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ADVANCED REVIEW

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Modulating osteoclasts with nanoparticles: A path for osteoporosis management?

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Abstract

Osteoclasts are the cells responsible for the bone resorption process during bone remodeling. In a healthy situation, this process results from an equilibrium between new matrix formation by osteoblast and matrix resorption by osteoclast. Osteoporosis (OP) is a systemic bone disease characterized by a decreased bone mass density and alterations in bone microarchitecture, increasing fracture predisposition. Despite the variety of available therapies for OP management there is a growing gap in its treatment associated to the low patients´ adherence owing to concerns related with long-term efficacy or safety. This makes the development of new and safe treatments necessary. Among the newly developed strategies, the use of synthetic and natural nanoparticles to modulate osteoclasts differentiation, activity, apoptosis or crosstalk with osteoblasts have arisen. Synthetic nanoparticles exert their therapeutic effect either by loading antiresorptive drugs or including molecules for osteoclasts gene regulation. Moreover, this control over osteoclasts can be improved by their targeting to bone extracellular matrix or osteoclast membranes. Furthermore, natural nanoparticles, also known as extracellular vesicles, have been identified to play a key role in bone homeostasis. Consequently, these systems have been widely studied to control osteoblasts and osteoclasts under variable environments. Additionally, the ability to bioengineer extracellular vesicles has allowed to obtain biomimetic systems with desirable characteristics as drug carriers for osteoclasts. The analyzed information reveals the possibility of modulating osteoclasts by different mechanisms through

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nanoparticles decreasing bone resorption. These findings suggest that controlling osteoclast activity using nanoparticles has the potential to improve osteoporosis management.

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extracellular vesicles, nanoparticles, osteoclasts, osteoporosis

1 | INTRODUCTION

Osteoporosis (OP), a progressive skeletal disorder, is characterized by a reduction in bone mass and a bone tissue microarchitecture deterioration, leading to a high fracture predisposition (Anam & Insogna, 2021; Clynes et al., 2020; Muraca & Cappariello, 2020; Noh et al., 2020; Pouresmaeili et al., 2018; Rosen, 2020; Rozenberg et al., 2020). Although hip and vertebral fractures are the most prevalent fractures of proximal humerus, ribs, wrist or ischiopubic branches may also occur, resulting in a substantial reduction in patients´ quality of life (Rinonapoli et al., 2021). OP is the most widespread chronic metabolic bone disease according to recent statistics (Noh et al., 2020). In this way, OP affects around 200 million individuals worldwide and causes 9 million fractures annually. In other words, a fragility fracture is taking place every 3 s (Muraca & Cappariello, 2020; Noh et al., 2020; Rinonapoli et al., 2021). Despite the preventive measures and the available treatments, fracture rates have risen every year since 2015 (Rosen, 2020). This trend has been linked to the presence of atypical femur fractures, a side effect in patients undergoing therapy with denosumab or bisphosphonates (Rosen, 2020), and with the aging of the global population (Clynes et al., 2020; Noh et al., 2020). Because of this osteoporosis is considered a major health concern and a burden to healthcare systems (Muraca & Cappariello, 2020).

OP exhibits, in general, a higher prevalence in elderly women than in men (Rosen, 2020), which may be caused by several factors including decreased estrogen levels and lower bone diameter (Rinonapoli et al., 2021; Rosen, 2020). In addition, fracture incidence exhibits a wide variation, both globally and among individual countries (Clynes et al., 2020). In this way, northern countries tend to exhibit a higher risk of fracture, due to the population's vitamin D status (Clynes et al., 2020). Besides, ethnicity also plays a significant role in OP development (Clynes et al., 2020; Pouresmaeili et al., 2018), since Asian and African American populations have been reported to exhibit a lower fracture risk, compared to Caucasian individuals (Pouresmaeili et al., 2018). This phenomenon may be associated with factors such as the higher bone mineral density (BMD) present in African American individuals, the higher trabecular volumetric BMD or the higher cortical thickness and density displayed by Asian populations, together with the shorter hip axis lengths present in both races regarding Caucasians (Cauley, 2011).

OP management relies on the use of different antiresorptive and anabolic drugs, producing a decrease in bone resorption or an increase in bone formation, respectively (Ng et al., 2022). Thus, antiresorptive drugs, including orally administered bisphosphonates such as alendronate, ibandronate and risendronate, or intravenously administered zoledronate and pamidronate are first-line treatments for the prevention and treatment of OP (Ayub et al., 2021; Kanis et al., 2019). These compounds display a high affinity for bone apatite and tend to accumulate in areas of active bone remodeling, reducing bone resorption by decreasing osteoclasts activity (Anam & Insogna, 2021; Ayub et al., 2021; Kanis et al., 2019). Moreover, RANKL inhibitors, including the monoclonal antibody denosumab, are alternative antiresorptive drugs for the treatment of osteoporotic postmenopausal women who cannot tolerate bisphosphonates (Anam & Insogna, 2021; Ayub et al., 2021; Kanis et al., 2019). Denosumab increases BMD and decreases bone resorption and fracture risk by inducing osteoclast apoptosis and reducing osteoclastogenesis (Anam & Insogna, 2021; Kodama & Kaito, 2020). Another antiresorptive drug employed is raloxifene, a selective estrogen receptor modulator that decreases the risk of vertebral fractures, prevents bone loss and increases BMD (Anam & Insogna, 2021, Ayub

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et al., 2021, Kanis et al., 2019). Finally, intranasal or injectable salmon calcitonin can also be employed to decrease the osteoclasts activity and reduce the risk of vertebral fractures in osteoporotic women who have been postmenopausal for at least 5 years (Anam & Insogna, 2021).

Among the anabolic drugs used to promote bone formation are teriparatide, abaloparatide and romosozumab (Anam & Insogna, 2021; Ayub et al., 2021). Teriparatide, a human parathyroid hormone, promotes osteoblasts activity, thus increasing bone formation and, therefore, decreasing the risk of fractures (Ayub et al., 2021). Conversely, the mechanism of action of abaloparatide, an analog of the human parathyroid hormone-related peptide (PTHrP), is not fully understood (Makino et al., 2021). Romosozumab, a humanized antibody against sclerostin, promotes bone formation by binding to sclerostin, an inhibitor of bone formation (Anam & Insogna, 2021, Ayub et al., 2021). However the administration of antiresorptive drugs is required to maintain bone density after the treatment with these anabolic drugs (Anam & Insogna, 2021, Ayub et al., 2021).

Despite the array of available therapies, several authors highlight the existence of a growing gap in the treatment of OP (Ayub et al., 2021). The main reason behind this claim is the low adherence of patients due to concerns related to drug safety or long-term efficacy. For example, highly prescribed drugs including bisphosphonates and RANKL inhibitors, are associated with atypical femur fractures or osteonecrosis of the jaw (Ayub et al., 2021; McDonald et al., 2021). Therefore, the development of new, safe, and alternative treatments is necessary. On this basis, this review is intended to provide some insight into new potential nanoparticle-based treatments for OP, that, by altering the behavior of cells involved in bone remodeling, may have a substantial impact on the course of the disease in the coming years.

2 | ROLE OF OSTEOBLASTS AND OSTEOCLASTS IN OP

Bone remodeling is a natural process within the body which takes place throughout the human's entire life and includes generation of new bone and resorption of the mature one (He et al., 2021). This process involves skeletal cells such as osteoblasts, derived from bone marrow stem cells (BMSCs) and responsible for bone formation, and osteoclasts, derived from hematopoietic macrophage or monocyte precursors and responsible for bone resorption together with osteocytes; regulating both bone formation and resorption (He et al., 2021; Kong & Penninger, 2000; Liu, Kou, et al., 2018). Furthermore, T and B cells, dendritic cells, megakaryocytes and monocytes are also involved in bone remodeling (He et al., 2021; Liu, Kou, et al., 2018).

Communication between these cells is essential for bone remodeling and can be produced by direct contact or via soluble mediators (Liu, Kou, et al., 2018). A relevant example of communication through soluble mediators is the receptor activator of nuclear factor κ-B (RANK)/osteoprotegerin (OPG)/RANK-ligand (RANKL) system (He et al., 2021, Liu, Kou, et al., 2018), which carefully regulates bone remodeling in physiological conditions as shown in Figure 1. Osteoblasts and osteocytes produce RANKL after stimulation with different compounds such as vitamin D, prostaglandin 2 (PGE2) or parathyroid hormone (PTH) (Ming et al., 2020). Then, the released RANKL binds to the RANK present in the membrane of preosteoclasts, triggering osteoclastogenesis and bone resorption (Ming et al., 2020). On the other hand, osteoblasts and osteocytes produce also OPG, a decoy receptor for RANKL, which inhibit the RANK pathway and, therefore, osteoclasts maturation and bone resorption (Ming et al., 2020). In addition, the immune system is also involved in the bone remodeling process (Caetano-Lopes et al., 2009). Thus, immune cells, such as T and B cells, monocytes, or dendritic cells, can release RANKL, triggering osteoclasts´ differentiation and bone resorption. Furthermore, immune cells are also able to secrete different cytokines that can exert both anti-osteoclastogenic or pro-osteoclastogenic effects (Caetano-Lopes et al., 2009).

However, a dysregulation of this pathway can occasionally lead to an excessive osteoclastic activity, causing an imbalance in bone homeostasis and leading to pathogenic conditions. For example, the decrease in estrogen levels present in postmenopausal women triggers a decrease in OPG levels and, therefore, an increase in RANKL activity, which promotes osteoclastogenesis and bone resorption, resulting in bone loss and OP (Kong & Penninger, 2000; Rao et al., 2018).

3 | SYNTHETIC NANOPARTICLES FOR OP MANAGEMENT

The aforementioned gap in OP management has prompted the screening for novel, cutting-edge therapies aimed at improving current treatments. These treatments are generally systemically administered at high concentrations increasing the risk of undesirable side-effects (Mora-Raimundo et al., 2017). There are several delivery systems designed to

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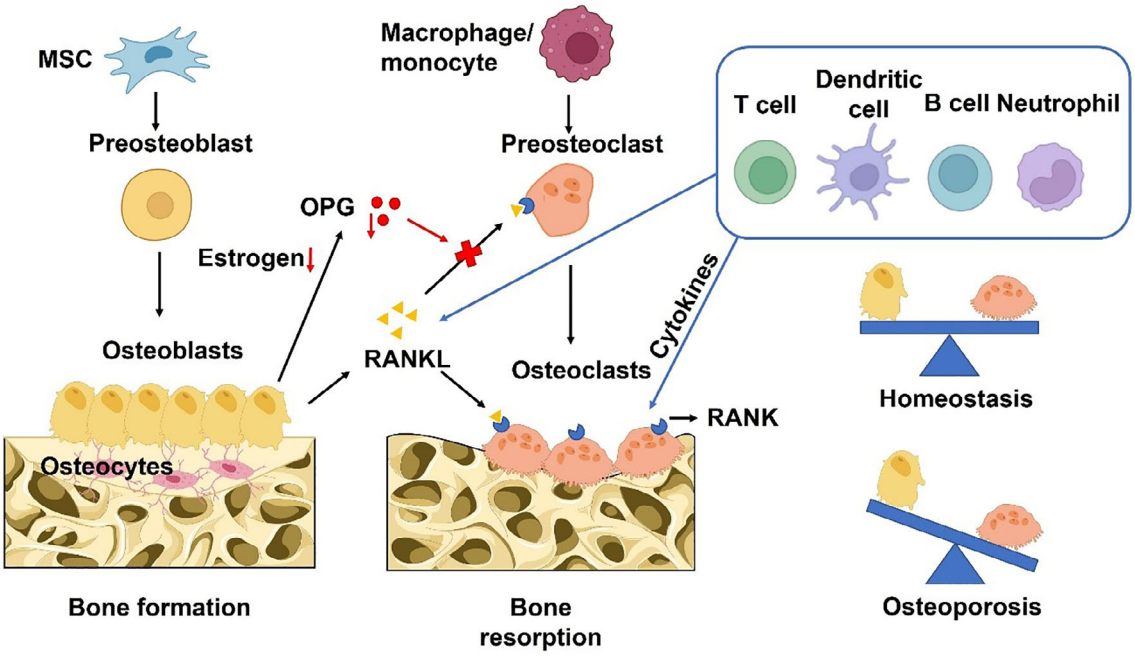

FIGURE 1 Communication between osteoblasts, osteoclasts and immune cells during bone remodeling through the RANK/OPG/RANKL pathway and the activity balance between osteoblasts and osteoclasts in normal and disease conditions. OPG, osteoprotegerin; RANK, nuclear factor κ-B; RANKL, nuclear factor κ-B-ligand.

enhance drug efficacy and therapeutic index while minimizing side effects and toxicity and protecting the drugs from degradation (Elsharkasy et al., 2020). In this regard, nanoparticles have emerged as a suitable alternative due to their ability to accumulate at the target site, in this case, bone, while ensuring local therapeutic doses (Higino & França, 2022). This section is focused on the synthetic nanoparticulated systems developed so far for OP management.

Generally, nanoparticles can be divided into two mayor types: organic or inorganic, being the first ones composed by polymers, lipids and/or proteins, and the latter ones formed by ceramics or metallic constituents (Higino & França, 2022). These nanoparticle systems can be functionalized with moieties that have a high affinity for the bone extracellular matrix (ECM), such as tetracycline and bisphosphonates, to achieve their bone-specific accumulation. Moreover, acidic amino acid oligopeptides such as eight aspartate (Asp8) or six repetitive sequences of aspartate, serine, serine (AspSerSer)6 have shown affinity for bone-resorption or bone-formation surfaces, respectively. This effect is related to their specific affinity for the different hydroxyapatite (HAp) crystallization phases (Liu et al., 2014; Sawamoto et al., 2020).

Among the inorganic nanoparticles developed for OP management, hydroxyapatite (HAp) nanoparticles are of particular interest due to their similarity in mineral structure to the extracellular matrix of bone and their demonstrated osteoinductive properties. HAp nanoparticles with calcium ions partially substituted by cobalt ions showing small diameter (63 and 71 nm) were synthesized hydrothermally. These systems were locally implanted in a critical bone fracture in glucocorticoid induced osteoporotic rats. After 24 weeks of implantation, a higher vascularization and bone formation was observed for those animals treated with the developed particles compared to the empty defect (Ignjatović et al., 2013). Moreover, HAp has been used to coat magnetite nanoparticles to obtain systems with improved in vitro osteoinductive properties in a osteoblast culture treated for 21 days with a 200 μg/mL nanoparticle dispersion (Tran & Webster, 2011). These nanoparticles were obtained also by wet chemistry and presented a higher mean diameter showing a mean value of 170 nm.

Silica nanoparticles, another type of inorganic nanoparticles, are able to induce the osteogenic differentiation of stem cells (Barry et al., 2016). Mesoporous silica nanoparticles synthesized following a modification of the Stöber method have been used for small interfering RNA (siRNA) delivery targeting the Wnt/β-catenin signaling pathway. More specifically, the SOST gene was silenced by loading anti-SOST siRNA on poly(ethylenimine) coated mesoporous silica nanoparticles. SOST also known sclerostin is, as previously mentioned, a bone formation inhibitor protein that induces an enhancement in the osteoclasts number and activation state (Delgado-Calle et al., 2017). The developed

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nanoparticles presented a mean diameter of 150 nm and zeta potential (ZP) of −21.5 mV at pH = 7. Their intra-bone marrow administration of 50 μL of a nanoparticle dispersion at 0.8 mg/mL in ovariectomized (OVX)-induced osteoporotic rats led to an increase in the expression of osteogenic markers after 5 days of administration (Mora-Raimundo et al., 2019).

On the other hand, poly (lactic-co-glycolic acid) (PLGA) has been used to develop simvastatin loaded tetracycline grafted nanoparticles leveraging the tetracycline's high affinity for bone ECM. The nanoparticles, prepared by a solvent emulsification method, showed a mean diameter of 224.9 ± 10.3 nm and a polydispersity index (PdI) below 0.3. When intravenously injected at 0.5 mg/kg every 2 days for 2 months in OVX-induced osteoporotic rats the treatment with the developed nanoparticles indeed promoted an increased in BMD (Deng, Wang et al., 2015). Hybrid lipid-PLGA nanoparticles have been screened as carriers for oligonucleotides to silence the expression of sfrp1 which is implicated in the inhibition of the pro-osteogenic effect of bone morphogenic protein 2 (BMP-2). Therefore, its inhibition potentiates the osteogenic effect of this growth factor. Moreover, these systems were functionalized with a BMSCs-specific aptamer to achieve a bone specific targeting. The designed hybrid nanoparticles were obtained by a modified nanoprecipitation and showed an average diameter of 168.2 ± 22.89 nm and low PdI (<0.3). The potential osteoinductive effect of the nanoparticles was then evaluated in OVX induced osteoporotic mice. To this end, nanoparticles were intravenously injected once a month for 3 months at 3 mg/mL. The in vivo data showed an increased bone density and improved bone microarchitecture for those mice treated with the nanoparticles when compared to the saline solution group (García-García et al., 2022).

Liposomes have been of particular interest as drug delivery systems in recent decades because they can incorporate water-soluble molecules in their core and lipid-soluble drugs within their membrane. These systems modulate the drug pharmacokinetics and protect them from degradation in the bloodstream (van der Koog et al., 2022). Liposomes obtained by thin film evaporation–extrusion and loaded with the estrogen ethinylestradiol, have been developed for OP management. These systems showed an average diameter that ranged from 175.9 ± 2.6 nm to 235.1 ± 1.9 nm and were characterized by a negative surface charge between −28.3 ± 2.1 mV and − 13.8 ± 1.5 mV. The intraperitoneal administration of the developed liposomes at 2 μg/kg every day for 1 month in OVX-induced osteoporotic rats stimulated bone calcium deposition and mineral content (Lu et al., 2011). On the other hand, (AspSerSer)6 functionalized cationic liposomes loaded with the siRNA against casein kinase-2 interacting protein-1 (Plekho1), a suppressor of bone formation, were prepared by a lyophilization/rehydration method. These systems indeed presented a positive ZP about 10 mV and an average particle size of 140 nm. The treatment of OVX-induced osteoporotic rats once per week for 9 weeks with 3.75 mg/kg of the developed systems by intravenous administration increased bone mass and improved the bone trabecular architecture (Zhang, Guo et al., 2012).

3.1 | Nanoparticulated systems controlling osteoclasts

The importance of the osteoclastic function on bone resorption turns osteoclasts into suitable cells for targeted drug delivery though nanoparticulated systems. In this regard, several authors have reported successful osteoclast-targeting or osteoclast activity-targeting nanoparticulated drug delivery systems as summarized in Table 1.

One of the most commonly employed strategies for targeting osteoclasts is the utilization of gold nanoparticles (GNPs), which have been shown to possess intrinsic inhibitory properties on osteoclastic differentiation through the blockade of the RANKL signaling pathway (Sul et al., 2010). Heo et al. developed GNPs functionalized with beta-cyclodextrin-curcumin complexes and obtained exceptional results in inhibiting the differentiation of bone marrow monocytes (BMMs) to osteoclasts in vitro, and in an ovariectomy (OVX)-induced osteoporosis mouse model in terms of inhibition of osteoclast differentiation and preservation of trabecular bone volume while avoiding toxicity. The treatment protocol consisted of intragastric administration of 50 or 500 μM loaded CGNPs (50 μL) once a day for 9 weeks (Heo et al., 2014). Similarly, Lee et al. prepared alendronate-conjugated GNPs to enhance the targeted delivery of the nanoparticles to bone. The results demonstrated a synergistic effect of alendronate (ALD) and gold nanoparticles. In vitro, the differentiation of BMMs to osteoclasts was inhibited, and in vivo, a greater inhibition of bone resorption was achieved using these nanoparticles compared to the individual agents alone in an OVX-induced osteoporosis mouse model. The treatment protocol consisted of oral administration of 500 μM (0.5 mL) GNPs-ALD once a day for 12 weeks (Lee, Heo, et al., 2016). In contrast, Bai et al. employed GNPs which surface had been chemically modified through carboxylation. These particles, instead, inhibited the capacity of osteoclasts for creating an acidic environment, preventing

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TABLE 1 Published studies describing the effect of synthetic nanoparticles designed to control osteoclasts behavior for the treatment of OP.

System	Elaboration method	Targeting strategy	Effect in OCs	Cargo	Diameter (nm)	ZP (mV)	PdI	Ref.
CD-GNPs	Citrate reduction	GNPs	Inhibition of OCs differentiation	CUR	20–40	NE	NE	(Heo et al., 2014)
GNPs	Citrate reduction	ALD, GNPs	Inhibition of OCs differentiation	ALD	32.9 ± 0.7	−40.75±2.71	0.262 ± 0.08	(Lee, Heo, et al., 2016)
Carboxylated GNPs	Citrate reduction	Surface carboxylation, GNPs	Inhibition of OCs differentiation	None	22.45 ± 2.38	−33.14±0.42	0.312	(Bai et al., 2020)
Fullerenol	Alkaline reaction	Fullerenol	Inhibition of osteoclastogenesis	None	32.12 ± 8.78	−25.94±7.18	NE	(Chen et al., 2020)
HAp-ALD	-	HAp, ALD	Block resorption	None	61.5 ± 12.6	−21.1±4.2	NE	(Hwang et al., 2016)
HAp-ZOL	Sol–gel	HAp, ZOL	Block resorption	ZOL	100–130	NE	NE	(Khajuria et al., 2015)
HAp-SCT	Chemical precipitation	HAp, SCT	Disruption of cytoskeletal organization	SCT	90.9 ± 12.3	−24.5 ± 0.7	0.197 ± 0.04	(Kotak & Devarajan, 2020)
ZnHAp	Sol–gel method	HAp, RIS	Block resorption	RIS	18.08	NE	NE	(Khajuria et al., 2016).
HAp-IO	Microwave-stimulated hydrothermal method	HAp	Reduced TRAP, Inhibition of osteoclasts activity, Thermolysis	miR-21 miR-124	25–56	NE	NE	(Marycz et al., 2021)
HAp-IO	Co-precipitation	HAp	Inhibition of osteoclastogenesis	None	60	−24	NE	(Li, Fu, et al., 2021)
Mesoporous SiO_2	Modified sol –gel	-	Inhibition of ROS	Nanoceria	80 ± 10	−30 ± 0.4	NE	(Pinna et al., 2021)
MBG	Combination of stabilizing condensation and dynamic self-assembly	-	Inhibition of RANK expression	siRNA for *RANK*	100	+19.1	NE	(Kim, Singh et al., 2016)
Cerium NPs	Microemulsion	ALD-PEG600	Bone resorption surfaces	None	15.2 ± 0.4	NE	NE	(Dou et al., 2021)
Dex-IO	Co-precipitation	ALD	Thermolysis	ALD	20	NE	NE	(Lee, Su, et al., 2016)
DOTAP-based liposome	Lyophilization/ rehydration	Asp8	Inhibition of osteoclastogenesis	AntagomiR-148a	150	−10	NE	(Liu, Li, et al., 2015)
HPMA-D-Asp8	Radical copolymerization	Asp8	Silencing of *sema4D* in OCs increases Osteoblast differentiation	siRNA for *sema4D*	NE	NE	NE	(Zhang et al., 2015)

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TABLE 1 (Continued)

System	Elaboration method	Targeting strategy	Effect in OCs	Cargo	Diameter (nm)	ZP (mV)	PdI	Ref.
PLGA-CHT-CD ALG-ALD	Ionic gelation	ALD	Block resorption	ALD	200–400	NE	NE	(Jing et al., 2021)
CHT-TPP	Ionic gelation	-	Decreased osteoclastogenesis	RLX	174.8 ± 10.2	NE	NE	(Saini et al., 2015)
CHT	Ionic gelation	RIS	Block resorption	RIS	415.6 ± 0.24	NE	0.27	(Santhosh et al., 2019)
CHT-TPP	Ionic gelation	HA, CGRPR, TRAP peptide	Specific delivery to mature or precursor OCs	None	190.8 ± 4	−17.20±0.35	NE	(Zhang, Zhao, et al., 2022)

Abbreviations: ALD, alendronate; Asp8, eight aspartate; CD, *β*-cyclodextrin; CGRPR, calcitonin gene-related peptide receptor; CHT, chitosan; CUR, curcumin; Dex, dextran; GNPs, gold nanoparticles; HA, hyaluronic acid; HAp, hydroxyapatite; HPMA, N-(2-hydroxypropyl)methacrylamide; IO, iron oxide; MBG, bioactive glass nanospheres; miR-21, microRNA-21; miR-124, microRNA-124; MSCs, mesenchymal stem cells; NE, not evaluated; OCs, osteoclasts; PdI, polydispersity index; PLGA, poly(lactic-co-glycolic acid; RANKL, nuclear factor κ-B-ligand; RIS, risendronate; RLX, raloxifene hydrochloride; ROS, reactive oxygen species; SCT, salmon calcitonin; sema4D, semaphoring 4D; TPP, tripolyphosphate; TRAP, tartrate-resistant acid phosphatase; ZnHAp, zinc-hydroxyapatite; ZOL, zoledronic acid; ZP, zeta potential.

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bone erosion both in vitro and in vivo in an LPS-induced bone erosion mice model. In this case, nanoparticles were intraperitoneally administered at 40 mg/kg along with 5 mg LPS to mice every 72 h for 15 days (Bai et al., 2020).

Aside from gold nanoparticles, other types of nanoparticulated systems have been used to target osteoclasts. Chen et al. managed to exert anti-osteoclastic activity with a fullerenol nanoparticulated system both in vitro, inhibiting the BMMs differentiation to osteoclasts, and in vivo. These nanoparticles demonstrated efficacy as an anti-osteoporotic treatment in both a lipopolysaccharide (LPS)-induced bone erosion mouse model and an OVX-induced OP rat model. This was achieved by modifying the podosome patterning of osteoclasts, while not impacting osteoblastic activity and with no significant toxicity being observed (Chen et al., 2020). Furthermore, HAp nanoparticles loaded with the bisphosphonate zoledronate were found to promote bone formation in osteoporotic rats, likely due to the antiresorptive properties of the loaded bisphosphonate (Hwang et al., 2016). Moreover, the same effect was observed for HAp nanoparticles loaded with the bisphosphonate zoledronate have been proven to promote bone formation in OVX-induced osteoporotic rats after a single intravenous injection (100 μg/kg) (Khajuria et al., 2015). Salmon calcitonin, another antiresorptive drug already described above, has also been loaded in HAp nanoparticles. Their daily sublingual administration (200 IU) showed an increase in bone mass and mechanical strength compared to the parenterally administered drug alone in an OVX OP rat model (Kotak & Devarajan, 2020).

Furthermore, HAp can be doped with other components such as zinc and iron oxide to increase its bioactivity. As an example, risendronate functionalized zinc-HAp nanoparticles showed an ameliorated bone loss, improving trabecular disorganization and bone quality in OVX osteoporotic rats after a single intravenous injection at 500 μg/kg (Khajuria et al., 2016). HAp coated superparamagnetic iron oxide nanocomplexes were also able to be accumulated in the bone marrow, promoted BMSCs osteoblastic differentiation and inhibited BMMs osteoclast differentiation in vitro. This effect led to a bone loss inhibition in OVX-induced osteoporotic mice when intravenously injected at 10 mg/kg every 4 days for 3 months (Li, Fu, et al., 2021). Similarly, iron oxide-HAp nanoparticles can be loaded with miR-21-5p and miR-124-3p, microRNAs recognized to regulate bone formation through osteoclasts inhibition allowing for a magnetically driven release. The experimental data showed an in vitro enhanced osteogenesis together with a decreased preosteoclasts differentiation in the pre-osteoblast cell line MC3T3-E1 and the macrophage cell line Raw 264.7 (Marycz et al., 2021).

Mesoporous silica nanoparticles loaded with the reactive oxygen species (ROS)-scavenger nanoceria have been tested for OP management. These systems show in vitro osteogenic and anti-osteoclastogenic properties in an osteoblast-macrophage co-culture (Pinna et al., 2021). Similarly, mesoporous bioactive glass nanospheres have been used as carriers for siRNAs loaded by complexation to suppress osteoclastogenesis by silencing the RANK expression. In vitro data showed that these nanoparticles successfully inhibited the expression of RANK, thus downregulating osteoclastogenesis in Raw 264.7 macrophages (Kim, Haney, et al., 2016).

Alternatively, cerium nanoparticles with selective oxidative activity at the acidic pH environment created by mature osteoclasts in the resorption area were synthesized. These nanoparticles were functionalized with alendronate. In vivo results in OVX-induced osteoporotic mice showed that they were more efficient in preserving bone mass than alendronate alone, decreasing the viability of mature osteoclasts while maintaining preosteoclasts function (Dou et al., 2021).

Dextran coated magnetic Fe_3O_4 nanoparticles were conjugated with alendronate, aiming at the thermolysis of osteoclasts for OP management. The developed nanoparticles showed potential as magnetic resonance imaging contrast agents in vivo, but no therapeutic utility in OP management was tested (Lee, Su, et al., 2016).

Moving to lipid nanoparticles Liu et al developed a DOTAP-based liposomes functionalized with Asp8 peptide and containing a specific miRNA antagonist (antagomiR-148a), a bone resorption inhibitor. These functionalized liposomes showed effective bone biodistribution in an OVX-induced osteoporotic mouse model and seemed a promising delivery system to inhibit osteoclastic disorders (Liu, Dang, et al., 2015). Similarly, the same peptide was used to target N-(2-hydroxypropyl)methacrylamide (HPMA) nanoparticles to osteoclasts. These nanoparticles contained a siRNA targeted to semaphorin 4D, inhibiting its expression. This inhibition would induce osteoblast maturation and restore bone homeostasis in an osteoporosis scenario. This delivery system showed an increase in bone mass in healthy mice and the recovery of bone loss in OVX mice (Zhang et al., 2015).

Polymeric nanoparticles obtained by the ionic cross-linking between PLGA, β-cyclodextrin-modified chitosan, and alendronate modified alginate were obtained for OP management showing high affinity for bone ECM and antiresorptive properties (Jing et al., 2021). Chitosan has also been used to develop raloxifene loaded nanoparticles for intranasal administration. Although the impact on bone tissue was not evaluated, the synthesized nanoparticles exhibited a higher plasma concentration of raloxifene than the drug administered orally (Saini et al., 2015). Chitosan

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nanoparticles have been as well functionalized with risedronate and tested in OP rats. The injection of these nanoparticles was able to increase BMD and improve bone microarchitecture (Santhosh et al., 2019).

However, while mature osteoclasts are significant for their bone-resorbing activity, preosteoclasts have also gained attention due to their role in promoting angiogenesis in bone and supporting osteoblast differentiation and function, thus playing an essential role in osteogenesis (Boyce, 2013; Kusumbe & Adams, 2014; Xie et al., 2014). Preosteoclasts are mononucleated cells that, if do not get to multinucleate, express mature osteoclasts phenotype but are not able to completely work as bone resorbing cells (Kodama & Kaito, 2020). Thus, preosteoclast multinucleation may be a promising target for limiting bone resorption while preserving their role in osteogenesis (Kodama & Kaito, 2020). Regarding this, nanoparticulated delivery systems have been developed.

Zhang et al. designed a delivery system able to target two stages of osteoclast development, early and mature osteoclasts. In brief, they developed two types of dual-targeting chitosan-tripolyphosphate nanoparticles both grafted with hyaluronic acid: one was functionalized with calcitonin gene-related peptide receptor (CGRPR), targeting early osteoclasts, while the other was functionalized with TRAP (Tartrate-resistant acid phosphatase) peptides, targeting the mature ones. These particles showed a successful bone biodistribution and an effective targeting to the resorption areas in BALB/c mice. Furthermore, in vitro assays showed the differences in cell targeting for these nanoparticles (Zhang, Zhao, et al., 2022).

From the described data it is gathered that most of the therapeutic molecules used for the traditional management OP have been included into nanoparticulated systems of variable nature. This incorporation has led to an enhancement in the therapeutic activity of the selected drugs. Interestingly, none of the studies reported any toxic effect, this fact can be associated to either an efficient accumulation of the nanoparticles at the target tissue decreasing the undesired side effects or to the relatively short-time exposure of the animals to the nanoparticulated systems limiting the identification of toxicity.

4 | NATURAL NANOPARTICLES

Extracellular vesicles (EVs) are complex phospholipidic structures secreted by different cells that can be classified according to their size and biogenesis in microvesicles (100–1 μm), apoptotic bodies (50–2 μm) and exosomes (30–150 nm) (Zaborowski et al., 2015). Apoptotic bodies and microvesicles are formed by plasma membrane gemination (Koritzinsky et al., 2017), while exosomes formation is endosome mediated, obtained via fusion of multivesicular bodies and the plasma membrane (Zhang, Bi, et al., 2020). EVs have a great potential as natural nanoparticles for drug delivery owing to their lipid bilayer structure, low immunogenicity, good plasma stability and high penetration capacity in cells and tissues (Jeyaram & Jay, 2017). Also, their surface and content can be modified to target specific cell types. EVs are released by several cells, such as immune cells, mesenchymal stem cells, platelets, tumor cells or cardiomyocytes (Luo et al., 2021; Simpson et al., 2008). They have been found in different body fluids such as plasma, saliva, nasal secretions, spinal fluid, milk or blood (Hannafon et al., 2016; Lasser et al., 2011). EVs play an active role in cell–cell communication, homeostasis, immune responses, antigen presentation, programed cell death, organ development or even tumor progression (Becker et al., 2016; Gurunathan et al., 2021; Kalluri & LeBleu, 2020).

4.1 | Roles of extracellular vesicles on bone remodeling

EVs are known to act as mediators in intercellular communication by encapsulating active cargoes able to modify cell phenotype, constituting an essential communication mechanism in bone remodeling (He et al., 2021; Liu, Sun, & Zhang, 2018). Among the active cargoes that can be loaded onto nanoparticles, two types can be distinguished: canonical cargoes, which are molecules that are commonly associated with the function of the parent cells, and special cargoes, which are molecules that specifically reflect the unique function of the parent cells (Liu, Sun, & Zhang, 2018). Canonical cargoes linked with vesicle trafficking and biogenesis include enzymes (GAPDH, ATPase), stress proteins (as heat-shock proteins), cytoskeletal proteins (as tubulin), membrane trafficking proteins (as annexins) or tetraspanins (Liu, Sun, & Zhang, 2018). Alternatively, among the special cargoes carried by EVs in the bone remodeling microenvironment are microRNAs or miRNAs (as miR-143-3p or miR-218), mRNAs involved in the transcription regulation or kinase activity (as BDP1 or ZEB2), proteins involved in osteoclast differentiation (as RANK or RANKL), non-collagenous matrix proteins (as osteocalcin) and osteogenic proteins (such as bone morphogenic proteins or BMPs)

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(Liu, Sun, & Zhang, 2018). In addition, circular RNAs (CircRNAs) and long noncoding RNAs (LncRNAs) can also be present in these EVs (Zhang, Huang, et al., 2022). Examples of the interaction between cells involved in bone remodeling by means of EVs loaded with these active compounds will be discussed below.

4.1.1 | Osteoblast communication with surrounding cells

Communication among osteoblast during bone remodeling is triggered by EVs. As an example, osteoblast-derived small EVs containing miR-143-3p, were able to suppress osteoblast differentiation by inhibiting the Runt-related transcription factor 2, an important regulator of osteoblastogenesis, leading to a reduced bone formation (Uenaka et al., 2022). The communication between osteocytes and osteoblasts can also be modulated by means of EVs. In this way, myostatin treated osteocytes have been reported to produce exosomes targeting osteoblast precursors, leading to a reduction in osteoblasts differentiation by downregulating the Wnt signaling pathway (Qin et al., 2017). This effect might be related with the decreased presence of miR-218 in the vesicles extracted from the treated cells (Qin et al., 2017). EVs are also implied in MSCs-osteoblast communication. Exosomes released by mineralizing preosteoblasts can trigger BMSCs differentiation to osteoblasts, via Wnt signaling activation (Cui et al., 2016). Conversely, other works suggest that osteoblast-derived EVs obtained from patients suffering from osteoporosis or osteoarthritis, alter cell metabolism and have a negative impact on the osteogenic differentiation of BMSCs (Niedermair et al., 2020). Osteoclast–osteoblast communication can also be regulated by EVs, some of which have been found to be derived from lipopolysaccharide (LPS)-induced osteoclasts in inflammatory conditions. These exosomes promote osteoblasts activity and bone formation by delivering LncRNA LIOCE, which upregulates Osterix expression, an osteogenic transcription factor (Ren, Zeng, et al., 2022). Conversely, osteoclast-derived exosomes containing miR-214-3p inhibit osteoblastic activity and bone formation after being transferred to osteoblasts (Hannafon et al., 2016; Li et al., 2016).

4.1.2 | MSCs communication with surrounding cells

Communication between MSCs is also mediated by EVs. BMSCs-derived EVs containing miR-22-3p have been shown to enhance the osteogenic differentiation of other MSCs by inactivating the MYC/PI3K/AKT pathway, which is expected to increase bone formation (Zhang, Wang, et al., 2020). Similarly, EVs obtained from mid to late stage MSCs during osteoblastic differentiation promote the osteogenic differentiation of BMSCs, encouraging bone formation (Wei et al., 2019). Likewise, exosomes obtained from osteogenic MSCs have been reported to induce the osteogenic differentiation of other MSCs in vivo and in vitro (Narayanan et al., 2018). Therefore, it can be inferred that MSC-derived extracellular vesicles have the capability to efficiently regulate the behavior of undifferentiated MSCs toward osteoblastic differentiation. An example of crosstalk between immune cells and MSCs is the extracellular vesicles obtained from pro-inflammatory M1 macrophages, which are enriched in miR-155. These vesicles have been shown to decrease the osteogenic differentiation of MSCs by downregulating the BMP pathway, thereby hindering bone regeneration in a rat calvaria defect model (Kang, Huang et al., 2020). Whereas M2 macrophage-derived EVs containing miR-378a produced the opposite effect (Kang, Huang et al., 2020). Furthermore, MSCs are also able to secrete EVs conditioning immune cells behavior. Thus, BMSCs-derived apoptotic EVs inhibit macrophage polarization into proinflammatory phenotypes via AMPK/SIRT1/NF-κB pathway (Ye et al., 2022).

4.2 | Effect of EVs on osteoblasts and osteoclasts activity and implications in bone-related diseases

As previously mentioned, osteoclasts and osteoblasts are skeletal cells playing a crucial role in bone remodeling and their function can be effectively regulated by means of synthetic and natural nanoparticles (He et al., 2021). In this way, Table 2 summarizes the effect of EVs obtained from diverse cell types on osteoclasts and osteoblasts activity, together with their impact on the bone status.

According to several studies, EVs secreted by osteoblasts have been reported to efficiently modulate osteoclasts activity. As an example, osteoblastic matrix vesicles containing miR-125-b inhibit osteoclast formation in vitro. Specifically, treatment of mouse BMMs with these vesicles (1 μg protein/ml) has been shown to decrease osteoclastogenesis in

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TABLE 2 Published studies describing the effect of EVs secreted by a wide variety of cells on osteoclasts and osteoblasts differentiation and activity, together with their impact on bone remodeling.

Source	Function	Bioactive factor	In vivo effects on bone	Ref.
Epimedium-treated MSCs	Promotion of osteogenic differentiation	miR-27a-5p	Improvement of bone mass and microstructure	(Li, Chen, et al., 2021)
Osteoblasts	Promotion of osteoblasts differentiation	Undetermined	Attenuation of bone loss	(Wei et al., 2019)
Endothelial progenitor cells	Reduction of the corticoid-induced osteoblast damage	Undetermined	Improved trabecular thickness and connectivity Reduction in the formation of femoral necrotic tissue	(Lu et al., 2019)
Osteoblasts	Promotion of osteoclasts differentiation and survival	RANKL	Appearance of TRAP-positive cells in RANKL−/− mice	(Deng, Wang, et al., 2015, Cappariello et al., 2018)
Osteoblasts	Promotion of osteoclasts differentiation and activity	CircRNA_0008542	Promotion of bone resorption	(Wang, Qiao, et al., 2021)
Osteoblastic/osteoclastic prostate cancer cell lines	Promotion of osteoclast differentiation	miR-92a-1-5p	Bone resorption Decreased bone formation	(Yu et al., 2021)
Multiple myeloma cells	Promotion of osteoclast differentiation Increased bone resorption	Endoplasmic reticulum-associated unfolded protein response (UPR)-related molecules	Not evaluated	(Raimondi et al., 2020)
Osteosarcoma cells	Promotion of osteoclast differentiation Pre-osteoclast migration	miR-19a-3p	Bone destruction and osteopenia	(Luo et al., 2021)
Osteosarcoma cells	Promotion of osteoclast differentiation and resorption activity	miR-148a miR-21-5p	Not evaluated	(Raimondi et al., 2020)
Bone metastatic mammary tumor cells	Enhancement of mature osteoclast survival Increased bone resorption	miR-92a-3p	Not evaluated	(Uehara et al., 2022)
Erythroblastic cells	Promotion of osteoclastogenesis	Peroxiredoxin-2 protein (PRDX2)	Not evaluated	(Sadvakassova et al., 2021)
Osteoblasts	Inhibition of osteoclasts differentiation	miR-125-b	Inhibition of bone resorption	(Minamizaki et al., 2020)
Osteoblasts	Inhibition of osteoclasts differentiation	miR-503-3p	Not evaluated	(Wang, Shen, et al., 2021)
Endothelial cells	Inhibition of osteoclast differentiation and activity	miR-155	Inhibition of bone resorption	(Song et al., 2019)
Adipose-derived MSCs	Suppression of osteoclasts inflammatory response	Undetermined	Improvement on bone mineral loss	(Zhang, Wang, et al., 2021)
M2-like macrophages	Inhibition of osteoclasts differentiation Promotion of osteogenic differentiation	IL-10 mRNA	Attenuation of bone resorption	(Chen et al., 2022)

(Continues)

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TABLE 2 (Continued)

Source	Function	Bioactive factor	In vivo effects on bone	Ref.
Orofacial MSCs	Inhibition of osteoclasts differentiation Promotion of osteoblasts differentiation	miR-206-3p	Increase in bone mass	(Li, Guo et al., 2021)
Systemic circulation	Inhibition of osteoclasts activity Improvement of osteogenic cells´ function	Ubiquitin ligase RNF146 miR-328-3p	Increased bone formation Improvement of bone mineral density	(Liu, Kou, et al., 2018)
Osteoclasts	Inhibition of osteoclastogenesis	RANK	Not evaluated	(Huynh et al., 2016)
Human umbilical cord MSCs	Inhibition of osteoclastogenesis	CLEC11A	Inhibition of bone resorption Increased bone formation	(Hu et al., 2020)
Child gut microbiota	Inhibition of osteoclastogenesis Promotion of osteoblastogenesis	Undetermined	Maintenance of bone mass and strength	(Yu et al., 2021)
MSCs	Inhibition of osteoclastogenesis Promotion of osteoblastogenesis	mRNA-27a	Improved bone density and structure	(Wang, Zhou, & Wang, 2022)
Urine-derived stem cells	Suppression of osteoclastogenesis Inhibition of osteoclasts function Promotion of osteoblasts activity	Collagen triple-helix repeat containing 1 (CTHRC1) OPG miR-26a-5p	Prevention of diabetic osteoporosis Improvement of bone strength Attenuation of bone loss	(Hu, Xu, et al., 2019, Zhang, Du, et al., 2022)
Human umbilical cord blood	Inhibition of osteoclastogenesis Promotion of osteogenic differentiation	miR-3960	Attenuation of bone loss	(Hu et al., 2019)

Abbreviation: MSCs, mesenchymal stem cells.

the presence of RANK Ligand (RANKL) (Minamizaki et al., 2020). Furthermore, it has been reported that OVX mice overexpressing miR-125-b in osteoblasts improved bone mass in comparison with wild type mice, demonstrating the protective effects of this matrix vesicle cargo, which can be related with their ability to degrade Prdm1 in osteoclast precursors, a transcriptional repressor of anti-osteoclastogenic factors (Minamizaki et al., 2020). Similarly, exosomes containing miR-503-3p hinder osteoclast differentiation from progenitor cells in vitro by downregulating the expression of an heparanase gene (Wang, Shen, et al., 2021). As proven by the co-culture of Raw 264.7-derived osteoclast precursors and osteoblast-derived exosome pellets in a 100 μg/mL concentration (Wang, Shen, et al., 2021). Conversely, osteoblast derived EVs containing RANKL have been found to exert a pro-osteoclastic effect (Deng, Wang et al., 2015, Cappariello et al., 2018). In this way, the daily intraperitoneal administration of a dose ranging from 30,000 to 120,000 vesicles for 5 days, was able to induce, in all cases, the appearance of TRAP positive cells in $RANKL^{-/-}$ mice, a model lacking the osteoclast lineage, without causing any toxic effects (Cappariello, Loftus, et al., 2018). Similarly, exosomes transporting the circular RNA CircRNA_0008542 enhanced osteoclast differentiation by promoting RANK gene expression, leading to an increased bone resorption in vivo, after the intravenous administration of 100 μg EVs per week, for 8 weeks, to female mice (Wang, Shen, et al., 2021). Interestingly, osteoblasts have also been reported to modulate their own behavior. To this regard, it has been shown that EVs derived from osteoblasts in the mid-late stages of their differentiation process present bone targeting potential and ameliorate OP in an OVX osteoporotic mice model by promoting

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osteoblasts differentiation, after the weekly intravenous administration of a dose of 3 mg EVs/kg for 4 weeks (Wei et al., 2019).

Alternatively, endothelial cells can also influence osteoclasts and osteoblasts behavior. As an example, EVs obtained from bone marrow-derived endothelial progenitor cells have found to be useful to alleviate steroid-induced osteoporosis in mice by reducing the corticoid-induced osteoblast damage via inhibition of the ferroptotic pathway (Lu et al., 2019). The intravenous administration of 50 μg of these EVs once a week during a month, led to a reduction in the formation of femoral necrotic tissue, together with an improved trabecular thickness and connectivity (Lu et al., 2019). In addition, miR-155-loaded exosomes extracted from endothelial cells inhibit osteoclastic differentiation by targeting Spi1, Mitf, and Socs (Song et al., 2019), attenuating bone loss in an OVX-induced OP mouse model after the intraperitoneal administration of 100 μg of EVs in 100 μL of PBS twice a week for 6 weeks, without causing any toxic effect (Song et al., 2019). However, it has also been found that hematopoietic cells-derived exosomes transferring Peroxiredoxin-2 protein (PRDX2), a cytosolic antioxidant protein, might be able to promote osteoclastogenesis in vitro and in vivo (Sadvakassova et al., 2021). Thus, conditioned medium obtained from primary mouse erythroblasts or commercial erythroleukemia cells, effectively promoted osteoclastogenesis in RANKL-primed osteoclast precursors. This link between erythropoiesis and osteoclastogenesis was further confirmed using an anemia mice model (Sadvakassova et al., 2021).

Osteoclasts are also known to regulate their own behavior, as RANK-containing exosome-like EVs isolated from osteoclasts and administered in a dose of 5×10^7 EVs/ml on days 1, 4 and 6, have been found to hinder osteoclastogenesis in vitro in $1,25(OH)_2D_3$-treated bone marrow (Huynh et al., 2016). On the other hand, MSCs can also modulate osteoclastic activity. Hence, orofacial MSCs-derived exosomes containing miR-206-3p, a downstream factor of Gata 4 (involved in the osteoclastic function regulation), hinder osteoclasts differentiation, enhancing bone mass. These effects were observed after the injection of 20 μg of EVs, diluted in 10 μL, into the buccal periosteum of Wnt1-Cre; Gata4fl/fl mice, a knockout model showing inhibited bone formation and active bone resorption. Mice were injected twice every 3 days (Guo et al., 2021). Also, MSCs-derived EVs containing miR-27a, have been found to modulate both osteoblasts and osteoclasts differentiation by targeting the miR-7a/DKK2/Wnt/ß-catenin signaling pathway. Leading, in this way, to an improved BMD and structure in ovariectomized OP mice, and therefore to an improved osteoporosis prognosis, after their injection through the femur periosteum, twice a week, for 1 week (Wang, Zhou, & Wang, 2022). Similarly, an inhibitory effect on osteoclastogenesis was noted with the administration of human umbilical cord MSCs-derived EVs containing CLEC11A (C-type lectin domain family 11 member A), a potent pro-osteogenic protein (Hu et al., 2020). The intravenous administration of these EVs to OVX mice (100 μg in 100 mL PBS for 2 month) and to a hindlimb-unloading osteoporosis mice model (100 μg in 100 mL PBS for 21 days) has been found to reduce marrow fat accumulation, improve bone strength and attenuate bone loss, by inhibiting osteoclast formation and by promoting the osteogenic differentiation of BMSCs (Hu et al., 2020). Likewise, exosomes obtained from adipose-derived MSCs have been reported to improve bone mineral loss and to ameliorate OP in a rat diabetic model, by suppressing the NLRP3 inflammasome activation in osteoclasts. Results were noticeable after 1 month of exosome intravenous treatment, administering a dose of 1.6 mg EVs/kg every 2 days (Zhang, Wang, et al., 2021).

Moreover, EVs derived from cancer cells have been reported to play a role in bone resorption. As an example, osteosarcoma cell-derived EVs containing the microRNAs miR-19a-3p or miR-148a and miR-21-5p, have been found to enhance osteoclastogenesis and bone resorption in vitro, when administered to Raw 264.7 cells for 4 days in a dose of 15 μg/mL, and 6 days in a dose of 25 μg/mL, respectively (Luo et al., 2021; Raimondi et al., 2020). Furthermore, the higher presence of miR-19a-3p-loaded EVs is likely to be responsible for the bone destruction and osteopenia observed in an osteosarcoma mice model (Luo et al., 2021). Similarly, exosomes obtained from both osteoblastic and osteoclastic prostate cancer cells, and bone metastatic mammary tumor cells transporting miR-92a-1-5p and miR-92a-3p promote osteoclast differentiation and favor mature osteoclasts survival by reducing type I collagen expression and via the Akt signaling pathway, respectively (Uehara et al., 2022; Yu et al., 2021). In this way, the treatment of osteoclast precursors such as Raw 264.7 cells, resulted in an increased osteoclastogenesis and bone resorption in vitro, after the administration of 100 ng/mL of miR-92a-3p-loaded EVs (Uehara et al., 2022). Similarly, the treatment of Raw 264.7 cells or bone marrow macrophages with 20 μg/mL of prostate cancer-cell derived exosomes containing miR-92a-1-5p for 6 days have found to promote osteoclast differentiation in vitro (Yu et al., 2021). Furthermore, the treatment of BALB/c mice with the prostate cancer derived exosomes by performing an intravenous administration of 10 μg of EVs in 100 μL of PBS, three times a week, resulted in osteolysis in vivo after 4 weeks (Yu et al., 2021). Instead, myeloma cells exert their pro-osteoclastic effect by releasing exosomes containing endoplasmic reticulum-associated unfolded protein response (UPR)-related molecules through the activation of the XBP1/IRE1 axis, leading to an in vitro increased bone resorption

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in Raw 264.7 cells and human primary osteoclasts when treated with 25 μg/mL of EVs for 6 days (Raimondi et al., 2020).

On the other hand, child gut microbiota (CGM) has found to be an interesting source of EVs able to fight bone conditions such as osteoporosis. In this way, CGM-EVs act as mediators between gut microbiota and bone, and were found to maintain bone mass and strength in an ovariectomized OP mice model by promoting osteogenic activity and hindering osteoclastogenesis after their oral administration during 2 months (1.2×10^{10} vesicles, twice a week) (Yu et al., 2021). In addition, the weekly systemic administration of circulating apoptotic bodies (4×10^{6} in 200 μL of PBS) containing RNF146 Ubiquitin ligase and miR-328-3p during 1 month has found to be able to upregulate the Wnt/β-catenin pathway and to be effective to treat OP in an OVX OP mouse model, by improving the osteogenic function and inhibiting osteoclasts activity (Liu, Kou, et al., 2018).

The pretreatment of cells, such as MSCs, with compounds such as Epimedium, a traditional Chinese medicine known to improve bone remodeling, leads to the production of exosomes with therapeutic properties (Li, Chen, et al., 2021). In this way, exosomes extracted from Epimedium-treated MSCs, containing miR-27a-5p, were administered intravenously in a dose of 100 ng/mL during 12 weeks, leading to an improved bone mass and microstructure in an ovariectomized OP rat model, by promoting osteogenic differentiation (Li, Chen, et al., 2021).

Therefore, EVs obtained from a wide variety of cell types and sources constitute a promising tool to improve the management of bone-related diseases such as OP, by efficiently modulating osteoclasts and osteoblast's function. Furthermore, these vesicles present several additional advantages such as good stability, ease of storage or low ability to trigger an immune response (Ren, Chen, et al., 2022), as demonstrated by the good tolerability reported by the studies included in this section after their in vivo administration. However, despite their promising features, the clinical translation of the EVs has been hindered during the last years by issues related with their isolation and purification (Ramasubramanian et al., 2019). Also, considering their heterogeneous composition and the variety of effects that are able to trigger, some authors claim that further investigation is required before the clinical use of EVs in OP (Liang et al., 2022).

4.3 | Drugs/protein encapsulation in EVs for drug delivery in bone regeneration

The limitations of synthetic nanoparticles such as restricted ability to cross biological barriers, off-target accumulation in organs and activation of the innate immune response (Elsharkasy et al., 2020; Sercombe et al., 2015; van der Koog et al., 2022) make it necessary to find suitable alternatives. Thus, the active role of EVs in bone homeostasis and their ability to cross complex biological barriers and to interact with different cell receptors, make them a promising alternative to deliver therapeutic molecules (Alvarez-Erviti et al., 2011; Elsharkasy et al., 2020; Liu, Dang, et al., 2015; van der Koog et al., 2022). These EVs can be loaded with therapeutic molecules before (endogenous) or after (exogenous) their isolation as shown in Figure 2. Both procedures will be detailed below.

4.3.1 | Therapeutic molecules endogenous encapsulation

EVs endogenous modification can be performed by two strategies, the first one is based on the cell incubation with the drug to be encapsulated. This approach has been used to increase the effectivity and specificity of antitumor drugs such as paclitaxel and doxorubicin (Pascucci et al., 2014; Smyth et al., 2015; Tian et al., 2014). This method is simple, and no significant effects have been reported on EVs structure and content (Li, Guo et al., 2021).

The second strategy consists on the modification of the mother cells protein and/or gene expression (Hung & Leonard, 2016; Kanuma et al., 2017), and requires the use of specific transfection vectors with certain DNAs, siRNAs, miRNAs, and so forth. An example is BMP-2 transfected MSCs to obtain osteoinductive EVs (Huang, Kang et al., 2020). To this end, cells were transduced with a lentiviral vector containing a plasmid for BMP-2 expression under the control of the elongation factor 1-alpha (EF1α) promoter. Modified EVs were afterwards isolated from the cell culture media. The obtained EVs either alone or embedded in an alginate hydrogel were administered at a concentration of 5×10^{8} EVs per defect in a rat calvarial defect model. After 4 weeks, rats treated with both strategies showed significantly increased bone formation compared to controls (Huang, Kang et al., 2020, Huang, Kang, et al., 2021). Similarly, other authors have transfected MSCs with a plasmid encoding both BMP-2 and a reporter protein (GFP) using lipofectamine as the transfection agent. The obtained exosomes with a mean size of 150 nm and a ZP of −5 mV accelerated bone

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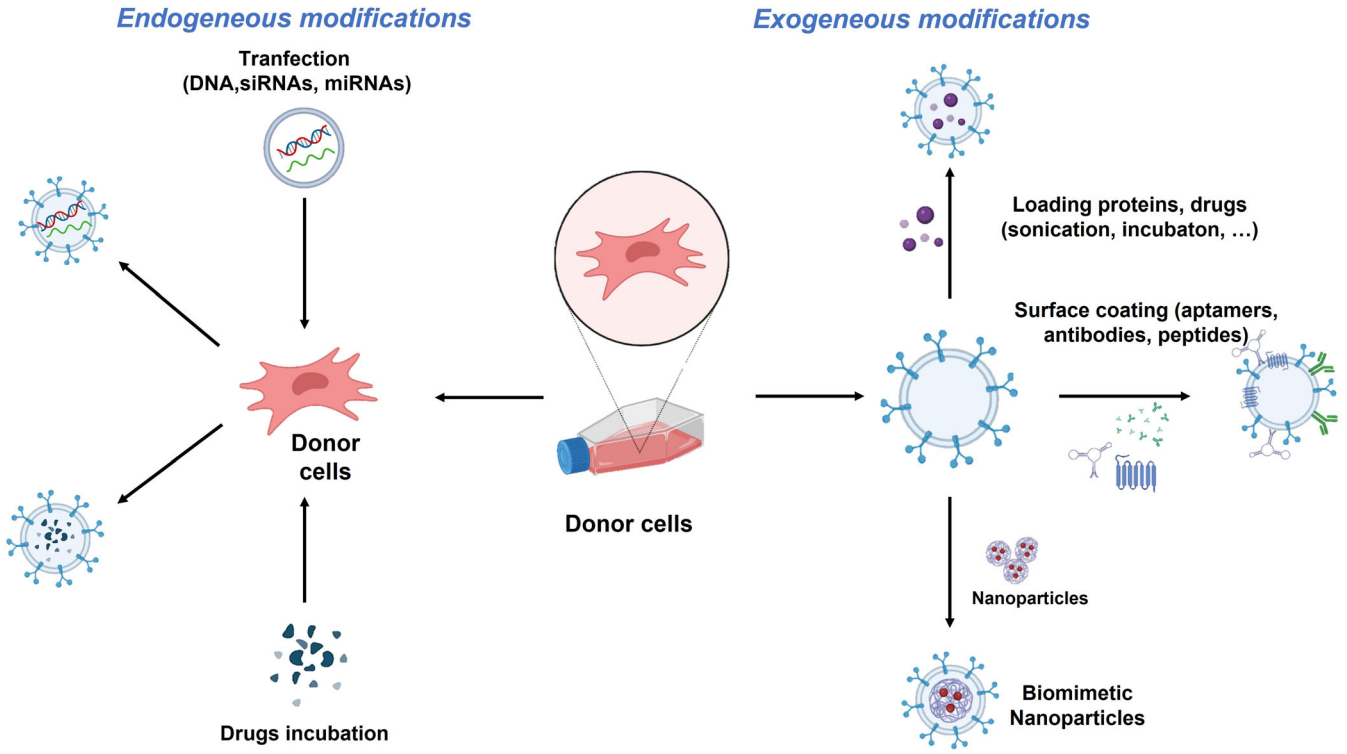

FIGURE 2 Strategies used for drugs/protein encapsulation in extracellular vesicles (EVs). miRNAs, microRNAs; siRNAs, small interfering RNAs.

healing in a mouse model of femoral defect when locally administered at a dose of 50 μg per mouse every week for 1 month. Moreover, the developed systems tend to accumulate at the bone tissue for at least 48 h after both locally and systemically administration (Li et al., 2022). Following a similar strategy MSC-derived EVs enriched with glycoprotein non-melanoma clone B (GPNMB) also known as osteoactivin were obtained. In this case, MSCs were transfected with lentiviral particles carrying GPNMB. The isolated EVs from the cell culture media supernatant were intravenously injected in OVX osteoporotic rats once a week for 8 weeks using 100 μg per animal. The obtained data showed the higher osteogenesis capacity of the modified EVs compared to control EVs (Huang, Su, et al., 2021).

Another example of MSC transfection to enrich exosomes with specific cargo is the work developed by Zhang and coworkers. In their study, MSC-derived exosomes were doped with miRNA-935 by transfecting the cells with this miRNA using lipofectamine. Exosomes were obtained by ultracentrifugation, with a size between 40 and 100 nm in diameter. Afterwards, they were administered via intracavitary injections twice a week for 3 weeks to OVX-osteoporotic rats. The experimental data showed an improvement in the bone quality with an increase in BMD, number and thickness of trabeculae (Zhang, Cao, et al., 2021). MSCs were also used as the cell type target for their transfection with the expression vectors of CIBN-CD9 (pUC-EFS > CIBN/3xGGGGS/hCD9-PGK > Neo) and CRY2-ZEB1 using lipofectamine. These vectors were selected based on their angiogenesis-dependent bone induction. This strategy was followed aiming at obtaining cells able to secrete EVs containing both vectors. Moreover, the surface modification of these EVs could also be achieved by the incubation of the mother cells with DSPE-PEG-c(RGDfC) before EVs isolation. In this way the loaded and surface modified EVs shown a particle size range within 30–150 nm. These systems were loaded into Beta-tricalcium phosphate (β-TCP)/ Poly-L-lysine/hyaluronic acid scaffolds by immersion and implanted in a critical-size bone defect in diabetes. As expected, defects treated with the loaded scaffolds showed improved bone repair ability (Tao, Li, et al., 2022).

4.3.2 | Therapeutic molecules exogeneous encapsulation

The exogenous modification includes several strategies as: incubation, electroporation, sonication, surfactant treatment, freeze–thaw cycles and extrusion (Elsharkasy et al., 2020; Fu et al., 2020; Vader et al., 2016). Incubation is the simplest

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method to encapsulate drugs within EVs and has been used to encapsulate curcumin increasing its in vitro stability and bioavailability (Vader et al., 2016). Other examples are exosome incubation with paclitaxel and doxorubicin (Garofalo et al., 2018; Haney et al., 2020; Wang et al., 2019). In these cases, the incubation of EVs with the drugs using a gentle sonication enhanced drug incorporation (Kim, Haney, et al., 2016). Follow this strategy Yerneni and coworkers loaded BMP-2 into macrophage derived EVs using two techniques; electroporation and sonication. None of the methods tested significantly modified the EVs morphological properties. Moreover, the osteoinductivity of the modified EVs was tested in a mouse muscle pocket model. Animals treated with 150 ng EVs containing 5 ng BMP2 for 4 weeks showed heterotopic ossification (Yerneni et al., 2021). Similarly, Zha and coworkers loaded VEGF plasmid into chondrogenic progenitor cell-derived exosomes by electroporation. These EVs were isolated by centrifugation and presented a spherical morphology with a size of 114.2 nm and −32 mV of ZP. The modified exosomes were loaded into a 3D printed polycaprolactone scaffold previously coated with amino groups and functionalized with the CP05 exosomal anchor peptide through the carbodiimide reaction. The final scaffolds were implanted in a rat radial defect model inducing vascularized osteogenesis (Zha et al., 2021).

4.3.3 | Tailor made and bioengineered EVs

Numerous efforts have been undertaken to bioengineer the components of EVs in order to enhance their circulation time, targeting specificity, and delivery efficacy. These "à la carte" EVs can be obtained by: (1) EVs surface coating with aptamers, antibodies or peptides; (2) Coating nanoparticles with cell membranes that is, biomimetic nanoparticles (Lu & Huang, 2020).

Following systemic administration, EVs tend to accumulate in the liver, kidneys, and spleen. They are subsequently rapidly eliminated through renal and biliary excretion or phagocytosis by the reticuloendothelial system, resulting in limited accumulation in the intended target tissues or organs. (Xitong & Xiaorong, 2016). To selectively target EVs they can be surface-modified with peptides, proteins, antibodies, aptamers or other molecules as folic acid (Zhang, Wang, et al., 2020, Zhang, Huang, et al., 2022). The functionalized EVs could be able to successfully deliver their cargo to the target cell. Some examples are aptamer-targeted exosomes for bone therapy, where the surface modification is achieved by the incubation of the Schwann cell-derived exosomes with PEG-COOH for 24 h followed by the addition of PS-aptamer. EDC is then incorporated thus leading to a condensation reaction between the aptamer and the modified exomes. These vesicles were incorporated in a PCL electrospun film and implanted in a cranial bone defect model leading to an increase in bone growth and blood vessels formation after 8 weeks of implantation (Su et al., 2022). Another example are BMSCs-derived exosomes functionalized with a specific BMSCs targeting aptamer for OP bone regeneration. Aldehyde modified aptamers were used to react with the amino group containing molecules on the exosomes surface leading to a covalent functionalization. Modified exosomes depicted a diameter of approximately 35–105 nm. Moreover, the intravenous injection of these systems at a dose of 100 μg per animal once per week for 2 months enhanced bone mass in OVX osteoporotic mice and accelerated bone healing (Luo et al., 2019). Other authors used similar strategies to functionalize MSCs derived exosomes with a bone-specific peptide. In this approach a modified lipid containing the SDSSD peptide was synthesized. The isolated exosomes and the modified peptide were then mixed and allowed to interact for an overnight period. Additionally, the functionalized exosomes were loaded by electroporation with Shn3 siRNA leading to nanoparticles of 118 ± 42 nm. These nanoparticles intravenously injected once a week for 2 months to OVX osteoporotic mice decreased osteoclastogenesis (Cui et al., 2022).

Biomimetic nanoparticles combine synthetic nanoparticles with natural components as cell membranes. The preparation of these particles typically involves the physical mixing of the isolated natural components with synthetic nanoparticles through sonication or co-extrusion (Fathi et al., 2021). Among this type, biomimetic particles exhibiting a synthetic nanocore camouflaged by a natural cell-derived membrane (Xiao et al., 2021; Yong et al., 2019) stand out as nanoparticles doped with the cell selectivity observed for EVs. Although biomimetic nanoparticles have mainly been studied for cancer therapy, there are limited studies investigating their use in the treatment of OP (Xiao et al., 2021). Hu and coworkers developed hybrid nanoparticles of 135.7 nm and −14.2 to −18.8 mV by combining exosomes isolated from CXCR4 transfected fibroblasts and liposomes that were extruded through 100 nm polycarbonate membranes. These particles showed an increased bone accumulation after 4 h since their intravenous injection (200–800 μg/animal) which was attributed to the presence of CXCR4 on their membrane. In another study, biomimetic nanoparticles were engineered to encapsulate antagomir-188, and their osteoinductive properties were evaluated in an age-related OP mouse model through a once-weekly intravenous injection. The results obtained after 8 weeks of treatment showed a

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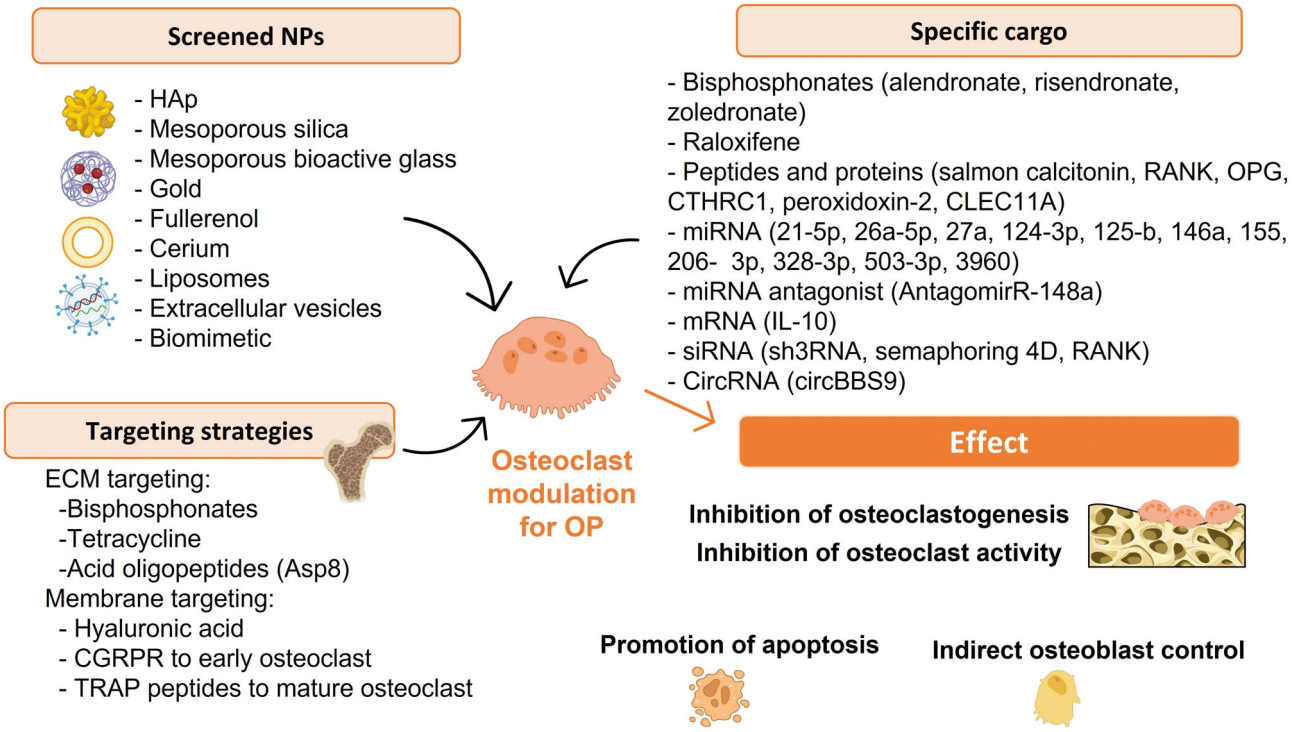

FIGURE 3 Schematic diagram of the strategies used to modulate osteoclasts for OP management using nanoparticles. Asp8, eight aspartate; CGRPR, calcitonin gene-related peptide receptor; CircRNAs, circular RNA; CLEC11A, C-type lectin domain family 11, member a; CTHRC1, collagen triple-helix repeat containing 1; HAp, hydroxyapatite; miRNA, microRNA; OP, osteoporosis; OPG, osteoprotegerin; RANK, nuclear factor κ-B; siRNA, small interfering RNA; TsmRNA, messenger RNA; RAP, tartrate-resistant acid phosphatase.

reduction in cortical bone porosity and an improvement in cortical bone quality, as well as a stimulation of the osteogenic differentiation of MSCs (Hu et al., 2021).

4.3.4 | Bioengineered EVs for osteoclasts targeting

Bone is a dynamic tissue in constant remodeling, which implies matrix breakdown and synthesis of new bone. There are scarce studies focused on osteoclasts targeted EVs in OP management, and even fewer using engineered EVs. Xiao et al. isolated exosomes from MSCs that were stimulated by mechanical stress. These exosomes were found to inhibit osteoclastogenesis of BMMs in vitro when present at a concentration of 25 μg/mL after 2 days of culture. Moreover, these particles that show a size peak at 105 nm administered at 5 mg/kg twice a week for 4 weeks via tail vein injection led to an improved bone loss in an hindlimb-unloading OP model (Xiao et al., 2021). In contrast, exosomes isolated from adipose derived MSCs previously transfected with miR-146a and deplaying a diameter size range of 50–100 nm were found to decrease the expression of proinflammatory cytokines (IL-18, IL-1β and TNF-α) and ameliorate BMD loss and bone resorption in diabetic osteoporotic rats. This effect was achieved when exosomes were administered (1.6 mg/kg) every 2 days through the tail vein by targeting osteoclasts (Zhang, Wang, et al., 2022). Using a similar approach, 4-(bromomethyl)phenylboronic acid and poly[(2-N,N-diethyl) aminoethyl acrylate] (B-PDEAEA) nanoparticles loaded with RNA interfering against circBBS9 were coated with osteoclasts and macrophage membranes to obtain biomimetic nanoparticles. The successful coating was achieved by mixing a suspension of nanoparticles with the cell membranes and subjecting the mixture to sonication and extrusion. Nanoparticles with an average diameter of 141.9 nm and negative zeta potential (−26.2 mV) were obtained. These nanoparticles were able to block the multicleation of BMMs and their consequent nucleation in vitro and avoid bone loss in an OVX-induced OP mouse model when intravenously administered every 3 days for 3 weeks. Moreover, the biodistribution studies show an exceptional accumulation in bone with no appreciable organ toxicity (Wang, Wang, et al., 2022).

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An additional strategy to obtain bone-targeted EVs similar to previously reported for synthetic nanoparticles is to functionalize them with bisphosphonates. In this regard, Wang and coworkers (Wang et al., 2020) developed alendronate-modified MSC-derived EVs by modifying the EVs surface with an alkynyl (DBCO) group and incorporating an azide group to the alendronate molecule. The modified EVs can then be obtained via copper catalyzed acetylene-azide cycloaddition reaction. These systems promoted cell growth and osteogenic differentiation of MSCs after 48 h of culture. Moreover, the modified EVs showed affinity for bone in vivo, avoiding toxic effects and leading to an improved bone microarchitecture in OVX OP rats.

Despite the significant potential of biomimetic nanoparticles and engineered EVs, their clinical translation is still a distant prospect. Reaching this step would require the standardization of isolation and characterization procedures to obtain reliable systems for clinical use. Moreover, the high frequency of administration and dose required for these therapies hinder their application to chronic pathologies such as OP. Therefore, future research should focus on developing an appropriate dosing strategy.

5 | CONCLUSION

Several nanoparticulated systems have been screened for OP management as gathered from the discussed literature, similarly than for conventional OP therapy, they are mainly focused on either inhibiting bone resorption or promoting bone formation. To this end, nanoparticles are mainly aimed at displaying their therapeutic effect on osteoclasts or osteoblasts. Subsequently, modifications in the bone characteristics, usually BMD, trabecular thickness and strength are observed.

Therefore, targeting osteoclasts with either natural or synthetic nanoparticles has been considered to be an alternative to control osteoclasts behavior and useful to manage OP. As summarized in Figure 3, these systems are focused on modulating osteoclasts differentiation, activity, apoptosis or crosstalk with osteoblasts. These nanoparticles exert their therapeutic effect mainly by loading antiresorptive molecules or including molecules for osteoclasts gene regulation. Additionally, nanoparticles have also been developed to control osteoclasts cell survival. The effects at the osteoclasts level can decrease bone resorption, increase bone mineral density and, remarkably, they do not show the undesirable effects observed for the conventional antiresorptive drugs. Some strategies, such as the usage of gold nanoparticles, functionalization with bisphosphonates, tetracycline or acidic peptides, are useful to directly target osteoclasts or to indirectly target them though their environment, namely bone ECM. These strategies open the possibility for novel approaches to manage systemic diseases as OP. The data available in the literature led us to conclude that the control of osteoclasts behavior is indeed useful in controlling bone resorption and OP diminished bone quality. Moreover, the use of natural nanoparticles, EVs, has great potential in bone remodeling and can be of excellent utility for OP management. Although this field is still in its initial steps, the key roles of EVs in bone homeostasis suggest that future research will focus on using these types of nanoparticles for OP management. Additionally, the EVs cargo identified as modulators to control osteoclasts behavior can be used as therapeutic molecules incorporated into synthetic nanoparticles. Moreover, the possibility of designing and controlling the EVs constituents opens new ways to benefit from the full potential of EVs. Remarkably, the development of biomimetic nanoparticles has been shown to achieve an outstanding biodistribution to bone and, therefore, could be a good path to follow for OP.

Grounding of these findings the research field would in the near future most likely be focused on the design of biomimetic nanoparticles including both the traditional therapeutic molecules for OP management and novel therapeutic molecules recently identified to control bone remodeling. These strategies would be useful in the development of therapies devoted to modify the disease progression decreasing the fracture risk and improving bone quality while showing minimal side effects. The diminished undesired effects would be associated to the specific accumulation of the nanoparticles in the bone. Moreover, these advanced therapies aim for retuning the bone homeostasis to a healthy bone environment controlling at once most of the cells implicated in the regeneration process rather than only one cell phenotype. In this sense, the control of osteoclasts differentiation, activity and viability can lead to an efficient management over both bone anabolism and catabolism.

AUTHOR CONTRIBUTIONS

Helena Rouco: Conceptualization (supporting); writing – original draft (equal); writing – review and editing (equal). **Patricia García-García:** Conceptualization (supporting); writing – original draft (equal); writing – review and editing (equal). **Erik Briffault:** Conceptualization (supporting); writing – original draft (equal); writing – review and editing

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(supporting). **Patricia Diaz-Rodriguez:** Conceptualization (lead); funding acquisition (lead); writing – original draft (equal); writing – review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

Authors declare there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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