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Zinc based biodegradable metals for bone repair and regeneration: Bioactivity and molecular mechanisms

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ABSTRACT

Bone fractures and critical-size bone defects are significant public health issues, and clinical treatment outcomes are closely related to the intrinsic properties of the utilized implant materials. Zinc (Zn)-based biodegradable metals (BMs) have emerged as promising bioactive materials because of their exceptional biocompatibility, appropriate mechanical properties, and controllable biodegradation. This review summarizes the state of the art in terms of Zn-based metals for bone repair and regeneration, focusing on bridging the gap between biological mechanism and required bioactivity. The molecular mechanism underlying the release of Zn ions from Zn-based BMs in the improvement of bone repair and regeneration is elucidated. By integrating clinical considerations and the specific bioactivity required for implant materials, this review summarizes the current research status of Zn-based internal fixation materials for promoting fracture healing, Zn-based scaffolds for regenerating critical-size bone defects, and Zn-based barrier membranes for reconstituting alveolar bone defects. Considering the significant progress made in the research on Zn-based BMs for potential clinical applications, the challenges and promising research directions are proposed and discussed.

1. Introduction

Skeletal defects—mainly caused by trauma (bone fracture), infections (osteomyelitis and periodontal disease), osteoporosis, tumors, and other skeletal disorders—represent a global public health issue that imposes a serious economic burden [1,2]. These serious diseases are challenging to treat due to factors such as patient age, defect size, and the compromised self-healing abilities of bone tissue [3,4]. However, in response to these challenges, the field of skeletal tissue engineering has witnessed a surge in the application and development of

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biomaterials-based strategies tailored to accommodate the specific type and severity of the ailment [5,6]. Various biomaterials—ranging from metals, polymers, ceramics, to composites—have emerged as viable options for bone regeneration owing to their exceptional biocompatibility [7,8]. In the field of tissue engineering, there has been a notable shift in the ongoing development of biomaterials, transitioning from bioinert to bioactive matrices [9,10]. Metallic biomaterials exhibit remarkable advantages, including superior biocompatibility, robust mechanical strength, inherent bioactivities, and cost-effective manufacturing processes, making them highly promising for applications in bone tissue engineering. While traditional metallic biomaterials like stainless steel, titanium alloys, and cobalt-chromium alloys offer excellent wear resistance, biocompatibility, and mechanical strength, they also have several drawbacks, including stress shielding effects, the emergence of metal artifacts, and the need for additional surgery for their removal [11,12]. Resorbable polymers have emerged as an alternative orthopedic implant material, but their inferior mechanical properties can generate local acid degradation products, leading to noninfectious inflammatory reactions [13,14].

Liu and colleagues defined biodegradable metals (BMs) have been defined as "*metals expected to corrode gradually in vivo, with an appropriate host response elicited by released corrosion products, which can pass through or be metabolized or assimilated by cells and/or tissue, and then dissolve completely upon fulfilling the mission to assist with tissue healing with no implant residues*" [15]. The development of BMs has focused on the use of magnesium (Mg), iron (Fe), and zinc (Zn), as well as their alloys/composites. The utilization of degradable metallic implants in orthopedic surgeries can mitigate the challenges linked to the subsequent removal of non-degradable metallic implants. The Mg-based implants offer the additional benefits superior mechanical properties [16], promotion of bone metabolism [17], and potential treatment for medication-related osteonecrosis of the jaw [18]. Nevertheless, the rapid degradation and release of hydrogen remain areas of concern. In contrast, Fe and its alloys exhibit relatively slow degradation with insoluble degradation products remaining in physiological environments [19]. The research and development of Zn-based BMs for bone fixation applications can be traced back to the early 2010s, proposed by Vojtech et al. [20]. Over the last decade, pure Zn and Zn-based alloys/composites have emerged as a new generation of BMs because of their acceptable biocompatibility, moderate biodegradation, superior mechanical properties, and potential bioactivity [21,22].

When assessing the primary advantages of Zn-based BMs, it is important to note that the intrinsic corrosion profile of Zn ranges between those of Mg and Fe. Unlike Mg-based BMs, the degradation of Zn-based BMs does not involve hydrogen release, thereby avoiding potential adverse effects and supporting tissue healing [23]. A recent study investigated the *in vivo* comparative performance of additively manufactured Mg-based and Zn-based scaffolds during the healing process. The main finding revealed that Mg scaffolds led to delayed yet complete defect healing with bone ingrowth, while Zn scaffolds facilitated early healing and the formation of high-quality new bone, despite the restricted calcification of osteoid within the Zn scaffold [24]. In contrast to Fe-based BMs, the degradation products released from Zn-based BMs can be safely metabolized and eliminated, at least in animal models *in vivo* [21,25]. Zn-based implants for bone repair and regeneration, such as internal fixation materials, scaffolds, and barrier membranes, have been developed and examined using various fabrication techniques, including casting, powder metallurgy, extrusion, and additive manufacturing [26,27]. To date, numerous *in vivo* studies have been conducted to evaluate the effectiveness and safety of Zn-based BMs for bone repair and regeneration. Particularly noteworthy is the registration of clinical trials for Zn-based implants, aimed at treating maxillofacial bone fractures (ChiCTR2100051050) and anterior cruciate ligament reconstruction (ChiCTR2300072163). Therefore, Zn-based BMs exhibit potential beneficial effects for bone repair and regeneration, offering unprecedented advantages over conventional treatment alternatives for bone defects.

Recent reviews have concentrated on aspects such as fabrication techniques [28,29], mechanical properties [29–31], surface modification [32,33], and potential clinical applications of Zn-based BMs [23, 34–36]. However, the bioactive effects of Zn-based implants for bone applications remain unclear. Our review aims to focus on the state of the art in terms of Zn-based implants for bone repair and regeneration, with a specific emphasis on bridging the gap between biological mechanisms and the required bioactivity. First, this review elucidates the molecular mechanisms behind the promotion of bone regeneration by Zn ions. Considering disease types and clinical application, the required bioactivity and biofunctionality of implant materials is highlighted. As illustrated in Fig. 1, based on the clinical applications (i.e., internal fixation, tissue scaffold, and barrier membrane), this review summarizes the bioactivity of Zn-based metals for bone repair and regeneration. Finally, the challenges and prospects are proposed to provide insights for the clinical translation of Zn-based metals.

2. Underlying mechanisms for the improvement of bone repair and regeneration through Zn-based metals

Considering bone repair and regeneration, the degradation products of Zn-based implants contribute to the sophisticated bone microenvironment. Over the process of bone healing, complex interactions between various Zn degradation products and major skeletal cell populations (*i.e.*, osteoblasts, osteoclasts, endothelial cells, and immune cells) can occur, determining the host response to the Zn-based implant [37,38]. To date, previous *in vivo* studies have demonstrated acceptable *in vivo* biocompatibility of Zn and its alloys/composites during long-term degradation processes [39–41]. Although no systematic toxic effects were reported, it is evident that fibrous encapsulation around Zn-based implants occurs during long-term degradation, probably attributable to the local release of degradation products [42]. As shown in Fig. 2, various animal bone defect models have demonstrated that Zn-based plates/screws accelerated bone fracture repair [43], Zn-based scaffolds enhanced new bone formation [44], and Zn-based membranes promoted bone regeneration [45]. The bioactivity and biofunctionality of Zn-based BMs are mainly based on their degradation resulting in Zn ions release. Understanding of the temporal and spatial distribution of Zn ions is critical for the success prediction of clinical applications. Herein, the impact of extracellular Zn ions on cellular function and the molecular mechanism underlying the beneficial effects of Zn-based implants are summarized systematically and in detail. In general, Zn ions have multiple functions in bone repair and regeneration, including the promotion of osteogenic differentiation of mesenchymal stem cells (MSCs), the enhancement of osteoblastic bone formation, and the inhibition of osteoclastic bone resorption [46,47]. Also, Zn ions may stimulate angiogenesis and induce immunomodulation, leading to new bone formation [48].

2.1. Zn-mediated osteogenic differentiation

A common finding shows that Zn ions promote cell proliferation, alkaline phosphatase (ALP) activity, osteogenic differentiation, and calcium deposition of MSCs in both primary and established cell lines [49–53]. In the early stage of osteogenic differentiation, extracellular Zn ions upregulate the expression of specific genes in MSCs, such as ALP, runt-related transcription factor 2 (RUNX2), and type 1 collagen [49, 54]. During middle and late differentiation stages, Zn-supplementation significantly enhances expression of late osteogenic markers by MSCs (*i.e.*, osteocalcin and osteopontin) [55,56]. More importantly, the impact of Zn on cell function regulation is dominated by dose-dependent effects. Extracellular Zn concentrations have double-edged effects on bone marrow-derived MSCs (BMSCs). Low Zn ion concentration (2–5 μg mL^{-1}) can enhance the initial adhesion and proliferation of BMSCs and subsequently regulate Zn transportation to induce osteogenic

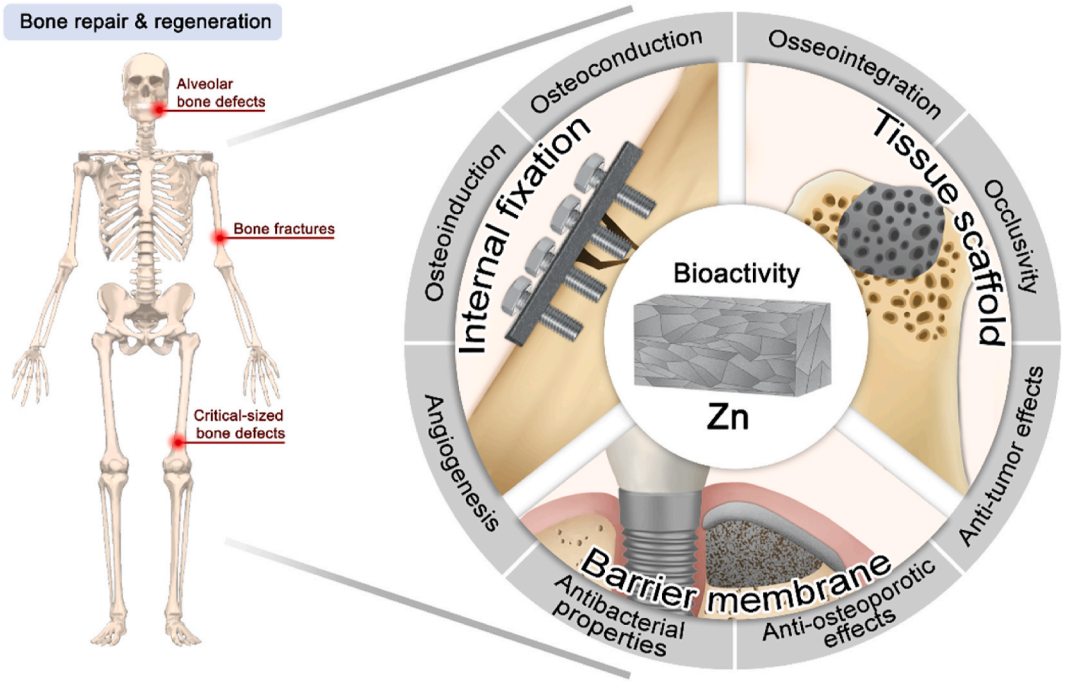

Fig. 1. Schematic illustration of possible clinical applications of Zn-based biodegradable metals, including Zn-based internal fixation for bone fractures, Zn-based scaffold for critical-sized bone defects, and Zn-based barrier membrane for alveolar bone defects.

differentiation. On the contrary, an excessively high Zn ion concentration (5 μg mL^{-1}) reduces cell adhesion and proliferation and inhibits subsequent osteogenic differentiation because of the resulting Zn homeostasis imbalance [51]. Low-concentrated Zn extracts containing degradation products of Zn-based BMs, led to enhanced osteogenic differentiation of periosteal stem cells, whereas high-concentrated Zn extracts inhibit calcium deposition [49]. Similarly, the *in vitro* study by Xiong et al. [57] showed that a Zn ion concentration of 10.91–27.15 μM can promote the ALP activity in BMSCs and increase the expression of osteogenesis-related genes, while a Zn concentration exceeding 128 μM significantly inhibits the ALP activity of BMSCs [57].

The underlying molecular mechanism of Zn-mediated regulation is based on the activation of the mitogen-activated protein kinase (MAPK) pathway, the protein kinase B (AKT) pathway, the protein kinase A (PKA) pathway, and the transforming growth factor β (TGF-β) pathway [58]. MAPK signal transduction is considered as one of the main signaling pathways in the osteogenic process [59]. Zhu et al. demonstrated that implant-derived Zn ions promoted osteogenic differentiation via activation of the MAPK pathway and the Gαq-PLC-AKT pathway (Fig. 3A) [58]. In particular, Zn ions released from Zn-based BMs have been incorporated into human MSCs through receptors/channels GPR39/ZnR and TRPM7. As soon as they are internalized, Zn ions activate the cAMP-PKA pathway and the parallel Gαq-PLC-AKT pathway, triggering intracellular calcium (Ca) ion responses. This leads to the activation of MAPK and AKT pathways, which regulate related gene expression, resulting in MSC survival, growth, and differentiation. Park et al. [60] demonstrated that Zn ions promote osteogenic differentiation through the activation of RUNX2 in the downstream signaling pathways of the cAMP-PKA-CREB axis. Zn ions increase intracellular cAMP levels in a dose-dependent manner. Further, PKA activity can be enhanced following increased intracellular cAMP levels, leading to the promotion of osteogenic differentiation in human BMSCs [60]. Additionally, activation of the TGF-β/BMP signaling pathway has been associated with Zn-mediated osteogenic differentiation of MSCs [61–63]. Gao et al. reported that Zn-containing BMs can upregulate the TGF-β pathway, according to the Kyoto Encyclopedia of Genes and Genomes [63]. Cho et al. [64] noted that Zn deficiency in osteoblasts causes Smad-1 activation and downregulation of RUNX2 expression through the BMP-2 signaling pathway, suppressing osteoblast differentiation, as shown in Fig. 3B. In contrast, with Zn level increased in media, both RUNX2 expression and Smad-1 activation can be enhanced, demonstrating that Zn ions promote RUNX2 via canonical BMP-2 signaling [64].

High concentrations of zinc ions have been shown to exert an inhibitory effect on osteogenic differentiation, and the pivotal process through which undifferentiated mesenchymal stem cells evolve into bone-forming cells [49,57]. One molecular mechanism contributing to this inhibition entails zinc ions interfering with crucial signaling pathways and regulatory molecules that orchestrate osteogenic differentiation. Specifically, elevated zinc concentrations can perturb the balance of intracellular signaling pathways, including those involved in promoting cell apoptosis, such as the Wnt/β-catenin pathway, which plays a critical role in fostering osteoblast differentiation and bone formation [65].

2.2. *Zn-mediated inhibition of osteoclastic bone resorption*

Zn, as an essential trace metallic element, plays a critical role in osteoclastogenesis [66]. Previous *in vitro* studies demonstrated that Zn ions are able to inhibit osteoclastogenesis in a dose-dependent manner [67–71]. Existing literature is inconsistent in terms of the effective Zn ion concentration. While certain studies reported inhibiting effects on osteoclastogenesis by Zn ion concentrations above 1 μM [71,72], other studies reported similar effects occurring at sub-nanomolar Zn concentration [73]. Zn substitution can modulate osteoclast activity by inhibiting tartrate-resistant acid phosphatase activity, which represents an early marker during osteoclast maturation [71,74,75]. However, Zn can also induce osteoclast apoptosis. Culturing of osteoclasts on Zn-containing tricalcium phosphate (TCP) discs has been shown to induce osteoclast apoptosis with increasing Zn concentrations [76].

The underlying mechanisms for Zn-mediated inhibition of osteoclastogenesis involve the nuclear factor (NF) of activated T-cells cytoplasmic 1 (NFATc1) signaling pathway and the NF-κB signaling pathway. NFATc1 activity is considered as a master transcription

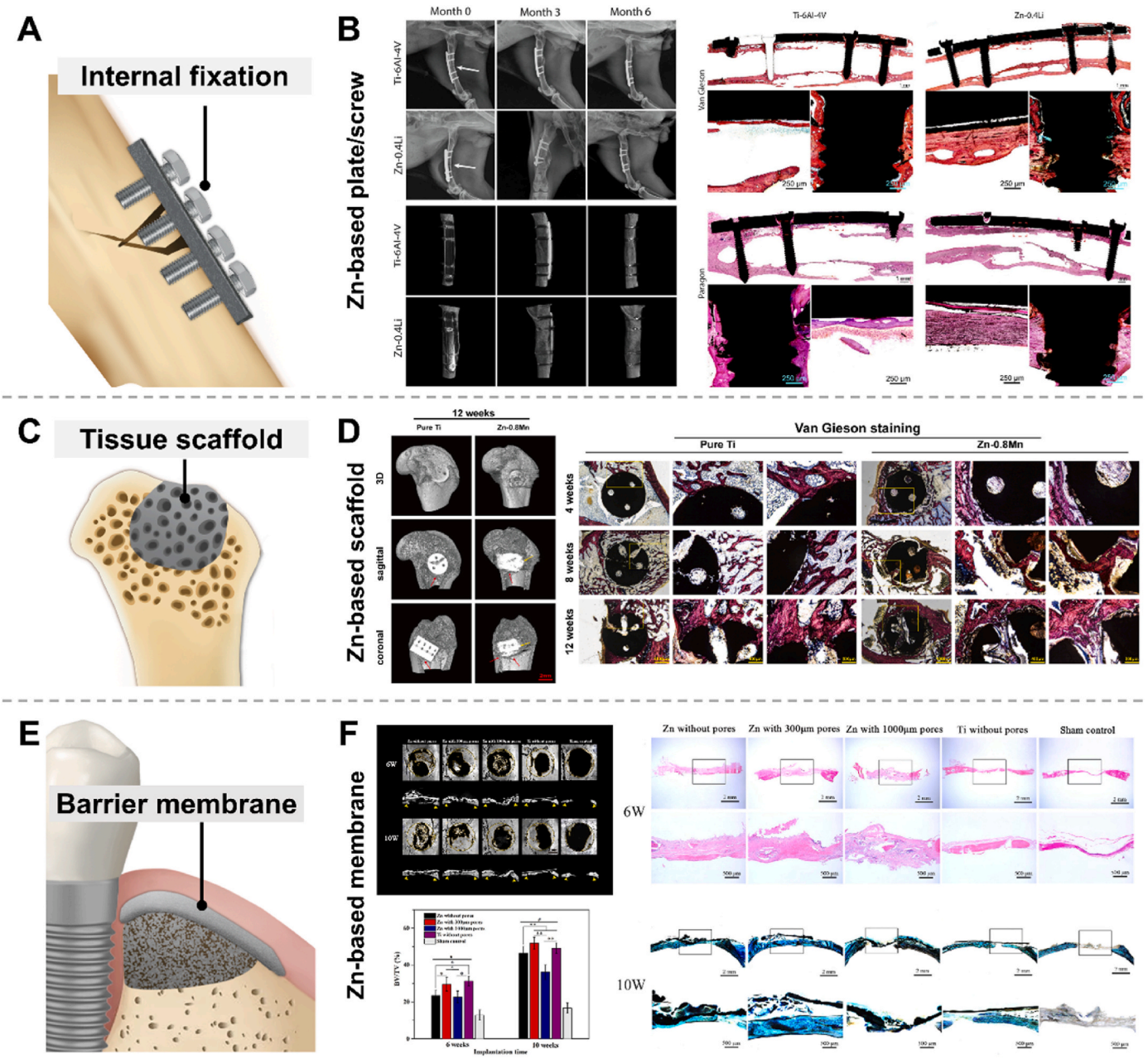

Fig. 2. Various applications of Zn-based implants for bone repair and regeneration. A) Schematic illustration of internal fixation for bone fractures. B) Representative X-ray images and histological examination showing Zn-based plate/screw accelerated bone fracture repair (reproduced with permission from Ref. [132]). C) Schematic illustration of tissue scaffold for critical-sized bone defects. D) Representative X-ray images and histological examination showing Zn-based scaffold enhanced new bone formation (reproduced with permission from Ref. [44]). E) Schematic illustration of barrier membrane for alveolar bone defects. F) Representative X-ray images and histological examination showing Zn-based guided bone regeneration (GBR) membrane effectively promoting bone regeneration (reproduced with permission from Ref. [45]).

regulator of osteoclastogenesis [77,78]. Park et al. [68] reported the inhibitory effect of Zn ions during osteoclastogenesis via the Ca^{2+}-calcineurin-NFATc1 signaling pathway. Their work demonstrated that Zn ions can suppress calcineurin at an early stage and inhibit calcium oscillations by blocking calcium influx from extracellular space during the middle or late stages of osteoclast differentiation (Fig. 3C). Furthermore, Zn-mediated inhibition of osteoclastogenesis was associated to the antagonism of NF-κB activation [70]. Specifically, Zn ions can suppress the induction of receptor activator of nuclear factor κB ligand (RANKL) of an NF-κB-luciferase reporter in osteoclast differentiation [95]. Similarly, an *in vivo* study by Hie and Tsukamoto [71] showed that Zn administration can inhibit osteoclastogenesis by downregulating receptor levels of NF-κB (RANK) by suppressing the production of reactive oxygen species and extracellular signal-regulated kinase (ERK). In addition, Zn ions can trigger crosstalk between osteoblast metabolism and osteoclastic differentiation by regulating the RANK/RANKL/OPG pathway [79,80]. However, Zn-stimulated crosstalk between osteogenesis and osteoclastogenesis requires further examination.

2.3. *Zn-stimulated angiogenesis contributes to the process of osteogenesis*

The mutual dependence of both processes of angiogenesis and osteogenesis is critical considering that an impaired angiogenic ability

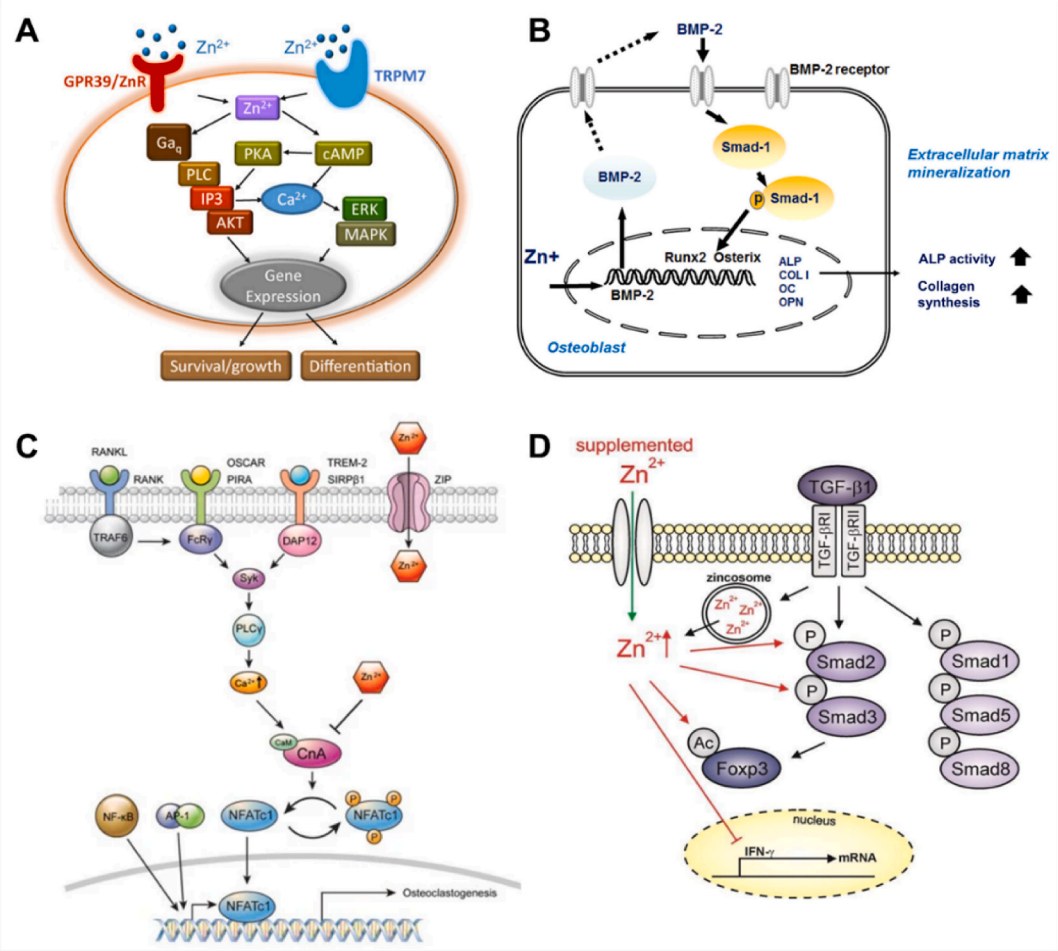

Fig. 3. Zn-stimulated cellular reactions and underlying molecular mechanisms. A) Zn implant-mediated signaling pathway in human mesenchymal stem cells (reproduced with permission from Ref. [58]). B) Zn contribution to the bone morphogenetic protein-2 (BMP-2) signaling pathway in osteoblasts (reproduced with permission from Ref. [64]). C) Inhibitory effects of Zn on RANKL-induced osteoclastogenesis (reproduced with permissions from Ref. [68]). D) Effects of Zn supplementation on the activation of the Smad signaling pathway in T cells (reproduced with permission from Ref. [103]).

can increase the occurrence of nonunions and delay bone repair [81,82]. Zn ions have been shown to have *in vitro* pro-angiogenic effects but in a strongly dose-dependent manner. This means that a high concentration (140 μM) of Zn ions has adverse effects on the angiogenic behavior of endothelial cells. In contrast, low concentrations of Zn ions (60 μM) can effectively promote the viability, proliferation, and migration of endothelial cells and upregulate related gene expression [83]. In an *in-ovo* chorioallantoic membrane test, endothelial cultures were exposed to Zn ion gradients, and the results showed that an appropriate Zn ion concentration of 50 μM can promote angiogenesis and significantly increase the levels of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) [84].

As already mentioned for MSCs, a physiologically relevant concentration of 25 μM activates the Zn ion-sensing receptor coupled with the G protein-receptor (ZnR/GPR39), triggering Gαq signaling pathways. Subsequently, activation of PLC, AKT, MAPK, phosphoinositide 3 (PI3), and extracellular signal-related kinase (ERK)1/2 signaling pathways follows. These pathways initiate physiological cell functions (*i.e.*, survival, proliferation, inflammation, and angiogenesis) in vascular endothelial cells [85]. HIF-1α is a transcription factor that regulates VEGF expression via the NF-κB pathway activation [86,87]. Expression of hypoxia-inducible factor 1 alpha (HIF1-α) can be stabilized by hypoxic conditions and increased lactate levels [88]. High Zn ion concentrations can downregulate HIF-1α levels, thereby suppressing VEGF expression under hypoxic conditions [87].

2.4. Zn-mediated immunomodulation may couple osteogenesis

The immune system has a significant impact on bone tissue regeneration [89]. Immune cells, mainly including macrophages and T cells, can indirectly promote bone repair and regeneration through cytokine secretion, which interacts with various bone-related cells [90]. The ability to timely resolve acute inflammation through the innate immune system is critical for normal bone healing. This is coupled with the transition of pro-inflammatory M1 macrophages into pro-regenerative M2 macrophages [91,92], or/and with the activation of the Th2 phenotype and of regulatory T cells [93,94]. The crosstalk between immune cells and bone niche cells can promote the process of bone repair and vascularization, bone deposition, and remodeling [90]. To date, biomaterial-based immunomodulatory strategies for tissue regeneration have largely focused on the activation of macrophage phenotypes and the balance of T lymphocytes [95,96].

Zn-containing biomaterials can boost the M1/M2 macrophage switch and induce the secretion of anti-inflammatory and osteogenic cytokines, thus creating a microenvironment favorable for bone healing [97–99]. Zn ions released from Zn silicate incorporated into calcium phosphate cement were shown to downregulate inflammatory-related gene expression (*i.e.*, interleukin-1, interleukin-6, and tumor necrosis factor) and to upregulate anti-inflammatory gene expression (interleukin-10) of mouse bone marrow MSCs, subsequently suppressing inflammation and osteoclastogenesis of RAW264.7 cells [100]. Furthermore, exosomes secreted by macrophages cultured under Zn ion

supplementation (4 μM) were able to enhance the ALP activity of osteoblasts, indicating that compounds of the exosomes mediate Zn-induced immunomodulatory functions [101]. Nevertheless, the effect of Zn ions on the potential molecular mechanisms of macrophages should be investigated in future research.

Zn supplementation was demonstrated to induce the polarization of regulatory T cells (Treg). Specifically, in an experimental set-up with mixed lymphocyte cultures treated with a Zn ion concentration of 50 μM, the Th1-cytokine (particularly IFNγ) production was inhibited and the number of Treg cells increased [102]. Regarding the potential mechanisms of Treg polarization, Zn ions can augment the activation of the TGFβ1-induced signaling pathway in association with an increased Smad 2/3 activation, thus increasing forkhead box P3 expression stabilization (Fig. 3D) [103]. Nevertheless, the long-term impact of Zn-based BMs on the adaptive immune response remains obscure.

3. Zn-based internal fixation for bone fractures

Bone fracture refers to the abrupt disruption of the continuity of bone tissue, caused by injury, trauma, stress, and bone diseases (*i.e.*, osteoporosis) [104,105]. The basic goal of fracture treatment is the restoration of the disrupted osseous anatomy and sufficient stabilization of the fractured bone [106]. Compared with non-operative treatment, the implementation of standardized fracture fixation techniques with bone plates can achieve the benefits of skeletal stabilization in complex and no-fusion fractures. This approach was proposed by the Swiss "Arbeitsgemeinschaft für Osteosynthesefragen" group [107]. The utilization of an appropriate biomaterial plays a critical role in the internal fixation of bone fracture. However, conventional bone plate/screws, fabricated from non-absorbable metallic materials (*i.e.*, stainless steel or titanium-based alloy), still have limitations including implant fracture/loosening, stress shielding, and the need for their removal by secondary surgeries [4,108]. Therefore, the development of novel internal fixation is crucial.

3.1. Bone fracture healing: biological mechanism and required bioactivity

Bone fracture healing involves a series of complex physiological processes, triggering interactions between various cellular and biomechanical factors. The whole process of bone fracture healing can be divided into four stages: hematoma formation and inflammation, fibrocartilaginous callus formation, bony callus formation, and bone remodeling [81,109,110]. First, hematoma formation around the fracture site is induced immediately following the fracture by blood vessels that supply the bone and periosteum. The secretion of pro-inflammatory cytokines and interleukins initiates cascades of cellular events and attracts monocytes, macrophages, and lymphocytes [111]. Subsequently, mesenchymal stem cells are recruited to the fracture site where they are further differentiated into fibroblasts, chondroblasts, and osteoblasts, leading to hematoma tissue replacement by cartilage callus [112]. During the stage of bony callus formation, cartilaginous callus begins to show endochondral ossification. Also, intramembranous ossification occurs, leading to the formation of a hard callus in the subperiosteal area [112,113]. Specifically, osteoblasts and osteoclasts are differentiated, leading to the resorption of cartilaginous callus and the formation of new bone tissue [113]. Finally, with the balance of resorption by osteoclasts and new bone formation by osteoblasts, the hard callus undergoes repeated remodeling, called coupled remodeling. The hard callus is ultimately replaced by compact bone and lamellar bone, and substantial remodeling of the vasculature occurs over a period of several months [109].

In general, the treatment principle of healing bone fractures is reduction and fixation, followed by rehabilitation treatment [114]. The specific management depends on the cause, type, and location of bone fractures. Operative fracture fixation is an essential treatment option for complex bone fractures, such as comminuted fracture, intra-articular fracture, and craniomaxillofacial fracture. The classical principles of operative fracture fixation include: 1) restoration of the disrupted osseous anatomy, 2) sufficient stabilization of the fractured bone, 3) adequate blood supply to the fracture area, and 4) permission of early motion without pain, which ultimately results in an optimal functional recovery of the fractured bone [105,114]. The core concept of fracture treatment can be summarized as 'early functional rehabilitation'. Thereby, stable fixation plays a critical role in eliminating motion at the fracture site and allowing early mobilization of the injured extremity. In terms of fracture fixation, stability refers to either absolute stability (where any micro-motion is abolished at the fracture zone under physiological loading) or relative stability (where most motion is prevented at the fracture zone, although micro-motion may still exist) [105]. The simplest and most effective way to achieve absolute stability is the exertion of interfragmentary compression via plate/screw fixation. In addition, to achieve relative stability, biological plate fixation (*i.e.*, indirect reduction and bridge plates), external fixation, and intramedullary nail fixation can be used in the absence of interfragmentary compression [105,115].

Based on the mechanism of bone fracture healing and relevant clinical considerations, the required bioactivity and biofunctionality of internal fixation can be summarized as follows: 1) Osteoinduction: Osteoblastic differentiation and mineralization are promoted, and bone fracture healing is accelerated. 2) Osseointegration: Bone screws/nails should avoid fibrous encapsulation, as this would lead to loosening and adverse effects on stability [116,117]. 3) Angiogenesis: materials should allow and promote angiogenesis, facilitating blood supply to the fracture area. 4) Antibacterial properties: materials should prevent infection at implantation sites, especially at the initial healing stage. 5) Anti-osteoporotic properties are important for the treatment of osteoporotic bone fractures.

3.2. Bioactivity of Zn-based biodegradable metals for internal fixation

In 2011, Vojtěch et al. [118] published the first study reporting on the feasibility of bioabsorbable bone fixation by using Zn-based alloys. They found that the *in vitro* degradation rates of Zn–Mg alloys ranged in the order of tens of microns per year, and suggested that Zn doses and toxicity may be tolerated. In 2013, a landmark study by Bowen et al. [119] demonstrated that pure Zn exhibits ideal physiological corrosion behavior in the abdominal aorta of Sprague-Dawley rats. Also, Li et al. [120] reported good biocompatibility of Zn-1X binary alloys with nutrient alloying elements in the bony environment, identifying Zn-based alloys as promising materials for bone applications. In the following years, research focused on Zn-based BMs for internal fixation, mainly including the development of novel Zn-based alloys with high mechanical properties, the exploration of *in vivo* biocompatibility and bioactivity of Zn-based alloys, and the investigation of biodegradation in the bony physiological environment. As summarized in Table 1, Zn-based internal fixation applications (*i.e.*, plates, screws, and nails) have been extensively studied in different animal models, such as the medullary cavity model, the bone fracture model, and the infection-prevention model. Herein, the recent research status of Zn-based internal fixations is further summarized and analyzed in terms of required bioactivity and biofunctionality.

Zn-based internal fixation has been demonstrated to promote new bone formation, which has been reported by most *in vivo* studies [22,120,122–124,127,132,134,135,145]. Compared to conventional materials (*i.e.*, poly-*l*-lactic acid and Ti-based alloy), Zn-based BMs exhibit better osteoinduction [127,132]. Specifically, Wang et al. [127] examined a Zn alloy osteosynthesis implant for the mandibular bone fracture model. The histomorphometric results showed that in the Zn alloy group, new bone formation was clearly higher than in the poly-*l*-lactic acid group. In addition, a Zn-0.4Li fixation plate and screw were placed in a rabbit femoral shaft fracture model. After implantation for 6 months, more new cortical bone had formed at the fracture site in the

Table 1
Representative *in vivo* studies on Zn-based biodegradable metals for internal fixations.
(1) Osteoinduction

Zn-based BMs (wt%)	Working history	Designed implant	Animal model	Implant site	Control	Duration	Degardation rate	Main key findings	Ref.
Zn–1Mg, Zn–1Ca, Zn–1Sr	Rolled	Nail	C57BL/6 mice	Medullary cavity of femoral shaft	Sham control	8 w	0.17 mm/year 0.19 mm/year 0.22 mm/year	The new bone thickness of the Zn–1Mg, Zn–1Ca, and Zn–1Sr pin groups is significantly higher than that of the sham control group.	[120]
Zn-0.05 Mg	Extruded	Pin	Rabbit	Femoral shaft	Pure Zn	24 w	N. A.	The Zn and Zn-0.05 Mg alloy can promote the formation of new bone tissue at the interface between the implant material and the bone tissue.	[121]
Zn-5HA	Spark plasma sintering	Pin	Sprague-Dawley rat	Femur condyle	Pure Zn	8 w	Volume loss: 3.2 %	The osteogenesis performance of the Zn-5HA composite was better than that of pure Zn, and the former did not lead to obvious inflammatory reactions.	[122]
Zn–5Mg composite	Spark plasma sintering	Pin	Sprague Dawley rat	Femoral condyle	Pure Zn	8 w	Volume loss: 2.4 %	The Zn–5Mg composite exhibited higher osteogenesis and osseointegration abilities than pure Zn.	[123]
Zn	Extruded	Pin	Sprague-Dawley rat	Femur condyle	N. A.	4 w	N. A.	New bone formation was observed around the Zn implant, whereas some fibrotic and collagenous tissues could be found between the implants and newly formed bone.	[124]
Zn	N. A.	Pin	Sprague-Dawley rat	Femur condyle	N. A.	4 w	N. A.	Some connective tissues can be formed at the bone tissue–implant interface. Moreover, the degradation products were not uniformly distributed on the Zn surface.	[125]
Zn–2Mg	Extruded	Hemispherical implant	Wistar rats	Rat's cranium	Mg–4Y–3RE alloy	12 w	0.10 mm/year	Zn–2Mg had no adverse effects on the behavior or physical condition of rats and did not cause inflammatory reactions. The corrosion of Zn–2Mg was relatively slow and uniform.	[126]
Zn-(0.001 % < Mg < 2.5 %, 0.01 % < Fe < 2.5 %)	Extruded	Plate and screw	Beagles	Mandibular fracture	Poly-*l*-lactic acid and Ti alloy	24 w	0.095 ± 0.009 mm/year	The Zn alloy possesses good mechanical properties that support fracture healing. The new bone formation around the Zn alloy was significantly higher than that detected in the poly-*l*-lactic acid group.	[127]
Zn-0.4Cu Zn-2.0 A g Zn-0.4Li Zn-0.4Fe Zn-0.1Sr Zn-0.8 Mg Zn-0.8Ca Zn-0.1Mn	Extruded	Pin	Rat	Femur shaft	Pure Zn	8 w	Ranged from 0.13 to 0.26 mm/year	New bone tissue formed around all the Zn-based implants. Larger amounts of new bone tissue continuously formed were observed around the implants in the Zn-0.4Li, Zn-0.1Mn, Zn-0.8 Mg, Zn-0.8Ca, and Zn-0.1Sr alloys. Adding the Mg, Ca, Sr, and Li elements to Zn could improve its osteogenic abilities and osseointegration.	[22]
Zn–2Cu	Extruded	Nail	Sprague-Dawley rat	Femur infection-prevention model	Blank, Ti	6 w	N. A.	The Zn–2Cu alloy showed an effective antibacterial ability and inhibited the inflammatory and toxic side effects induced by MRSA bacteria in the rat femur.	[128]
Zn–Li	ZrO_2 -nanofilm coated (Extruded)	Pin	Sprague-Dawley rat	Femur condyle	Zn–Li alloy (without coating)	12 w	N. A.	Thicker and denser new bone formation was found in the surroundings of the coated Zn-based implants. The ZrO_2 nanofilm-coated Zn effectively promotes osseointegration and controls its biodegradation behavior.	[129]
Zn–2Ag	Extruded	Pin	Sprague-Dawley rat	Osteomyelitis prevention model in rat femur	Blank, Ti	6w	N. A.	The Zn–2Ag alloy group showed significant antibacterial activity against MRSA.	[130]
		Extract	C57BL/6 mice	Ti particle-induced cranial osteolysis model	Blank, Pure Zn	2 w	N. A.	The Zn–2Ag alloy extract effectively inhibited osteoclast bone resorption, showing outstanding *anti*-osteolytic properties.	
		Screw	New Zealand rabbits	Femoral condylar split fractures	Ti-based alloy (Ti–6Al–4V)	24 w	N. A.	The Zn–2Ag-based screws showed reliable performance in bone fracture fixation in a femoral condyle fracture rabbit model.	
Pure Zn	N. A.	Screw	New Zealand rabbits	Anterior cruciate ligament (ACL) reconstruction	Mg screw Ti screw	12 w	Volume loss: 2.0 %	The screw-released Zn element effectively attenuated bone tunnel enlargement after anterior cruciate ligament reconstruction in rabbits.	[131]

(*continued on next page*)

Table 1 (*continued*)

Zn-based BMs (wt%)	Working history	Designed implant	Animal model	Implant site	Control	Duration	Degardation rate	Main key findings	Ref.
Zn-0.4Li	Extruded	Rod	Sprague-Dawley rat	Femurs shaft	Pure Zn	8 w	0.15 mm/year	The volume of new bone formed around the Zn-0.4Li alloy implant was significantly higher than that formed around the pure Zn implant.	[132]
		Plates/screw	New Zealand rabbits	Shaft fracture model	Ti-based alloy (Ti–6Al–4V)	24 w	N. A.	The Zn-0.4Li-based plates/screws exhibited a bone fracture fixation performance comparable to that of their Ti–6Al-4 V counterparts.	
Zn-0.8Li-0.1Sr	Extruded	Intramedullary nail	Sprague-Dawley rat	Osteoporotic femur bone fracture	Pure Zn	24 w	N. A.	The Zn-0.4Li alloy implants showed significantly higher osteogenic effects and higher performance for the treatment of osteoporotic bone fracture than the pure Ti implants.	[133]
Zn-0.05 Mg-(0, 0.5, 1 wt%)Ag	N. A.	Rod	New Zealand rabbits	Bilateral distal femur	Pure Zn	24 w	N. A.	The Zn–Mg–Ag alloy showed no toxicity to visceral organs and promoted new bone formation around the implant.	[134]
Zn–0.8Mg–0.2Sr	Extruded	Screw	New Zealand rabbits	Tibia bone	N. A.	120 days	13.5 μm/year	The material did not induce any inflammatory reaction or systemic toxicity. Periosteal apposition and the formation of new bone with a regular structure were frequently observed near the implant surface.	[135]
Zn–0.8Mg–0.2Sr	Extruded	Screws	New Zealand rabbits	Tibia bone	N. A.	360 days	N. A.	The alloy showed good biocompatibility and resulted in a low inflammatory response. The released degradation products were fully incorporated into the new tissue.	[136]
Zn-0.5Mn	Extruded	Rod	Sprague-Dawley rat	Tibia bone	Stainless steel (SUS304)	120 days	N. A.	The Zn-0.5Mn implant can promote the restoration of the medullary damage and causes no inflammation or damage to the liver and kidneys.	[137]
Zn-0.5Mn	Extruded	Rod	Sprague-Dawley rat	The proximal end of the tibia	Stainless steel (SUS304)	12 days	N. A.	The unimodal ultrafine-grained Zn-based implants could induce bone formation and revascularization in the early period. Moreover, the implant does not cause inflammatory responses or damage to the liver and kidneys *in vivo*.	[138]
Zn-0.8Li-0.5Ag	Extruded	Intramedullary nail	Sprague-Dawley rat	MRSA-induced osteomyelitis model	Blank, Ti	6 w	N. A.	The Zn-0.8Li-0.5Ag alloy implants showed good antibacterial and osteogenic properties. Furthermore, the alloy is biologically safe for *in vivo* use.	[139]
Zn-(0.001 % < Mg < 2.5 %, 0.01 % < Fe < 2.5 %)	Extruded	Plate and screw	Beagles	Mandibular fracture	N. A.	12 m	0.183 mm/year at 3 months, 0.065 mm/year at 12 months	Zn-based implants had good *in vivo* biocompatibility. The zinc content of the bone significantly increased in the 3–6-month post-implantation period. A trend of replacement of degradation products by bone was observed.	[140]
Zn-0.7Sr Zn–4Sr	Extruded	Pin	Sprague-Dawley rat	Femoral tissue	Pure Zn	12 w	0.028 mm/y 0.035 mm/y 0.020 mm/y	The Zn-0.7Sr implant promoted the new bone formation and increased osseointegration ability compared to that of the other two implants.	[141]
Zn	N. A.	Pin	Sprague-Dawley rat	*Staphylococcus aureus* (*S. aureus*)-infected rat model	Ti	8 w	N. A.	Zn is favorable for the formation of neutrophil extracellular traps by regulating the release of DNA fibers and granule proteins in a reactive oxygen species-dependent manner, thereby promoting osseointegration in *S. aureus*-infected rat femurs.	[142]
Zn-0.5 V, Zn-0.5Cr, Zn-0.5Zr	Extruded	Pin	Sprague-Dawley rat	Femoral tissue	Zn	12 w	Ranged from 0.03 to 0.05 mm/year	Zn alloys induced significantly higher bone-implant contact ratios, indicative of a higher osseointegration ability.	[143]
Zn–La, Zn–Ce, Zn–Nd	Extruded	Pin	New Zealand rabbits	Tibia bone	Zn	12 w	N. A.	Three alloys, Zn–La, Zn–Ce and Zn–Nd, exhibit superior *in vivo* biocompatibility and the improved osseointegration in the animal tibia model compared to that of the pure Zn.	[144]

N. A.: not available, MRSA: methicillin-resistant *Staphylococcus aureus*.

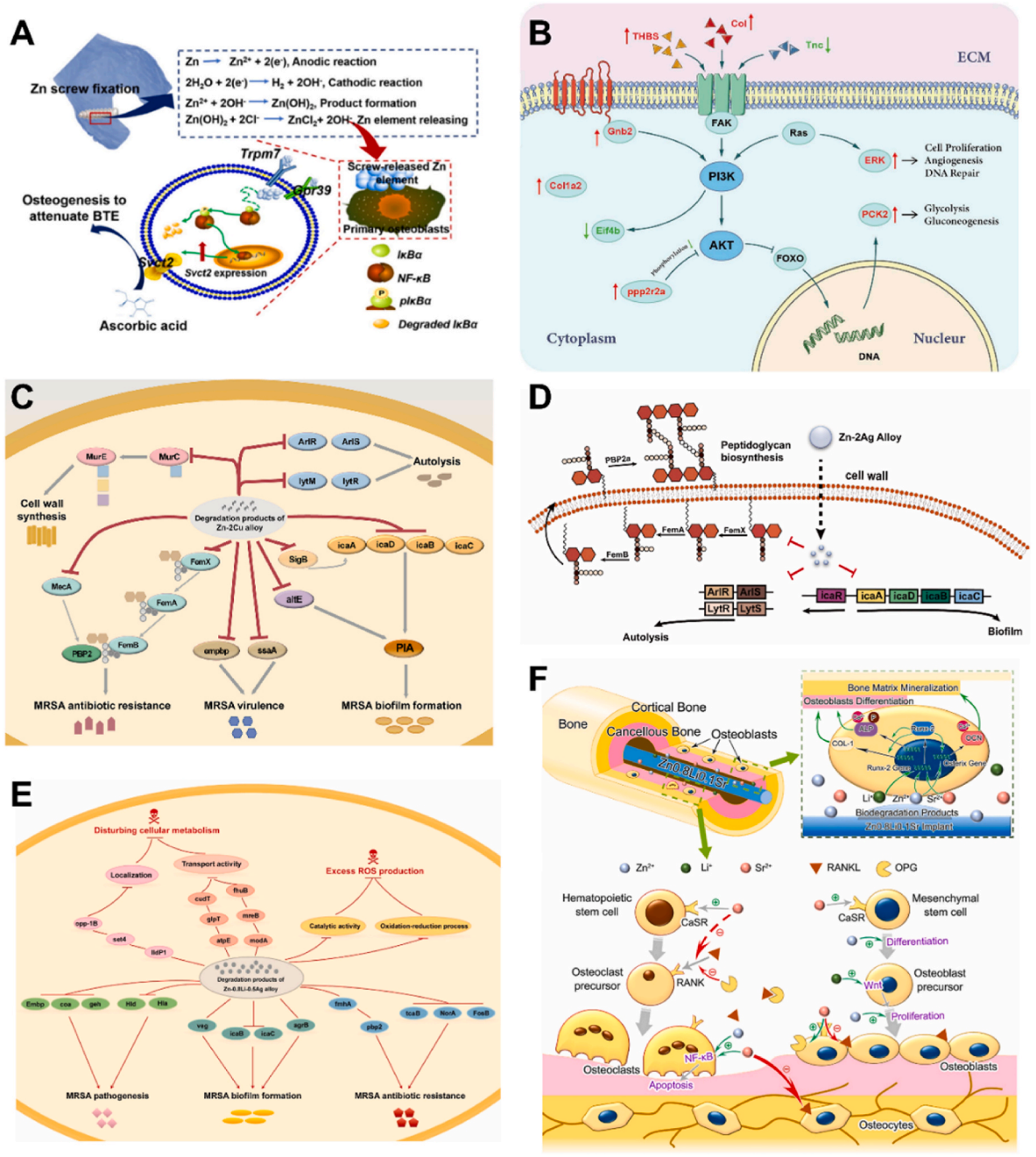

Fig. 4. Possible molecular mechanisms of Zn-based internal fixation materials. A) Zn ions released from pure Zn screws induce Svct2 expression in primary osteoblasts via the NF-κB pathway (reproduced with permission from Ref. [131]). B) Zn-0.4Li based alloy induces the PI3K-AKT signaling pathway during bone repair (reproduced with permission from Ref. [132]). C) Suggested underlying anti-microbial mechanism of Zn–2Cu alloys against methicillin-resistant *Staphylococcus aureus* (MRSA; reproduced with permission from Ref. [128]). D) Possible bactericidal mechanism of Zn–2Ag alloys (reproduced with permission from Ref. [130]). E) Proposed underlying mechanism for the antimicrobial properties of Zn-0.8Li-0.5 A g alloys against MRSA (reproduced with permission from Ref. [139].) F) Mechanism of gene-induction by Zn^{2+}, Li^{+}, and Sr^{2+} ions in osteoblasts, which further influences bone remodeling (reproduced with permission from Ref. [133]).

Zn-0.4Li alloy group, compared with the Ti-based alloy group [132]. These better osteopromotive properties of Zn-based BMs are partly the result of the osteoinductive effects of Zn ions released from implants during their degradation [127,132]. Notably, as shown in Table 1, previous *in vivo* studies showed that Zn-based alloys or composites have better osteoinductive properties than pure Zn. Yang et al. [22] implanted different Zn-based pins in rat femurs. Their histological analysis showed that addition of the elements Mg, Ca, Sr, and Li to pure Zn can improve the new bone area, probably because of the release of alloy elements. Regarding the potential mechanism of Zn-based implants, screw-released pure Zn ions upregulated the expression of sodium vitamin C transporter 2 (Svct2) in primary osteoblasts via the NF-κB pathway; this induced the expressions of RUNX2 and osterix, which promote osteogenic differentiation, as illustrated in Fig. 4A [131]. Yang et al. [132] explored the potential mechanism of Zn–Li alloy implants by bioinformatics analysis. As shown in Fig. 4B, the degradation products released from the Zn-0.4Li alloy could induce new bone formation through the activation of the PI3K-AKT pathway and stimulation of metallothionein proteins (MT1 and MT2).

(2) Osseointegration

To improve osseointegration of bone screws, direct bone bonding to implants can facilitate the stabilization of the osteosynthesis system for fractured bone healing [146]. An important finding of previous *in vivo* studies is that pure Zn implanted in the bony environment leads to the formation of fibrous connective tissue between implants and newly formed bones, probably adversely affecting the stabilization of implants [22,123–125,129]. This phenomenon can contribute to the fact that a rapid release of Zn ions leads to local mass accumulation around the implant. Such an excessive release of Zn ions may interfere with the formation and remodeling of new bone [123–125,129]. Thus, to improve the osseointegration of pure Zn, Zn ion release and accumulation can be controlled via different approaches, such as alloying/composite treatments and surface modifications. Yang et al. [22] suggested that Zn-based alloys (*e.g.*, Zn-0.8 Mg, Zn-0.8Ca, Zn-0.4Li, and Zn-0.1Sr alloys) should have a higher bone-implant contact ratio than pure Zn. The optimal bone-implant contact ratio is determined by the uniform corrosion mode with appropriate degradation rates of Zn-based alloys. Similar to these results, compared to pure Zn, osseointegration can be enhanced with Zn–Mg composites, which can be explained by the synergic biological effect of the co-release of Zn ions and Mg ions through preferential corrosion of a sacrificial Mg-rich phase. In addition, rare earth elements (RE) such as lanthanum (La), cerium (Ce), and neodymium (Nd) can serve as alloying elements for the biodegradable binary Zn alloys. A recent *in vivo* study has shown that implantation of Zn–La, Zn–Ce, and Zn–Nd alloys resulted in significantly improved osseointegration compared to pure Zn. This observation can be attributed to the slower degradation process of the Zn-RE alloys, leading to enhanced mechanical integrity for longer periods of implantation [144]. In another strategy, proposed by Yuan et al. [123,129], Zn-0.1Li alloys were modified as a barrier layer of the ZrO_2 nanofilm by the atomic layer deposition technique. The ZrO_2 nanofilm-coated Zn-0.1Li alloy demonstrated better *in vivo* osseointegration, which can be attributed to the decreased Zn ion release, enabling the beneficial effect of efficient Zn ion doses on new bone formation. In summary, pure Zn implants showed delayed osseointegration *in vivo*, which can be optimized in Zn composites to prevent rapid Zn ion release.

(3) Angiogenesis

Adequate blood supply to the fracture area is critical for bone healing [105,114]. Internal fixation materials that promote local angiogenesis facilitate the blood supply of the fracture area. To date, Zn-based BMs have been suggested to promote angiogenesis and induce revascularization [84,138,147,148]. Firstly, the release of Zn ions from pure Zn and Zn–Mg alloys has been shown to promote the proliferative and angiogenic properties of endothelial cells, resulting in an up-regulation of the expression of angiogenesis-related genes (*i.e.*, HIF-1, VWF, VEGF, and CD31). However, the angiogenesis process is not influenced by Mg ions released from Zn–Mg alloys [147]. Qian et al. [148] developed an organic-inorganic collagen entrapped Ca/Zn phosphate coating to control the Zn ion release of Zn substrates within a suitable concentration range. This hybrid coating was shown to enhance angiogenic properties in terms of viability, migration, and tube formation of endothelial cells. A short-term biocompatibility *in vivo* study demonstrated that unimodal ultrafine-grained Zn-0.5Mn alloy can induce revascularization in the early period of post-implantation, indicating its angiogenic potential [138]. Nevertheless, the effect of the angiogenic properties of Zn-based internal fixation on the fractured bone site remains obscure, and the underlying mechanism needs to be further examined.

(4) Antibacterial properties

Antibacterial properties of internal fixation materials can prevent post-operative infections. Theoretically, certain metal ions (such as Zn, Cu, and Ag) are regarded as antibacterial agents [149,150]. Previous *in vitro* studies demonstrated that the synergistic effects of Zn ions and Cu ions released from Zn–Cu alloys exhibit effective antibacterial activity towards *Staphylococcus aureus* (*S. aureus*) [151,152]. Notably, more than half of all orthopedic implant-related infections are caused by *S. aureus*, especially by methicillin-resistant *S. aureus* (MRSA) [153,154]. To mimic implant-related infections, an MRSA-induce osteomyelitis model was established to evaluate the anti-bacterial ability of Zn–2Cu alloys [128], Zn-0.8Li-0.5 A g alloys [139], and Zn–2Ag alloys [130]. Zn-based alloys could effectively control MRSA-induced femoral osteomyelitis *in vivo*. Nevertheless, the underlying antibacterial mechanisms of Zn-based alloys are based on various molecular mechanisms. Firstly, Zn–2Cu alloys can significantly downregulate the expression of genes related to cell wall synthesis (MurC, MurE, and saeR), bacterial adhesion (clfA, and atlE), and biofilm formation (icaA, icaB, icaC, icaD, icaR, SigB, and Spa), autolysis (SarA, lytM, lytR, ArlR, and ArlS), MRSA antibiotic resistance (PBP2a, MecA, FemA, FemB, and FemX), and bacterial virulence (empbp and ssaA), as shown in Fig. 4C [128]. The bactericidal mechanism of the Zn–2Ag alloy involves the expression of MRSA genes associated with autolysis (atlE, ArlS, lytM, lytR, and atlE), biofilm formation (icaA, icaB, icaC, icaD, Luxs, and fbe), and drug resistance (FemA, FemB, and FemX), as shown in Fig. 4D [130]. Thirdly, Zn-0.8Li-0.5 A g alloys kill MRSA mainly by disturbing its cellular metabolism (by inhibiting localization, transport, and transporter activity) and inducing the production of reactive oxygen species; this alloy could also inhibit biofilm formation and virulence of MRSA and alleviate drug resistance (Fig. 4E) [139].

(5) Anti-osteoporotic effects

With the aging of the global population, osteoporotic bone fractures have become a significant challenge for orthopedic surgeons [112]. While several internal fixation materials with anti-osteoporotic effects have been proposed and developed [155,156], further research on Zn-based internal fixation with anti-osteoporotic properties is still required. Zhang et al. [133] fabricated and investigated a novel Zn-0.8Li-0.1Sr (Zn–Li–Sr) alloy with high mechanical strength to treat osteoporotic bone fractures. Zn–Li–Sr intramedullary nails were placed in the femur bone of ovariectomized rats and their osteoporotic-bone-fracture treatment effects were evaluated and compared to Ti-based nails. The results demonstrated that the Zn–Li–Sr implant possesses superior osteogenesis-inducing and osteoporotic-bone-fracture treatment effects, compared to pure Ti implants. As shown in Fig. 4F, the osteoporotic-bone-fracture treatment mechanism of the Zn–Li–Sr implant is related to the synergic effects of Zn^{2+}, Li^{+}, and Sr^{2+} ion release, stimulating the expression of four osteogenic genes (ALP, COL-1, osteocalcin, and RUNX2) [133]. Specifically, Zn^{2+} ions can stimulate bone formation by the Wnt/β-catenin pathway [157] and inhibit bone resorption by the NF-κB pathway [158]. Li^{+} ions can promote the proliferation of osteoblasts via the Wnt/β-catenin pathway [158]. Sr^{2+} ions can stimulate bone formation by upregulating the expression of osteoprotegerin and by inhibiting bone resorption by the NF-κB pathway [159,160].

4. Zn-based bone tissue engineering scaffold for critical-sized bone defects

Large critical-sized bone defects (typically >2 cm, depending on the anatomical site) caused by traumatic injury, congenital defects, degenerative diseases, or surgical removal of tumors remain one of the primary challenges [161]. Undoubtedly, bone autografting is the gold standard for the treatment of critical-sized bone defects, but its main drawbacks are limited supply, requirement for additional operation, and donor site morbidity [162]. To overcome these limitations, tissue engineering has been first proposed by Professor Robert Langer through three-dimensional porous scaffolds [163,164]. After 30 years of development, bone tissue engineering (BTE) strategies have been extensively investigated and are being used in clinical treatment [165,166]. Current scaffold materials are made of ceramics, polymers, and bioinert metals.

The main shortcoming of ceramic-based scaffolds is their brittleness, while polymer-based scaffolds have relatively unfavorable mechanical properties [167]. Additionally, bioinert metallic biomaterials (*i.e.*, Ti and its alloys) have also been used for BTE scaffolds. However, bioinert BTE scaffolds may cause long-term endothelial dysfunction, permanent physical irritation, and chronic inflammatory local reactions [168]. Therefore, a novel bioactive material for BTE scaffolds needs to be developed.

4.1. Bone tissue engineering: biological mechanism and required bioactivity

In principle, the goal of bone tissue engineering is to produce artificial bone constructs and micro-environments that mimic both the structural characteristics and physiological behavior of autogenous bone to facilitate the regeneration and re-growth of bone tissue [169,170]. Bone tissue consists of characteristic cell types, an extracellular matrix (ECM), and biologically active molecules that are produced by cells and integrated into the ECM [171,172]. The extracellular bone matrix is a natural nanocomposite of inorganic Ca phosphate minerals and organic collagenous components (mainly type I collagen), and noncollagenous matrix components [173,174]. Inspired by the hierarchical structure of bone and its natural formation processes, tissue engineering strategies should meet three principles: make scaffolds osteoconductive, achieve osteoinduction through growth factors, and equip cells with osteogenic capability [175,176] (Fig. 5).

The three-tiered approach, i.e., osteoconductivity, osteoinductivity, and osteogenicity, are proposed and introduced to the BTE treatment. Specifically, osteoconductivity is defined as the ability to serve as a scaffold for new bone formation and vascular ingrowth [43]. The BTE scaffold typically serves as a mimicking ECM, as it provides mechanical support and a suitable environment for the attachment, proliferation, and differentiation of cells. Bone-related cells can be either recruited from surrounding native bone tissue after implantation or seeded onto the BTE scaffold before implantation [177]. Osteoinductivity is the presence of growth factors (signaling molecules) that attract bone-forming stem cells and encourage their differentiation into osteoblasts [178]. Regulatory signals, such as biological, biochemical, and biophysical factors within the ECM, can directly influence cellular activities and the fates of cells surrounding the ECM, eventually promoting bone regeneration [179]. Osteogenicity is determined by the presence of viable osteogenic cells that can promote bone formation [169,180]. The strategy of cell-based BTE combines living osteogenic cells with BTE scaffolds *ex vivo* to enable the development of a cell-seeded scaffold, that can be transferred to the bone defect site to stimulate new bone formation [181,182].

BTE strategies are considered by disease-related and patient-related factors. First, high vascular density in certain bone regions (*i.e.*, metaphysis) makes them prone to osteomyelitis infection, thereby potentially requiring antibacterial properties [183]. Additionally, bone defects can be caused by the surgical removal of tumors, such as osteosarcoma. A new multifunctional scaffold with anti-tumor effects has been proposed and developed [184,185]. BTE treatments for use in pediatric patients must consider biodegradation properties or dynamic structural properties that facilitate their tissue remodeling to aid the ongoing growth of the skeleton [186,187]. Conversely, regarding elderly individuals, the effects of natural aging on the bone microstructure should be integrated, such as anti-osteoporotic effects [188,189].

Considering the BTE mechanism and its clinical considerations, the required bioactivity and biofunctionality of BTE scaffolds can be summarized as follows: 1) Osteoconduction is defined as the ability of a scaffold to support tissue ingrowth, osteoprogenitor cell growth, and further development for bone formation [190,191]. 2) Osteoinduction is defined as the induction of osteoblastic differentiation and mineralization, which promotes new bone formation [192]. 3) Materials should promote angiogenesis, thus ensuring sufficient blood supply [193]. 4) Scaffolds should have antibacterial properties and should prevent infection during tissue healing [194,195]. 5) For anti-osteoporotic

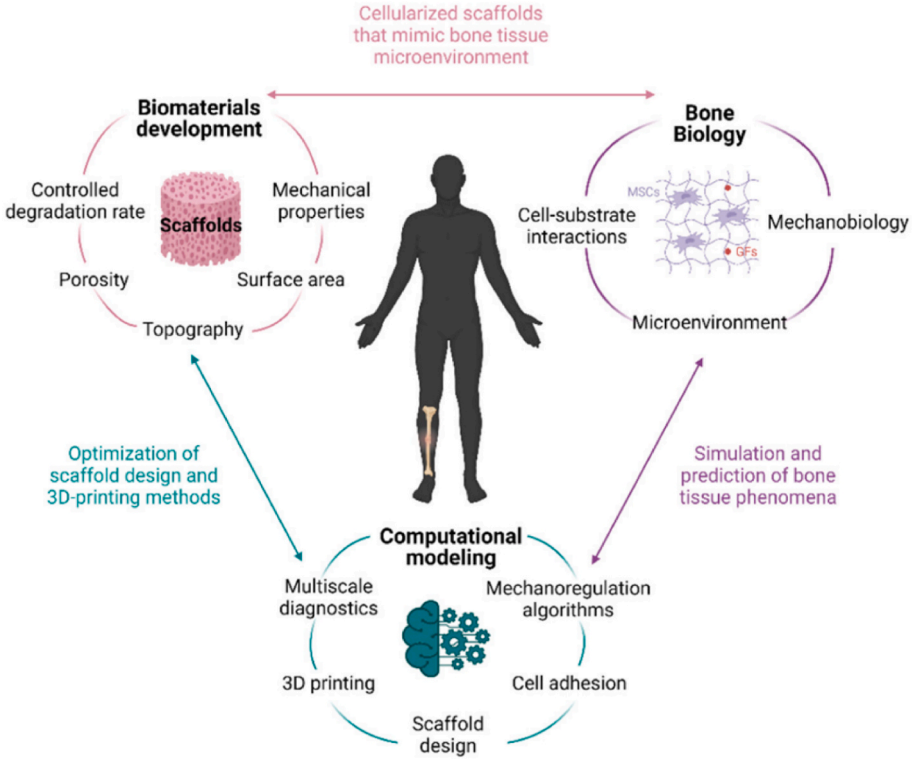

Fig. 5. Bone physiology and the principle of bone tissue engineering. Scheme for integrating biomaterials development, bone biology, and computational modeling towards bone tissue engineering (reproduced with permission from Ref. [176]).

Table 2
Representative *in vivo* studies on the use of Zn-based BMs as BTE scaffolds.
(1) Osteoconduction

Zn-based BMs (wt%)	Working history	Pore size (mm)	Porosity (%)	Animal modes	Implant site	Control	Duration	Main key findings	Ref.
Zn-0.8Mn	Extrusion and laser cauterization	0.5	30.85	Rat	Femoral condyle defect repair model	Pure Ti	12 w	The formation of abundant new bone tissue was observed around the Zn-0.8Mn alloy scaffold, indicating its superior osteogenic properties.	[44]
CaP coated Zn–3Cu–1Mg alloy	Hot press sintering	0.2–0.35	65–75	Rabbit	Cranial bone defect model	Pure Ti Pure Zn Zn alloy	6 m	New bone formation in the coated Zn group was significantly higher than that in other groups, indicating that the Ca–P coating could effectively promote osseointegration.	[206]
Zn-0.8Li-0.1Ca	Extrusion and laser cauterization	0.5	23.27	New Zealand rabbits	Radial bone defect model	Pure Ti	24 w	The new bone tissue integration and growth were significantly better on the Zn0·8Li0·1Ca alloy scaffold than on pure Ti.	[207]
Zn-0.8Sr	Extrusion and laser cauterization	0.5	30.83	Rat	Femoral condyle defect repair model	Pure Ti	12 w	The *in vivo* test confirmed the biosafety and considerable osteogenic properties of the Zn-0.8Sr alloy scaffold, which significantly promoted bone defect repair.	[208]
Zn Zn–1Mg	Additive manufacturing (L-PBF)	0.6	67 %	New Zealand rabbit	Femurs	Pure Zn	12 w	The newly formed bone was in better contact with the Zn–1Mg scaffold than with pure Zn, demonstrating its better biocompatibility and osteogenic effects.	[209]
Zn–2Ag–0.04 Mg	Air pressure infiltration method	0.5–0.6	80	New Zealand rabbit	Trochlea of femur bone defect	Pure Zn Zn–2Ag	6 m	The newly formed bone grew in the pores and was in direct contact with the surface of the Zn–2Ag-0.04 Mg scaffold, indicating good biocompatibility and bone regeneration ability.	[210, 211]
Zn–2Cu	Air pressure infiltration method	0.275	68.7	Sprague-Dawley rat	Femur bone defect	Pure Zn	6 m	Pure zinc and Zn–2Cu porous scaffolds showed excellent biocompatibility and normal inflammatory responses.	[212]
Pure Zn	Additive manufacturing (L-PBF)	0.6	N. A.	New Zealand rabbit	Femur condyle critical size bone defec	N. A.	24 w	The pure Zn porous scaffold showed good biocompatibility and osteogenic promotion ability *in vivo*.	[213]

L-PBF: laser powder bed fusion, N. A.: not available.

effects, agents should be used that are capable to promote bone regeneration in osteoporotic skeletal defect sites [189,196]. 6) For anti-tumor effects, scaffolds should kill or inhibit residual tumor cells to achieve bone tumor therapy [197,198].

4.2. Bioactivity of Zn-based biodegradable metals for scaffolds

Compared to bulk Zn-based BMs, porous Zn-based scaffolds have been proposed and examined for bone tissue regeneration over the years [199]. Porous Zn scaffolds can be fabricated through various techniques, including air pressure infiltration [199,200], powder metallurgy with spark plasma sintering [201,202], infiltration casting [203,204], hot-pressing sintering [204], and additive manufacturing [205]. Additionally, novel Zn-based scaffolds have been developed and explored *in vivo*, as summarized in Table 2. The current status of Zn-based scaffolds is further summarized based on the required bioactivity and biofunctionality for bone tissue regeneration.

The osteoconduction capacity of the scaffold can promote the growth of bone cells on its surface, which is an essential prerequisite for bone tissue engineering. Zn-based alloy BTE scaffolds have good osteoconductive capacity (osseointegration), as reported by most *in vivo* studies [44,206–209,213]. Alloying can optimize this capacity. Specifically, Xia et al. assessed the *in vivo* biocompatibility of a porous scaffold consisting of pure Zn [213]. After week 4 of implantation, a fibrous tissue layer had emerged between the bone tissue and the Zn scaffold, indicating that the relatively high concentrations of degradation products exceeded the tolerance level of the body at this early stage. In a study by Qin et al. [209] Zn–1Mg porous scaffolds were implanted in rabbit femurs. After 12 weeks, the Zn–1Mg porous scaffolds were in direct contact with the surrounding bone while pure Zn porous scaffolds were still surrounded by fibrous connective tissue. The degradation products of Zn–1Mg scaffolds were composed of Zn, O, C, Ca, and P, plus the addition of Mg. The better osteoconductive capacity of Zn–1Mg scaffolds may be attributed to the released Mg ions, which facilitate bone regeneration [209]. As depicted in Fig. 6A, a recent study revealed that additively manufactured Zn-based scaffolds were implanted in a bone defect, while being compared to Ti-based and Mg-based scaffolds. In the case of Zn scaffolds, the anterior region was already enveloped by a porous, mineralized bone layer by week 5. Furthermore, by week 25, the overall bone mass surrounding the Zn scaffold surpassed that surrounding the Mg scaffold (Fig. 6B). The in-vivo comparative study of bioresorbable Mg and Zn scaffolds demonstrated that Mg scaffolds resulted in delayed but complete defect healing with bone ingrowth, while Zn scaffolds promoted early healing and the formation of high-quality newly-formed bone, notwithstanding limited calcification of osteoid inside the scaffold, as illustrated in Fig. 6C [24].

(2) Osteoinduction and angiogenesis

Compared with Ti-based scaffolds, Zn-based BTE scaffolds have been identified as excellent osteoinductive materials for new bone formation [44,206–208]. Jia et al. [44] have shown that the implantation of a Zn-0.8Mn alloy scaffold in a bone defect achieved increased new bone formation and thickness of trabecular bones, compared to Ti-based scaffolds at the same time points after implantation. The superior osteogenic properties of the Zn-0.8Mn alloy *in vivo* can be contributed to appropriate mechanical support and slow co-release of Zn and Mn ions [44]. Additionally, Zhang et al. also examined Zn-0.8Li-0.1Ca alloy scaffolds for critical-sized bone defect regeneration at load-bearing sites. Better integration and growth conditions of new bone tissue were observed around the Zn–Li–Ca scaffold, compared to pure Ti scaffold. This result can be explained by the physiological functions of Zn, Li, and

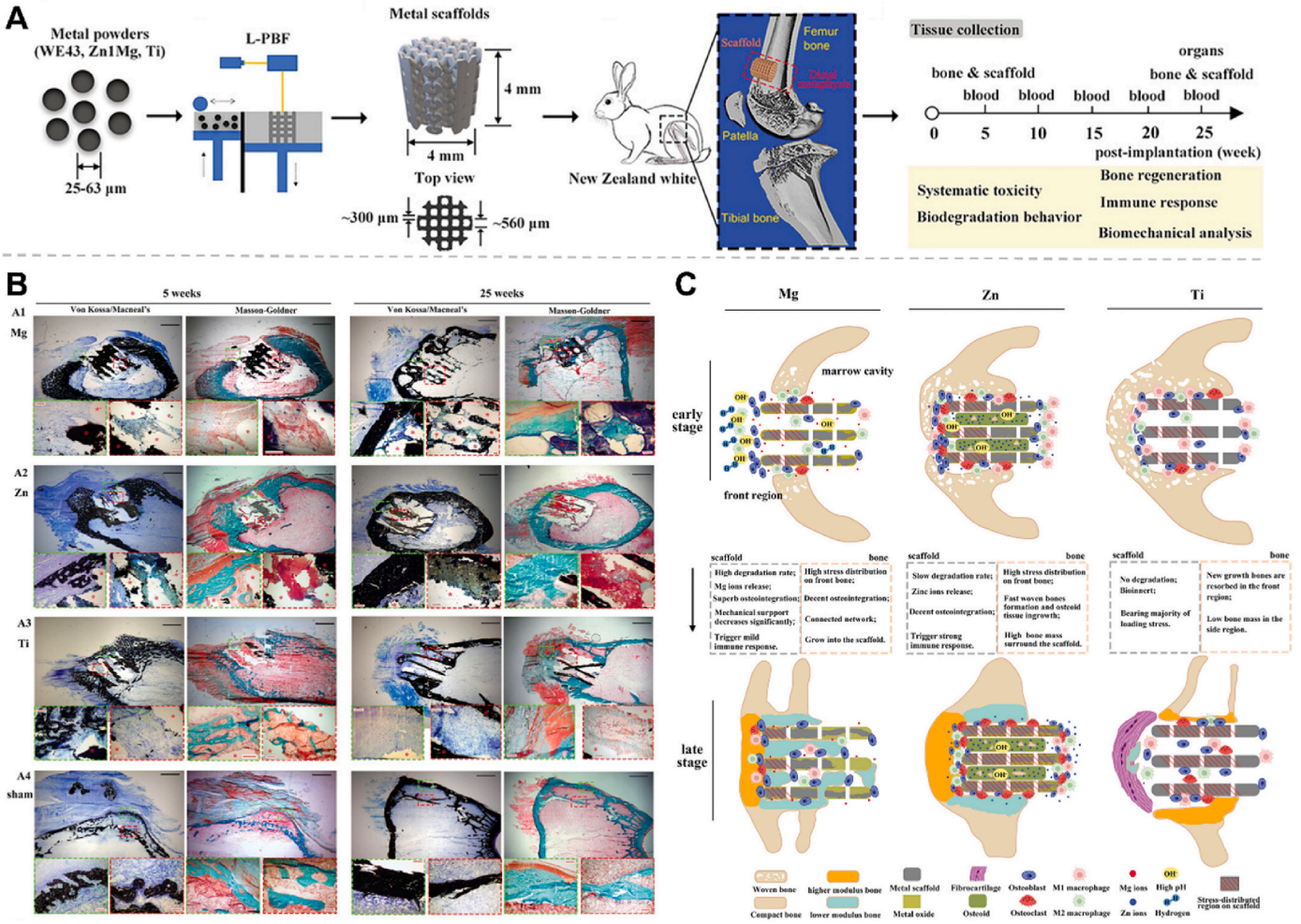

Fig. 6. Osteoconductive and osteoinductive effects of Zn-based scaffolds. A) Scheme for Mg and Zn scaffolds implanted into the distal femur of New Zealand white rabbits. B) Histological analysis of Mg scaffold, Zn scaffold, Ti scaffold, and sham control after 5 and 25 weeks of implantation, determined by Von Kossa/Macneal's staining and Masson-Goldner staining. C) The schematic diagram of the interaction between scaffolds and bone regeneration (reproduced with permission from Ref. [24]).

Ca ions, which are favorable for bone formation and inhibit bone resorption [207]. Jia et al. [208] examined the *in vivo* repair properties of Zn–Sr alloy scaffolds and explored the underlying cellular mechanism. The Zn-0.8Sr alloy was confirmed to promote bone regeneration and bone ingrowth. RNA-sequencing results associated with the osteogenic activity showed that the expression of genes involved in the processes of bone mineralization, bone morphogenesis, ossification, and bone remodeling were significantly upregulated in the Zn–Sr alloy group. Because the expression of key proteins (Wnt3a, β-catenin, Akt, *p*-Akt, and Erk) of signaling pathways was significantly elevated in the Zn-0.8Sr alloy group, induction of the downstream osteogenic proteins (osteocalcin and RUNX2) promotes osteogenesis and angiogenesis [208]. Apart from the released degradation products, a recent study showed that additively manufactured Zn-0.8Li-0.1 Mg alloy scaffolds with varying porosities significantly influenced biocompatibility and osteogenic ability. These findings reveal the critical role of alloying design and porosity in shaping the biocompatibility and osteogenic potential of Zn-based scaffolds [214].

(3) Antibacterial properties, anti-osteoporotic effects, and anti-tumor effects

Antibacterial properties of BTE scaffolds can avoid post-operative infection during tissue healing. Bulk Zn-based metals, Zn–Ag, Zn–Mg, and Zn–Cu alloys have been shown to possess good antibacterial effects [153,154,215,216]. Similarly, porous Zn–Cu based scaffolds showed effective antibacterial properties against *S. aureus* and *Escherichia coli*, and the antibacterial effects was higher with higher Cu content [212]. However, the *in vivo* antibacterial ability and the underlying mechanism in porous Zn-based scaffolds remain obscure.

Anti-osteoporotic effects facilitate bone regeneration at osteoporotic skeletal defect sites. As discussed in Section 3.2., Zn-based internal fixation materials with anti-osteoporotic effects have been fabricated and their feasibility validated [133,157]. However, further study and understanding of the anti-osteoporotic effects of porous Zn-based scaffolds are still lacking.

In osteosarcoma patients post clinical resection, efforts have been directed towards creating porous scaffolds with inherent anti-tumor properties aimed at eradicating recurrent tumors [217,218]. However, as of now, only a limited number of *in vitro* studies have presented findings on biodegradable Zn-based alloys with antitumor capabilities, illustrating their high toxicity towards osteosarcoma cells and low toxicity towards human dental pulp stem cells [219]. As such, further research is warranted to substantiate the presence of anti-tumor effects and elucidate the biological mechanisms underlying the use of Zn-based alloys for porous scaffolds.

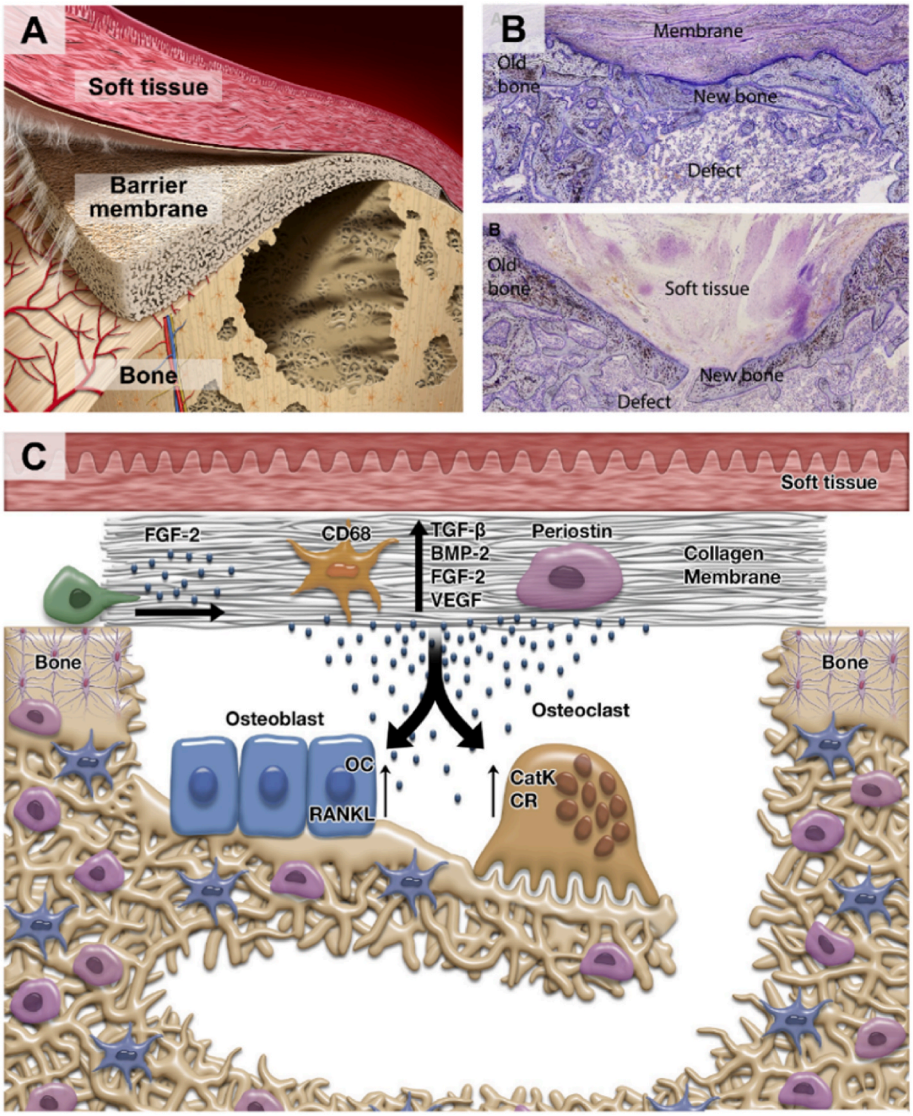

Fig. 7. Schematic overview of the GBR treatment. A) The supposed position of a GBR membrane as physical barrier. B) The GBR membrane promotes the structural reconstitution of the defect with newly formed bone tissue compared with the untreated sham defect where soft tissue infiltrates the defect site. C) Supposed underlying cellular and molecular mechanisms during GBR treatment (reproduced with permission from Ref. [222]).

5. Zn-based barrier membrane for alveolar bone defects

To address alveolar bone defects, guided bone regeneration treatment has been introduced. These techniques involve the application of barrier membranes to prevent the infiltration of non-osteogenic cells from surrounding soft tissues [220]. However, the currently available GBR membranes cannot meet clinical requirements mainly because of their low mechanical strength [221,222]; thus, novel barrier membrane materials must be developed.

5.1. Guided bone regeneration: biological mechanism and required bioactivity

The basic mechanism of GBR is to prevent undesirable cells from non-osteogenic tissues to interfere with bone regeneration [222,223]. To achieve this, a physical barrier membrane is placed between the bone defect and surrounding soft tissue (Fig. 7A). Compared to bone tissue, soft tissue (such as fibroblasts and epithelial cells) grows relatively fast, occupies available space, and builds up soft connective tissue [224]. For GBR treatment, the barrier membrane creates a shielded space for bone formation and natural healing in an undisturbed or protected manner. However, instead of merely being a physical passive barrier, the GBR barrier membrane (porcine collagen) promotes regenerative processes within the underlying defect by activating host cells, and allow them to communicate which each other to promote undisturbed new bone formation, as shown in Fig. 7B [222,225,226]. The schematic images provide an overview of the position of the GBR membrane and the underlying cellular and molecular mechanisms involved (Fig. 7A–C). Firstly, monocytes, macrophages, and osteoprogenitors can migrate from surrounding tissue into the membrane. Cells migrate into the membrane to express and secrete factors for bone formation and bone remodeling (*i.e.*, BMP-2, TGF-β, VEGF, and FGF-2). Both cellular and molecular activities inside the GBR membrane can regulate the pro-osteogenic (*i.e.*, RANKL) and osteoclastic (*i.e.*, cathepsin K and calcitonin receptor) molecular patterns at the defect site [222].

Considering the GBR mechanism and associated clinical considerations, the required bioactivity and biofunctionality of the barrier membrane is summarized as follows: 1) The barrier membrane should feature occlusivity (occlusiveness and cell occlusion), where the material should act as a barrier to exclude undesirable cell types from entering the secluded space adjacent to the bone defect. 2) The top side (soft tissue) of the barrier membrane should feature soft tissue

integration to promote the rapid growth of epithelial cells on the material surface and facilitate primary wound closure. 3) The back side (bone tissue) of the barrier membrane should feature osteoinduction, induce osteoblastic differentiation and mineralization, and accelerate new bone formation. 4) The materials of the barrier membrane should promote angiogenesis, thus facilitating blood supply of the bone regeneration area. 5) The barrier membrane should feature antibacterial properties, and materials should prevent infection during the healing stage.

5.2. Bioactivity of Zn-based biodegradable metals for use as barrier membrane

Recently, Zn-based barrier membranes have gained increasing attention for GBR [35,227]. In 2020, Guo et al. [45] were the first to examine the *in vitro* and *in vivo* performances of pure Zn for potential GBR membrane application. To date, most previous studies on Zn-based GBR membranes used *in vitro* tests, including alloy fabrication, and evaluations of mechanical properties, degradation behavior, cytocompatibility, and antibacterial properties [228–232]. Only a few studies have been focused on the bioactivity of Zn-based GBR membrane. The research status of Zn-based barrier membranes is summarized and illustrated in Table 3.

Occlusiveness, osteoinduction, soft tissue integration, angiogenesis, and antibacterial properties are optimal bioactive and biofunctional effects of barrier membranes. Previous *in vivo* studies demonstrated that a Zn membrane with 300 μm pores can promote bone formation and prevent soft tissue infiltration. This is comparable to Ti-based membranes, indicating prominent osteogenic capability and occlusivity [45]. In addition, considering clinical applications, the antibacterial effects of implants must be effective against infection-related microorganisms. Regarding GBR treatment, postoperative oral infections involve multiple

Table 3
Summary of the mechanical properties, biodegradation, and biocompatibility of the reported Zn-based GBR membranes.

Mateirals		Mechanical properties			Biodegradation		Biocompatibility and bioactive effects		Ref.
		Tensile yield strength (MPa)	Ultimate tensile strength (MPa)	Elongation (%)	Methods (solution)	Main findings	Models (cell/ animial)	Main findings	
Pure Zn membranes	As rolled	92.2 ± 8.8	108.0 ± 4.9	42.8 ± 2.7	Immersion test (Hank's solution)	The DR of the Zn membrane with 300 μm pores was 0.046 ± 0.004 mm/ year, whereas the Zn membrane with 1000 μm pores degraded quickly.	Extract test (MC3T3-E1)	Undiluted extracts had noticeable toxicity, whereas cell viability was improved when cells were cultured in the diluted extracts.	[45]
							Rat calvarial bone defect model	The Zn membrane with 300 μm pores displayed favorable osteogenic ability *in vivo*.	
Pure Zn	As extruded	N. A.	N. A.	N. A.	Immersion test (artificial saliva)	The DR of the pure Zn reached 31.42 μm/year after 28 days of immersion.	Extract test and direct test (HGF)	The 50 % Zn extracts and pre-treated Zn surface presented acceptable *in vitro* HGF cytocompatibility.	[228]
					Electrochemical test	DR was 3.96 μm/ year on day 28.	Antibacterial tests	Zn shows high antibacterial activity against *Porphyromonas gingivalis*.	
Zn Zn-0.5Cu Zn-0.5Cu-0.1Fe Zn-0.5Cu-0.2Fe Zn-0.5Cu-0.4Fe	As extruded	67.1 113.1 115.7 152.3 182.1	127.3 164.2 176.0 202.3 240.1	27.2 35.5 43.9 41.2 20.5	Immersion test and electrochemical test (α-MEM medium and artificial saliva)	The addition of Fe accelerated the degradation of the Zn-0.5Cu-xFe alloys, which were corroded relatively uniformly.	Extract test (L929, Saos-2, TAg)	The extracts of the Zn-0.5Cu-0.2Fe alloy had no apparent cytotoxic effects.	[233]
							Antibacterial tests	The Zn-0.5Cu-0.2Fe surfaces could inhibit the initial adhesion of *Streptococcus gordonii* and oral mixed bacteria.	
Zn-0.8Li Zn-0.8Li-0.2 Mg Zn-0.8Li-0.2 A g	As rolled	183.5 253.7 196.2	238.1 341.3 254.7	75.0 30.6 97.9	Immersion test and electrochemical test (Ringer's solution)	The DRs in Ringer's solution for 35 days were ranked as Zn–Li–Mg (0.17 mm/year) > Zn–Li (0.12 mm/year) > Zn–Li–Ag (0.11 mm/year).	Extract test (L929, BMSCs)	Sample extracts had good cytocompatibility, and the Zn–Li–Ag alloy was of Grade 0–1.	[234]
Zn-0.5Fe membrane	As-sintered As extruded Membrane	NA 101.31 110.20	100.53 150.92 168.8	0.51 19.93 16.25	Electrochemical tests (Ringer's solution)	The DRs of the as-sintered, as-extruded, and as-rolled Zn-0.5Fe alloys were 0.146 mm/year, 0.125 mm/year, and 0.115 mm/year, respectively.	Extract test (MC3T3-E1)	Diluted extracts (12.5 % and 25 %) showed no apparent cytotoxic effects.	[235]
Zn–Ti–Cu–Ca–P	Sintering	Compressive strength: 214 MPa Micro-hardness: 79.34 HV			Electrochemical test (Hank's solution	The DR was 0.18 mm/year.	Extract test (Vero cells)	The alloy had no cytotoxic effects.	[232]

N. A.: not available, DR: degradation rate, MC3T3-E1: mouse osteoblastic cell line, HGF: human gingival fibroblast, L929: mouse fibroblasts, Saos-2: human osteosarcoma cells, TAg: human immortalized cranial periosteal cells, BMSCs; bone marrow mesenchymal stem cells: BMSCs.

species of bacteria, such as *Streptococci*, anaerobic Gram-negative rods, and anaerobic Gram-positive cocci [236,237]. Previous studies investigated the antibacterial properties of Zn-based membranes using *Porphyromonas gingivalis*, *Streptococcus gordonii*, and a mix of oral bacteria [228,229]. Compared to a Ti-based samples and pure Zn, the surfaces of Zn–Cu–Fe alloys show higher antibacterial effects towards *S. gordonii* and mixed oral bacteria, which is probably due to the increased release of Zn ions and a shift towards alkaline pH levels [229]. Additionally, pure Zn exhibited high antibacterial activity against P. gingivalis, which can be attributed to the release of Zn ions [228]. The angiogenic capacity of the GBR barrier membrane can facilitate bone regeneration [238,239]. However, the effect of Zn-based barrier membranes on angiogenesis remains unknown. Additionally, considering primary wound closure, a fast soft tissue integration by migration of gingival fibroblasts can prevent exposure and infection of the barrier membrane [240–242]. Nevertheless, it is still not clear how Zn-based surfaces influence wound closure.

6. Challenges and perspectives

Recent studies have investigated and demonstrated the bioactivity and biofunctionality of Zn-based BMs for the repair and regeneration of bone tissue. Nevertheless, the existing knowledge concerning Zn-based BMs remains inadequate, leading to obstacles in the commercial availability of Zn-based implants. Previous studies have primarily concentrated on the biocompatibility, mechanical properties, biodegradation, and bioactivity of Zn-based BMs for internal fixation, tissue scaffolding, and barrier membranes. In consideration of bioactivity related to clinical aspects, significant challenges and future research endeavors in this field revolve around the intricate interplay between long-term biodegradation, host response, and material optimization", as illustrated in Fig. 8.

6.1. Long-term biodegradation: corrosion mechanism of Zn-based BMs in physiological environments

Considerable efforts have been directed to examine the corrosion mechanisms of Zn-based BMs *in vivo*; however, several important aspects are not yet completely understood. After implantation, the main body fluid is human interstitial fluid, which includes inorganic ions (*e.g.*, Cl^-, CO_3^{2-}, and HPO_4^{2-}), organic components (*e.g.*, proteins, glucose, and amino acids), and buffering agents. Zn degradation is associated with oxygen consumption, and with less than 5 %; in the bony environment, physiological oxygen levels are relatively low. Undoubtedly, it would be a substantial advance to clarify how these physiological elements influence the degradation process of Zn-based implants. Additionally, the *in vivo* long-term degradation of Zn-based bone implants is also worth studying. Passivation layers can retard the degradation kinetics of Zn-based implants during the degradation process. To the best of our knowledge, there are currently no reports showing that Zn-based bone-related implants can be fully degraded and absorbed during the limited observation period.

The problem that the resistance of Zn-based implants to internal fixation in human-body-fluid-assisted fractures (*e.g.*, stress corrosion cracking) may adversely affect the mechanical integrity of implants must be overcome. Further, the mechanical integrity of the BTE scaffold is mainly determined by the corrosion fatigue of the porous Zn-based scaffold during the long-term degradation. Another important issue is crevice-induced corrosion which can occur in Zn-based implants, probably causing failure of their mechanical integrity [243]. Therefore, a deeper understanding of corrosion-induced mechanical integrity has to be reached.

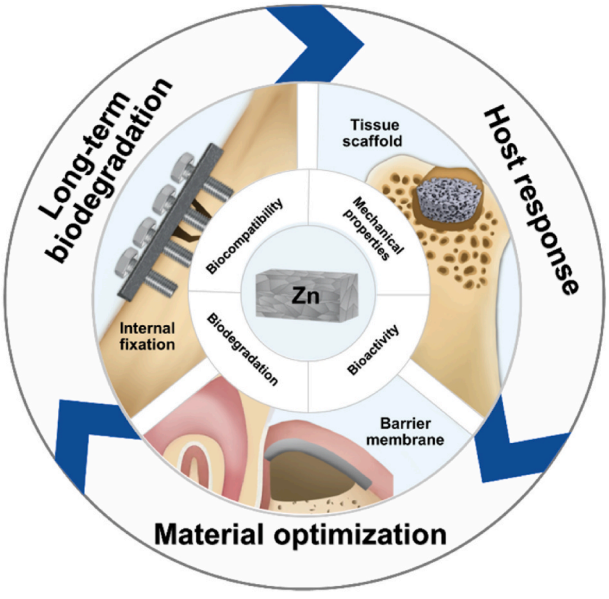

Fig. 8. Schematic illustration of challenges and perspectives of Zn-based biodegradable metals for bone repair and regeneration, showcasing the cyclic interrelation between long-term biodegradation, host response, and material optimization.

6.2. Host response: new insights into the biological mechanisms behind Zn-based BMs

According to the current *in vivo* animal tests, Zn-based BMs have not exhibited systemic toxic effects. However, it is crucial to further investigate the long-term biosafety of the resulting degradation products, particularly pertaining to their absorption, distribution, metabolism, and excretion, as well as the implications of Zn ion release from the orthopedic implant during prolonged degradation. This detailed examination is essential for a comprehensive understanding of the potential long-term impacts on human health and safety arising from the breakdown of these Zn-based implants.

Regarding the degradation products, previous studies focused on the effects of initial degradation products (*i.e.*, Zn ions and hydroxide ions) on biological functions. Theoretically, Zn-based bone implants degrade gradually into different degradation products within the physiological environment [244]. The intermediate products on the surface of Zn-based implants, mainly zincite, Zn hydroxide, and Zn phosphate, can also participate in physiological activities and functions. Released soluble or insoluble degradation products can trigger the host innate and/or adaptive immune responses [245].

Bone formation represents a complex, multifaceted process involving the interplay of various factors and cellular interactions. It encompasses the orchestrated involvement of signaling molecules, cellular differentiation, extracellular matrix deposition, and the regulation of mineralization. Importantly, research has demonstrated the critical influence of the nervous system, particularly peripheral and central nerves, on bone formation [246,247]. Understanding this neural involvement is essential not only for comprehending the mechanisms behind divalent metal cations (Mg^{2+}, Zn^{2+}, and Cu^{2+})-induced bone formation by sensory nerves but also for recognizing the intricate and interconnected nature of bone physiology [248]. In future research, exploring the intricate role of the nervous system in bone formation induced by Zn-based implants can provide valuable insights into the broader understanding of bone remodeling and regeneration processes.

Based on the recent advancements in evidence-based biomaterials/medicine research, standardized tests for the biosafety of Zn-based BMs should be further established and verified [249]. These should include the optimization of biological evaluation, the selection of animal models, and the standardization of pre-clinical protocols. Overall, an in-depth and mechanistic understanding of the host response to Zn-based implants has not been reached.

6.3. Material optimization: controllable Zn ion release synergistically mediates bioactivity

The bioactivity and biofunctionality of Zn-based implants with regard to the process of osteogenesis, angiogenesis, immunomodulation, and their antibacterial effects are mainly determined by the release of Zn ions. A local high concentration of Zn ions can significantly inhibit osteogenesis, leading to fibrous encapsulation around the implant. In contrast, low concentrations of Zn ions can induce osteogenic differentiation, thus promoting new bone regeneration. Clearly, outstanding work has been conducted in the design of novel Zn-based bone implants (*e.g.*, surface modification and composite/alloying) to achieve controllable release of Zn ions. Various techniques have been utilized to enhance biodegradability and biocompatibility, including chemical modifications (*e.g.*, micro-arc oxidation, anodic oxidation, chemical deposition), physical modifications (*e.g.*, magnetron sputtering, dip coating), and mechanical surface treatments [250,251]. However, it has been observed that the ranges of tolerable Zn ion concentrations vary depending on the cell type. The observation that a specific concentration of Zn ions can effectively inhibit bacterial adhesion and growth and simultaneously induce cell apoptosis suggests that features such as cell tolerance and bacterial toxicity have to be further analyzed. These features will provide important information for the optimization of the Zn-based implant design to guarantee.

In conclusion, the usage of Zn-based biodegradable materials for BTE scaffolds, internal fixation, and GBR membranes, has great potential for bone repair and regeneration, owing to their exceptional bioactivity. However, to ensure their safety and efficacy needed for the clinical translation, further research is required to improve our understanding of their biological mechanisms.

CRediT authorship contribution statement

Ping Li: Conceptualization, Investigation, Methodology, Writing – original draft. **Jingtao Dai:** Validation, Writing – original draft. **Yageng Li:** Methodology, Writing – review & editing. **Dorothea Alexander:** Validation, Writing – review & editing. **Jaroslav Čapek:** Conceptualization, Writing – review & editing. **Jürgen Geis-Gerstorfer:** Conceptualization, Writing – review & editing. **Guojiang Wan:** Conceptualization, Investigation, Writing – review & editing. **Jianmin Han:** Project administration, Writing – review & editing. **Zhentao Yu:** Funding acquisition, Project administration, Writing – review & editing. **An Li:** Funding acquisition, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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