Bone Grafts and Bone Substitutes for Bone Defect Management

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Keywords Bone defect · Segmental bone defect · Orthopedic trauma · Bone regeneration · Musculoskeletal healing · Bone grafting · Bone substitute · Autologous bone graft · Allogenic bone graft · Bone ceramic · Bone cement · Bioactive glass · Bone growth factors · Bioinorganic ions · 3D printing · Tissue engineering

1 Introduction

Bone grafting is one of the most commonly used surgical methods to augment bone regeneration in orthopedic procedures [1]. In 2000, annual bone-grafting procedures been performed worldwide have exceeded two million [2]. This makes it the second most frequent tissue transplantation following blood transfusion [3]. Autologous bone is considered the gold standard of available clinical biological materials, since it combines all necessary properties required in bone regeneration in terms of osteoconduction, osteoinduction, and osteogenesis [4]. However, there remain concerns of limited supply and donor site complications. Bone allografts dominantly share the second most popular option for orthopedic surgeons; nearly one-third of all bone grafts used in North America are allografts [5], since they are available in various forms and large quantities. They are primarily osteoconductive, while reduced osteoinductivity is retained only in demineralized bone matrix (DBM) preparations [6]. Nevertheless, inferior healing was observed compared to the use of autologous grafts and potential for transmission of infectious agents or even diseases were also reported [7, 8]. More importantly, the available amount of natural bone graft that is traditionally used is far from sufficient to meet the

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B. Li, T. Webster (eds.), *Orthopedic Biomaterials*,
https://doi.org/10.1007/978-3-319-73664-8_18

clinical demands, especially in light of the impending global pandemic of aging and obesity [9].

To address these limitations, the emergence of synthetic bone substitutes in recent decades have provided tremendous alternatives and options, since the bone grafts and substitutes (BGS) are one of the most promising markets in the orthopedic industry. It has also been reported that the revenue from the global BGS market was over two billion U.S. dollars in 2013 [10]. Bone grafting procedures are gradually shifting from using natural grafts to using synthetic bone substitutes and biological factors [10]. Among these synthetic bone substitutes and biological factors, the most widely used (either alone or in combination) are calcium phosphate (CaP)-based biomaterials (e.g. hydroxyapatite (HAp), CaP cements and ceramics), and recombinant human bone morphological proteins (rhBMPs, e.g. rhBMP-2 and rhBMP-7) [11]. The former bone substitutes are generally osteoconductive only and are mainly applied in the reconstruction of large bone defects; the rhBMPs are basically osteoinductive and are capable of enhancing fracture healing [1]. However, clinical applications of BMPs as off-label drugs have been a concern due to supraphysiological dosage, adverse clinical outcomes and cost issues [10, 12, 13]. In addition, the application of stem cell therapy and natural bioinorganic ions as well as musculoskeletal tissue engineering approaches have been extensively investigated [14–18].

2 Bone Grafts and Substitutes for Bone Defect Treatments

Bone grafts and substitutes (Fig. 1) mainly serve the combined functions of mechanical support and osteoregeneration [19, 20], which involve three important biological properties: osteoconduction, osteoinduction and osteogenesis [21]. *Osteoconduction* refers to the ability to support the attachment of osteoblast and osteoprogenitor cells, and allow the migration and ingrowth of these cells within the three-dimensional architecture of the graft [21]. *Osteoinduction* is the possess by

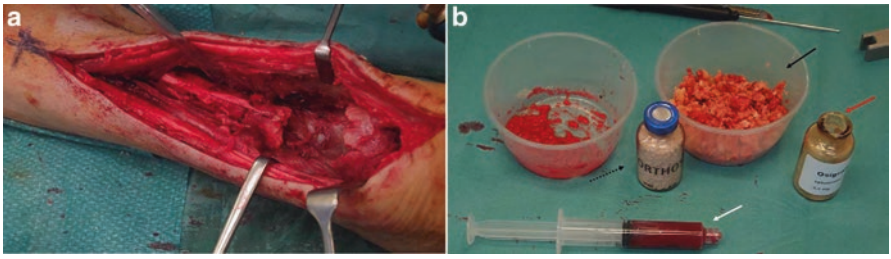

Fig. 1 (**a**) A typical bone defect site after the debridement of the necrotic tissue and (**b**) the representatives of the bone grafts and substitutes: black arrow, cancellous autograft mixed with allograft; dotted arrow, bone substitute; red arrow, BMP-7; white arrow, bone marrow aspirate (Reprinted from [24], Copyright (2011), with permission from Elsevier)

which the graft induces the primitive, undifferentiated, pluripotent cells to develop into the bone-forming cell lineage by which osteogenesis is induced [21, 22]. *Osteogenesis* refers to osteo-differentiation and the subsequent new bone formation by donor cells derived from either the host or grafts [4, 23]. Besides, *osteoinduction* is also an important criterion in evaluating the result of bone healing. It occurs when an implant anchors itself by forming the bony tissue around itself at the bone-implant interface without forming fibrous tissue [21]. The biological properties of some commonly used bone grafts and substitutes are listed in Table 1.

2.1 Natural Bone Grafts

2.1.1 Autologous Bone Grafts

An autologous bone graft is a procedure in which an osseous graft is harvested from an anatomical site and transplanted to another site within the same individual [23, 25]. This type of bone graft can integrate into the host bone more rapidly and completely, since it possesses osteoconductive, osteoinductive and osteogenic properties [25]. It is therefore regarded as the gold standard in the treatment of bone defects, and the benchmark in evaluating other bone grafts and substitutes. However, the drawbacks of the autograft have been extensively reported, and are related to the harvesting process, including donor site complication and pain, increased blood loss, increased operative time, potential for donor site infection and limited volume of material available [19, 23, 26, 27].

The development of the reamer-irrigator-aspirator (RIA) system offers an alternative to traditional autologous bone graft options such as iliac crest bone graft, in which the graft can be harvested from the intramedullary canal of the femur or tibia [28]. In a systematic review covering over 6000 patients, the use of an RIA device was found to reduce the complication rate to 6% as compared to 19.37% from iliac crest bone [29]. Bone volume increased from 15 to 20 mL with harvested iliac crest bone to over 40 mL with RIA [30, 31]. In a comparison of bone grafts harvested from different areas of the same patient, genes associated with vascular, skeletal and hematopoietic tissues had higher levels of expression in the RIA samples than in those from iliac crest bone; stem cells and growth factors in the RIA samples were also more abundant [32]. Documented complications of RIA primarily include iatrogenic fracture, anterior cortical perforation, exsanguination, and heterotopic ossification [29, 33].

Cancellous autografts are the most commonly used form of autologous bone grafting. Since the occurrence of ischemia during transplantation results in the survival of only a few osteoblasts and osteocytes, but abundant mesenchymal stem cells (MSCs), osteogenic potential is maintained along with the ability to generate new bone from the graft [34, 35]. In addition, the large surface area of a cancellous autograft facilitates superior revascularization and incorporation of the graft locally to the host bone [19]. Graft-derived proteins, which are attributed to the osteoinduc-

Table 1 Summary of biological properties of bone grafts and bone substitutes in clinical application (Reprinted from [19], Copyright (2001), with permission from Wolters Kluwer Health Inc., and from [33], Copyright (2012), with permission from Elsevier

	Autologous bone grafts		Allogeneic bone grafts			Synthetic bone substitutes					
	Autologous Cancellous	Autologous cortical	Allogeneic Cancellous	Allogeneic cortical	DBM	Calcium sulfate	HAp	Calcium phosphate ceramic	Calcium phosphate cement	Bioactive glass	PMMA
Clinical applications	Bone defect, delayed union/ non-union, discectomy, arthroplasty	Bone defects, discectomy	Bone defects, delayed union/ non-union, discectomy, arthroplasty	Bone defects, discectomy	Bone defects, discectomy, arthroplasty	Bone void filler, bone graft extender	Same as above	Same as above	Same as above	Same as above	Masquelet technique, arthroplasty, vertebraplasty and kyphoplasty
Disadvantages	Limited availability and donor site morbidity	Same as above	Risk of disease transmission and immune reaction	Sam as above	Variable osteoinductivity associated with donors and processing methods	Rapid resorption, osteo conductive only	Slow resorption, osteo conductive only	Osteo conductive only	Osteo conductive only	Bioactive osteo conductive only	Inert, exothermic, monomer-mediate toxic
Structural support	–	+++	–	+++	–	+	++	++	+		+++
Osteo-integration	+++	+	++	+	++	++	–	+	+	–	–
Osteo-genesis	+++	+	–	–	–	–	–	–	–	–	–
Osteo-induction	+++	+	+	–	++	–	–	–	–	–	–
Osteo-conduction	+++	+	+	+	+	+	+	+	+	+	–

tion of the graft, are also preserved and present when the autografts are appropriately treated [19, 25]. In the early phase of autograft transplantation, MSCs contribute to the rapid formation of hematoma and inflammation, aiding in the creation of fibrous granulation tissue. Meanwhile, macrophages slowly eliminate the necrotic graft tissue and neovascularization occurs. Next, during the incorporation of the autograft, seams of osteoid are produced by a line of osteoblasts surrounding the necrotic tissue; this is concurrent with the formation of new bone by accumulated hematopoietic cells within the transplanted bone [19, 23, 25]. This process, which leads to the complete resorption and replacement of the graft, usually takes 6–12 months [36].

Cortical autografts possess excellent structural integrity and are mechanically supportive, due to their limited number of osteoprogenitor cells [23]. Unlike the autologous cancellous graft, the creeping substitution of a cortical autograft is primarily mediated by osteoclasts, following the rapid formation of hematoma and the inflammatory response that occurs in the early phase of bone regeneration. The revascularization and remodeling processes are hampered by the dense architecture [25]. Consequently, the appositional bone growth over a necrotic core is the primary means by which the cortical autograft is incorporated following osteoclast resorption [37, 38]. This process may take years, depending on the graft size and implantation site [19, 25].

2.1.2 Allogeneic Bone Grafts

In allogeneic bone grafting, bony tissue from one individual is harvested and transplanted to a genetically different individual of the same species [23, 25]. In light of the limitations of autologous bone grafts, a bone allograft is considered the best alternative. It has been used effectively in clinical practice in many circumstances, especially for patients with poor healing potential, established non-unions, and extensive comminution after fractures [25, 34]. The allograft may be machined and customized; it is therefore available in a variety of forms, including cortical, cancellous and highly processed bone derivatives (i.e., a demineralized bone matrix) [23]. Compared to autografts, allografts are immunogenic and demonstrate a higher failure rate, which may be due to the activation of major histocompatibility complex (MHC) antigens [8]. If failure occurs, the initial osteoinduction phase is destroyed by an immune response and inflammatory cells, which quickly surround the neovascular tissue and cause the necrosis of osteoprogenitor cells [39–41]. The exact mechanism of immune responses in bone allograft incorporation is not clear; studies have found that allograft acceptance is improved when the allograft is modified to diminish differences in immunogenicity, thereby reducing immunogenicity [25]. While the risk of viral transmission was once an issue, it has been significantly improved by the development of modern tissue banks [19] and improvement in processing technology [42]. For these reasons, the application of fresh allografts is always limited and preserved, modified allografts are usually preferred in clinical practices [43].

Cancellous allografts are the most common types of commercial allogeneic grafts and are supplied predominately in the form of cuboid blocks [23]. Due to the weak mechanical property they confer and their relatively poor ability to promote healing, preserved, modified cancellous allografts are used primarily in spinal fusion augmentation, as filler material for cavitary skeletal defects, and similar situations [19, 25]. Compared to autografts, a similar but slower sequence of events occurs during incorporation of allografts [25]. However, osteointegration may be delayed by a host inflammatory response which leads to the formation of fibrous tissue around the graft, this was found in less than 10% of cases [44]. Meanwhile, the allografts remain entrapped and are never completely resorbed many years after transplantation [19, 26].

Cortical allografts confer rigid mechanical properties and are mainly applied in spinal augmentation for filling large skeletal defects where immediate loading-bearing resistance is required [23]. Frozen or freeze-dried products that are free of marrow and blood are commonly transplanted, in the light of possible immune responses and for safety [25]. The incorporation of a cortical allograft is also preceded by creeping substitution, which is similar to its autogenous counterpart. In general, the process is initiated by osteoclastic resorption, and followed by a process of osteoconduction resulting in the sporadic formation of new appositional bone [25, 34].

Demineralized bone matrix (DBM) is a kind of highly processed allograft derivative, in which at least 40% of the mineral content of the bone matrix is removed by mild acid, while collagens, non-collagenous proteins, and growth factors remain [45]. DBM is mainly used for filling bone defects, due to inferior structural integrity and mechanical properties [46]. The osteoconductivity of the DBM is conferred by providing a framework for cell population and the generation of new bone after the demineralization treatment [19]. The osteoinductive property of DBM is mainly determined by the remaining growth factors, which are directly correlated with preparation methods. Much of the commercially available DBM uses 0.5–0.6 M of hydrochloric acid as a demineralizing agent. The incorporation of the DBM is similar to that of the autogenous graft, with growth factors triggering an endochondral ossification cascade and culminating in new bone formation at the site of implantation [19].

2.2 Synthetic Bone Graft Substitutes

As discussed above, the serious shortage of natural bone grafts and the little chance of supply meeting demand in an aging population [47] have triggered the tremendous growth in the bone graft and substitute (BGS) market [48]. Calcium sulfate, calcium phosphate (CaP) ceramics, CaP cements, bioactive glass, and combinations thereof are the most commonly available synthetic bone substitutes [49].

2.2.1 Calcium Sulfate

Calcium sulfate, also known as plaster of Paris, is a kind of osteoconductive and biodegradable ceramic that has been used to fill void defects since 1892 [50]. It is prepared by heating gypsum with a patented alphahemihydrate crystal structure and can be made in different forms, such as hard pellets or injectable viscous fluids that harden in vivo [23]. Although lacking a macroporous structure, calcium sulfate still has a rapid resorption rate and weak internal strength. This implies that it can only be used to fill small bone defects with rigid internal fixation; the ingrowth of neovascular and new bone occurs during resorption of the graft [51]. Niu et al. [52] reported that the fusion rate of calcium sulfate was not optimal in spinal arthrodesis, primarily due to faster degradation than bone deposition in the early phase of bone regeneration. However, easy preparation and a relatively low cost have made calcium sulfate a useful choice when combined with other synthetic bone substitutes and/or growth factors [47].

One promising approach is to load antibiotics to this biomaterial. From June 2015 to November 2015, Glombitza and Steinhausen [53] used vancomycin-loaded calcium sulfate/hydroxyapatite to treat chronic osteomyelitis caused by multi-resistant bacterial for 7 patients. Rapid control of infection was achieved in 6 patients. However, as can be expected, new bone did not replace the composite in a uniform manner. More recently, Jing et al. [54] used calcium sulfate to modify the traditional Masquelet technique, which is a commonly used method for treating massive bone defects, in the hope of rendering the technique a one-step surgery. This case report described an open fracture of the calcaneus at the right foot that was reconstructed. They found the formation of the induced membrane with the implantation of calcium sulfate by X-ray images and a computed tomography scan. However, this trial was then stopped by the patient and the calcium sulfate was replaced by autologous iliac crest bone grafts; and further characterization of the induced membrane and bone regeneration was not possible [54].

2.2.2 Calcium Phosphate Ceramics (CaP Ceramics)

Calcium phosphate ceramics are constituted by calcium hydroxyapatites. These are chemical compositions similar to the mineral phase of calcified tissues [49]. They are synthetic mineral salts and are usually produced by sintering at high temperatures with the exclusion of water vapor and subsequently molded by high-pressure compaction [51]. Common commercially available forms include porous implants, non-porous dense implants and granular particles with pores. As they are bioabsorbable ceramics with excellent osteoconductivity, CaP ceramics have received great attention and have been examined extensively in clinical studies [55–59]. Unlike the calcium-to-phosphate (Ca/P) ratio of biphasic calcium phosphate (BCP), it is possible to identify the ratio of HAp and tricalcium phosphate (TCP), which are being most widely used in orthopedics. Several key parameters of CaP ceramics, such as absorption rate and mechanical properties, are strictly related to the Ca/P ratios. In

addition, the crystal and porous structure is a very important factor in choosing CaP ceramics.

Hydroxyapatite (HAp) is a natural occurring mineral form of calcium apatite with the formula of $Ca_{10}[PO_4]_6[OH]_2$. It comprises about 50% of the weight of the bone, which accounts for its excellent osteoconductive and osteointegrative properties [23, 34]. HAp has similar initial mechanical properties compared to cancellous bone—it is brittle and weak under tension and shear but resistant to compressive loads [49], and may decrease by 30–40% in situ after being implanted for several months [60]. The macroporosity (pore with diameters >100 μm) and pore interconnectivity of synthetic HAp allow for the adhesion, proliferation, and differentiation of osteoprogenitor cells, as well as the revascularization, and subsequently ingrowth of new bone, when implanted in vivo [61, 62]. However, the relatively high Ca/P ratio and crystallinity delay the resorption rate of HAp; this process is predetermined by giant cells and macrophages [63]. It has been demonstrated that when porous hydroxyapatite cylinders were implanted in the cancellous bone of rabbits, only a 5.4% volume reduction was observed after 6 months, whereas the number for tricalcium phosphate ceramic was 85.4% under the same conditions [61]. Consequently, the decrease in the aforementioned mechanical properties would mean the remaining hydroxyapatite grafts within the host bone would compromise the intrinsic strength of the bone at the callus site [51]. Therefore, HAp alone is more often applied as a coating on implants and external fixator pins or in sites with low mechanical stress [34, 64].

The recent development of nanocrystalline HAp (nano-HAp) may help in overcoming these drawbacks, since it confers a larger surface to volume ratio. This great surface both significantly reduced the sintering temperature of HAp ceramics and led to the increased resorption rate [65]. However, this increase is not noticeable in clinical observation [66]. Efforts have also been made to enhance the mechanical performance of nano-HAp by incorporating carbon nanotubes (CNTs) [65, 67, 68]. While the addition of CNTs increased the open porosity from about 2.52% (pure nano-HAp) to a maximum of 7.93% (with an additional 2 wt.% of CNTs), fracture toughness with a value of 1.88 MPa $m^{1/2}$ (similar to that of the human cancellous bone) was achieved when the additional amount was 1 wt.% [68]. Enhanced bone formation was also observed in a rabbit distal femur bone defect model, whereas toxicity was not exhibited in the liver or kidney. Nevertheless, the resorption rate of this nanocomposite was not fully investigated and the enhanced mechanical properties are insufficient to extend the application of HAp in the clinic.

Tricalcium phosphate (TCP), especially the rhombohedral β-form, β-tricalcium phosphate (β-TCP), has attracted increased attention since it was first reported in 1920 by Albee [69]. With the chemical formula of $Ca_3(PO_4)_2$, β-TCP has a Ca/P ratio of 1.5. This ratio, which is lower than that of hydroxyapatite, may partially accelerate its degradation and absorption [35]. Like HAp, TCP has even more interconnected porous structures that can directly benefit fibrovascular invasion and bony replacement [34], but at the same time weaken mechanical properties [70]. A portion of TCP would inevitably convert into hydroxyapatite after implantation due to the thermodynamically unstable physiological pH. While this would partially

hamper the degradation of TCP [71], the bulk would be resorbed by phagocytosis after 6–24 months, with some remaining for years [72]. This makes the TCP effective for filling bone defects caused by trauma and benign tumors; however, its unpredictable biodegradation profile means it is not a favored bone-graft substitute [46].

Recent research has begun to focus on enhanced angiogenesis, in which the tricalcium phosphate was applied to augment bone defects [73, 74]. By comparing the in vitro neovascularization capacity of four different types of CaP ceramics, namely HAp, BCP-1 (HAp: β-TCP = 70/30), BCP-2 (HAp: β-TCP = 30/70) and β-TCP, they found that human umbilical vein endothelial cells (HUVECs) demonstrated significantly up-regulated proliferation and angiogenesis when cultured with BCP-2, which contain a higher amount of the β-TCP phase, and pure β-TCP [73]. In the mouse intramuscular implantation model, CaP ceramics containing a larger amount of β-TCP also induced higher microvessel density [73]. Several hypotheses have been proposed to explain the mechanism, such as the porous structure [75–77], the effects of ionic transfer upon degradation of CaP ceramics and homeostasis [78–80], and potential strains imposed on CaP during degradation [81, 82]. Nevertheless, the mechanism has not been fully investigated and further study is required.

Biphasic calcium phosphate (BCP) is another widely used commercial ceramic obtained by mixing hydroxyapatite and tricalcium phosphate in different concentrations for the purpose of combining the advantages of both calcium salts [83]. By adjusting the formulation, the dissolution rate and mechanical properties can be controlled within the desired range and subsequently applied in bulk or as an implant coating [84].

2.2.3 Calcium Phosphate Cements (CPC)

Unlike CaP ceramics, calcium phosphate cements (CPCs) usually involve two compounds, one of which is an aqueous curing agent. They were invented by Brown and Chow in the 1980s [85, 86] for the purpose of extending the adaptability and moldability of CaP bone substitutes. They were approved by the U.S. Food and Drug Administration (FDA) [49] in 1996. They can be injected to fill defects in various shapes; they are subsequently solidified by mixing with an aqueous phase through isothermic reaction. Self-hardened CPCs are generally highly microporous, biocompatible, and mechanically supportive with low bending strength [87]. However, they can only degrade one layer at a time, as predetermined by the dissolution in human in vivo physiological conditions and osteoclast resorption activity. As a result, an ingrowth of neovascular and bone tissue is theoretically hampered compared to the CaP ceramics that support interconnected macroporosity [49]. Apatitic CPCs and brushite CPCs can be identified according to their composition. Their properties, with regard to feasibility, setting reaction and biodegradation rates, are highly related to their compositions. Apatitic CPCs are viscous, indicating relatively poor injectability; however, a setting reaction can occur at the physiological pH value and the mechanical properties are slightly stronger than brushite CPCs. Due

to the low crystalline structure of the calcium-deficient-hydroxyapatite obtained after hardening, a higher degradation rate was demonstrated but still incomplete [88]. The brushite CPCs are feasible for injection and solidify quickly at a low pH value (< 6) [87]. While they demonstrate higher degradability, unpredictable degradation was reported due to the favorable kinetic transformation to hydroxyapatite [71]. Based on their flow behavior before setting, CPCs are clinically favored for bone replacement, especially in percutaneous vertebroplasty [89–91] and kyphoplasty [92, 93], but not as bone substitutes.

Like CaP ceramics, the preparation of nanostructured CaP was developed in order to promote the mechanical properties and biological performances of CPCs. Even though the up-regulated cell attachment and proliferation, as well as the in vivo bone regeneration, was achieved over the course of several studies [94–96], the motivation of applying nanostructured CPCs is mainly attributed to the fact that the native architecture of bone tissue is at the nano-scale [97–100]; however, the cellular and molecular mechanism has not been fully elaborated [95]. The incorporation of fibers, which is also inspired by the hierarchical nanostructure of bone, is another approach that is being widely investigated to enhance the mechanical strength of CPCs [101]. However, there is still insufficient evidence that these modifications benefit clinical practice [102].

Phase separation, which refers to the separation of the powder and liquid components during injection, is another important concern associated with the clinical application of CPCs [103]. Given the abundant research over the past two decades, several methods have achieved clinical success in some applications by weakening other crucial properties of CPCs [104–108]. However, recent research has tended to elucidate the relationship of the critical parameters of CPCs by theoretical calculations and analysis alone, due to the extremely difficult isolation of those parameters by solely experimental work [109–115]. Since these studies are not yet reflected in real experimental practice, which would affect the clinical application of CPCs, they will not be highlighted in this review [116].

2.2.4 Bioactive Glass

Bioactive glass, also known as bioglass, refers to a group of synthetic silicate-based ceramics. It was originally constituted of silicon dioxide (SiO_2), sodium oxide (Na_2O), calcium oxide (CaO), and phosphorus pentoxide (P_2O_5) when first developed in the 1970s [117]. This was later modified to a more stable composition, by the addition of potassium oxide (K_2O), magnesium oxide (MgO), and boric oxide (B_2O); the key component, silicate, constitutes 45–52% of its weight [34]. The optimized constitutions led to a strong physical bonding between bioglass and host bone. This phenomenon, called bioactivity, was first found on BGS [118]. This bone-binding property is believed to be caused by leaching and the accumulation of silicon ions when exposed to body fluids upon implantation and the subsequent formation of hydroxyapatite coating on the surface of bioglass [119]. This thin hydroxyapatite coating absorbs proteins and attracts osteoprogenitor cells. In

addition, this biological apatite layer is partially replaced by bone through a creep substitution process in long-term implantation [120]. The porosity and relatively fast resorption rate in the first two weeks of implantation allows an ingrowth of neovascular following deposition of the new bone [11, 35]. One study has demonstrated that bioglass fiber scaffolds can be completely resorbed in 6 months in vivo with little inflammatory response [121]. Like the other ceramics, the mechanical properties of bioglass were reported to be brittle and weak. Therefore, it has been applied primarily in the reconstruction of facial defects [122, 123] when combined with growth factors [124, 125].

Bioglass 45S5 (46.1 mol.% SiO_2, 24.4 mol.% Na_2O, 26.9 mol.% CaO and 2.6 mol.% P_2O_5, now sold by NovaBone Products LLC, US) and S53P4 (53.8 mol.% SiO_2, 22.7 mol.% Na_2O, 21.8 mol.% CaO and 1.7 mol.% P_2O_5, now sold by BonAlive Biomaterials, Finland) are the two most recognized commercially available bioglasses that can be used as bone graft substitutes. They are made using the standard melt-quenching technique under high temperature (usually above 1300 °C); thus, they cannot be fabricated into amorphous scaffolds due to the crystallization that occurs during sintering at that temperature. One exception is 13–93, with a composition of 54.6 mol.% SiO_2, 6 mol.% Na_2O, 22.1 mol.% CaO, 1.7 mol.% P_2O_5, 7.9 mol.% K_2O and 7.7 mol.% MgO; this composition does not crystallize during sintering. However, the bioactivity of 13–93 was significantly reduced in the form of prolonging the formation of hydroxyapatite layer in the stimulated body fluid (SBF) immersion tests, from 8 h on the surface of Bioglass 45S5 to 7 days on the 13–93 [126]. Several clinical trials demonstrated similarly good contact with the host bone when the S53P4 and Bioglass 45S5 was applied to treat bone defects, respectively [127–129], whereas reduced resorption of S53P4 was exhibited due to the higher silica content [123, 130]. Additionally, inferior healing results were also reported when compared to autologous grafts [131, 132].

The development of sol-gel processing offers another route to produce bioactive glass with a porous structure ranging from mesopores to macropores [133–135], in which 58S (60 mol.% SiO_2, 36 mol.% CaO and 4 mol.% P_2O_5) and 77S (80 mol.% SiO_2, 16 mol.% CaO and 4 mol.% P_2O_5) are representatives. In a study involving the management of critical-sized defects at the femoral condyle of rabbits, the bone regeneration ability and in vivo degradation of melt-derived Bioglass 45S5 and sol-gel-derived bioglass 77S and 58S were compared [136]. Due to the nanoporosity and enhanced surface area, the sol-gel-derived bioglass demonstrated faster degradation speed compared to Bioglass 45S5 between 4 and 24 weeks after implantation, while the bone defect filled with Bioglass 45S5 contained more bone than those filled with 77S or 58S at 8 weeks post-operation; they then equalized after implantation for 12 weeks [136]. It seems the fast degradation of bioglass may lead to an elevated pH value and accumulated ions in the microenvironment; since this is not favored by cells, it thus jeopardized the bone ingrowth [123].

2.2.5 Poly(Methyl Methacrylate) (PMMA) Bone Cement

First employed by orthopedic surgeons 60 years ago [137], PMMA remains a key component of modern practice and may be one of the most enduring materials in orthopedic surgery [138]. It is non-biodegradable and non-resorbable, which makes it more like grout rather than cement, and thus it cannot be considered a bone substitute material even though it is the most commonly used synthetic material used in clinics [139]. A two-part self-polymerizing PMMA bone cement has been widely used in total joint replacement for the fixation of components [140] and percutaneous vertebroplasty, due to its high mechanical properties and feasibility for handling [141, 142]. Antibiotic-loaded acrylic cement was developed in response to infection from prosthetic joints. It is considered to be part of antimicrobial prophylaxis in primary arthroplasty [138]. However, the drawbacks of PMMA cement are clear. The polymerization of PMMA is exothermic and may potentially damage adjacent tissues [143, 144]. Moreover, aseptic loosening caused by monomer-mediated bone damage [145], mechanical mismatch, and inherent inert property [146], was reportedly inevitable during long-term wearing and thus led to the failure of arthroplasties when using PMMA cement [147]. Other than its application in total joint replacements and percutaneous vertebroplasty, PMMA has also been widely used as a temporary cement spacer in the Masquelet technique [148]. As illustrated in Fig. 2, the PMMA was originally used to fill a defect after bone debridement. It induced the formation of an organized, richly vascularized pseudosynovial membrane [149]. About 6–8 weeks later, the PMMA was gently removed upon opening the membrane and the defect was filled again with autogenous cancellous bone graft so as to allow bone healing. Although some studies have explored the possibility of

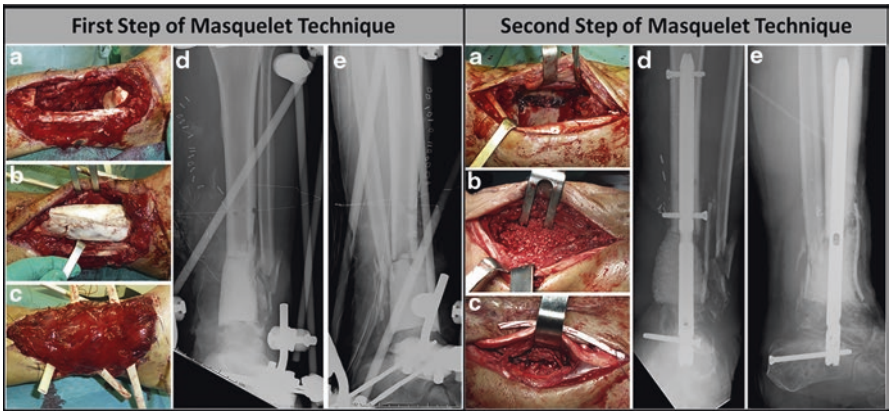

Fig. 2 Scheme of a typical Masquelet technique in treating a tibial bone defect about 6 cm long. The first step included a (**a**) bone debridement; (**b**) PMMA cement spacer bridging tibial and talus; and (**c**) Soft tissue coverage with gracilis free flap. (**d**) and (**e**) X-ray images of the cement spacer. The second step included (**a**) Opening of the defect and removal of the cement spacer; (**b**) Bone graft implantation in the cavity; (**c**) Closure of the membrane. (**d**) and (**e**) X-ray images graft and nail (Reprinted from [156], Copyright (2014), with permission from Elsevier)

combining periosteum and bone graft [150, 151], or biodegradable biomaterials [54, 152–154] as the "space filler" in the hope of streamlining the Masquelet technique into a single-stage technique, PMMA is still the primary option for a cement spacer [155].

3 The Adoption of Growth Factors on Bone Defect Management

Most bone graft substitutes, especially synthetic ceramics and cements, do not possess any osteoinductive property. The ability of those bone substitutes to enhance bone healing mainly relies on osteoconductive means [23]. In general, the osteoconduction of a bone substitute would facilitate migration and support the attachment of progenitor cells, which would then secrete growth factors to stimulate bone formation [34]. However, if the ideal environment for callus formation is disturbed and the secretion of growth factors is missing, the environment is thereby predisposed to a delayed union or even non-union [157]. The presence of osteoinductive factors during bone healing is also critically important. Therefore, direct application of growth factors, some of which are involved in the natural healing process of bone injury, has also been extensively studied and accepted as a kind of therapeutic strategy in the clinic [158]. It must be noted that only a few biological factors, such as BMPs, fibroblast growth factors (FGF) vascular endothelial growth factors (VEGF), PTH and platelet-rich plasma (PRP), have undergone rigorous preclinical tests and clinical trials (see Table 2) [159].

3.1 Bone Morphogenetic Proteins (BMPs)

Bone morphogenetic proteins (BMPs), especially BMP-2 (including recombinant human BMP-2, rhBMP-2), and BMP-7 (including recombinant human BMP-7, rhBMP-7), are members of the transforming growth factor beta (TGF-β) superfamily with superior osteoinductive properties. They are possibly the most extensively investigated growth factors in treating skeletal defects [159]. BMP-2 is able to induce osteoblastic differentiation from mesenchymal stem cells, and BMP-7 can directly promote angiogenesis. The largest trial in the use of BMPs was in treating open tibial fractures [162]. This trial, known as BMP-2 Evaluation in Surgery for Tibial Trauma (BESTT), involved multiple clinical centers. In the trial, 450 patients were randomly divided into three groups. One group received BMP-2 at 0.75 mg/mL, the second group received 1.5 mg/mL, and the third was the control group. An intramedullary nail was applied universally. Twelve months after surgery, patients treated with 1.5 mg/mL rhBMP-2 displayed quicker bone callus formation and wound closure with lower infection and less pain, compared to the control group.

Table 2 Selected growth factors and their functions in fracture healing (Reprinted from [23, 159, 160], with permission from Wolters Kluwer Health Inc.)

	Source	Receptors class/target cells	Functions	Clinical applications in orthopedics
BMPs	Osteoprogenitor cells, osteoblasts, bone extracellular matrix	Serine/ threonine kinase receptors, stem cell and chondrocyte	Promotes differentiation of mesenchymal stem cells/ osteoprogenitor cells into chondrocytes and osteoblasts, influences skeletal pattern formation	rhBMP-2 is used for the treatment of anterior lumbar spinal fusion and open tibial fractures, and rhBMP-7 is used for posterolateral lumbar spine fusion
FGFs	Macrophage, mesenchymal cells, chondrocytes, osteoblasts	Tyrosine kinase receptors	Mitogenic for mesenchymal stem cells, chondrocytes, and osteoblasts. Increases collagen deposition and angiogenesis [23]	
VEGF	Platelets, chondrocytes in callus	Vascular endothelial cells	Increases angiogenesis and vascular development	
PTH	Parathyroid glands	Stem cell, chondrocyte and osteoblast	Increased callus size, bone mass and mineral content	The full length PTH(1–84) and a segment, PTH(1–34), is used to increase the cancellous bone mass and reduce the risk of vertebral and non-vertebral fracture of patients with osteoporosis
PRP	Blood	Variety cell types	Cocktail of growth factors	Mainly applied in orthopedics and sports medicine to help hemostasis and musculoskeletal healing [161]

BMPs bone morphogenetic proteins; *FGFs* fibroblast growth factors; *VEGF* vascular endothelial growth factor; *(rh)PTH* (recombinant human) parathyroid hormone; *PRP* platelet-rich plasma

This indicates the efficiency of BMP-2 in treating tibial open fractures, although there is a dosage-dependent effect. In an earlier study by Friedlaender et al. [163], 124 tibial non-unions were fixed by an intramedullary rod before randomly receiving either rhBMP-7 in a collagen sponge or iliac crest autografting at revision surgery. Nine months later, 81% of patients in the rhBMP-7 group and 85% of those in the autograft group were able to bear full weight without significant pain. At a final follow-up of 2 years, no statistically significantly differences were observed between these two groups. The use of a rhBMP-2 or rhBMP-7 soaked collagen sponge in treating tibial non-unions demonstrated results equivalent to autologous iliac crest

grafting, while also reducing persistent donor pain. After being tested in numerous animal models [164] and clinical trials [165–168], rhBMP-2 (INFUSE™, Medtronic, US) has been approved by the FDA and the European Medicines Evaluation Agency (EMEA) for application in anterior lumbar spinal fusion and open tibial fractures [26, 157], while rhBMP-7 (OP-1™, Stryker, US) has received approval in treatment of posterolateral lumbar spine fusion [167, 168]. However, since commercially available forms of BMPs lack osteoconductivity, they are always combined with an osteoconductive carrier, such as collagen, allograft, or even an autologous bone graft, to enhance efficiency.

Off-label usage has increased dramatically, triggered by the clinical evidence of quicker bone formation stimulated by BMPs; this was reported to account for 85% of lumbar fusion procedures in 2008 [169]. Other than the approved application in tibial non-union, the studies in treating other long-bone non-unions, such as humerus pseudarthrosis [170], lower-limb pseudarthrosis [171–173], clavicle [174] and ulna [175], have also been reported sporadically; results were poor or relied on insufficient evidence [176]. In a prospective and randomized clinical trial reported by Ekrol et al. in 2008 [177], 30 patients with symptomatic malunion of the distal radius received a corrective osteotomy, either autogenous iliac crest bone grafting (AICBG, 16 patients) or direct application of rhBMP-7 (without any carrier, 14 patients). Due to the loss of fixation, an external fixation system was applied in 4 patients from the rhBMP-7 group and 6 patients in the AICBG group, respectively, before the internal fixation system was used in the remaining patients (10 patients in each group). Although this change makes the result difficult to explain, inferior healing and union percentage was demonstrated in the group receiving rhBMP-7 treatment. It was believed that their results would have been significantly different if a carrier had been applied. Several researchers have also applied BMPs to improve foot or ankle arthrodesis fusion in patients with poor surgical healing [178–180] and an effective adjuvant effect was exhibited [181]; however, there remains a lack of randomized controlled trials.

It is generally accepted that the equivalent clinical outcome would be achieved if BMPs were used to treat some complex bone defects, such as spinal fusion and tibial open fractures, rather than iliac crest autologous bone grafts, however, the high rate of complications remains a concern [176]. BMPs are especially soluble proteins and have a tendency to dissipate from their intended locations [23], which can lead to several complications. As demonstrated in the previous literature, there tends to be a dose-dependent effect in the application of BMPs [182]. The dissipation of proteins dilutes their local concentration, and, in turn, their efficiency. In addition, BMPs can influence several cell types and organs, which subsequently cause heterotopic bone formation. Boraiah et al. reported ten cases of ectopic bone formation out of 17 complex proximal tibia fractures treated with rhBMP-2; four of them needed extra surgical excision [183]. In some extreme conditions, such as one case reported by Ritting et al., the use of BMP-2 in an ulnar non-union in a 9-year-old patient led to a persistent inflammatory response and finally caused osteolysis [184]. In addition, cost effectiveness is another important issue when using BMPs [185].

3.2 Fibroblast Growth Factors (FGFs)

Twenty-two members of the fibroblast growth factors family and four fibroblast growth factor receptors (FGFRs) have been identified and are secreted by monocytes, macrophages, mesenchymal stem cells, osteoblasts, and chondrocytes, starting with the early stages of fracture healing and lasting throughout the entire healing process [186]. While the role of FGFs in fracture healing is not well understood, it has been demonstrated that FGFs both play a critical role in angiogenesis [187–189] and have potent mitogenic effects on mesenchymal progenitor cells [190]; all of these are mediated by FGFs/FGFRs signaling. Among all these FGFs and FGFRs, numerous studies have found that FGF1, FGF2 and FGFR1–3 are closely related to bone regeneration [191–194]. Furthermore, these studies have found that FGFR1 and FGFR2 have stronger expressions in osteoprogenitors and osteoblasts, whereas FGFR3 is more closely related to chondrogenesis [195]. Hence, the efficacy of FGF2 in treating bone defects was investigated by numerous in vivo animal studies [196, 197], including two non-human primate studies [198, 199]. Results also showed it promoted fracture healing, although this effect is dose- and time-dependent [195, 200]. Kawaguchi et al. performed a representative clinical trial of rhFGF in treating tibial shaft fractures in 70 patients [201]. After fixation by an intramedullary nailing system, patients were randomly injected with either a gelatin hydrogel (placebo, 24 patients), 0.8 mg rhFGF-2 in a gelatin hydrogel (low dosage group, 23 patients), or 2.4 mg rhFGF-2 in a gelatin hydrogel (high dosage group, 23 patients) at the fracture site. Radiographic analysis demonstrated accelerated fracture healing and higher facture unions in both rhFGF treated groups compared to the hydrogel-only group, while no difference between the low dosage group and high dosage group was displayed. However, due to our limited understanding of the spatiotemporal expression patterns of FGF/FGFR signaling in fracture healing, further studies are required before clinical trials may begin. In addition, the results of FGFs in treating bone fractures compared to autologous and BMPs are still missing.

3.3 Vascular Endothelial Growth Factor (VEGF)

Local vascularity at the fracture site is recognized as one of the most significant parameters affecting bone regeneration. There are two main hormonal pathways controlling angiogenesis, the VEGF pathway and the angiopoietin pathway; the VEGF is dominant [202, 203]. Except for angiogenesis, VEGF has also been demonstrated to be osteogenic [204]. In the bone fracture healing process, VEGF is initially released from hematoma and promotes the development of endothelial cells to induce vascular invasion [23] under the hypoxia environment [205]. Consequently, during the endochondral ossification process, VEGF is secreted by hypertrophic chondrocytes in the epiphyseal growth plate to promote the blood vessel invasion of cartilage and blood flow that facilitates new bone formation [204, 206]. Numerous

animal studies have shown the effectiveness of exogenous VEGF in promoting bone fracture healing [207–211]. In one study reported by Kaigler et al. [208], rodents with critical-sized cranial bone defect were treated by either bioglass alone or VEGF-containing bioglass. Increased vascularization and bone quality were observed in the VEGF-containing group, but no significant difference was found when the quantity of the newly formed bone was compared. A similar result was documented in other research published from the same laboratory [209], which implied that VEGF tends to contribute to bone maturation but does not enhance the amount of new bone formation [204]. In a rabbit model, either VEGF or an autograft was compared to a carrier-alone group in treating experimental fracture nonunions [210]. Compared to the control group, significant new bone formation and enhanced mechanical properties were observed from a radiological evaluation and bio-mechanical testing, respectively, while no significant difference was demonstrated in the blood flow or vascularity. All the evidence points to the importance of the collaboration of angiogenesis and osteoinductive factors in bone regeneration [212]. Although the cornerstone role of VEGF in angiogenesis during fracture healing has been confirmed and promising bone regeneration outcomes have been demonstrated in preclinical research, VEGF is in fact very unstable and short-lived in vivo, so a gene delivery vehicle is usually employed. In addition, there is a risk of haemangiomas or recurrence of tumors that are stimulated by VEGF, especially for patients who have had radiotherapy or tumor excision. The application of VEGF in clinical trials and its direct effect on human fracture healing is strictly limited [211]; the application of VEGF must be very accurate in terms of dosology [204].

3.4 Parathyroid Hormone (PTH)

Parathyroid hormone (PTH) is a naturally occurring endocrine containing 84 amino acids. It functions as a mediator of calcium and phosphate homeostasis in humans [213]. It has also been demonstrated to increase bone mass, bone strength, and reduce bone loss; the structure-function analysis of PTH has suggested that these activities are mainly attributed to the N-terminal fragment (encompassing amino acids 1–34 and called PTH(1–34)) [214]. Thus, there are two PTH-derived products available nowadays, the full-length protein PTH(1–84), with a commercial name of Natpara™ (Shire-NPS Pharmaceuticals, US), and a segment of protein PTH(1–34), which was licensed by the FDA in 2002 under the name Teriparatide (Forteo™, Lilly LLC, US) [213]. They have been developed as a drug to increase the cancellous bone mass and reduce the risk of vertebral and non-vertebral fracture of patients with osteoporosis. Although the detailed mechanism is not yet fully understood, it was found that several signaling pathways were involved. The anabolic effect of PTH was exerted mainly through inhibiting the apoptosis of pre-osteoblasts; this, in turn, increased osteoblast function and lifespan, thus increasing the number of these bone-making cells [215].

Since PTH increases bone mass and prevents fracture in osteoporotic bone [216], a growing number of studies have suggested the ability of PTH to accelerate fracture healing even though most of the studies were focused on animals. In a diaphysial femoral fracture model involving 270 male Sprague Dawley rats, either a placebo or 5 μg/kg or 30 μg/kg PTH(1–34) was injected daily subcutaneously for 35 days [217]. Significantly, torsional strength, stiffness, bone mineral content, bone mineral density, and cartilage formation were observed in the callus from the group treated with 30 μg/kg PTH compared to that of the control group over 21 days; no difference in osteoclast density was detected. Other animal experiments confirmed the positive effects of PTH on fracture healing in different species, locations and under various pathological conditions [218]. In short, these studies conducted on animal models confirmed that intermittent treatment with PTH has anabolic effects on bone and thus leads to recovery of bone mass and increased mechanical property; however, continuous exposure to PTH causes bone loss [214, 219–222].

Aspenberg et al. conducted a prospective, randomized clinical trial employing 102 postmenopausal female patients with distal radial fractures in 2010 [223]. They were randomized to receive a placebo, a 20 μg (ordinary osteoporosis dosage) or 40 μg PTH injection daily (double dosage). No difference was found between the 20 μg group and the 40 μg group, however, shorter times for the first radiographic evidence of cortical bridging were documented, which were 9.1, 7.4, and 8.8 weeks in the placebo, 20 μg group, and 40 μg group, respectively. Further analysis demonstrated that PTH would mainly increase early callus formation with a dose-dependent pattern, whereas the cortical bridging is not necessarily stimulated by PTH [224]. In another study involving pelvic ramus fractures in 65 osteoporotic women, radiographic bridging of cortical bone was found to be shortened from 12.6 weeks in the control group to 7.8 weeks in the PTH(1–84)-treated group [225]. More recently, a randomized clinical trial was performed to examine the effect of Teriparatide in treating elderly patients with a pertrochanteric hip fracture as compared to those using risedronate, which is a bisphosphonate drug [226, 227]. In 171 patients, 86 received 20 μg Teriparatide every day and others received 35 mg risedronate once per week, starting two weeks after surgery. After 78 weeks, several outcomes were comprehensively analyzed, including the BMD at the lumbar spine (LS), femoral neck (FN) and total hip (TH), functionality (through timed up-and-go (TUG) test), hip pain (Charnley score and 100 mm visual analog scale (VAS)), quality of life, radiology outcomes, and safety. A significantly greater increase in LS and FN BMD, less pain, and a faster TUG results were recorded when patients were treated with Teriparatide as compared to those with risedronate [227]. In conclusion, there is little doubt that PTH has a positive influence on fracture healing; however, it must be noted that PTH is not a differentiation factor and is unlikely to help if fracture healing has not already properly begun. Additionally, the robust evidence observed in animal studies has not been demonstrated beyond a reasonable doubt in humans [214].

3.5 Platelet-Rich Plasma (PRP)

The investigation of platelet-rich plasma (PRP) for bone regeneration represents attempts to harness the power of the cascade of growth factors released by the aggregation and degranulation of platelets in a native fracture hematoma [228]. PRP is mainly produced by using commercially available devices to isolate and concentrate platelets from peripheral blood. It is the plasma fraction of autologous blood having a platelet concentration above baseline [229]. It contains various key mitogenic and chemotactic growth factors, including platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), fibroblast growth factors (FGFs), transforming growth factor-beta (TGF-β), and VEGF [230]. For those patients receiving conservative orthopedic treatment caused by aging and degeneration, such as knee pain and tennis elbow, PRP is frequently used and demonstrates good clinical outcomes [231–233]. However, when investigating the effects of PRP on bone healing, especially in humans, the clinical results are conflicting and strong supportive evidence is lacking [26]. Calori et al. (2008) conducted a prospective, randomized trial comparing the treatment effect of rhBMP-7 and PRP in 120 patients with long bone non-unions [234]. They found a union occurring in 68.3% of cases (41 of 60 patients) in the PRP-treated group, and 86.7% of cases (52 of 60 patients) in the rhBMP-7 group. The mean time to clinical healing was 4 months in the PRP group compared to 3.5 months in the rhBMP-7 group. These results implied significantly inferior healing when treated with PRP. Another study investigated the efficacy of PRP in treating 132 patients with delayed union when long bone fractures were surgically treated at the Military Medical Institute in Warsaw between 2009 and 2012 [235]. A bone union was established in 108 patients (81.8%) after PRP administration, whereas 24 patients (18.2%) showed no improvement. They also concluded that the location-dependent efficacy of PRP following 100% union (on average, 3.5 months) was exhibited at the proximal tibial, whereas the union at the proximal humerous was only 63.64% (on average, 3.2 months). A more recently report described the efficacy of PRP in treating the non-union of long bone fractures among 94 patients [236]. Autologous PRP (> 2,000,000 platelets/μL) with a dose of 15–20 mL was injected directly into the defect sites and the bridging was radiologically evaluated by X-ray at monthly intervals for 4 months. Union occurred in 82 patients (87.23%) at the end of 4 months and no complication was documented. Nonetheless, the negative effect of PRP on bone healing was not rare [237, 238]. Ranly et al. reported that PRP may inhibit bone formation through the prevention of osteoinduction in mice models [239, 240].

While faster bone healing was demonstrated in this limited number of human clinical trials involving the usage of PRP to treat orthopedic defects, its efficacy was still found to be inferior to that of BMPs. Nevertheless, it is still insufficient to support its routine use in orthopedic trauma; well planned, randomized control trials are still needed [241, 242]. Meanwhile, it must be noted that platelet activity is influenced by many factors related to the individual whose blood is collected [243];

therefore, the standardized concentration and biological quantification of PRP in treating bone healing requires further study.

4 The Adoption of Bioinorganic Ions on Bone Regeneration

Safety issues have become a concern as the result of negative attention and adverse events regarding off-label usage of growth factors [10, 12, 244]. Alternatively, incorporation and/or local delivery of bioinorganic ions, which is also a natural but safer approach, has been highlighted [245]. Inspired by observing nutritional deficiency or excess, bioinorganic ions have long been applied in a variety of therapies, even when little was known of their mechanisms [18]. In recent years, the role of metallic ions in the human body has been gradually unraveled (see Table 3 and Fig. 3). Bioinorganic ions, such as silicon, magnesium, strontium, zinc, and copper, can still be regarded as essential cofactors of enzymes, coenzymes or prosthetic groups. Additionally, they are actively involved in ion channels or in the process of secondary signaling, either on direct stimulation or as an analog [18]. Incorporation of these ions confers low cost, longer shelf life and perhaps lower risk compared to growth factors [246]. While the therapeutic use of bioinorganic ions, especially some heavy metal ions, seems counter-intuitive, the words of Paracelsus are pertinent: “Everything is poisonous and nothing is non-toxic, only the dose makes something not poisonous” [247]. Consequently, the challenge in using bioinorganic ions in bone healing is also quite clear and has been described succinctly by Ash and Stone: “It is indeed a narrow path between poison and nutrition” [248].

4.1 Silicon (Si)

Silicon is the second most abundant element on earth. Since silicate (a silicate is a silicon-containing anion) is rich in foods and water, deficiency in humans is rare and its pathology is unknown. But in an animal model, chickens on a silicon-depleted diet showed deformed bone development, low collagen formation, and stunted growth [268]. Research found that silicon is rich in bone and connective tissue as an integral component of glycosaminoglycan and their protein complexes [269], which may subsequently affect bone formation and maintenance [270]. In research performed by Carlisle, an electron micro-probe was applied to locate silicon in the tibial bones of young mice and rats. Silicon was detected in the early stages of the bio-mineralization process at an active calcification site, increasing in parallel with calcium at low calcium concentrations, and diminishing when the mineral composition approached hydroxyapatite [271]. These observations have confirmed that silicon is associated with calcium in bone metabolism [272].

While testing the effect of aqueous silicon on cellular activity, dose-dependent enhancement of osteoblast proliferation, differentiation, and collagen production

Table 3 Roles of selected bioinorganic ions and their proposed mechanisms of action (Reprinted from [18], Copyright (2011), and [246], Copyright (2013), with permissions from Elsevier)

	Role	Mechanism of action	Documented efficiency dosage	mg per day
Si^{4+}	Angiogenesis, osteogenesis	Silicon has been shown to induce angiogenesis by upregulating NOS leading to increased VEGF production at low concentration when cultured with human dermal fibroblasts Osteogenic mechanism is not well understood. However, Si^{4+} at higher concentrations has been shown to play a vital role in the mineralization process	MG-63, HCC1 and human osteoblast-like cells: 10–20 μM [249]; human MSCs: Less than 100 μg/mL [250]	None, N/A
Sr^{2+}	Osteogenesis	Strontium promotes the activity of bone-forming osteoblastic cells, while inhibiting the bone resorbing osteoclasts It activates CaSR and downstream signaling pathways. It increases the OPG production and decreases RANKL expression. This promotes osteoblast proliferation, differentiation, and viability and induces the apoptosis of osteoclasts that result in the decrease of bone resorption	Rat BMSCs and primary osteoblasts, less than 1 mM [251, 252]	N/A
Mg^{2+}	Osteogenesis, angiogenesis, neural stimulation	Magnesium induces HIF and activates PGC-1α production in undifferentiated and differentiated hBMSCs, respectively. This stimulates the production of VEGF Mg^{2+} enters into DRG neurons and promotes the release of CGPR and then stimulates the PDSCs to express the genes contributing to osteogenic differentiation	Mouse pre-osteoblasts and hTMSCs, 50–150 ppm [253–255]; human BMSCs, 5–10 mM [256, 257]	Male adult: 420 RAD, 350 UL Female adult: 320 RAD, 350 UL
Zn^{2+}	Osteogenesis	Zinc has been found to be involved in the structural, catalytic or regulation of ALP expression in which it plays an important role in osteogenesis and mineralization. It is also believed that zinc is able to suppress the osteoclastic resorption process	Mouse pre-osteoblast: 10^{-5} M [258–260]; Rat BMSCs: 10^{-5} M [261]	Male adult: 11 RAD, 40 UL Female adult: 8 RAD, 40 UL

(continued)

Table 3 (continued)

	Role	Mechanism of action	Documented efficiency dosage	mg per day
Cu^{+}	Angiogenesis, osteogenesis	Copper is reported to be a hypoxia-mimicking factor leading to the induction of angiogenesis. The immune microenvironment induced by Cu^{2+} may indirectly lead to robust osteogenic differentiation of BMSCs via the activation of the Oncostation M (OSM) pathway	Human BMSCs: Less than 50 ppm [262]; Mouse pre-osteoblasts: Less than 50 ppb [263]	Male and female adult: 0.9 RDA, 10 UL
Li^{+}	Osteogenesis	Lithium is able to inhibit the GSK3 expression, which is a negative regulator of the Wnt signaling pathway. Other investigations demonstrated that lithium is able to improve fracture healing by serving as an agonist of Wnt/β-catenin signaling	Mice: 0.02 M daily in drinking water [264]	Adult with 70 kg: 1 RDA
Co^{2+}	Angiogenesis	The Co^{2+} ion is believed to induce the formation of a hypoxia cascade, with which stabilizes HIF-1α. Then, the cells will compensate this hypoxic environment by expressing genes (such as VEGF and EPO) that promote neovascularization and angiogenesis	Human BMSCs: 100 μM [265], 20 mg/L [266, 267]	N/A

VEGF vascular endothelial growth factor; *CaSR* calcium sensing receptor; *OPG* osteoprotegerin; *RANKL* receptor activator of nuclear factor kappa beta ligand; *NOS* nitric oxide synthase; *ROS* reactive oxygen species; *GSK3* glycogen synthase kinase 3; *HIF-1α* hypoxia-inducible factor-1α; *EPO* erythropoietin; *HCC1* human early osteoblastic cell line; *hTMSCs* human TERT-immortalized mesenchymal stem cells; *BMSCs* bone marrow stem cells; *ALP* alkaline phosphatase; *RDA* recommended dietary allowance; *UL* tolerable upper intake

were observed in vitro [249, 273]. Human osteoblast cells demonstrated 1.8-, 1.5- and 1.2-fold increases in type I collagen, alkaline phosphatase and osteocalcin activity, respectively, when cultured with a conditioned medium supplemented with 0–1.4 ppm (50 μM Si^{4+}) of orthosilicic acid [249]. In another study, human osteoblast-like cells were incubated with 0.1–100 ppm (3.6 mM) Si^{4+} for 48 h, and a dose-dependent increase in proliferation and osteogenic differentiation mediated through the up-regulation of transforming growth factor beta (TGF-β) was reported [273]. In a recently published paper [250], bioactive silicate nanoplatelets with a concentration of 100 μg/mL triggered osteogenic differentiation of human mesenchymal stem cells (hMSCs) without any osteoinductive factor; this effect dropped when the concentration exceeded 1 mg/mL (Fig. 4). However, the mechanism of inducing osteogenic differentiation of hMSCs has not been fully explained. Numerous studies on bone healing involving the application of Si-substituted CaPs, including Si-HA and Si-TCP, have documented superior biological performance of their stoichiometric counterparts [274]. However, a critical review pointed out that

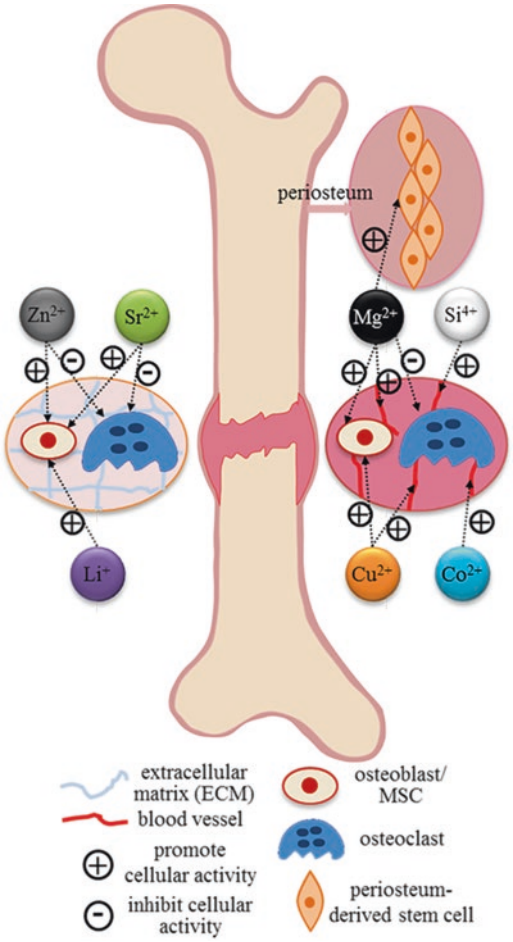

Fig. 3 Most common specific targets of relevant bioinorganic ions in their role of therapeutic agents revealed by current research

no direct evidence can link the improved biological performance of Si-substituted CaPs to the released Si [272], since the substitution of silicon not only alters the Si release but may also change the dissolution rate of ceramics [275, 276], grain size in structural composition [277, 278], protein conformation at the material surface [279], and surface topography [278, 280]. Since bioglass is a kind of silica-based synthetic bone substitute widely used in orthopedic applications, it cannot be ignored when discussing the effects of silicon on bone regeneration. As mentioned above, there is speculation that the bioactivity of bioglass can be attributed to the leaching and accumulation of silicon ions when exposed to body fluids upon implantation and the subsequent formation of the hydroxyapatite coating on the surface [119]. Nevertheless, it is accepted that the hydroxyapatite coating, but not the leaching silicon ions, played an active role in the processes leading to new bone formation [18].

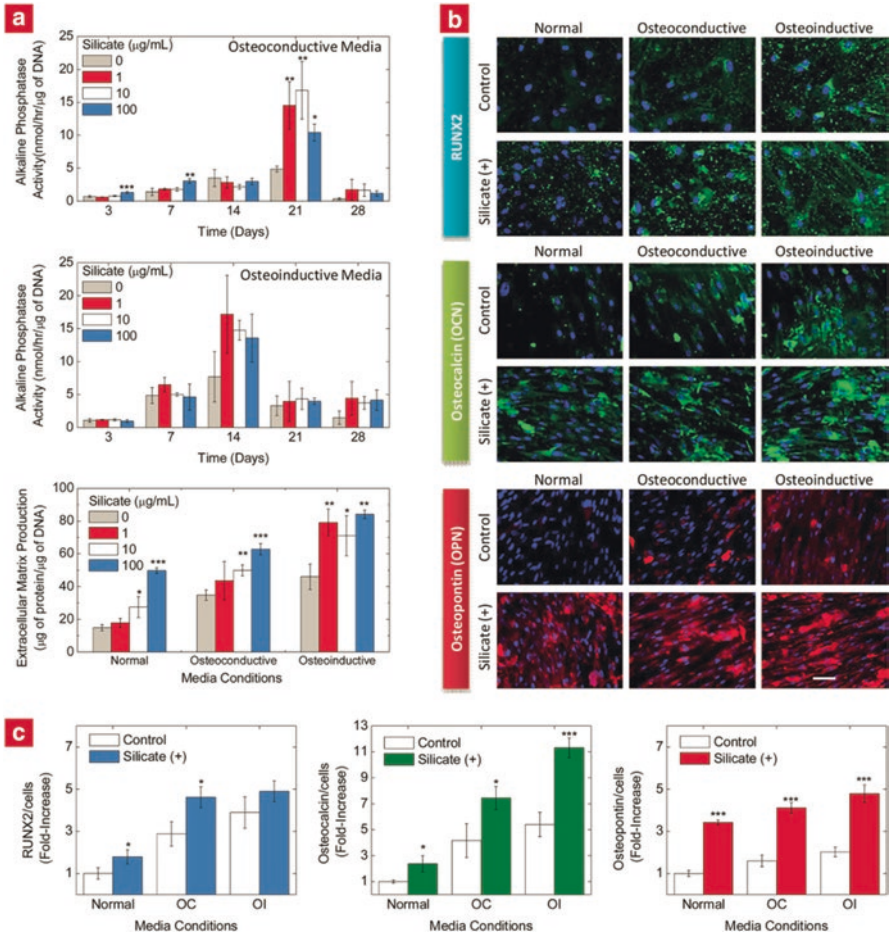

Fig. 4 Effect of silicate nanoplatelets on hMSCs differentiation. (**a**) The addition of silicate nanoplatelets up-regulate the alkaline phosphatase (ALP) activity of hMSCs. (**b**) The increase in the RUNX2 (green) and production of bone-related proteins such as osteocalcin (OCN, green), and osteopontin (OPN, red) was observed due to the addition of silicates. Cells in normal media without silicate particles act as a negative control, whereas cells in an osteoinductive medium serve as a positive control. Cell nuclei were counterstained with DAPI (blue) (scale bar = 200 μm). (**c**) The protein production was quantified using image analysis from the fluorescence images. The intensity of protein per cell was quantified and later normalized by the control (hMSCs in normal growth media with no silicate particles) to obtain a fold-increase in the production of the protein ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) (Reprint with permission from [250])

4.2 Strontium (Sr)

Strontium is a bone-seeking element, 98% of which can be found in the skeleton [281]. It accounts for 0.035% of mineral content in the skeletal system [246]. Its size and behavior are similar to those of calcium, since they are in the same periodic

group. As a non-essential element, a clinical case regarding the deficiency of strontium is rare; however, the over-feeding of strontium in rats produced rickets by disrupting calcium absorption, vitamin D synthesis, and subsequent mineralization [282]. It is predicted the mechanism can be attributed to the similarity between strontium and calcium, which allows Sr to share some osteoblast-mediated processes dominated by calcium in bone metabolism as shown in Fig. 5. Briefly, strontium activates the calcium sensing receptor (CaSR) in an osteoblast [283, 284] to stimulate the production of osteoprotegerin (OPG) [285, 286], which then suppresses the expression of the receptor activator of nuclear factor kappa beta ligand (RANKL), thus inhibiting RANKL-induced osteoclastogenesis [287]. In one in vitro experiment, bone marrow mesenchymal stem cells (BMMCSs) from rats were cultured in an osteogenic medium supplemented with 0.1 or 1 mM Sr^{2+} (8.7 mg/L or 87 mg/L) for two weeks. Proliferation of BMMCSs was significantly inhibited, while osteoblastic differentiation was promoted dose-dependently [252]. In another cell culture experiment, the dose-dependent effects of strontium on rat primary osteoblasts in terms of nodule formation and mineralization were observed compared to the Sr-depleted control. In the conditioned medium supplemented with a low dosage of Sr (0.5 and 1 μg/mL), nodule formation was reduced, while mineralization was intact. In the conditioned medium supplemented with an intermediate dosage of Sr (2 and 5 μg/mL), neither of these processes was affected. In the conditioned medium supplemented with a high dosage of Sr (20 and 100 μg/mL), nodule formation was not affected but mineralization was reduced, indicating that the formation of hydroxyapatite was inhibited [251]. Strontium's ability to reduce bone resorption [288] and osteoclast activity [289] was also observed when cultured with rat osteoclasts and primary mature rabbit osteoclasts, respectively. Given the dual roles of strontium on bone formation, one strontium salt, strontium ranelate, has been used clinically as a prescriptive treatment for postmenopausal women with osteoporosis in Europe [290].

Attempts have also been made to incorporate strontium into synthetic mineral ceramics; mechanisms may involve an exchange of ions on an apatite surface or heteroionic substitution [291]. Data showed that tricalcium phosphate was able to host up to 20 wt.% of strontium [292, 293] and 12 wt.% in hydroxyapatite [294, 295], without provoking rearrangement of the unit cell. The biological activity of those strontium-substituted mineral ceramics has been documented in numerous studies, demonstrating pronounced apatite layer formation [296], increased attachment, proliferation, and differentiation when cultured with osteoprecursor cells [297] and human osteoblasts MG-63, and suppressed osteoclast proliferation [298]. Similar results were displayed in animal studies [299–301]. Enhanced new bone formation was presented on the surface of strontium-containing mineral ceramics, while the resorptive activity of osteoclasts was inhibited. Nevertheless, in analyzing these in vivo characterizations, it must be noted that strontium substitution not only releases Sr^{2+} into the microenvironment but also alters the other physico-chemical properties, and these effects cannot be isolated from the final results.

Recently, concerns have arisen about the adverse side effects of strontium ranelate in patients with an established history of cardiovascular events and venous

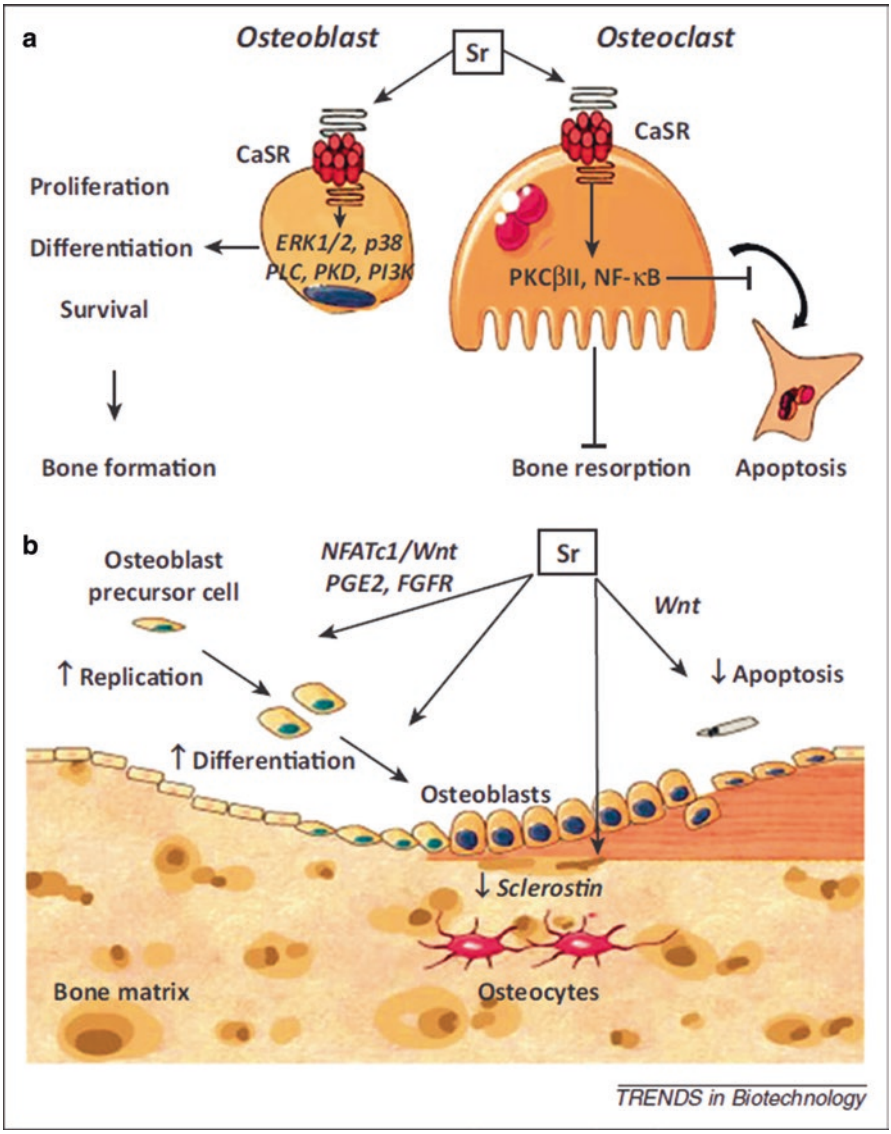

Fig. 5 (**a**) A schematic showing the dual mechanism of strontium (Sr): the stimulatory role on bone-forming osteoblast cells and the inhibitory role on bone-resorbing osteoclast cells. (**b**) A schematic showing how Sr activates osteoblastogenesis. Abbreviations: *CaSR* calcium sensing receptor; *ERK1/2* extracellular signal-regulated kinases 1/2; *P38* a mitogen-activated protein kinases; *PLC* phospholipase C; *PKD* protein kinase D; *PI3K* phosphatidylinositide 3-kinases; *PKCβII* protein kinase C βII; *NF-kB* nuclear factor kappa beta; *NFATc* nuclear factors of activated T cells; *PGE2* prostaglandin, E2; and *FGFR* fibroblast growth factor receptor (Reprinted from [246, 302], Copyright (2012), with permissions from Elsevier)

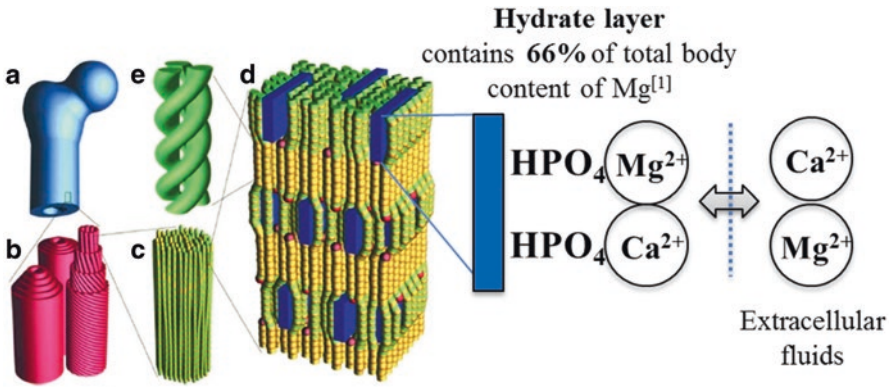

Fig. 6 Schematic diagram of the hierarchical structure in bone and a proposed mechanism of ion-exchange behavior. (**a**) Macroscopic bone. (**b**) Haversian osteons in cortical bone, consisting of several concentric lamellar layers that are built from parallel collagen fibers. (**c**) Fine structure of collagen fiber, consisting of collagen fibrils. (**d**) Collagen molecular packing with mineral in the fibril. Collagen molecules are shown as green and yellow rods. Mineral crystals are shown as blue tiles. (**e**) Single molecule triple helix. Reproduced with permission of the International Union of Crystallography [318]

thrombosis [303]. In 2013, an increased risk of myocardial infarction in postmenopausal women was reported when using strontium, thus leading to the suspension of this drug [304]. More importantly, this drug has not yet been approved by the FDA.

4.3 Magnesium (mg)

Magnesium is the fourth most abundant cation in the body [305], equal to about 1 mol (24 g) in an adult human body [306]; over 60% is accumulated in bone and teeth [307]. Studies have shown that the majority of Mg that accumulates in bone tissue is concentrated on the hydrated surface layers of apatite crystals and is not incorporated into the lattice structure of bone crystals, as shown in Fig. 6. This would allow rapid exchange of Mg^{2+} between blood and extracellular fluid, leading to ion homeostasis [308–310]. As an essential element in the human body, magnesium has been found to be cofactor for various enzymatic reactions involved in energy metabolism, protein and nuclei acid synthesis, functional maintenance of parathyroid glands, and vitamin D metabolism that are strictly related to bone health [311, 312]. Several researchers studying the effects of a Mg-depleted diet on rats showed decreased systemic bone density [313], inhibition of growth in the proximal end of the tibia [314], and even development of osteoporosis [315]. A higher intake of magnesium has been proven to efficiently prevent reduction of bone mineral density (BMD) in patients with osteoporosis [316]. However, toxic symptoms induced by magnesium in excess, such as metabolic alkalosis, hypokalemia, and

paralytic ileus [317], are rarely reported since the Mg concentration is strictly mediated by the kidneys through urine excretion [311].

Our previous studies [253, 254] suggested that when the magnesium ion concentration fell to an appropriate range (i.e., 50–100 ppm), it was able to up-regulate the viability of mouse pre-osteoblasts. The specific alkaline phosphate activity of osteoblasts cultured with Mg ion-supplemented media was found to be significantly higher compared with the control. The real-time RT-PCR study also exhibited higher levels of ALP and runt-related transcription factor-2 (Runx2) expression after stimulation with a suitable amount of Mg ions. The highest levels of Type I collagen (Colla 1) and osteopontin (Opn) expression were found on Day 3 from the cells cultured with a conditioned medium. In other research, magnesium was doped into various kinds of materials, including hydroxyapatite, tricalcium phosphate, and collagen, and the biological activities of those materials were investigated and compared to a non-doped control. Interestingly, when the apatite in the collagen was totally replaced by magnesium, a toxic effect was demonstrated and the formation of extracellular matrix (ECM) was inhibited [319]. However, when the amount of magnesium being doped was controlled in a suitable range [320], densification as well as osteoblastic cellular attachment, proliferation, and ALP production improved [256, 257, 321], and greater osteogenic properties were also observed in vivo [322]. Meanwhile, the osteoclast formation, polarization, and osteoclast bone resorption were suppressed in vitro [323]. These results are similar to the observations in our previous in vivo studies. High dosage (high-Mg/PCL, 0.6 g Mg in 1 g PCL), low dosage (low-Mg/PCL, 0.1 g Mg in 1 g PCL) Mg/PCL and pure PCL were implanted at the lateral epicondyle of rats. Superior newly formed bone was observed in the low-Mg/PCL group after 2 months, whereas bone regeneration in the high-Mg/PCL group was even worse than that of the control (unpublished data). These phenomena again highlight the importance of dosage when utilizing magnesium in bone healing.

Although the mechanism of magnesium ions on fracture healing has not been yet fully explained, recent studies are bridging this gap. Research conducted by S Yoshizawa et al. [256, 257] hypothesized that the osteo-regenerative effect of Mg^{2+} on undifferentiated human bone marrow stromal cells (hBMSCs) and osteogenic hBMSCs could likely be attributed to the subsequent orchestrated responses of activating hypoxia-induced factor 2α (HIF-2α) and peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α, respectively. The hypothesized intracellular signaling cascades are exhibited in Fig. 7. Zhang et al. found that the rat bone marrow stem cells (BMSCs) displayed significantly up-regulated integrin α5ß1 expression when cultured with 5%-Mg-incorporated calcium phosphate cement (5MCPC). The BMSCs thus promoted osteogenic differentiation, whereas this effect was not observed when cultured with 10MCPC and 20MCPC [325]. More recently, Lin et al. demonstrated that the magnesium ions may stimulate the accumulation of neuronal calcitonin gene-related polypeptide-α (CGRP) in both the peripheral cortex of the femur and the ipsilateral dorsal root ganglia (DRG), thereby promoting fracture healing in rat animal models. This mechanism is depicted in Fig. 8 [324]. This research revealed an undefined role of Mg^{2+} in CGRP-mediated osteogenic

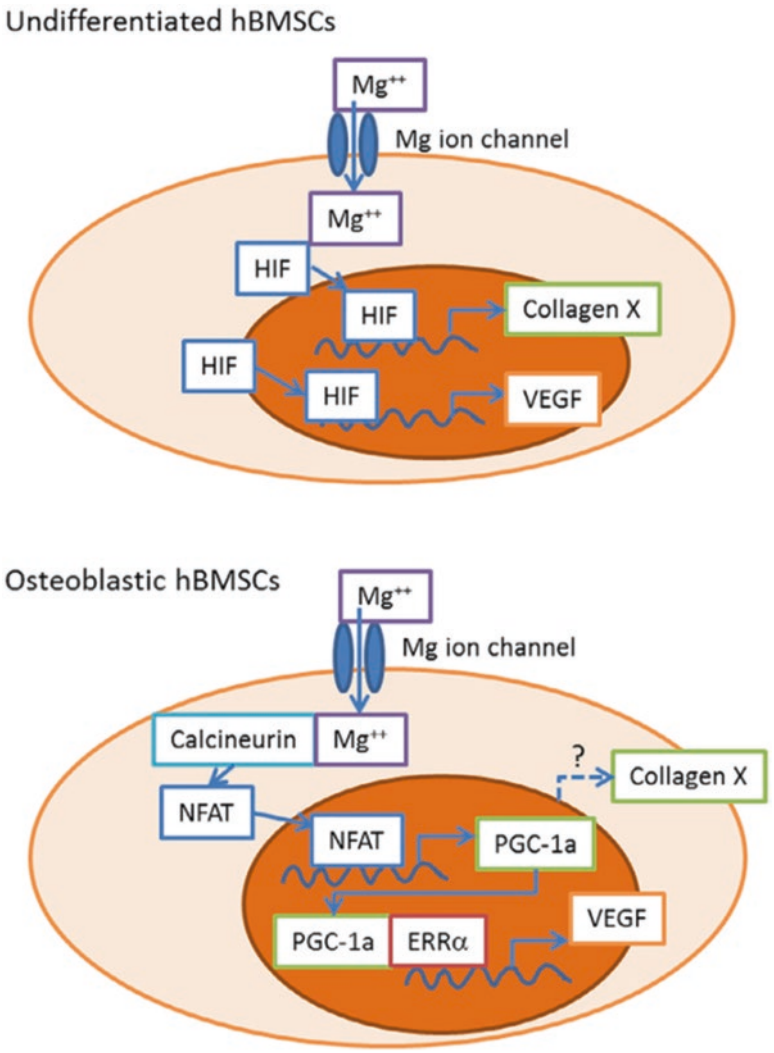

Fig. 7 Schematic of the hypothesized intracellular signaling cascades by Mg ion stimulation of human bone mesenchymal stem cells (hBMSCs). Addition of $MgSO_4$ will increase intracellular Mg concentration in undifferentiated hBMSCs. HIFs are then translocated into the cell nucleus and induce production of Collagen X-α1 and VEGF. In differentiated hBMSCs, Mg ion activates PGC-1a production, which induces the production of VEGF. Abbreviations: HIF, hypoxia-inducible factor; NFAT, nuclear factor of activated T-cells; PGC-1α, peroxisome proliferation-activated receptor gamma, coactivator 1α; ERRα, estrogen-related receptor α; and VEGF, vascular endothelia growth factor (Reprinted from [257], Copyright (2014), with permission from Elsevier)

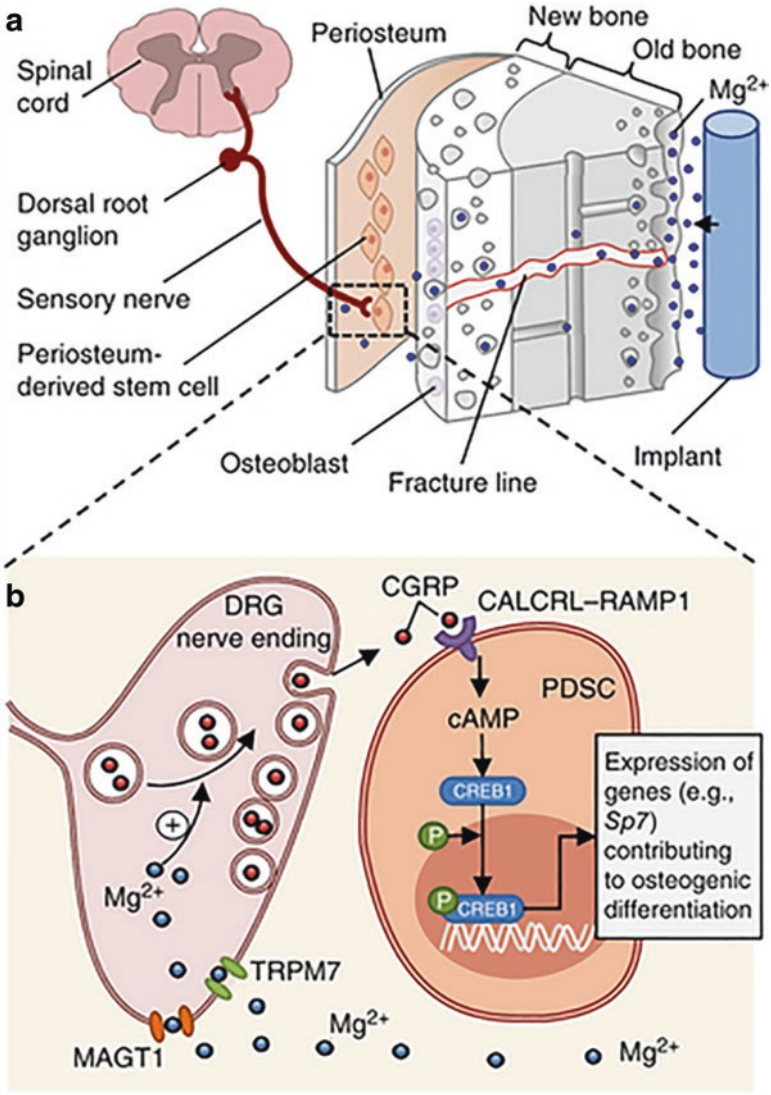

Fig. 8 Schematic diagram showing (**a**) diffusion of Mg^{2+} across the bone toward the periosteum, which is innervated by DGR sensory neurons and enriched with PDSCs undergoing osteogenic differentiation into new bone. (**b**) The released Mg^{2+} enters DRG neurons via Mg^{2+} transporters or channels and promotes CGRP-vesicle accumulation and exocytosis. The DRG-released CGRP, in turn, activates the CGRP receptor in PDSCs, which triggers phosphorylation of CREB1 via cAMP and promotes the expression of genes contributing to osteogenic differentiation. Abbreviations: DGR, dorsal root ganglia; PDSCs, periosteum-derived stem cells; CREB1, cAMP-responsive element binding protein 1; cAMP: cyclic adenosine monophosphate (Reprinted by permission from Macmillan Publishers Ltd: Nature Medicine [324], copyright (2016))

differentiation. In another study, while investigating the long-term in vivo degradation mechanism of the Mg alloy, Lee et al. found that the existence of Mg may facilitate the crystallization of calcium phosphate in a rabbit femoral condyle defect model [326]. All these recent findings again highlighted the importance of magnesium in fracture healing and suggest the therapeutic potential in the orthopedic clinics.

4.4 Zinc (Zn)

Zinc is also an essential trace element in the human body with a total weight of about 1.4–2.3 g. This number is between 150–250 μg/g in bone ash (0.015–0.025 wt.%), which is higher than other tissues [327]. It is involved in the structural, catalytic, or regulatory action of several important metalloenzymes, including alkaline phosphatase (ALP). It was found that ALP not only generates phosphates by hydrolyzing pyrophosphates, but also creates an alkaline environment that favors the precipitation and subsequent mineralization of these phosphates onto the extracellular matrix, which were produced by osteoblasts [246]. A far as we know, ALP is a zinc enzyme with three closely spaced metal ions (two Zn ions and one Mg ion) presented at the active center [328]. Further investigation suggested that inactivation of ALP is caused by the dissociation of an active center Zn; preventing the dissociation of this active center Zn can stabilize the enzyme and increase its half-life [329]. Given its important role in the skeletal system, zinc deficiency is reportedly associated with decreased bone age [18], whereas high zinc levels may lead to the suppression of the immune system, reduction of high-density lipoprotein (HDL) cholesterol, and hypocupremia by retarding the intestinal absorption of copper [317, 330]. These findings support some cell culture experiments. Yamaguchi et al. [258] showed significantly increased Runx2, OPG, and regucalcin mRNA expressions in osteoblastic MC3T3-E1 cells when zinc supplementation was in the concentration of 10^{-5}–10^{-4} M (0.65 mg/L–6.5 mg/L). Kwun et al. [259] observed a negative effect of zinc deficiency on the osteogenic activity of the same cell type, whereby bone marker gene transcription and ECM mineralization were reduced through inhibited and delayed Runx2 expression and inhibition of ALP activity in osteoblasts, respectively. However, these regulation effects were not displayed when rat bone marrow stem cells were cultured with Zn^{2+} supplemented ($1*10^{-5}$ and $4*10^{-5}$ M, eq. to 0.65 mg/L and 2.6 mg/L) osteogenic medium [261]. Suppression effects of zinc on bone resorption and osteoclastogenesis from bone marrow-derived osteoclasts were recently shown in the tissue culture system [331].

Zinc has also been found to stabilize the crystal lattice of β-tricalcium phosphate, thus making the dissolution of TCP predictable and complete [292]. In the rabbit femoral defect model, zinc-containing HA/TCP either with a high concentration (about 0.6 wt.%, high-Zn-HA/TCP) or a low concentration (about 0.3 wt.%, low-Zn-HA/TCP) was applied. Low-Zn-HA/TCP demonstrated increased bone apposition, and high-Zn-HA/TCP led to increased resorption of the host bone [332].

However, in another in vitro study, ceramics containing 0.6 wt.% of zinc displayed inhibited resorptive activity in mature osteoclasts [333]. Still, the gaps between the in vitro studies and in vivo assessments are huge, and controversial results have been reported.

4.5 Copper (Cu)

Copper is an essential element, and 90% of copper in plasma is presented in ceruloplasmin [18]. Copper's function in the human body was first identified in iron metabolism; copper deficiency usually leads to iron overload in brain, liver, and other tissues [334]. Following this early research, copper was recognized as a cofactor for several other enzymes in body. One of these related to the musculoskeletal system, is lysyl oxidase. This enzyme catalyzes the formation of aldehyde-based crosslinks from lysine residues in collagen and elastin precursors [18]. Consequently, 300% higher collagen solubility was found to lead to copper-deficiency and brittle bone [335]. Recently, having been discovered as an essential element in angiogenesis [336] and the initiation of endothelia cells toward angiogenesis [337, 338], the application of copper ions has attracted increasing attention as an alternative therapeutic agent in promoting vascularization [338–341]. Since vascularization plays a critical role in bone healing [342], it is reasonable to conduct the relevant research. Several studies have demonstrated rapid and enhanced vascularization in copper-doped porous scaffolds as well as increased extracellular matrix (ECM) formation; the collagen formation, in turn, supports further blood vessel formation in vivo physically [343, 344]. Interestingly, instead of utilizing the traditional biomaterial-assisted concept (that is, to accelerate bone ingrowth from the periphery), copper-doped biomaterials have demonstrated a tendency to accelerate bone formation throughout the defect. This is likely attributable to the angiogenic effect of Cu^{2+} [18]. Recently, it was predicted that the copper ion would have a positive effect on osteogenic differentiation of bone marrow stem cells [262, 345] and mouse MC3T3-E1 pre-osteoblasts [263], when doped into mesoporous silica nanosphere and stainless steel, respectively. Nevertheless, the consumption of high levels of copper in drinking water or beverages usually leads to gastrointestinal illness, such as abdominal pain, cramps, nausea, diarrhea, and vomiting [317], while excessive intake of copper can lead to serious liver disease and neurological issues such as idiopathic copper toxicosis (ICT) [346, 347]. Again, dosage is critical.

4.6 Other Ions

Lithium (Li) has attracted attention due to its role in osteogenesis [246]. A study involving 75 lithium-treated patients reported that the mean bone mineral density in several areas in a treated group was significantly higher than in normal participants;

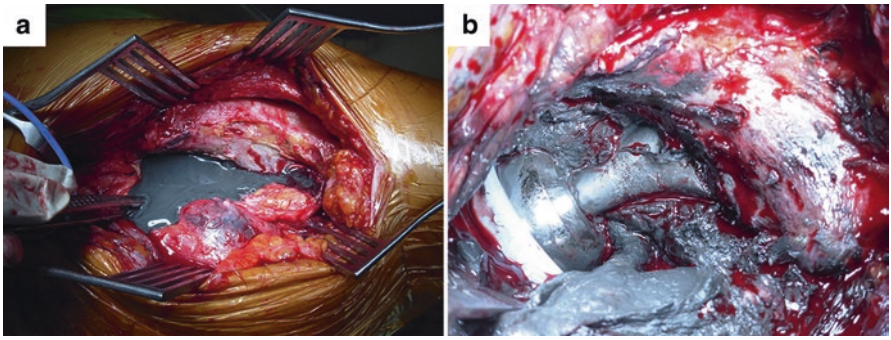

Fig. 9 A case of extreme cobalt toxicity in total hip replacement surgery. (**a**) After incision of the skin and soft tissue, clear metallic black fluid was observed; (**b**) soft tissue and peripheral bone were stained black with the metal debris after draining the fluid (Reprint from [355], with permission from Wolters Kluwer Health Inc.)

this was possibly due to a lower bone turnover in lithium-treated patients [348]. Another case-control study compared 231,778 fracture cases and found that the current use of lithium showed a decreased risk of fracture, while an increased risk of fracture was observed among past users [349]. The mechanism of lithium behind osteogenesis is predicted by inhibiting glycogen synthase kinase 3 (GSK3), which is a negative regulator of the Wnt signaling pathway [350, 351]. In addition, Li^+ has been shown to activate β-catenin signaling by mediating osteoblast proliferation, differentiation, and maturation [352] during bone and cartilage fracture healing [264]. The excess intake of lithium was found to cause stomach or intestinal symptoms and nervous system problems [353].

Cobalt (Co) is an integral part of vitamin B12, which stimulates the production of red blood cells [248]. Like copper, cobalt was recently shown to stimulate angiogenesis [245]. Significantly up-regulated VEGF was expressed when bone marrow stem cells were treated in a 100 μM $CoCl_2$ supplemented culture medium, and these $CoCl_2$-treated cells subsequently promoted vascularization and osteogenesis when implanted in vivo with a collagen scaffold [265]. This effect was recently found to associate with the hypoxia-mimicking capacity of Co ions. Studies demonstrated that mesoporous bioactive glass (MBG) scaffolds doped with a suitable amount of cobalt induced the hypoxia cascade, by which hypoxia inducible factor-1 (HIF-1) was activated [266] and stabilized [267]. This increased the expression of HIF-α target genes, such as VEGF and erythropoietin (EPO) [354], thus leading to a higher degree of vascularization. Nevertheless, cobalt intoxication has become a concern in recent years due to the wide use of metal hip prostheses, in which most of the metal-on-metal articulations are made of cobalt-chromium alloy [354, 355]. Several randomized, prospective trials [356–358] demonstrated that this was highly related to the elevated serum cobalt concentration caused by the debris upon wearing (see Fig. 9), which raised it at most 50 times higher as compared to the ceramic-on-polyethylene/ceramic control group. The symptoms

included but were not limited to bone tissue absorption, neurological toxicity, weight loss, reduced mobility, and pain [359]. Furthermore, patients generally felt improved after changing the metal hip implants to non-metallic ones or undergoing cobalt chelation therapy [354, 360].

5 Conclusion and Future Directions

Large segmental bone defects may result in delayed union or even non-union if improperly treated. Hence, surgical interventions together with bone grafting techniques are commonly considered in the treatment process. Even though the emergence of various synthetic bone substitutes offers various options, the treatment outcome cannot be compared to the approach of autologous bone graft in terms of bone healing quality and time. One of the issues leading to inferior bone regeneration when synthetic substitutes are used is due to inferior osteoinductivity. The incorporation of recombinant human growth factors (e.g. rhBMP-2) with bone allograft and other substitutes have been considered and widely applied clinically. Their clinical outcomes have been extensively reported as well. The efficacy of the treatment has been approved. Post-operative complications and the controversy of off-label applications and high application costs have been also documented. The complications can be attributed to the uncontrolled release of growth factor that collaterally interferes with the un-targeting cells. Alternatively, the incorporation of bioinorganic ions such as magnesium, strontium, silicon, copper, and cobalt into bone graft materials provides an economical and feasible solution for bone defect repair. The safety issues related to the use of these bioinorganic ions has been investigated by a number of studies over these years. When the therapeutic effect and working mechanisms of these ions have been clearly understood, it may be possible to implement a human clinical trial. With additional new discoveries, bioinorganic ions can be considered for use in the combination of growth factors for bone defect treatment that may induce synergistic effect in terms of new bone formation.

The 3D–printing technology in particular to the capability to reproduce the microarchitecture of bony tissue has been advanced by the latest development of biomaterials and therefore shed light on the next generation of synthetic bone substitutes [361]. In addition, when the 3D printed scaffolds are fabricated together with mesenchymal stem cells, this approach may induce new bone formation in the treatment of bone defects. However, challenges including suboptimal resolution, relatively slow printing speed and limited selection of bio-inks are concerned. When all these issues have been resolved, it is believed that the demand for bone graft and autograft will become lesser in the future.

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