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Review Article

Extracellular derivatives for bone metabolism

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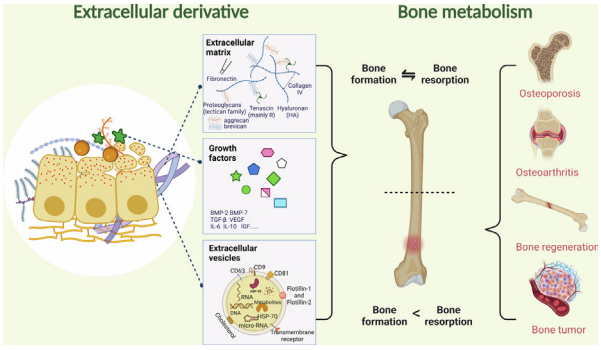
HIGHLIGHTS

- The sources of producing extracellular derivatives are extensive, that together affect bone growth and metabolism.
- Extracellular derivatives act as targeted drug carriers and offer a fresh perspective in the treatment landscape.
- Orthopedics witnessed innovative approaches centered around extracellular vesicles, revealing their promise in bone healing and regeneration.
- The exact mechanisms by which extracellular derivatives influence bone health are complex and subtle, marking an exciting area of exploration and study.
- Extracellular derivatives have attractive potential for organoid applications, and their combination could usher in a new era of organoid-based research and therapy.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Bone metabolism can maintain the normal homeostasis and function of bone tissue. Once the bone metabolism balance is broken, it will cause osteoporosis, osteoarthritis, bone defects, bone tumors, or other bone diseases. However, such orthopedic diseases still have many limitations in clinical treatment, such as drug restrictions, drug tolerance, drug side effects, and implant rejection.
Aim of review: In complex bone therapy and bone regeneration, extracellular derivatives have become a promising research focus to solve the problems of bone metabolic diseases. These derivatives, which include components such as extracellular matrix, growth factors, and extracellular vesicles, have significant therapeutic potential. It has the advantages of good biocompatibility, low immune response, and dynamic demand for bone tissue. The purpose of this review is to provide a comprehensive perspective on extracellular derivatives for bone metabolism and elucidate the intrinsic properties and versatility of

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Clinical application

extracellular derivatives. Further discussion of them as innovative advanced orthopedic materials for improving the effectiveness of bone therapy and regeneration processes.

Key scientific concepts of review: In this review, we first listed the types and functions of three extracellular derivatives. Then, we discussed the effects of extracellular derivatives of different cell sources on bone metabolism. Subsequently, we collected applications of extracellular derivatives in the treatment of bone metabolic diseases and summarized the advantages and challenges of extracellular derivatives in clinical applications. Finally, we prospected the extracellular derivatives in novel orthopedic materials and clinical applications. We hope that the comprehensive understanding of extracellular derivatives in bone metabolism will provide new solutions to bone diseases.

Contents

Introduction

Bone as the hardest organ of vertebrates, mainly provides exercise, support, protection, and storage to the body, so bone metabolism is an activity that organisms carry out all lives. Bone metabolism refers to the continuous process of bone formation (osteogenesis) and bone resorption (osteolysis) that occurs throughout life [1]. It mainly consists of four parts: bone formation, bone absorption, calcium homeostasis, and hormone regulation. Osteoblasts are responsible for the synthesis and deposition of new bone matrix and are essential for bone development, fracture healing, and remodeling of old bone or damaged bone tissue [2]. Bone resorption releases minerals back into the bloodstream. This process helps maintain calcium and phosphorus levels, allows bone remodeling and repair, and promotes the release of growth factors stored in the bone matrix [3,4]. Between cells in bone tissue, hormones, and signaling factors are produced that regulate the activity of bone cells, affect bone renewal, and maintain overall bone health [5]. Imbalances in bone metabolism can lead to diseases such as osteoarthritis, osteoporosis, bone tumors, and fracture healing difficulties, which may increase the risk of other complications.

At present, the commonly used treatment methods for bone metabolic diseases are drug therapy, surgical treatment, and physical therapy [6]. Physical therapy cannot play the main role, mainly in relieving the pain caused by bone diseases and auxiliary treatment. Drug treatment is usually the use of anti-bone resorption drugs, hormone-regulating drugs, and calcium supplements. However, drug treatment varies not all patients have significant improvement or benefit, and drugs may be taken for a long time, resulting in a heavy physiological and economic burden on patients [7]. Common complications of gastrointestinal problems may also occur. Surgery is not appropriate for older postmenopausal women and those at high risk for diabetes [8]. Persistent bone metabolism imbalance caused by osteoporosis, osteoarthritis bone tumors, and other diseases, the use of current treatment to treat the symptoms, cannot solve the problem from the root. Therefore, there is an urgent need for a safe and efficient treatment that can fundamentally regulate bone metabolism and promote bone tissue regeneration.

In recent years, more attention has been paid to the application of extracellular derivatives in disease treatment and their potential for further clinical application. Extracellular derivatives are mainly divided into three types: extracellular matrix (ECM), growth factors, and extracellular vesicles (EVs). ECM is a reservoir of bioactive molecules such as extracellular growth factors, cytokines, and enzymes, and provides a dynamic microenvironment and structural support for surrounding tissue [9,10]. The microenvironment of ECM can support the endogenic growth of blood vessels and bone cells, provide a scaffold for new bone formation, and promote

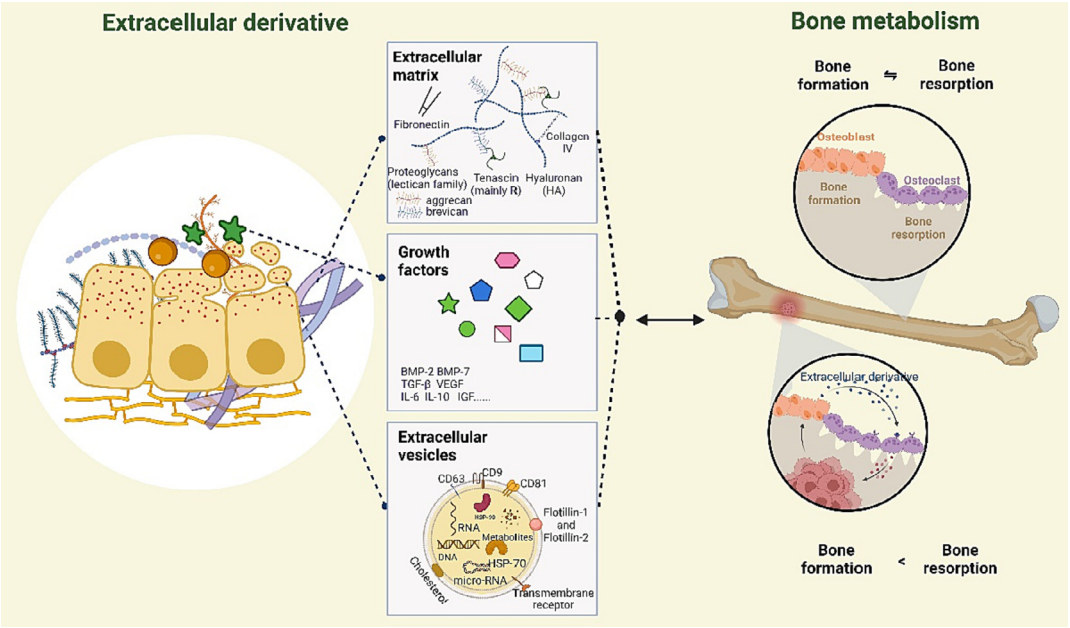

Fig. 1. Diagram of the types and structure of extracellular derivatives and bone metabolism. Three main extracellular derivatives: ECM, growth factors, and EVs. The common function of the three is to participate in cell communication and signal transmission in bone metabolism. The difference is that ECM can also provide mechanical properties and signal storage functions for cells and tissues, growth factors can directly act on related cells of bone metabolism, and EVs can carry nucleic acids of source cells. The dynamic balance of bone regeneration and bone resorption maintains a good bone metabolism process, whereas the extracellular derivatives also change when the imbalance occurs, which can regulate bone metabolism through extracellular derivatives. The figure was created with BioRender.com.

the repair and regeneration of damaged bone [2,11]. ECM contains growth factors that stimulate stem cells to differentiate into osteoblasts and induce bone formation. The specific role and function of growth factors in bone may vary depending on the microenvironment, cell type, and stage of bone development. The interaction between growth factors and their downstream signaling pathways contributes to the complex regulation of bone remodeling and homeostasis. Growth factors can be localized or transported throughout the body along blood vessels [12]. EVs also can be transported through the blood. EVs are important mediators of intercellular communication in the bone microenvironment and contain a variety of nucleic acids, proteins, lipids, signaling molecules, and other bioactive molecules used for signaling [13]. Different from ECM, EVs can transfer regulatory microRNAs that affect gene expression in recipient cells [14]. EVs are involved in communication between bone cells and other cell types in the bone microenvironment, such as immune cells and endothelial cells [15]. Understanding the signal transduction mechanism of extracellular bone derivatives helps develop new drugs and materials for the treatment of bone-related diseases. In conclusion, extracellular derivatives are important substances that regulate bone metabolic homeostasis and are also the most obvious markers of bone metabolic abnormalities (Fig. 1).

In this review, an overview of the types, sources, and functions of extracellular derivatives is summarized. Then, special attention is focused on extracellular derivatives from different cell origins in bone metabolic disease treatment. Finally, the advantages and challenges of extracellular derivatives in clinical applications are concluded, and extracellular derivatives in new orthopedic materials are prospected.

Types and functions of extracellular derivatives

Extracellular derivatives refer to products produced by cells and released into their surroundings with certain biological activity, including ECM, growth factors, and EVs. They can influence cellular behavior, regulate tissue homeostasis, promote tissue repair and regeneration, and modulate immune responses. Extracellular derivatives can load cargo and affect target cells far from the location where the cell was produced (Fig. 2).

Extracellular matrix

ECM is a three-dimensional protein structure interspersed among cells in every tissue and organ. It not only provides the necessary physical scaffolding for cellular components but also supports biochemical substances for cell differentiation and tissue homeostasis. ECM provides the integrity and elasticity of tissues. As cells undergo physiological transformations, the array of receptors, cytokines, and growth factors housed within the ECM modulates accordingly. This dynamic adaptation ensures the balanced homeostasis, growth, and functional integrity of tissues and organs [2,13]. This interplay becomes especially evident during tissue development, where intricate biochemical and biophysical dialogues ensue between diverse cellular entities and the continually evolving cellular and protein microenvironments.

The principal constituents of the ECM are proteins and polysaccharides. Their various combinations bestow upon cells a distinct compositional and topological character [16,17]. The collagens most abundant in ECM in bone are type I, III, and V collagens, whose main function is to serve as a mechanical support and scaffold for bone cells [18,19]. 90 % of collagen in bone tissue is type I collagen, which forms collagen fibril. It then interacts with other collagen and non-collagen proteins to assemble into highly ordered fiber bundles [20]. The functions of type III and V collagen are mainly to regulate the formation of type I collagen. Cross-linking between collagen proteins can provide mechanical properties for tissues, cross-linking strength and collagen quality determine bone strength [21]. A lack of collagen can cause changes in the ECM, which can increase the risk of fracture [22,23].

The polysaccharide of ECM is mainly the residue after the degradation of glycosaminoglycan (GAG) which is continuously processed by enzymes, including hyaluronic acid, heparin sulfate, chondroitin sulfate, dermal sulfate, and keratin sulfate. In addition,

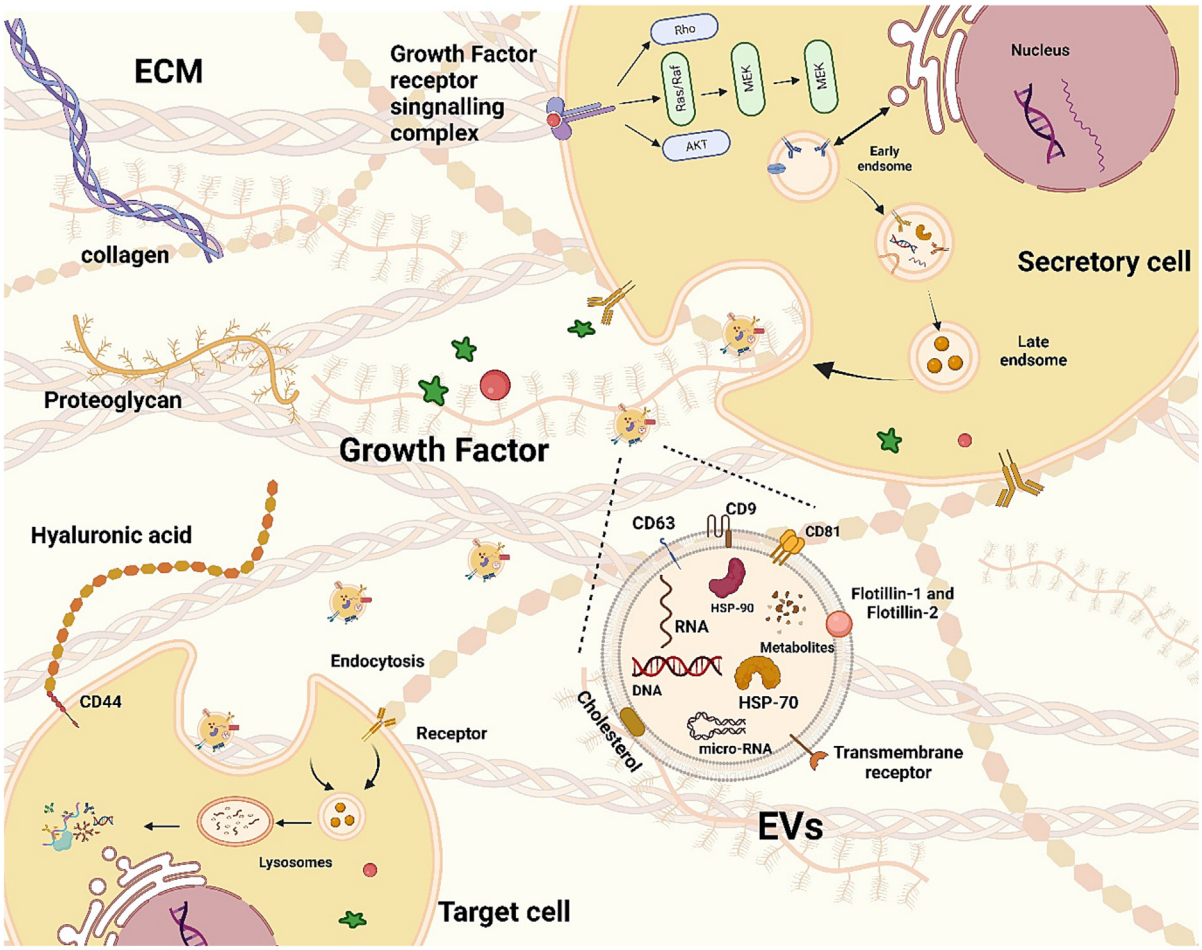

Fig. 2. Schematic diagram of the types, generation, and effects of extracellular derivatives. i) The complex network of cells secreting collagen, hyaluronic acid, and proteoglycan into the extracellular space is ECM, which provides structural support, nutrients, and regulating signal pathways for tissues and organs. ii) Growth factors are usually produced by cells in response to stimuli or during specific stages of development or tissue repair. They can be secreted locally or transmitted throughout the body, bind to specific receptors on the cell surface, initiate intracellular signaling cascades, and regulate gene expression, protein synthesis, and cellular response. iii) EVs are formed through the plasma membrane inward budding or outward foaming, resulting in the cell components being encapsulated in EVs. They can carry various bioactive molecules such as nucleic acids, proteins, and lipids from the parent cells. EVs also can be absorbed by the recipient cells through mechanisms such as endocytosis and receptor binding, leading to the transfer of its cargo and subsequent cell function regulation. The figure was created with BioRender.com.

glycoproteins and signaling proteins are the main regulatory factors, which are crucial for regulating the behavior of bone metabolism. Among the glycoproteins of ECM, osteonectin is a common representative, also known as an acid-rich cysteine-secreting protein. Osteonectin has the highest expression in osteoblast differentiation and is widely present in mineralized tissues. By binding collagen and hyaluronic acid crystals, it releases pivotal calcium regulators, thereby influencing collagen mineralization [24].

In mature mineralized tissues, there is osteopontin (OPN), dentin matrix protein-1 (DMP-1), stromal extracellular phosphoglycoprotein (MEPE), and bone sialoprotein (BSP). OPN has a high base sequence of serine and aspartic acid, which helps to inhibit the potential phosphorylation site of mineralization and can be highly expressed in osteoblasts. It is an important marker of bone formation and mineralization during bone metabolism. In addition, OPN also regulates osteoclast differentiation and promotes bone resorption [25]. Both DMP-1 and MEPE, majorly synthesized by osteoblasts (with some contribution from bone marrow cells and odontoblasts), are pivotal in phosphate metabolism and bone matrix mineralization [26,27]. BSP is a highly glycosylated non-collagenous phosphorylated protein, which plays a regulatory role in osteoblast differentiation and matrix mineralization and is mainly expressed in the early stage of connective tissue mineralization. The absence of BSP can significantly reduce the length of long bone, cortical thickness, bone deposition rate, and bone formation rate [28].

Every cellular and tissue phase necessitates intimate interaction with the ECM. It equips cells with both the interstitial matrix and the basement membrane. While the basement membrane furnishes support to cell layers and tissue architectures, the interstitial matrix facilitates signal transduction through cell and receptor adhesion. Consequently, the ECM not only dictates cellular positioning but also orchestrates vital biological processes, including cell differentiation, proliferation, polarity, and migration.

Growth factors

The proteoglycan component of ECM regulates the activity of many growth factors, which are proteins and cytokines that regulate bone metabolism and are essential for cell signaling and tissue development [29]. All growth factors work together to participate in the bone metabolism process and participate in the various stages of bone regeneration. The bone healing process after injury involves catabolism and anabolism, including all the links of bone metabolism to form new and complete bone tissue. Bone injury is

accompanied by the destruction of the integrity of local soft tissue and vascular tissue, resulting in inflammation [30].

In early inflammation, ECM of degranulated platelets and macrophages can store and release a variety of pro-inflammatory cytokines, including tumor necrosis factor (TNF), bone morphogenetic protein (BMP), typical cytokines associated with inflammation (IL-1, IL-6), macrophage colony-stimulating factor, and transforming growth factor (TGF-β) [31–33]. TNF-α is primarily secreted by macrophages, which can induce apoptosis in stem cells and repair fractures by allowing muscle cells to gather [34]. The main marker of proinflammatory response is the local activation of IL-1β, which in turn activates other secondary inflammatory mediators. So, IL-6 can be used to fight inflammation and produce acute proteins. A key function of TGF-β is to regulate inflammation, and a sudden increase in its expression is mostly associated with malignancy, as well as with defects in the cell growth-inhibiting response to TGF-β. BMPs are the largest subfamily of TGF-β, which are the major growth factors that induce bone and cartilage formation, including BMP-2, BMP-4, BMP-6, and BMP-7 [35,36].

Another important factor affecting bone remodeling is vascular remodeling, as blood vessels provide outlets for nutrient and gas exchange and breakdown products. During intramembranous bone formation, angiogenesis into osteoblast progenitors provides access. In addition, blood vessels transmit circulating factors such as vitamin D and parathyroid hormone (PTH) that regulate bone regeneration throughout the body [37]. In intraoral bone healing, angiogenesis is also involved in chondrocyte apoptosis and cartilage degradation [38,39]. Pro-angiogenic factors include placental growth factor (PLGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF-2), and BMP, also blood vessels provide paracrine signals to promote bone growth. The most important growth factor in vascular remodeling is vascular endothelial growth factor (VEGF), which will directly balance bone metabolism, and too much or too little will cause the biological function of osteoblast or osteoclast. Abu-Amer et al. [40] investigated whether VEGF was involved in promoting osteolysis. The core process of osteolysis was shown to be around the angiogenesis of the prosthesis, as blocking VEGF function with neutralizing antibodies prevents angiogenesis and osteolysis. There are two reasons for this phenomenon, one is the pro-lysis effect of VEGF, and the other is the physical effect of VEGF and its receptors [41]. In addition, angiogenesis can prevent or slow down bone loss. Blood vessels transport lots of inflammatory cells and related proteins and growth factors, which help promote the formation of osteoclasts and inhibit osteoblasts.

At the later stage of the bone regeneration process, the early callus of cartilage is absorbed to form a harder callus, and the mechanical properties of bone are gradually restored. At this stage, chondrocytes become hypertrophic, release calcium, and undergo apoptosis. The reabsorption of mineralized cartilage is driven by a series of BMP factors secreted, which in turn are involved in bone metabolism signaling pathways, such as Wnt, BMP, receptor activator of nuclear factor-κB (RANK) [42,43]. Wnt refers to a family of secreted signaling proteins that play critical roles in tissue homeostasis. Wnt proteins act as ligands that bind to specific cell surface receptors called Frizzled receptors, initiating a complex signaling cascade known as the Wnt signaling pathway. Understanding Wnt signaling is crucial for advancing our knowledge of both normal development and disease pathogenesis, potentially leading to the development of therapeutic interventions. The receptor activator of NF-kB ligand (RANKL) is a transmembrane protein that is primarily produced by osteoblasts, binds to the RANK, and can differentiate osteoclast precursors into mature osteoclasts after activation [44]. In summary, the key role of growth factors in bone metabolism is to stimulate the activity of osteoblasts, enhance matrix synthesis, participate in the regulation of osteoclast activity, promote angiogenesis, and accelerate bone healing.

Extracellular vesicles

EVs are small membrane-wrapped structures secreted by all types of cells, which are important in transmitting and regulating cells. There are two kinds of EVs: ectosomes and exosomes. Exosomes are produced directly from the plasma membrane to the outward budding, and the diameters of the micro-vesicles, micro-particles, and large vesicles produced by exosomes range from 50 nm to 1 μm. In contrast, exosomes are about 100 nm in diameter and originate from endosomes. As a subset of EVs, exosomes have no essential difference in composition [45]. They communicate and regulate information by carrying bioactive molecules inside cells, and are present in almost all cells and organs. The process from production to reception of EVs covers all aspects of pathogenic information transmission and tissue remodeling in physiological tissues.

EVs contain source cell nucleic acids, lipids, membrane proteins, and other cell-specific proteins involved in biomolecules essential for bone metabolism [46]. EVs cargo associated with bone formation contains two classes of substances, divided into typical and special species. Typical is the general substance involved in the production and transport of vesicles, such as enzymes, cytoskeletal proteins, and specific stress proteins. Special is the bone-associated EVs special cargo composed of specific osteogenic proteins and non-collagen matrix proteins, such as OPN, osteocalcin (OCN), alkaline phosphatase (ALP), osteonectin (ON), BMP, and eukaryotic initiation factor 2 (EIF2) [47–49], which all function as parent cells. Specific species can also be EVs that contain cargoes associated with osteoclast differentiation, including receptor activators of RANK and RANKL. Another important component of EVs is miRNAs and ncRNAs. The miRNAs play a regulatory role in embryonic development, tissue differentiation, and signaling pathway control. Most EVs-mediated regulatory effects triggered in cells and organs are mediated by ncRNAs. The most representative categories of ncRNAs are "antisense RNA" and "lncRNA" [50,51].

In bone metabolism, EVs derived from different cell types in the bone, such as osteoblasts, osteoclasts, and mesenchymal stem cells (MSCs), contain specific cargo that can modulate bone cell behavior. For example, EVs derived from osteoblasts or osteocytes can transport factors involved in bone mineralization, osteoblast proliferation, and matrix synthesis. These EVs can also transfer regulatory microRNAs that can influence gene expression in recipient cells. EVs act as immunomodulatory messengers mediating immune stimulation or suppression, and EVs secreted by mesenchymal stem cells (MSCs-EVs) provide a variety of immunomodulators, including TGF-β, galactin-1, and programmed death ligand-1 [52]. Moreover, EVs have been implicated in the communication between bone cells and other cell types present in the bone microenvironment, such as immune cells and endothelial cells. This intercellular crosstalk mediated by EVs helps coordinate bone homeostasis and repair processes [53]. Additionally, EVs derived from osteoclasts or MSCs can carry factors that regulate osteoclast differentiation and activity. They can deliver receptor activators of RANKL, a key molecule involved in osteoclast formation, to target cells and promote bone resorption. Osteoclasts are involved in bone resorption and mineralization, and osteoblasts are involved in bone remodeling through bone matrix synthesis.

Bone mineralization is an important step in bone formation, during which endothelial cells present in the developing bone growth plate produce a type of EVs that plays an important role and has attracted increasing attention. EVs are responsible for transporting calcium and phosphate between cells, and the

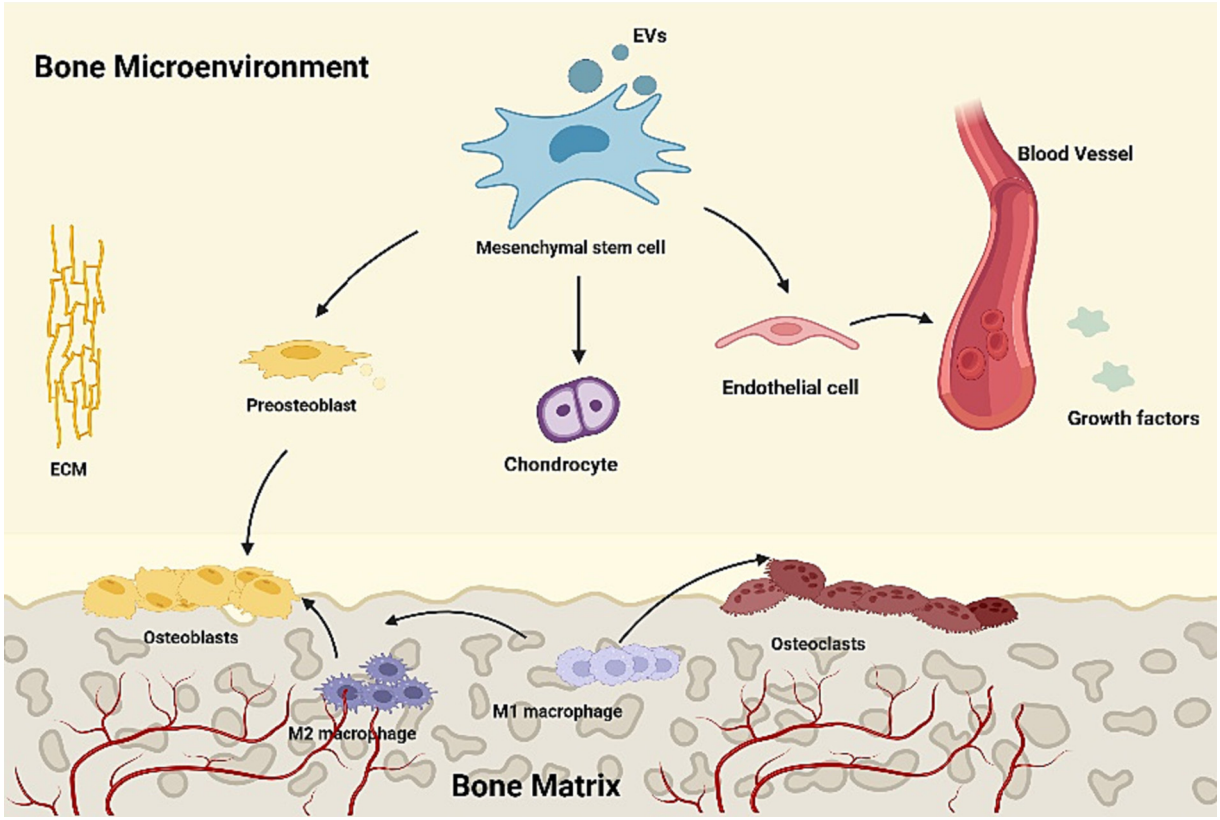

Fig. 3. Diagram of cells associated with bone formation units, bone resorption units, and hematopoietic units involved in bone metabolism. MSCs have multidirectional differentiation potential and can differentiate into osteoblast precursors, chondrocytes, and endothelial cells. Osteoblasts and osteoclasts work in homeostasis to ensure proper bone remodeling. Osteoblasts deposit new bone matrix, while osteoclasts absorb old or damaged bone, and hematopoietic stem cells provide a continuous supply of osteoclast precursors and other blood cell lineages for bone development, repair, and maintenance. Different cells produce their specific extracellular derivatives, and the interaction and communication between cells are strictly regulated and coordinated to maintain bone homeostasis. The figure was created with BioRender.com.

reaction results in the formation of hydroxyapatite crystals, which are released into the ECM with EVs and calcified directly after collagen calcification. At this time, a new bone matrix begins to form, the EVs are located at the initial site of hydroxyapatite deposition [54,55]. Osteoblast-derived EVs can dynamically mediate mineralization and changes in vesicle content and morphology, resulting in developmental changes during stromal tissue.

The process of bone metabolism requires the participation of blood vessels, and EVs are closely related to the formation of blood vessels. EVs can significantly increase collagen synthesis as well as the occurrence of newly formed and mature blood vessels at the wound site. EVs can enhance the secretion of collagen and elastin in fibroblasts, promote the proliferation and migration of human fibroblasts and human umbilical vein endothelial cells, and aid vascular reconstruction. Vascular reconstruction will directly affect the rate of bone metabolism, forming new blood vessels during bone development, repair, and diseases [56]. Overall, EVs are a bridge between cells, delivering bioactive molecules, and influencing the behavior of different cell types involved in bone formation and absorption. Understanding EV-mediated signaling mechanisms in bone could help develop new therapeutic strategies for bone-related diseases [57].

EVs from parasites, bacteria, and plants transmit bone metabolic cytokines affecting bone growth. Nematode EVs modulate macrophage activation and immune responses. Worm EVs can promote immunity against infections, and the cytokine IL-33 plays a pivotal role in this process, especially in its interactions with macrophages [58,59]. IL-33 binding to the IL-33 receptor is a key interaction that triggers allergic and infectious responses. Another strong correlation of IL-33 is with macrophages, which can be strongly polarized into another activated phenotype when stimulated to form immunity to infection [60,61]. Parasite-derived EVs not only infect host cells but also influence them to produce EVs, making these EVs potential targets for drug delivery and biomarker identification [62].

Plant EVs, similar to mammalian ones, can deliver drugs. They often contain nutrients beneficial to humans, like folic acid and vitamin C, which combat stem cell oxidation and aging [63]. Specifically, ginger EVs can address bacteria associated with periodontitis [64,65]. Bacterial EVs, due to their nanoscale structure, present advantages for drug delivery [66]. Liu et al. [67] developed bone-targeting, drug-loaded materials using EVs for potential osteoporosis treatment. While parasite, plant, and bacterial EVs relate to bone metabolism, their exact roles and mechanisms remain to be fully understood.

Functions of extracellular derivatives from different cells

Bone metabolism is a series of complex biological events, including bone formation, bone resorption, Ca^{2+} regulation, and bone remodeling, all of which maintain dynamic balance. The process of bone metabolism mainly involves MSCs, osteoblasts, osteoclasts, and endothelial cells (Fig. 3). The statistics of functions of extracellular derivatives from different cells are shown in Table 1. There is a close interaction between these cells to regulate bone metabolism and maintain the balance and function of bone tissue. Therefore, an in-depth study of the interrelationships between

Table 1
Functions of extracellular derivatives from different cells.

Cell types	Characterizations			Effects	References
	Extracellular matrix	Growth factors	Extracellular vesicles		
Mesenchymal stem cell	Contain interleukin, TNF	Generate FGF-2, IGF-1, TGF-β1, PDGF	• CD13, CD29, CD44, CD73 and CD105 receptors expressed • miR-199b, miR-218, miR-148a, miR-135b, miR-203, and miR-219 are upregulated	• Induce MSC to differentiate into osteoblasts • Promote osteoblast proliferation • Transmit signal • Transport specific protein • Activate the osteogenic signaling pathway	[52,53,69–73]
Osteoblast	More Type I collagen, osteopontin, osteocalcin and ALP	Generate parathyroid hormone, prostaglandin E2, insulin-like growth factor, TGF-β, BMP	• miR-667-3p, miR-6769b-5p, miR-7044-5p, miR-7668-3p are upregulated • Contain RANKL	• Activate the OPG/RANKL/RANK signaling pathway • Promote osteoblast differentiation into osteoblast • Conducive to mineralization • Coordinate osteoclast differentiation	[71–74,80–84]
Osteoclast	• Contain M-CSF, PDGF-BB, and more transmembrane protein	Geberate TNF-α, PDGF-BB	• Contains CD63, TSG101, HSP70, β-actin, RANK protein • miR-21、miR-23a、miR-24、miR-93、miR-100、miR-122a expression	• Stimulates osteoclast formation • Target osteoblasts Regulation of Ca^{2+}	[80,88–92]
Endothelial cell	Contain PDGF-BB, TGF, and more fibronectin	Generate PDGF-BB, RANKL, VEGF, BMP2, matrix Gla protein, bone protective protein	• Contains CD31, CD34, and vascular endothelial growth factor receptor 2	• Promote osteoblast proliferation and differentiation • Promote cell migration • Promote angiogenesis	[98–103]
Tumor cell	More HA and HA enzymes	Generate EGF, TGF, IGF	• Contains CyclinD1, MMP-9, VEGF	• Activate the RANK signaling pathway • Promote angiogenesis • Regulate the cholesterol content of bone marrow MSCs	[15,105–107]

Abbreviations: TNF: tumor necrosis factor; PDGF: platelet-derived growth factor; FGF: fibroblast growth factor; IGF: insulin-like growth factor; ALP: alkaline phosphatase; TGF: transforming growth factor; EGF: epidermal growth factor; RANK: receptor activator of nuclear factor-κB; OPG: osteoprotegerin; MSCs: mesenchymal stem cells; M-CSF: macrophage delivery stimulator; RANKL: receptor activator of nuclear factor-κB ligand; VEGF: vascular endothelial growth factor; BMP: bone morphogenetic protein.

these cell types will help us better understand and treat bone diseases.

Mesenchymal stem cells

MSCs, possess both a self-renewal capability and a diverse differentiation potential. These cells can mature into osteoblasts, chondrocytes, or adipocytes, playing a pivotal role in bone metabolism regulation and repair [68,69]. Hede et al.[70] evaluated MSCs-EVs on bone marrow stimulation combined with bone inward growth in focal cartilage defects of the knee to optimally repair damaged cartilage while enhancing subchondral bone healing. Maiborodin et al. [71] observed that utilizing MSCs-EVs during dental implantation in the proximal tibial condyle of rabbits augmented the bone tissue density near the implants. The bone fragments generated during this procedure primarily fused among themselves and with the regenerated bone.

Moreover, the MSCs-EVs exhibit immunomodulatory properties, potentially attenuating inflammation. There's a noted reduction in bone vasodilation and leukocyte infiltration in the surrounding soft tissue. Li et al. [72] highlighted the distinctions when culturing primary bone marrow mononuclear cells (BMM) on polystyrene dishes versus the extracellular matrix (ECM) developed with bone marrow mesenchymal stem cells (BM-MSCs). The findings suggested that BMMs when co-cultured with ECM, could not differentiate into osteoclasts due to the interference of ECM on RANK-RANKL signal and the attenuation of ROS. MSCs-derived ECM studies have demonstrated that acellular biomimetic scaffolds, derived from ECM, exhibit biological activities comparable to natural bone, ensuring minimized graft rejection and enhanced bone regeneration. Qin et al. [73] confirmed that the osteogenic efficacy of human induced pluripotent stem cells (hiPSC-MSCs-EVs) correlates with EV concentration, leading to heightened osteogenic gene expression and ALP activity.

In addition, coral-MSCs-BMP-2 composite scaffolds loaded with growth factor BMP-2 and seed cells MSCs cultured in the iliac bone marrow had better osteogenic ability [74]. Immunohistochemical examination showed that MSCs induced by composite scaffolds were partly derived from ECM. Qin et al. [50] revealed that exogenous BMSCs-EVs primarily target the Golgi apparatus, not lysosomes. These EVs mediate bone regeneration through miRNA-196a, and when delivered via hydrogel systems, they significantly amplify in vivo bone formation. Adipose-derived stem cells (ADSCs) EVs counteract osteoblast aging in osteoarthritis.

Ho et al. [75] demonstrated that ADSCs-EVs mediate osteoblasts by affecting the function of autophagy, MAPK, and Rap-1 signaling pathways. Its influence on the function of osteoblasts contributes to bone regeneration based on ADSCs-EVs. ADSCs can be collected in larger quantities and have higher proliferation capacity during in vitro culture, which is conducive to the collection of more EVs and is more conducive to clinical application. The miRNA embedding activity of EVs from chondrocytes, adipose tissue, and BM-MSCs has great immunomodulatory potential to promote bone formation. These extracellular derivatives partake in an intricate intracellular regulatory mechanism, determining their composition and function. Once released into the extracellular milieu, they wield the potential to regulate physiological tissue impairment and organ remodeling.

Osteoblasts

Osteoblasts in bone metabolism are responsible for the synthesis and secretion of bone matrix, involved in the formation of new bone tissue. The production of osteoblasts is regulated by multiple pathways, which are surrounded by ECM and extend long processes (dendrites). They allow growth factors, protein signals, and EVs to be passed from one cell to another [76,77]. Osteoblasts are intermediate cells in the osteoblastic process of MSCs, which secrete bone-like mineralization of ECM, growth factors, and EVs in the process of continuous differentiation [78]. Osteoblasts need the surface to synthesize substrates, the raw material for which is supplied by collagen in the ECM, and for the manufacture of new substrates.

The collagen of osteoblast ECM can significantly affect cell behavior, for example, the tough form of type I collagen inhibits osteoblast-like cell proliferation and can stimulate osteoblast-like differentiation. The tough form of type I collagen inhibits osteoblast-like cell proliferation and can stimulate osteoblast-like differentiation. ECM without type III collagen will affect the differentiation of osteoblasts, decrease the mineralization ability of osteoblasts, and reduce the activities of bone salivary protein (BSP), ALP, and OCN [23,79]. Hence, collagen in ECM not only anchors cells to provide a matrix, but also can regulate the growth and osteogenic properties of osteoblasts, and participate in the formation of new bone tissue. Extracellular derivatives of osteoblasts regulate bone morphology and bone metabolism by interacting with other signaling molecules, such as growth factors, hormones, and proteins. These derivatives can promote the proliferation and differentiation of bone cells and affect the formation and repair of bone tissue.

The activated proteins BSP and OPN in ECM and EVs are the three main makers involved in osteogenic differentiation. BSP appears after ALP and is the main regulator of calcium ion binding in ECM, promoting the nucleation activity of hydroxyapatite and enhancing the formation of calcium nodules and bone mineralization. OCN appears at the end of osteoblast differentiation and is a sign of advanced osteogenesis [80–82]. OPN is an abundant non-collagenous protein produced by osteoblasts that prevents excessive osteogenesis by blocking the expression of BMP-2 and simultaneously regulates phosphate content to regulate osteoblast mineralization [25]. Consistent with OCN, OPN produced by osteoblasts is both a marker and an inhibitor of osteogenic mineralization.

In the ECM of osteoblasts, MGP, R-spondin2, and periosteum protein, as the Wnt pathway, are important regulators of bone formation, regulating the mineralization and differentiation of osteoblasts [79,83,84]. The specific response of osteoblasts to growth factors and hormones, developmental regulation and selective response of osteoblast phenotypic genes. EVs carry osteoblast bioactive molecules, such as proteins, RNA, and cytokines, which play an important role in intercellular communication. EVs of osteoblasts contain RANKL, which can be transferred to the precursor of osteoclasts [85]. Cappariello et al.[86] reported that the use of osteoblast-derived EVs was able to effectively target bone and induce bone formation through RANKL, bisphosphonate, and tyrosine kinase inhibitors, opening up a pathway for the biotechnology of bone disease treatment. EVs-specific protein receptors of osteoblasts can stimulate the RANKL-RANK signaling pathway and promote osteoclast formation. During mineralization, osteoblasts EVs can transmit miR-667-3p, miR-874-3p, miR-6769b-5p, miR-7044-5p, and miR-7668-3p, which can regulate the Wnt signaling pathway between osteoblasts and further promote the expression of β-catenin in ECM [87]. Osteoblasts EVs can also assist in the transmission of eukaryotic initiation factor 2 (EIF2), integrin signal, and mammalian target of rapamycin (mTOR) signal between osteoblasts to promote bone formation. EIF2 signal is involved in bone growth factor BMP-2 inducing osteoblast differentiation [88]. Integrin signaling promotes angiogenesis, which facilitates bone formation, remodeling, and fracture healing. Activation of mTOR signaling can contribute to the bone by inhibiting peroxisome proliferator-activated receptor-γ [89].

Osteoclasts

Osteoclasts are multinucleated cells with 2–50 nuclei that are formed mainly from monocytes and macrophages in the blood or bone marrow. They are responsible for the absorption and remodeling of aging or damaged bone tissue and play a key role in bone reconstruction. Macrophage delivery stimulator (M-CSF) promotes osteoclast progenitor cell proliferation, while RANKL, a ligand secreted by osteoblasts, binds to osteoblast receptors and activates osteoclast formation and activity [83]. Osteoclasts attach to the ECM and interact with the bone surface through the ECM, which plays an important role in the differentiation, migration, and bone resorption of osteoclasts. Osteoclasts are directly bound to bone integrins expressed on the cell surface in a process mediated by $\alpha_v\beta_3$ [90]. The integrin-ECM interaction is achieved by binding $\alpha_v\beta_3$ to the RGD peptide in the ECM component, which polarizes the osteoclast initiates the formation of actin rings and produces characteristic fold boundaries [91]. Interfering with integrin or potential peptide binding sites alters the ability of osteoclasts to absorb bone. In the absence of $\alpha_v\beta_3$ integrin subunits, the cytoskeleton does not effectively reabsorb tissue, leaving actin rings and wrinkly membranes either unable to form or abnormally formed, eventually leading to hypocalcemia and osteolytic phenotypes [91–93]. Similarly, a lack of osteopontin leads to resistant bone loss, reflecting the importance of ECM function around osteoclasts. RGD is a protein surface peptide with cell adhesion, and RGD of OPN and BSP interacts with $\alpha_v\beta_3$ integrin to initiate osteoclast adhesion to bone matrix and polarize actin rings in osteoclasts [94].

OPN stimulates osteoclast activity and makes a critical difference in sealing the ECM surrounding osteoclasts. OPN has a variety of ligands, which can be calcium, surface receptors, and heparin. In addition to bone metabolism, OPN also regulates the immune system. BSP main effect on ECM can stimulate osteoclast activity and behavior to promote bone resorption, but can also prevent the migration of preosteoclasts and mature osteoclasts [95]. There are three main modes of interaction between EVs released by osteoclasts and other cells, namely signaling protein 4D in EVs binding plexin-B1, EVs binding ephrin-B4 on ephB2, and RANK binding RANKL in EVs [96–98]. Signaling proteins 4D and ephrinB2 were transferred to the surface of osteoblasts via EVs as signaling factors derived from osteoclasts and transported the osteoblast gene microRNA-214-3p [96]. RANK is an important signaling pathway of bone metabolism. RANKL is present on osteoblasts and

osteoblasts, binds to RANK on osteoclasts, stimulates signaling pathways, and causes RANK activation [99,100]. EVs derived from osteoclasts can be attached to the membrane of target cells and induce intracellular signaling through corresponding receptors. EVs also have physiological and pathological effects similar to osteoclasts in intercellular communication.

Endothelial cells

Endothelial cells are the main constituent cells of the inner wall of blood vessels and participate in the formation and maintenance of bone vessels. They promote the growth of new blood vessels and blood supply by secreting angiogenic factors and cell adhesion molecules and provide nutrients and oxygen for bone cells. Endothelial cells in bone tissue come into direct contact with the pericytes of blood vessels, thereby establishing a closer connection with the peripheral connective tissue. Endothelial cells transmit and secrete various cytokines and growth factors through ECM and EVs to promote osteogenesis and osteoclast formation and participate in the regulation of bone metabolism. Endothelial cells secrete varieties of signaling molecules including platelet-derived growth factor (PDGF-BB), RANKL, VEGF, BMP2, matrix Gla-protein, and bone protective protein (OPG) through paracrine interactions. The derivatives around endothelial cells secrete growth factors such as BMP-2, MMP, RANKL, and other activated proteins. It is also an important key chemokines and VEGF series, attracting mononuclear/macrophages to the site of inflammation and preventing bone cell apoptosis [101–103]. In addition, PDGF of endothelial cells and their ECM release an appropriate amount of PDGF-BB to aggregate LepR + and Nestin + periosteal cells on the surface to form periosteal bone. PDGF-BB can recruit peripheral progenitor cells expressing PDGFR-β to new bone regions, allowing attached cells to stabilize newly formed vascular structures. The PI3K signal contained in PDGF-BB can promote the proliferation of MSCs [104,105]. In addition to proliferation, PDGF-BB and PDGFR-β also increase the migration ability of MSCs but strongly inhibit the osteogenic differentiation of MSCs.

Tumor cells

In addition to cells related to bone formation and bone resorption, extracellular derivatives of tumor cells also have a great influence on bone metabolism. Most patients with advanced breast cancer tend to have bone metastasis. EVs play an important role in bone metastasis with other diseases. EVs secreted by breast tumor cells contain heparin, interleukin, parathyroid hormone, prostaglandin, and other biological factors that activate osteoclasts [106]. Loftus et al. [15] found that EVs (MDA-EVs) of breast cancer cells can mediate the physiological activities of endothelial cells in bone tissue. When osteoblasts and MDA-EVs were co-cultured, the proliferation and early mineralization ability of osteoblasts decreased. This was because MDA-EVs decreased the expression of cyclin D1 in osteoblasts and affected the osteoblast differentiation pathway, but increased the expression of the RANKL signal and the pro-angiogenic factors IL6I and L1-β in osteoclasts. MDA-EVs target osteoclasts and stimulate them to differentiate into polynucleated osteoclasts.

Then, Stephen et al. [107] found that prostate cancer cells can mediate the cholesterol balance of bone marrow cells. EVs of prostate cancer cells can activate RANK signaling, and promote osteoclast differentiation and bone resorption. After the occurrence of cancer in the human body, it is easy to transfer to the bone. Bone marrow cells produce a large amount of cholesterol, and through cancer cells EVs to resist the increase of cholesterol in bone marrow cells can maintain the balance of bone metabolism.

In addition, Baglio et al. [108] demonstrated that EVs produced by osteosarcoma (OS) can generate a proto-metastatic inflammatory via physiological alterations in MSCs. EVs from metastatic OS cells carry a membrane-associated form of TGF-β, which "tumor-educated" MSCs (TEMSCs) produce IL-6. When injected in preclinical mouse models, TEMSCs promote OS growth and lung metastasis formation. Therefore, the co-administration of therapeutic IL-6 receptor antibodies eliminates the cancer-promoting effects of TEMSCs, and IL-6 and TGF-β are reasonable targets for therapeutic intervention in patients with OS.

The applications of extracellular derivatives in bone disease

Regenerative medicine in a broad sense refers to the technical and surgical operation of the study of the regeneration of human tissues and organs, and is also a discipline that studies the mechanism of tissue or organ regeneration [109]. At present, the main research directions in the field of regenerative medicine are repairing damaged tissues by transplantation of cells, artificial tissues or organs can be implanted into lesions by preparing biomimetic materials in vitro, and regeneration of defective tissue is induced by external stimulation, such as drugs or electrical signals. The major directions of regenerative medicine research all require the deep involvement of extracellular derivatives, which provide nutrients and Bridges between tissues. Extracellular derivatives carry many bioactive substances that can regulate the function and metabolic process of the recipient cell. Therefore, they have potential applications in bone metabolic prevention and treatment, such as osteoporosis, osteoarthritis, bone fracture, and bone tumor. The role of major biomaterials prepared from extracellular derivatives in the treatment of bone metabolic diseases is summarized in Fig. 4. Specifically, the therapeutic effects of different extracellular derivatives in bone metabolic diseases are shown in Table 2.

Osteoporosis

Osteoporosis is simply a disease of decreased bone density as a symptom, mainly related to age and metabolism, increasing the risk of fragility fractures. Osteoporosis involves a variety of growth factors and signaling pathways, such as BMP, PI3K/AKT, TGF-β/BMP, receptor activators of RANKL/RANK/OPG system, and Wnt/β-catenin signaling pathways. Osteoporosis has traditionally been treated with hormones, selective estrogen receptor modulators, calcitonin, and bisphosphonates. However, drug therapy has obvious side effects and limited effectiveness.

EVs offer an alternative to therapeutic drugs. Lu et al. [115] successfully treated osteoporosis using MSCs-EVs, highlighting the role of miRNA-21, miRNA-29, and miRNA-221 in targeting the PI3K/AKT pathway, which enhances metabolism and osteoblast growth. Mutations in Col10a1 affect chondrocyte maturation, causing bone issues [116]. Meanwhile, Col2a1 is ECM-specific, and its deletion results in bone loss and spinal epiphyseal dysplasia. [117,118]. Liu et al. [119] conducted bone remodeling studies in a blue medaka model, analyzing bone cells' transcriptomes for osteoporosis treatment. Their findings revealed strong MMP13 expression in osteoblast ECM, influenced by RANKL. This expression suggests MMP13 promotes osteoclast activity under osteoporosis. MMP13-positive osteoblasts reshape the ECM, influencing osteoblast aggregates, and osteoblast-derived MMP13 affects osteoclast maturation [120].

Additionally, MSCs-EVs, with low immune response risks, offer potential alternative treatments for various diseases and provide a foundation for new therapeutic research. MSC-EVs cannot target bone tissue in mice unless combined with alendronate in the treatment of osteoporosis. Hu et al. [121] genetically engineered

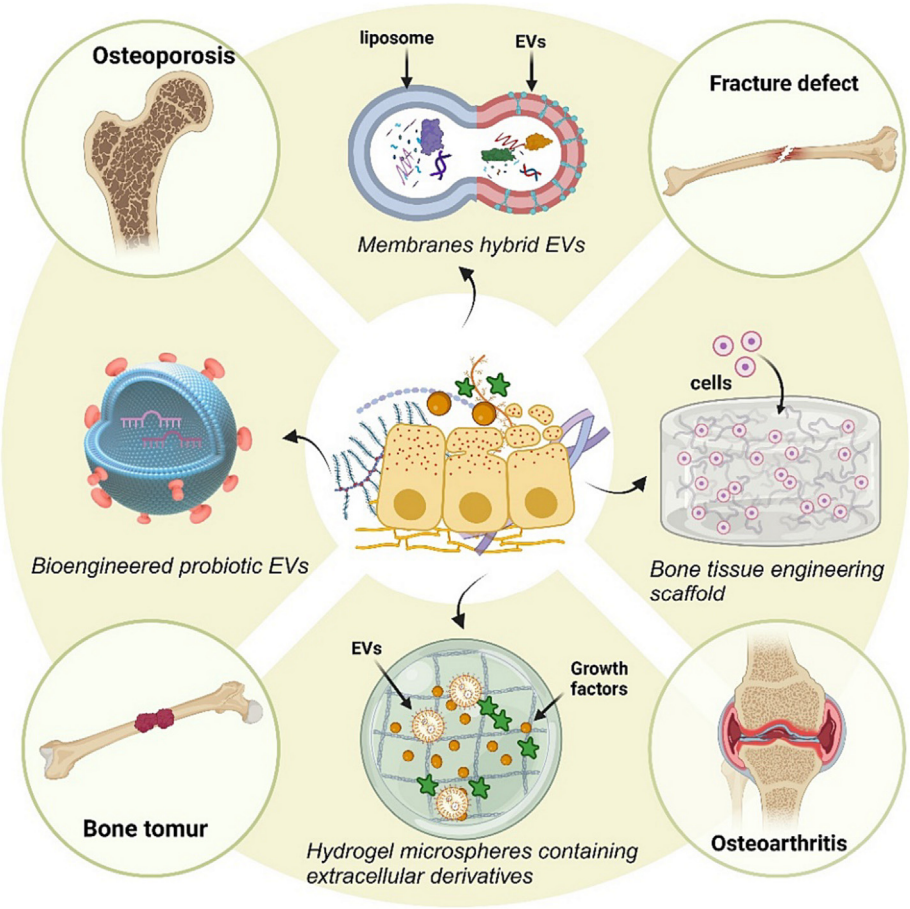

Fig. 4. Schematic diagram of biomaterials prepared by extracellular derivatives for treating bone diseases. The figure was created with BioRender.com.

NIH-3T4 cells highly expressing CXCR3 and collected their exosomes, which had CXCR4 protein on their surface and fused with antagomiR-188 carrying liposomes to obtain hybrid nanoparticles with bone-targeting and anti-miR-188 capabilities. Hybrid nanoparticles can aggregate at specific bone sites significantly reverse age-related trabecular bone loss and reduce cortical bone porosity by inhibiting lipogenesis and promoting bone formation of BMSCs in older mice. In addition to modifying EVs to have bone targeting, endothelial EVs naturally have bone targeting. Song et al. [122] demonstrated that EVs of endothelial cells can efficiently deliver miR-155 to reduce bone resorption.

Osteoarthritis

Osteoarthritis (OA) is a chronic degenerative disease caused by the breakdown of articular cartilage and underlying bones, the main causes of which are aging, trauma, mechanical load, and obesity [123]. The most effective treatment is joint surgery, such as joint replacement surgery, but subsequent joint life expectancy is shortened and revision surgery is required. Rheumatoid arthritis (RA) is an inflammatory autoimmune disease, which is essentially caused by the destruction of the balance of immune tolerance, producing many kinds of matrix degradation enzymes, pro-inflammatory cytokines, and autoantibodies, eventually resulting in joint damage and synovial inflammation [124]. However, the drug treatment of OA and RA is mainly to relieve pain and reduce inflammation and has little effect on the treatment of osteoarthritis [125]. In pathological arthritis processes, the collagen network of cartilages undergoes irreversible degradation, and that can be used as markers for ECM recombination and early diagnosis of diseases [126].

Cartilage healing strategies are therefore necessary for many joint disorders. Many methods have been used to replace cartilage at the lesion site, but traditional cartilage repair strategies still have some problems, such as failure to produce consistent and transparent cartilage [127]. Cell therapy is emerging as a potential OA solution. Liu et al. [128] utilized miR-223-loaded EVs from human umbilical cord mesenchymal stem cells (hUC-EVs) and modified them for better targeting. These EVs effectively hindered chondrocyte death, enhancing OA conditions. With OA progression, there is reduced collagen and supportive protein due to increased enzyme expressions [84]. MSCs derivatives can counteract this degradation. MSCs-EVs significantly promoted the proliferation and mineralization of chondrocytes, limiting the synthesis of ECM proteins [129]. MSCs-EVs foster chondrocyte growth but restrict ECM protein synthesis due to specific cytokines and miRNAs. EVs from synovial mesenchymal stem cells (SMSCs-EVs) with mir-140-5p increased chondrocyte proliferation and migration [130]. Moreover, ECM formation of chondrocytes was not affected, and SMSCs-EVs could effectively treat OA in vivo [131]. Zhou et al. [132] showed that EVs transmitting the miR-126-3p gene can inhibit cartilage degeneration, with overexpression leading to chondrocyte growth and reduced inflammatory protein expressions. Synovial fibroblasts with miR-126-3p showed potential in OA treatment.

The imbalance of M1/M2 macrophages represents the imbalance of immune regulation causing OA. In the OA model, activating the M1 macrophages can accentuate inflammation and cartilage

Table 2
Different extracellular derivatives used in the treatment of bone diseases.

Diseases	Strategy	Effects	References
Osteoporosis	MSCs-EVs	· Activate PI3K/AKT pathway · Col10a1 and Col2a1 up-regular to bone matrix repair · Find new therapeutic microRNAs	[111–114]
	HSF-EVs, Osteoblasts-EVs, ECs-EVs	· Modulates Ca^{2+} signaling · MMP-1, -13, -10 promote tissue repair ·MMP-1, -14 promote angiogenesis	[52,116,117]
	ECs-EVs	· Secretion of IL-33 drives BMSCs differentiation · Promote osteoblast proliferation and migration · Promote angiogenesis	[56]
	Engineered EVs	· Bone targeting · Transfer BMP to facilitate bone formation · Promote angiogenesis	[110,118]
Osteoarthritis	MSCs-EVs	· Carry a cytokine · Activate Wnt signaling pathway · Reduce the expression of pro-inflammatory interleukins · Promote proliferation of chondrocytes · Decreased expression of IL-1β, IL-6, TNF-α proteins	[65,121,122]
	SMSCs-EVs	· Help the ECM formation of chondrocytes · Enhance proliferation and migration of articular chondrocytes	[127]
	Engineered EVs	· Bone targeting · Inhibit chondrocyte scorch death	[124,132]
	EVs of synovial mucus	· Chondrocyte proliferation and differentiation · Cartilage repair · Increase mineralization	[128]
	Chitosan stabilizes platelet growth factors	· Produce PDGF, FGF, IGF and TGF-β · Promote soft tissue repair	[111]
Bone defect	MSCs-EVs	· Promote the transfer of osteogenic genes and growth factors · Promote osteogenic differentiation of MSCs · Reduced immune response · Promote the transfer of osteogenic genes and growth factors · Promote osteogenic differentiation of MSCs · Reduced immune response · Bone tissue engineering material raw material	[50,75,76,142]
	SHED-EVs	· Deliver growth factors · Promote bone mineralization ability combine β-TCP · Promote angiogenesis regulating Ca^{2+}	[141]
	OS-EVs	· Promote osteoblast proliferation significantly · The activity of M2 macrophages was decreased · Promote angiogenesis	[144]
	Platelet plasma hydrogel	· Simulated fracture hematoma microenvironment · Promote the proliferation and differentiation of MSCs · Promote angiogenesis	[112]
	BMP/ECM composite scaffold	· Good biocompatibility · Facilitate cell adhesion · Produc more EVs · Promote angiogenesis	[135,136]
Bone tumor	BMSCs-EVs	· Promote osteoblast production · Reduced immune response	[149]
	Modifica OS-EVs	· Inhibit cancer cell metastasis	[152]
Fracture union disorder	MSCs-EVs	· Transfer bone growth factor · Modulates the osteogenic signaling pathway	[153]
	BMSCs-EVs	· Activate the osteogenic pathway · Decrease osteoclast anchoring protein	[155]
Congenital bone disease	Collagen supplement	· Store and deliver TGF-β · Promote the production of Col1A1 or Col1A2 protein	[158]
	Muscle-EVs	· Promote myoblast differentiation · Promote soft tissue regeneration	[113,114]

Abbreviations: ECM: extracellular matrix; EVs: extracellular vesicles; MSCs: mesenchymal stem cells; HSF: human skin fibroblasts; EC: endothelial cells; BMSCs: bone marrow derived mesenchymal stem cells; BMP: bone morphogenetic protein; TNF: tumor necrosis factor; SMSCs: skeletal muscle satellite cells; TGF: transforming growth factor; PDGF: platelet-derived growth factor; IGF: insulin growth factor; SHED: stem cells from human exfoliated deciduous teeth; β-TCP: β-tricalcium phosphate; OS: osteosarcoma; FGF: fibroblast growth factor.

injury, while M2 macrophages induce decreased repair and remodeling activity [133]. Macrophage polarization is because microRNA transmitted by tissue cell derivatives around OA targets TLR4 through RANK, and then inhibits STAT3 to increase M2 polarization [134]. It is worth noting that miR-24-3p, miR-34a-5p, miR-146a-5p, miR-181a-5p, and miR-222-3p delivered by extracellular derivatives can promote M2-polarized macrophages, reduce immune rejection of cartilage grafts, and promote joint repair [135–137]. The extracellular derivatives of MSCs contain a variety of growth factors and microRNA. It can produce anti-inflammatory interleukin IL-10, reduce the expression of pro-inflammatory interleukin (IL-1α, IL-1β, IL-6, IL-8, and IL-17). They can enhance the activity of chondrocytes in OA, and promote chondrocyte proliferation. The expression of anti-inflammatory and inflammatory factors can affect the apoptosis of chondrocytes promote chondrogenesis, and reduce the senescence of osteoblasts [138].

Bone defect

Bone defects usually occur after infection, bone tumors, and trauma. A bone defect of a critical size, the smallest intra-bone wound in a particular bone or species, can never heal in the life of an animal. Clinical strategies for repairing bone defects include autotransplantation, allotransplantation, and synthetic biomaterial transplantation. With the development of new bone repair materials, bone tissue engineering (BTE) is the most promising treatment for bone defect repair among all orthopedic disease models [139]. BTE refers to the reproduction of seed cells in vitro and planting in three-dimensional biomaterials (including surface and interior), the interaction between cells and biomaterials. The secretion of a series of extracellular derivatives and the surrounding tissues after implantation of BTE to produce biological reactions, that repair bone tissue damage. The electrospun PLLA nanofiber scaffold loaded with BMP-2 can enhance the osteoinductivity of the scaffold [140]. Collagen mimetic peptide GFOGER has been coated on PCL composite scaffolds, which can bind to the integrin receptor $\alpha_2\beta_1$ involved in osteogenesis, up-regulate osteoblast differentiation, and lead to increased bone formation [141].

In recent years, many bone tissue engineering materials combined with scaffolds, MSCs, and their derivatives have been designed. Loading EVs in hydrogel scaffolds is the most attractive research hotspot, and the cell sources used are usually MSCs, BMSCs, and osteoblasts [71,142,143]. BMSCs-EVs significantly upregulated the expression of BMP-2, BMP-7, BSP, Col I, Runx2, and OCN [144]. Human adipose-derived mesenchymal stem cells (hADSC) have great potential to treat ischemic diseases, suggesting that also hADSC-EVs may work in bone regeneration by promoting angiogenesis [145]. Stem cells from human exfoliated deciduous teeth (SHEDs) derived from secrete a large number and variety of growth factors, which have stronger proliferation ability and promote bone mineralization ability. The combination of SHEDs-EVs and tricalcium phosphate (β-TCP) can enhance alveolar bone regeneration via osteogenesis and angiogenesis [146].

The effect of EVs is influenced by their quantity, with 3D-cultured cells secreting more EVs than flat-surface cultured cells. Yu et al. [147] found that using EVs from periodontal ligament stem cells (PDLSC) within a hydrogel 3D environment enhanced bone repair. MSCs in 3D environments produce more EVs, and when combined with an alginate saline gel, they promote cell migration, proliferation, and osteogenesis. Hydrogels, serving as natural substrates for extracellular derivatives, provide storage and slow-release effects. Adding specific bioactive substances in hydrogels can boost damage repair and enhance molecule targeting. Zhang et al. [148] integrated umbilical cord MSCs-EVs into a complex scaffold for rat skull defect repair. MSCs-EVs within this scaffold promote angiogenesis, essential for rapid bone repair. Swanson et al.[149] designed a 3D scaffold using polylactic-glycolic acid (PLGA) and polyethylene glycol (PEG) for controlled OS-EVs release, derived from human dental pulp stem cells (hDPSC). This approach accelerated bone healing in 8 weeks. In critical bone defects, controlled EVs release from scaffolds aids bone formation without needing seeded cells to multiply within the scaffold.

Bone tumor

Benign or malignant tumors can occur in the bone, such as osteosarcoma (OS) and metastatic bone cancer. OS is a malignant tumor that originates in connective tissue. The occurrence and development of OS are related to the imbalance of bone metabolism. So the researchers needed to address the effects of EVs secreted by OS cells in the tumor microenvironment [150]. OS-EVs contain many specific microRNAs and osteoclast metabolites, promoting the expression of osteoclast genes and the differentiation of macrophages into osteoclasts [151,152]. In addition to affecting bone remodeling, extracellular derivatives can also regulate tumor angiogenesis [12]. In other words, the dispersion and signal transmission of OS-EVs will greatly promote the signal exchange between OS and endothelial cells, resulting in the release of pro-angiogenic growth factors. More EVs and growth factors are generated after accelerated angiogenesis, and faster signal transmission efficiency, thus forming a positive cycle.

In addition, OS-EVs trigger a pro-metastatic inflammatory ring by altering the physiology of MSCs. OS-derivatives can also induce M2 phenotype in alveolar macrophages to support tumors, which contributes to immune escape in tumors [108,153]. Tumor cells produce bones or osteoids in it and most often metastasize to the lungs. Circular RNA (circRNA) is a closed-loop RNA produced by the end-to-end connection of RNA transcripts during transcription. It is abnormally expressed in epithelial cancers such as lung cancer, OA, and OS [154,155]. CircRNAs are rich in EVs, which are members of the small ncRNA family. A large amount of evidence shows that EVs can participate in the mechanism of tumorigenesis by transmitting circRNAs. Circ-0000190 in EVs is a new biomarker and has potential value in the diagnosis of OS. Li et al.[156] found that OS cells endocytosis EVs released by normal cells containing circ-0000190. It is possible to load circ-0000190 with exogenous EVs and use circ-0000190 to mediate the communication of EVs during the carcinogenesis of OS. By inducing miR-767-5p to regulate TET1, the development of OS is hindered.

The OS may affect any bone and often produce a variety of ECM while showing varying degrees of differentiation. The non-coding RNA activated by DNA damage (NORAD) is high in OS cells and surrounding tissues, which may be involved in the progression and metastasis of OS. He et al. [157] found that EVs isolated from BMSCs can transfer NORAD from BMSC to OS cells, which can inhibit the migration and proliferation of OS cells to other sites. There are special binding relationships between NORAD and microRNA-30c-5p (miR-30c-5p) and between miR-30c-5p and Krueppel-like factor 10 (KLF10). Mechanistically, NORAD acts as a delivery platform for miR-30c-5p, helping up-regulate KLF10, while its mimics reduce NORAD-induced cancer cell effects. OS cells were injected into mice to establish a tumor growth and metastasis model. After the experiment, it was found that the injection of BMSCs-EVs increased the expression of NORAD and KLF10, but decreased the expression of miR-30c-5p, thereby inhibiting tumor growth and lung metastasis.

Fracture union disorder

Recovery from fracture involves a very precise regulation of bone metabolism. In this process, a variety of cells such as periosteal cells, stem/progenitor cells, and osteoblast chondrocytes are involved and work together. The healing of a fracture is usually organized and includes early hematoma formation and inflammation, the development of cartilage and callus, and the final stage of bone remodeling. Fracture union disorders are mainly caused by bone metabolism disorders, which can lead to persistent bone defects. It can also be associated with bone tissue loss of function, instability, and discomfort. Treatment of fracture healing disorders, MSCs are the most promising therapeutic cells among seed cells added in bone tissue engineering [158,159]. Hu et al. [160] found that the EVs derived from bone marrow mesenchymal stem cells (BMSCs-EVs) carrying miR-335 can help up-regulate miR-335 in osteoblasts and reduce VapB, thus enabling osteoblasts to be efficiently utilized in the initial stage of osteogenesis in fracture repair. The increase of MSCs or differentiation factors can induce MSCs to differentiate into osteoblasts at the fracture site. The expression of VapB increased during osteoclast formation, and VapB knockdown

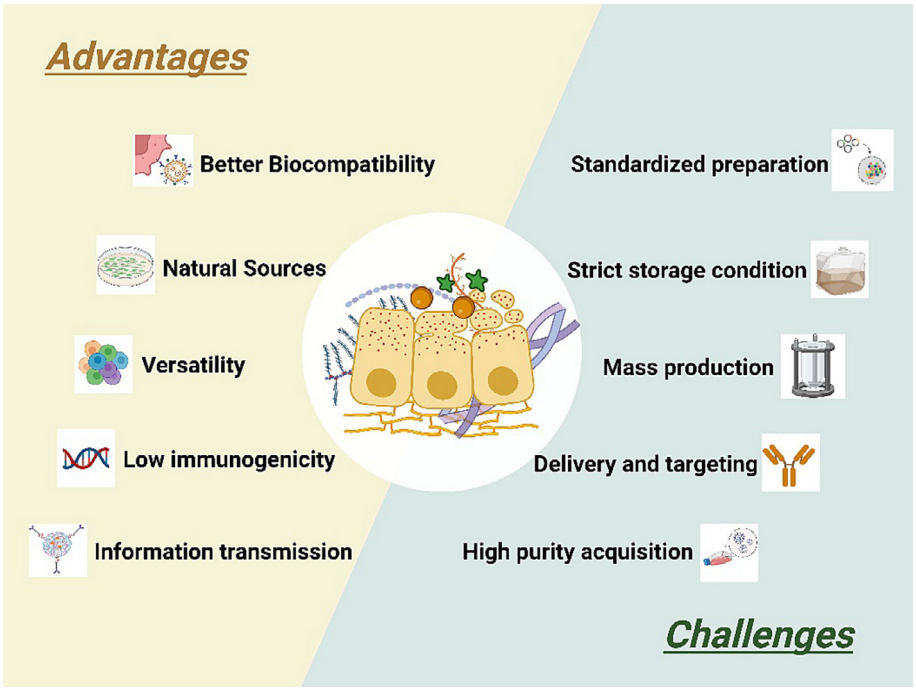

Fig. 5. Advantages and challenges of extracellular derivative for clinical application. The figure was created with BioRender.com.

resulted in inhibition of bone resorption. Activation of the Wnt/β-catenin pathway results in decreased VapB expression and increased OCN, FGF-2, α-SMA, and GDF-10 content, promoting osteogenic differentiation and fracture healing.

Congenital bone disease

Hereditary genetic defects in congenital rare bone metabolic diseases alter the correct bone modeling and remodeling in terms of bone synthesis or bone resorption. The main symptoms are bone tissue defects that are prone to long bone bending, stress fractures, or brittle fractures. Bone fragility may be caused by excessive osteoclastic-driven bone resorption that is not in balance with the corresponding amount of bone. Excessive bone mass loss leads to the formation of osteoporosis, dysfunctional mineralization leads to the pathological condition of osteomalacia and rickets in children. Osteoblast-driven enhanced mineralized bone deposition or reduced osteoclast uptake activity leads to osteopenia [161,162].

Osteogenesis imperfecta (OI) is a genetic disease of inborn bone metabolic dysfunction, which mainly affects the formation of connective tissue and is prone to frequent fractures. The most common cause is a dominant mutation in one of two genes, Col1A1 and COL1A2. By storing TGF-β signals in the ECM of OI-deficient bone tissue, it acts as a central coordinator of bone remodeling by coupling the activity of osteoclasts and bone-forming osteoblasts. Collagen type I in the ECM helps TGF-β from osteoblasts to be produced and deposited into the bone matrix, which can be released and activated by osteoclasts during bone resorption. Grafe et al. [163] found that the binding of type I collagen to decorin, a small proteoglycan rich in leucine, a known regulator of TGF-β activity, was reduced in recessive OI mice. Anti-TGF-β treatment with neutralizing antibody 1D11 can correct the bone phenotype of OI and improve lung abnormalities in mice. Therefore, the change of TGF-β stromal cell signal is the main mechanism of osteogenesis imperfecta, which may hold promise as an effective treatment for OI.

Advantages and challenges

Although extracellular derivatives have many advantages, they still face some challenges in practical applications (Fig. 5). With further research and technological development, these challenges are expected to be overcome, leading to the widespread use of extracellular derivatives in clinical therapy.

Advantages

There are many kinds of signal proteins and bioactive substances in extracellular derivatives, which can regulate cell adhesion, migration, proliferation, and differentiation. Extracellular derivatives are active substances released by living cells and therefore have a natural source and composition. They do not need to go through complex preparation processes such as artificial synthesis or genetic modification [164,165]. Due to their natural origin, extracellular derivatives are generally more biocompatible and can reduce immune rejection and other side effects. Compared with cell therapy, extracellular derivatives have lower immunogenicity and do not cause significant immune response, which is conducive to clinical application and long-term treatment. At present, various growth factors have been widely used in clinics, such as BMP-2 and BMP-7 approved by FDA [166,167]. The use of BMP can promote bone integration and improve the success rate of surgery. There is also an FDA-approved bioactive molecule for bone regeneration, a peptide called P-15, which was found to be effective, safe, and similar to autograft 2 years after surgery for cervical radiculopathy caused by cervical disc degenerative disease [167]. In China, several startups are preparing external vesicles for use in the cosmetics and medical beauty fields. In addition to therapeutic use, extracellular derivatives carry lots of proteins, nucleic acids, and cytokines that also reflect the state and function of their mother cells. They are envisioned as potential biomarkers for disease diagnosis and disease early warning [168,169]. Existing extracellular derivatives containing tumor-specific, liver, kidney, and bone metabolic disease RNAs and proteins have been used as

markers for cancer diagnosis [170,171]. There are many metabolites in bone metabolism, which are distributed locally in bone tissues or body fluids and detected in cell derivatives. Bone metabolic balance can be observed through their detection, and bone metabolic diseases can be diagnosed.

Challenges

At first, although there is much research on extracellular derivatives, the main preparation method is achieved by ultra-low temperature gradient centrifugation. But, the standards for collecting EVs are not uniform, it is necessary to establish a more standardized and standardized preparation process to ensure the consistency and quality of products [172]. Secondly, obtaining an ECM of high quality and purity from the human body or other sources may have certain technical challenges, including extraction, handling, and preparation processes, to avoid the presence of contaminants affecting its function and stability [172,173]. Thirdly, extracellular derivatives can be targeted through engineering modification, but how to achieve the precise delivery and targeting of extracellular derivatives in vivo is still difficult. However, they can effectively reach the site of the disease and have a therapeutic effect, need to be further studied and improved [174]. In addition, during the modification process, the biological activity of extracellular derivatives may be affected by environmental conditions and degradation. Maintaining its function and stability when combined with other materials still needs to be studied. Finally, it remains challenging to produce extracellular derivatives at scale and cost-effectively in quantity and quality for clinical needs.

Conclusion

In this paper, the types, sources, functions, and applications of extracellular derivatives related to bone metabolism were reviewed, and the advantages and challenges of their clinical application were summarized. When living is healthy, extracellular derivatives maintain bone metabolic homeostasis, while triggering complex pathways in the case of metabolic abnormalities has the opposite effect on tissue health. The phenotype of extracellular derivatives is highly dependent on the derived cell, so each cell derivative may affect bone metabolism, and bone histiocytes are also potential therapeutic targets for diseases with impaired bone regulation.

Extracellular derivatives still have a long way to go in clinical application. Due to the strict requirements and long process of clinical approval of drugs and implanted devices, many extracellular derivatives have not yet been marketed and are in preclinical research. The most established clinical application is the growth factor, which the FDA approved in 2017 for fibroblast growth factor to treat neurotrophic keratitis and VEGF for eye drops like pegaptanib, bevacizumab, ranibizumab, and bortezomib to treat age-related macular degeneration, diabetic retinopathy, diabetic macular edema, and other eye diseases [175,176]. Except for growth factors, there are no clinically available approved ECM or EVs treatments. There are no commercially available treatments for ECM, but the polymers it contains, such as hyaluronic acid, are widely used in drug delivery, cosmetics, medicine, and food.

Extracellular derivatives have shown potential clinical applications in several areas, particularly in regenerative medicine and drug delivery systems. In regenerative medicine, extracellular derivatives can be used to promote tissue repair and regeneration, for example, to support heart disease and fracture treatment. The bionic bone microenvironment scaffold is prepared by combining ECM with hydrogel to improve osteogenic performance [147,177–179]. Simulated ECM prepared by biomaterials can manipulate the interaction between macrophages and endothelial cells, regulate immunity, and promote vascular regeneration [180]. As drug carriers, extracellular derivatives can naturally carry and protect drug molecules, including small molecule drugs, proteins, and RNA, allowing them efficiently delivered to target cells. EVs can be combined with drug delivery, which can be used as a carrier, through surface modification to make it targeted, and then loaded with substances with therapeutic effects [121]. As the drug, EVs also can be used as drugs and are secreted by cells of related origin that have specific miRNA and protein delivery. They have bone therapeutic functions and are directly used to treat disease [86,181]. Extracellular derivatives can also be used in vaccine development to carry specific antigens to immune cells to provoke an immune response against infectious diseases or cancer.

In addition to being a therapeutic drug, extracellular derivatives can also be used as probes for disease detection and prevention. EVs biomarkers have been studied for cancer diagnosis, neurodegenerative diseases, cardiovascular disease, and other diseases [182]. Abnormal levels of collagen fragments or glycosaminoglycans in ECM may indicate kidney disease or cartilage degeneration [183,184]. It is used as a non-invasive biomarker to monitor disease status or response to treatment. There are even more applications based on extracellular derivatives from patients, and customized personalized medical solutions, to provide more efficient, safer, and predictable treatment strategies. Also, extracellular derivatives are used to deliver modified RNA or DNA to treat specific genetic diseases.

Extracellular derivatives play an important role in regenerative medicine, among which organoids are the most potential applications. The extracellular derivatives produced by the organoids characterize the organoid microenvironment and use the organoids to predict and formulate therapeutic strategies through drug therapy and simulated transplantation environment. Organoids are based on simulating the tissue microenvironment by carrying extracellular derivatives of biomaterials in vitro. The concept of the liver-bone axis, gut-bone axis has been proposed, that is through the gut and liver affect bone metabolism [185–187]. Our team has accumulated rich research experience on extracellular derivatives in orthopedic diseases, and now focuses on the therapeutic effects of organoid extracellular derivatives, hoping that these organoid extracellular derivatives will lead to the development of new therapeutic strategies for bone diseases [188–191].

Since extracellular derivatives have not been commonly employed for practical clinical applications, more in-depth research is required into the mechanisms by which to regulate finely tuned signaling networks within cells and tissues. The active development of the application function of extracellular derivatives will promote their clinical approval process, and strive to provide patients with more efficient, safe, and convenient treatment options.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

CRediT authorship contribution statement

Yan Wu: Writing – original draft, Writing – review & editing. **Peiran Song:** . **Miaomiao Wang:** . **Han Liu:** Conceptualization. **Yingying Jing:** Conceptualization, Project administration. **Jiacan Su:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision.

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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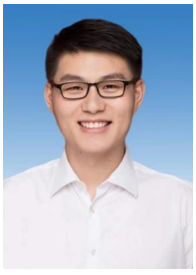

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Jiacan Su , the Principal scientist and director of Translational Medicine Research Institute, Shanghai University. He is also the chief orthopedic physician of the Department of Orthopedics at Shanghai Xinhua Hospital and the chief scientist of key projects of the Science and Technology Commission of Military Commission. His research focuses on the basic and clinical research of bone and joint degenerative diseases, the development and application of new biomaterials, and the development and transformation of bone/cartilage organoids.