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Review Article

Perceiving the connection between the bone healing process and biodegradation of biodegradable metal implants through precise bioadaptability principle

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ABSTRACT

The implants made of metallic biomaterials help healing the bone fracture but also affect the bone repair process. As proposed in Matter 4 (2021) 2548–2650 by Wang et al., a precisely adaptable biomaterial ought to recapitulate the targeted tissue with spatiotemporal precision and hierarchical accuracy, ranging from atoms and molecules (genes, proteins, etc.) to cells (including organelles) and to tissues and organs. In comparison to traditional bio-inert metallic bone implants such as Co-based alloys and Ti alloys, biodegradable metal (Mg and Zn alloys) bone implants had been developed and might arise many unexpected variables in the bone repair, due to their bioactive nature. In this paper, the bone repair without and with the presence of metallic implants is compared. Thereafter, the perspectives concerning the interactions between the bone tissues and biodegradable metal implants are put forward, and how to better mimic *in vivo* biodegradation by *in vitro* experiments is proposed for further research and development of biodegradable metals.

1. Introduction

Bone fractures are the common health issues worldwide and of great concern to the aging demographic. Despite the bone is one of the few tissues that possesses the natural regenerative and self-repair capacity sufficient for healing small sites of damage without forming a fibrous scar, the bone repair may fail regarding to large segmental bone defects [1], which can only be repaired with the help of the bone grafting [2]. In the United States, over a million surgeries are performed to repair fractured bones annually [3]. An upsurge in the bioimplant market is predicted to exceed 116 billion dollars in 2020 [4]. Currently, the bone fixation with biomaterials owning ideal mechanical performance and biological properties becomes the hot spot of research. In the early 19th century, the first attempt to repair the damaged bone using bio-inert metallic materials as the bone grafts was reported [5]. Then, a large variety of materials such as polymers, bioceramics, biomedical metals and their combinations emerge as promising candidates for bone-engineering applications in subsequent years.

Initially, the polymer materials with excellent biological and degradable properties provide inspiration for the novel approach to bone-engineering applications. Owing to the insufficient structural support for polymer biomaterials, metallic biomaterials including stainless steels, titanium alloys and cobalt-chromium alloys with superior mechanical properties appear on the scene thereupon and are recently predominately used for the bone fixation and replacement [6]. Nevertheless, these bio-inert metallic biomaterials would be retained as permanent implants in the host and require a secondary surgical operation to be removed. Besides, the stress shielding complications as a consequence of mismatching in the elastic modulus between implants and natural bones could lead to insuf-

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ficient mechanical stimuli to the bone, evoking unhealthy growth of the surrounding bones [7]. In addition, the systemic toxicity of released metal ions from bio-inert metallic biomaterials as a consequence of wear and erosion is reported [8], further hindering the application of these bio-inert implants.

Entering the 21st century, biodegradable metals (BMs) that can induce appropriate host responses, provide sufficient initial mechanical integrity and degrade over time to offer a frame for the new tissue formation before being completely replaced by natural tissues are greatly explored as promising alternatives. Several key issues for BMs have been widely investigated over the last decade, including the selection of alloying elements, adjustment for microstructural and mechanical properties, biodegradation mechanisms and their influencing factors, control of degradation mode and rate and metal ion release behavior, and *in vitro* and *in vivo* biocompatibilities of BMs. Major approaches to control the biodegradation rate of BMs to match the healing rate of the host tissues involve various surface modification techniques and composite designs.

As well-known, the introduction of the foreign material might make a difference to the bone repair on the macroscopic level and on the cellular and molecular levels [9], and introduce a perfusion of intricate physical or chemical interactions taking place in the bone-metallic biomaterial implant interface. Bone repair can be significantly affected by metallic biomaterial implants in desirable or unexpected ways. Hence, the narration of bone fracture healing in the presence of metallic biomaterials will be established in this paper and perspectives on the development of BMs are proposed. It aims to appeal more attention to the precise bioadaptability between the bone tissue healing and the biodegradation of metallic biomaterial implants, and provide strategies for the design of animal tests and *in vitro* material characterization tests of metallic biomaterial.

2. Natural bone healing without incorporating biomaterials

As a highly dynamic tissue, the bone will undergo a highly complicated process to repair the fractures. There is a traditional four-stage procedure of the bone repair in which it can be conventionally partitioned into the inflammatory response, soft callus formation, hard callus formation and remodeling, as shown in Fig. 1. Notably, each stage is characterized by a specific set of cellular and molecular events, and significant overlap often exists among different stages regarding the timeline.

(i) *Stage I.* In general, the bone fracture involves the damage to cells and tissues, interruption to the normal vascular function inside the bone and the surrounding soft tissue and the distortion of the marrow architecture [10]. In such circumstance, with the vasodilatation and increased vascular permeability, the blood plasma and leukocytes consecutively exudate as a consequence. The fibrinogen will be converted into the fibrin and contribute to the formation of hematoma, which is typically characterized by low pH value and hypoxia. More importantly, the inflammatory response necessary for the bone healing is progressed. The hematoma serves a temporary scaffold to house the inflammatory cells and the neutrophils recruited by dead cells and the debris will be the first group of cells to arrive to the fracture sites [11]. During the first hours after injury, the neutrophils promptly accumulate and recruit monocytes or macrophages infiltrating to the same site by secreting inflammatory and chemotactic mediators [12]. The arrived macrophages are capable of removing the necrotic cells and provisional fibrin matrix via phagocytosis, while monocytes can partially differentiate into macrophages [9]. The mediators secreted by two waves of inflammatory cells, i.e., neutrophil and macrophage, will then initiate the recruitment of fibroblasts, mesenchymal stem cells (MSCs) and osteoprogenitor cells [12–14]. The fibroblasts migrating to the fracture sites will lead to the production of collagen and can create the fibrin meshwork. As time goes by, the granulation tissue rich in proliferating mesenchymal cells and vascularisation in the unorganized extracellular collagen matrix will be formed and eventually take the place of the hematoma.

(ii) *Stage II.* The proliferation and differentiation of MSCs from the surrounding soft tissues, cortex, periosteum and bone marrow raise the population of chondrogenic cells and osteogenic cells, contributing to the formation of the cartilage. Along with the fibrotic tissues, the cartilage tissue is commonly known as the soft callus. It can provide the initial mechanical stability for the fracture and serve as the scaffold for the following bone formation [15].

(iii) *Stage III.* There is an abundance of proliferative chondrocytes undergo mitosis. The chondrocytes become hypertrophic and go onto apoptosis. Later the proliferation of cells declines and hypertrophic chondrocytes become the dominant cell type [16]. Then the hypertrophic chondrocytes secrete calcium and mediators, and lead to the calcified cartilage extracellular matrix. Once the cartilage is calcified, it becomes the target for the extensive ingrowth of the blood vessels. In the meantime, the recruited MSCs and osteoprogenitor cells differentiate into osteoblasts, contributing to the woven bone deposited on the cartilage scaffold [12]. The mineralized cartilage will be resorbed with time and the primary soft callus is gradually replaced by the hard callus, which is more solid and mechanically rigid [17].

(iv) *Stage IV.* To fully restore the biomechanical properties of bones tissues, the hard callus needs to be remodeled into a lamellar bone structure. This remodeling phase is carried out with the activities of osteoclast and osteoblast. The osteoclasts derived from monocytes coming from the new blood vessels are able to resorb the necrotic bone fragments and necrotic ends of the fractured bones [9]. Accordingly, the woven bone and the cartilage matrix would be removed by osteoclasts, and at the same time, the lamellar bones are continuingly deposited in presence of osteoblasts [1]. The balance between two types of cells would finally result in the remodeled bone tissue and this process usually takes a long period to complete.

3. Metallic biomaterial designed for promoting bone healing

3.1. Traditional bio-inert metallic biomaterials

Titanium-based alloys, cobalt chromium alloys and stainless steels are the major non-degradable metallic biomaterials for load-bearing applications such as intervertebral fusion devices, joint replacements, craniomaxillofacial reconstruction, bone screw and plate systems owing to their high mechanical strength and excellent biocompatibility. Ti-based alloys, with low modulus, superior corrosion resistance and high capacity to join with the bone tissue, fast emerge as the first choice for the majority of load-bearing applications [18]. According to studies performed in the rabbit, baboon and rat models, implants made of pure Ti and Ti–6Al–4V alloy displayed excellent corrosion resistance and biocompatibility and had similar biomechanical anchorage [19]. They underwent acceptable osseointegration *in vivo* and both exhibited high level of direct bone-implant contact without apparent adverse tissue response. Therefore, commercial pure Ti and Ti–6Al–4V alloy are now widely used in dentistry and orthopaedics, respectively.

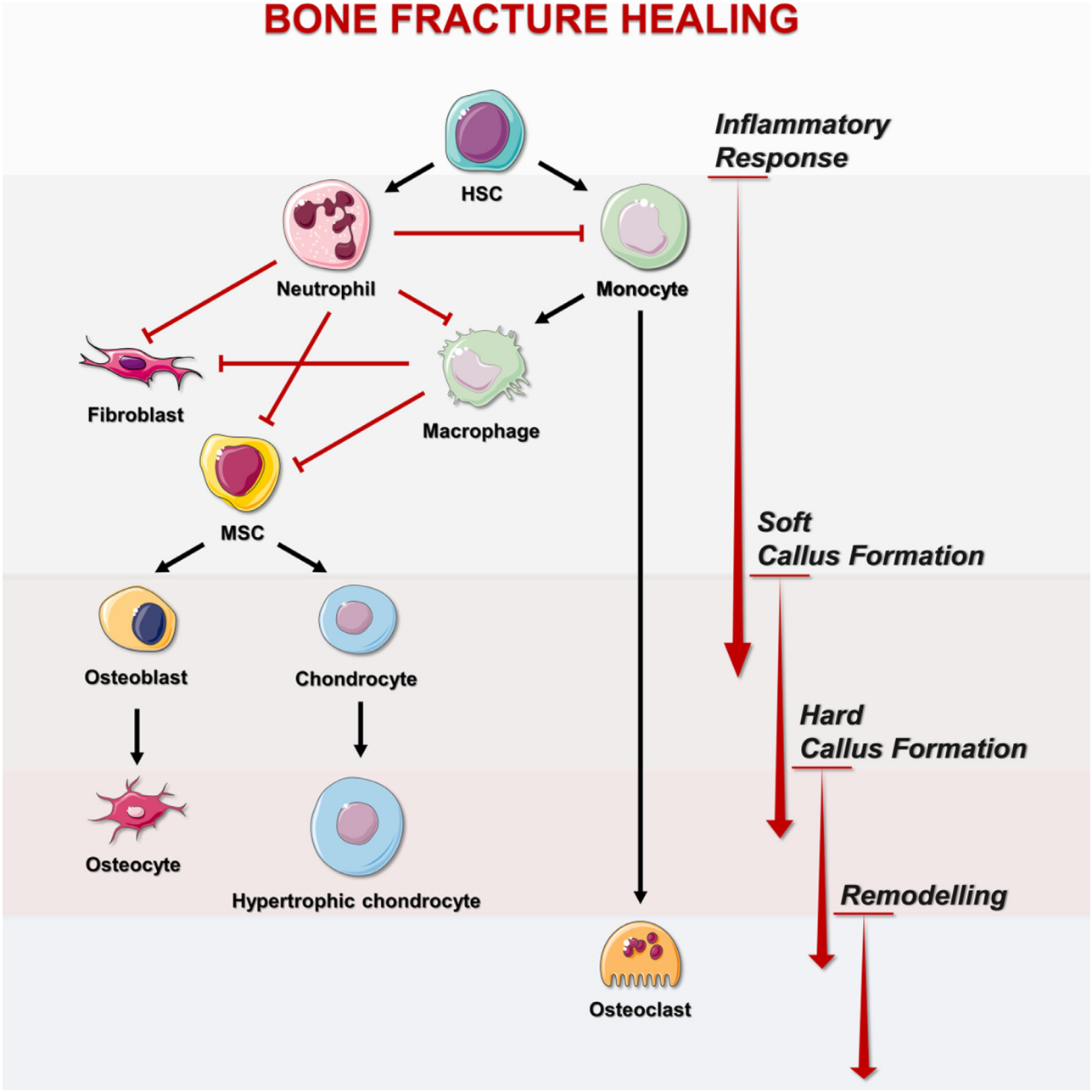

Fig. 1. Cellular illustration of bone fracture healing in four-stage model: the location of cells in the diagram corresponds to the time when they arrive or function at the fracture site. Black arrows indicate the differentiation of cells, red lines indicate the promotion on the proliferation, differentiation or migration of pointed cells by secreting mediators.

3.2. Biodegradable metals

Unlike the bio-inert metals, the implants made of biodegradable metals are not only designed to offer mechanical support to the growing bone tissue and enhance the bone formation, but also experience biodegradation process during the bone repair. The research of biodegradable implants conspicuously challenges the existing knowledge about the bone repair and is under a great amount of research currently.

3.2.1. Fe-based BMs

Iron-based biodegradable metals have emerged as a topic of interest thanks to their degradability and excellent mechanical properties rival the stainless steels [8]. The usage of Fe-based BMs is dominantly believed to be within blood vessel by the majority of the biodegradable metal society, due to the fact that Fe ions mainly exist in the red blood cells meanwhile no Fe ions being detected in the bone, yet in the past years there are few reports on this topic. A study investigated the degradation performance of pure Fe pins and two Fe-based biodegradable alloy pins in a growing rat skeleton over 1 year. It turned out that the degradation process caused no harm to the surrounding tissues and there existed no severe inflammatory reactions or local toxicity. The implant could be well integrated into the bone but sheathed by a narrow capsule of connective tissue. Moreover, it showed signs of slow degradation and exhibited no pronounced reduction in volume or mass loss for the whole year. A dense layer of degradation products was formed on the surface, substantially hindering the oxygen transport and further slowing down the corrosion, despite P and Ca on the outer layer of degradation products indi-

cated the great bio-conductivity of Fe-based BMs [20]. Recently, Trincă et al. [21] claimed that FeMnSiCa alloy could provide necessary mechanical support on the tibia of rabbit model, improve the growth of the newly connective tissue and facilitate the osteoid formation and mineralization. FeMnSiCa alloy can also promote the osteoinduction and osseointegration. However, the slow degradation rate still raised significant questions about their usage for the temporary bone repair implants. Thereby, the marked drawbacks of Fe-based BMs compel them to make room for more proper candidates, i.e., Mg-based BMs and Zn-based BMs.

3.2.2. Mg-based BMs

Owing to their high specific strength and similar Young's modulus to that of human bones, as well as adjustable biodegradability, good biocompatibility and osteo-promotive property, Mg and its alloys have been considered as a revolutionary biomedical material in the past decades. The element Mg plays an essential part in the construction of bone and soft tissue [22]. It possesses the unique osteo-promotive capability that can promote the new bone formation, enhance the osteoblast adhesion and temporarily inhibit the osteoclastic activity [23–25]. It can increase the proliferation of endothelial cells and promote the growth of new blood vessels near the implantation sites, encouraging the recruitment of osteoprogenitor cells and eventually accelerating the bone repair [26]. It is reported that the bone regeneration rate and the quality of the newly formed bone tissues are closely associated with the release profile of Mg^{2+} [27, 28]. During the early inflammation phase, Mg^{2+} facilitates the recruitment and activation of monocytes towards matured macrophages and can stimulate macrophages to a cytokine mixture tailored for the bone regeneration, leading to the formation of a pro-osteogenic immune microenvironment [29]. However, it also revealed that in the later remodeling phase, the continued stimulation of Mg^{2+} may result in the over-activation of NF-κB signaling in macrophages, increase the number of osteoclastic-like cells and inhibit the calcification of the extracellular matrix, decelerating the bone maturation as a consequence [29].

A series of *in vivo* assays of biodegradable Mg-based BMs intended for biomedical bone fixation applications are summarized and depicted in Fig. 2. Despite diverse and multifaceted roles of Mg^{2+} in the bone healing, the insertion of HP Mg screws for the fixation of rabbit femoral intracondylar fracture verified the osteoinductivity of Mg as a conclusion of increased bone volume and bone mineral density at the fracture gap. It also revealed that the implants degraded uniformly, and offered sufficient bending force and rigid fixation to the host, ultimately leading to enhanced bone fracture healing [30]. Furthermore, Castellani et al. [31] reported that Mg alloy rods even had the advantage in the osseointegration over Ti–6Al–7Nb alloy controls and can yield significantly higher bone-implant interface strength. Except the trace of new bone formation along the Mg–Y–Nd–HRE alloy pins inserted in the medullar cavity of rat, there was no evidence of fibrous tissue layers surrounding the implant at any time point and the degradation of implants induced no systemic inflammatory response and barely affected the cellular blood composition. In addition, Lee et al. [32] applied Mg–Ca–Zn alloy screws to fix 53 radius fracture cases in a long-term clinical study. It turned out that Mg implants can be completely replaced by the newly formed bones within 1 year, effectively avoiding the second surgery to remove the remaining implant and accomplishing the ultimate goal of biodegradable materials successfully.

Recently, progress has been achieved that two kinds of Mg-based BMs (WE43 alloys and MgCaZn alloys) medical devices, i.e., bone screws and pins, obtain the approval from Conformité Européene (CE) and the Korea Food and Drug Administration (KFDA), respectively [33]. For now, the great potential of Mg-based BMs for bone implant applications have been confirmed. To further fulfill two critical requirements of bone implants, meaning sufficient interfacial strength and enhanced bone response, researchers are now persevering in their attempts to improve the corrosion resistance, mechanical properties and biocompatibility of Mg-based BMs.

3.2.3. Zn-based BMs

Zn-based BMs have received fast-growing attention owing to their sui' mechanical properties and corrosion resistance, in good accordance with the requirements for ideal biodegradable implants [33]. Biodegradable Zn-based alloys intended for biomedical bone fixation applications are listed in Fig. 3. Besides, element Zn has a stimulatory effect on the osteogenesis and mineralization and is able to suppress the differentiation of osteoclast [34, 35]. It is reported that several designated Zn alloys can promote the formation of new bone tissue while causing no harm to the function and histology of important organs of hosts [36–39]. Compared to PLLA (poly-L-lactic acid) and titanium alloys, Wang et al. [40] found that the novel biodegradable Zn-based alloys are endowed with enough mechanical strength to support the fracture healing, adequate facilitation on the healing of the fractured bone with good biosafety and an acceptable degradation rate in the canine mandibular fracture model during a 24-week observation period, and thus might be promising candidates for the new generation of osteosynthesis system. Moreover, an *in vivo* study inserting pure Zn and Zn–0.05Mg alloy into a rabbit model for 24 weeks revealed the osseointegration around the implant within 12 weeks. The newly formed bones were in close contact with the implant and integrated well with the implant surface. The interface between bones and Zn-based BM implant remained tight and the bone trabecula was formed in 24 weeks [41]. Yang et al. [42] also observed the formation of the new bone surrounding pure Zn and Zn-HA biocomposites in the femur condyle of rats after 4 weeks, and plenty of osteocytes existed in the new bone tissue. However, a thin layer of fibrous connective tissue primarily containing fibroblasts was present and it separated the bone tissue from implants. A mild inflammatory response with the local infiltration of lymphocytes and macrophages was observed, as well. Nevertheless, the fibrous connective tissue can be replaced by the newly formed bone with time and the gap between the implant and new bone tissue was reduced after 8 weeks.

4. Bone healing process in presence of metallic biomaterial implants

4.1. Material-dependant bone healing process

It should be noted that regarding the normal bone repair occurring in the animal models covering rats and rabbits, the acute inflammatory response usually peaks within the first day and might last for about a week [22]. The soft callus formation might start by week 1 [43] and can reach its peak at 7–9 days [1]. The hypertrophy of chondrocytes might take place after approximately 10–14 days of proliferation [44]. The peak of hard callus formation generally occurs by week 2 [1] and can last for several weeks [43]. Eventually, the remodeling will be initiated 3–4 weeks after the bone fracture in animal or human models, and it might take years to ultimately restore the normal form and the integrity of bone [45]. Today, the application of metallic biomaterials is pretty common in the clinical treatment and proved beneficial for the bone repair. However, previous studies showed that the process of bone repair was affected by implants in unexpected ways.

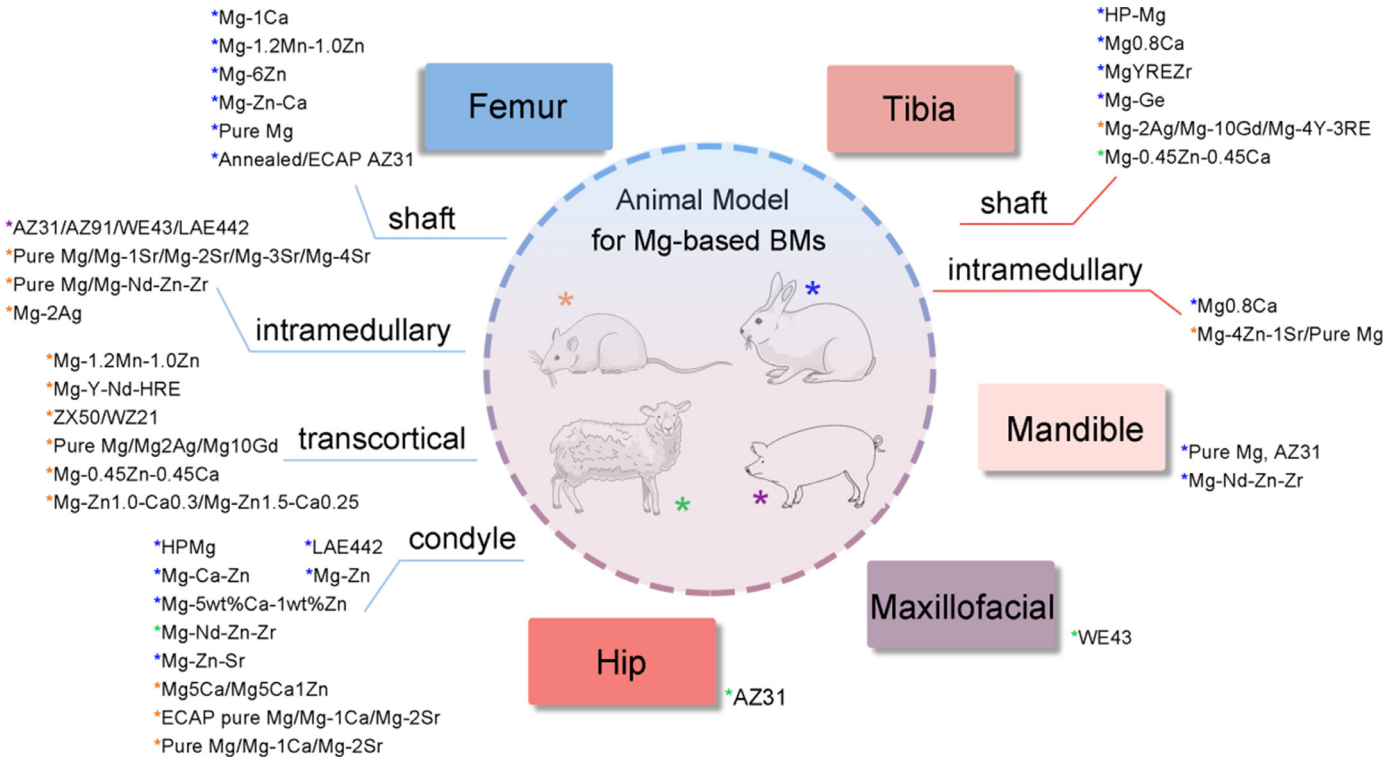

Fig. 2. Animal tests of biodegradable Mg-based alloys for bone applications. The stars with different colors ahead of alloys correspond to the animal model marked with the same colors.

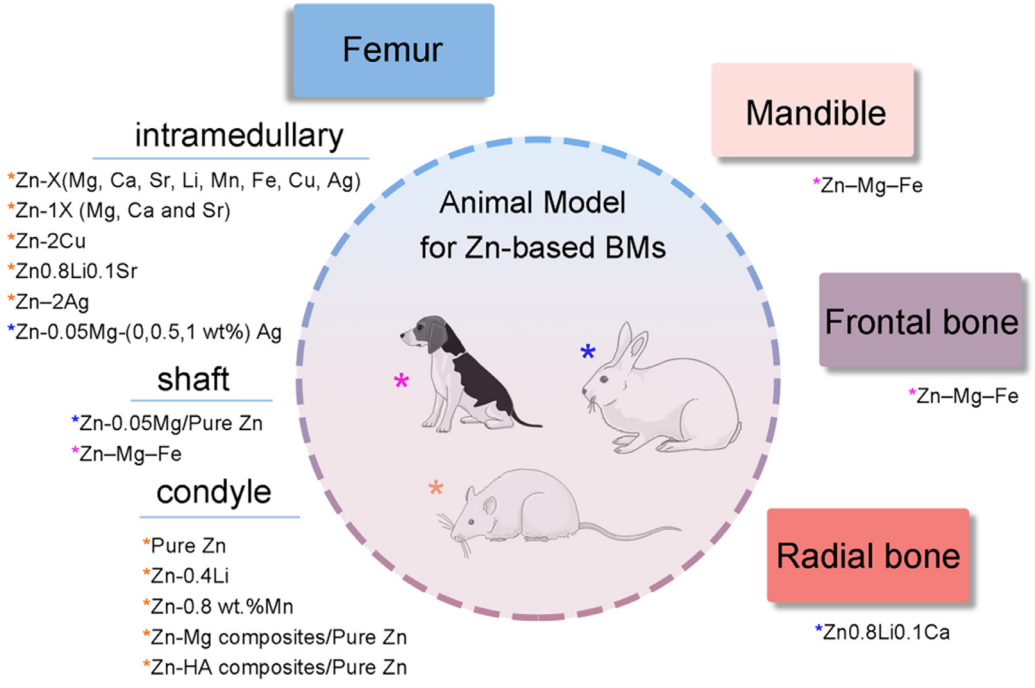

Fig. 3. Animal tests of biodegradable Zn-based alloys for bone applications. The stars with different colors ahead of alloys correspond to the animal model marked with the same colors.

4.1.1. Bone healing process in presence of bio-inert metallic biomaterial implants

The bone repair with the presence of titanium has been extensively investigated, and as a matter of fact, most of the current knowledge of the osseointegration stemmed from research on dental implants and limb prostheses made of Ti-based alloys [46]. Despite detailed mechanisms of the bone fracture healing in the presence of implants made of bio-inert metallic biomaterial implants are incompletely understood yet, it is widely accepted that it also involves the inflammatory response, angiogenesis, and recruitment of diverse progenitor cells as the normal bone healing does [9]. Immediately after the insertion of titanium implant, the surgical trauma and the underlying bone injury might elicit the inflammatory response within 3 days [46]. In comparison to the

stainless steels, fewer macrophages and lower degree of inflammation can be traced on the surface of Ti-based materials [47]. Then, the angiogenesis might take place within the peri–implant gap during the first week and the woven bones can be formed in 2 weeks in the presence of osteoblasts. The study inserting the titanium implant into the mandible of minipigs unveiled a layer consisting of a cell layer and mineralized bone tissue formed at the bone-implant interface [48]. The implant imposed no disturbance to the viability of osteoblasts, and fibronectins, fibronectin receptors and osteonectin were well synthesized by cells attached to the surface of implant. The fibronectin and fibronectin receptor at the cell protrusions implied the stable attachment between the cell and the implant [48]. Thereafter, the trabecular bone might be formed around day 10 and provide active biological fixation. At 3 months post-implantation, the woven bones and lamellar bones might be distributed around the implant [46], and actually after 6–48 weeks of implantation in the rat tibiae, the newly formed bone can be found around the Ti–15Zr–4Nb–4Ta alloy implants placed in the bone marrow [49].

4.1.2. Bone healing process in presence of biodegradable metals

For biodegradable metals, no severe inflammatory responses were reported after 2–4 weeks of implantation under most circumstance [30,50-52]. Nevertheless, for pure Mg screws implanted into the rabbit tibiae, inflammatory cells around the implants were observed at week 4 and the inflammatory response gradually subsided at week 12 [53]. Besides, after implanting AZ31 magnesium alloy into the tibia, head, back, abdominal cavity and femur of rats, the histological analysis of tissues surrounding the implants revealed the existence of immature granulation and infiltration by inflammation-associated cells at about 1–2 weeks. At 2–4 weeks, the granulation tissue maturated and well-developed granulation tissues emerged, accompanied by significant proliferation of fibroblasts and capillary growth, and formation of collagen fibers. At 4 weeks post-operation, large areas of collagen fibrils were present and the quantity of capillaries and fibroblasts decreased [54]. Moreover, it is reported that a continuous fibroblast band was formed between Mg–1.2Mn–1.0Zn alloy rods and the bone tissues after 2-week implantation in the rabbit femoral shaft, and the band became thinner at 3 weeks [55]. What is more, lymphocytes were identified after 2-month implantation of Mg–1Ca alloy pins in the femoral shaft of rabbits, with no visible evidence of multinucleated giant cells [56]. It came to a conclusion that the biological process involved in the normal fracture healing normally functioned even in the presence of degradable materials. However, compared to 1-week inflammation that occurred in the normal bone repair process, inflammatory responses with degradable implants present proceeds in the similar manner but last for a significantly extended period, as shown in Fig. 4, perhaps due to unceasing released ions, hydrogen gas and production of corrosion product throughout the implantation [57].

The formation of new bones can be basically characterized by a sequence of events, beginning with the commitment of osteoprogenitor cells and then their differentiation into osteoblasts to synthesize the bone matrix and regulate the mineralization [58]. It turned out that the highly active osteoblasts can be observed after inserting Mg–1.2Mn–1.0Zn [55] and Mg–1Ca [56] alloys into the femoral shaft of rabbits for 1 month. The osteoid tissue can be found after 3 weeks and the bone matrix can be observed at week 4 [55]. The active osteocytes were distributed without organization after 2 months, and then aligned in rows after 3 months [56]. More importantly, the osteoblasts responsible for the synthesis and mineralization of bone, play an essential role not only in the initial bone formation stage, but also in the later bone remodeling [58]. It is reported that osteoblasts and osteoid can be observed regularly around the corroding Mg screws even after 3 and 6 months in the hip bone of sheep [59]. For pure Mg in the rabbit tibiae, the orderly osteoblasts can be found at the bone-implant interface after 26 weeks. However, for Mg–Zn–Ca alloy in the femur shaft of rabbit, osteoblasts were revealed at 18 weeks [60].

The callus formation is an important clue in the bone formation and provides much information on the biocompatibility of degradable magnesium. Jahn et al. [61] claimed that the callus was already fully developed by day 14 in the fractured femora of mice stabilized by the Mg2Ag alloy pin and the fracture healing was successfully finished with a complete removal of the callus by day 133. The callus formation seems to keep in pace with the normal bone formation in this case. However, regarding ZX50 alloy inserted into the transcortical femoral of SD rats, callus formation took place at week 4 and week 8 [62]. In addition, for AZ31 screws implanted into the hip bone of sheep, the newly formed microcallus exhibited direct contact with screws after 3 months of implantation, most of which got replaced by the lamellar cancellous bones at 6 months [59]. Accordingly, since the soft callus formation in the normal bone fracture healing mostly peaked within 10 days, biodegradable metal implants might prolong the process under some circumstances.

In the meantime, it is claimed that new bone formation and bone resorption occurred simultaneously during the bone remodeling [62]. In the studies of Mg-based BMs, mostly, the newly formed bones were observed within 4 weeks in diverse animal models, occupied the material surface progressively and integrated well with implants [30,53,62-65]. The bone trabecular can be observed at about week 4 and get replaced by the lamellar bone later on [53,60]. In the meanwhile, the osteoclast, the highly specialized cell uniquely capable of bone resorption, is another key player in the bone remodeling [66]. In the studies of Mg–Zn–Ca alloy inserted in the femur shaft of rabbits, the appearance of osteoclasts was reported and can be tracked after 12 weeks [60].

To summarize it briefly, the bone formation with biodegradable implants proceeded in the same manner as the normal bone repair did, while the implant could change the timeline of the healing process. The crucial cells and events involved in the bone repair behaved normally even with biodegradable implants present.

4.2. Implantation site-dependent biodegradation and bone healing process

To evaluate the biocompatibility, mechanical properties, degradation and by-product of investigated materials in the bone environment, *in vivo* studies are greatly performed in small or big animals covering rats, rabbits, sheep and pigs. The classic implantation sites primarily encompass femur, tibia, mandible, maxillofacial and hips. Note, the natural bones form distinctly in the living body and differ in the organic and inorganic phases, as well as blood flow conditions and mechanical properties. It can be generally classified into the cortical bone and cancellous bone [67]. The cortical bone is a dense tissue with mainly mechanical function, and the cancellous bone with low density and mechanical strength but high surface area is endowed with vital metabolic function [68]. The mechanical property of the natural bone can actually vary among hosts, bones and even regions in the same bone [69]. Thus, it is hardly surprising that the participation of biodegradable metals in the bone repair can be dependent on the implantation sites.

(i) *Different regions in the same bone:* By characterizing the femur implanted with an intramedullary as-rolled Mg–2Sr alloy, Gu et al. [63] found that the implant corroded dissimilarly in different regions of the same femur. A higher corrosion rate was observed in the distal with the trabecular bone than in the proximal femur filled with bone marrow cavity. It might be explained by the rich blood supply in the trabecular bone region

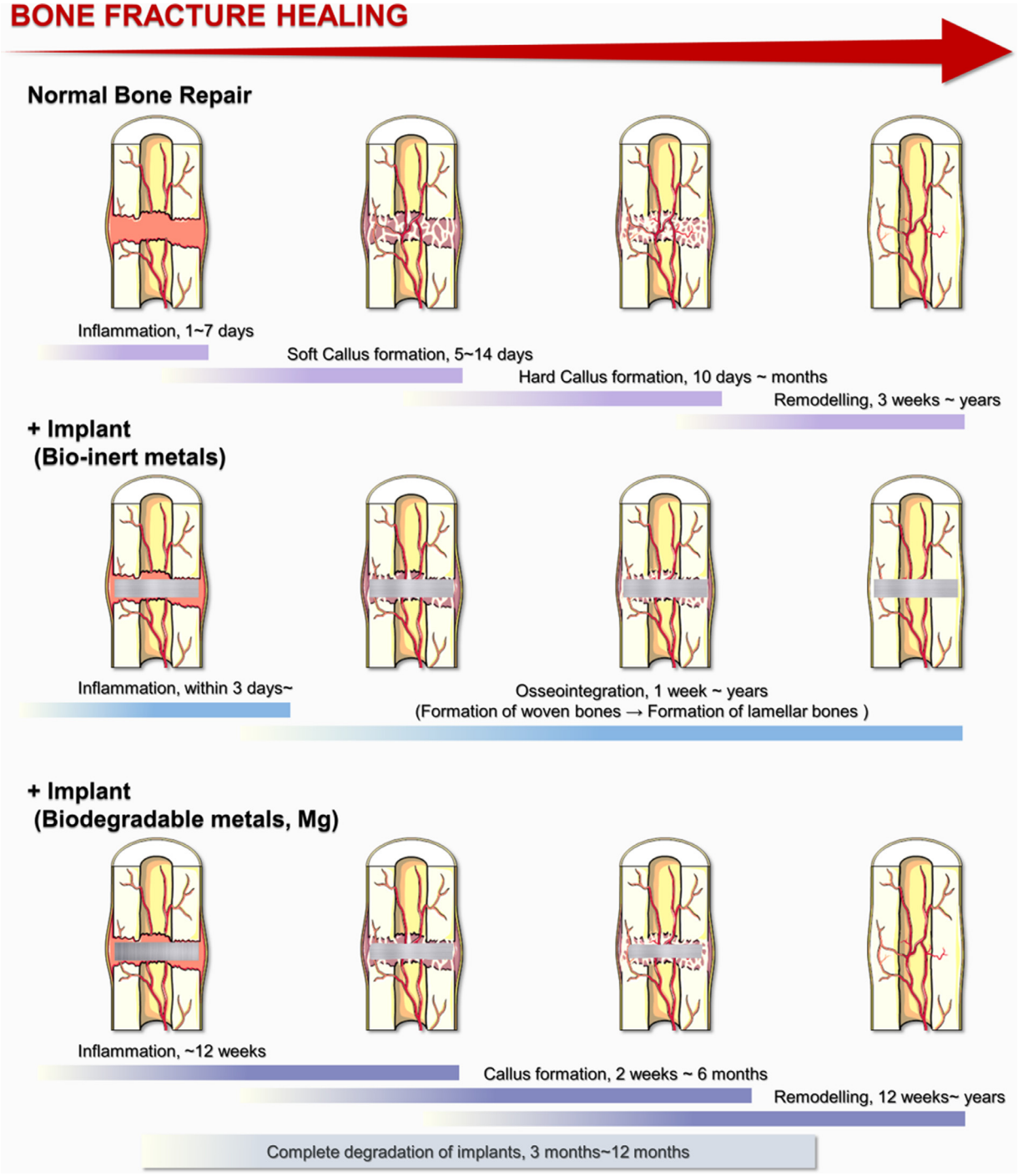

Fig. 4. Schematic illustration of bone healing process involving metallic implants: overlap exists among different stages in each case regarding the timeline.

as the circulation of surrounding tissue fluids and the exudate from blood vessels can both promote the dissolution of metal [54]. Meanwhile, Cihova et al. [52] observed increased dissolution of Mg–Zn–Ca alloy in the interface of the bone marrow to the cortical bone subjecting to the strong bone remodeling, the interface of the cortical bone to soft tissue and muscles subjecting to the mechanical stimuli, and within the medullary cavity in the rat femurs model. Besides, as shown in Fig. 5, the position "a, b, c" can be characterized by distinct amount of trabecular bone, cortical bone, marrow and hematopoietic tissue. By implanting M–Zn alloy in these selected positions in the femoral condyle of rabbits, Han et al. [68] found that the degradation of Mg–Zn alloy varied in three implantation sites on account of diverse components and biological functions. The corrosion rates decreased in the following order: soft tissue, less trabecular bone, more trabecular bone and cortical bone. Similar to the findings in the femur, after implanting MgCa0.8 alloy screws into the tibiae of rabbits, Erdmann et al. [70] found that the thread gradually corroded within the marrow cavity while the volume of part within the cortex barely reduced during 8-week implantation. In comparison to those in the cortical bone, the screws in the medullary cavity were in close contact with blood vessels and body fluid, and the corrosion of Mg was promoted as a result. The animal studies performed

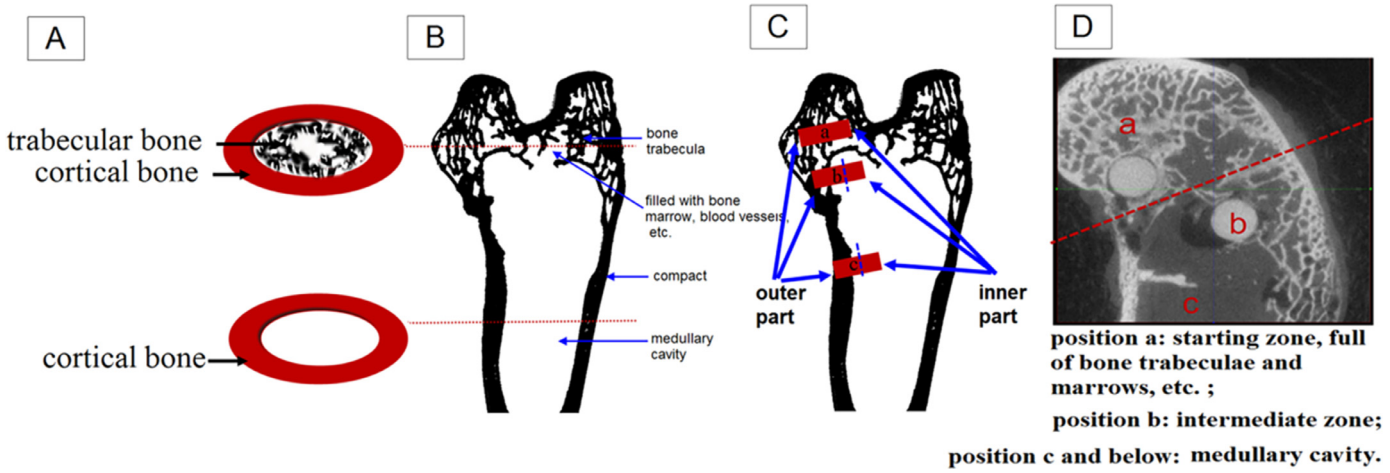

Fig. 5. (A) Schematic plot of the cross section of condyle and shaft, the red dashed lines pointed at the corresponding position in (B); (C, D) Schematic sketch of different regions in one condyle [68].

in the rabbit mandible further confirmed that the AZ31 alloy screws degraded faster in the bone marrow compared to the cortical space [71]. The AZ31 alloy screws inserted in the hip bone of sheep revealed that different biological environments can lead to different outcomes in biodegradation even in big animal models. After 3 months of implantation, the screw head covered by muscles and connective tissue suffered from severe surface corrosion on the convex outer areas because of higher water content and blood flow, but minor corrosion attack was shown on the threads placed in the bone [72].

(ii) *Different bones:* The biodegradation and osseointegration of implants at distinct bones vary a lot, as well. Cheng et al. [73] inserted high-purity magnesium pins into the femoral shaft and condyle of New Zealand rabbits. It turned out that HP Mg exhibited similar corrosion rates in two bones, but the distribution of contact osteogenesis centers and biological properties of peri-implant bone tissues were different. In the femoral condyle, the osseointegration initiated from the contact osteogenesis center in the periosteum and the cancellous bone. The newly formed bone gradually accumulated on the HP Mg pins and the trabecular bone covered the whole pins after 16 weeks. In the femoral shaft, the origin of contact osteogenesis centers was periosteum and the bone accumulation and remodeling were observed in the cortical bone surrounding HP Mg pins, along with empty cavities in marrow cavity. In the meantime, the bone volume to total bone volume (BV/TV) and bone mineral density (BMD) of peri-implant bone tissues in the femoral condyle were above those of normal bone tissues, while BV/TV and BMD in the femoral shaft were lower than normal. By evaluating the biodegradation behavior of AZ31 magnesium alloy in several implantation sites in the rats, Sato et al. [54] revealed that the volume loss of implants was the highest in the abdomen, followed by head, back, tibia, and femur, owing to distinct tissue blood flow, water content and adjacent tissue mobility.

5. Inspiring future experiment design on BMs guided by precise bioadaptability principle

In 2016, Wang [74] proposed the concept of bioadaptability of biomaterials. This concept describes the three most important aspects that can determine the performance of biomaterials in tissue repair: 1) the adaptability of the micro-environment created by biomaterials to the native micro-environment in situ; 2) the adaptability of the mechanical properties of biomaterials to the native tissue; 3) the adaptability of the degradation properties of biomaterials to the new tissue formation. The concept of bioadaptability emphasizes both the material's characteristics and biological aspects within a certain micro-environment and molecular mechanism.

Recently, growing realization of bioadaptability, the spatiotemporally specific tenet that hinges on the precise and dynamic interactivity between hosts and biomaterials [75], offers a great opportunity for the development of biodegradable metals. Desirably, BMs with precise bioadaptability can recapitulate targeted tissue with spatiotemporal precision and hierarchical accuracy, ranging from atoms and molecules to cells and to tissues and organs, and dynamically and actively respond to biological milieus/signals or externally applied triggers with spatial and temporal precision [75]. Thus, to meet the requirements of precise bioadaptability for bone fixation applications, the biocompatibility, corrosion properties and mechanical properties of BMs need to be tailored and balanced to match with the tissue repair procedure as the function of time and spatial location [76].

In the research of BMs, the *in vivo* animal tests and *in vitro* material characterization tests provide massive amounts of valuable information to evaluate the essential properties of materials and play essential roles in promoting the design of BMs. Herein, learning the fact the bone repair process can be largely affected by BMs and the outcomes might be site-dependent, the suggestions in order to characterize the property of BMs more precisely and more effectively are proposed. In short, the animal model for the *in vivo* tests should be selected according to specific scenarios and requirements, and *in vitro* experiments should also be elaborately designed to keep pace with the bone repair as the function of time and space.

5.1. *Purpose-oriented design of* in vivo *animal tests*

In the animal studies, the location of implant can decide the type of bones and tissues it contacting with, indicating distinct blood flow and mechanical stimulus, as well as other physicochemical parameters around materials, e.g., ion concentrations, cells, proteins, pH and oxygen, which can greatly affect the biodegradation of BMs and the bone repair. Take the mostly studied implantation site femur for example, as shown in Fig. 6. The implant inserted into the femoral shaft will be exposed to the cortical bone and bone marrow, and the one in the femoral condyle will be surrounded by the cortical bone, the cancellous bone and cartilage [73]. Different regions in the same bone exhibit unlike

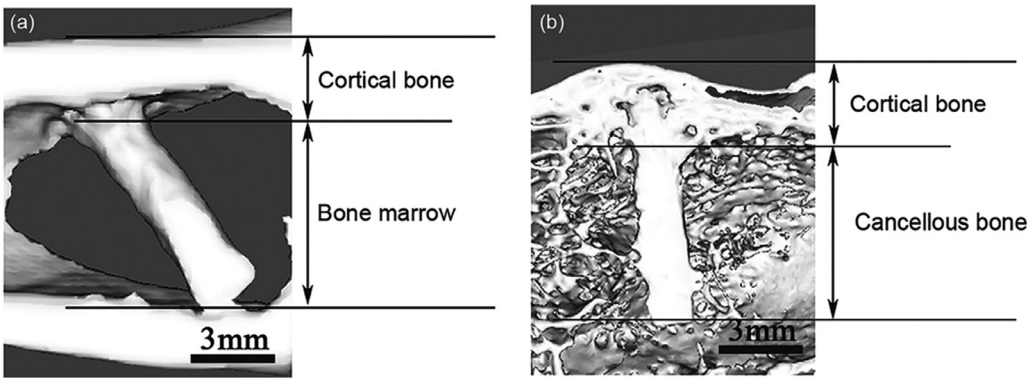

Fig. 6. 3-D reconstruction of peri–implant bone tissues and HP Mg pins (cortical portion and bone marrow portion) in femoral shaft and (b) femoral condyle at 16 weeks [73].

features, not to mention different bones even if in the same animal. Moreover, in the case that the implant is placed in the site contacting with several kinds of tissues, it will exhibit multiple corrosion modes and undertake a distinct degree of osteointegration in different areas. The implant is highly likely to lose integrity ahead of time due to non-uniform corrosion and fail the test. The conclusion about whether the material is eligible for the bone application actually manifests insufficiently convincing.

Therefore, in designing and selecting the animal models to characterize the BMs, a variety of factors should be considered to hit the mark well and truly. To emphatically assess the corrosion resistance of materials, the bone implants can be placed in more corrosive sites such as bone marrow and trabecular bones. The bone tissues with more active new bone formation can better translate the osteoconduction, osteoinduction or osteogenesis of materials, and the bones that bear higher loads can evaluate the mechanical properties of implants in more effective ways. Moreover, in order to fix the material tightly and avoid failures of animal tests, the transcortical implantation into the bone can be a wise choice although undesired nonuniform degradation and divisional bone formation might emerge as a result. Although, upon most occasions, the animal models cannot fully satisfy the requirements possibly restricted to the size of implant or animal, difficulty of surgical operation, budget, etc., the selection of animal models should be as discreet and scientific as possible, and take a full account of the influence factors raising from implantation sites to precisely estimate the materials. Satisfying the use in the corrosive environment does not mean that it is also satisfied in the actual environment. Therefore, the implant site should be selected as close to the actual situation as possible for *in vivo* tests, but not more corrosive sites.

5.2. Precise design of in vitro *biodegradation test to mimic the* in vivo *biodegradation*

5.2.1. Factors influencing the precise design of in vitro *biodegradation test*

The biodegradation of BMs cannot only decide the retention time of implants, but also affect their efficacy and safety by altering the mechanical property and biocompatibility during the bone repair. The characterization of degradation behavior is thus a matter of the utmost importance to evaluating and developing BMs. The hierarchical structure of the natural bone is quite sophisticated, featuring inorganic minerals, multiple types of stem cells, proteins and biological molecules integrated in the extracellular matrix [77,78]. After the surgical insertion, the implant will mostly get exposed to the bone tissue, which can be highly corrosive to biodegradable metals. Herein, the critical determinants of the biodegradation of BMs during the bone repair are summarized, as shown in Fig. 7.

(i) *Biology:* During the bone repair, the spatiotemporally orchestrated events occur at scales ranging from atomic, molecular and cellular regimes to tissue, organ, and system levels, and in the meantime, within the time frames spanning from seconds to months and years [74]. The inflammatory cytokines, growth factors, pro-osteogenic factors and angiogenic factors will play crucial roles at the molecular level [10], and at the same time, diverse types of cells covering inflammatory cells, osteochondral progenitors, vascular cells, fibroblasts, osteoblasts and osteoclasts get involved at the cellular level. Thereinto, multitudes of small biomolecules, proteins and cells affect the biodegradation of BMs in various ways, involving the expression of thousands of genes and biologically intertwined with inflammatory reactions and immune response [12].

Firstly, the adsorption of proteins takes place on the surface of implant immediately after implantation and can regulate the adhesion, activation, migration and proliferation of cells [79–81]. The interactions between protein and implant also involve desorption and re-adsorption processes, though the adsorption of proteins on the surface can directly determine the biocompatibility of implant [82] and improve the osteoconductivity [83]. The denatured proteins might be transformed into a film on the surface then, which can be found on many retrieved artificial joints, and possibly inhibit or promote the corrosion of metals depending on the type of proteins and materials [84]. In addition, metal ions and proteins might form the colloidal organometallic complexes, and the transportation of them away from the interface can increase the dissolution rate and accelerate the metal corrosion [84]. For Mg–Nd–Zn–Zr alloy, the layer containing proteins can be formed on the surface in the presence of fetal bovine serum (FBS). The layer can act as a barrier to slow down the ion exchange between surface and corrosive medium, contributing to accumulated OH^- and changing the composition of corrosion product [85]. For pure Zn, the addition of FBS in simulated body fluid also inhibited the Zn corrosion and induced the localized corrosion [86]. Albumin, the protein with the highest concentration in the blood serum, can promote the adsorption of biomolecules and stimulate the nucleation of hydroxyapatite. It can also slow down the corrosion of Mg1.5Ca alloy and act as a corrosion inhibitor by enhancing the corrosion resistance of the surface film in 0.9 wt% NaCl solution [87]. For biodegradable Zn-based alloys, albumins can affect the chemical composition, surface morphology and compactness of the protective film, decreasing the corrosion current and promoting the passivation as a consequence [88]. Fibrinogens, recognized as the key mediators of inflammatory response, leukocyte bind-

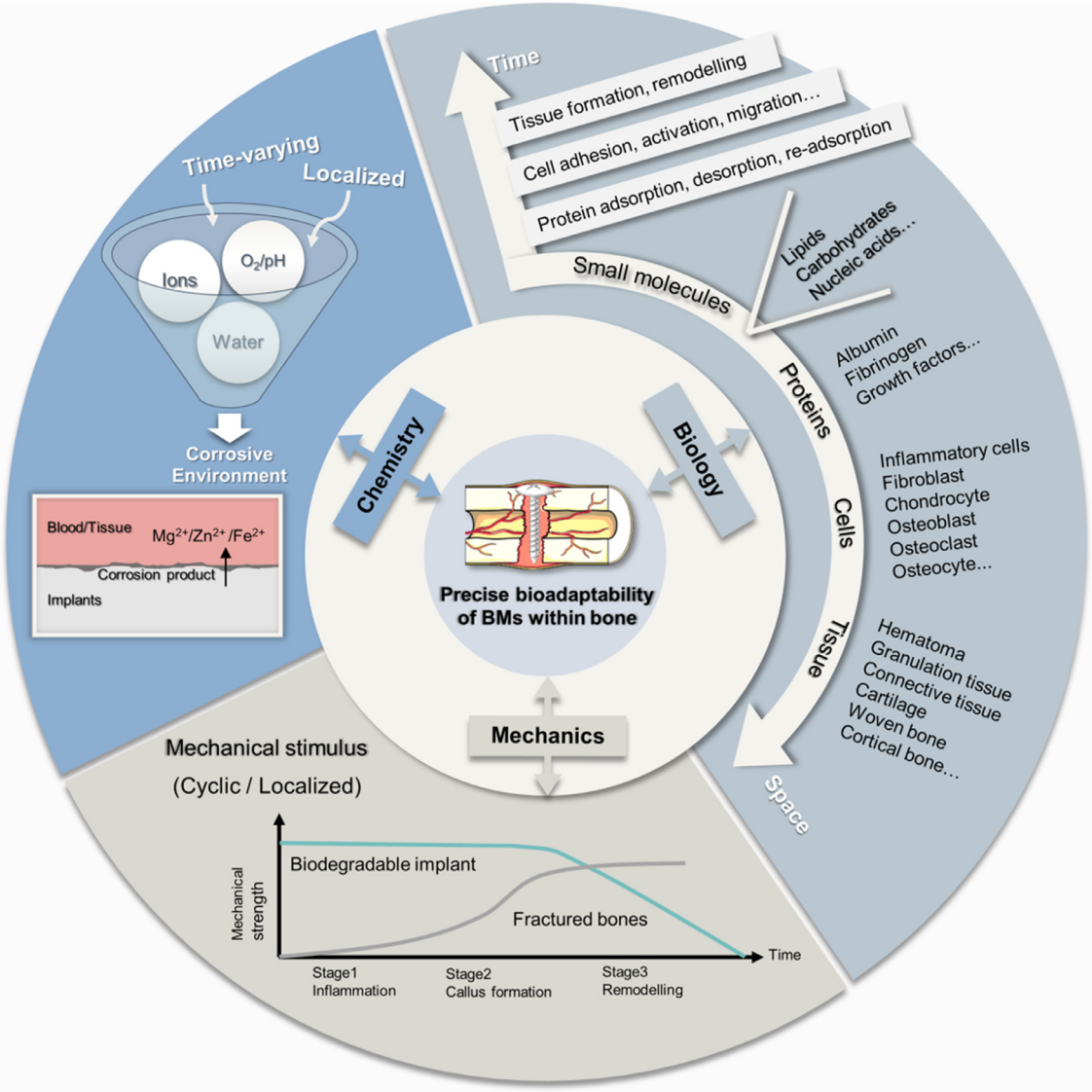

Fig. 7. Biodegradation of BMs during bone repair.

ing, platelet activation and blood coagulation [79], also change the property of the passive film and differently affect the metal corrosion from albumins due to distinct structure [89].

In the meanwhile, there are plenty of cells getting involved in the bone repair and functioning in a fixed sequence to accomplish their vocations. The bone implants provide the necessary scaffolds for cell adhesion, proliferation and differentiation, and are capable of modulating cell activity and function. When the inflammation takes place, immunocytes will be assembled around the implant [90]. Inflammatory cells in the peri-implant environment, particularly leukocyte and macrophage, are able to generate and release highly oxidative chemicals known as reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), nitric oxide and chloramines [91,92]. The inflammatory cells can use both ROS secretion and acid to attack the foreign bodies [93], not only causing tissue destruction but also creating a localized corrosive environment around the implant [94]. The direct corrosion induced by the activated inflammatory cells, such as osteoclasts on the stainless steel, titanium alloys and cobalt-based alloys, was revealed by the *in vitro* tests [47,66,95], and accelerated dissolution of Ti caused by macrophages was reported [96].

With the reduction in the inflammatory response, the surrounding tissue adheres to the implant consisting of cells, including osteoblasts in the bone tissue and fibroblasts in the connective tissue, and body fluid filling the tissue comprising various inorganic ions and organic molecules is formed [90]. The study concerning the effect of primary human osteoblast on the degradation interfaces of pure Mg, Mg–2Ag and Mg–10Gd alloys found that the metabolic activity of osteoblasts was correlated with the formation and release of the lactate into surrounding environment. The cells can alter the chemical composition of degradation interfaces, and change the degradation rates of pure Mg, Mg–2Ag and Mg–10Gd alloys [97]. The influence of fibroblasts on the corrosion of permanent implant metals made of Ti6Al4V alloy, Co-based alloy and 316L stainless steel was also studied. It turned out that fibroblasts might consume the oxygen and prevent the diffusion of dissolved oxygen near cells, changing the corrosion of metals [90].

(i) *Chemistry:* The vascular nature of the bone guarantees that the first tissue in contact with the endosseous implant is the blood. In addition to the numbers of proteins and organic species involved in the rapid adsorption process and altering the surface characteristics of implant, water, inorganic ions, pH and O_2 con-

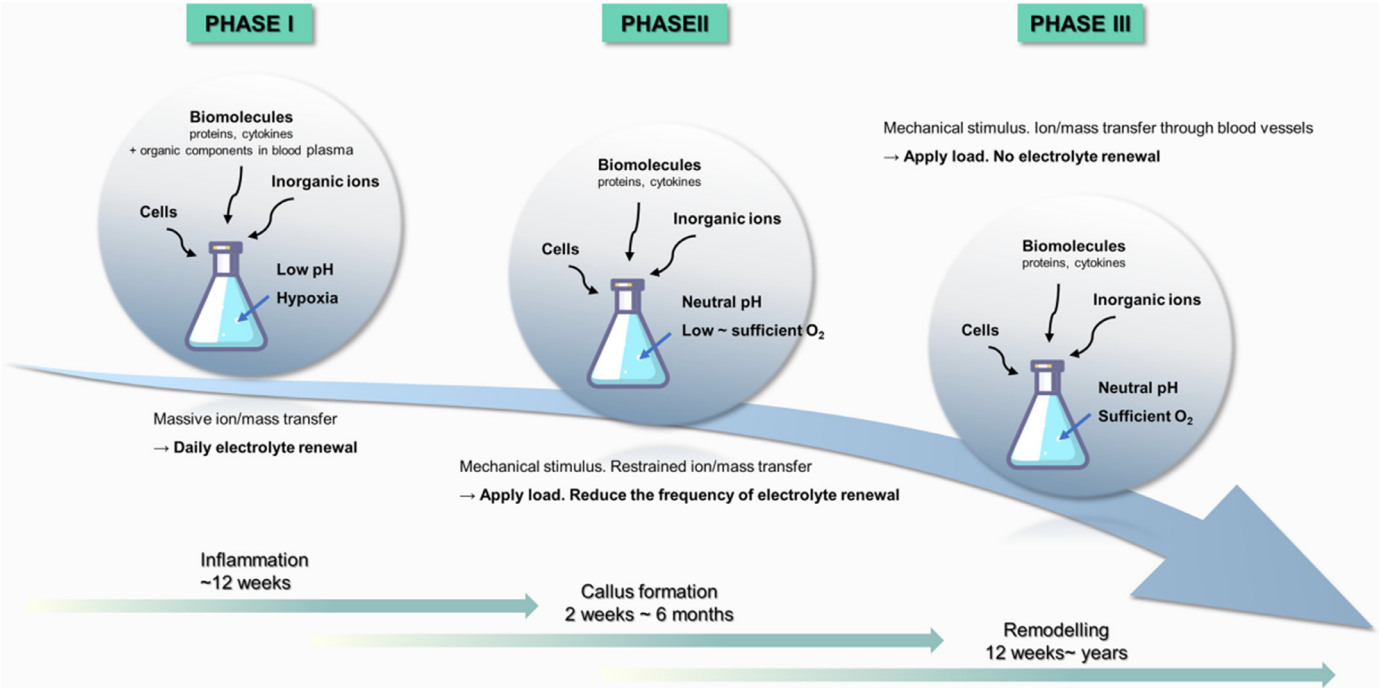

Fig. 8. Time-varying design of *in vitro* biodegradation test.

tained in the blood all contribute to the corrosive environment. Besides, the blood vessels in the bone are highly active and can act as the passive source for the delivery of O_2, nutrients, growth factors and circulating cells. The circulation of blood can affect the ion diffusion, mass transfer, local pH and O_2, and distribution of proteins and cells during the bone repair. The biodegradation of implants will be inevitably affected by these factors. The study found that the corrosion product on the titanium implant preferred to be distributed around the blood vessels [98]. More importantly, the blood flow in the bone tissue is site-specific and can dynamically change in response to trauma, metabolic demands and aging [99], implying time-varying and localized corrosive environment around the implants.

(ii) *Mechanics:* During the bone repair, the sophisticated bone structures will undergo dynamic changes to restore its function when biodegradable implants ideally keep its integrity to provide adequate mechanical support or fixation for a period. The implant needs to exhibit a dynamic degradation with decreasing load-bearing support, and the newly formed bone at the fracture site will bear growing mechanical load bit by bit, instead of jumping to the stress stimulation at physiological level directly. The way to gradually restore the original load-bearing function is more beneficial to shaping the new bone tissue [22], while the dynamically-changing load on the implant raises concerns in term of corrosion. It should be noted that the stress applied to devices can change the mechanical and corrosion profile of implants at the same time, and lead to premature rupture potentially. The stress-induce corrosion is much concerned, not only in the field of biomedical materials, but also in the application of industrial materials. The load can significantly raise the vulnerability of metals to corrosion, accelerating the corrosion of Mg-based BMs and inducing the stress corrosion cracking [100], so as to Zn-based BMs [101]. It should be noted that the mechanical properties of the natural bone vary among hosts, bones and even regions in the same bone [69]. In some cases, the mechanical stimulus from the host can also be cyclic, making the biodegradation of implants much more complicated.

5.2.2. Inspiring experiment design of in vitro *corrosion test with precise bioadaptability*

For the *in vitro* characterization tests, immersion test, electrochemical experiment and hydrogen evolution test are commonly conducted to evaluate the corrosion behavior of BMs. The simulated body fluids such as NaCl solution, phosphate borate solution (PBS), Hank's solution, simulated body fluid (SBF) and cell culture medium such as Dulbecco's modified Eagles' medium (DMEM) are frequently used as the corrosive media. They are designed to replicate the chemical composition of blood or tissue fluid, and can mimic the physiological environment around the implant, to a certain extent. Besides, a singular medium is utilized throughout the whole test, which might lead to the accumulation of released ions and particles, the consumption of free ions, organic components and oxygen, and diverged solution pH [102]. As a result, the *in vivo* degradation of BMs is actually quite different from that in the *in vitro* tests [103,104]. The time-variant ultrastructure of bones during the bone repair has not been taken into serious consideration in designing the *in vitro* corrosion tests yet, as well as the site-dependent characteristics of peri–implant environment, as shown in Fig. 8.

It should be noted that the initial stage of inflammation manifests low pH and hypoxia. A decrease in the pH value from 7.35 to 5.2 will be caused by inflammatory cells. When the inflammation gets reduced, pH might restore to its normal level and the hypoxia will be relieved after vascularisation. The concentration of O_2 is associated with the implantation sites and might be non-uniformly distributed around the implants. The change in O_2 concentration will affect the oxygen reduction reaction, alter the formation and dissolution of corrosion product and finally change the biodegradation of BMs [105]. What is more, the occurrence of vascularisation might affect the corrosion of metal by altering the transportation of mass and ions. With respect to the participation of organic components, the serum albumin might be the first one to arrive at the surface of implant owing to its high concentration. With prolonged time, multiple types of proteins will join the party one by one and make difference to the biodegradation of BMs. Moreover, with a layer of proteins formed on the surface, cells will then in-

teract with implants [106]. Likewise, the presence of cells might keep pace with the bone repair and affect the biodegradation of BMs one after another. With the formation of tissues, the biological and chemical reactions on the implants will be changed. The fibrous tissue surrounding the implants is composed of dispersed cells separated by connective voids, and thus the mass transfer will be driven mainly via diffusion caused by concentration gradients and the degradation of implants is altered accordingly [106]. At the same time, the effect of dynamic, localized and cyclic mechanical stimulus on the biodegradation should also be taken into consideration.

Therefore, after tracing and analysing the distribution of essential factors influencing the biodegradation of BMs during the bone repair, the idea of designing time-varying *in vitro* corrosion tests is proposed, as shown in Fig. 8. The usage of several simulated body fluids at different time points, advanced equipment to control the O_2 concentration and the mechanical loads matching the bone environment will make a step forward to better comprehend and predict the *in vivo* biodegradation. In the future, the site-targeted simulated body fluid based on the time-varying design might be the next step to better mimic the hierarchical organization and microenvironments. The evaluation of the biodegradation of implants will be achieved across spatial dimensions and timescales, and precisely characterize the BMs *in vitro*.

Similarly, a precise design of *in vitro* biological test to mimic the *in vivo* biodegradation can also be proposed. On the one hand, we can collect the extracts produced by time-varying *in vitro* corrosion, on the other hand, we can change the cell lines or do the co-culture of various cell lines. It will be more complex, but the results will be more inspiring. For example, to understand the impact of biodegradable metals which are intended to be used as bone graft materials, not only the interaction with bone-forming osteoblasts and bone-resorbing osteoclasts is worth investigating, but also the influence on osteocytes should be studied. The *in vitro* triple cultures of human primary osteoblasts, osteocytes and osteoclasts can potentially help to analyze the effect of drugs and degradation products of biomaterials as a model for native bone tissue. Bernhardt et al. [107], analysed the effect of Mg degradation products on primary osteocytes, found that transition of osteoblasts to osteocytes is not hampered by Mg degradation products and hypothesized an accelerated transition due to the significantly decreased ALPL expression in presence of the Mg degradation products. The decreased mRNA expression of the osteocyte markers PHEX and MEPE, in contrary, suggests a slower osteocytic differentiation in the presence of Mg extracts. Additional future experiments, possibly involving also osteocytic cell lines, will be necessary to unravel potential effects of Mg degradation products on osteocyte differentiation and signaling.

6. Concluding remarks

As the interactions between the bone tissues and metallic biomaterial implants are mutual, the bone repair is significantly affected by metallic biomaterial implants in desirable or unexpected ways, and the performance of implants is also changed by multiple physico-chemical parameters. Therefore, the time-varying and site-dependent narration of the bone repair in presence of BM implants are firstly established in this review. The factors influencing the biodegradation of BMs are summarized as a function of time and space, and suggestions about the design of *in vitro* experiments are proposed. The time-varying simulated body fluid containing essential factors might be a nice attempt to understand the fracture healing and characterize the biodegradation of implants in the future.

Declaration of Competing Interest

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

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