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Review article

The impact of copper on bone metabolism

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ABSTRACT

Copper is an essential trace element for the human body. Abnormalities in copper metabolism can lead to bone defects, mainly by directly affecting the viability of osteoblasts and osteoclasts and their bone remodeling function, or indirectly regulating bone metabolism by influencing enzyme activities as cofactors. Copper ions released from biological materials can affect osteoblasts and osteoclasts, either directly or indirectly by modulating the inflammatory response, oxidative stress, and rapamycin signaling. This review presents an overview of recent progress in the impact of copper on bone metabolism.

Translational potential of this article: The impact of copper on bone metabolism can provide insights into clinical application of copper-containing supplements and biomaterials.

1. Copper homeostasis and physiological functions

1.1. Copper homeostasis

Copper (Cu) is an essential trace element involved in numerous of biological processes including enzymatic reactions, nucleic acid synthesis, antioxidant defense, iron metabolism and immune function. Copper deficiency may result in impaired bone and cholesterol metabolism and cardiovascular disorders [1]. Therefore, copper homeostasis is pivotal for the normal functioning of multiple organ systems of the body.

The total amount of copper 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 [2]. Copper cannot be produced and synthesized in the body and thus needs to be obtained in food. The amount of diet copper is the primary factor influencing both absorption and excretion of copper. The World Health Organization recommends an upper limit of 2–3 mg copper for adults every day [3]. Copper in diet is mainly absorbed into the body through the stomach and small intestine mucosa [4], and transported via the portal blood to the liver. Much of that is incorporated into ceruloplasmin, released into the blood, and ultimately delivered to tissues to synthesize copper-containing proteins. More than 90 different enzymes and proteins have been found to contain copper to various extents [5]. Several conditions and diseases influence whole-body copper metabolism, including metabolic defects such as Menkes syndrome (systemic copper uptake is deficient) and Wilson disease (copper excretion from the body is blocked), pregnancy, inflammation, and numerous diseases [6].

The cellular needs for Cu are met by a tightly regulated interconnected network of proteins that include the Cu transporters, the Cu chaperones, and Cu-ATPases (ATP7A and ATP7B). In mammalian cells, extracellular Cu^{2+} is first reduced to Cu^{+} by metal reductase on the plasma membrane, and then crosses the plasma membrane into cytosol via the Cu transporter 1(CTR1). Entering copper is either binds directly or is retrieved from CTR1 by copper chaperones, a collection of proteins that carry copper to specific intracellular sites and enzymes [7]. These chaperones help to prevent the presence of free Cu ions by binding them and releasing them directly to their target proteins. Copper chaperone for Superoxide dismutase distributes copper to Cu/Zn-Superoxide dismutase (SOD1) in cytoplasm and mitochondria. Cytochrome c oxidase copper chaperone takes it to the mitochondria, where it is required for Cytochrome C Oxidase (CCO). Antioxidant protein1 transfers copper to the Cu-ATPases located in the trans Golgi network. Cu-ATPases transport copper to the secretory pathways for incorporation into cuproenzymes and mediate copper excretion by sequestering excess copper in vesicles. Some of the entering Cu will also associate with cytosolic metallothioneins [8].

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1.2. Physiological functions of copper

Copper, as a trace element for humans, takes part in the metabolism and synthesis of over 30 coenzymes. A main function of copper is the constitution of oxidases that transfer electrons to achieve the reduction of molecular oxygen, and is therefore essential for energy metabolism at the cellular level [9]. The role of copper enzymes in the formation/degradation of reactive oxygen species (ROS) is obvious from the presence of copper in the enzymes CCO and Superoxide dismutase (SOD). Copper also interacts with iron status as ferroxidases, which oxidize ferrous (Fe^{2+}) iron to the ferric form (Fe^{3+}). The oxidation of ferrous iron is required for iron binding to its carrier protein transferrin [10].

Increasing copper-containing biomaterials have been developed and explored in the field of medical implants in recent years [11–13]. Related studies suggested that Cu could increase the expression of VEGF, thereby stimulating the proliferation and migration of endothelial cells to promote the formation of new blood vessels [14,15]. The addition of copper in bioactive glass nanofibers spontaneously improves the vascularization during new bone formation by promoting osteoblastic and endothelial cell activity [16]. Besides, researchers have demonstrated that Cu ions released from the biomaterials possess strong and broad-spectrum antibacterial abilities [17]. This is due to their ability to damage bacterial cell walls and cell membranes, adsorb electrons from bacteria, and generate ROS, resulting in severe damage to and death of bacteria and fungi [18,19], including *Staphylococcus haemolyticus* [20], *Escherichia coli* [21], and *Candida albicans*.

2. Abnormal copper homeostasis induces bone defects

Copper maintains a delicate and dynamic homeostasis in the body, and deviations from optimal copper levels can result in physiological abnormalities. Systemic abnormalities of copper metabolism usually manifest as abnormalities of bone metabolism.

2.1. Copper deficiency

Copper deficiency may contribute to bone defects, such as decreased bone mineral density (BMD) and increased bone fragility [22].

In epidemiological studies, serum copper levels were found to be significantly lower in patients with osteoporosis and lumbar osteopenia (96.6 ± 39 μg/ml) than in healthy controls (112.6 ± 28.2 μg/ml) [23]. Patients with femoral neck fracture also exhibit lower serum copper levels (20.5 μmol/L) than their healthy controls (26 μmol/L) [24]. Furthermore, low serum copper levels have also been considered as a risk factor for age-related osteoporosis. A cross-sectional study demonstrated that serum copper levels were positively associated with BMD in the range of 98.5–134 μg/dL [1]. And another study showed that patients suffering from advanced tooth wear had reduced spine BMD along with local enamel copper deficits (19.861 ± 13.171 μg/L) compared to healthy controls (36.673 ± 22.661 μg/L) [25]. In terms of dietary considerations, a low-copper diet is thought to be associated with osteoporosis [26]. Researchers have found that copper intake was positively correlated with increased BMD in adults and negatively related to the risk of osteoporosis [27]. A longitudinal intervention trial showed that biomarkers of bone resorption were significantly increased when copper was lacking (0.7 mg/d) in the diet [28].

The importance of copper in normal skeletal development has been demonstrated by histologic examination [29,30]. Osteoporosis developed in the spongiosa of both mandible and maxilla of the copper deficient animals, with remaining trabeculae being surrounded by numerous osteoclasts. The marrow spaces were significantly enlarged and had lost most of their myeloid cellular elements. In addition, condyles of the copper deficient animals exhibited distinct features, including widened cartilaginous layer containing several resting lines. Besides, there was a prominent presence of hypertrophied cells in the widened cartilaginous layer. Additionally, a marked reduction in both size and number of trabeculae was observed, which were characterized by a wide calcified cartilage core covered either by flattened spindle-shaped cells or an extremely thin layer of bone.

2.2. Copper excess

It was found that copper has direct toxicity to cartilage and bone in chicken embryo bone tissue [31], and an abundance of serum copper has been found to cause a decrease in bone size and bone density in C57 mice [32]. It was also found that excessive copper produces large amounts of free radicals that cause lipid peroxidation and interfere with bone metabolism, leading to a decrease in bone cortex and bone strength. Oxidation can also, in itself, promote aging and reduce bone strength [33–36]. In Wilson's disease, characterized by copper deposition, epidemiological studies show that the BMD of cortical and cancellous bone are normal, whereas the risk of fracture is increased [37]. A nationally representative cross-sectional study enrolled participants from the National Health and Nutrition Examination Survey (2011–2014) in the United States demonstrated that higher serum copper levels (>134 μg/dL) are significantly associated with increased total fracture, especially in men [1]. In addition, excessive copper and decreased bone strength are also associated with other disease manifestations such as rickets and abnormal osteophytes [34]. Blood glucose also affects the relationship between copper and bone, but the results are controversial. The levels of copper increased in diabetic patients with periodontitis (176.78 ± 6.67 μg/dL) compared to healthy individuals with periodontitis (68.53 ± 7.03 μg/dL) [38]. Moreover, the concentration and tissue content of copper was increased significantly in the femur of diabetic rats [39]. However, a copper-containing engineered implant significantly improved the viability and osteogenic activity of osteoblasts under high glucose microenvironment [40]. It was also found that femoral BMD in diabetic patients was significantly lower than that in healthy control. But no obvious difference was observed in relative content of copper [41].

From the above studies, both Cu deficiency and excess lead to abnormal bone metabolism. Low copper intake, serum copper level and local tissue copper content cause BMD reduction and bone structural changes, while excessive copper also has direct toxicity to bone tissue or reduces bone strength by affecting lipid peroxidation, resulting in increased bone fractures.

3. Copper effects on osteoblasts

3.1. Abnormal endogenous copper metabolism could affect osteoblast behavior

Menkes disease (MD) is a copper metabolism disorder that is caused by a loss-of-function of a major copper transporter, ATP7A. Bone abnormality is a typical phenotype in MD patients. Bone abnormalities in Menkes patients include osteoporosis, metaphyseal spurs, diaphyseal fractures, and wormian occipital bones. Kim et al. [42] found that the dysfunction of copper utilization in MD gives rise to delayed mesenchymal stem cell (MSC) development and impaired osteogenesis, proved by low alkaline phosphatase (ALP) activity, reduced calcium mineralization and decreased expression of osteogenic marker genes. Wilson's disease (WD), also named hepatolenticular degeneration, is an autosomal recessive disorder associated with abnormal copper metabolism caused by ATP7B gene mutation. ATP7B mutation can disrupt the functions of its encoding enzyme and cause excessive copper deposition. Patients with WD typically exhibit low bone density and osteoporosis. The expression of osteogenic genes and the mineralization level were significantly lower in the WD osteoblasts, indicating a lower osteogenesis activity. Liu et al. [43] demonstrated that this osteogenesis impairment may be caused by aberrant β-catenin signaling pathway.

In addition to systemic metabolism, copper could also affect

osteoblasts as an important enzyme cofactor. Lysyl oxidase (LOX) is a secreted copper-dependent amine oxidase which modifies the bone extracellular matrix by using lysin and hydroxylysine in collagen and elastin as substrates to produce intermolecular cross-links necessary for the development of bones [44]. The primary function of the LOX family is to oxidize primary amine substrates to reactive aldehydes and catalyze the covalent crosslinking of collagen and elastin in the extracellular matrix, thereby increasing insoluble matrix deposition and tensile strength [45]. Copper ion binding to pro-LOX is necessary for LOX activation, meaning copper can affect the formation of extracellular matrix of bone tissue. The expression of LOX increases during the early stage of osteoblast differentiation, and the expression pattern of COL1A2, which encodes the α2-chain of mouse collagen type I, the principal constituent of the organic matrix of bones, is similar to that of LOX. At the same time, the temporal pattern of total amine oxidase activity during osteoblast differentiation parallels the LOX protein levels, indicating that the amine oxidase activity originated from the LOX protein. Inhibition of the amine oxidase activity of LOX significantly suppressed both mineral nodule formation (later stage) and the expression of osteoblast marker genes during osteoblast differentiation of the primary calvaria cells, suggesting that LOX plays an essential role in regulating osteoblast differentiation through the amine oxidase activity required for the formation of collagen fibers [46]. However, researchers also found that tumor-derived LOX inhibited the formation

Table 1
Effect of copper-containing biomaterials on osteoblasts.

Materials	Description	Concentration	Conclusion	Reference
Cu–TiO_2 Microporous Coatings on Titanium	microporous copper-titanium dioxide coating prepared on the surface of titanium by microarc oxidation	0.06M copper acetate in the basic electrolyte solution	Promote cell adhesion, proliferation, osteogenic differentiation and has a better osseointegration effect.	[49]
TiCu	Cu-alloyed titanium	5 wt %	Accelerate bone formation, enhance BMD, and reduce the risk of refracture in the osteoporotic bone environment.	[50]
Cu-SS	copper-bearing stainless steel	4.5 wt %	Promote osteogenesis, possess good biocompatibility, and inhibit inflammation.	[4]
PG-Cu@MSNs composite scaffold	Copper-loaded mesoporous silica nanoparticles (Cu@MSNs) were incorporated into the poly (lactic-co-glycolic acid)/gelatin (PLGA/Gel, denoted as PG) fiber matrix	Not mentioned	Promote newborn tissue ingrowth and resist connective tissue infiltration in guided bone regeneration, to induce favorable periodontal bone regeneration with repaired bone tissue.	[51]
Copper containing nanocomposite scaffold	Nanocomposite scaffold containing gelatin–collagen- and copper-doped nanofibrilar 45S5 bioglass	1 wt % CuO	Promote growth of osteoblasts.	[52]
PLGA/Cu(I)@ ZIF-8 nanocomposite scaffold	Copper-loadedZIF-8 nanoparticles and poly (lactide-co-glycolide) (PLGA) were combined to fabricate porous PLGA/Cu(I)@ZIF-8 scaffolds using three-dimensional printing technology	10 wt % Cu(I)@ZIF-8 nanoparticles	Promote the proliferation of MSCs and facilitate cell adhesion and spreading, as well as inducing osteoblastic differentiation.	[53]
Cu (II)-loaded brushite scaffolds	Macro-porous and biodegradable brushite scaffolds infiltrated with low doses of copper sulphate	0.56 μg/cm^2	Enhance the viability and proliferation of osteoblastic cells on calcium phosphate cements and positively affect the expression of bone sialo protein and osteocalcin.	[54]
Cu/Ti oxide composite layer	Implantation of a copper salt into a titanium implant	1 μg/mm^2	Promote the proliferation of MSCs and enhance osteogenic differentiation in a narrow concentration range around 0.1 mM.	[55]
Co–Cr–Mo–Cu Alloys	Co–Cu alloys with different Cu content (1–4 wt. %) were prepared using an investment casting method	2 wt %	Induce osteoblast proliferation and differentiation and inhibit osteoblast apoptosis by generating ROS and activating mTOR signaling pathway.	[56]
Cu-doped 45S5 bioactive glass		0.1 wt %	Enhance lipid peroxidation associated with mild growth enhancement of osteoblast-like cells.	[57]
Cu-doped 45S5 bioactive glass		5 wt %	Induce an early osteogenic differentiation of hMSCs, promote the expression of anti-inflammatory interleukin, and reduce proinflammatory interleukin expression.	[59]
Cu–CaP	Cu-containing CaP-based coatings on Ti and novel Ti–40Nb alloy	0~0.4 at. %	Promote high motility of hAMMSCs on the implant/cell interface and subsequent cell ability to differentiate into osteoblasts.	[61]
Copper-enriched diamond-like carbon coatings	Multifunctional Cu/a-C:H thin coating depositing on the Ti–6Al–4V alloy (TC4)	>37 wt %	Higher osteogenesis activities.	[62]
PCL/RGO Cu	Copper nanoparticle decorated reduced graphene oxide (RGO_Cu) hybrid particles in polycaprolactone (PCL) matrix	0.6 wt %	Promote osteogenic activity of osteoblasts by increasing mineral deposition.	[63]
MgO–Cu nanoparticles	Multi-functional nanocomposites by precise loading of Cu^{2+} onto MgO nanoparticles	0.3 wt% 3 wt% 25 wt %	Promote osteoblast proliferation (0.3 wt %, 3 wt % and 25 wt %) and differentiation (3 wt % and 25 wt %).	[64]
Cu–TiO_2 coatings on Ti	micro-arc oxidation (MAO) technique was employed to fabricate Cu containing ceramic coatings on titanium substrates	2 mM	Exhibit enhanced macrophage-mediated osteogenesis and bactericidal capacity.	[65]
Cu-Hier-Ti surface	Cu-containing micro/nano-topographical bio-ceramic surface	Not mentioned	Activate pro-inflammatory M1 macrophages by Cu-transport signaling and enhance the proliferation and differentiation of osteoblasts.	[66]
Pure Cu	Disk of pure copper	99.99 %	Have severe cytotoxicity toward osteoblasts.	[68]
Pure Cu	Copper wires	Not mentioned	Induce an arrest of the osteoblastic growth in a dose- and time-dependent manner.	[70]
Cu^{2+} deposited into calcium phosphate	Copper was homogenously deposited into calcium phosphate films in varying concentrations	0.1 μM 1 μM 10 μM	Inhibit osteoblast proliferation in a concentration dependent way.	[71]

and mineralization of osteoblastic bone nodules [47].

In vivo cytoplasmic superoxide caused a distinct weakness in bone stiffness and decreased BMD, aging-like changes in collagen cross-linking, and transcriptional alterations in the genes associated with osteogenesis. SOD1 catalyzes the disproportionation of the superoxide radical to produce molecular oxygen and hydrogen peroxide. The surface areas of both osteoblasts and osteoclasts were decreased significantly in the SOD1-deficient mice, indicating the occurrence of low-turnover osteopenia. Nojiri et al. [48] found SOD1 deficiency impairs the viability of osteoblasts via redox imbalance. The expression levels of essential genes (COL1A1, ALP) for osteogenesis and osteoblast maturation were suppressed significantly in SOD1deficient cells. Runt-related transcription factor 2 (Runx2) expression also tended to decrease in $SOD1^{-/-}$ mice. In vitro experiments confirmed that intracellular oxidative stress induced cell death and reduced the proliferation in primary osteoblasts.

3.2. Exogenous copper from biomaterials has bidirectional effects on osteoblasts

Copper has been extensively studied in orthopedic and dental implant materials due to its angiogenic and antimicrobial capabilities. The released copper ions have bidirectional effects on osteoblasts. The effect of copper-containing biomaterials on osteoblasts is shown in Table 1.

3.2.1. Promotion

Microporous Cu–TiO_2 coating on the titanium surface can promote the osseointegration of titanium implants [49]. A new class of Cu-alloyed titanium (TiCu) alloys greatly improved the bone mass and bone structure parameters, which was conductive to improve the bone strength around the implants. The addition of copper could also alleviate the reduced implantation stability caused by osteoporosis. Histological analysis shows TiCu alloy could significantly promote osteogenesis, its remodeling toward trabecular structure around the implants [50]. Copper-bearing stainless steel (Cu-SS) has more new bone tissue formed around the implant with more stable bone-to-implant contact than 317L SS [4]. A Cu-functionalized bi-layered scaffold shows significantly reduced angular resorption in the apical direction of periodontal bone tissue, increased new bone volume and mineralization degree in bone defect model. Histological evaluation shows the scaffold with copper induced favorable periodontal bone regeneration with repaired bone tissue, fully replacing the defect and well integrating with the surrounding tissues [51].

The addition of copper in biomaterials could promote the proliferation of osteoblasts, so as to markedly enhance the bone formation activity and accelerate the grow and repair of bone defect [49,50,52–55]. Duan et al. [56] found the addition of Cu element in the Co–Cr–Mo alloys could stimulate ROS production in osteoblasts at a certain Cu ion concentration (2 wt%) thus promote cell proliferation. Furthermore, the addition of copper ions to the alloy resulted in decreased Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) and p-AMPK level, suggesting that Cu ions could activate mammalian target of rapamycin (mTOR) signaling by downregulating AMPK phosphorylation, leading to the induction of osteoblast proliferation. The reduction of Caspase-3 protein levels was also observed, indicating that osteoblasts apoptosis was inhibited by the incorporation of copper. It is also found that copper containing bioactive glass could enhance lipid peroxidation associated with mild growth enhancement of osteoblast-like human osteosarcoma cells [57]. On the other hand, some researchers have found that the addition of Cu inhibits the proliferation of osteogenic precursor cells and promotes their osteogenic differentiation. Rodriguez et al. have found that copper added to the culture medium modified both the differentiation and the proliferative activity of MSCs obtained from postmenopausal women. Copper (50 mM) diminished the proliferation rate of MSCs, increasing their ability to differentiate into the osteogenic lineages [58]. Copper loaded bioactive glass also showed lower cell proliferation due to hMSC osteogenic differentiation [59].

Osteoblasts in differentiation accumulated many mineral elements, including calcium, copper, iron, manganese, magnesium, and zinc [60]. The promoting effect of copper on osteogenesis is also manifested in that it can promote osteoblast differentiation and calcium deposition [61,62]. The addition of copper to varies biomaterials exhibited favorable osteogenic properties, proved by higher ALP enzyme activity (the early-stage osteogenic differentiation of bone MSCs), substantially enhanced osteocalcin (OCN) expression (a late-stage osteogenic marker), and promotion of the expression of osteogenic-related genes (Runx2, ALP, collagen I (COLI), OCN, osteopontin (OPN) and bone morphogenetic protein (BMP)) [4,51,56]. Cu-loaded bioactive glass even induced an inductive effect on ALP expression in a basal medium without osteogenic factors [59]. Copper could also induce higher expression of collagen I, osteoprotegerin, osteopontin and finally mineralization of the cells [55,63], which indicates that copper is able to stimulate the development to fully functional osteoblast from osteogenic precursor cells. Duan et al. [56] demonstrated that proper concentration of Cu ions (2 wt%) could accelerate the extracellular matrix mineralization of osteoblasts. The mineralization assay showed an apatite-rich layer was formed on the surface of a copper-loaded porous scaffold [53]. It is worth noticing that Rodríguez et al. [58] reported that Cu^{2+} significantly inhibited the ALP activity in MSCs, while it could induce a shift in the ALP activity expression to earlier days and accelerate bone-like nodule formation. Thus, the inhibited ALP activity in Cu^{2+}-treated osteoblasts is also considered as a marker for enhanced osteoblastic differentiation [64].

The mechanism by which copper ions regulate osteogenic differentiation is usually related to the regulation of inflammatory responses, but the relevant findings have been conflicting. Rau et al. [59] found that the presence of copper in bioactive glass coatings induces an early differentiation of hMSC through osteoblast phenotype, promotes the expression of anti-inflammatory interleukin (IL-10), and reduces pro-inflammatory interleukin (IL-1β) expression. Ren et al. [4] have found the release of Cu^{2+} ions from Cu-SS was able to suppress the inflammatory response with lower TNF-a expression in the bone tissue post implantation. Contrary to their opinion, Huang et al. [65,66] advocates that copper modulates a favorable inflammatory microenvironment for osteogenic differentiation. They have found that Cu^{2+} released from Cu-containing micro/nano-topographical bio-ceramic surface could polarize macrophages to pro-inflammatory M1 phenotype by activating Cu-transport signaling (CTR1 and ATP7A) in macrophages, evidenced by up-regulated gene expressions of M1 surface markers (CD86 and CD11c) and pro-inflammatory cytokines (TNF-α, IL-6, iNOS and IL-1β). The inflammatory microenvironment created by macrophages treated by Cu^{2+} could enhance the proliferation and differentiation of SaOS-2 cells. Therefore, the mechanism behind the regulation of osteogenic differentiation by copper still needs further investigation.

3.2.2. Inhibition

Qi et al. [67] have found that osteoblasts treated with $CuCl_2$ exhibited promoted ROS production, and inhibited SOD and Glutathione peroxidase activities. At the same time, the concentration of ALP and the mRNA expression of OCN and Col1 decreased with $CuCl_2$ dose, indicating that copper induces the structure impairment in osteoblasts by oxidative stress. In addition, they have also found that Cu^{2+} induced inflammation cytokine (IL-1, TNF-α, IL-6) by inactivating transforming growth factor-β1/Smad3 pathway, leading to the reduction of the core binding factor α-1 and BMP, which resulted in the osteogenesis impairment of osteoblasts.

In addition, copper itself has shown severe cytotoxicity toward osteoblast-like cells [68]. Femurs from 9-day-old chick embryos cultured in the presence of 1 uM copper sulfate showed atrophied trabecular and a thinner layer of uncalcified osteoid tissue around it,

which represents decreased bone matrix [69]. Pure Cu induced an arrest of the osteoblastic growth in a dose- and time-dependent manner. It also decreased the ALP, a specific marker of osteoblastic phenotype [70,71].

It can be found that Cu exhibits two opposite effects on osteogenesis. Bioimplant materials that possess an excellent corrosion resistance with much small amount release of copper ions generally have better osteogenic properties, while cells cultured with copper containing solution or directly on pure copper show significant cytotoxicity and inhibition of osteogenesis, which might be caused by excessive copper ion concentrations.

4. Copper effects on osteoclasts

4.1. Copper affects osteoclast formation and viability

As an important enzyme cofactor, copper may affect osteoclasts by influencing enzyme activity. Copper is an important enzyme cofactor of Copper Metabolism MURR1 Domain-containing protein (COMMD1) [72], which could effectively suppress the activation of NF-κB pathway to block osteoclastogenesis [73].

LOX is a secreted copper-dependent amine oxidase that modifies the extracellular matrix of bone. In a previous study, LOX was shown to increase receptor activator of nuclear factor-κB ligand (RANKL) expression in osteoblasts and consequently promote osteoclastogenesis [74]. Reynaud et al. [47] found that tumor-secreted LOX and IL-6 were acted in concert to promote the generation of differentiated osteoclasts induced by RANKL. LOX overexpression induced a robust production of IL-6, which has an apparent function of promoting osteoclastic bone resorption. In addition, LOX promoted a greater nuclear localization of nuclear factor-activated T cell 1 (NFATc1), the master regulator of osteoclastogenesis.

Copper deficiency may reduce the activity of the antioxidant enzyme superoxide dismutase, thereby increasing osteoclast activation and bone resorption [75]. SOD catalyzes the disproportionation of the superoxide radical to produce molecular oxygen and hydrogen peroxide. Copper/-zinc superoxide dismutase, encoded by the SOD1 gene is located in the cytoplasm. Nojiri et al. [48]. found that both the number of osteoblasts and osteoclasts were decreased in the bones of the SOD1-deficient mice. SOD1 deficiency impairs the viability and function of osteoblasts via redox imbalance, while it does not directly impair osteoclast function and viability. Impaired osteoblast viability suppresses RANKL/Macrophage colony-stimulating factor osteoclastogenic signaling, resulting in the suppression of osteoclastogenesis in bone.

Copper has been widely used in the study of orthopedic implant biomaterials for its superior osteogenic, angiogenic and antimicrobial properties. In the process, researchers have discovered that copper may affect osteoclasts by modulating the inflammatory response. It has been reported that copper modified cobalt-chromium particles could down-regulate the expression of inflammatory factors TNF-a, IL-6, and IL-1β, and reduce the expression of NF-κB as well as downstream osteoclast related specific transcription marker genes, such as tartrate resistant acid phosphatase (TRAP), NFATc1 and Cathepsin K (CTSK) [3]. Researchers have demonstrated that the addition of copper to biomaterials may reduce the gene expression of pro-inflammatory factors by inhibiting the formation of M1-type macrophages, while up-regulate the gene expression of anti-inflammatory factors by promoting the proliferation and differentiation of M2-type macrophages [3,76].

Biomaterials models containing copper were often used to study the role of copper in bone homeostasis. The effect of copper-containing biomaterials on osteoclasts is shown in Table 2. Researchers have found that the addition of copper in bioactive glass nanofibers induced a decrease in cell proliferation of treated osteoclast precursors. MgO nanoparticles loaded with Cu^{2+} exhibited a dose-dependent inhibitory effect on osteoclast formation, as evidenced by the decrease in osteoclast size and number [64]. And Cu-bearing Co29Cr9W particles could reduce the wear particle-induced osteolysis by decreasing the resorptive area and number of osteoclasts [3]. Cu ions released from the Ti6A14V–6Cu alloys decreased the TRAP activity of osteoporotic osteoclasts, as well as the expression of osteoclast differentiation genes (CTSK, NFATc1, TRAP, NK-κB) and protein (NK-kB and TRAP) [76]. However, it is also reported that copper chelator ammonium tetrathiomolybdate decreased the expression of RANKL in osteoblasts and osteocytes, subsequently suppressing osteoclast differentiation [77]. This might be due to the inhibition of LOX activity by the copper chelator. The local release of copper ions by implantation of biomaterials may lead to the opposite conclusion to the regulation of systemic copper metabolism. Therefore, the choice of research model is also crucial for studying the relationship between copper and bone metabolism.

4.2. Copper affects osteoclastic bone resorption

1981, Wilson [78] cultivated mouse calvarial tissue in the presence and absence of Cu^{2+} and showed a dose-dependent decrease in osteoclastic resorption. It was reported that the concentrations of 1×10 (-14) mol/L - 1×10 (-4) mol/L copper ion can inhibit osteoclastic resorption on dental slices [75].

In a study of the effect of inorganic additives on the behavior of osteoblasts and osteoclasts, primary rat osteoclasts cultured on calcium phosphate films containing copper showed a significantly decreased resorptive activity which is evidenced by the significant decrease in the number of resorption pits. However, the total number of osteoclasts attached on mineral films or cell viability wasn't affected [71]. In a study in 2017 conducted by Bernhardt [79], resorption of mineralized extracellular bone matrix was strictly inhibited when osteoclasts were differentiated in the presence of Cu^{2+} (0.027–0.305 mM). However, they have also found that the reduced resorption in the presence of Cu^{2+} was only demonstrated in the case of osteoclasts which were treated with Cu^{2+} during osteoclastogenesis. In contrast, copper doesn't alter bone resorption when cultivated with mature osteoclasts, indicating an effect of Cu^{2+} on osteoclast formation rather than osteoclast function

Table 2
Effect of copper-containing biomaterials on osteoclasts.

Materials	Description	Concentration	Conclusion	Reference
Co29Cr9W3Cu particles	Copper modified cobalt-chromium alloy	Not mentioned	Reduce osteoclast formation and decrease the amount of osteoclast and osteolysis area of murine calvaria.	[3]
Copper-modified Ti6Al4V	Selective laser melting (SLM)-fabricated Ti6Al4V–Cu alloy	6 wt %	Suppress the inflammatory response of osteoporotic macrophages and osteoclast formation.	[76]
MgO–Cu nanoparticles	Multi-functional nanocomposites by precise loading of Cu^{2+} onto MgO nanoparticles	3 wt % 25 wt %	Inhibit osteoclast formation.	[64]
Cu^{2+} deposited into calcium phosphate	Copper was homogenously deposited into calcium phosphate films in varying concentrations	0.1 μM 1 μM 10 μM	Decrease the resorptive activity of primary osteoclasts in a dose dependent manner.	[71]
Brushite forming calcium phosphate cements (CPC) doped with Cu^{2+}	Brushite-forming calcium phosphate cements modified with low doses of Cu^{2+}	10 mM/g	Have cytotoxic effect on osteoclasts during direct cultivation. Indirect cultivation with diluted extracts did not provoke cytotoxic effects but strictly inhibited bone resorption.	[79]

[79].

Bone resorption is associated with an increase in acid phosphatase activity. The sensitivity of this osteoclastic acid phosphatase activity to copper may be related to its ability to inhibit bone resorption. Hammarstrom [80] found that the vitamin D-stimulated acid phosphatase activity in bone was almost completely inhibited when copper was present in the incubation medium (0.1 mM). The sensitivity of the vitamin D-induced acid phosphatase to copper may be correlated with in vivo experiments in mice, showing that copper could counteract the effect of parathyroid hormone. Besides, copper sulfate produced results that acid phosphatase in osteoclasts was inhibited more than the enzyme in the forming cells of hard tissue, indicating that osteoclastic acid phosphatase is sensitive to copper.

5. Conclusion

This review describes the effects of copper ions on bone metabolism and focuses on the effects of copper levels on the proliferation and differentiation of osteoblasts and

osteoclasts, as well as their ability of bone deposition and resorption. Both copper deficiency and copper excess can affect the differentiation of osteoblasts and osteoclasts, leading to an imbalance between bone formation and bone resorption, and ultimately to abnormal bone metabolism. Systemic abnormalities of copper metabolism like Menkes disease and Wilson's disease show impaired osteogenesis and lower osteogenesis activity. Inside the cell copper usually affects cell as an important enzyme cofactor. Copper-dependent amine oxidase LOX and SOD1 play an essential role in regulating both osteoblast differentiation and osteoclast formation. Copper is widely used in the study of orthopedic implantable biological materials because of its angiogenic and antimicrobial activity, copper ions released from the materials could affect osteoblasts and osteoclasts at the implantation site. Most studies have concluded that copper ions promote osteogenesis and inhibit osteoclastogenesis, but others have held the opposite conclusion. In addition, the mechanism by which exogenous copper ions regulate local osteogenic and osteoclastic differentiation and their ability of bone formation and bone resorption has not been clarified. In summary, researchers still have a long way to go in terms of the effects of both endogenous copper ion metabolism and exogenous copper ion release on bone metabolism.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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