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Review article

Overview of methods for enhancing bone regeneration in distraction osteogenesis: Potential roles of biometals

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ABSTRACT

Background: Distraction osteogenesis (DO) is a functional tissue engineering approach that applies gradual mechanical traction on the bone tissues after osteotomy to stimulate bone regeneration. However, DO still has disadvantages that limit its clinical use, including long treatment duration
Methods: Review the current methods of promoting bone formation and consolidation in DO with particular interest on biometal.
Results: Numerous approaches, including physical therapy, gene therapy, growth factor-based therapy, stem-cell-based therapy, and improved distraction devices, have been explored to reduce the DO treatment duration with some success. Nevertheless, no approach to date is widely accepted in clinical practice due to various reasons, such as high expense, short biologic half-life, and lack of effective delivery methods. Biometals, including calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), and cobalt (Co) have attracted attention in bone regeneration attributed to their biodegradability and bioactive components released during in vivo degradation.
Conclusion: This review summarizes the current therapies accelerating bone formation in DO and the beneficial role of biometals in bone regeneration, particularly focusing on the use of biometal Mg and its alloy in promoting bone formation in DO. Translational potential: The potential clinical applications using Mg-based devices to accelerate DO are promising. Mg stimulates expression of multiple intrinsic biological factors and the development of Mg as an implantable component in DO may be used to argument bone formation and consolidation in DO.

1. Overview of DO in bone regeneration

Hippocrates was the first to propose the placement of traction forces to aid in bone healing more than 2000 years ago [1]. In the modern era, it was Codivilla who first applied bone elongation techniques in 1905, publishing a case report of a femoral distraction osteogenesis [2]. Since being developed in the 1950s, the Ilizarov transportation technique—also known as "DO technique"—has become an important technique in the fields of oral, maxillofacial, and orthopedic surgeries [3–6].

The mechanism of DO, which was reported through tension stress, has attracted great attention in both research and clinical domains since the 1950s [3,7]. It is reported that both appropriate mechanical stimuli and adequate angiogenesis are required for successful bone formation during DO [8–10]. However, overly rapid distraction could cause localized ischemia, thereby inhibiting bone formation. It has also been demonstrated that new bone formation is closely linked to angiogenesis during DO as well [11,12].

The procedure of DO is composed of three sequential phases including latency, distraction, and consolidation [2]. In the latency phase, bone segments are fixed for 5–7 days after the osteotomy, as suggested by Ilizarov [4]. The expression of interleukin 1 (IL-1), interleukin 6 (IL-6), bone morphogenic protein-2 (BMP-2), and bone

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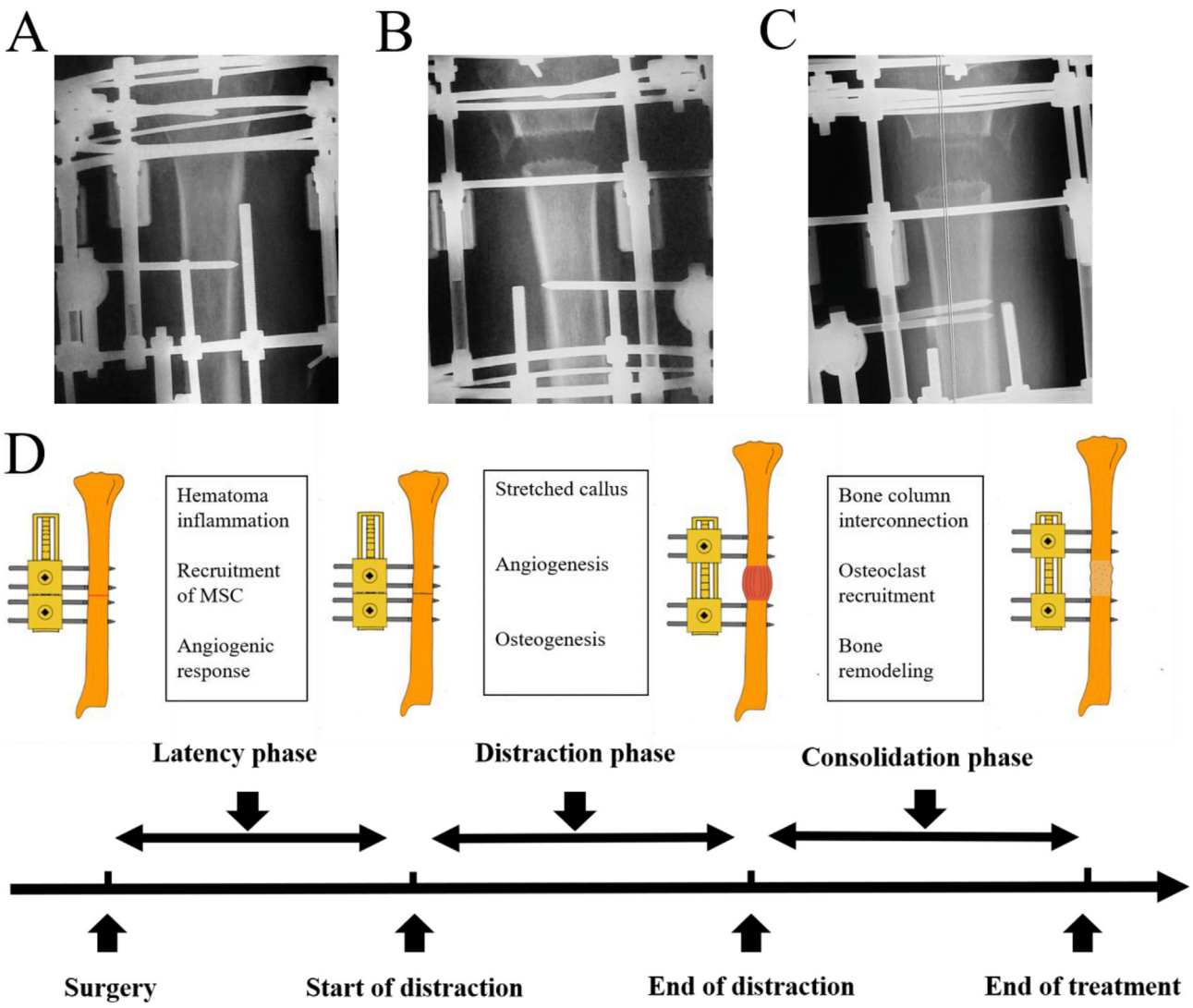

Figure 1. The process of distraction osteogenesis (A) Latency phase (B) Distraction phase (C) Consolidation phase (D) Schematic diagram of the distraction process. The biological processes of latency phase include hematoma inflammation, recruitment of mesenchymal stem cells and angiogenesis response. The biological processes of distraction phase include callus formation, angiogenesis and osteogenesis. The biological processes of consolidation phase include bone formation, osteoclast recruitment and bone remodeling.

morphogenic protein-4 (BMP-4) are up-regulated during the latency phase and subsequently return to baseline [13]. Then, distraction is performed at a controlled rate (1.0–1.5 mm/day) and frequency (2–4 times/day) until the desired lengthening is obtained [4]. During the distraction phase, the expression of interleukin 6 (IL-6) is up-regulated again, which plays a role in intramembranous ossification by promoting the differentiation of cells into the osteoblastic lineage [13,14]. The receptor activator of nuclear factor-kb ligand/osteoprotegerin (RANKL/OPG) ratio remains high during the early distraction phase, helping facilitate the resorption of the newly formed mineralized cartilage during the latency phase [13]. The expression of bone morphogenic protein-6 (BMP-6) is high during the early stage of the distraction phase [13]. In response to distraction tension, the expression of BMP-2, BMP-4, and TGF-β peak in this phase to stimulate new bone formation [15]. The direct effect of mechanical tension in enhancing osteoblast activity and promoting osteoblastic differentiation of bone marrow mesenchymal stem cells (BMSCs) has been demonstrated in various studies [16,17]. Additionally, it is also reported that the tension caused by distraction could upregulate the expression of neurotrophic (nerve growth factor, brain-derived neurotrophic factor, and neurotrophic-3) and their receptors (tropomyosin-related kinases A, B, and C) to enhance osteogenesis during DO [18], suggesting the importance of tension-induced neural response during DO. The expression of IGF-1 and β-fibroblast growth factor (βFGF) are also increased during this phase [13]. In addition, vascular endothelial growth factor (VEGF) and angiopoietin-1 and -2 expression are up-regulated, stimulating new vessel formation and enhancing the plasticity of existing larger vessels [15]. The consolidation phase usually takes half to one year or longer to accomplish and contains a long period of immobilization as the distracted callus becomes mature with the mechanical support from the fixation device, keeping the callus stable and preventing cartilaginous formation in-between. During the consolidation phase, bone remodeling starts by allowing the formation of lamellar bone with bone marrow elements to help form a better remodeling structure, which can provide mechanical support over a long period of time [2]. The biological processes involved in the consolidation phase consist of bone columns interconnecting, osteoclast recruitment, and bone remodeling [13]. The expression of BMP-2, BMP-4, and βFGF gradually decreases in the consolidation phase [13]. Toward the end of the consolidation phase, the expression TNF-α is significantly increased, suggesting that it plays an important role in bone consolidation (Fig. 1) [13].

Table 1
Advantages and disadvantages of DO in bone regeneration.

Authors	Advantages	Disadvantages
Aronson et al. (1997)	Induction of local bone formation with a minimally invasive procedure	Inflammation surrounding the pin track caused by mechanical or thermal damage, cellulitis, abscess, or local osteomyelitis
Nakase et al. (2009) [21]	Stimulating correction of coronal, sagittal, and rotational defects with shortening in the lower limbs	Complications were as follows: superficial pin tract infection, deep infection, and transient decrease of range of motion of the nearby joint
Barakat et al. (2010) [22]	Enhancing the regeneration of soft tissues such as skin, muscle, tendon and neurovascular structures	Complications included pin tract infection
Borzunov et al. (2012) [24]	Bone loss is compensated for by distraction regeneration and results in consolidation at the docking site of the transported bone fragment	Requires several stages and takes a long time in cases of extensive bone defects
Kempton et al. (2014) [20]	Restoring length after digital amputations and relatively technically easy with no donor-site morbidity	Long duration of treatment and high complication rates
Suzanne et al. (2020) [25]	The application of force over time for the generation of all tissues: skin, muscle, nerves, blood vessels and bone	Force-related complications including misshaped regenerate, tipping of the regenerate and open bite
Dogra et al. (2020) [26]	Correcting the gross mandibular asymmetry	Scar formation and requirement of frequent patient follow up

2. The advantages and disadvantages of DO in bone regeneration

In comparison to other methods, the DO technique has several advantages (Table 1). The main advantage of DO is to induce endogenous bone formation [19,20]. Kempton et al. [20] reported that DO offered succinct advantages in restoring length and was relatively easier to handle. According to the report by Nakase et al. [21], DO technique can correct deformities in coronal, sagittal, and rotational planes with shortening in the lower limbs which is corrective in a variety of skeletal disorders. More importantly, the DO technique can stimulate regeneration of surrounding soft tissues such as skin, muscle, tendon, and neurovascular structures at the same time of bone formation, where other methods can hardly achieve [22]. DO technique has 3–5 times decreased primary disability for the treatment of post-traumatic non-unions compared to other treatments, which means more patients can return to work sooner, hence leading to beneficial social and economic impacts [23].

Despite the advantage of the DO technique, there are still challenges that need to be solved to make the DO technique more accessible (Table 1). The DO technique usually needs lengthy treatment duration which can lead to high complication rates [20,24]. Complications of DO include: risks of infection (5% overall) including superficial pin, tract, and deep infections; transient decreasing range of motion of the nearby joint; premature or delayed consolidation, non-union, delayed union, axial deviation, late twisting, or fracture; and failure for the bone to grow in the desired direction [11]. The pin track inflammation is commonly caused by mechanical stimuli, thermal damage, cellulitis, abscess, or local osteomyelitis [11]. In addition, joint complications may cause joint mobility to be lost temporarily or permanently [11].

3. Enhancement of bone formation in DO

To reduce the complications of DO, extensive research over the past two decades has focused on improving surgical technique, fixator and distraction devices, physical stimulation, and the use of biological agents.

Improvement in surgical technique, fixator and distraction devices, and physical stimulation.

Developments in surgical technique, as well as fixator and distraction devices continue in DO, and these developments are summarized in Table 2.

Intramedullary nailing: Lengthening over intramedullary nailing (IMN) can give several benefits including reduction of the duration of external fixation time, prevention of refracture, and earlier rehabilitation [27]. It was also reported that adjuvant IMN or lengthen-and-then-nailing (LATN) can allow early removal of the external fixator, gaining popularity in adults for cosmetic surgery, limb

Table 2
Methods to accelerate bone formation in DO.

Methods	Authors	Methods	Clinical study/ Models	Main conclusion	Disadvantages/limitations
Intramedullary nailing	Jager et al. [29]; Popkov et al. [30]	Elastic stable intramedullary nailing (ESIN)	Clinical study	Reducing external fixator wearing time	Risk of deep intramedullary infection
	Gubin et al. [3] ; Lan et al. [28]	Intramedullary nailing (IMN)	Clinical study	Allowing early removal of the external fixator	Risk of deep intramedullary infection
External fixation pin coating	Caja et al. [63];	Hydroxyapatite (HA) coating	Clinical study	Reducing pin loosening	No influence on infection and malunion
Automated continuous devices	Kessler P et al. [36]	Motor-driven hydraulic pump	Pigs	Speeding regeneration	Economic burden, inflexibility
Physical stimulation	Chan et al. [39]	Low-intensity pulsed ultrasound (LIPUS)	Rabbits	Increasing endochondral formation	Economic burden
	Miloro et al. [40]	Low-level laser (LLL)	Rabbits	Enhancing new bone formation	Unknown mechanism and efficiency
	Hagiwara et al. [42]	Electrical stimulation (ES)	Rabbits	Enhancing new bone formation	Unknown mechanism and efficiency
Gene therapy	Sun et al. [53]	Micro-RNA-503	Rats	Promoting bone formation	Safety issues
	Ashinoff et al. [48]	Adenoviral-mediated delivery of BMP-2	Rats	Improving bone deposition	Safety issues
Cytokine-based therapy	Zhao et al. [55]	Osteogenic growth peptide (OGP)	Rabbits	Promoting the new bone formation	Short biologic half-life
	Sailhan et al. [54]	Bone Morphogenic Protein-2 (BMP-2)	Rabbits	Enhancing consolidation	High expense
Stem-cell based therapy	Yang et al. [52]	Transplantation of allogeneic MSCs	Rats	Significantly increased bone volume fraction	Lack of efficient delivery methods

Table 3
The biological functions of biometals and their proposed role in bone regeneration [94,95].

Biometal	Body content	Blood content	Biological functions	Signaling molecules and their proposed role in bone regeneration
Ca	1.0–1.5 Kg [96]	8.8–10.4 mg/dL [96]	Enzyme co-factor, maintaining skeletal framework, signaling molecule [64]	Enhances the effects of BMP-2 on *Osteocalcin*, *Runx2* and *Osteria* expression via SMAD signaling (7.5 mM Ca^{2+}) [97]
Mg	24–25 g [98,99]	1.5–2.5 mg/dL [99]	Enzyme co-factor, composition of chlorophylls [94]	Stimulates CGRP-mediated osteogenic differentiation of stem cells (Mg rod) [100]; Promotes angiogenesis and prevents vessel leakage (10 mM Mg^{2+}) [101]; Inhibits osteoclast differentiation (10 mM Mg^{2+}) through regulating Ca^{2+} signal [101];
Zn	2–3 g [81]	6.3 mg/L [102]	Enzyme co-factor (nucleic-acids polymerases), involved in cell division [94]	Stimulates osteoblast bone formation (15 uM Zn^{2+}) [83]; Inhibits osteoclast differentiation [84]; Increases alkaline phosphatase activity (1.0 mg Zn^{2+}/100 g body weight) [85];
Cu	80–120 mg [81]	0.8–1.6 mg/L [102]	Transportation of oxygen, redox reactions [94]	Promotes angiogenesis, osteostimulation and antibacterial activity of bioactive glass (5%) [88];
Mn	12 mg [103]	4-15 ug/L [104]	Enzyme co-factor (superoxide dismutase, pyruvate kinase), metabolism of fats [94]	Accelerates fracture healing in a rat model (0.125 mg/kg) [90]; Enhances osteogenesis (0.55%) [105];
Co	3 mg [103]	0.39 ug/L [102]	Hematopoiesis (vitamin B12) [94]	Upregulates anti-inflammatory, osteogenic, and proangiogenic factors (1 ppm) [91]

length discrepancy, and sequelae of poliomyelitis [3,28]. Jager et al. [29] reported that elastic stable intramedullary nailing (ESIN) can reduce external fixator wearing time with no additional complications. However, many problems that limit the clinical application of intramedullary nailing still exist including the risk of deep intramedullary infection and blockade of the ideal positions of pins for external fixator [30].

External fixation pin coating: The application of pin coatings such as hydroxyapatite (HA), titanium, and silver to enhance fixation and reduce infection has been studied for many years [31,32]. HA, constituting 65% of the human bone mineral component, is widely accepted as a bone substitute and prosthetic coating [31]. Piza et al. reported that HA-coating could reduce pin loosening, while no significant difference was found in the infection rate between groups with or without HA-coating [31,33]. Pieske et al. demonstrated that HA-coating improved bone fixation with no difference in rates of infection [34]. Therefore, the antimicrobial properties of the coatings need to be further improved in future studies.

Automated continuous devices: Novel distraction devices that can lengthen automatically and continuously have been developed. The automated distraction is of clinical significance since it can eliminate the need for patient compliance and diminish the frequency of postoperative care [2]. There are three types of automated devices for DO: hydraulic power, motor-driven, and spring-mediated devices [35]. Continuous distraction may be carried out at a rate of up to 2 mm per day, with relatively good bone quality [36]. Despite promising results, automated devices are quite expensive and not widely accepted for routine clinical use [35]. Problems with currently available automated devices include risk of infection, device breakage, economic burden, inflexibility for adjustment during the treatment, limited range of lengthening, and the need for multiple surgical procedures [35]. However, further development of automated devices continues to improve their reliability, adjustability, and affordability.

Physical stimulation: Physical therapies including low-intensity pulsed ultrasound (LIPUS) [37–39], low-level laser (LLL) [40,41], electrical stimulation (ES) [42,43], and pulsed electromagnetic field stimulation [44,45] had been investigated to accelerate bone formation in the consolidation phase during DO. Shimazaki et al. reported the positive effects of the low-intensity ultrasound during DO in the rabbit model, which may stimulate osteoblastic cells to synthesize extracellular matrix [37]. Moreover, it was reported that LIPUS could increase endochondral formation in a dose-dependent manner during rapid DO [39]. Miloro et al. applied the low-level laser (820 nm) with an output of 400 mW of 6.0 J × 6 transmucosal sites in a rabbit DO model [40]. Radiographical and histological results showed that new bone formation and ossification were more obvious for the low-level laser-treated group [40]. Hübler et al. also reported improved bone structure using low-level laser on the newly formed bone [41]. Hagiwara et al. applied electrical current stimulation (10 A) to two of the screws used as electrodes during DO and observed an increased bone formation at 10 and 20 days after distraction [42]. The beneficial effect of electrical stimulation might be attributed to the decreased partial pressure of oxygen, increased pH, and increased vascularization which are all favorable for new bone formation [42,46]. A pulsed electromagnetic field with 75 Hz frequency was applied by Felipe et al. at 10 days after surgery for 8 h/day to promote bone formation and consolidation [45]. Li et al. reported that the pulsed electromagnetic field could reduce the healing time of regenerated bone in a rabbit DO model [44]. However, the mechanisms and efficiency of the physical stimulation methods in accelerating DO bone formation still need further elucidation.

4. Biological agents

Multiple studies applied biological agents to enhance DO (Table 2). These included gene therapy [47–49], cytokine-based therapy [50], and stem-cell-based therapy [51,52].

Gene therapy: Local gene therapies of bone morphogenic protein (BMP) have been reported to induce sustained and relatively high levels of BMP production at regenerates during DO. Local adenoviral-mediated delivery of BMP-2 could improve bone consolidation [48]. BMP-7-mediated *ex vivo* gene transfer based on MSCs promoted callus formation and bone consolidation during DO [49]. Sun et al. reported that Micro-RNA-503 could promote bone formation in DO through suppressing Smurf1 expression in a rat DO model [53]. Despite many promising results of gene therapy, safety issues and selection of optimal dose, timing, and delivery methods still require further investigation [48,50].

Growth factor-based therapy: Local or systematic administration of growth factors have been reported to promote bone formation including BMP [54], osteogenic growth peptide (OGP) [55], platelet-derived growth factor (PDGF) [9], VEGF [9], nerve growth factor (NGFβ), and calcitonin gene-related peptide (CGRP). Among these growth factors, BMP plays the most important role in bone healing through regulating osteogenic differentiation of MSCs and synergistic effects with VEGF signaling [2]. Local application of BMP-2 in the distraction phase effectively enhances consolidation in DO [54]. Intravenous systemic application of OGP enhances bone formation in a rabbit DO model [55]. VEGF, PDGF, and angiopoietins are important for new blood vessel formation in the distraction regenerate during DO [9]. Apart from applying angiogenic and osteogenic factors to accelerate bone formation in DO, other factors such as NGFβ had also been applied to shorten the consolidation phase in DO [56]. The local injection of neuropeptide CGRP accelerated DO bone formation via the enhancement of angiogenesis [57]. Despite many beneficial results of growth factor therapies, their application in clinical practice is limited by high expense, short biologic half-life, and

the lack of efficient delivery methods [48,50].

Stem-cell based therapy: With capacity in producing regenerative cytokines, differentiating into different cell types of the tissue or organ and self-renewal, mesenchymal stem cells (MSCs) are applied to enhance bone formation in DO [52,58–62]. Studies have demonstrated that applying autologous or allogeneic MSCs to the distraction regenerates shortens the treatment time of DO [51,52,60]. The selection of a cost-effective treatment protocol, a suitable cell type and the development of apposite carrier materials for the delivery of cells, still need further examination [51].

5. Overview of biometals in bone regeneration

Biometals—including Ca, Mg, Zn, Cu, Mn, and Co—are termed as metals that have a biological function [64]. The biological functions of these biometals and their proposed roles in bone regeneration are summarized in Table 3.

Calcium is the main component of human bones and teeth, and 99% of which is present as HA [$Ca_{10}(PO_4)_6(OH)_2$] crystals [65]. Calcium acts as co-factor of enzyme and plays an important role in maintaining skeletal framework [64]. It is reported that Calcium can improve the effects of BMP-2 on *Osteocalcin*, *Runx2* and *Osteria* expression through SMAD signaling. Compared with the limited use of Calcium-based alloys, Calcium phosphate is more widely used in bone regeneration applications because of its osteoconductive and osteoinductive properties. The poor strength and fatigue resistance of Calcium phosphate-based biomaterials makes them unsuitable for load-bearing parts of the human body. However, the surface functionalization of metallic implants with HA coatings shows promise in improving the performance of bone implants [63,66–68].

Mg is one of the major mineral components of bone matrix, and 53% of body Mg is stored in bone [69]. As the second most abundant intracellular cation, 95% of Mg in cells are bound to negatively charged molecules such as ribosomes, plasma membrane phospholipids, and adenosine triphosphate (ATP) [70]. Mg ion (Mg^{2+}) functions as a cofactor of more than 300 enzymes and their activities all exhibit a similar bell-shaped curve for dependence on Mg^{2+} [70]. The degradation products of Mg-based implants include Mg^{2+} [71–74], hydrogen [75,76], and elevated local pH [77–79] which have all been demonstrated to promote osteogenesis and angiogenesis. The Mg^{2+} generated during Mg metal degradation can stimulate the osteogenic differentiation of stem cells and enhance the migration of endothelial cells, ultimately inducing osteogenesis and angiogenesis in many *in vitro* studies [71–74]. Hydrogen therapy can decrease the volume of infarction and suppress injuries caused by ischemia via reducing oxidative stress [75,76]. The alkaline environment has been recognized to increase osteoblastic mineral deposition and suppress osteoclastic activities, suggesting the therapeutic value of elevated local pH in bone regeneration [77–79].

Zn is an essential mineral for the growth and development of mammals [80], and around 2–3 g of Zn in the human body forms an integral part of more than three hundred important enzymes, including enzymes that are involved in regulating gene expression [64,81]. Recently, biodegradable Zn has been developed into novel alloy systems with outstanding mechanical strength [82]. Zn and its alloys exhibit distinct advantages in promoting bone regeneration due to their capacity to stimulate osteoblast bone formation, increase alkaline phosphatase activity, and inhibit osteoclast differentiation [82–85]. However, the underlying mechanism of Zn action in these activities has not been elucidated.

Cu is an important element for maintaining normal health and for survival, and there is around 80–120 mg Cu in the human body [64]. Cu functions as a cofactor for enzymes involved in regulating many physiological processes, including maintaining energy production in the human body [64]. Cu^{2+} can stimulate the proliferation of endothelial cells [86], enhance the activity and proliferation of osteoblasts [87], and promote the osteostimulation and antibacterial activities of bioactive glass [88]. Therefore, Cu shows great potential in enhancing bone regeneration.

Mn is an important mineral, which is required for the development of brain and nervous tissues [64]. Mn is an important cofactor for enzymes that regulate carbohydrate and fat metabolism, and promote the synthesis of sex hormones [64]. Mn is associated with the maintenance of bone structure and in regulating bone metabolism [89]. The addition of Mn can significantly enhance osteogenesis. Additionally, it has been shown that local treatment of Mn^{2+} can accelerate fracture healing in a rat model via amplifying early angiogenesis [90]. Therefore, the local administration of Mn^{2+} is a potential therapeutic method for bone regeneration.

Co is an important element that forms an integral part of vitamin B12 (cyanocobalamin) and is involved in the formation of hemoglobin [64]. However, the inconsistent effect of cobalt, possibly attributable to differences in tested concentrations, has produced controversy regarding the application of Co-based biomaterials in bone regeneration [91]. The 1 ppm concentration of cobalt is reported to have optimal bone regeneration outcomes via upregulating anti-inflammatory, pro-osteogenic, and pro-angiogenic factors [91]. Hence, the application of Co-based alloys for the development of cost-effective lengthening devices to reduce the treatment duration of DO shows great potential.

The biometals Mg, Zn, Cu, Mn, and Co have all been shown to have beneficial effects in bone regeneration. Among the above biometals, Mg and its alloys have been intensively investigated in recent years as a new class of biodegradable materials due to their suitable mechanical properties and low mass density, as well as angiogenic and osteogenic properties [71–74,92,93]. Therefore, we discuss the advantages and disadvantages of Mg in bone regeneration and the potential of using biodegradable Mg to shorten the treatment duration of DO.

Table 4
Advantages and disadvantages of using Mg to enhance bone regeneration.

Advantage	Disadvantage
Desirable mechanical strength Osteogenic ability Angiogenic ability Anti-microbial activity High biocompatibility Degradability in the biological environment, thereby avoiding the economic cost and risk of physical or psychological complications from a second surgery.	If degradation rate is too fast, then it may lead to loss of mechanical strength of the implant for intended long-term bone regeneration applications; Localized alkaline environment during degradation; Gas formation and accumulation due to the rapid degradation process, leading to the displacement of surrounding tissues and a decrease in the implant-bone contact area

6. Advantages and disadvantages of Mg in bone regeneration

The advantages of Mg in bone regeneration have been indicated in several prior studies [106–112] (Table 4). Mg has attracted great attention for bone repair because of its suitable mechanical strength [113], the capacity of promoting osteogenesis [100], angiogenesis [114], degradability [115], and antimicrobial potential [116]. The anti-microbial property is especially useful for DO implications that are associated with infection [116,117]. The degradation characteristic of Mg in the biological environment may also avoid a second surgery for implant removal [111]. Additional advantages include the low density of the biodegradable Mg alloy and the observation that its degradation products (Mg ions, hydrogen and elevated local pH value) increased expression of multiple endogenous biological agents continuously, resolving the problem of inefficient delivery methods for growth factor therapy, therefore making it a good candidate for accelerating DO [93,117].

There are still a number of challenges that have restricted Mg implants as a suitable material in bone tissue engineering, most notably the rapid *in vivo* degradation rate and alkaline degradation products, which trigger an acute and unfavorable excessive inflammatory response [118].

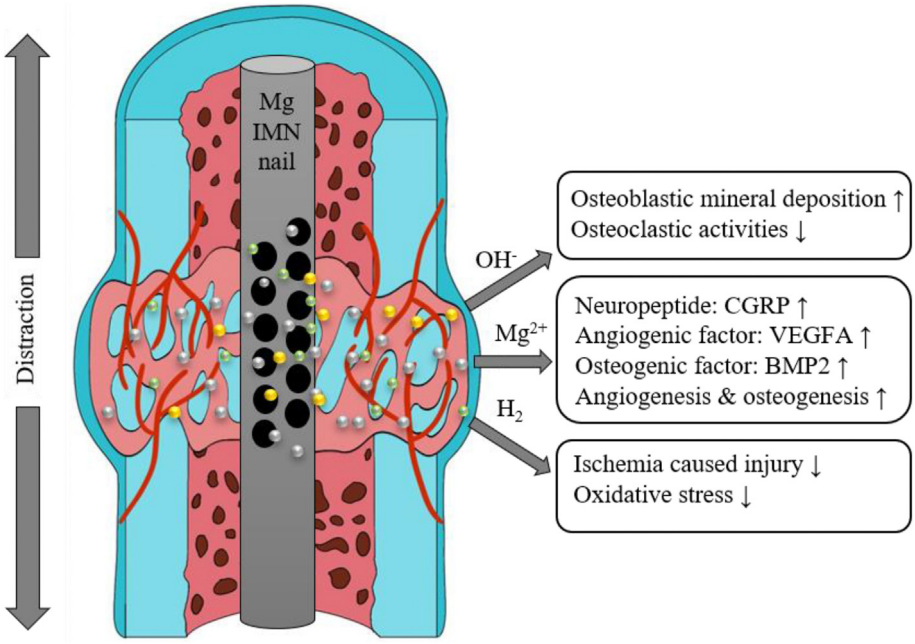

Figure 2. Diagram illustrating the potential application of Mg-based IMN to accelerate DO.

Besides that, extensive gas formation due to the rapid degradation process could lead to displacement of the surrounding tissues and a decrease in the implant-bone contact area, eventually hampering bone regeneration [119]. These disadvantages may be overcome by changing the composition, microscopic structure, grain size, texture orientation, and incorporating a protective coating [107].

The potential of using biodegradable Mg to promote bone formation in DO.

The problems of Mg-based implants could be solved by using alloying, coating, or high-purification technologies to provide higher corrosion resistance, suitable mechanical properties and various biofunctions [118].

Alloy design of Mg has been investigated for dozens of years with a focus on biodegradability, as well as desirable mechanical and osteopromotive properties. So far, two Mg alloys have proven effective in humans. A Mg alloy ((MgYREZr)) screw, developed by Waizy et al. is comparable to the titanium one in treating hallux valgus abnormalities [120]. Lee et al. have developed a Mg–Ca–Zn alloy implant, and they found that the implant could facilitate early bone healing and could be completely replaced by new bone within one year of implantation [121]. As one of the most popular magnesium alloys with aluminum, biodegradable AZ31 Mg alloy provides a low mass density and good mechanical properties. It has been investigated as an external device by Wang et al. in a mandibular DO canine model and the results suggest that AZ31 Mg alloy is equivalent to the stainless steel device in terms of fixation stability [93]. The alloy shows a certain degradation rate in the mandible and does not have a negative effect on the kidney or liver [93]. However, the efficiency of the AZ31 Mg alloy in reducing the DO treatment period has not been explored.

With the development of metallurgy technology, high-purity Mg (99.99%) has been developed to improve corrosion resistance during *in vivo* application [118]. A 3D-printed pure Mg incorporated scaffold has been developed for bone defect repair, showing good osteogenesis, angiogenesis, and suitable mechanical properties while simultaneously upregulating the expression VEGFA and BMP2 [114]. It is reported that intramedullary nailing can reduce external fixator wearing time with no additional complications [27]. Mg and its alloys may also be designed as part of the intramedullary nail (hybrid device) for promoting bone consolidation when exchanging the external fixator. An innovative, pure Mg-containing intramedullary nail has been developed to promote fracture repair in an ovariectomy-induced osteoporosis rat model via upregulating the release of CGRP by Zhang et al. in *Nature Medicine* [100]. CGRP can also promote angiogenesis by promoting endothelial cell migration and tube formation [122]. Moreover, CGRP has been reported to promote bone formation via enhancing angiogenesis during DO [57]. The pro-angiogenic effect of Mg may be attributable to CGRP-mediated signaling. Hence, the use of a Mg-based metal for development of a cost-effective intramedullary nailing or coating could enhance bone formation and ultimately reduce the treatment duration of DO due to its capacity to continuously upregulate the expression of multiple endogenous agents that promote angiogenesis, osteogenesis and neuronal regeneration. These diverse effects may themselves be driven by Mg degradation products, including local release of Mg ions, elevated pH value, and hydrogen (Fig. 2).

7. Conclusion

Current methods including physical therapy, gene therapy, growth factor-based therapy, stem-cell-based therapy, and improved distraction devices to shorten the treatment duration of DO have been proven effective in animal models. However, further development is still needed to improve their reliability, adjustability, and affordability. Mg and its alloys are promising biomaterials that may be applied in DO to promote bone formation due to their suitable mechanical strength, osteogenic and angiogenic potential, degradability and antimicrobial ability. The ability of Mg to upregulate the expression of multiple endogenous biological agents continuously solves the problems of lacking an efficient delivery method and short biologic half-life for growth factor therapy, making it a good candidate for accelerating DO. The use of pure Mg metal as an intramedullary nail for application in the distraction regenerate may shorten the treatment duration of DO by upregulating the expression of osteogenic and angiogenic factors as well as enhancing bone formation. In conclusion, this review summarizes the various methods for promoting bone formation in DO, with focus on the exploration of the translational potential of biodegradable Mg and its alloys.

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