



Biological applications of copper-containing materials

Peng Wang^{a,1}, Yonghui Yuan^{b,1}, Ke Xu^a, Hongshan Zhong^a, Yinghui Yang^d, Shiyu Jin^a,
Ke Yang^{c,**}, Xun Qi^{a,*}

^a Key Laboratory of Diagnostic Imaging and Interventional Radiology of Liaoning Province, Department of Radiology, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning, 110001, China

^b Clinical Research Center for Malignant Tumor of Liaoning Province, Cancer Hospital of China Medical University Liaoning Cancer Hospital & Institute, Shenyang, Liaoning, 110042, China

^c Institute of Metal Research, Chinese Academy of Science, Shenyang 110016, China

^d Suzhou Silvan Medical Co., Ltd, Suzhou 215006, China

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ABSTRACT

Copper is an indispensable trace metal element in the human body, which is mainly absorbed in the stomach and small intestine and excreted into the bile. Copper is an important component and catalytic agent of many enzymes and proteins in the body, so it can influence human health through multiple mechanisms. Based on the biological functions and benefits of copper, an increasing number of researchers in the field of biomaterials have focused on developing novel copper-containing biomaterials, which exhibit unique properties in protecting the cardiovascular system, promoting bone fracture healing, and exerting antibacterial effects. Copper can also be used in promoting incisional wounds healing, killing cancer cells, Positron Emission Tomography (PET) imaging, radioimmunological tracing and radiotherapy of cancer. In the present review, the biological functions of copper in the human body are presented, along with an overview of recent progress in our understanding of the biological applications and development of copper-containing materials. Furthermore, this review also provides the prospective on the challenges of those novel biomaterials for future clinical applications.

1. Introduction

Trace elements are elements present in the body but at levels less than one-thousandth of the body weight in humans. There are more than 70 different trace elements in the human body. Although their levels in the body are very low, they have important biological effects [1–3].

Copper (Cu), a trace element that is indispensable in organisms, cannot be produced and synthesized in the body and thus needs to be obtained in food. The World Health Organization (WHO) recommends an upper limit of 2–3 mg Cu for adults every day [4,5]. Cu plays important roles in the growth and development of the body, as well as in maturation of the nervous, hematopoietic, bone, and other systems [6,7]. Cu is also an important component of enzymes involved in the metabolism of glucose, amino acids, and cholesterol, and plays a unique role in various catalytic reactions [8,9]. As with other trace elements, it is necessary for Cu to be maintained in equilibrium in the body.

Abnormal Cu metabolism or content can cause numerous diseases, such as those associated with deteriorated immune function, diabetes, coronary heart disease, and osteoporosis [10].

In recent years, based on the important roles of Cu in the body, an increasing number of researchers have attempted to use the advantageous effects of Cu to develop new biomedical materials to benefit the human body. More and more studies showed that Cu-containing metallic biomaterials showed excellent properties in protecting the cardiovascular system, exerting antibacterial effects, and promoting bone fracture healing [5,11,12].

In this review, we summarize the current biological functions of Cu in the human body and focus on the development and biological applications of Cu-containing biomaterials. We hope that this review can help researchers from different fields to know the attractive properties of copper-containing biomaterials in biological applications so as to further expand the applications of those novel biomaterials.

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* Corresponding author.

** Corresponding author.

E-mail addresses: kyang@imr.ac.cn (K. Yang), qixun716@hotmail.com (X. Qi).

¹ Peng Wang and Yonghui Yuan equally contributed to this work.

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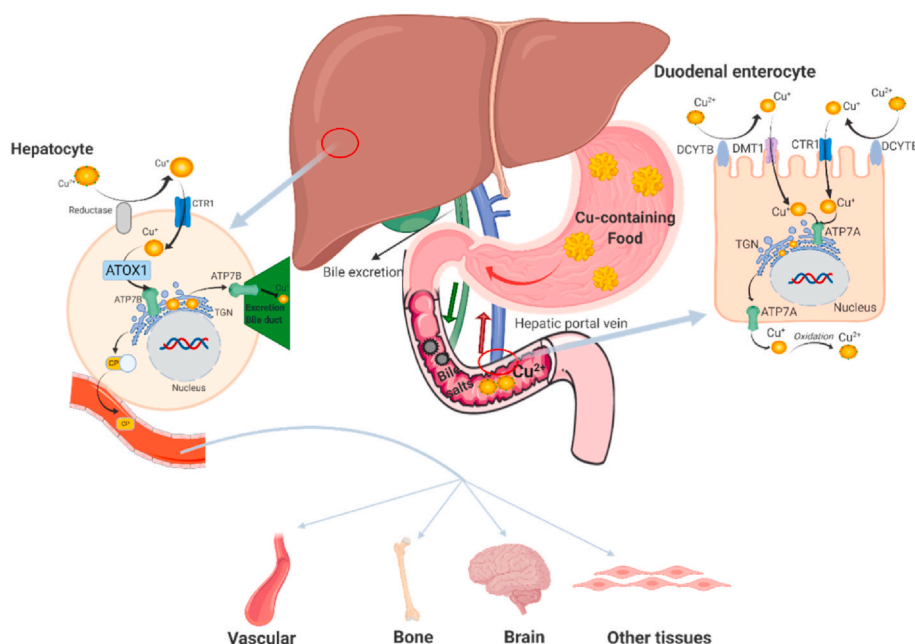


Fig. 1. Copper metabolism in human body. Copper is supplied entirely by food in the human body, which is mainly absorbed in stomach and small intestine, especially in duodenum. In the duodenal enterocyte, Cu^{2+} requires reduction prior to absorption, which may be mediated by DCYTB (duodenal cytochrome B). After reduction, Cu^+ is transported into enterocytes by CTR1 (copper transporter 1) or DMT1 (divalent metal-ion transporter 1). Then copper is pumped into the TGN (trans Golgi network) by ATP7A, supporting cuproenzyme synthesis, or exported from enterocyte, Cu^+ is spontaneously oxidized into Cu^{2+} by dissolved oxygen in blood, then mainly bounded to albumin and $\alpha 2$ -macroglobulin in the portal blood and finally delivered to the liver. In the hepatocyte, firstly, Cu^{2+} requires a reduction, which may be mediated by reductase. After reduction, Cu^+ is taken up into hepatocytes via CTR1. Then, ATOX1 (antioxidant protein 1) delivers Cu^+ to ATP7B, which transports Cu^+ into TGN for incorporation into CP (cuproenzymes). ATP7B also transports excessive Cu^+ across the canalicular membrane into bile for excretion. CP, which is released into blood, is transported to blood vessels, brain, bone and other tissues for biological functions.

2. Cu, an essential trace element

The total amount of Cu in normal adults is 50–150 mg, of which 50%–70% is distributed in muscle and bone, 20% in liver, and 5%–10% in blood. Cu mainly exists in the ceruloplasmin and cuprase *in vivo*, and there are few free Cu ions in the body [4,13]. Cu in the diet is mainly absorbed in the stomach and small intestine, especially the duodenum, which absorbs about 40% of the total [11,14]. After absorption, Cu is transported into the liver and throughout the body through the blood. Apart for some Cu-containing proteins are stored in the liver, and the rest are synthesized in liver or other human tissues, such as cytochrome oxygenase and monoamine enzyme [8,15]. More than 90 different enzymes and proteins have been found to contain Cu to various extents [16,17]. Cu metabolism is also regulated according to the physiological demand, but the mechanisms involved have yet to be elucidated [13,17–20]. There is a complex system of Cu transport and regulation in the human body, the most important of which is to maintain the balance of Cu in the body by intestinal absorption and biliary excretion [21,22]. Especially in the biliary tract, the concentrations of Cu are controlled by excretion into the bile; the biliary Cu is complexed with bile salts and thus cannot be reabsorbed in the intestinal tract [23] (See Fig. 1).

It is becoming increasingly understood that the homeostasis of Cu, which is regulated by complex mechanisms in the human body, is important in human health. Both the overload and the deficiency of Cu can influence human health through multiple mechanisms [24,25]. Disorders featuring overload of Cu mainly include two aspects: (1) acute Cu toxicity (the acute toxic reaction caused by exposure to a large number of copper-containing substances in a short period of time) and (2) chronic Cu toxicity (the chronic toxic reaction caused by long-term repeated entry of low-dose copper containing substances into the body). With regard to acute Cu toxicity, nausea, vomiting, headache, diarrhea, hemolytic anemia, gastrointestinal hemorrhage, and liver and kidney failure, as well as death, may occur with an increase of the Cu dose [26,27]. In the case of chronic Cu toxicity, this can cause Wilson disease [28]. This background underlines the fact that Cu is a trace element that is essential for life and good health. There are multiple clinical symptoms caused by Cu deficiency in humans [20,29]. Early and common signs of acquired Cu deficiency are hematological manifestations such as anemia, leukopenia, neutropenia, and pancytopenia [30]. Bone abnormalities including osteoporosis, bone fractures, and bone

malformations are also often observed in Cu-deficient low-birthweight infants and young children [31].

As Cu is an indispensable trace element with key biological functions in the human body, there are various biological applications of Cu-containing metallic biomaterials, as presented below [19,21,32,33].

3. Beneficial effects of Cu on the cardiovascular system

Early in 1980, McAuslan and Gole showed that Cu can stimulate blood vessel formation by introducing CuSO_4 into anterior chamber implants at micromolar levels [34]. Later studies further suggested that Cu could not only stimulate the proliferation and migration of endothelial cells to promote the formation of new blood vessels, but also inhibit the proliferation of vascular smooth muscle cells and thrombosis [22,35]. These results prompted more intense research on Cu in vessels. Vascular endothelial growth factor (VEGF) is the most effective growth factor for promoting angiogenesis. The VEGF signaling pathway can significantly promote the proliferation, migration, and chemotaxis of endothelial cells in various tissues and organs. Rigracciolo et al. [36] proposed that Cu ions could activate the EGFR/ERK/c-Fos pathway to increase the expression of VEGF in cancer cells. Fibroblast growth factor (FGF) can promote the growth of fibroblasts and is a strong growth factor of connective tissue cells. There are many isomers of FGF, and the active center of some isomers requires the participation of Cu [37]. In recent years, studies have shown that hypoxia can cause microvascular formation through promoting the expression of VEGF [38], especially in the tumor environment, and that hypoxia inducible factor-1 (HIF-1) is a regulatory factor of angiogenesis under hypoxia, which has a wide range of target gene profiles [39,40]. Cu can not only activate HIF, but also promote its expression, which increases vascularization [41,42]. In addition, Li et al. [43] found that 5Cu-BG/ESM films containing 5 mol% Cu can stimulate angiogenesis by improving VEGF and HIF-1 α protein secretion as well as the expression of angiogenesis-related genes [VEGF, HIF-1 α , VEGF receptor 2 (KDR), and endothelial nitric oxide (eNOS)] of human umbilical vein endothelial cells (HUVECs). Nitric oxide (NO) is an important vasorelaxant produced along with L-citrulline from L-arginine in a reaction catalyzed by endothelial nitric oxide synthase (eNOS) [44], and it can relax blood vessels, and promote the differentiation, proliferation, and migration of endothelial cells by activating NO synthetase and the MAPK signaling pathway [45]. Demura et al. found that only when the concentration of Cu^{2+}

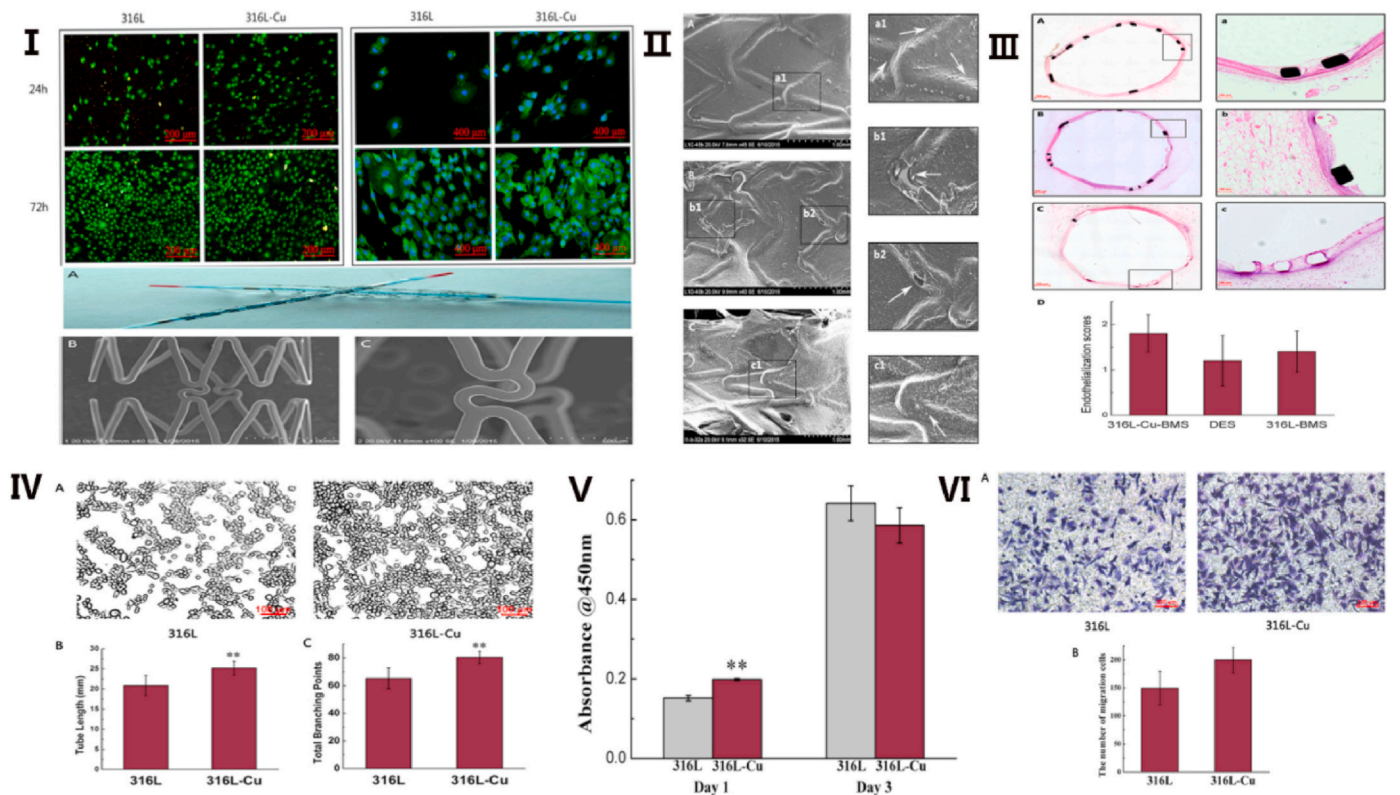


Fig. 2. Structure of 316-Cu stent and it promotes vascular endothelialization. **I.** The vWF staining and F-actin filament staining of HUVECs seeded on 316L and 316L-Cu, respectively. **II.** Scanning electron micrographs of surface characterization of explanted endovascular stent. **III.** Hematoxylin-eosin stain of explanted stents of 316L-Cu-BMS (A a), DES (B b), and 316L-BMS (C c). **IV.** Tube formation induced by the extracts of 316L and 316L-Cu, respectively. **V.** CCK-8 proliferation assay of HUVECs cultured on different substrates. **VI.** Cell migration induced by the extracts of 316L and 316L-Cu, respectively. Reproduced with permission from Ref. [15], copyright 2017, Nature.

was higher than 10^{-6} M could it significantly increase the eNOS activity in human pulmonary artery endothelial cells [46] (See Fig. 2).

The above research results show that Cu, as an indispensable trace element in the human body, can promote the endothelialization of blood vessels and inhibit the proliferation of smooth muscle cells and thrombosis. In recent years, therefore, increasing research has focused on how to add Cu to medical materials to treat cardiovascular disease. Atherosclerotic occlusive diseases, the main forms of which include coronary disease, apoplexy, and peripheral artery disease, are the major causes of death in humans [47]. Stent implantation is one of the most important therapeutic modalities for these diseases, which can dredge blood vessels and restore blood flow through a minimally invasive approach [48]. Stent restenosis is the main complication after stent implantation, and its incidence has gradually increased in recent years [49]. The main factors causing stent restenosis include: (1) that the vascular endothelium is seriously damaged when the stenosis or occlusion is opened; and (2) after stent implantation, smooth muscle cells proliferate and a thrombus forms [50]. However, increasing research on Cu-containing stents has indicated that the addition of an appropriate amount of Cu in the stent material may be a promising approach for reducing the incidence of stent restenosis. Xu et al. found that Cu-bearing stainless steel (SS) could promote the adhesion and proliferation of vascular endothelial cells (VECs), and reduce the early apoptosis rate of these cells through Cu-bearing SS inoculated with VECs and incubated for 1, 2, or 3 days [51]. Subsequently, Jin et al. obtained almost the same results when HUVECs were incubated with an L605-Cu cobalt alloy [5]. Furthermore, they demonstrated that L605-Cu significantly increased the mRNA expression of VEGF in HUVECs. However, it had no effect on the secretion of NO or mRNA expression of eNOS, which may have been because the concentration of released Cu ions was relatively low. Besides promoting the proliferation of vascular

endothelial cells [17,22], studies showed that Cu-bearing SS or Ti alloy (Ti-Cu) could inhibit the proliferation of vascular smooth muscle cells, thus reducing the formation of thrombosis to in turn lower the occurrence of in-stent restenosis [52,53]. Subsequent studies shifted the focus from *in vitro* to *in vivo*. For example, Liu et al. [54] suggested that the time to complete endothelialization was significantly shorter than that of bare stents after plating a copper film on the surface of metal stents. They further found that vascular smooth muscle cells and vascular endothelial cells tended to undergo apoptosis to some degree when the Cu concentration was over $7.5 \mu\text{g/ml}$. Moreover, Jin et al. implanted 316L-Cu bare metal stents (BMS), drug-eluting stents (DES), and 316L BMS into swine to evaluate the re-endothelialization ability *in vivo*; their results demonstrated that 316L-Cu BMS showed the best effect on endothelialization along with good biosafety [15]. Previous studies have shown that copper containing metal materials with copper content of 3-5 wt% could promote the endothelialization and inhibit the growth of smooth muscle cells [5,48,49,51,52]. Consequently, Cu-containing metal stents are considered promising for treating atherosclerotic occlusive diseases. The main beneficial effects of Cu-containing materials on the cardiovascular system are summarized in Table 1.

4. Cu-containing materials promoting bone fracture healing

Besides the beneficial effects of Cu on the cardiovascular system, recently, research has indicated that a certain amount of Cu^{2+} could induce the differentiation of mesenchymal stem cells and osteoblastic cells [55,56], indicating that Cu-containing biomaterials may help to accelerate the bone fracture healing [14,57,58].

The widespread use of motorized transport, along with natural disasters and increases in industrial accidents, has led to a dramatic increase in trauma and bone fractures [59,60]. However, the

Table 1
Beneficial effects of Cu-containing materials on cardiovascular system.

Effect	Materials	Functions	Targets
Protecting the cardiovascular system	Cu ions [36,46]	Increasing the expression of VEGF [36], increase the eNOS activity [46]	Activating EGFR/ERK/c-fos pathway [36]
	5Cu-BG/ESM [43]	Stimulating proangiogenesis [43]	Improving VEGF and HIF-1a protein secretion, activating angiogenesis-related gene expression (VEGF, HIF-1a and eNOS) [43]
	Cu-bearing SS [51,52]	Promoting the adhesion, proliferation of VEC, reducing the early apoptosis ratio of VEC [51], inhibiting the proliferation of VSMC [52]	–
	L605-Cu [5]	Promoting the adhesion, proliferation of HUVECs [5]	Increasing the mRNA expression of VEGF [5]
	Ti-Cu [53]	Inhibiting the proliferation of VSMC, reducing the formation of thrombosis [53]	–
	316L-Cu stent [15]	Showing the best effect on endothelialization with good biosafety [15]	Improving the angiogenesis-related gene expression of VEGF and eNOS [15]

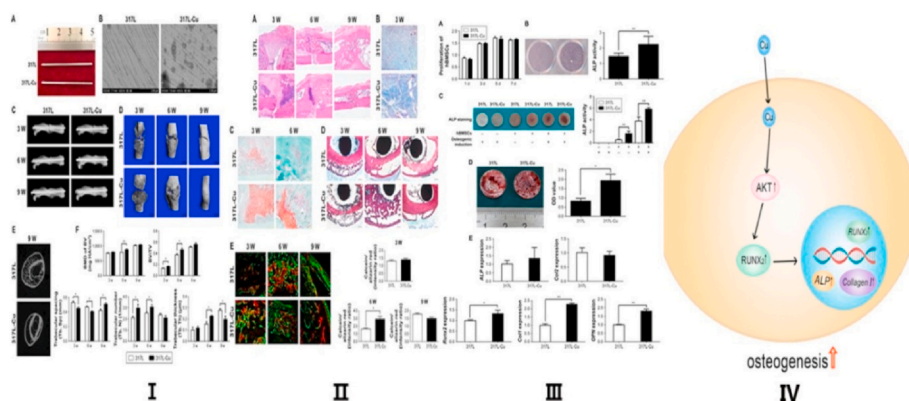


Fig. 3. Promoting osteogenesis and its mechanism of copper-containing implants. I. General view and scanning electron microscope view of 317L SS and 317L-Cu SS (A and B); The fracture line is clearly observed in X-ray (C); Micro-CT showed the callus formation stage (D and E); Callus bone mineral density in the 317L-Cu SS group is higher than that of 317L SS group (F). II. H&E staining displays the general evolution of 317L-Cu SS group calluses and 317L SS group, respectively (A); Masson staining showed the amount of fibrous tissue of 317L-Cu SS group calluses and 317L SS group, respectively (B); Safranin O/fast green staining (C); Van Gieson staining (D); Calcein/alizarin red double labeling (E). III. 317L-Cu stainless steel significantly promoted the osteogenic differentiation of human bone

mesenchymal stem cells *in vitro*. Reproduced with permission from Ref. [63], copyright 2017, Dove Press. IV. Schematic diagram for copper-containing implants stimulated osteogenesis. Reproduced with permission from Ref. [4], copyright 2019, Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

development of surgical techniques and implant materials has greatly improved the prognosis of fractures [61,62]. Nonetheless, it was reported that 5%–10% of fractured bones ended in nonunion and/or incomplete healing, which could even lead to disability in some cases [63]. The principles for treating bone fractures mainly involve early treatment, anatomic reduction, and rigid fixation [64,65]. Open fractures, fractures with failed manual reduction, and obsolete fractures without ideal function are treated surgically. This always requires fixation devices, such as screws, intramedullary nails, and internal plates, to fix the fracture broken end or assist in functional recovery [66,67]. The implant materials should ideally create the optimal healing environment for the injured bones. Therefore, conferring osteogenicity to existing metal implants is crucial for improving the prognosis of bone fractures and reducing postoperative complications [68–70] (See Fig. 3).

Based on research and development of metal-manufacturing process, there are three main methods of fabricating Cu-containing biomaterials: (1) melting metals with the addition of Cu; (2) depositing Cu coating on the surface of metals; and (3) incorporating Cu nanoparticles into metals. For the first method, Ren et al. developed a new Cu-bearing 317L SS (317L-Cu) by directly immobilizing an appropriate amount of Cu into medical 317L SS during the metallurgical process, which could enable the continuous release of trace amount of Cu²⁺ ions from the surface of the steel. The results demonstrated that Cu²⁺ ions could promote osteogenic differentiation by stimulating alkaline phosphatase enzyme activity and osteogenic gene expression (type I collagen, runt-related transcription factor 2, and osteopontin), and enhancing the adhesion and proliferation of osteoblasts cultured on the steel surface [11]. In a further study, Yuan et al. found that Cu²⁺ ions released from 316L-Cu stainless steel could enhance the gene expression of *collagen I*, *ALP*, and *Runx2*, and showed that the protein expression of Runx2 was

stimulated by an Akt cell signaling pathway [4]. Moreover, Liu et al. indicated that a Ti–Cu alloy could upregulate the expression of osteogenesis-related genes including alkaline phosphatase, collagen I, osteopontin, and osteocalcin, but had no significant impact on the adhesion, proliferation, and apoptosis of osteoblasts cultured with Ti–Cu alloy [7]. The difference in experimental results may be related to the release of Cu ions. Large amount of copper should not be added to ensure the original properties of the material, therefore, the concentration of Cu ions released from Cu-containing alloy is usually finite due to the limited content in the alloy.

In terms of the second method of fabricating Cu-containing biomaterials, most researchers used micro-arc oxidation technology, which relies on the instantaneous high temperature and high pressure effect generated by discharge, to make copper ions to get into the surface of aluminum, magnesium, titanium and their alloys in the electrolyte solution, and the ceramic coating dominated by matrix metal oxides [71–73]. Zhao et al. demonstrated that magnesium–copper–fluorine-containing titanium coatings might promote osteogenesis through ERK1/2 signaling [72]. In addition, Huang et al. employed the micro-arc oxidation technique to fabricate Cu-containing ceramic coating on a titanium substrate, and then cultured macrophages on the surface. They found that the integration of Cu into the material's surface enhanced macrophage-mediated osteogenesis and bactericidal capacity [73]. Other studies employed this method to fabricate Cu-containing materials and obtained the same positive results for improving bone fracture healing [74–76]. Compared with the method of melting metals with the addition of Cu, this method does not change the hardness and toughness of the graft. However, the Cu containing layer might lead to the massive release of Cu ions or tiny Cu particles, which might cause a relatively severe and persistent foreign body reaction *in vivo* [77]. To overcome this limitation, some researchers innovatively used the

sustained-release properties of nanomaterials to prepare copper-containing materials via screening and analysis of nanoparticle size [78–80]. For example, Wang et al. developed a novel nano-copper-bearing SS with nano-sized copper-precipitation (317L-Cu SS) to explore the effect of 317L-Cu SS on bone fracture healing *in vivo*. Their results demonstrated that 317L-Cu SS not only promoted osteogenic differentiation and the expression of osteogenesis-related genes, but also upregulated lysyl oxidase activity [63]. Subsequently, Huang et al. employed a Cu-containing micro/nano-topographical bioceramic surface (Cu-Hier-Ti surface) as a material model to explore the role played by Cu^{2+} release or the material surface in regulating macrophage-mediated osteogenic and bactericidal effects [6]. They found that M1 macrophages activated by Cu^{2+} exhibited a stimulatory effect on osteoblast maturation. Besides, focusing on Cu-containing alloy, Zhang et al. fabricated aqueous soluble graphene oxide-copper nanocomposites (GO-Cu) [80], which were used to coat porous calcium phosphate scaffolds for the regeneration of vascularized bone. The results demonstrated that the GO-Cu coating enhanced the adhesion and osteogenic differentiation of rat bone marrow stem cells and upregulated the expression of Hif-1 α by activating the Erk1/2 signaling pathway *in vitro*. Furthermore, they found that the GO-Cu-coated calcium phosphate cement scaffolds significantly promoted angiogenesis and osteogenesis. Moreover, Wang et al. investigated a method based on the controlled delivery of Cu ions from borate bioactive glass scaffolds for stimulating angiogenesis and osteogenesis in a rodent calvarial defect model *in vivo* [81]. They found that the controlled delivery of Cu ions from borate bioactive glass implants is a promising approach for the healing of bone defects. More studies to utilize nanotechnology to fabricate Cu-containing materials indicated basically similar results that Cu^{2+} could promote osteoblast proliferation and accelerate bone fracture healing [82–85]. Li et al. found that at a concentration of 10 μM lower, Cu^{2+} was safe for mouse osteoblastic cell line (MC3T3-E1) and beneficial for its proliferation in a dose-dependent manner. The half maximal inhibitory concentration (IC50) was about 134.6 μM and the inhibition ratio rapidly rose to 100% when concentration of Cu^{2+} ranged from 111 μM to 333 μM ⁸⁶. The above-mentioned results clearly show that different methods using Cu-containing materials can promote bone fracture healing. Some examples of Cu-containing materials promoting bone fracture healing are summarized in Table 2.

5. Antibacterial activity of Cu-containing alloys

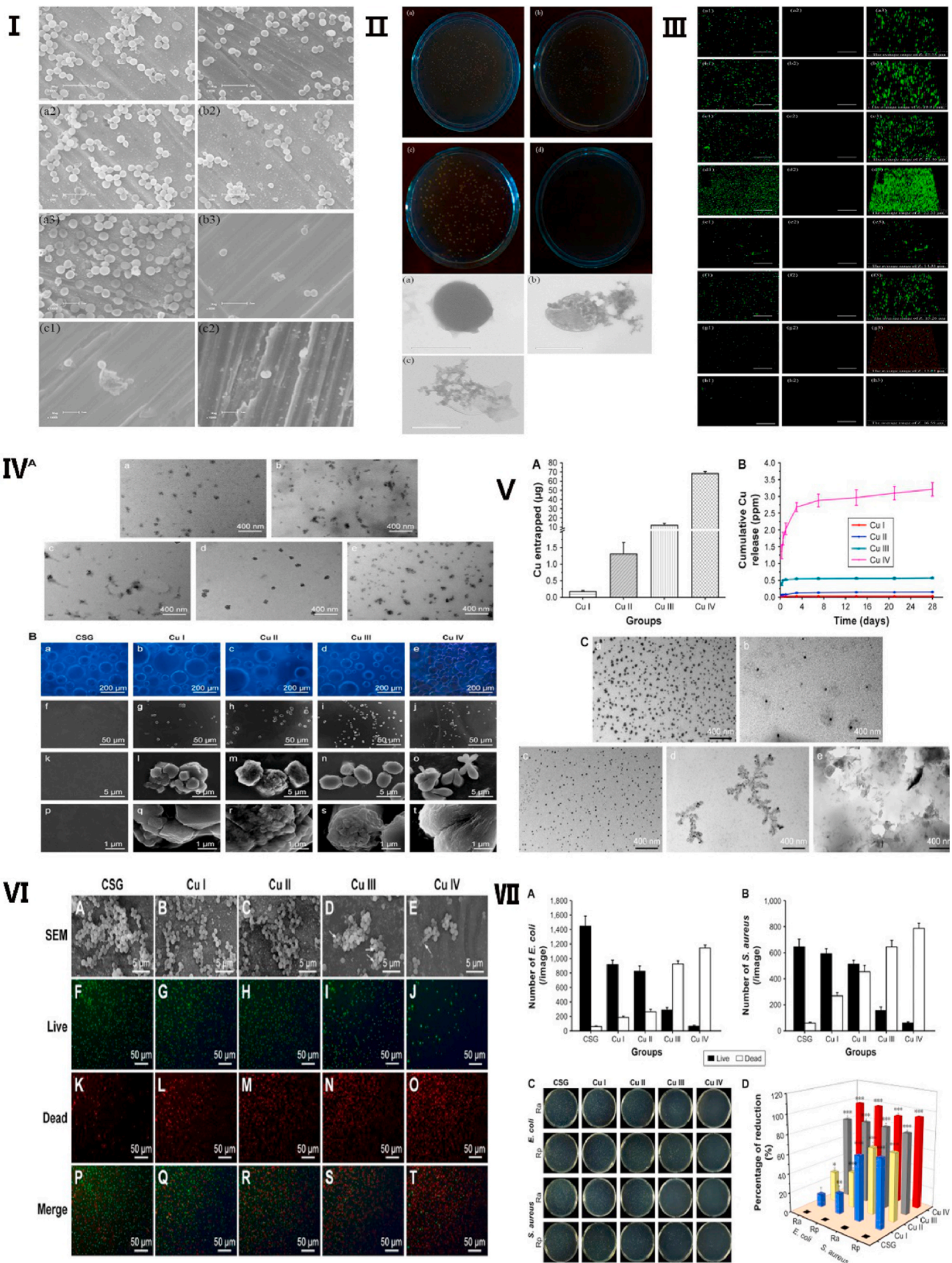
Infection remains one of the most common postoperative complications to be resolved in all kinds of surgical operations, even in minimally invasive interventional surgery, although modern medicine has advanced aseptic management and developed effective broad-spectrum antibiotics [87–91]. In addition, implant-related infection is a devastating complication following the application of a medical

implant. For example, implant-related infection in orthopedics has become a common problem against the background of a markedly increasing number of implant surgeries being performed [92–94]. In the United States, implant-related infection is the main cause of total knee arthroplasty, accounting for more than 20% of all cases, and severe infection may lead to amputation [95,96]. The treatment of implant-related infection is complicated; preventing such infection from occurring at all is obviously the ideal solution, but complete prevention is typically not possible. Surgeons routinely perform a variety of strategies to avoid infection, such as applying strict aseptic techniques, wound dressing, and administering antibiotics [97,98]. However, these measures can only provide short-term protection and inhibition of infection. Some studies have shown that residual bacteria on implants or distant bacterial infection cause opportunistic infection of implants, which damages implant functions and can even lead to bacteremia [99,100]. With the advances of materials for biomedical applications, studies have demonstrated that biofilm formation on the surfaces of materials is the main cause of implant-related infections and has serious consequences [101,102]. Increasing research has now focused on developing new implant materials or modifying the surfaces of implants to inhibit persistent biofilm-related infections. To ensure biosafety, almost all studies in this field need to meet the following requirements: (1) the material for the implant must be biocompatible; (2) the stability and properties of the implant should not be weakened; and (3) the antibacterial effect of new implants should have a broad spectrum. Originally, some studies used antibiotic-coated metallic materials to prolong the prevention of infection through surface modification technology [103,104]. However, there are two major problems to this approach. On the one hand, the bonding between the coating and the metal surface is usually unstable. On the other hand, long-term antibiotic release increases the risk of drug resistance emerging [105,106]. Reddy et al. performed a novel microscopic physical surface modification method (Sharklet) for preventing bacterial colonization and migration [107]; their results showed that the Sharklet micropattern was effective at inhibiting bacterial colonization and migration. Unfortunately, manufacturing of the Sharklet micropattern is too complicated to apply it to metal implants.

Based on the strong antibacterial performance of Cu, which is one of the most essential trace elements present in the human body [108], researchers have increasingly focused on fabricating Cu-containing implantable materials to inhibit the occurrence of infections. Stainless steel is one of the most commonly used materials in the biomedical field [9,109]. Combined with the above conditions, Nisshin Steel (Tokyo, Japan) first developed antibacterial Cu-containing SS in the 1990s¹⁰². Related research demonstrated that the strong and broad-spectrum antibacterial ability of Cu-containing SS is mainly due to the Cu ions released from the steel's surface, which damage bacterial cell walls and cell membranes, adsorb electrons from bacteria, and generate reactive

Table 2
Cu-containing materials promoting bone fracture healing.

Effect	Materials	Functions	Targets
Promoting bone fracture healing	317L-Cu [11]	Promoting the osteogenic differentiation, enhancing the adhesion and proliferation of osteoblasts [11]	Stimulating the Alkaline phosphatase enzyme activity and osteogenic gene expressions [11]
	316L-Cu [4]	Enhancing the gene expression of <i>collagen I</i> , <i>ALP</i> and <i>Runx2</i> [4]	Activating Akt signaling pathway [4]
	Ti-Cu [7]	Upregulate the expressions of alkaline phosphatase, Collagen I, osteopontin and osteocalcin [7]	–
	Magnesium-copper-fluorine containing titanium coatings [72]	Promoting osteogenesis [72]	Activating ERK1/2 signaling [72]
	Cu-containing ceramic coatings on titanium [73]	Enhancing macrophage-mediated osteogenesis [73]	–
	Nano-copper-bearing 317L SS ⁶³	Promoting osteogenic differentiation and the expression of osteogenesis-related genes, upregulating the lysyl oxidase activity [63]	–
	Cu-Hier-Ti [6]	Regulating macrophage-mediated osteogenic [6]	Activating M1 macrophages [6]
	GO-Cu [80]	Enhancing the adhesion and osteogenic differentiation [80]	Activating the Erk1/2 signaling pathway [80]



(caption on next page)

Fig. 4. Antibacterial activity and its mechanism of copper-containing materials. I. Scanning electron microscope image of *S. aureus* cells on 317L-Cu SS surface. II. Scanning electron microscope images showed the deformation process of *S. aureus*, which incubated with 317L-Cu SS. III. Confocal laser scanning microscopy images of the *S. aureus* biofilm on 317L SS. Reproduced with permission from Ref. [116], copyright 2016, Nature Publishing Group. IV. Transmission electron microscopy images (A) and fluorescence images (B) of copper-containing coatings. V. Cu entrapment and release of the copper-containing coatings. VI. Field emission scanning electron microscopy images and Live/Dead stain fluorescent images showed that copper-containing coatings inhibit proliferation of *E. coli*. VII. copper-containing coatings may inhibit proliferation of *E. coli* and *S. aureus*. Reproduced with permission from Refs. [82], copyright 2017, Dove Press.

oxygen species (ROS), resulting in severe damage to and death of bacteria and fungi [24,110,111], such as *Staphylococcus haemolyticus* [112,113], *Escherichia coli* [114], and *Candida albicans* [111,115]. Subsequently, Yang et al. from the Institute of Metal Research, Chinese Academy of Sciences (Shenyang, China), proposed a new concept of a bio-functional design for metallic biomaterials, focusing on the development of new metallic biomaterials with the combination of excellent mechanical properties, corrosion resistance, and antibacterial ability [21]. 317L SS, which has a good balance of biocompatibility and corrosion resistance, has been applied in various implantable and non-implantable medical devices [102]. Therefore, Yang et al. added an appropriate amount of Cu in 317L SS in steelmaking to fabricate a novel Cu-bearing 317L SS (317L-Cu SS) [21]. Sun et al. showed that 317L-Cu SS (4.46% Cu) exhibited satisfactory antibacterial ability against *Staphylococcus haemolyticus*, and its antibacterial rate reached 98.3% after 24 h of treatment *in vitro* [116]. Similarly, Yang et al. indicated that 317L-Cu SS achieved a 99% inhibition rate of sessile *Staphylococcus aureus* cells after 5 days of incubation [102]. Guan et al. found that 317L-Cu SS could effectively kill *Escherichia coli* on the material surface without having an adverse effect on osteoblasts *in vitro* [117]. Moreover, *in vitro* and *in vivo* studies by Chai et al. demonstrated that 317L-Cu SS possessed a strong antibacterial effect to prevent implant-related infection [118].

Besides 317 L-Cu SS, other Cu-containing materials also exhibited satisfactory antibacterial abilities [24]. For example, Ren et al. fabricated a Cu-bearing 316L SS (316L-Cu SS) containing 3.77% Cu, with the aim of preventing implant-related infection (IRI) [12]. The results demonstrated that 316L-Cu SS exhibited a broad-spectrum antibacterial effect against *Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus epidermidis*, with bacteriostatic rates of 95.2%, 94.8%, and 94.1%, respectively, *in vitro*. Furthermore, 316L-Cu SS showed lower bone infection when 316L-Cu SS nails were used to treat a rat model. Compared with the conventional 304 SS, Cu-bearing 304 SS (304 SS-Cu) could effectively kill *Porphyromonas gingivalis*, indicating that it may inhibit the occurrence of peri-implantitis [119]. Ma et al. [120] showed that the Ti-5Cu alloy exhibited excellent antibacterial property and corrosion resistance, providing a great potential in clinical application for dental implants. Li et al. [121] demonstrated that 420-Cu SS presented excellent antibacterial performance against the mixed bacteria, including *E. coli* and *S. aureus*, with an approximately 99.4% of antibacterial rate. Yan et al. [122] and Liu et al. [123] also successfully prepared a Mg-Cu alloy, which showed excellent antibacterial effects both *in vitro* and *in vivo*.

In recent years, Cu-containing titanium alloys have attracted increasing attention since titanium alloy is one of the most commonly used materials in orthopedics [124–126]. For example, Jin et al. demonstrated that 316L SS coated with a Ti-Cu layer (316L/Ti-Cu) showed an effective reduction of 99.9% of *Escherichia coli* within 12 h¹²⁷. In addition, Ma et al. developed a Cu-containing antibacterial titanium alloy (Ti-5Cu), which possessed strong antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus* [120]. Moreover, the research of Liu et al. revealed antimicrobial/antibiofilm activities of Ti-Cu alloy against the orally specific bacterial species *Streptococcus mutans* and *Porphyromonas gingivalis* [128]. Furthermore, Cu-bearing mesoporous bioglass could continuously release Cu ions, which effectively decreased bacterial viability and inhibited biofilm formation [41,129,130]. Gosau et al. also established a silicone material coated with Cu (silicone/Cu) and found that these new breast implants had

anti-adherence and antibacterial effects against *Staphylococcus epidermidis* [131]. Lee et al. [132] found that it was possible to tune the antibacterial property of Ti-Cu_x alloys by changing the Cu concentration and aging, which could help to optimize the antibacterial properties of copper-containing materials. Li et al. found that when concentration of Cu²⁺ was over 37 μM, it showed inhibition ratio suddenly increased and approached to 100% at 111 μM on both *E. coli* and *S. aureus*. The *in vitro* study also showed that the proliferation of human umbilical vein endothelial cells (HUVECs) was promoted when concentration of Cu²⁺ was lower than 222 μM. At a concentration of 10 μM lower, Cu²⁺ was safe for MC3T3-E1 and beneficial for its proliferation. Therefore, Cu²⁺ performed on both cells and bacteria yields good osteogenic, angiogenic and antibacterial activity without cytotoxicity [86] (See Fig. 4).

Although many studies have shown that Cu-containing materials exhibited strong antibacterial effects and good biocompatibility, the related antibacterial mechanisms of Cu²⁺ are still unclear [21]. Recent research showed that, as a result of the carboxyl group of lipoproteins, the surface of bacteria has a negative charge, which causes bacteria to attract Cu ions given their positive charge. Upon contacting Cu ions, the permeability of bacterial cell membranes changes and these cells are damaged. When Cu ions enter bacterial cells, the enzymes are out of activities, and DNA, RNA, and proteins along with cytoplasm leak out [133]. This could result in the bacteria dying (See Figs. 5 and 6). Therefore, in contrast to the traditional antibacterial mechanism, Cu-containing materials possess long-lasting antibacterial effects. Some antibacterial effects of Cu-containing materials are summarized in Table 3.

6. Other effect of copper

In addition to the above-mentioned functions of copper-containing biomaterials, Ghosh Deepanjan et al. found that delivery of ionic copper from sutures could promote incisional wounds healing, and copper-eluting fibers may have translational potential for accelerating repair on surgical and trauma wounds [134]. L. A. Volodina et al. developed ointment containing copper nano-particles (NPs), which could provide a high level of repair of skin wounds [135].

Some studies suggest that copper-containing materials have a certain role in the treatment of cancer. Based on the previous studies, colloidal Cu NPs have been proven to be one of the effective inorganic materials against various cancer cell lines. As reported by Valodkar et al. [136,137], Cu NPs was toxic to human lung carcinoma cell line (A549)¹³⁷, human liver hepatoma (HepG2), Chinese hamster ovary (CHO), human osteosarcoma (Saos), and mouse embryonic fibroblast (3T3L1) cells in a dose-dependent manner [136]. The study revealed that the capped Cu NPs by nontoxic aqueous extract of latex could be directly used for administration/*in vivo* delivery for cancer therapy [136]. In another study, Harne et al. [138] reported excellent viability against HeLa, A549 and BHK21 cells even at 120 μM concentration of Cu NPs. Anticancer studies demonstrated the *in vitro* cytotoxicity values of Cu NPs against the tested human colon cancer Caco-2 cells, human hepatic cancer HepG2 cells and human breast cancer MCF-7 cells, which can be used as a photothermal treatment to kill cancer cells [139].

Copper has several radioisotopes, and ⁶⁰Cu, ⁶¹Cu, ⁶²Cu, ⁶⁴Cu and ⁶⁷Cu are particularly interesting for radiotherapy and imaging applications. Decay characteristics of copper radionuclides make them suitable for numerous medical applications, such as Positron Emission

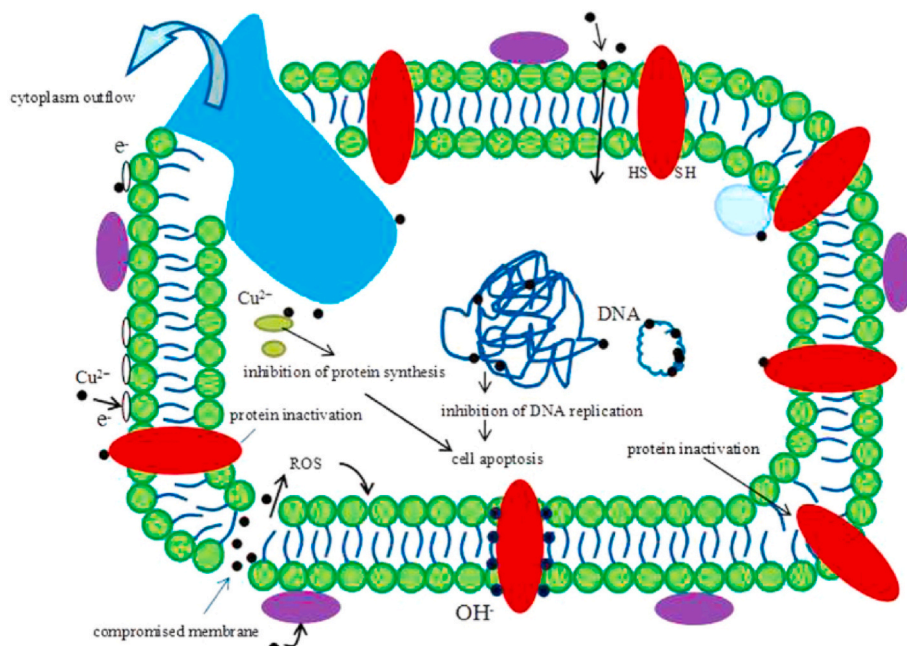


Fig. 5. The schematic image of hypothesis about the antibacterial mechanism of Cu^{2+} . Reproduced with permission from Ref. [128], copyright 2016, Nature Publishing Group.

Tomography (PET) imaging, radioimmunological tracing and radiotherapy of cancer [140].

7. Conclusion

In the last few decades, considerable efforts have been expended in clarifying the functions of Cu^{2+} in the human body, as well as in exploring the biological applications of Cu-containing materials. As a trace element in the human body, Cu is an important component and catalytic agent of many enzymes and proteins. Therefore, Cu possesses multiple important bio-functions, such as protecting the cardiovascular system, promoting bone fracture healing, and exerting antibacterial effects. With the greater understanding of the biological effects and biosafety of Cu, more attention has been focused on developing Cu-containing materials in order to treat diseases and improve human health. In recent years, more and more substantial progress has been made in this field. For example, coronary stents made of Cu-containing

stainless steel have been shown to prevent the occurrence of in-stent restenosis, while orthopedic grafts made of Cu-containing materials can promote osteoblast proliferation and inhibit bacterial adhesion so as to promote bone fracture healing and reduce the risk of infection. Despite the important progress that has been made, further investigation is needed to understand the mechanisms by which Cu-containing materials act in order to create a wide range of applications. With the increased focus on achieving further progress with Cu-containing materials, it is believed that the addition of an appropriate amount of Cu to biomaterials may become a novel and beneficial approach to improve current biomedical material applications.

8. Future prospective

Bioactivity is a future pursuit for development and application of biomaterials, making them to have better biocompatibility and therapeutic effect, and many efforts have been devoted to realize this target.

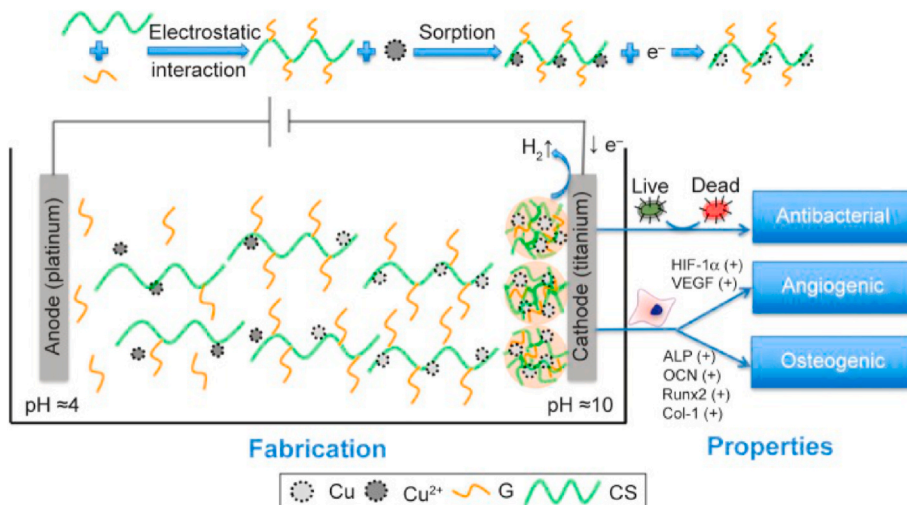


Fig. 6. Schematic diagram for fabrication and properties of copper-containing coating materials. Reproduced with permission from Refs. [82], copyright 2017, Dove Press. Abbreviations: G, gelatin; CS, chitosan.

Table 3
Antibacterial effects of Cu-containing materials.

Effect	Materials	Functions	Targets
Antibacterial activity	Cu-containing SS [110–112,114,115]	Resulting in the fatal damage and death of the bacteria and fungus [110–112,114,115]	Generating the reactive oxygen species [110,111]
	317L-Cu [102,116–118]	Inhibiting and killing <i>Staphylococcus haemolyticus</i> , <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> [102,116–118]	–
	316L-Cu [12,119]	Exhibiting a broad-spectrum antibacterial effect [12,119]	–
	Mg-Cu [122,123]	Showing an excellent antibacterial effect both <i>in vitro</i> and <i>in vivo</i> [122,123]	–
	316L/Ti-Cu [127]	Inhibiting <i>Escherichia coli</i> [127]	–
	Ti–5Cu ¹²⁰	Strong antibacterial activity against both <i>Escherichia coli</i> and <i>Staphylococcus aureus</i> [120]	–
	Ti-Cu [128]	Inhibiting <i>Streptococcus mutans</i> and <i>Porphyromonas gingivalis</i> [128]	–
	Cu-bearing mesoporous bioglass [41,129,130]	Decreasing bacterial viability [41,129,130]	Inhibiting biofilm formation [41,129,130]
	Silicone/Cu [131]	Inhibiting <i>Staphylococcus epidermidis</i> [131]	–

Cu-containing biomaterials, including metals and ceramics, have been found to possess multiple bio-functions through continuous release of trace amount of Cu ions from the materials in the biological environment, which can be a prospective way for the development of bioactive materials. However, there still exist many challengers for future applications of those novel biomaterials, such as optimization of Cu content in materials to better present the bio-functions, optimization of comprehensive properties (bio-function, bio-safety, mechanical property, corrosion, processing ability, etc.), relevant mechanisms of bio-functional behaviors, and final clinical applications. More studies on these issues will help to have deep understanding on this novel class of biomaterials and to solve those technical problems for applications. It is much expected that the Cu-containing biomaterials will realize their clinical applications and benefit the majority of patients.

Authorship contribution statement

Ke Yang and Xun Qi supervised the whole work and revised the manuscript, Peng Wang and Yonghui Yuan made literature search and draft the manuscript of this review, Hongshan Zhong, Ke Xu, Yinghui Yang and Shiyu Jin edited the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

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

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