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Biodegradable metals for bone defect repair: A systematic review and meta-analysis based on animal studies

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ABSTRACT

Biodegradable metals are promising candidates for bone defect repair. With an evidence-based approach, this study investigated and analyzed the performance and degradation properties of biodegradable metals in animal models for bone defect repair to explore their potential clinical translation. Animal studies on bone defect repair with biodegradable metals in comparison with other traditional biomaterials were reviewed. Data was carefully collected after identification of population, intervention, comparison, outcome, and study design (PICOS), and following the inclusion criteria of biodegradable metals in animal studies. 30 publications on pure Mg, Mg alloys, pure Zn and Zn alloys were finally included after extraction from a collected database of 2543 publications. A qualitative systematic review and a quantitative meta-analysis were performed. Given the heterogeneity in animal model, anatomical site and critical size defect (CSD), biodegradable metals exhibited mixed effects on bone defect repair and degradation in animal studies in comparison with traditional non-degradable metals, biodegradable polymers, bioceramics, and autogenous bone grafts. The results indicated that there were limitations in the experimental design of the included studies, and quality of the evidence presented by the studies was very low. To enhance clinical translation of biodegradable metals, evidence-based research with data validity is needed. Future studies should adopt standardized experimental protocols in investigating the effects of biodegradable metals on bone defect repair with animal models.

1. Introduction

Bone provides mechanical support and protection of internal organs in the human body [1,2]. Bone tissue is capable of self-repair and regeneration [3]. However, large volume of bone loss caused by serious trauma, infection, tumor resection and other injuries, might exceed the self-repair capacity, leading to bone defects [4]. Autogeous bone graft has been the "gold standard" for bone defect treatment, given its excellent performance in osteogenesis, osteoconduction and osteoinduction [5–8]. However, the wide application is limited by the incidence of infection, pain and hematoma at the defect site [9]. Allogeneic or heterogenous grafts have limitations including potential disease transmission and host immune rejection [10,11]. In the United States and Europe, more than half a million patients need to repair bone defects every year, with an estimated cost of over 3 billion US dollars [12]. About 2.2 million autogeous bone grafts are carried out in clinical practice every year around the world, making it one of the most transplanted tissues, second only to blood [13]. Hence, it is urgent to develop bone grafts and bone substitutes to meet the clinical need of bone defect treatment [14].

An ideal bone graft material should be biocompatible, biodegradable, osteoconductive, osteogenesis and osteoinductive. The strength and modulus of bone graft materials should be comparable to the replaced bone tissue [15]. In addition, it should be affordable and accessible to meet the clinical needs [13,16]. Traditional bone graft materials include calcium phosphate, calcium sulfate, bioactive glass and natural polymers such as collagen [15,17]. However, the mechanical properties of calcium phosphate and bioactive glass [18,19], the

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rapid absorption rate of calcium sulfate and natural polymers [17,20], and the adverse tissue reactions related to degradation of synthetic biodegradable polymers [21] make the above-mentioned bone graft materials less than ideal. As a result, their clinical applications have been constrained.

In recent years, ions such as magnesium (Mg), strontium (Sr) and zinc (Zn) are considered as osteogenic biological factors [22–26]. Increasing attention has been given to Mg, Zn, Sr and their alloys in bone defect repair. Pure Mg and Mg alloys have both higher strength and modulus than those of biodegradable polymers, and lower modulus than traditional non-degradable metallic materials [27]. The elastic modulus of Mg is close to cortical bone, effectively reducing the occurrence of stress shielding [28]. Compared with bioceramics, Mg and its alloys exhibit higher toughness [27] along with good biocompatibility [29,30]. In addition, Magnesium ions released by alloy degradation can stimulate the growth of osteoblasts and bone regeneration [31–33]. Meanwhile, magnesium ions can be excreted through kidney by urination, reducing the risk of toxic reactions caused by concentrated ions [34]. Although pure Mg may degrade rapidly at the physiological pH with the release of hydrogen and the loss of mechanical integrity before tissue healing [29], the degradation rate of pure Mg can be controlled by limiting the impurity content in Mg and its alloy through fine-tuned material processing, coating and other methods [35,36]. Considering the comprehensive and desirable properties of Mg and its alloys, including biodegradability and osteogenesis, they hold great promise to be the ideal biomaterials for bone defect repair. A recent study [25] also strongly supports the use of zinc-based biomaterials for bone-related diseases and bone defect repair. As an active area in the field of orthopedic biomaterials, the research on biodegradable metals includes design of alloy composition, optimization and developing new processing methods, evaluation of simulated in vitro degradation, assessment of biocompatibility, animal studies and clinical translation [37,38].

Compared with animal studies, in vitro simulated degradation tests and biocompatibility evaluation such as cytotoxic tests, cannot represent the complex physiological environment [39]. Proof of concept in bone regeneration studies can only be demonstrated with the help of animal models. None of the in vitro methods can mimic the complexity of an in vivo environment sufficiently nor predict clinical efficacy. Therefore, synthetic bone grafts must be strictly evaluated in preclinical animal models during their translation. As a result, animal studies play a pivotal role in evaluating the efficacy and safety of synthetic bone grafts.

Previous studies on biodegradable metals have investigated their in vivo biocompatibility, degradation, osteogenesis and ability of bone defect repair in different animal models. Limitations of previous studies include over-simplified animal models, short observation durations, single evaluation criterion and inconsistent results. Therefore, it is necessary to review and analyze existing literature on animal studies with an evidence-based approach before more ambitious animal studies are conducted. Such an effort can provide suggestions and references for future animal studies, which could provide further evidence for later clinical translation [40].

A systematic review is a literature review [41] that aims at tackling a specific research question by searching, identifying, selecting, evaluating and synthesizing all existing evidence related to the question. By combining and analyzing the homogeneity of the included research data (i.e., meta analysis), the quantitative assessment of the average level of research effects can be achieved, with the effective improvement of the test efficiency [42]. Compared with narrative reviews, the systematic review follows a more structured approach [43], and evaluates evidence in a more objective manner [44]. Since the initiation of the research method in the 1990s [45], it has been widely used in clinical, public health and even health policy fields, known as the highest level of evidence in medical research [46]. In 2002, *Lancet* published an influential review [47], calling for a strict and standardized systematic review of animal studies evidence before clinical trials. The systematic review of animal studies is of great significance to improve the transparency of the research process. It not only prevents the waste of health resources and the repeated use of experimental animals, but improves the quality of animal studies and the outcome of future translation. The systematic review is also considered as a precondition for the design of subsequent trials. Although animal studies are widely adopted in biomaterial and translational research, their quality of evidence has rarely been systematically evaluated.

With the systematic review method, this study aimed at a comprehensive analysis of the published animal studies of bone defect repair with biodegradable metals in comparison with traditional biomaterials, which include non-degradable metals, biodegradable polymers, bioceramics, and autogenous or allogeneic bone grafts. We reviewed the composition of materials, shape of implants, animal models, anatomical sites, construction of bone defect models, follow-up duration, repair and healing of critical size defects (CSD), and degradation properties of implants. We then evaluated efficacy and safety of biodegradable metals on bone defect repair, assessed feasibility, benefits and risks of clinical translation, and established a reference for potential future clinical trials.

2. Materials & methods

2.1. Objectives

This research intended to conduct the systematic review on animal models of biodegradable metals for bone repair. Searching strategies and screening methods were adopted to cover comprehensive animal study results on biodegradable metals for repairing both bone defects and bone fractures. Due to the differences in the repair models, clinical manifestations and treatment principles between bone fractures and bone defects, this study only systematically reviewed evidence related to bone defect repair. A relevant systematic review on bone fracture repair was reported separately [48].

2.2. Quality assurance

The systematic review was carried out in strict accordance with *Cochrane Handbook for Systematic Reviews of Interventions* [49], and the PRISMA checklist of the review was provided as Appendix 1. All the researchers who participated in the systematic review had received rigorous training sessions, including those on PICOS, inclusion and exclusion criteria, study selection and data extraction, assessment tool (s) for the risk of bias in animal studies (SYRICE), and for quality of the evidence (GRADE and CERqual). After the training, 10% of the preliminary literature was randomly selected for the trainees' graduation test, which included independent literature screening, data extraction and quality assessment. Before the researchers could participate, they had to past the test of consistency on their results. If the Kappa value was $\geq$0.8, then the trainees were qualified and became part of the research team.

2.3. Inclusion/exclusion criteria

This study strictly follows the PICOS guideline, and extracts data after carefully searching the title, abstract and full text of each article. Only studies that meet the following criteria are included in the final systematic review and meta-analysis.

2.3.1. Population

Studies that include animal models of bone defects, with no limitations on the animal species nor modeling methods.

2.3.2. Intervention

Degradable metals and their alloys, modified degradable metals and their alloys (composites, coating and surface modification).

2.3.3. Comparison

① Non-degradable metals, such as titanium, titanium alloy, stainless steel and cobalt chromium alloy; ② Degradable polymers, such as polylactic acid; and ③ Other materials, such as calcium phosphate ceramic, autogenous bone, allogeneic bone, or degradable composites used in traditional clinical applications (e.g. ceramic-polymer composites).

2.3.4. Outcome

2.3.4.1. Outcome measures for bone defect repair. ① New bone formation: Increased density shadows (of trabecular bone, epiphysis, etc.) are detected in or around the defect site by imaging methods; New bone tissue and osteoblasts are observed by tissue section; ② Defect repair: New bone formed and the size of bone defect decreases until healing is detected by imaging methods; and ③ The percentage of bone volume/tissue volume around the implant (BV/TV): The ratio is calculated by Micro-CT. Secondary outcome measures include ④ Bone implant contact (BIC): The percentage of peri-implant contact area with the surrounding bone tissue is calculated by imaging and histological analysis.

2.3.4.2. Implant-related outcome measures. ① Degradation: Detection of degradation/corrosion of implants through image analysis (Micro-CT) and histological analysis (tissue section); ② Hydrogen generation: Observation of gas shadows through image analysis, or detection of bubbles around the implants through histological analysis.

Given the different species of animals in the included studies, for instance, rabbits, rats, mice, sheep and pigs, there must be differences in the defect healing time. To facilitate the analysis of the outcome measures, we divided the whole follow-up process of the included studies into four measurement periods, which were T1, the initial period ($0<T_1 \leq 1/4T$), T2, the mid-term period ($1/4T < T_2 \leq 2/4T$), T3, the long-term period ($2/4T < T_3 \leq 3/4T$), and T4, the terminal period ($3/4T < T_4 \leq T$), with "T" representing the whole follow-up time.

In addition, the main objective of this study is to systematically evaluate the in vivo performance of biodegradable metals to repair bone defects. The outcome measures in this study were carefully identified to reflect this research objective after thoroughly investigating literature and consulting with field experts of clinicians, biomaterials scientists and evidence-based research scientists. Such performance was evaluated via two simultaneous approaches with one addressing bone defect repair of animals and the other addressing implants of biodegradable metals. The systemic reactions and blood biochemical indicators were not identified as the outcome measures because they are related to the safety aspects other than in vivo performance of biodegradable metals for bone defects repair.

2.3.5. Study design

Controlled studies were included, with no restriction on whether they were randomly grouped. In order to ensure the quality of included studies, self-control studies were excluded because metallic ions from both experiment and control groups may influence each other in terms of their effects on bone defects repair [22,50].

2.4. Search strategy

We searched the PubMed (1966–August 2019), Ovid-Embase (1980 to August 2019), Cochrane Library (1989 to August 2019), Web of science (from inception to August 2019), China National Knowledge Infrastructure or CNKI (from inception to August 2019), Wanfang Data Knowledge Service Platform (from inception to August 2019), Chinese Scientific Journal Database or VIP (from inception to August 2019), and China Biomedical Literature Database or CBM (from inception to August 2019). Supplementary search included Scopus (from inception to August 2019). In addition, the references of included studies were checked. Authors of studies with incomplete data were contacted to obtain the required information. The retrieval method was a combination of free words and medical subject heading (MeSH). Table 1 shows the PubMed search strategy, and see Appendix 2 for the search strategies for Chinese and English literature. In order to update the references of systematic reviews in a timely fashion, we also conducted a supplementary search on October 19, 2020.

2.5. Study selection and data extraction

Two trained researchers (Z. S. and Y. J.) selected the papers and extracted the data in strict accordance with the inclusion/exclusion criteria, and cross-checked them. In case of disagreement, a third party (B. M.) would decide. Data was extracted according to the pre-established full-text data extraction checklist, including: ① Basic parameters of the included studies: Including the animal species, age, weight, sample size, bone defect model, defect size, CSD, repair method, types of interventions, and follow-up durations of the experimental animals; ② Outcome measures: 1) Outcome measures for defect repair: new bone formation, defect healing, BV/TV and BIC; 2) Outcome measures for implant degradation: degradation and hydrogen generation.

2.6. Assessment of risk of bias

Based on SYRCLE's risk of bias tool for animal studies [51], two trained review authors (B. Z. and M. J.) independently evaluated and cross-checked the inherent risk of bias in the included studies, covering selection bias, implementation bias, measurement bias, follow-up bias, report bias, and other biases from a list of ten questions. A difference in opinions were negotiated or decided by a third review author (J. Z.). The answer to the assessment questions should be either "yes" that indicated low risk of bias, or "no" that indicated high risk of bias. For unclear items, an answer with "unclear" was assigned.

2.7. Assessment of quality of evidence

The quality of the evidence decides if the results of systematic review of animal studies could support its clinical translation. The CERQual tool (Confidence in the Evidence from Reviews of Qualitative Research) [52,53] developed by Cochrane Collaboration for the grading and evaluation of evidence was used to assess the qualitative outcomes in the

Table 1
Search strategies for PubMed.

Search subject	Keywords	Result
#1 Type of study	"Fractures, Bone"[Mesh] OR fracture*[tiab] OR Bone defect*[tiab] OR "Fracture Healing"[Mesh] OR "Fracture Healing"[tiab] OR "fracture fixation"[Mesh] OR ("fracture"[tiab] AND "fixation"[tiab]) OR "fracture fixation"[tiab] OR Bone repair*[tiab] OR bone heal[tiab] OR bone healed[tiab] OR bone heals[tiab] OR bone healing[tiab] OR Bone fill*[tiab] OR "Bone Screws"[Mesh] OR "Bone Screws"[tiab] OR "Bone Plates"[Mesh] OR "Bone Plates"[tiab] OR "Bone Nails"[Mesh] OR "Bone Nails"[tiab] OR intramedullary nail*[tiab] OR "pins"[tiab]	491704
#2 Object of study	Search filter for animal studies [165]	6834740
#3 Intervention	(biodegradable metal OR degradable metal OR biodegradable alloy OR degradable alloy OR absorbable metal) OR ((biodegradable implants OR biodegradable fixation OR absorbable implants OR bioabsorbable implants OR biodegrading implants) AND (metal OR alloy OR magnesium OR Mg OR zinc OR Zn OR Iron OR Fe))	4746
#4 Combination of all search key words	#1 AND #2 AND #3	322

following four aspects. ① Methodological limitations; ② Correlation; ③ Consistency of results; and ④ Adequacy of data. The review authors evaluated the above four criteria individually, and then the results on each criterion were combined to decide on the final quality of the evidence, which would be high, medium, low, or very low [53]. The GRADE evidence grading system was adopted to evaluate the quality of the evidence quantitatively [54]. Grades were given in five areas. ① Limitations of the study; ② Inconsistency of results; ③ Indirectness; ④ Imprecision; and ⑤ Publication bias. The results on each criterion were averaged for a quality score of high, medium, low, or very low [54].

2.8. Statistical analysis

The meta-analysis was conducted through Revman5.3 software. Meta analysis is a statistical analysis of results from separate studies, examining sources of differences in results among studies, and leading to a quantitative summary of the results if the results are judged sufficiently similar to support such synthesis. The continuous variables took the mean difference (MD) as the effect index, while the categorical variables were analyzed by risk ratio (RR), both with 95% confidence interval (95% CI). The diamonds in the forest plot represent the combined effect size. Heterogeneity in the included studies was analyzed using the chi-square test, with the significance level of $\alpha = 0.1$, and the heterogeneity was quantitatively determined using I^2. If there was no statistically significant heterogeneity between the outcomes, a fixed-effects model was applied for meta-analysis. Otherwise, the sources of heterogeneity were further analyzed. If there was still heterogeneity, a random-effect model would be run, with the significance level of $\alpha = 0.05$. If the heterogeneity was difficult to eliminate between studies, a qualitative description method was used to comprehensively describe the evidence.

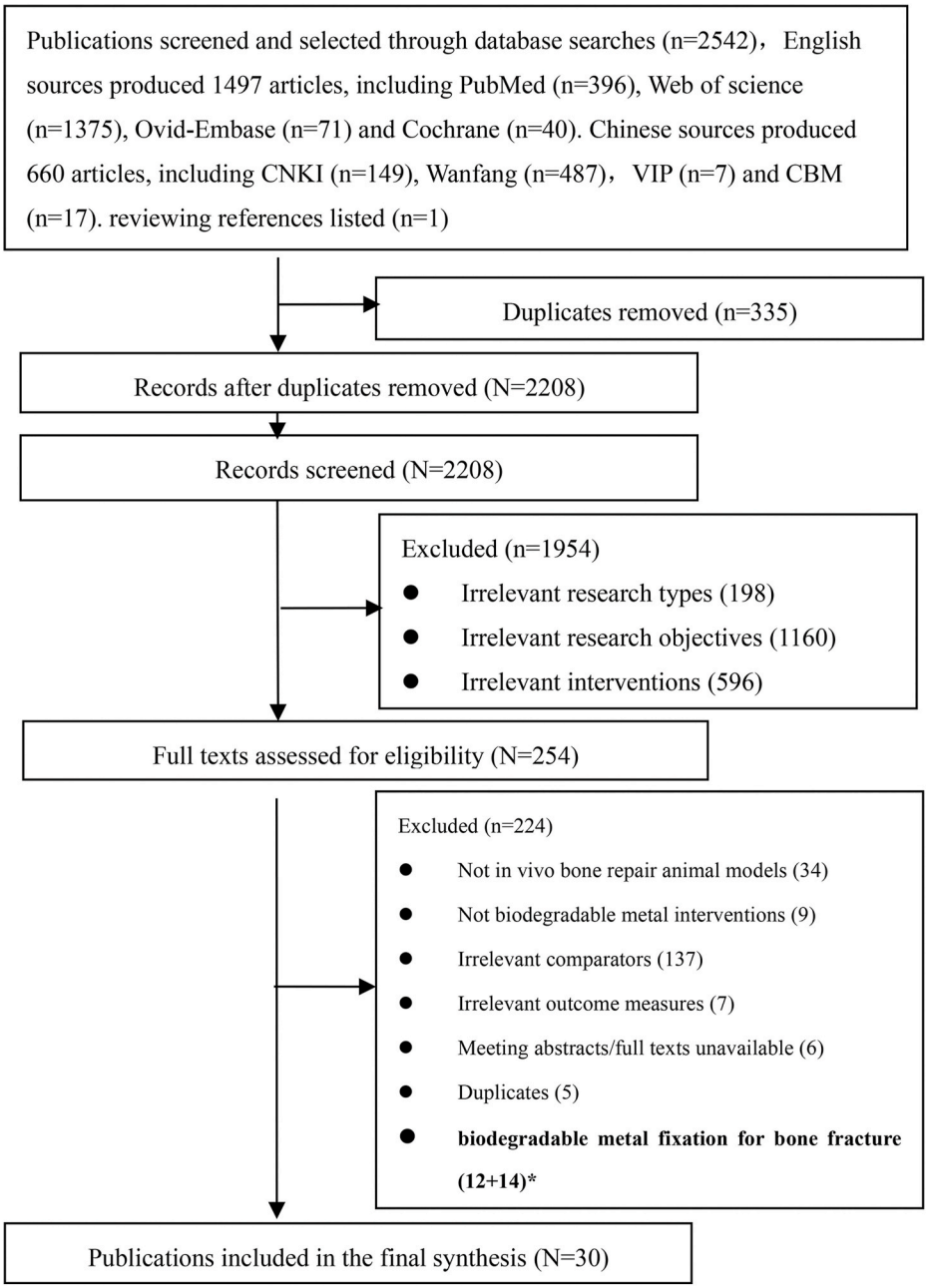

Fig. 1. The study screening and selection process (* is reported in a separate review [48]).

3. Results

3.1. Included studies

A total of 2543 studies were obtained from five English databases and four Chinese databases as well as reviewing related references, of which 660 were in Chinese and 1883 in English. After excluding duplicates, 2208 of them underwent preliminary screening. 198 reviews, commentaries and secondary research articles were excluded due to inconformity in research types. 1160 clinical, in vitro and other research articles were excluded for inconformity in research objectives. 596 studies with calcium phosphate cement and degradable PLA bone grafting materials as the intervention measure were excluded, leaving a total of 254 valid studies for the review.

After close reading of full texts, 34 in vitro studies and nine studies with biodegradable polymers as the intervention were excluded. 137 studies (22 self-control, 80 with degradable metals as control, 16 with blank control, 14 without control groups and 5 in which control groups were not clearly identified) were excluded for inconformity in control (See Appendix 3). Seven studies were excluded for failing to report on any of the six outcome measures for the systematic review. After excluding six conference abstracts, five duplicate studies and 26 studies with fracture animal model as research objective, 30 animal studies of bone defect repair with biodegradable metals were included, with 25 in English [55–79] and five in Chinese [80–84] (Fig. 1).

In recent years, animal studies of bone defect repair with biodegradable metals have been widely carried out. The 30 controlled studies included were published between 2008 and 2020 by eight countries including Germany, Austria, Ukraine, China, the United States, the Czech Republic, Switzerland and Romania. The biodegradable metals included pure magnesium, magnesium alloys, pure Zn and Zn alloys. Six different animal models were employed, including rabbits (in 13 studies), rats (in 11 studies), mice (in one study), sheep (in two studies), beagles (in one study) and pigs (in two study).

3.2. Summary of results in included studies

This study carried out a systematic search on the effects of biodegradable metals in bone defect repair. After rigorous screening, animal studies of bone defect repair with the following two kinds of biodegradable metals were included, pure magnesium [62,70–72,76] and magnesium alloy [55–61,63–69,71–73,75,77,78,80–84], and pure Zn [74] and Zn alloys [79]. The 30 included studies consisted of 20 randomized controlled studies [55,58–60,63–67,71,74–78,80–84] and ten controlled studies [56,57,61,62,68–70,72,73,79]. The animal models in the included studies were rabbits [55,57,58,60,61,63,65,70,72,73,75,80,82,84], rats [56,59,64,69,71,74,76,79,81,83], mice [62], sheep [66,68], beagles [77], guinea pigs [78] and pigs [67]. Ages were mostly between five weeks [56,64] and 12 months [77] and weights between 2.0 kg [61,80,82] and 4.0 kg [60]. The sample sizes were between three [72] and 56 [70]. Bone defect models included tibial defect [55,57,59,60,63,69,70] (23%), femoral defect [56,61,64,70,71,73,78,81–83] (33%), mandibular defect [58,65,67,72,76,80,82] (23%), femoral condyle defect [68,79] (6%), skull defect [62,74,75] (10%), ulna defect [84] (3%) and orbital defect [77] (3%). CSD models included skull defect model [62,74] (6%) and ulna defect model [84] (3%) (see Fig. 2). The follow-up duration ranged from four weeks [69] to eighteen months [65]. The detailed information of included studies is shown in Tables 2 and 3.

The outcome measures and measurement time points in the included studies were different. There was also a large difference in the measuring methods and assessment criteria of the outcome measures. There were five measures of bone defect repair. ① New bone formation: This outcome was reported in all 30 studies [55–84], with varied experimental animals of rabbits [55,57,58,60,61,63,65,70,72,73,75,80,82,84], rats [56,59,64,69,71,74,76,79,81,83], mice [62], sheep [66,68],

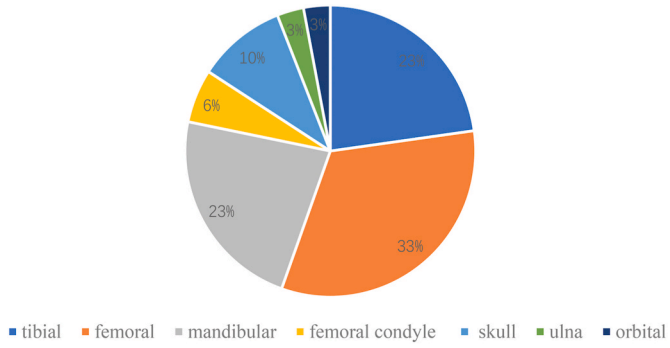

Fig. 2. Proportion of bone defect sites.

beagles [77], guinea pigs [78] and pigs [67]). The implants were pure magnesium [62,70–72,76], magnesium alloy [55–61,63–69,71–73,75,77,78,80–84], pure Zn [74] and Zn alloys [79].). The measuring methods were X-ray [55,66], CT [59,61–64,71–73,75–78,81] and histological analysis [56–58,60,61,65,68–73,75,76,80,82–84]). The measurement time points were between four weeks [69] and eighteen months [65]. ② Bone defect healing: This outcome was reported in five studies [59,61,73,80,84]. Though the experimental animal (rabbits) was the same, and the implants were all magnesium alloy, differences existed in measurement time points (between six weeks [59]- and 180 days [80]) and in measuring methods (X-ray [84], Micro-CT [59,61,73] and general observation [80]). ③ BV/TV: This outcome was reported in seven studies [61,64,67,70,74,75,79] all through Micro-CT, with high homogeneity of animal model in each intervention (pure magnesium vs. control group: NZW [70]; magnesium vs. non-degradable metal: mini-pig [67]; magnesium vs. biodegradable polymer: rats [64], magnesium vs. Bioceramics: NZW [61]; magnesium vs. GRB: NZW [75]; pure Zn vs. non-degradable metal: Rats [74]; Zn vs. non-degradable metal: Rats [79]). ④ Bone implant contact: This outcome was reported in three studies [60,62,67], with variance in experimental animals (mice [62], rabbits [60] and mini-pigs [67]), implant compositions (pure magnesium [62] and magnesium alloy [60,67]), measurement time points (between eight-four days [62] and twenty-four weeks [60,67]) and measuring methods (histological analysis [60] and Micro-CT [62,67]).

Two implant degradation measures were included. ⑤ Degradation: This measure was reported in 21 studies [56,59–66,68–72,74,78,79,81–84], with varied experimental animals (rats [56,59,64,69,71,74,79,81,83], rabbits [60,61,63,65,70,72,82,84], mice [62], sheep [66,68], guinea pigs [78]), implant components (pure magnesium [62,70–72], magnesium alloy [56,59–61,63–66,68,69,71,72,78,81–84], pure Zn [74] and Zn alloys [79]), and measurement time points (between six weeks [59,71] and eighteen months [65]) used by different research institutions. There were also differences in the measuring methods of implant degradation (histological analysis [56,68], CT [65,66,70], Micro-CT [59–63,71,72,78,79,81,84], scanning electron microscope [69,74,82], X-ray [83] and optical microscope observation [64]). ⑥ Hydrogen generation: This measure was reported in 18 studies [55–57,59–61,66–71,73,77,78,80,81,84], with different experimental animals (rabbits [55,57,60,61,70,73,80], sheep [66,68], rats [56,59,69,71,81], mini-pigs [67], beagles [77], guinea pigs [78]), implant compositions (pure magnesium [70,71] and magnesium alloy [55–57,59–61,66–69,73,77,78,80,81,84]), measurement points (between 8 weeks [55,61] and six months [56,57]), and measuring methods (general observation [55,68,84], X-ray [55,66,67], Micro-CT [60,61,69–71,73,77,78,81] and histological analysis [56,57,59,68,80]).

A meta-analysis could only be conducted on the BV/TV measure in this review. The other five measures, new bone formation, bone defect healing, degradation and hydrogen generation, presented only qualitative data in the original studies. Although analyzed with quantitative data, the bone implant contact outcome was substantially

Table 2
Characteristics of included animal studies.

Implant type	Author(year)	Country	Study design	Species	Sample size (T/C)	Gender	Age	Body weight	Model	Model size	CSD	Follow-up time	Included in Meta-analysis
Mg	Grau M 2017 [62]	Germany	Ctrl	Mice	8/8	F	8 weeks	/	Right skull	3 mm × 3 mm square	Y	84 d	N
	Wang, Jiali 2017 [70]	China	Ctrl	NZW	56/56	M	6 Mos.	/	Both the femur and tibia	Ø: 2.5 mm	U	16 Weeks	Y
	Yu, K 2018b [71]	China	Ran	Rats/SD	12/12	/	/	230–270g	Left distal femora	Ø: 1.5 mm × 4 mm	U	6 Weeks	N
	Henderson, S. E 2014a [72]	USA	Ctrl	NZW	3/3?	F	12 Mos.	5–7 kg	Rabbit mandible	Ø: 0.85 mm	U	6 Weeks	N
	He, Wei 2020 [76]	China	Ran	Rats/SD	18/18	F	3 Mos.	/	alveolar bone	Ø: 1.4 mm	U	6 Weeks	N
Magnesium alloy	Erdmann, N 2010 [55]	Germany	Ran	NZW	24/16	F	Adult	3.81 ± 0.84 kg	Both tibiae	Ø: 3.5 mm	U	8 Weeks	N
	Celarek, A 2012 [56]	Austria	Ctrl	Rats/SD	/	M	5 weeks	140–160 g	Each distal femur	Ø: 1.5 mm	U	6 Mos.	N
	Bondarenko, A 2014 [57]	Ukraine	Ctrl	NZW	40/6	F	Adult	3.5 kg	Right tibiae	Unclear	U	6 Mos.	N
	Guan, Xingmin 2014 [58]	China	Ran	NZW	10/10	/	Adult	2.5–2.7 kg	Bottom of mandible bone	L: 2 mm	U	7 Mos.	N
	Berglund, I. S 2016 [59]	USA	Ran	Rats/SD	6/6	M	Adult	250–300 g	Proximal tibial metaphyses	Ø: 0.8 mm	U	6 Weeks	N
	Diekmann, J 2016 [60]	Germany	Ran	NZW	18/18	F	6 Mos.	3.8 ± 0.2 kg	Left tibiae	Ø: 2.7 mm	U	24 Weeks	N
	Dong, J. H 2018a [61]	China	Ctrl	NZW	4/2	/	Adult	2.0–3.0 kg	Both femoral	Ø: 6.0 mm D:9 mm	U	8 Weeks	Y
	Dong, J. H 2018b [61]	China	Ctrl	NZW	4/2	/	Adult	2.0–3.0 kg	Both femoral	Ø: 6.0 mm D: 9 mm	U	8 Weeks	Y
	Levorova, J 2018 [63]	CZ	Ran	NZW	8/8	M	9 weeks	2.22–2.75 kg	Right tibia/two defects	Ø: 2 mm D: 3 mm	U	16 Weeks	N
	Lindtner, R. A 2013 [64]	Austria	Ran	Rats/SD	36/36	M	5 weeks	120–140 g	Each Femur	/	U	24 Weeks	Y
	Niu, J. L 2016 [65]	China	Ran	NZW	10/10	/	Adult	2.5–3.0 kg	Bottom of mandible bone	L: 2 mm	U	18 Mos.	N
	Rössig, Christina 2015 [66]	Germany	Ran	Sheep	5/5	F	Adult	92.45 ± 13.38 kg	Right tibia	Ø: 10 mm	U	24 Weeks	N
	Schaller, B 2016a [67]	Switzerland	Ran	Mini-pig	6/2	/	30–36 Mos.	53 ± 7 kg	Left and right mandible	Ø: 2.5 mm, 2.6 mm	U	24 Weeks	Y
	Schaller, B 2016b [67]	Switzerland	Ran	Mini-pig	6/2	/	30–36 Mos.	53 ± 7 kg	Left and right mandible	Ø: 2.5 mm, 2.6 mm	U	24 Weeks	Y
	Thormann, U 2015 [68]	Germany	Ctrl	Sheep	5/2	F	/	/	Femur condyle	Ø: 8 mm,	U	12 Mos.	N
	Trincă, Lucia Carmen 2015 [69]	Romania	Ctrl	Rats/Wistar	5/5	M	6 weeks	230 g	Tibiae	Ø: 1.7 mm	U	4 Weeks	N
	Yu, K 2018a [71]	China	Ran	Rats/SD	12/12	/	/	230–270 g	Left distal femora	Ø: 1.5 mm × 4 mm	U	6 Weeks	N
	Henderson, S. E 2014b [72]	USA	Ctrl	NZW	3/3	F	12 Mos.	5–7 kg	Rabbit mandible	Ø: 0.85 mm	U	6 Weeks	N
	Chen, Junxiu 2019 [73]	China	Ctrl	NZW	4/4	/	/	2.0 kg	Left distal femur	Ø: 6 mm L: 9 mm	U	2 Mos.	N
	Guo, Yu 2019 [75]	China	Ran	NZW	14/14	M	/	3.0–3.5 kg	Calvarial	Ø: 6 mm	U	12 Weeks	Y
	Zhang, D. 2020 [77]	China	Ran	Beagles	/	/	>1 year old	/	Orbital	Ø: 10 mm	U	6 Mos.	N
	Witte, F. 2005 [78]	Germany	Ran	Guinea pigs	8/8	F	/	568–768 g	Both femoral	Ø: 1.5 mm	U	18 Weeks	N
	Hong, Y 2008a [80]	China	Ran	NZW	6/6	M	Adult	2.0–2.5 kg	Rabbit mandible	10 mm × 2 mm	U	180 d	N

(*continued on next page*)

Table 2 (*continued*)

Implant type	Author(year)	Country	Study design	Species	Sample size (T/C)	Gender	Age	Body weight	Model	Model size	CSD	Follow-up time	Included in Meta-analysis
	Hong, Y 2008b [80]	China	Ran	NZW	6/6	M	Adult	2.0–2.5 kg	Rabbit mandible	15 mm × 15 mm	U	180 d	N
	Qi, Z 2013a [81]	China	Ran	Rats/SD	6/6	M	2 Mos.	235–266 g	Femur	Ø: 2 mm	U	26 Weeks	N
	Qi, Z 2013b [81]	China	Ran	Rats/SD	6/6	M	2 Mos.	235–266 g	Femur	Ø: 2 mm	U	26 Weeks	N
	Sun, W 2010a [82]	China	Ran	Rabbits	15/15	M	Adult	2.0–3.0 kg	Femur, mandible	Ø: 2 mm L: 7 mm	U	3 Mos.	N
	Sun, W 2010b [82]	China	Ran	Rabbits	15/15	M	Adult	2.0–3.0 kg	Femur, mandible	Ø: 2 mm L: 7 mm	U	3 Mos.	N
	Sun, W 2010c [82]	China	Ran	Rabbits	15/15	M	Adult	2.0–3.0 kg	Femur, mandible	Ø: 2 mm L: 7 mm	U	3 Mos.	N
	Zhu, Z 2013a [83]	China	Ran	Rats/SD	6/6	M	3 Mos.	200 ± 30 g	Left femora	Ø: 1 mm L: 30 mm	U	6 Mos.	N
	Zhu, Z 2013b [83]	China	Ran	Rats/SD	6/6	M	3 Mos.	200 ± 30 g	Left femora	Ø: 1 mm L: 30 mm	U	6 Mos.	N
	Zhang, N 2018a [84]	China	Ran	NZW	24/24	M, F	Adult	2.71 ± 0.32 kg	Ulna	L: 15 mm	Y	12 Weeks	N
	Zhang, N 2018b [84]	China	Ran	NZW	24/24	M, F	Adult	2.71 ± 0.32 kg	Ulna	L: 15 mm	Y	12 Weeks	N
Pure Zn	Guo, H. 2020 [74]	China	Ran	Rats/SD	8/8	M	ten-week	/	Calvaria	Ø: 6 mm	Y	10 Weeks	Y
Zn alloy	Jia, Bo 2020 [79]	China	Ctrl	Rats	15/15	M	12-week	293 g ± 23.5 g	Femoral condyle	Ø: 3 mm L: 4 mm	U	12 Weeks	N

CSD: Critical-sized defect; Ran: Randomized; Ctrl: Controlled; NZW: New Zealand White Rabbits; F: female; M: male; Mos.: months; ø: diameter; D: depth; L: length; Y: Yes; U: Unclear; N: No.

heterogeneous between different studies, with variance in experimental animals (mice, rabbits, mini-pigs), implant compositions (pure magnesium and magnesium alloy) and measuring methods (histological analysis and Micro-CT). Hence, only a descriptive analysis was possible for the above five measures.

3.3. Bone defect repair with pure magnesium [62,70–72,76]

Only five included studies [62,70–72,76] explored bone defect repair with pure magnesium, all of which were compared with non-degradable metals. However, the bone defect models used were different, which included skull [62], femur [70,71], tibial [70] and mandible defect [72,76], with only one study [62] on critical size defects (CSD). In addition, the composition and morphology of implants used in each control group were also different, including Porous Ti6Al4V (3 mm × 3 mm × 1.2 mm basic body with a pore size and strut width of 0.6 mm) [62], Ti (3 mm in diameter and 8 mm in Length [70], 1.4 mm in diameter and 5 mm in Length [76], stainless steel (1 mm in diameter [63], 1 mm in diameter and 2 mm in thread length [72]). The shape of the implant in one study [62] was a scaffold, while the rest were screws [70,72] and rods [71,76].

In addition, there were differences in sample sizes (six [72], sixteen [62] twenty-four [71], 36 [76] and 112 [70]), animal species (New Zealand White Rabbits [70,72], Sprague-Dawley Rats [71,76] and BALB/c Mice [62]), ages (8-week-old [62], 3-month-old [76], 6-month--old [70] and 12-month-old [72],with one study [71] noting report the age of experimental animals), and weights (230–270g [71] and 5–7 kg [72]. Two studies [62,70] did not report the weights of experimental animals). There were also different follow-up durations (six weeks [71,72,76], eighty-four days [[62]] and sixteen weeks [70]).

The healing effect of pure magnesium on bone defects (see Figs. 3–5 and Appendix 4) was as follows.

1) New bone formation

This measure was reported in five studies. The results in Fig. 3 showed ① Initial period bone defect healing. Wang et al. [70] showed that the mineral deposition rate of bone tissues around the screws in the pure magnesium group was better than that in the control group with more eminent new bone formation. ② Mid- and long-term period bone defect healing. Wang et al. [70] showed that the mineral deposition rate of bone tissues around the screws in the pure magnesium group was not statistically different from that in the control group. The results of the study [70] showed that there was no statistical difference in the mineral deposition rate of the bone tissue around the screw between the pure magnesium group and the control group. Another study [76] showed the bone volume around the pure magnesium implant is larger than that of the control group; ③Long-term period bone defect healing: Wang et al. [70] showed that there was no statistical difference in the mineral deposition rate of the bone tissue around the screw between the pure magnesium group and the control group; ④ Terminal period bone defect healing. Studies [71,72,76] revealed that the quantity of new bone formation in the pure magnesium group was better than that in the control group. There was, however, no statistical difference in the quantity of bone formation and osteoid between the pure magnesium group and the control group [62]. On the other hand, Wang et al. [70] yielded an entirely opposite finding, in which the mineralization rate of bone tissues around the screws in the control group was better than that in the pure magnesium group.

2) BV/TV

Wang et al. [70] reported on the BV/TV outcome. Meta-analysis revealed that there was no significant difference in the BV/TV

Table 3
Materials and implants of the included animal studies.

Implant type	Author (year)	Biodegradable metal implant	Control implant	Biodegradable metal implant components	Coating	Implant shape		Implant specifications	
						T	C	T	C
Mg	Grau M 2017 [62]	Pure magnesium	Porous Ti6Al4V	Pure magnesium	PCL	Scaffolds	Scaffolds	3 mm × 3 mm × 1.2 mm, width 0.6 mm	3 mm × 3 mm × 1.2 mm, width 0.6 mm
	Wang, Jiali 2017 [70]	High purity Mg	Ti	99.99 wt%Mg	/	Screws	Screws	Ø:3 mm L:8 mm	Ø:3 mm L:8 mm
	Yu, K 2018b [71]	Pure Mg	Stainless Steel	99.9% pure,	/	Rod	Rod	Ø:1 mm	Ø:1 mm
	Henderson, S. E 2014a [71]	Pure Mg	Stainless Steel	99.9% pure,	/	Screws	Screws	Ø:1 mm Thread:M0.25 L: 2 mm	Ø:1 mm Thread:M0.25 L: 2 mm
	He, Wei 2020 [76]	Pure Mg	Ti	99.99 wt%Mg	/	Rod	Rod	Ø:1.4 mm L:5 mm	Ø:1.4 mm L:5 mm
Magnesium alloy	Erdmann, N 2010 [55]	MgCa0.8	Stainless Steel 316L	MgCa0.8 wt%	/	Screws	Screws	Ø:4 mm L:6 mm	Ø:4 mm L:6 mm
	Celarek, A 2012a [56]	ZX50	PHB	5% Zn, 0.25% Ca, 0.15% Mn. [wt.%]	/	Pins	Pins	Ø:1.6 mm L:8 mm	Ø:1.6 mm L:8 mm
	Celarek, A 2012b [56]	WZ21;	PHB	2% Y, 1.0% Zn, 0.25% Ca, 0.15% Mn [wt.%]		Pins	Pins	Ø:1.6 mm L:8 mm	Ø:1.6 mm L:8 mm
	Celarek, A 2012c [56]	Mg BMG	PHB	29% Zn; 5% Ca [at. %]		Pins	Pins	Ø:1.6 mm L:8 mm	Ø:1.6 mm L:8 mm
	Bondarenko, A 2014 [57]	MgCa0.8; LAE442; LANd442; ZEK100	TiAl6V4	/	/	Pins	Pins	Ø:2.5 mm L:25 mm	Ø:2.5 mm L:25 mm
	Guan, Xingmin 2014 [58]	JDBM	316L Stainless steel	Mg-2.8 wt%Nd-0.2 wt%Zn-0.4 wt %Zr	brushite	Screws	Screws	Ø:2 mm Thread length: 4.6 mm	Ø:2 mm Thread length: 4.6 mm
	Berglund, I. S 2016 [59]	Mg–1.0Ca–0.5Sr alloy	PLLA	Mg–1.0 wt% Ca–0.5 wt%Sr	/	Pins	Pins	Ø:0.8 mm	Ø:1.1 mm
	Diekmann, J 2016 [60]	MgYREZr	Ti6Al4V	MgYREZr	/	Screws	Screws	Ø:2.6 mm L:10 mm Thread pitch: 0.8 mm	Ø:2.6 mm L:10 mm Thread: 0.8 mm
	Dong, J. H 2018a [61]	Mg–Sr alloy	TCP	Mg-1.5sr	High temperature treatment	Pins	Pins	2.7 mm × 2.2 mm	/
	Dong, J. H 2018b [61]	Mg–Sr alloy	TCP	Mg-1.5sr	/	Pins	Pins	2.7 mm × 2.2 mm	/
	Levorova, J 2018 [63]	WE43	Titanium	Mg–4Y-3RE-Zr	/	Screws	Screws	Head Ø:3 mm outer thread Ø:1.5 mm core:1.1 mm L:3 mm	Head Ø:3 mm outer thread Ø:1.5 mm core:1.1 mm L:3 mm
	Lindtner, R. A 2013 [64]	Mg–Y-ND-HRE	PLGA	Mg–Y-ND-HRE	/	Pins	Pins	Ø:1.6 mm L:7 mm	Ø:1.6 mm L:7 mm
	Niu, J. L 2016 [65]	JDBM	316L Stainless steel	Mg-2.8 wt%Nd-0.2 wt%Zn-0.4 wt %Zr	brushite	Screws	Screws	Ø:2 mm Thread length:4.6 mm	Ø:2 mm Thread length:4.6 mm
	Rössig, Christina 2015 [66]	LAE442	Stainless austenitic steel (1.4441LA)	Li 3.7 wt%; Al 3.62 wt%; Rare earths: Nd 0.16 wt%, Ce 0.73 wt%, La 0.38 wt%, Pr 0.03 wt%	/	Nails/ screws	Nails /screws	Nails: Ø:9 mm; L:130 mm Screws: shank Ø:3.8 mm; thread Ø:3.5 mm; core Ø:2.9 mm; L:15–40 mm; Thread pitch:1.25 mm	Nails: Ø:9 mm; L:130 mm Screws: shank Ø:3.8 mm; thread Ø:3.5 mm; core Ø:2.9 mm; L:15–40 mm; Thread pitch:1.25 mm
	Schaller, B 2016a [67]	WE43	Ti	Mg–Y-Nd	Plasma electrolytic coating	Screws	Screws	outer Ø: 2.43 mm, inner Ø: 2.1 mm, L: 6.0 mm, thk.: 0.165 mm; outer Ø: 2.53 mm, inner Ø: 2.2 mm, L: 6.0 mm, thk.: 0.165 mm	outer Ø: 2.2 mm, inner Ø: 2.1 mm, L: 6.0 mm, thk.: 0.05 mm;
	Schaller, B 2016b [67]	WE43	Ti	Mg–Y-Nd	/	Screws	Screws		

(*continued on next page*)

Table 3 (*continued*)

Implant type	Author (year)	Biodegradable metal implant	Control implant	Biodegradable metal implant components	Coating	Implant shape		Implant specifications	
						T	C	T	C
								The same as Schaller, B 2016a	The same as Schaller, B 2016a
	Thormann, U 2015 [68]	W4	Titanium	96 wt.-%Mg,4 wt.-% Yttrium, <0.25 wt.-% rare earth	MAO(Ca–P)	Screws	Screws	8 × 23 mm	8 × 23 mm
	Trincă, Lucia Carmen 2015 [69]	Mg–Ca–Si alloy	Zr	Mg–0.4%Ca–0.5% Si	/	Pins	Pins	Ø:2 mm L:4 mm	Ø:2 mm L:4 mm
	Yu, K 2018a [71]	Mg–Ag–Y	Stainless Steel	Mg-1 wt% Ag- 1 wt % Y	/	Rod	Rod	Ø:1 mm	Ø:1 mm
	Henderson, S. E 2014b [72]	AZ31	Stainless Steel	Mg-2.5–3.5 wt% Al, 0.6–1.4 wt% Zn and 0.2–1.0 wt% Mn	/	Screws	Screws	Ø:1 mm Thread:M0.25 L: 2 mm	Ø:1 mm Thread:M0.25 L: 2 mm
	Chen, Junxiu 2019 [73]	$Mg_{69}Zn_{27}Ca_4$ metal glass	β-TCP	69.45 at.%Mg-26.60 at%Nd-3.95 at%Ca	/	Cylinder	Cylinder	Ø:3.0 mm L:3.0 mm	Ø:3.0 mm L:3.0 mm
	Guo, Yu 2019 [75]	Mg3Gd	Heal-All	/	Chitosan	Membranes	Membranes	/	/
	Zhang, D. 2020 [77]	Mg–Zn-Gd	Ti	/	Ca–P	Scaffolds	Scaffolds	/	/
	Witte, F. 2005a [78]	AZ31	SR-PLA96	/	/	Rods	Rods	Ø:1.5 mm L:20.0 mm	Ø:1.5 mm L:20.0 mm
	Witte, F. 2005b [78]	AZ91	SR-PLA96	/	/	Rods	Rods	Ø:1.5 mm L:20.0 mm	Ø:1.5 mm L:20.0 mm
	Witte, F. 2005c [78]	LAE442	SR-PLA96	Mg-51 wt%Ce-22 wt%La-16 wt%Nd-8 wt%Pr	/	Rods	Rods	Ø:1.5 mm L:20.0 mm	Ø:1.5 mm L:20.0 mm
	Witte, F. 2005d [78]	WE43	SR-PLA96	Mg-71 wt%Nd-8 wt%Ce-6 wt%La	/	Rods	Rods	Ø:1.5 mm L:20.0 mm	Ø:1.5 mm L:20.0 mm
	Hong, Y 2008a [80]	AZ31B	Titanium alloy	Mg 95.0–97.0, A1 2.50–3.50, Zn 0.60–1.40, Mn 0.20–1.00, Si ≤ 0.10, Fe ≤ 0.005, Cu ≤ 0.05, Ni ≤ 0.005	/	Plates	Plates	L: 10 mm, four holes in the middle, Ø: 2 mm	L: 10 mm, four holes in the middle, Ø: 2 mm
	Hong, Y 2008b [80]	AZ31B	Titanium alloy	Mg 95.0–97.0, A1 2.50–3.50, Zn 0.60–1.40, Mn 0.20–1.00, Si ≤ 0.10, Fe ≤ 0.005, Cu ≤ 0.05, Ni ≤ 0.005	/	Plates	Plates	Bottom L: 20 mm, Height: 15 mm, porous area: 165 mm^2, pore Ø: 1 mm, 3 pores at the edge, Ø: 2 mm	Bottom L: 20 mm, Height: 15 mm, porous area: 165 mm^2, pore Ø: 1 mm, 3 pores at the edge, Ø: 2 mm
	Qi, Z 2013a [81]	ZK60	PLLA	Zn 5.5 wt%,Zr 0.4 wt%,(balance)	MAO	Screws	Screws	Ø 2 × 6 mm	Ø 2 × 6 mm
	Qi, Z 2013b [81]	ZK60	PLLA	Zn 5.5 wt%,Zr 0.4 wt%,Mg(balance)	/	Screws	Screws	Ø 2 × 6 mm	Ø 2 × 6 mm
	[a] Sun, W 2010a [82]	AZ31B	Titanium alloy	Al:2.50–3.50, Zn:0.60–1. , Mn:0.20–1.00,Si = 0.10,Fe = 0.005, Cu = 0.05,Ni = 0.005	/	Screws	Screws	Ø:2.0 mm L:7.0 mm	Ø:2.0 mm L:7.0 mm
	Sun, W 2010b [82]	AZ31B	Titanium alloy	Al:2.50–3.50, Zn:0.60–1.40, Mn:0.20–1.00,Si = 0.10,Fe = 0.005, Cu = 0.05,Ni = 0.005	F	Screws	Screws	Ø:2.0 mm L:7.0 mm	Ø:2.0 mm L:7.0 mm
	Sun, W 2010c [82]	AZ31B	Titanium alloy	Al:2.50–3.50, Zn:0.60–1.40, Mn:0.20–1.00,Si = 0.10,Fe = 0.005, Cu = 0.05,Ni = 0.005	Ca–P	Screws	Screws	Ø:2.0 mm L:7.0 mm	Ø:2.0 mm L:7.0 mm
	Zhu, Z 2013a [83]	JDBM	Ti	/	CaP	Pins	Pins	1 mm × 30 mm	1 mm × 30 mm
	Zhu, Z 2013b [83]	JDBM	Ti	/	/	Pins	Pins	1 mm × 30 mm	1 mm × 30 mm
	Zhang, N 2018a [84]	Mg–Zn–Ca	Autogenous bonegraft	2.5 wt%~3.0 wt% Ca,0.7 wt%~1.3	MAO	Scaffolds	Scaffolds		

(*continued on next page*)

Table 3 (*continued*)

Implant type	Author (year)	Biodegradable metal implant	Control implant	Biodegradable metal implant components	Coating	Implant shape		Implant specifications	
						T	C	T	C
				wt%Zn,0.2 wt% Mnand pure Mg				L: 15 mm, inner Ø:3 mm, outer Ø: 5 mm	L: 15 mm, inner Ø:3 mm, outer Ø: 5 mm
	Zhang, N 2018b [84]	Mg–Zn–Ca	Autogenous bonegraft	2.5 wt%~3.0 wt% Ca,0.7 wt%~1.3 wt%Zn,0.2 wt% Mnand pureMg	/	Scaffolds	Scaffolds	L: 15 mm, inner Ø:3 mm, outer Ø: 5 mm	L: 15 mm, inner Ø:3 mm, outer Ø: 5 mm
Pure Zn	Guo, H. 2020a [74]	Zn without pores	Ti without pores	Pure Zn	/	Membranes	Membranes	9×9 mm^2 in area	9×9 mm^2 in area
	Guo, H. 2020b [74]	Zn with 300 μm pores	Ti without pores	Pure Zn	/	Membranes	Membranes	9×9 mm^2 in area	9×9 mm^2 in area
	Guo, H. 2020c [74]	Zn with 1000 μm pores	Ti without pores	Pure Zn	/	Membranes	Membranes	9×9 mm^2 in area	9×9 mm^2 in area
Zn alloy	Jia, Bo 2020 [79]	Zn-0.8Mn	Pure Ti	Zn-0.8 wt% Mn	/	Scaffolds	Scaffolds	Ø:3 mm L:4 mm	Ø:3 mm L:4 mm

Ø: diameter; L:length; thk.: thickness.

[a] Two experiments in the study (Sun, W 2010) on the degradation and osteogenesis of biodegradable metals were described together.

measure around the pure magnesium implant in all periods of bone defect healing between the two groups (Fig. 5, initial period; MD-0.06 [95% CI-0.16, 0.04]; P = 0.22 mid-term period; MD 0.00 [95% CI-0.10, 0.10]; P = 1.00 long-term period; MD 0.07 [95% CI-0.05, 0.19]; P = 0.25 terminal period; MD 0.00 [95% CI-0.11, 0.11]; P = 1.00). The effects of pure magnesium and the non-degradable metals on bone defect repair were similar.

3) Bone implant contact

Only one study [62] reported on bone implant contact in the terminal period of healing. The results showed that bone implant contact in the pure magnesium group was lower than that of the control group in the terminal period of bone defect healing, see Fig. 3.

4) Degradation

The implant degradation outcome was reported in four studies [62,70–72]. The results showed that implant degradation in the pure magnesium group was better than that in the control group [62,70–72], see Fig. 4.

5) Hydrogen generation

This measure was presented in two studies [70,71]. The results indicated that: ① During the initial period of bone defect repair there was gas generation around the pure magnesium implants [71], which led to premature loss of mechanical strength. ② In the terminal period of bone defect healing there was no gas concentration around the implants [70] in both the pure magnesium group and the control group, see Fig. 4.

3.4. Bone defect repair with magnesium alloy [55–61,63–69,71,72,75,77,78,80–84]

25 included studies [55–61,63–69,71,72,75,77,78,80–84] investigated bone defect repair with magnesium alloys. Among them, 16 studies [55,57,58,60,63,65–69,71,72,77,80,82,83] compared magnesium alloy material with non-degradable metals, five [56,59,64,78,81] with biodegradable polymers, two with bioceramics [61,73],one with autogenous bone [84], and one with guided bone regeneration membrane [75]. However, the bone defect models involved were quite different, including tibial defect [55,57,59,60,63,66,69], femoral defect [56,61,64,71,73,78,81–83], skull defect [75], mandibular defect [58,65,67,72,80,82], femoral condylar defect [68], ulnar defect [84] and Orbital defect [77]. Only one study [84] reported on CSDs. In addition, different implant materials were used in each control group, including stainless steel [55,58,65,66,71,72], titanium alloy [57,60,80,82], titanium [63,67,68,77,83], Zr [69], PHB [56], PLLA [59,81], PLGA [64], PLA [78], TCP [61,73], guided bone regeneration membrane [75] and bone graft [84]. The diameter of the implants was between 1 mm [71,72] and 4 mm [55], and the length was between 2 mm [72] and 130 mm [66]. The shapes of the implants included mostly screws [55,58,60,63–68,72,73,81,82], rods [71,78] and pins [56,57,59,61,69,83]. Only in one study [80], the shapes of the implants were strips and porous flakes. In two studies [77,84], the implant was a scaffold. In another study [75] the implant was a membranes.

Moreover, differences existed in sample sizes (between six [72] and seventy-two [64]), animal species (including rabbits/NZW [55,57,58,60,61,63,72,73,80,84], rabbits [65,82], rats/SD [56,59,64,71,75,81,83], rats/Wistar [69], sheep [66,68], mini-pig [67], beagles [77] and guinea pigs [78]), ages (mostly between five weeks [56] and six months [60]), weights (mostly between 120g [64] and 4.0 kg [60]), and follow-up durations (between four weeks [69] and eighteen months [65]).

*3.4.1. **Magnesium alloys vs. non-degradable metals** [55,57,58,60,63,65–69,71,72,77,80,82,83] (see Figs. 3, 4 and 6 and Appendix 4)*

1) New bone formation

16 studies [55,57,58,60,63,65–69,71,72,77,80,82,83] reported new bone formation. The results in Fig. 3 were as follows. ① In the initial period of bone defect healing, studies [55,60] showed new bone formation around the implants in both groups. No statistical comparison, however, was conducted between the groups. One study [58] found that there was better new bone formation in the magnesium alloy group than in the control group, with osteoid and osteoblasts forming around the screws, and good bone trabeculae arrangement. Another study [82] observed new bone formation around the implants in the magnesium alloy group, but the results in the control group were not described. ② In the mid-term period of bone defect healing, studies [55,60,69,80] revealed that there were new bone formation around the implants in both groups. No statistical comparison, however, was carried out between the groups. Studies [57,66,77,80,82] also observed that the quantity of new bone tissues around the magnesium alloy implants was better than that of the control group. However, some research [67] reported just the opposite results, with the bone volume around the magnesium alloy implant being significantly smaller than that of the control group. ③ In the long-term period of bone defect healing, studies [55,58] showed that there were new bone formation and bone cells around the screws in both groups, with no statistical comparison conducted between the groups. Research [82] pointed out that the number

	Outcome measures	New bone formation				bone defect healing				bone implant contact			
Implant type	measurement periods	initial	mid-term	long-term	terminal	initial	mid-term	long-term	terminal	initial	mid-term	long-term	terminal
Mg	Grau M 2017 [62]				=								<
	Wang, Jiali 2017 [70]	>	=	=	<								
	Yu, K 2018b [71]				>								
	Henderson, S. E 2014a [72]				>								
	He, Wei 2020 [76]		>		>								
Magnesium alloy	Erdmann, N 2010 [55]	?	?	?	?								
	Celarek, A 2012a [56]	?											
	Celarek, A 2012b [56]	?	?										
	Celarek, A 2012c [56]			?									
	Bondarenko, A 2014 [57]		>		>								
	Guan, Xingmin 2014 [58]	>		?									
	Berglund, I. S 2016 [59]	?		?	>				>				
	Diekmann, J 2016 [60]	?	?		?					<	=		?
	Dong, J. H 2018a [61]				>				>				
	Dong, J. H 2018b [61]				>				>				
	Levorova, J 2018 [63]				>								
	Lindtner, R. A 2013 [64]	>	=		=								
	Niu, J. L 2016 [65]				?								
	Rössig, C.2015 [66]		>		>								
	Schaller, B 2016a [67]		<		<						<		<
	Schaller, B 2016b [67]		<		<						<		<
	Thormann, U 2015 [68]				=								
	Trincă, L. C. 2015[69]		?										
	Yu, K 2018a [71]				>								
	Henderson, S. E 2014b [72]				>								
	Chen, Junxiu 2019 [73]				>				?				
	Guo, Yu 2019 [75]		=		=								
	Zhang, D. 2020 [77]		>		?								
	Witte, F. 2005 [78]		>		>								
	Hong, Y 2008a [80]		>		>		=						
	Hong, Y 2008b [80]		>		?		?		<				
	Qi, Z 2013a [81]		>		>								
	Qi, Z 2013b [81]		>		>								
	Sun, W 2010a [82]	?	>	>	>								
	Sun, W 2010b [82]	?	>	>	>								
	Sun, W 2010c [82]	?	>	>	>								
	Zhu, Z 2013a [83]				>								
	Zhu, Z 2013b [83]				>								
	Zhang, N 2018a [84]	=	<	>	?	=	<	<	=				
	Zhang, N 2018b [84]	=	<	>	?	=	<	<	<				
Pure Zn	Guo, H. 2020a [74]			?	?								
	Guo, H. 2020b [74]			?	?								
	Guo, H. 2020c [74]			?	?								
Zn alloy	Jia, Bo 2020 [79]		?	>	>								

Fig. 3. Summary diagram of qualitative descriptive outcomes (new bone formation related outcomes)
“>“, green: the effect of the biodegradable metal group is superior to the control group;
”<“: red: the effect of the control group is superior to the biodegradable metal group;
” = “: blue: there is no difference between the biodegradable metal group and the control group;
“?“: yellow: there is no comparison conducted between the biodegradable metal group and the control group, or only outcomes of the biodegradable metal group were reported;
“/“: blank: there is no outcome for this period
The follow-up processes of the included studies are divided to four periods, which are the initial period, the mid-term period, the long-term period and the terminal period.

of new bone tissues and osteoblasts in the magnesium alloy group was better than that in the control group. ④ In the terminal period of bone defect healing, studies [55,60,68,69,80] reported that there was new bone formation around the implants in both groups, with no statistical comparison. Another study [65,77] showed that the new bone in the magnesium alloy group was closely attached to the degradation layer with good bone integration and significant bone cells observed in bone tissues, whereas no description was yielded for the control group. Studies [57,63,66,71,72,80,82,83] revealed that the new bone formation of the magnesium alloy group was better than that of the control group, while others [67], on the contrary, found that the quantity of bone around the magnesium alloy implants was significantly smaller than that of the control group.

2) Bone defect repair

Only one study [80] reported bone defect healing. The results in Fig. 3 were as follows. ① In the mid-term period of bone defect healing, the study [80] showed that the bone defects in the strip magnesium alloy group and the control group had been completely healed, while the study [80] showed that the bone defects in the porous flake magnesium alloy group and the control group had only been partially repaired, with no statistical difference between the groups. ② In the terminal period of bone defect healing,the study [80] revealed that there were irregular osteogenic and osteolytic areas under the porous flake magnesium alloy group, and the bone defects were not well repaired, whereas the bone defects in the control group were well repaired.

3) BV/TV

Only one study [67] reported BV/TV. The meta-analysis revealed that, in the mid-term and terminal period of bone defect healing, the BV/TV of magnesium alloy group was lower than that of the control group (Fig. 6a, mid-term period; MD-48.63 [95% CI-64.03, - 33.24]; P = 0.04 terminal period; MD-40.92 [95% CI-50.72, - 31.12]; P = 0.04). The difference was statistically significant.

	Outcome measures	Degradation				Gas formation			
Implant type	measurement periods	initial	mid-term	long-term	terminal	initial	mid-term	long-term	terminal
Mg	Grau M 2017 [62]				>				
	Wang, Jiali 2017 [70]				>				=
	Yu, K 2018b [71]				>	>			
	Henderson, S. E 2014a [72]			>					
	He, Wei 2020 [76]					>			
Magnesium alloy	Erdmann, N 2010 [55]					>	>	>	>
	Celarek, A 2012a [56]	>	>		>				>
	Celarek, A 2012b [56]	>	>		>				
	Celarek, A 2012c [56]		>						
	Bondarenko, A 2014 [57]								>
	Guan, Xingmin 2014 [58]								
	Berglund, I. S 2016 [59]	>	>		>	>	>		=
	Diekmann, J 2016 [60]					>	>		=
	Dong, J. H 2018a [61]				?				?
	Dong, J. H 2018b [61]				?				?
	Levorova, J 2018 [63]	=	=	=	>				
	Lindtner, R. A 2013 [64]				>				
	Niu, J. L 2016 [65]				>				
	Rössig, C. 2015 [66]				=		>		>
	Schaller, B 2016a [67]					>	>		
	Schaller, B 2016b [67]					>	>		
	Thormann, U 2015 [68]	>			>	>			>
	Trincă, L. C. 2015 [69]		>				>		?
	Yu, K 2018a [71]				>	>			
	Henderson, S. E 2014b [72]		=	>					
	Chen, Junxiu 2019 [73]								?
	Zhang, D. 2020 [77]						=		=
	Witte, F. 2005 [78]				>	>			
	Hong, Y 2008a [80]								
	Hong, Y 2008b [80]						>		
	Qi, Z 2013a [81]	?	?		?	>	>		>
	Qi, Z 2013b [81]	?	?		?	>	>		>
	Sun, W 2010a [82]	>	>	>	>				
	Sun, W 2010b [82]	=	>	>	>				
	Sun, W 2010c [82]	=	>	>	>				
	Zhu, Z 2013a [83]				>				
	Zhu, Z 2013b [83]				>				
	Zhang, N 2018a [84]				?	>	>	>	>
	Zhang, N 2018b [84]				?	>	>	>	>
Pure Zn	Guo, H. 2020b [74]				>				
Zn alloy	Jia, Bo 2020 [79]		>	>	>				

Fig. 4. Summary diagram of qualitative description outcomes (implant degradation related outcomes)
">", green: the effect of the biodegradable metal group is superior to the control group;
"<": red: the effect of the control group is superior to the biodegradable metal group;
" = ": blue: there is no difference between the biodegradable metal group and the control group;
"?": yellow: there is no comparison conducted between the biodegradable metal group and the control group, or only outcomes of the biodegradable metal group were reported;
"/": blank: there is no outcome for this period
The follow-up processes of the included studies are divided to four periods, which are the initial period, the mid-term period, the long-term period and the terminal period.

4) Bone implant contact

Two studies [60,67] reported bone implant contact. The results in Fig. 3 were as follows. ① In the initial period of bone defect healing, Diekmann et al. [60] observed lower bone implant contact in the intermediate and medial locations in the magnesium alloy group than that in the control group, and the difference reached statistical significance. However, there was no statistical difference between the groups in the bone implant contact in lateral locations. ② In the mid-term period of bone defect healing, Diekmann et al. [60] found that the bone implant contact in the magnesium alloy group was lower than that in the control group in lateral, intermediate and medial locations. The difference, however, was not statistically significant. Schaller et al. [67] showed less bone implant contact in the magnesium alloy group than the control group and the difference was statistically significant. ③ In the terminal period of bone defect healing, Diekmann et al. [60] exhibited that the bone implant contact of screws in the medial locations in the magnesium alloy group was lower than that in the control group, with statistically

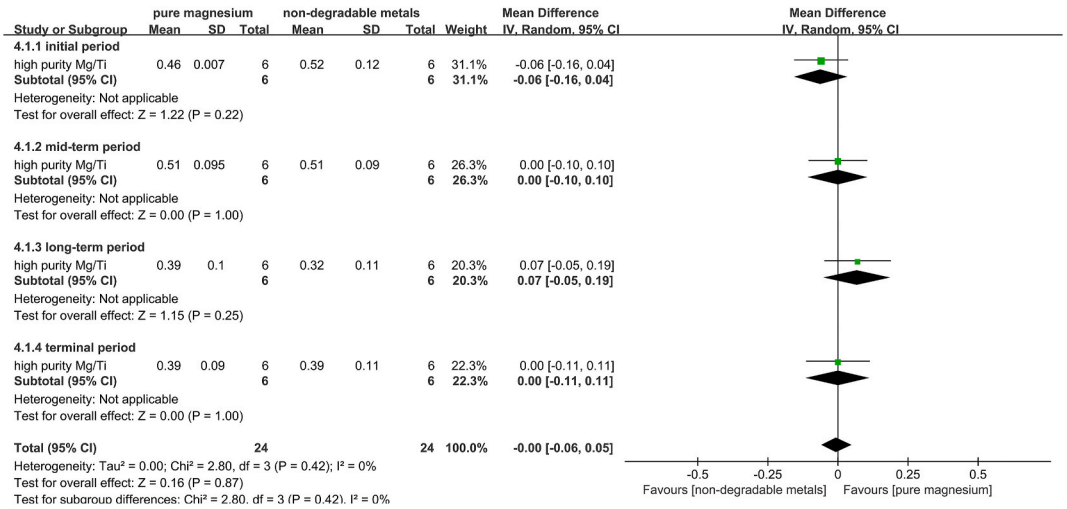

Fig. 5. Forest plot of BV/TV for pure magnesium versus non-degradable metals.

significant difference. In the same study, it was found that the bone implant contact in the lateral and intermediate locations in the magnesium alloy group was higher than that of the control group. The difference, however, was not statistically significant. In the study [67], bone implant contact in the magnesium alloy group was smaller than that of the control group, with statistically significant difference.

5) Degradation

Nine studies [60,63,65,66,68,71,72,82,83] reported implant degradation. The results in Fig. 4 were as follows. ① In the initial period of bone defect healing, some studies revealed that the implants of the magnesium alloy group and the control group did not degrade [63,82], while others showed that the magnesium alloy implant decreased in volume and irregular degradation occurred on the surface [60,68,82]. ② In the mid-term period of bone defect healing, studies [63,72] observed that implants of the magnesium alloy group and control group did not degrade, but others [60,69,82] identified implant degradation in the magnesium alloy group. ③ In the long-term period of bone defect healing, one study [63] found no degradation in the magnesium alloy group and the control group, while others [72,82] observed degradation in the magnesium alloy group and null in the control group. ④ In the terminal period of bone defect healing, studies [60,63,65,68,71,72,82,83] revealed better performance in implant degradation in the magnesium alloy group than that in the control group. Another study [66] found only slight degree of degradation in the magnesium alloy with no significant difference between the two groups.

6) Hydrogen generation

Ten studies [55,57,60,66–69,71,77,80] reported hydrogen generation. The results in Fig. 4 were as follows. ① In the initial period of bone defect healing, it was observed that hydrogen was produced around the implants [55,60,68,71] in the magnesium alloy group, while others [67] observed no hydrogen generation around the implants in the magnesium alloy group nor the control group. ② In the mid-term period of bone defect healing, studies [55,60,66,67,69,80] found hydrogen generation around the magnesium alloy implants, while hydrogen was not observed around the implant [77]. ③ In the long-term period of bone defect healing, only one study [55] found gas generation around magnesium alloy screws. ④ In the terminal period of bone defect healing, Diekmann et al. [60] observed little hydrogen generation around the magnesium alloy implants. No hydrogen production around the implant was observed in the study by Zhang et al. [77], whereas others [55,57,66,68,69] confirmed hydrogen generation around the magnesium alloy implants.

3.4.2. Magnesium alloy vs. biodegradable polymers [56,59,64,78,81] (see Figs. 3, 4, Fig. 6 and Appendix 4)

1) New bone formation

Five studies [56,59,64,78,81] reported new bone formation. The results in Fig. 3 were as follows. ① In the initial stage of bone defect healing, the implants in the magnesium alloy group and the control group showed new bone formation. There was, however, no statistical difference between the groups [56]. Berglund et al. [59] revealed scattered new bone formation around the magnesium alloy implants, while the outcome was not described in the control group. Larger quantity of new bone formation around the magnesium alloy implant was revealed in another study, as compared with the control group [64]. ② In the mid-term period of bone defect healing, Celarek [56] found fine bone growth on the surface of the magnesium alloy implants, whereas the outcome was not described for the control group. Lindtner et al. [64] pointed out that there was no statistical difference in new bone formation between the magnesium alloy group and the control group. Yet another study [78,81] showed better new bone formation in the magnesium alloy group than the control group. ③ In the long-term period of bone defect healing, new bone formation was observed around the magnesium alloy implants with no description of the control group [59]. ④ In the terminal period of bone defect healing, studies [59,78,81] found larger quantity of new bone formation in the magnesium alloy group than the control group, while no statistical difference in the quantity of new bone formation was observed between the two groups in another study [64].

2) Bone defect healing

Only one study [59] reported bone defect healing. The results in Fig. 3 revealed that in the terminal period of bone defect healing, the magnesium alloy group performed better than the control group.

3) BV/TV

Only one study [64] reported BV/TV. The meta-analysis showed that the BV/TV around the implants in the magnesium alloy group was higher than that in the control group in the initial period of bone defect healing, and the difference was statistically significant. However, there was no significant difference in BV/TV around the magnesium alloy implants in the mid-term and terminal periods of bone defect healing

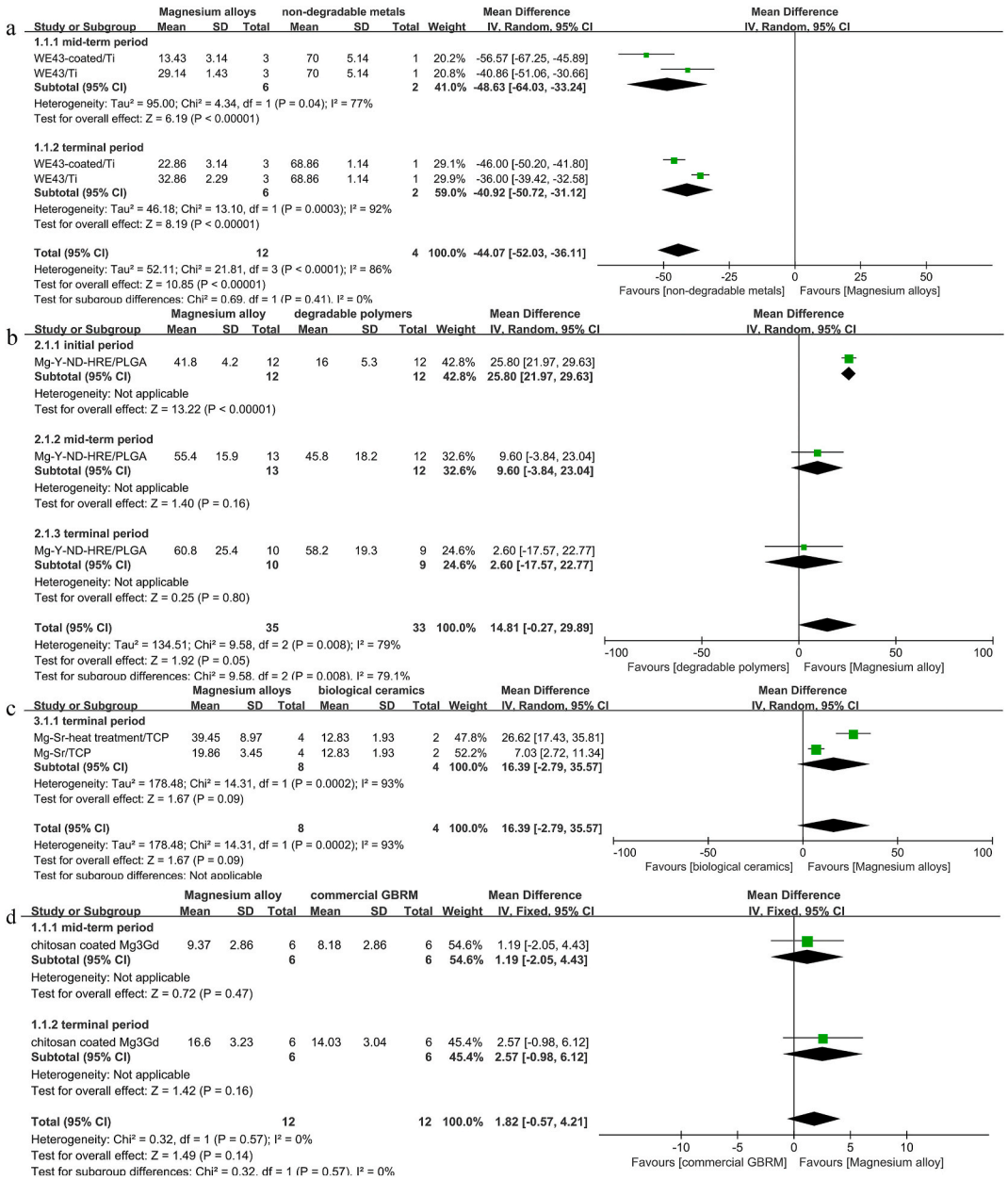

Fig. 6. Forest plot of BV/TV for magnesium alloy versus control group.

between the two groups (Fig. 6b, initial period; MD 25.80 [95% CI 21.97, 29.63]; P < 0.01 mid-term period; MD 9.60 [95% CI-3.84, 23.04]; P = 0.16 terminal period; MD 2.60 [95% CI-17.57, 22.77]; P = 0.80). The meta-analysis revealed that in the initial period of bone defect healing, magnesium alloy excelled at promoting the regeneration of bone tissue and bone defect healing than biodegradable polymers. Then in the subsequent periods of bone defect healing, the two materials showed similar ability in bone defect healing.

4) Degradation

Five studies [56,59,64,78,81] reported implant degradation. The results in Fig. 4 were as follows. (1) In the initial period of bone defect healing, the magnesium alloy implants substantially degraded, causing the loss of mechanical stability prematurely [56] while the control group showed no sign of degradation. Studies [56,59,81] reported degradation of magnesium alloy implants, with no description of the control group. ② In the mid-term period of bone defect healing, one study [56] identified substantial degradation of magnesium alloy implants, while no sign of degradation was observed in the control group. Another study [81] found irregular-shaped magnesium alloy caused by palpable degradation, while the degradation of the control group was not described. ③ In the long-term period of bone defect healing, one study [59] found substantial degradation of magnesium alloy with some broken parts, but no degradation in the control group. ④ In the terminal period of bone defect healing, it was found that the condition of magnesium alloy degradation was better than that of the control group [56,59,64,78,81].

5) Hydrogen generation

Four studies [56,59,78,81] reported gas generation. The results in Fig. 4 were as follows. ① In the initial, mid-term and long-term periods of bone defect healing, hydrogen cavity was observed only around the magnesium alloy implants [59,78]. ②In the mid-term period of bone defect healing, one study by Qi et al. [81] found that hydrogen cavity

was only generated around the magnesium alloy implant; ③In the long-term period of bone defect healing, one study by Berglund et al. [59] also found hydrogen cavity was only generated around the magnesium alloy implant; ④In the terminal period of bone defect healing, Berglund et al. [59] found there was no hydrogen generation around the implants in both the magnesium alloy group and the control group, while the other studies [56,81] found that hydrogen was still seen in the magnesium alloy group, which affects the healing process of bone defects.

3.4.3. *Magnesium alloy vs. bioceramics [61,73] (see Figs. 3, 4 and 6 and Appendix 4)*

Two studies [61,73] compared the effects of magnesium alloy and bioceramics on bone defect repair. The results were as follows.

1) New bone formation

In the terminal period of bone defect healing, the quantity and thickness of bone trabeculae, as well as the quantity of mature bone around the implants in magnesium alloy group were better than those in the control group [61]. The cancellous bone of the magnesium alloy group was in close contact with the implant, and the control group only formed new cartilage, and no mature bone was seen. The contact condition was worse than that of the magnesium alloy group [73], see Fig. 3.

2) Bone defect healing

In the terminal period of bone defect healing, the remaining size of bone defect in the magnesium alloy group was smaller than that in the control group, with statistically significant difference [61]. In another study, the diameter of the remaining bone defect in the magnesium alloy group was 3.35 mm, and the diameter of the bone defect in the control group was 4.14 mm, but no statistical analysis was not performed [73], see Fig. 3.

3) BV/TV

Only one study [61] reported BV/TV. The meta-analysis revealed that there was no significant difference in BV/TV around the implants between the two groups in the terminal period of bone defect healing (Fig. 6c, MD 16.39 [95% CI-2.79, 35.57]; P = 0.09). Although the included animal models in the study were all NZW, the heterogeneity was high (Q = 14.31, P = 0.0002; I^2 = 93%). The meta-analysis indicated that the healing effects of magnesium alloy and bioceramics were similar.

4) Degradation

Only one study [61] reported implant degradation. In the terminal period of bone defect healing, partial corrosion occurred to the implants of the magnesium alloy group, but not to the control implants, see Fig. 4.

5) Gas generation

In the terminal period of bone defect healing, a small amount of gas generation was observed for the magnesium alloy implants in one study by Dong et al. [61]. However, large amount of gas generation was found for the magnesium alloy implants, whereas the condition of the control group was not described in the study [61]. For another study [73], there was no obvious gas generation around the magnesium alloy implant, but the control group did not show any related results, see Fig. 4.

3.4.4. *Magnesium alloy vs. autogenous bone [84] (see Figs. 3 and 4 and Appendix 4)*

Only one study [84] compared the effects of magnesium alloy and autogenous bone graft on bone defect repair. The results were as follows.

1) New bone formation

① In the initial period of bone defect healing, no callus formation was observed in the two groups. ② In the mid-term period of bone defect healing, bone cells and a large quantity of chondrocytes were identified in both group. Callus formation in the autogenous bone graft group, however, was better than that of the magnesium alloy group. ③ In the long-term period of bone defect healing, more mature osteocytes were observed around the defect site in regular arrangements in both groups, while the quantity of chondrocytes of the autogenous bone graft group was significantly less than that of the magnesium alloy group. ④ In the terminal period of bone defect healing, mature osteocytes in the new bone around the scaffold were further increased in a more orderly arrangement in the magnesium alloy group. The fibrous membrane around the scaffold disappeared, and a small number of chondrocytes were observed around the scaffold. The condition of the control group was not described, see Fig. 3.

2) Bone defect healing

① In the mid- and long-term periods of bone defect healing, the healing in the autogenous bone graft group is better than that in the magnesium alloy group. ② In the terminal period, the healing effect of magnesium alloy with coating was equivalent to that of autogenous bone graft. However, the healing effect of uncoated magnesium alloy was still worse than the control, see Fig. 3.

3) Degradation

In the terminal period of bone defect healing, there was a small amount of scaffold residue of the coated magnesium alloy, and the scaffold disappeared in the uncoated magnesium alloy group. Condition of the control group was not described, see Fig. 4.

4) Hydrogen generation

① From the initial to the long-term period of bone defect healing, gradual increase of hydrogen generation and gas accumulation under the skin were observed in the magnesium alloy group. ② In the terminal period of bone defect healing, gas generation in the magnesium alloy group decreased, while no gas accumulation was observed in the control group, see Fig. 4.

3.4.5. *Mg alloy vs guided bone regeneration membrane [75] (see Figs. 3 and 6 and Appendix 4)*

Only one study [75] compared the effect of magnesium alloy and guided bone regeneration membrane on bone defect repair. The results showed the following:

1) New bone formation:

① In the mid-term period of bone defect healing, both the magnesium alloy group and the control group showed new bone formation, but there was no statistical difference in quantifying the area of new bone formation. ② In the terminal period of bone defect healing, both groups formed new bone from the edge to the center of the defect, but there was still no statistical difference in the area of new bone, see Fig. 3.

2) BV/TV: Meta-analysis results show that BV/TV around the implant in the magnesium alloy group is greater than that in the control group in the mid-term period of bone defect healing, but the difference is not statistically significant. In the terminal period of bone defect healing, there was no statistical difference of BV/TV between the control group around the magnesium alloy implant (Fig. 6d, mid-term; MD 1.19 [95%CI -2.05–4.43]; P = 0.47 final; MD 2.57 [95% CI -0.98 6.12]; P = 0.16). Meta analysis results show that magnesium alloy and traditional guided bone regeneration membranes have similar ability to guide bone

regeneration, see Fig. 4.

3.5. *Bone defect repair with pure zinc material [74] (see Figs. 3, 4 and 7 and Appendix 4)*

Only one included study [74] explored the effect of pure zinc material on bone defect repair in comparison with a non-degradable metal. The report uses critical skull defect. The material and shape of the control group are Ti and membranes (9 × 9 mm^2 in area), respectively.

The effects of pure zinc material on bone defect repair (see Figs. 3, 4 and 7 and Appendix 4) are summarized below.

1) New bone formation: ①In the long-term period of bone defect healing, clear new bone formation was seen on the edges of the three kinds of pure zinc membrane. The zinc membrane of 300 μm pores had the strongest osteogenic ability. The formation of new bone could also be clearly observed at the edge of the control group, but statistical analysis was not conducted. ②At the terminal period of bone defect healing, the zinc membrane of 300 μm pores formed the most amount of new bone, and the new bone formation of the zinc membrane of 1000 μm pores was the least. The control group also had new bone formation, but no statistical analysis was performed, see Fig. 3.
2) BV/TV: Meta-analysis results show that BV/TV around the implant in the pure zinc group is lower than that in the control group in the long-term period of the bone defect healing, and the difference is statistically significant. However, in the terminal period of the bone defect healing, there was no statistical difference in terms of BV/TV between the pure zinc implant and the control group (Fig. 7, long-term; MD -6.07 [95% CI -10.15–2.05]; P = 0.003 final; MD -4.27 [95% CI -13.32 4.77]; P = 0.003). Meta-analysis results show that Ti membranes have a better ability to promote new bone formation than pure Zn membranes in the long-term period of bone defect healing. However, in the subsequent terminal period of bone defect healing stage, the two groups show similar ability to repair bone defects.
3) Degradation: In the terminal period of bone defect healing, the zinc membrane of 300 μm pores showed a relatively uniform degradation pattern without obvious local corrosion, while the control group did not see any degradation, see Fig. 4.

3.6. *Bone defects repair with zinc alloy materials [79] (see Figs. 3, 4 and 8 and Appendix 4)*

Only one included study [79] explored the repair of bone defects with zinc alloys in comparison with non-degradable metals. The bone defect model was femoral condyle defect. In addition, the implant used in the control group is porous pure Ti scaffolds (3 mm in diameter and 4 mm in length).

The effects of the zinc alloys on bone defects repair (see Figs. 3, 4 and 8 and Appendix 4) are described below.

New bone formation: ① In the mid-term period of bone defect healing, a small amount of new bone formation was seen in both the zinc alloy and the control groups, but no statistical analysis was conducted. ② In the long-term period of bone defect healing, a large amount of new bone was seen around the implant in the zinc alloy group. There was a large amount newly formed bone tissue in the zinc alloy group. In addition, the newly formed trabecular bone is also thicker than that in the pure titanium group. ③ In the terminal period of bone defect healing, compared with the control group, there is more new bone tissue around the implant in the zinc alloy group, see Fig. 3.

2) BV/TV: Meta-analysis results show that BV/TV around the implant in the zinc alloy group was higher than that in the control group in the mid-term period of bone defect healing, but the difference is not statistically significant. While in both the long-term and terminal periods of bone defect healing, the BV/TV around the zinc alloy implant was higher than the control group, and the difference was statistically significant (Fig. 8 mid-term; MD 3.96 [95% CI -0.40 8.32]; P = 0.08 long-term; MD 9.07 [95% CI 4.69 13.45]; P < 0.0001 terminal; MD 9.78 [95% CI 4.73 14.83]; P = 0.0001). Meta-analysis results show that in the mid-term period of bone defect healing, zinc alloy and pure Ti have similar effects on promoting new bone formation, while in the long-term and terminal periods of bone defect healing, zinc alloy shows a stronger ability to promote new bone formation.

3) Degradation: ①In the mid-term period of bone defect healing, degradation products appeared around the zinc alloy implant. ②In the long-term period of bone defect healing, the degradation products around the implant in the zinc alloy group increased, but there was no degradation product around the control group. ③In the terminal period of bone defect, a lot of degradation products were seen around the zinc alloy implants, and there was still no degradation product around the control group, see Fig. 4.

3.7. *Risk of bias and quality of evidence*

The assessment results of the risk of bias in the included studies are shown in Figs. 9 and 10. Of the 30 animal studies included, only 20 studies [55,58–60,63–67,71,74–78,80–84] were randomized controlled experiments. None of the studies revealed specific randomized grouping method or if random sequence generation was concealed. Although the 20 studies had balanced baseline characteristics, none of them reported if caregivers and researchers were blinded. Only four studies [59,64,68,

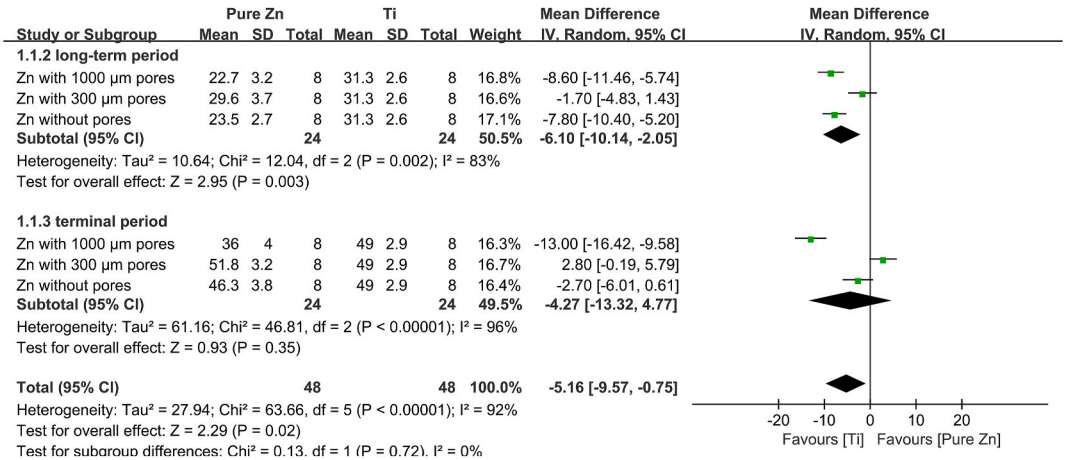

Fig. 7. Forest plot of BV/TV for pure Zn versus control group.

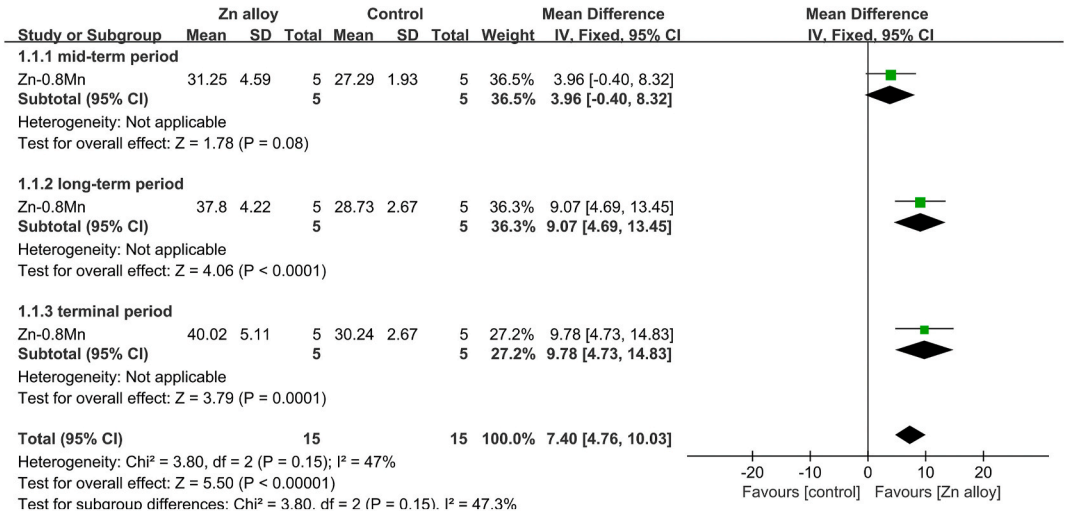

Fig. 8. Forest plot of BV/TV for Zn alloy versus control group.

76] reported that the animals were randomly selected in the outcome evaluation process. Only two studies [64,68] randomized placement of experimental animals. Only one study [84] reported blinding of outcome raters. The experimental animals of 13 studies [55,57,58,60,65,67,68,71,75,76,79,80,84] were included in the final analysis. Although no research protocol was available for any of the studies, all expected results were clearly reported, see Figs. 9 and 10.

The quality of the evidence was *very low* according to assessment of the six outcome measures. The reasons for poor quality of evidence included lack of internal validity, inconsistency of results, and difficulty in reproducing relevant results. See Tables 4 and 5 for more details.

4. Discussion

30 animal studies that qualified for the inclusion criteria were systematically reviewed, and only the BV/TV measure was analyzed quantitatively through meta-analysis. A high degree of heterogeneity existed in most of the outcome measures in the included studies. Variance was found in study design, animal species, age, defect model, type and composition of biodegradable metals, implant design and implantation time, measurement time point of outcome measures, and measuring methods and criteria for outcome measures. Therefore, only qualitative descriptions were performed. Overall, this review adopted a mixed method of qualitative and quantitative analysis in discussing the included studies.

4.1. Degradable Mg and its alloys for bone defect repair and degradation performance

In general, compared with traditional bone repair materials, degradable magnesium and its alloys show good ability to repair bone defects. However, some studies have shown that in the terminal period of bone defect healing, the amount of bone tissue around magnesium alloy implants is significantly lower than that of non-degradable metal groups [67]. The terminal results in Wang et al. [70] showed that the mineralization rate of bone tissue around the screws in the control group was better than that in the pure magnesium group, which may be related to the negative impact of gas generation on bone quality during metal degradation [67]. At the same time, the terminal results in the study by Hong et al. [67] exhibited that the healing effect in the porous flake magnesium alloy group was worse than that in the control group, which might be ascribed to the increased contact area of the porous flake magnesium alloy with the surrounding tissue fluid, which accelerated its degradation [29]. This also led to PH value and high concentration of magnesium ions, excessive secretion of BMP-2, activation of osteoclasts and regional osteolysis [80]. Bone-implant contact in the magnesium alloy group was generally worse than that in control group, which may be related to implant displacement and hydrogen generation [62]. In addition, the research of Tanja Kraus et al. [85] and Wang, J., et al. [86] also showed that hydrogen generation led to perforation on bone tissue, which interfered with the initial healing process of bone defects. The rapid generation of hydrogen bubbles could also lead to obvious subcutaneous bubbles and even tissue necrosis [87]. Therefore, whether the initial gas generation by biodegradable metals could be controlled is crucial to the quality of bone defect healing and new bone formation [88]. In addition, Liu, C. et al. [89] observed that heat-treated magnesium alloy showed enhanced repair capability in that the remaining defect size was smaller than that of untreated magnesium alloy. This may be related to the improvement of corrosion resistance of magnesium alloy by heat treatment. This in turn contributed to an appropriate magnesium ions concentration to promote osteoblast adhesion and bone tissue growth [61]. Moreover, in the terminal period of bone defect healing, the biodegradable metal with MAO coating had similar healing effect as autogenous bone and the former also decelerated the degradation of magnesium alloy in the study by Zhang et al. [84]. This was related to the improvement of corrosion resistance and osteogenesis of magnesium alloy materials by MAO coating [90].

In terms of degradation, although most studies revealed that biodegradable metals were well degraded in the terminal periods. However, the magnesium alloy implant (ZX50) in the study by Celarek et al. [56] substantially degraded in the initial period of bone defect healing, which did not lead to the bone defect healing in the weight-bearing area. Compared with WZ21 (slow degradation, 2% Y, 1.0% Zn, 0.25% Ca, 0.15% Mn [wt.%] [85,91], the excessive degradation rate of ZX50 (5% Zn, 0.25% Ca, 0.15% Mn. [wt.%]) may be related to the lack of rare earth element Y in its alloy composition, which may leads to its rapid degradation rate. Studies showed that rare earth elements such as Y increased the corrosion resistance of magnesium alloy materials [92,93], and improved the mechanical properties and creep resistance of magnesium alloy [94]. In addition, many rare earth elements have anti-cancer properties [95,96]. At present, Mg-rare earth (RE)-based alloys have been studied extensively [97–101], showing good corrosion resistance. However, rare earth elements are mainly distributed in the implantation site and may not be tolerated by human body [102]. Some alloys are slightly cytotoxic [97]. Therefore, future research should report the in vivo degradation of Mg-rare earth (RE)-based alloys and the long-term in vivo safety of rare earth elements.

Regarding the surface modifications, although studies showed that

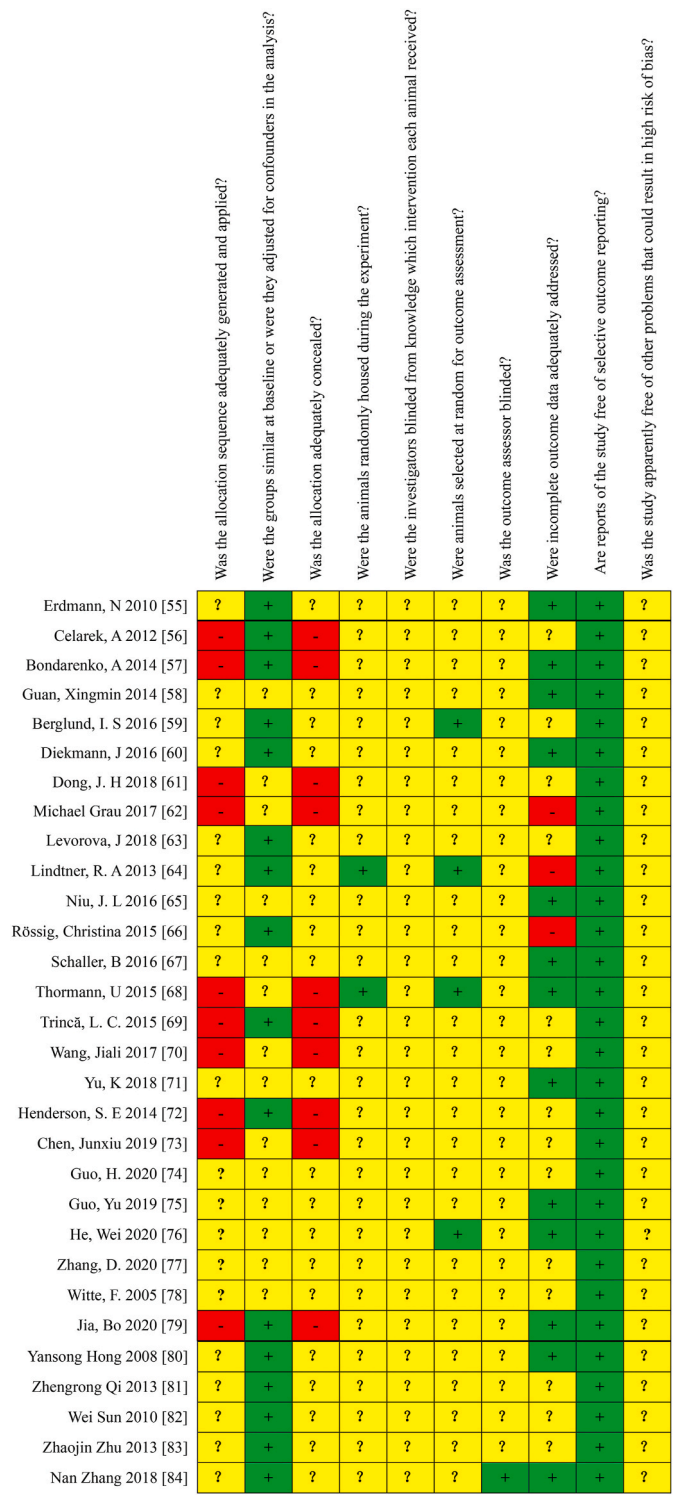

Fig. 9. Results of the risk of bias assessment of the 30 studies included in this systematic review [51].

heat treatment improved the corrosion resistance of magnesium alloy [103,104], only Ran, F. et al. [105] compared the effects of different heat treatment processes on the mechanical properties of biodegradable metals and the in vitro corrosion resistance. There is a serious lack of research on the effects of different heat treatment processes on in vivo bone defect repair. No consensus has been reached so far in the field regarding the most suitable heat treatment process for bone defect repair. Therefore, some unanswered key questions include how to improve the structure and properties of the alloy, how to optimize its composition design and processing, and how to combine it with feasible coating or surface modification technology to make the degradation rate controllable [106]. In addition, studies showed that biodegradable metal coating boosted alloy corrosion resistance [107–110], biocompatibility [111–113] and the ability to stimulate new bone formation [114]. However, there is a wide range of coatings available with no standard process for preparation [115]. Some key elements of the coating, such as corrosion rate, surface chemical property, adhesion, coating morphology and controllability of degradation, were not fully reported [116]. Therefore, it is necessary to investigate the key elements of biodegradable metal coating, and their impact on the efficacy and safety in specific cases of bone defect repair.

The matching between degradation rate of biodegradable metals and rate of bone defect repair is the critical factor for the product translation and clinical applications. At present, only Berglund et al. [59] attended to the problem of aligning the degradation rate of biodegradable metals and bone defect repair rate. It was observed that at the end of the follow-up, the degradation of magnesium alloy reached over 90% with the bone defect well repaired [59], avoiding retrieval of the implants. Most of the other studies only reported the degradation at different measurement time points, which could not reflect the degradation rate of degradable metals in the actual process of bone defect repair. Therefore, it is necessary for future research to observe and compare the degradation rate of the biodegradable metals in light of the bone defect healing rate, with a better understanding of the their value in bone defect repair.

The vast majority of studies only reported new bone formation but not bone defect healing. Some studies [55,60,68,69,80] did not carry out between-group statistical analysis on new bone formation, making it difficult to identify the comparative advantage. Therefore, future research should focus on the comparative analysis and report between-group differences, presenting more reliable experimental data for the clinical translation of biodegradable metals in bone defect repair.

4.2. Degradable zinc and its alloys for bone defect repair and degradation performance

The two currently included literatures show that the degradable pure zinc and its alloys have shown good bone defect repair capabilities in the terminal period of bone defect healing, and the degradable zinc alloys have shown better ability to promote new bone formation compared to pure zinc. On one hand, in the process of bone formation, Zn has dual effects of promoting bone formation with osteoblasts and inhibiting bone resorption with osteoclasts. At the same time, Zn can also promote the growth of cartilage [117]. This is in line with the effects of Zn on upregulating the activity of alkaline phosphatase (ALP) [118], promoting collagen synthesis [119], activating Runx-2 (runt-related transcription factor 2) and its expression of downstream genes to promote the proliferation of osteoblasts [120] which leads to the formation of new bone [121–123]. On the other hand, the corrosion rate of Zn is lower than that of Mg [124], and the degradation rate of Zn alloys is in between that of Mg alloy and Fe alloy [125]. In addition, preclinical in vivo studies have shown that Zn and its alloys have good biocompatibility [126,127], which makes Zn-based biodegradable metals as an emerging substitute for magnesium-based biodegradable metals. However, the low mechanical strength of Zn and its alloys limits its wide clinical applications [128].

The two articles included in this study show that pure Zn and Zn–Mn binary alloys have good bone defect repair capabilities. In addition, Zn–Mg, Zn–Ca, Zn–Sr binary alloys also suggest their bone defect repair potential [129]. A recently published study demonstrated that Zn-0.8Sr alloy has potential to repair critical-size bone defects in load-bearing situations [130]. Furthermore, Zn–2Cu alloy implants have also proved their potential in the treatment of orthopedic infections [131]. Taken together, the current preclinical in vivo studies on zinc-based

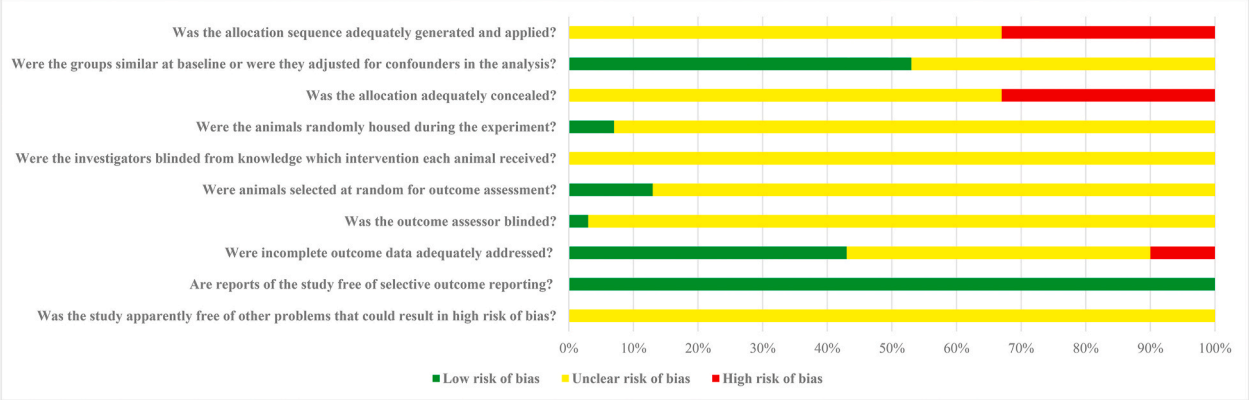

Fig. 10. Results of the risk of bias assessment of the 30 studies included in this systematic review [51].

biodegradable metals are limited to binary alloys such as Zn–Mg, Zn–Ca, Zn–Sr, Zn–Mn, Zn–Cu, etc. New Zn alloys such as those with addition of rare earth elements could be studied in the future to explore their potential bone repair abilities. Meanwhile, future studies may also need to address topics such as the processing methods of Zn-based biodegradable metals (for example, additive manufacturing, electron beam melting, or electrical casting), strengthening nano-additives such as nanodiamonds, nanocarbon nanotubes, or graphene, surface modification such as coating technologies as well as preclinical in vivo animal studies of Zn-based biodegradable metals to repair bone defects [126].

4.3. Animal model, anatomical site and critical size of bone defects

After reviewing previous literature on biodegradable metals in bone defect repair, it was found that the selection of animal model, the location of bone defect, CSD, and the follow up time points and durations had no correlation with the expected clinical application. This might lead to discrepancies in the repair effect of biodegradable metals in animal experiment and clinical trials [27].

Animal models are generally divided into small animal models (mice, rats and rabbits) and large animal models (dogs, goats, pigs and sheep). Small animal models are usually used as primary screening for in vivo evaluation of biomaterials [132]for ethical, economic and statistical considerations. In recent years, mice and rats have become increasingly popular as a model animals for bone defect and fracture healing research due to the availability of molecular analysis tools and transgenic models. Nonetheless, there are great limitations in the clinical translation of small animal models [133,134]. Rodent models cannot adequately mimic human bone regeneration for a number of reasons, among them a lack of cortical remodeling and the fact that cessation of growth occurs much later than in other mammals. Whereas initial screening and feasibility testing are popularly carried out in rodent models, large animal models whose bone regeneration is closer to the same processes in humans are essential to provide translational proof of concept. To mitigate the limitations [132], it is usually necessary to refer to internal fixation and biomaterial implants in large animals to deduce the effect on human body [135]. The Food and Drug Adminstration (FDA), for example, often requires the testing of bone therapies in both a small and large animal model before accepting a medical product for clinical trials [136]. Therefore, when an innovative bone graft material is introduced to the clinical field, it is necessary for the material to be qualified for translation through large animal models. Only 13.33% (4/30) of the 30 reviewed studies, however, investigated the effect of biodegradable metals on bone defect repair in large animal models. This is not beneficial to the evaluation of the practical value of biodegradable metals for their clinical translation.

Bone provides in vivo support and protection to human organs. Bone regeneration is complicated by the bones being load-bearing or not. Depending on the bones having load-bearing functions or not, the performance of biomaterials may be different [137]. For bone defect repair in non-/low load-bearing sites, skull is the ideal choice of model. For bone defect repair in load-bearing sites, not only the anatomical sites of bone defect model (such as femur, tibia, etc.) should be considered, but also the fact that the sites should have similar load-bearing functions as those in the human body. At present, most of the studies only focused on the performance of biodegradable metals in bone repair and degradation, and shed no light upon their application for anatomic sites where the load-bearing function is necessary. Therefore, it is crucial for future research to consider the specific load-bearing condition of biodegradable metals as bone graft materials. This is also very important for their clinical translations.

In the 30 included studies, only 10.00% (3/30) of the studies paid attention to CSD, while most of the studies did not even clarify this question. The definition of CSD has been modified several times. The notion generally refers to the smallest size of defect that cannot be self-repaired in the lifetime of the animal without medical intervention [138]. It is also referred to as a defect which shows less than 10% bone regeneration during the lifetime of the animal [139]. In non-CSD models, even if without bone graft material implanted, the self-healing ability of bone tissue itself will enable new bone formation and complete bone defect healing. The choice of such non-CSD bone defect model is not science-based, and bear little clinical significance in the research of orthopedic biomaterials for bone defect repair. Therefore, future research should pay special attention to the establishment of critical-sized defect animal models for the benefit of clinical application. This way, authentic and reliable preclinical research data can be generated for the clinical translation of biodegradable metals in bone defect repair.

In addition to different metabolism level and tissue response of implants in different bone defect animal models [90], variance in materials, models and evaluation methods adopted in the animal studies of biodegradable metals make it difficult to reproduce and compare between different studies. Even contradictory conclusions were reached. For instance, the osteogenic effect of biodegradable metals was found to be worse than that of Ti [67], but another study [68] revealed that the repair effect of the two was similar. Therefore, the material composition, microstructure-property relationships, animal models, anatomical sites, design features of implants, surgical procedures and measuring methods must be standardized to ensure cross-examination between different studies and results. This is helpful for solidifying the sustainable development of biodegradable metals through accumulation of reliable data.

4.4. Sources of heterogeneity, internal validity and quality of evidence

Based on the rigorous systematic review, our research found that the

Table 4
Summary of the confidence rating of outcomes (CERQual Qualitative Evidence Profile Table) [52,53].

Outcome measures	Number of included studies	Aspect 1: methodological limitations	Aspect 2:correlation	Aspect 3:Consistency of results	Aspect 4:Adequacy of data	Quality of the evidence (CERQual)
New bone formation	30 [55–84]	Selection bias; performance bias; detection bias; attrition bias	The clinical translation is limited by the location of defects, CSD, implant design of biodegradable metals, and duration of implantation	Among the included 30 studies, only the initial period of [58,70], the mid-term period of [57,64,58,76-78,80a,81,82], the long-term period of [79,82], and the terminal period of [57,59,61,63,66,71,72-73,76,78-79,80a,81-83] showed that the biodegradable metal is superior to the control group in terms of new bone formation. However, the mid-term period of [67,84], the long-term period of [84] and the terminal period of [67,70], showed just the opposite.	All study quantitatively measured new bone formation	⊕⊖⊖⊖ very low
Bone defect healing	5 [59,61,73,80,84]	Selection bias; performance bias; detection bias; attrition bias	The clinical translation is limited by the location of defects, CSD, implant design of biodegradable metals, and duration of implantation	Among the five studies, only the terminal measurement of [59,61] showed that the biodegradable metal is superior to the control group in terms of bone defect healing. However, the terminal measurement of [80b,84b] showed just the opposite.	Bone defec healing was quantitatively reported in 3 studies [61,73,84]. However, the bone defect model, the species, composition, specification, implantation duration and result data of the biodegradable metals were incomplete, making meta-analysis unfeasible.	⊕⊖⊖⊖ very low
Bone-implant contact area	3 [60,62,67]	Selection bias; performance bias; detection bias; attrition bias	The clinical translation is limited by the location of defects, CSD, implant design of biodegradable metals, and duration of implantation	There is no statistical difference between the biodegradable metal group and the control group.	All the studies quantitatively reported the bone-implant contact area, however, there was significant heterogeneity in animal species, age, body weight, bone defect model, material type and composition of biodegradable metals, outcome measurement methods and efficacy criteria. As a result, the data could not be synthesized and analyzed.	⊕⊖⊖⊖ very low
Implant degradation	21 [56,59–66,68–72,74,78,79,81–84]	Selection bias; performance bias; detection bias; attrition bias	The clinical translation is limited by the location of defects, CSD, implant design of biodegradable metals, and duration of implantation	Among the 21 included studies, it was shown that implant degradation of the biodegradable metals' group is more significant than that of the control group.	Implant degradation was qualitatively measured in all studies	⊕⊖⊖⊖ very low
Hydrogen generation	18 [55–57,59–61,66–71,73,77,78,80,81,84]	Selection bias; performance bias; detection bias; attrition bias	The clinical translation is limited by the location of defects, CSD, implant design of biodegradable metals, and duration of implantation	Among the 18 included studies, it was shown that hydrogen generation of the biodegradable metals group is more significant than that of the control group.	Hydrogen generation was qualitatively measured in all studies	⊕⊖⊖⊖ very low

Table 5
Quality of the evidence-GRADE [54].

Outcome measures	Number of included studies	Aspect 1: limitations in risk of bias	Aspect 2: inconsistency	Aspect 3: indirectness	Aspect 4: imprecision	Aspect 5: publication bias	Quality of the evidence (GRADE)
BV/TV	7 [61,64,67,70,74,75,79]	-1[a]	-1[b]	-1[c]	-1[d]	0	⊕⊖⊖⊖ very low

[a] No random sequence generation was concealed and no blinding.
[b] Point estimates vary widely from study to study.
[c] Differences in animal species and interventions (types, specifications, and implantation time of biodegradable metals).
[d] The confidence interval contains invalid values.

current quality of evidence for the effect of biodegradable metals on bone defect repair was very low, reducing the reliability of the experimental results, and increasing the risks of animal model results being translated into the clinical practice. Possible reasons are explained as follows.

There were significant differences in the animal species, bone defect models, measurement points, measuring methods, and efficacy criteria in the included studies. Consequently, meta-analysis could only be conducted for the BV/TV measure. For instance, there was a total of six different animal species and eight different defect models in the 30 included studies. The ideal animal study of biodegradable metals should be guided by the expected clinical indications. An animal evaluation model reflecting the product function should be established [27,140]. At the same time, the selected animal model should have similar physiological and pathological manifestations as those in the human body [141]. The observation of large and relevant data should be enabled in a relatively short period of time [142]. In addition, the cost of animal access and care, the availability of animals, social acceptability, tolerance for captivity and the ease of placement should also be considered [143]. Therefore, it was hard to perform the cross-reference and comparability of different studies.

Reichert et al. [144] recommended standardization of animal models, fixation devices, surgical procedures and measurement methods to gather reliable original data. Consideration should be given to the practical application of 3Rs (reduction, refinement and replacement) in experimental design and implementation [145]. Consideration should also be given to animal types, model-related parameters such as the load-bearing condition, size of defects and repair methods [146], sample sizes and evaluation methods [147]. CSD models should be used to investigate the practical value of biodegradable metals in bone defect repair [148].

The primary problems with the outcome measures were variance and inconsistency. For any outcome measure, there would be different numbers of studies involved. For instance, 19 of the studies [55–57,59–61,66,67,69–71,73,76–78,80,81,84] reported on gas generation, five on bone defect healing [59,61,73,80,84], three on bone implant contact [60,62,67], and seven on BV/TV [61,64,67,70,74,75,79]. Moreover, the outcome measures were captured by divergent approaches and methods. ① The same outcome measures were taken under different paradigms. For instance, new bone formation was quantitatively measured in one study [67], whereas a qualitative approach was used in another research [55]. ② The results of the same outcome measures came from different tools. To evaluate defect healing, one study [80] adopted general observation, while other studies [61] used Micro-CT, or X-ray [84]. Therefore, it is necessary to standardize the methods of outcome measurements to enhance the value of animal studies and avoid the waste of experimental animals.

In addition, only nine of the 30 included studies conducted quantitative analysis on BV/TV and bone-implant contact. Most of the studies relied only on the qualitative approach in presenting the outcome measures. As is widely known, quantitative analysis enables more complex prediction, test of significance, correlation strength and other complex analysis [149,150], which facilitates in-depth data mining and provides stronger data support for hypotheses. Moreover, meta-analysis of homogeneous quantitative data from different studies can greatly improve the level of precision from single studies [44,47,151]. More importantly, it is of significance to explore the possible causes of contradictory results among similar experiments. This may give rise to new hypotheses and provide scientific and evidence-based medical support for the development and design of subsequent experiments. Therefore, it is necessary for future studies to apply in-depth mining and report of quantitative data and provide substantial evidence with rich quantitative data support. This will also avoid and reduce the repeated use of experimental animals.

The study design of most of the included experiments was not scientifically rigorous. For example, the randomization process of the trials of all studies (30/30) was unspecified. None of the studies reported on whether random sequence generation was concealed. Baseline characteristics were uneven in 46.67% (14/30) of the studies [58,61,62,65,67,68,70,71,73–78]. Consequently, the probability of selection bias was high. In addition, most studies did not carry out blinding of caregivers/researchers or outcome assessors. Randomized and concealed allocation and blindness are important measures to reduce the risk of inherent bias in animal studies [152–155]. Strict control of various risks of bias will help reduce risk of clinical translation from animal study results. Compared with clinical trials, the sample size of most animal studies was small. For instance, among the 30 studies included in this systematic review, 18 [58,59,61–63,65–69,72–75,77,80,81,83] had fewer than thirty animal subjects. Some important differences in baseline characteristics will greatly affect the experimental results [51]. Therefore, future research would benefit from scientifically rigorous methods to estimate the viability of sample size [156], and comprehensively report the experimental details. This practice will improve the validity and reliability of animal study results.

Most experiments lacked quality control measures to reduce measurement and implementation bias. For example, none of the studies reported whether caregivers/researchers were blinded and only 3.33% (1/30) [84] outcome assessors/raters were blinded. Although animal blindness is not required in animal studies, most of the researchers are caregivers. Therefore, blindness should be adopted during intervention and outcome measurement to reduce implementation and measurement bias and increase data validity [157,158]. For example, the measurement of new bone formation and bone defect healing in the study of biodegradable metals for bone defect repair mainly relies on researcher observation of new bone formation and bone defect healing measures around the implants through imaging and histological methods. If researchers have knowledge of the interventions in advance, they may be biased when evaluating the osteogenesis or defect healing effect between groups, affecting the data validity. In addition, to capture outcome measures, especially those that depend on human judgment, it is imperative to implement effective blinding to avoid measurement bias on the results. Having qualified technicians is the key to ensure the inter/intra-rater consistency on different animals, and the accuracy of measurement calibration. These potential biases have an impact on the results to various degrees [159]. However, the 30 studies included in this systematic review did not report on the qualifications of the raters, nor the protocols and standards they followed for specific measurement processes.

Regarding unbiased report of experimental data, although all the included studies clearly reported all expected results in their methods and results sections, we could not obtain their original research protocols, and judge if they were implemented accordingly and all results were reported in an unbiased manner. Selective reporting of animal study results lead to publication bias, which may affect the reliability of systematic reviews, and even cause contradictory conclusions [160]. Government agencies and academic societies or associations should encourage prospective registration of animal studies to obtain raw data [161].

4.5. Publication bias

Experiments with positive results are usually more likely to be published than those with negative or null results [162]. Publication bias may be more severe in animal studies [163]. Therefore, if systematic reviews do not include unpublished studies, they are likely to produce overestimation of the effects of interventions. This present review did not evaluate the possibility of publication bias by statistical analysis. In the field of experimental research, it is important to take measures to promote data sharing and encourage journals to publish studies with negative or neutral results to avoid the "file-drawer problem" and reduce the impact of publication bias on their results [164].

4.6. Strengths and limitations of this study

To the best of our knowledge, this is the first systematic review of animal studies to assess the efficacy of biodegradable metals in bone defect repair. First, this review adopted the CERQual and GRADE tools to respectively evaluate the quality of evidence on both the qualitative and quantitative outcome measures. It provided an evidence-based assessment of the risk of translating preclinical results from animal studies to clinical trials. Second, the risk of bias in animal studies was assessed based on the internationally recognized SYRCLE tool. Third, the internal and external authenticity of the evidence was discussed in detail to objectively analyze the risk and feasibility of converting animal model results to clinical practice. However, there are two limitations for this systematic review. First, searching only Chinese and English databases might result in certain language bias. Second, failure to search gray literature and conference abstracts might cause publication bias.

4.7. Prospects for future research

After the comprehensive analysis of the basic information of the included literature, the inherent risk of bias, the quality of evidence, and the study outcomes, it is found that animal studies on the repair of bone defects with biodegradable metals have limitations. Future study should be oriented towards clinical indications to establish animal models that could reflect product performance. At the same time, in terms of bone defect models, standardized critical bone defect sizes should be established. The design of study protocol should consider randomization and blindness. In addition, during the process of study implementation and quality control, the data collection at time zero, the qualifications of the outcome measurers, and the standards and specific measurement processes during outcome measurement shall be reported. Furthermore, third-party evaluations could be cited. The methods and processes of outcome measurements should be standardized. Special attention should be paid to the quantitative report of outcome measurements to enhance the value and quality of the study. Finally, we recommend that the original data of literatures on animal studies be provided as an online appendix [161] in order to both improve the transparency of the entire process of animal studies, and to promote the translation and utilization of the results [48].

5. Conclusions

In summary, biodegradable metals have been widely used in the animal studies for bone defect repair. Biodegradable metals have shown healing effects for bone repair and degradation properties of materials. The results of this evidence-based research suggest that the quality of evidence for the efficacy of biodegradable metals on in vivo bone defect repair is still very low. There are inconsistent conclusions among existing studies in that some biodegradable metals did not show sound bone repair performance in animal models. At present, the animal models, anatomical sites and CSD in the included studies remain divergent, limited and non-standard. To support further clinical translation, animal studies need improvement in study design, outcome measurement and quality assurance to both reduce bias and scientifically examine the role of biodegradable metals in bone defect repair. Future animal studies should be designed in light of prospective clinical indications. A standardized framework for the animal studies should be established to assess the effect of biodegradable metals on bone defect repair. Meanwhile, more evidence-based research should be carried out to enhance clinical translation of biodegradable metals.

Declaration of competing interest

There are no conflicts to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2021.03.035.

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