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Exosome-based therapeutics in bone regeneration: from fundamental biology to clinical translation

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Abstract

Bone deficiencies are a major clinical issue for millions worldwide, with challenges to treatment because of donor site morbidity, immunological rejection, and limited integration. Exosomes are endogenously secreted extracellular vesicles and have potential as cell-free therapeutics. Exosomes derived from mesenchymal *stromal* cells (MSCs), bone marrow cells, and other cell-derived exosomes transmit bone morphogenetic proteins, growth factors, and immunoregulatory microRNAs to initiate osteogenic pathways. These exosomes stimulate and orchestrate vascularization and bone formation. Engineering strategies such as cargo optimization, surface functionalization, and cellular preconditioning further augment therapeutic promise. Clinical translation for exosome therapy has hurdles in manufacturing standardization and regulatory routes.

Highlights

- Exosomes deliver osteogenic proteins (BMP-2, RUNX2), angiogenic factors (VEGF, FGF-2), and immunoregulatory molecules (miR-21, TGF-β) for comprehensive bone healing.
- Surface functionalization, genetic modification, and cargo optimization techniques enhance exosome targeting specificity and therapeutic efficacy.
- Hydrogel-ceramic composites, electrospun nanofibers, and 3D-printed scaffolds provide controlled exosome release and mechanical support for bone regeneration.
- Cell-free exosome therapy offers reduced immunogenicity and eliminates risks associated with cellular therapies, including tumor formation and vascular occlusion.

Keywords Exosomes, Mesenchymal stem cells, Bone regeneration, Tissue engineering, Biomaterial scaffolds

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Background

Bone defects affect millions of people worldwide [1, 2]. In bone regeneration, the body must coordinate several processes, forming new bone, growing blood vessels, and managing immune responses [3]. Existing treatments are effective, though at a great disadvantage. Multiple approaches, such as fibular flap, autologous bone graft, allografts, stem cell therapy, and distraction osteogenesis, have been established to treat osseous defects [2, 4–7]. However, the barriers to these techniques include intricate surgical techniques, limited substitute sources, potential infection, tumorigenesis, and ethical issues [8]. FDA-approved bone regeneration therapies, including demineralized bone matrix (DBM) and recombinant human bone morphogenetic protein-2 (rhBMP-2, marketed as Infuse®), have been shown to be clinically effective [9, 10]. The clinical use of rhBMP-2 is restricted by its high cost and safety concerns, including ectopic bone formation, inflammatory reactions, and, in some reports, a possible increase in cancer risk [11, 12]. Demineralized bone matrix (DBM) also has important drawbacks: its quality can vary between batches, clinical outcomes are often inconsistent, and there is a residual risk of disease transmission [13, 14]. By comparison, exosome-based therapies offer a cell-free alternative with lower risks of immune rejection and tumor formation than stem cell transplantation. Exosomes naturally carry a wide range of osteogenic, angiogenic, and immunoregulatory molecules in physiologically balanced proportions [15, 16]. The potential to reduce costs and enhance safety in comparison to recombinant growth factor therapies has been further enabled by advancements in bioengineering, which have enabled the modification and production of exosomes on a larger scale [17, 18].

Exosome-based therapeutic approaches have demonstrated high regenerative potential across diverse organ systems, with successful preclinical and clinical applications documented in cardiovascular repair following myocardial infarction [19], neurological regeneration in stroke and spinal cord injuries [20], hepatic tissue regeneration [21], renal recovery after acute kidney injury [22], and accelerated wound healing in diabetic ulcers [23]. Within the specific context of bone regeneration, the exponential growth in research interest is demonstrated by bibliometric analysis of publications combining "exosome" and "bone regeneration" terms in PubMed databases from 2013 to 2025 (Fig. 1). 92% of the total publications within the recent six-year period (2019–2025). This rapid growth trend and the high potential of exosomes in bone regeneration indicate the existence of significant gaps in clinical evidence and translational pathways that require review and analysis.

The International Society for Cell & Gene Therapy (ISCT) now recommends the term "mesenchymal stromal cells (MSCs)" instead of "mesenchymal stem cells". This change in terminology is especially relevant for regenerative medicine because it highlights the diverse immunomodulatory properties of MSCs. These include their ability to regulate macrophage polarization (M1/M2), balance T-cell subsets such as Th1/Th2 and Th17/Treg, and foster anti-inflammatory microenvironments

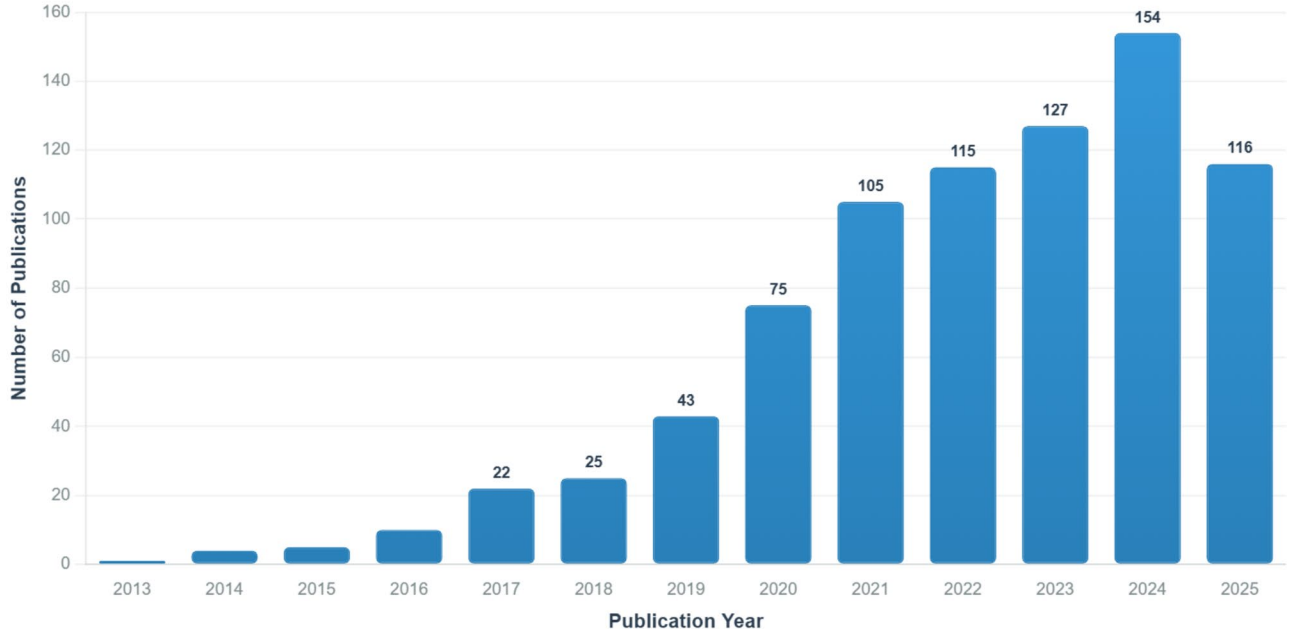

Fig. 1 Growth in exosome and bone regeneration research publications from 2013 to 2025

that aid cartilage repair and subchondral bone remodeling [24].

To our knowledge, this review is the first integration of exosome biology, engineering strategies, delivery platforms, and clinical translation pathways specifically for bone regeneration [25–30]. It provides a clear framework to understand the potential of exosome therapy in skeletal tissue engineering. In this article, the biology of exosomes, engineering and loading strategies, advanced delivery systems, preclinical evidence, and challenges of their clinical translation in bone tissue regeneration are reviewed and analyzed.

Review

Exosome extraction and production

Exosomes are tiny vesicles (30–150 nanometers) that cells release into their environment (Fig. 2) [31]. They are formed inside special compartments in the cell called multivesicular bodies, which fuse with the cell membrane to release them. This process is different from microvesicles that bud directly from the cell surface. For therapeutic use, exosomes are collected using methods such as differential ultracentrifugation, size exclusion chromatography, or tangential flow filtration. For therapeutic use, exosomes are collected using methods such as differential ultracentrifugation, size exclusion chromatography, or tangential flow filtration. Each of the three main size-based methods for exosome isolation comes with advantages and drawbacks. Differential ultracentrifugation is the most commonly used because it is simple and relatively inexpensive, but it is slow, less efficient, and not ideal for large-scale use. Size-exclusion chromatography is better at preserving the natural structure and function of exosomes, though it works slowly and can only process limited volumes. Ultrafiltration is quicker and produces higher yields, but problems like membrane fouling and vesicle loss can make results less consistent. To overcome these obstacles, hybrid approaches, such as the integration of tangential flow filtration (TFF) with SEC, have enhanced both cost-effectiveness and yield. Microfluidic platforms, including cascaded pulsatile filtration, are even more promising, as they can isolate purified exosomes from blood in less than 30 min. Collectively, these developments suggest a transition to more integrated and microfluidics-based methodologies that may surpass the constraints of conventional techniques [32].

The type of cell they come from matters greatly: bone marrow MSCs tend to produce exosomes with strong

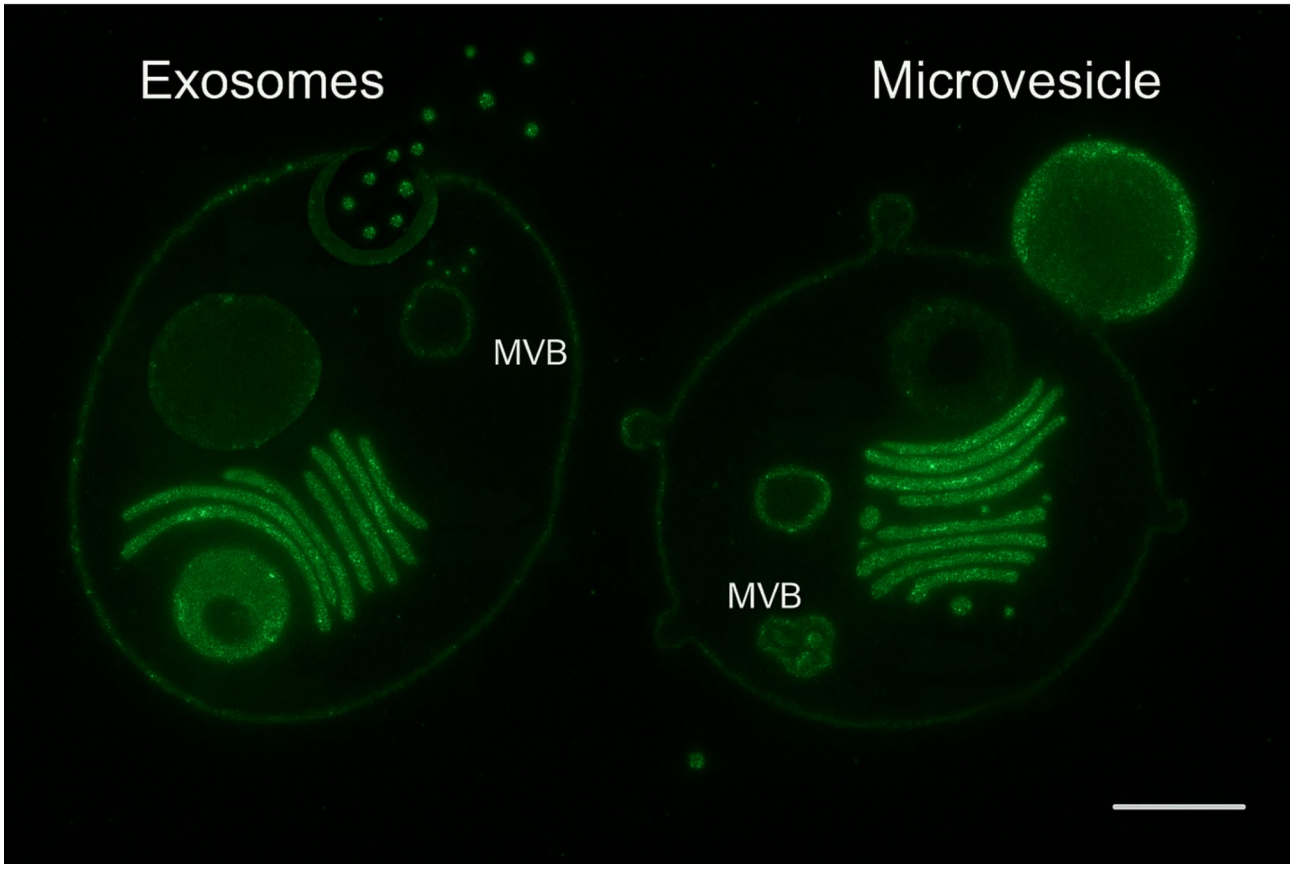

Fig. 2 Exosomes form inside multivesicular bodies, and microvesicles bud directly from the cell surface for cell communication

bone-forming potential, while adipose-derived MSCs produce exosomes with better immune-regulating effects. This makes choosing the right source important for bone regeneration therapies. As summarized in Table 1, bone marrow MSC-derived exosomes exhibit stronger regenerative potential, whereas adipose tissue MSC-derived exosomes are more associated with immune regulatory functions [33–39]. Exosome identification and characterization should follow the updated *MISEV2023* guidelines, which provide comprehensive standards for EV nomenclature, isolation, and characterization. These recommendations include the detection of canonical markers such as CD9, CD63, and CD81, along with confirmation of the absence of cellular contaminants, including calnexin, GM130, and cytochrome c [40].

Table 1 Comparative analysis of cellular sources for therapeutic exosomes in bone regeneration

Cellular source	osteogenic factors	Therapeutic advantages	Clinical limitations
Bone marrow MSCs (BMSCs)	BMP-2, RUNX2, OSX, miR-196a, miR-27a	• Gold standard with extensive characterization • Proven osteogenic efficacy • Established safety profile • Strong preclinical evidence	• Invasive harvesting procedure • Donor site morbidity • Age-related potency decline • Limited donor availability
Adipose-derived MSCs (AD-MSCs)	BMP-2, VEGF, TGF-β1, miR-451a, miR-21	• Minimally invasive procurement • Abundant yield potential • Superior anti-inflammatory properties • Better age-resistance	• Variable tissue quality • Donor BMI dependency • Less osteogenic vs. BMSCs • Standardization challenges
Umbilical cord MSCs (UC-MSCs)	BMP-2, PDGF, VEGF, miR-100-5p, miR-125b	• Standardized sourcing • No age-related decline • Superior expansion capacity • Reduced immunogenicity	• Limited tissue availability • Ethical considerations • Storage requirements • Regulatory complexity
Induced pluripotent stem cells (iPSCs)	BMP-2, VEGF, FGF-2, miR-302, miR-371	• Unlimited scalability • Patient-specific potential • High regenerative capacity • Genetic modification possible	• Complex differentiation protocols • Tumorigenicity concerns • Manufacturing complexity • Regulatory uncertainty
Endothelial cells (ECs)	VEGF, FGF-2, Angiopoietin-1, miR-126, miR-31	• Enhanced angiogenic• Type H vessel formation • Osteogenesis-angiogenesis coupling • Rapid vascularization	• Limited direct osteogenic potential • Shorter culture lifespan • Specialized culture requirements • Limited clinical data

Molecular cargo and therapeutic mechanisms

Exosomal cargo classification encompasses three functional categories, as illustrated in Fig. 3: osteogenic proteins that sustain bone formation, angiogenic factors that promote vascularization, and immunoregulatory molecules that create favorable healing environments [41]. Sphingomyelin makes the membrane more rigid, while cholesterol keeps it flexible and controls what can pass through, helping the vesicle stay intact during circulation and interaction with target cells. Lysophosphatidic acid (LPA) supports the growth and maturation of bone-forming cells, and sphingosine-1-phosphate (S1P) encourages bone formation while reducing bone breakdown. Cholesterol-rich membrane areas improve cell uptake and protect exosomes under stress. Osteogenic microRNAs, such as miR-22-3p and miR-196a, enhance differentiation and mineralization, while blocking miR-182-5p boosts bone formation. Exosomal lipids such as ceramide and cholesterol have structural roles in vesicle biogenesis, membrane stability, and uptake [42].

Long noncoding RNAs (lncRNAs) (greater than 200 nucleotides) act through mechanisms, such as altering patterns of gene expression and functioning as molecular sponges for miRNAs [43]. They may be transferred through cells with the help of exosomes to determine patterns of gene expression. MALAT1 is a long noncoding RNA found in exosomes containing stem cells that induce the formation of bone through competition with miR-143, which represses bone cell growth [44]. In a similar context, HOTTIP induces bone cell proliferation through Wnt pathways, and MEG3 regulates processes involved in removing bone [45]. Another type of regulatory exosomal molecule in the field of bone regeneration is circular RNAs (circRNAs) [46]. circRNA_0001105 induces the formation of bone by absorbing miR-143-3p to halt its inhibition of bone genes. circRNA_0000437 assists in blood vessel formation through VEGF pathways [47, 48].

One key way exosomes work is by changing recipient cells into bone-forming cells. They do this by carrying transcription factors, signaling molecules, and regulatory RNAs [49, 50]. In addition, exosomes can induce epigenetic modifications that result in sustained activation of osteogenic gene networks [51].

Advanced delivery systems

Hydrogels

Hydrogels are good materials for delivering exosomes in bone regeneration because they are water-rich, three-dimensional structures that closely resemble the bone's natural extracellular matrix (ECM) [52–54]. Hydrogels have limited mechanical strength and can not provide wound stability during the new bone formation phase. Since wound stabilization is essential for new bone

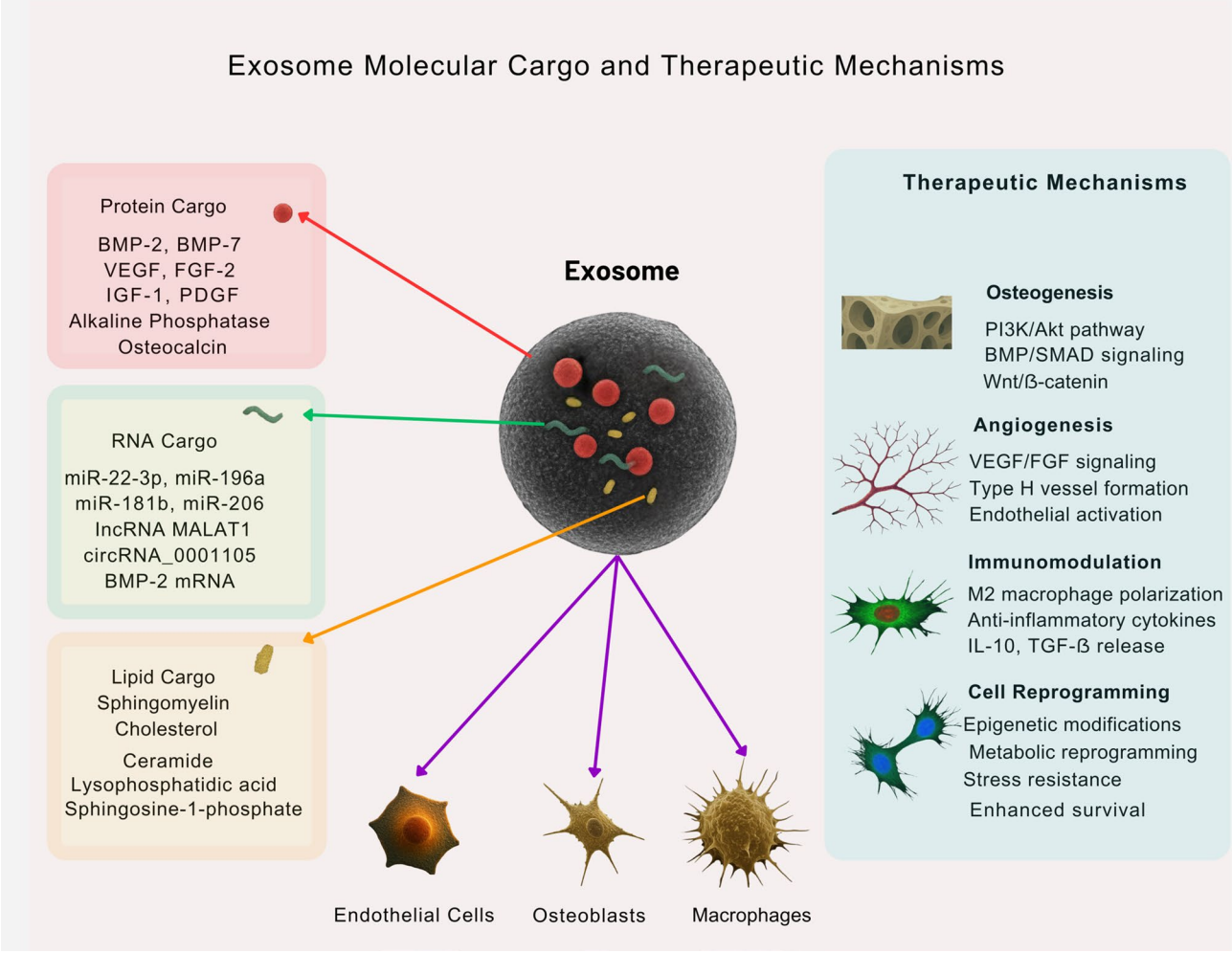

Fig. 3 Exosome-mediated bone regeneration mechanisms: from molecular cargo to therapeutic outcomes

Table 2 Comparative analysis of delivery platforms

Delivery platform	Characteristics	Release profile	Advantages	Limitations
Systemic injection	Rapid clearance, accumulation in liver/spleen	Minutes to hours	Non-invasive	Poor target site accumulation [64]
Hydrogel systems	Sustained release	10–16% daily (chitosan), up to 30 days total	Injectable, ECM-mimicking	Limited mechanical strength [54, 56]
Ceramic scaffolds	Osteoconductive support	~50% in 8 days	High mechanical strength	Limited bioactivity alone [58, 61]
Composite scaffolds	Biphasic release profiles	Variable, 68% in 14 days (PAA/triCaPNPs)	Combined advantages	Complex manufacturing [58, 65]
Smart materials	Stimuli-responsive	Condition-dependent	Targeted delivery	Limited clinical data [62]

formation, hydrogel-ceramic composite systems have emerged as the preferred approach (Table 2) [55, 56].

Ceramic and composite scaffolds

Ceramic scaffolds such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) have osteoconductive structural support and controlled porosity conducive to both cellular integration and exosome retention [57, 58]. Combining ceramics with hydrogels or other polymers merges the mechanical strength of ceramics with the controlled release features of hydrogels(Fig. 4) [54]. pH-responsive materials use the acidic environment at bone-remodeling sites to trigger targeted release. Temperature-responsive poly(N-isopropylacrylamide) allows injectable delivery that gels at body temperature [59]. Electrospun nanofibers can encapsulate exosomes in a core–shell structure, and 3D-printed gradient scaffolds enable precise spatial placement of exosomes [60–63].

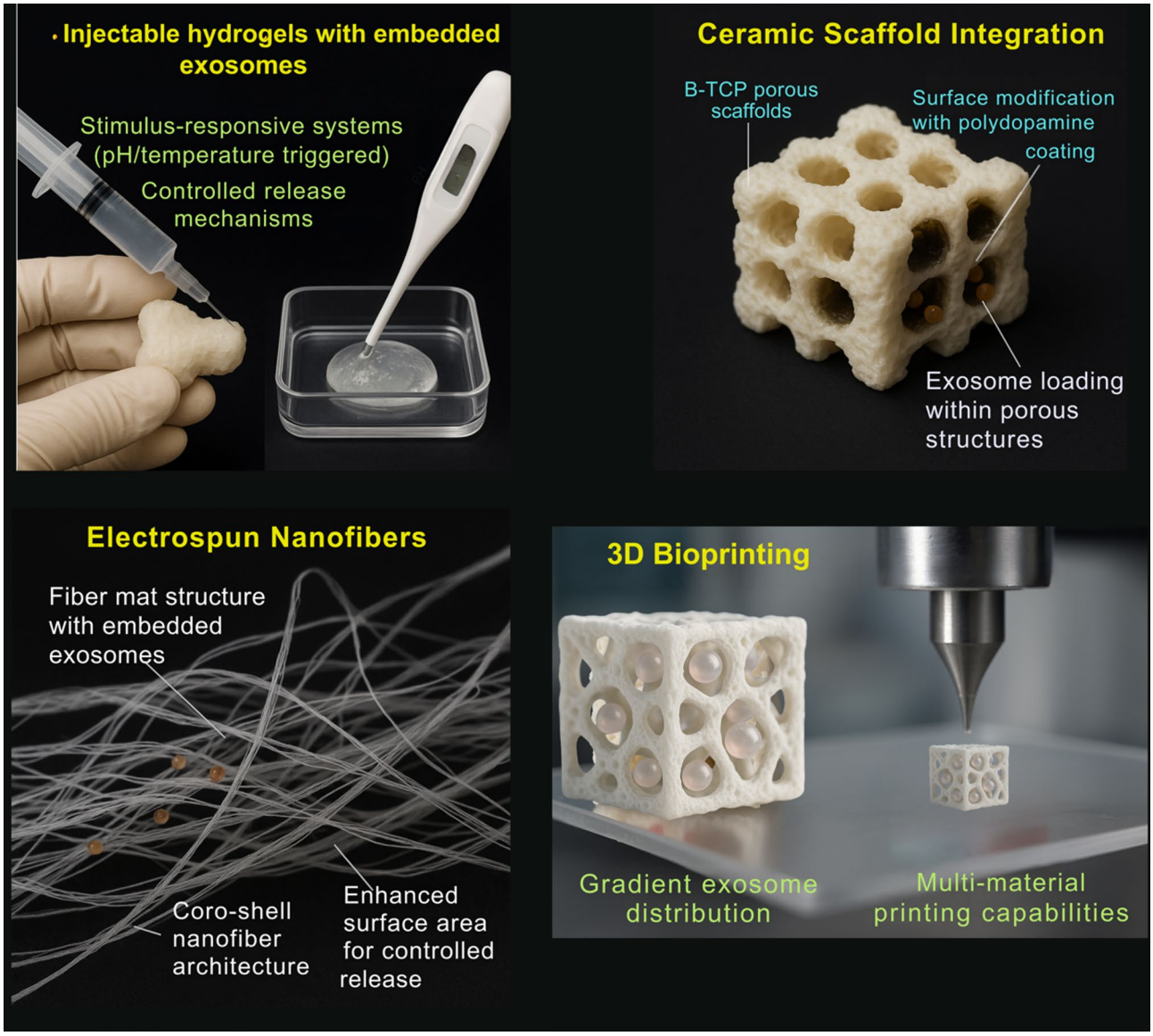

Fig. 4 Advanced delivery systems for exosome-based bone regeneration therapeutics

Nanofiber and electrospun material systems

The large surface area, adjustable fiber size and alignment, and ability to mimic the architecture of native tissue are benefits of electrospun nanofiber systems [66].

Three main strategies, including post-fabrication surface binding, blend electrospinning, and coaxial electrospinning, are used to incorporate exosomes into nanofiber systems [17]. Post-fabrication surface binding attaches exosomes to fibers after they are made, reducing their exposure to damaging processing conditions. However, this method can lead to weaker attachment and a quicker release. In blend electrospinning, exosomes are mixed directly into a polymer solution before fiber formation. Coaxial electrospinning uses a double-needle system [60]. This method can also produce fibers with more than one compartment to hold different therapeutic agents with various release speeds. The polymer core can act as a reservoir for slow and steady exosome release, while the shell can be modified to promote cell attachment, growth, or differentiation. The surface chemistry of the fibers controls how exosomes bind, load, and release, through processes such as electrostatic attraction or ligand-specific binding [67]. The diameter and curvature of the fibers affect how exosomes attach, and the fiber alignment influences how easily cells and exosomes interact. Biodegradable polymers allow timed release in line with the stages of bone healing [68, 69].

Three-dimensional Bioprinting applications

3D bioprinting allows for controlled exosome spatial distribution in modular scaffold constructs [53]. Bioprinting exosome-loaded material with living cells generates platforms that cohesively integrate cellular therapy and cell-free exosome delivery. High-resolution printing techniques like two-photon polymerization can build scaffolds with sub-micron dimensions to tightly manage exosome loading and delivery [70].

Engineering strategies for enhanced efficacy

Engineering strategies use surface modifications, genetic engineering of producer cells, biomimetic surface engineering, cargo optimization, cellular preconditioning, and synthetic biology approaches [71, 72].

Chemical modification links targeting molecules to exosome surface proteins through covalent bonds using established conjugation methods [73]. These procedures are based on N-hydroxy succinimide (NHS)-ester reactions to prepare stable amide bonds between carboxyl groups found on targeting molecules and primary amine groups found on exosome surface proteins, click chemistry strategies using very efficient and selective cycloaddition reaction chemistry to prepare sturdy linkages, and maleimide-thiol conjugation procedures based on the reactivity between maleimide functional groups and cysteine residues or other thiol-containing molecules [74].

Avidin-biotin systems are particularly favored because they can allow for the modular addition of various targeting molecules after isolation of exosomes without the necessity for genetic reengineering of cells. Lipid insertion is a simpler approach in which exosomes are incubated with modified lipids containing targeting groups [75]. The insertion efficacy can be customized by optimizing and selecting the most suitable incubation and anchor sequence conditions.

Bone-targeting surface modifications are an advanced method to improve the therapeutic performance of drug delivery systems in skeletal applications. A number of classes of bone-targeting agents have been widely studied in the past, and these include bisphosphonates, tetracycline derivatives, and bone-targeting peptides with mechanisms of selective skeletal localization (Fig. 5) [76].

Conjugating exosome surfaces with the nitrogen-containing bisphosphonate alendronate is an example of this targeting modality due to its great preference for binding to bone mineral surfaces. Increased bone targeting with alendronate conjugation has afforded greater therapeutic outcomes in experimental models of osteoporosis than its untargeted counterparts with delivery systems, with greater bioavailability and a reduced extent of systemic exposure [36].

The underlying mechanism for this increased targeting is through the chelating of calcium ions in the bone matrix by the bisphosphonate moiety to form stable complexes that are not cleared promptly from skeletal locations.

Genetic modification of producer cells can add targeting molecules and therapeutic cargo during exosome formation [77]. Methods like CRISPR, viral vectors, and transfection enhance protein or miRNA content and cell resilience [78, 79].

Cell membrane coating enhances exosome targeting and extends systemic circulation by preventing immune clearance, improving accumulation at target sites [80].

Natural exosomes are limited by their cargo capacity and loading efficiency [81].

Exosomes can be designed to specifically reach bone tissue using genetic or chemical methods. For example, bone marrow stem cell–specific aptamers can guide exosomes to the marrow, bisphosphonates can help them bind to hydroxyapatite in bone, and lipid-linked peptides can direct them to target cells [36, 82]. Therapeutic molecules can be loaded into exosomes through methods such as electroporation, sonication, extrusion, incubation, or saponin-assisted loading [83].

Therapeutic molecules can be loaded into exosomes through methods such as electroporation, sonication, extrusion, incubation, or saponin-assisted loading (Fig. 6) [84]. Electroporation is effective for nucleic acids and water-soluble drugs, though it may cause clumping, which can be reduced using trehalose buffers. Sonication and extrusion can load proteins and small molecules by temporarily opening the membrane and letting it close again for slow release. Incubation works for fat-soluble molecules but usually gives lower and less consistent results. The success of loading depends on the type of cargo, the chosen method, and experimental conditions [85–87].

Cellular preconditioning approaches include environmental modification, drug treatment, and genetic improvement that collectively boost exosome yield, therapeutic cargo content, and biological activity. Hypoxic preconditioning (1–5% O_2 for 24–72 h) enhances the angiogenic potential of exosomes by upregulating VEGF, HIF-1α, and pro-angiogenic miRNAs [88]. Mechanical stimulation via cyclic strain, shear stress, or vibration increases BMP-2, RUNX2, and osteogenic miRNAs in exosomes [89]. Three-dimensional culture systems, such as spheroids, organoids, and biomaterial scaffolds, can improve exosome quality by increasing cell-to-cell contact and guiding natural cell differentiation [90]. Adding growth factors or metabolic modulators can further enhance the cargo content [91]. Drugs like lithium chloride and valproic acid stimulate bone-forming signals and help load therapeutic factors, while BMP-2 or VEGF supplementation increases bone- and vessel-promoting cargo. Anti-inflammatory agents such as dexamethasone

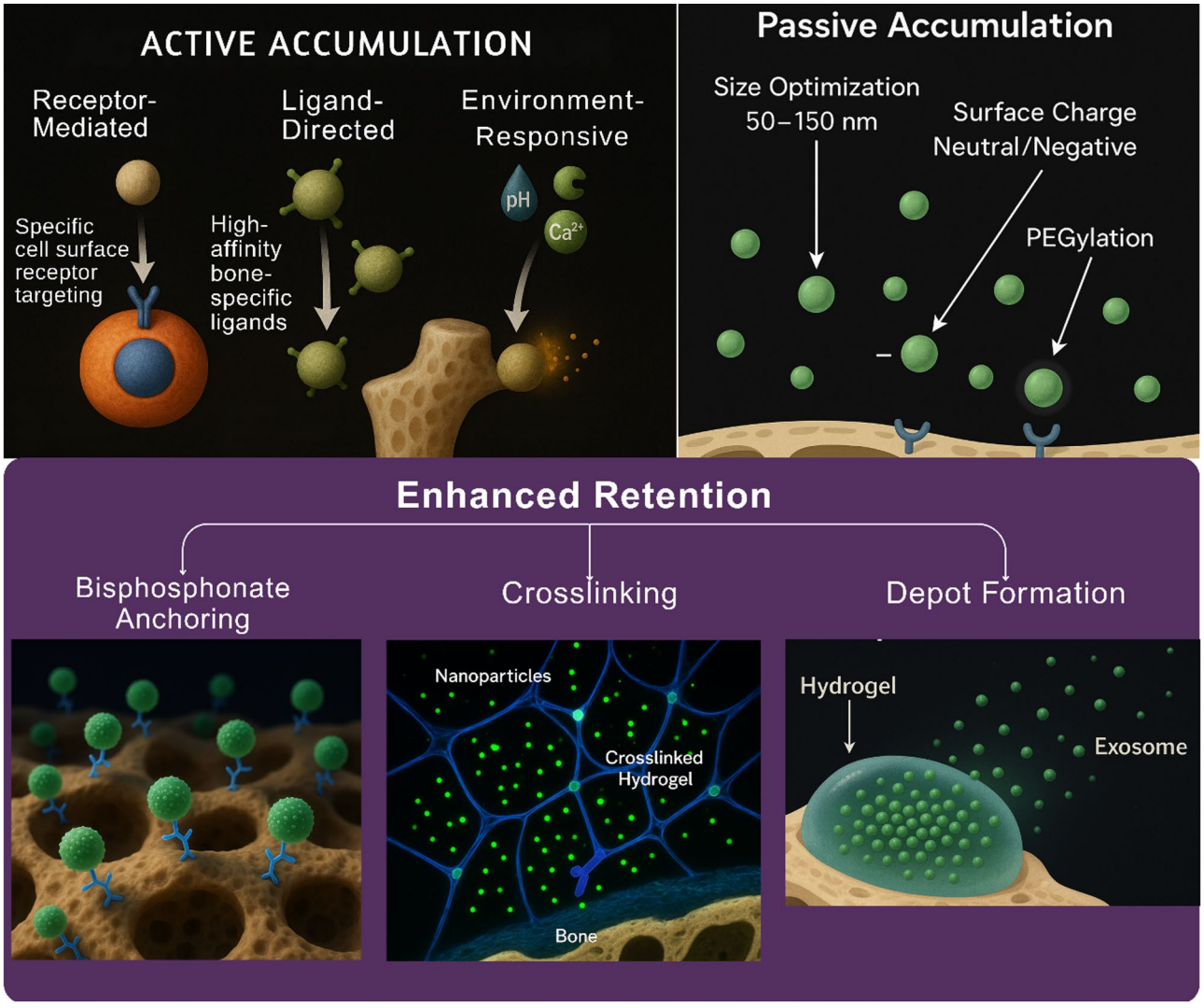

Fig. 5 Strategies for exosome targeting and retention in bone regeneration applications

or anti-TNF-α improve the immune-regulating properties of exosomes, and metabolic modulators such as metformin or rapamycin support cell health, extend lifespan, and increase exosome yield (Fig. 7) [92–94].

Mechanistic and immunological concerns in exosome engineering

Natural exosomes have low immunogenicity, but engineering them can change their behavior. These modifications might disrupt their molecular networks, create new antigens that trigger immune reactions, or alter their biodistribution patterns. Engineered exosomes may also move differently through the body, be absorbed by cells in new ways, or accumulate in tissues where natural exosomes typically would not [95]. Specific evidence of immunological concerns has been documented for engineered exosomes. Electroporation-modified exosomes exhibit altered surface protein profiles, potentially triggering complement activation and enhanced macrophage uptake compared with native vesicles [86]. Loading procedures using electroporation create transient membrane pores that may permanently alter vesicle surface characteristics, while sonication-based methods can cause membrane damage and particle aggregation [78]. Genetic modification of producer cells carries additional risks, as viral vector-mediated engineering may introduce foreign genetic elements that could trigger host immune responses [78].

To mitigate these risks, several evidence-based strategies have been developed. Systematic optimization of modification parameters through dose-escalation studies can establish maximum tolerable modification ratios while preserving therapeutic efficacy. Comprehensive preclinical safety assessment protocols should include complement activation assays, cytokine profiling, and long-term biodistribution studies [84]. Alternative

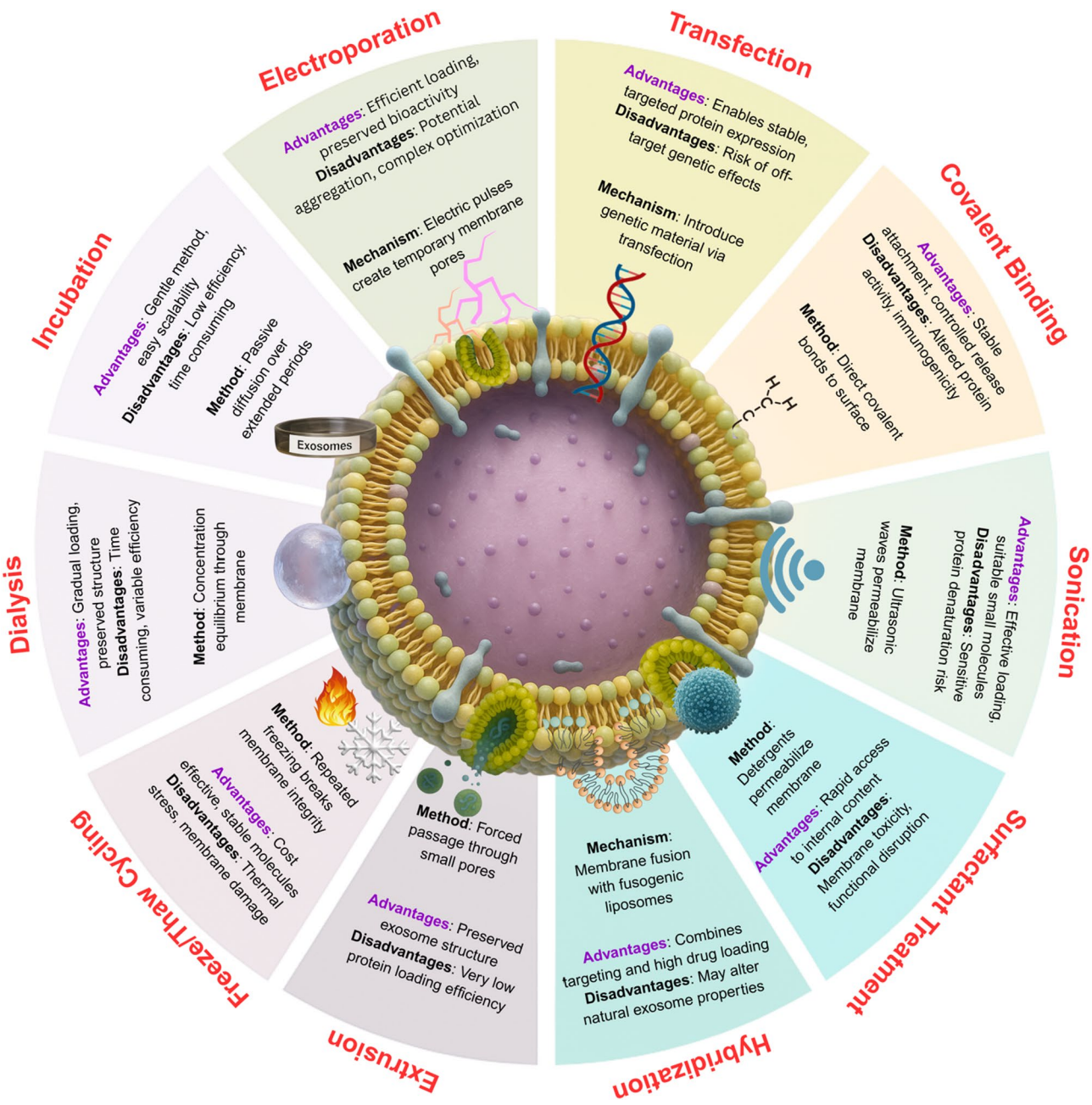

Fig. 6 Exosome loading methods for bone regeneration therapeutics

approaches, such as gentle loading methods using incubation techniques, may reduce membrane damage compared to electroporation [86].

A major limitation in current research is the lack of systematic toxicological evaluation of engineered exosomes. Genetic engineering methods, including CRISPR editing and viral vector modification, raise risks of insertional mutagenesis. Preclinical studies report integration events that may cause abnormal gene expression or malignant transformation [78]. CRISPR-modified producer cells require genomic analyses to detect off-target al.terations that may affect exosome cargo, while viral vector–based modifications necessitate evaluation of integration patterns and replication competence. Immunogenicity following repeated dosing remains insufficiently studied, despite evidence that frequent administration of vesicle-based products can stimulate complement activation and adaptive immune memory, potentially reducing therapeutic efficacy over time [86]. Off-target biodistribution further complicates safety assessment, as exosomes tend to accumulate in the liver, spleen, and lungs, raising the possibility of unintended tissue responses

Fig. 7 Engineering strategies for enhanced exosome efficacy in bone regeneration

[64]. Addressing these gaps will require systematic dose-escalation and long-term safety studies in large-animal models, incorporation of biodistribution imaging and immune monitoring into preclinical protocols, and harmonization of toxicological testing guidelines tailored for extracellular vesicle–based therapeutics. Establishing standardized toxicological protocols, including dose–response studies, safety margins, and validated monitoring assays, will be essential for regulatory approval and safe clinical translation [84, 96].

Preclinical evidence and animal models

Preclinical studies show that exosome-based therapies can increase bone formation and blood vessel growth [97, 98]. However, differences in isolation methods, storage, and outcome assessment make it hard to compare results between studies [99]. Most follow-up periods are only 8 to 12 weeks, which is too short to assess long-term safety [61]. Rodents also heal differently from humans. The use of young and healthy animals reduces clinical relevance because it does not reflect the complex conditions of real patients, including other diseases, medications,

and lifestyle factors [65]. These limitations show the need for standardized preclinical protocols and large-animal studies. Rodent calvarial defect models are commonly used because they are cost-effective and easy to handle. However, their cranial bone architecture and density differ greatly from those of humans. Rodent calvaria are thinner, heal faster, and lack the diploë structure found in human cranial bone, which limits their translational value [100–102]. Large-animal models such as canine, ovine, and porcine calvarial defects more closely resemble human cranial bone in density, thickness, and remodeling behavior [103, 104]. These similarities allow for a more accurate assessment of graft integration, vascularization, and biomechanical stability [105]. Moreover, large-animal models permit evaluation of clinically relevant defect sizes and healing timelines that better reflect human bone regeneration [106]. Therefore, future preclinical research should increasingly incorporate large-animal calvarial defect models to bridge the gap between small-animal experiments and human clinical applications, thereby strengthening the translational pathway of exosome-based bone regeneration therapies.

Engineered exosomes bring extra safety concerns that are hard to evaluate in short-term animal studies. Natural exosomes have low immunogenicity, but modifications can change their properties, trigger immune reactions, or damage their membranes. Genetic engineering may cause inflammation, autoimmune reactions, or abnormal tissue formation. These issues may appear months or years later and remain undetected in 8 to 12-week studies [107]. The effects of repeated doses and the chance of modified genetic material entering host cell DNA are also not well studied, making longer safety evaluations necessary before clinical use (Table 3; Fig. 8) [97, 108].

Comparative analysis with alternative approaches

MSCs carry risks including tumor formation, immune rejection in allogeneic settings, and vascular occlusion upon intravenous administration [49, 112]. In contrast, exosome therapy is less immunogenic and lacks the vascular delivery complications linked to cellular aggregates [50].

Recombinant growth factor therapy, most notably BMP-2 and PDGF, is the standard clinical approach to bone regrowth acceleration, approved by the FDA for certain applications such as spine fusion and fracture repair. Exosome-transferred growth factors have physiologically balanced concentration intervals with augmented bioactivity through shielded delivery and long-term release with fewer side effects than in the case of recombinant protein therapy. Gene therapy for bone regeneration often uses viral vectors, lipofection, or electroporation to deliver genes like BMP-2 or VEGF directly into target cells. In contrast, exosome-based delivery carries therapeutic mRNAs and miRNAs through natural cellular pathways, which helps reduce safety risks linked to viral vectors and permanent genetic modifications [31, 50, 113–116].

Clinical translation

Differential ultracentrifugation (dUC) is still the most common method for isolating exosomes. However, it has some drawbacks, including low recovery rates, contamination with lipoproteins, and possible vesicle clumping [95]. Standardization challenges in exosome research have been partially addressed by the recent MISEV2023 guidelines, which provide updated recommendations for EV nomenclature, isolation methods, and characterization protocols to ensure reproducibility across different laboratories and clinical settings [40]. Size exclusion chromatography (SEC) can produce purer exosome preparations by separating them from proteins based on size, giving higher specificity than ultracentrifugation while keeping their structure and activity intact [117]. Tangential flow filtration (TFF) is a scalable method that can process large volumes continuously and cause minimal damage to exosomes. Exosome identification includes detecting specific markers (CD9, CD63, and CD81) and the absence of cellular contaminants like calnexin, GM130, or cytochrome c [118, 119]. Their size and concentration are measured with nanoparticle tracking analysis (NTA). Morphology is examined by transmission electron microscopy (TEM), and size uniformity is checked using dynamic light scattering (DLS) [119, 120]. Purity is assessed by the particle-to-protein ratio, with values above 1×10^{10} particles per microgram of protein considered high quality. Safety tests include measuring endotoxin levels, which should be below 5 EU/kg body weight for parenteral use, and sterility checks following pharmacopoeia standards [96, 121]. In the United States, the FDA regulates all exosomes as biological products. In Europe, exosomes that are not extensively modified are treated as biological medicines, similar to the US system. However, if they are highly modified, the EMA may classify them as Advanced Therapy Medicinal Products (ATMPs) and, as a result, require stricter approval procedures [96, 121, 122]. Addressing these regulatory challenges requires implementing specific operational frameworks. Good Manufacturing Practice compliance necessitates controlled cell culture environments with validated isolation and characterization procedures [121]. Quality control frameworks must incorporate standardized methods for marker detection, contamination assessment, and morphological evaluation [40]. Sterility protocols should follow pharmacopoeia standards with appropriate endotoxin testing [120]. Standardization requires establishing consensus on quantification methods emphasizing particle enumeration and protein

Table 3 Preclinical studies on engineered Exosomal platforms for bone tissue engineering

Study	Intervention	Animal model	Groups (in vivo)	Effect	Analysis
[109]	Scaffold: CHA/SF/GCS/DF-PEG hydrogel Exosomes: hucMSC(human umbilical cord MSCs) Binding: without any special binding Dose: 25 & 50 µg/ml exosomes per defect Delivery: Local implantation Release: Sustained exosome release up to 30days; ~78.2%±0.36% Note: -bone defect volume ~ 0.05 ml -Coralline hydroxyapatite (CHA), silk fibroin (SF), glycol chitosan (GCS)	Species: Sprague-Dawley (SD) rats (♂, 4&8w), suckling C57BL/6 mice (♂, 24 h) Sample size: $n = 18$ per group Defect: Femoral condyle defect (diameter: 4 mm, depth: 4 mm) Maximum Follow Up: 90days	1. Blank (PBS) 2. CHA/SF/GCS/DF-PEG 3. CHA/SF/GCS/DF-PEG + Exos	↑ Enhanced osteogenesis + Best bone regeneration: "CHA/SF/GCS/DF-PEG + Exos"	In Vitro Characterisation: TEM, SEM, XRD Biocompatibility: MTT assay, FDA/PI staining Cell Interaction: 3D culture, contact angle Migration & Proliferation: Scratch assay, CCK-8 Osteogenesis: ALP, Alizarin Red In Vivo Bone Regeneration: X-ray, Micro-CT (BV/TV, BMC), 3D reconstruction Histology: H&E, Masson's trichrome Immunohistochemistry: BMP-2, CD34
[110]	Scaffold: GNP (gelatine nanoparticle) hydrogel Exosomes: ADSC (Adipose-Derived Stem Cells) Binding: Electrostatic Dose: 0.8 mg ADSC-Exos per 1mL PBS mixed with 0.1 g GNPs powder Delivery: Local injection (GNPs), Local implantation (GNP-Exos) Release: Sustained controlled release up to ≥ 7 days; ~10–16% daily exosome release	Species: SD rats (8w) Sample size: $n = 12$ per group Defect: Calvarial defect (diameter: 5 mm) Maximum Follow Up: 8w	1. Blank (no scaffold) 2. GNPs 3. GNP + Exos	↑ Bone regeneration + Best bone regeneration: "GNP-Exos"	In Vitro Characterisation: TEM, NTA, DLS, WB (CD9, CD63) Gene & Protein Expression: RT-qPCR, WB (iNOS, CD86, CD206, MIF) Exosome Uptake: PKH67 fluorescence labelling, Confocal microscopy Macrophage Polarisation: LPS/IFN-γ stimulation Mechanism Analysis: miRNA microarray In Vivo Bone Regeneration: Micro-CT (BV/TV, Tb.Th, Tb. N, Tb.Sp), 3D reconstruction Histology: H&E staining, Semi-quantitative histological scoring Immunofluorescence: CD206, iNOS, F4/80 Biocompatibility
[111]	Scaffold: 3D-fabricated Ca-Si/PCL Exosomes: hTMSCs (Human tonsil-derived MSCs) Binding: Electrostatic + pDA stabilization Dose: 100 µg/mL exosomes per scaffold Delivery: Local implantation Release: Not reported	Species: C57BL/6 (♂, 6w) Sample size: $n = 4$ (calvarial); $n = 3$ (subcutaneous) per group Defect: calvarial defect (diameter: 5 mm) Maximum Follow Up: 8w	1. Blank (PCL) 2. 10% Ca–Si/PCL 3. 20% Ca–Si/PCL 4. PCL + Exos 5. 10% Ca–Si/PCL + Exos 6. 20% Ca–Si/PCL + Exos	↑ Bone regeneration + Best bone regeneration: "10% Ca–Si/PCL + Exos" + pDA enhances exosome binding & scaffold stability	In Vitro Characterisation: SEM Scaffold Interaction: PKH26 fluorescence microscopy, Confocal microscopy, pDA surface coating Biocompatibility: CCK-8, DAPI & Phalloidin staining Migration: Boyden chamber assay Osteogenesis: ALP activity, Alizarin Red, S staining, WB (OCN, OPN), Exosome marker (CD9) In Vivo Bone Regeneration: Micro-CT (BV/TV, Tb.Th, Tb. N, Tb.Sp), 3D reconstruction Histology: H&E staining, Masson's Trichrome Immunofluorescence: CD44, CD73, OCN Signaling Pathway: WB (PI3K/AKT/mTOR)

Table 3 (continued)

Study	Intervention	Animal model	Groups (in vivo)	Effect	Analysis
[65]	Scaffold: PAA/triCaP-NPs (Poly (acrylic acid)/tricalcium phosphate nanoparticles) Exosomes: UC-MSCs (umbilical cord MSCs) Binding: Electrostatic + physical absorption Dose: 1 µg/µL, 150 µg exosomes per rat Delivery: Local implantation Release: ~68% exosome in 14 days	Species: Wistar rats (♂, ~ 300 ± 30 g, 10w) Sample size: $n = 8$ per group Defect: calvarial defect (diameter: 8 mm) Maximum Follow Up: 12w	1. blank (no scaffold) 2. PAA/triCaPNPs 3. PAA/triCaPNPs + Exos	↑ Bone regeneration + Best bone regeneration: "PAA/triCaPNPs + Exos"	In Vitro Characterisation: WB (CD9, CD81) Scaffold Interaction: SEM, DiI-labelled uptake (fluorescence microscopy) Degradation: Weight variation in PBS In Vivo Bone Regeneration: Micro-CT (BV/TV, BMD, Tb.Th), 3D reconstruction Histology: H&E staining, Masson's Trichrome Osteogenesis & Angiogenesis: Immunohistochemistry (OCN, CD31), Quantification via ImageJ
[61]	Scaffold: 3D-printed HA (hydroxyapatite) or TCP (tricalcium phosphate) Exosomes: BMSCs (rabbit bone marrow MSCs) Binding: Not reported Dose: 30–50µL Delivery: Local implantation Release: ~50% of exosomes in 8 d (slower in TCP)	Species: NZW rabbits (♀, 26w) Sample size: Not reported Defect: calvarial defect (diameter: 6 mm) Maximum Follow Up: 4w	1. HA 2. TCP 3. HA + Exo 4. TCP + Exo	No effect	In Vitro Characterisation: NTA, LC-MS/MS proteomics (GO/STRING), ExoCarta reference matching Gene Expression: RT-qPCR (ALPL, RUNX2) Migration: Transwell assay, Jonckheere–Terpstra trend test Scaffold Interaction: SEM, exosome retention analysis Biocompatibility & Metabolism: Alamar Blue assay In Vivo Bone Regeneration: Micro-CT (bone ingrowth, bone-to-implant contact, augmentation volume) Histology: Toluidine Blue staining Scaffold Performance: Comparison of Gyroid vs. Lattice architecture, HA vs. TCP materials
[83]	Scaffold: HA-coated PLGA Exosomes: engineered EMs (exosome mimetics) from hMSC Binding: Electrostatic, Dropwise seeding Dose: Not reported Delivery: Local implantation Release: Not reported	Species: CD-1 nude mice Sample size: Not reported Defect: calvarial defect (diameter: 3 mm) Maximum Follow Up: 12w	1. Blank (no scaffold) 2. EMs 3. ALD-EM 4. ALD-EM-SAG	↑ Bone regeneration + Best bone regeneration: "ALD-EM-SAG"	In Vitro Characterisation: TEM, DLS, ζ-potential analysis, WB (CD63, Calnexin, Cytochrome c) Scaffold Interaction: Confocal microscopy (DiI, FITC-DBCO labelling), SEM, EDX (HA/PLGA scaffold surface) Biocompatibility: cell proliferation imaging Osteogenesis: ALP staining, Alizarin Red S staining, qRT-PCR (ALP, RUNX2, OCN) In Vivo Bone Regeneration: Micro-CT (BV/TV, BMD, Tb.N), 3D reconstruction Histology: H&E staining, Masson's Trichrome Osteogenesis: Immunohistochemistry (RUNX2, OCN) Biodistribution: fluorescence imaging, ex vivo organ-level retention analysis

Table 3 (continued)

Study	Intervention	Animal model	Groups (in vivo)	Effect	Analysis
[61]	Scaffold: 3D-printed porous PCL Exosomes: ATDC5-derived, engineered with VEGF plasmid Binding: CP05 anchor peptide Dose: 10 µg exosomes per scaffold Delivery: Local implantation Release: Not reported	Species: SD rats (♂, ~180 g) Sample size: Not reported Defect: radial segmental defect (5 mm long) Maximum Follow Up: 12w	1. Blank (no scaffold) 2. PCL 3. PCL + CP05 4. PCL + CP05 ~ EXOs 5. PCL + CP05 ~ EXOs-VEGF	↑ Bone regeneration + Best bone regeneration: "PCL + CP05 ~ EXOs-VEGF"	In Vitro Characterisation: TEM, NTA, DLS, WB (CD63, TSG101) Gene Expression: qRT-PCR, ELISA, EGFP fluorescence Angiogenesis: Tube formation (HUVEC) Scaffold Interaction: Confocal microscopy (Z-stack), SEM, DiI-labelled uptake Biocompatibility: CCK-8, Cell adhesion imaging Osteogenesis: ALP staining, Alizarin Red staining, OCN immunofluorescence, qRT-PCR (ALP, Runx2, OCN) In Vivo Bone Regeneration: Micro-CT (BV, BV/TV, Tb.Th), 3D reconstruction Histology: H&E staining, Masson's Trichrome, CD31 immunofluorescence Mechanics: Compressive strength

content rather than mass-based measurements [118]. Regulatory harmonization demands consistent classification frameworks distinguishing minimally manipulated from extensively modified products [96].

Economic factors may represent an important barrier to the clinical translation of exosome-based bone regeneration. Large-scale production is likely to require considerable investment in GMP facilities and specialized purification systems, making costs potentially higher than for standard drugs [121]. Isolation methods such as ultracentrifugation and SEC often demand skilled staff and costly equipment. Storage may add further expense, since exosomes usually need cryoprotectants, low-temperature freezers, and cold-chain distribution systems [120]. To improve economic feasibility, several approaches should be investigated: developing more efficient isolation methods, establishing centralized manufacturing to achieve economies of scale, implementing automated workflows to reduce labor costs, and developing stable formulations to reduce storage complexity [40]. Economic analysis comparing exosome therapies with established treatments like rhBMP-2 will be essential for demonstrating commercial viability and healthcare system adoption.

A search in PubMed, Scopus, Web of Science, and ClinicalTrials.gov using the terms "exosome," "extracellular vesicle," "bone regeneration," "bone repair," "bone augmentation," and "clinical trial," covering publications up to August 14, 2025, revealed no reported human clinical trials on exosome-based bone regeneration. This highlights the urgent need for standardized protocols, guided by the MISEV2023 framework, to ensure reproducibility and regulatory compliance [40]. The absence of clinical trials reflects several barriers. Standardization of manufacturing remains the foremost challenge, as current isolation methods lack scalability and reproducibility, with batch-to-batch variability compromising consistent dosing [121]. Regulatory uncertainty remains a major barrier. Exosomes are classified differently depending on whether they are minimally modified or extensively engineered. This creates unpredictable approval pathways and increases research costs [96]. Economic barriers also play a role, since GMP-compliant production facilities and lengthy regulatory processes require substantial investment, while intellectual property protection for naturally derived vesicles remains limited [40]. To overcome these challenges, international collaborations are needed to establish standardized protocols and shared reference materials, while regulatory science initiatives should provide clearer guidance on exosome classification and approval routes [40]. Dosage standardization poses a critical challenge in exosome research, as studies often employ inconsistent reporting units that hinder comparative analysis. Current literature exhibits variability in measurement approaches, including protein content, particle number, and total exosome weight, without established correlations between these methods [40, 123]. This inconsistency is evident in preclinical studies presented in Table 3, where different research groups report doses using varying metrics and concentrations. The absence of standardized potency assays compounds this challenge, as therapeutic activity may depend on multiple factors beyond simple protein or particle quantification [118]. Addressing this standardization gap requires developing consensus reporting standards that incorporate both quantitative measurements and functional assessments relevant to therapeutic applications [120, 123].

Fig. 8 animal models for bone augmentation

Future perspectives

Organ-on-a-chip technologies provide microengineered platforms that more closely resemble human bone settings than 2D cultures or animal models. Recent bone-on-a-chip systems have even replicated osteoporotic and healthy bone niches, allowing scientists to examine osteogenesis in a controlled environment. Integrating exosomes into these models could enable a more precise evaluation of their regenerative capacity and safety, thereby facilitating the development of individualized

bone regeneration therapies [124, 125]. Future research priorities must include long-term stability studies evaluating membrane integrity following loading techniques such as electroporation and sonication, as current literature lacks comprehensive data on extended stability profiles.

Conclusion

Exosomes can help bone regeneration by inducing new bone growth, improving blood supply, and balancing the immune response. Before they can be used in patients, we need better ways to produce them in large amounts. Future studies should focus on making them more effective, lowering costs, and running strong clinical trials. If these steps are taken, exosomes could become a safe and advanced option for repairing damaged bones.

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Author contributions
F.T., S.G., F.S., A.E.H.C., N.R., A.M., and A.R. conceptualized the study and drafted the manuscript. F.T. designed the figures. S.G. and F.S. performed a bibliometric analysis. M.H.H. supervised the study and provided critical revisions. All authors reviewed and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable.

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