# Development of degradable magnesium-based metal implants and their function in promoting bone metabolism (A review)

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## ARTICLE INFO

## ABSTRACT

*Background:* Use of degradable magnesium (Mg)-based metal implants in orthopaedic surgeries can avoid drawbacks associated with subsequent removal of the non-degradable metallic implants, reducing cost and trauma of patients. Although Mg has been applied in the clinic for orthopaedic treatment, the use of Mg-based metal implants is largely in the research phase. But its application is potentially beneficial in this context as it has been shown that Mg can promote osteogenesis and inhibit osteoclast activity.
*Methods:* A systematic literature search about "degradable magnesium (Mg)-based metal implants" was performed in PubMed and Web of Science. Meanwhile, relevant findings have been reviewed and quoted.
*Results:* In this review, we summarize the latest developments in Mg-based metal implants and their role in bone regeneration. We also review the various molecular mechanisms by which Mg ions regulate bone metabolic processes, including osteogenesis, osteoclast activity, angiogenesis, immunity, and neurology. Finally, we discuss the remaining research challenges and opportunities for Mg-based implants and their applications.
*Conclusion:* Currently, establishment of the in vitro and in vivo biological evaluation systems and phenotypic modification improvement of Mg-based implants are still needed. Clarifying the functions of Mg-based metal implants in promoting bone metabolism is beneficial for their clinical application.
*The Translational potential of this article:* All current reviews on Mg-based implants are mainly concerned with the improvement of Mg alloy properties or the progress of applications. However, there are few reviews that provides a systematic narrative on the effect of Mg on bone metabolism. This review summarized the latest developments in Mg-based metal implants and various molecular mechanisms of Mg ions regulating bone metabolism, which is beneficial to further promote the translation of Mg based implants in the clinic and is able to provide a strong basis for the clinical application of Mg based implants.

## 1. Introduction

Traditionally orthopaedic clinical implants are made of non-degradable metals. These metals include stainless steel titanium (Ti) alloy and cobalt–chromium (Co–Cr) alloys. Although these metals exhibit excellent biocompatibility, high wear resistance and sufficient mechanical strength [1], they nonetheless have critical limitations. Firstly, they are often incompatible with the mechanical properties of natural bone, they display mechanical wear, and importantly they can induce inflammation due to their corrosion [2]. Also their use requires the presence of a permanent drilled hole given the long-term implantation they require.

This requirement can limit the use of additional surgery if the patient needs further repair. Also, these limitations may lead to the necessity to remove the internal fixation if complications, such as broken nails, pain or immobility, arise. But such additional surgery greatly increases the pain the patient may suffer, as well as raising medical expenses. But even if metallic implants do not require secondary surgery, they produce stress shielding at the implant position due to the high Young's modulus of the current metals used. And finally, the decrease in peripheral bone tissue density and loss of mechanical strength due to the presence of the implant increases the risk of secondary fracture. To overcome some of these limitations, bone implants made of biodegradable materials,

including Mg-based metal implants, have been developed and used clinically [3].

Mg-based metal implants have become particularly of interest as it has been shown that they can promote osteogenesis, angiogenesis and neuroregeneration [2], while inhibiting osteoclast activity and inflammation [4,5]. Thus, Mg-based metal implants can promote bone healing of skeletal disease [6]. However, it is still unclear how Mg affects bone metabolism and remodeling.

Here, we review the development of degradable Mg-based metal implants and the mechanisms by which they promote bone metabolism. We also discuss the challenges and opportunities of Mg-based implants and their applications. By highlighting these advances and hurdles, we hope such insights and its discussion will help facilitate the translation of degradable Mg-based metal implants from the bench to the bedside.

## 2. Application of Mg-based metal implants

Mg is an essential element of human metabolism. It is also one of the essential elements for bone anabolism, as excess bone resorption and osteoporosis can occur when Mg is deficient. As the second-largest cation element in the human body, the Mg ion concentration in the human blood is 0.75–1.25 mmol/L, and 60% of the total Mg exists in the bones. The recommended daily intake of Mg for adults is 310–420 mg, with any excess excreted by the kidneys through the urine [7](Fig. 1). The density of Mg metal is 1.74–1.84 g/cm$^3$ and its elastic modulus is 41–45 GPa, which are close to the density and elastic modulus of human bone tissue (elastic modulus (E) = 15–25 GPa) [8]. These properties provide a good foundation for the use of Mg metals in orthopaedic implants. In addition, Mg-based alloys have mechanical properties similar to natural bone (density, yield strength, tensile strength, fracture elongation, and elastic modulus), which facilitates reducing stress shielding between natural bone and metal implants [9]. In patients with bone disease, Mg metals can be chemically combined with body fluids, and are gradually degraded to ionic states through corrosion. This property avoids the risk of the requirement for secondary surgery. Further, the Mg ions released by the Mg-based metal implantation can promote bone regeneration and accelerate healing, while promoting blood vessel and bone formation [10]. Therefore, research into biodegradable metals has become of keen interest, especially in China.

After implantation, Mg ions released by Mg-based metal implants can diffuse through either a natural channel (Haversian system) or along an artificial pathway, such as a fracture line or gap (Fig. 2). Mg can also diffuse through the bone marrow to the periosteum, where it promotes bone regeneration and accelerates bone healing [11]. Along these lines,
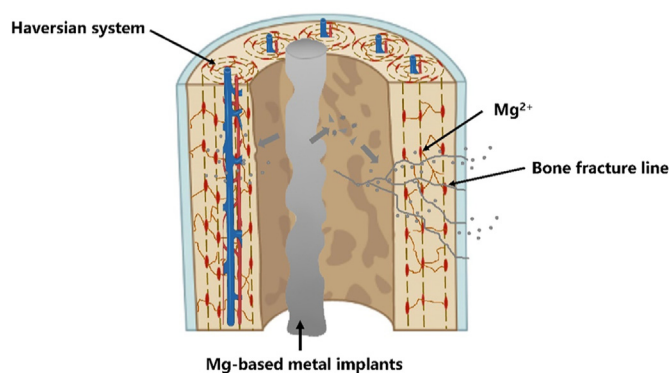


**Fig. 2.** The means of diffusion of Mg ions after release by Mg-based metal implants [11].

Zhang et al. [12] found that Mg can promote calcitonin gene-related peptide (CGRP)-mediated osteogenic differentiation and thus contributes to improved femoral fracture healing. Further, Mg can promote osteogenesis and blood vessel formation, while inhibiting osteoclast activity [4], and thus Mg-based metal implants have a beneficial effect on osteogenesis and angiogenesis [13,14]. Given these effects, Mg-based metal implants offer clear advantages in the treatment of challenging bone diseases compared to traditional metal implants.

The clinical application of Mg alloys dates back one and half centuries. Dr. Huse and Erwin Payr used pure Mg to prepare bone plates for fracture treatment in 1878 and 1892, respectively [15]. Although some clinical results of Mg-based implants have been achieved, the rate of the degradation of Mg-based implants is still not effectively controlled. Thus, most physicians choose implants utilizing more traditional metals. Because of this hesitancy, Mg metal as a clinical application and its research has stalled since the beginning of the last century. However, with recent progress into the research of biodegradable implants and the improvement of material technology, Mg-based metal implants have garnered renewed interest.

## 3. Functional improvement of Mg-based metal implants

In recent years studies regarding biomedical magnesium have been published in international journals by research groups from Germany, Switzerland, Japan, Australia, the USA, the UK, New Zealand, Turkey, and China [16–20]. What has become clear is that the biocompatibility,
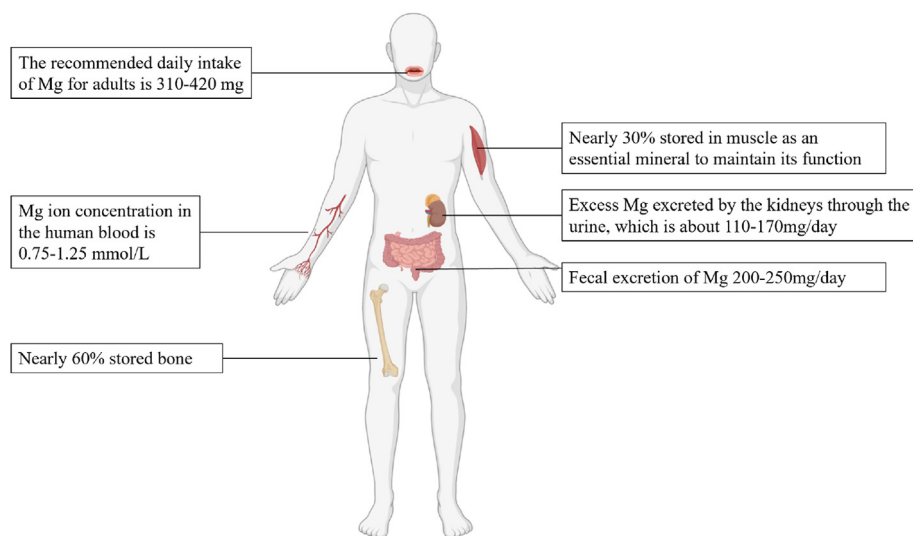


**Fig. 1.** Metabolism and distribution of Mg in the body [7].

rapid degradation, and high corrosion rate of degradable Mg alloys in the human body remain the main barriers to their clinical use. Currently, there is no unified system for the biological evaluation of degradable metal implants, both nationally and internationally. And the available Mg alloys degrade too rapidly to remain sufficiently stable after implantation. Such degradation can lead to the formation of hydrogen cavities, rapid loss of mechanical integrity of the implant and adverse host tissue reactions, such as local swelling and significant pain, within the first week after surgery. Therefore, intensive research into the design of corrosion-resistant and biocompatible Mg-based alloys compatible with stable implantation is still required. To overcome these drawbacks, there are many avenues for improvement, such as incorporation of alloying elements and surface modification. Numerous studies have shown that adding alloying elements, such as zinc, neodymium, calcium, tin, manganese and germanium, to Mg can effectively improve the mechanical performance, biocompatibility and corrosion resistance of Mg, and reduce hydrogen production [21–24]. Furthermore, the biocompatibility of these alloy elements on bone-related cells is an important factor. Alloy implants have been extensively tested *in vitro* and *in vivo* to determine their cytotoxicity [25,26].

Recent studies have also reported the improved corrosion behavior and biocompatibility of Mg alloy through surface modification [27]. One approach is to significantly improve the corrosion resistance of Mg alloys with both sol–gel and synthetic polyester-based coatings. For example, Zhang et al. developed a novel Mg ion-incorporating dual-crosslinked hydrogel by photocrosslinking gelatin methacryloyl (GelMA), thiolated chitosan (TCS) and modified polyhedral oligomeric silsesquioxane (POSS) nanoparticles, followed by incorporation of Mg ions via coordination bonds of MgS [28]. Zhang et al. coated 3D gel-printed porous magnesium scaffold with dibasic calcium phosphate dihydrate. They used more than two approaches to improve the corrosion behavior and biocompatibility of Mg-based implants successfully, opening up a new technical route for the preparation of degradable Mg-based implants [29]. Li et al. modified the inner nano-coated Mg(OH)$_2$ layer by introducing stearic acid (SA), which improved the adhesion strength of the interface between the outer and inner layer, avoiding the penetration of the corrosion medium [30]. Likewise, to improve the corrosion resistance and biocompatibility of Mg alloys Cheng et al. tested a Mg–Al layered double hydroxide (LDH) coating on the surface of pure Mg and showed not only more corrosion resistance but also enhanced osteogenic, angiogenic and anti-inflammatory behavior *in vitro* and better osteointegration *in vivo* than naked Mg or Mg coated with Mg(OH) [31].

The use of other nano coatings to improve the biocompatibility and degradation resistance of Mg alloy has been reviewed elsewhere [32]. But briefly, Lin et al. used a nano-coating of TiO$_2$/Mg$_2$TiO$_4$ on the outer layer of a Mg alloy to improve its biocompatibility and bacterial infection resistance properties [33]. Likewise, Liu et al. found that the lithium-bound nanoporous coating formed by micro arc oxidation gives Mg alloy enhanced corrosion resistance and promotes angiogenesis and bone formation [34]. Further, Gao et al. improved the biocompatibility and degradation resistance of Mg alloy by calcium phosphate coating [35]. And Razavi et al. expanded on this approach by using a novel bioceramic nanocomposite coating, which resulted in reduced hydrogen bubbling around the implant site, reduced the corrosion rate and improved the new bone formation while reducing inflammation in the interface of the implants and the surrounding tissue [36]. Meanwhile, Safari et al. achieved superior degradation resistance and bactericidal activity by incorporating graphene nanoparticles into the Mg alloy [37].

Numerous studies have also highlighted the improvement of the corrosion performance of natural polymer coatings through the incorporation of synthetic polymers. In addition, sol–gel and synthetic polyester coatings have shown the ability to act as local drug delivery platforms, and attempts have been made to coat the surface of Mg alloys with drugs [13,14]. Chen et al. fabricated a zoledronate loaded coating on AZ31 Mg alloy. The results showed that it had better corrosion resistance and zoledronate can also play its role which proved it can

easily and efficiently used in clinic [38]. But research in this area is very limited. There have also been attempts into making alloys into block metal glass to improve corrosion resistance [39,40]. With the advancement of material science, more investigators have used nanocomposites to enhance the corrosion resistance and biocompatibility of Mg alloys [41]. The key problem is the dynamic balance between the rate of degradation and tissue repair (Table 1).

The mechanical strength, corrosion resistance and biocompatibility of Mg alloys can be improved by surface modification. However, many reports have not included cytotoxicity tests. For non-degradable metals, there is a standard procedure for cytotoxicity testing. The metal is placed in a culture medium for some time and the target cells are subsequently cultured in that medium to determine cytotoxicity. Obviously, a degradable metal does not apply to this criterion as they can degrade in the culture medium. Thus, metal compounds make it difficult to determine whether the metal itself has effects. From our review of the literature, we found some reports in which cells were placed on metal surfaces to observe the cytotoxicity effects of the metal, but there is no consensus on this methodology. Therefore, we found unlike inert metal, there is not unified evaluation standard for degradable metals. We need to establish a new *in vitro* and *in vivo* biological evaluation system for degradable metals. To mimic the *in vivo* animal test, *in vitro* corrosion test can use culture medium with serum which containing similar components to human blood and avoid immersing for long time. Different methods of evaluating corrosion rate should base on the real implant situation and too long immersion time should be abandoned. It's better to use dynamic corrosion [42]. When it comes to *in vivo* animal test, the time point for examine the concentration of decomposition products needs to be unified. Only the blood concentration were observed in many studies. The effects of liver and kidney should be further observed necessarily. The method of observing tissue changes where the implants placed needs further exploration.

## 4. Effect of Mg-based metal implants on bone regeneration

Previous experimental studies [31,43,44] have shown that Mg-based metal implants have beneficial effects on bone metabolism, vascular formation, neuroregeneration, and immunity (Fig. 3). However, it is difficult to test for effects on cell growth during *in vitro* experiments due to the rapid degradation of Mg. Further, it is difficult to replicate *in vitro* the *in vivo* environment, and, thus, the function of metallic materials used in implants *in vivo* remains unknown. As such, the role of materials derived from Mg-based alloys in bone formation and the mechanisms involved in their promotion of bone regeneration and accelerated bone healing during bone diseases have been of keen interest. We discuss below the recent findings along these lines.

### 4.1. Effects of Mg-based metal implants on osteogenesis

More and more studies found that Mg can promotes osteogenesis(Fig. 4). Recent studies have indicated that the main signaling pathways involved in Mg-mediated bone metabolism includes the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway [45,46], the osteoprotegerin (OPG)-receptor activators of nuclear factor-κB (RANK)-RANK ligand (RANKL) signaling pathway [47], the TRPM protein pathway [48], and the Wnt signaling pathway. The OPG-RANK-RANKL signaling pathway mainly affects osteoclast biogenesis and differentiation, which is discussed below.

The TRPM protein pathway mainly regulates intracellular Mg ion concentrations through TRPM6 and TRPM7 [49]. It promotes the differentiation and proliferation of osteoblasts by regulating the balance of Mg ions [50]. Through the TRPM7-PI3K signaling pathway, the expression of Runx2 and alkaline phosphatase (ALP) can be up-regulated by Mg ions, leading to the enhancement of osteogenic activity of human osteoblasts [10] (Fig. 5).

The main pathways affecting osteogenic function are the PI3K-Akt

**Table 1**
Summary of methods to improve Mg alloys.

| Method | Mg-based implant | Control group | Animal species | Endpoint | Degradation rate | Cytotoxicity tests | biocompatibility | Ref. |
|---|---|---|---|---|---|---|---|---|
| Alloy | Mg–5Zn-2Nd-0.13Y-0.35Zr | Ti–6Al–4V alloy | Rats | 11 weeks | Accelerated | NA | No significant adverse effects | [22] |
| | Mg–Sr binary alloys | Different concentrations of Sr non-implanted | Mice | 4 weeks | Slowest in all groups | NA | No significant adverse effects | [24] |
| | Mg–Zn–Ca alloy | | Mice | 4 weeks | MEM containing 10% FBS is the slowest | non-toxic to MG63 cells | No significant adverse effects | [41] |
| | Mg–1Zn–1Sn-xSr (x = 0, 0.2, 0.4, and 0.6 wt%) alloy | Between group comparisons | Rats | 3, 7, 15 and 30 days | (0.2 wt% Sr)have lowest degradation rate | NA | Enhanced the viability, adhesion, and spreading of MC3T3-E1 cells | [27] |
| | Mg alloy with graphene nanoparticles(Gr) | Mg alloy without Gr | NA | 10 h, examined every 2 h | Reduced approximately 4-fold | NA | Had high cytocompatibility and superior osteogenic properties | [37] |
| Coating | SrHPO4 coating on Mg-Nd-Zn-Zr alloy | Mg-Nd-Zn-Zr alloy without coating | Rats | 4,8,16 weeks | Slowest | NA | No significant adverse effects | [26] |
| | SA (stearic acid) @PLG A[poly(1,3-trimethylene carbonate)]coating | AZ31, AZ31-OH, and AZ31-OH&SA | Rats | 30 days | The volume loss of AZ31-OH&SA@PTMC was the lowest, | NA | 1. Enhanced the proliferation and adhesion of rBMSCs, angiogenic of HUVEC. 2. Induced macrophagepolarizese to M2 phenotype (anti-inflammatory). | [30] |
| | Mg–Al layered double hydroxide coating | bare Mg and Mg(OH)2 coated Mg | Rats | 4.8 weeks | Mg-LDH showed the best corrosion resistance | NA | Enhanced osteogenic differentiation of MC3T3-E1 | [31] |
| | TiO(2)/Mg(2)TiO(4) nano-layer | Ti control and untreated WE43 group | Rats | 12 weeks | Mg– TiO(2)/Mg(2)TiO(4) nano-layer showed best corrosion resistance | No cytotoxicity to the MC3T3-E1 | No significant adverse effects | [33] |
| | Li-micro arc oxidation (MAO) nanoporous -coating on AZ91 Mg. | AZ91 with MAO-Mg and bare AZ91 | Rats | 8 weeks | The AZ91 group exhibited higher corrosion rate and no significant difference was found about the corrosion resistance between MAO and Li-MAO groups. | NA | Enhanced the proliferation and adhesion of rBMSCs, angiogenic of HUVEC。 | [34] |
| | CaP on AZ60 Mg alloy | AZ60 Mg alloy without CaP | Rats | 4.12weeks | The control group is three times higher than the trial groups | Grade 0 cytotoxicity in the first 5 days and grade 1 cytotoxicity on day 7 (MC3T3-E1 cell) | Had better biocompatibility | [35] |
| | MAO/EPD on Mg-aluminum-zinc alloy (AZ91) | Without coating | Rats | 2.8 weeks | The corrosion rate of AZ91 significantly reduced to 1.2 and 0.00005 mm/year | NA | No significant adverse effects | [36] |
| Hydrogel | GelMA/TCS/POSS-Mg | NA | Rats | NA | NA | NA | Exhibited favorable osteogenic activity and superior angiogenic activity | [28] |
| | 3D gel-printed porous magnesium scaffold coated with dibasic calcium phosphate dihydrate | implanted with β-TCP scaffold and the model group was only drilled for modeling. | Rats | 6, 12 and 24 weeks | Much slower than uncoated one | No cytotoxicity to the MC3T3-E1 | Did not cause an increase in Mg ion concentration *in vivo* and no toxic damage in the liver or kidney. | [29] |
| Nanocomposites | Mg–Zn–Si/HA nanocomposites | Without HA nanocomposites or different ratios of Mg–Zn–Si | NA | 21 days | Trial groups show a better surface morphology with the absence of deep pits and cracking | Revealed a level 0 cytotoxicity after 24 h of incubation of MC3T3E1 cells | Had the best biocompatibility | [41] |

Notes: full name of the abbreviated forms used above
NA: not applicable

signaling pathway and the Wnt signaling system, with the former pathway having been investigated comprehensively (Fig. 6). Its effect on osteogenesis is mainly through the activation of downstream Akt signaling via PI3K. The target proteins downstream of Akt are important in bone metabolism. The P13K-Akt pathway regulates the levels of ATP

and UTP to affect osteoblast proliferation and differentiation. Meanwhile, this pathway can also stimulate the macrophage growth factor receptor on the surface of Mesenchymal Stem Cells (MSCs) to promote their differentiation into osteoblasts [51]. Further, appropriate concentrations of Mg ions can activate downstream target proteins by regulating
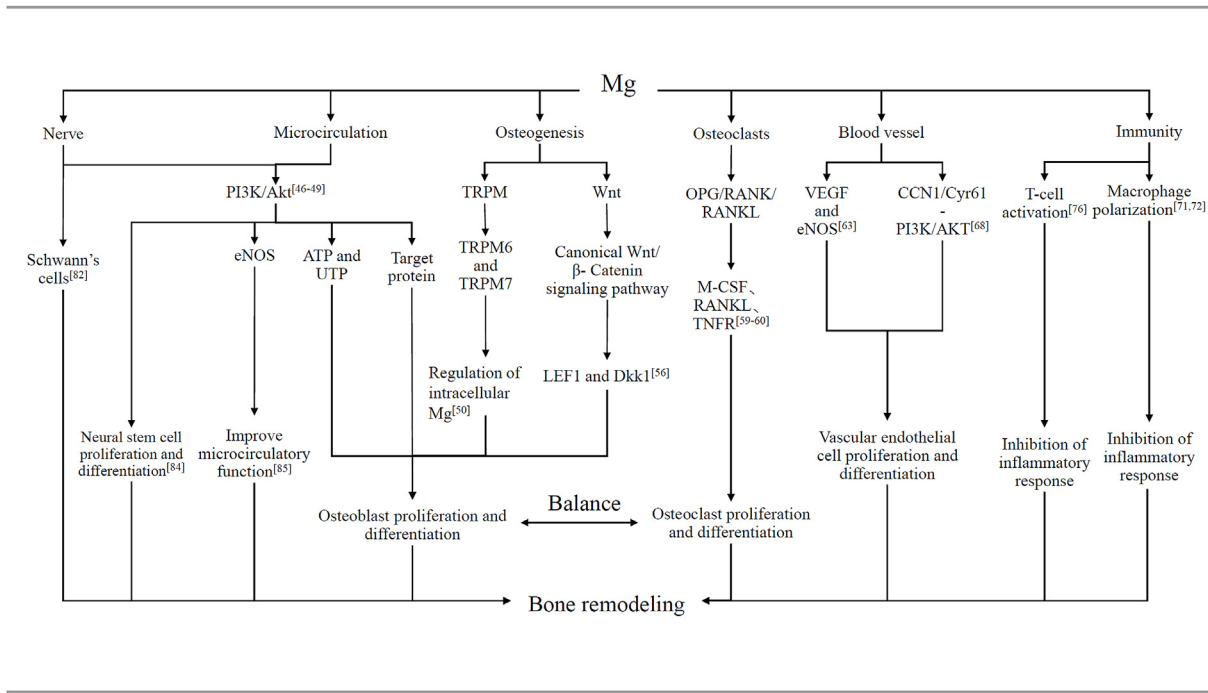
**Fig. 3.** Mg ions can regulate bone metabolic processes, including osteogenesis, osteoclast activity, angiogenesis, immunity, and neurology. They can promote osteogenesis, angiogenesis and neuroregeneration, while inhibiting osteoclast activity and inflammation. This figure summarized mechanisms of Mg on bone remodeling.(Relevant reference have been marked in the figure.)
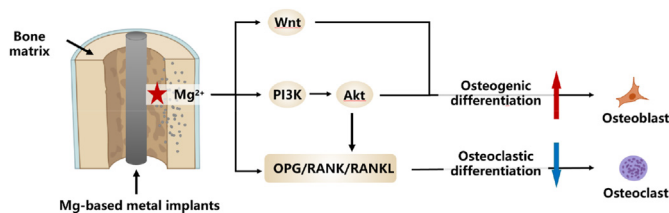


**Fig. 4.** The mechanism of Mg ions promoting osteogenesis. Mg ions can promote osteogenic differentiation through PI3K-Akt and Wnt signaling pathway and inhibit osteoclastic differentiation through OPG/RANK/RANKL signaling pathway.
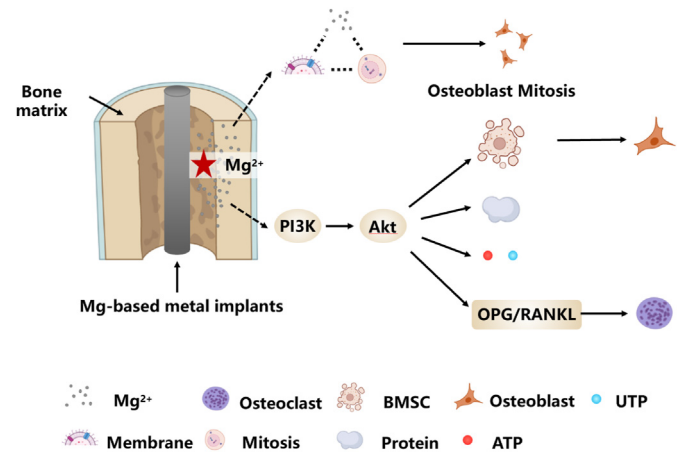


**Fig. 6.** The signaling pathways regulated by Mg ions to promote osteogenesis summarized from published work [46–49].
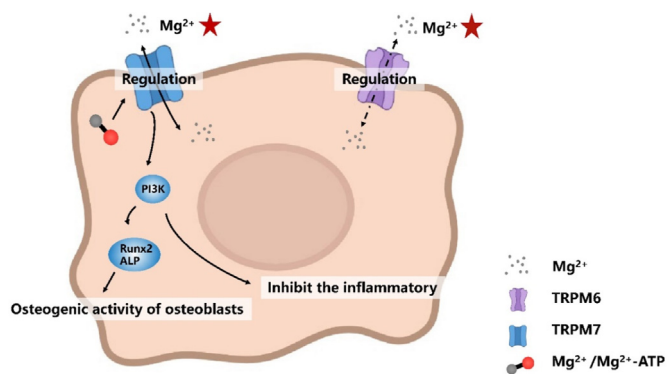


**Fig. 5.** The mechanism of Mg ions promoting osteogenesis and inhibiting inflammatory via TRPM protein pathway signaling pathway summarized from the published work [10,43,44].

Akt expression, thus promoting osteogenesis. Qi et al. discovered that Mg ions produced through biodegradation regulate the expression of genes and proteins related to osteogenesis, which can activate multiple

signaling pathways, enhance autophagic activity and regulate pH in the microenvironment [52].

Wnt is a newly discovered osteogenesis-related signaling pathway in recent years. Wnt signaling is generally classified into the classical Wnt-β-catenin pathway and into non-classical Wnt signaling [53], and it plays a role in physiology and disease [54]. Classical Wnt-β-catenin signaling can promote osteogenic activity by activating the Src-ERK and PI3K-Akt signaling pathways [55]. The previous regulatory mechanism of Mg ions on the Wnt signaling pathway has not been clarified, making it an active research topic in recent years. Hung et al. revealed that Mg ions induce osteogenic activity in the bone marrow space through the activation of the classical Wnt signaling pathway, which causes the differentiation of bone marrow stromal cells into the osteoblast lineage [56]. They showed that Mg ions can promote the accumulation of β-catenin in the cytoplasm by blocking its proteosomal degradation, before it then enters the nucleus

to bind to and promote the transcriptional activity of the T cell factor (TCF)-lymphatic enhancer factor (LEF) complex, leading to activation of the transcription of downstream target genes that promote osteoblast differentiation (Fig. 7). However, the current study of the Wnt signaling pathway has been expanded. Hamushan et al. suggested that high-purity Mg implants can enhance osteoporosis in tensile osteogenic applications [57]. This process may regulate the Ptch protein to activate the selective Wnt signaling pathway by activating Hedgehog pathway. Hung et al. observed and quantified the mineral deposition in the bone marrow space via the rabbit ulna fracture model [56]. They focused on the expression of LEF1 and Dkk1, the downstream target genes directly controlled by active β-linked proteins. They indicated that $Mg^{2+}$ induced osteogenic effects in the bone marrow space through activation of the classical Wnt signaling pathway.

### 4.2. Effect of Mg-based metal implants on osteoclast activity

The OPG-RANK-RANKL signaling pathway mainly controls osteoclast differentiation. Osteoclasts and mature osteoblasts express RANK on their cell surface. The ligands macrophage colony-stimulating factor (M-CSF) and RANKL are essential in regulating gene transcription during the initiation of osteoblast differentiation and in triggering osteoclast differentiation [47,58]. NF-κB and NFATc1 are suppressed by Mg ions and inhibit the differentiation of osteoclast precursors [59]. However, OPG which acts as a decoy receptor for RANKL is expressed on the osteoblast surface, where it can compete with RANK binding to RANKL, and thus reduces the production of osteoclasts. Therefore, OPG is known as an osteoprotective factor. Mg ion deficiency leads to increased bone resorption by regulating this cytokine system, as the Mg ion concentration influences the serum levels of OPG and RANKL. A previous study suggested that the effects of calcium on bone can be effectively rescued by Mg supplementation as Mg can increase serum OPG levels to promote bone formation [60] (Fig. 8).

### 4.3. Effect of Mg-based metal implants on angiogenesis

The effects of Mg ions on blood vessel formation have been investigated mainly by experimental studies *in vitro*, and these studies have focused on vascular endothelial cell changes in orthopaedics or dentistry after the application of Mg-based implants, which increase the concentration of Mg ion [61,62]. Gu et al. found that Mg concentrations could increase the expression levels of the angiogenic biomarkers vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) in *in vitro* studies of Mg-containing TCP scaffolds [63]. Liu et al. demonstrated improved angiogenesis by shuffling *in vitro* experiments on Mg alloys [34]. Liu et al. found that Mg promoted the secretion of
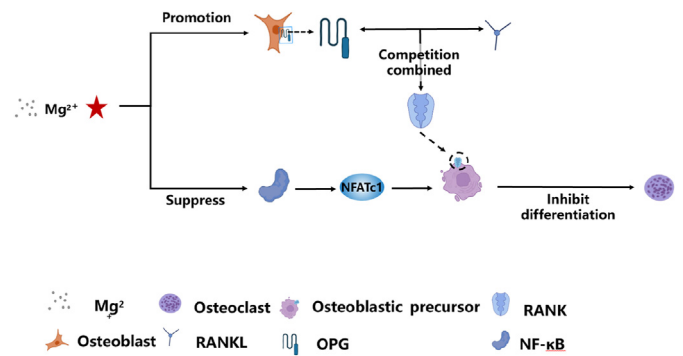
Fig. 8. The mechanism of Mg ions affecting osteoblast functions via the OPG-RANK-RANKL signaling pathway summarized from published work [42,52–54].

PDGF-BB by MC3T3-E1 cells, which could improve not only the osteogenic differentiation of osteoblasts, but also the angiogenic ability of HUVECs [64]. These studies suggest that Mg ions can promote angiogenesis in surrounding tissues by increasing the secretion of angiogenic substances, such as VEGF, eNOS and PDGF-BB. Xu et al. analyzed the angiogenic response under different concentrations of Mg and oxygen content and found a general negative effect on early (migration) and late (tubule) angiogenesis in the case of increased normoxic and Mg concentrations [65]. However, this effect is eliminated under hypoxia. As the degradation of Mg-based implants is an oxygen-dependent process, hypoxic conditions may be a necessary factor for testing the cytocompatibility of materials. However, experiments *in vitro* are not able to provide exactly the same environment compared with that *in vivo*. Thus, it is important to follow the results of *in vivo* tests. Gao et al. performed *in vivo* experiments on Mg-based metal implants. HUVECs cultured with Mg-coated Ti6Al4V showed significantly higher expression of angiogenesis-related genes (HIF-1α and VEGF). Experiments *in vivo* used microangiography, which showed that the Mg-coated Ti6Al4V scaffold significantly enhanced angiogenesis [66]. Han et al. showed that vascular-mediated bone-producing mechanisms enhanced tissue regeneration via the 3D confocal imaging of the vasculature within skeletal tissue [67].

In conclusion, both *in vitro* and *in vivo* experiments fully affirmed the promoting effect of Mg ions on angiogenesis, and that the stimulation of VEGF generation is the main mechanism for this effect. Interestingly, Di et al. found that CCN1-Cyr61-PI3K-AKT signaling can promote oxygen-induced retinal neovasculogenesis in retinopathy [68]. The PI3K-AKT signaling pathway is also important for bone metabolism, as noted above. Whether Mg ions can promote the growth of blood vessels around bone tissue is currently being investigated (Fig. 9).
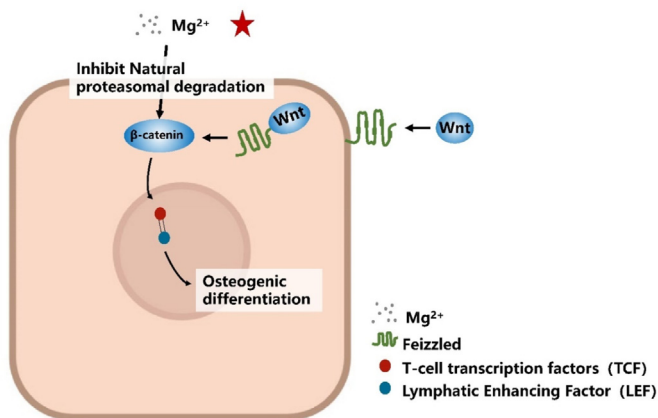
Fig. 7. The mechanism of Mg ions promoting osteogenesis via Wnt signaling summarized from published work [54].
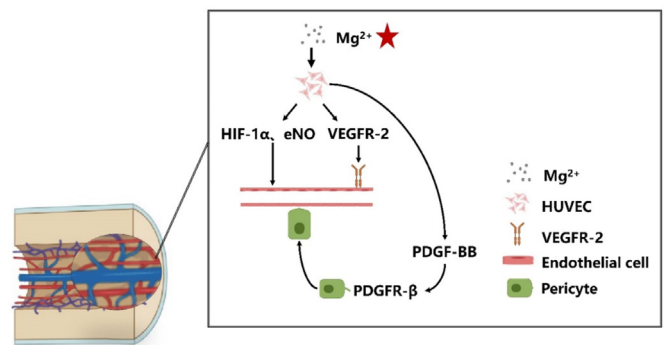
Fig. 9. The mechanism of Mg ions promoting angiogenesis summarized from published work [64,66].

## 4.4. Effects of Mg-based metal implants on immune function

Recent studies in China and elsewhere have shown that Mg-based metal implants can play a crucial role in bone regeneration by regulating immunity [69]. Immune cells are the central regulators of the bone immune microenvironment. They influence osteogenesis, bone fragmentation and fibrosis during bone regeneration by secreting various cytokines into the bone regeneration microenvironment [70].

It has been hypothesized that Mg ions can regulate immune function by affecting macrophages and T cells. Therefore, the release of Mg ions from Mg-based metal implants after implantation can have an impact on the tissue immune response. As macrophages are the main cells involved in the immune response, biomaterial implants should affect their polarization into the different macrophage phenotypes; namely, M1, which is generally proinflammatory, and M2, which is generally pro-regeneration and anti-inflammatory. These two types of macrophages release different factors and cytokines that interact with other coordinating factors of bone healing, such as endothelial cells, mesenchymal cells, osteoclasts and osteoblasts [71,72]. Costantino et al. found that Mg extracts can be quite compatible with macrophages [44]. Mg extracts stimulated macrophage M1 and M2 lineages, while Mg–2Ag and Mg-10Gd inhibited the M1 lineage and stimulated the M2 lineage. Thus, Mg-based biomaterials can induce faster inflammatory regression and improve tissue repair. The appropriate concentration of $Mg^{2+}$ (100 mg/L) promoted osteogenic differentiation of MSCs, while the normal Mg ion concentration in the body is between 18 and 30 mg/L. A high concentration $Mg^{2+}$ promotes the secretion of BMP2 by macrophages, which enhances the osteogenic differentiation of MSCs by activating the BMP2-Smad signaling pathway. Mg2+ release concentrations (~100 mg/L) could overcome the deleterious bone immunomodulatory properties of Mg-based biomaterials and make them more favorable to the bone marrow [73].

Intracellular free Mg regulates the cytotoxic function of natural killer (NK) and CD8+ T cells. Mg can inhibit NF-kB to reduce the production of inflammatory cytokines by monocytes. T cells can suppress innate immune cell-mediated inflammatory responses by suppressing TNF production by macrophages via NK cells [74,75]. In a recently published study, Lötscher et al. showed that the cell surface molecule LFA-1 requires Mg to adopt its active conformation on CD8+ T cells [76]. T cell receptor (TCR) stimulation rapidly activates LFA-1, which provides unique LFA-1-dependent signaling to promote T cell activation [77], which results in specific cytotoxicity. Accordingly, Mg-sufficiency sensed via LFA-1 translated to the superior performance of pathogen- and tumor-specific T cells, enhanced effectiveness of bi-specific T cell engaging antibodies, and improved CAR T cell function. According to the above studies, only low doses of Mg ions (but much higher than the concentration of Mg particles in human blood) can exert its induced differentiation effect on macrophage polarization and activation of T cells. In a study of COVID-19, DiNicolantonio and O'Keefe found an important relationship between vitamin D activation and Mg, which in turn pointed to the importance of Mg in the immune system [78]. Indeed, Mg deficiency can lead to immune dysfunction. The lack of Vitamin D and Mg also have a major impact on the production of the cytokine storm that often occurs with COVID-19 infection [79]. Jin et al. found that degradation products of Mg-based alloys (especially Mg) can inhibit the inflammatory response through activation of the TRPM7-PI3K-AKT1 signaling pathway [80]. Therefore, Mg-based biomaterials can induce faster inflammatory regression and improve tissue repair (Fig. 10).

## 4.5. Effects of Mg-based metal implants on neuroregeneration

In a recent study, it was found that Mg ions not only promote osteogenesis and angiogenesis and inhibit immune-inflammatory responses, but also are beneficial to the body's nerves [81]. Mg has been shown to induce nerve growth factor secretion by Schwann cells and
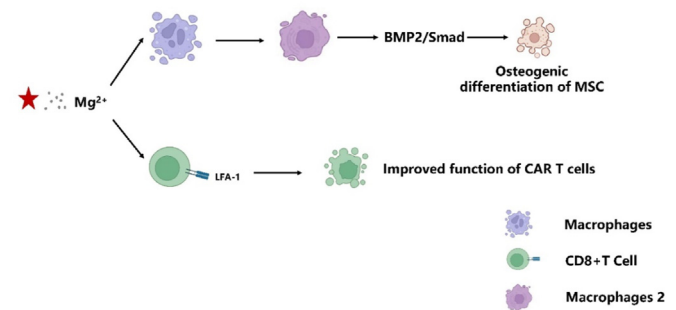


**Fig. 10.** The mechanism of Mg ions affecting osteogenesis and immune function summarized from published work [71,72].

promote the regeneration of nerve axons after central nervous system injury [43,82]. Soluble Mg ions and hydrogen can be released during degradation of Mg-based metal implants, which have neuroprotective and antioxidant effects [81]. Meanwhile, Monfared et al. reported that MSCs proliferation can be inhibited by excessive Mg [82]. Shen et al. prepared Mg-zinc metal–organic skeletons on titanium implants [83]. They found it inhibited bacterial infection and promoted bone regeneration. Zhang et al. found that Mg lithospermate B (MLB) could enhance local neurogenesis and that its effects on neural stem cell proliferation and differentiation were through PI3K-Akt signaling [84]. Liu *et al.* found that MLB could restore microcirculation dysfunction through activation of eNOS [85], which enhances the generation of vascular nitric oxide. This is caused by the activation of the MLB-induced pathway. Overall, the promotion of neural stem cell differentiation, stimulation of local neural regeneration and improvement of the microcirculation are all associated with the PI3K-AKT pathway, which coincides with the signaling pathway of Mg ions in promoting osteogenesis.

## 5. Challenges and outlook

Bone metabolism includes both bone formation and bone destruction. It is the dynamic balance of osteoblasts and osteoclasts that maintains the stability of bone tissue. Currently, the pathways mainly affecting osteoclast differentiation and osteoresorption are PI3K-Akt signaling and OPG-RANK-RANKL signaling. Recent studies have indicated that inhibition of PI3K-Akt signaling activity attenuates osteoclast resorption [86]. While different Mg ion concentrations can regulate PI3K-Akt signaling in osteoblasts, it has been found that 6 and 10 mmol/L Mg ions upregulate expression levels of p-Akt, while an 18 mmol/L concentration decrease its expression levels [87]. However, the OPG-RANKL-RANK signaling pathway promotes bone resorption [60]. Gene transcription for osteoclast differentiation requires M-CSF and RANKL, which are released by osteoblasts [88,89]. This suggests that some degree of osteoblast differentiation and functional activity promotes osteoclast activity. Overall, Mg-based implants release Mg ions, resulting in an increase in local concentrations. When the appropriate concentration is achieved, the PI3K-Akt signaling pathway is activated to promote osteogenic differentiation. Osteoblast differentiation results in the secretion of RANK and M-CSF to induce osteoclast differentiation, which explains the coexistence of bone formation and bone absorption during the early stages after implantation of Mg-based implants. However, more experiments are still needed to prove this speculation. In contrast, we believe that the coexistence of bone formation and bone resorption accelerates bone remodeling and ultimately promotes bone formation, which allows for an increased rate of bone disease healing.

Mg-based metal implants can promote tissue repair and healing during degradation. It can avoid secondary surgery, and thus reduce patient pain and reduce treatment costs. Mg can promote osteogenesis, angiogenesis and neuroregeneration, while inhibiting osteoclast activity and inflammation. Overall, the current clinical transformation all wants

to use the advantages of Mg such as promoting osteogenesis and angiogenesis. Current researches have gradually shifted from simple applications to solve problems in practice. For example, Zhu *et al.* placed the Mg-based metal implants into the rat which has Medication-related osteonecrosis of the jaw. They found that biodegradable Mg implant could alleviate the development of MRONJ-like lesion. Thus, it proved the translational potential of Mg-based metal implants [90]. Some researcheres tried to use Mg-based screws in anterior cruciate ligament reconstruction, but the strength of Mg-based screws is still a question. J R Mau *et al.* analyzed the stresses in the screw head and improve screw design successfully according to that information. That solved the key point of the clinical use of Mg-based screws [91]. Mg-based metal implants also show advantages of in repairing bone fracture healing due to aging and osteoporosis and refractory bone defects. Lin *et al.* added magnesium particles into clinically used poly(methylmethacrylate) (PMMA) bone cement to make it possess osseointegrative, angiogenic and antiinfective properties [92]. Bioactive Mg metal powder also was incorporated into the composite bioactive scaffold which is to repair large bone defects [6]. However, the clinical application of Mg-based metal implants is still in the research and exploration stage. The metabolism of Mg ions in bone is incompletely elucidated and thus needs further examination. In particular, there is currently no clear biological evaluation system for biodegradable implants. Thus, the application of Mg alloy has been limited in the clinic.

Mg-based metal implants have been used in many clinical fields, such as cardiovascular, musculoskeletal, and general surgery. German BIO-TRONI has reported the results of a series of animal and clinical experiments on fully degraded vascular stents based on WE43 Mg alloy since 2003 [93].

In the field of orthopaedics, there have been many *in vivo* animal studies on Mg-based implants over the years. Lab animals have evolved from mice and rats to large animals, such as goats and pigs. Small animals are mainly subjected to *in vivo* toxicity tests, *in vivo* corrosion tests and observation of effects on various systems. Whether the Mg-based implants have an excellent mechanical strength can be analyzed by implanting them into large animals [9,94], which can simulate the stress state after implantation into the human body. More and more new techniques have been applied to analyze the *in vivo* effects of Mg-based metal implants, such as state-of-the-art fetal-mouse metatarsal assays [67], CS model analysis [95], and so on. MAGNEZIX compression screw (Syntellix AG, Hannover, Germany) is the first commercial Mg screw, which is compositionally similar to WE43. It is classified as a MgYREZr alloy according to DIN EN 1753. Its European CE mark approval has been received in 2013 for the treatment of hallux valgus surgery [96,97]. In 2021, 58 countries or regions around the world have agreed to register these series of compression screws for more fracture types [98]. Vascularized bone grafting fixed by a biodegradable Mg screw was the first Mg-based implant in China, which was for treating osteonecrosis of the femoral head in 2016 [99]. Patients with medial malleolar fractures treated with coated JDBM screws were also reported recently [100].

Overall, the problems that still need to be solved for the clinical application of Mg-based implants are several. Firstly, the degradation rate of Mg-based implants needs to be controlled and desirable mechanical properties need to be maintained throughout the treatment period to support tissue healing, especially for sites with a large load bearing. Secondly, *in vivo* experiments of limb animals still cannot replicate the stress assessment needed for implantation into humans. Use of non-human primates for such implantation tests should be encouraged in the future to achieve better evaluation data. Thirdly, the question of how to reduce the gas production of Mg-based implants *in vivo* still requires further research. Fourthly, there still is not a proper *in vitro* and *in vivo* biological evaluation system in place for degradable metals.

With the gradual improvement of *in vitro* and *in vivo* studies, especially stress studies in large animals and phenotypic modification of Mg-based implants, the above problems will be gradually resolved. Indeed, great progress in the clinical translation of in Mg-based implants has been achieved in the recent years, and should continue to progress in the future.

## 6. Conclusions

In conclusion, this Review presented the recent research and developments of Mg-based metal implants. The cellular and molecular mechanisms by which Mg-based implants affect bone metabolism was summarized and discussed, including the conclusion that the coexistence of bone formation and bone destruction in animal experiments likely explains the promotive effects of Mg ions on the osteogenic and osteoclastic systems. A series of clinical trials achieved by Mg-based implants have also provided a solid foundation for their large-scale clinical application. Although there are many challenges that remain for the clinical application of Mg-based implants, they are also being gradually solved. It is worth mentioning that the establishment of the *in vitro* and *in vivo* biological evaluation systems for Mg-based implants has become an urgent requirement. We hope to manufacture the Mg-based orthopaedic implants that can be used for clinical applications as soon as possible.

## Ethical statement

No human and animal subjects are covered in this review.

## Declaration of competing interest

The authors have declared that no competing interests exist.

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