**Review**
https://doi.org/10.1631/jzus.B2600208

# Plant-derived exosome-like nanovesicles for orthopaedic diseases: mechanisms, delivery strategies, and translational perspectives

Zicheng DENG[1], Kailun WU[2], Ju-Sheng SHIEH[3], Moli HUANG[4,5], Hong LIN[6,7], Jiong Jiong GUO[1,4,8]✉

[1]*Department of Orthopedics and Sports Medicine, The First Affiliated Hospital of Soochow University, Suzhou 215006, China*

[2]*Department of Orthopedics, The Fourth Affiliated Hospital of Soochow University/Suzhou Dushu Lake Hospital, Suzhou Industrial Park, Suzhou 215125, China*

[3]*Department of Periodontology, School of Dentistry, Tri-Service General Hospital and Defense Medical Center, Taipei 11490, China*

[4]*MOE Key Laboratory of Geriatric Diseases and Immunology, School of Basic Medical Sciences, Suzhou Medical College of Soochow University, Suzhou 215123, China*

[5]*Jiangsu Key Laboratory of Drug Discovery and Translational Research for Brain Diseases, School of Basic Medical Sciences, Soochow University, Suzhou 215123, China*

[6]*Department of Orthopedics, Zhongshan Hospital, Fudan University, Shanghai 200032, China*

[7]*Department of Orthopedics, Shanghai Geriatric Medical Center, Fudan University, Shanghai 201104, China*

[8]*MOE China-Europe Sports Medicine Belt and Road Joint Laboratory, Soochow University, Suzhou 215006, China*



**Abstract:** Orthopaedic diseases, including osteoporosis, osteoarthritis, and bone defects, are characterised by dysregulated tissue microenvironments driven by inflammation, oxidative stress, vascular dysfunction, and impaired cell communication. Strategies targeting single pathways often provide limited durable benefit, highlighting the need for coordinated microenvironmental modulation. Plant-derived exosome-like nanovesicles (PENs) have emerged as operationally defined bioactive nanovesicles with relevance to orthopaedic research. While PENs share structural features with mammalian extracellular vesicles, including nanoscale size and lipid bilayer membranes, they are not biogenetically equivalent to mammalian exosomes. Their diverse cargos, low immunogenicity, abundant availability, and intrinsic biological activity may enable the modulation of osteogenic differentiation, osteoclast activity, inflammatory responses, and angiogenesis. This review synthesises current knowledge on PEN biology, disease-specific therapeutic roles, delivery strategies, and translational barriers in osteoporosis, osteoarthritis, and bone defect repair. We emphasise that PEN function and feasibility vary across indications: systemic osteoporosis faces challenges with chronic dosing

✉ Jiong Jiong GUO, drjjguo@163.com; guojiongjiong@suda.edu.cn
Jiong Jiong GUO, https://orcid.org/0000-0002-3751-5791
Zicheng DENG, https://orcid.org/0000-0002-2315-8658

Received Mar. 31, 2026; Revision accepted May 24, 2026;
Crosschecked xxx. xx, 20xx

and standardisation, whereas local applications in osteoarthritis and bone defect repair may offer more tractable translational routes. Engineering approaches, including hydrogels and scaffolds, are key to improving localisation, retention, and controlled presentation. Overall, PENs are best understood as complementary bioactive nanovesicles for modulating the orthopaedic microenvironment rather than as direct plant analogues of mammalian exosomes.

**Key words:** Plant-derived exosome-like nanovesicles; Osteoporosis; Osteoarthritis; Bone defects; Drug delivery; Translational medicine

## 1. Introduction

Orthopaedic diseases, including osteoporosis (OP), osteoarthritis (OA), and bone defects, impose a substantial and steadily increasing global health burden (Hak et al., 2014; Yao et al., 2023; Liu Y et al., 2025b). In the context of population ageing and extended life expectancy, chronic musculoskeletal disorders have become a leading cause of disability, diminished quality of life, and escalating healthcare expenditure worldwide (Qiu et al., 2025). Despite notable advances in pharmacological treatments, biomaterials, and surgical interventions, the long-term clinical outcomes for many orthopaedic conditions remain unsatisfactory. A key challenge is that, rather than arising from a single pathological trigger, these disorders are driven by complex, dynamic dysregulation of local tissue microenvironments that evolve over prolonged periods (Hao S et al., 2023; Xu et al., 2025). Bone is a highly active and adaptive tissue that undergoes continuous remodelling through the coordinated actions of osteoblasts, osteoclasts, immune cells, vascular endothelial cells, and stromal components. This finely balanced process preserves skeletal integrity and enables functional adaptation to mechanical demands, whereas its disruption initiates pathological cascades (Wang L et al., 2022). OP is characterised not only by reduced bone formation but also by excessive bone resorption, chronic low-grade inflammation, oxidative stress, and altered bone–vascular coupling (Wang et al., 2023; Ensrud and Crandall, 2024). Likewise, OA extends far beyond isolated cartilage degeneration to involve inflammatory signalling, aberrant chondrocyte metabolism, subchondral bone remodelling, and synovial dysfunction (Kloppenburg et al., 2025; Liu Y et al., 2025b; Tang et al., 2025). In the context of bone defects, effective healing requires precise temporal coordination of inflammation resolution, angiogenesis, osteogenic differentiation, and extracellular matrix remodelling (Duda et al., 2023; Zhao et al., 2025). Collectively, these interdependent processes render orthopaedic diseases intrinsically resistant to therapeutic strategies that target a single molecule, pathway, or cell type.

While existing therapeutic approaches have improved symptom control and short-term outcomes, their limitations become increasingly evident in chronic or severe orthopaedic disorders. Pharmacological agents used in OP and OA primarily aim to slow disease progression or alleviate pain but rarely restore long-term tissue homeostasis (Wang et al., 2023; Fuggle et al., 2025; Liu W et al., 2025). Regenerative strategies based on growth factors or bioactive biomaterials can enhance local repair, but their effects are often transient and

highly sensitive to dosing, delivery mode, and patient-specific variables (Lei et al., 2023). Cell-based therapies, particularly mesenchymal stem cell (MSC) transplantation, have demonstrated encouraging regenerative and immunomodulatory effects in preclinical and early clinical studies. However, challenges related to cell survival, immune compatibility, manufacturing scalability, cost, and regulatory oversight continue to hinder widespread clinical translation (Zhang et al., 2023; Chen et al., 2024; Jiang et al., 2025). In this context, extracellular vesicles (EVs) have emerged as an attractive cell-free therapeutic paradigm. EVs are nanoscale, membrane-enclosed vesicles released by nearly all cell types and are now recognised as critical mediators of intercellular communication. By transporting diverse bioactive cargo (including proteins, lipids, nucleic acids, and metabolites), EVs can simultaneously modulate multiple signalling pathways in recipient cells (Nieuwland et al., 2018; van Niel et al., 2018; Xiang et al., 2024). Importantly, accumulating evidence suggests that many of the therapeutic benefits initially attributed to MSC transplantation are primarily mediated by paracrine mechanisms, with EVs playing a central role (Kim et al., 2024; Ngo et al., 2025). This conceptual shift has positioned EVs as promising alternatives to cell therapy, particularly for disorders requiring coordinated modulation of complex tissue microenvironments. In orthopaedic research, mammalian cell-derived EVs have been shown to promote osteogenesis, inhibit osteoclast differentiation, modulate inflammatory responses, and enhance angiogenesis (Yin et al., 2022; Man et al., 2023; Song et al., 2023; Wan et al., 2024; Staubli et al., 2026). Recent studies have expanded this concept by highlighting exosomes as bioactive mediators of inflammatory microenvironments, engineered drug carriers, and regenerative nanoplatforms across oncology and tissue repair settings (Xiong et al., 2019; Hao Z et al., 2023; He et al., 2025; Zhang et al., 2026).

However, translational progress remains uneven and context-dependent, reflecting persistent challenges related to donor variability, large-scale production, storage stability, and cost-effectiveness. These translational bottlenecks have stimulated increasing interest in identifying alternative EV sources that could offer improved scalability and practicality without compromising biological relevance. In this context, plant-derived exosome-like nanovesicles (PENs) have recently attracted attention as a novel class of bioactive nanomaterials (Feng et al., 2023; Li J et al., 2025; Xia et al., 2025). PENs are naturally occurring nanovesicles isolated from a wide range of edible and medicinal plants and share key structural characteristics with mammalian EVs, including nanoscale dimensions and lipid bilayer membranes. Unlike conventional plant extracts or isolated phytochemicals, PENs encapsulate complex cargos that can be delivered in a protected and biologically active form (Cao et al., 2023). Plant-derived active ingredients have also been investigated in tendon–bone healing (Li Y et al., 2025a), but evidence for isolated phytochemicals should not be conflated with PEN-specific activity. From a translational perspective, PENs offer several distinctive advantages, including abundant, sustainable sources, low immunogenicity, and minimal risk of transmission of mammalian pathogens. However, their biological behaviour is unlikely to fully recapitulate that of mammalian EVs, and PENs should instead be viewed as a complementary class of bioactive nanovesicles whose functional properties depend on plant origin and biological context. Growing preclinical evidence indicates that PENs influence osteogenic differentiation, osteoclast activity, inflammatory signalling, and oxidative stress, all of which are critical processes in orthopaedic tissue development and repair (Hwang et al., 2023; Park et al.,

2023; Zhan et al., 2023; Liu Y et al., 2025a; Zhao et al., 2025; Xi et al., 2026). This review provides a comprehensive and critical overview of PENs' therapeutic potential in orthopaedic diseases, with a particular focus on OP, OA, and bone defects. We summarise their fundamental characteristics and methodological challenges, synthesise current preclinical evidence across different disease contexts, and highlight bone defect repair as a particularly promising translational direction.

## 2. Fundamental characteristics of PENs

Precise terminology is particularly important in the context of plant-derived vesicles, as nomenclature in this field has evolved rapidly over the past decade (Kim et al., 2022; Feng et al., 2023). In mammalian systems, the collective term "EVs" is widely accepted to encompass a heterogeneous population of membrane-enclosed particles (including exosomes, microvesicles, and apoptotic bodies) that differ in size, biogenesis, and molecular composition. Exosomes are strictly defined by their endosomal origin and typically range from 30 to 150 nm in diameter (Ng et al., 2025; Yadav et al., 2026). In contrast, nanoscale vesicles isolated from plant tissues are typically characterised by size, morphology, and cargo content, yet direct evidence of their precise biogenetic pathways remains limited. Consequently, an increasing number of studies have adopted the term "plant-derived exosome-like nanovesicles" to acknowledge their structural and functional similarities to mammalian exosomes without implying identical origins (Feng et al., 2023; Jung et al., 2025; Shen et al., 2025). Table 1 provides a comparative overview clarifying the conceptual and translational distinctions between PENs and mammalian cell-derived EVs. This review uses the term "PENs" to describe nanoscale extracellular vesicles isolated from plant sources that share common physical and biological features with mammalian exosomes, while recognising that their biogenesis may involve distinct or multiple intracellular routes. Importantly, from a translational orthopaedic perspective, functional reproducibility, biocompatibility, and production feasibility are often more practically relevant than definitive classification based on intracellular origin. By explicitly defining this conceptual boundary, the use of "PENs" facilitates discussion of biological activity and therapeutic potential without overstating mechanistic certainty.



Table 1 Comparison between plant-derived exosome-like nanovesicles (PENs) and mammalian cell-derived extracellular vesicles (EVs)

| Category | PENs | Cell-derived EVs | References |
|---|---|---|---|
| Source | Plant tissues or juices; isolated directly from edible or medicinal plants | Conditioned media from cultured mammalian cells or body fluids | (van Niel et al., 2018; Feng et al., 2023) |
| Biogenesis | Heterogeneous and incompletely defined; multiple plant-specific intracellular routes | Relatively well-characterised endosomal and plasma-membrane pathways | (van Niel et al., 2018; Cui et al., 2020; Man et al., 2020) |
| Terminology | Operationally defined as exosome-like nanovesicles | Classified as EVs/exosomes under ISEV/MISEV guidance | (Lian et al., 2022; Feng et al., 2023) |
| Surface markers | No universally conserved markers across plant species | Established markers include CD9, CD63, and CD81 | (Nieuwland et al., 2018; Feng et al., 2023) |
| Cargo composition | Lipids, proteins, RNAs, and metabolites with plant-specific signatures | Proteins, lipids, mRNA, and miRNA reflecting the donor cell type | (Cai et al., 2018; Kim et al., 2022) |
| Production scalability | High yield and relatively low cost without cell-culture expansion | Limited by cell-culture capacity and production cost | (Lian et al., 2022; Feng et al., 2023) |
| Immunogenicity and safety | Generally low immunogenicity and no risk of mammalian pathogen transmission | Safety depends on the donor cell source and GMP-compliant production | (Man et al., 2020; Jung et al., 2025) |
| Stability | Often stable under gastrointestinal or ambient conditions | Sensitive to enzymatic degradation and storage conditions | (Fang and Liu, 2022; Jung et al., 2025) |

| Category | PENs | Cell-derived EVs | References |
|---|---|---|---|
| Delivery routes | Oral, local, and biomaterial-assisted delivery are feasible | Predominantly intravenous, intra-articular, or local injection | (Fang and Liu, 2022; Shen et al., 2025) |
| Engineering compatibility | Compatible with hydrogels, scaffolds, and composite constructs | Engineering is feasible but constrained by yield and cost | (Cong et al., 2022; Shen et al., 2025) |
| Standardisation | High heterogeneity and no unified quality standards | More advanced standardisation guided by MISEV | (Lian et al., 2022; Liu H et al., 2025) |

PENs, plant-derived exosome-like nanovesicles; EVs, extracellular vesicles; ISEV, International Society for Extracellular Vesicles; MISEV, Minimal Information for Studies of Extracellular Vesicles; CD, cluster of differentiation; mRNA, messenger RNA; miRNA, microRNA; GMP, Good Manufacturing Practice.

Unlike mammalian EVs, which arise from relatively well-characterised endosomal or plasma membrane–associated pathways, plant-derived vesicles appear to originate from diverse intracellular compartments, reflecting the structural and functional complexity of plant cells (Man et al., 2020). Vesicle formation in plants has been associated with multivesicular bodies, vacuoles, and other endomembrane systems, and the relative contributions of these pathways may vary by species, tissue type, developmental stage, and physiological conditions. Consequently, intrinsic heterogeneity is a defining feature of PENs rather than merely a technical limitation. This heterogeneity is further amplified by differences in plant species, the specific tissue or organ used for isolation (such as roots, leaves, fruits, or rhizomes), and environmental factors that influence plant metabolism (Jimenez-Jimenez et al., 2019; Jain et al., 2022). At the molecular level, PENs contain a diverse spectrum of bioactive cargos, including lipids, proteins, various RNA species, and small metabolites (Cai et al., 2018; Feng et al., 2023). Importantly, their biological effects are unlikely to be driven by a single dominant component; instead, they are more plausibly understood as resulting from multiple cargos acting in combination. This multimodal organisation is particularly pertinent in orthopaedic contexts, where disease progression and tissue repair depend on the interplay among inflammation, oxidative stress, angiogenesis, and lineage-specific cellular responses (Xia et al., 2025). While individual studies have proposed specific candidate molecules within PENs as mediators of particular effects, assigning causality to any single cargo remains challenging. Strictly reductionist interpretations thus risk overlooking the broader, system-level regulatory capacity that underlies PENs' therapeutic relevance.

This heterogeneity has practical biological consequences, as illustrated by specific examples of diverse outcomes across plant sources. PENs obtained from yam, plum, *Pueraria lobata*, *Morinda officinalis*, Rhizoma drynariae, ginseng, garlic, sea buckthorn, and lotus root have been reported to preferentially affect different endpoints, including osteoblast differentiation, osteoclast inhibition, autophagy activation, MAPK signalling, ERα-associated osteogenesis, angiogenic coupling, or anti-inflammatory modulation (Hwang et al., 2023; Park et al., 2023; Seo et al., 2023; Zhan et al., 2023; Cao et al., 2024; Zhao et al., 2024; Liu Y et al., 2025a; Zhao et al., 2025; Xi et al., 2026). Variation can also emerge within a single plant source because multiple variables, including cultivation conditions, maturity, storage, tissue selection, juicing or homogenisation intensity, differential centrifugation speed, density-gradient purification, filtration pore size, and sterilisation procedures, can alter particle yield, size distribution, purity, RNA/protein/lipid composition, and residual non-vesicular contaminants (Cong et al., 2022; Fang and Liu, 2022; Lian et al., 2022; Cao et al., 2023; Feng et al., 2023; Jung et al., 2025; Liu H et al., 2025; Shen et al., 2025; Wang and Kong, 2025). Batch

effects should therefore not be interpreted merely as analytical noise; they may shift potency across osteogenic, anti-osteoclastic, immunomodulatory, and antioxidative readouts. Future studies should report source metadata and isolation parameters in sufficient detail to enable potency-linked comparisons across batches of the same botanical material.

Methodological considerations constitute another important aspect of PEN research, particularly regarding isolation, characterisation, and standardisation. Most studies isolate plant-derived vesicles using combinations of differential centrifugation, density gradient separation, or filtration-based approaches, followed by characterisation with transmission electron microscopy, nanoparticle tracking analysis, and size distribution profiling (Cui et al., 2020; Lian et al., 2022). While these techniques provide reasonable confirmation of vesicle-like structures and nanoscale dimensions, they do not yield uniform preparations across different laboratories (Shen et al., 2025). Consequently, direct quantitative comparison across studies remains challenging, and batch-to-batch variability is difficult to eliminate. From a translational perspective, however, it should be recognised that orthopaedic applications often fundamentally differ from systemic therapeutic scenarios. Many orthopaedic interventions rely on local delivery, surgical implantation, or biomaterial-assisted retention, which may tolerate a degree of heterogeneity that would be unacceptable for intravenous administration. Furthermore, PENs have consistently demonstrated favourable biocompatibility profiles, with preclinical studies reporting low immunogenicity and minimal cytotoxicity across a range of in vitro and in vivo models (Shen et al., 2018; Hwang et al., 2023; Zhan et al., 2023; Zhou et al., 2024; Xi et al., 2026). Their inherent tendencies towards anti-inflammatory and antioxidative modulation further align with the requirements of orthopaedic tissue repair, where prolonged inflammatory activation and oxidative stress can impair regeneration. When combined with local delivery systems such as hydrogels or scaffolds, PENs can achieve a sustained presence within the target microenvironment, thereby mitigating some of the limitations associated with incomplete standardisation (Li L et al., 2025). Collectively, these fundamental characteristics—conceptual flexibility, intrinsic heterogeneity, multimodal cargo composition, and practical compatibility with local orthopaedic delivery—provide a rational basis for exploring the roles of PENs across different orthopaedic disease contexts, as discussed in the following sections.



## 3. Therapeutic applications of PENs in orthopaedic diseases

Fig. 1 schematically summarises the key mechanisms discussed in this section, providing an integrated overview of how PENs modulate orthopaedic tissue microenvironments in a disease-dependent manner, while Fig. 2 integrates common and distinct PEN-associated pathways across orthopaedic contexts. Table 2 provides an overview of representative preclinical studies investigating PENs across major orthopaedic diseases.

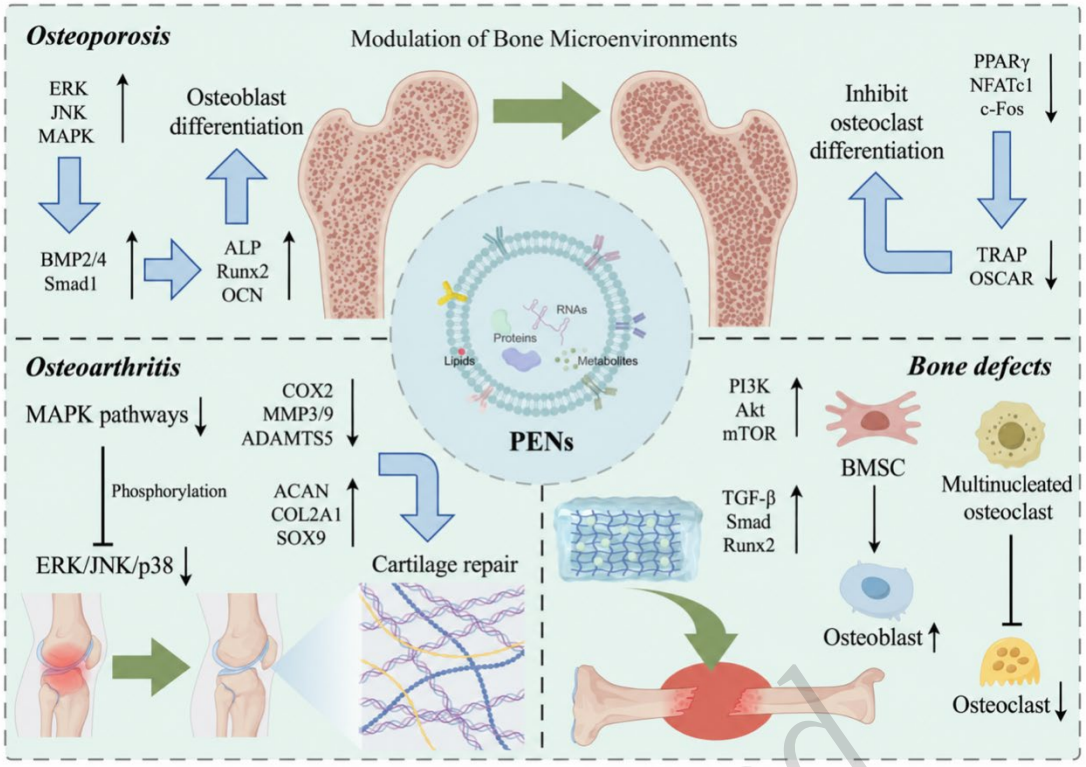


Fig. 1 PENs act as multimodal regulators of orthopaedic tissue microenvironments in osteoporosis, osteoarthritis, and bone defects by promoting osteogenic differentiation, suppressing osteoclastogenesis and catabolic signalling, and supporting cartilage repair and bone regeneration through disease-specific intracellular pathways. PENs, plant-derived exosome-like nanovesicles; ALP, alkaline phosphatase; RUNX2, runt-related transcription factor 2; OCN, osteocalcin; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; BMP, bone morphogenetic protein; Smad, suppressor of mothers against decapentaplegic; PPARγ, peroxisome proliferator-activated receptor γ; NFATc1, nuclear factor of activated T cells 1; TRAP, tartrate-resistant acid phosphatase; OSCAR, osteoclast-associated receptor; COX2, cyclooxygenase 2; MMP, matrix metalloproteinase; ADAMTS5, a disintegrin and metalloproteinase with thrombospondin motifs 5; ACAN, aggrecan; COL2A1, collagen type II alpha 1 chain; SOX9, SRY-box transcription factor 9; BMSC, bone marrow mesenchymal stem cell; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; mTOR, mechanistic target of rapamycin;

TGF-β, transforming growth factor β.

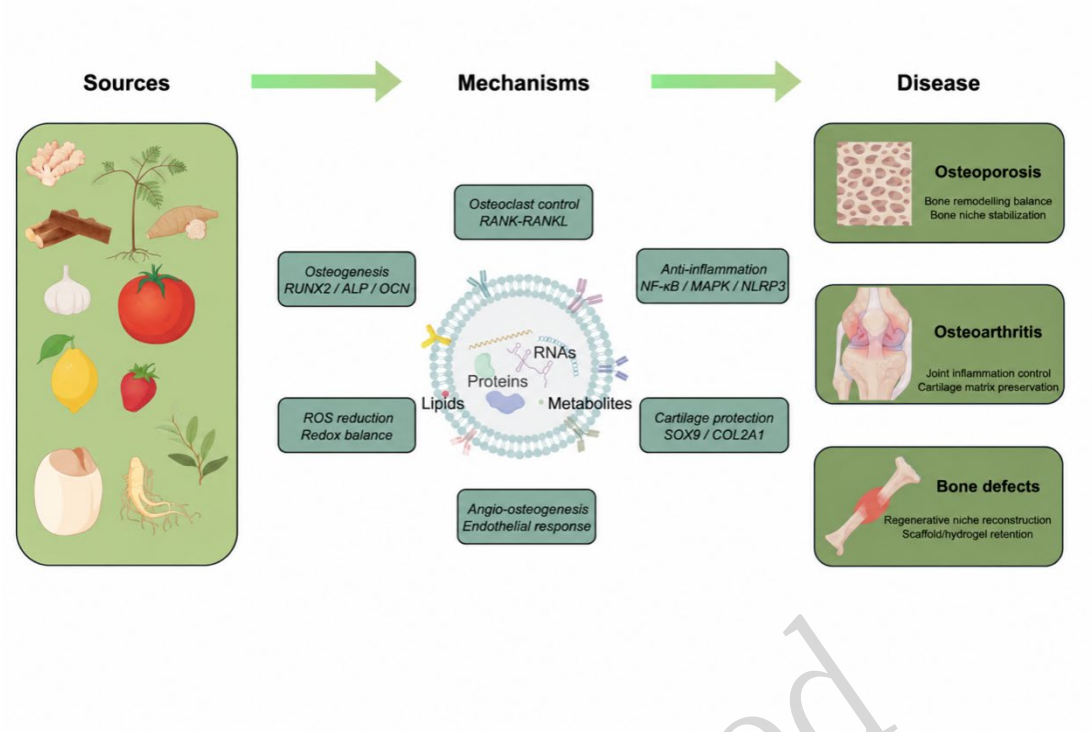


Fig. 2 Integrated schematic of PEN sources, shared mechanisms, and disease-specific orthopaedic applications. Representative plant sources yield PENs containing RNAs, proteins, lipids, metabolites, and membrane-associated components. These cargos may regulate osteogenesis, osteoclast activity, inflammation, redox balance, cartilage protection, and angio-osteogenesis, thereby supporting disease-specific outcomes in osteoporosis, osteoarthritis, and bone-defect repair. PENs, plant-derived exosome-like nanovesicles; RNA, ribonucleic acid; RUNX2, runt-related transcription factor 2; ALP, alkaline phosphatase; OCN, osteocalcin; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; NLRP3, NOD-like receptor family pyrin domain-containing 3; ROS, reactive oxygen species; SOX9, SRY-box transcription factor 9; COL2A1, collagen type II alpha 1 chain.



Table 2 Representative preclinical studies of plant-derived extracellular vesicles in orthopaedic diseases

| Plant source | EV designation | Disease context | Main effects | Key mechanisms | References |
|---|---|---|---|---|---|
| Plum | PENVs | Osteoporosis | Increased osteogenesis; reduced osteoclastogenesis | BMP2–Smad1; p38/JNK | (Park et al., 2023) |
| Yam | YNVs | Osteoporosis | Prevented bone loss | BMP2–p38–RUNX2 | (Hwang et al., 2023) |
| Apple | Apple NVs | Osteoporosis | Increased ALP activity and mineralisation | ERK/JNK; BMP2/4 | (Sim et al., 2023) |
| *Pueraria lobata* | PELNs | Osteoporosis | Enhanced osteogenesis | Autophagy; AMPK–mTOR | (Zhan et al., 2023) |
| *Cissus quadrangularis* | Callus EVs | Osteoporosis | Promoted osteogenic differentiation | Phenotype-based evidence | (Gupta et al., 2023) |
| *Morinda officinalis* | MOEVLPs | Osteoporosis | Bone-targeted anti-osteoporotic effects | MAPK signalling | (Cao et al., 2024) |
| Rhizoma Drynariae | RDNVs | Osteoporosis | Restored bone microarchitecture | ERα signalling | (Zhao et al., 2024) |
| Epimedium | EELNs | Osteoporosis/alveolar bone defects | Supported alveolar bone regeneration | Osteogenic and anti-osteoporotic activity | (Shen et al., 2018) |
| Epimedium | Exosome-loaded GelMA | Bone defects | Enhanced osteogenesis with hydrogel retention | PI3K/Akt | (Hu et al., 2025) |
| Ginseng | GDNs | Osteoporosis | Inhibited osteoclast differentiation | RANKL–NF-κB/MAPK | (Seo et al., 2023) |
| Sea buckthorn | SAEVs | Bone defects | Enhanced bone regeneration | aau-miR168/LBH/RUNX2 signalling | (Zhao et al., 2025) |
| Tomato/lemon | TELVs/LELVs | Osteoarthritis | Increased chondrogenic markers | SOX9/COL2A1 | (Yıldırım et al., 2024) |
| Strawberry | SELNs | Bone/joint-relevant stromal-cell stress | Prevented oxidative stress | Antioxidative effects | (Perut et al., 2021) |

EV, extracellular vesicle; PENVs, plum-derived exosome-like nanovesicles; YNVs, yam-derived nanovesicles; NVs, nanovesicles; ALP, alkaline phosphatase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; BMP, bone morphogenetic protein; RUNX2, runt-related transcription factor 2; PELNs, Pueraria lobata-derived exosome-like nanovesicles; AMPK, AMP-activated protein kinase; mTOR, mechanistic target of rapamycin; MOEVLPs, Morinda officinalis-derived extracellular vesicle-like particles; MAPK, mitogen-activated protein kinase; RDNVs, Rhizoma Drynariae-derived nanovesicles; ERα, oestrogen receptor α; EELNs, Epimedium-derived exosome-like nanovesicles; GelMA, gelatin methacryloyl; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; GDNs, ginseng-derived nanovesicles; RANKL, receptor activator of nuclear factor-κB ligand; NF-κB, nuclear factor-κB; SAEVs, sea buckthorn-derived extracellular vesicles; LBH, limb-bud and heart development protein; miRNA, microRNA; TELVs, tomato-derived exosome-like vesicles; LELVs, lemon-derived exosome-like vesicles; SOX9, SRY-box transcription factor 9; COL2A1, collagen type II alpha 1 chain; SELNs, strawberry-derived exosome-like nanoparticles.

To reduce ambiguity in mechanistic interpretation, the currently reported and plausible cargo–function relationships are summarised in Table 3. Because most studies have linked cargo classes or pathway activation to biological effects rather than proving single-cargo causality, these relationships should be regarded as context-dependent and incompletely conserved across PEN types.

Table 3 Reported or plausible molecular mediators linking PEN cargos to orthopaedic effects

| Cargo or mediator | Representative PEN source | Orthopaedic relevance | Interpretive limitation | References |
|---|---|---|---|---|
| Plant miRNAs/small RNAs (e.g., aau-miR168) | Sea buckthorn-derived EVs | May influence osteogenic differentiation and reparative signalling through cross-kingdom RNA-mediated regulation | Sequence conservation, uptake efficiency, and direct mRNA targets require further validation | (Zhao et al., 2025) |
| Autophagy-related bioactivity | *Pueraria lobata*-derived nanovesicles | May protect osteogenic cells from stress and support bone-remodelling balance | Autophagy may be a downstream response rather than a cargo-specific mechanism | (Zhan et al., 2023) |
| Phytoactive metabolites and lipid-associated cargos | Multiple edible or medicinal plant PENs | May contribute to anti-inflammatory, antioxidative, and membrane-interaction effects | Cargo composition is source- and extraction-dependent; active species are rarely isolated individually | (Kim et al., 2022; Lian et al., 2022; Feng et al., 2023; Huang et al., 2025) |
| Protein- and membrane-associated components | Multiple characterised PEN preparations | May support cellular uptake, vesicle stability, and modulation of immune or stromal responses | Plant vesicle markers are not standardised, limiting batch comparability | (Kim et al., 2022; Feng et al., 2023; Huang et al., 2025; Shen et al., 2025) |
| Pathway-level outputs (MAPK, ERα, PI3K/Akt) | *Morinda officinalis*-, garlic-, Rhizoma Drynariae-, and Epimedium-derived vesicles | Provide tractable readouts for anti-inflammatory, osteogenic, and osteoclast-modulating effects | Pathway activation does not prove direct cargo-mediated causality | (Cao et al., 2024; Zhao et al., 2024; Hu et al., 2025; Liu Y et al., 2025a) |

PEN, plant-derived exosome-like nanovesicle; miRNA, microRNA; EV, extracellular vesicle; mRNA, messenger RNA; MAPK, mitogen-activated protein kinase; ERα, oestrogen receptor α; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B.

## 3.1. Osteoporosis

OP is a systemic skeletal disorder characterised by progressive loss of bone mass and deterioration of bone microarchitecture, ultimately leading to increased fracture risk and impaired mechanical competence (Ensrud and Crandall, 2024; Xia et al., 2025). While initially framed as an imbalance between osteoblast-mediated bone formation and osteoclast-driven bone resorption, OP is now widely recognised as a consequence of broader, more persistent dysregulation of the bone microenvironment (Salhotra et al., 2020; Cheng et al., 2022). Ageing, oestrogen deficiency, metabolic alterations, and chronic inflammatory activation collectively reshape the cellular and molecular landscape of bone tissue over time. These changes include sustained low-grade inflammation, increased oxidative stress, impaired angiogenesis, and disrupted coupling between vascular and osteogenic compartments (Wang L et al., 2022; Wang T et al., 2022). Importantly, rather than operating in isolation, these processes interact dynamically, reinforcing one another to gradually

erode skeletal integrity. This evolving understanding helps to explain why therapies that selectively inhibit osteoclast activity or stimulate osteoblast differentiation, although clinically valuable, often fail to fully restore long-term bone homeostasis, particularly in postmenopausal and senile OP (Luo et al., 2024). Within this expanded disease framework, OP can be more appropriately conceptualised as a disorder of bone niche stability rather than a purely cell-autonomous defect. Chronic inflammatory mediators released by immune cells within the bone marrow promote osteoclastogenesis while simultaneously suppressing osteoblast differentiation and function, while the excessive accumulation of reactive oxygen species interferes with osteogenic signalling and accelerates cellular senescence (Tian et al., 2025; Zhao Y et al., 2026). Alterations in bone-associated vasculature further exacerbate these effects by limiting nutrient delivery, oxygen diffusion, and progenitor cell recruitment, thereby disrupting angiogenesis–osteogenesis coupling (Zhang et al., 2020; Qin et al., 2022). Collectively, these microenvironmental perturbations create a self-perpetuating cycle of bone loss that is difficult to interrupt through single-target intervention. Consequently, there is increasing interest in therapeutic strategies that can simultaneously modulate multiple components of the bone microenvironment, with the aim of stabilising bone remodelling dynamics rather than maximising any single anabolic or anti-resorptive effect.

In this context, accumulating preclinical evidence suggests that PENs possess biological properties that align closely with the multifactorial pathophysiology of OP. Figure 2 integrates these effects into a unified conceptual framework that distinguishes shared PEN functions, such as anti-inflammatory and antioxidative modulation, from context-specific outputs, including osteogenesis, osteoclast inhibition, and angiogenesis-related coupling. In vitro studies across a range of experimental systems have consistently demonstrated that PENs can enhance osteogenic differentiation of mesenchymal stem cells and pre-osteoblastic cell lines, as evidenced by increased alkaline phosphatase activity, matrix mineralisation, and upregulation of osteogenic markers (Shen et al., 2018; Gupta et al., 2023; Hwang et al., 2023; Park et al., 2023; Sim et al., 2023; Zhan et al., 2023). Concurrently, several independent studies have reported that PENs exert inhibitory effects on osteoclast differentiation or activity, indicating that they can influence both arms of the bone remodelling process (Park et al., 2023; Seo et al., 2023). Notably, these dual effects have been consistently observed across PENs derived from distinct plant species and tissues, despite substantial heterogeneity in vesicle composition and isolation protocols. This convergence at the functional level supports the interpretation that PENs operate through coordinated modulation of remodelling dynamics rather than through a single dominant osteoanabolic pathway (Park et al., 2023). Beyond their direct influence on osteoblasts and osteoclasts, PENs appear to exert broader regulatory effects on key microenvironmental drivers of osteoporotic bone loss. Chronic inflammatory activation, often conceptualised as inflammageing, plays a central role in OP by enhancing osteoclastogenesis and suppressing osteogenic potential. Several preclinical studies suggest that PENs attenuate pro-inflammatory signalling in cellular and animal models, thereby reducing the inflammatory burden within bone tissue (Zhan et al., 2023; Cao et al., 2024). Furthermore, these effects are frequently accompanied by reductions in oxidative stress markers, suggesting concurrent modulation of redox-sensitive pathways known to impair osteoblast function (Gómez-Puerto et al., 2016). Although the precise molecular mediators responsible for these effects remain incompletely defined, the multimodal cargo composition of

PENs, including lipids, proteins, various RNA species, and small metabolites, provides a plausible mechanistic basis for their combined immunomodulatory and antioxidative actions. Importantly, this indirect stabilisation of the bone microenvironment may be particularly relevant for chronic conditions such as OP, where the long-term maintenance of tissue homeostasis is as critical as the direct stimulation of bone formation.

In vivo evidence, predominantly derived from ovariectomised rodent models, further supports the potential relevance of PENs in osteoporotic disease. In these models, the administration of PENs has been associated with preservation of trabecular bone volume, improvements in microarchitectural parameters, and partial recovery of biomechanical properties, as assessed by micro-computed tomography and histological analyses (Zhan et al., 2023; Cao et al., 2024; Zhao et al., 2024). Notably, these beneficial outcomes have been reported across studies using varied dosing regimens, delivery routes, and vesicle preparation methods. This robustness, observed across vesicles derived from distinct plant species and preparation protocols, suggests that functional outcomes may represent a more reliable benchmark for evaluating PEN efficacy than strict molecular uniformity, particularly in the context of a complex, system-level disease such as OP. Nevertheless, important limitations must be considered when interpreting the current body of evidence. Most existing studies remain confined to short- to medium-term preclinical experiments, with limited exploration of long-term safety, dose optimisation, or treatment durability. In addition, OP represents a chronic and systemic disorder, raising practical questions regarding systemic delivery (Ensrud and Crandall, 2024), biodistribution, and cumulative exposure that have yet to be comprehensively addressed for PEN-based interventions. Unlike with localised orthopaedic conditions, systemic administration imposes higher requirements for standardisation, reproducibility, and regulatory oversight. Accordingly, while current data support the capacity of PENs to modulate key drivers of osteoporotic bone loss, their most realistic near-term role may lie in complementary or adjunctive strategies, rather than as stand-alone replacements for established anti-resorptive or anabolic therapies.



### 3.2. Osteoarthritis

OA, traditionally regarded as solely a consequence of mechanical wear, is increasingly recognised as a degenerative joint disorder driven by sustained microenvironmental disturbances and inflammatory dysregulation within the joint microenvironment (Dang et al., 2025; Tang et al., 2025). Inflammatory mediators released from chondrocytes, synoviocytes, and infiltrating immune cells create a catabolic milieu that accelerates cartilage matrix breakdown and disrupts tissue homeostasis (De Roover et al., 2023). Simultaneously, oxidative stress and metabolic alterations impair chondrocyte function and viability, while changes in subchondral bone structure feed back into cartilage degeneration (Riegger et al., 2023; Wei et al., 2023). These interconnected processes unfold locally within the joint space and develop over long periods, rendering OA particularly resistant to interventions that target cartilage alone or focus exclusively on symptom relief. Current pharmacological treatments primarily address pain and inflammation but do not halt disease progression, whereas regenerative strategies for cartilage repair face substantial challenges due to cartilage's avascular nature and the hostile inflammatory environment of the osteoarthritic joint (Liu W et al., 2025). Within this localised and inflammation-dominated disease context, PENs have emerged as potential modulators of the

joint microenvironment rather than direct agents of cartilage regeneration. Preclinical studies indicate that PENs can attenuate inflammatory signalling in chondrocytes and synovial cells, reduce the expression of catabolic enzymes, and mitigate oxidative stress–induced cellular damage (Yıldırım et al., 2024; Liu Y et al., 2025a; Rashidi et al., 2025). These effects appear to converge on the preservation of cartilage matrix integrity and the stabilisation of joint homeostasis, rather than on the induction of de novo cartilage formation (You et al., 2023). Importantly, such outcomes have been reported for PENs derived from various plant sources, suggesting that the observed biological effects reflect shared functional properties rather than plant-specific molecular signatures. From a mechanistic perspective, the multimodal cargo composition of PENs provides a plausible basis for their ability to dampen inflammatory cascades and modulate stress responses within the joint. However, as with OP, it remains challenging to attribute these effects to individual cargos or signalling pathways with certainty, underscoring the need to interpret PEN activity at the system level.

The translational relevance of PENs in OA is further shaped by the disease's anatomical and clinical features. Unlike systemic skeletal disorders, OA is inherently localised, making it amenable to intra-articular delivery strategies that can achieve high local concentrations while minimising systemic exposure (Gao et al., 2022; Song et al., 2022). A preclinical study locally administered garlic-derived PENs into the joint space and reported reduced synovial inflammation, preserved cartilage structure, and improved histological outcomes (Liu Y et al., 2025a). These findings highlight a key distinction between OA and other orthopaedic conditions: in OA, the therapeutic value of PENs lies primarily in their capacity to modulate the inflammatory and catabolic joint environment, rather than in driving tissue regeneration. Accordingly, rather than as stand-alone regenerative therapies, PENs are best positioned as disease-modifying adjuncts that may complement existing anti-inflammatory or symptom-relieving treatments. The feasibility of local delivery, combined with favourable biocompatibility profiles, further enhances the translational appeal of PENs for OA, particularly when integrated with biomaterial-based retention systems or controlled-release formulations (Yang et al., 2024). Nevertheless, important limitations and open questions remain. Most available evidence is derived from short-term preclinical models, and the durability of PEN-mediated effects in the context of chronic joint degeneration has yet to be established. Optimal dosing regimens, treatment frequency, and long-term safety following repeated intra-articular administration remain largely unexplored. Furthermore, while PENs appear to stabilise cartilage integrity and suppress inflammatory activity, they are unlikely to reverse advanced structural damage in late-stage OA. Careful patient stratification and disease staging will therefore be essential for any future translational application. Collectively, the current evidence supports a role for PENs as local modulators of joint inflammation and microenvironmental stress in OA. Their greatest potential lies not in cartilage regeneration per se, but in slowing disease progression and preserving joint function by restoring a more balanced intra-articular milieu.



### 3.3. Bone defects

Bone defects constitute a distinct class of orthopaedic conditions characterised by a loss of structural continuity and mechanical competence arising from trauma, tumour resection, infection, congenital anomalies, or revision surgery (Loi et al., 2016; GBD 2019 Fracture Collaborators, 2021). Unlike chronic skeletal disorders such as OP or OA, bone defects present a clearly defined anatomical and clinical problem, where

the primary objective is to restore load-bearing structure through repair processes that may, under favourable biological conditions, progress towards true tissue regeneration (Zhu et al., 2021; Zhou et al., 2023). The intrinsic regenerative capacity of bone is strongly dependent on defect size, anatomical location, vascular supply, and the local biological milieu. Critical-sized defects, in particular, exceed the natural healing potential of bone and invariably require surgical intervention (Zhou et al., 2023). Autologous and allogeneic bone grafts remain the clinical gold standard, yet their use is constrained by donor-site morbidity, limited availability, risk of immune rejection or infection, and inconsistent integration with host tissue (Schmidt, 2021; Rohilla et al., 2022). Synthetic substitutes and engineered scaffolds offer structural support but often lack sufficient biological activity to guide coordinated repair (Li Y et al., 2025b). Collectively, these limitations highlight a fundamental challenge in bone defect management: successful reconstruction depends not only on mechanical stabilisation, but also on the precise orchestration of inflammation, angiogenesis, osteogenic differentiation, and matrix remodelling within the defect microenvironment.

Within this biologically demanding context, PENs have emerged as promising adjunctive regulators of the bone defect milieu rather than as direct replacements for structural grafts. Preclinical evidence suggests that PENs can influence multiple, tightly coupled processes essential for defect healing (Hu et al., 2025; Zhao et al., 2025; Xi et al., 2026). In vitro studies consistently demonstrate that PENs enhance osteogenic differentiation of mesenchymal progenitors and pre-osteoblastic cells, promoting matrix mineralisation and osteogenic gene expression (Hwang et al., 2023; Park et al., 2023). Additionally, PENs have been shown to modulate endothelial cell behaviour, supporting angiogenic responses that are indispensable for nutrient delivery, waste removal, and the recruitment of reparative cells (Zhao et al., 2025; Xi et al., 2026). These osteogenic and angiogenic effects are complemented by PENs' capacity to attenuate excessive inflammatory signalling and oxidative stress, thereby preventing the persistence of a hostile microenvironment that would otherwise derail the repair cascade. Importantly, despite substantial heterogeneity in plant sources, vesicle composition, and isolation methods, the functional outcomes reported across studies exhibit notable convergence. This suggests that the biological activity of PENs is best interpreted as the integrated effect of their multimodal cargo (comprising lipids, proteins, RNAs, and metabolites) rather than the action of any single dominant molecule or pathway. Such system-level modulation closely aligns with the complex, multistage nature of bone defect repair, where coordinated regulation, rather than maximal stimulation of a single process, is critical for successful healing.

From a translational perspective, bone defects represent the most clinically actionable and pragmatic entry point for the application of PEN-based strategies. Defect repair is inherently localised and typically requires surgical exposure, enabling direct delivery of PENs to the target site at therapeutically relevant concentrations while minimising systemic distribution. This local accessibility facilitates the integration of PENs into existing reconstructive workflows, either through direct application or by incorporating them into implantable biomaterials such as hydrogels, scaffolds, or composite constructs (Li L et al., 2025). Engineering strategies that immobilise or gradually release PENs within the defect environment address the inherent limitation of free vesicles, namely rapid diffusion and clearance, without overshadowing their biological

function. In this setting, PENs function as bioactive modulators that complement the mechanical and structural roles of grafts or scaffolds, rather than replace them. Nevertheless, important challenges remain. Most supporting evidence is derived from small animal models, and translation to large, load-bearing defects in humans will require careful optimisation of dosing, timing, and delivery kinetics. Issues related to standardisation, batch-to-batch variability, and regulatory classification also remain unresolved. Accordingly, PENs should not be viewed as stand-alone regenerative solutions, but as facilitators that enhance endogenous repair processes when deployed within well-defined surgical and material frameworks. Taken together, a clear clinical objective, a localised and accessible treatment site, and a strong biological rationale for microenvironmental modulation position bone defects as the most realistic and compelling context for the translational development of plant-derived exosome-like nanovesicles in orthopaedic practice. However, this positioning remains a translational hypothesis rather than clinical proof. The most relevant preclinical studies remain primarily limited to small-animal systems, local implantation models, or non-load-bearing defects; efficacy, retention, and mechanical integration in critical-sized load-bearing defects in large animals or humans remain unproven.

## 4. Engineering strategies and delivery systems for PENs

The therapeutic application of PENs in orthopaedic contexts is fundamentally constrained by their physicochemical behaviour and biological fate when administered in free form. As nanoscale vesicles, PENs are inherently susceptible to rapid diffusion away from the target site, enzymatic degradation, and clearance through local vascular and lymphatic pathways (Chai et al., 2025; Jung et al., 2025; Li L et al., 2025; Liu H et al., 2025; Shen et al., 2025). In mechanically dynamic tissues such as bone and joint structures, these processes are further exacerbated by interstitial fluid flow, cyclic loading, and continuous tissue remodelling (Xia et al., 2025). These limitations are not merely technical inconveniences; they have direct biological consequences. Insufficient local retention reduces the duration and intensity of interactions between PENs and resident or recruited cells, thereby limiting their capacity to influence multistage repair processes that unfold over extended periods. In orthopaedic settings, key biological events (e.g., inflammation resolution, angiogenic ingrowth, osteogenic differentiation, and matrix maturation) occur over weeks to months rather than hours or days (He et al., 2023). Transient exposure to bioactive cues is therefore unlikely to induce sustained therapeutic effects. From this perspective, engineering strategies should not be viewed as optional enhancements to PEN delivery but as essential enablers that align vesicle bioavailability with the temporal and spatial requirements of musculoskeletal repair (Cong et al., 2022; Feng et al., 2023). The primary goal of engineering is not to amplify or modify the intrinsic biological activity of PENs, but to preserve, localise, and present that activity in a manner compatible with the biological constraints of orthopaedic tissues. Localised delivery systems have thus emerged as the most pragmatic and clinically relevant approach for translating PEN-based strategies, particularly in surgically accessible scenarios such as bone defects and intra-articular interventions. Biomaterial-assisted delivery provides a way to confine PENs within a defined anatomical space, thereby prolonging their residence time and enabling sustained interaction with the local microenvironment. Hydrogels, porous matrices, and composite constructs have all been explored as PEN carriers that provide spatial confinement, mechanical compatibility, and controlled release while allowing the

vesicles to supply the principal biological cues (Hu et al., 2025; Li L et al., 2025; Xi et al., 2026; Zhao M et al., 2026). Crucially, the effectiveness of such systems does not depend on maximal material sophistication, but on the degree to which release kinetics are matched to the biological processes being targeted. Early inflammatory modulation may benefit from relatively rapid PEN availability, whereas angiogenesis and osteogenesis require prolonged exposure during later stages of repair (Saberi et al., 2023). Engineering approaches that accommodate these temporal dynamics can enhance biological relevance without requiring excessive dosing or repeated administration. In addition, incorporating PENs into materials that already serve structural or defect-filling functions enables the seamless integration of biological modulation into established surgical workflows. This is particularly important for orthopaedic translation, where new technologies are more readily adopted when they complement, rather than complicate, existing clinical procedures. In this sense, engineering serves as a translational bridge connecting biological potential and surgical practicality, rather than as an independent layer of technological novelty.

Beyond improving retention and release, engineering strategies also play a critical role in enhancing the safety, controllability, and reproducibility of PEN-based interventions (Jung et al., 2025). Localised delivery inherently limits systemic exposure, thereby reducing the risk of off-target effects and unintended interactions with non-skeletal tissues, which is an important consideration given the current uncertainties surrounding long-term biodistribution and clearance of plant-derived vesicles (Huang et al., 2025). Confinement within defined material constructs also enables more predictable dosing and reduces the variability associated with free administration. However, engineering also introduces additional variables that must be carefully managed. Interactions between PENs and carrier materials can influence vesicle stability, cargo integrity, and biological availability, while fabrication, sterilisation, and storage processes may inadvertently compromise vesicle function. Furthermore, batch-to-batch variability in PEN preparations remains a fundamental challenge that cannot be fully mitigated by delivery design alone (Cong et al., 2022; Fang and Liu, 2022; Lian et al., 2022; Cao et al., 2023; Feng et al., 2023; Jung et al., 2025; Shen et al., 2025; Wang and Kong, 2025). From a translational standpoint, there is also a risk that increasingly complex material systems may outpace their clinical utility, adding manufacturing and regulatory burdens without proportional therapeutic benefit. In orthopaedic practice, simplicity, robustness, and reproducibility are often more valuable than maximal functional sophistication. Accordingly, the most promising engineering strategies for PEN delivery prioritise biological alignment, manufacturability, and surgical compatibility over technological complexity. When viewed through this lens, engineering and delivery systems should be regarded not as separate innovations, but as enabling frameworks that facilitate the safe, consistent realisation of PENs' intrinsic biological properties within clinically relevant orthopaedic settings.

## 5. Challenges and future perspectives

Despite the growing body of preclinical evidence supporting the therapeutic potential of PENs in orthopaedic applications, several fundamental challenges must be addressed before meaningful clinical translation can be realised. Foremost among these is the issue of standardisation. PENs are inherently heterogeneous; their physicochemical properties and biological activities are influenced by plant species, tissue source, growth conditions, and isolation protocols (Cao et al., 2023). Unlike mammalian cell-derived EVs,

for which consensus guidelines for characterisation and reporting are beginning to emerge, plant-derived vesicles remain subject to substantial methodological variability (Liu H et al., 2025). Differences in isolation techniques, purification stringency, and analytical readouts complicate cross-study comparison and hinder reproducibility. From a translational perspective, this variability creates a significant obstacle to regulatory evaluation, as batch-to-batch consistency is a prerequisite for clinical-grade products. The lack of universally accepted markers for plant-derived vesicles further complicates efforts to define product identity and quality (Kim et al., 2022). Addressing these challenges will require coordinated efforts to establish minimum reporting standards, reference materials, and functionally relevant quality control metrics. Importantly, standardisation should prioritise parameters that correlate with biological performance rather than exhaustive molecular profiling, thereby balancing scientific rigour with translational feasibility.

A second major challenge is the incomplete understanding of the mechanisms by which PENs exert their biological effects on orthopaedic tissues. While numerous studies have demonstrated functional outcomes such as enhanced osteogenesis, reduced inflammation, or improved defect healing, the underlying molecular and cellular pathways remain only partially defined (Park et al., 2023; Zhan et al., 2023; Hu et al., 2025). This limitation is not unique to PENs and reflects a broader challenge in EV research, where multimodal cargo composition complicates mechanistic dissection (Kim et al., 2024; Ngo et al., 2025). In the context of orthopaedic diseases, where multiple cell types and signalling pathways interact within a dynamic microenvironment, reductionist approaches may fail to capture the full spectrum of biological activity. Nevertheless, a lack of mechanistic clarity can raise concerns regarding predictability, safety, and rational optimisation. Future studies should therefore aim to integrate functional assays with targeted mechanistic analyses that focus on pathway-level modulation rather than single-molecule attribution. In addition, disease-stage specificity warrants increased attention, as the effects of PENs may differ substantially between the early inflammatory phases and later regenerative stages (Duda et al., 2023). Addressing these knowledge gaps will be essential for both scientific understanding and informing dosing strategies, treatment timing, and patient selection in potential clinical applications.

Safety considerations constitute another critical dimension in the translational pathway of PEN-based therapies. Although plant-derived vesicles are generally regarded as biocompatible and exhibit low immunogenicity in preclinical models, systematic evaluation of long-term safety remains scarce. Issues such as chronic exposure, accumulation in non-target tissues, and potential interactions with host immune responses have not yet been comprehensively explored. These concerns are particularly relevant for systemic administration, as opposed to local delivery strategies that confine PENs to defined anatomical sites. Furthermore, plant-derived vesicles may carry bioactive cargos that exert unintended effects under certain conditions, especially when delivered repeatedly or at high doses (Jung et al., 2025). Rigorous toxicological studies, including long-term in vivo assessments and biodistribution analyses, will therefore be indispensable. From a regulatory standpoint, clear classification of PENs—as biological products, drug delivery systems, or combination products—will also influence safety evaluation frameworks. Early engagement with agencies such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the National Medical Products Administration (NMPA) will be needed to determine whether a specific PEN product

should be regulated as a biological product, a drug or nanomedicine, or a combination product when incorporated into a scaffold or hydrogel. The most relevant guidance would likely involve EV or biologics quality control, nanomedicine characterisation, advanced therapy or tissue-engineered product frameworks, and combination-product requirements for device-associated delivery systems. Concrete strategies to reduce batch-to-batch variability include (i) defining botanical source information, including species, cultivar, plant tissue, maturity, geographic origin, and growth/storage conditions; (ii) adopting harmonised isolation workflows with predefined centrifugation, filtration, density-gradient, and sterilisation parameters; (iii) establishing reference materials or internal benchmark batches; (iv) applying orthopaedic potency assays as batch-release criteria, such as osteogenic differentiation, osteoclast inhibition, macrophage inflammatory polarisation, chondrocyte catabolic marker suppression, or endothelial tube formation; and (v) using chemometric or multi-omics approaches to correlate particle size distribution, lipid/protein/RNA/metabolite fingerprints, and functional potency. These measures would shift quality control from descriptive characterisation toward function-linked release testing.

The prioritisation of clinically realistic application scenarios will be crucial for advancing PEN-based strategies beyond proof-of-concept studies. Among the orthopaedic indications discussed in this review, bone defect repair stands out as the most tractable and compelling entry point for translation. The combination of a well-defined clinical problem, localised delivery, and surgical accessibility offers a favourable environment in which the biological effects of PENs can be harnessed with minimal systemic risk. In contrast, chronic systemic conditions such as OP pose greater challenges for long-term administration, dosing control, and regulatory oversight, and may therefore require more extensive preclinical validation prior to clinical consideration. OA occupies an intermediate position in which local delivery is feasible, but disease heterogeneity and progression stage complicate patient stratification. The strategic prioritisation of indications based on feasibility rather than breadth will be essential to avoid diluting research efforts and to generate clinically meaningful outcomes within a reasonable timeframe. Future research should also emphasise integrating PENs into interdisciplinary therapeutic strategies that combine biological modulation with established orthopaedic interventions. Rather than being positioned as stand-alone solutions, PENs' greatest value may lie in their ability to complement surgical reconstruction, biomaterial implantation, or mechanical stabilisation. Such combination approaches reflect the realities of orthopaedic practice, where successful outcomes often depend on the interplay between mechanical and biological factors. In this context, the engineering strategies discussed in the preceding section play a pivotal role in bridging experimental promise and clinical applicability. However, the pursuit of increasingly complex delivery systems should be tempered by considerations of manufacturability, reproducibility, and cost-effectiveness. Simpler systems that reliably deliver biological benefit may ultimately prove more translatable than highly sophisticated designs with limited scalability. A plausible first-in-human scenario would therefore be a locally delivered PEN-containing hydrogel or scaffold used as an adjunct during surgical management of a contained bone defect, such as an alveolar, craniofacial, or non-load-bearing metaphyseal defect, where dosing can be confined to the operative site, and safety monitoring is feasible. Before such testing, the minimum preclinical package should include Good Manufactur-

ing Practice-compatible production, identity and potency assays, sterility and endotoxin testing, biodistribution and degradation studies, local and systemic toxicology, immunogenicity assessment, and efficacy/safety evaluation in a relevant large animal defect model that more closely approximates human defect volume, vascularisation, and mechanical constraints.

Finally, the future development of PEN-based orthopaedic therapies will depend on close cross-disciplinary collaboration among the fields of materials science, cell biology, orthopaedic surgery, and regulatory science. Establishing shared frameworks for experimental design, outcome reporting, and translational benchmarking will reduce redundancy and facilitate more efficient knowledge transfer. Comparative studies that evaluate PENs alongside mammalian extracellular vesicles or other bioactive agents may also elucidate their relative advantages and limitations, thereby informing rational therapeutic selection. As the field matures, a shift away from exploratory demonstrations towards hypothesis-driven, clinically informed research will be essential. By systematically addressing challenges related to standardisation, mechanism, safety, and prioritisation, the translational potential of PENs in orthopaedic medicine can be more accurately defined and, ultimately, more effectively realised.

## 6. Conclusions

Plant-derived exosome-like nanovesicles (PENs) represent an emerging class of bioactive nanomaterials with increasing relevance to orthopaedic research, primarily due to their capacity to modulate complex tissue microenvironments through the coordinated actions of diverse molecular cargos. Unlike conventional pharmacological agents or single-factor regenerative strategies, PENs exert multimodal regulatory effects that align well with the multifactorial nature of orthopaedic disorders, in which inflammation, oxidative stress, vascular dysfunction, and impaired intercellular communication collectively shape disease progression and repair outcomes. Importantly, the functional roles and translational prospects of PENs are not uniform across disease contexts. In systemic conditions such as OP, PENs primarily function as modulators of bone remodelling dynamics and microenvironmental stability, although challenges related to chronic administration, standardisation, and long-term safety remain substantial. Current evidence regarding OA supports a more restrained positioning of PENs as local disease-modifying agents that stabilise the inflammatory joint milieu rather than directly inducing cartilage regeneration. In contrast, bone defects emerge as the most clinically actionable and translationally favourable application scenario, owing to the presence of a clearly defined repair objective, surgical accessibility, and compatibility with local delivery and biomaterial-assisted strategies. Nevertheless, translating PEN-based therapies into clinical practice will require careful navigation of unresolved challenges, including reproducible manufacturing, clarification of mechanisms at the systems level, and rigorous evaluation of safety and regulatory pathways.

Crucially, future progress in this field will depend on resisting the temptation to overgeneralise the capabilities of PENs, instead integrating them rationally into established orthopaedic treatment paradigms that combine mechanical stabilisation with biological modulation. Collectively, current evidence supports the view that PENs constitute a promising yet still evolving bioactive platform for orthopaedic applications, the clinical impact of which will ultimately be determined by the prioritisation of realistic indications, thoughtful engineering of local delivery strategies, and a cautious, translation-oriented research approach.

## Data availability statement

No new data were generated or analysed in this review. All information synthesised in this article is available in the cited publications.


## Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 62475181), the China-Europe Sports Medicine Belt and Road Joint Laboratory, Ministry of Education of the People's Republic of China (No. 2023297), and the Project of the MOE Key Laboratory of Geriatric Diseases and Immunology (No. KJS2607). The funders had no role in the conception, literature analysis, preparation, review, approval, or decision to submit this manuscript.

We dedicate this article to the memory of Ms Lingfen Zhou, the mother of Dr Jiong Jiong GUO, who made important contributions to the development and realisation of this project.


## Author contributions

CRediT statement: Zicheng DENG contributed to conceptualisation, literature investigation, visualisation, and writing of the original draft. Kailun WU contributed to literature investigation, data curation, and writing of the original draft. Ju-Sheng SHIEH contributed to validation and writing—review and editing. Moli HUANG contributed to literature investigation, visualisation, and writing—review and editing. Hong LIN contributed to supervision, validation, and writing—review and editing. Jiong Jiong GUO contributed to conceptualisation, supervision, project administration, funding acquisition, and writing—review and editing. All authors have read and approved the final manuscript and take responsibility for its content.

## Compliance with ethics guidelines

Zicheng DENG, Kailun WU, Ju-Sheng SHIEH, Moli HUANG, Hong LIN, and Jiong Jiong GUO declare that they have no conflicts of interest. This review does not contain any studies involving human participants or animals performed by any of the authors; therefore, ethics approval, informed consent, and clinical trial registration are not applicable.

## Declaration on the use of generative AI tools

During preparation of this revision, the authors used OpenAI Codex (GPT-5) from 20 May to 16 September 2026 to assist with language revision, citation-consistency checking, table and figure-caption formatting, and adaptation of the manuscript to the journal template. Key prompts requested revision in response to reviewers and editors, verification and conversion of citations, and standardisation of tables and figures according to Journal of Zhejiang University-SCIENCE B requirements. Relevant records and the modification process have been preserved and can be provided for editorial review on request. All output was critically reviewed and edited by the authors, who take full responsibility for the accuracy, originality, and final content of the submitted manuscript.

## References


Cai Q, Qiao L, Wang M, et al., 2018. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science, 360:1126–9.
https://doi.org/10.1126/science.aar4142

Cao M, Diao N, Cai X, et al., 2023. Plant exosome nanovesicles (PENs): Green delivery platforms. Mater Horiz, 10:3879–94.
https://doi.org/10.1039/D3MH01030A

Cao Y, Tan X, Shen J, et al., 2024. *Morinda officinalis*-derived extracellular vesicle-like particles: Anti-osteoporosis effect by regulating MAPK signaling pathway. Phytomedicine, 129:155628.
https://doi.org/10.1016/j.phymed.2024.155628

Chai M, Gao B, Wang S, et al., 2025. Leveraging plant-derived nanovesicles for advanced nucleic acid-based gene therapy. Theranostics, 15:324–39.
https://doi.org/10.7150/thno.104507

Chen Y, Huang Y, Li J, et al., 2024. Enhancing osteoporosis treatment with engineered mesenchymal stem cell-derived extracellular vesicles: Mechanisms and advances. Cell Death Dis, 15:119.
https://doi.org/10.1038/s41419-024-06508-w

Cheng C-H, Chen L-R, Chen K-H, 2022. Osteoporosis due to hormone imbalance: An overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. Int J Mol Sci, 23:1376.
https://doi.org/10.3390/ijms23031376

Cong M, Tan S, Li S, et al., 2022. Technology insight: Plant-derived vesicles—how far from the clinical biotherapeutics and therapeutic drug carriers? Adv Drug Deliv Rev, 182:114108.
https://doi.org/10.1016/j.addr.2021.114108

Cui Y, Gao J, He Y, et al., 2020. Plant extracellular vesicles. Protoplasma, 257:3–12.
https://doi.org/10.1007/s00709-019-01435-6

Dang Y, Liu Y, Zhang B, et al., 2025. Aging microenvironment in osteoarthritis focusing on early-stage alterations and targeted therapies. Bone Res, 13:84.
https://doi.org/10.1038/s41413-025-00465-6

De Roover A, Escribano-Núñez A, Monteagudo S, et al., 2023. Fundamentals of osteoarthritis: Inflammatory mediators in osteoarthritis. Osteoarthritis Cartilage, 31:1303–11.
https://doi.org/10.1016/j.joca.2023.06.005

Duda GN, Geissler S, Checa S, et al., 2023. The decisive early phase of bone regeneration. Nat Rev Rheumatol, 19:78–95.
https://doi.org/10.1038/s41584-022-00887-0

Ensrud KE, Crandall CJ, 2024. Osteoporosis. Ann Intern Med, 177:ITC1–16.
https://doi.org/10.7326/AITC202401160

Fang Z, Liu K, 2022. Plant-derived extracellular vesicles as oral drug delivery carriers. J Control Release, 350:389–400.
https://doi.org/10.1016/j.jconrel.2022.08.046

Feng J, Xiu Q, Huang Y, et al., 2023. Plant-derived vesicle-like nanoparticles as promising biotherapeutic tools: Present and future. Adv Mater, 35:2207826.
https://doi.org/10.1002/adma.202207826

Fuggle N, Laslop A, Rizzoli R, et al., 2025. Treatment of osteoporosis and osteoarthritis in the oldest old. Drugs, 85:343–60.
https://doi.org/10.1007/s40265-024-02138-w

Gao J, Xia Z, Mary HB, et al., 2022. Overcoming barriers for intra-articular delivery of disease-modifying osteoarthritis drugs. Trends Pharmacol Sci, 43:171–87.
https://doi.org/10.1016/j.tips.2021.12.004

GBD 2019 Fracture Collaborators, 2021. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990-2019: A systematic analysis from the Global Burden of Disease Study 2019. Lancet Healthy Longev, 2:e580-92.
https://doi.org/10.1016/S2666-7568(21)00172-0

Gómez-Puerto MC, Verhagen LP, Braat AK, et al., 2016. Activation of autophagy by FOXO3 regulates redox homeostasis during osteogenic differentiation. Autophagy, 12:1804–16.
https://doi.org/10.1080/15548627.2016.1203484

Gupta R, Gupta S, Gupta P, et al., 2023. Establishing the callus-based isolation of extracellular vesicles from cissus quadrangularis and elucidating their role in osteogenic differentiation. J Funct Biomater, 14:540.
https://doi.org/10.3390/jfb14110540

Hak DJ, Fitzpatrick D, Bishop JA, et al., 2014. Delayed union and nonunions: Epidemiology, clinical issues, and financial aspects. Injury, 45:S3–7.
https://doi.org/10.1016/j.injury.2014.04.002

Hao Z, Ren L, Zhang Z, et al., 2023. A multifunctional neuromodulation platform utilizing Schwann cell-derived exosomes orchestrates bone microenvironment via immunomodulation, angiogenesis and osteogenesis. Bioact Mater, 23:206–222. https://doi.org/10.1016/j.bioactmat.2022.10.018

Hao S, Wang M, Yin Z, et al., 2023. Microenvironment-targeted strategy steers advanced bone regeneration. Mater Today Bio, 22:100741.
https://doi.org/10.1016/j.mtbio.2023.100741

He S, Deng H, Li P, et al., 2023. Arthritic microenvironment-dictated fate decisions for stem cells in cartilage repair. Adv Sci, 10:2207715.
https://doi.org/10.1002/advs.202207715

He C, Yu W, Zhao Q, et al., 2025. Exosomes shape inflammatory tumor microenvironment. Chin Chem Lett, 111900.
https://doi.org/10.1016/j.cclet.2025.111900

Hu W, Xie X, Xu J, 2025. Epimedium-derived exosome-loaded GelMA hydrogel enhances MC3T3-E1 osteogenesis via PI3K/Akt pathway. Cells, 14:1214.
https://doi.org/10.3390/cells14151214

Huang D, Chen J, Zhao M, et al., 2025. Plant-derived extracellular vesicles: Composition, function and clinical potential. J Transl Med, 23:1065.
https://doi.org/10.1186/s12967-025-07101-1

Hwang J-H, Park Y-S, Kim H-S, et al., 2023. Yam-derived exosome-like nanovesicles stimulate osteoblast formation and prevent osteoporosis in mice. J Control Release, 355:184–98.
https://doi.org/10.1016/j.jconrel.2023.01.071

Jain N, Pandey M, Sharma P, et al., 2022. Recent developments in plant-derived edible nanoparticles as therapeutic nanomedicines. J Food Biochem, 46(12):e14479.
https://doi.org/10.1111/jfbc.14479

Jiang T, Su S, Tian R, et al., 2025. Immunoregulatory orchestrations in osteoarthritis and mesenchymal stromal cells for therapy. J Orthop Transl, 55:38–54.
https://doi.org/10.1016/j.jot.2025.08.009

Jimenez-Jimenez S, Hashimoto K, Santana O, et al., 2019. Emerging roles of tetraspanins in plant inter-cellular and inter-kingdom communication. Plant Signal Behav, 14:e1581559.
https://doi.org/10.1080/15592324.2019.1581559

Jung D, Kim N-E, Kim S, et al., 2025. Plant-derived nanovesicles and therapeutic application. Pharmacol Ther, 269:108832.
https://doi.org/10.1016/j.pharmthera.2025.108832

Kim J, Li S, Zhang S, et al., 2022. Plant-derived exosome-like nanoparticles and their therapeutic activities. Asian J Pharm Sci, 17:53–69.
https://doi.org/10.1016/j.ajps.2021.05.006

Kim HI, Park J, Zhu Y, et al., 2024. Recent advances in extracellular vesicles for therapeutic cargo delivery. Exp Mol Med, 56:836–49.
https://doi.org/10.1038/s12276-024-01201-6

Kloppenburg M, Namane M, Cicuttini F, 2025. Osteoarthritis. Lancet, 405:71–85.
https://doi.org/10.1016/S0140-6736(24)02322-5

Lei C, Song J, Li S, et al., 2023. Advances in materials-based therapeutic strategies against osteoporosis. Biomaterials, 296:122066.
https://doi.org/10.1016/j.biomaterials.2023.122066

Li Y, Wang W, Xu W, 2025a. Mechanisms and new advances in the efficacy of plant active ingredients in tendon-bone healing. J Orthop Surg Res, 20:106.
https://doi.org/10.1186/s13018-025-05483-y

Li L, Ye C, Wu Z, et al., 2025. Plant exosome-loaded intelligent hydrogels for osteoporotic bone regeneration: Mechanisms and applications. Int J Nanomedicine, 20:15863–81.
https://doi.org/10.2147/IJN.S567471

Li J, Luo T, Wang D, et al., 2025. Therapeutic application and potential mechanism of plant-derived extracellular vesicles in inflammatory bowel disease. J Adv Res, 68:63–74.
https://doi.org/10.1016/j.jare.2024.01.035

Li Y, Li W, Li L, et al., 2025b. Treating critical bone defects by using core-shell biological scaffold to regulate fibrosis–osteogenic homeostasis. Mater Today Bio, 31:101560.
https://doi.org/10.1016/j.mtbio.2025.101560

Lian MQ, Chng WH, Liang J, et al., 2022. Plant-derived extracellular vesicles: Recent advancements and current challenges on their use for biomedical applications. J Extracell Vesicles, 11:12283.
https://doi.org/10.1002/jev2.12283

Liu Y, Nie M, Li X, et al., 2025a. Garlic-derived exosomes alleviate osteoarthritis through inhibiting the MAPK signaling pathway. Appl Biochem Biotechnol, 197:518–33.
https://doi.org/10.1007/s12010-024-05047-6

Liu W, Guo N, Wang J, et al., 2025. Osteoarthritis: Mechanisms and therapeutic advances. MedComm, 6:e70290.
https://doi.org/10.1002/mco2.70290

Liu H, Dong T, Dong C, et al., 2025. Plant-derived exosome-like nanovesicles: a novel therapeutic perspective for skin diseases. J Nanobiotechnology, 23:640.
https://doi.org/10.1186/s12951-025-03715-1

Liu Y, Huang X, Tang K, et al., 2025b. Prevalence of osteoporosis and associated factors among Chinese adults: A systematic review and modelling study. J Glob Health, 15:04009.
https://doi.org/10.7189/jogh.15.04009

Loi F, Córdova LA, Pajarinen J, et al., 2016. Inflammation, fracture and bone repair. Bone, 86:119–30.
https://doi.org/10.1016/j.bone.2016.02.020

Luo Y, Liu H, Chen M, et al., 2024. Immunomodulatory nanomedicine for osteoporosis: Current practices and emerging prospects. Acta Biomater, 179:13–35.
https://doi.org/10.1016/j.actbio.2024.03.011

Man F, Wang J, Lu R, 2020. Techniques and applications of animal- and plant-derived exosome-based drug delivery system. J Biomed Nanotechnol, 16:1543–69.
https://doi.org/10.1166/jbn.2020.2993

Man K, Eisenstein NM, Hoey DA, et al., 2023. Bioengineering extracellular vesicles: Smart nanomaterials for bone regeneration. J Nanobiotechnology, 21:137.
https://doi.org/10.1186/s12951-023-01895-2

Ng JQ, Abu Yazid NA, Tan SC, et al., 2025. Extracellular vesicles as delivery vehicles and therapeutic agents for glioblastoma treatment: A systematic review of in vitro and in vivo preclinical studies. Asian J Pharm Sci, 20:101043.
https://doi.org/10.1016/j.ajps.2025.101043

Ngo JM, Williams JK, Zhang C, et al., 2025. Extracellular vesicles and cellular homeostasis. Annu Rev Biochem, 94:587–609.
https://doi.org/10.1146/annurev-biochem-100924-012717

Nieuwland R, Falcon-Perez JM, Soekmadji C, et al., 2018. Essentials of extracellular vesicles: Posters on basic and clinical aspects of extracellular vesicles. J Extracell Vesicles, 7:1548234.
https://doi.org/10.1080/20013078.2018.1548234

Park Y-S, Kim H-W, Hwang J-H, et al., 2023. Plum-derived exosome-like nanovesicles induce differentiation of osteoblasts and reduction of osteoclast activation. Nutrients, 15:2107.
https://doi.org/10.3390/nu15092107

Perut F, Roncuzzi L, Avnet S, et al., 2021. Strawberry-derived exosome-like nanoparticles prevent oxidative stress in human mesenchymal stromal cells. Biomolecules, 11:87.
https://doi.org/10.3390/biom11010087

Qin Q, Lee S, Patel N, et al., 2022. Neurovascular coupling in bone regeneration. Exp Mol Med, 54:1844–9.
https://doi.org/10.1038/s12276-022-00899-6

Qiu K, Wang C, Mo X, et al., 2025. The global macroeconomic burden of musculoskeletal disorders. Int J Surg, 111:7857–66.
https://doi.org/10.1097/JS9.0000000000003072

Rashidi N, Liu C, Guillot PV, et al., 2025. Isolation, Characterization, and In Vitro Cell Studies of Plant-Based Exosome-like Nanovesicles for Treatment of Early Osteoarthritis. Int J Mol Sci, 26:2211.
https://doi.org/10.3390/ijms26052211

Riegger J, Schoppa A, Ruths L, et al., 2023. Oxidative stress as a key modulator of cell fate decision in osteoarthritis and osteoporosis: A narrative review. Cell Mol Biol Lett, 28:76.
https://doi.org/10.1186/s11658-023-00489-y

Rohilla R, Sharma PK, Wadhwani J, et al., 2022. Prospective randomized comparison of bone transport versus masquelet technique in infected gap nonunion of tibia. Arch Orthop Trauma Surg, 142:1923–32.
https://doi.org/10.1007/s00402-021-03935-8

Saberi A, Kouhjani M, Mohammadi M, et al., 2023. Novel scaffold platforms for simultaneous induction osteogenesis and angiogenesis in bone tissue engineering: A cutting-edge approach. J Nanobiotechnology, 21:351.
https://doi.org/10.1186/s12951-023-02115-7

Salhotra A, Shah HN, Levi B, et al., 2020. Mechanisms of bone development and repair. Nat Rev Mol Cell Biol, 21:696–711.
https://doi.org/10.1038/s41580-020-00279-w

Schmidt AH, 2021. Autologous bone graft: Is it still the gold standard? Injury, 52:S18–22.
https://doi.org/10.1016/j.injury.2021.01.043

Seo K, Yoo JH, Kim J, et al., 2023. Ginseng-derived exosome-like nanovesicles extracted by sucrose gradient ultracentrifugation to inhibit osteoclast differentiation. Nanoscale, 15:5798–808.
https://doi.org/10.1039/D2NR07018A

Shen Y, Liang L, Zhang S, et al., 2018. A Comprehensive Study on Herba Epimedium-derived extracellular nanovesicles as a prospective therapy for alveolar bone regeneration in postmenopausal osteoporosis. Nanoscale, 10:1622–30.
https://doi.org/10.1039/C7NR08636A

Shen H, Li S, Lin L, et al., 2025. Advancements in plant-derived exosome-like vesicles: Versatile bioactive carriers for targeted drug delivery systems. J Pharm Anal, 15:101300.
https://doi.org/10.1016/j.jpha.2025.101300

Sim Y, Seo H-J, Kim D, et al., 2023. The effect of apple-derived nanovesicles on the osteoblastogenesis of osteoblastic MC3T3-E1 cells. J Med Food, 26:49–58.
https://doi.org/10.1089/jmf.2022.K.0094

Song P, Cui Z, Hu L, 2022. Applications and prospects of intra-articular drug delivery system in arthritis therapeutics. J Control Release, 352:946–60.
https://doi.org/10.1016/j.jconrel.2022.11.018

Song X, Xu L, Zhang W, 2023. Biomimetic synthesis and optimization of extracellular vesicles for bone regeneration. J Control Release, 355:18–41.
https://doi.org/10.1016/j.jconrel.2023.01.057

Staubli F, Zhou Y, Vader P, et al., 2026. Harnessing extracellular vesicles for endochondral bone regeneration: Mechanisms and applications. Acta Biomater, 212:168–186.
https://doi.org/10.1016/j.actbio.2026.01.012

Tang S, Zhang C, Oo WM, et al., 2025. Osteoarthritis. Nat Rev Dis Primers, 11:10.
https://doi.org/10.1038/s41572-025-00594-6

Tian K, Xiong T, Zeng D, et al., 2025. Impact of reactive oxygen species on bone regeneration in diabetes: Mechanisms and therapeutic strategies. Redox Biol, 88:103941.
https://doi.org/10.1016/j.redox.2025.103941

van Niel G, D'Angelo G, Raposo G, 2018. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol, 19:213–28.
https://doi.org/10.1038/nrm.2017.125

Wan R, Liu S, Feng X, et al., 2024. The revolution of exosomes: From biological functions to therapeutic applications in skeletal muscle diseases. J Orthop Transl, 45:132–9.
https://doi.org/10.1016/j.jot.2024.01.001

Wang L, You X, Zhang L, et al., 2022. Mechanical regulation of bone remodeling. Bone Res, 10:16.
https://doi.org/10.1038/s41413-022-00190-4

Wang T, Huang S, He C, 2022. Senescent cells: A therapeutic target for osteoporosis. Cell Prolif, 55:e13323.
https://doi.org/10.1111/cpr.13323

Wang H, Luo Y, Wang H, et al., 2023. Mechanistic advances in osteoporosis and anti-osteoporosis therapies. MedComm, 4:e244.
https://doi.org/10.1002/mco2.244

Wang J, Kong Z, 2025. Plant-derived vesicle-like nanoparticles in food crops: Emerging insights into nutritional biofortification and biomedical applications. Plant Biotechnol J, 23(8):3260–3282.
https://doi.org/10.1111/pbi.70074

Wei G, Lu K, Umar M, et al., 2023. Risk of metabolic abnormalities in osteoarthritis: A new perspective to understand its pathological mechanisms. Bone Res, 11:63.
https://doi.org/10.1038/s41413-023-00301-9

Xi Y, Huang C, Qiu B, et al., 2026. Multifunctional hydrogel platform encapsulating lotus root-derived extracellular vesicles stimulate osteogenesis-angiogenesis coupling for accelerated bone regeneration. Chem Eng J, 528:171906.
https://doi.org/10.1016/j.cej.2025.171906

Xia X, Zhu J, Xu X, et al., 2025. Current progress of plant-derived exosome-like nanovesicles on the regulation of osteoporosis and osteoarthritis. Ann Med, 57:2549524.
https://doi.org/10.1080/07853890.2025.2549524

Xiang H, Bao C, Chen Q, et al., 2024. Extracellular vesicles (EVs)' journey in recipient cells: From recognition to cargo release. J Zhejiang Univ Sci B, 25(8):633–55.
https://doi.org/10.1631/jzus.B2300566

Xiong F, Ling X, Chen X, et al., 2019. Pursuing specific chemotherapy of orthotopic breast cancer with lung metastasis from docking nanoparticles driven by bioinspired exosomes. Nano Lett, 19:3256–3266.
https://doi.org/10.1021/acs.nanolett.9b00824

Xu M, Liu H, Zhang J, et al., 2025. Functionalized zeolite regulates bone metabolic microenvironment. Mater Today Bio, 31:101558.
https://doi.org/10.1016/j.mtbio.2025.101558

Yadav A, Sharma A, Moulick M, et al., 2026. Labeling, isolation, and characterization of cell type-specific exosomes derived from murine skin tissue. Nat Protoc, 21(3):1192–1234.
https://doi.org/10.1038/s41596-025-01238-5

Yang L, Li W, Zhao Y, et al., 2024. Stem cell recruitment polypeptide hydrogel microcarriers with exosome delivery for osteoarthritis treatment. J Nanobiotechnology, 22:512.
https://doi.org/10.1186/s12951-024-02765-1

Yao Q, Wu X, Tao C, et al., 2023. Osteoarthritis: Pathogenic signaling pathways and therapeutic targets. Signal Transduct Target Ther, 8:56.
https://doi.org/10.1038/s41392-023-01330-w

Yıldırım M, Ünsal N, Kabataş B, et al., 2024. Effect of *Solanum lycopersicum* and *Citrus limon*–derived exosome-like vesicles on chondrogenic differentiation of adipose-derived stem cells. Appl Biochem Biotechnol, 196:203–19.
https://doi.org/10.1007/s12010-023-04491-0

Yin B, Ni J, Witherel CE, et al., 2022. Harnessing tissue-derived extracellular vesicles for osteoarthritis theranostics. Theranostics, 12:207–31.
https://doi.org/10.7150/thno.62708

You B, Zhou C, Yang Y, 2023. MSC-EVs alleviate osteoarthritis by regulating microenvironmental cells in the articular cavity and maintaining cartilage matrix homeostasis. Ageing Res Rev, 85:101864.
https://doi.org/10.1016/j.arr.2023.101864

Zhan W, Deng M, Huang X, et al., 2023. *Pueraria lobata*-derived exosome-like nanovesicles alleviate osteoporosis by enhacning autophagy. J Control Release, 364:644–53.
https://doi.org/10.1016/j.jconrel.2023.11.020

Zhang J, Pan J, Jing W, 2020. Motivating role of type H vessels in bone regeneration. Cell Prolif, 53:e12874.
https://doi.org/10.1111/cpr.12874

Zhang Z, Zhao S, Sun Z, et al., 2023. Enhancement of the therapeutic efficacy of mesenchymal stem cell-derived exosomes in osteoarthritis. Cell Mol Biol Lett, 28:75.
https://doi.org/10.1186/s11658-023-00485-2

Zhang Z, Li X, Wang X, et al., 2026. Naringenin-functionalized mesenchymal stem cell-derived exosomes alleviate sepsis-induced cardiac injury by restoring mitochondrial homeostasis and attenuating inflammation. Chin Chem Lett, 112677.
https://doi.org/10.1016/j.cclet.2026.112677

Zhao Q, Feng J, Liu F, et al., 2024. Rhizoma drynariae-derived nanovesicles reverse osteoporosis by potentiating osteogenic differentiation of human bone marrow mesenchymal stem cells via targeting ERα signaling. Acta Pharm Sin B, 14:2210–27.
https://doi.org/10.1016/j.apsb.2024.02.005

Zhao M, Chen X, Wang W, et al., 2025. Sea buckthorn-derived extracellular vesicles foster bone regeneration through aau-miR168-mediated pathways. Stem Cell Res Ther, 16:281.
https://doi.org/10.1186/s13287-025-04373-8

Zhao M, Zhang X, Xiao C, et al., 2026. Biomimetic mineralized hydrogels for bone regeneration. Adv Mater, 38(9):e20380.
https://doi.org/10.1002/adma.202520380

Zhao Y, Xu K, Wu K, et al., 2026. The roles of the nerve-immune axis in modulating bone regeneration. Bone Res, 14:2.
https://doi.org/10.1038/s41413-025-00476-3

Zhou J, See CW, Sreenivasamurthy S, et al., 2023. Customized additive manufacturing in bone scaffolds—the gateway to precise bone defect treatment. Research, 6:0239.
https://doi.org/10.34133/research.0239

Zhou X, Xu S, Zhang Z, et al., 2024. Gouqi-derived nanovesicles (GqDNVs) inhibited dexamethasone-induced muscle atrophy associating with AMPK/SIRT1/PGC1α signaling pathway. J Nanobiotechnology, 22:276.
https://doi.org/10.1186/s12951-024-02563-9

Zhu G, Zhang T, Chen M, et al., 2021. Bone physiological microenvironment and healing mechanism: Basis for future bone-tissue engineering scaffolds. Bioact Mater, 6:4110–40.
https://doi.org/10.1016/j.bioactmat.2021.03.043