Applications of Bioactive Ions in Bone Regeneration

Si Han LIN[1,2,3], Wen Jie ZHANG[1,2,3], Xin Quan JIANG[1,2,3]

The repair of large bone defects remains a huge challenge for bone regenerative medicine. To meet this challenge, a number of bone substitutes have been developed over recent years to overcome the drawbacks of traditional autograft and allograft therapies. Thus, the improvement of the osteoinductive ability of these substitutes has become a major focus for research in the field of bone tissue engineering. It has been reported that some metallic ions play an important role in bone metabolism in the human body, and that bone repair could be enhanced by incorporating these ions into bone substitutes. Moreover, it is well documented that ions released from these substitutes such as magnesium, zinc, and strontium can increase the osteogenic and angiogenic properties of bone repair scaffolds. However, the mechanisms of action of these ions on cellular bioactivity are currently unclear. Therefore, in the present article, we highlight the recent use of bioactive ions in bone tissue engineering and discuss the effects of these ions on osteogenesis and neovascularisation.

Key words: *bioactive ions, bone regeneration, tissue engineering, osteogenesis, angiogenesis*
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Bones perform numerous vital functions, including bearing the body's weight, protecting the internal organs from harm and providing a framework to support the shape of the body[1]. Although bones have the capacity for regeneration, they may be unable to heal under certain conditions such as critical-size bone defects[2,3]. Currently, bone defects are primarily caused by tumours, injuries, trauma and congenital deformities; these defects may decrease quality of life, lead to mental illness and shorten the lifespan. Studies have shown that millions of bone graft treatments are carried out annually worldwide, and that the demand for bone grafts is increasing steadily. Moreover, owing to the growth of the ageing population, global healthcare costs on bone fractures are expected to increase by 25% in the next 10 years[1,4,5]. In response to this, various approaches to bone regeneration have been explored. Currently, autografts are the gold standard for clinical applications due to their distinctive regeneration capacity, including their osteoinductive, osteogenic and osteoconductive properties. However, autografts have some disadvantages. The harvest of autografts requires sufficient healthy bone tissue from another part of the body (known as the 'donor site') – usually from the iliac crest or fibula. These second surgical interventions increase the risk of infection and other early or late complications. Additionally, the amount and characteristics of bone grafts are not always suitable for the recipient site[6-8]. These drawbacks limit the clinical applications of autografts, while allografts and xenografts face the problems of host rejection and disease transmission. Thus, extensive studies are required to develop new and more effective alternative treatments.

1 Department of Prosthodontics, Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, P.R. China.
2 Shanghai Engineering Research Center of Advanced Dental Technology and Materials, Shanghai, P.R. China.
3 Shanghai Key Laboratory of Stomatology and Shanghai Research Institute of Stomatology; National Clinical Research Center of Stomatology, Shanghai, P.R. China.

Corresponding authors:
Prof Xin Quan JIANG, Department of Prosthodontics, Shanghai Ninth People's Hospital, College of Stomatology, Shanghai Jiao Tong University School of Medicine, 639# Zhizaoju Road, HuangPu District, Shanghai 200011, China; Tel: 86-21-23271699; Fax: 86-21-63136856; Email: xinquanj@aliyun.com;
Dr Wen Jie ZHANG, Department of Prosthodontics, Shanghai Ninth People's Hospital, College of Stomatology, Shanghai Jiao Tong University School of Medicine, 639# Zhizaoju Road, HuangPu District, Shanghai 200011, China; Tel: 86-21-23271699; Fax: 86-21-63136856; Email: zhangwenjie586@126.com.

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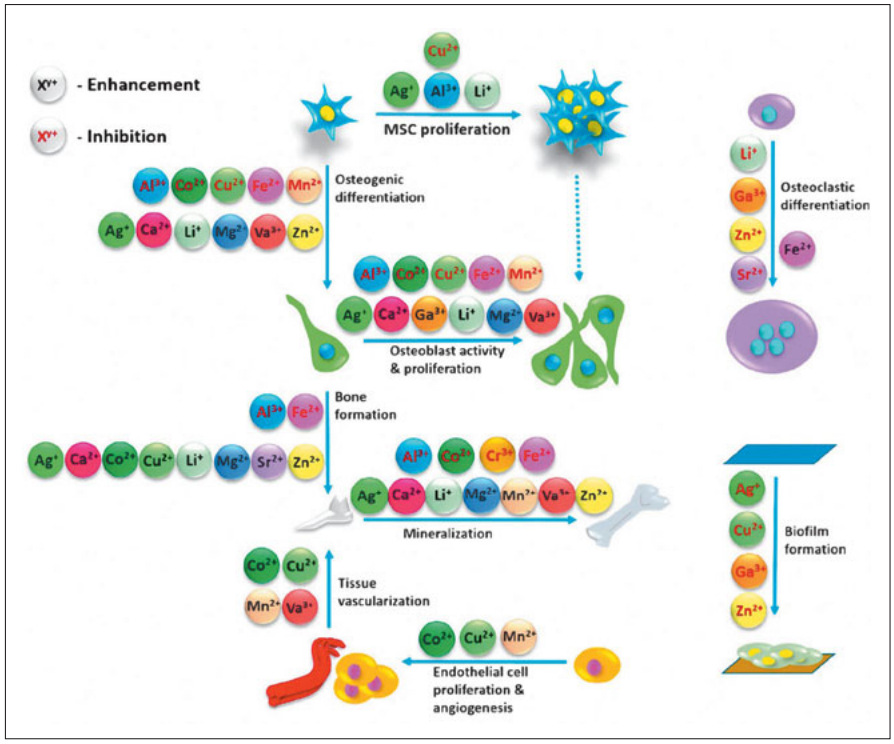

Fig 1 Effects of ions in the process of bone healing: Mesenchymal stem cell (MSC) (adapted from Glenske et al[37]).

Through remarkable achievements in tissue engineering, a large number of bone substitutes have been developed[1,9]. Biocompatible scaffolds, bioactive growth factors, and seed cells are three key elements in bone tissue engineering[10]. The process of bone regeneration is quite complex and delicate, and depends on the interactions between biomaterials and seed cells. In engineered bone substitutes, materials act as scaffolds to elicit bone ingrowth and provide an environment for seed cells and endogenous stem- and osteoblastic-progenitor cells to proliferate and differentiate[11,12]. Thus, it is very important to improve the osteoinductive and osteogenic properties of these materials[13]. Numerous relevant bone-forming growth factors have been investigated and have proven to have specific effects in enhancing the bioactivity of scaffolds. These growth factors include transforming growth factor-beta (TGF-β), fibroblast-like growth factor (FGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)[11]. Although these growth factors contribute to osteogenesis, side effects such as ectopic or unwanted bone formation have led to doubts regarding their safety[14,15].

Bones are composed of about 20% collagen, while the majority of bone mass derives from minerals (about 70%). Other organic materials such as proteins, polysaccharides and lipids make up only a small part of bones[5,12]. Bone minerals contain various major and trace elements such as magnesium, calcium, zinc and strontium. As shown in Figure 1, these bioactive ions are involved in multiple processes related to bone regeneration[10]. Hence, the incorporation of these natural bioactive ions with scaffolds may provide a safer alternative strategy for bone regeneration. It was recently reported that several ions, namely magnesium, strontium and lithium, could stimulate the formation of new bones[16,17]. Compared with growth factors, the incorporation of bioactive ions into bone substitutes is a simpler and safer method to enhance bone regeneration at a relatively low cost. However, the specific mechanisms of the effects of these ions on bone formation remain unclear. In this review, we explore the physiological function of bioactive ions and their applications in bone tissue engineering, and discuss possible mechanisms through which they affect bone formation.

Magnesium

Role of magnesium in bone

Magnesium ion (Mg^{2+}) is an essential element and the fourth most abundant metallic ion in the human body. More than half of the total magnesium ions in the body

are stored in the bones and teeth (0.44% of enamel, 1.24% of dentine and 0.72% of bone [w/w]). Magnesium plays an important role in multiple physiological reactions such as the regulation of intracellular cations, deoxyribonucleic acid (DNA) replication, enzyme activation and immune defence[18]. Thus, magnesium deficiencies can result in numerous health problems. In bone metabolism, it has been shown that magnesium deficiencies may lead to decreased bone mass, reduced bone growth, osteoporosis and increased susceptibility of the skeleton to fractures; these may be linked to impaired bone formation due to the reduced secretion of parathyroid hormone (PTH) and calcitriol as well as the enhancement of bone resorption. Increased levels of substance P, tumor necrosis factor (TNF)-α and receptor activator of nuclear factor kappa-B ligand (RANKL) are reported to be involved in enhanced bone resorption[19-21].

Applications of magnesium in bone tissue engineering

In addition to its fundamental effects in bone metabolism, magnesium has similar effects on the mechanical properties of natural bone and could reduce bone resorption caused by stress shielding[22,23]. Unlike titanium, which is currently the most widely used implantation metal, magnesium is biodegradable *in vivo* and possesses a balance between degradation and strength[24]. Thus, magnesium is regarded as an ideal implantation material for the treatment of bone defects. However, the corrosion rate of pure magnesium is too rapid to provide a stable mechanical support *in vivo*; thus, various magnesium-based alloys have been developed. Previous work has revealed that treatment with magnesium can accelerate osseointegration with surrounding bone tissue, encourage the recruitment of bone marrow stromal stem cells (BMSCs) towards peri-implantation bone tissue and enhance the attachment of cells to the implantation surface[24-26]. Notably, the degradation of magnesium alloys is accompanied by the uncontrollable release of hydrogen gas and the development of an alkaline environment, which is harmful for osteogenesis[27]. In general, three methods have been developed to solve these problems. One method is to modify magnesium alloys with different coatings. Microarc oxidation (MAO) and electrophoretic deposition (EPD) are two of the most commonly used techniques[28] that enhance the surface roughness of magnesium alloys, making it easier for cells to attach and expanding the interface between implant and bone by increasing the surface area. Coatings with higher corrosion resistance and wear resistance make magnesium alloys more durable, and the release of other elements from the coatings has a synergistic effect on osteogenesis as well as antibacterial effects[28-31]. Introducing magnesium ions into titanium implants is a second treatment strategy. In a recent study, Okuzu et al[25] introduced Mg^{2+} into titanium implants using the alkali and heat treatment method. *In vitro* experiments showed that Mg^{2+} released from implants promoted the proliferation and osteogenic differentiation of MC3T3 cells. After the implantation of these magnesium-containing implants to rabbit tibial defects, greater bone-implant contact was obtained compared with those with calcium-containing implants, especially at the early stage (4 to 8 weeks). No significant changes in the serum concentration of Mg^{2+} were observed after implantation, suggesting the biocompatibility of the magnesium-containing implants. Magnesium-containing bioceramics are also widely used. Bioceramics, usually referred to as calcium phosphate (CaP) ceramics, are one of the most successful materials used in bone regenerative medicine due to the high similarity of their chemical composition to natural bone. Incorporation of the magnesium dopant with CaP ceramics can provide a stable and efficient ion delivery system that is favorable for bone formation[10,32]. Magnesium-containing bioceramics include the MgO-P_2O_5 binary system, the CaO-MgO-P_2O_5 ternary system and the MgO-SiO_2 binary system[33-36]. Wu et al[34] introduced magnesium phosphate cement (MPC) into calcium phosphate cement (CPC) to form a novel calcium-magnesium phosphate cement (CMPC). Their results suggested that the incorporation of MPC significantly reduced the setting time and enhanced the mechanical properties of CPC. Cell culture results indicated that CMPC was biocompatible and promoted the attachment and proliferation of MC3T3 cells. An *in vivo* study showed that the introduction of MPC into CPC enhanced the efficiency of new bone formation in rabbits with bone defects[34]. It is worth noting that the osteogenic inductive effects of Mg^{2+} may be dose dependent[37]. Previous studies found that low extracellular Mg^{2+} (≤ 0.1 mM) significantly inhibited the expression of osteogenic genes in rat BMSCs cultured in osteogenic medium; in addition, high Mg^{2+} (≥ 18 mM) also had an inhibitory effect on osteoblast activity[38,39]. Hence, the correct concentration of extracellular Mg^{2+} is essential for bone regeneration.

Bone is a highly vascularised tissue. Nutrient and oxygen exchange between individual cells and blood vessels in bone is limited to distances of within 500 μm[40]. Therefore, it is crucial to rebuild the vascular system in engineered scaffolds for bone rehabilitation, especially for large bone defect repairs. To investigate the effects of magnesium supplementation on angiogenesis, Maier et al[41] cultured human umbilical vein

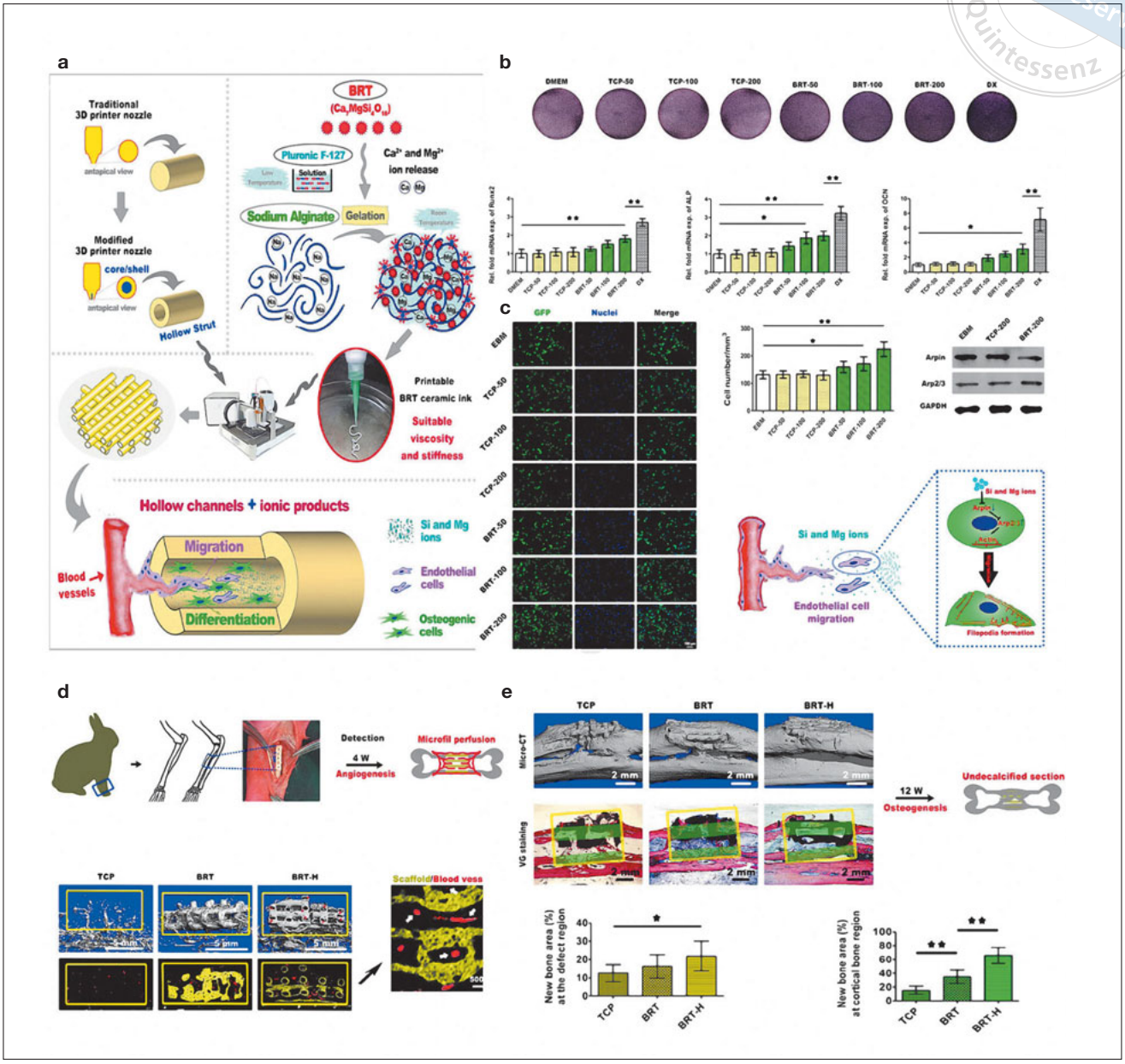

Fig 2 Hollow-pipe-packed bioceramic scaffolds incorporated with magnesium and other bioactive ions improved vascularised bone regeneration (adapted from Zhang et al[43]). (a) The fabrication of 3D-printed silicate bioceramic (BRT-H) scaffolds. Vascularised bone regeneration was enhanced by the synergistic effects of hollow structures and bioactive ions. (b) The dissolution products of BRT-H stimulated the osteogenic differentiation of the BMSCs by promoting ALP activity and the expression of osteogenic-related genes. (c) Ionic products of BRT induced the migration of endothelial cells, possibly by increasing the expression of Arp 2/3 proteins and decreasing the expression of Arpin. (d) Micro-CT was used to detect newly formed blood vessels in the bone defect areas (yellow rectangles). (e) Radiological and histological findings show that the BRT-H scaffolds enhanced bone formation and remodelling. The yellow rectangles indicate the entire defect regions and the green indicates the cortical bone.

endothelial cells in media with concentrations of Mg^{2+} ranging from 2 to 10 mM and compared them with the corresponding controls (1 mM Mg^{2+}). Their results showed increased endothelial proliferation and an enhanced cellular response to angiogenic factors with increasing Mg^{2+} concentrations. Furthermore, a high concentration of magnesium (5 mM) not only enhanced the synthesis of nitric oxide by acting as the signalling molecule in angiogenesis, but also up-regulated the secretion of angiogenic factors such as VEGF[10,41,42].

Recently, new research has been done on the formation of vascularised bone tissue. As shown in Figure 2, a hollow-pipe-packed bioceramic scaffold using coaxial 3D-printing technology was successfully fabricated. The bioactive ions, including magnesium, were added to the printing scaffolds in a layer-by-layer manner controlled by a computer. The promotion of osteogenesis and angiogenesis was observed both *in vitro* and *in vivo* owing to the synergistic effect of the hollow-pipe structure and released bioactive ions[43].

Possible mechanisms of magnesium for bone regeneration

A number of studies have been done on the effects of magnesium on osteogenic cells *in vitro*[44-47]. It has been determined that concentrations of Mg^{2+} ranging from 2.5 to 10 mM can increase the expression of osteogenic markers (including osteocalcin, osteopontin and collagen type X), enhance alkaline phosphatase (ALP) activity and stimulate the proliferation and migration of rat BMSCs[48-51]. However, the molecular mechanisms underlying the effects of magnesium on cell behavior are still poorly understood. A recent study revealed that ion channels such as magnesium transporter 1 (MAGT1) are involved in the bone healing process mediated by neuronal calcitonin gene-related polypeptide-α (CGRP) (Fig 3). Apart from this, the knockdown of MAGT1 has been shown to attenuate magnesium inhibition during the osteogenic differentiation of MSCs[46]. Additionally, previous work has revealed that Mg^{2+} can enter into cells through these ion channels and then stimulate downstream signalling pathways, including the Wnt/β-catenin pathway and the PI3K/AKT pathway, both of which contribute to osteogenic differentiation[39,52,53].

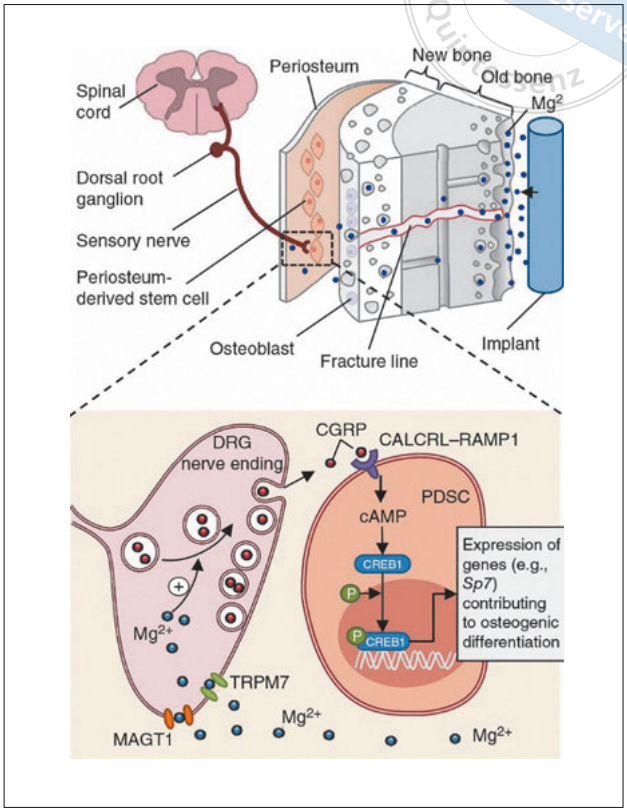

Fig 3 Implant-derived magnesium enhanced bone regeneration via inducing the production of CGRP (adapted from Zhang et al[53]). The released Mg^{2+} enters dorsal root ganglion (DRG) neurons via magnesium transporters such as magnesium transporter 1 (MAGT1) and transient receptor potential cation channel subfamily M member 7 (TRPM7), which promotes the production of CGRP. The DRG-released CGRP, in turn, activates the CGRP receptor in periosteum-derived stem cells, which results in the upregulation of the expression of genes contributing to osteogenic differentiation.

Strontium

Role of strontium in bone

Strontium (Sr^{2+}) is not an essential element in the human body, and makes up only 0.035% of the mineral content in the human skeletal system. However, strontium appears in a strong bone-seeking element, of which over 90% is deposited in bones and teeth, thus suggesting its high affinity to the mineral content of bone[54]. Additionally, strontium is similar to calcium in terms of its structure and chemical properties, which implies that it could displace calcium in bone metabolic processes[10,16]. Recently, strontium ranelate was successfully used to treat osteoporosis due to its dual effects on osteoblasts and osteoclasts[55]. As a result, the effect of strontium on bone cells has attracted much attention.

Applications of strontium in bone tissue engineering

Strontium is widely used to enrich the osteoinductive properties of biomaterials such as bioceramics, hydrogels and metallic implants[56-58]. MAO is the most widely used technique to fabricate strontium coatings on an implantation surface, while additional manufacturing technologies make it possible to control the spatial distribution of strontium in scaffolds[59]. It has been reported that the presence of strontium can enhance the proliferation and osteogenic differentiation of osteoblastic cells and inhibit osteoclast activity *in vitro*. For example, 5% strontium-substituted glass remarkably promoted

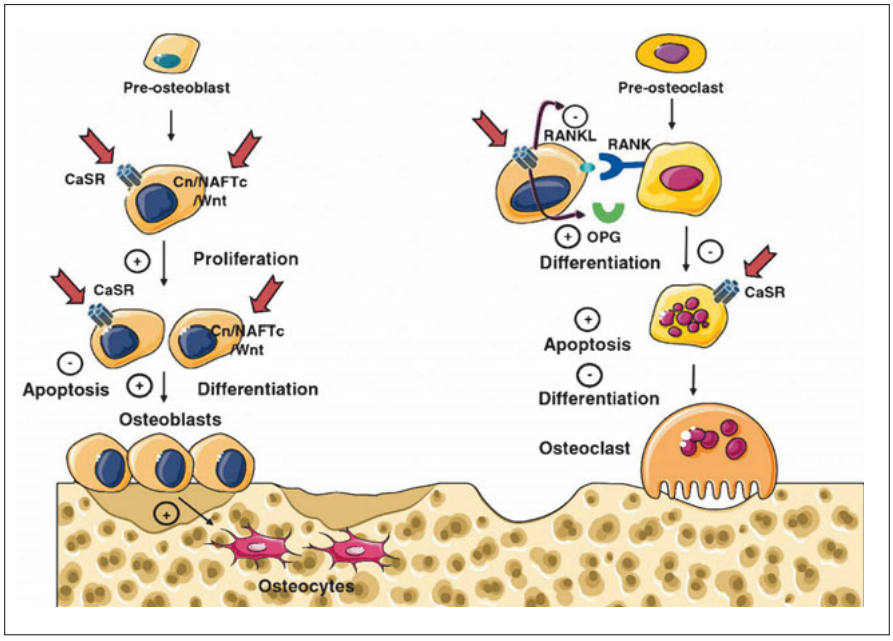

Fig 4 Possible mechanisms of action of strontium (Sr^{2+}) on osteoblasts and osteoclasts (adapted from Marie et al[65]). Briefly, strontium promotes pre-osteoblast replication and osteoblast differentiation and survival. Strontium has inhibitory effects on the differentiation, function and survival of osteoclasts and may affect these processes via calcium-sensing receptor (CaSR), nuclear factor of activated T-cells (NFATc)/Wnt signalling and by modulating the osteoprotegerin (OPG)/RANKL ratio.

the ALP activity and mineralisation of MC3T3 cells *in vitro*, and incubation with 10 mM strontium ranelate significantly accelerated the mineralisation of osteoblasts and simultaneously decreased the differentiation of osteoclasts in a co-culture model[56,60]. In addition, the local strontium-enriched environment created by the sustained release of strontium from implants promoted bone formation and bone-to-implant contact *in vivo*; this may prevent the side effects caused by the systemic use of strontium ranelate[56-58]. As already mentioned, neovascularisation is pivotal to bone regeneration. Kargozar et al[61] incorporated Sr^{2+} (47.2 mol %) and Co^{2+} (0.6 mol %), another promising element known to enhance angiogenesis, into bioactive glasses (BGs) seeded with human umbilical cord perivascular cells. After implantation of these scaffolds into critical bone defects in rabbits, an accelerated bone healing process was achieved due to enhanced osteogenic and angiogenic activities[61].

Possible mechanisms of strontium for bone regeneration

It has been elucidated that strontium can stimulate bone formation in a dual regulation pattern by increasing osteoprotegerin (OPG) production and down-regulating RANKL expression in osteoblasts through the calcium-sensing receptor (CaSR)[60,62-64]. Moreover, OPG can exert inhibitory activity on RANKL-induced osteoclast differentiation by acting as a decoy receptor for RANKL[54]. Thus, this change in the OPG/RANKL system can impair osteoclast differentiation and weaken bone resorption. Figure 4 shows a schematic of the possible molecular mechanisms of strontium[65]. However, further research is required to determine how strontium enters the cells. Research is also required to determine the influence of intracellular strontium levels on cell signalling.

Copper

Physiological effect of copper

Copper (Cu^{2+}) is an essential trace element that is most abundant in the liver. Normally, copper is one of the most important ions for humans as a cofactor and an important component of numerous enzymes. It is well known that copper is required for various metabolic processes such as electron-transfer reactions and oxygen and metallic ion transportation[66,67]. Copper has a major effect on bone integrity. Studies have shown that copper deficiencies resulted in decreased mechanical strength and mineralisation of bone, probably due to the decrease in collagen crosslinking and lysyl oxidase activity[68]. Copper enhances angiogenesis through the up-regulation of VEGF[69]. Therefore, there is a growing interest in copper for bone regeneration due to its osteoinductive properties and stimulatory effects on angiogenesis, which is a vital factor for tissue ingrowth[70,71].

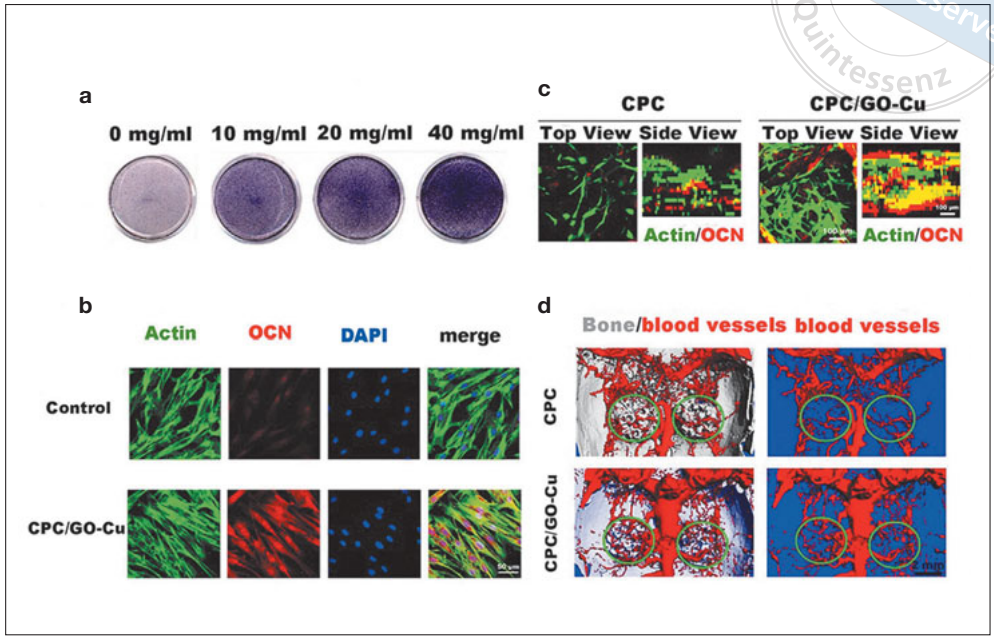

Fig 5 Calcium phosphate (CaP) scaffolds with graphene oxide-copper (GO-Cu) coatings stimulate vascularised bone regeneration (adapted from Zhang et al[76]). (a) ALP staining of BMSCs after being cultured with a different concentration of GO-Cu materials for 3 days. (b) After incubation with 40 μg/mL GO-Cu materials for 7 days, the expression of osteocalcin (OCN) in rat BMSCs was detected by immunofluorescence. (c) Three-dimensional (3D) reconstruction of confocal images of OCN immunofluorescence staining. (d) Micro-CT analysis of bone regeneration and revascularisation. Green circles indicate bone defect regions.

Applications of copper in bone tissue engineering

Copper supplementation has been used to improve the antibacterial activity and angiogenesis of biomaterials[72-75]. Wang et al[71] found that the addition of 0.5% to 3.0% copper (w/w) to BGs had no cytotoxic effects on human bone marrow stem cells (hBMSCs). The ALP activity of hBMSCs increased with increasing BG copper content. A micro-computed tomography (micro-CT) evaluation and histological analysis revealed that BGs doped with 3.0% cupric oxide (CuO) (w/w) showed an improved ability to promote angiogenesis and osteogenesis in rat cranial defects compared with BGs without CuO. Moreover, the incorporation of reduced graphene oxide and copper (0.6% [w/w]) significantly promoted the antibacterial activity of Poly (ε-caprolactone) scaffolds[70]. Our previous study also proved that enhanced vascularised bone regeneration could be achieved by incorporating copper into scaffolds. As shown in Figure 5, the combination of copper and other bioactive materials such as graphene oxide may have synergistic effects on osteogenesis and angiogenesis[76]. Furthermore, copper was reported to promote the chondrogenic differentiation of stem cells. Another study showed that, compared with pure chondrogenic medium, chondrogenic medium supplemented with 100 μM Cu^{2+} significantly increased glycosaminoglycan deposition and the expression of chondrogenic genes in MSCs *in vitro*. Researchers then added alginate powder to 10 ml 100 μM $CuSO_4$ solution or pure water as a control. Mixtures were processed by freeze drying to fabricate scaffolds. *In vivo* experiments showed that Cu-containing MSC-laden alginate scaffolds were more effective than pure MSC-laden alginate scaffolds in terms of cartilage regeneration[77].

Possible mechanisms of copper for bone regeneration

Copper is antibacterial because it can destroy the cytoderm of bacteria and generate reactive oxygen species (ROS), which induce oxidative damage to DNA[78,79]. Studies have revealed that copper could regulate the activation of hypoxia-inducible factor 1 (HIF-1)-α, an extensively expressed transcriptional factor regulating the oxygen homeostasis[80,81]. Activated HIF1-α promotes the expression of downstream gene production involving angiogenic factors such as VEGF and FGF-2, thus stimulating neovascularisation[69].

Zinc

Role of zinc in bone

Similar to copper, zinc (Zn^{2+}) is another essential trace element involved in numerous physiological processes in the human body. Notably, zinc is relatively abundant in bone, and the majority of zinc in the body is stored in bone tissue. Zinc is vital for the development and maintenance of healthy bones and has a similar effect

to strontium in the formation and resorption of bones[82]. Thus, zinc has been implemented into biocompatible scaffolds in dental and orthopaedic treatments.

Applications of zinc in bone tissue engineering

Similar to magnesium, zinc and its alloys have similar mechanical properties to those of natural bone, which makes them a promising implantation material for clinical applications. It has been found that, through the incorporation of zinc, implants showed a significant improvement in osteogenesis both *in vitro* and *in vivo*. For example, to investigate the effects of zinc on osteogenesis, Luo et al[83] fabricated TCP scaffolds with various concentrations of zinc (0 to 45 mmol $ZnCl_2$/100 g TCP powder). *In vitro* experiments showed that hBMSCs cultured on the TZ45 scaffolds (45 mmol $ZnCl_2$/100 g TCP powder) exhibited higher proliferation and osteogenic differentiation than those on unmodified TCP scaffolds. Subsequent *in vivo* experiments displayed that *de novo* bone formation increased with an increasing zinc content to TCP ratio at 12 weeks after implantation of the Zn-TCP scaffolds in the paraspinal muscles of canines. Additional evidence has suggested that zinc may affect the bioactivity of cells dose-dependently. Zinc ions released from scaffolds promoted cell adhesion as well as proliferation at concentrations of 1.1 ppm, while a concentration of 2.7 ppm zinc in the culture medium had negative effects on cell proliferation[84]. Furthermore, because of advances in manufacturing technologies such as 3D printing, more efficient zinc loading is possible to facilitate bone regeneration[85]. Furthermore, the release of zinc ions from bone substitutes can exert antimicrobial activity as well as suppress inflammation[83,86,87].

Possible mechanisms of zinc for bone regeneration

Zinc is a pivotal component and regulator of several enzymes. APL is one factor that is of great importance to the maturation of bone. ALP creates an alkaline environment that benefits the precipitation and subsequent mineralisation of phosphates, and therefore accelerates the process of bone maturation[10]. Zinc ion has the ability to kill bacteria by neutralising the bacterial surface and generating electron holes on the cell membrane[88]. Moreover, it was shown that zinc sulfate at concentrations between 10 and 250 μM significantly suppressed RANKL-induced nuclear factor-κB (NF-κB) activity in RAW 264.7 cells, a signalling pathway that is necessary for osteoclastogenesis but suppresses osteogenesis. In addition, concentrations of zinc sulfate ranging from 10 to 100 μM enhanced the mineralisation of MC3T3 cells *in vitro*[89]. These findings suggest that zinc could stimulate bone formation through stimulating osteogenesis and simultaneously inhibiting osteoclastogenesis.

Lithium

Lithium is a non-essential element that is widely used in the treatment of psychological disorders[90-92]. Interestingly, lithium administration may result in hyperparathyroidism and hypercalcemia, which are highly related to bone metabolism[93,94]. Therefore, lithium has received much attention as a potential bone substitute. The correlation between lithium and bone formation might result from its inhibitory effects on glycogen synthase kinase-3 (GSK-3). Previous studies have reported that lithium could replace magnesium ions in GSK-3, which may impair the phosphoryl transfer mechanism and disrupt

Table 1 Summary of bioactive ions released from scaffolds and their effects on bone regeneration.

Bioactive ions	Osteogenic effects	Angiogenic effects	References
Calcium	Yes	-	31, 33, 34
Magnesium	Yes	Yes	24–50
Copper	Yes	Yes	69–81
Zinc	Yes	-	83, 85, 87
Strontium	Yes	Yes	54–65
Lithium	Yes	-	98–100
Boron	Yes	-	101
Cobalt	Yes	Yes	61, 104
Fluoride	Yes	-	103
Niobium	Yes	-	102

the combination of GKS-3 with pre-phosphorylated substrates[95]. The inhibition of GSK-3 facilitates the activation of the Wnt/β-catenin signalling pathway that is stimulated by differentiation inducers, promoting osteoblast proliferation and differentiation[96]. A further *in vivo* study also revealed that oral administration of lithium chloride (LiCl) increased the bone volume of mice with femoral distal metaphysis[97]. Zhu et al[98] confirmed that human MSCs treated with 5 mM lithium proliferated more rapidly *in vitro* than untreated cells, and the results of flow cytometry showed that the proportion of cells in the S phase was significantly elevated in lithium-treated groups. These results suggest that lithium may be applied to strengthen the efficacy of MSC transplantation therapy. Arioka et al[99] further examined the effects of the local administration of lithium on bone formation *in vivo*. A Matrigel basement membrane matrix with or without 10 mM Li_2CO_3 was placed in the tibial defects of rats. Subsequent imaging and histological analysis showed that Li_2CO_3 accelerated bone regeneration after 14 days of implantation. Based on these findings, applications of lithium in bone tissue engineering using new technologies such as 3D printing have been developed in recent years[100]. Notably, lithium is a fairly new biomaterial used in bone regeneration and its exact mechanisms of action need to be identified.

Summary

Bioactive ions are of great importance for numerous physiological reactions during life, and each one of them plays a pivotal role in the formation and maintenance of healthy bone tissue. The incorporation of bioactive ions rather than growth factors into bone substitutes is an alternative, cheaper and more efficient way to enhance osteoinductive ability. Table 1 summarises the effects of bioactive ions released from scaffolds on bone regeneration.

In this article, we have highlighted the uses of several bioactive ions in bone regeneration and provided insight into how they may affect bone formation. Notably, in addition to the ions discussed here, there are quite a few ions such as boron, fluoride, niobium, cobalt and calcium that are reported to possess osteogenic and angiogenic abilities[103-104]. Nevertheless, there are several problems that need to be solved before the utilisation of bioactive ions can progress from the laboratory to the clinic, including how to control the release of ions of biomaterials, how to minimise the side effects of high local ion concentrations as well as the specific mechanisms of these ions on bone regeneration. These issues highlight the urgent need for further studies on the applications of bioactive ions for bone regeneration.

Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Dr Si Han LIN collected the literature and prepared the manuscript; Prof Xin Quan JIANG and Dr Wen Jie ZHANG designed and revised the manuscript.

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