability to be injected as liquid and form gel at physiological conditions, so that they facilitate the *in situ* filling of irregularly shaped lesion sites and can form a close physical integration with the adjacent tissue. From a rheological viewpoint, an injectable hydrogel for applications in tissue regeneration should behave as a free-flowing liquid during injection (a state wherein storage modulus (G') is lower than loss modulus (G'')), and upon ejection, it should quickly transform into non-flowable gel state wherein $G' > G''$, representing an elastic behavior. Injectable hydrogels are typically shear-thinning, and their shear viscosity decreases by increasing the shear rate.

An ideal injectable hydrogel should form at mild, physiologically compatible gel formation conditions and within a reasonable time frame after injection into the desired site. This is necessary to ensure uniform mixing of the precursors and prevent or minimize the extravasation of precursor polymers into the neighboring tissues or avoid hydrogel-forming liquids become diluted by the body fluid, which may hinder hydrogel formation or influence the hydrogel properties.[8, 10] A very slow gelation can result in rapid material dispersion after injection which can lead to cargo loss, while very quick gelation would impair diffusion-regulated mixing of precursor polymers and leaves regions of heterogeneity within the hydrogel polymer matrix. Indeed, so fast gelation can lead to segregation of hydrogel structure with the endogenous pool of progenitor cells and nutrients and it does not allow encapsulation and immobilization of nutrients/cells within hydrogel structures. The premature gel formation might also occur in the syringe as a result of rapid gelation, which may cause network defects, and in turn, influence the gel mechanical properties and release/retention of cargo, and impair

injectability.[82] A gelation time in the range of 5 to 9 min has been found to be optimal for a wide range of clinical uses.[62, 83]

Shear-thinning behavior allows delivering a preformed gel with favorable physical characteristics through needles without clogging, thereby enabling homogenous encapsulation of cargo molecules and cells.[84, 85] Shear-thinning hydrogels flow or thin under applied shear stress and rapidly self-heal upon cessation of mechanical load. As these hydrogels are preformed ex-vivo, comprehensive characterization can be performed before injection, and the local environment has minimal influence on crosslinking.[6] Furthermore, in comparison to other types of hydrogels, shear-thinning hydrogels recover their physical properties faster after injection, which minimize the risk of leakage into the surrounding tissues. Shear-thinning hydrogels have been vastly used in 3D printing due to their non-Newtonian behavior and stress-relaxation properties.[86] By evaluating the rheological properties of a shear-thinning hydrogel under increasing shear stress, it is possible to determine if a biomaterial shows a yield stress behavior; the stress at which significant structural breakdown takes place and materials start to flow.[74] The self-healing property of these hydrogels are also being evaluated to understand to what extent the materials can restore their elastic property upon being subjected to alternating high and low shear stress. These hydrogels, however, could be mechanically less stable than the *in situ* covalently crosslinked hydrogels as they are often formed by physical crosslinking.[87]

In spite of the promising properties of injectable hydrogels, rendering them suitable in various biomedical applications, issues associated with their mechanical properties, swelling and degradation kinetics as well as bioactivity cannot be overlooked. Towards tackling these issues, considerable efforts have been made to reinforce or strengthen these hydrogels by the incorporation of inorganic particles or fibrillar materials, and/or formation of double-network (DN) hybrid hydrogels.[88-92] Inorganic fillers, such as BGs, can be incorporated into the hydrogels to tune these properties and extend their clinical applicability. Here, we will highlight the influence of BG inclusions on gelling behavior, rheological, mechanical, hydration, degradability and biological properties of *in situ* forming and shear-thinning hydrogels. Table 1 summarizes the properties of BG-reinforced hydrogels.

Table 1: Summary of BG-reinforced injectable hydrogels developed for tissue regeneration.

Polymer	BG composition	BG size/ morphology	BG ratio	Chemistry/ Gelling factor	Mechanical properties	Gelation time	Gelation temperature (°C)	Porosity (%)/ pore size (µm)	Application	Reference
CS/ SF	95 SiO_2-5 CaO, 95 SiO_2-2.5 CaO-2.5 CuO mol.%	~460 nm (Spherical) ~490 nm (Spherical)	0.3 and 1% (w/v)	β-GP	-	472-284 s	~35-31	-	Bone regeneration	[93]
CS/ Collagen	80 SiO_2-15 CaO-5 P_2O_5 mol.%	87 ± 5 nm (Spherical)	1 and 2 wt.%	β-GP	-	3.57-3.35 min	~37	100-900 µm	Bone regeneration	[94]
CS	46 SiO_2-24 CaO-24 Na_2O-6 P_2O_5 mol.%	748 ± 10 nm	0.5-2% (w/v)	$NaHCO_3$	-	~19.30-20.10 min	-	-	Bone regeneration	[95]
CS/ GEL	60 SiO_2-36 CaO-4 P_2O_5 mol.%	15-75 nm (Spherical)	10 wt.%	β-GP	-	218-172 s	34.6-36.7	73.1-79.1%	Bone regeneration	[96]
Glycol CS/ Dibenzaldehyde-functionalised PEG/ALG	60 SiO_2-36 CaO-4 P_2O_5 mol.%	300 nm (Spherical)	3 wt.%	Benzoic-imine dynamic covalent bonding and ionic interaction	Compressive modulus ~0.05-0.10 MPa Fracture energy ~47-24 kJ/m^3	140-110 s	-	-	Osteochondral defect repair	[97]
ALG-Poloxamer/ SF	85 SiO_2-15 CaO mol.%	227.2 ± 10.3 and 264.7 ± 12.1 nm (Spherical)	1 and 2 wt.%	Horseradish peroxidase-catalyzed reaction	-	11.75-9.75 min	36.1-34.6	~200 µm	Bone regeneration	[98]
ADA/ GEL	45S5 Bioglass	2 µm	0.1 and 0.5% (w/v)	Schiff-base	-	45-4 min	-	-	Bone regeneration	[99]
ADA/ GEL	55 SiO_2-40 CaO-10 P_2O_5, 55 SiO_2-30 CaO-5 P_2O_5-10 SrO mol.%	40–100 nm	0.1 and 0.5% (w/v)	Schiff-base	-	<1-25 min	-	-	Bone regeneration	[100]
ADA/ GEL	45S5 Bioglass	2 µm	1 and 5% (w/v)	Schiff-base	Compressive strength 514-908 kPa Compressive modulus 135-417 kPa	~25-3 min	-	~90-30% ~200-100 µm	Bone regeneration	[101]

Table 1: Continued.

Polymer	BG composition	BG size/ morphology	BG ratio	Chemistry/ Gelling factor	Mechanical properties	Gelation time	Gelation temperature (°C)	Porosity (%)/ pore size (µm)	Application	Reference
GEL/ Oxidized chondroitin sulfate	85 SiO_2-15 CaO mol.%	~100 nm (Spherical)	5, 10, 15 wt.%	Schiff-base/ Borate complexation	Compressive strength at 90% strain ~1.95-9.05 MPa Compressive modulus ~6-58 KPa	640-10 s	-	~50-217 µm	Bone regeneration	[102]
GelMA	80 SiO_2-16 CaO-4 P_2O_5 mol.%	Nano-BG: 460.9 nm GelMA-modified BG: 249.1 nm (Spherical)	1, 3 and 5 wt%	Photo-crosslinking	Compressive modulus Unmodified BG ~21-30 kPa GelMA-modified BG ~21-38 kPa	-	-	150 µm	Bone regeneration	[103]
GelMA	40 SiO_2-45 CaO-15 P_2O5 mol.%	400 nm (Spherical)	0, 2.5, 5 and 10% (w/v)	Photo-crosslinking	Compressive modulus 237.9-153.6 kPa	-	-	90-200 µm	Bone regeneration	[104]
BP-functionalised hyaluronan	45S5 Bioglass	Microparticles (Spherical)	15 to 120 mg mL^{-1}	Non-covalent physical bonds	Compressive strength and elastic modulus ~12 kPa	-	-	-	Bone regeneration	[105]
PEG/ CPBA-grafted PVA	60 SiO_2-36 CaO-4 P_2O_5 mol.%	300 nm (Spherical)	10, 20 and 40 wt.%	Dynamic-covalent bond-based double network	Compressive modulus 0.5-4.1 MPa Compressive strength 20-90 MPa Fracture energy 20-75 kJ m-2 Elongation at break 600%-170% obtained from tensile test	Immediately	-	-	Bone regeneration	[106]
Au-based 4-arms thiol terminated PEG	70 SiO_2-30 CaO mol.%	100 nm (Spherical)	5-20 wt.%	Thiol-disulfide exchange reaction	Compressive stress at 30 % deformation 1-16 kPa	<1 min	-	-	Bone regeneration	[107]
Poloxamer 407-based poly(ether urethane)	85 SiO_2-13 CaO-2 CuO mol.%	150-200 nm and 1-5 µm (Spherical)	20 mg mL^{-1}	Self-assembly	-	5-3 min	27-29	-	Bone regeneration and wound healing	[108]

Table 1: Continued.

Polymer	BG composition	BG size/ morphology	BG ratio	Chemistry/ Gelling factor	Mechanical properties	Gelation time	Gelation temperature (°C)	Porosity (%)/ pore size (µm)	Application	Reference
Catechol-modified quaternized CS/ PLLA-b-PEG-b-PLLA	80 SiO_2-16 CaO-4 P_2O_5 mol.%	780 nm (Spherical)	2 wt.%	Self-assembly	-	-	39-32.6	~72%	Wound healing	[109]
ADA/ Adipic acid dihydrazide-modified γ-polyglutamic acid	45S5 Bioglass	Microparticles	0.5, 1 and 1.5 wt.%	Schiff-base	-	<1 min-8 min	-	-	Wound healing	[110]
ALG/ PEG diacrylate	SiO_2-CaO-P_2O_5, SiO_2-CaO-P_2O_5-CuO	Nanoparticles	1, 2 and 3 mg mL^{-1}	Photo-crosslinking	Compressive strength ~3-6.1 kPa Tensile strength at 65% strain ~125-200 Pa	5 min	-	-	Wound healing	[111]
Elastin-like polypeptides	45S5 Bioglass	10 µm	0.25, 0.5 and 1 % (w/v)	Schiff-base	Storage moduli after 1 h gelling at 37 °C 300-4500 Pa	~500-100 s	-	-	Wound healing	[112]
Human serum albumin/ Succinimidyl succinate-modified PEG	45S5 Bioglass	10 µm	0.05, 0.25, 0.5 and 1 wt.%	Carbodiimide Chemistry	Compressive strength 273-265 kPa Tensile strength ~40 kPa	1100-15 s	-	-	Wound healing	[113]
Dibenzaldehyde-terminated F-127 (F127-Phe-CHO)/ F127-ε-poly(L-lysine)	Polydopamine-functionalised 60 SiO_2-36 CaO-4 P_2O_5 mol.%	~300 nm (Spherical)	1 wt.%	Schiff-base	-	55 s at 37 °C	30.5-27.8	-	Wound healing	[114]

Abbreviations: ALG = alginate, ADA = alginate dialdehyde, CS = chitosan, GEL = gelatin, GelMA = gelatin methacryloyl, PEG = poly(ethylene glycol), PVA = polyvinyl alcohol, SF = silk fibroin, BG = bioactive glass, CPBA = 4-carboxyphenylboronic acid.

3.1 Effect of BGs on gelling behavior, rheological and mechanical properties of hydrogels

3.1.1 Overview

Several studies have reported the role of BGs as a regulator to control the gelation, rheological, and mechanical behavior of injectable hydrogels. The mechanism that BGs employ to influence these properties not only varies depending upon the precursor polymers but also can be as a result of factors such as BG particle size, composition and concentration, and thus these parameters need to be thoroughly investigated prior to starting to design an injectable hydrogel.

The particle size of BGs plays a major role on final properties of composite hydrogels. Mechanical properties, bioactivity and cellular response of such composites can be significantly influenced by changing the size of BG particulates. As the size of particles becomes smaller, the surface area increases, which enhances their integration within the polymer matrices during composite fabrication and induces apatite nucleation more rapidly upon contact with body fluids.[115-117] It is also well-documented that mechanical properties of such composite materials increase in line with a reduction in size of BG particles.[118-120] Understanding the gelation and rheological behavior of hydrogels as the particle size change can open a new window toward developing multifunctional hydrogels with favorable response during and after injection (See Table 1 for additional details). In a noteworthy example, Pontremoli and co-workers studied the effect of copper (Cu)-doped mesoporous bioactive glass (MBG) spheroidal nanoparticles (MBG_Cu 2%_SG) and microspheres (MBG_Cu 2%_SD) on the gelation kinetics of Poloxamer 407-based poly(ether urethane) hydrogel.[108] Addition of MBGs to the hydrogel resulted in a small decrease in gelation time at 37 °C, from 5 min for unloaded hydrogel to 3 min and 4 min for MBG_Cu 2%_SG and MBG_Cu 2%_SD, respectively. This reduction was attributed to a rise in viscosity of mixture with MBG incorporation, which is common in colloidal suspensions. The more rapid gelation of hydrogel with MBG_Cu 2%_SG was due to the smaller size of the particles (150-200 nm vs 1-5 μm) and their more homogenous distribution into the polymer matrix. However, an opposite trend was observed for Lower Critical Gelation Temperature (LCGT), which increased for hybrid hydrogels (from 27 °C to 29 °C); this suggests that MBGs retard the micelle formation towards a mature gel as the temperature increases. The injectability of the composite hydrogels were compared at three varied temperatures, i.e., 5, 25, and 37 °C, using three different needles, i.e., G22, G18, G14. All the samples were easily injectable at 5 °C through all the examined needles; however, their injectability was impaired by rising temperature. Notwithstanding that both composite hydrogels could be injected through the largest needle (G14) at both 25 and 37 °C, the composite hydrogel loaded with nano-sized BGs was not injectable through G18 and G22. This phenomenon was assigned to the quicker gel formation and higher viscosity of MBG_Cu 2%_SG-loaded hydrogel. Notably, Cu^{2+} was sustainably released from the hydrogels during 14 days of soaking in Tris HCl, with expectedly higher concentration for the hydrogel incorporated with nano-MBG. Normalization of Cu^{2+} concentration released from hydrogel to those of pure BG powder indicated comparable release profile for both systems, suggesting that release properties of embedded BG particles were retained.[108]

In another study by Douglas *et al.*, two calcium-rich sol-gel ternary BGs ($40SiO_2$–$54CaO$–$6P_2O_5$ in mol%) with particle sizes of < 45 μm and 2.5 μm were used to reinforce hydrogels composed of two different pectins derived from citrus peels (CA) and apple pomace (AA).[121] The rheometric results demonstrated that the storage modulus of hydrogel containing BGs with smaller particle sizes reached a plateau markedly faster (1 min) than those

consisting BGs with particles of size < 45 μm, CA (5 mins) and AA (10 mins), when tested at 37 °C. Given that aqueous solutions of pectin readily turn to gel by rising the pH of solution (in a pH range of 3.2-5.3), the release of Ca ions from the BG phase was found to bring about the internal gelation of pectins through ionic interactions, which removes the need for adding a pH modifier like NaOH or $NaHCO_3$.[46, 122] It was further explained that the smaller BG particles tend to release greater amounts of Ca ions faster due to their higher surface area, which also gives rise to more rapid gelation.[121]

It is also imperative to tailor the composition of BGs prior to incorporating into the hydrogels to suit the application of interest. Composition-wise, BGs are generally categorized into three main classifications: silicate, borate/borosilicate, and phosphate. The chemical nature of BGs can have direct implications on physicochemical and biological properties of these particles and their composites. For instance, it has been reported that porous structure and surface area of BGs can be controlled by manipulating Ca/Si ratio in the way that by increasing this ratio the BG nanospheres became less porous and the surface area significantly decreased.[123] It has also been shown that the dissolution rate can be modulated through modification of BG composition. For example, by replacing SiO_2 with B_2O_3 in silicate-based glasses (e.g., 45S5 or 13-93), the degradation rate can be altered within a wide range, and can be tailored to match the bone growth rate *in vivo*.[124, 125] The ability of BGs to form a surface apatite layer are also closely connected with their chemical compositions. Incorporation of specific metallic cations, such as Cu^{2+}, Ag^{+}, Zn^{2+}, Ga^{3+}, etc., into the BGs cannot only regulate the apatite-forming ability of BG systems, but can also improve osteogenesis, impart angiogenic and/or antibacterial activity to the BG-containing materials; in these cases, the therapeutic effects depend on the ion release process that should be properly controlled.[24, 126]

The impact of BG composition on the gelation and rheological properties of hydrogels have been explored by Wu *et al.* who prepared a series of hydrogels composed of CS/silk fibroin (SF)/β-GP and BG nanoparticles doped or not with Cu.[93] Incorporation of BGs, regardless of the composition, shortened the gelling time of the hydrogel, with a markedly shorter gelling time for the hydrogels containing Cu-BGs. An increase in the Cu-BG content from 0.3 to 1 w/v % could even further lessen the gelation time from 472 s for unfilled hydrogel to 284 s for the hydrogel loaded with 1 w/v % Cu-BGs. The rheology results revealed that addition of BGs decreased the sol-gel transition temperature (from ~35 °C to ~31 °C), indicating a need to optimize the BG concentration in order to obtain a hydrogel that undergoes a change near the physiological temperature. As obtained from frequency-dependence spectra, G′ was much higher than G″ over the entire frequency range (0 to 100 Hz), illustrating that all the gels were elastic. The composite hydrogels filled with Cu-BGs showed higher G′ that increased proportionally with the BG content. Assessment of viscosity variation against shear rate elucidated that all hydrogels had very low viscosity at room temperature even though applying shear could further decrease the viscosity. At 37 °C; however, the hydrogels were highly viscous initially, and their viscosity steadily decreased as a function of shear rate. These hydrogels were readily injectable at ambient temperature when tested using a syringe with a 23G needle.[93]

It has also been reported that doping a nano-sized BG (55 SiO_2-40 CaO-10 P_2O_5, mol.%) with Sr can further shorten the onset of gelation from ~5 min to <1 min for the alginate dialdehyde (ADA)-GEL hydrogels containing 0.5% (w/v) Sr-free BG and Sr-doped BG, respectively, presumably because substituting Sr for Ca enhanced the rate of glass dissolution and ion release which can subsequently accelerate the crosslinking rate of the hydrogel.[100, 127] It is worth noting that the gel viscosity increased in line with BG content and in a time-dependent manner

likely due to the enhanced interaction between the free L-guluronic acid units of polymers and the released Ca^{2+} and/or Sr^{2+} from BG particles. [100]

It is a common knowledge that mechanical properties of hydrogels increase by addition of BG particles, often up to a threshold concentration depending on the hydrogel matrix, because of the stiffening effect of these particles.[128, 129] However, the impact of BGs on gelation and rheological properties of injectable hydrogels is not always the same. Some studies have shown that increasing BG concentration in the hydrogel can accelerate gelation process. For example, the rheological properties of an *in situ* self-crosslinked hydrogel based on GEL and oxidized chondroitin sulfate (OCS) was modulated by adding borax and incorporating MBG nanospheres (85 SiO_2-15 CaO mol.%; ~100 nm) into the hydrogel matrix.[102] Sweep rheology experiment at 37 °C indicated that gelation time was significantly decreased with increasing the MBG content; from 640 s to 10 s for unloaded and 15 wt.% MBG-loaded hydrogels, respectively. As the MBG concentration rose, the gap between the G′ and G″ curves diminished, revealing the role of MBG nanoparticles in enhancement of hydrogel elasticity. The authors speculated that a range of physical and covalent interactions were involved in the formation of composite hydrogel and their accelerated gelation (Figure 2). Firstly, the pH increment resulted from the release of alkaline ions (i.e., Ca^{2+}) from the MBG nanoparticles highly promoted the reaction of Schiff-base formation. In addition, in aqueous solution the amino and carboxyl moieties of the polymers could link with the silanol groups on the surface of MBG through hydrogen bonding. It is also expected that the released Ca ions from MBG phase enhanced the alkalinity and could contribute to ionic gelation of OCS. As such, the synergistic effect of multi-interactions between the components of this hydrogel may facilitate gelation. A significant enhancement in compressive behavior of these hydrogels was observed as a function of BG content (Table 1). Indeed, by increasing the alkalinity of the system, MBGs facilitate formation of hydrogen bonding and OCS ionic gelation, leading to a remarkably enhanced compressive strength and modulus. It should be noted that all the composite hydrogels exhibited shear-thinning behavior so that applying high shear stress led to decreased viscosity and enabled them to be injected facilely through the syringe. While the gelation time of these hydrogels could be controlled by adding different amounts of MBG nanoparticles, their injectability was not influenced by incorporation of up to 15% (w/w) of MBGs.[102]

Figure 2

In another study, knowing the pH-dependency of amide formation (in the range of 7.0 to 10.5) in a hydrogel made of human serum albumin and succinimidyl succinate-modified PEG, Zhou and co-workers studied the impact of various contents of 45S5 BG microparticles (10 μm) on its gelation time.[113] Gelling time dropped appreciably in line with increasing BG content from nearly 1100 s for unloaded hydrogel to 360 s and 15 s for the composites filled with 0.05% and 1% w/v BG. The authors explored the changes in pH in order to better understand the role of BGs during gelation. The results disclosed that the solution pH increased faster as BG content rose due to the release of metal ions such as Na^+ and Ca^{2+}, resulting in a more rapid gelation of composite hydrogels. Given that the hydrogel forms as a result of the reaction between the amine moieties of the human serum albumin and succinimidyl active esters moieties of PEG, the gelling time highly relies on the nucleophilicity of the amine functionalities, which, in turn, can be largely influenced by the pH of the solution. It was therefore inferred that the amount of BG microparticles in the mixture can modulate the gelation time through changing the pH. The composite hydrogels showed good injectability;

however, no significant difference was observed between the composite containing small amounts of BG particles (i.e., 0.5% w/v) and the unloaded hydrogel with respect to their mechanical properties.[113]

Likewise, Zeng and co-workers developed a thermoreversible and self-healable hydrogel with tunable gelation behavior and mechanical properties using aldehyde-modified ELP and different amounts of 45S5 BG microparticles (average diameter of 10 μm).[112] The hydrogels were formed as a result of Schiff-base reaction between primary amines and aldehydes. Since formation of the Schiff-base is accelerated at higher pH, a rise in BG content induced faster gelation, ~500 s vs 100 s for the hydrogels filled with 0.25 and 1 % (w/v) BG, respectively (Figure 3). In addition, storage modulus of the gels, after 1 h gelling at 37 °C, significantly enhanced as the BG content increased (from 300 Pa for the hydrogel containing 0.25 % BG to 4500 Pa for that filled with 1 % BG), which was attributed to quicker rate of reaction between amines and aldehydes. The hydrogels remained in liquid form at room temperature and were quickly turned to gel at 37 °C, as revealed by rheological test. The sol-gel transition was partly due to the ELP thermo-responsiveness, so that constituent proteins of EPL (e.g., E125 and K125) tended to aggregate at the physiological temperature, resulting in faster crosslinking reaction. Notably, the hydrogels exhibited self-healing property due to the reversible nature of imine bonds. The rheology results showed that, while the storage modulus of hydrogels was higher than loss modulus at low strain, the trend changed at higher strains (γ = 100%) due to the network rupture. Nonetheless, the hydrogels had the capability to rapidly recover upon removal of high strain, which explains the self-healing phenomenon.[112]

Figure 3

The effect of 45S5 BG microparticles (~2 μm) on the gelation time of an ADA/GEL hydrogel was investigated by Sarker and co-workers.[101] Gelation time of the hydrogel was notably decreased as a function of BG content, such that the composite hydrogels with 1 and 5 % (w/v) BG showed 4- and 8-times shorter gelation time than the control (~7.5 and 3 min vs ~25 min), respectively. In order to understand the rationale behind this behavior, the authors assessed and compared the crosslinking degree of all the hydrogels, elucidating that incorporation of BG microparticles into the hydrogel could significantly enhance the degree of crosslinking.[99] It was further explained that the ions released from BG phase could increase the alkalinity of the hydrogel mixture, which is favorable for Schiff-base reaction. Additionally, released Ca^{2+} might likely contribute in ionic crosslinking of the ADA, thereby accelerating gelation. Incorporation of BG remarkably reduced the porosity (~90 to 30%) and pore size (~200 to 100 μm) of the ADA/GEL hydrogel whilst enhanced its compressive strength (514 to 908 kPa) and modulus (135 to 417 kPa). It should be pointed out that despite the higher porosity of ADA/GEL hydrogel than ALG/GEL the mechanical strength was higher which was thought to be due to the formation of covalent crosslinking between ADA and GEL.[101] Similarly, gelation time of a dual-network hydrogel of ALG-poloxamer/SF was accelerated by rising the amount of binary MBG nanoparticles (Si/Ca with 85:15 mol%) from 11.75 min for unfilled sample to 10.5 and 9.75 min for composites with 1 and 2 wt.% MBG. However, gelation temperature was insignificantly decreased by addition of BGs, from 36.1 °C to 34.6 °C.[98] The study of shear-dependent viscosity revealed that the viscosity decreased in line with rising shear rate. The hydrogels, regardless of the composition, had low viscosity (70 Pa.s) at room temperature, which enables an easy injectability. In addition, inclusion of MBGs into the

hydrogels had no notable influence on gel strength, and the G′/G″ was >15 for all the hydrogels indicating that these gels are highly elastic.

In another recent study, Ganter and co-workers evaluated the rheological properties of gold-based 4-arms thiol terminated PEG (Au-(PEGSH)4) hydrogel with different amounts of agglomerated BG (70 % SiO_2-30 % CaO, mol.%; 100 nm), from 5 to 20 wt.%.[107] All the hydrogels formed within 1 min after mixing the precursors, which, according to the authors, necessitated the use of a dual-syringe for their delivery. The frequency sweep test indicated that G′ was greater than G″ at all frequencies, confirming the formation of free-standing hydrogels. Notably, G′ values enhanced with an increase in the loading level of BGs. The small stiffness enhancement at higher BG loading was assigned to the increasing polymer entanglements rather than formation of chemical or electrostatic interactions between the BGs and polymer. This effect was more noticeable at neutral pH in comparison with the pH 10, verifying the pH-dependent rheological properties. The unloaded hydrogels demonstrated a frequency-dependent stiffness behavior at both tested pH in the sense that, at lower frequencies, tan(δ) (G″/G′ ratio) was increased, whilst at higher frequencies this value showed a decrease, which was associated with the inadequate rearrangement time for the polymer chains, a characteristic of dynamic hydrogels. The reversible dynamic bonds in these hydrogels would enable the permanent rearrangement of materials, thereby inducing self-healing and frequency-dependent stiffness properties. The latter allows the mechanical properties of the hydrogels to recover upon damage and become compliant with the external environment.[130, 131] The composite hydrogels experienced this behavior only at pH 10 since the gold-thiolate/disulfide (Au-S/SS) exchange reaction was deaccelerated with BG particles. In spite of the slower Au-S/SS exchange rate, all the composite hydrogels showed self-healing ability at pH 7, albeit both G′ and G″ diminished for the healed composite hydrogels. Moreover, the compressive stress of the hydrogels enhanced by the inclusion of BG particles (from 1 to 16 kPa at 30% deformation), indicating the stiffening effect of BGs. However, the nanocomposite hydrogels were found to suffer fracture at larger deformation where the compressive stress dropped noticeably.[107]

The addition of MBG nanoparticles (80% SiO_2, 16% CaO, and 4% P_2O_5 mol.%) to the photo-crosslinkable GelMA hydrogel also led to the improvement of mechanical properties.[103] Indeed, the compressive modulus of GelMA hydrogel steadily enhanced by the inclusion of up to 3 wt% MBGs from 21 MPa to 30 MPa and 38 MPa for the hydrogels incorporated with plain MBGs and GelMA-grafted MBGs, respectively. However, when the concentration of both MBGs increased to 5 wt% the compressive modulus of the hydrogel considerably decreased, which was thought to be due to the aggregation of particles within the GelMA phase. In a different study, it was found that the mechanical properties of GelMA/BG nanocomposite hydrogels were markedly increased (about 4 folds) after incubating the hydrogels at 4 °C prior to UV crosslinking process as compared to the unincubated samples.[104] Surprisingly, the compressive modulus of GelMA hydrogel was diminished as a function of BG content. The authors of this study speculated that this might be because the BG particles impeded the formation of physical networks and enhanced the UV light reflection, thereby reducing the crosslinking density.

On the other hand, a few studies have highlighted the gelation retardant effect of BGs. For instance, incorporation of 45S5 BG microparticles into a hydrogel synthesized by the Schiff-base reaction of ADA and adipic acid dihydrazide-modified γ-polyglutamic acid delayed the gelation time in a concentration-dependent manner (from less than 1 min for unfilled hydrogel to 8 min with addition of 1.5 wt.% BG) as the ions released from BG phase shifted the pH of solution towards the weak alkaline.[110] As alleged by the authors, the neat hydrogel was

formed too rapidly (<1 min) at the acidic pH of hydrogel solution; therefore, the use of a dual syringe was required to allow proper processing. Hence, the inclusion of BGs enabled them to adjust the gelation time of the hydrogel and provided a greater time window for the assessment to be performed.

In another study, Liu and co-workers generated a biphasic DN hydrogel from glycol CS and dibenzaldhyde-functionalized PEG network, as well as ALG and $CaCl_2$ network.[97] The authors reported a prolonged gelling time by adding 3 wt.% of 58S BG nanoparticles (300 nm) into the hydrogel, from 110 s to 140 s. Additionally, a slight decrease in shear modulus was observed after loading nano-sized BG into the hydrogel. These observations were assigned to the barrier effect of BGs which reduces the chance for the polymer chains to come into close proximity, thereby hampering the crosslinking reaction. However, due to reinforcing function of BGs, the compressive modulus of the composite hydrogel was higher than that of the neat hydrogel (0.10 vs 0.05 MPa). Noticeably, fracture energy was significantly lower for the composite hydrogel (~24 vs 47 kJ/m^3), verifying that BG inclusion turn the hydrogel to a more brittle material. The hydrogels showed self-healing property which enabled fabrication of double-layered hydrogel from a combination of pure and composite hydrogels. It is interesting to mention that inclusion of BG particles into the hydrogel did not influence the self-healability and injectability.[97] The same group also constructed an injectable tough DN hydrogel through interpenetration of a composite network formed by hydrogen bonded PEG and 58S BG nanoparticles (300 nm) into a hydrogel constituted of Ca ionically-crosslinked carboxyphenylboronic acid-grafted PVA.[106] As the hydrogel tended to form immediately after mixing the precursors, a dual-syringe kit was necessary for the injection. The authors found that the addition of nano-sized BG can slightly prolong the gelation time while significantly enhancing the shear modulus of the composite. Hydrogels with high shear moduli require higher force to be injected and, as such, can deteriorate the injection efficiency and clinical usability. In order to tackle this problem, PEG was used to plasticize the gel and lower the shear moduli. Therefore, the DN hydrogel benefited from enhanced elastic modulus and injection coefficient (100%) in comparison to the gel without PEG (injection coefficient of 35%). Importantly, the crosslinking degree of the PVA network was markedly diminished by the presence of large BG particles (~4 μm). In addition, the large free intermolecular distance between the BG particles in such composites unable the particles to form internal friction under compression, which justifies the lower modulus of PVA/BG hydrogel even than that of pure PVA. As can been seen in Table 1, Not only did increasing the BG content at a constant PEG ratio of 40 % w/v tremendously improve the compressive modulus and strength as well as fracture energy (Figure 4), but the DN hydrogel with 40 %of BG also retained its deformability (>95 %).[106]

Figure 4

DN hydrogels were also fabricated from PEGDA, ALG and Cu-free and -doped nano-BGs.[111] The main network of the hydrogels was formed through photo-crosslinked PEGDA (by irradiation under UV light for 5 min) and second network was built by the dynamic ionic interactions between the ALG and divalent metal ions (i.e., Ca^{2+}or Cu^{2+}) released from the BG nanoparticles (Figure 5). The G′ and G″ of the pristine polymeric hydrogel and hydrogels containing nano-BG with and without Cu showed a nonlinear rheological behavior, which increased as a function of shear stress. The hydrogels exhibited elastomeric behavior and the storage modulus as well as tensile and compressive strength were remarkably enhanced by

inclusion of BG particles, as shown in Table 1. However, at low BG concentration (1 mg mL^{-1}), these properties slightly decreased which was thought to be because of uneven distribution of the BG nanoparticles and reduced crosslinking degree of the polymer phase. Conversely, high content of BG (3 mg mL^{-1}) brought about aggregation within the hydrogel, which led to inferior tensile, compressive and elastomeric properties of the composite hydrogels. The composite hydrogels could be also readily injected and showed a rapid and superior self-healing than the unloaded hydrogel as a consequence of strong ionic bonds that was formed between Ca^{2+}/Cu^{2+} and ALG. The self-healing ability of the hydrogels was evident from the insignificant difference between the storage modulus of the hydrogels after self-healing at different time points, and also from the colors of reattached parts which diffused together over a course of 48 h.[111]

Figure 5

Another series of studies have investigated the multicomponent and multifunctional hydrogels generated by co-incorporation of organic nanofillers and BGs or inclusion of biopolymer-functionalized BG particles in the hydrogel matrix. These systems have been developed with the aim to modulate the rheological properties, gelation behavior, injectability and biological response of the BG-reinforced hydrogels. For example, incorporation of Sr-doped borosilicate BG microparticles (47.12 SiO_2-6.73 B_2O_3-6.77 CaO-22.66 Na_2O-1.72 P_2O_5-5 MgO-10 SrO, mol.%; 13–50 μm) into ALG-GEL hydrogel led to 20-fold increase in viscosity, which necessitated a high shear stress during bioprinting procedures.[132] The hydrogels displayed shear-thinning behavior, recognizable from the viscosity reduction as a function of increasing shear rate and temperature. The high viscosity of BG-containing hydrogels was considered as a drawback, since it can result in a phase separation between the liquid and BG during the printing process. In order to overcome this complication and preserve the embedded cells, in particular osteosarcomas Saos-2 cells, from high shear forces during bioprinting, a small quantity (0.25 % w/w) of wood-based cellulose nanofibrils (CNFs) was co-incorporated along with BG microparticles. The hydrogels containing CNFs showed a more stable rheological properties in correlation with shear rate, confirming the flow-modulating role of these nanofibrils and enhanced hydrogel printability. It was hypothesized that, under applied shear stress, the CNFs align as the bioink flow through the nozzle during printing, thereby enhancing the flowability. Notably, the highest storage modulus was reported for the hydrogel incorporated solely with BG.[132]

The effect of co-incorporation of organic and inorganic fillers on the rheological properties of a shear-thinning hydrogel developed based upon chitin and poly(butylene succinate) (PBSu) was also investigated by Priya and co-workers after inclusion of fibrin nanoparticles (FNPs) and a magnesium-doped BG (60 SiO_2-30 CaO-10 MgO, mol.%).[133] In general, the G′ of the hydrogels decreased by the addition of FNPs and increased when BG was incorporated into the hydrogel; the gel with higher BG content (i.e., 5 wt.%) and in the absence of FNPs had the highest elastic modulus (268 kPa). Viscosity of all the hydrogels showed a decrement as a function of shear stress, which is a common characteristic of shear-thinning hydrogels, and they all showed good temperature stability within the range of 25-40 °C and good injectability regardless of the amount of BG.[133]

Functionalization of BG particles with a polymer consisting of active functional groups has shown to be an effective strategy to improve particle distribution within the hydrogel matrix

network, leading to a composite with customized functionality. To this end, a temperature-responsive and shear-thinning hydrogel was prepared by Zhou and co-workers through Schiff-base reaction between dibenzaldehyde-terminated F-127 (F127-Phe-CHO), F127-ε-poly(L-lysine) copolymer (FEPL) and polydopamine-functionalized nano-BG (BGN@PDA).[114] Temperature sweep test indicated that the crossover points between G′ and G″ occurred at 30.5 °C for the hydrogel without BGN@PDA, while this temperature dropped to 27.8 °C after incorporation of BGN@PDA into the hydrogel, implying formation of further crosslinking between PDA and -NH_2 of FEPL. The mixture remained in the form of sol at room temperature (25 °C), and turned to gel state at physiological temperature in 55 s, signifying the temperature responsiveness of this nanocomposite hydrogel (Figure 6). Overall, both G′ and G″ of the nanocomposite hydrogel were greater than those of the neat hydrogel. Moreover, under high shear strain (1000%), the nanocomposite hydrogel network was disrupted and G′ decreased to lower values than G″; however, it was instantaneously recovered when the shear strain returned to low levels (1%), suggesting that the hydrogel has shear-thinning and self-healing properties. These behaviors allowed the nanocomposite hydrogel to be facilely injected without clogging of the needle during injection.[114]

Figure 6

Availability of multivalent metallic cations such as Ca ions in the composition of BGs has enabled researchers to exploit them for preparation of injectable self-healing hydrogels. Bisphosphonate (BP)-functionalized polymers have shown strong affinity toward Ca ions of BGs. In this regard, a series of shear-thinning hydrogels were prepared by using BP-functionalized hyaluronan and 45S5 BG microparticles.[105] The rheological measurements indicated that by increasing BG content from 15 to 30 mg mL^{-1} the G′ remarkably enhanced, yet yield strength substantially decreased, while further rise in the BG content resulted in insignificant changes in the elasticity of hydrogels. The authors hypothesized that crosslinking played the major role in alteration of gel elasticity while network brittleness was the main contributor in yield strength of the hydrogels. As observed from the compression test results, the hydrogels had the capability to fully recover their mechanical properties after healing process was completed. Under applied shear stress, the hydrogels demonstrated a liquid-like characteristic, and upon relieving the strain the elasticity was entirely recovered. The recovery of G′ had a direct relation with the proportion of BG microparticles in the hydrogels and enhanced in line with an increase in BG content. This phenomenon was attributed to the confined ability of BG particles, when their concentration is low, to regenerate non-covalent links with polymers as they were fully coated with BP-functionalized hyaluronan chains during gel destruction. On the other hand, applying high shear at higher concentration of BG resulted in more homogenous distribution of BG particles within the hydrogel, thereby leading to an increase in healing efficiency.[105] The same group also developed a colloidal hydrogel from BP-functionalized GEL nanoparticles and micro-sized 45S5 BG.[134] Similar to their first study, the inclusion of BG particles into the colloidal GEL gels gave rise to a considerable enhancement of elastic modulus, from 0.1 kPa for pure GEL to 10000 kPa for the composite loaded with 25 wt.% of BG (Figure 7). Indeed, more elastic gels were produced when BG/GEL ratio was less than 1, yet the tan(δ) rose remarkably as the BG content further increased, suggesting the hydrogels behave like a viscous liquid at high BG concentrations. It is worth noting that the presence of BP was only effective when the concentration of BG microparticles was very low (<0.1 wt.%). For higher concentrations, there was no appreciable difference between the gels made of BP-functionalized and non-functionalized GEL nanoparticles. The

samples were able to entirely self-heal at low concentration of BG particles in less than 7 s; however, the G′ recovery decreased progressively as a function of BG content and reached less than 1% when the amount of BG particles was 25 wt.%.[134]

Figure 7

3.1.2 Summary and discussion

Overall, the ability of BGs to degrade and release ions to the solution in which they are dispersed make it possible to regulate the gelation kinetics of hydrogels by simply controlling their compositions, dimensions (i.e., micro- vs. nano-sized) and concentrations. Release of certain metal cations such as Ca^{2+}, Cu^{2+} or Sr^{2+} can contribute in ionic crosslinking of polymers such as pectin, CS and ALG to induce gelation, while changing of the solution pH can speed up or slow down the gelation of pH-dependent systems.[93, 100, 101, 121, 135, 136] As they typically release alkaline cations, BGs play the role of gelling accelerator where the higher pH favors hydrogel formation, and they exert gelling retardant effect where the gel formation is accelerated at more acidic pH.[110, 113] In addition, the size of BG particles could have a profound impact on gelation kinetics of hydrogels as smaller particles with higher surface area dissolve in a faster rate and release more ions into the surroundings. The shortened gelling time and enhanced elasticity of composite hydrogels support the hypothesis that further molecular interactions could occur during the process of gelation by incorporation of BGs into the polymeric hydrogels. The viscosity of hydrogels was found to enhance with increasing BG content, and the injectability would be likely impeded at high BG concentrations.[95, 96, 101, 108] The results reported to date indicate that, at low concentrations, BGs have often insignificant implication on gelation temperature of hydrogels with a marginal difference of some 1-2 °C.[94, 96, 98, 109] Despite the major role that BGs' composition, size and morphology can play on the gelation behavior of hydrogels, they have received little attention in recent studies; thereby the impact of such parameters on hydrogel optimization is still to be fully explored.

A major limiting factor with injectable hydrogels is their rather poor mechanical properties. The studies reviewed here suggest that incorporation of BGs into the hydrogels can be an effective approach for improving the mechanical properties of the polymeric hydrogels (see Table 1).[101, 102, 106] A key step in the development of BG-incorporated hydrogel systems with robust mechanical properties is the homogenous distribution of BG particles within the hydrogel matrix in order to maximize their interactions with the polymer chain. At high concentration, BG particles tend to aggregate, which can diminish the particle active sites available for formation of crosslinking bonds in the composite network. Furthermore, the particle aggregation can lead to non-uniform crosslinking density in the hydrogel and subsequently non-homogeneous mechanical properties. Comparatively, small amounts of BG particles can result in insignificant enhancement in mechanical properties of hydrogels. Functionalization of BG particles with a biocompatible material and suitable functional groups may pave the way for preparation of uniform composites by covalently binding the BG particles with the polymer matrix and further enhances the degree of crosslinking within the hydrogel: in this way, a mechanically more robust hydrogel would be obtained without compromising their injectable properties. It has also been shown that the viscosity and rigidity of these composite hydrogels could be regulated by addition of organic fillers such as cellulose nanofibrils.[132] However, more research efforts are still needed to investigate the effect of

co-incorporation of BGs and organic fillers on the rheological and mechanical properties of injectable hydrogels. We envision that further investigation into these systems will give rise to unique multifunctional biomaterials for efficient tissue regeneration. Further in-depth studies on the fracture toughness of BG-reinforced hydrogels will also be important to understand the service life of the hydrogels and to set their operation limit. Indeed, numerous injectable hydrogels are restricted by their low toughness and high creep. We anticipate that small to moderate amounts of BG inclusion could improve the fracture toughness of these materials by distributing stress and dissipating energy throughout the hydrogel, and, thus, can impede or delay their failure.

3.2 Effect of BGs on swelling ratio and degradability

3.2.1 Overview

The highly hydrophilic nature of hydrogels enables them to absorb and hold substantial amounts of liquid within their structures with respect to their mass. This interesting property provides cells with an ECM-like environment, which renders hydrogels particularly suitable for cell-based therapeutics and tissue engineering applications.[18, 34, 137] However, it is important to consider that extensive hydrogel swelling can induce several adverse effects *in vivo* since a highly swollen hydrogel possesses lower mechanical stability and can generate compression on surrounding healthy tissues when they are applied, for instance, for dural or socket preservation applications.[138, 139] It can also result in bulging of the hydrogel from the injection site and causing cellular irritation. On the other hand, the transfer of oxygen, nutrient and waste from cells, the release of the encapsulated drugs, growth factors, and cells as well as the cellular infiltration upon *in vivo* implantation are highly relied on the hydration and degradation degree of the hydrogel scaffolds.[140-142] The more hydrophilic the polymer forming the hydrogel is, the higher its ability to absorb water and swell, which results in an alteration of the degradation kinetics of the hydrogel.[143] Understanding the physiological degradability of hydrogels is particularly vital in designing a tissue-engineered construct. An ideal tissue scaffold should degrade at a compatible rate with tissue regeneration, physically create open space for neo-tissue growth until full regeneration is achieved.[10] A hydrogel scaffold with slow degradation rate can impede neo-tissue ingrowth.[144] The hydrogel degradation normally occurs as a result of three basic biological processes; hydrolysis, dissolution and enzymatic cleavage.[145, 146] The degradation rate of hydrogels depends on several factors including crosslinking density and nature, polymer molecular weight, structural properties of hydrogels (i.e., porosity percentage and pore morphology), as well as the physiological pH. Furthermore, the degree of hydrogel swelling and degradation can be partly governed by inclusion of inorganic fillers (e.g., BGs) into their structure. Upon contact of BG composites with body fluid, rapid ion-leaching/proton-exchange occurs for alkali elements of BGs, resulting in the formation of new interfaces which enhance the hydration degree, thereby altering the hydrogel degradation kinetics.[147, 148] Incorporation of BGs into polymeric hydrogels with acidic degradation by-products, like polyesters, can regulate the degradation profile by buffering the local pH of nearby solution.[149] Notably, in order to ensure that these composite hydrogels can preserve their structural and mechanical stability *in vivo*, degradation rates of both organic and inorganic phases need to be matched.

The hydration and degradation behavior of such composite hydrogels can be dramatically changed by the size and concentration of BG particles. For example, it has been reported that as the concentration of a sol-gel-derived nano-BG (40 SiO_2-45 CaO-15 P_2O_5, mol.%) increases

in a GelMA hydrogel, the degradation and swelling rates of this hydrogel decrease significantly, as a result of formation of ionic bonds between the Ca ions released from the BG phase and carboxyl moieties of GEL as well as electrostatic interactions between the negatively charged surface silanol groups of BG particles and amino groups of the GEL.[104] A decrease in the free carboxyl groups of GelMA as well as the enhanced crosslinking degree in the composite hydrogels have been found to be the primary reasons for diminished hydrophilicity and water absorption. These changes in crosslinking degree of the hydrogel have had a knock-on effect on degradation profile of hydrogels where the remaining mass of composite hydrogels increased with a rise in the loading of BG particles. It was also observed that the incorporation of BG particles into the GelMA network yielded a scaffold with thicker pore wall, which could impede the process of collagenase solution penetration.[104] Inclusion of a nanoscale spherical MBG (85 SiO_2-15 CaO mol.%) into an injectable GEL/OCS hydrogel has also led to a reduction in the degradation rate as a function of BG content, and the time of degradation was prolonged from 21 to 36 days for the unloaded and 15 wt.% loaded hydrogels, respectively. Similar to the above study, the enhanced crosslinking degree after addition of BG nanoparticles was found to be the main cause of such phenomenon.[102] The degradation degree of a hydrogel composed of GEL and ADA was found to be modulated by the addition of 45S5 BG microparticles.[99] As the concentration of BG increased, the degradation rate reduced because of the enhanced crosslinking degree between the polymer components. The authors also speculated that the reactivity of Schiff base bonds can be appreciably enhanced by a rise in the pH of the mixture, arisen from released ions from BG particles. The complex formation between the silanol groups of micro-sized BGs and hydroxyl groups of ADAs was suggested to be another reason for the enhanced crosslinking degree.

Surface modification of BG nanoparticles has also been shown to be an effective approach to regulate structural stability of composite hydrogels. Incorporation of GelMA-modified nano-sized MBG into GelMA matrix has resulted in a marked decrease in swelling ratio (Figure 8).[103] Comparison of the composite hydrogels containing unmodified and modified MBG particles demonstrated that degradation of the latter composite occurs more slowly in PBS solution due to the formation of further crosslinking between the GelMA and glass using carbodiimide chemistry, and increasing the BG concentration could interestingly speed up the degradation rate.

Figure 8

The accelerated degradation rate of composite hydrogels after inclusion of Cu-doped MBG particles have been proved in another study where amphiphilic PEU based on Poloxamer 407 was filled with two MBGs of different morphologies, i.e., spheroidal nanoparticles and microspheres.[108] Regardless of the MBG morphology, the mass loss increased over the incubation time in Tris HCl buffer solution and a significantly higher weight loss was found as compared to the non-loaded hydrogels (~80% vs ~40%). The fast degradation of these composites can be primarily attributed to the fast dissolution rate of MBGs, resulted from their high surface area. The released Cu ions was postulated to catalyze the oxidative degradation of hydrogel networks, which can facilitate controlling the degradation rate of the composite hydrogel at the site of pathology.[150] Another recent study also illustrated the faster degradation of an injectable hydrogel composed of PEGDA and ALG with incorporation of a nano-BG.[111] Indeed, the swelling ratio of this hydrogel enhanced with an increase in BG content, from 168.2% for the neat hydrogel to 220.0% for the hydrogel incorporated with 3 mg

mL^{-1} BG nanoparticles. The authors assumed that this phenomenon could be due to the formation of strong bonds between the ALG and BG nanoparticles, which would likely decrease the crosslinking density in the PEGDA network, and consequently bring about the quicker degradation of the composite hydrogels.

Evaluation of the influence of BG particle size on degradation behavior of a gellan gum hydrogel manifested that weight loss of the hydrogels containing nano-sized BG was significantly higher than the microcomposite counterparts after 5 days of immersion in citric acid buffer, pH 3.[151] This difference was thought to stem from the enhanced degree of crosslinking in the hydrogels containing the micron-sized BGs which release remarkably more Ca ions. In author's opinion, the method of preparation was the influential parameter in solubility of BG particles. While BG microparticles were synthesized by sol-gel approach, flame-spraying technique, which is a high temperature process, was employed for the synthesis of BG nanoparticles. Indeed, although the apparent outer surface area of BG nanoparticles is larger than that of BG microparticles, the latter has an extra-area associated to the presence of mesopores, which are inherent of the sol-gel process and thus, more ions can be released from the BG microparticles. Consequently, as obtained from ICP-OES analysis, the nano-BG released less Ca^{2+} and thus the hydrogel was less crosslinked. Another hypothesis posed in this study was that the hydrogel networks were disturbed by the BG nanoparticles to a greater extent due to more even distribution of these particles in comparison to micro-sized BG: therefore, the hydrogel became more susceptible to degradation in the presence of nano-BG.[151]

3.2.2 Summary and discussion

In summary, BG particles, irrespective of their size, morphology or composition, can be utilized to modulate the hydration and degradative properties of hydrogel biomaterials. Nonetheless, the size control of BG particles could have a profound influence on their dissolution and distribution within the hydrogel matrix. Moreover, the method of BG preparation could yield BGs with different porosity and solubility, so that the highly porous sol-gel-derived BGs dissolve much faster than the melt-derived BGs when incorporated into the hydrogel system, and thus can release reasonably more ions to the system.[152] As such, they can have a greater influence on pH sensitive hydrogels or networks. The enhanced network formation within the hydrogels can often alleviate the hydration and degradation rates. Even though the effects of BG particles on degradation and swelling rates of hydrogels have been reported in several studies, more research efforts are still needed to address the challenges related to the discrepancy in the degradation rate of BG particles and the polymeric matrix, which could often lead to instability of the hydrogel scaffold and migration of the particles *in vivo*.[26] It is also important to comprehensively assess the degradation behavior of these composite hydrogels *in vivo* since *in vitro* studies alone can just partially model the degradation process of hydrogels in the human body. Indeed, *in vitro* studies are often carried out under static mechanical conditions and considered the influence of only one parameter or enzyme which fails to truly reliably simulate the mechanically dynamic environment and complex mechanisms that synergistically contribute to degradation of hydrogels *in vivo*. For instance, the degradation products of hydrogels can promote an inflammatory response, resulting in the recruitment of immune cells to the inflammation site and consequently the release of reactive oxygen species, which can accelerate the degradation of hydrogels.

4. Biomedical applications of BG-reinforced hydrogels

The significance of tissue regeneration has markedly risen with extended life expectancy. The physical, mass transport and biological properties should be considered precisely during selection of an appropriate hydrogel material for a specific application. In other words, the characteristics of the natural tissue for which one is designing a hydrogel biomaterial should be carefully regarded, as in our body different tissues have distinctive properties and functions. A hydrogel for tissue engineering must fulfil a series of criteria to function properly and promote new tissue formation once implanted into the body. For example, it must be biodegradable, biocompatible to cells and adjacent tissues, induce no immune response, be capable to transport cells and other cargos to the damaged tissue, facilitate the bidirectional diffusion of nutrient, oxygen and metabolites, and provide support for neo-tissue development until it matures. Although myriads of non-toxic hydrogels have been synthesized which do not activate a chronic immune response, most of them lack bioactivity to promote cell adhesion. In fact, the hydrophilic nature of hydrogels reduces the adsorption of ECM proteins like fibronectin, laminin and vitronectin, which play key roles in cell attachment and function.[153, 154] A promising strategy to improve the bioactivity of hydrogels is to add bioactive fillers, such as BGs, into their matrices. This gold combination can result in multifunctional materials, which can resemble the structure and properties of native tissues. Notwithstanding that BGs were initially developed for hard tissue (bone) regeneration, their application in soft tissue regeneration such as wound healing or skin regeneration are growing rapidly as these "magic" materials can act as an outstanding delivery platform for therapeutic ions and biomolecules, which can stimulate tissue regeneration through regulating antibacterial activity, angiogenesis and immune response. In this section, we will look into the application of BG-reinforced composite hydrogels in bone tissue regeneration as well as skin wound healing.

4.1 Bone and osteochondral tissue engineering

4.1.1 Overview

Bone defects resulting from infections, trauma, skeletal diseases, or non-unions represent a substantial challenge to clinicians. The current gold standard for treatment of bone defects, i.e., autografts and allografts, have limitations relating to infection, donor site morbidity, disease transmission and chronic pain.[155] The use of an artificial material, which outperforms the current gold standards, to mediate bone formation is the primary goal of bone tissue engineering. Bone or osseous tissue is considered as a hard tissue with the capacity to self-repair and regenerate; hence, hydrogel scaffolds should have similar features in order to be able to withstand and respond to the mechanical stress. It is well-documented that the viscoelastic properties of hydrogels, such as stress relaxation, can direct the cell responses and enable cellular remodeling of these scaffolds.[156] These scaffolding materials serve as a temporary ECM for mineralized matrix deposition, cellular adhesion, proliferation, differentiation, and migration.[157] Extensive studies have reported the mechanism of bone formation and strategies for designing suitable bone-regenerative biomaterials.[158, 159] An ideal biomaterial for bone tissue engineering should possess the ability to induce both osteogenesis and angiogenesis and desirably have pore size larger than 100 μm, with an optimum of 200-350 μm for tissue ingrowth.[160] Nonetheless, this concept might be valid for the scaffolds composed of rigid materials such as metals, ceramics or glasses, whereas hydrogels often swell and degrade faster than these stiff materials in physiological conditions, and thus can create larger pores over time. A desirable biomaterial should also possess mechanical properties

comparable to the tissue intended to be treated, *e.g.,* Young's modulus (0.1-2 GPa) and compressive strength (2-20 MPa) of trabecular bone. [161] However, the poor mechanical properties of hydrogels limit their application in bone tissue engineering to non-load-bearing sites. Incorporation of fillers, in particular BGs, is a viable and simple approach to improve their mechanical properties, besides imparting bioactivity characteristics to the systems, thereby promoting scaffold/host integration and the rate and quality of bone formation. These hydrogels have been used both with and without encapsulated cells. The effect of BG particles on bone formation ability of such hydrogels is discussed in the following paragraphs.

Recently, much research interest has been generated in the study of pH-induced injectable CS-based hydrogels as CS can undergo sol-gel transition simply by tuning the environmental pH.[162] These hydrogels have also exhibited thermosensitivity around the body temperature, which is considered a pivotal characteristic in biomedicine. For instance, a series of injectable drug-loaded CS hydrogels were developed with and without BG nanoparticles (46 SiO_2, 24 CaO, 24 Na_2O, 6 P_2O_5 mol.%, ~748 nm) by employing $NaHCO_3$ as gelling agent.[95] Incorporation of nano-BG into the CS hydrogel was found to be effective in control of the initial burst release profile of encapsulated raloxifene hydrochloride (RLX). Indeed, the amount of RLX release was reduced in correlation with increasing BG content, attributed to the enhanced crosslinking density of the gel by formation of higher electrostatic interactions between the CS and BG nanoparticles. The unloaded CS hydrogel was compared with that containing 1% (w/v) BG nanoparticles in terms of bone formation ability *in vivo* after implantation in rat tibia non-critical bone defect models. Surprisingly, an accelerated bone regeneration was observed for the pure CS hydrogel group, which was attributed to a more rapid RLX release at early stages. On the contrary, the bone healing effect of the BG-loaded hydrogel was detected only after 6 weeks post-implantation. The authors speculated that the bone healing function of the BG-filled hydrogel would be more pronounced if tested in critical-sized bone defects, which usually require extended time to heal.[95]

In another recent study, Moreira and co-workers synthesized a rBMSC-laden thermosensitive hydrogel by combination of CS, GEL, 58S BG nanoparticles and β-GP.[163] The proliferation of encapsulated cells exhibited an increasing trend over 7 days of culture for the sample containing BG nanoparticles and it was significantly higher than the pristine polymeric hydrogel. *In vivo* studies displayed no inflammatory response in subcutaneous and liver tissues of Swiss mice treated with the hydrogels. The group treated with the composite hydrogel illustrated a more efficacious bone regeneration 30 days after implantation in the rat tibial defect than the untreated group. However, the neat polymeric hydrogel was not examined in this study for its ability to stimulate osseous tissue formation and thus it is not possible to firmly conclude to what extent the superior bone forming ability of these hydrogels can be correlated to the presence of BG nano-inclusions.[163]

Angiogenesis is an indispensable process during tissue formation as blood vessels are the means by which nutrients, oxygen and even osteoprogenitor cells are supplied to the neo-tissues.[164] Cu ions are potent angiogenic stimulators, in the sense that they promote angiogenesis through upregulation of a range of provasculogenic genes.[165] To this end, Wu and co-workers, explored the bone regeneration ability of a thermally responsive hydrogel constituted of CS/SF/β-GP and incorporated with Cu-free (95 SiO_2-5 CaO mol.%, ~460 nm) and Cu-doped (95 SiO_2-2.5 CaO-2.5 CuO mol.%, ~490 nm) BG nanoparticles by comparison of their angiogenic and osteogenic abilities.[93] *In vitro*, all the gels were able to support the cell growth and promoted the expression of osteogenesis- and angiogenesis-related genes such as ALP, VEGF, bFGF and bFGFR in MC3T3-E1 and human umbilical vein endothelial cells

(HUVECs) at a significantly higher level for the hydrogels loaded with Cu-BG nanoparticles. Eight weeks after *in vivo* implantation into critical-sized rat calvarial bone defects, the group treated with the Cu-BG-reinforced hydrogels (1 mol.% Cu) were fully recovered in the absence of growth stimulators like growth factors and/or cells, which was evidenced from the formation of neovascularization and deposition of mineralized collagen, thanks to the biological effects of the ions released from BGs. Nevertheless, a negligible amount of regenerated osteoid tissue was detectable in the bone defects treated with the unfilled hydrogel.[93]

Engineering osteochondral tissue is considered challenging as it requires a scaffold which can resemble both cartilage and bone. The use of bilayered scaffolds as an emerging strategy has aroused considerable interest in recent years.[166] However, the most challenging part in the development of such scaffolds is the choice of host biomaterials which can adequately bond together and minimize the risk of poor integration between the regenerated cartilage and subchondral bone at the interface.[167] In this respect, an injectable, self-healing, DN hydrogel consisting of glycol chitosan and dibenzaldehyde-functionalized PEG network as well as ALG and calcium chloride network was fabricated, which exploited the favorable formation of a continuous matrix.[97] The lower layer was reinforced with 3 wt.% 58S BG nanoparticles (300 nm) to fulfil the unique requirement for subchondral bone regeneration, whilst the upper layer remained unfilled aiming to promote chondrogenesis. After 24 weeks post-implantation into full-thickness osteochondral defects in rabbit models, the defects treated with both hydrogels, with and without BG nanoparticles, were fully recovered and the neo-cartilage tissues were well-integrated with the surrounding cartilage. Surprisingly, the group treated with pure BG powder failed to regenerate the cartilage and only a thin layer of fibrocartilaginous-like tissue was observed at the end of the experiment. However, bone formation was markedly stimulated by the hydrogels containing BG nanoparticles. Newly dense subchondral bone was formed in the three groups of BGs only, biphasic scaffold with TGF-β and/or BG, and they were connected with the neighboring host osseous tissue, whereas the deep space of the defects remained empty for the group treated with the hydrogel without BG nanoparticles. In contrast to the BGs only group, the volume of new trabecular bone formed in the defects filled by the biphasic hydrogels was lower, implying that ion release from BG nanoparticles was delayed when incorporated into the hydrogel. The authors believed that this phenomenon could promote the cartilage repair in long term as the slowly formed neo-bone can provide a mechanically stable platform for cartilage crawling, and lower the risk of cartilage collapse.[97]

In another noteworthy study, an injectable continuous bilayer-stratified scaffold was developed using a rBMSC-encapsulated ALG/Bioglass (20 μm) hydrogel to be used for subchondral bone regeneration and an ALG/agarose (AG) thermosensitive hydrogel loaded with rBMSCs/rat articular chondrocytes (ACs) co-cultures to induce cartilage regeneration.[168] As both hydrogels consisted of ALG in their composition a continuous phase was created without any clear interface barrier. While released ions from BG phase of the ALG/BG/rBMSC hydrogel stimulated the osteogenesis of rBMSCs inside and outside the scaffold by upregulating the expression of ALP and collagen type-I, the ALG/AG/rBMSCs+ACs hydrogel was able to maintain the chondrogenic phenotype of ACs inside the hydrogel and enhanced the chondrogenesis of rBMSCs outside the hydrogel by upregulating important chondrogenic markers such as collagen type-II and aggrecan (Acan), demonstrating the potential of this bilayer scaffold in regeneration of osteochondral tissue. The implantation of the bilayered scaffold into a rat osteochondral defect model led to the formation of matured subchondral bone and hyaline-like cartilage with appropriate integration with the adjacent tissues 12 weeks post-surgery. However, the time for subchondral bone regeneration was increased when the

ALG/AG/rBMSCs+ACs was solely used, and the regenerated subchondral bone and cartilage had a different structure than the natural tissues.[168]

A similar approach was used to develop an injectable hydrogel based on ALG/AG and incorporated with Bioglass and quercetin (an anti-inflammatory drug) to regulate the host inflammatory response and prevent the failure of cartilage repair.[169] Indeed, cartilage tissue has a limited self-repair ability because it lacks vascularization and co-incorporation of BG and quercetin into the hydrogel matrix can exert a synergistic effect in accelerating cartilage regeneration. BGs have shown the ability to promote the differentiation of macrophages to the M2 type and together with the quercetin could maintain the chondrocytic phenotype, prevent ECM degradation by reducing the expression level of matrix metalloproteinase-13 (MMP13), matrix metalloproteinase-1 (MMP1) and inducible nitric oxide synthase (iNOS), and delay the progression of osteoarthritis, thereby enhancing the cartilage regeneration capacity of the hydrogel. Moreover, the gene tracks showed significant upregulation of ECM and chondro-related genes such as Acan, SRY-box 9 (SOX9) and collagen type II alpha 1 chain (COL2A1) in the presence of BG/quercetin-containing hydrogel extract. Twelve weeks post-implantation in rat full-thickness articular cartilage defects, significantly higher collagen type-II was observed in the group treated with the hydrogel co-incorporated with BG and quercetin in comparison to the hydrogel filled with only BG and control groups. In addition, the defects were filled with a thicker neo-cartilage tissue and boundary between the defects and the neighbouring tissues were almost disappeared.[169] Micro-CT imaging demonstrated that the BG/quercetin-containing hydrogel could also promote the regeneration of subchondral bone more efficiently than the other groups, 12 weeks post-surgery. In another similar study, ALG/AG/BG hydrogel was combined with another anti-inflammatory drug, i.e. naringin, which resulted in similar outcomes in terms of cartilage regeneration.[170]

Hybrid hydrogels of ALG-GEL have been made to address the drawbacks of individual materials. Not only this approach can result in an enhancement in ALG bio-interfacial interactions with cells, but it can also improve the stability of GEL in physiological conditions.[171, 172] Promising results have been obtained from the covalently crosslinked ADA and GEL. The findings revealed that this injectable hydrogel is highly biodegradable, and can significantly enhance human dermal fibroblast (HDF) adhesion, viability, spreading and proliferation as compared to the pristine ALG hydrogel.[173] Most notably, cell growth has been optimum when equal quantities of the polymer precursors were mixed. Lack of bioactivity has, however, limited the application of this hybrid hydrogel in bone tissue regeneration and has been the motive for development of their composites by inclusion of BG particles of various compositions and sizes into their structures. In a recent study, the ability of a series of hydrogels with and without 45S5 BG (2 μm), namely pure ALG, ALG-GEL, ADA-GEL, and ADA-GEL with 1 and 5 wt.% BG microparticles, were compared in terms of induction of osteogenic differentiation of murine bone marrow stromal cells (ST-2 cells) *in vitro*.[101] Unexpectedly, incorporation of micro-BG in ADA-GEL led to a remarkable decrease in cell viability. It was thought that increased degree of crosslinking between the polymer components after addition of BG particles played a major role in this phenomenon. On the one hand, with an increment of crosslinking degree, the GEL release rate decreases, resulting in retention of cell adhesion motifs within the scaffolds, on the other hand, the degradation rate reduces which can negatively influence cell viability as the space required for cell proliferation and migration diminishes. The release of ions in excess of cytotoxic concentration level from BG phase was said to be another reason for reduced cell viability. The cells tended to form a cluster on the surface of pure ALG and ALG-GEL scaffolds while the surface of ADA-GEL composite with 1 wt.% BG was covered with the flattened cells. Due to the cytotoxic effect of high release of

ions; however, a noticeable decrease in the number of cells was observed on the composite loaded with 5 wt.% BG microparticles. It is worth mentioning that addition of BG, particularly at high concentration, decreased the degradability rate and, in turn, the degree of cell migration and osteogenic differentiation.[101] Nevertheless, Reakasame and co-workers reported that when the amount of 45S5 BG microparticles is as low as 0.1 and 0.5 % (w/v), the ALP activity of MG-63 cells encapsulated in ADA-GEL microcapsules increases in line with rising the BG content.[99] Therefore, it can be inferred from these observations that released ions from BGs can upregulate gene expression in osteoprogenitor cells when the concentration of ions is within the therapeutic range. This is consistent with previous literature on BGs.[174] Notably, due to the rapid degradation of composite hydrogels in the first 3 days of culture, cell viability appreciably increased from day 3 to day 7. However, it was still significantly lower than the unloaded hydrogel, which was said to be presumably due to enhanced pH of the matrix induced by BG particles. In order to have a better picture of the role of BG particles on biocompatibility and angiogenic potential of these hydrogels, *in vivo* studies was conducted by subcutaneous implantation of ADA-GEL with and without 45S5 BG nanoparticles (20–60 nm) in rats.[175, 176] Inclusion of a small quantity of BG (0.1% w/v) into the hydrogel only marginally increased the cell survival and no noticeable inflammatory reaction was observed 4 weeks post-implantation. Scaffolds containing BG nanoparticles also promoted angiogenesis and it was markedly enhanced by rat bone marrow-derived mesenchymal stem cell (rBMSC) encapsulation in comparison to the cell-free scaffolds. This was evident by the higher number of lectin-positive endothelial cells appeared in the nanocomposite hydrogels.[176]

The biocompatibility of ALG-GEL hydrogel with a variety of mammalian cells and its tunable rheological properties have made it a potential candidate for use as a bioink to fabricate 3D printed cell-laden constructs for use in bone regeneration.[177, 178] In a recent study, Leite and co-workers investigated the influence of BG nanoparticles with and without Sr (i.e., 55 SiO_2-40 CaO-5 P_2O_5 and 55 SiO_2-30 CaO-5 P_2O_5-10% SrO, mol.%) at two different concentrations of 0.1 and 0.5 % (w/v) on the ability of ADA-GEL hydrogel to preserve the viability of embedded MG-63 cells during printing.[100] Cell viability in both BG-loaded and unloaded hydrogels was comparable, suggesting that nano-BG, irrespective of the composition, have no adverse impact on the viability of encapsulated cells. What is more, all formulations containing BG nanoparticles promoted the formation of an apatite layer on their surface after immersion in SBF and indicated high potential for sustained release of ibuprofen. As the amount of nano-BG increased the release of ibuprofen became slower, likely owing to the formation of a hydrogen bonding between the carboxylic acid moieties of the ibuprofen and silanol groups of BG particles.[100]

In another interesting study, Ojansivu and co-workers compared the sensitivity of two cell types i.e., Saos-2 and hBMSC cells, to the applied shear stress during the printing process of a multicomponent hydrogel of ALG-GEL incorporated with CNF and a borosilicate BG (47.12 SiO_2-6.73 B_2O_3-6.77 CaO-22.66 Na_2O-1.72 P_2O_5-5 MgO-10 SrO, mol%; 13–50 μm), with potential to be used in bone tissue engineering.[132] While inclusion of a small amount of CNF to the hydrogel enhanced the printability of hydrogels, the presence of BG microparticles promoted the osteogenic activity of both cells. The results disclosed that the Saos-2 cells survived during the printing process and well-proliferated in BG-free gels, whilst their viability and proliferation significantly reduced in the gels containing BG microparticles (Figure 9). The authors further explained that the high viscosity of the gels incorporated with BG microparticles has necessitated the application of high shear stress during printing, resulting in an extensive drop in cell viability. In contrast, hBMSCs embedded gels containing both CNF and BG microparticles were found to better sustain the printing-induced shear forces, but also

limited proliferation over 14 days. This study highlighted the importance of viscosity and printing-induced shear forces in the fate of cells in both short and long term.[132]

Figure 9

In a bid to develop a bone-tissue engineered 3D scaffold with enhanced mechanical properties and cellular response, human adipose-derived mesenchymal stem cells (hASCs) encapsulated ALG-GEL bioink was bioprinted alongside PCL paste with and without 13-93B3 BG (~20 μm) by means of a solvent-based extrusion 3D bioprinting technique.[179] As expected, co-printing the bioink with PCL/BG resulted in enhanced mechanical properties and bioactivity. Both direct (i.e., BGs added to the bioink) and indirect (i.e., BGs added to the PCL) approaches brought about a marked decrease in cell viability on day 0 predominantly due to the initial pH shock. However, the cell viability improved for the direct approach after 7 days of culture, which was attributed to a shift in the pH to neutral values as the media changed frequently. This study strengthens the hypothesis that the solvent-based bioprinting process could be considered as a potential technique for development of cellularized scaffolds.

The positive effect of a nano-sized BG (55 SiO_2-40 CaO-5 P_2O_5 mol.%, 55 nm) on biomineralization of a series of hydrogels composed of bone-related SaOS-2 cells-laden ALG-GEL and polyphosphate-Ca^{2+}-complex, and/or silica, or biosilica was highlighted in a recent study.[180] The authors found that the incorporation of BG nanoparticles into the 3D printed constructs led to a substantial enhancement in the mineralization potency of the embedded cells as evaluated by Alizarin Red staining, while they had an insignificant influence on the cell proliferation.

In another study, a triple crosslinked injectable hydrogel was developed by Bai *et al.* for cranial bone repair based upon a double modified-ALG and F127 crosslinked-chondroitin sulfate and was reinforced with 45S5 BG microparticles (20–50 μm).[136] The results of *in vitro* cytotoxicity study demonstrated a comparatively higher cell viability for the control group (without scaffold) than the group tested with the composite hydrogel. *In vivo* implantation of the composite hydrogel into critical-sized cranial defect in rats; however, elucidated formation of drastically more bone-like tissue in comparison to PBS control. Unfortunately, the bone formation ability of the hydrogel without BG was not assessed in this study.

Composites of gellan gum and BGs have shown high potential for bone tissue engineering. In this respect, the antibacterial activity and cellular biocompatibility of a series of injectable gellan gum hydrogels filled with BGs of various sizes and compositions were evaluated in a recent study.[151] The hydrogels containing both calcium-rich and calcium-poor BG microparticles synthesized through sol-gel techniques showed remarkable cytotoxicity towards MG63 osteoblast-like cells at all tested time points up to 7 days which was attributed to the high Ca^{2+} release from the BG phase that could surpass the cytotoxic level in cell culture medium. Nonetheless, the cell viability for the hydrogel incorporated with BG nanoparticles produced by flame synthesis reached approximately 80% of tissue culture plate control, which was ascribed to the less solubility and thus ion release from BG nanoparticles into the cell culture media. Though the former hydrogels showed improved rBMSC proliferation relative to the control, obtained from LDH assay, after incubation of the samples in PBS for a week, the ALP activity was significantly higher with inclusion of BG nanoparticles. The hydrogels filled with the sol-gel derived BG microparticles demonstrated superior antibacterial activity

against Methicillin-resistant Staphylococcus aureus (MRSA) bacterium than the nanocomposite counterpart, most likely due to the higher ion release rate, which could enhance the ionic strength and the pH of bacteria solution.[151] Surprisingly, incorporation of the calcium-rich BG microparticles doped with a small amounts of antimicrobial Zn into the gellan gum hydrogel resulted in insignificant enhancement of antibacterial activity against MRSA relative to the undoped BG-reinforced hydrogel.[181] This phenomenon was ascribed to the high affinity of gellan gum for divalent ions (i.e., Zn^{2+}) which can cause a decrease in Zn^{2+} release into the medium.

Osteogenic differentiation of embedded cells in hydrogels could be induced by the ionic dissolution products of BGs. In a study, hydrogels were developed by mixing hASC-embedded gellan gum with a BG extract (12.1 Na_2O-14 K_2O-19.8 CaO-2.5 P_2O_5-1.6 B_2O_3-50 SiO_2 wt.%) and/or cationic spermidine and compared with a cell-laden collagen hydrogel.[182] The viability, proliferation and osteogenesis of hASCs were evaluated in two different media, i) osteogenic medium (OM) and ii) OM with BG extracts (BG-OM). It was found that osteogenic differentiation of hASCs was remarkably promoted for the gellan gum hydrogel crosslinked with BG ionic dissolution products cultured in the presence of BG-OM supplements with respect to the typical OM. In addition, a notably higher mineralization of encapsulated hASCs was observed in the gellan gum hydrogel crosslinked with BG extract compared to that with spermidine, mainly because of high Ca^{2+} content of the BG phase, suggesting that the need for a separate cationic crosslinker like spermidine might be eliminated for the development of gellan gum hydrogels where BGs are present. Comparison of both types of hydrogels revealed that while the cells remained well viable on all the hydrogels, the cell proliferation and expression of osteogenic marker genes, such as DLX5, OSX and RUNX2, were significantly higher in the collagen hydrogel either in OM or BG-OM than the gellan gum/spermidine hydrogels. This study, however, did not assess the influence of crosslinking time and BG extracts of different concentrations on the encapsulated cells.[182]

The inclusion of BGs has shown to be a promising approach towards modulating collagen scaffold degradation as well as enhancing the structural stability and mineralization potential of the pure collagen hydrogel. A recent study indicated that the incorporation of 45S5 BG microparticles into the collagen hydrogel can induce osteoinductive properties in the absence of osteogenic cells.[183] In this regard, gel aspiration-ejection technique was deployed to prepare an injectable dense collagen hydrogel with and without BG microparticles and their ectopic bone formation was extensively studied after injection into subcutaneous sites of adult rats. As depicted by Micro-CT analysis, a markedly higher mineralized phase was deposited on the composite gel than the pure collagen gel, 21 days after surgery and no signs of inflammation was seen in both groups at the end of the experiment. While neovascularization was observed in the group treated with the composite gel at 1-week post-implantation, it was only evident after 3 weeks in the group treated with pure collagen gel. Notably, collagen remodeling into woven bone-like tissue was detected with second harmonic generation imaging. The authors pointed out the potential of this technique for production of tissue scaffolds at large-scale and low-cost with insignificant rise in processing time that make it probable to outperform the existing techniques.[183]

Composite hydrogels of GEL have been formed by mixing with BG particles, which demonstrated enhanced physiological stability, bioactivity and biological functionality. To this end, a series of injectable self-crosslinked composite hydrogels comprised of GEL, OCS and varied amounts of spherical MBG nanoparticles (85 SiO_2-15 CaO mol.%; ~100 nm) were prepared to be used for bone regeneration.[102] The compressive strength (at 90% strain from

~1.95 to 9.05 MPa) and pore size (from ~50 to 217 μm) of hydrogels were favorably enhanced as a function of BG content and placed in the range reported for human trabecular bone. *In vitro*, the rBMSC proliferation rate was significantly higher on the composite hydrogels after 7 days of culture and enhanced with increasing MBG content. The considerably higher osteogenic potential of the composite hydrogels relative to the unfilled hydrogel was evident from the enhanced expression of osteogenic biomarkers including ALP, OCN, OPN, RunX-2, and Col-I as well as greater calcium biomineralization. The composite hydrogels demonstrated a noticeably higher bone regenerative capacity than the pure polymeric hydrogel after 6 weeks implantation in rat critical-sized cranial bone defects. Mature new bone was formed around the margin of the defects treated with the composite hydrogels, while the groups implanted with the unfilled hydrogel showed limited neo-bone formation. The *in vitro* and *in vivo* studies verified the bone-forming ability of the composite hydrogel arisen from the presence of MBG nanoparticles.[102]

BGs have been also exploited to improve the bioactivity and bone regeneration potential of GelMA hydrogels. For example, Zheng and co-workers reported the development of a series of composite hydrogels by mixing GelMA and various amounts of BG nanoparticles (40 SiO_2-45 CaO-15 P_2O_5, mol.%; ~400 nm) from 0 to 10 % w/v with potential to be used as bone tissue scaffolds.[104] The *in vitro* studies indicated that, at early time points (i.e., 1 and 4 days), mouse bone mesenchymal stem cell (mBMSC) viability was significantly higher for the unloaded hydrogel, while there was no noticeable difference between all the groups of hydrogels at day 7. It was assumed by the authors that the increase in the pH of culture medium as a consequence of fast release of Ca ions could interfere in cell proliferation at early time points of the experiment, the phenomenon which could be mediated at longer culture time. Likewise, ALP activity was lower for the mBMSCs cultured on BG-filled hydrogels at day 5, which was attributed to the smaller number of cells at early time points; however, the composite hydrogels markedly stimulated the ALP activity at day 10 and increased with the increase in BG content.[104] Noticeably, the composite hydrogels showed pore size in the range of 90-200 μm, satisfying the requirements for bone tissue engineering applications.

In order to enhance the performance of hydrogels, a few studies explored the impact of functionalized BG particles on the biological activity of the GelMA hydrogel. In an attempt to improve the structural stability and osteoconductivity of GelMA hydrogel for artificial periosteum to accelerate bone regeneration, Xin and co-workers produced a co-crosslinked composite hydrogel by combining surface modified-MBG nanospheres (80% SiO_2, 16% CaO, and 4% P_2O_5 mol.%) using GelMA with the GelMA matrix.[103] Similar pore size was observed for all the hydrogels (~150 μm) while at the same concentrations of MBG the compressive modulus of the hydrogels containing GelMA-grafted MBG was markedly higher than those filled with unmodified MBG. Nevertheless, increasing MBG content to >3 wt% led to a reduction in mechanical properties predominantly as a result of MBG agglomeration. *In vitro* studies disclosed that MC3T3-E1 cell adhesion and proliferation as well as osteogenic differentiation were promoted more significantly with the co-crosslinked nanocomposite hydrogel than the pure GelMA and uncrosslinked GelMA/MBG composite hydrogels. Besides the known biological function of both GelMA and MBG nanoparticles discussed earlier, chemical co-crosslinking approach could modulate the degradation rate of MBGNs embedded in the hydrogel and consequently made the local environment pH more stable which is more desirable for cell adhesion and differentiation. *In vivo*, the co-crosslinked nanocomposite hydrogels demonstrated a more rapid neo-bone formation and the greatest amount of mature lamellar bone after 8 weeks of implantation into critical-size calvarial bone defects in rats. Additionally, a remarkably greater number of positive-stained endothelial cells was observed

in the group treated with co-crosslinked nanocomposite hydrogel in the results of immunohistochemical CD31 staining, proven that this hydrogel is more effective in stimulation of neovascularization.[103]

Although the specific structural characteristics of MBGs can facilitate formation of surface HCA and regulate the expression of osteogenic associated genes, it takes some times until an HCA layer forms and the autocrine activity of osteoblasts being induced by the secreted growth factors like bone morphogenetic proteins (BMPs).[184, 185] In order to overcome this limitation, MBG nanoparticles grafted with recombinant human bone morphogenetic protein-2 (rhBMP-2) was employed to improve the bone forming ability of the GelMA hydrogel. BMPs are indispensable growth factors during tissue repair and remodeling upon injury and play a vital role in promoting cell proliferation and differentiation.[186] This strategy was found to be effective in controlling the release rate and safer use of rhBMP-2 *in vivo,* meanwhile, the hydrogel matrix protects the MBG nanoparticles (MBGNs) from being washed away by body fluids.[187] The findings of *in vitro* studies indicated that the hydrogels containing rhBMP-2 showed significantly higher cell proliferation and osteogenesis-related genes expression compared to the pure GelMA and GelMA with MBG nanoparticles. While there was no statistically significant difference between rBMSC adhesion on the hydrogels containing rhBMP-2-grafted MBGNs (GelMA/MBGNs-rhBMP-2) and that wherein the rhBMP-2 and MBGNs were added separately (GelMA/MBGNs/rhBMP-2), the number of cells was appreciably higher on the GelMA/MBGNs-rhBMP-2 surface (Figure 10). Additionally, on day 7, the expression of ALP was more pronounced on GelMA/MBGNs/rhBMP-2, due to the burst release of rhBMP-2, whereas GelMA/MBGNs-rhBMP-2 illustrated a remarkably higher mineralization ability than other groups after 21 days of culture. In the long-term, GelMA/MBGNs-rhBMP-2 hydrogel demonstrated a superior osteogenic potential than the GelMA/MBGNs/rhBMP-2 counterpart. Most notably, all the hydrogels possessed pore diameter of approximately 150 μm, which is considered to be suitable for supporting tissue ingrowth. In contrast to the other groups, the rat calvarial critical-sized defects were nearly completely filled with new-formed bone when treated with GelMA/MBGNs-rhBMP-2 hydrogel 8 weeks after surgery, highlighting the importance of controlled and sustained release of rhBMP-2. Overall, the expression of genes of osteoblastic cells was promoted at early stages by the release of rhBMP-2, and then by the long-term release of therapeutic ions from MBGNs, resulting in a programmed long-term bone healing effect.[187]

Figure 10

Colloidal gels are another appealing class of injectable biomaterials which have been widely utilized in tissue engineering in recent years. To this end, injectable and self-healing colloidal hydrogels were prepared from non-functionalized and BP-functionalized GEL nanospheres and 45S5 BG microparticles (~2 μm), and characterized for their antiosteoporosis effects.[134] *In vitro* mineralization study manifested that the composite gels, with a minimum BG/GEL ratio of 0.5, stimulated calcium deposition significantly at a higher level in the presence and absence of MC3T3 cells in the cell culture medium. The osteogenic differentiation of cells progressively increased for the bisphosphonate-containing hydrogels incorporated with the highest BG content (i.e., BG/GEL ratio = 0.5) up to day 20 when it reached the peak level of osteoblast maturation, and thereafter declined upon mineralization. *In vivo*, composite hydrogels were able to form apparently more new vascularized bone in femoral condyles of osteoporotic rats after 8 weeks implantation, mainly owing to the angiogenic potential of BG

particles. Formation of considerably more bone in the adjacent tissue in the groups treated with the hydrogels containing bisphosphonate confirmed the good antiosteoporosis effect of bisphosphonate molecules.[134]

The effects of direct and indirect techniques on viability and proliferation MC3T3-E1 osteoblast-like cells in the presence of a series of injectable, self-gelling composite hydrogels comprised of two different particle sizes (2.5 μm and <45 μm) of a BG (40 SiO_2-54 CaO-6 P_2O_5, mol.%) and two types of low-esterified amidated pectins derived from citrus peels and apple pomace were assessed in a recent effort.[121] As expected, the composites made of smaller BG microparticles (20% w/v) showed higher apatite-forming ability upon immersion in SBF. Due to the low stiffness of all the hydrogels, the cells were found to be poorly adherent to and proliferating on the scaffolds when tested in direct method, although the cells showed good viability and proliferation in the vicinity of the scaffolds. As well as the stiffness effect, the presence of large amounts of BG particles in the composites could release high concentration of Ca^{2+} beyond the cytotoxic level (10 mM), which could impair the cellular responses. Notably, there was no statistically significant difference between the cellular response on the hydrogels made of a same pectin and various sizes of BG particles in direct method. However, the indirect approach unveiled that, upon culturing the cells in the presence of 5 or 10× diluted extracts of the composites, cell proliferation significantly increased as compared to the undiluted extracts, and was comparable to the TCPS control after 24 h of culture.[188] The strong antibacterial effect of the composite scaffolds with respect to the silicone disc control against MRSA was principally ascribed to the enhanced alkalization of the bacterial culture medium with Ca^{2+} release from the BG phase.[121]

A sol-gel derived micron-sized BG doped with magnesium (60 SiO_2-30 CaO-10 MgO mol.%) was used along with fibrin nanoparticles (FNPs, ~250 nm) to promote the osteogenic and angiogenic properties of the injectable chitin/poly(butylene succinate) hydrogel.[133] While the protein absorption enhanced as the BG content increased, hydrogels with higher concentration of BG microparticles (5 wt.%) demonstrated a toxic effect towards both rASCs and HUVECs at day 1. Assessment of angiogenic potential of hydrogels with aortic ring and tube formation assays showed notably more sprouting when the BG-containing hydrogels were incorporated with FNPs, which are inherently angiogenic.[189] The presence of BG microparticles and FNPs together in the hydrogel enhanced the expression of early osteogenic markers (i.e., ALP and osteocalcin) even in the absence of osteogenic media, indicating the bone regeneration potential of this composite hydrogel.[133]

Recent advances in hydrogel technologies have enabled scientists to create DN hydrogels, which offer promise for treating periarticular fractures. To this end, a recent study compared the bone forming ability of tough injectable and preformed hydrogels developed by interpenetration of 58S nano-BG-embedded PEG into Ca crosslinked carboxyphenylboronic acid-grafted PVA.[106] The evaluation of mechanical properties revealed that the hydrogels had high compressive modulus (0.5-4.1 MPa) and strength (20-90 MPa), and thus could met the mechanical requirements of human cancellous bone. Interestingly, during the mineralization process in SBF, the mechanical properties of the DN hydrogel remarkably increased due to self-strengthening phenomenon, whereas the pure PVA hydrogel became weaker over the incubation time. The nontoxicity of DN hydrogels towards L929 cells was proven when the cells exposed to the extracts of the hydrogels collected at different time points up to 72 h. The cell proliferation on the composite hydrogels was also enhanced over the culture time. *In vivo*, injection of the composite hydrogel in a rabbit femoral supracondylar bone defect resulted in faster and higher-quality bone regeneration than the pure BG particles and the

preformed composite hydrogel, so that 12 weeks post-treatment the defects implanted with the injectable hydrogel were almost entirely filled with new bone. However, the neo-bone formation was observed only around the border of the defects treated with the neat BG particles. This was thought to be likely due to the confinement of particles in the polymer chain and their immobilization in the defect site, which in turn can promote the process of mineralization. The outperformance of injectable gel relative to the preformed hydrogel could be perhaps as a result of the formation of firm interface between the injectable gel and the rim of fracture, which facilitate the transmission of cargo to the implant site and improve stress transmission, thereby leading to enhanced bone regeneration.[106]

In order to attain a scaffold with sufficient mechanical properties and osteogenic potential for bone tissue engineering, Gao *et al.* exploited the poly(ethylene glycol) dimethacrylate (PEGDMA), with capacity to be simultaneously polymerized during bioprinting.[190] This study explored the potential of thermal inkjet printing approach in co-printing of hBMSCs suspended in PEGDMA with 45S5 BG microparticles (20 μm) and/or hydroxyapatite nanoparticles (HAp NPs, <200 nm). The presence of HAp NPs led to a significant increase in hBMSCs osteogenesis and ECM production with negligible cell toxicity. Comparatively, reduced cell viability and remarkably slower osteogenic differentiation were observed for the hydrogels filled with the BG microparticles. Interestingly, an intermediate cellular response was resulted from the co-incorporation of HAp NPs and BG microparticles into the hydrogel, so that the values placed in-between what were observed for PEGDMA/HAp and PEGDMA/BG alone.[191] Nevertheless, the effect of BG and HAp particle sizes on cellular behavior were not discussed in this study. It is highly likely that the nano-sized HAp particles with significantly higher reactive surface area could exert greater influence on the fate of cells than the BG microparticles counterpart.[190]

4.1.2 Summary and discussion

Injectable BG-reinforced hydrogels offer advantages over the preformed hydrogels in bone regeneration not only due to the ease of administration but also for the promotion of more rapid and higher quality of bone regeneration arisen from their ability to form a desired shape that is more coherent with the adjacent tissues, thereby improving stress transmission as well as transport of nutrients and ions. The ionic dissolution products of BGs can have a profound influence on the fate of embedded and surrounding cells. Biocompatibility of BG-reinforced hydrogels could be compromised by the high concentrations of ions that are released from BG phase. *In vitro*, the released ions from BGs can cause toxicity primarily due to the drastic pH change or pH shock in early days, an issue that has led to minimal concern *in vivo* where the body fluid constantly dilute the ionic species released from BGs. Using hydrogel matrices as a delivery platform of BG particulates can maximize the potential of BGs in tissue engineering as it can modulate the ion release rate while preventing them from being washed away upon implantation *in vivo*. To further enhance the efficiency of these hydrogels, surface of BG particles can be grafted with growth factors (e.g., rhBMP-2) or polymers (e.g., GelMA) before inclusion into the hydrogel matrix to achieve an ideal scaffold with enhanced mechanical properties, tuned degradation time, stabilized local pH environment and promoted osteogenesis and angiogenesis. Furthermore, co-incorporation of BG with HAp particles was shown to be a potentially highly promising approach to enhance osteogenesis and ECM production. BGs have also been incorporated into self-healable hydrogels to develop a biphasic construct with controllable mechanical and biological performance for treatment of osteochondral defects. Taking into account the experimental evidence reported in this article, several BG-reinforced

hydrogels have not been examined in animals, and thus their cytocompatibility with human body tissues remain somewhat vague. In addition, more attention should be paid to the development of BG-reinforced injectable hydrogels containing synthetic polymers as this class of polymers possesses outstanding mechanical performance and water absorption capacity, and in combination with ion-releasing BGs can give rise to desirable scaffolds for bone regeneration.

4.2 Skin wound healing

4.2.1 Overview

The skin is considered as the largest organ of the human body that covers the entire external surface, and accounts for nearly 15 % of the total body mass.[192] It consists of three main layers: the outermost epidermis layer, the mid-dermis layer beneath the epidermis and the innermost hypodermis or subcutaneous layer, and protects the internal organs from external harmful environment.[193] When an injury occurs to the skin, a series of events progressively take place to regenerate the damaged tissues. The cutaneous wound healing process occurs in four sequential, overlapping phases: hemostasis (clot formation), inflammation (activation of the body's immune response and prevention of infection), proliferation or re-epithelialization (new tissue formation), and remodeling (the maturation of granulation tissue into scar). These stages are explained in details elsewhere.[194-196] While minor injuries or superficial wounds can undergo self-repair with the help of topical medication, treating deep and chronic/non-healing wounds (e.g., diabetic ulcers) are more complex and might take months or years to heal that impose a huge burden on the individual and the healthcare system as a whole. Skin autografting has been found to be an effective therapy for chronic wounds; however, it might be inappropriate where there are large area wounds.[197, 198] It is therefore necessary to look for alternative artificial materials which are effective in treating wounds of different types and extents.

Wound dressings have been deployed in varied forms of membrane, sponge, micro or nanofibers and hydrogels. Among which, injectable hydrogels have shown high promise for the treatment of the wounds with complex irregular geometries and varied depths that traditional bulk hydrogels cannot fill.[15] The high-water content and porous structure of these hydrogels assist in maintaining the wound environment moist, absorbing abundant wound exudates, facilitating the exchange of gases and inhibiting exogenous microorganism invasion.[199, 200] Hydrogel dressings with pore size in the range of 20-125 μm and elastic modulus in the range of human skin (2.9 to 150 MPa) could be optimal for skin tissue regeneration.[201, 202] An ideal hydrogel dressing should also have good adhesive property to avoid shedding and prevent bacterial penetration. More importantly, it should possess antibacterial effect and angiogenic potential in order to promote the healing of chronic wounds, what is absent in most purely polymeric hydrogels.[203-205] As such, BGs with the ability to release therapeutic ions are regarded as fascinating candidates to be incorporated into the hydrogels so as to accelerate the process of wound healing. It is documented that ionic species of BGs can produce a marked increment in the secretion of critical angiogenic growth factors (e.g., VEGF, EGF and bFGF) from fibroblasts, increase the synthesis of collagen type-I and fibronectin in the ECM of fibroblast, and stimulate the migration ability of fibroblasts and differentiation into myofibroblasts as well as promoting angiogenesis, thereby accelerating wound healing.[206, 207] It is also worth noting that highly-ordered MBGs have shown significant potential to promote hemostasis due to their unique textural properties (i.e., high

surface area and porosity) which enable them to absorb large amounts of water from the blood and concentrating the clotting factors and platelets, besides their outstanding ion-releasing capacity.[208] The potential of BGs for skin regeneration has been recently reviewed elsewhere in detail.[209] A range of studies have recently investigated the effect of injectable hydrogels embedded with BG particles of different compositions on skin wound healing.

Diabetic wounds are hard to heal and remain a critical clinical concern worldwide. Wound healing is delayed in the diabetic patients due to the disruption of the normal wound healing process (discussed above), thereby leading to impaired angiogenesis, poor neutrophil antimicrobial ability, hypoxia and over-generation of reactive oxygen species (ROS).[210-212] Even though cell and growth factor-based treatment strategies have shown promising results, these methods are generally prohibitively costly and difficult to translate to clinical practice.[213, 214] To address this constraint, an injectable composite hydrogel based upon ALG was proposed by Kong and co-workers, in which desferrioxamine (DFO) and 45S5 BG microparticles (20.28 μm) were encapsulated simultaneously to promote the angiogenesis.[215] It was found that in comparison to the hydrogel filled with either DFO or BG microparticles alone, the co-incorporation of both materials into the ALG hydrogel could have a synergistic effect in improving the HUVECs migration and tube formation *in vitro* as well as upregulation of VEGF expression, which plays a key role in revascularization. *In vivo* studies in diabetic rat skin defect model indicated the fastest wound healing for the group treated with the hydrogel containing the mixture of DFO and BG microparticles and the wound surface was covered with the newly formed skin within 20 days (Figure 11). Moreover, an increase of neovascularization was observed after 12 days of implanting this composite hydrogel, which stemmed from the significant upregulation of HIF-1α and VEGF. Nonetheless, at day 20, a significantly higher number of newly formed and mature blood vessels were observed for the control group. The authors hypothesised that this phenomenon could be because the proliferative phase was nearly completed in the group treated with DFO-BG co-incorporated hydrogel and advanced to the ECM remodeling phase, when the small blood vessels are steadily shrunk and disappeared, whilst the angiogenesis was slow at early stages for the control group and only elevated at later stages, i.e., 20 days after injection.[215] In fact, this study reinforced the hypothesis that, in addition to the vascularization effect of DFO, the Si ions released from the BG phase can upregulate the secretion of proangiogenic factors such as hypoxia inducing factor 1α (HIF-1α) by stabilizing it via blocking HIF-prolyl hydroxylase 2 in HUVECs, and DFO and BG microparticles can work synergistically towards promoting angiogenesis.[216, 217]

Figure 11

In recent years, growing attention has been devoted to the use of Cu^{2+} ions as prominent inorganic angiogenic factors in production of wound care products due to their low cost, high stability and antibacterial activity as well as their potential to stimulate the proliferation of endothelial cells and induction of angiogenesis by up-regulation of VEGF expression.[30, 218, 219] In general, Cu-doped BGs proved to be highly appealing for advanced biomedical therapies.[220] In this respect, Li and co-workers proposed an injectable and self-healing composite hydrogel, in which the organic phase was constituted of PEGDA and ALG and the inorganic phase was comprised of a nanoscale Cu-doped BG, for diabetic wound healing.[111] *In vitro* studies clearly showed the remarkably higher cell viability, proliferation and tube formation of endothelial progenitor cells (EPCs) in the presence of the composite hydrogel

including Cu-doped BG nanoparticles as compared to the control groups, followed by the Cu-free BG containing hydrogel. *In vivo* testing revealed formation of abundant thick granulation tissue with neo-epidermis, and accelerated collagen deposition and remodeling in the full-thickness cutaneous wounds of Institute of Cancer Research (ICR) mice treated with the Cu-BG-filled hydrogel at day 21, markedly higher than the other examined groups. The assessment of angiogenesis *in vivo* confirmed that the sustained release of Cu ions could up-regulate the expression of HIF-1α, VEGF and VEGFR2, and therefore enhanced angiogenesis and neovascularization, which are essential for accelerating the diabetic wound healing. Notably, the hydrogel containing Cu-doped BG nanoparticles exhibited repeatable antibacterial properties against both *S. aureus* and *E. coli,* which was predominantly ascribed to the release of Cu^{2+}.[111]

The dressings with multifunctional properties such as anti-skin cancer, anti-infection and wound healing have been of substantial interest in wound management. For this purpose, a nanocomposite hydrogel was developed based on dibenzaldehyde-terminated F-127 (F127-Phe-CHO), F127-ε-Poly(L-lysine) copolymer (FEPL) and polydopamine-modified BG nanoparticles (BGN@PDA) with capability to be injected and rapidly self-heal.[114] Not only did the inclusion of BG nanoparticles into the hydrogel give rise to insignificant cytotoxicity towards A375 and C2C12 cells, but it also led to photothermal effect under near-infrared laser irradiation (rising to approximately 40 °C within 9 min), resulting in noticeable A375 cancer cell killing (>90%), and demonstrated a significant inhibitory effect towards the tumor growth (~ 94%) in a subcutaneous skin-tumor model after 18 days of treatment. The mouse full-thickness skin wounds treated with nanocomposite hydrogel was nearly entirely healed 14 days post-treatment with remaining wound area of 1.2%, which was dramatically smaller than the unloaded hydrogel group (4.3%), and the area was filled with plenty of granulation tissue with approximate thickness of 240 μm. Furthermore, considerably more collagen deposition and blood vessel formation were observed for the nanocomposite hydrogels as compared to the control or the unloaded hydrogel groups. The hydrogels also exhibited antibacterial activity against multidrug-resistant bacteria such as *S. aureus*, *E. coli* and *MRSA in vitro* and *in vivo*, mainly due to the use of an antimicrobial polypeptide like poly-L-lysine, in the synthesis of the hydrogels. While the effectiveness of BG nanoparticles in wound healing has been proven in several studies, this study furthers an understanding of their significance in development of injectable and anti-tumor hydrogel dressing.[114]

In another study, Zhou and co-workers deployed the bifunctional role of the released ions from 45S5 BG (10 μm) to modulate the gelling time of a hydrogel composed of human serum albumin and succinimidyl succinate-modified PEG, which could be accelerated in the alkaline environment, and to promote vascularization and wound healing.[113] This study too, employed the microdialysis technique to quantitatively measure the concentration of released ions from the composite hydrogel *in vivo* during the process of wound healing. In contrast to the radiolabeling technique, which requires to sacrifice the animal for sampling, microdialysis technique allows *in situ* sampling and continuously measuring the concentrations of intended substances while eliminate the need to animal sacrifice.[221] The assessment of ion release indicated similar trends for Ca and Si ions released from the composite hydrogels both *in vitro* and *in vivo*, yet the concentrations of both ions were significantly lower *in vivo*, likely because of the continuous dilution of the ions with the flowing body fluid. These findings support the hypothesis that *in vitro* ion release experiments could be utilized to project the ion release behavior of the BG-based materials *in vivo*. Implantation of the hydrogel containing 0.5% w/v BG microparticles in a full-thickness excisional wound model in mice resulted in a considerably more blood vessel formation and collagen deposition than the control and

unloaded hydrogel 14 days post-treatment. The composite hydrogel was also able to stimulate the fibroblasts to differentiate into myofibroblasts and promote keratinocyte migration and proliferation in the wound site. The authors thought the improved wound contraction and angiogenesis in the group implanted with the composite hydrogel might be because of the release of Si ions from the BG microparticles to the wound area.[113]

Bacterial infection is a major source of complications connected with chronic wounds. The high permeability of hydrogels to small molecules has restricted their utilization against infection.[222] As such, incorporation of antimicrobial agents into hydrogel dressings has become prevalent. Zinc-based biomaterials have gained wide attention due to their broad-spectrum of antibacterial activity.[223, 224] To this end, spherical-shaped zinc-doped BG nanoparticles (60 SiO_2-30 CaO-5 ZnO-5 P_2O_5, mol.%; 50−100 nm) incorporated into an injectable hydrogel based on succinyl CS and ADA showed a considerable potential to be used as wound dressing.[225] The composite hydrogel exhibited bactericidal activity against *S. aureus* and *E. coli* due to the synergistic effect of the released Zn^{2+} ions and CS, so that no viable bacteria was observed in the culture media treated with this hydrogel after 2 h. The cytocompatibility of the hydrogels with mouse fibroblast L929 and embryonic fibroblast 3T3 cells was confirmed after 24 h incubation with various concentrations of the hydrogel, ranging from 80 to 320 μg/ml. This study also revealed that the inclusion of epidermal growth factor (EGF) along with Zn-doped BG nanoparticles into the hydrogel can accelerate the healing rate of a mouse full-thickness wound, deduced from the enhanced neo-granulation tissue and blood vessel formation as well as significantly more collagen deposition and lower inflammation in this group compared to the groups treated with gauze, blank hydrogel, BG-loaded and EGF-loaded hydrogels.[225]

In recent years, a good deal of attention has been dedicated to the design of adhesive hydrogels based upon mussel-inspired catechol chemistry for use as wound dressing.[226] Zheng and co-workers, for example, produced such a thermoresponsive hydrogel by incorporating a catechol-modified quaternized CS (QCS-C) into triblock poly(D,L-lactide)-b-poly(ethylene glycol)-b-poly(D,L-lactide) (PLEL).[109] A nanoscale BG (80 SiO_2, 16 CaO and 4 P_2O_5 mol%; 780 nm) was further added to the hydrogel to improve its angiogenic potential. Unsurprisingly, the composite hydrogel included 1 wt.% QCS-C exhibited significantly higher adhesive strength than those without it, and this property was highly improved at 37 °C when compared to the lower temperatures (Figure 12). The viability of L929 cells exposed to the various concentrations of hydrogel extracts (12.5-100%) was found to be higher than 85 %, and presence of QCS-C appreciably enhanced the adhesion of L929 fibroblasts and HUVEC cells as well as the antibacterial efficacy of the hydrogel against *S. aureus* and *E. coli*. Comparatively, the released ions from BG nanoparticles were essential for stimulating the secretion of b-FGF and VEGF for angiogenesis as well as migration of HUVEC cells, so that the *in vitro* scratch assay indicated the disappearance of the scratch after 12 h treating with highly diluted extract (1/60) of the ternary composite hydrogel, suggesting that this hydrogel can improve the endothelial cells migration *in vivo,* where the released ions are continually diluted by the body fluid. *In vivo*, the ternary hydrogel outperformed the typical suture and fibrin glue. The hydrogel treated a partial-thickness laceration model in mice showed remarkably lower inflammatory reaction as well as enhanced vascularization, collagen synthesis and neo-epidermis formation relative to the other groups, thereby leading to accelerated re-epithelization and wound repairing.[109]

Figure 12

In another recent effort, Gao and co-workers exploited the multifunctional properties of BGs to endow an injectable hydrogel based on ADA and adipic acid dihydrazide-modified γ-polyglutamic acid with bioactivity and adhesiveness.[110] The authors speculated that the improved adhesiveness of the composite hydrogels to tissues could stem from the enhanced alkalization of the microenvironment by the release of ions from BG phase, which tends to boost the Schiff-base formation between the aldehyde groups of the hydrogel and amino moieties of the neighboring tissues. As such, the adhesive strength increased in proportion with rising the BG content. It was also stated that the released Ca ions from the BG phase chelate the carboxyl functional groups in the polymer constituents to provide the hydrogels with enhanced adhesiveness to implantable biomaterials. This interesting property can make the proposed composite hydrogel functional as coating for fixing implants or enable it to be utilized to avoid implant dislocation at the site of surgery. The good adhesive strength of the composite hydrogels was further proven *in vivo*, where there were no signs of wound leakage or infection in the skin wounds of the mice treated with composite hydrogels and an intact epidermis layer was detected at the lacerated sites, whereas insufficient tissue adhesion and no neo-epidermal layer was seen for the control and unfilled hydrogel groups (Figure 13). The composite hydrogel containing 1 wt.% of 45S5 BG particles displayed significantly higher secretion of VEGF in the HUVEC and HDF cells after 7 days of culture *in vitro*. *In vivo* studies revealed a remarkably enhanced neovascularization in the group treated with the composite hydrogel at all tested time points than the other groups. Enhanced collagen type-I deposition and keratinized layer formation were detectable in the wounds injected with the composite hydrogel. In the authors' opinion, the enhanced wound healing efficiency of composite hydrogels was likely due to the release of Si ions from the BG phase.[110]

Figure 13

The significance of the role that macrophages can play during wound healing process has been emphasized in a number of studies.[227, 228] It is thought that macrophages secrete cytokines and growth factors to stimulate and modulate matrix protein deposition, angiogenesis, and re-epithelialization.[229] The depletion of macrophages in the wound site has been found to be one of the main causes of impaired wound healing.[228, 230] A recent study has reported that macrophages can be activated towards M2 phenotype and stimulated to secret more anti-inflammatory and angiogenic growth factors by the ionic dissolution products of BGs.[231] However, the high local pH generated by the use of pristine BG powders in the wound site may cause pain to the patients. To avoid such hurdles, Zhu and co-workers, embedded BG particles into the ALG/GDL injectable hydrogel and its ability to stimulate expression of macrophages and skin wound healing was evaluated.[232] *In vitro*, the composite hydrogel could promote the expression of some particular pro-regeneration cytokines and chemokines, including VEGF, TGF-β and bFGF, from the M2 macrophages, which found to be imperative for stimulating the migration of endothelial cells and fibroblasts as well as angiogenesis and vascularization. Comparison of this composite hydrogel implanted in normal and macrophage-depleted mouse models with full-thickness excisional wounds elucidated that the wound healing was impaired in the absence of macrophages, so that the fibroblasts and endothelial cells were hardly recruited in the wound site, and cell migration, ECM synthesis as well as angiogenesis was retarded. This study clarified the pivotal role of macrophages in promotion of wound healing.[232]

4.2.2 Summary and discussion

Overall, smart bioactive dressings based on BGs have recently stimulated much interest in the management of acute and chronic wounds, thanks to their high compositional and processing versatility, which enable these potent materials to act as multifunctional platforms for delivery of biomolecules and release of therapeutic ions. Hydrogels can be a potential formulation for the controlled delivery of therapeutic agents using BG particles. In fact, besides providing a moist environment, hydrogels can control the ion release from BG particles during the wound healing process. The ionic species released from the BGs can modulate the macrophages to recruit fibroblast and endothelial cells to the wound area to regulate angiogenesis and ECM expression, thereby leading to the promotion of granulation tissue formation. However, the role of individual ions released from BGs on the interactions of BG-reinforced hydrogels and immune system is not fully understood and further research into this critical biological process is needed. Impaired angiogenesis is the main complication associated with diabetic wound healing. BGs can significantly enhance the angiogenic properties of hydrogels when are doped with angiogenic elements such as Cu or used in combination with an angiogenic drug (e.g., DFO) or a growth factor (e.g., EGF). Sustained release of Cu and Si ions has been found to be essential for stimulating the expression of b-FGF, TGF-β, HIF-1α, and VEGF factors which play a key role in promotion of angiogenesis and neovascularization, while release of Zn ions can inhibit bacterial growth. Biomaterials containing these elements have shown efficiency for the treatment of the diabetic wounds, multidrug resistant infection and skin tumor. In the authors' opinion, there still is a lack of research evaluating phosphate or borate BGs-embedded hydrogels in the management of wounds and this is even more surprising as a marketed borate glass (DermaFuse™/Mirragen™) has been shown to be highly effective in the treatment of diabetic wounds.[233]

4.3 Regeneration of other types of tissues

BG-reinforced injectable hydrogels have also been scoped for regeneration of dental pulp and myocardial infarction. For example, these hydrogels have been used as pulp-capping materials in the treatment of diffuse pulpitis. In this direction, in a recent study, the thermosensitive CS/β-GP hydrogel was loaded with an Ag-doped 58S BG to modulate dental pulp inflammation and to enhance the dental tissue regeneration.[234] This study showed that the composite hydrogel downregulated the inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) to a significantly larger extent than did mineral trioxide aggregate (MTA) and increased the expression of odontogenic markers such as dentin sialophosphoprotein (DSPP) and bone sialoprotein (BSP) in the presence of inflamed dental pulp stem cells (iDPSCs). Eight weeks post-implantation of the composite hydrogel in a rat pulpitis model, the inflammatory status of the tissue was modulated and a calcified barrier was formed underneath the defect site, while the inflammatory dental pulp tissue was mostly degenerated in the MTA-treated group. This study, however, did not investigate the effect of Ag^+ release from BG phase on the inhibition of dental bacteria growth.

BG-containing injectable hydrogel systems have also shown promise for cardiac tissue regeneration. Indeed, cardiac tissue has limited intrinsic regenerative capacity in adults mainly owing to the non-proliferative nature of cardiomyocytes.[235] The stimulation of angiogenesis is a pivotal step that may reduce heart failure after myocardial infarction. It is postulated that

the ionic dissolution products of BG-containing hydrogels can induce angiogenesis by increasing VEGF expression and stimulate the differentiation of human endometrial stromal cells (HESCs) into endothelial lineage *in vitro*.[236] In a recent study, Qi and co-workers reported the enhanced vascularization effect of an ALG-based injectable hydrogel by incorporation of Bioglass (~20 μm).[237] The expression of VEGF and bFGF was upregulated and notably higher vessel density was observed 4 weeks after injection of the composite hydrogel in a rat myocardial infarction model as compared to the other tested groups. Furthermore, a considerable reduction in the size of an infarct scar and myocardial apoptosis was observed in the group treated with the composite hydrogel. In another study, an injectable and self-healing hydrogel was fabricated from γ-polyglutamic acid /CS and 45S5 BG (15.9 μm) to deliver MSCs to the infarcted myocardium to promote the intercellular interaction between endogenous cardiomyocytes and MSCs, and facilitate cardiac tissue repair.[238] The co-culturing of cardiomyocytes and MSCs in the presence of the composite hydrogel extract resulted in a remarkable increment in the expression of VEGF and cardiac-related genes, such as cTnT and GATA4, in comparison to the mono-cultured cardiomyocytes or MSCs as well as co-cultured MSCs and cardiomyocytes in normal medium. Therefore, it was suggested that the stimulation of angiogenesis could be assigned to the activation of both MSCs and cardiomyocytes to express VEGF. Moreover, the results of TUNEL staining showed that the MSC-loaded composite hydrogel significantly reduced myocardial apoptosis in a rat model of acute myocardial infarction 4 weeks post-surgery, and the hydrogel markedly decreased fibrotic tissue formation. This study revealed that a combination of bioactive hydrogels and MSCs may lead to a substantial improvement of overall cardiac function in relation to the use of MSCs alone.[238]

5. Conclusions and future perspectives

Recent advances in BG-reinforced hydrogels hold substantial promise to revolutionize the arena of regenerative medicine. Key factors that should be controlled and optimized when producing a tissue-like hydrogel are gelation kinetics, biodegradability, mechanical compatibility with the host tissue and cytocompatibility of the formulation. The introduction of BG particles transfers new functionalities to the hydrogels. This gold combination could overcome the limitations and drawbacks relating to the use of individual materials. Multifunctional BGs and their composites are progressively developing in terms of their compositions, method of synthesis and structural properties to tackle the unmet needs for the controlled release of therapeutic ions into the defect area in order to facilitate tissue regeneration process. Nearly all the studies reviewed within the context of this work demonstrate that BG particles can provide the hydrogels with stiffening effect and induce bioactivity to non-bioactive polymeric biomaterials in the form of apatite-forming ability. There are, however, some studies reporting that the inclusion of BG particles into the hydrogels can impair injectability and increase the injection force as a consequence of enhanced hydrogel viscosity. In order to modulate the viscosity and enhance the printability of these composite hydrogels, a proposed solution is to co-incorporate organic fillers such as CNFs. This strategy was found to be effective to maintain the cells viable during bioprinting of the gels. In addition, the gelation mechanism of hydrogels can be largely influenced with incorporation of BG particles since the BG surface silanol groups and the released divalent metal ions (e.g., Ca^{2+}, Cu^{2+} and Sr^{2+}) from these particles can strengthen the crosslinking within the hydrogel networks. The ionic dissolution products of BGs could, for example, lead to crosslinking of the ALG-based hydrogels in a time-dependent and dynamic manner such that the physicochemical

properties of these gels could be altered over time, leading to enhanced biological capability of the hydrogels.

A major difference between the BGs and the majority of other fillers used in reinforcing hydrogels is their ability to release ionic species to their surroundings. Ionic dissolution of BGs not only can endow the materials apatite-forming bioactivity, but can also stimulate angiogenesis and osteogenesis through activation of related genes such as ALP, VEGF, bFGF, bFGFR and Col-I. The release profile of ions from BG particles is highly relied on their compositional, morphological and structural properties as well as the hydrophilicity of polymer matrix in which they are embedded. The controlled release of ionic species from BG phase is imperative in order to achieve desirable cell responses or avoid the concentration of these ions surpasses the cytotoxic level at which they could have detrimental effects on mammalian cell viability, proliferation and differentiation. To this end, it is of crucial importance to measure the concentration of released ions from BG-containing biomaterials prior to proposing them as potential tissue-regenerative products. Having said this, it is rather hard to predict *in vivo* release prolife of these materials via the current popular *in vitro* closed-system tests, mainly because *in vivo*, the flowing body fluid constantly dilutes the released ions. In order to overcome this limitation, a recent study has proposed employing microdialysis technique to quantify the rate of released ions *in vivo, in situ*, which can pave the way for future studies to more precisely evaluate the cytotoxic effect of such materials. By the use of indirect technique, whereby the cells culture in the presence of the material extracts, we can also relatively project the influence of various concentrations of ions on the growth of cells. However, it is important to point out that utilizing the extracts does not completely emulate the *in vivo* condition since the factors such as implant stiffness, surface roughness or electric charge, and porosity characteristics are ignored.

Chemical interactions between BG particles and hydrogel matrix can be of marked significance for better controlling the composite properties. Functionalization of BG particles with biopolymers like polydopamine and including these modified particles into the hydrogel has considered as a potent approach to tune the degradation behavior, mechanical integrity and sustained release of ions from these particles as well as introducing further functionalities and potentials to the system, resulting in a material with a desirable biological response. BGs are also suitable carriers for growth factors like BMPs and could modulate their release, or in synergy with TGF-β, EGF or other medication such as DFO or RLX could remarkably promote tissue growth and wound healing. However, this area of research is still ripe for investigation and further research on the exploration of other biomolecules such as aptamers may lead to new therapeutic advances.

Generally, the mechanical properties of injectable hydrogels are poor and considerably inferior to the performance of load-bearing tissues. BG inclusions can result in an enhancement of the strength and elastic modulus of these hydrogels, yet, the mechanical properties of most reported hydrogels are far lower than the ideal for most natural tissues. To bring these materials one step closer towards mimicking the mechanical function of tissues, few studies have evaluated the development of DN hydrogels by using BG particles. DN hydrogels benefit of possessing high strength and toughness. Although the mechanical performance of these hydrogels has shown to be enhanced by inclusion of BG particles, these improvements often seem to be less than expected for a DN hydrogel, and also a decrease in mechanical properties has been reported for the hydrogels containing high concentration of BGs as a result of particle agglomeration. In our opinion, this research field is still immature and more studies are required to fully

understand the role of BGs in design of DN hydrogels based on reversible dynamic covalent bonds such as disulfide, imine or Diels-Alder bonds, which have stronger binding affinity than non-covalent bonds, and could give rise to mechanically robust hydrogels with self-healing properties suitable for load-bearing tissue engineering applications.

An extensive effort has also been devoted to develop self-healing hydrogels in recent years. Similar to living tissues, these hydrogels can self-heal to their initial state in response to damages. Interestingly, most studies have demonstrated that incorporation of small amounts of BG particles into the hydrogels either enhance or do not significantly influence the self-healing ability of the composite hydrogels. This appealing property has enabled some researchers to develop bi-layered hydrogels for osteochondral tissue engineering. We conjecture that further advance in this area will unveil many new and exciting paradigms in tissue engineering.

The need for advanced multifunctional and adaptive materials has pushed scientists to investigate and design new BGs doped with active metal ions such as Cu, Sr and Zn so as to exploit the therapeutic potential of these ions to attain favorable biological outcomes in response to a specific host environment. Based on the analysis of the literature in this study, there are clear indications of gaps in the research into the use of BGs doped with other therapeutic elements, e.g., Ag, Ga or Fe or the use of borate or phosphate-based BGs, in injectable hydrogels in order to control their solubility, release rate of elements and biological response. We also envision that only by taking full control over gelation mechanism of hydrogels and gaining detailed insights into the fate of such multifunctional composite hydrogels upon *in vivo* implantation, we can be optimistic to advance the design of functional hydrogel biomaterials that have potential to be translated to the clinic. Indeed, despite the high promise of the BG-reinforced injectable hydrogels, a major challenge that has not been adequately addressed by the current literature is, in particular, the long-term assessment of these systems in vivo. The true understanding of the potential cytotoxic effects and immune response of such composite hydrogels over an extended time frame post-implantation may open a new paradigm for the safe use of these biomaterials in the current and emerging tissue engineering applications, and can bridge the gap between the laboratory and the clinic. The advances in BG-reinforced injectable hydrogels are forecast to enable tissue engineers to further expand the application fields of these unique materials in contact with soft tissues, for example, in vascular, muscular, pulmonary and neural tissue engineering.

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Conflict of interest

The authors declare no conflicts of interest.

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