



## Antibacterial metals and alloys for potential biomedical implants

Erlin Zhang<sup>a,b,1,\*</sup>, Xiaotong Zhao<sup>a,1</sup>, Jiali Hu<sup>a</sup>, Ruoxian Wang<sup>a</sup>, Shan Fu<sup>a</sup>, Gaowu Qin<sup>a,b</sup>

<sup>a</sup> Key Lab. for Anisotropy and Texture of Materials, Education Ministry of China, School of Materials Science and Engineering, Northeastern University, Shenyang, 150819, China

<sup>b</sup> Research Center for Metallic Wires, Northeastern University, Shenyang, 110819, China

### ARTICLE INFO

#### Keywords:

Antibacterial metals and alloys  
Antibacterial alloying elements  
Antibacterial stainless steel  
Antibacterial titanium alloy  
Antibacterial magnesium alloy

### ABSTRACT

Metals and alloys, including stainless steel, titanium and its alloys, cobalt alloys, and other metals and alloys have been widely used clinically as implant materials, but implant-related infection or inflammation is still one of the main causes of implantation failure. The bacterial infection or inflammation that seriously threatens human health has already become a worldwide complaint. Antibacterial metals and alloys recently have attracted wide attention for their long-term stable antibacterial ability, good mechanical properties and good biocompatibility *in vitro* and *in vivo*. In this review, common antibacterial alloying elements, antibacterial standards and testing methods were introduced. Recent developments in the design and manufacturing of antibacterial metal alloys containing various antibacterial agents were described in detail, including antibacterial stainless steel, antibacterial titanium alloy, antibacterial zinc and alloy, antibacterial magnesium and alloy, antibacterial cobalt alloy, and other antibacterial metals and alloys. Researches on the antibacterial properties, mechanical properties, corrosion resistance and biocompatibility of antibacterial metals and alloys have been summarized in detail for the first time. It is hoped that this review could help researchers understand the development of antibacterial alloys in a timely manner, thereby could promote the development of antibacterial metal alloys and the clinical application.

### 1. Introduction

Metals and alloys, including stainless steel, titanium and its alloys and cobalt alloys, have been widely used clinically due to their high strength, good wear resistance, good corrosion resistance, high fatigue properties and good biocompatibility, such as dental implants, hip and knee replacements, bone plates and screws. It was reported that up to 2.5 million dollars medical devices were made of metals and alloys.

The medical devices greatly improve the life quality of patients, but the device related infection or inflammation is still a main complaint despite strict antiseptic operating procedures, including systemic prophylaxis. It was reported that surgical site infection represents the most common nosocomial infection, accounting for 15% of all nosocomial infections [1]. Oral perimucosal implants pose a very high risk of infection, as they breach the epithelial barrier and are thereby permanently exposed to the oral microflora. It was reported that 90% of all implants showed signs of inflammation and 50% of all implants showed

signs of irreversible tissue destruction [2]. Nevertheless, the ten-year survival rates of endosseous implants were reported to be around 90–96% [2]. For dental implants, the most frequent cause of failure of dental implants is peri-implant inflammatory diseases such as peri-implantitis, which normally causes a circumferential bone loss and in turn may compromise the longevity of an implant. An analysis based on 11 studies showed a mean prevalence of peri-implantitis of 22% and a positive relationship between insertion time and prevalence [3] in which 5–11% of dental implants failed and must be removed [4]. Peri-implantitis affected about 10% of implants and 20% of patients during a 5–10 year observation period after implant placement [5]. For orthopedic replacement, with sterile operation and perioperative antibiotic defenses, the risk of infection after internal fixation ranged between 0.4% and 16.1% depending on the extent of fracture [6,7]. To be more precise, the infection rates of periprosthetic joint infections were 0.5%–2% [8], 2%–9% [9], and 0.3%–1.7% [10] after total joint replacement of the knee, hip and ankle, respectively.

Peer review under responsibility of KeAi Communications Co., Ltd.

\* Corresponding author. Key Lab. for Anisotropy and Texture of Materials, Education Ministry of China, School of Materials Science and Engineering, Northeastern University, Shenyang, 150819, China.

E-mail address: [zhangel@atm.neu.edu.cn](mailto:zhangel@atm.neu.edu.cn) (E. Zhang).

<sup>1</sup> These authors contributed equally to this work.

<https://doi.org/10.1016/j.bioactmat.2021.01.030>

Received 12 November 2020; Received in revised form 11 January 2021; Accepted 27 January 2021

2452-199X/© 2021 The Authors. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC

BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The bacterial infection or inflammation that seriously threatens human health has already become a worldwide complaining issue [11]. So research on antibacterial technologies [12], including mechanical clearance, surface modification [13] and antibacterial materials is urgently necessary. Taking dental implants for example, the biofilm has to be removed mechanically, which should not change the micromorphology of the implant surface. Mechanical *in vitro* and *in vivo* treatments with an air powder abrasion device or with a titanium brush left around 10% bacterial residues on rough sand blasted titanium surfaces [14,15]. Surface modification can effectively prohibit the biofilm formation on the implant materials, such as developing a surface which causes cell damage to the adhered bacteria or anti-adhesive properties that inhibits bacterial adhesion in the first place. Various approaches have been used to convert the surfaces of biomedical devices into antimicrobial surfaces and lately reviews have been published on these approaches [4].

Antibacterial metals and alloys are a kind of metallic materials, which exhibits strong ability to inhibit the adhesion, the growth and the proliferation of a bacterium by element alloying and proper metal forming and heat treatment. Recently, Cu- and Ag-containing antibacterial metal alloys have been reported to exhibit good antibacterial ability against lots of bacteria, such as antibacterial stainless steel [16], antibacterial titanium [17,18], antibacterial magnesium and alloys [19] and antibacterial cobalt alloy [20,21]. The alloying elements mainly are Ag and Cu elements, which have been reported to have broad spectrum antibacterial ability. Nowadays, antibacterial metal alloys have attracted much attention worldwide and lots of new progresses have been achieved, which will promote the clinical application of antibacterial metal alloys. Fig. 1 shows the publication numbers on antibacterial or antimicrobial research searched by Web of Science. There are about 56,000 publications on this area in the past two decades with a 6–16% increase in the past decade. But research on “Antibacterial Metal” shows a much higher increase, especially on “Antibacterial Titanium”, which even increased by as much as 40% in 2016.

This paper summarizes the recent research results on antibacterial metals and alloys for the first time and Fig. 2 overviews the main framework of this review. It is hoped this review could benefit the flourishing development of antibacterial metals and metal alloys.

## 2. Interaction between bacterium and metal alloy

In order to develop antibacterial metals and alloys, it is very necessary to understand the interaction between a bacterium and materials. The interaction between a bacterium and materials, such as metallic materials can be roughly divided into four steps:

1) *The primary step is the bacterial adhesion to a material surface.* As pointed out by Campoccia [12], bacterial adhesion on materials surfaces is critically influenced by numerous variables, including the

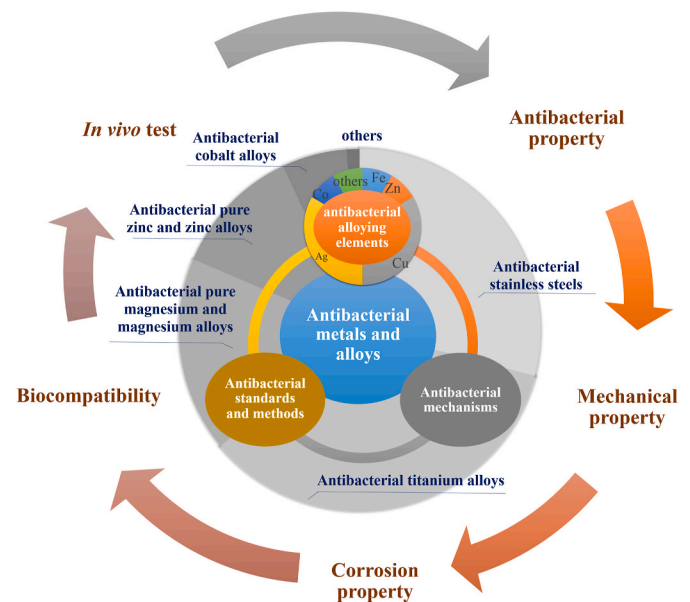


Fig. 2. The main framework of this review.

type of pathogen, the nature of physiological fluids, the surface morphometry and the surface physico-chemical properties of materials. The bacterial adhesion process is reversible.

- 2) *The secondary step is the colonization of the bacterium on the material surface.* During this process, bacteria accumulate on the surface and colonize. The colonization is influenced by specific molecular and cellular interaction (adhesion proteins, proteinaceous appendages and extracellular polymeric substance production) [22]. On the other hand, bacterial colonization also changes the surface chemistry of the substrate by virtue of its metabolic byproducts. This process is irreversible.
- 3) *The third step is the biofilm formation and maturation.* When bacteria colonize on the surface, bacterial microcolonies will form and bacteria will produce exopolymeric substances (mainly polysaccharides and other macromolecules), which contribute to biofilm formation. Once a biofilm is formed on the surface, it will protect bacteria in self-produced polysaccharidic matrix from both fluid shear stress and the action of systemic pharmacological therapies [22].
- 4) *The final step is the proliferation of bacteria.* With the protection of the biofilm, bacteria begin to proliferate on the surface. As a result, the whole surface will be covered by bacteria.

From the interaction between a bacterium and materials, it can be easily deduced that the strategies to reduce bacteria can be also roughly

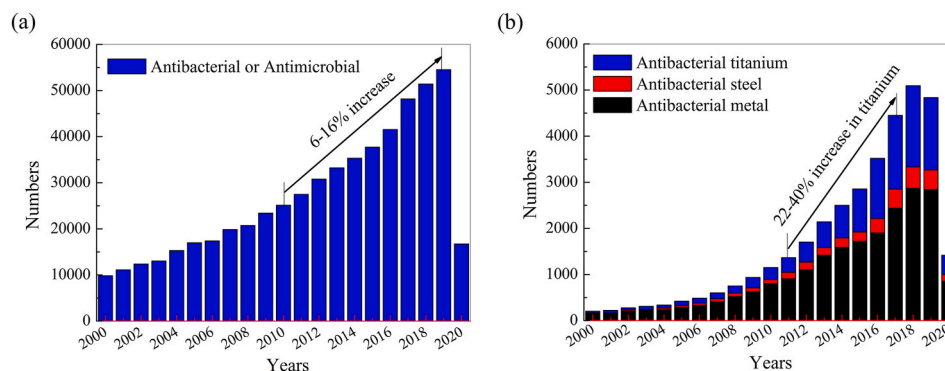


Fig. 1. Publications on antibacterial research searched by Web of Science. a) key words: antibacterial or antimicrobial in topic, b) keywords: antibacterial metal, antibacterial steel and antibacterial titanium in topic. (Updated at 2020-05-21).

divided into four steps corresponding to the above four-step interaction, as shown in Fig. 3:

- 1) Strategies to inhibit the adhesion of a bacterium on a metal surface, or anti-adhesion.
- 2) Strategies to inhibit the colonization of a bacterium on a metal surface, or anti-colonization.
- 3) Strategies to inhibit the biofilm formation on a metal surface, or anti-biofilm.
- 4) Strategies to inhibit the proliferation of a bacterium on a metal surface, or anti-proliferation.

As stated above, the biofilm will protect the bacteria from external adversity. Therefore, the strategies mainly focus on the inhibition of the bacterium adhesion, the colonization and the biofilm formation. Lots of factors affect the bacteria adhesion, including the surface morphometry and physical-chemical properties of materials, environmental conditions as well as pathogen. Many surface technologies have been developed to change the surface properties, such as polymer coating, drug-eluting coating and charged surface [12]. Low adhesiveness of antifouling surfaces could possibly hinder tissue adhesion and integration of the implant. Nanostructure surface topography can determine a different bacterial behavior not just in terms of adhesion, but also in terms of cell metabolism, finally resulting bioactive. Smoothness down to nanometric levels was found to be associated *in vitro* to the lowest adhesion of both Gram-positive and Gram-negative bacteria [23].

It has been demonstrated that Ag- or Cu-containing surface coatings could resist the cell adhesion, the colonization as well as the biofilm formation due to the metal ion release [22,24–31]. Recently, researches on antibacterial metal alloys also indicated that Cu- and Ag-containing metal alloys also show anti-adhesion and anti-biofilm function due to the metal ion release [32,33].

### 3. Advantages and disadvantage of antibacterial metals and alloys

Antibacterial surface modifications on metal could provide strong antibacterial ability but also good biocompatibility, including but not limited to the following measurements:

- 1) Antibacterial metal-implanted surfaces, including silver-containing (particle or ion) [34–37] and Cu-containing [38–40] or Zn-containing [41–43] antibacterial surface.
- 2) Nanoscale surfaces, including Ag-containing nanoparticle (NPs) [44], Cu-containing NPs [45] or Zn-containing NPs [46–48], gold-containing [49] surfaces due to the strong antibacterial property of metal nanoparticle (NP), even if confronts against drug-resistant bacteria [50,51].
- 3) UV-activatable or photo-sensitive antibacterial surfaces. Ti<sub>2</sub>O coating becomes bactericidal under near ultraviolet light. It was reported that these surfaces require up to 80 min of UV exposure to

eliminate 75–95% of bacteria [4,52]. However, it is unclear if the technology would be effective on osseointegrated implants or in deep peri-implant pockets due to limitations in the penetration of light. Recently, it has been discovered that TiO<sub>2</sub> nanostructures could produce superior antibacterial activity *in vitro* and *in vivo* under the irradiation of 808 nm near-infrared (NIR) light that human body can withstand for a short time [53]. Wu [54–56] reported some novel coatings, such as red phosphorus/IR780/arginine-glycine-aspartic acid-cysteine, polydopamine/IR780/MnO<sub>2</sub> and red phosphorus/graphene oxide film on titanium implants utilizing NIR light, exhibiting excellent antibacterial property. Meanwhile, other photo-sensitive materials, such as hydrogels [57] and chitosan/Ag/MoS<sub>2</sub> [58], have also used to prepare antibacterial surfaces.

- 4) Drug-loaded surfaces. The drugs include conventional antibiotics such as amoxicillin [59], vancomycin [60–62], gentamicin [63], tetracycline [64], minocycline [59,65], cephalotin [66] and chlorhexidine [59,67].
- 5) Antibacterial peptide surfaces (AMPs). Compared with antibiotics, AMPs have drawn increasing attention as novel antibacterial agents to combat bacterial invasion due to their special mechanisms of action, non-inducible bacterial resistance and lack of detrimental effects on humans [68–70].

However, on the other hand, there are some potential disadvantages in the antibacterial surface modification:

- 1) *The risk of bacterial resistance and cell toxicity.*
- 2) Finite duration of antimicrobial activity.
- 3) Coating delaminating on implants.

In comparison with the antibacterial coatings, the antibacterial metals and alloys are of following advantages:

- 1) *Long-term antibacterial ability.* The whole alloy is of antibacterial activity, thus the antibacterial ability will not be lost due to the machining, implantation, wear or abrasion, corrosion, etc. On the contrary, wear, abrasion or machining will produce a fresh surface, which in turn enhances the antibacterial activity. But for antibacterial coating, including Ag- and Cu-containing coating, wear or abrasion will destroy the coating and significantly reduce the antibacterial efficiency.
- 2) *Easy control and easy preparation.* Normal metal and alloy processing, including casting, metal forming and powder metallurgy, etc. can be used to prepare and produce antibacterial metals and alloys with different shapes including bar, sheet and even complex structure. The metal and alloy can also be machined to produce implants with complex structure without reduction of antibacterial properties. Normal sterilization treatments also can be used on the antibacterial metal and alloy devices without reduction of antibacterial properties.

Inhibiting the adhesion of bacterium	Inhibiting the colonization	■ Inhibiting the biofilm formation	● Inhibiting the proliferation
<ul style="list-style-type: none"> <li>□ hydrophilic surface</li> <li>□ highly hydrated surface</li> <li>□ non-charged surface</li> <li>□ superhydrophobic surface</li> <li>□ smoothness to nanometric levels</li> <li>□ drug diluted coating</li> <li>□ Ag/Cu doped coating</li> <li>□ antibacterial metal alloy</li> </ul>	<ul style="list-style-type: none"> <li>□ drug diluted coating</li> <li>□ Ag or Cu containing coating</li> <li>□ photocatalysis coating</li> <li>□ antibacterial metal alloy</li> </ul>	<ul style="list-style-type: none"> <li>□ drug diluted coating</li> <li>□ Ag/Cu doped surface coating</li> <li>□ photocatalysis coating</li> <li>□ antibacterial metal alloy</li> </ul>	<ul style="list-style-type: none"> <li>□ drug diluted coating</li> <li>□ Ag/Cu doped surface coating</li> <li>□ photocatalysis coating</li> <li>□ antibacterial metal alloy</li> </ul>

Fig. 3. Antibacterial Strategies for metal alloys.

3) *No potential drug-resistance.* The antibacterial ability of the metals and alloys is mainly attributed the antibacterial mechanism of alloying element, such as Ag, Cu and Zn elements which have been proven to have broad spectrum antibacterial ability to nearly all bacteria and no drug-resistance has been reported so far. More recently, it was demonstrated that the antibacterial properties were mainly controlled by the precipitation of Ag-or Cu-containing phase rather than the Ag or Cu ion release [71].

However, there are also some drawbacks of antibacterial metals and alloys:

- 1) *Mechanical properties and corrosion property.* Ag and Cu are not main alloying elements for antibacterial stainless steel, titanium alloy and magnesium alloy. The addition of these antibacterial elements will change the microstructure characteristic, and thus change the mechanical properties potentially as well as corrosion resistance. For example, Cu element is considered as one of impurity elements for magnesium alloy, which can significantly deteriorate the corrosion resistance or accelerate the degradation of magnesium alloy [72]. Optimization of the mechanical property, the corrosion resistance and the antibacterial ability of antibacterial alloys will be a big challenge. Especially for long-term application, the effect of the antibacterial alloying elements on the fatigue strength will be significantly important.
- 2) *Potential toxicity of the metal ion release.* It has been widely accepted that antibacterial ability of metals and alloys comes mainly from the released metal ions, including Ag<sup>+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>. In order to obtain high antibacterial activity, metal ion release concentration has to be high enough, which might cause local toxicity and sometimes accumulation in distant target organs. Thus, it is always critical to find out a correct balance between bactericidal effects and biocompatibility properties, especially as far as cytotoxicity, cytocompatibility and immunocompatibility are concerned. However, it has been shown recently that titanium alloys with low Ag ion and Cu ion release also exhibited strong antibacterial ability, which reduces the toxicity of metal ions.
- 3) *Function of surface modification.* For biomedical application, the metal surface is normally modified by surface coatings technology, such as bone morphogenetic protein (BMP) containing coating, to improve the surface cell bioactivity. Some surface coatings separate the bacteria from antibacterial metal alloy, and reduce or eliminate the antibacterial property as a result. However, sand blasting, sand-blasting and acid etch (SLA), alkaline and heat treatment and micro-arc oxidation (MAO) have been proven to have no influence on the antibacterial activity of antibacterial titanium [73–76].

#### 4. Selection of antibacterial alloying element

Antibacterial agents can be broadly classified into three major categories: organic, inorganic and natural. To develop antibacterial metal alloys, the selection of antibacterial alloying elements is very important. So far, it is widely accepted that Ag and Cu are normally alloying elements in the development of antibacterial metal alloys, in which Ag has been described as one of the earliest materials to be intentionally used in surgery for its bactericidal properties [77]. Actually, other metals also exhibit antibacterial property to some extent.

Sreekumari et al. [78] reported the ability of different metals to resist bacterial attachment, including Zn, Ni, Pb, Co, Mo, Zr, Cu, Sn and Ti. Except Sn and Ti, all other metals showed profound antibacterial property against *E. coli* and *S. aureus* by reducing the bacteria colony forming units (CFU) on their surface to less than 10<sup>1</sup> from 10<sup>6</sup> within a period of 24 h. It was also reported that Pb followed by Co and Cu were most effective metals those resisted bacterial attachment and growth by causing mortality [78].

Kawakami [79] assessed the antibacterial property of 21 metallic

elements against *E. coli* and *S. aureus* by a plate counting method. Besides Ag, Cu and Zn, other elements, including Al, Co, Ni, Mo, Pd and W also exhibited antimicrobial activity (R > 2.0) against both *E. coli* and *S. aureus*, and Pt and Pb only against *E. coli*, and V and Zr only against *S. aureus*. In addition, the bactericidal efficiency (K value) of Ag and Cu was significantly higher than the values of other metals, indicating strong biocidal activities of Ag and Cu.

Miyano [80] also evaluated the antibacterial ability of some pure metals by two different methods: plate count method and shaking flask method (JIS Z2802:2000). The plate count method results after 24 h incubation indicated that pure Co, Ni, Cu, Zn, Zr, Mo and Pb exhibited strong antibacterial ability (more than 2log<sub>10</sub> reduction in total viable cell, cfu/mL) against *S. aureus* and Ti, Co, Ni, Cu, Zn, Zr, Mo and Pb showed strong antibacterial ability (more than 2log<sub>10</sub> reduction in total viable cell, cfu/ml) against *E. coli*. On the contrary, the shaking flask test after 24 h incubation indicated that only Pb showed strong antibacterial ability against *S. aureus* and Zn and Co against *E. coli*. In addition, their plate count results indicated the antibacterial efficiency was in the following order from high to low: Pb > Cu > Co > Zn > Ni > Zr > Mo.

Heidenau et al. [81] evaluated the antibacterial ability of several metal ions against *S. epidermis* by the plate count method. Their results confirmed that Hg<sup>+</sup>, Ag<sup>+</sup> and Cu<sup>2+</sup> exhibited very strong antibacterial property but Co<sup>2+</sup> and Zn<sup>2+</sup> exhibited no significant antibacterial effect at the investigated concentrations. Table 1 summaries the minimum inhibitory concentration (MIC) of several metal ions for different bacteria. Significant difference was found in MIC among different metal ions and for different bacteria. For example, MIC value of Cu ion for *S. Typhimurium S9* was as high as 14000 μM while the value for *P. phosphoreum* was just 31.2 μM. Different researchers reported different MIC values due to the different testing methods.

Metal ion provides strong antibacterial ability, it on the other hand might lead to cytotoxicity or poor cytocompatibility. Heidenau et al. [81] evaluated cell toxicity of several metal ions by a growth inhibition test with L929 cell line. Their results showed that Ag<sup>+</sup>, Zn<sup>2+</sup> and Hg<sup>2+</sup> ions exhibited very strong cytotoxicity at low concentrations. Co<sup>2+</sup> showed intermediate cytotoxicity whereas tissue cells tolerated relatively high concentrations of Cu<sup>2+</sup> and Al<sup>3+</sup>. Table 2 summaries the LD50 value (a value which causes 50% cell death) of several metal ions to different cells. Although large difference in LD50 value was found,

**Table 1**  
Minimum inhibitory concentration (MIC) for different bacteria.

Elements	MIC (μM)	Bacterium	Ref.	
Ag <sup>+</sup>	37.4	<i>E. coli</i>	[82]	
	37.4	<i>S. choleraesuis</i>		
	74.7	<i>S. aureus</i>		
Co <sup>2+</sup>	40	<i>S. epidermis</i>	[81]	
	240 *	<i>S. epidermis</i>		
	>508.5	<i>P. phosphoreum</i>	[83]	
	384	<i>P. phosphoreum</i>		
Cu <sup>2+</sup>	10000	<i>S. Enteritidis SE</i>	[84]	
	12000	<i>S. Typhimurium S19</i> ;		
		<i>S. Typhimurium S20</i>		
	14000	<i>S. Typhimurium S9</i>		
	7000	<i>S. aureus</i>	[82]	
	4000	<i>E. coli</i>		
	4000	<i>S. choleraesuis</i>		
		31.2	<i>P. phosphoreum</i>	[83]
		110	<i>S. epidermis</i>	
	Fe <sup>3+</sup>	0.89	<i>P. phosphoreum</i>	[83]
Mn <sup>2+</sup>	26812.3	<i>E. coli</i> ; <i>S. choleraesuis</i>	[82]	
	29143.9	<i>S. aureus</i>		
Ni <sup>2+</sup>	>6779	<i>P. phosphoreum</i>	[83]	
Zn <sup>2+</sup>	11743	<i>S. aureus</i> ; <i>E. coli</i> ; <i>S. choleraesuis</i>	[82]	
	>45.8	<i>P. phosphoreum</i>		
	>300	<i>S. epidermis</i>	[81]	
	<139.5	<i>S. aureus</i>	[85]	
	155	<i>S. aureus</i>	[86]	

Note: MIC data have been transferred into μM for comparison purpose.

**Table 2**  
Cell toxicity of metal ions (LD50).

Metal ion	Medium	Test method	LD50 ( $\mu\text{M}$ )	Cell line	Ref.
Ag <sup>+</sup>	DMEM with 10% FBS	MTT	11.0	human osteosarcoma cell line MG-63	[87]
	DMEM with 10% FBS	MTT	9.0	tumour-derived mouse macrophage cell line	
	L929: MEM with 10 vol% FBS	10 vol% Giemsa staining	4.25	L929	[88]
	MC3T3-E1: $\alpha$ -MEM with 10 vol% FBS		2.77	MC3T3-E1	
	RPMI 1640 with 10 vol% FCS	WST-1 test assay	3.5	L929	[81]
Co <sup>2+</sup>	Same as [88] above	10 vol% Giemsa staining	11.2 81.2	MC3T3-E1 L929	[88]
	RPMI 1640 with 10 vol% FCS	WST-1 test assay	34	L929	[81]
Cr <sup>3+</sup>	Same as [88] above	10 vol% Giemsa staining	12.7 743	MC3T3-E1 L929	[88]
	Cu <sup>2+</sup>	Same as [88] above	10 vol% Giemsa staining	15.9 41.5	MC3T3-E1 L929
RPMI 1640 with 10 vol% FCS		WST-1 test assay	230	L929	[81]
Fe <sup>3+</sup>	Same as [88] above	10 vol% Giemsa staining	328 5420	MC3T3-E1 L929	[88]
	Fe <sup>2+</sup>	Same as [88] above	10 vol% Giemsa staining	583 6950	MC3T3-E1 L929
Ni <sup>2+</sup>		Same as [88] above	10 vol% Giemsa staining	52.2 106	MC3T3-E1 L929
	Ta <sup>5+</sup>	Same as [88] above	10 vol% Giemsa staining	2060 1880	MC3T3-E1 L929
Ti <sup>4+</sup>		Same as [88] above	10 vol% Giemsa staining	871 1090	MC3T3-E1 L929
	Zn <sup>2+</sup>	Same as [88] above	10 vol% Giemsa staining	90 92.8	MC3T3-E1 L929
RPMI 1640 with 10 vol% FCS		WST-1 test assay	3.6	L929	[81]
serum-free medium		MTT	300	Primary human aortic endothelial cells	[89]
serum-free medium		MTT	280	Primary human aortic smooth muscle cells	[89]
complete cell-culture medium		MTT	215	Primary human endometrial epithelial cells	[90]
	serum-free medium: 2 h + medium with serum: 24 h	MTT	185	Neuronal PC12 cells	[91]

Note: a) MEM: minimum essential medium, b) DMEM: Dulbecco's modified Eagle's medium, c) FBS: fetal bovine serum, d) FCS: fetal calf serum, e) RPMI: Roswell Park Memorial Institute-a cell culture medium.

even for same cell line, Ag<sup>+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup> and Ni<sup>2+</sup> showed low LD50 value while Ti<sup>4+</sup>, Ta<sup>5+</sup>, Fe<sup>2+</sup> and Fe<sup>3+</sup> showed high LD50 value, indicating that Ag<sup>+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup> and Ni<sup>2+</sup> show potential higher cell toxicity than the others.

Metal ion provides strong antibacterial ability, it on the other hand might lead to cytotoxicity or poor compatibility. Heidenau et al. [81] evaluated cell toxicity of several metal ions by a growth inhibition test with L929 cell line. Their results showed that Ag<sup>+</sup>, Zn<sup>2+</sup> and Hg<sup>2+</sup> ions

exhibited very strong cytotoxicity at low concentrations. Co<sup>2+</sup> showed intermediate cytotoxicity whereas tissue cells tolerated relatively high concentrations of Cu<sup>2+</sup> and Al<sup>3+</sup>. Table 2 summaries the LD50 value (a value which causes 50% cell death) of several metal ions to different cells. Although large difference in LD50 value was found, even for same cell line, Ag<sup>+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup> and Ni<sup>2+</sup> showed low LD50 value while Ti<sup>4+</sup>, Ta<sup>5+</sup>, Fe<sup>2+</sup> and Fe<sup>3+</sup> showed high LD50 value, indicating that Ag<sup>+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup> and Ni<sup>2+</sup> show potential higher cell toxicity than the others.

According to the general antibacterial mechanism, the strong antibacterial ability of metal is due to the accumulation of metal ion, thus high metal ion concentration will result in high antibacterial property. But on the other hand, the high metal ion concentration will also lead to cell toxicity. With above considerations, the alloying elements with low MIC and high LD50 will be the best choice for developing antibacterial metal alloys, such as Fe<sup>3+</sup>, Ag<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>. Ag<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> have been widely used as antibacterial agent but Fe<sup>3+</sup> and Co<sup>2+</sup> have not yet been reported to be an antibacterial agent.

#### 4.1. Ag

Silver has a strong inhibitory and bactericidal effect on a range of bacteria, fungi and viral pathogens [92]. Silver ions and silver-based compounds are highly toxic to microorganisms [93] showing strong biocidal effects on as many as 16 species of bacteria [94]. The antibacterial activity of silver was first demonstrated in the 19th century. Now, the use of various forms of silver as a topical agent is commonplace [92,95]. Silver is certainly the metal most commonly used with the purpose to confer anti-infective properties to biomedical devices for its oligo dynamic antibacterial activity, i.e. exhibiting bactericidal/bacteriostatic activity at very low concentration [12] for dental and orthopedic implant applications [96,97]. Widely documented use of silver is prophylactic treatment of wounds, water disinfection, pleurodesis, cauterization etc. Silver was used as a biocide to sterilize recycled drinking water aboard of the NASA space shuttle and MIR space station [98]. However, the use of silver as bulk material in medical devices has progressively been ceasing. On the other hand, the utilization of Ag element in antibacterial surface modification has progressively been flourishing, such as nanoparticles loaded thin film [99,100], doped solid coatings [101], hydrogel materials [102], micro- and nanoparticles reinforced antibacterial glasses [103] and antibacterial polymer material [50]. Meanwhile, Ag element has also been used as an alloying element in preparation of antibacterial metal alloys [104,105].

It was reported that silver at low concentrations was not cytotoxic for osteoblast *in vitro* [106]. Controversially, debate still persists over the possible inactivation of silver mediated antibacterial activity in physiological fluids and over the low biocompatibility index of silver determined by the low threshold concentration for cytotoxic effects, especially inform of nanoparticles. Li [107] showed that nano-Ag had the potential to induce embryo cytotoxicity, although the toxicity was lower than Ag<sup>+</sup> ion.

#### 4.2. Co

Although some researchers reported that Co metal exhibited strong antibacterial ability, as mentioned above, Co<sup>2+</sup> showed a high MIC value to several bacteria as listed in Table 1, and low LD50 value to cells as listed in Table 2, which indicates that Co and Co<sup>2+</sup> are not a proper antibacterial alloying element. So far, Co or Co<sup>2+</sup> has not been used as an alloying element or antibacterial agent in the development of antibacterial materials and coating.

#### 4.3. Ce

Recently, Jing [108] reported for the first time that Ce-containing stainless steels with 0.11 wt% Ce and 3.25 wt% exhibited very strong

antibacterial ability (>99% reduction). In comparison with antibacterial agent Cu and Ag elements, Ce element showed a high antibacterial efficiency, only 0.11 wt% Ce in steel provided with strong antibacterial properties. As for the antibacterial mechanism, it was suggested that Ce ion released from the Ce-containing alloy might have similar antibacterial effects as Cu ion and Ag ion. However, Ce release concentration was not detected in their report.

#### 4.4. Cu

Long time ago, copper was used to clean up wounds on the chest and to purify water, to treat local skin ulcers in the legs and skin diseases, syphilis and tuberculosis. After copper was certified by the US Environmental Protection Agency (EPA) as having antibacterial properties in 2008 [109], its potential application for antibacterial activity has aroused more and more commercial interest. It has been reported that a copper surface can complete kill *MRSA* and *E. coli* within a few hours [110,111]. Many researchers have studied the antibacterial and bactericidal concentrations of Cu ions against different types of bacteria. Copper-sensitive bacteria, e.g., *K. aerogenes* were inhibited in their growth and survival in a range of  $10^{-8}$ – $10^{-6}$  M  $\text{Cu}^{2+}$  ion concentrations [112]. When the  $\text{Cu}^{2+}$  concentration was  $10^{-5}$  M, the sterilization rate to *S. aureus* was 92% while the sterilization rate to *E. coli* was 93% at  $5 \times 10^{-6}$  M  $\text{Cu}^{2+}$  [113–115]. Recently, Cu has been widely used to develop antibacterial materials, and some reviews have been published, including Cu-containing ceramic [116,117], Cu-containing polymer composites [118], and Cu-containing metal alloys [119,120].

In addition, copper is an essential metal ion to the proper functioning of organs and metabolic processes, which is incorporated into a variety of proteins and enzymes of organisms, such as cytochrome oxidase, superoxide dismutase, internal plasma copper blue protein, dopamine,  $\beta$ -hydroxylase, etc. [121]. Cu deficiency is harmful for human health, which may not only result in anemia, cardiac hypertrophy, coronary heart disease, arthritis, and osteoporosis, etc., but also influence the human secretion system and immune function, especially cardiovascular system [122]. The World Health Organization (WHO) reports that the minimal intake to prevent copper deficiency is 0.9 mg/day [123]. Cu ion can promote osteoblast proliferation, differentiation and migration [124–129]. Cu ion also plays an important role in the cardiovascular system and has beneficial biomedical functions in promoting endothelial cell growth, inhibiting excessive proliferation of arterial smooth muscle, and reducing thrombosis [120,130–133]. Zhang [134] recently proposed that in-stent restenosis might be prevented by the constant release of Cu ion due to inhibition of migration and proliferation of vascular smooth muscle cells by Cu ion. Due to the biofunction of Cu ion, it is proposed that the Cu-containing materials, including metal alloys might have both biofunction and antibacterial activity by special alloy design.

However, high concentrations of Cu ions will induce an inhibition of growth and have a toxic effect on humans [16]. Many researchers reported that the toxicity of Cu depended on the existing form of Cu element. Thit [135] argued that all the three existing forms of Cu including poly-CuO, CuO NPs or  $\text{Cu}^{2+}$  increased cell death and altered cell cycle progression, but poly-CuO caused the most severe effects. Bondarenko [136] indicated dissolution of CuO particles was the key factor triggering the reactive oxygen species (ROS) and DNA damage responses in bacteria. It was also reported that Cu ion might be related to Alzheimer disease [137].

#### 4.5. Fe

Fe is a necessary trace element for human being, which is involved in many Fe-containing enzymes and proteins [138,139]. Fe plays significant roles in the human body, including the transport, storage and activation of molecular oxygen, and the reduction of ribonucleotides and dinitrogen [138]. WHO permissible maximum tolerable daily Fe intake is < 56 mg [140].

Wang's results [83] on the toxicity of some metal ions against *P. phosphoreum* indicated that  $\text{Fe}^{3+}$  showed the highest inhibition, followed by  $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Co}^{3+} > \text{Cr}^{3+} > \text{Ni}^{2+}$ . It started to inhibit the bioluminescence at the concentration of 0.01 mg/L (0.18  $\mu\text{M}$ ) and reached complete inhibition when the concentration was only 0.05 mg/L (0.89  $\mu\text{M}$ ). However, pure Fe and Fe alloy do not exhibit any antibacterial properties.

So far, Fe or Fe ion has not been used as an alloying element or antibacterial agent in the development of antibacterial materials and coating. However, previous studies have revealed that pure Fe and Fe-based alloys were “safe” degradable implantable materials because they did not seem to be associated with inflammation, neointimal proliferation or thrombotic events [141–144]. Moreover, Fe and Fe-based alloys do not have the issue of gas release during degradation compared with Mg and Mg-based alloys [145].

#### 4.6. Ga

Recently, it was reported that gallium-doped specimens showed the best ALP synthesis and antibacterial properties [146]. In the report, Ga-doped coating was produced on titanium substrate by an anodic spark deposition (ASD) method. Ga-doped specimen reduced the bacterial viability by 70–80% against multi drug resistant (MDR) pathogen *A. baumannii* (DSM 30007) as well as two *A. baumannii* clinical isolates (AB1 and AB2). The antibacterial ability of Ga-doped specimen was much stronger than Ag-doped specimen.

Although it was reported that the presence of anatase formed during ASD process might confer antibacterial activity to all the ASD coatings, this effect was enhanced significantly by the presence of Ga. It was thought that the antimicrobial activity of the Ga-doped surface was mainly related to competition with iron in a “Trojan horse” mechanism: in bacterial metabolism, the role of  $\text{Fe}^{3+}$  ions is crucial, and is mediated by intra-membrane transport via siderophores; gallium ions ( $\text{Ga}^{3+}$ ) can effectively compete with  $\text{Fe}^{3+}$  for binding to siderophores, thus interrupting crucial Fe-dependent metabolic pathways in the bacterium. Moreover,  $\text{Ga}^{3+}$  can act intracellularly affecting iron homeostasis leading to different pathways damage.

#### 4.7. La

It was reported addition of only 0.42 wt% La into 316L SS content could obtain excellent antibacterial activity (>99% reduction) against *E. coli* and *S. aureus* [147]. Another research also indicated that La- and Ce-containing 316L-4.36Cu alloy showed antibacterial activity to the sessile sulfate reducing bacteria (SRB) at the first 7 days due to the addition of rare elements La and Ce but the antibacterial activity disappeared at 21st day [148].

#### 4.8. Sn

Many researchers reported the dopant Sn played a vital role in enhancing the antimicrobial activities [149–151]. Verissimo's research [149] on *S. aureus* growth on Ti–35Nb–3Sn showed for the first time that adding Sn to  $\beta$ -type Ti alloys strongly decreased the adhesion of *S. aureus*, but also increased mechanical properties. As for the antibacterial mechanism, it was proposed that the addition of Sn might change the wettability which may have changed surface properties to repel bacteria. It was also reported that the bacteria attachment decreased with the presence of Sn in the Ti oxide layer due to the change in the physical and chemical properties, such as wettability, surface architectures and chemical composition ( $\text{SnO}_2$ ). However, no negative sample was used in their studies. Research on the influence of Sn doping on the antibacterial activity of ZnO films found positive relationship between Sn doping level and antibacterial activity [151].

#### 4.9. Sr

In recent years, strontium (Sr) has been incorporated into dental and orthopedic biomaterial to reduce microbial contamination and the antimicrobial activity of Sr<sup>2+</sup> ions can be used to enhance the use of medical devices by inhibiting bacterial growth and reproduction and impeding permeability of cytoplasmic membrane, cell wall synthesis, replication of bacterial chromosomes and cell metabolism [152–154]. In addition, Sr is one of trace minerals (0.00044% of body mass) predominantly present in the calcified animal tissue. Shorr and Carter [155] discovered the usefulness of low doses of Sr in the treatment of osteoporosis in 1952. Several *in vitro* and *in vivo* studies on Sr-substituted hydroxyapatite (HA), biological glass and bio-ceramics revealed that they promoted osteoblast proliferation and down-regulated osteoclastogenesis in both normal and osteopenic cells, with an increase in the alkaline phosphatase activity.

Guida's research [156] suggested that the bactericidal action of strontium was more significant than that of fluoride. It was also suggested that the fast degradation of Sr-containing ceramic increased the local pH, which might inhibit the bacteria [157]. The antibacterial properties of HA nanoparticles against *E. coli*, *S. aureus* and *Lactobacillus* were reported to be improved after the calcium was half or totally substituted by strontium and the antibacterial ability was strongly dependent on the Sr concentration [158]. However, Sr<sup>2+</sup> in a concentration of 0.19 mol/L to 1.11 mol/L did not exhibit any antibacterial function to *A. viscosus*, *A. naeslundii*, *A. odontolyticus*, *S. mutans*, *S. salivarius*, *S. sorbinus*, *L. caesi*, *L. acidophilus*, *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* [159]. It was also suggested that Sr release concentrations above 0.16 mmol/L (14 ppm) did not further improve bactericidal action [160].

Sr has not been used as an alloying element for titanium, steel and cobalt alloy design as biomedical materials, but has been used in magnesium alloys [161–169].

#### 4.10. Zn

Zinc is used as an antibacterial agent in dental and formulated into oral health products to control plaque such as mouth rinses and toothpaste [170,171]. Based on this knowledge, Zn ion has been widely used in surface modification of metal implant as antibacterial agent to provide good antibacterial ability [172–175].

However, the antibacterial mechanism of Zn containing coating has not been systematically elaborated [174]. Numerous studies have been conducted to clarify the antibacterial mechanism of ZnO, including some reviews [176,177], while the dispute remains existing. Antibacterial mechanism of ZnO is publicly recognized as ROS generation [178, 179] and Zn<sup>2+</sup> ion release [180], but most researchers maintain that the antibacterial property of ZnO mainly attributes to ROS generation. Li [178] considered ROS generation was the main antimicrobial factor for the photoexcited ZnO, because ROS concentration produced by

nano-ZnO and their bacterial mortality rate kept linear relationship. Hu [179] observed no obviously high concentration of zinc ions when researched Zn-incorporated TiO<sub>2</sub> coatings on titanium, thus it was believed the generation of ROS played a leading role in this work. In addition, for antibacterial mechanism of ZnO nanomaterials, increase membrane dysfunction [181], and internalization between the ZnO particles and the bacteria caused by electrostatic forces [182] are taken into account as well.

Compared with Ag and Cu, although Zn possesses a relatively weaker antibacterial activity [173], Zn ion shows non-cytotoxicity in a ranges from 10<sup>-6</sup> M to 10<sup>-5</sup> M, as illustrated in Table 3. In addition, Zn plays important roles in DNA synthesis, enzyme activity, nucleic acid metabolism, biomineralization and hormonal activity, and it has long been recognized that zinc can stimulate bone formation [173,183–186].

### 5. Standards or methods

So far, there is only one standard to assess the antibacterial activity of metals and alloys, SN/T 2399-2010 China, but there are several methods and standards to measure the antibacterial activity of plastic materials, as listed in Table 4. The standard SN/T 2399-2011 is based on JIS Z 2801. In all test methods, glass wear such as Petri dish or culture plate is normally selected as a negative sample. Also, a well-known metals and alloys that do not show antibacterial ability such as pure titanium is selected as a control sample.

#### 5.1. Agar diffusion plate test

Specimens of the materials to be tested are placed on two-layer agar plates. The lower layer consists of a culture medium free from bacteria and the upper layer is inoculated with the selected bacteria. The specimens are tested on both sides. The level of antibacterial activity is assessed by examining the extent of bacterial growth in the contact zone between the agar and the specimen and, if present, the extent of the inhibition zone around the specimen. Thus, the width of the inhibition zone represents the antibacterial ability: the larger the inhibition zone is and the strong the antibacterial activity is.

According to the Standard ISO 20645: 2004 (BS EN ISO 20645: 2004 or GB/T 20944.1), the hot agar solution is first spread evenly with a Conrage stick on the sterilized Petri dishes and is allowed to congeal under sterile conditions. A suspension of bacterium, such as *S. aureus* or *E. coli*, is sprayed evenly over the total area of each Petri dish. A test sample, a control sample and a positive sample are separately placed and contact with the agar closely in the Petri dish. After this, the Petri dishes with samples are incubated immediately at (37 ± 1) °C for 18 h–24 h. The antibacterial activity is accessed by the width of an inhibition zone (H) around the sample calculated by the following formula

$$H = (D - d)/2 \quad (1)$$

**Table 3**  
Zinc ion cytotoxicity.

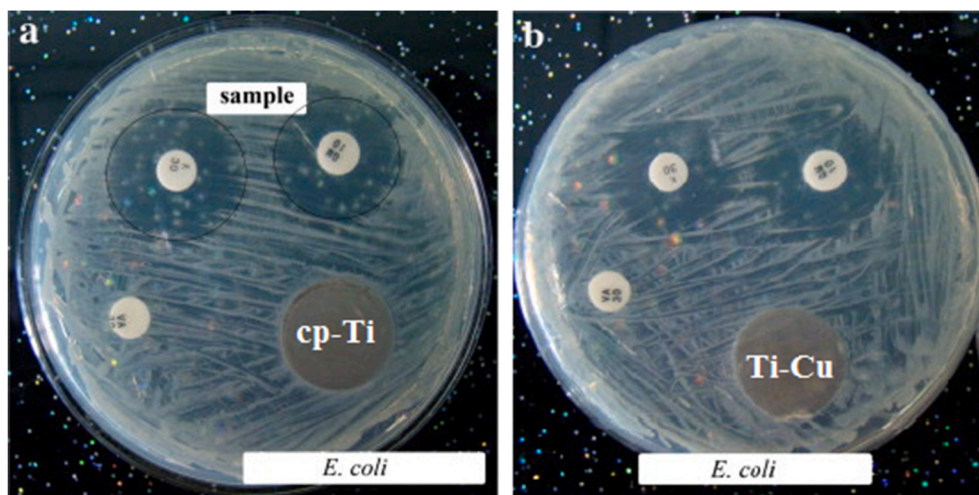
Concentration of Zn (μM)	Medium	Cell	Exposition time (h)	Test	Result	Ref
20–80	serum-free medium	Rat retinal cells	24	LDH	20–40 μM: little cell death; 50 μM: 30%–40% cell death; 60–80 μM: almost 100% death	[187]
1–100	serum-free medium	Human proximal tubular cells	24–96	MTT	1–10 μM: ≥ 80% viability at 24–96 h; 50 μM: ≥ 80% viability until 48 h while then 30%–40% cell death; 100 μM: significant cell reduction at 24–96 h	[188]
2–300	DMEM/MEM +5%FBS	U–2OS (human cell line derived from osteosarcoma)	24	WST-1	2–120 μM: > 70% viability, 180–300 μM: nearly 100% cell death	[189]
2–300	DMEM/MEM +5% FBS	L929	24	WST-1	2–80 μM: > 70% viability, 120–300 μM: nearly 100% cell death	[189]

Note: a) MEM: minimum essential medium, b) DMEM: Dulbecco's modified Eagle's medium, c) FBS: fetal bovine serum.

**Table 4**  
Some Standards for the measurement of the antibacterial activity of solid materials.

Country/Organization	Standards No.	Title	Remarks
China	SN/T 2399-2010	Evaluation method for antibacterial metallic materials	Based on JIS Z 2801–2000
ISO	ISO 22196:2016	Measurement of antibacterial activity on plastics and other non-porous surfaces	ISO 22196:2007 is Based on JIS Z 2801–2000
China	GB/T 31402-2015	Plastics-Measurement of antibacterial activity on plastics surfaces	ISO 22196:2007, IDT
Japan	JIS Z 2801–2000	Antimicrobial products—Test for antimicrobial activity and efficacy	
UK	BS ISO 22196:2016	Measurement of antibacterial activity on plastics and other non-porous surfaces	ISO 22196:2016
USA	ASTM E3031-15	Standard Test Method for Determination of Antibacterial Activity on Ceramic Surfaces.	Ref. ISO-22196
USA	ASTM G21–15	Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi	
ISO	ISO 20645:2004	Text fabrics- Determination of antibacterial activity- Agar diffusion plate test	
China	GB/T 20944.1-2007	Textiles—Evaluation for antibacterial activity—Part 1: Agar diffusion plate method	ISO 20645:2004

where, H is the inhibition zone in mm, D is the total diameter of sample and inhibition zone in mm and d is the diameter of the sample in mm, respectively. After measuring the inhibition zone, the specimens will be removed from the agar with a pair of tweezers and the bacterial growth in the nutrient medium under the specimen with a microscope at 20 times magnification. Inhibition zone up to 1 mm with no bacteria growth under specimen or no inhibition zone with no bacteria growth means good antibacterial effect of the specimen. As an example, Fig. 4 shows the inhibition zones around samples in the agar diffusion plate



**Fig. 4.** Inhibition zones around different samples against *E. coli* (the white sample is the positive sample). a) cp-Ti and b) Ti-Cu samples [18], copyright 2013, Elsevier B.V.

test. An inhibition zone was clearly observed around the white positive sample, indicating that the positive sample exhibited strong antibacterial ability against *E. coli*. On the other hand, there was no inhibition zone around cp-Ti and Ti-Cu alloy, indicating that both cp-Ti and Ti-Cu alloy did not kill the surrounding bacteria.

## 5.2. Plate-count method

Although the plate-count method is intended to evaluate the antibacterial activity of antibacterial plastics (JIS Z 2801–2000, ISO 22196-2016, GB/T31402-2015) and other non-porous surface of products (ISO 22196-2016), this method has been widely adopted to assess the antibacterial activity of metals and alloys. The experiment process has been described in detail in JIS Z 2801–2000, ISO 22196 or GB/T31402-2015. Briefly, samples are placed in Petri dishes separately. Then, the bacterial suspension (0.4 mL in the standard, not less than 0.1 mL) is dripped onto the sample, including the test sample, the control sample and the negative sample. After this, the sample is covered by a sterile polyethylene film with a dimension to make sure that the suspension spreads to, but does not leak beyond the edges of the film, as shown in Fig. 5. Then the samples with bacterium are incubated at  $(35 \pm 1)^\circ\text{C}$  for 24 h under a humidity of  $\geq 90\%$ . After the incubation, 10 mL or more sterilized physiological saline solution is added to the Petri dish to wash the sample and the polymer film completely to make sure that no bacterium is left on the sample and the film. Then 0.1 mL the above washing solution is inoculated onto nutrient agar plates and incubated at  $(35 \pm 1)^\circ\text{C}$  for 24 h under a humidity of  $\geq 90\%$ . The active bacteria colonies are counted. Three samples are assessed for each type of samples. The antibacterial activity, R, is calculated by the following formula:

$$R = \frac{N_{control} - N_{sample}}{N_{control}} \times 100\% \quad (2)$$

where,  $N_{control}$  and  $N_{sample}$  are the average numbers of the bacterial colony on the control sample and the tested sample, respectively.

## 5.3. Live/dead stain

Live/dead stain is another method which has been widely used to observe the bacterium adhesion, biofilm and dead bacterium in order to assess the antibacterial properties. In this method, as shown in Fig. 6, bacterial suspension is dripped onto the tested samples or the sample is immersed in a bacterium suspension, and then incubated at  $37^\circ\text{C}$  for 24 h. After incubation, the sample surface is washed gently for three times by using phosphate buffer saline (PBS) to remove the traces of culture



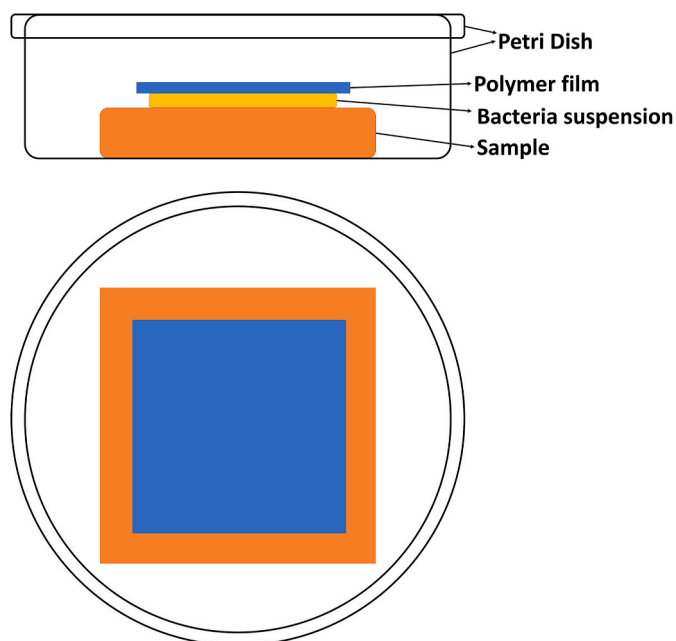


Fig. 5. Schematic diagram of the plate-count method.

medium. Then, two fluorescent dyes, LIVE/DEAD® BacLight™ Bacterial Viability Kits L7012 containing SYTO-9 dye and PI dye are used to stain the bacteria on the sample surface according to the instruction in dark at room temperature. After that, the dyed sample is observed under a confocal Laser scanning microscope (CLSM) at 514/488 nm in an argon laser. The bacteria with green color mean living bacteria and the ones with red color are dead bacteria. The inserted figures in Fig. 6 shows *S. aureus* and *E. coli* on 317L stainless steel and 317L-Cu stainless steel after incubation at 37 °C for 24 h [190]. Most of the bacteria on the surface of 317L stainless steel were stained in green whereas bacteria on the surface of 317L-Cu stainless steel were stained in both red and green, and the red color was significantly dominated.

#### 5.4. Immersion test

In some cases, the antibacterial activity against both the adhered bacteria and the planktonic bacteria properties need to be investigated, thus the immersion test method will be used. In the immersion test, as shown schematically in Fig. 7, the sample is immersed in a bacterium suspension, and incubated at 37 °C for 24 h (or for different time to

assess the dynamic antibacterial properties), then the sample is taken out and washed gently by sterilized SBF solution to remove non-adherent bacterial cells. After this, the sample is gradually dehydrated and gold coated for SEM evaluation, as shown in Fig. 7b or the sample is stained with biological stain (400  $\mu$ L Triton X-100 and 0.4  $\mu$ L DAPI solution for example) for fluorescence microscope observation, as shown in Fig. 7c, or the sample is Live/dead stained as described above. By this way, the bacterium adhesion or biofilm formation can be clearly observed, which can directly reflect the anti-adhesion and anti-biofilm property of metal and alloy. Sometimes, the adherent bacteria cells on the sample are detached and resuspended into PBS by a two-step vortexing procedure at 4 °C for 1 min for each step to avoid unnecessary cell deaths. Serial dilutions ( $10^{-2}$  and  $10^{-3}$ ) of the suspensions are plated on BHI agar plates and incubated overnight, followed by visual counting of colony forming units (CFUs) [191], as schematically shown in Fig. 7d. The antibacterial activity can be calculated by Eq. (2).

Meanwhile, 100  $\mu$ L of the bacteria suspension is taken onto the agar for further plate culture at 37 °C for 24 h and the bacteria colony is counted to assess the antibacterial properties of the sample against the planktonic bacteria by Eq. (2) [192], as shown in Fig. 7d.

#### 5.5. In vivo test

In fact, antibacterial metal implants are in continuous contact with physiological fluids, including plasma in the human body and saliva in the oral cavity. Thus, *in vivo* microbial adhesion and plaque formation on biomaterial surfaces is influenced by factors such as multi-species oral pathogens, “host adhesions” (e.g. fibrinogen, fibronectin, collagen, and plasma albumin), host cells (cells of the innate and adaptive immune system) and the pellicle, a rapidly created conditioning film which covers a newly implanted device [2]. Another important limitation of *in vitro* studies is the protein concentration in artificial body fluids. This is because simply using a single host protein or a small selection of these cannot reflect the highly complex oral conditions *in vivo* [2].

However, no standard on the *in vivo* test method has been set up so far. Recently, an *in vivo* test was used to determine the *in vivo* antibacterial activity of Ti–Cu alloy [195]. Briefly, sample with bacterium was implanted in the muscle of a rabbit. Postoperatively, the general observation, the white blood cell test, the plate-count method and the routine pathological examination were conducted to examine the inflection or inflammation situation surrounding the implantation site to reveal the *in vivo* antibacterial activity, as illustrated in Fig. 8.

## 6. Antibacterial stainless steels

Stainless steels have been widely used as a biomaterial for fabricating

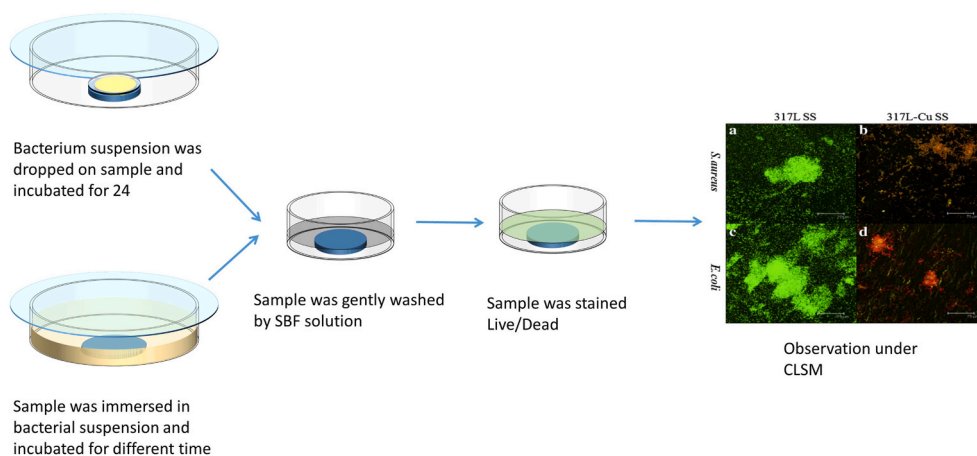
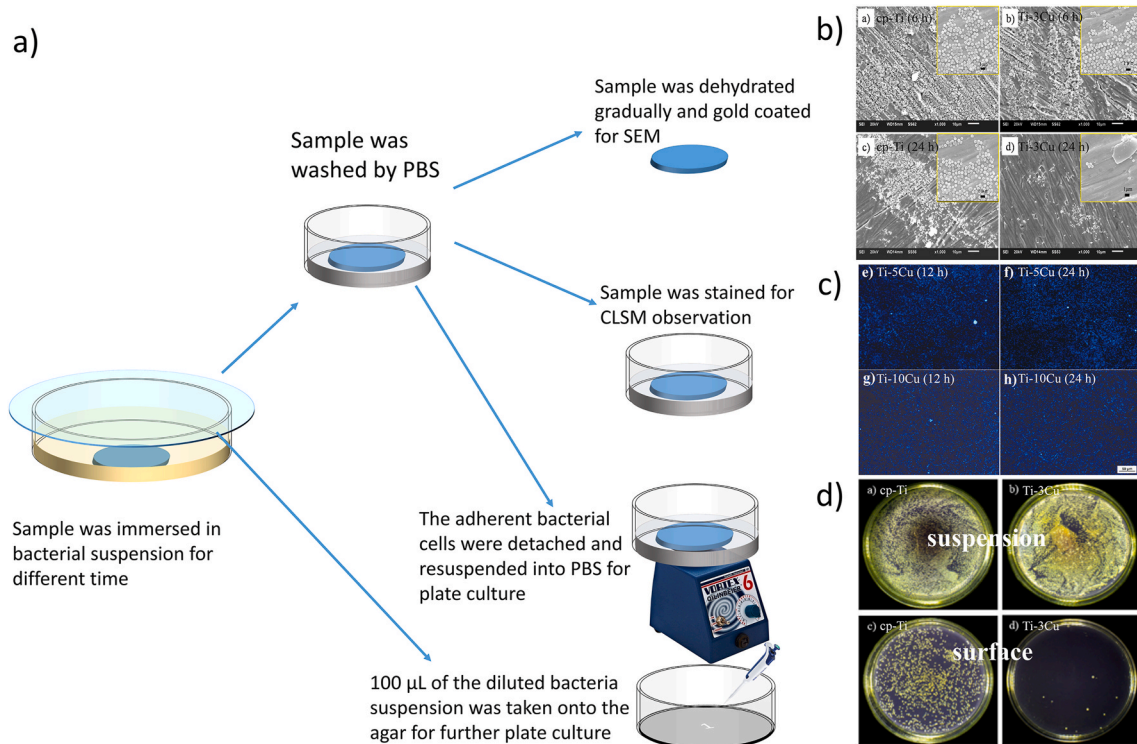
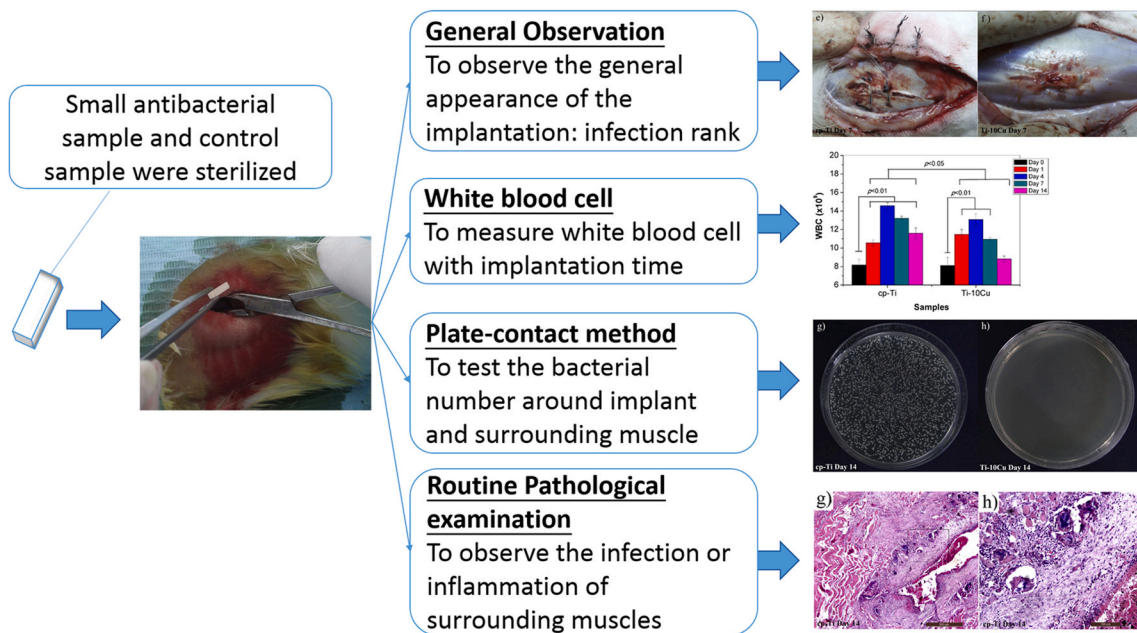


Fig. 6. Live/Dead stain method used to observe the surface bacterium. The inserted Fluorescent images show *S. aureus* and *E. coli* on surfaces of 317L stainless steel and 317L-Cu stainless steel after incubation at 37 °C for 24 h [190], copyright 2012, Elsevier B.V.



**Fig. 7.** (a) Schematic diagram of the immersion method. (b) cell adhesion and biofilm under SEM [193], copyright 2018, Elsevier B.V.; (c) DAPI staining *P. gingivalis* on cp-Ti and Ti-10Cu alloy [194], copyright 2016, The Japanese Society for Dental Materials and Devices; (d) typical bacterium colonies from sample surface and the suspension [193], copyright 2018, Elsevier B.V.



**Fig. 8.** Illustration of *in vivo* test of antibacterial titanium alloy. The inserted images show the general appearance, the blood cell test, the typical bacterium colonies and the pathological examination *in vivo* [195], copyright 2019, Elsevier B.V.

cardiovascular stents/valves, orthopedic prosthesis, dentistry and other devices and implants used in biomedicine due to their good mechanical properties, corrosion resistance, biocompatibility and low price [196]. However, the lack of antibacterial properties is their great weakness in biomedical application. Nisshin Steel (Tokyo, Japan) developed the first copper-containing antibacterial stainless steel in the late 1990s [197, 198]. Since the beginning of this century, lots of researches have been

carried out and various types of copper-containing or silver-containing antibacterial stainless steel have been investigated so far including ferritic antibacterial stainless steel, austenitic antibacterial stainless steel, martensitic antibacterial stainless steel, as well as duplex antibacterial stainless steels [199,200]. Antibacterial alloying elements such as Cu and Ag are added into traditional stainless steel, which provides traditional stainless steel with antibacterial function and therefore is

expected to provide a new approach to solve the problem of peri-implantitis for biomedical application [16].

### 6.1. Antibacterial properties

There are lots of factors that have influence on the antibacterial properties of stainless steel. As listed in Table 5, Cu-containing stainless steels exhibit excellent broad-spectrum antibacterial properties against lots of bacteria, such as *S. aureus*, *B. coil*, *C. albicans*, *S. epidermis*, *S. lutea*, *E. coli*, *S. marcescens*, *B. pumilus*, *E. faecalis*, *S. flexneri*, *P. aeruginosa*, *P. sp* as well as *P. gingivalis*, etc. However, antibacterial stainless steel shows different resistant ability to different bacteria. Nan [201,202] reported that antibacterial stainless steel had relatively stronger effect to Gram-negative bacteria than to Gram-positive bacteria, possibly because Gram-negative bacteria possessed thinner cell wall and were more susceptible to be damaged. The antibacterial stainless steel currently developed is mainly targeted at *E. coli* and *S. aureus* which are most common causes of implant-associated infections.

**Table 5**  
Antibacterial stainless steels.

Alloy system	Alloy	Bacteria (antibacterial rate[Ref.])
Ferritic stainless steel	430-1.8Cu	<i>S. aureus</i> , <i>E. coli</i> , <i>B. coil</i> , <i>C. albicans</i> , <i>S. epidermis</i> , <i>S. lutea</i> , <i>S. flexneri</i> and <i>P. aeruginosa</i> (all > 99% [199,201]); <i>B. subtilis</i> (80% [199]); <i>S. marcescens</i> , <i>B. pumilus</i> , <i>E. faecalis</i> (98.2%, 97.7%, 91.0%, respectively [201])
Austenitic stainless steel	304-xCu (x = 1.5–5.5, 9,18 wt%)	<i>S. aureus</i> (23.4%–99.9% [199,203,204]); <i>E. coli</i> (20%–99.9% [204–207]); <i>B. coil</i> (10.6%–99% [200]); <i>C. albicans</i> , <i>S. epidermis</i> , <i>S. lutea</i> , <i>S. flexneri</i> and <i>P. aeruginosa</i> (all ≥ 99% [199,201]); <i>P. gingivalis</i> (100% after 12 h [16]); <i>B. subtilis</i> (80% [199]); <i>S. marcescens</i> , <i>B. pumilus</i> , <i>E. faecalis</i> (99.5%, 92.4%, 93.6%, respectively [201]); planktonic bacteria in Tap Water (33.3%, 96%, 76.7% at 24 h, 48 h, 72 h [208])
	304-xAg (x = 0.039–0.3 wt%)	<i>E. coli</i> (70.8–99.9% [209]); <i>S. aureus</i> (99.7%–99.9% [209]); <i>P. sp</i> [210]
	304-xCe (x = 0.01–3.25 wt%)	<i>E. coli</i> (12.3–99.0% [108]); <i>S. aureus</i> (74.0%–99.4% [108])
	316-xCu (x = 2.46–4.5 wt%)	<i>S. aureus</i> (95.2%–99.9% [211,212]); <i>E. coli</i> (25%–99% [211–213]); <i>S. epidermidis</i> (94.1% [212])
	316L-xLa (x = 0.05–0.42 wt%)	<i>S. aureus</i> (15%–99.6% [147]); <i>E. coli</i> (9%–99.9% [147])
	317-4.5Cu	<i>E. coli</i> (99.9% <i>in vitro</i> and <i>in vivo</i> [214], 92.7% in the artificial urine for 24 h and 90.3% in the human urine for 6 h [215]); <i>S. aureus</i> (99.0% [32,214,216], <i>in vivo</i> [214])
Martensitic stainless steel	410-xCu (x = 1.52–4.74 wt%)	<i>E. coli</i> (96–99.99% [217])
	420-xCu (x = 0.4–5 wt%)	<i>S. aureus</i> (35.4%–99.9% [197,198,218–220]); <i>E. coli</i> (43.6%–99.9% [218])
	Cr17Ni4–Cu (x = 3.4–5.06 wt%)	<i>S. aureus</i> (82%–99% [221])
Duplex stainless steels	200-xCu (x = 1.45–3.57 wt%)	<i>S. aureus</i> (42.8%–93.6% [202,222]); <i>E. coli</i> (56.1%–97.1% [202,222])
	2205-0.2Ag	<i>E. coli</i> (100% [223]), <i>S. aureus</i> (99.6% [223])
	2205–3.02Cu	<i>P. aeruginosa</i> (33.1% [224], 99.5% [225])
	Cr26Ni5Mo2-3.17Cu-0.07Ag	<i>E. coli</i> (>99% at 3 h and 6 h [226])

Note: 24 h incubation as default.

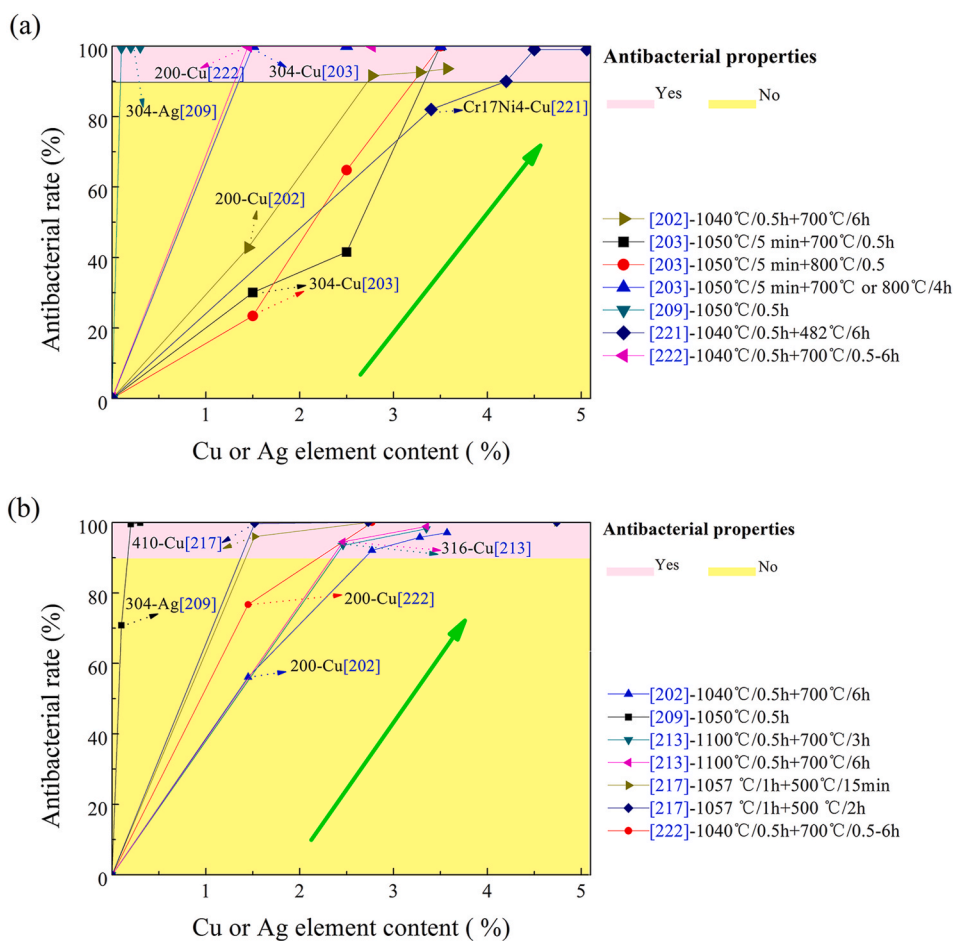
The content of antibacterial alloying element in stainless steels plays a very important role in the antibacterial properties. Normally, high content leads to strong antibacterial ability. However, high Cu content for example on the other hand might have side effect on the mechanical properties, the processing parameter and the corrosion resistance. Cu content in stainless steels is normally in a range of 1.52–5.5 wt% to obtain strong antibacterial ability. Fig. 9 summarizes the effect of Cu or Ag content on the antibacterial rate of some antibacterial stainless steels. 304 stainless steel containing 1.5–2.5 wt% Cu did not exhibit good antibacterial ability (<65%) in aging state, but increasing the Cu content to 3.5 wt% improved the antibacterial rate greatly to as high as > 99.99% [203]. Nan [222] reported that 200 stainless steel with 1.45 wt % Cu showed only 78.7% reduction against *E. coli* and >99.99% against *S. aureus*, but 200 stainless steel with 2.77 wt% Cu showed strong antibacterial activity (>99.99%) against both *E. coli* and *S. aureus*, indicating Cu content in 200 stainless steel has to be higher than a critical value, such as 2.77% in their study, to get good antibacterial ability. Wang [221] also revealed that the Cu content in a Cr17Ni4-(3.4–5.06)Cu had to be 4.2 wt% in order to get good antibacterial activity (>90%) or 4.5% Cu to obtain strong antibacterial ability (>99.9%).

Ag element has much stronger antibacterial ability than Cu, thus the Ag content in stainless steel is significantly lower than Cu content, about 0.039–0.3 wt% Ag. 2205-0.2Ag double-phase stainless steel exhibited an excellent bacteria-inhibiting effect on *E. coli* and *S. aureus* with an antibacterial rate of 100% and 99.5%, respectively [223]. Liao [209] found 304 austenitic stainless steel with 0.2 wt% Ag had an excellent antibacterial rate above 99%. With the consideration of reducing corrosion resistance due to the Cu-rich precipitate, small amount of Ag was also added to a Cu-containing dual-phases stainless steel to prepare an antibacterial dual-phase stainless steel. The alloy exhibited strong antibacterial ability (>99.4% reduction) against *E. coli* at 3 h incubation [226].

It was also reported the more the Ce content in the stainless steel was, the stronger the antibacterial ability of the steels appeared and 304 stainless steel with merely 0.11% Ce (304–0.11Ce) has strong antibacterial activity (98.9% reduction against *E. coli* and 99.1% against *S. aureus*) [108]. The addition of La also contributed to the antibacterial activity in a dose dependent method [147]. When the La content in 316L was about 0.05%, no antibacterial activity was found. With the increasing of La content increased to 0.11 wt%, 0.19 wt% and 0.42 wt%, the antibacterial rate increased to 54–62%, 78–89% and >99.6% against *E. coli* and *S. aureus*, respectively. However, all these alloys exhibited antibacterial function only to the sessile bacteria rather than surrounding planktonic bacteria.

The processing process of antibacterial stainless steel also greatly influences the final antibacterial properties. 304 stainless steel prepared by strip casting and cold rolling only caused 65% of *E. coli* bacteria death upon direct contact for 24 h even if the copper content was as high as 18 wt%, much less than the recommended value of 90% [205]. As-received Cu-containing stainless steels did not have antibacterial ability and solid solution treatment could not provide with strong antibacterial ability [213]. Only the sample underwent solid solution and subsequent ageing treatment to possess precipitated phase, showed a distinct antibacterial function [200,218]. It has been widely accepted that the antibacterial function of Cu-containing stainless steels was attributed mainly to the ε-Cu precipitate during heat treatment. Solid solution dissolves Cu element in matrix completely, thus the bacteria will not contact with Cu-rich compound, which will reduce the antibacterial activity significantly. After ageing treatment, nano-scale Cu-rich precipitates from the matrix and will be contact with bacteria during incubation, and kill the bacteria by some ways [220].

Further research displayed that aging temperature and duration had influence on the antibacterial properties. The aging temperature should be high and the aging duration should be long enough for the complete precipitation of nano-scale Cu-rich phase from the matrix and thus



**Fig. 9.** Relationship between Cu content and antibacterial rate of stainless steel. a) against *S. aureus* (data from Refs. [202,203,209,221,222]) b) against *E. coli* (data from Refs. [202,209,213,217,222]).

resulting in a high antibacterial activity. In Wang's study [219], after being aged at 500 °C for 4 h, 420-3.5Cu alloy only had an antibacterial rate of 69.2% against *S. aureus*, while the ageing treatment at 500–800 °C for 6 h improved the antibacterial rate significantly to as high as > 94%. Similar results were also reported elsewhere, when the aging duration was extended to 240 min at 700 or 800 °C, even the addition of as low as 1.5 wt% Cu generated enough Cu-containing precipitates by long-time diffusion, to make the antibacterial rate reach at > 99.99% against *S. aureus* [203].

It was thought that solid solution treatment could provide strong antibacterial ability for Ag-containing stainless steels [209,223]. Ag and Fe have a low mutual solubility in both the solid and the liquid states, and the silver solidified and reacted with impurities forming a silver-rich compound during solidification to obtain strong antibacterial ability [223]. Compared to the conventional Cu- or Ag- bearing stainless steels, the Ce-bearing stainless steels exhibited good antibacterial ability after solid solution treatment, which was attributed to the precipitation of Ce-rich zones in the solid solution treated sample due to the low solubility of Ce [108].

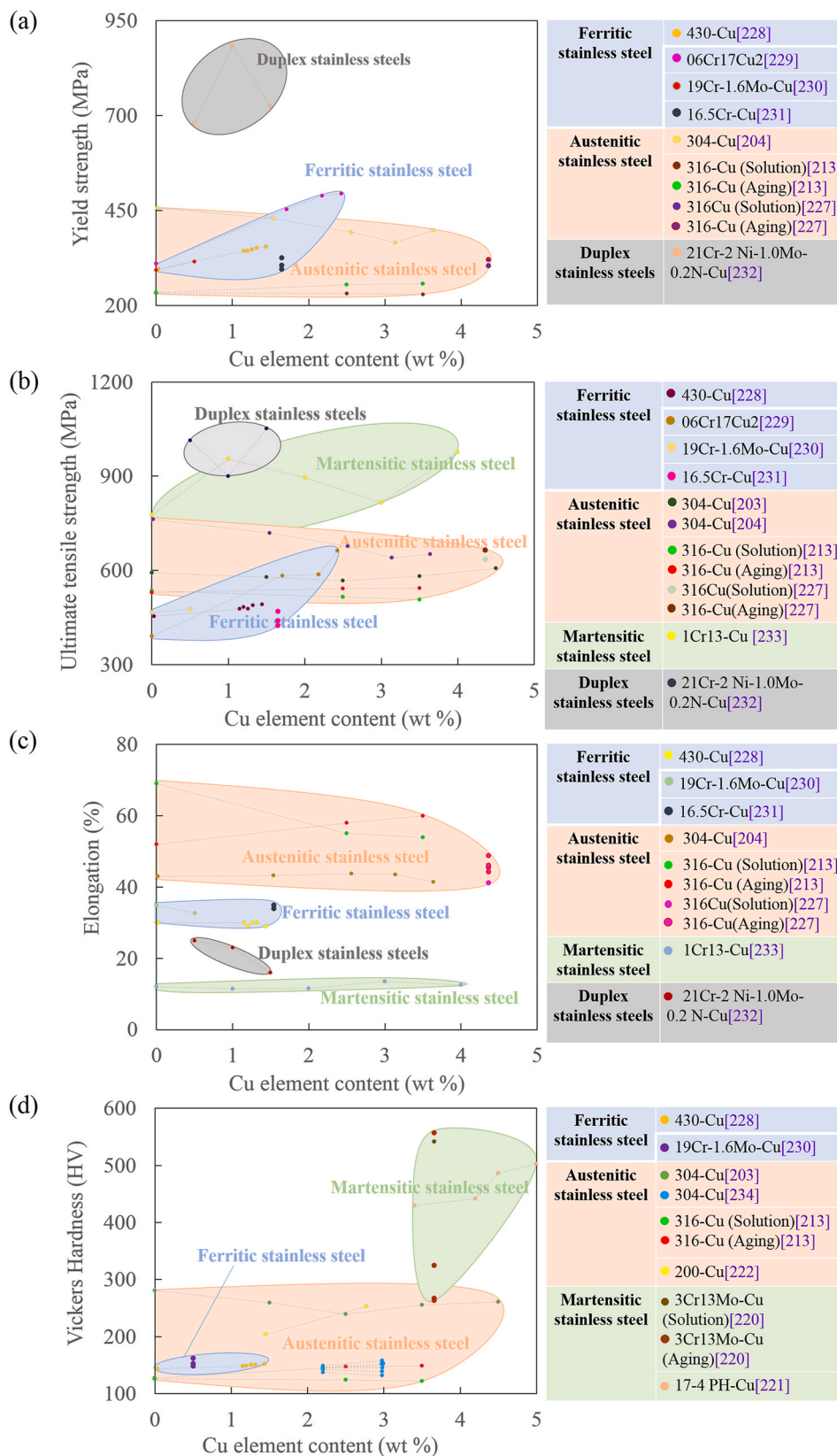
## 6.2. Mechanical properties

Cu and Ag elements have very low solid solubility in Fe matrix at room temperature. Cu and Ag normally do not form intermetallic phase with Fe matrix and exist in Fe matrix as solid solution state or Cu- or Ag-rich phase. It was reported addition of sufficient Cu could increase mechanical properties (including strength and hardness) such as adding over 2.5 wt% Cu into 304 [203]. Xi [213] reported the strengthening

effect of Cu was limited when the Cu content reached at 2.5% for 316L-Cu no matter solution or aging treated, indicating Cu addition with 2.5 wt% was enough to precipitate sufficient Cu-rich phase for precipitation hardening. It was reported that low Cu addition (1.45 wt %) did not bring influence on hardness during 700 °C aging treatment, but high Cu content (2.77 wt%) significantly increased the hardness due to the precipitation of Cu rich phase [222]. In another research, the Vickers hardness of 17-4 PH-Cu stainless steel was positively related to the Cu content (3.4–5.06 wt%) [221]. Fig. 10 summaries the relationship between mechanical properties of antibacterial stainless steels and the Cu or Ag content.

It was also suggested that the addition of Cu had tiny influence on the mechanical properties of solution treated stainless steel, which were mainly attributed to the synergistic effect of the softening effect by increase of stacking fault energy (SFE) and strengthening effect by the solid solution strengthening [213]. Nano-scale Cu-rich precipitation caused by the saturated Cu in the steel in the aging treatment played a positive role in mechanical properties [213,222]. The yield strength of 316L-Cu was almost similar to that of 316L after solution treatment, whereas the aging treatment obviously increased the yield strength of 316L-Cu due to the formation of tiny Cu-rich precipitates [213,231].

Extension of the aging time after solution treatment can enhance the mechanical properties of antibacterial Cu-bearing stainless steels without impairing antibacterial properties, while hardly changes mechanical properties under exceeding critical ageing time [219,220]. As the aging time extended, the hardness of 200–2.77Cu increased rapidly and approached a peak value after 3 h, and then hardly changed [222]. In Xi's study [231], the mechanical property of 316L-4.36Cu remained



**Fig. 10.** Relationship between Cu content and mechanical properties of stainless steel (data from Refs. [203,204,213,220–222,227–234]). a) yield strength b) tensile strength, c) elongation d) hardness.

steady with the aging time increasing from 20 min, further even up to 15 h, implying the modest precipitation hardening effect of Cu-rich precipitates.

It was reported the aging temperature could also affect the mechanical properties of antibacterial Cu-bearing stainless steel. As the aging temperature increased within 500–800 °C, the softening effect and the dislocation density decreased rapidly, resulting in a rapid decreased in the hardness of the 420-Cu steel [219]. Similarly, when the aging temperature increased from 500 °C to 800 °C, the strengthening and hardness of 3Cr13Mo–Cu gradually decreased, which was attributed to the Cu segregation zone in steel evolved into precipitated particles, and the pinning effect of Cu-rich phase on dislocation is weakened [220].

Wang [211] fabricated a novel type of low stiffness porous 316L-4.5Cu by selective laser with similar stiffness (3–20 GPa) with bone to prevent stress shielding. Such low stiffness porous structures, especially coupled with the addition of antimicrobial Cu, may provide a new direction for medical stainless steels.

### 6.3. Corrosion properties

Long-lasting and broad-spectrum antibacterial activity of antibacterial stainless steel is inseparable from corrosion resistance. It is widely accepted the metal ion release, including Cu and Ag ion, controls mainly the antibacterial activity [206,235]. Thus, it is reasonable to believe that high metal ion release will lead to high antibacterial ability. The high metal ion release concentration on the other hand might cause cell toxicity. Therefore, one main challenge in the designing and manufacturing biomedical antibacterial stainless steels is how to optimize the chemical composition, especially the Cu content or Ag content, to balance the corrosion resistance, antibacterial property and cell compatibility. The research on the resistance of Cu-containing ASS to localized corrosion in humans (simulated with 0.9% NaCl solution or PBS buffer) is multiple, but has not been clarified sufficiently, and there are some controversial conclusions in different literatures [236]. Fig. 11 summaries the pitting corrosion potential ( $E_{pit}$ ) of antibacterial stainless steels.

The corrosion properties of Cu-containing antibacterial stainless steels are closely related to the heat treatment process. It was reported that the Cu addition enhanced the corrosion resistance in the solution state under the mild corrosion environment (simulated with 0.9% NaCl solution or PBS buffer, 37 °C), whereas the corrosion resistance was detrimental after aging treatment [213]. After solution treatment, dissolved Cu ions in the surface would form a protective  $\text{CuCl}_2$  film in the chloride media, and was beneficial to improvement in the corrosion resistance of 316L-Cu [213]. However, in order to obtain high antibacterial ability, the antibacterial stainless steel has to be aged to precipitate Cu-rich phase from the matrix. Many researchers insisted the

Cu-rich phase precipitated from a saturated amount of Cu in the steel matrix after the aging treatment formed galvanic corrosion with steel matrix and acted as a cathodic center, thereby accelerating the dissolution of the matrix [32,206,218]. The overall reaction was [32,221,239].



Cu-rich precipitates would destroy the uniform continuity and compactness of the passive film and act as the “weak points” on the steel surface which are susceptible to the local attack. As a result, the discontinuity of passive film caused by Cu-rich precipitates reduced the resistance to pitting corrosion and the ability of self-passivation and self-repairing [218,240]. Sun [32] reported that the addition of copper after aging treatment slightly decreased the pitting potential ( $E_{pit}$ ) of 317L, indicating the copper addition influenced the structure of the passivation film.

The aging time also has a certain effect on the corrosion resistance of the antibacterial stainless steel. Ren [241] reported that the radius of the capacitance arc in the Nyquist diagram of 304-3.9Cu was reduced gradually with the increase of the aging time, which indicates that formation of the passive film on surface of the steel was affected by the Cu precipitate in the steel, and thus the corrosion resistance of the steel was reduced to some extent. In another research, 304L-Cu possessed a higher intergranular corrosion tendency and a higher pitting corrosion rate with extended aging time [239].

The Cu content also has a great influence on corrosion performance of antibacterial stainless steels. It was reported that the increase of copper content in Fe–18Cr–Cu could increase the tendency toward chromium segregation at grain boundaries and thus the tendency toward intergranular corrosion of cast alloys [240]. In Xi's study [213], the corrosion resistance increased as the Cu content (0, 2.5%, 3.5%) increased for the solution-treated 316L, while opposed for the aged steel. In those higher Cu-content alloys that contain Cu-rich precipitates, the anticorrosion performance of the steel was greatly deteriorated, with the corrosion mechanism changing from pitting corrosion to selective corrosion [205]. Wang [221] reported the potentiodynamic polarization curve of Cr17Ni4–Cu in 3.5% NaCl solution shifted to the more negative direction, and  $E_{pit}$  and  $i_{pit}$  were more negative with increasing Cu content, which was believed to be related to Cu-rich phase formed after ageing treatment.

However, few researches indicated that the Cu had no adverse effects on the corrosion resistance of antibacterial stainless steels under the mild corrosion environment of 0.9% NaCl solution. The effect of trace Cu is similar to that of nickel, which will improve the corrosion resistance of weathering steel by forming a dense protective rust layer on the surface [119]. Nan [222] reported the passivity domains were very close between 200 and 1.45Cu with low copper content and 200–2.77Cu with

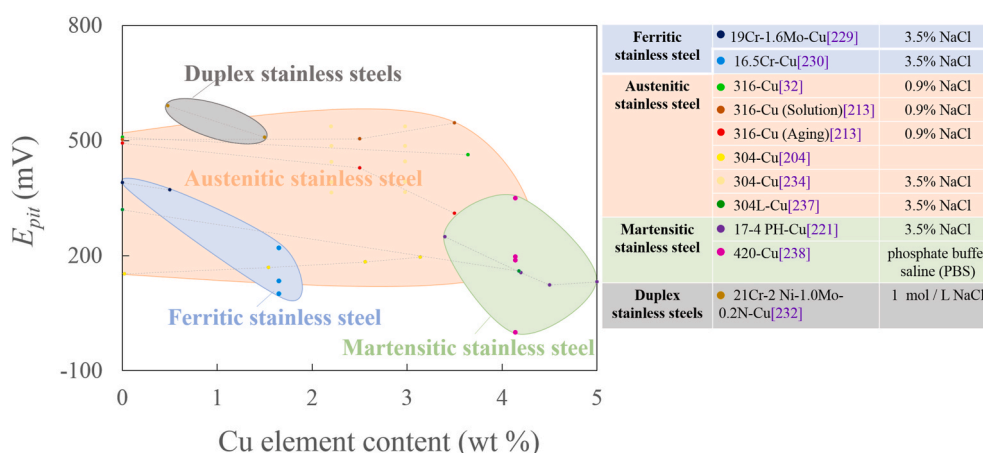


Fig. 11. Relationship between the Cu content and the pitting corrosion potential (data from Refs. [32,204,213,221,229,230,232,234,237,238]).

high copper content after aging treatment at 700 °C, indicating that addition of Cu in the 200 stainless steel did not have strong influence on the corrosion resistance.

Antibacterial stainless steels showed excellent corrosion behaviors in bacterial solution. The addition of Cu or Ag improves the pitting corrosion resistance of stainless steel [210,242,243]. It was suggested that Cu-rich precipitates will be changed to Cu<sup>2+</sup> partially due to the interaction between the materials and bacterium in the bacterial solution, which should reduce the conductivity of the surface of the steel, and result in a lower *i*<sub>corr</sub> of antibacterial stainless steels [207,243]. The presence of Cu in the 304L-Cu SS inhibited the pitting corrosion due to *E. coli* [242]. The pitting corrosion resistance of 2205-Cu was significantly better than that of 2205 in the presence of *P. aeruginosa* [243]. It was reported that Ag containing 304 (0.039Ag) restricted bacterial (*P. sp*) adhesion compared to the normal stainless steel, which reduced the pitting corrosion [210]. Yuan [244] reported the microbiological (*S. aureus*) corrosion resistance of 316L containing an appropriate amount silver was improved, but an excessive silver was prone to form segregation and degrade the corrosion resistance. In addition, La and Ce were believed to improve the anticorrosion performance of 316L in a medium containing sulfate reducing bacteria (*SRB*) [148].

#### 6.4. Biocompatibility

The metal ion release from antibacterial stainless steels plays a very important role in the antibacterial behavior. So far, the research results have demonstrated that Ag- or Cu-containing stainless steels have good biocompatibility *in vitro* similarly with traditional biomedical stainless steels, which offers a prerequisite for the *in vivo* (animal test) and provides an important judgement for the development and application of antibacterial stainless steels.

The results of cell compatibility of antibacterial stainless steel *in vitro* are summarized in Table 6, results clearly showed the cells viability of Ag- or Cu-containing antibacterial stainless steels was improved or not significantly different compared to stainless steels, indicating that Ag- or Cu-containing antibacterial stainless steels had no cytotoxicity to MG-63 cells [16,214], KB cells [16], rMSCs [211], UECs [215,245], SaOS-2 cells [246] and MC3T3-E1 cells [216]. Moreover, the apoptosis rates to MG-63 [16], KB cells [16], UECs [245], SaOS-2 [246] of Ag- or Cu-containing antibacterial stainless steel decreased or had no significant difference compared with the control stainless steel. It can be found 316L-Cu SS behaved the fast in cells migration, which was beneficial to the endothelialization [215]. In addition, Ren [246] reported 317L-Cu alloy could promote the osteogenic differentiation by stimulating the alkaline phosphatase enzyme activity and the osteogenic gene expressions (including Col1a1, OPN, and Runx2).

Compared with the conventional 316L, Cu-containing 316L was proved to inhibit the inflammation reaction caused by endothelial dysfunction through blockading the inflammatory factors (TNF- $\alpha$ , IL-1 $\beta$ , 6, 8) to reduce the in-stent restenosis after the stent implantation, which would reduce the recruitment and infiltration of leukocytes, rather than

have direct effect on leukocytes [247]. In addition, anti-fibrotic function of 316L-4.5Cu could reduce recurrence of ureteral stricture after stent implantation [248].

The continuous release of Cu<sup>2+</sup> ions from 316L-Cu stainless steel will contribute to the Cu/Zn superoxide dismutase viability, which steadily reduces toxicity and encrustation [245]. Some researchers [215,249] reported 316L-4.5Cu had satisfied dual functions with anti-encrustation and anti-infection.

#### 6.5. In vivo test

Antibacterial stainless steel has not been applied clinically, but 317L-4.5Cu and 316L-3.77Cu have been tested *in vivo*. The *in vivo* test results have demonstrated the good biocompatibility of antibacterial stainless steel, as listed in Table 7.

It was suggested from current *in vivo* results that Cu-containing ASS showed strong anti-infection and anti-inflammatory activity [212,214,246] as well as the ability of promoting osteogenesis [214,246,250] *in vivo*, as Fig. 12. Moreover, the push-out of Cu-containing ASS implant was doubled compared with that of 317L SS ( $p < 0.05$ ), suggesting Cu-containing ASS implant demonstrated superior bone-implant integration in animal model [246], as Fig. 12(f). Cu-containing stainless steel was also reported to promote fracture healing by accelerating the callus evolution process. However, the specific mechanism needed to be further explored [250]. Ren [246] further proposed Cu<sup>2+</sup> ions released from the Cu-containing stainless steel did have promotion effect on osteogenesis through three successive courses including osteoinduction, osteoconduction, and osseointegration, which are the most important factors to the osteogenic behavior for implants.

The excellent and unique antibacterial stainless steel is looking forward to being widely used in the biomedical application, such as orthopedics, dental and other hard tissue repair and replacement, cardiovascular stent intervention, ureteral stents, and manufacturing medical devices to ensure safety and hygiene. Another possible application of antibacterial stainless steel is surgical instruments, orthopedic furniture and outside surface of clinical equipment because of its excellent antibacterial activity against the biofilm, good biocompatibility with animals, and strong ability of corrosion resistance.

### 7. Antibacterial titanium alloys

Titanium and titanium alloy have been widely used in long-term implants, such as spine screw and spine fusion cage due to their good biocompatibility. In the long-term implantation, infection or inflammation will be a serious problem due to the good surface biocompatibility of titanium and titanium alloys, therefore, titanium alloy with antibacterial properties should have great potential. Here are some important turning points for the development of antibacterial titanium alloys, as illustrated in Fig. 13.

The first report on antibacterial titanium alloy was published in 2009 [17]. The results demonstrated that Ti-1Cu possessed excellent

**Table 6**  
The cell biocompatibility of antibacterial stainless steel.

Test	Alloy system	Year	Cell line	Method	Result (antibacterial stainless steel compared with stainless steel)	[Ref.]
Cell viability	317L-4.5Cu	2011	MG-63	MTT	significant cellular growth	[16,214]
		2015	SaOS-2	MTT	no significant difference	[246]
		2016	MC3T3-E1	real-time cell analysis (RTCA)	no significant difference	[216]
	304-3.9Cu 316-4.5Cu	2013	MG-63; KB	CCK-8	no significant difference	[16]
		2016	rMSC	MTT	no significant difference	[211]
		2016	UEC	MTT	no significant difference	[215,245]
Cell apoptosis	304-3.9Cu	2018	UEC	MTS	significant cellular growth	[215,245]
		2013	MG-63; KB	Annexin V-FITC/PI staining	no significant difference	[16]
	317L-4.5Cu	2015	SaOS-2	Annexin V-FITC/PI staining	significantly lower	[246]
		2018	UEC	Annexin V-FITC/PI staining	significantly lower	[215,245]
Cell differentiation cells migration	317L-4.5Cu	2015	SaOS-2	ALP	mildly higher	[246]
	316-4.5Cu	2016	UEC	wound healing assay	more cells migrated toward the wound	[215,245]

**Table 7**  
*In vivo* results of antibacterial stainless steel.

Year	Antibacterial stainless steel	Animal model	<i>In vivo</i> results	[Ref.]
2011	317L-4.5Cu	Japanese white rabbits	Strong antimicrobial activity, anti-inflammatory ability and strong promoting osteogenesis <i>in vivo</i> :  1) 317L-Cu screw tracts were negative for bacterial growth 2) The soft-tissues and bone connected the 317L-4.5Cu screws were observed without inflammation or gross infection. 3) 317L-Cu implanted screws showed strong osteoid while 317L screws showed weaker osteoid growth. 4) The expression of insulin-like growth factor-1 (IGF-1) in osteoblasts around 317L-4.5Cu screws tracts increased compared with 317L screws	[214]
2014	317L-4.5Cu	Sprague-Dawley rats	Strong anti-inflammatory ability, superior bone-implant integration and promoting osteogenesis <i>in vivo</i> :  1) No inflammation was found in the bone tissue post implantation compared with 317L. 2) The Cu-containing stainless steel implant demonstrated superior bone-implant integration. 3) Faster bone formation was found on the Cu-containing stainless steel samples as compared to the 317L SS.	[246]
2016	317L-4.5Cu	zebrafish embryos	No <i>in vivo</i> cytotoxicity:  1) The rate and time of hatching, mortality and touch response of the larval zebrafish were similar on 317L and 317L-Cu incubated samples. 2) There was no malformations on embryos and larval zebrafish incubated on the 317L-Cu SS.	[32]
2017	317L-4.5Cu	Sprague-Dawley rats	Promoting osteogenesis: 1) The callus evolution efficiency and the fracture healing speed were superior for 317L-Cu 2) Higher osteogenic efficiency at 6 weeks, and increased Runx2 expression were observed in the callus for 317L-Cu 3) The mechanical strength of the fracture site in the case of 317L-4.5 was much better than that of the control group	[250]
2020	316L-3.77Cu	Sprague-Dawley rats	Implant-related infection was also alleviated by 316L-Cu SS	[212]

osteoid-formation and no cell toxicity and thereby was promising to be a biomaterial [17]. The first report on the antibacterial properties of Ag-containing titanium alloys was published in 2012 [104]. Antibacterial Cu-containing and Ag-containing titanium alloys are presently two main alloy systems of antibacterial titanium alloys. During the past ten years, many great achievements have been obtained on the development of new alloy systems, the optimization of antibacterial activity and mechanical properties, cell biocompatibility *in vitro* and *in vivo* as well as antibacterial mechanism. Recently, antibacterial titanium alloy with low elastic modulus was reported, which exhibited strong antibacterial ability (>90% reduction) and low elastic modulus of 66 GPa, close to  $\beta$ - or near  $\beta$ -titanium alloy [256]. This alloy shows the potential ability to reduce the infection and “stress shield” for bone implant application.

Although the addition of copper or silver element provides with strong antibacterial activity against bacteria, the antibacterial titanium alloys are still inert to cells. Surface biomodification is still need to improve the surface cell adherence and proliferation even osseointegration. Surface acid etching (SAE) [74], sand blasting, sandblasting and acid etch (SLA) [75,76,104], anodization [259,260], micro-arc oxidation (MAO) [73] etc. have applied to antibacterial titanium alloy. So far, only several *in vivo* research reports have been published concerning the bone implant and dental implant. More *in vivo* researches should be carried out to provide more clinical evidences for the advantage of antibacterial titanium alloy instead of pure titanium and Ti-6Al-4V and promote the clinical application of antibacterial titanium alloy. Fig. 14 shows the research interests in the antibacterial titanium alloys.

### 7.1. Antibacterial properties

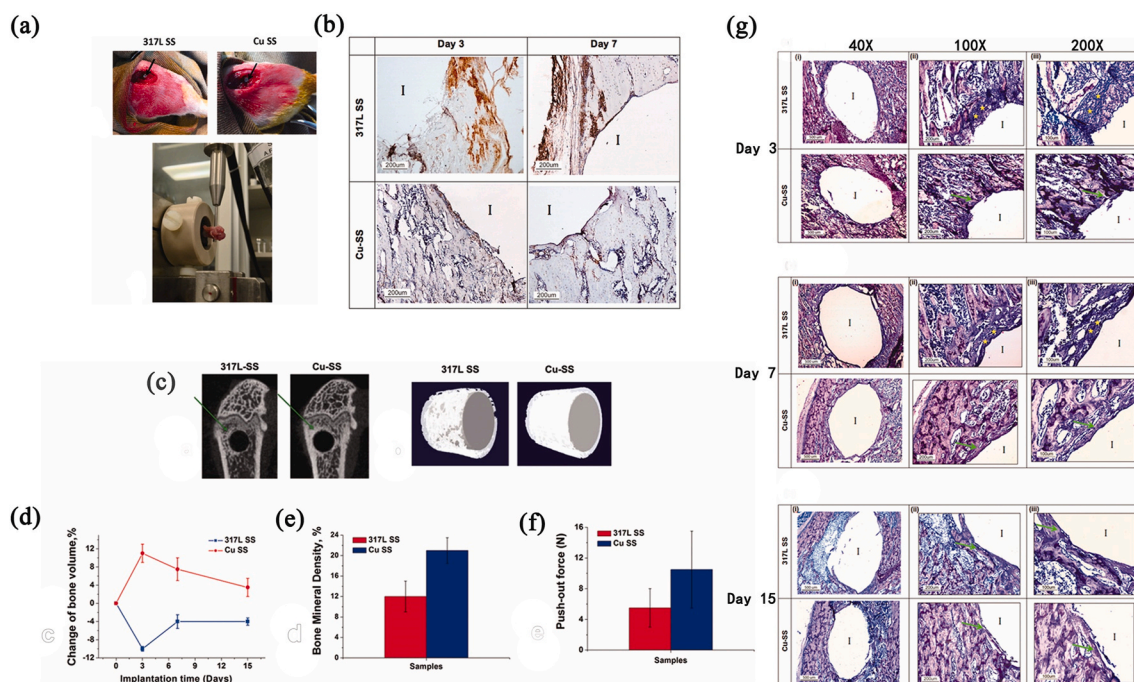
The research results of Cu-containing and Ag-containing antibacterial titanium alloys in recent years are listed in Table 8, suggesting the antibacterial titanium alloys exhibit excellent broad-spectrum antibacterial properties against lots of bacteria, such as *S. aureus*, *E. coli*, *P. gingivalis*, *S. epidermidis*, *S. mutans* as well as *S. sobrinus*. Antibacterial titanium alloy has different resistance to different bacteria. For example, *S. aureus* displayed markedly less adhesion on Ti-Cu antibacterial alloy compared with *E. coli* [17,254]. It was also noticed Ti-Cu sintered alloy showed certain anti-anaerobic bacteria activity, which is much less than their anti-aerobic bacteria abilities [194]. The antibacterial titanium alloy currently developed is mainly targeted at *E. coli* and *S. aureus* which are most common causes of implant-associated infections.

It is now widely accepted that Ag- or Cu-containing particles in Ti-Ag or Ti-Cu alloy control mainly the final antibacterial activity while Ag ion or Cu ion release also contributes the improvement in the antibacterial properties. According to this conclusion, any measure which could promote the formation or the precipitation of Cu- or Ag-containing compounds will enhance the antibacterial activity. Cu or Ag content influences the antibacterial property and high content normally results in a high antibacterial activity [105,194,262,267,273]. However, it is noted that no antibacterial property was not reported on the as-cast Ti-Cu alloys with up to 10 wt% Cu [252] or as-cast Ti-Ag alloys with up to 30 wt% Ag [268,269].

Although Ti-Ag and Ti-Cu are similar alloy system, but the solid solubilities of Cu and Ag element in  $\alpha$ -Ti are quite different. For example, the maximum solid solubility in  $\alpha$ -Ti is about 2.1 wt% of Cu at 790 °C and about 10 wt% of Ag at 855 °C, respectively. Recently study on Ti-Ag alloy demonstrated that nano-scale and homogeneously distributed Ti<sub>2</sub>Ag phases plays the key role in the antibacterial activity of Ti-Ag alloy [71]. Therefore, more Ag is need for Ti-Ag alloy to obtain Ti<sub>2</sub>Ag phase by ingot metallurgy and heat treatment. Some studies also demonstrated that certain surface treatments would further improve antibacterial properties of Ti-Ag alloy [74,259], in which one of the most representative technologies is acid etching, and the particles with high Ag contents are thought to play a key role in the antibacterial mechanism [74].

Heat treatment has great influence on the antibacterial properties of





**Fig. 12.** The *in vivo* results of copper-containing antibacterial stainless steel [246], copyright 2015, John Wiley and Sons, Inc. (Wiley Company). (a) Surgical procedures and set-up of the biomechanical push-out test. Black arrows show the implant positions (b) Immunohistochemical staining of TNF- $\alpha$  of bone tissue around the implants. Brown color represents positive staining and “I” the implant location. (c) Micro-CT images and Micro-CT 3D reconstruction models after 15 days of postoperation. (d, e) Bone volume, bone mineral density of the bones formed around 317L SS and Cu-SS implants. (f) Average maximum push-out forces of 317L SS and Cu-SS implants after 15 days of implantation obtained by biomechanical push-out test. (g) Histological photographs of Giemsa stained bone tissue formed around the implants.

Ti–Ag and Ti–Cu. Recently, more research results confirmed the influence of Ti<sub>2</sub>Cu phase or Ti<sub>2</sub>Ag phase on the antibacterial activity of Ti–Cu and Ti–Ag alloy, and found that high volume fraction of Ti<sub>2</sub>Cu phase (or Cu-rich phase) and Ti<sub>2</sub>Ag phase (or Ag-rich phase) normally results in a high antibacterial rate [71,105,252,264,266,268]. Heat treatment process can mainly change the existing form of Cu or Ag element, in turn affect the final antibacterial activity of titanium alloy. Cu or Ag element almost completely dissolved in matrix in an interstitial atoms condition after solution treatment followed by a fast cooling rate such as water cooling, thus the treatment had little improvement on antibacterial property of Ti–Cu or Ti–Ag alloy [252,268]. However, it was reported after solution treatment followed by a slow cooling rate such as air cooling or furnace cooling, the Ti–Cu alloy had antibacterial property against *E. coli*, *S. aureus*, *S. mutans* as well as *P. gingivalis*, which was because Ti<sub>2</sub>Cu precipitated from titanium matrix when the cooling rate was slow after solution treatment as Cu was a fast eutectoid element in Ti–Cu alloy [52,255,263]. The aging treatment significantly improved the antibacterial property due to large amount of nano-scale Ti<sub>2</sub>Cu or Ti<sub>2</sub>Ag precipitated from matrix [252,268]. Fortunately, it has been recognized Ti–Cu alloys and Ti–Ag alloys with sufficient Cu or Ag content had antibacterial property after heat treatment. The addition of 15 wt% Ag was enough for Ti–Ag alloy after solution treatment and aging treatment to have a much high antibacterial rate (>99.9% reduction against *S. aureus*) [268]. However, it is controversial whether Ti–3Cu alloy has antibacterial property in aging state. Hu [73] indicated Ti–3Cu alloy in the aging state showed no antibacterial activity against *S. aureus* with 60–80% antibacterial rate. But in previous researches, Ti–3Cu alloy in the aging state had antibacterial property against *S. aureus* (>90%) [193,264]. It has been now recognized that addition of 5 wt% Cu was enough for Ti–Cu alloy after proper heat treatment had excellent antibacterial property [52,252,255,263].

In powder metallurgy, Ag element mainly reacts with Ti to form Ti–Ag intermetallic compound and very little dissolve in Ti as interstitial atoms. Thus, it is possible to prepare antibacterial Ti–Ag alloy with low

Ag content by powder metallurgy. It was only required to contain 3 wt% Ag for Ti–Ag sintered alloys (10  $\mu$ m Ag powder) in order to obtain a strong and stable antibacterial property against *S. aureus* (98.2%), which was attributed the precipitation of a large amount of micro-scale Ti<sub>2</sub>Ag [105]. In addition, Chen [105] demonstrated the reduction in the Ag powder size contributed to the improvement in the antibacterial property, which was attributed mainly to the smaller particle size silver powder is easier to form fine and homogeneous Ti<sub>2</sub>Ag during sintering. Similarly, Cu content had to be  $\geq$  5 wt% for Ti–Cu sintered alloys in order to obtain strong and stable antibacterial property against *S. aureus* and *E. coli* due to the precipitation of a large amount of micro-scale Ti<sub>2</sub>Cu phase [252,262].

The antibacterial property of as-cast Ti6Al4V–Cu alloy has not been reported, and the heat treatment has an effect on the antibacterial property. The results indicated that appropriate solution treatment could make Ti6Al4V–Cu alloy have antibacterial properties [270,272]. Ma [270] reported Ti6Al4V–5Cu alloy solution treated at 810–1050  $^{\circ}$ C for 1 h and followed by water quenching had antibacterial activity against *S. aureus* (90.2%–98.6%), and the higher the solution temperature was and the stronger the antibacterial ability was. This probably because sterilized Cu ions was easier released from interstitial atoms compared with the large number of stable Ti<sub>2</sub>Cu phase precipitated during the aging process. Peng [272] investigated the Ti6Al4V5Cu treated at low solution temperature of 700  $^{\circ}$ C and 740  $^{\circ}$ C and followed by air quenching possessed good antibacterial property (95.4–97.5%), while treated at high solution temperature of 780–910  $^{\circ}$ C and followed by air quenching did not possess antibacterial ability (only 51.0%–77.2%). The authors speculated the larger globule Ti<sub>2</sub>Cu or bulk  $\beta$  phase in the alloy solution treated at low temperature, rather than the lathy Ti<sub>2</sub>Cu/ $\alpha$  lamellas in the alloy solution treated at high temperature, could efficiently inhibit the growth of bacteria that adhered on the surface of the alloy. In other researches [271,273], it was also reported Ti6Al4V–5Cu alloy displayed strong antibacterial ability (>99% against *S. aureus* and *E. coli*) after solid solution and subsequent aging treatment.

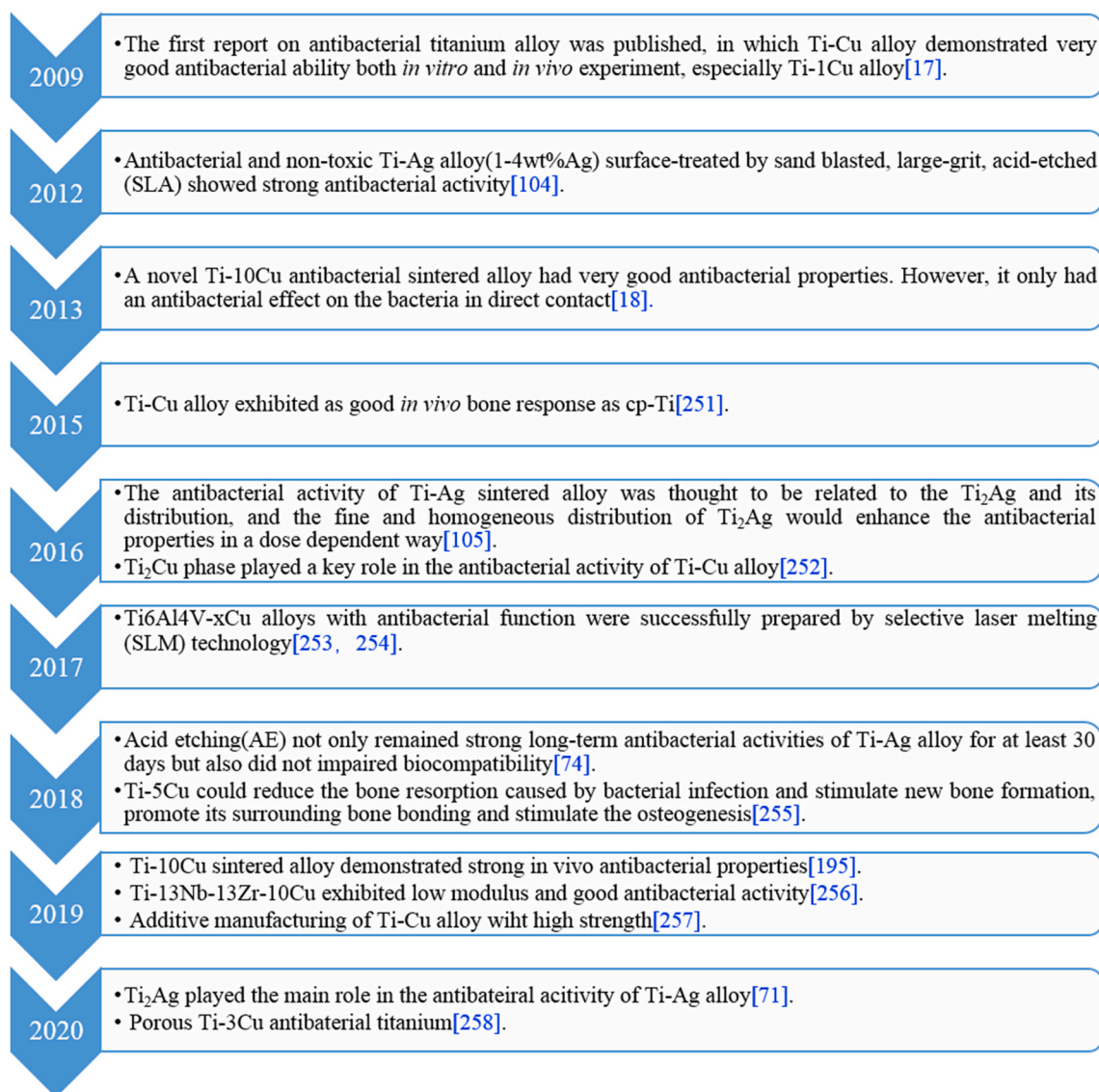


Fig. 13. Development flow chart of antibacterial titanium alloys [17,18,71,74,104,105,195,251–258].

Therefore, it was only required to contain 5 wt% Cu for Ti6Al4V–5Cu alloys after proper heat treatment to obtain a strong and stable antibacterial property.

Moreover, A series of antibacterial Ti6Al4V–Cu were successfully fabricated by selective laser melting (SLM) technology [253,254], which shows the possibility of Cu -containing antibacterial titanium by additive manufacturing. Research of Ti–6Al–4V–xCu prepared by SLM confirmed that the increase in Cu content enhanced the antibacterial activity and Cu content had to be 4 wt% or more in order to obtain high antibacterial activity against *E. coli* and *S. aureus* (>90%) [253]. However, in another research, Ti–6Al–4V–5Cu prepared by SLM had no antibacterial property (22% against *E. coli*), which might be due to the fact that only a small amount of  $Ti_2Cu$  was contained in the SLM materials manufactured in the study [274]. In addition, it appears that the addition of 0.5 wt% Ag is insufficient to provide antibacterial property (75% against *E. coli*) for SLM-titanium alloy [274].

Ag element was added to Ti–Nb alloy in order to obtain a titanium alloy with low modulus and antibacterial activity, and the antibacterial activity was attributed to nanoscale silver precipitates after solid solution and aging treatment [275,276]. It was reported that Ti–12Nb–1Ag alloy showed an antibacterial activity of 71.4% against *E. coli* [276]. But for Ti-27.5Nb alloy [275], the addition of 0.2–1.2 wt% Ag provided with

very strong antibacterial activity (>90% against both *S. aureus* and *E. coli*). With the increasing of Ag content, the antibacterial activity increased and reached at 100% at 1.2 wt% Ag.

## 7.2. Mechanical properties

The strength of cp-Ti is insufficient for certain biomedical application, such as dental implants and dental prostheses [277]. Element alloying with silver or copper can enhance the mechanical strength, hardness, and wear resistance, however, it is always accompanied with a reduction in ductility [262,272,277]. Ti–Ag and Ti–Cu alloy are both eutectoid alloy system, which means that both Ag and Cu elements have solid solution strengthening ability and precipitation/dispersion strengthening ability [252]. Thus, the addition of Cu or Ag and the precipitation of  $Ti_2Cu$  or  $Ti_2Ag$  will improve the hardness and strength but might reduce the ductility of titanium alloy [277]. The entanglement dislocations in the alloy matrix further explain the strengthening of strength and wear resistance [278]. Thus, developing antibacterial titanium alloy with comparatively high strength as well as satisfied ductility to simultaneously meet strength need and avoid brittle fracture in clinical application is always desired. Fig. 15 summaries the relationship between mechanical properties of antibacterial titanium alloys

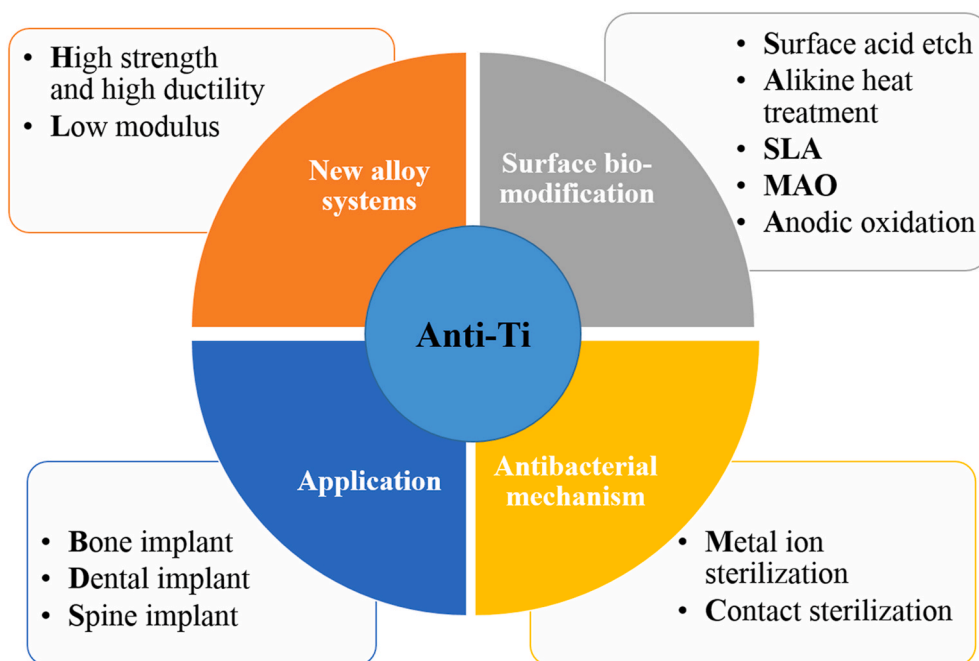


Fig. 14. Research activities in antibacterial titanium alloys.

Table 8

Cu-containing antibacterial titanium alloys.

Alloy system	Bacteria (antibacterial rate[Ref.])
Ti-xCu (x = 1–25 wt%, 57.03 wt%)	<i>S. aureus</i> (33%–100% [18,252,255,261–265], 100% after incubation for 12 h [258], No [17,193,195,262]); <i>E. coli</i> (49.3%–99.9% [18,255,261–263,265,266], 100% after incubation for 12 h [258], No [17,262]); <i>P. gingivalis</i> (54.4%–75.4% [194]); <i>S. epidermidis</i> (4%–27% at 6 h [267]); <i>S. mutans</i> , <i>P. gingivalis</i> [52]
Ti-xAg (x = 1–30 wt%)	<i>S. aureus</i> (3%–99.9% [71,74,105,268], 91%–98% after 30 days [259]); <i>S. mutans</i> , <i>S. sobrinus</i> [269]; <i>S. aureus</i> (51%–99.46% [6,253,254,270–272], No [273]); <i>E. coli</i> (22%–99% [253,254,274], No [273]); <i>E. coli</i> (75% [274]); <i>S. aureus</i> [256]
Ti-6Al-4V-xCu (x = 1–6 wt%)	<i>S. aureus</i> (51%–99.46% [6,253,254,270–272], No [273]); <i>E. coli</i> (22%–99% [253,254,274], No [273]); <i>E. coli</i> (75% [274]); <i>S. aureus</i> [256]
Ti-6Al-4V-0.5Ag	<i>S. aureus</i> [256]
Ti-13Nb-13Zr-10Cu	<i>S. aureus</i> [256]
Ti-Nb-xAg (x = 0.2–1.2 wt%)	<i>S. aureus</i> (92.66%–100% [275]); <i>E. coli</i> (71.4%–100% [275,276])

Note: 24 h incubation as default.

“No” means no antibacterial rate is shown in the literature.

and the Cu or Ag content.

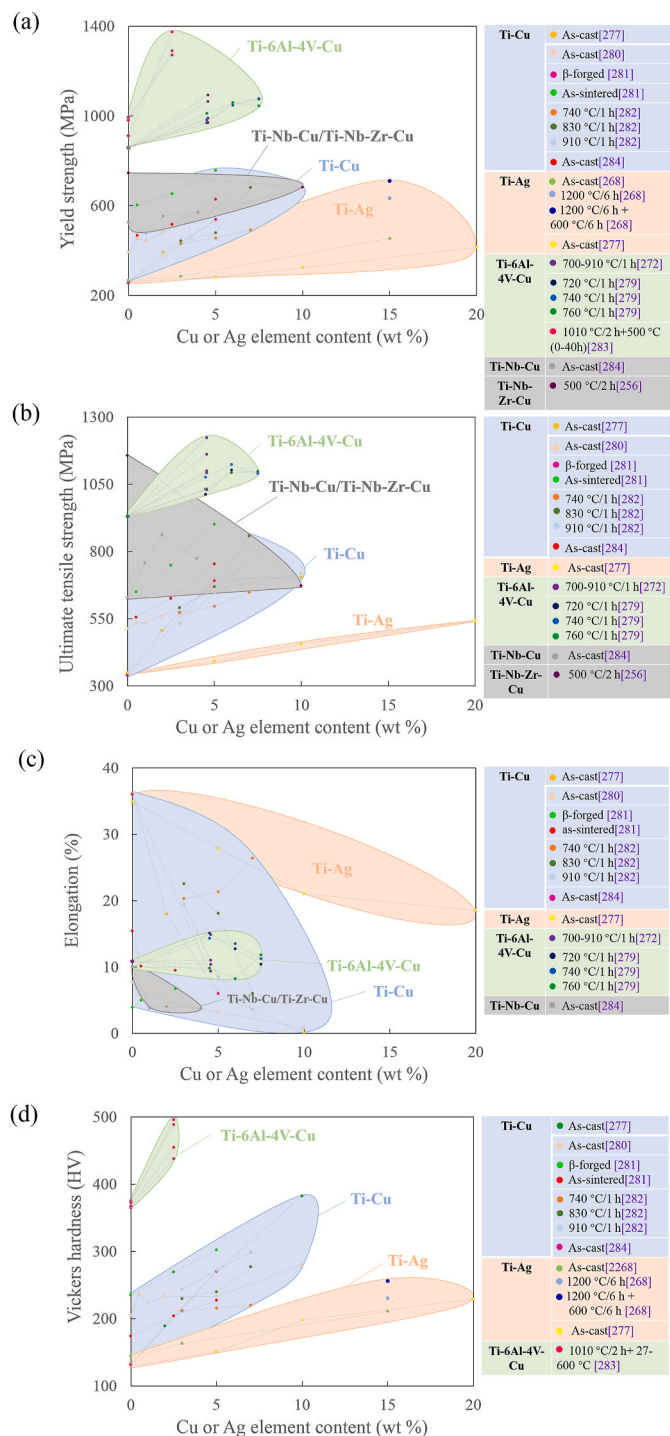
Alloying Cu or Ag element normally reinforce the as-cast cp-Ti matrix and enhance the hardness and the strength. The increasing in Cu or Ag content results in the improvement of strength and hardness of as-cast Ti-Cu alloy or as-cast Ti-Ag alloy due to the increasing in the volume fraction of Ti<sub>2</sub>Cu or Ti<sub>2</sub>Ag intermetallic particles while the ductility is opposite [252,277,285]. The hardness of as-cast Ti-Cu alloys are positively correlated with copper content (<10%) [252,277]. The tensile strength and hardness of as-cast Ti-Cu alloy increased with the increase of copper concentration. As the addition of copper increased from 5% to 10%, the tensile strength was basically unchanged, while the elongation declined from >6% to less than 1% [277]. It was suggested the addition of ≤20 wt% Ag improved the strength and hardness of as-cast titanium alloys while retaining relatively high elongation (≥19%) [277].

The heat treatment can improve the mechanical properties of Ti-Cu alloy and Ti-Ag alloy by changing the existence form and distribution of

alloying elements Ag or Cu. Both solution treatment and aging treatment enhance the strength and the hardness but deteriorate the ductility of Ti-Cu alloy and Ti-Ag alloy, displaying the strong solid solution strengthening ability of Cu (or Ag) element and the dispersion strengthening ability of nanoscale Ti<sub>2</sub>Cu (or Ti<sub>2</sub>Ag) [252,268,285]. Moreover, the strengthening increases with the increase of Cu (or Ag) content, which was attributed to the high Cu (or Ag) content resulted in a high volume fraction of Ti<sub>2</sub>Cu (or Ti<sub>2</sub>Ag) and high solid solution of Cu (or Ag) [252,268]. The proper heat treatment is very important for the mechanical properties of Ti-Cu and Ti-Ag alloys. For example, the strength and hardness of Ti-20Ag alloy solid solution treated at 1100 °C was greatly improved compared with that treated at 950 °C, even higher than 800Mpa, while the ductility was greatly reduced to less than 2%, indicating the alloy was not suitable for solution treatment at high temperature [285]. The tensile strength of Ti-3Cu alloy increased as the aging time increased from 16 h to 24 h, however, while hardly further increased when the aging time extended to 36 h, but the elongation had a greater decrease of 6.07%, which indicated too long aging time might make the grain coarsening and then reduced the properties [264].

Addition of 1% Cu into as-cast Ti-6Al-4V did not change the tensile strength and the elongation but further increasing of Cu decreased significantly the tensile strength and the elongation. Both elongation and tensile strength of the as-cast Ti-6Al-4V-Cu had negative correlation with copper content [286]. However, the mechanical properties of solution treated or aging treated Ti-6Al-4V-Cu were higher than that of similarly heat-treated Ti6Al4V alloy due to solid solution strengthening of Cu and the dispersion strengthening of Ti<sub>2</sub>Cu phase [272,283]. Hardness and tensile strength of Ti6Al4V-Cu alloy increased with increasing annealing temperature, but elongation was opposite [287]. Ti6Al4V-5Cu alloys showed good strength when treated at 700 °C–910 °C, while the elongation decreased from with the annealing temperature increased and only the alloys treated at 700 °C, 740 °C and 780 °C showed acceptable ductility [272]. Moreover, it was reported the hardness of Ti6Al4V-2.5Cu alloy increased with aging temperature up to 500 °C, indicating Ti<sub>2</sub>Cu was inadequate precipitation below 500 °C [283].

The metal preparation process and the metal forming process also have great influence on the mechanical properties. It is widely accepted Ti-Ag or Ti-Cu sintered alloy showed a much higher strength and



**Fig. 15.** Mechanical properties of antibacterial titanium alloys (data from Refs. [256,264,268,272,277,279–284]). a) yield strength, b) ultimate tensile strength, c) elongation, d) hardness.

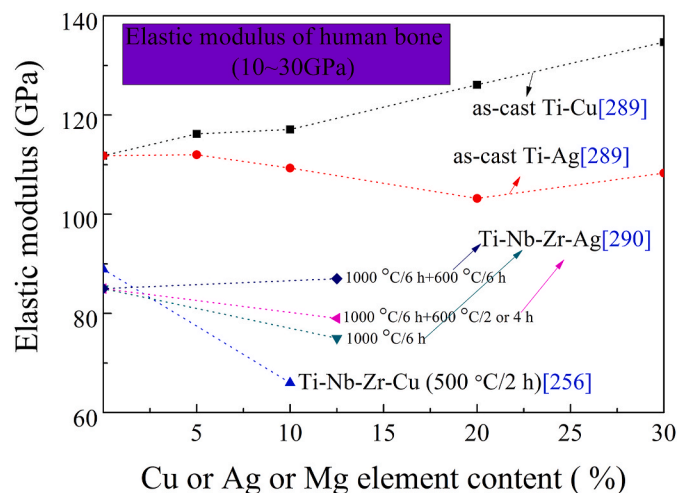
hardness than cp-Ti but greatly reduction in the ductility [18,105,262]. In antibacterial Ti–Ag (or Ti–Cu) sintered alloys, nearly all Ag (or Cu) element reacted with titanium to form  $Ti_2Ag$  (or  $Ti_2Cu$ ) phase during sintering process, and the high volume fraction of  $Ti_2Ag$  (or  $Ti_2Cu$ ) phase contributed to the high hardness and strength [262,268]. Recently, Ti-57.03Cu was prepared by a powder metallurgy in which large amount of TiCu and  $Ti_2Cu_3$  intermetallic phases with 23 nm grain size contributed to the ultra-high hardness of 10 GPa and acceptable toughness of  $8.14 MPam^{1/2}$ , which is much higher than other reported values in

titanium alloys [265]. Further research demonstrated that hot forming processing, such as the extrusion, refined the microstructure and densified the alloy, in turn improved the ductility and strength without reduction in antibacterial properties [288].

More recently, additive manufacturing technology has been used to prepare metal alloy with high mechanical properties. It was reported antibacterial Ti–6Al–4V–Cu alloy fabricated by selective laser melting led to a substantial increase of the ultimate tensile strength compared with Ti–6Al–4V alloy but led to a decrease in ductility [254,274]. Research on Ti–Cu alloy prepared by additive manufacturing demonstrated much high tensile strength of 1073 MPa and acceptable elongation of 5.5%, owing to the formation of an ultrafine eutectoid microstructure [257], showing the great potential application in antibacterial titanium alloy and related implants.

There are few reports on antibacterial titanium alloy with low elastic modulus, as shown in Fig. 16. Cu and Ag elements were added to Ti–Nb or Ti–Nb–Zr alloy in order to obtain a titanium alloy with low elastic modulus and antibacterial activity. It was reported that the addition of Nb and Cu produced a higher strength than Ti–Cu and Ti–Nb binary alloys due to the solid solution strengthening of two elements and the precipitation strength by the dual-phase, but the elongation of the ternary alloys was only about 4%, much lower than the binary alloy [284]. Ti–13Nb–13Zr–10Cu alloy exhibited slightly lower compressive strength and yield strength than those of Ti–13Nb–13Zr alloy, but significantly higher than those of cp-Ti. Moreover, the alloy had an enhanced antibacterial activity against *S. aureus* as well as a low elastic modulus of 66 GPa, which might reduce the bacteria infection and “stress shield” in bone implant [256]. Cai [290] designed a novel Ti–13Nb–13Zr–12.5Ag alloy in aging state with low elastic modulus of 79 GPa and strong antibacterial ability (>90% against *S. aureus*). But for Ti-27.5Nb alloy, the addition of 0.2–1.2 wt% Ag provided with very strong antibacterial activity (>90% against both *S. aureus* and *E. coli*). With the increasing of Ag content, the antibacterial activity increased but the microhardness decreased [275]. Ti–12Nb–1Ag alloy showed an antibacterial activity of 71.4% against *E. coli*. However, no modulus or strength was available in this report [276].

It was found that element alloying with silver or copper could improve the inherently poor grindability and wear resistance of titanium alloy due to the precipitation of eutectoid  $Ti_2Cu$  or  $Ti_2Ag$  phase [291–295]. Zhang [285] reported the wear resistance of Ti–Ag alloys increased with the increasing of Ag content (5–20% Ag). Kikuchi [291] indicated the grindability was significantly improved with an increase of the Cu content (0.5–10% Cu) in Ti–Cu alloy.



**Fig. 16.** Elastic modulus of Cu- or Ag-containing titanium alloys (data from Refs. [256,289,290]).

### 7.3. Corrosion properties

Good corrosion resistance is one of the most important reasons for titanium and titanium alloys to be applied as orthopedic and dental implants [296,297]. It is critical for antibacterial titanium alloys in biomedical application to judge whether the addition of antibacterial elements would affect the corrosion properties of titanium alloys in body environments. Fig. 17 summarizes the corrosion properties of reported antibacterial titanium alloys. The corrosion behavior of titanium alloys is mainly controlled by a galvanic corrosion between titanium matrix and secondary phase in matrix. Both Cu and Ag element have higher standard electrode potential than titanium element, thus Cu–Ti and Ag–Ti intermetallic compounds normally have a higher electrode potential than titanium matrix. As a result, Cu–Ti and Ag–Ti intermetallic compounds normally act as cathode and titanium matrix acts as anode during the galvanic corrosion.

Addition of Cu into titanium alloys to manufacture as-cast Ti–Cu alloy containing small amount of  $Ti_2Cu$  intermetallic compound would increase the strength and provide with strong antibacterial activity but do not facilitate the corrosion resistance due to galvanic corrosion [252, 299]. The corrosion resistance of as-cast Ti–Cu alloy decreased with increasing of Cu content (0–15 wt% Cu), which could be attributed to more  $Ti_2Cu$  phase leading to increasing of the galvanic corrosion effect [252,297]. However, it was reported that as-cast Ti–Ag alloys possessed higher corrosion resistance compared with cp-Ti [268,285,298]. In the as-cast Ti–Ag alloy, no or rare  $Ti_2Ag$  precipitated from matrix due to the large solubility of Ag in  $\alpha$ -Ti phase. Some researchers [298] attributed the enhancement in corrosion resistance to two reasons. Firstly, Ag is a nobler element than Ti thus can shift the corrosion potential of titanium alloys toward the noble direction. Secondly, corrosion will preferentially leach out Ti in preference to Ag, thus Ag atoms may accumulate on the surface of the Ti–Ag alloys and higher Ag content further increases the electrode potential of the alloys towards more positive values. But further increasing the Ag content to more than 20 wt%, the precipitated  $Ti_2Ag$  and/or TiAg intermetallic compounds preferentially dissolved in 0.9% NaCl solution, which deteriorated the corrosion resistance [300].

The heat treatment including solution treatment and aging treatment can improve the corrosion resistance of Ti–Ag and Ti–Cu antibacterial titanium alloy, especially aging treatment. After solution treatment,  $Ti_2Ag$  or  $Ti_2Cu$  phases were dissolved completely in matrix, which could decrease the galvanic corrosion effect and increase the general corrosion resistance [252]. After aging treatment, lots of nano-scale second phases precipitates from matrix and produce “envelopes effect”, which envelopes the Ti-rich (less noble) phase to prevent the alloy from contacting corrosive liquid and thus provide corrosion protection [301]. It was reported that solid solution state Ag/Cu and massively nano-sized  $Ti_2Ag/Ti_2Cu$  particles in Ti matrix would improve the corrosion resistance [264,268]. In another hand, nanoparticles dispersed on surface could enhance corrosion resistance due to that their small size and high surface area lead to greater barrier properties, small nano-particles sizes tend to enhance anti-corrosion properties [302]. The addition of Cu in Ti6Al4V alloy would not affect its corrosion resistance. Solution treated and aging treated Ti–6Al–4V–xCu (x = 1, 3 and 5%) possessed better corrosion resistance than commercial medical grade Ti–6Al–4V alloy [273]. Ti6Al4V–5Cu solution treated at 740 °C and 910 °C showed relatively lower  $i_{corr}$  and higher  $E_{corr}$  compared with Ti6Al4V alloy [272].

Metal alloys preparation processing also has influence on the corrosion properties. Ti–Ag and Ti–Cu alloy prepared by powder metallurgy exhibited better corrosion resistance than cp-Ti, and the corrosion resistance increased with increasing the addition of Ag content (1–5% Ag) or Cu content (2–25% Cu) [105,262]. However, the size, number, and morphology of pores would affect the corrosion behavior of sintered alloy [303]. For example, inhomogeneous distribution of  $Ti_2Ag$  phase in Ti–Ag sintered alloys prepared with Ag powder sized 10  $\mu m$  compared with 75  $\mu m$  resulted in more pores on the surfaces, accelerating corrosion rate [105]. However, in another research, the corrosion current density of Ti–57.03%Cu sintered alloy was about 3 times higher than that of cp-Ti [265]. Corrosion resistance of antibacterial Ti6Al4V–xCu (x = 0, 2, 4, 6 wt%) alloys fabricated by selective laser melting (SLM) technology were stronger than Cu-free alloy [253].

The metal ion release from antibacterial titanium alloy, such as Cu

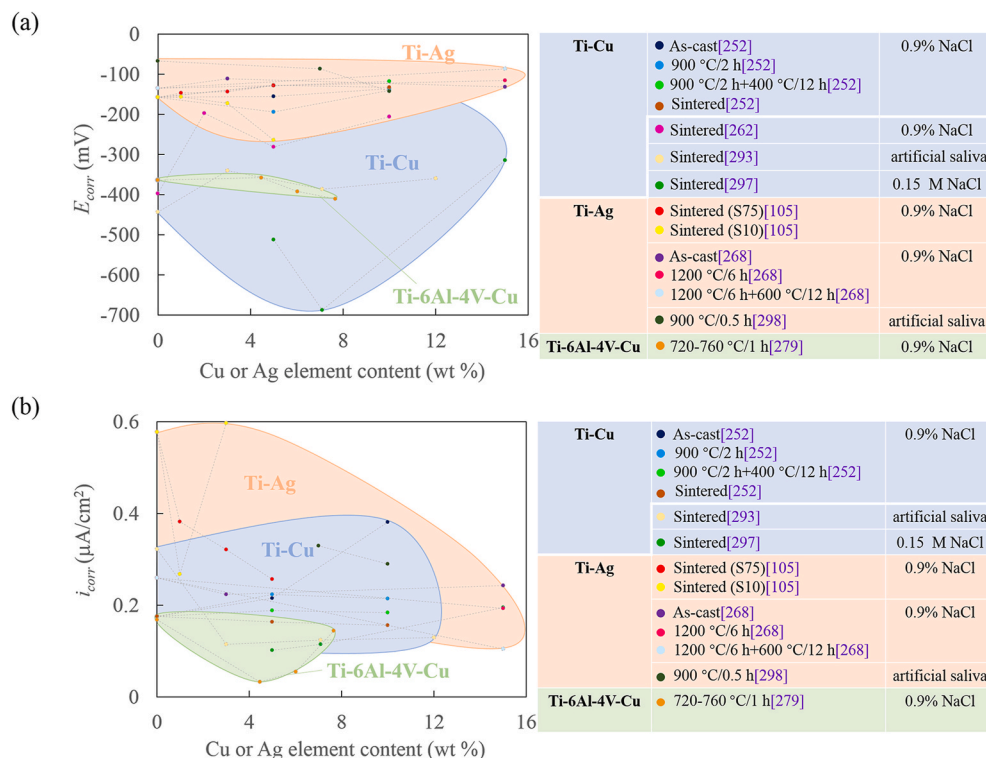


Fig. 17. Electrochemical properties of antibacterial titanium alloys(data from Refs. [105,252,262,268,279,293,297,298]). a)  $E_{corr}$  b)  $i_{corr}$ .

ion and Ag ion, affects the antibacterial activity but also influences the biocompatibility. *In vitro* results indicated that the release amount of Cu or Ag ions from antibacterial titanium alloy immersed in 0.9% NaCl solution were very low. It has been reported the Cu ion release of any antibacterial titanium alloys is much less than the recommended daily intake of Cu for an adult by WHO (2–3 mg). It was found that the existing forms of Cu element significantly affected the Cu ion release behavior in the following order from low to high: as-cast Ti–Cu < solution treated Ti–Cu < aging treated Ti–Cu < sintered Ti–Cu [252]. Ag or Cu ion release behavior is closely controlled by its alloying content and a high content normally corresponds to a high ion release [105,252,262]. It was also reported Ag ion concentrations from all Ti–Ag sintered alloys (1–3% Ag) after 24 h immersion were lower than 5 ppb, and Ag ion concentration release from Ti–5Ag sintered alloys was higher than that of Ti–1Ag and Ti–3Ag sintered alloys [105]. Zhang [252] reported high Cu concentrations from 7 ppb to 27.5 ppb were detected in Ti–5Cu alloys and Ti–10Cu alloys with different existing forms of Cu element in 24 h, and Cu ion concentration release from Ti–10Cu alloys was nearly 2 times higher than that of Ti–5Cu alloys. Moreover, the high release rate of Cu or Ag ions was only shown at the beginning of the immersion, which might be attributed to the formation of passive layer on the surface with the increasing immersion duration. Metal ion release rate decreases with the extension of the immersion duration. Zhang [18] reported Cu ion release concentration of sintered Ti–10Cu was 50 ppb after 72 h immersion. Liu [262] pointed out when the immersion time exceeded 72 h, the Cu ion release rate of the sintered Ti–Cu alloy decreased with the immersion time. Ma [304] reported Cu ion release rate of Ti–5Cu alloy in solution state was 8.3 ppb per day in the first 10 days but slowed down to 2.35 ppb per day from 10th to 30th day. Peng [272] pointed out that the total cumulative release of Cu ions from Ti6Al4V–5Cu alloys after 20 days immersion was only 3.108 ppb and 2.498 ppb, respectively. Ma [270] indicated Ti6Al4V–5Cu alloys after 90 days immersion released 244 ppb Cu ions.

In addition to 0.9% NaCl, the corrosion resistance of antibacterial titanium alloys in other solutions has also been widely studied. Varied fluoride-containing materials have been used in dental applications such as toothpaste and mouthwash to prevent dental caries [305,306]. However, fluoride ion is liable to react with titanium dioxide passivation film on the surface of titanium alloys, making the titanium potential more active and accelerating the corrosion rate [307,308]. Although artificial saliva solution containing fluoride ions significantly reduced the corrosion resistance of the Ti–Ag alloy, the corrosion resistance of the Ti–Ag alloy in as-cast and solid solution state was still higher than that of the pure titanium [285,298,309]. But it was reported Ti–Cu sintered alloy showed higher corrosion rate in artificial saliva containing  $F^-$  compared with cp-Ti and increased with the increase of Cu content (3–10% Cu) [310]. Lactic acid is a metabolite produced when anaerobic bacteria in the oral decompose glucose, which can reduce the pH of body fluid and may be harmful to corrosion resistance of metal alloy. Unlike the as-cast Ti–Ag alloy in the NaCl solution that  $Ti_2Ag$  was preferentially attacked, while in the lactic acid solution,  $Ti_2Ag$  was not preferentially dissolved, thus insoluble  $Ti_2Ag$  might reduce the speed of dissolution for the Ti–Ag alloys in the transpassive region [299,311]. Takahashi [299] reported that titanium with 20–25% Ag had excellent corrosion resistance in the lactic acid solution. After immersing in the lactic acid solution for 7 days, the amount of Ti ion release of the as-cast or the solution treated Ti–Ag alloy was significantly lower than that of cp-Ti, and the Ag ion was hardly released [285].

#### 7.4. Biocompatibility

A certain amount of Ag ions or Cu ions may cause cytotoxicity, therefore, research on cell compatibility is essential for silver-containing or copper-containing antibacterial titanium alloys as biomaterials. At present, the biocompatibility of antibacterial titanium alloys *in vitro* is mainly focused on Ti–Cu alloy and Ti–6Al–4V–Cu alloy and few reports

about Ti–Ag alloy.

The cells on the surfaces of Cu- or Ag-containing antibacterial titanium alloys exhibited a polygonal shape with a large number of filopodia and lamellipodia, indicating that the alloys exerted good cell spreading behavior [52,253,263,271]. Results from *in vitro* clearly showed antibacterial titanium alloys had no cytotoxicity (cell toxicity grade 0–1) to MG-63 cells [261,265,312], rBMSCs [52,253], L929 cells [273,285], MC3T3-E1 cells [73,263,270,271,313], V79 cells [17], NIH3T3 cells [285], as shown in Fig. 18.

Zhang [285] indicated addition of Ag element would not reduce the biocompatibility of titanium alloy. Ti–Ag alloys with 5–20% Ag, no matter in the as-cast condition or the solid solution condition, showed low cell toxicity to L929 and NIH3T3 cell lines (less than Grade 1). In Zhang's study [312], Ti–Cu sintered alloys showed no cytotoxicity to MG63 cells, even containing as high as 25% Cu, had no effect on cell proliferation and differentiation, including cell adhesion and spread, which indicating Ti–Cu sintered alloys had very good cell biocompatibility. Ma's research [270] indicated Ti–6Al–4V–5Cu alloy had heat treatment under different conditions performed non-cytotoxicity (>90%) but heat treatment affected the cytotoxicity of Ti–6Al–4V–5Cu antibacterial titanium alloy. Increasing the solution temperature up to 1050 °C could precipitate more  $\beta$  phases carried more interstitial Cu atoms leading to easy to release more Cu, which in turn compromised the viability of MC3T3-E1 cells on Ti–6Al–4V–5Cu alloy. However, the aging treatment after solution treatment further increased cell activity.

Current studies have shown that compared with cp-Ti, Ti–Cu alloy promotes or has no effect on cell differentiation. It was reported the AKP value of the Ti–10Cu sample at day 1 was significantly higher than the value of the cp-Ti value ( $p < 0.01$ ) [261]. While in another research [312], no difference in AKP value after incubation for 3 days or longer ( $p > 0.05$ ) was found between cp-Ti sample and Ti–Cu alloy (2–25 wt% Cu), indicating that even Cu content up to 25 wt% did not bring any influence on the differentiation of MG63.

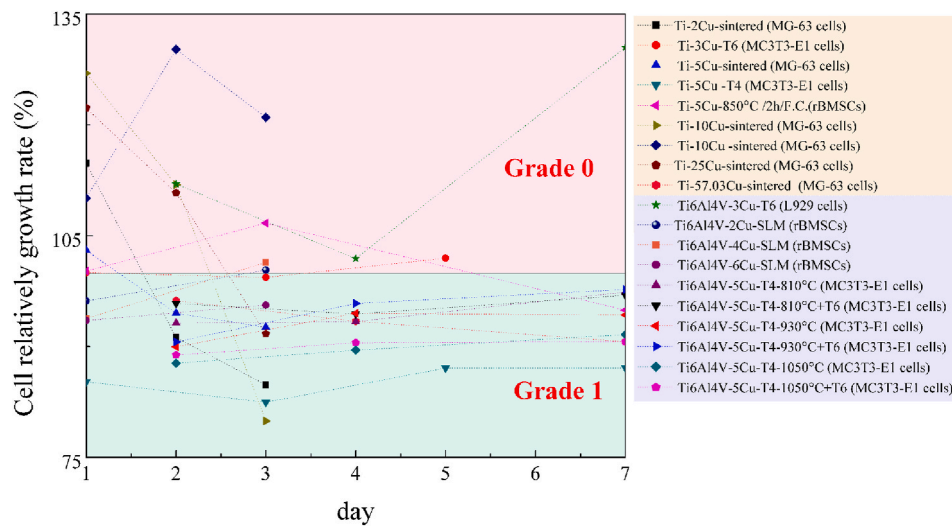
Moniri [265] discovered significant amounts of calcium and phosphorous deposited on Ti–Cu alloy compared to pure titanium, indicating that Ti–Cu alloy stimulated osteoblast formation and osseointegration due to the release of  $Cu^{2+}$  ions as well as to its nanostructural properties. Moreover, Ti–Cu [263] and Ti–Ag [285] alloy were reported to have good hemocompatibility with extremely low hemolysis rate (less than 1%) and less platelet adhesion.

#### 7.5. *In vivo* test

Only Ti–Cu alloy has been carried on *in vivo* experiments, as summarized in Table 9. *In vivo* results showed that Ti–Cu alloy had strong anti-infection and anti-inflammatory activity [17,195], as shown in Fig. 19(a–e). In addition, the osteoid formations on sintered Ti–10Cu alloy was as good as pure titanium [251]. In Liu's study, heat-treated Ti–5Cu alloy effectively reduced the bone resorption and stimulated the new bone formation compared with pure titanium, as shown in Fig. 19(f) [255]. In addition, it was also reported the postoperative blood copper level was not significantly different from preoperative, indicating Ti–Cu alloy mainly had local effects and could be safe for the whole body [17].

### 8. Antibacterial pure zinc and zinc alloys

Zn-based alloys have been turned focus towards an ideal degradation materials (such as cardiovascular stents) taking account of their moderate degradation rate in comparison with the fast biodegradation rate of Mg and Mg alloys and the low biodegradation rate of Fe-based materials due to different standard potentials of Mg, Zn and Fe (–2.372 V, –0.762 V and –0.440 V, respectively) [314]. However, the mechanical properties of Zn-based alloys are insufficient for most biomedical applications, and some antibacterial elements alloying may be effective to



**Fig. 18.** The cell viability of antibacterial titanium alloys (data from Refs. [52,73,253,261,263,265,270,273,285,312]). Note: The cell viability was expressed by a relatively growth rate (RGR). RGR = 75%–100% is cell toxicity grade 1 and RGR ≥ 100% is cell toxicity grade 0 (grade 0–1 means no cytotoxicity).

**Table 9**  
*In vivo* results of antibacterial Ti–Cu alloy.

Year	Ti–Cu alloy	Animal model	<i>In vivo</i> results	[Ref.]
2009	Ti–1Cu Ti–5Cu (No machining and treatment process)	Japanese white rabbits	1) Ti–1% Cu alloy significantly inhibited inflammation and infection. 2) The osteoid formations on Ti–1% Cu alloy was excellent as well as pure titanium, but was diminished in Ti–5% Cu alloy. 3) The postoperative blood copper level was not significantly different from preoperative.	[17]
2015	Ti–10Cu (Powder metallurgy)	New Zealand white rabbits	Ti–10Cu alloy had as good bone biocompatibility as cp-Ti, which brought no toxicity to the surrounding cell and tissue and no delayed to bone healing.	[251]
2018	Ti–5Cu (850 °C/ 2 h/furnace cooling)	Beagles	Ti–Cu implant not only effectively reduced the bone resorption but also stimulated the new bone formation compared to Ti.	[255]
2019	Ti–10Cu (Powder metallurgy)	New Zealand white rabbits	Ti–Cu alloy exhibited strong <i>in vivo</i> antibacterial properties while serious infection, inflammation and fester were observed in the cp-Ti.	[195]

solve the problem as well as improve the infection problem.

**8.1. Antibacterial properties**

Pure Zn did not show obvious antibacterial activity both in the agar diffusion plate test and in the plate method [314–316]. Tang [314] indicated the reason why pure Zn had no obvious antibacterial property was attributed to its high corrosion resistance in c-SBF solution and low release of Zn ions. The antibacterial rates of extruded Zn against both *E. coli* and *S. aureus* reached at 75.49% and 72.36%, respectively [316], and the inhibition zone for *S. aureus* of extruded Zn was less than 1 mm [314]. But in other studies, Zn showed strong antibacterial function as the antibacterial rate was over 98.1% [317] and inhibition zone was

about 6.19 mm [318]. Therefore, zinc alloys containing Cu, Mg or Ag etc. were designed to further improve the antibacterial property, as Table 10.

Addition of Cu significantly improved the antibacterial property of pure Zn and the antibacterial property increased gradually as the Cu content increased because Cu ions have stronger antibacterial ability than Zn ions and the addition of Cu accelerated the degradation rate of Zn and increased the Zn release concentration [314,316,318]. The satisfied antibacterial properties of Zn–Cu was obtained at ≥ 2 wt% Cu [314]. Another research also confirmed that Zn–4Cu could effectively inhibit bacteria adhesion and biofilm formation due to the antibacterial function of both Cu ion and Zn ion [322].

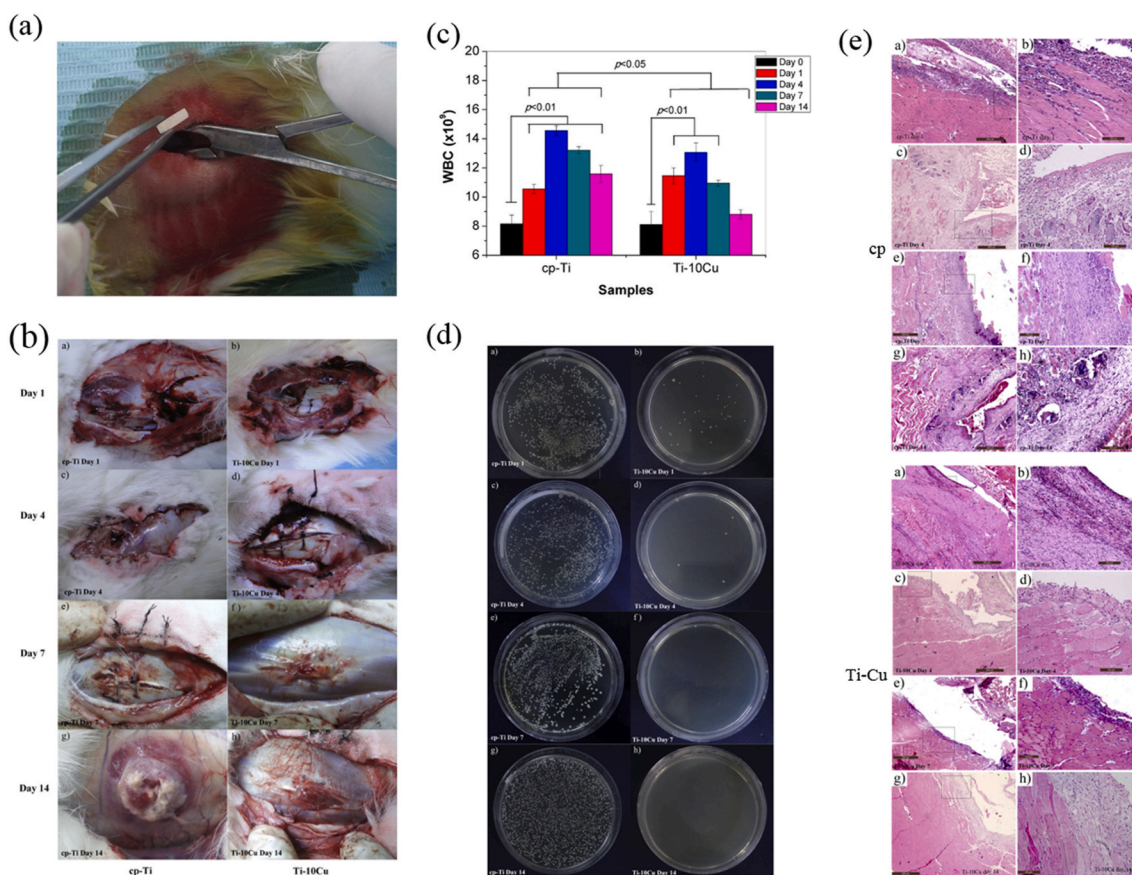
Alloying by Ag element can also enhance the antibacterial ability of Zn alloy in a content dependent way due to the strong and broad antibacterial function of Ag. Porous Zn–1Ag prepared by the air pressure infiltration method exhibited an antibacterial rate of 58.9% while porous Zn–3.5Ag was of nearly 100% antibacterial rate [315]. Zn–4Ag alloy was also reported to effectively inhibit bacterial adhesion and biofilm formation in comparison to Ti–6Al–4V alloy, demonstrating that adding Ag into pure zinc had a good antibacterial effect [323]. Zn-based with small amount of Cu or Ag (0.4%) also showed good antibacterial rate (>90%) against *E. coli* which was mainly due to the high Zn ion release [321].

Zn alloy with Mg as alloying element also exhibited good antibacterial activity. Zn–0.05 Mg alloy exhibited over 98.1% antibacterial function against both *E. coli* and *S. aureus* [317]. In another research, Zn–Al alloys with or without Mg both had antibacterial activity, while Zn–Al–Mg alloys indicated stronger inhibitive function against *E. coli* than Zn–0.5Al alloy [320].

The addition of Cu and Fe elements improved the antibacterial effect due to the high Cu ion and Zn ion release caused by fast degradation of Zn alloy. Zn–3Cu–0.2Fe and Zn–3Cu–0.5Fe alloys showed good antibacterial activity (>90% reduction) against both *E. coli* and *S. aureus* [316].

**8.2. Mechanical properties**

Zinc and zinc-based alloys are easier to cast and process due to their low melting points, low chemical reactivity and good machinability [324]. Similar to pure magnesium, the strength and ductility of pure Zn is poor, which could not meet the clinical requirements. The yield strength (YS) of pure Zn is from 10 MPa to 140 MPa, the ultimate tensile strength (UTS) is from 18 MPa to 180 MPa, the elongation is 0.32% to



**Fig. 19.** The *in vivo* results of copper-containing antibacterial titanium alloy. (a) Implantation of Ti-10Cu and cp-Ti implants with bacterial suspension and general observation of the implantation site after the implantation. (b) White blood cell (WBC) test results before and after implantation of Ti-10Cu and cp-Ti implants. (c) Typical photos of colonization by *S. aureus* taken from Ti-10Cu and cp-Ti implants which had been implanted with bacterial suspension in rabbit muscle. (d) Pathological photographs of the muscle surrounding the Ti-10Cu and cp-Ti implants. (e) Experimental design of Ti-5Cu and cp-Ti dental implants for *in vivo* animal tests and the radiographic analysis, Micro-CT, histological observations. (a–e): [195], copyright 2019, Elsevier. (f): [255], copyright 2018, Elsevier.

>60%, and the Vickers hardness is 25 HV to 44 HV [314,317,324–330], depending on the metal processing. Improvements in mechanical properties as well as antibacterial properties may be achieved by element alloying and appropriate thermomechanical treatment by principal solid solution and second-phase strengthening. Fig. 20 summarizes the mechanical properties of some zinc-based alloys.

Addition of Cu significantly improved the strength and the elongation [314]. Cu has a relatively high solid solubility of about 1.53 wt% in Zn alloys, thus CuZn<sub>5</sub> phase will precipitate after homogenized heat treatment when Cu content is higher than the critical [331]. CuZn<sub>5</sub> phase could also contribute to grain refinement during subsequent hot extrusion. Therefore, the strengthening mechanism of Cu-containing zinc alloy could be attributed to CuZn<sub>5</sub> precipitation hardening, as well as solid-solution strengthening of Cu element [318,322].

Interestingly, Mg has also been reported as an alloying element for Zn-based alloy [317,320,332,333]. The solid solubility of magnesium in zinc is low (0.15 wt%) and the Mg<sub>2</sub>Zn<sub>11</sub> intermetallic phase forms in the structure even at small amounts of magnesium [326], thus the mechanical properties of Mg-containing zinc alloys can considerably be improved by the presence of fine and hard Mg<sub>2</sub>Zn<sub>11</sub> intermetallic particles [329], but alloying with high magnesium concentrations would cause brittleness of such alloys due mainly to brittle eutectic phase [326]. It is proposed that optimal Mg concentration falls in between 0.01 wt% and 3 wt% [328]. Adding Mg element into Zn–Al alloy also improved the mechanical properties, which was attributed to that the presence of Mg<sub>2</sub>(Zn, Al)<sub>11</sub> phase impeded the grain boundary motion and grain growth of ternary Zn–Al–Mg alloy [320]. In addition, Niu [322] considered adding trace Mg in Zn–Cu alloy had the potential as a

biomaterial. Similar, adding the alloying elements such as Ca and Sr was also effective on the improvement of the mechanical property zinc alloys [321,330].

The metal preparation process and the metal forming process also have great influence on the mechanical properties. Extrusion, rolling, forging, annealing, etc. could improve its mechanical properties significantly [334–336]. It is widely accepted extrusion not only eliminates brittleness of the as-cast alloys but also significantly improves their strengths [318,321,328]. Extruded zinc alloy consisted of very refined Zn matrix, and the refined microstructure characteristics could be closely related to the high strength and ductility [317].

### 8.3. Corrosion properties

As a biodegradable biometallic alloy, Zn attracts much attention due to its moderate degradation rate in comparison with magnesium alloy and iron. However, it is still desired to accelerate the degradation rate of Zn to some extent. The corrosion rate of pure Zn in c-SBF solution was as low as  $22.1 \pm 4.7 \mu\text{m}/\text{year}$ , which is much lower compared with the reported Mg alloys and mainly be attributed to the higher standard potential of Zn compared with Mg-based alloys [314]. It was reported that the generated Zn(OH)<sub>2</sub> and other corrosion products like ZnCO<sub>3</sub> and Zn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> could form a compact surface layer, and thus restrained the degradation of Zn alloys [328]. Alloying elements have influence on the corrosion properties of Zn alloys dependent on the element and content. Table 11 lists the corrosion properties of some reported antibacterial zinc alloys.

Cu and Ag have high standard electrode potential than Zn. The



**Table 10**  
Antibacterial zinc and zinc alloys.

Alloy system	Bacteria (antibacterial rate[Ref.])
Pure Zn	<i>S. aureus</i> ( <sup>a</sup> 72.36%–>98.1% [316,317], <sup>b</sup> 0.0 ± 0.3 mm [314], <sup>b</sup> 6.19 ± 0.42 mm [318]); <i>E. coli</i> ( <sup>a</sup> 5%–>98.1% [315–317]); <i>Streptococcus gordonii</i> ( <sup>a</sup> about 64% for 12 h incubation [319]) Mixed oral bacteria ( <sup>a</sup> about 65% for 12 h incubation [319])
Zn-0.5Al	<i>E. coli</i> ( <sup>b</sup> 1.9 ± 0.1 mm [320])
Zn-0.8Mn	<i>E. coli</i> ( <sup>a</sup> 81.3% [321])
Zn-xCu (x = 1–4 wt%)	<i>S. aureus</i> ( <sup>a</sup> 81.86% [316], <sup>b</sup> 0.5 ± 0.4mm–3.5 ± 0.2 mm [314]; <sup>c</sup> [322]); <i>E. coli</i> ( <sup>a</sup> 88.77% [316])
Zn-xAg (x = 1–4 wt%)	<i>E. coli</i> ( <sup>a</sup> 58.9%–100% [315]); <i>S. gordonii</i> - <sup>d</sup> [323]
Zn-0.05 Mg	<i>E. coli</i> and <i>S. aureus</i> ( <sup>a</sup> > 98.1% against both bacteria [317])
Zn-0.8Mn-0.4Cu/Ag/Ca	<i>E. coli</i> ( <sup>a</sup> 93.7%, <sup>a</sup> 91.8%, <sup>a</sup> 84.5%, respectively [321])
Zn-xCu-yFe (x = 0.5, 3 wt%)	<i>S. aureus</i> ( <sup>a</sup> 90.17%–93.60% [316]); <i>E. coli</i> ( <sup>a</sup> 92.99%–96.46% [316]) <i>Streptococcus gordonii</i> ( <sup>a</sup> about 76% for 12 h incubation [319]) Mixed oral bacteria ( <sup>a</sup> about 84% for 12 h incubation [319])
Zn-1Cu-0.1Ti	<i>S. aureus</i> ( <sup>b</sup> 6.19 ± 0.42 mm–6.99 ± 0.33 mm [318])
Zn-0.5Al-xMg (x = 0.1–0.5 wt%)	<i>E. coli</i> ( <sup>b</sup> 3.3 ± 0.2mm–6.3 ± 0.2 mm [320])

Note: 24 h incubation as default.

<sup>a</sup> Measured by the plate count method.

<sup>b</sup> Measured by the Agar diffusion plate test.

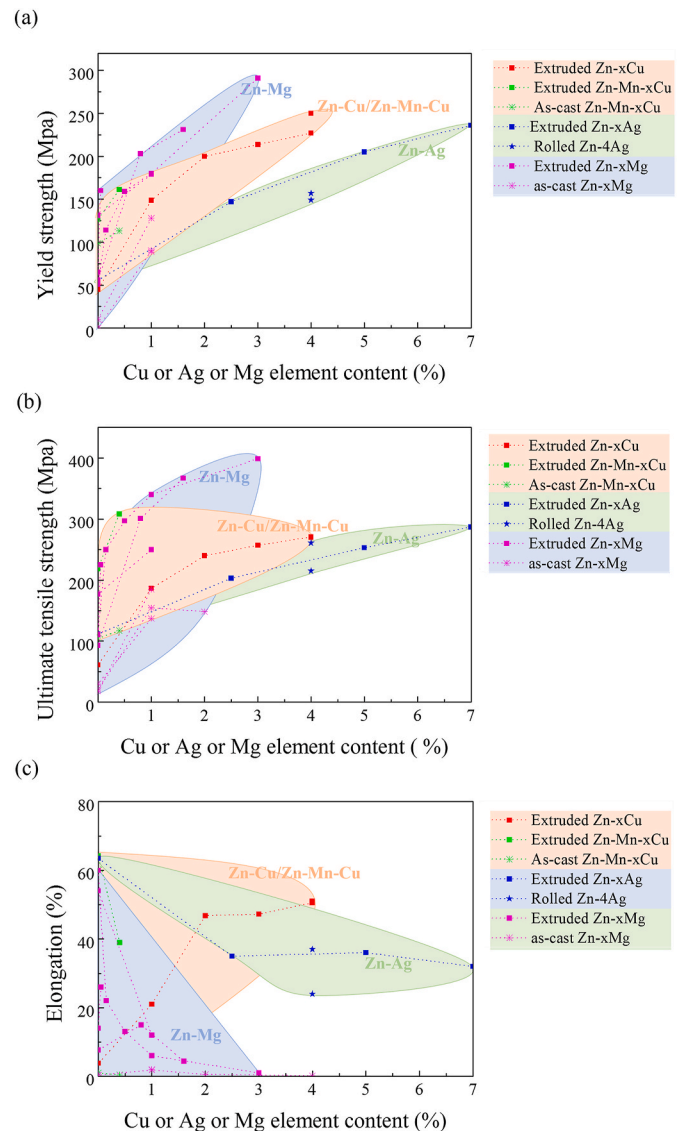
<sup>c</sup> Measured by Live/Dead staining.

<sup>d</sup> Measured by crystal violet staining.

intermetallic phase between Cu–Zn or Ag–Zn normally acts as cathodic and enhances the corrosion of zinc matrix. The degradation rates of antibacterial Zn–Cu alloys showed a little increase compared with pure Zn due to galvanic corrosion between CuZn<sub>2</sub> phase and matrix, but there was no significant difference among Zn–Cu alloys with different Cu contents, suggesting the standard potential of CuZn<sub>5</sub> phase might be close to that of Zn matrix, and thus no severe galvanic corrosion occurred. On the other hand, fine and uniform corrosion pits may also confirm that severe galvanic corrosion did not occur [314]. The addition of Ag element clearly decreased the corrosion resistance of Zn–Ag alloy, which might be related to the formation of AgZn<sub>3</sub> phase, inducing micro-galvanic corrosion, which finally leads to a decreased corrosion resistance of Zn–Ag alloys [315,323,325]. But in another research, addition of 0.4 wt% Cu/Ag/Ca accelerated corrosion of Zn-0.8Mn alloy, among which the effect of Ag was the most prominent [321].

It was reported the stable corrosion rate of Zn-0.05 Mg alloy immersed in the SBF slightly increased compared to that of Zn [317], but the addition of Mg had a positive effect on the corrosion resistance of Zn–Al alloys [320]. It was further reported that the corrosion resistance increased with increasing Mg content in Zn–Al–Mg alloys due to the homogeneously dispersed Mg<sub>2</sub>(Zn, Al)<sub>11</sub> secondary phase in the matrix, which leads to the formation of a uniform surface film of Mg-containing corrosion products, postpones the occurrence of localized corrosion and can effectively protect the Zn-based alloy [320].

Metal produce processing also significantly influences the corrosion rate of Zn alloy. In Lin's study [318], the as-cast Zn–Cu–Ti alloy showed the best corrosion resistance, and hot rolling deteriorated the corrosion resistance. It was thought that second phases of TiZn<sub>16</sub> and ε-CuZn<sub>5</sub> mainly distributed along the grain boundaries and formed a network in the matrix in the as-cast alloy and protected the alloy from fast corrosion. The hot-rolling broke the network and accelerated the corrosion by serious galvanic corrosion.



**Fig. 20.** Mechanical properties of antibacterial zinc alloy (data from Refs. [314, 317,321–323,325–330]). a) yield strength, b) ultimate tensile strength, c) elongation.

#### 8.4. Biocompatibility

The stimulation for osteogenesis by zinc ion has been approved by numerous researches [337,338]. Generally, low concentrations of Zn<sup>2+</sup> promoted viability, proliferation, adhesion, and migration, while high concentrations of Zn<sup>2+</sup> were just the opposite [339]. The current standard for cytotoxicity evaluation is not suitable for biodegradable metals, and a minimal 6 times to a maximal 10 times dilution of extracts to better simulate the *in vivo* environment were recommended for the *in vitro* cytotoxicity evaluation of biodegradable Mg-based materials [340]. The dilution of extracts may also be applicable for biodegradable Zn-based materials though further study is required [314,322].

100% extracts of pure Zn exhibited a low cell viability to the EA.hy926 [314], MC3T3-E1 [318], and MG-63 cells [318], however, when the extract was diluted to a certain concentration, it had no toxicity to cell viability. The concentration of released metal ions higher than the ion threshold for cell proliferation may have adverse effects on cells, therefore, it is desirable to maintain a low concentration of Zn<sup>2+</sup> in the local tissue by controlling the slow degradation rate of zinc alloy would be very crucial for the biological properties of antibacterial zinc alloy

**Table 11**  
The corrosion properties of antibacterial zinc alloys.

Alloys	Condition	Solution	$E_{corr}$ (V)	$i_{corr}$ ( $\mu\text{A}/\text{cm}^2$ )	Corrosion rate ( $\mu\text{m}/\text{year}$ )	Ref.
Zn–4Cu	as-cast+380 °C/9 h + extrusion	Hank's	-	4.1 ± 0.2	9.41 ± 1.34	[322]
Zn–1Cu	as-cast+360 °C/8 h + extrusion	c-SBF	-	-	33.0 ± 1.0	[314]
Zn–2Cu			-	-	about 27	
Zn–3Cu			-	-	about 30	
Zn–4Cu			-	-	about 25	
Zn-2.5Ag	as-cast+410 °C/6 h and 12 h/W.C.+extruded at 250 °C	Hanks' modified solution	-1.12 ± 0.01	9.2 ± 0.9	137 ± 21	[325]
Zn-5Ag			-1.12 ± 0.02	9.7 ± 0.7	144 ± 7	
Zn-7Ag			-1.14 ± 0.04	9.9 ± 0.6	147 ± 18	
Zn-0.5Al	as-cast	SBF	-1.074 ± 0.012	20.4 ± 1.3	150 ± 10	[320]
Zn-0.5Al-0.1 Mg			-1.065 ± 0.013	17.3 ± 1.1	130 ± 10	
Zn-0.5Al-0.3 Mg			-1.034 ± 0.011	11.2 ± 0.7		
Zn-0.5Al-0.5 Mg			-1.018 ± 0.012	9.5 ± 0.3	110 ± 10	
Zn-0.05 Mg	N.A	SBF	-	-	728	[317]
Zn-0.8Mn	As-cast	SBF	-1.070 ± 0.02	6.76 ± 0.35	101 ± 9	[321]
Zn-0.8Mn-0.4Ag			-1.190 ± 0.01	11.22 ± 0.79	168 ± 21	
Zn-0.8Mn-0.4Cu			-1.180 ± 0.01	8.91 ± 0.67	133 ± 15	
Zn-0.8Mn-0.4Ca			-1.160 ± 0.01	10.72 ± 1.28	160 ± 19	
Zn-1Cu-0.1Ti	As-cast	Hanks'	-1.025 ± 0.264	21.5 ± 0.4	315 ± 6	[318]
Zn-1Cu-0.1Ti	Hot-rolling		-1.123 ± 0.185	111.2 ± 0.9	1628 ± 13	
Zn-1Cu-0.1Ti	Hot-rolling + cold rolling		-1.100 ± 0.201	67.7 ± 0.5	991 ± 7	

[318]. It is now recognized that adding appropriate amount of Ag, Cu and Mg to zinc showed good cytocompatibility, as shown in Table 12.

Research indicated addition of Ag into pure Zn would not lead to potential cytotoxicity, because the metabolic activities and the proliferation activities of L929 cells and Saos-2 cells always reached 100% when the extract of Zn–4Ag was diluted to only less than 33% and 16.7%, respectively [323].

In the study [314,322], the EA. hy926 cells showed good viability

cultured in 50% and 10% extracts of Zn-xCu alloys (cell viability higher than 90%), indicating good cytocompatibility of Zn-xCu alloys. Pure Zn with Cu or Cu plus Fe could facilitate the growth of endothelial cells and promote the completion of endothelial cell function and inhibit the adhesion growth of smooth muscle cells [316]. Another research indicated the extract of Zn–1Cu-0.1Ti antibacterial alloy at a concentration  $\leq 25\%$  showed no significant cytotoxicity to MC3T3-E1 and MG-63 cells [318].

**Table 12**  
The cell viability of antibacterial pure zinc and zinc alloy.

Alloy system	Metal or alloy	Cell	Cell viability (Cell culturing time)	[Ref.]
Pure Zn	Zn	EA.hy926	100% extract: cytotoxicity (1, 3, 5 days); 50% and 10% extracts: >90% (1, 3, 5 days)	[314]
	Zn	MC3T3-E1	100% extract: < 55% (1, 3, 5 days); 25% extract: 64.48% (1 day); 12.5% extract: > 90% (1, 3, 5 days)	[318]
		MG-63	100% extract: < 55% (1, 3, 5 days); 25% extract: 87.30% (1 day); 12.5% extract: > 90% (1, 3, 5 days)	[318]
Ag-containing	Zn–4Ag	L929	100% extract: < 40% (1 day); 33.3% extract: approximately 40% (1 day); 16.7% and 10% extracts: approached 100% (1 day)	[323]
		Saos-2	100% extract: < 40% (1 day); 33.3%, 16.7% and 10% extracts: approached 100% (1 day)	[323]
Cu-containing	Zn-xCu (x = 1–4 wt%)	EA.hy926	100% extract: cytotoxicity (1, 3, 5 days); 50% and 10% extracts: >90% (1, 3, 5 days)	[314]
		Zn–4Cu	100% extract: only reaches 10–20% (1, 3, 5 days); 50% and 10% extracts: approaching 100% (1, 3, 5 days)	[322]
	Zn–3Cu–Fe	EA.hy926	100% extract: < 75% (3 days); 75% extract: 75–90% (3 days); 50% and 10% extracts: > 100% (3 days)	[316]
	A7r5	75% and 100% extracts: < 75% (3 days); 50% extract: >75% (3 days); 10% extract: > 100% (3 days)	[316]	
	Zn–1Cu-0.1Ti	MC3T3-E1	100% extract: < 55% (1, 3, 5 days); 25% extract: 82.74% (1 day); 12.5% extract: > 90% (1, 3, 5 days)	[318]
Mg-containing	Zn-0.8 Mg	U-2 OS	100% extract: < 55% (1, 3, 5 days); 25% extract: 65.45% (1 day); 12.5% extract: > 90% (1, 3, 5 days)	[318]
		L929	75% and 100% extracts: < 20% (1 days); 50% extract: >80% (1 days); 10% extract: > approaching 100% (1 days)	[327]
	Zn-0.5Al–Mg (0.1–0.5 wt%)	MC3T3-E1	50%, 75% and 100% extracts: < 20% (1 days); 25% extract: > 100% (1 days) extraction medium: 102–108% (7 days)	[327]
				[320]

The 50% extract of Zn-0.8 Mg alloy exhibited acceptable cell viability U-2 OS cells (cell viability higher than 80%), while it needed to be diluted to 25% could make L929 cells viability reach 100% [327]. Addition of Mg to Zn-0.5Al alloy could improve the cells viability of MC3T3-E1 cells from  $101 \pm 1\%$  to  $108 \pm 1\%$  and the cells viability increase with increasing Mg content, possibly because Mg contributed to decreasing in the dissolution rate of  $Zn^{2+}$  and pH values in physiological environments [320].

In addition, zinc alloy exhibit lower hemolysis rates, less platelet adhesion, and adherent platelets remain ellipsoidal morphology without irreversible transformation, and the addition of Cu or Cu plus Fe element has no adverse effect on the hemocompatibility of pure Zn [316].

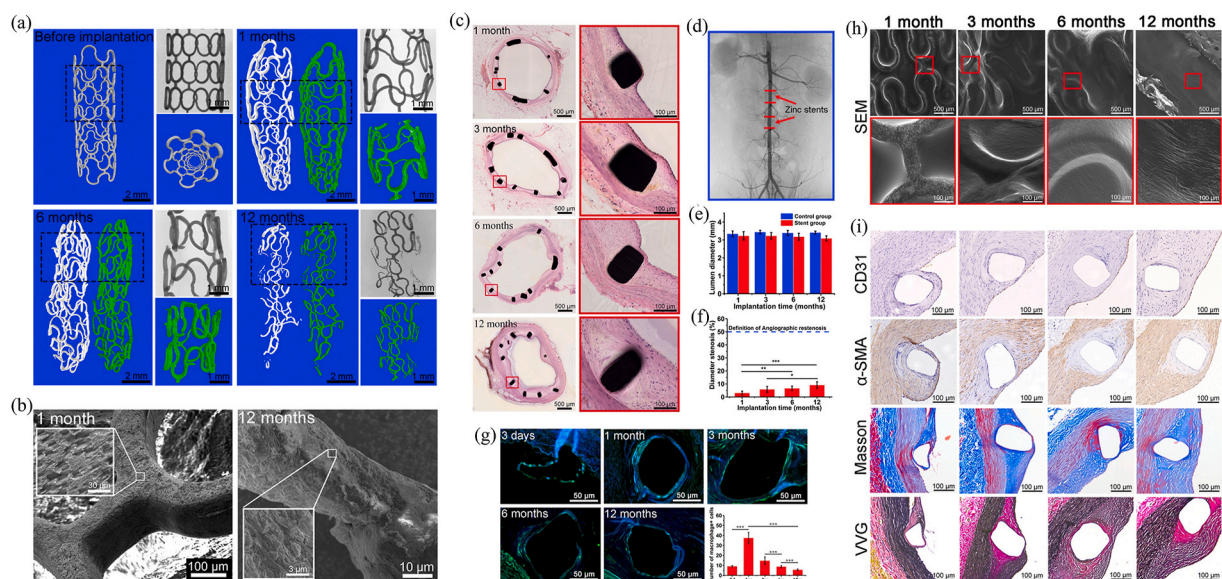
### 8.5. *In vivo* test

So far, limited data on *in vivo* experiments of antibacterial pure zinc and zinc alloy was reported, as summarized in Table 13. The results indicated that Zn and Zn-0.05 Mg alloy as vascular stent and bone implant performed well *in vivo* antibacterial activity and compatibility.

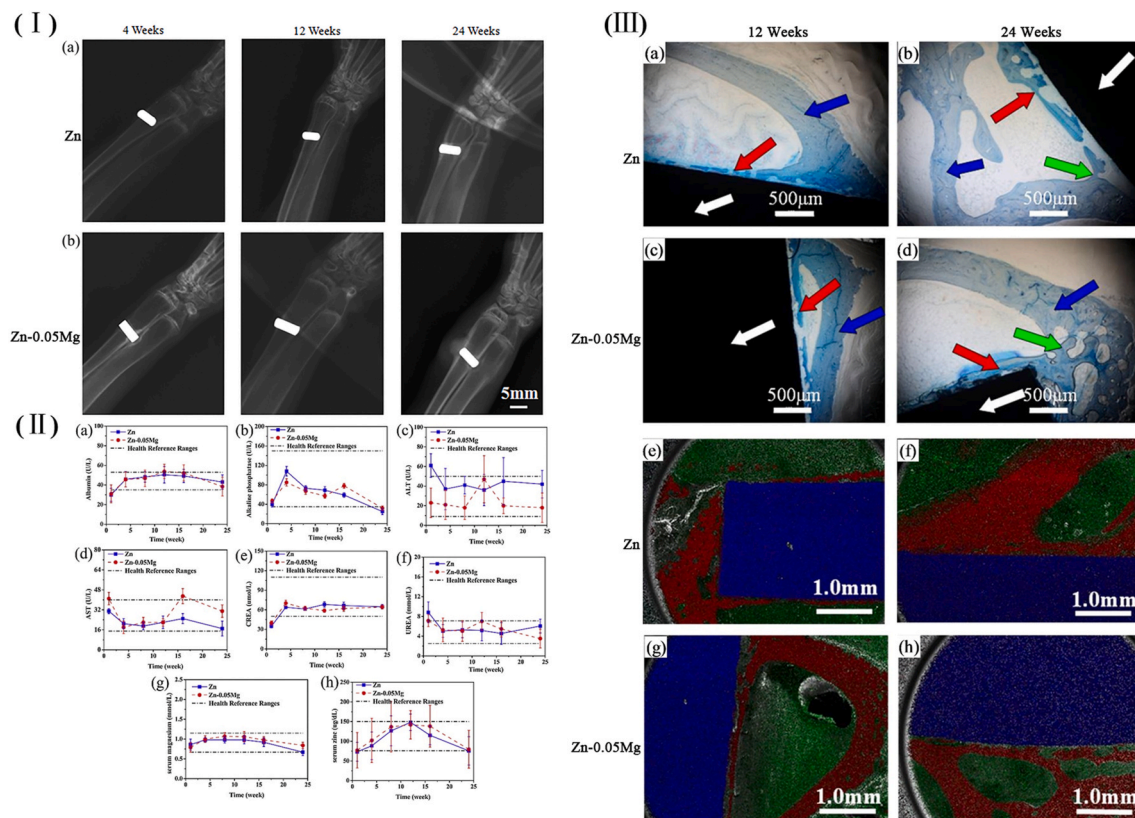
**Table 13**

*In vivo* results of antibacterial pure zinc and zinc alloy.

Year	Pure Zn and Zn alloy	Animal model (position)	Application	<i>In vivo</i> results	[Ref.]
2015	Zn	Sprague Dawley rats (abdominal aorta)	Endovascular stent	Zn did not promote restenosis responses and might suppress the activities of inflammatory and smooth muscle cells	[341]
2017	Zn	Japanese rabbits (abdominal aorta)	Endovascular stent	No severe inflammation, platelet aggregation, thrombosis formation or obvious intimal hyperplasia was observed after 12 months post implantation (as Fig. 21).	[342]
2018	Zn and Zn-0.05 Mg	Sprague Dawley rats (bilateral tibial shaft)	load-bearing implant	Zn alloy could promote the formation of new bone tissue at the interface between the implant material and bone tissue <i>in vivo</i> , and the degradation had no harm to the function and histology of important organs (as Fig. 22).	[317]



**Fig. 21.** The *in vivo* results of pure zinc as endovascular stent [342], copyright 2017, Elsevier Ltd. (a) Selected 2D and 3D micro-CT images of zinc stents after 0, 1, 6 and 12 months implantation. Each time point is composed of three images: the left one is a 3D reconstruction of the zinc stent, among which the white one is the residue zinc stent and the green one represents corrosion products. (b) Corrosion morphologies of zinc stents after 1 and 12 months *in vivo*. (c) Representative low- and high-magnification photomicrographs of hematoxylin-eosin (H&E) stained sections of abdominal aorta after 1, 3, 6, and 12 months implantation of zinc stents. The asterisk marked region at 12 months indicates the neointima without stent struts left. (d) Angiography of rabbit abdominal aorta after 12 months implantation of zinc stents. The stented segments are marked by red arrows. (e) Lumen diameter and (f) diameter stenosis of stented segments after 1, 3, 6 and 12 months implantation calculated from quantitative vessel angiography (QVA) measurements. (g) Representative immunofluorescence staining images of macrophage antibody after 3 days, 1, 3, 6 and 12 months implantation and the number of macrophages per strut at each time point. (h) Representative low- and high-magnification SEM images of endothelial coverage on zinc stents. (i) Immunohistochemical staining images of CD31 antibody and  $\alpha$ -SMA antibody, Masson's trichrome and Verhoeff-Van Gieson (VVG) staining images.



**Fig. 22.** The *in vivo* results of Zn and Zn-0.05 Mg alloy as bone implant [317], copyright 2018, Elsevier Ltd. (I) Plain anteroposterior radiographs of pure Zn and Zn-0.05 Mg pins implanted in femoral shafts of rabbits. (II) Blood biochemical indicators: (a) Albumin (b) alkaline phosphatase (c) ALT (d) AST and (e) CREA (f) UREA (g) serum magnesium (h) serum zinc. The black dot lines in (a)–(h) represent the health reference ranges. (III) (a)–(d): Representative histology of cross-sections of bone-implant interface stained with Toluidine blue and observed by microscopy. There are normal cortical bones (blue arrow); implant (white arrow); newly formed bone fractions around the implant (red arrow); bone junction between cortical bone and new bone formation (green arrow). (e)–(h): element mapping analysis on a cross-section of bone/implant interface. Concentration of Zn (blue area); concentration of C (green area), medullary cavity regions and surrounding tissue; concentration of Ca (red area). Implants with a high Zn concentration, the newly formed bone fractions around the implants showing enriched Ca.

### 9.1. Antibacterial properties

Mg showed an antibacterial activity that was comparable to a fluoroquinolone antibiotic against *E. coli*, *P. aeruginosa* and *S. aureus* [345]. A significant amount of researches also indicated Al-containing magnesium alloys such as AZ31, AZ91 and Re-containing magnesium alloys such as Mg-4Y showed strong antibacterial activity [346–348]. It was widely accepted that the antibacterial ability was mainly caused by the pH raise with the degradation of Mg metal in the incubation process [174,345,346].

However, the antibacterial effect of Mg might be insufficient in a dynamic environment like human body, because the degradation rate of pure Mg and magnesium alloys *in vivo* is not as fast as the rate *in vitro* and a high pH cannot be maintained to achieve long-term inhibition to bacteria [349,350]. In addition, some studies have shown that corrosion products formed a deposition layer on the surface of magnesium, which provides a favorable place for bacterial adhesion. Therefore, the antibacterial activity *in vivo* will be significantly reduced [351]. *In vivo* mouse model also indicated magnesium was effective against bacteria during *in vitro* assays or during surgery, but bacteria thrived in the presence of magnesium after implantation [352]. Therefore, magnesium alloys containing Ag, Zn or Cu etc. were designed as implant materials to combine the favorable properties of magnesium with the well-known antibacterial property of antibacterial elements [87,353,354]. Table 14 summaries some reported antibacterial pure magnesium and magnesium alloys in recent years.

Mg-Cu alloys possessed excellent and prolonged antibacterial effects against *E. coli* because of the high pH value as well as sustain release of

Cu ion (50–100 ppb after 24 h and 100–200 ppb after 72 h [358]. Addition of Cu above 0.1 wt% could efficiently enhance the antibacterial activity against *C. albicans* (almost 100% reduction) [353] as well as *S. aureus* [362]. Similar, the extruded Mg-Zn-Y-Nd-xCu alloy with at least 0.1 wt% Cu could ensure the alloy had an antibacterial rate of more than 99% against *E. coli* and *S. aureus* [366]. Li [72] further confirmed the *in vivo* antibacterial activity of Mg-Cu alloys in a rabbit model of chronic tibial osteomyelitis and measurement with imaging examination, microbiological cultures.

Mg-Ag alloys (2–6% Ag) at solution treatment demonstrated higher antibacterial activity than pure Mg [87]. In addition, extrusion treatment without solution treatment would optimize antibacterial properties of Mg-Ag alloys [360]. Zhan et al. [363] found that the implantation of new magnesium alloy materials, Mg-3Zn, Mg-3Zn-1Ag and Mg-3Zn-3Ag, showed obvious antibacterial effect on *S. aureus* or *E. coli* infection, and the addition of silver in magnesium alloy could enhance its antibacterial ability. Li [365] also proved that the antibacterial rate of Mg-Zn-Y-Nd-xAg alloy increased with the increase of Ag content, while Mg-Zn-Y-Nd alloy did not have antibacterial property.

Mg-Nd-Zn-Zr displayed a similar but higher antibacterial activity than pure magnesium, which was caused by the combination or synergy of increased pH value with the degradation and the antibacterial function of both Zn and Zr element [355]. In another research, Zn-containing magnesium alloys Mg-1Ca-0.5Sr-xZn ( $x = 0, 2, 4, 6$ ) contained at least 4% Zn to insure high antibacterial property (>96.6% reduction) and the good antibacterial property of the alloy was attributed to the antibacterial efficiency of both Sr ion and Zn ion [354].

In addition, it was also pointed out that the addition of trace Ga and

**Table 14**  
Antibacterial pure Mg and Mg based alloys.

Alloy system	Bacteria (antibacterial rate)
Pure Mg	<i>E. coli</i> (>99.9% after 6 h [346], planktonic bacteria: 58.3% [355]/59.9% [356] and on the surface: 59.4% [355]/70.3% [356], <5% [72], No [345,347]); <i>S. aureus</i> (>99.9% after 6 h [346], 80% after 12 h and 100% after 24 h [357], 100% after 72 h in the environment of normal pH values and no antibacterial effect in the neutral environment [358], planktonic bacteria: 50.3% [355]/34.5% [356] and on the surface: 51% [355]/60.7% [356], both <i>in vitro</i> and <i>in vivo</i> [351], No [345,352]); <i>P. aeruginosa</i> (both <i>in vitro</i> and <i>in vivo</i> [352], No [345]); <i>MRSA</i> (planktonic bacteria: 68.9% and on the surface: 62.8% [350], <i>in vivo</i> [72,350], <10% [72], No [359]); <i>C. albicans</i> (>98% [346], about 90% [353]) <i>S. epidermidis</i> (planktonic bacteria: 50.8% [355]/37.7% [356] and on the surface: 49.8% [355]/56.8% [356], <5% [72]) <i>S. aureus</i> : <i>S. epidermidis</i> = 1:1 (49.7% after 15 h [360])
AZ31	<i>S. aureus</i> (>99.9% after 6 h [346]); <i>E. coli</i> (>99.9% after 6 h [346], No [347])
AZ91	<i>A. baumannii</i> (>99.9% after 18 h <i>in vitro</i> , <i>in vivo</i> [361]);
Mg-4Y	<i>E. coli</i> [347]
Mg-Ga	<i>E. coli</i> (planktonic bacteria: 51.8%; on the surface: 67.8%) [356]; <i>S. aureus</i> (planktonic bacteria: 48.3%; on the surface: 63.0%) [356]; <i>S. epidermidis</i> (planktonic bacteria: 46.5%; on the surface: 63.9%) [356]
Mg-xCu	<i>S. aureus</i> (100% [362], 100% after 12 h [353], 100% after 72 h both in the normal pH values and neutral environment [358]); <i>E. coli</i> (about 33%–>90%), <i>S. epidermidis</i> , and <i>MRSA</i> (>90%) [72]; <i>C. albicans</i> (>99.9% [353])
Mg-xAg	<i>S. aureus</i> : <i>S. epidermidis</i> = 1:1 (72.5%–83.5% after 15 h [87], 81.4%–>95% after 15 h [360]);
Mg-3Zn	<i>E. coli</i> and <i>S. aureus</i> [363,364]
Mg-xSr	<i>E. coli</i> (planktonic bacteria: 48.0% and on the surface: 63.5% [356]); <i>S. aureus</i> (80% after 12 h and 100% after 24 h [357], planktonic bacteria: 29.1% and on the surface: 59.1% [356]) <i>S. epidermidis</i> (planktonic bacteria: 35.6% and on the surface: 58.7% [356])
Mg-3Zn-xAg	<i>E. coli</i> and <i>S. aureus</i> [363,364]
Mg-2Zn-0.5Ca	<i>MRSA</i> [359]
Mg-4Zn-1Sr	<i>MRSA</i> [359]
Mg-Ca-Sr	<i>E. coli</i> (planktonic bacteria: 53.1%, on the surface: 66.5% [356]); <i>S. aureus</i> (>90% [354], planktonic bacteria: 40.9%, on the surface: 64.0% [356]); <i>S. epidermidis</i> (planktonic bacteria: 44.9%; on the surface: 68.0%) [356]
Mg-1Ca-0.5Sr-xZn	<i>S. aureus</i> (76.9%–>96.6%) [354]
Mg-Nd-Zn-Zr	<i>E. coli</i> (planktonic bacteria: 59.4%–83.1%; on the surface: 74.8%–94.8%) [355]; <i>S. aureus</i> (planktonic bacteria: 51%–79.1%; on the surface: 74.2%–88.4%) [355]; <i>S. epidermidis</i> (planktonic bacteria: 49.8%–79.8%; on the surface: 69.5%–89.5%) [355]
Mg-Zn-Y-Nd-xAg	<i>E. coli</i> and <i>S. aureus</i> (both >99.9% [365], No [19])
Mg-Zn-Y-Nd-xCu	<i>E. coli</i> and <i>S. aureus</i> (both >99.9%) [366]
Mg-Ca-Mn-Zn-xAg	<i>E. coli</i> and <i>S. aureus</i> [367]

Note: 24 h incubation on the surface as default.

“No” means no antibacterial rate is shown in the literature.

Sr (0.1 wt%) could improve the antibacterial activity of pure Mg [356]. Mg-0.1Ga, Mg-0.1Sr and Mg-0.1Ga-0.1Sr alloy induced a less pronounced increase in pH but stronger toxicity to stains than pure Mg, as such the latter could be attributed to the release of Ga<sup>3+</sup> and Sr<sup>2+</sup> ions [356].

## 9.2. Mechanical and corrosion properties

Pure magnesium is extremely susceptible to corrosion, leading to

losses of strength and toughness, which limits their biomedical application. While element alloying is an effective measurement to improve both the corrosion resistance and mechanical properties of magnesium [368]. The most common biomedical magnesium alloys contain aluminum and/or rare earth (RE) elements and it is known that Al and Re additions could improve the mechanical properties and corrosion resistance of Mg alloys [163].

As-cast Mg–Ag alloys with 2%–6% Ag continuously enhanced mechanical properties including hardness and strength meanwhile continuously reduced the corrosion resistance against DMEM and DMEM with FBS, which was attributed to the increase of Mg<sub>4</sub>Ag phases and dendrite structure [369]. In Tie’s another study [87], heat treatment, including solution treatment and aging treatment, could significantly improve both mechanical properties and corrosion resistance of the as-cast Ti–Ag, and the heat-treated Mg–Ag alloys showed a significant better mechanical properties and lower corrosion rate than pure magnesium. Feng [19] suggested both the increase of Ag content and extrusion process improved the microhardness of magnesium alloys. Moreover, the degradation rate could be reduced by eliminating the precipitates in Mg–Ag alloys via solid solution treatment after extrusion process [360]. Li [365] found that a new phase, Ag<sub>51</sub>Nd<sub>14</sub>, was formed in the as-cast Ag-containing Mg–Zn–Y–Nd alloy. Due to the potential difference between the magnesium matrix and Ag<sub>51</sub>Nd<sub>14</sub>, a primary cell was formed between the magnesium matrix and Ag<sub>51</sub>Nd<sub>14</sub>, which increased the corrosion rate of the alloy. At the same time, the microhardness, tensile strength and compressive strength all showed an increasing tendency. Another study has shown addition of Ag (0.5–3% Ag) improved the microhardness of Mg–3Zn alloys, while only addition of 0.5% Ag improved the compressive strength and elongation of Mg–3Zn, and the addition of Ag content was not conducive to the corrosion performance [364].

The addition of Cu into as-cast magnesium alloy forming Mg<sub>2</sub>Cu intermetallic phases increased the hardness and strength while significantly accelerated the degradation rate of magnesium in the physiological environment due to galvanic corrosion between Mg matrix and Mg<sub>2</sub>Cu intermetallic phase [72,358]. Chen [353] indicated that finely and continuously distributed second phase could more effectively retard the corrosion when the content of Cu was low, while large and thick Mg<sub>2</sub>Cu intermetallic formed along the grain boundaries accelerated the corrosion when the content of Cu was high. For instance, the corrosion rate of Mg-0.3Cu alloy was almost 10 times faster than that of Mg-0.1Cu alloy. Yan [362] found that the solution treatment after extrusion of low Cu-containing magnesium alloy eliminated the second phase and reduced the grain size significantly by recrystallization.

Zn is known to be a good solid solution and precipitation strengthening agent in Mg alloys and thereby to be one of the most commonly used alloying elements in Mg [370,371]. In addition, zinc is able to elevate the corrosion potential of magnesium alloys, and thus reduce the corrosion rate [372]. Cai [368] indicated the tensile properties and corrosion properties of Mg–Zn alloys strongly depended on the volume fraction and existence form of secondary phases. Solution treatment enhanced corrosion resistance while aging treatment decreased the corrosion resistance [373]. In Qin’s study [355], element alloying with Zn, Zr and Nd dramatically improved the corrosion resistance of pure Mg and the novel degradable as-cast Mg–Nd–Zn–Zr alloy has been proven to possess proper mechanical properties and enhanced corrosion resistance. Brar [163] reported that the increasing of Zn content (2–6% Zn) increased the corrosion rate of aging-treated Mg–Zn-0.5Sr.

Furthermore, adding a small amount of Sr and Ca could improve the mechanical properties of pure magnesium or magnesium alloy by the grain refinement mechanism, while decreased the corrosion resistance to generate a more rapid degradation, potentially because more second phase precipitates enhanced micro-galvanic corrosion [163,374]. But Berglund [375] further found that low amounts of alloying elements enhanced the corrosion resistance properties of as-cast Mg-xCa-ySr system (x = 0.5–7.0 wt%, y = 0.5–3.5 wt %), with an optimal

composition of 1% Ca and 0.5% Sr.

### 9.3. Biocompatibility

Good bone tissue response was observed around pure Mg implant in comparison with cp-Ti implant, such as high bone mineral content (BMC) and bone mineral density (BMD) and low porosity [351]. On the other hand, the raise of pH due to magnesium alloy degradation caused hemolysis and cell toxicity [376]. Zhen [376] indicated when the pH value was higher than 10.3, the hemolysis was higher than 5% (the recommended value by standard), and when the pH value was 10.72, the hemolysis was as high as 47.77%. Zhen's results [376] also confirmed that severe death of L929 cell line was induced when pH raised over 9. It was reported the cytotoxicity of Mg was ascribed to high alkalinity and  $Mg^{2+}$  concentration caused by its high degradation rate, thereby adversely affecting cell adhesion and growth [376]. It is unrealistic for pure magnesium to rely on a high pH to achieve antibacterial effect, even if without cytotoxicity, because high osmolality or magnesium ion concentration causes osmotic shock in human cells [377], while alloying of antibacterial elements can improve the biocompatibility of magnesium without compromising antimicrobial properties.

It was reported human primary osteoblasts could survive on the surface of extruded Mg–6Ag alloy and solution-treated Mg–8Ag alloy but died on extruded Mg–8Ag alloy (without solution treatment), indicating that addition of proper Ag content and solution treatment improved the cytocompatibility of magnesium alloy [360]. In another research [87], Mg–Ag alloys after solution treatment were non-cytotoxic to MG-63 and RAW 264.7 (survival rates between 95% and nearly 100%) by a long-term cytotoxicity and cell adhesion test over 14 days, and lower release silver ion concentrations than LC50 (lethal concentration LC50, where 50% of the cells survive) also verified this point of view. Bakhsheshi-Rad [367] confirmed that Mg–Ca–Mn–Zn–xAg with 0–0.5 wt% Ag was more favorable for the cell viability and cell adhesion of MG-63 cells than that with 1–2.0 wt% Ag.

The release of bioactive Mg and Cu ions into the biological environment during the degradation of the as-cast Mg–Cu alloys could induce osteogenesis and stimulate angiogenesis without cytotoxicity towards HUVECs and MC3T3-E1 cells [358]. In the report [72], the

as-cast Mg–Cu alloys were non-cytotoxic towards Balb/c 3T3 cells compared with cp-Ti. Similarly, the cytotoxicity towards rBMSC cells of Mg-0.1Cu alloy is grade 0 [362].

Addition of Nd, Zn and Zr improved the cytocompatibility towards hBMSC cells of magnesium but the alloy still had lower cytocompatibility than cp-Ti, which may be associated with the different pH value (the corresponding pH value of the medium was 7.26 for Ti, 9.08 for Mg and 8.24 for Mg–Nd–Zn–Zr [355]. He [354] also indicated that addition of Zn into Mg–1Ca–0.5Sr alloy exhibited a better biocompatibility with no obvious toxicity to MC3T3-E1 cells (cytotoxicity grade 0–1).

### 9.4. In vivo test

Three kinds of biodegradable Mg implants have been already approved by Conformite Europeene (CE) or Korea Food and Drug Administration (KFDA) [378]. It is worth mentioning that more and more Chinese surgeons and researchers are trying a lot in the research and development of antibacterial Mg based implants to accelerate clinical application, as listed in Table 15.

The *in vivo* experiments results suggested even if Mg implants were pre-cultured with bacterial suspension, Mg implantation could effectively protect bone and surrounding tissues from infection, and inhibit the growth of bacterial on the surface of Mg and bone tissue [351,352]. However, Mg implants exhibited insufficient antibacterial effect *in vivo* against MRSA compared to the high antibacterial rate *in vitro* [350,379]. Bacteria could be detected on the surface of the Mg implant surface as well as peri-implant tissue. Moreover, inflammatory cells filled cavities in the cortical bone of the femur after 2 and 4 weeks of implantation [350]. Similarly, AZ91 magnesium alloy was not able to maintain its antimicrobial effects during a one-week implantation period in a rat humeral head [361].

Mg alloys with proper number of alloying elements effectively enhanced antimicrobial properties and biocompatibility of Mg. For example, a novel Mg–Nd–Zn–Zr magnesium alloy showed enhanced the antibacterial activity *in vivo* compared with cp-Ti and Mg, moreover, the alloy had a small amount of gas in the medullary cavity, mild medullary cavity swelling deformation and unobscure reconstruction of cortical bone compared with Mg [355]. Li [72] designed a series of

**Table 15**  
*In vivo* results of antibacterial pure magnesium and magnesium alloy.

Year	Metal and alloy	Animal model (position)	Co-cultured with bacterial	<i>In vivo</i> results	[Ref.]
2014	Mg	Sprague Dawley rats (right femur cavities)	Mg implants precultured with MRSA ( $10^6$ CFU/mL) with an $OD_{600}$ of 0.1 for 24 h	1) Mg inhibited the growth of MRSA on the surface of Mg nails and bone tissue. 2) Mg protected bone and surrounding tissues from MRSA infection	[351]
2015	Mg	Balb/c mice (lower tail artery)	Mg implants precultured in a 1 mL suspension of <i>P. aeruginosa</i> culture with an $OD_{600}$ of 0.1 for 5 min.	Mg implants showed an enhanced resistance to bacterial colonization.	[352]
2015	Mg–Nb–Zn–Zr	Sprague Dawley rats	<i>S. aureus</i> in a concentration of $10^5$ CFU/mL was injected into the implant position.	1) Mg–Nb–Zn–Zr could reduce the risk of implant-related infection compared with Mg and Ti. 2) Mg–Nb–Zn–Zr had a small amount of gas in the medullary cavity, mild medullary cavity swelling deformation and unobscure reconstruction of cortical bone compared with Mg.	[355]
2016	Mg	Sprague Dawley rats (femoral medullary cavity)	50 $\mu$ l bacterial suspension of MRSA was injected into the implant position.	1) Mg showed inadequate antibacterial effect against MRSA <i>in vivo</i> compared with <i>in vitro</i> . 2) Obvious bone infections, numerous cavities and destruction of the cortical bone as well as inflammatory cells were observed after 2 and 4 weeks implantation.	[350]
2016	Mg	murine	5 $\mu$ l bacterial suspension was injected into the implant position.	Bacterial agglomerates could be detected on the implant surface and peri-implant tissue.	[379]
2016	Mg-0.25Cu	New Zealand White rabbits (proximal tibial metaphysis)	0.15 mL of 5% morrhuate sodium was injected into the implant position.	1) Mg-0.25Cu alloy reduced MRSA-induced osteomyelitis infection <i>in vivo</i> compared with Ti. 2) No microscopic differences in the various organs of rabbits implanted with Mg-0.25Cu nails, and no obvious deposition of $Cu^{2+}$ or $Mg^{2+}$ ion complexes were observed in their organs and tissues.	[72]
2018	AZ91	Long-Evans rats (humeral head)	$10^5$ CFU (0.1 cc) of <i>A. baumannii</i> was injected into the implant position.	No significant differences in the antibacterial activity from the cp-Ti and AZ91 implants after a week implantation.	[361]

Mg–Cu alloys that combine the favorable properties of Mg with the antibacterial properties of Cu and the *in vivo* results, as Fig. 23, indicated Mg-0.25Cu alloy reduced MRSA-induced osteomyelitis infection compared with Ti. On the other hand, the Mg-0.25Cu alloy induced no local or systemic side effects and deposited no obvious Cu<sup>2+</sup> or Mg<sup>2+</sup> ion complexes in the organs or tissues.

### 10. Antibacterial cobalt alloys

Co-based alloys have been widely used in hip implants and knee implants due to their excellent wear resistance and corrosion resistance, and in stents due to their high stiffness and ductility and excellent corrosion resistance. Implant related infection or inflammation is still a serious clinical complaint.

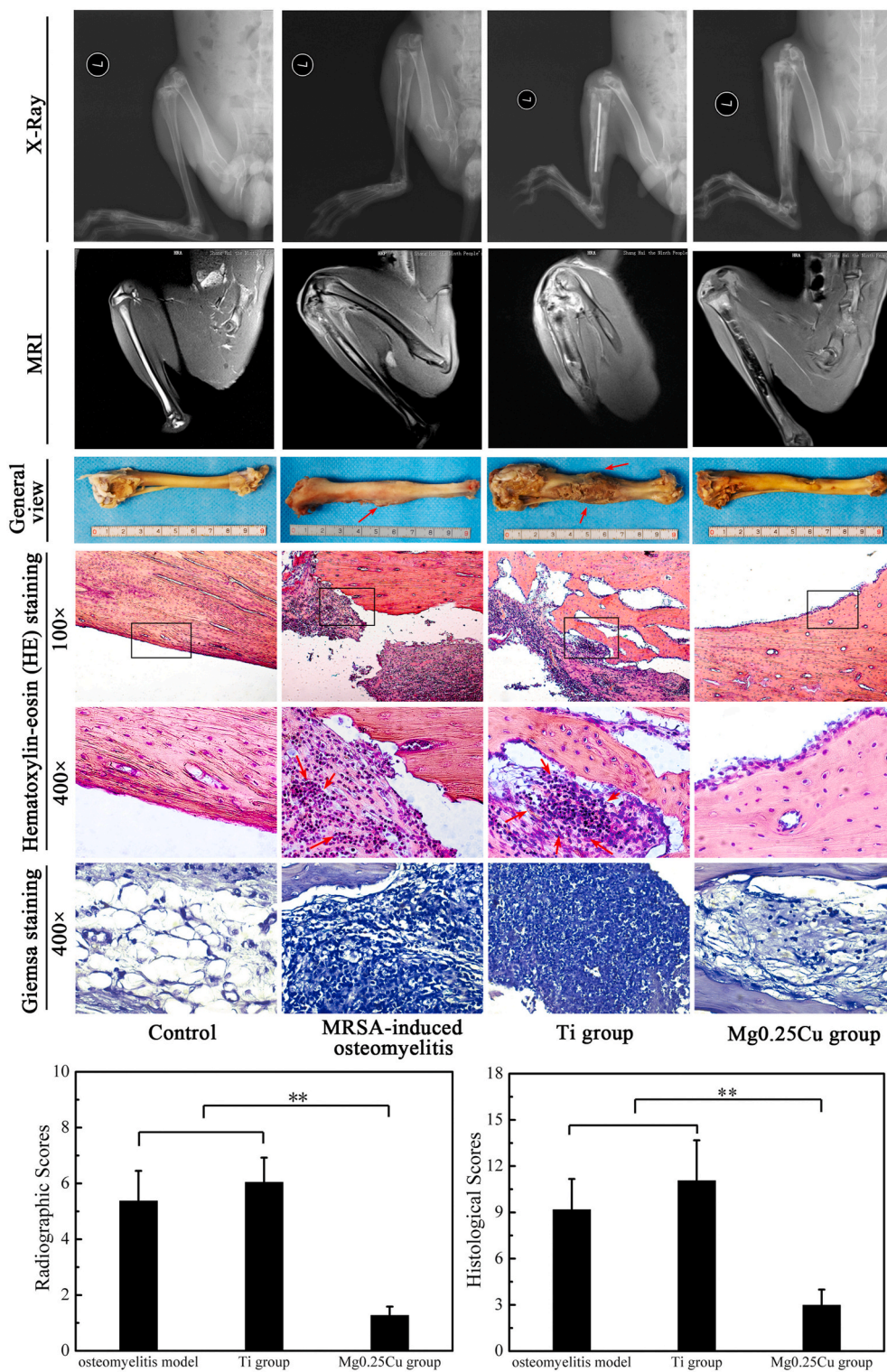


Fig. 23. The *in vivo* results of Mg-0.25Cu [72], copyright 2016, Elsevier Ltd. Digital X-ray and MR images, surface topography photographs, hematoxylin-eosin (HE) staining, Giemsa staining of longitudinal sections, radiographic and histological scores of the left tibia of the healthy control group, 4 weeks after osteomyelitis induction group, and 4 weeks after implantation of Ti and Mg-0.25Cu intramedullary nails group.

### 10.1. Antibacterial properties

The development of antibacterial cobalt alloys mainly focuses on Cu-containing Co-based alloy, as shown in Table 16. It was reported that the addition of Cu did provide cobalt alloy with antibacterial property and the antibacterial ability of cobalt alloy was Cu content dependent. In the study of antibacterial performance of CoCrW–Cu alloy against *E. coli*, it was clear that much less bacterial colonies were observed in the group of CoCrW–Cu alloy compared with the group of CoCrW matrix alloy [21]. Meanwhile, when 2.13 wt% Cu was added into Co19Cr10Ni14W, the antibacterial rates against *E. coli* and *S. aureus* were only 63.3% and 51.1%, respectively. With the increasing of the Cu content to 4.39 wt%, the antibacterial rate sharply increased to >90% [380]. In another study, it was shown that 1 wt% Cu provided very good antibacterial property (94.2% reduction) for Co29Cr6Mo, and further increasing in Cu content enhanced the antibacterial property, 98.5% and 99.6% reduction at 2 wt% and 4 wt% Cu, respectively [20]. Ag element has also been added into Co–Cr alloy to fabricate antibacterial Co–Cr–Ag alloy. Ag significantly enhanced the antibacterial activity of Co–Cr alloys, with antibacterial rates of 90.5% and 72.6% against *E. coli* and *S. aureus*, respectively [381].

### 10.2. Mechanical and corrosion properties

The addition of Cu element could increase the antibacterial property, but also have an influence on the mechanical and corrosion properties. Cu element has very high solubility in Co matrix at high temperature but low solid solubility at room temperature, about 19.7 at% at 1367 °C and nearly 0% at room temperature, which means that the solid solution strengthening ability of Cu element in Co-based alloy is very weak [20]. In the research, the hardness and strength of Cu-rich phase is lower than that of Co-matrix, which suggests that the dispersion strengthening ability of Cu-rich phase would be very low. Due to these two main reasons, the addition of Cu element and the precipitation of Cu-rich during the process of Cu-containing Co-based alloy do not contribute to the improvement of mechanical properties of Co-based alloys. In the research [20], the addition of Cu reduced the microhardness of Co–Cr–Mo alloy in a content dependent way, and thus might reduce the wear resistance. The tensile strength of Co–Cr–Mo–Cu alloy decreased with the addition of Cu element up to 2 wt% and then increased with

**Table 16**  
Antibacterial Co-based alloys.

Alloy system	Bacteria (antibacterial rate[Ref.])
Co–Cr–Mo	<i>S. aureus</i> , <i>S. epidermidis</i> , CNS (No [382])
Co29Cr6Mo-xCu	<i>S. aureus</i> (>90% [20])
Co(28–29)Cr9W-xCu	<i>S. aureus</i> (>99.99% [383]); <i>E. coli</i> (>90% [21,383], No [384])
Co19Cr10Ni14W-xCu	<i>S. aureus</i> (51.1%- > 90% [380]); <i>E. coli</i> (63.3%- > 90% [380])
Co-30at.%Cr-5at.% Ag	<i>S. aureus</i> (72.6% [381]), <i>E. coli</i> (90.5% [381])

Note: 24 h incubation as default.

**Table 17**  
Microhardness and tensile properties of Co29Cr6Mo-xCu alloys [20].

Alloys	Microhardness (HV)	Tensile strength (MPa)		Elongation (%)
		0.2% YS	UTS	
Co29Cr6Mo-0Cu	298.33 ± 29.41	423.61 ± 18.21	576.92 ± 50.36	10.7 ± 1.8
Co29Cr6Mo-1Cu	292.69 ± 28.63	310.38 ± 41.43	447.83 ± 24.85	18.5 ± 3.7
Co29Cr6Mo-2Cu	279.67 ± 26.79	432.73 ± 12.86	499.13 ± 21.65	11.5 ± 3.8
Co29Cr6Mo-4Cu	274.64 ± 26.05	440.53 ± 36.47	620.56 ± 15.24	15.2 ± 0.1

further increasing of Cu content, but the elongation was improved by the addition of Cu element [20], as listed in Table 17. It was reported that the addition of 3% Cu reduced the bonding strength between Co–Cr–W–Cu alloy and porcelain in comparison with Co–Cr–W system, but the bonding strength between Co–Cr–W–Cu alloy and porcelain was still higher than the recommended value in Standard ISO 9693-1999 [385]. The research on CoCrWNi–Cu alloy also indicated that the addition of Cu slightly reduced the microhardness but had a limited influence on the compression properties of CoCrWNi alloy [386].

One of advantages of Co-based alloys as a biomedical material is their high corrosion resistance against body fluids. However, the addition of Cu slightly increases the corrosion current density of CoCrW–Ni–Cu in a Cu content dependent mode. For example, the corrosion current densities of CoCrWNi base alloy, CoCrWNi-2.13Cu and CoCrWNi-4.39Cu were  $5.39 \times 10^{-7}$  A/cm<sup>2</sup>,  $6.59 \times 10^{-7}$  A/cm<sup>2</sup> and  $1.02 \times 10^{-6}$  A/cm<sup>2</sup>, respectively [380]. It was thought that the Cu-rich precipitate acted as a cathodic center, thereby enhancing the dissolution of the matrix. Another investigation on the change in the corrosion property of as-cast CoCrWNi–Cu alloy proved that the addition of Cu slightly reduced the corrosion resistance as a result of the galvanic corrosion caused by dendritic segregation [386]. Research on SLM Co–Cr–Cu alloy also indicated that the influence of Cu on the electrochemical data of SLM Co–Cr alloy in 0.9% NaCl was negligible [383]. It must be pointed out the change in the corrosion properties of Co-based due to the addition of Cu element slightly decreased the corrosion resistance but the difference was very limited [20]. Co-based with and

**Table 18**  
Antibacterial Cu based alloys.

Alloy system	Bacteria	Reference
Cu	<i>E. coli</i> ; <i>S. aureus</i> ; MRSA; <i>L. monocytogenes</i> ; <i>M. tuberculosis</i> strain R432 and R267; <i>C. albicans</i> ; <i>K. pneumoniae</i> ; <i>P. aeruginosa</i> ; <i>A. baumannii</i> ; <i>Salmonella</i> ; <i>Enteritidis</i> ; <i>S. Typhimurium</i> DT193 S9, DT120 S19 and DT66S20; <i>S. typhimurium</i> ; <i>Streptococcus</i> sp. BY1; <i>Enterococcus</i> sp. BY2; <i>B. cereus</i> BY3	[110,111, 393–396]
Cu-xZn	<i>E. coli</i> ; <i>L. monocytogenes</i> ; MRSA; <i>C. albicans</i> ; <i>K. pneumoniae</i> ; <i>P. aeruginosa</i> ; <i>A. baumannii</i> ; <i>M. tuberculosis</i> strain R432 and R267; <i>Salmonella</i> ; <i>Enteritidis</i> ; <i>S. Typhimurium</i> DT193 S9, DT120 S19 and DT66S20	[84,110, 393–395]
Cu–Ni	<i>E. coli</i> ; <i>Salmonella</i> ; <i>Enteritidis</i> ; <i>S. Typhimurium</i> DT193 S9, DT120 S19 and DT66S20	[84,393]
Cu–5Sn	<i>E. coli</i>	[84,393]
Cu–3Si	<i>L. monocytogenes</i>	[394]
Cu–Ni–Zn	<i>E. coli</i> ; <i>L. monocytogenes</i> ; MRSA; <i>C. albicans</i> ; <i>K. pneumoniae</i> ; <i>P. aeruginosa</i> ; <i>A. baumannii</i> ; <i>M. tuberculosis</i> strain R432 and R267	[84, 393–395]
Cu–15Ni–8Sn	<i>E. coli</i>	[393]
Cu–2Ni–8Al	<i>E. coli</i>	[393]
Cu–10Ni–1.4Fe	<i>L. monocytogenes</i>	[394]
Cu–3Al–2Si	<i>E. coli</i>	[393]
Cu–1Mn–2Si	<i>E. coli</i>	[393]
Cu–23Sn–3Al	<i>E. coli</i>	[393]
Cu-22.5Zn-2.5 Pb	<i>C. albicans</i> ; <i>K. pneumoniae</i> ; <i>P. aeruginosa</i> ; <i>A. baumannii</i> ; MRSA; <i>S. aureus</i> ; <i>M. tuberculosis</i> strain R432 and R267	[395]
Cu–Zn–Sn–Pb	<i>E. coli</i>	[111]
Cu–Zn–Sn–Al	<i>E. coli</i> ; <i>P. stewartii</i> ; <i>A. johnsonii</i> ; <i>S. panni</i> ; <i>P. oleovorans</i> ; <i>S. haemolyticus</i> ; <i>S. epidermidis</i> ; <i>S. warnerii</i> ; <i>B. conglomeratum</i> ; <i>M. luteus</i> ; <i>K. marina</i> ; <i>K. palustris</i>	[393,401]
Cu-2.8Al-1.8Si-0.4Co	<i>L. monocytogenes</i>	[394]



without Cu element still keep a very low corrosion rate in simulated body fluid.

The addition of Cu also has influence on the martensite transformation [387], which in turn affect the final deformation behavior. Co-based alloy is normally subjected to hot deformation to refine the microstructure and improve the mechanical properties. The hot deformation behavior is closely related to the microstructure and the phase constitute. In Co-based alloy, fcc  $\gamma$ -Co phase provides very good ductility and deformability while hcp  $\epsilon$ -Co phase shows low ductility and deformability. The addition of Cu element effectively inhibited the martensite transformation from  $\gamma$ -Co to hcp  $\epsilon$ -Co phase, thus, Cu containing alloys showed good ductility and also good thermal deformation ability [20].

### 10.3. Biocompatibility

Cobalt (Co) is an essential trace element for human and an important component of cyanocobalamin. As we all know, cyanocobalamin is an essential vitamin (vitamin B12) which is required for the production of red blood (RBC) cells and the prevention of pernicious anemia [388]. Co was historically used to treat certain types of anemia as a result of its ability to stimulate hemoglobin and RBC production [389]. What should be noted is the recommended daily intake of Vitamin B12 in the US is 2.4 mg/d, corresponding to approximately 0.10 mg of Co [389]. There is ample evidence to indicate that Co exposures in highly elevated blood Co concentrations (>300  $\mu\text{g/L}$ ) can lead to certain endocrine, hematological, cardiovascular, and neurological effects in some animals and/or humans [390]. Human subchronic and chronic oral exposure to Co has been closely linked with effects on the hematological, thyroid and cardiovascular systems. The average measured Co blood concentrations in these cohorts ranged from 2.1 to 26  $\mu\text{g/L}$  [389]. Pettersson [391] evaluated the cytotoxic effect and the inflammatory response of macrophages to Ti, Co Cr and Mo in a range of 0–800  $\mu\text{M}$ . Cytotoxic effects was reported for all ions except Cr, but no differences in the release of IL-1 $\beta$  was found with exposure to Co and Cr ions. However, no detail data was available in this reference.

In the antibacterial CoCr alloy system, the addition of Cu and Ag could enhance antibacterial property, but also affect cytotoxicity of

alloy. The effect of Cu on cytotoxicity of CoCrW alloy is in a Cu-content dependent feature. There was no cytotoxicity for Cu-bearing CoCrW alloys when the Cu content was less than 4 wt% [384]. In the study of ultrafine-grained Co-30at.%Cr-5at.%Ag alloy, the addition of Ag also significantly enhanced the antibacterial activity of Co–Cr alloys but showed no cytotoxicity [381].

### 11. Antibacterial pure copper and copper alloys

Copper has been registered at the U.S. Environmental Protection Agency as the first solid antibacterial material [392]. It is suggested pure copper has excellent antibacterial properties against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium*, *L. monocytogenes*, *Streptococcus* sp. BY1, *Enterococcus* sp. BY2 and *B. cereus* BY3, etc. [393–396]. Moreover, Yousuf [396] suggested the Gram-negative cells were more resistant than the Gram-positive cells. The high antibacterial property was anticipated as that copper ions are able to inhibit the survival of several microorganisms [112–115], many also ascribed it to the mechanistic of contact killing bacteria [392]. However, the poor corrosion resistance and strength made pure copper itself unsuitable for use as biomedical implant even though it has such strong antibacterial properties. Some antibacterial pure copper or copper alloys reported in recent years as illustrated in Table 18, including pure copper, brasses, bronzes, copper-nickels and copper-nickel-zinc alloys.

It was reported the brasses were harder and more durable than copper, Cu–Ni and Cu–Ni–Zn had much greater corrosion resistance than copper and all of these alloys showed excellent antibacterial activity at room temperatures (20 °C) [393]. Zhu [84] revealed that the antimicrobial activity of copper alloys against *S. enterica* and *S. Typhimurium* increased as the copper content (60–99.9 wt% Cu) of the alloys increased, even the copper resistant strains only survived for short periods of time on the high copper content alloys. Pure Cu demonstrated both growth inhibition of 98% and 88% against two *M. tuberculosis* strains, R267 and R432, respectively, while the other copper alloys with >55 wt% Cu also showed effective inhibition effect against both nosocomial pathogens and *M. tuberculosis* [395]. In Yousuf’s study [396], the cells exposed to rougher surface of rolled-out copper plates were killed more effectively and faster due to the faster Cu ion release from rougher

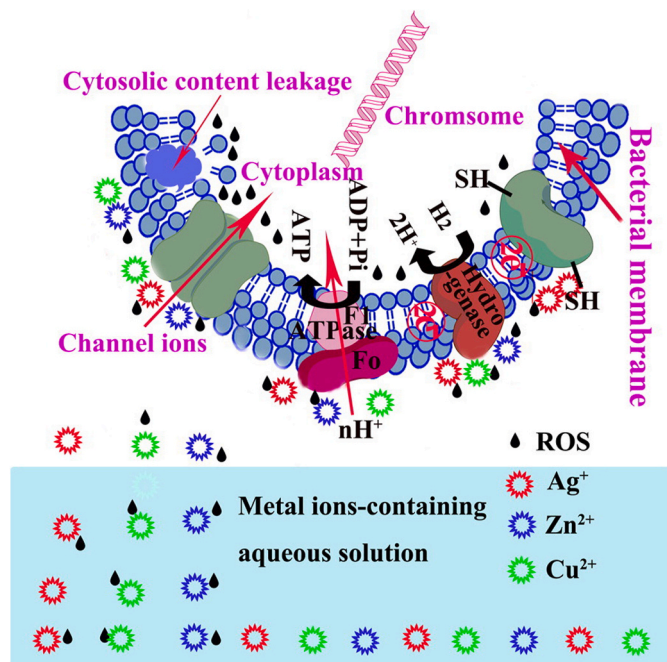


Fig. 24. Antibacterial mechanism of metal ion [115], copyright 2016, Elsevier Inc.

Table 19

Cu or Ag ions release concentration Cu or Ag ions release concentration and related antibacterial properties from reported antibacterial alloy.

Ion release concentration	The immersion solution	Antibacterial (%)	Bacteria	ref
22.5 ppb Cu	0.9% NaCl	98.3	<i>S. aureus</i>	[216]
11.5 ± 4.7 ppb Cu	artificial seawater	33.1	<i>P. aeruginosa</i>	[224]
25.7 ± 7.1 ppb Cu		56.0		
37.8 ± 8.9 ppb Cu		70.3		
1000 ppb Cu	supernatant of media cultured	>90	<i>E. coli</i> ; <i>S. aureus</i>	[214]
2.0 ppb Cu	tap water	40	microorganism	[208]
7.1 ppb Cu		95.9		
8.1 ppb Cu		75		
about 4 ppb Ag	0.9% NaCl	<21	<i>S. aureus</i>	[74]
0.63 ppb Ag	0.9% NaCl	38.0	<i>S. aureus</i>	[71]
29.657 ± 3.926 ppb Ag	antibacterial test solution			
0.78 ppb Ag	0.9% NaCl	57.0		
36.519 ± 3.340 ppb Ag	antibacterial test solution			
1.06 ppb Ag	0.9% NaCl	67		
49.961 ± 8.081 ppb Ag	antibacterial test solution			
1 day: < 5 ppb, 7 day: 105 ppb	0.9% NaCl	99.9	<i>S. aureus</i>	[105]

Note: The default antibacterial method is the plate counting method for one day.

surface.

Touch surfaces in hospitals, such as door handles, touch plates, bed rails, call buttons, toilet seats, etc., can be highly contaminated with microbes. Thus in several clinical studies, copper has been evaluated for use on touch surfaces, attempting to curb nosocomial infections [392, 397, 398]. Palza's clinical trial results [399] firstly showed that it was a viable route to add copper to organic materials for applying the antimicrobial effects of copper, such as plastic waiting room chairs with embedded copper metal nanoparticles, and metal hospital IV pools coated with an organic paint with nanostructured zeolite/copper particles. Borkow [400] even imagined possible future applications of Cu alloy include filtration devices capable of deactivating viruses in solutions, such as contaminated blood products and breast milk.

## 12. Antibacterial metallic glasses

The emergence of bulk metallic glasses (BMGs) offers a new opportunity to achieve biomaterials with the desirable traits of excellent mechanical, chemical properties, process ability as well as low cytotoxicity, which make them as potential candidates for medical implants [402–405]. Fe-based, Ni-based and Cu-based metallic glasses were firstly reported in 2012 to inhibit growth of *E. coli* [406]. Therefore, it is promising to explore applications of antibacterial metallic glasses in healthcare ranging from hospital public facilities to surgical instruments and implants.

BMGs frequently includes heavy metal elements to tune various material properties, for instance, Cu and Ag is commonly used in Zr-based BMGs to attain good glass formation ability [407, 408]. However, the presence of these biocidal elements implies that there may be an antimicrobial potential of BMGs. In Huang's study [191], although bacteria killing function of Zr-based BMGs on *S. aureus* was observed in the direct contact test, while the alloys lacked antibacterial effect in the more aggressive dynamic immersion experimental settings, which possibly suggested the killing efficacy was directly related to the release of Cu ions.

Thin film metallic glasses (TFMGs) have recently emerged as alternative advanced surfaces for many applications [409]. It was reported the strong antibacterial ability of TFMG was ascribed to their amorphous rough surface, hydrophobic properties and released antibacterial element ions [192]. In particular, Zr-based TFMGs exhibit promising antibacterial properties [192, 410–417].

## 13. Antibacterial mechanisms

Antibacterial metals and alloys are not a new concept. However, antibacterial mechanism of antibacterial metals and alloys is still unclear, including the dissolution of metal ions such as Ag, Cu and Zn, the rising of pH value, the direct contact killing mechanism, the electron transfer, and the surface structure, etc.

### 13.1. Antibacterial mechanism of metal ions

Antibacterial metal ions such as silver ( $\text{Ag}^+$ ), zinc ( $\text{Zn}^{2+}$ ), and copper ( $\text{Cu}^{2+}$ ) are well known for their strong broad-spectrum antibacterial

**Table 20**

The relationship between antibacterial ability and Mg ion concentration.

Solution	Mg ion concentration (mM)	Bacterium	Antibacterial ability	Ref
MH broth + $\text{MgCl}_2$ salt	0.16	<i>E. coli</i> ; <i>S. aureus</i> ;	No	[345]
MH broth + NaCl salt	5.95	<i>P. aeruginosa</i>	No	
LB medium + magnesium granules + adjust to the pH of 7	$5.5 \pm 1.1$ $7.7 \pm 0.7$ $9.7 \pm 0.9$	<i>P. aeruginosa</i>	No	[352]

Note: a) MH broth: Mueller Hinton Broth, b) LB medium: Luria-Bertani medium.

**Table 21**

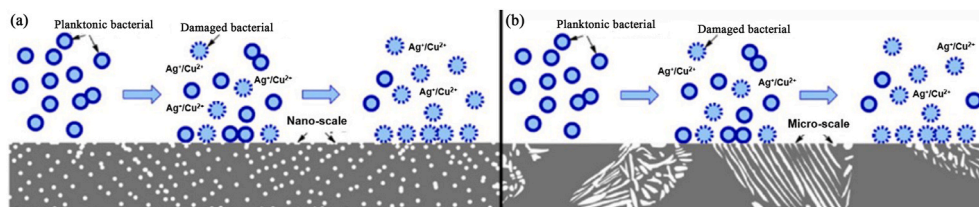
The relationship between antibacterial ability and pH value.

Solution	pH value	bacterium	Antibacterial ability	Ref
MH broth + NaOH	8	<i>E. coli</i> ;	No	[345]
	9	<i>P. aeruginosa</i> ;	Yes	
	10	<i>S. aureus</i>	Yes	
Pure Mg + bacterial suspensions	8.5	<i>E. coli</i> ;	Yes (>99.9%)	[346]
	10	<i>S. aureus</i>		
Pure AZ31 + bacterial suspensions	8.2			[352]
	9.5			
LB culture medium + NaOH	7	<i>P. aeruginosa</i>	No	[352]
	7.5			
	8			
	8.5			
Pure Mg + broth	9		Yes (restrict bacterial proliferation)	[355]
	9.5		Yes (further restrict)	
pure Mg + PBS	7.4	<i>E. coli</i> ;	70.5%, 63.8%,	[358]
	(0–120 h)	<i>S. epidermidis</i> ;	65.2%, respectively	
pure Mg + PBS	12	<i>S. aureus</i>	88.8%, 83%, 81.4%, respectively	
			No	

Note: a) MH broth: Mueller Hinton broth, b) LB culture medium: Luria-Bertani culture medium.

activity [93, 113]. It has been widely accepted that metal ions, such as  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , kill bacteria by inducing the production of reactive oxygen species (ROS) [113]. ROS is the reduction products of oxygen, such as peroxides, superoxides, hydroxyl radicals and singlet oxygen, which destroy the cell membrane of bacteria, as illustrated in Fig. 24.

Antibacterial activity of the materials that release metal ions ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^+$  ... etc.) involves main distinct parts, as following: firstly, metal ions on the surface form strong bonds with amino and carboxyl groups of bacterium membrane and proteins, and disable them and lead to structural changes. Secondly, a bacterial membrane with structural changes presents an important permeability increase, making the



**Fig. 25.** The direct contact killing mechanism [268], copyright 2017, Elsevier B.V. a) nano-scale and b) micro-scale Ag- or Cu containing particles on the surface resist the bacteria adhesion, the formation of biofilm and kill the adhered bacteria.

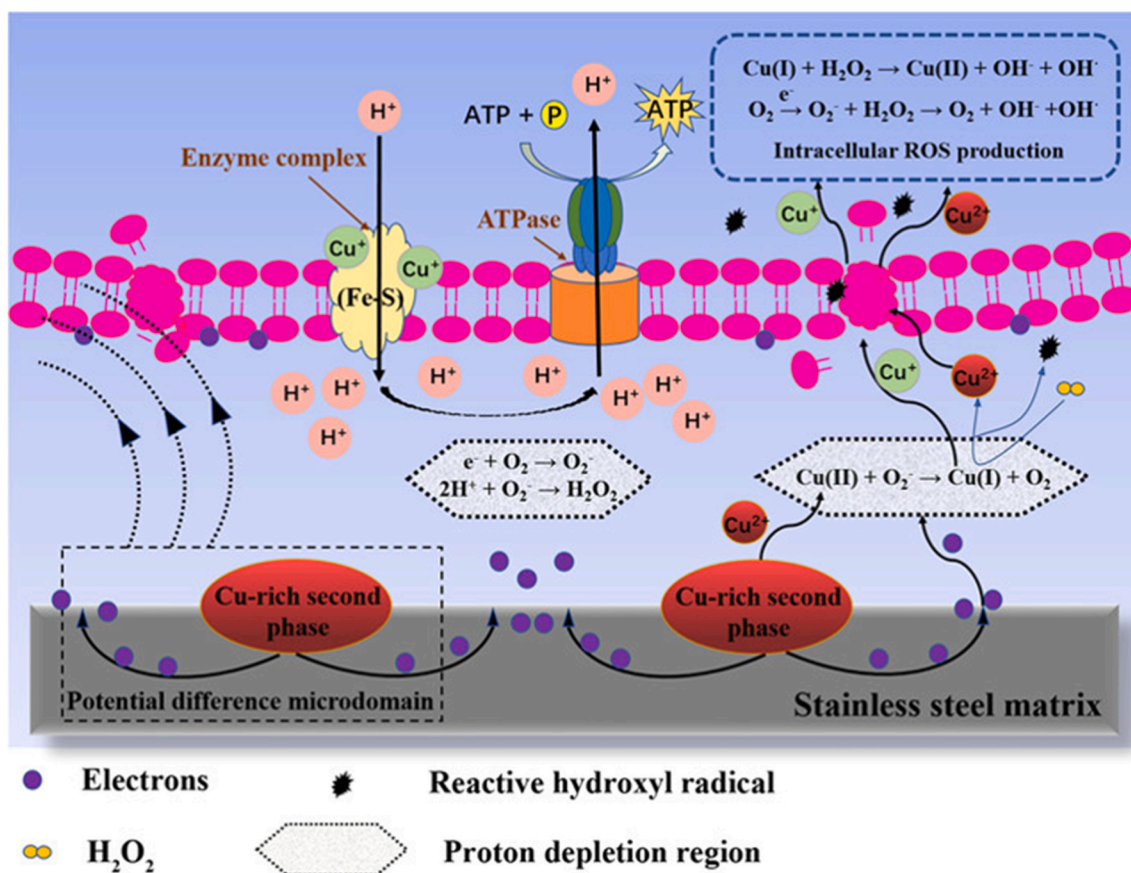


Fig. 26. Schematic diagram of antibacterial process by electron transfer [436], copyright 2019, American Chemical Society.

bacterium unable to monitor properly the transport through the plasma membrane. In the final stage, metal ions interact with nucleic acids from microorganisms preventing replication and causing cell death.

The minimum inhibitory concentrations (MICs) of silver ions against *S. aureus* and *E. coli* were both in a range of 25.4–2040 µg/L [113]. Ning et al. [113] suggested that the optimal concentration of zinc ions was 65 µg/L. To kill the bacteria, the minimum bactericidal concentrations (MBCs) of Zn<sup>2+</sup> was 650 µg/L. Researches on Cu- or Ag- containing metal alloys, including stainless steels, titanium alloys and cobalt alloys, have indicated that the dissolved copper ions or silver ions played an important role for the antibacterial effect, which inhibits bacterial adhesion or the formation of biofilm, or leads to the collapse of some lipopolysaccharide (LPS) patches of the cell surface, and consequently alters the permeability and functionality of the outer cell membrane [418]. It was also pointed out that the more the Cu addition, the more the release of Cu ions from alloy [380]. The antibacterial rates increased with increasing Cu<sup>2+</sup> solution concentrations. The minimum inhibitory concentrations (MICs) of Cu<sup>2+</sup> ions were in a range of 64–7104 µg/L [113]. Nan's results [206] strongly demonstrated that DNA of *E. coli* was not obviously damaged when *E. coli* was in contact with Cu-containing stainless steel, but cell walls were seriously changed and lots of contents in the cells were leaked, which means that the steel killed the bacterium by damaging the cell membrane rather than damaging the DNA of inner cells.

### 13.2. The direct contact sterilization mechanism

According to the antibacterial mechanism of metal ions, high metal ions should be released from alloy in order to obtain high antibacterial ability. However, the metal ion release from antibacterial alloy is very low. For example, the release amount of Cu ions from antibacterial

Ti6Al4V–5Cu alloy after 20 days immersion in 0.9% NaCl solution was  $2.498 \pm 0.755$  µg/L [272] and the Ag ion release from antibacterial Ti–3Ag (S) after 24 h immersion in 0.9% NaCl solution was only 1.9 µg/L [105], much less than MIC for Cu<sup>2+</sup> of 64–7104 µg/L and for Ag<sup>+</sup> of 25.4–2040 µg/L [113,419,420]. Table 19 summaries Cu or Ag ions release and reported antibacterial properties. Actually, previous studies have demonstrated that metallic copper [421], 316L-Cu-La-Ce stainless steel [148], La-containing antibacterial stainless steel [147] and Ti–Cu sintered alloy [18] could only kill the bacteria on the surface and had no antibacterial ability to the surrounding bacteria.

Meanwhile, more research results have shown that the antibacterial ability of titanium alloy is closely related to the precipitation of Ag- or Cu-containing compounds. Zhang et al. [252] believed that the Ti<sub>2</sub>Cu phase played a key role in the antibacterial mechanism according to the research of Ti–Cu alloys. Peng et al. [272] discovered that the antibacterial performance of larger globule Ti<sub>2</sub>Cu and bulk β phases was better than the small ones. Chen et al. [268] suggested that the size and amount of Ag-containing compounds had a significant influence on the antibacterial properties and concluded that the antibacterial mechanism of different Ti–Ag alloys was a mixture of the direct contact antibacterial mechanism by Ti<sub>2</sub>Ag phases and Ag ions antibacterial mechanism. More recently, Shi et al. [71] confirmed that nearly 90% antibacterial ability of Ti–Ag alloys came from the precipitation of Ti<sub>2</sub>Ag in a contact sterilization mode and 10% antibacterial ability came from Ag ion sterilization.

In summary, Cu- or Ag-containing particles in nano-scale or micro-scale size, resists the adhesion of bacterium [193,272], the formation of biofilm [255] and kill the bacterial on the surface, as an example shown in Fig. 25. However, the exact antibacterial mechanism in the contact sterilization mode is still unclear.

### 13.3. Rising of pH value

Biodegradable magnesium and magnesium alloys with Ag or Cu elements also show strong antibacterial activity. The major soluble corrosion products of magnesium and magnesium alloys in aqueous physiological solutions are magnesium ions and hydroxide ions, thereby, the increase in solution pH and the increase in Mg ion concentrations caused by fast degradation of magnesium alloy and the formation of  $Mg(OH)_2$  on the surface might possibly contribute to the antibacterial property of magnesium and magnesium alloys [347]. As an example, Table 20 lists the relationship between antibacterial rate and Mg ion concentration reported in literatures, which indicates the addition of  $Mg^{2+}$  alone would not inhibit bacterial growth [345,352].

On the contrary, more researches suggested there existed a relationship between pH value and the antibacterial ability, as summarized in Table 21. These results demonstrate that bacteria can be alive usually in an environment with pH range of 6.0–8.0 while both acid and alkali environments are not suitable for the survival of bacteria. When the corrosion supernatants were adjusted to a neutral pH, the inhibition effect of the bacteria was completely abolished [352,358]. In Qin's study [355], the antibacterial effect of Mg increased with the extended degradation time, which indicated the antibacterial activity was proportional to the pH value. In suitable pH environment, bacteria can maintain a cytoplasmic pH that is compatible with the optimal functional and structural integrity of the cytoplasmic proteins [422,423]. Due to the fast degradation rate of magnesium and magnesium alloys, pH value can reach at 11.0 or even higher, thus the rising in pH value should be response to the high antibacterial ability of magnesium and magnesium alloys. Although it is believed that the alkalinity contributed to bacteria death, there may be other factors affect antibacterial properties. For example, magnesium hydroxide and magnesium oxide particles formed during magnesium degradation may contribute to bacteria death [424,425].

### 13.4. The electron transfer

Besides above mentioned antibacterial mechanisms, electron transfer in bacterial activities is another widely accepted antibacterial mechanism [30,426–431]. In fact, electron transfer is a crucial step in many bacterial activities. For instance, by means of electron transfer, bacteria complete respiration on the cell membrane to supply energy for cell growth, proliferation and maintenance [432–434], but on the other hand, disturbing electron transfer in bacteria can raise the production of reactive oxygen species (ROS) to hinder growth [435], as schematically shown in Fig. 26.

Many studies have shown that electron transfer plays an important role in destroying electron transport in bacteria. Wang et al. [431] discovered that the antibacterial effects of Ag-NPs@Ti relied on a conductive substrate. Electrons transfer took place between the Ag-NPs and Ti substrate and produced ROS in the solution to elevate the oxidative stress at the interface. In the research of capacitive carbon-doped  $TiO_2$  nanotubes, Wang et al. [437] confirmed that electron transfer observed during early contact contributed to the surface-dependent antibacterial process. In addition, a lot of researches have realized the sterilization effect by constructing charged material surface. Van [438] have reported that a positively charged carbon surface can reduce the viability of bacteria. Positive or negative surface charges have also been found to promote the antibacterial efficiency of chitosan and inhibit adherence of Gram-negative bacteria on polymeric materials [439,440]. Further mechanistic studies indicated that surface charges can disrupt the membrane potential of bacterial cells producing irreversible damage in the membrane structure [441,442].

The electron transfer between metal ions in aqueous solution and catalytic enzyme affects the performance of bio-electronic systems (BESs). Some metal ions produced ROS by Fenton reactions, and other metal ion bond protein sulfhydryl group on bacterial membrane, which

can maintain the integrity of bacterial DNA and cause the destruction of bacterial membrane, so as to achieve the effect of antibacterial effect [443,444].

## 14. Future

Strong and long-term antibacterial ability of antibacterial metals and alloys as well as good corrosion resistance and mechanical properties all demonstrate the potential application of antibacterial metal alloy in the future. However, there are still some concerns before the clinical application:

- (1) *The antibacterial mechanism.* Although several antibacterial mechanisms have been discussed in detail so far, the antibacterial mechanism is still not clear. Due to this reason, the design and preparation of antibacterial metal alloy still depends mainly on element alloying by the addition of proper Cu or Ag element.
- (2) *Potential toxicity of Ag ion or Cu ion release.* Lots of *in vitro* investigation have demonstrated that metal ion release from antibacterial alloy is very low and the antibacterial alloys exhibit good cell compatibility. *In vivo* results also confirm the biocompatibility of antibacterial metal alloys. However, the metal ion release, especially Ag ion and Cu ion, might cause long-term cytotoxicity or side-effect to human body.
- (3) *Surface bioactivity.* Antibacterial stainless steel, antibacterial titanium alloys and antibacterial cobalt alloys are still inert to cells and tissues. Surface biomodification to improve or enhance the cell response is still necessary, which might cause reduction in antibacterial activity on the other hand. Therefore, selection of surface biomodification and its influence on the cell response and antibacterial activity should be investigated in detail.

## 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.

## Acknowledgements

The authors would like to acknowledge the financial support from National Natural Science Foundation of China (no. 31971253/C1002). Authors are also thankful to Xuefeng Song, Shuo Liu, Jiayu Wang, Shuang Cao and Ming Lu for their help in the preparation of this manuscript. Special thanks for the instrumental analysis of the Analytical and Testing Center, Northeastern University.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioactmat.2021.01.030>.

## References

- [1] D.E. Reichman, J.A. Greenberg, Reducing surgical site infections. A review, *Rev. Obstet. Gynecol.* 2 (2009) 212–221.
- [2] J. Grischke, J. Eberhard, M. Stiesch, Antimicrobial dental implant functionalization strategies —a systematic review, *Dent. Mater. J.* 35 (2016) 545–558.
- [3] K. Duske, L. Jablonowski, I. Koban, R. Matthes, B. Holtfreter, A. Sckell, et al., Cold atmospheric plasma in combination with mechanical treatment improves osteoblast growth on biofilm covered titanium discs, *Biomaterials* 52 (2015) 327–334.
- [4] P.A. Norowski, J.D. Bumgardner, Biomaterial and antibiotic strategies for peri-implantitis: a review, *J. Biomed. Mater. Res. B Appl. Biomater.* 88B (2009) 530–543.
- [5] A.D. Pye, D.E.A. Lockhart, M.P. Dawson, C.A. Murray, A.J. Smith, A review of dental implants and infection, *J. Hosp. Infect.* 72 (2009) 104–110.

- [6] M. Li, Z. Ma, Y. Zhu, H. Xia, M.Y. Yao, X. Chu, et al., Toward a molecular understanding of the antibacterial mechanism of copper-bearing titanium alloys against *Staphylococcus aureus*, *Adv. Healthcare Mater.* 5 (2016) 557–566.
- [7] D.L. Helfet, N.P. Haas, J. Schatzker, P. Matter, R. Moser, B. Hanson, AO philosophy and principles of fracture management - its evolution and evaluation, *J. Bone Joint Surg. Am.* 85A (2003) 1156–1160.
- [8] R.R. Laffer, P. Graber, P.E. Ochsner, W. Zimmerli, Outcome of prosthetic knee-associated infection: evaluation of 40 consecutive episodes at a single centre, *Clin. Microbiol. Infect.* 12 (2006) 433–439.
- [9] C.L. Wu, X.H. Qu, F.X. Liu, H.W. Li, Y.Q. Mao, Z.N. Zhu, Risk factors for periprosthetic joint infection after total hip arthroplasty and total knee arthroplasty in Chinese patients, *PLoS One* 9 (2014).
- [10] B. Kessler, P. Sendi, P. Graber, M. Knupp, L. Zwicky, B. Hintermann, et al., Risk factors for periprosthetic ankle joint infection: a case-control study, *J. Bone Joint Surg. Am.* 94A (2012) 1871–1876.
- [11] Y.L. Liao, Y.C. Yao, Y.L. Yu, Y. Zeng, Enhanced antibacterial activity of curcumin by combination with metal ions, *Colloid Interface Sci. Commun.* 25 (2018) 1–6.
- [12] D. Campoccia, L. Montanaro, C.R. Arciola, A review of the biomaterials technologies for infection-resistant surfaces, *Biomaterials* 34 (2013) 8533–8554.
- [13] L.Z. Zhao, P.K. Chu, Y.M. Zhang, Z.F. Wu, Antibacterial coatings on titanium implants, *J. Biomed. Mater. Res. B Appl. Biomater.* 91B (2009) 470–480.
- [14] P. Sahrmann, V. Ronay, D. Hofer, T. Attin, R.E. Jung, P.R. Schmidlin, In vitro cleaning potential of three different implant debridement methods, *Clin. Oral Implants Res.* 26 (2015) 314–319.
- [15] G. John, J. Becker, F. Schwarz, Rotating titanium brush for plaque removal from rough titanium surfaces - an in vitro study, *Clin. Oral Implants Res.* 25 (2014) 838.
- [16] D. Zhang, L. Ren, Y. Zhang, N. Xue, K. Yang, M. Zhong, Antibacterial activity against *Porphyromonas gingivalis* and biological characteristics of antibacterial stainless steel, *Colloids Surf. B Biointerfaces* 105 (2013) 51–57.
- [17] T. Shirai, H. Tsuchiya, T. Shimizu, K. Ohtani, Y. Zen, K. Tomita, Prevention of pin tract infection with titanium-copper alloys, *J. Biomed. Mater. Res. B Appl. Biomater.* 91B (2009) 373–380.
- [18] E.L. Zhang, F.B. Li, H.Y. Wang, J. Liu, C.M. Wang, M.Q. Li, et al., A new antibacterial titanium-copper sintered alloy: preparation and antibacterial property, *Mater. Sci. Eng. C* 33 (2013) 4280–4287.
- [19] Y.S. Feng, S.J. Zhu, L.G. Wang, L. Chang, Y.C. Hou, S.K. Guan, Fabrication and characterization of biodegradable Mg-Zn-Y-Nd-Ag alloy: microstructure, mechanical properties, corrosion behavior and antibacterial activities, *Bioact. Mater.* 3 (2018) 225–235.
- [20] E.L. Zhang, C. Liu, A new antibacterial Co-Cr-Mo-Cu alloy: preparation, biocorrosion, mechanical and antibacterial property, *Mater. Sci. Eng. C* 69 (2016) 134–143.
- [21] Y.J. Lu, L. Ren, S.Q. Wu, C.G. Yang, W.L. Lin, S.L. Xiao, et al., CoCrWCu alloy with antibacterial activity fabricated by selective laser melting: densification, mechanical properties and microstructural analysis, *Powder Technol.* 325 (2018) 289–300.
- [22] S. Ferraris, S. Spriano, Antibacterial titanium surfaces for medical implants, *Mater. Sci. Eng. C* 61 (2016) 965–978.
- [23] N. Mitik-Dineva, J. Wang, V.K. Truong, P. Stoddart, F. Malherbe, R.J. Crawford, et al., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* attachment patterns on glass surfaces with nanoscale roughness, *Curr. Microbiol.* 58 (2009) 268–273.
- [24] Y.P. Wang, S.Y. Zhao, G.Q. Li, S.F. Zhang, R.F. Zhao, A. Dong, et al., Preparation and in vitro antibacterial properties of anodic coatings co-doped with Cu, Zn, and P on a Ti-6Al-4V alloy, *Mater. Chem. Phys.* (2020) 241.
- [25] X.J. He, G.N. Zhang, X. Wang, R.Q. Hang, X.B. Huang, L. Qin, et al., Biocompatibility, corrosion resistance and antibacterial activity of TiO<sub>2</sub>/CuO coating on titanium, *Ceram. Int.* 43 (2017) 16185–16195.
- [26] M. Hadidi, A. Bigham, E. Saebnoori, S.A. Hassanzadeh-Tabrizi, S. Rahmati, Z. M. Alizadeh, et al., Electrophoretic-deposited hydroxyapatite-copper nanocomposite as an antibacterial coating for biomedical applications, *Surf. Coating Technol.* 321 (2017) 171–179.
- [27] H. Cao, X. Liu, F. Meng, P.K. Chu, Biological actions of silver nanoparticles embedded in titanium controlled by micro-galvanic effects, *Biomaterials* 32 (2011) 693–705.
- [28] J.B. Li, Y.Q. Qiao, Z.H. Ding, X.Y. Liu, Microstructure and properties of Ag/N dual ions implanted titanium, *Surf. Coating Technol.* 205 (2011) 5430–5436.
- [29] F. Zhang, G.K. Wolf, X.H. Wang, X.H. Liu, Surface properties of silver doped titanium oxide films, *Surf. Coating Technol.* 148 (2001) 65–70.
- [30] H.L. Cao, Y.Q. Qiao, X.Y. Liu, T. Lu, T. Cui, F.H. Meng, et al., Electron storage mediated dark antibacterial action of bound silver nanoparticles: smaller is not always better, *Acta Biomater.* 9 (2013) 5100–5110.
- [31] D. Wojcieszak, M. Mazur, M. Kalisz, M. Grobelny, Influence of Cu, Au and Ag on structural and surface properties of bioactive coatings based on titanium, *Mater. Sci. Eng. C* 71 (2017) 1115–1121.
- [32] D. Sun, D.K. Xu, C.G. Yang, J. Chen, M.B. Shahzad, Z.Q. Sun, et al., Inhibition of *Staphylococcus aureus* biofilm by a copper-bearing 317L-Cu stainless steel and its corrosion resistance, *Mater. Sci. Eng. C* 69 (2016) 744–750.
- [33] Y.F. Zheng, B.B. Zhang, B.L. Wang, Y.B. Wang, L. Li, Q.B. Yang, et al., Introduction of antibacterial function into biomedical TiNi shape memory alloy by the addition of element Ag, *Acta Biomater.* 7 (2011) 2758–2767.
- [34] X.G. Hou, D. Mao, H.Y. Ma, Y.K. Ai, X.L. Zhao, J.H. Deng, et al., Antibacterial ability of Ag-TiO<sub>2</sub> nanotubes prepared by ion implantation and anodic oxidation, *Mater. Lett.* 161 (2015) 309–312.
- [35] X.G. Hou, H.Y. Ma, F. Liu, J.H. Deng, Y.K. Ai, X.L. Zhao, et al., Synthesis of Ag ion-implanted TiO<sub>2</sub> thin films for antibacterial application and photocatalytic performance, *J. Hazard Mater.* 299 (2015) 59–66.
- [36] H. Qin, H.L. Cao, Y.C. Zhao, G.D. Jin, M.Q. Cheng, J.X. Wang, et al., Antimicrobial and osteogenic properties of silver-ion-implanted stainless steel, *ACS Appl. Mater. Interfaces* 7 (2015) 10785–10794.
- [37] J.H. Li, X.Y. Liu, Y.Q. Qiao, H.Q. Zhu, C.X. Ding, Antimicrobial activity and cytocompatibility of Ag plasma-modified hierarchical TiO<sub>2</sub> film on titanium surface, *Colloids Surf. B Biointerfaces* 113 (2014) 134–145.
- [38] X.L. Zhang, W.J. Wang, Y.J. Zhang, T. Zeng, C.H. Jia, L.X. Chang, Loading Cu-doped magnesium oxide onto surface of magnetic nanoparticles to prepare magnetic disinfectant with enhanced antibacterial activity, *Colloids Surf. B Biointerfaces* 161 (2018) 433–441.
- [39] X.H. Yao, X.Y. Zhang, H.B. Wu, L.H. Tian, Y. Ma, B. Tang, Microstructure and antibacterial properties of Cu-doped TiO<sub>2</sub> coating on titanium by micro-arc oxidation, *Appl. Surf. Sci.* 292 (2014) 944–947.
- [40] W. Zhu, Z.X. Zhang, B.B. Gu, J.Y. Sun, L.X. Zhu, Biological activity and antibacterial property of nano-structured TiO<sub>2</sub> coating incorporated with Cu prepared by micro-arc oxidation, *J. Mater. Sci. Technol.* 29 (2013) 237–244.
- [41] Y.Q. Yu, G.D. Jin, Y. Xue, D.H. Wang, X.Y. Liu, J. Sun, Multifunctions of dual Zn/Mg ion co-implanted titanium on osteogenesis, angiogenesis and bacteria inhibition for dental implants, *Acta Biomater.* 49 (2017) 590–603.
- [42] G.D. Jin, H.L. Cao, Y.Q. Qiao, F.H. Meng, H.Q. Zhu, X.Y. Liu, Osteogenic activity and antibacterial effect of zinc ion implanted titanium, *Colloids Surf. B Biointerfaces* 117 (2014) 158–165.
- [43] K.F. Huo, X.M. Zhang, H.R. Wang, L.Z. Zhao, X.Y. Liu, P.K. Chu, Osteogenic activity and antibacterial effects on titanium surfaces modified with Zn-incorporated nanotube arrays, *Biomaterials* 34 (2013) 3467–3478.
- [44] H. Qin, H.L. Cao, Y.C. Zhao, C. Zhu, T. Cheng, Q.J. Wang, et al., In vitro and in vivo anti-biofilm effects of silver nanoparticles immobilized on titanium, *Biomaterials* 35 (2014) 9114–9125.
- [45] I. Burghardt, F. Luthen, C. Prinz, B. Kreikemeyer, C. Zietz, H.G. Neumann, et al., A dual function of copper in designing regenerative implants, *Biomaterials* 44 (2015) 36–44.
- [46] M. Roknian, A. Fattah-alhosseini, S.O. Ghashti, M.K. Keshavarz, Study of the effect of ZnO nanoparticles addition to PEO coatings on pure titanium substrate: microstructural analysis, antibacterial effect and corrosion behavior of coatings in Ringer's physiological solution, *J. Alloys Compd.* 740 (2018) 330–345.
- [47] M. Mirak, M. Alizadeh, M. Ghaffari, M.N. Ashtiani, Characterization, mechanical properties and corrosion resistance of biocompatible Zn-HA/TiO<sub>2</sub> nanocomposite coatings, *J. Mech. Behav. Biomed. Mater.* 62 (2016) 282–290.
- [48] Q.M. Luo, H.L. Cao, L.Y. Wang, X.H. Ma, X.Y. Liu, ZnO@ZnS nanorod-array coated titanium: good to fibroblasts but bad to bacteria, *J. Colloid Interface Sci.* 579 (2020) 50–60.
- [49] T.T. Yang, S. Qian, Y.Q. Qiao, X.Y. Liu, Cytocompatibility and antibacterial activity of titania nanotubes incorporated with gold nanoparticles, *Colloids Surf. B Biointerfaces* 145 (2016) 597–606.
- [50] C.J. Shuai, W. Guo, P. Wu, W.J. Yang, S. Hu, Y. Xia, et al., A graphene oxide-Ag co-dispersing nanosystem: dual synergistic effects on antibacterial activities and mechanical properties of polymer scaffolds, *Chem. Eng. J.* 347 (2018) 322–333.
- [51] K.B.A. Ahmed, T. Raman, A. Veerappan, Future prospects of antibacterial metal nanoparticles as enzyme inhibitor, *Mater. Sci. Eng. C* 68 (2016) 939–947.
- [52] R. Liu, K. Memarzadeh, B. Chang, Y.M. Zhang, Z. Ma, R.P. Allaker, et al., Antibacterial effect of copper bearing titanium alloy (Ti-Cu) against *Streptococcus mutans* and *Porphyromonas gingivalis*, *Sci. Rep.* 6 (2016).
- [53] X.Y. Zhang, G.N. Zhang, M.Z. Chai, X.H. Yao, W.Y. Chen, P.K. Chu, Synergistic antibacterial activity of physical-chemical multi-mechanism by TiO<sub>2</sub> nanorod arrays for safe biofilm eradication on implant, *Bioact. Mater.* 6 (2021) 12–25.
- [54] B. Huang, L. Tan, X.M. Liu, J. Li, S.L. Wu, A facile fabrication of novel stuff with antibacterial property and osteogenic promotion utilizing red phosphorus and near-infrared light, *Bioact. Mater.* 4 (2019) 17–21.
- [55] Q. Zhang, X.M. Liu, L. Tan, Z.D. Cui, Z.Y. Li, Y.Q. Liang, et al., An UV to NIR-driven platform based on red phosphorus/graphene oxide film for rapid microbial inactivation, *Chem. Eng. J.* 383 (2020) 123088.
- [56] X.F. Teng, X.M. Liu, Z.D. Cui, Y.F. Zheng, D.F. Chen, Z.Y. Li, et al., Rapid and highly effective bacteria-killing by polydopamine/IR780/MnO<sub>2</sub>-Ti using near-infrared light, *Prog. Nat. Sci.: Mater. Int.* 30 (2020) 677–685.
- [57] D.L. Han, Y. Li, X.M. Liu, B. Li, Y. Han, Y.F. Zheng, et al., Rapid bacteria trapping and killing of metal-organic frameworks strengthened photo-responsive hydrogel for rapid tissue repair of bacterial infected wounds, *Chem. Eng. J.* (2020) 125194.
- [58] M. Zhu, X.M. Liu, L. Tan, Z.D. Cui, Y.Q. Liang, Z.Y. Li, et al., Photo-responsive chitosan/Ag/MoS<sub>2</sub> for rapid bacteria-killing, *J. Hazard Mater.* (2020) 383.
- [59] S.W. Park, D. Lee, Y.S. Choi, H.B. Jeon, C.-H. Lee, J.-H. Moon, et al., Mesoporous TiO<sub>2</sub> implants for loading high dosage of antibacterial agent, *Appl. Surf. Sci.* 303 (2014) 140–146.
- [60] H.Z. Zhang, Y. Sun, A. Tian, X.X. Xue, L. Wang, A. Alquhali, et al., Improved antibacterial activity and biocompatibility on vancomycin-loaded TiO<sub>2</sub> nanotubes: in vivo and in vitro studies, *Int. J. Nanomed.* 8 (2013) 4379–4389.
- [61] J.J. Tu, M.F. Yu, Y. Lu, K. Cheng, W.J. Weng, J. Lin, et al., Preparation and antibiotic drug release of mineralized collagen coatings on titanium, *J. Mater. Sci. Mater. Med.* 23 (2012) 2413–2423.
- [62] Z.H. Liu, Y.Z. Zhu, X.M. Liu, K.W.K. Yeung, S.L. Wu, Construction of poly (vinyl alcohol)/poly (lactide-glycolide acid)/vancomycin nanoparticles on titanium for enhancing the surface self-antibacterial activity and cytocompatibility, *Colloids Surf. B Biointerfaces* 151 (2017) 165–177.

- [63] K.C. Popat, M. Eltgroth, T.J. Latempa, C.A. Grimes, T.A. Desai, Decreased *Staphylococcus epidermidis* adhesion and increased osteoblast functionality on antibiotic-loaded titania nanotubules, *Biomaterials* 28 (2007) 4880–4888.
- [64] H. Davidson, M. Poon, R. Saunders, I.M. Shapiro, N.J. Hickok, C.S. Adams, Tetracycline tethered to titanium inhibits colonization by Gram-negative bacteria, *J. Biomed. Mater. Res. B Appl. Biomater.* 103 (2015) 1381–1389.
- [65] H.B. Lv, Z. Chen, X.P. Yang, L. Cen, X. Zhang, P. Gao, Layer-by-layer self-assembly of minocycline-loaded chitosan/alginate multilayer on titanium substrates to inhibit biofilm formation, *J. Dent.* 42 (2014) 1464–1472.
- [66] S. He, P. Zhou, L.X. Wang, X.L. Xiong, Y.F. Zhang, Y. Deng, et al., Antibiotic-decorated titanium with enhanced antibacterial activity through adhesive polydopamine for dental/bone implant, *J. R. Soc. Interface* 11 (2014), 20140169.
- [67] M.C. Cortizo, T.G. Oberti, M.S. Cortizo, A.M. Cortizo, M.A. Fernandez Lorenzo de Mele, Chlorhexidine delivery system from titanium/polybenzyl acrylate coating: evaluation of cytotoxicity and early bacterial adhesion, *J. Dent.* 40 (2012) 329–337.
- [68] C.L. Li, J.H. Zhu, Y.Q. Wang, Y.Y. Chen, L.Y. Song, W.M. Zheng, et al., Antibacterial activity of AI-Hemocidin 2, a Novel N-Terminal peptide of hemoglobin purified from arca inflata, *Mar. Drugs* 15 (2017).
- [69] W.C. Wimley, Describing the mechanism of antimicrobial peptide action with the interfacial activity model, *ACS Chem. Biol.* 5 (2010) 905–917.
- [70] M.H. Semreen, M.I. El-Gamal, S. Abdin, H. Alkharaji, L. Kamal, S. Hammad, et al., Recent updates of marine antimicrobial peptides, *Saudi Pharmaceut. J.* 26 (2018) 396–409.
- [71] A.Q. Shi, C.S. Zhu, S. Fu, R.X. Wang, G.W. Qin, D.F. Chen, et al., What controls the antibacterial activity of Ti-Ag alloy, Ag ion or Ti<sub>2</sub>Ag particles? *Mater. Sci. Eng. C* 109 (2020) 110548.
- [72] Y. Li, L.N. Liu, P. Wan, Z.J. Zhai, Z.Y. Mao, Z.X. Ouyang, et al., Biodegradable Mg-Cu alloy implants with antibacterial activity for the treatment of osteomyelitis: in vitro and in vivo evaluations, *Biomaterials* 106 (2016) 250–263.
- [73] J.L. Hu, H.X. Li, X.Y. Wang, L. Yang, M. Chen, R.X. Wang, et al., Effect of ultrasonic micro-arc oxidation on the antibacterial properties and cell biocompatibility of Ti-Cu alloy for biomedical application, *Mater. Sci. Eng. C* (2020) 110921.
- [74] Z.M. Lei, H.Z. Zhang, E.L. Zhang, J.H. You, X.X. Ma, X.Z. Bai, Antibacterial activities and biocompatibilities of Ti-Ag alloys prepared by spark plasma sintering and acid etching, *Mater. Sci. Eng. C* 92 (2018) 121–131.
- [75] H. Liu, R. Liu, I. Ullah, S.Y. Zhang, Z.Q. Sun, L. Ren, et al., Rough surface of copper-bearing titanium alloy with multifunctions of osteogenic ability and antibacterial activity, *J. Mater. Sci. Technol.* 48 (2020) 130–139.
- [76] R. Liu, Y.L. Tang, H. Liu, L.L. Zeng, Z. Ma, J. Li, et al., Effects of combined chemical design (Cu addition) and topographical modification (SLA) of Ti-Cu/SLA for promoting osteogenic, angiogenic and antibacterial activities, *J. Mater. Sci. Technol.* 47 (2020) 202–215.
- [77] D. Campoccia, L. Montanaro, C.R. Arciola, A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces, *Biomaterials* 34 (2013) 8018–8029.
- [78] K.R. Sreekumari, Y. Sato, Y. Kikuchi, Antibacterial metals - a viable solution for bacterial attachment and microbiologically influenced corrosion, *Mater. Trans.* 46 (2005) 1636–1645.
- [79] H. Kawakami, K. Yoshida, Y. Nishida, Y. Kikuchi, Y. Sato, Antibacterial properties of metallic elements for alloying evaluated with application of JIS Z 2801:2000, *ISIJ Int.* 48 (2008) 1299–1304.
- [80] Y. Miyano, K. Koyama, K.R. Sreekumari, Y. Sato, Y. Kikuchi, Evaluation of antibacterial ability of some pure metals, *Tetsu to Hagane-J. Iron Steel Inst. Jpn.* 93 (2007) 57–65.
- [81] F. Heidenau, W. Mittelmeier, R. Detsch, M. Haenle, F. Stenzel, G. Ziegler, et al., A novel antibacterial titania coating: metal ion toxicity and in vitro surface colonization, *J. Mater. Sci. Mater. Med.* 16 (2005) 883–888.
- [82] W.L. Du, S.S. Niu, Y.L. Xu, Z.R. Xu, C.L. Fan, Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions, *Carbohydr. Polym.* 75 (2009) 385–389.
- [83] D.L. Wang, Z.F. Lin, T. Wang, Z.F. Yao, M.N. Qin, S.R. Zheng, et al., Where does the toxicity of metal oxide nanoparticles come from: the nanoparticles, the ions, or a combination of both? *J. Hazard Mater.* 308 (2016) 328–334.
- [84] L. Zhu, J. Elguindi, C. Rensing, S. Ravishanker, Antimicrobial activity of different copper alloy surfaces against copper resistant and sensitive *Salmonella enterica*, *Food Microbiol.* 30 (2012) 303–310.
- [85] G.D. Jin, H.L. Cao, Y.Q. Qiao, F.H. Meng, H.Q. Zhu, X.Y. Liu, Osteogenic activity and antibacterial effect of zinc ion implanted titanium, *Colloids Surf. B Biointerfaces* 117 (2014) 158–165.
- [86] N.M. Franklin, N.J. Rogers, S.C. Apte, G.E. Batley, G.E. Gadd, P.S. Casey, Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl<sub>2</sub> to a freshwater microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility, *Environ. Sci. Technol.* 41 (2007) 8484–8490.
- [87] D. Tie, F. Feyerabend, W.D. Müller, R. Schade, K. Liefeth, K.U. Kainer, et al., Antibacterial biodegradable Mg-Ag alloys, *Eur. Cell. Mater.* 25 (2013) 284–298.
- [88] A. Yamamoto, R. Honma, M. Sumita, Cytotoxicity evaluation of 43 metal salts using murine fibroblasts and osteoblastic cells, *J. Biomed. Mater. Res.* 39 (1998) 331–340.
- [89] J.E. Schaffer, E.A. Nauman, L.A. Stanciu, Cold drawn bioabsorbable ferrous and ferrous composite wires: an evaluation of in vitro vascular cytocompatibility, *Acta Biomater.* 9 (2013) 8574–8584.
- [90] J. Wu, L.Y. Wang, J. He, C.H. Zhu, In vitro cytotoxicity of Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup> and their mixtures on primary human endometrial epithelial cells, *Contraception* 85 (2012) 509–518.
- [91] P.K. Wong, Mutagenicity of heavy metals, *Bull. Environ. Contam. Toxicol.* 40 (1988) 597–603.
- [92] R.J. White, An historical overview of the use of silver in wound management, *Br. J. Nurs.* 10 (2001) S3–S8.
- [93] R.M. Slawson, M.I. Van Dyke, H. Lee, J.T. Trevors, Germanium and silver resistance, accumulation, and toxicity in microorganisms, *Plasmid* 27 (1992) 72–79.
- [94] T.J. Berger, J.A. Spadaro, S.E. Chapin, R.O. Becker, Electrically generated silver ions: quantitative effects on bacterial and mammalian cells, *Antimicrob. Agents Chemother.* 9 (1976) 357–358.
- [95] I. Chopra, The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? *J. Antimicrob. Chemother.* 59 (2007) 587–590.
- [96] M. Riaz, R. Zia, A. Ijaz, T. Hussain, M. Mohsin, A. Malik, Synthesis of monophasic Ag doped hydroxyapatite and evaluation of antibacterial activity, *Mater. Sci. Eng. C* 90 (2018) 308–313.
- [97] A. Ansari, S. Pervez, U. Javed, M.I. Abro, M.A. Nawaz, S.A.U. Qader, et al., Characterization and interplay of bacteriocin and exopolysaccharide-mediated silver nanoparticles as an antibacterial agent, *Int. J. Biol. Macromol.* 115 (2018) 643–650.
- [98] R.M. Patabi, M. Patabi, Antibacterial applications of silver nanoparticles, *Mater. Sci. Forum* 754 (2013) 131–142.
- [99] V.S. Calderon, I. Ferreri, M. Henriques, J.T.M. De Hosson, A. Cavaleiro, S. Carvalho, Nano-galvanic coupling for enhanced Ag<sup>+</sup> release in Zn/CN-Ag films: antibacterial application, *Surf. Coating. Technol.* 298 (2016) 1–6.
- [100] P.N. Catalano, M. Pezzoni, C. Costa, G.J.D.A. Soler-Illia, M.G. Bellino, M. F. Desimone, Optically transparent silver-loaded mesoporous thin film coating with long-lasting antibacterial activity, *Microporous Mesoporous Mater.* 236 (2016) 158–166.
- [101] S. Durdu, S.L. Aktug, K. Korkmaz, E. Yalcin, S. Aktas, Fabrication, characterization and in vitro properties of silver-incorporated TiO<sub>2</sub> coatings on titanium by thermal evaporation and micro-arc oxidation, *Surf. Coating. Technol.* 352 (2018) 600–608.
- [102] M. Rasoulzadehali, H. Namazi, Facile preparation of antibacterial chitosan/graphene oxide-Ag bio-nanocomposite hydrogel beads for controlled release of doxorubicin, *Int. J. Biol. Macromol.* 116 (2018) 54–63.
- [103] S. Ferraris, M. Miola, A. Cochis, B. Azzimonti, L. Rimondini, E. Preneri, et al., In situ reduction of antibacterial silver ions to metallic silver nanoparticles on bioactive glasses functionalized with polyphenols, *Appl. Surf. Sci.* 396 (2017) 461–470.
- [104] M.K. Kang, S.K. Moon, J.S. Kwon, K.M. Kim, K.N. Kim, Antibacterial effect of sand blasted, large-grit, acid-etched treated Ti-Ag alloys, *Mater. Res. Bull.* 47 (2012) 2952–2955.
- [105] M. Chen, E. Zhang, L. Zhang, Microstructure, mechanical properties, bio-corrosion properties and antibacterial properties of Ti-Ag sintered alloys, *Mater. Sci. Eng. C* 62 (2016) 350–360.
- [106] J. Hards, A. Streitburger, H. Ahrens, T. Nusselt, C. Gebert, W. Winkelmann, et al., The influence of elementary silver versus titanium on osteoblasts behaviour in vitro using human osteosarcoma cell lines, *Sarcoma* 2007 (2007) 26539.
- [107] P.W. Li, T.H. Kuo, J.H. Chang, J.M. Yeh, W.H. Chan, Induction of cytotoxicity and apoptosis in mouse blastocysts by silver nanoparticles, *Toxicol. Lett.* 197 (2010) 82–87.
- [108] H.M. Jing, X.Q. Wu, Y.Q. Liu, M.Q. Lu, K. Yang, Z.M. Yao, et al., Antibacterial property of Ce-bearing stainless steels, *J. Mater. Sci.* 42 (2007) 5118–5122.
- [109] Agency UEP. <http://www.epa.gov/pesticides/factsheets/copper-alloy-products.htm>.2008.
- [110] J.O. Noyce, H. Michels, C.W. Keevil, Potential use of copper surfaces to reduce survival of epidemic methicillin-resistant *Staphylococcus aureus* in the healthcare environment, *J. Hosp. Infect.* 63 (2006) 289–297.
- [111] J.O. Noyce, H. Michels, C.W. Keevil, Use of copper cast alloys to control *Escherichia coli* O157 cross-contamination during food processing, *Appl. Environ. Microbiol.* 72 (2006) 4239–4244.
- [112] L.P.T.M. Zevenhuizen, J. Dolfin, E.J. Eshuis, I.J. Scholtenkoerselman, Inhibitory effects of copper on bacteria related to the free ion concentration, *Microb. Ecol.* 5 (1979) 139–146.
- [113] C. Ning, X. Wang, L. Li, Y. Zhu, M. Li, P. Yu, et al., Concentration ranges of antibacterial cations for showing the highest antibacterial efficacy but the least cytotoxicity against mammalian cells: implications for a new antibacterial mechanism, *Chem. Res. Toxicol.* 28 (2015) 1815–1822.
- [114] X. Wang, Study on Antibacterial Properties and Mechanism of Metal Ions, South China university of technology, 2015.
- [115] X.L. Wang, S.X. Liu, M. Li, P. Yu, X. Chu, L.H. Li, et al., The synergistic antibacterial activity and mechanism of multicomponent metal ions-containing aqueous solutions against *Staphylococcus aureus*, *J. Inorg. Biochem.* 163 (2016) 214–220.
- [116] S. Kargozar, M. Montazerian, S. Hamzehlou, H.W. Kim, F. Baino, Mesoporous bioactive glasses: promising platforms for antibacterial strategies, *Acta Biomater.* 81 (2018) 1–19.
- [117] J.S. Fernandes, P. Gentile, R.A. Pires, R.L. Reis, P.V. Hatton, Multifunctional bioactive glass and glass-ceramic biomaterials with antibacterial properties for repair and regeneration of bone tissue, *Acta Biomater.* 59 (2017) 2–11.
- [118] L. Tamayo, M. Azócar, M. Kogan, A. Riveros, M. Páez, Copper-polymer nanocomposites: an excellent and cost-effective biocide for use on antibacterial surfaces, *Mater. Sci. Eng. C* 69 (2016) 1391–1409.
- [119] E.L. Zhang, S. Fu, R.X. Wang, H.X. Li, Y. Liu, Z.Q. Ma, et al., Role of Cu element in biomedical metal alloy design, *Rare Met.* 38 (2019) 476–494.

- [120] P. Wang, Y.H. Yuan, K. Xu, H.S. Zhong, Y.H. Yang, S.Y. Jin, et al., Biological applications of copper-containing materials, *Bioact. Mater.* 6 (2021) 916–927.
- [121] I.F. Scheiber, J.F. Mercer, R. Dringen, Metabolism and functions of copper in brain, *Prog. Neurobiol.* 116 (2014) 33.
- [122] S.J. Jin, L. Ren, K. Yang, Bio-functional Cu containing biomaterials: a new way to enhance bio-adaptation of biomaterials, *J. Mater. Sci. Technol.* 32 (2016) 835–839.
- [123] R. Squitti, F. Tecchio, M. Ventriglia, The role of copper in human diet and risk of dementia, *Cur. Nutr. Rep.* 4 (2015) 114–125.
- [124] N. Gargiulo, A.M. Cusano, F. Causa, D. Caputo, P.A. Netti, Silver-containing mesoporous bioactive glass with improved antibacterial properties, *J. Mater. Sci. Mater. Med.* 24 (2013) 2129–2135.
- [125] H.M. Lin, J. Zhang, F.Y. Qu, J.J. Jiang, P. Jiang, In vitro hydroxyapatite-forming ability and antimicrobial properties of mesoporous bioactive glasses doped with Ti/Ag, *J. Nanomater.* 2013 (2013) 24.
- [126] Y. Zhu, X. Li, J. Yang, S. Wang, H. Gao, N. Hanagata, Composition-structure-property relationships of the CaO-MxOy-SiO<sub>2</sub>-P<sub>2</sub>O<sub>5</sub> (M = Zr, Mg, Sr) mesoporous bioactive glass (MBG) scaffolds, *J. Mater. Chem.* 21 (2011) 9208–9218.
- [127] J.C. Zhang, Y.P. Li, K.N. Yang, X.H. Hao, Effects of Cu<sup>2+</sup> and Cu<sup>+</sup> on the proliferation, differentiation and calcification of primary mouse osteoblasts in vitro, *Chin. J. Inorg. Chem.* 26 (2010) 2251–2258.
- [128] C.T. Wu, Y.H. Zhou, M.C. Xu, P.P. Han, L. Chen, J. Chang, et al., Copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity, *Biomaterials* 34 (2013) 422–433.
- [129] F. Shi, Y.M. Liu, W. Zhi, D.Q. Xiao, H.Y. Li, K. Duan, et al., The synergistic effect of micro/nano-structured and Cu<sup>2+</sup>-doped hydroxyapatite particles to promote osteoblast viability and antibacterial activity, *Biomed. Mater.* 12 (2017), 035006.
- [130] L.M. Klevay, Copper in legumes may lower heart disease risk, *Arch. Intern. Med.* 162 (2002) 1780.
- [131] G.F. Hu, Copper stimulates proliferation of human endothelial cells under culture, *J. Cell. Biochem.* 69 (2015) 326–335.
- [132] G. Giavaresi, P. Torricelli, P.M. Fornasari, R. Giardino, R. Barbucci, G. Leone, Blood vessel formation after soft-tissue implantation of hyaluronan-based hydrogel supplemented with copper ions, *Biomaterials* 26 (2005) 3001–3008.
- [133] X.Y. Zhang, J.F. Li, X. Wang, Y.Y. Wang, R.Q. Hang, X.B. Huang, et al., Effects of copper nanoparticles in porous TiO<sub>2</sub> coatings on bacterial resistance and cytocompatibility of osteoblasts and endothelial cells, *Mater. Sci. Eng. C* 82 (2018) 110–120.
- [134] Y. Zhang, X.Y. Wang, Z.L. Ma, B. Bai, J. Liu, L. Yang, et al., A potential strategy for in-stent restenosis: inhibition of migration and proliferation of vascular smooth muscle cells by Cu ion, *Mater. Sci. Eng. C* 115 (2020) 111090.
- [135] A. Thit, H. Selck, H.F. Bjerregaard, Toxicity of CuO nanoparticles and Cu ions to tight epithelial cells from *Xenopus laevis* (A6): effects on proliferation, cell cycle progression and cell death, *Toxicol. Vitro* 27 (2013) 1596–1601.
- [136] O. Bondarenko, A. Ivask, A. Käkinen, A. Kahru, Sub-toxic effects of CuO nanoparticles on bacteria: kinetics, role of Cu ions and possible mechanisms of action, *Environ. Pollut.* 169 (2012) 81–89.
- [137] C.J. Sarell, S.R. Wilkinson, J.H. Viles, Substoichiometric levels of Cu<sup>2+</sup> ions accelerate the kinetics of fiber formation and promote cell toxicity of amyloid-beta from Alzheimer disease, *J. Biol. Chem.* 285 (2010) 41533–41540.
- [138] M. Fontecave, J.L. Pierre, Iron: metabolism, toxicity and therapy, *Biochimie* 75 (1993) 767–773.
- [139] A. Purnama, H. Hermawan, J. Couet, D. Mantovani, Assessing the biocompatibility of degradable metallic materials: state-of-the-art and focus on the potential of genetic regulation, *Acta Biomater.* 6 (2010) 1800–1807.
- [140] S.B. Goldhaber, Trace element risk assessment: essentiality vs. toxicity, *Regul. Toxicol. Pharmacol.* 38 (2003) 232–242.
- [141] M. Peuster, P. Wohlsein, M. Brüggmann, M. Ehlerding, K. Seidler, C. Fink, et al., A novel approach to temporary stenting: degradable cardiovascular stents produced from corrodible metal - results 6-18 months after implantation into New Zealand white rabbits, *Heart* 86 (2001) 563–569.
- [142] R. Waksman, R. Pakala, R. Baffour, R. Seabron, D. Hellinga, F.O. Tio, Short-term effects of biocorrosible iron stents in porcine coronary arteries, *J. Intervent. Cardiol.* 21 (2008) 15–20.
- [143] M. Peuster, C. Hesse, T. Schloo, C. Fink, P. Beerbaum, C. von Schnakenburg, Long-term biocompatibility of a corrodible peripheral iron stent in the porcine descending aorta, *Biomaterials* 27 (2006) 4955–4962.
- [144] M. Peuster, P. Wohlsein, M. Brüggmann, M. Ehlerding, K. Seidler, C. Fink, et al., A novel approach to temporary stenting: degradable cardiovascular stents produced from corrodible metal-results 6-18 months after implantation into New Zealand white rabbits, *Heart* 86 (2001) 563–569.
- [145] Q. Zhang, P. Cao, Degradable porous Fe-35wt.%Mn produced via powder sintering from NH<sub>4</sub>HCO<sub>3</sub> porogen, *Mater. Chem. Phys.* 163 (2015) 394–401.
- [146] A. Cochis, B. Azzimonti, C. Della Valle, E. De Giglio, N. Bloise, L. Visai, et al., The effect of silver or gallium doped titanium against the multidrug resistant *Acinetobacter baumannii*, *Biomaterials* 80 (2016) 80–95.
- [147] J.P. Yuan, W. Li, C. Wang, Effect of the La alloying addition on the antibacterial capability of 316L stainless steel, *Mater. Sci. Eng. C* 33 (2013) 446–452.
- [148] H.W. Liu, D.F. Xu, K. Yang, H.F. Liu, Y.F. Cheng, Corrosion of antibacterial Cu-bearing 316L stainless steels in the presence of sulfate reducing bacteria, *Corrosion Sci.* 132 (2018) 46–55.
- [149] N.C. Verissimo, B.M. Geilich, H.G. Oliveira, R. Caram, T.J. Webster, Reducing *Staphylococcus aureus* growth on Ti alloy nanostructured surfaces through the addition of Sn, *J. Biomed. Mater. Res. A* 103A (2015) 3757–3763.
- [150] Y. Kang, J. Park, D.W. Kim, H. Kim, Y.C. Kang, Controlling the antibacterial activity of CuSn thin films by varying the contents of Sn, *Appl. Surf. Sci.* 389 (2016) 1012–1016.
- [151] M. Vasanthi, K. Ravichandran, N. Jabena Begum, G. Muruganatham, S. Snega, A. Panneerselvam, et al., Influence of Sn doping level on antibacterial activity and certain physical properties of ZnO films deposited using a simplified spray pyrolysis technique, *Superlattice. Microsc.* 55 (2013) 180–190.
- [152] G.A. Fielding, M. Roy, A. Bandyopadhyay, S. Bose, Antibacterial and biological characteristics of silver containing and strontium doped plasma sprayed hydroxyapatite coatings, *Acta Biomater.* 8 (2012) 3144–3152.
- [153] D. Leung, D.A. Spratt, J. Pratten, K. Gulabivala, N.J. Mordan, A.M. Young, Chlorhexidine-releasing methacrylate dental composite materials, *Biomaterials* 26 (2005) 7145–7153.
- [154] Y.G. Lin, Z.R. Yang, J. Cheng, L.S. Wang, Synthesis, characterization and antibacterial property of strontium half and totally substituted hydroxyapatite nanoparticles, *J. Wuhan Univ. Technol.-Materials Sci. Ed.* 23 (2008) 475–479.
- [155] E. Shorr, A.C. Carter, The usefulness of strontium as an adjuvant to calcium in the remineralization of the skeleton in man, *Bull. Hosp. Jt. Dis.* 13 (1952) 59–66.
- [156] A. Guida, M.R. Towler, J.G. Wall, R.G. Hill, S. Eramo, Preliminary work on the antibacterial effect of strontium in glass ionomer cements, *J. Mater. Sci. Lett.* 22 (2003) 1401–1403.
- [157] M. Looney, H. O'Shea, L. Gunn, D. Crowley, D. Boyd, An evaluation of the processing conditions, structure, and properties (biaxial flexural strength and antibacterial efficacy) of sintered strontium-zinc-silicate glass ceramics, *J. Biomater. Appl.* 27 (2013) 937–947.
- [158] N.D. Ravi, R. Balu, T.S.S. Kumar, Strontium-substituted calcium deficient hydroxyapatite nanoparticles: synthesis, characterization, and antibacterial properties, *J. Am. Ceram. Soc.* 95 (2012) 2700–2708.
- [159] F. Dabsie, G. Gregoire, M. Sixou, P. Sharrock, Does strontium play a role in the cariostatic activity of glass ionomer? *J. Dent.* 37 (2009) 554–559.
- [160] D.S. Brauer, N. Karpukhina, G. Kedia, A. Bhat, R.V. Law, I. Radecka, et al., Bactericidal strontium-releasing injectable bone cements based on bioactive glasses, *J. R. Soc. Interface* 10 (2013).
- [161] M. Bornapour, M. Celikin, M. Pekguleruz, Thermal exposure effects on the in vitro degradation and mechanical properties of Mg-Sr and Mg-Ca-Sr biodegradable implant alloys and the role of the microstructure, *Mater. Sci. Eng. C* 46 (2015) 16–24.
- [162] M. Bornapour, N. Muja, P. Shum-Tim, M. Cerruti, M. Pekguleruz, Biocompatibility and biodegradability of Mg-Sr alloys: the formation of Sr-substituted hydroxyapatite, *Acta Biomater.* 9 (2013) 5319–5330.
- [163] H.S. Brar, J. Wong, M.V. Manuel, Investigation of the mechanical and degradation properties of Mg-Sr and Mg-Zn-Sr alloys for use as potential biodegradable implant materials, *J. Mech. Behav. Biomed. Mater.* 7 (2012) 87–95.
- [164] X.N. Gu, X.H. Xie, N. Li, Y.F. Zheng, L. Qin, In vitro and in vivo studies on a Mg-Sr binary alloy system developed as a new kind of biodegradable metal, *Acta Biomater.* 8 (2012) 2360–2374.
- [165] X.N. Gu, Y.F. Zheng, Y. Cheng, S.P. Zhong, T.F. Xi, In vitro corrosion and biocompatibility of binary magnesium alloys, *Biomaterials* 30 (2009) 484–498.
- [166] Y.M. Shangquan, L.N. Sun, P. Wan, L.L. Tan, C.Y. Wang, X.M. Fan, et al., Comparison study of different coatings on degradation performance and cell response of Mg-Sr alloy, *Mater. Sci. Eng. C* 69 (2016) 95–107.
- [167] Y.M. Shangquan, P. Wan, L.L. Tan, X.M. Fan, L. Qin, K. Yang, Investigation of the inner corrosion layer formed in pulse electrodeposition coating on Mg-Sr alloy and corresponding degradation behavior, *J. Colloid Interface Sci.* 481 (2016) 1–12.
- [168] C.Y. Zhao, F.S. Pan, L. Zhang, H.C. Pan, K. Song, A.T. Tang, Microstructure, mechanical properties, bio-corrosion properties and cytotoxicity of as-extruded Mg-Sr alloys, *Mater. Sci. Eng. C* 70 (2017) 1081–1088.
- [169] K. Chen, X.H. Xie, H.Y. Tang, H. Sun, L. Qin, Y.F. Zheng, et al., In vitro and in vivo degradation behavior of Mg-2Sr-Ca and Mg-2Sr-Zn alloys, *Bioact. Mater.* 5 (2020) 275–285.
- [170] M.M. Almoudi, A.S. Hussein, M.I. Abu Hassan, N. Mohamad Zain, A systematic review on antibacterial activity of zinc against *Streptococcus mutans*, *Saudi Dental J.* 30 (2018) 283–291.
- [171] R.J. Lynch, Zinc in the mouth, its interactions with dental enamel and possible effects on caries; a review of the literature, *Int. Dent. J.* 61 (Suppl 3) (2011) 46–54.
- [172] P. Petrini, C.R. Arciola, I. Pezzali, S. Bozzini, L. Montanaro, M.C. Tanzi, et al., Antibacterial activity of zinc modified titanium oxide surface, *Int. J. Artif. Organs* 29 (2006) 434–442.
- [173] X.Y. Zhang, H.Z. Wang, J.F. Li, X.J. He, R.Q. Hang, X.B. Huang, et al., Corrosion behavior of Zn-incorporated antibacterial TiO<sub>2</sub> porous coating on titanium, *Ceram. Int.* 42 (2016) 17095–17100.
- [174] R.Y. Wang, X.J. He, Y. Gao, X.Y. Zhang, X.H. Yao, B. Tang, Antimicrobial property, cytocompatibility and corrosion resistance of Zn-doped ZrO<sub>2</sub>/TiO<sub>2</sub> coatings on Ti6Al4V implants, *Mater. Sci. Eng. C* 75 (2017) 7–15.
- [175] L. Zhang, Q. Gao, Y. Han, Zn and Ag Co-doped anti-microbial TiO<sub>2</sub> coatings on Ti by micro-arc oxidation, *J. Mater. Sci. Technol.* 32 (2016) 919–924.
- [176] K.Z. Qi, B. Cheng, J.G. Yu, W.K. Ho, Review on the improvement of the photocatalytic and antibacterial activities of ZnO, *J. Alloys Compd.* 727 (2017) 792–820.
- [177] A.B. Djuricic, Y.H. Leung, A.M. Ng, X.Y. Xu, P.K. Lee, N. Degger, et al., Toxicity of metal oxide nanoparticles: mechanisms, characterization, and avoiding experimental artefacts, *Small* 11 (2015) 26–44.
- [178] Y. Li, J.F. Niu, W. Zhang, L.L. Zhang, E.X. Shang, Influence of aqueous media on the ROS-mediated toxicity of ZnO nanoparticles toward green fluorescent protein-

- expressing *Escherichia coli* under UV-365 irradiation, *Langmuir* 30 (2014) 2852–2862.
- [179] H. Hu, W. Zhang, Y. Qiao, X. Jiang, X. Liu, C. Ding, Antibacterial activity and increased bone marrow stem cell functions of Zn-incorporated TiO<sub>2</sub> coatings on titanium, *Acta Biomater.* 8 (2012) 904–915.
- [180] X. Bellanger, P. Billard, R. Schneider, L. Balan, C. Merlin, Stability and toxicity of ZnO quantum dots: interplay between nanoparticles and bacteria, *J. Hazard Mater.* 283 (2015) 110–116.
- [181] T. Kavitha, A.I. Gopalan, K.P. Lee, S.Y. Park, Glucose sensing, photocatalytic and antibacterial properties of graphene-ZnO nanoparticle hybrids, *Carbon* 50 (2012) 2994–3000.
- [182] L. Zhang, Y. Ding, M. Povey, D. York, ZnO nanofluids - a potential antibacterial agent, *Prog. Nat. Sci.* 18 (2008) 939–944.
- [183] M. Yamaguchi, H. Oishi, Y. Suketa, Zinc stimulation of bone protein synthesis in tissue culture: activation of aminoacyl-tRNA synthetase, *Biochem. Pharmacol.* 37 (1988) 4075–4080.
- [184] M. Yamaguchi, H. Oishi, Y. Suketa, Stimulatory effect of zinc on bone formation in tissue culture, *Biochem. Pharmacol.* 36 (1987) 4007–4012.
- [185] M. Yamaguchi, K. Inamoto, Y. Suketa, Effect of essential trace metals on bone metabolism in weanling rats: comparison with zinc and other metals' actions, *Res. Exp. Med.* 186 (1986) 337–342.
- [186] M. Yamaguchi, R. Yamaguchi, Action of zinc on bone metabolism in rats: increases in alkaline phosphatase activity and DNA content, *Biochem. Pharmacol.* 35 (1986) 773–777.
- [187] M.H. Yoo, J.Y. Lee, S.E. Lee, J.Y. Koh, Y.H. Yoon, Protection by pyruvate of rat retinal cells against zinc toxicity in vitro, and pressure-induced ischemia in vivo, *Invest. Ophthalmol. Vis. Sci.* 45 (2004) 1523–1530.
- [188] V. Rodilla, A.T. Miles, W. Jenner, G.M. Hawksworth, Exposure of cultured human proximal tubular cells to cadmium, mercury, zinc and bismuth: toxicity and metallothionein induction, *Chem. Biol. Interact.* 115 (1998) 71–83.
- [189] J. Kubasek, D. Vojtech, E. Jablonska, J. Pospisilova, J. Lipov, T. Ruml, Structure, mechanical characteristics and in vitro degradation, cytotoxicity, genotoxicity and mutagenicity of novel biodegradable Zn-Mg alloys, *Mater. Sci. Eng. C* 58 (2016) 24–35.
- [190] L. Ren, K. Yang, L. Guo, H.W. Chai, Preliminary study of anti-infective function of a copper-bearing stainless steel, *Mater. Sci. Eng. C* 32 (2012) 1204–1209.
- [191] L. Huang, E.M. Fozo, T. Zhang, P.K. Liaw, W. He, Antimicrobial behavior of Cu-bearing Zr-based bulk metallic glasses, *Mater. Sci. Eng. C* 39 (2014) 325–329.
- [192] J.H. Chu, J. Lee, C.C. Chang, Y.C. Chan, M.L. Liou, J.W. Lee, et al., Antimicrobial characteristics in Cu-containing Zr-based thin film metallic glass, *Surf. Coating Technol.* 259 (Part A) (2014) 87–93.
- [193] Z.M. Zhang, G.T. Zheng, H.X. Li, L. Yang, X.Y. Wang, G.W. Qin, et al., Antibacterium influenced corrosion effect of antibacterial Ti-3Cu alloy in *Staphylococcus aureus* suspension for biomedical application, *Mater. Sci. Eng. C* 94 (2019) 376–384.
- [194] B. Bai, E.L. Zhang, J.C. Liu, J.T. Zhu, The anti-bacterial activity of titanium-copper sintered alloy against *Porphyromonas gingivalis* in vitro, *Dent. Mater. J.* 35 (2016) 659–667.
- [195] X.Y. Wang, H. Dong, J. Liu, G.W. Qin, D.F. Chen, E.L. Zhang, In vivo antibacterial property of Ti-Cu sintered alloy implant, *Mater. Sci. Eng. C* 100 (2019) 38–47.
- [196] A. Bekmurzayeva, W.J. Duncanson, H.S. Azevedo, D. Kanayeva, Surface modification of stainless steel for biomedical applications: revisiting a century-old material, *Mater. Sci. Eng. C* 93 (2018) 1073–1089.
- [197] Morihiro H, Katsuhisa M, Naoto O, Sadayuki N. Stainless steel excellent in antibacterial property and designing property. In: Nisshin Steel Co L, editor. Japanese Laid-Open Patent Publication. Japan1996.
- [198] Naoto O, Sadayuki N, Morihiro H. High strength martensitic stainless steel excellent in antibacterial characteristic. In: Nisshin Steel Co L, editor. Japanese Laid-Open Patent Publication. Japan1996.
- [199] S.H. Chen, M.Q. Lu, J.D. Zhang, J.S. Dong, K. Yang, Microstructure and antibacterial properties of Cu-contained antibacterial stainless steel, *Acta Metall. Sin.* 40 (2004) 314–318.
- [200] K. Yang, M.Q. Lu, Antibacterial properties of an austenitic antibacterial stainless steel and its security for human body, *J. Mater. Sci. Technol.* 23 (2007) 333–336.
- [201] L. Nan, Y.Q. Liu, W.C. Yang, H. Xu, Y. Li, M.Q. Lv, et al., Study on antibacterial properties of copper-containing antibacterial stainless steel, *Acta Metall. Sin.* 43 (2007) 1065–1070.
- [202] L. Nan, K. Yang, Effect of Cu addition on antibacterial property of type 200 stainless steel, *Mater. Technol.* 31 (2016) 44–47.
- [203] I.T. Hong, C.H. Koo, Antibacterial properties, corrosion resistance and mechanical properties of Cu-modified SUS 304 stainless steel, *Mater. Sci. Eng. A* 393 (2005) 213–222.
- [204] W.J. Qiu, G. Lin, L.Z. Jiang, G. He, Effect of Cu on properties of antibacterial austenitic stainless steel, *Iron Steel* 44 (2009) 81–84.
- [205] B. Guan, S.H. Hong, C. Schulz, N. Stanford, The microstructure, antimicrobial properties, and corrosion resistance of Cu-bearing strip cast steel, *Adv. Eng. Mater.* 22 (2020).
- [206] L. Nan, W.C. Yang, Y.Q. Liu, H. Xu, Y. Li, M.Q. Lu, et al., Antibacterial mechanism of copper-bearing antibacterial stainless steel against *E.coli*, *J. Mater. Sci. Technol.* 24 (2008) 197–201.
- [207] L. Nan, K. Yang, Cu ions dissolution from Cu-bearing antibacterial stainless steel, *J. Mater. Sci. Technol.* 26 (2010) 941–944.
- [208] M.J. Li, L. Nan, D.K. Xu, G.G. Ren, K. Yang, Antibacterial performance of a Cu-bearing stainless steel against microorganisms in tap water, *J. Mater. Sci. Technol.* 31 (2015) 243–251.
- [209] K.H. Liao, K.L. Ou, H.C. Cheng, C.T. Lin, P.W. Peng, Effect of silver on antibacterial properties of stainless steel, *Appl. Surf. Sci.* 256 (2010) 3642–3646.
- [210] K.R. Sreekumar, K. Nandakumar, K. Takao, Y. Kikuchi, Silver containing stainless steel as a new outlook to abate bacterial adhesion and microbiologically influenced corrosion, *ISIJ Int.* 43 (2003) 1799–1806.
- [211] Q. Wang, L. Ren, X.P. Li, S.Y. Zhang, T.B. Sercombe, K. Yang, Antimicrobial Cu-bearing stainless steel scaffolds, *Mater. Sci. Eng. C* 68 (2016) 519–522.
- [212] Y.F. Zhuang, S.Y. Zhang, K. Yang, L. Ren, K.R. Dai, Antibacterial activity of copper-bearing 316L stainless steel for the prevention of implant-related infection, *J. Biomed. Mater. Res. B Appl. Biomater.* 108 (2020) 484–495.
- [213] T. Xi, M.B. Shahzad, D.K. Xu, Z.Q. Sun, J.L. Zhao, C.G. Yang, et al., Effect of copper addition on mechanical properties, corrosion resistance and antibacterial property of 316L stainless steel, *Mater. Sci. Eng. C* 71 (2017) 1079–1085.
- [214] H.W. Chai, L. Guo, X.T. Wang, Y.P. Fu, J.L. Guan, L.L. Tan, et al., Antibacterial effect of 317L stainless steel contained copper in prevention of implant-related infection in vitro and in vivo, *J. Mater. Sci. Mater. Med.* 22 (2011) 2525–2535.
- [215] J. Zhao, Z.Q. Cao, L. Ren, S.S. Chen, B.C. Zhang, R. Liu, et al., A novel urethral stent material with antibacterial and reducing encrustation properties, *Mater. Sci. Eng. C* 68 (2016) 221–228.
- [216] D. Sun, D.K. Xu, C.G. Yang, M.B. Shahzad, Z.Q. Sun, J. Xia, et al., An investigation of the antibacterial ability and cytotoxicity of a novel Cu-bearing 317L stainless steel, *Sci. Rep.* 6 (2016).
- [217] M. Bahmani-Oskooee, S. Hossein Nejad, A. Samadi, E. Kozeschnik, Cu-bearing, martensitic stainless steels for applications in biological environments, *Mater. Des.* 130 (2017) 442–451.
- [218] Y.Q. Liu, L. Nan, D.M. Chen, K. Yang, Study of a Cu-containing martensitic antibacterial stainless steel, *Rare Met. Mater. Eng.* 37 (2008) 1380–1383.
- [219] S. Wang, C. Yang, M. Shen, K. Yang, Effect of aging on antibacterial performance of Cu-bearing martensitic stainless steel, *Mater. Technol.* 29 (2014) 257–261.
- [220] S. Wang, C.G. Yang, D.K. Xu, M.G. Shen, L. Nan, K. Yang, Effect of heat treatment on antibacterial performance of 3Cr13MoCu martensitic stainless steel, *Acta Metall. Sin.* 50 (2014) 1453–1460.
- [221] S. Wang, K. Yang, M. Shen, C.G. Yang, Effect of Cu content on antibacterial activity of 17-4 PH stainless steel, *Mater. Technol.* 30 (2015) B115–B119.
- [222] L. Nan, J.L. Cheng, K. Yang, Antibacterial behavior of a Cu-bearing type 200 stainless steel, *J. Mater. Sci. Technol.* 28 (2012) 1067–1070.
- [223] S.M. Yang, Y.C. Chen, Y.T. Pan, D.Y. Lin, Effect of silver on microstructure and antibacterial property of 2205 duplex stainless steel, *Mater. Sci. Eng. C* 63 (2016) 376–383.
- [224] Y.T. Lou, L. Lin, D.K. Xu, S.Y. Zhao, C.G. Yang, J.L. Liu, et al., Antibacterial ability of a novel Cu-bearing 2205 duplex stainless steel against *Pseudomonas aeruginosa* biofilm in artificial seawater, *Int. Biodeterior. Biodegrad.* 110 (2016) 199–205.
- [225] J.L. Liu, R. Jia, E.Z. Zhou, Y. Zhao, W.W. Dou, D.K. Xu, et al., Antimicrobial Cu-bearing 2205 duplex stainless steel against MIC by nitrate reducing *Pseudomonas aeruginosa* biofilm, *Int. Biodeterior. Biodegrad.* 132 (2018) 132–138.
- [226] H. Xiang, D. Liu, X. Chen, H. Cao, X. Dong, On the microstructure and mechanical properties of silver-bearing antibacterial CD4MCu duplex stainless steels: solid solution temperature, *Mater. Express* 9 (2019) 1067–1075.
- [227] G. Lin, J.C. Shen, R.M. Wang, The effect of copper on the properties of ferritic antibacterial stainless steel, *Funct. Mater.* 42 (2011) 549–551.
- [228] Q. Xie, Microstructure and Properties of Ferritic Stainless Steel Containing Copper, Northeastern University, 2011.
- [229] W.F. Zhao, J. Shu, H.Y. Bi, Effect of copper on the mechanical property and corrosion behavior of 19Cr-1.6Mo ferritic stainless steel, *J. Iron Steel Res.* 24 (2012) 45–50.
- [230] Z.X. Zhang, Microstructure and Properties of Antibacterial Stainless Steel Containing Copper (Nitrogen), Shanghai Jiaotong University, 2007.
- [231] T. Xi, M. Babar Shahzad, D.K. Xu, J.L. Zhao, C.G. Yang, M. Qi, et al., Copper precipitation behavior and mechanical properties of Cu-bearing 316L austenitic stainless steel: a comprehensive cross-correlation study, *Mater. Sci. Eng. A* 675 (2016) 243–252.
- [232] Q.X. Ran, J. Li, Y.L. Xu, X.S. Xiao, H.F. Yu, L.Z. Jiang, Novel Cu-bearing economical 21Cr duplex stainless steels, *Mater. Des.* 46 (2013) 758–765.
- [233] R.D. Zhao, Effect of Copper Content on Microstructure and Properties of Low-Carbon Martensitic Antibacterial Stainless Steel, Liaoning University of Engineering and Technology, 2004.
- [234] F.F. Luo, Z.H. Tang, S.F. Xiao, Y.L. Xiang, Study on properties of copper-containing austenitic antibacterial stainless steel, *Mater. Technol.* 34 (2019) 525–533.
- [235] L. Nan, Y.Q. Liu, M.Q. Lu, K. Yang, Study on antibacterial mechanism of copper-bearing austenitic antibacterial stainless steel by atomic force microscopy, *J. Mater. Sci. Mater. Med.* 19 (2008) 3057–3062.
- [236] T. Ujiro, S. Satoh, R.W. Staehle, W.H. Smyrl, Effect of alloying Cu on the corrosion resistance of stainless steels in chloride media, *Corrosion Sci.* 43 (2001) 2185–2200.
- [237] J. Jiang, D. Xu, T. Xi, M.B. Shahzad, M.S. Khan, J. Zhao, et al., Effects of aging time on intergranular and pitting corrosion behavior of Cu-bearing 304L stainless steel in comparison with 304L stainless steel, *Corrosion Sci.* 113 (2016) 46–56.
- [238] Y.L. Huang, J.L. Zhao, J.R. Zhang, C.G. Yang, Y. Zhao, K. Yang, Optimized antibacterial treatment for the Cu-bearing 420 stainless steel, *Mater. Technol.* 33 (2018) 699–708.
- [239] J. Jiang, D.K. Xu, T. Xi, M.B. Shahzad, M.S. Khan, J. Zhao, et al., Effects of aging time on intergranular and pitting corrosion behavior of Cu-bearing 304L stainless steel in comparison with 304L stainless steel, *Corrosion Sci.* 113 (2016) 46–56.



- [240] J. Banas, A. Mazurkiewicz, The effect of copper on passivity and corrosion behaviour of ferritic and ferritic-austenitic stainless steels, *Mater. Sci. Eng. A* 277 (2000) 183–191.
- [241] L. Ren, L. Nan, K. Yang, Study of copper precipitation behavior in a Cu-bearing austenitic antibacterial stainless steel, *Mater. Des.* 32 (2011) 2374–2379.
- [242] L. Nan, D.K. Xu, T.Y. Gu, X. Song, K. Yang, Microbiological influenced corrosion resistance characteristics of a 304L-Cu stainless steel against *Escherichia coli*, *Mater. Sci. Eng. C* 48 (2015) 228–234.
- [243] J. Xia, C.G. Yang, D.K. Xu, D. Sun, L. Nan, Z.Q. Sun, et al., Laboratory investigation of the microbiologically influenced corrosion (MIC) resistance of a novel Cu-bearing 2205 duplex stainless steel in the presence of an aerobic marine *Pseudomonas aeruginosa* biofilm, *Biofouling* 31 (2015) 481–492.
- [244] J.P. Yuan, W. Li, Antibacterial 316L stainless steel containing silver and niobium, *Rare Met. Mater. Eng.* 42 (2013) 2004–2008.
- [245] Z.Q. Cao, J. Zhao, K. Yang, Cu-bearing stainless steel reduces cytotoxicity and crystals adhesion after urethral epithelial cells exposing to calcium oxalate monohydrate, *Sci. Rep.* 8 (2018) 14094.
- [246] L. Ren, H.M. Wong, C.H. Yan, K.W. Yeung, K. Yang, Osteogenic ability of Cu-bearing stainless steel, *J. Biomed. Mater. Res. B Appl. Biomater.* 103 (2015) 1433–1444.
- [247] J.Z. Li, L. Ren, S.Y. Zhang, G.G. Ren, K. Yang, Cu-bearing steel reduce inflammation after stent implantation, *J. Mater. Sci. Mater. Med.* 26 (2015) 114.
- [248] J. Zhao, L. Ren, M.X. Liu, T. Xi, B.C. Zhang, K. Yang, Anti-fibrotic function of Cu-bearing stainless steel for reducing recurrence of urethral stricture after stent implantation, *J. Biomed. Mater. Res. B* 106 (2018) 2019–2028.
- [249] J. Zhao, L. Ren, B.C. Zhang, Z.Q. Cao, K. Yang, In vitro study on infectious urethral encrustation resistance of Cu-bearing stainless steel, *J. Mater. Sci. Technol.* 33 (2017) 1604–1609.
- [250] L. Wang, G.Y. Li, L. Ren, X.D. Kong, Y.G. Wang, X.G. Han, et al., Nano-copper-bearing stainless steel promotes fracture healing by accelerating the callus evolution process, *Int. J. Nanomed.* 12 (2017) 8443–8457.
- [251] B. Bai, E.L. Zhang, H. Dong, J. Liu, Biocompatibility of antibacterial Ti-Cu sintered alloy: in vivo bone response, *J. Mater. Sci. Mater. Med.* 26 (2015) 265.
- [252] E.L. Zhang, X.Y. Wang, M. Chen, B. Hou, Effect of the existing form of Cu element on the mechanical properties, bio-corrosion and antibacterial properties of Ti-Cu alloys for biomedical application, *Mater. Sci. Eng. C* 69 (2016) 1210–1221.
- [253] S. Guo, Y.J. Lu, S.Q. Wu, L.L. Liu, M.J. He, C.Q. Zhao, et al., Preliminary study on the corrosion resistance, antibacterial activity and cytotoxicity of selective-laser-melted Ti6Al4V-xCu alloys, *Mater. Sci. Eng. C* 72 (2017) 631–640.
- [254] P. Krakhmalev, I. Yadroitsev, I. Yadroitsava, O. de Smidt, Functionalization of biomedical Ti6Al4V via in situ alloying by Cu during laser powder bed fusion manufacturing, *Materials (Basel)* 10 (2017).
- [255] R. Liu, Y.L. Tang, L.L. Zeng, Y. Zhao, Z. Ma, Z.Q. Sun, et al., In vitro and in vivo studies of anti-bacterial copper-bearing titanium alloy for dental application, *Dent. Mater. : Off. Publ. Acad. Dental Mater.* 34 (2018) 1112–1126.
- [256] Z.Y. Ke, C.B. Yi, L. Zhang, Z.Y. He, J. Tan, Y.H. Jiang, Characterization of a new Ti-13Nb-13Zr-10Cu alloy with enhanced antibacterial activity for biomedical applications, *Mater. Lett.* 253 (2019) 335–338.
- [257] D.Y. Zhang, D. Qiu, M.A. Gibson, Y.F. Zheng, H.L. Fraser, D.H. StJohn, et al., Additive manufacturing of ultrafine-grained high-strength titanium alloys, *Nature* 576 (2019) 91–95.
- [258] S.C. Tao, J.L. Xu, L. Yuan, J.M. Luo, Y.F. Zheng, Microstructure, mechanical properties and antibacterial properties of the microwave sintered porous Ti-3Cu alloys, *J. Alloys Compd.* 812 (2020) 152142.
- [259] X.W. Liu, A. Tian, J.H. You, H.Z. Zhang, L. Wu, X.Z. Bai, et al., Antibacterial abilities and biocompatibilities of Ti-Ag alloys with nanotubular coatings, *Int. J. Nanomed.* 11 (2016) 5743–5755.
- [260] X.G. Wang, J.W. Qiao, F.X. Yuan, R.Q. Hang, X.B. Huang, B. Tang, In situ growth of self-organized Cu-containing nano-tubes and nano-pores on Ti90-xCu10Alx (x=0, 45) alloys by one-pot anodization and evaluation of their antimicrobial activity and cytotoxicity, *Surf. Coating. Technol.* 240 (2014) 167–178.
- [261] J. Liu, X.X. Zhang, H.Y. Wang, F.B. Li, M.Q. Li, K. Yang, et al., The antibacterial properties and biocompatibility of a Ti-Cu sintered alloy for biomedical application, *Biomed. Mater.* 9 (2014).
- [262] J. Liu, F.B. Li, C. Liu, H.Y. Wang, B.R. Ren, K. Yang, et al., Effect of Cu content on the antibacterial activity of titanium-copper sintered alloys, *Mater. Sci. Eng. C* 35 (2014) 392–400.
- [263] Z. Ma, M. Li, R. Liu, L. Ren, Y. Zhang, H. Pan, et al., In vitro study on an antibacterial Ti-5Cu alloy for medical application, *J. Mater. Sci. Mater. Med.* 27 (2016) 91, 2016;27:91.
- [264] M.M. Bao, Y. Liu, X.Y. Wang, L. Yang, S.Y. Li, J. Ren, et al., Optimization of mechanical properties, biocorrosion properties and antibacterial properties of wrought Ti-3Cu alloy by heat treatment, *Bioact. Mater.* 3 (2018) 28–38.
- [265] S. Moniri Javadhesari, S. Alipour, M.R. Akbarpour, Biocompatibility, osseointegration, antibacterial and mechanical properties of nanocrystalline Ti-Cu alloy as a new orthopedic material, *Colloids Surf. B Biointerfaces* 189 (2020) 110889.
- [266] J.H. Wu, K.K. Chen, C.Y. Chao, Y.H. Chang, J.K. Du, Effect of Ti2Cu precipitation on antibacterial property of Ti-5Cu alloy, *Mater. Sci. Eng. C* 108 (2020) 110433.
- [267] L. Fowler, O. Janson, H. Engqvist, S. Norgren, C. Ohman-Magi, Antibacterial investigation of titanium-copper alloys using luminescent *Staphylococcus epidermidis* in a direct contact test, *Mater. Sci. Eng. C* 97 (2019) 707–714.
- [268] M. Chen, L. Yang, L. Zhang, Y. Han, Z. Lu, G.W. Qin, et al., Effect of nano/micro-Ag compound particles on the bio-corrosion, antibacterial properties and cell biocompatibility of Ti-Ag alloys, *Mater. Sci. Eng. C* 75 (2017) 906–917.
- [269] K. Nakajo, M. Takahashi, M. Kikuchi, Y. Takada, O. Okuno, K. Sasaki, et al., Inhibitory effect of Ti-Ag alloy on artificial biofilm formation, *Dent. Mater. J.* 33 (2014) 389–393.
- [270] Z. Ma, L. Ren, R. Liu, K. Yang, Y. Zhang, Z.H. Liao, et al., Effect of heat treatment on Cu distribution, antibacterial performance and cytotoxicity of Ti-6Al-4V-5Cu alloy, *J. Mater. Sci. Technol.* 31 (2015) 723–732.
- [271] Z. Ma, M. Yao, R. Liu, K. Yang, L. Ren, Y. Zhang, et al., Study on antibacterial activity and cytocompatibility of Ti-6Al-4V-5Cu alloy, *Mater. Technol.* 30 (2015) B80–B85.
- [272] C. Peng, S.Y. Zhang, Z.Q. Sun, L. Ren, K. Yang, Effect of annealing temperature on mechanical and antibacterial properties of Cu-bearing titanium alloy and its preliminary study of antibacterial mechanism, *Mater. Sci. Eng. C* 93 (2018) 495–504.
- [273] L. Ren, Z. Ma, M. Li, Y. Zhang, W.Q. Liu, Z.H. Liao, et al., Antibacterial properties of Ti-6Al-4V-xCu alloys, *J. Mater. Sci. Technol.* 30 (2014) 699–705.
- [274] A. Macpherson, X.P. Li, P. McCormick, L. Ren, K. Yang, T.B. Sercombe, Antibacterial titanium produced using selective laser melting, *JOM (J. Occup. Med.)* 69 (2017) 2719–2724.
- [275] K.L. Ou, C.C. Weng, Y.H. Lin, M.S. Huang, A promising of alloying modified beta-type Titanium-Niobium implant for biomedical applications: microstructural characteristics, in vitro biocompatibility and antibacterial performance, *J. Alloys Compd.* 697 (2017) 231–238.
- [276] J.K. Du, C.Y. Chao, K.Y. Chiu, Y.H. Chang, K.K. Chen, J.H. Wu, et al., Antibacterial properties and corrosion resistance of the newly developed biomaterial, Ti-12Nb-1Ag Alloy, *Metals* 7 (2017).
- [277] M. Takahashi, M. Kikuchi, Y. Takada, Mechanical properties and microstructures of dental cast Ti-Ag and Ti-Cu alloys, *Dent. Mater. J.* 21 (2002) 270–280.
- [278] M. Kikuchi, Y. Takada, S. Kiyosue, M. Yoda, M. Woldu, Z. Cai, et al., Mechanical properties and microstructures of cast Ti-Cu alloys, *Dent. Mater.* 19 (2003) 174–181.
- [279] C. Peng, Y. Liu, H. Liu, S.Y. Zhang, C.G. Bai, Y.Z. Wan, et al., Optimization of annealing treatment and comprehensive properties of Cu-containing Ti6Al4V-xCu alloys, *J. Mater. Sci. Technol.* 35 (2019) 2121–2131.
- [280] M. Kikuchi, Y. Takada, S. Kiyosue, M. Yoda, M. Woldu, Z. Cai, et al., Mechanical properties and microstructures of cast Ti-Cu alloys, *Dent. Mater.* 19 (2003) 174–181.
- [281] Y. Alshammari, F. Yang, L. Bolzoni, Low-cost powder metallurgy Ti-Cu alloys as a potential antibacterial material, *J. Mech. Behav. Biomed.* 95 (2019) 232–239.
- [282] J.W. Wang, S.Y. Zhang, Z.Q. Sun, H. Wang, L. Ren, K. Yang, Optimization of mechanical property, antibacterial property and corrosion resistance of Ti-Cu alloy for dental implant, *J. Mater. Sci. Technol.* 35 (2019) 2336–2344.
- [283] P. Mukhopadhyay, V. Singh, A. Bhattacharjee, A.K. Gogia, Effect of nano Ti2Cu precipitates in Ti6Al4V2.5Cu alloy, *Mater. Today: Proc.* 2 (2015) 3580–3585.
- [284] M. Takahashi, M. Kikuchi, Y. Takada, Mechanical properties and microstructures of dental cast Ti-6Nb-4Cu, Ti-18Nb-2Cu, and Ti-24Nb-1Cu alloys, *Dent. Mater. J.* 35 (2016) 564–570.
- [285] B.B. Zhang, Microstructure and Biocompatibility of Ti-Ag Based Alloys, Harbin Engineering University, Harbin, 2011.
- [286] T. Aoki, I.C.I. Okafor, I. Watanabe, M. Hattori, Y. Oda, T. Okabe, Mechanical properties of cast Ti-6Al-4V-xCu alloys, *J. Oral Rehabil.* 31 (2004) 1109–1114.
- [287] M.T. Jovanović, S. Tadić, S. Zec, Z. Mišković, I. Bobić, The effect of annealing temperatures and cooling rates on microstructure and mechanical properties of investment cast Ti-6Al-4V alloy, *Mater. Des.* 27 (2006) 192–199.
- [288] E.L. Zhang, S.Y. Li, J. Ren, L. Zhang, Y. Han, Effect of extrusion processing on the microstructure, mechanical properties, biocorrosion properties and antibacterial properties of Ti-Cu sintered alloys, *Mater. Sci. Eng. C* 69 (2016) 760–768.
- [289] M. Kikuchi, M. Takahashi, O. Okuno, Elastic moduli of cast Ti-Au, Ti-Ag, and Ti-Cu alloys, *Dent. Mater.* 22 (2006) 641–646.
- [290] D.G. Cai, X.T. Zhao, R.X. Wang, G.W. Qin, D.F. Chen, E.L. Zhang, A novel biomedical titanium alloy with high antibacterial property and low elastic modulus, *J. Mater. Sci. Technol.* 81 (2021) 13–25.
- [291] M. Kikuchi, Y. Takada, S. Kiyosue, M. Yoda, M. Woldu, Z. Cai, et al., Grindability of cast Ti-Cu alloys, *Dent. Mater.* 19 (2003) 375–381.
- [292] M. Koike, Z. Cai, Y. Oda, M. Hattori, H. Fujii, T. Okabe, Corrosion behavior of cast Ti-6Al-4V alloyed with Cu, *J. Biomed. Mater. Res.* 73 (2005) 368–374.
- [293] V.G. Pina, V. Amigó, A.I. Muñoz, Microstructural, electrochemical and tribo-electrochemical characterisation of titanium-copper biomedical alloys, *Corrosion Sci.* 109 (2016) 115–125.
- [294] S. Wang, Z. Ma, Z.H. Liao, J. Song, K. Yang, W.Q. Liu, Study on improved tribological properties by alloying copper to CP-Ti and Ti-6Al-4V alloy, *Mater. Sci. Eng. C* 57 (2015) 123–132.
- [295] M.R. Akbarpour, S.M. Javadhesari, Wear performance of novel nanostructured Ti-Cu intermetallic alloy as a potential material for biomedical applications, *J. Alloys Compd.* 699 (2017) 882–886.
- [296] L. Ottria, D. Lauritano, M.A. Bassi, A. Palmieri, V. Candotto, et al., Mechanical, chemical and biological aspects of titanium and titanium alloys in implant dentistry, *J. Biol. Regul. Homeost. Agents* 32 (2018) 81–90.
- [297] W.R. Osório, A. Cremasco, P.N. Andrade, A. Garcia, R. Caram, Electrochemical behavior of centrifuged cast and heat treated Ti-Cu alloys for medical applications, *Electrochim. Acta* 55 (2010) 759–770.
- [298] B.B. Zhang, Y.F. Zheng, Y. Liu, Effect of Ag on the corrosion behavior of Ti-Ag alloys in artificial saliva solutions, *Dent. Mater.* 25 (2009) 672–677.
- [299] M. Takahashi, M. Kikuchi, Y. Takada, T. Okabe, O. Okuno, Electrochemical behavior of cast Ti-Ag alloys, *Dent. Mater.* 25 (2006) 516–523.

- [300] Y. Takada, H. Nakajima, O. Okuno, T. Okabe, Microstructure and corrosion behavior of binary titanium alloys with beta-stabilizing elements, *Dent. Mater.* 20 (2001) 34–52.
- [301] H. Wei, Y.H. Wei, L.F. Hou, N. Dang, Correlation of ageing precipitates with the corrosion behaviour of Cu-4wt.% Ti alloys in 3.5wt.% NaCl solution, *Corrosion Sci.* 111 (2016) 382–390.
- [302] B. Ramezanzadeh, M.M. Attar, Studying the effects of micro and nano sized ZnO particles on the corrosion resistance and deterioration behavior of an epoxy-polyamide coating on hot-dip galvanized steel, *Prog. Org. Coating* 71 (2011) 314–328.
- [303] K.H.W. Seah, R. Thampuran, X. Chen, S.H. Teoh, A comparison between the corrosion behaviour of sintered and unsintered porous titanium, *Corrosion Sci.* 37 (1995) 1333–1340.
- [304] Z. Ma, M. Li, R. Liu, L. Ren, Y. Zhang, H.B. Pan, et al., In vitro study on an antibacterial Ti-5Cu alloy for medical application, *J. Mater. Sci. Mater. Med.* 27 (2016).
- [305] G. Rolla, E. Saxegaard, Critical evaluation of the composition and use of topical fluorides, with emphasis on the role of calcium fluoride in caries inhibition, *Dent Res* 69 (1990) 780–785.
- [306] G.K. Stookey, Critical evaluation of the composition and use of topical fluorides, *Dent Res* 69 (1990) 805–812.
- [307] L. Reclaru, J.M. Meyer, Effects of fluorides on titanium and other dental alloys in dentistry 19 (1998) 85–92.
- [308] M. Nakagawa, S. Matsuya, T. Shiraiishi, M. Ohta, Effect of fluoride concentration and pH on corrosion behavior of titanium for dental use, *J. Dent. Res.* 78 (1999) 1568–1572.
- [309] H.M. Shim, K.T. Oh, J.Y. Woo, C.J. Hwang, K.M. Kim, Corrosion Resistance of titanium-silver alloys in an artificial saliva containing fluoride ions, *J. Biomed. Mater. Res. B Appl. Biomater.* 73B (2005) 252–259.
- [310] I. Mutlu, Electrochemical Corrosion Behavior of TiN-coated biomedical Ti-Cu alloy foam in fluoride containing artificial saliva, *Metall. Mater. Trans.* 45 (2014) 3640–3649.
- [311] M. Takahashi, M. Kikuchi, Y. Takada, Corrosion behavior of Ti-Ag alloys used in dentistry in lactic acid solution, *Met. Mater. Int.* 17 (2011) 175–179.
- [312] E.L. Zhang, L.L. Zheng, J. Liu, B. Bai, C. Liu, Influence of Cu content on the cell biocompatibility of Ti-Cu sintered alloys, *Mater. Sci. Eng. C* 46 (2015) 148–157.
- [313] Y. Li, Y. Zhang, J. He, K. Ma, M. Wang, S. Li, Effects of different copper contents on the adhesion and migration of osteoblasts, *Chin. J. Pract. Stomatol.* 10 (2017) 354–359.
- [314] Z.B. Tang, J.L. Niu, H. Huang, H. Zhang, J. Pei, J.M. Ou, et al., Potential biodegradable Zn-Cu binary alloys developed for cardiovascular implant applications, *J. Mech. Behav. Biomed. Mater.* 72 (2017) 182–191.
- [315] Y. Xie, L.C. Zhao, Z. Zhang, X. Wang, R. Wang, C.X. Cui, Fabrication and properties of porous Zn-Ag alloy scaffolds as biodegradable materials, *Mater. Chem. Phys.* 219 (2018) 433–443.
- [316] R. Yue, J.L. Niu, Y.T. Li, G.Z. Ke, H. Huang, J. Pei, et al., In vitro cytocompatibility, hemocompatibility and antibacterial properties of biodegradable Zn-Cu-Fe alloys for cardiovascular stents applications, *Mater. Sci. Eng. C* 113 (2020) 111007.
- [317] C. Xiao, L.Q. Wang, Y.P. Ren, S.N. Sun, E.L. Zhang, C.N. Yan, et al., Indirectly extruded biodegradable Zn-0.05wt%Mg alloy with improved strength and ductility: in vitro and in vivo studies, *J. Mater. Sci. Technol.* 34 (2018) 1618–1627.
- [318] J.X. Lin, X. Tong, Z.M. Shi, D.C. Zhang, L.S. Zhang, K. Wang, et al., A biodegradable Zn-1Cu-0.1Ti alloy with antibacterial properties for orthopedic applications, *Acta Biomater.* 106 (2020) 410–427.
- [319] W.T. Zhang, P. Li, G. Shen, X.S. Mo, C. Zhou, D. Alexander, et al., Appropriately adapted properties of hot-extruded Zn-0.5Cu-xFe alloys aimed for biodegradable guided bone regeneration membrane application, *Bioact. Mater.* 6 (2021) 975–989.
- [320] H.R. Bakhsheshi-Rad, E. Hamzah, H.T. Low, M. Kasiri-Asgarani, S. Farahany, E. Akbari, et al., Fabrication of biodegradable Zn-Al-Mg alloy: mechanical properties, corrosion behavior, cytotoxicity and antibacterial activities, *Mater. Sci. Eng. C* 73 (2017) 215–219.
- [321] Z.Z. Shi, J. Yu, X.F. Liu, H.J. Zhang, D.W. Zhang, Y.X. Yin, et al., Effects of Ag, Cu or Ca addition on microstructure and comprehensive properties of biodegradable Zn-0.8Mn alloy, *Mater. Sci. Eng. C* 99 (2019) 969–978.
- [322] J.L. Niu, Z.B. Tang, H. Huang, J. Pei, H. Zhang, G.Y. Yuan, et al., Research on a Zn-Cu alloy as a biodegradable material for potential vascular stents application, *Mater. Sci. Eng. C* 69 (2016) 407–413.
- [323] P. Li, C. Schille, E. Schweizer, F. Rupp, A. Heiss, C. Legner, et al., Mechanical characteristics, in vitro degradation, cytotoxicity, and antibacterial evaluation of Zn-4.0Ag alloy as a biodegradable material, *Int. J. Mol. Sci.* 19 (2018).
- [324] G. Katarivas Levy, J. Goldman, E. Aghion, The prospects of zinc as a structural material for biodegradable implants—a review paper, *Metals* 7 (2017) 402.
- [325] M. Sikora-Jasinska, E. Mostaed, A. Mostaed, R. Beanland, D. Mantovani, M. Vedani, Fabrication, mechanical properties and in vitro degradation behavior of newly developed ZnAg alloys for degradable implant applications, *Mater. Sci. Eng. C* 77 (2017) 1170–1181.
- [326] D. Vojtěch, J. Kubásek, J. Šerák, P. Novák, Mechanical and corrosion properties of newly developed biodegradable Zn-based alloys for bone fixation, *Acta Biomater.* 7 (2011) 3515–3522.
- [327] J. Kubásek, D. Vojtěch, E. Jablonská, I. Pospíšilová, J. Lipov, T. Ruml, Structure, mechanical characteristics and in vitro degradation, cytotoxicity, genotoxicity and mutagenicity of novel biodegradable Zn-Mg alloys, *Mater. Sci. Eng. C* 58 (2016) 24–35.
- [328] H. Gong, K. Wang, R. Strich, J.G. Zhou, In vitro biodegradation behavior, mechanical properties, and cytotoxicity of biodegradable Zn-Mg alloy, *J. Biomed. Mater. Res. B Appl. Biomater.* 103 (2015) 1632–1640.
- [329] E. Mostaed, M. Sikora-Jasinska, A. Mostaed, S. Loffredo, A.G. Demir, B. Previtali, et al., Novel Zn-based alloys for biodegradable stent applications: design, development and in vitro degradation, *J. Mech. Behav. Biomed. Mater.* 60 (2016) 581–602.
- [330] H.F. Li, X.H. Xie, Y.F. Zheng, Y. Cong, F.Y. Zhou, K.J. Qiu, et al., Development of biodegradable Zn-1X binary alloys with nutrient alloying elements Mg, Ca and Sr, *Sci. Rep.* 5 (2015) 10719.
- [331] D. Ma, Y. Li, S.C. Ng, H. Jones, Unidirectional solidification of Zn-rich Zn-Cu peritectic alloys—I. Microstructure selection, *Acta Mater.* 48 (2000) 419–431.
- [332] C. Yao, Z. Wang, S.L. Tay, T. Zhu, W. Gao, Effects of Mg on microstructure and corrosion properties of Zn-Mg alloy, *J. Alloys Compd.* 602 (2014) 101–107.
- [333] X.W. Liu, J.K. Sun, F.Y. Zhou, Y.H. Yang, R.C. Chang, K.J. Qiu, et al., Micro-alloying with Mn in Zn-Mg alloy for future biodegradable metals application, *Mater. Des.* 94 (2016) 95–104.
- [334] S.N. Sun, Y.P. Ren, L.Q. Wang, B. Yang, H.X. Li, G.W. Qin, Abnormal effect of Mn addition on the mechanical properties of as-extruded Zn alloys, *Mater. Sci. Eng. A* 701 (2017) 129–133.
- [335] H. Kabir, K. Munir, C. Wen, Y. Li, Recent research and progress of biodegradable zinc alloys and composites for biomedical applications: biomechanical and biocorrosion perspectives, *Bioact. Mater.* 6 (2021) 836–879.
- [336] W. Pachla, S. Przybysz, A. Jarzębska, M. Bieda, K. Sztwiertnia, M. Kulczyk, et al., Structural and mechanical aspects of hypoeutectic Zn-Mg binary alloys for biodegradable vascular stent applications, *Bioact. Mater.* 6 (2021) 26–44.
- [337] S.F. An, Q.M. Gong, Y.H. Huang, Promotive effect of zinc ions on the vitality, migration, and osteogenic differentiation of human dental pulp cells, *Biol. Trace Elem. Res.* 175 (2017) 112–121.
- [338] Y.P. Qiao, W.J. Zhang, P. Tian, F.H. Meng, H.Q. Zhu, X.Q. Jiang, et al., Stimulation of bone growth following zinc incorporation into biomaterials, *Biomaterials* 35 (2014) 6882–6897.
- [339] J. Ma, N. Zhao, D.H. Zhu, Endothelial cellular responses to biodegradable metal zinc, *ACS Biomater. Sci. Eng.* 1 (2015) 1174–1182.
- [340] J.L. Wang, F. Witte, T.F. Xi, Y.F. Zheng, K. Yang, Y.S. Yang, et al., Recommendation for modifying current cytotoxicity testing standards for biodegradable magnesium-based materials, *Acta Biomater.* 21 (2015) 237–249.
- [341] P.K. Bowen, R.J. Guillory 2nd, E.R. Shearier, J.-M. Seitz, J. Drelich, M. Bocks, et al., Metallic zinc exhibits optimal biocompatibility for bioabsorbable endovascular stents, *Mater. Sci. Eng. C* 56 (2015) 467–472.
- [342] H.T. Yang, C. Wang, C.Q. Liu, H.W. Chen, Y.F. Wu, J.T. Han, et al., Evolution of the degradation mechanism of pure zinc stent in the one-year study of rabbit abdominal aorta model, *Biomaterials* 145 (2017) 92–105.
- [343] M.P. Staiger, A.M. Pietak, J. Huadmai, G. Dias, Magnesium and its alloys as orthopedic biomaterials: a review, *Biomaterials* 27 (2006) 1728–1734.
- [344] N. Sezer, Z. Evis, S.M. Kayhan, A. Tahmasebifar, M. Koç, Review of magnesium-based biomaterials and their applications, *J. Magnes. Alloys* 6 (2018) 23–43.
- [345] D.A. Robinson, R.W. Griffith, D. Shechtman, R.B. Evans, M.G. Conzemius, In vitro antibacterial properties of magnesium metal against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, *Acta Biomater.* 6 (2010) 1869–1877.
- [346] L. Ren, X. Lin, L.L. Tan, K. Yang, Effect of surface coating on antibacterial behavior of magnesium based metals, *Mater. Lett.* 65 (2011) 3509–3511.
- [347] J.Y. Lock, E. Wyatt, S. Upadhyayula, A. Whall, V. Nunez, V.I. Vullev, et al., Degradation and antibacterial properties of magnesium alloys in artificial urine for potential resorbable ureteral stent applications, *J. Biomed. Mater. Res.* 102A (2014) 781–792.
- [348] L. Nan, D.K. Xu, T.Y. Gu, X. Song, K. Yang, Microbiological influenced corrosion resistance characteristics of a 304L-Cu stainless steel against *Escherichia coli*, *Mater. Sci. Eng. C* 48 (2015) 228–234.
- [349] F. Witte, J. Fischer, J. Nellesen, H.A. Crostack, V. Kaese, A. Pisch, et al., In vitro and in vivo corrosion measurements of magnesium alloys, *Biomaterials* 27 (2006) 1013–1018.
- [350] P. Hou, C.L. Zhao, P.F. Cheng, H.L. Wu, J.H. Ni, S.X. Zhang, et al., Reduced antibacterial property of metallic magnesium in vivo, *Biomed. Mater.* 12 (2016) 015010.
- [351] Y. Li, G.W. Liu, Z.J. Zhai, L.N. Liu, H.W. Li, K. Yang, et al., Antibacterial properties of magnesium in vitro and in an in vivo model of implant-associated methicillin-resistant *Staphylococcus aureus* infection, *Antimicrob. Agents Chemother.* 58 (2014) 7586–7591.
- [352] M.I. Rahim, R. Eifler, B. Rais, P.P. Mueller, Alkalization is responsible for antibacterial effects of corroding magnesium, *J. Biomed. Mater. Res. A* 103 (2015) 3526–3532.
- [353] J. Chen, W. Peng, L. Zhu, L. Tan, I.P. Etim, X. Wang, et al., Effect of copper content on the corrosion behaviors and antibacterial properties of binary Mg-Cu alloys, *Mater. Technol.* 33 (2018) 145–152.
- [354] G.P. He, Y.H. Wu, Y. Zhang, Y. Zhu, Y. Liu, N. Li, et al., Addition of Zn to the ternary Mg-Ca-Sr alloys significantly improves their antibacterial properties, *J. Mater. Chem. B* 3 (2015) 6676–6689.
- [355] H. Qin, Y.C. Zhao, Z.Q. An, M.Q. Cheng, Q. Wang, T. Cheng, et al., Enhanced antibacterial properties, biocompatibility, and corrosion resistance of degradable Mg-Nd-Zn-Zr alloy, *Biomaterials* 53 (2015) 211–220.
- [356] Z.H. Gao, M.S. Song, R.L. Liu, Y.S. Shen, L. Ward, I. Cole, et al., Improving in vitro and in vivo antibacterial functionality of Mg alloys through micro-alloying with Sr and Ga, *Mater. Sci. Eng. C* 104 (2019) 109926.

- [357] C. Liu, Design and Biomedical Function of New Degradable Magnesium Alloy for Bone Implantation, Nanjing University of Science & Technology, 2016.
- [358] C. Liu, X.K. Fu, H.B. Pan, P. Wan, L. Wang, L.L. Tan, et al., Biodegradable Mg-Cu alloys with enhanced osteogenesis, angiogenesis, and long-lasting antibacterial effects, *Sci. Rep.* 6 (2016).
- [359] C. Zhang, J. Lin, N.Y.T. Nguyen, Y. Guo, C. Xu, C. Seo, et al., Antimicrobial bioresorbable Mg-Zn-Ca alloy for bone repair in a comparison study with Mg-Zn-Sr alloy and pure Mg, *ACS Biomater. Sci. Eng.* 6 (2019) 517–538.
- [360] Z. Liu, R. Schade, B. Luthringer, N. Hort, H. Rothe, S. Muller, et al., Influence of the microstructure and silver content on degradation, cytocompatibility, and antibacterial properties of magnesium-silver alloys in vitro, *Oxid Med Cell Longev* 2017 (2017) 8091265.
- [361] E.K. Brooks, R. Ahn, M.E. Tobias, L.A. Hansen, N.R. Luke-Marshall, L. Wild, et al., Magnesium alloy AZ91 exhibits antimicrobial properties in vitro but not in vivo, *J. Biomed. Mater. Res. B* 106 (2018) 221–227.
- [362] X.D. Yan, P. Wan, L.L. Tan, M.C. Zhao, L. Qin, K. Yang, Corrosion and biological performance of biodegradable magnesium alloys mediated by low copper addition and processing, *Mater. Sci. Eng. C* 93 (2018) 565–581.
- [363] Z. Wang, J. Yang, J.J. Li, Antibacterial properties of new magnesium alloy implanted in vivo, *Res. Tissue Eng. China* 22 (2018) 3451–3455.
- [364] M. Razzaghi, M. Kasiri-Asgarani, H.R. Bakhsheshi-Rad, H. Ghayour, In vitro degradation, antibacterial activity and cytotoxicity of Mg-3Zn-xAg nanocomposites synthesized by mechanical alloying for implant applications, *J. Mater. Eng. Perform.* 28 (2019) 1441–1455.
- [365] S. Li, Study on the Tissue and Antibacterial Properties of Degradable Mg-Zn-Y-Nd-Ag Alloy for Bone Implantation, Zhengzhou University, Henan, 2016.
- [366] C.C. Qu, Microstructure and Properties of Extruded Mg-Zn-Y-Nd-Xcu Alloy for Biological Use, Zhengzhou University, Henan, 2017.
- [367] H.R. Bakhsheshi-Rad, E. Dayaghi, A.F. Ismail, M. Aziz, A. Akhavan-Farid, X. Chen, Synthesis and in-vitro characterization of biodegradable porous magnesium-based scaffolds containing silver for bone tissue engineering, *Trans. Nonferrous Metals Soc. China* 29 (2019) 984–996.
- [368] S.H. Cai, T. Lei, N.F. Li, F.F. Feng, Effects of Zn on microstructure, mechanical properties and corrosion behavior of Mg-Zn alloys, *Mater. Sci. Eng. C* 32 (2012) 2570–2577.
- [369] D. Tie, F. Feyerabend, N. Hort, D. Hoeche, K.U. Kainer, R. Willumeit, et al., In vitro mechanical and corrosion properties of biodegradable Mg-Ag alloys, *Mater. Corrosion-Werkstoffe Und Korrosion* 65 (2014) 569–576.
- [370] J.B. Clark, Transmission electron microscopy study of age hardening in a Mg-5 wt. % Zn alloy, *Acta Metall.* 13 (1965) 1281–1289.
- [371] J. Buha, Reduced temperature (22–100°C) ageing of an Mg-Zn alloy, *Mater. Sci. Eng. A* 492 (2008) 11–19.
- [372] Z.M. Shi, G.L. Song, A. Atrons, Corrosion resistance of anodised single-phase Mg alloys, *Surf. Coating. Technol.* 201 (2006) 492–503.
- [373] X.B. Liu, D.Y. Shan, Y.W. Song, E.H. Han, Effects of heat treatment on corrosion behaviors of Mg-3Zn magnesium alloy, *Trans. Nonferrous Metals Soc. China* 20 (2010) 1345–1350.
- [374] P. Yin, N.F. Li, T. Lei, L. Liu, C. Ouyang, Effects of Ca on microstructure, mechanical and corrosion properties and biocompatibility of Mg-Zn-Ca alloys, *J. Mater. Sci. Mater. Med.* 24 (2013) 1365–1373.
- [375] I.S. Berglund, H.S. Brar, N. Dolgova, A.P. Acharya, B.G. Keselowsky, M. Samtiranont, et al., Synthesis and characterization of Mg-Ca-Sr alloys for biodegradable orthopedic implant applications, *J. Biomed. Mater. Res. B* 100 (2012) 1524–1534.
- [376] Z. Zhen, X.L. Liu, T. Huang, T.F. Xi, Y.F. Zheng, Hemolysis and cytotoxicity mechanisms of biodegradable magnesium and its alloys, *Mater. Sci. Eng. C* 46 (2015) 202–206.
- [377] J. Fischer, D. Profrock, N. Hort, R. Willumeit, F. Feyerabend, Reprint of: improved cytotoxicity testing of magnesium materials, *Mater. Sci. Eng.*, B 176 (2011) 1773–1777.
- [378] Y. Sun, H.L. Wu, W.H. Wang, R. Zan, H.Z. Peng, S.X. Zhang, et al., Translational status of biomedical Mg devices in China, *Bioact. Mater.* 4 (2019) 358–365.
- [379] M.I. Rahim, M. Rohde, B. Ravis, J.M. Seitz, P.P. Mueller, Susceptibility of metallic magnesium implants to bacterial biofilm infections, *J. Biomed. Mater. Res. A* 104 (2016) 1489–1499.
- [380] S. Wang, C.G. Yang, L. Ren, M.G. Shen, K. Yang, Study on antibacterial performance of Cu-bearing cobalt-based alloy, *Mater. Lett.* 129 (2014) 88–90.
- [381] F.L. Jiang, W.W. Zhu, C.C. Zhao, Y.L. Li, F.Z. Ren, A strong, wear- and corrosion-resistant, and antibacterial Co-30 at.% Cr-5 at.% Ag ternary alloy for medical implants, *Mater. Des.* 184 (2019) 108190.
- [382] A.H. Hosman, H.Cvd Mei, S.K. Bulstra, R. Kuijter, H.J. Busscher, D. Neut, Influence of Co-Cr particles and Co-Cr ions on the growth of staphylococcal biofilms, *Int. J. Artif. Organs* 34 (2011) 759–765.
- [383] L. Ren, K. Memarzadeh, S. Zhang, Z. Sun, C. Yang, G. Ren, et al., A novel coping metal material CoCrCu alloy fabricated by selective laser melting with antimicrobial and antibiofilm properties, *Mater. Sci. Eng. C* 67 (2016) 461–467.
- [384] Y.J. Lu, L. Ren, X.C. Xu, Y. Yang, S.Q. Wu, J.S. Luo, et al., Effect of Cu on microstructure, mechanical properties, corrosion resistance and cytotoxicity of CoCrW alloy fabricated by selective laser melting, *J. Mech. Behav. Biomed. Mater.* 81 (2018) 130–141.
- [385] Y.J. Lu, C.Q. Zhao, L. Ren, S. Guo, Y.L. Gan, C.G. Yang, et al., Preliminary assessment of metal-porcelain bonding strength of CoCrW alloy after 3 wt.% Cu addition, *Mater. Sci. Eng. C* 63 (2016) 37–45.
- [386] R.X. Wang, R.X. Wang, D.F. Chen, G.W. Qin, E.L. Zhang, Novel CoCrWNI alloys with Cu addition: microstructure, mechanical properties, corrosion properties and biocompatibility, *J. Alloys Compd.* 824 (2020) 153924.
- [387] R.X. Wang, G.W. Qin, E.L. Zhang, Effect of Cu on Martensite Transformation of CoCrMo alloy for biomedical application, *J. Mater. Sci. Technol.* 52 (2020) 127–135.
- [388] D.G. Barceloux, Cobalt, *J. Toxicol. Clin. Toxicol.* 37 (1999) 201–206.
- [389] D.J. Paustenbach, B.E. Tvermoes, K.M. Unice, B.L. Finley, B.D. Kerger, A review of the health hazards posed by cobalt, *Crit. Rev. Toxicol.* 43 (2013) 316–362.
- [390] B.E. Tvermoes, D.J. Paustenbach, B.D. Kerger, B.L. Finley, K.M. Unice, Review of cobalt toxicokinetics following oral dosing: implications for health risk assessments and metal-on-metal hip implant patients, *Crit. Rev. Toxicol.* 45 (2015) 367–387.
- [391] M. Pettersson, A. Johansson, M.M. Thorén, Inflammatory response by Ti, Co-Cr-Mo in cultures of human macrophages, *Dent. Mater.* 28 (Supplement 1) (2012) e32.
- [392] G. Grass, C. Rensing, M. Solioz, Metallic copper as an antimicrobial surface, *Appl. Environ. Microbiol.* 77 (2011) 1541–1547.
- [393] S.A. Wilks, H. Michels, C.W. Keevil, The survival of *Escherichia coli* O157 on a range of metal surfaces, *Int. J. Food Microbiol.* 105 (2005) 445–454.
- [394] H.T. Michels, S.A. Wilks, J.O. Noyce, C.W. Keevil, Copper alloys for human infectious disease control, in: *Material Science and Technology Conference, Editor. Copper for the 21st Century Symposium, 2005. Pittsburgh, PA. September 25–28.*
- [395] S. Mehtar, I. Wiid, S.D. Todorov, The antimicrobial activity of copper and copper alloys against nosocomial pathogens and *Mycobacterium tuberculosis* isolated from healthcare facilities in the Western Cape: an in-vitro study, *J. Hosp. Infect.* 68 (2008) 45–51.
- [396] B. Yousuf, J.J. Ahire, L.M.T. Dicks, Understanding the antimicrobial activity behind thin- and thick-rolled copper plates, *Appl. Microbiol. Biotechnol.* 100 (2016) 5569–5580.
- [397] F. Marais, S. Mehtar, L. Chalkley, Antimicrobial efficacy of copper touch surfaces in reducing environmental bioburden in a South African community healthcare facility, *J. Hosp. Infect.* 74 (2010) 80–82.
- [398] A. Mikolay, S. Huggett, L. Tikana, G. Grass, J. Braun, D.H. Nies, Survival of bacteria on metallic copper surfaces in a hospital trial, *Appl. Microbiol. Biotechnol.* 87 (2010) 1875–1879.
- [399] H. Palza, M. Nuñez, R. Bastías, K. Delgado, In situ antimicrobial behavior of materials with copper-based additives in a hospital environment, *Int. J. Antimicrob. Agents* 51 (2018) 912–917.
- [400] G. Borkow, J. Gabbay, Copper, an ancient remedy returning to fight microbial, fungal and viral infections, *Curr. Chem. Biol.* 3 (2009) 272–278.
- [401] C.E. Santo, P.V. Morais, G. Grass, Isolation and characterization of bacteria resistant to metallic copper surfaces, *Appl. Environ. Microbiol.* 76 (2010) 1341–1348.
- [402] M.D. Demetriou, A. Wiest, D.C. Hofmann, W.L. Johnson, B. Han, N. Wolfson, et al., Amorphous metals for hard-tissue prosthesis, *JOM* 62 (2010) 83–91.
- [403] S. Buzzi, K. Jin, P.J. Uggowitzer, S. Tosatti, I. Gerber, J.F. Löffler, Cytotoxicity of Zr-based bulk metallic glasses, *Intermetallics* 14 (2006) 729–734.
- [404] Y. Sun, Y. Huang, H. Fan, Y. Wang, Z. Ning, F. Liu, et al., In vitro and in vivo biocompatibility of an Ag-bearing Zr-based bulk metallic glass for potential medical use, *J. Non-Cryst. Solids* 419 (2015) 82–91.
- [405] J. Horton, D. Parsell, Biomedical potential of a zirconium-based bulk metallic glass, *MRS Proc.* (2011) 754.
- [406] B. Lin, R. Mu, L. Yang, X. Bian, Antibacterial effect of metallic glasses, *Chin. Sci. Bull.* 57 (2012) 1069–1072.
- [407] J. Latuch, P. Blyskun, T. Kulik, Influence of Silver on the Glass Forming Ability and Mechanical Properties in Cu-Zr-Ti-Ag Bulk Metallic Glasses, *Wydawnictwo SIGMA-NOT*, 2012.
- [408] S. Lesz, R. Babilas, R. Nowosielski, Influence of copper addition on glass forming ability, thermal stability, structure and magnetic properties of Fe-Co-Based BMGs, *Solid State Phenom.* 203–204 (2013) 296–301.
- [409] J.P. Chu, J.S.C. Jang, J.C. Huang, H.S. Chou, Y. Yang, J.C. Ye, et al., Thin film metallic glasses: unique properties and potential applications, *Thin Solid Films* 520 (2012) 5097–5122.
- [410] P.T. Chiang, G.J. Chen, S.R. Jian, Y.H. Shih, J.S.C. Jang, C.H. Lai, Surface antimicrobial effects of Zr61Al7.5Ni10Cu17.5Si4 thin film metallic glasses on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *acinetobacter baumannii* and *Candida albicans*, *Fooyin Journal of Health Sciences* 2 (2010) 12–20.
- [411] J.P. Chu, T.Y. Liu, C.L. Li, C.H. Wang, J.S.C. Jang, M.J. Chen, et al., Fabrication and characterizations of thin film metallic glasses: antibacterial property and durability study for medical application, *Thin Solid Films* 561 (2014) 102–107.
- [412] H.W. Chen, K.C. Hsu, Y.C. Chan, J.G. Duh, J.W. Lee, J.S.C. Jang, et al., Antimicrobial properties of Zr-Cu-Al-Ag thin film metallic glass, *Thin Solid Films* 561 (2014) 98–101.
- [413] B. Subramanian, S. Maruthamuthu, S.T. Rajan, Biocompatibility evaluation of sputtered zirconium-based thin film metallic glass-coated steels, *Int. J. Nanomed.* 10 (Suppl 1) (2015) 17–29.
- [414] Y. Liu, J. Padmanabhan, B. Cheung, J.B. Liu, Z. Chen, B.E. Scanley, et al., Combinatorial development of antibacterial Zr-Cu-Al-Ag thin film metallic glasses, *Sci. Rep.* 6 (2016).
- [415] P.H. Tsai, Y.Z. Lin, J.B. Li, S.R. Jian, J.S.C. Jang, C. Li, et al., Sharpness improvement of surgical blade by means of ZrCuAlAgSi metallic glass and metallic glass thin film coating, *Intermetallics* 31 (2012) 127–131.
- [416] M. Apreutesei, P. Steyer, L. Joly-Pottuz, A. Billard, J. Qiao, S. Cardinal, et al., Microstructural, thermal and mechanical behavior of co-sputtered binary Zr-Cu thin film metallic glasses, *Thin Solid Films* 561 (2014) 53–59.

- [417] A. Etienneble, C. Der Loughian, M. Apreutesei, C. Langlois, S. Cardinal, J. M. Pelletier, et al., Innovative Zr-Cu-Ag thin film metallic glass deposited by magnetron PVD sputtering for antibacterial applications, *J. Alloys Compd.* 707 (2017) 155–161.
- [418] Y.M. Zhang, Y.Q. Cheng, W.C. Li, X.H. Jie, Effects of antibacterial heat treatment on cold workability of SUSXM7 Cu-bearing stainless steel, *Hot Work. Technol.* 42 (2013) 181–183.
- [419] K.Q. Li, C. Xia, Y.Q. Qiao, X.Y. Liu, Dose-response relationships between copper and its biocompatibility/antibacterial activities, *J. Trace Elem. Med. Biol.* 55 (2019) 127–135.
- [420] Z.M. Xiu, J. Ma, P.J.J. Alvarez, Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions, *Environ. Sci. Technol.* 45 (2011) 9003–9008.
- [421] D. Quaranta, T. Krans, C.E. Santo, C.G. Elowsky, D.W. Domaille, C.J. Chang, et al., Mechanisms of contact-mediated killing of yeast cells on dry metallic copper surfaces, *Appl. Environ. Microbiol.* 77 (2011) 416–426.
- [422] E. Padan, E. Bibi, M. Ito, T.A. Krulwich, Alkaline pH homeostasis in bacteria: new insights, *Biochim. Biophys. Acta Biomembr.* 1717 (2005) 67–88.
- [423] R.A. Burne, R.E. Marquis, Alkali production by oral bacteria and protection against dental caries, *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 193 (2000) 1–6.
- [424] C.X. Dong, J. Cairney, Q.H. Sun, O.L. Maddan, G.H. He, Y.L. Deng, Investigation of Mg(OH)<sub>2</sub> nanoparticles as an antibacterial agent, *J. Nanoparticle Res.* 12 (2010) 2101–2109.
- [425] T. Jin, Y.P. He, Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens, *J. Nanoparticle Res.* 13 (2011) 6877–6885.
- [426] S. Chernousova, M. Epple, Silver as antibacterial agent: ion, nanoparticle, and metal, *Angew. Chem. Int. Ed.* 52 (2013) 1636–1653.
- [427] F. Paladini, M. Pollini, A. Sannino, L. Ambrosio, Metal-based antibacterial substrates for biomedical applications, *Biomacromolecules* 16 (2015) 1873–1885.
- [428] AF de Faria, D.S.T. Martinez, S.M.M. Meira, A.C.M. de Moraes, A. Brandelli, A.G. S. Filho, et al., Anti-adhesion and antibacterial activity of silver nanoparticles supported on graphene oxide sheets, *Colloids Surf. B Biointerfaces* 113 (2014) 115–124.
- [429] J.H. Li, G. Wang, H.Q. Zhu, M. Zhang, X.H. Zheng, Z.F. Di, et al., Antibacterial activity of large-area monolayer graphene film manipulated by charge transfer, *Sci. Rep.* 4 (2014) 4359.
- [430] J.H. Li, H.J. Zhou, S. Qian, Z.W. Liu, J.W. Feng, P. Jin, et al., Plasmonic gold nanoparticles modified titania nanotubes for antibacterial application, *Appl. Phys. Lett.* 104 (2014).
- [431] G.M. Wang, W.H. Jin, A.M. Qasim, A. Gao, X. Peng, W. Li, et al., Antibacterial effects of titanium embedded with silver nanoparticles based on electron-transfer-induced reactive oxygen species, *Biomaterials* 124 (2017) 25–34.
- [432] S.Y. Reece, D.G. Nocera, Proton-coupled electron transfer in biology: results from synergistic studies in natural and model systems, *Annu. Rev. Biochem.* 78 (2009) 673–699.
- [433] D.R. Lovley, J.D. Coates, E.L. Blunt-Harris, E.J.P. Phillips, J.C. Woodward, Humic substances as electron acceptors for microbial respiration, *Nature* 382 (1996) 445–448.
- [434] M.E. Hernandez, D.K. Newman, Extracellular electron transfer, *Cellular and Molecular Life Sciences CMLS* 58 (2001) 1562–1571.
- [435] J.A. Lemire, J.J. Harrison, R.J. Turner, Antimicrobial activity of metals: mechanisms, molecular targets and applications, *Nat. Rev. Microbiol.* 11 (2013) 371.
- [436] X.R. Zhang, C.G. Yang, K. Yang, Contact killing of Cu-bearing stainless steel based on charge transfer caused by the microdomain potential difference, *ACS Appl. Mater. Interfaces* 12 (2020) 361–372.
- [437] W.J. Qiu, Process and Properties of Copper-Containing Austenite Antibacterial Stainless Steel, Shanghai Jiaotong University, 2009.
- [438] H.C. van der Mei, J. Atema-Smit, D. Jager, D.E. Langworthy, D.I. Collias, M. D. Mitchell, et al., Influence of adhesion to activated carbon particles on the viability of waterborne pathogenic bacteria under flow, *Biotechnol. Bioeng.* 100 (2008) 810–813.
- [439] Qi LF, Xu ZR, Jiang X, Hu CH, Zou XF. Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydr. Res.* 339:2693-27002004.
- [440] B. Gottenbos, D.W. Grijpma, H.J. Busscher, H.C. van der Mei, J. Feijen, Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria, *J. Antimicrob. Chemother.* 48 (2001) 7–13.
- [441] H. Murata, R.R. Koepsel, K. Matyjaszewski, A.J. Russell, Permanent, non-leaching antibacterial surfaces—2: how high density cationic surfaces kill bacterial cells, *Biomaterials* 28 (2007) 4870–4879.
- [442] H. Strahl, L.W. Hamoen, Membrane potential is important for bacterial cell division, *Proc. Natl. Acad. Sci. U.S.A.* 107 (2010) 12281–12286.
- [443] Y.M. Zhang, C.O. Rock, Membrane lipid homeostasis in bacteria, *Nat. Rev. Microbiol.* 6 (2008) 222–233.
- [444] C.E. Santo, E.W. Lam, C.G. Elowsky, D. Quaranta, D.W. Domaille, C.J. Chang, et al., Bacterial killing by dry metallic copper surfaces, *Appl. Environ. Microbiol.* 77 (2011) 794.