

Exosomes in Bone Generation and Repair: Focusing on Bone Microenvironmental Crosstalk and Engineering Biomaterial Designs

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Abstract: The development of effective therapeutic strategies for bone regeneration and repair has proven to be highly challenging due to the sluggish and unpredictable nature of the healing process. Under pathological conditions, impaired cellular function can lead to poor biomineralization and compromised bone healing, resulting in various failures. Exosomes, as potent intercellular communicators capable of delivering diverse bioactive cargo, offer significant therapeutic promise. However, the lack of comprehensive understanding of their roles in the bone healing microenvironment and biomaterial design poses challenges for exosome-based therapies. This review provides the essential biological context for exosome application in bone regeneration, with a dual focus. First, we elucidate the pivotal roles of exosomes in mediating bone microenvironmental crosstalk, emphasizing their critical involvement in immunomodulation (eg, macrophage polarization), osteogenesis-angiogenesis coupling, osteoclast-osteoblast balance, neuro-skeletal communication, and dynamic extracellular matrix remodeling, rather than merely listing cell-specific functions. Second, building on this foundation, we summarize the rationale for engineering exosomal biomaterial designs. This includes strategies for exosome optimization (eg, targeting modifications, cargo loading, parental cell stimulation) and their integration with functional scaffolds to modulate the identified crosstalk pathways and create a conducive microenvironment. By delineating exosome functions within the bone microenvironmental network and outlining corresponding biomaterial engineering strategies, this review offers a holistic perspective essential for advancing exosome-based therapies.

Keywords: exosome, osteogenic microenvironments, biomaterials, intercellular interaction

Introduction

The repair and reconstruction of damaged bone tissue continue to pose significant clinical challenges, as bone regeneration is a complex and coordinated process that necessitates the collaboration of various specialized cells and a wide array of bioactive factors.^{1,2} Furthermore, the intrinsic self-repair capacity of bone tissue is limited, and its natural regenerative pace is often insufficient to satisfy the clinical requirements. Consequently, external interventions are essential to accelerate bone regeneration, particularly when the inherent self-repair capabilities of the tissue are exceeded.

Over the past two decades, traditional design strategies for bone replacement materials have typically focused on activating osteoblastic lineage cells and fabricating single-function biomaterials to stimulate osteogenesis. However, bone formation is a complex process linked to angiogenesis, immunomodulation, and even neural modulation, both temporally and spatially. It is evidently not a single event; rather, it depends on the superposition of multiple steps. A re-evaluation of interactions within the microenvironment is ultimately necessary to design a biomimetic bone material with improved performance. Within the bone microenvironment, osteogenic cells and immune cells engage in reciprocal communication through diverse signaling pathways and cytokine networks, thereby sustaining the dynamic equilibrium of bone

metabolism.^{3,4} The often-ignored immune response is one of the main reasons leading to undesirable bone repair outcomes. The concept of the “bone immune system” was introduced by Arron and Choi in 2000⁵ and has been gradually recognized. Within this framework, immune cells are crucial for maintaining bone health. For example, under physiological conditions, immune cells can secrete osteoprotegerin, which counteracts the production of osteoclasts and thereby inhibits osteopenia.⁶ In contrast, in patients with bone metabolic disorders, the inflammatory activation of immune cells results in the secretion of osteoclast-promoting factors, such as tumor necrosis factor-alpha (TNF- α) and receptor activator of nuclear factor kappa-B ligand (RANKL).⁷ Interestingly, recent research has shown that macrophages can modulate the inflammatory state, transforming an inflamed milieu into an environment that promotes bone healing through the transition of their phenotypes.^{8–13} Of particular interest is the mechanism of communication between immune cells and bone cells. Moreover, bone marrow-derived exosomes shuttle between the bone and immune environments, triggering macrophage plasticity and modulating bone formation processes.¹⁴ Furthermore, exosomes derived from type 2 macrophages (M2) facilitate osteogenesis while reducing adipogenesis in bone marrow-derived stem cells (BMSCs).¹⁵ These findings support the hypothesis that exosomes play a critical role in intercellular communication between bone and the immune system.

The vascular environment plays a critical role in bone regeneration, as angiogenesis serves as both the initiating and key step in the bone healing process. Extensive evidence indicates that complications such as delayed healing and nonunion are more frequently attributed to failures in vascular reconstruction rather than a deficiency in osteogenic potential.^{16,17} The progression of osteogenesis is facilitated by angiogenesis, in which the blood supply stimulates osteoblast migration and promotes bone tissue mineralization. Recent studies have shown that exosomes derived from BMSCs enhance fracture healing by promoting angiogenesis.¹⁸ Furthermore, vascular endothelial cells release exosome-derived miR-5p-72106_14, which plays a significant role in influencing the fate determination of BMSCs.¹⁹ Notably, a specific class of microRNAs, known as angio-miRNAs, has been shown to be essential for modulating vascular development and angiogenesis.^{20,21} Accumulating evidence suggests that functional RNAs can be encapsulated within exosomes and transported from donor cells to recipient cells. This significant feature has led to the proposal of exosome-RNAs as a novel category of intercellular regulatory molecules.²² Within the complex network of the bone microenvironment, the balance between osteoblasts and osteoclasts is a well-known critical element during bone healing. Research has indicated that osteoblastic bone generation may be regulated by exosomes released by active osteoclasts.²³ Therefore, elucidating the role of exosomes within intricate cell communication networks is pivotal for developing novel therapeutic strategies for bone regeneration.

Over the past decade, the field of stem cell therapy has undergone rapid development, especially regarding the use of BMSCs, which have strong immunomodulatory properties in immunomodulatory bone therapy. However, the application of stem cell therapy is constrained by several factors, including inherent heterogeneity, the potential for immune rejection, the low viability of transplanted cells, the risk of tumorigenesis, and uncontrolled differentiation leading to teratoma formation. Consequently, a pressing need for safer and more effective therapeutic options exists. Notably, research has shown that the effectiveness of mesenchymal stem cell (MSC)-based treatments is attributed to their exosome-mediated autocrine and paracrine effects.^{24,25} As master regulators of cellular signaling, exosomes exhibit advantages such as a smaller size, excellent penetration, high biodistribution *in vitro*, and low immunogenicity. Therefore, exosome treatment may be a preferred therapeutic option.

Bone healing is contingent upon adequate proliferation and osteogenic differentiation, as well as functional modifications within a multicomponent microenvironment. Consequently, elucidating the roles of exosomes in shuttling intercellular signals and triggering various osteogenesis-related interactions is of paramount importance, as it will enable the development of novel and more effective treatment strategies.

The Formation and Characteristics of Exosomes

Exosomes, which have garnered significant attention in the context of bone formation, are extracellular vesicles released by a variety of cell types.^{26,27} The process of exosome formation is finely tuned and consists of four sequential steps: initiation, endocytosis, the formation of multivesicular bodies, and the subsequent secretion of exosomes (Figure 1).²⁸ These vesicles originate from endosomes and are characterized by a lipid bilayer structure with diameters ranging from 30 to 150 nm. They

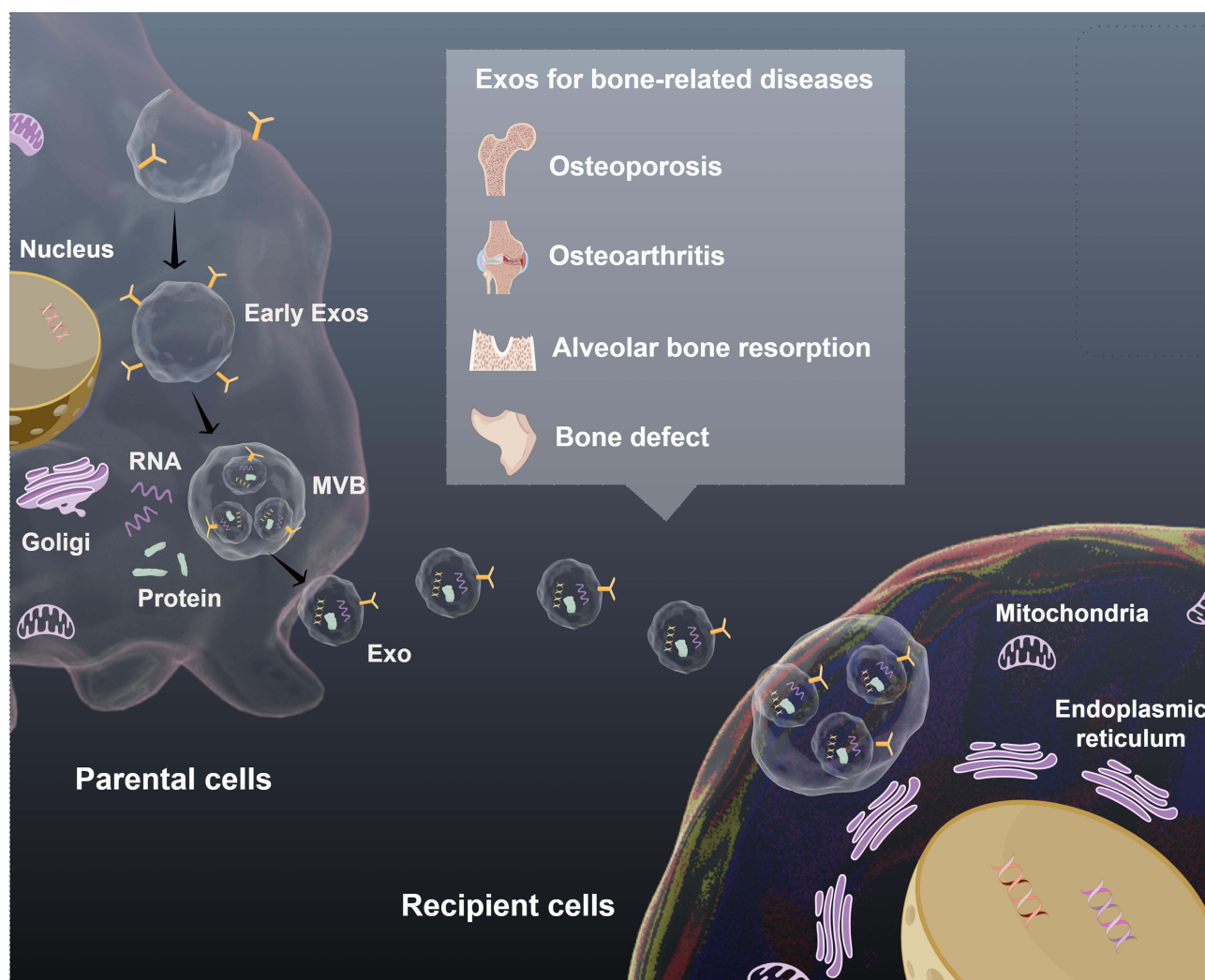


Figure 1 The formation and characteristic of exosome and bone related diseases. Exosomes (Exos) are small extracellular vesicles that facilitate intercellular communication by transferring bioactive molecules, such as RNA, proteins, and lipids. They are secreted from parental cells into the extracellular space through the formation of multivesicular bodies, which fuse with the plasma membrane to release exosomes. These exosomes can influence recipient cells, contributing to bone-related diseases such as osteoporosis, osteoarthritis, alveolar bone resorption, and bone defects. The image illustrates the process of exosome formation, cargo packaging, and delivery to recipient cells, highlighting their involvement in various bone pathologies. The figure was created by Figdraw (www.figdraw.com).

Abbreviations: Exos, exosomes; RNA, Ribonucleic acid; MVB, Multivesicular body.

encapsulate a diverse array of bioactive molecules, including nucleic acids and proteins.²⁹ The protective layer and compact size of exosome-carried RNA enable its transport between donor and target cells through fusion with the membranes of target cells. This mechanism is essential in cell communication and the modulation of multiple physiological functions with high efficiency and stability. To date, according to the ExoCarta database (www.exocarta.org), independent studies have identified 2838 miRNAs, 3408 mRNAs, and 9769 proteins in exosomes derived from various organisms.

Accumulating evidence shows that exosomes are highly heterogeneous, and their biological activities depend strongly on the cellular origin and physiological context of donor cells. Exosomes derived from mesenchymal stem cells, osteoblasts, osteoclasts, macrophages, endothelial cells, chondrocytes and Schwann cells exhibit distinct RNA, protein and lipid signatures, which translate into markedly different effects on osteogenesis, angiogenesis, immune regulation and neuro-skeletal coupling.^{30–32} Importantly, even exosomes from the same cell type display substantial intra-population heterogeneity. Subsets differ in tetraspanin composition (CD9/CD63/CD81), size, membrane lipids, and sorting of RNA–protein cargo, which are shaped by variable stimuli such as hypoxia, mechanical loading, inflammation, or metabolic stress.^{33,34} Recognizing these layers of heterogeneity is essential for accurately interpreting exosome-

mediated communication in the bone microenvironment and provides a biological foundation for understanding their divergent effects across different stages of bone regeneration.

Foundational Biology of Exosomes for Bone Generation and Repair

Unique Contents of Exosomes Associated with Osteogenesis

Researchers have identified a total of 1536 proteins present in osteoblast-derived exosomes, with 172 of these proteins overlapping with those in the bone database.³⁵ Notably, exosomal ephrinB1, BMP receptor type-1, lipoprotein receptor-related protein 6, transforming growth factor beta receptor 3, and Smad ubiquitylation regulatory factor-1 have been identified to potentially play significant roles in bone formation. These proteins may be involved in various osteogenesis-related signaling pathways, such as the BMP–SMAD–RUNX2 and Wnt/ β -catenin pathways. Furthermore, proteins derived from osteoblast exosomes also play a role in the signaling pathway of eukaryotic initiation factor 2 (EIF2), which is essential for the differentiation process of osteoblasts.³⁶

MicroRNAs (miRNAs) are short noncoding RNAs (19–24 nt) that regulate gene expression post-transcriptionally and are essential for multiple stages of bone development.³⁷ When encapsulated in exosomes, miRNAs are protected from RNases and environmental stress, enabling stable transfer between bone-resident cells and long-range regulation within the osteogenic microenvironment.³⁸ Multiple studies have demonstrated that osteoblast-, osteocyte-, macrophage-, and MSC-derived exosomes modulate BMSC proliferation, osteogenic differentiation, angiogenesis, and bone regeneration through miRNA-mediated signaling pathways. Representative exosomal miRNAs, such as miR-26a,³⁹ miR-29a,⁴⁰ miR-1260a,⁴¹ miR-451a,⁴² miR-21-5p,⁴³ miR-126,⁴⁴ miR-668-3p,⁴⁵ miR-133b-3p,⁴⁶ and miR-144-5p,⁴⁷ have been shown to exert pro-osteogenic effects through pathways such as Wnt/ β -catenin, PI3K–Akt, YAP1, MIF, and TGF- β /Treg signaling, collectively supporting osteoblast lineage commitment and extracellular matrix formation. Other exosomal miRNAs (eg, miR-140-3p,⁴⁸ miR-218,⁴⁹ miR-125⁵⁰ and miR-885-5p⁵¹) exert anti-osteogenic effects by repressing key transcriptional and signaling mediators, including RUNX2, DLX5, BMP2, and SMAD1. These findings indicate that both upregulated and downregulated exosomal miRNAs collectively shape osteogenesis by fine-tuning lineage specification, mechano-transduction, and osteoimmune interactions. A comprehensive summary of currently reported osteogenesis-related exosomal miRNAs, including their cellular sources, molecular targets, regulatory effects, and expression patterns, is presented in Table 1.

Table 1 Exosomal miRNAs Implicated in Osteogenic Differentiation and Bone Regeneration

miRNA	Exosome Source	Expression	Main Target/Pathway	Effect on Osteogenesis	Reference
miR-26a	BMSC-Exos	↑	RUNX2/ALP	Enhances osteogenic differentiation + mineralization	Lai et al 2023 ³⁹
miR-29a	BMSC-Exos	↑	Osteogenic & angiogenic pathways	Promotes angiogenesis + osteoblast differentiation	Lu et al, 2020 ⁴⁰
miR-1260a	BMSC-Exos	↑	Wnt/angiogenic pathways	Promotes osteogenesis + angiogenesis	Wu et al 2021 ⁴¹
miR-451a	ADSC-Exos	↑	MIF	Induces M2 polarization to enhance bone healing	Li et al, 2022 ⁴²
miR-21-5p	ESC-Exos	↑	YAP1	Promotes osteoblast differentiation	Huang et al 2024 ⁴³
miR-126	Hypoxic MSC-Exos	↑	VEGF signaling	Enhances fracture healing and angiogenesis	Liu et al 2020 ⁴⁴
miR-668-3p	BMSC-Exos	↑	Not specified	Promotes osteoblast proliferation + differentiation	Qiu et al 2024 ⁴⁵
miR-133b-3p	Exosomes in osteoporosis models	↑	TGF- β 1/Treg axis	Improves osteoporosis via immunomodulation	Zhao et al 2025 ⁴⁶
miR-144-5p	Macrophage-Exos	↑	Smad1	Impairs fracture healing	Zhang et al 2021 ⁴⁷
miR-140-3p	BMSC-Exos	↓	Pixnbl-RhoA/ROCK	Restores osteogenesis in diabetic bone loss	Wang et al 2022 ⁴⁸
miR-218	Osteocyte-Exos	↓	Wnt pathway	Promotes osteogenesis	Qin et al 2017 ⁴⁹
miR-125	OA chondrocyte- Exos	↑	Not specified	Disrupts subchondral bone homeostasis	Guan et al 2025 ⁵⁰
miR-885-5p	hBMSC-derived exosomes	↓	BMP2-RUNX2 signaling	Suppresses osteogenic differentiation	Xu et al 2014 ⁵¹

Circular RNA (circRNA), recognized for its stable noncoding RNA form generated through backsplicing and characterized by covalently closed loop structures, has recently emerged as a crucial regulator of osteogenesis.^{52,53} CircRNAs are enriched in exosomes and have been reported to interact with miRNAs related to osteogenesis. Shaoyang Ma et al discovered that circHIPK3 is abundant in BMSC-derived exosomes and enhances the osteogenic differentiation of MC3T3-E1 cells by modulating mitophagy through the miR-29a-5p/PINK1 pathway.⁵² Few reports have been published describing how exosomal circRNAs impact mitophagy during osteogenesis, and further studies are needed to explore this hypothesis in the future. Messages are pragmatically conveyed between cells through exosomes not only in various physiological contexts but also in pathological situations. Exosomes derived from circ-Rtn4-altered BMSCs mitigate TNF- α -induced cytotoxicity and apoptosis in mouse MC3T3-E1 cells by acting as sponges for miR-146a.⁵⁴ The cellular sources of exosomes have been the focus of extensive research in recent years. A particularly intriguing phenomenon identified by stomatologists is the increased expression of circLPAR1 in exosomes derived from dental pulp stem cells (DPSCs) during osteogenic differentiation. Furthermore, they confirmed that these exosomal circLPAR1 molecules induce an osteogenic effect on recipient homotypic DPSCs by upregulating SATB2 expression through a competitive interaction with hsa-miR-31.⁵⁵ Compared with other sources of MSCs, DPSCs exhibit unique characteristics, as they are more accessible with minimal trauma, potentially representing a novel approach for the use of exosomes in the treatment of bone deficiency (Figure 2). Recent studies have revealed that the expression of circRNAs is negatively correlated with their osteogenic differentiation ability. For example, Feng Li et al reported a significant negative correlation between elevated levels of circFAM63B in exosomes from postmenopausal patients with osteoporosis and their reduced bone density.⁵⁶ Liangkun Xie et al demonstrated that exosomal circRNAs exhibited

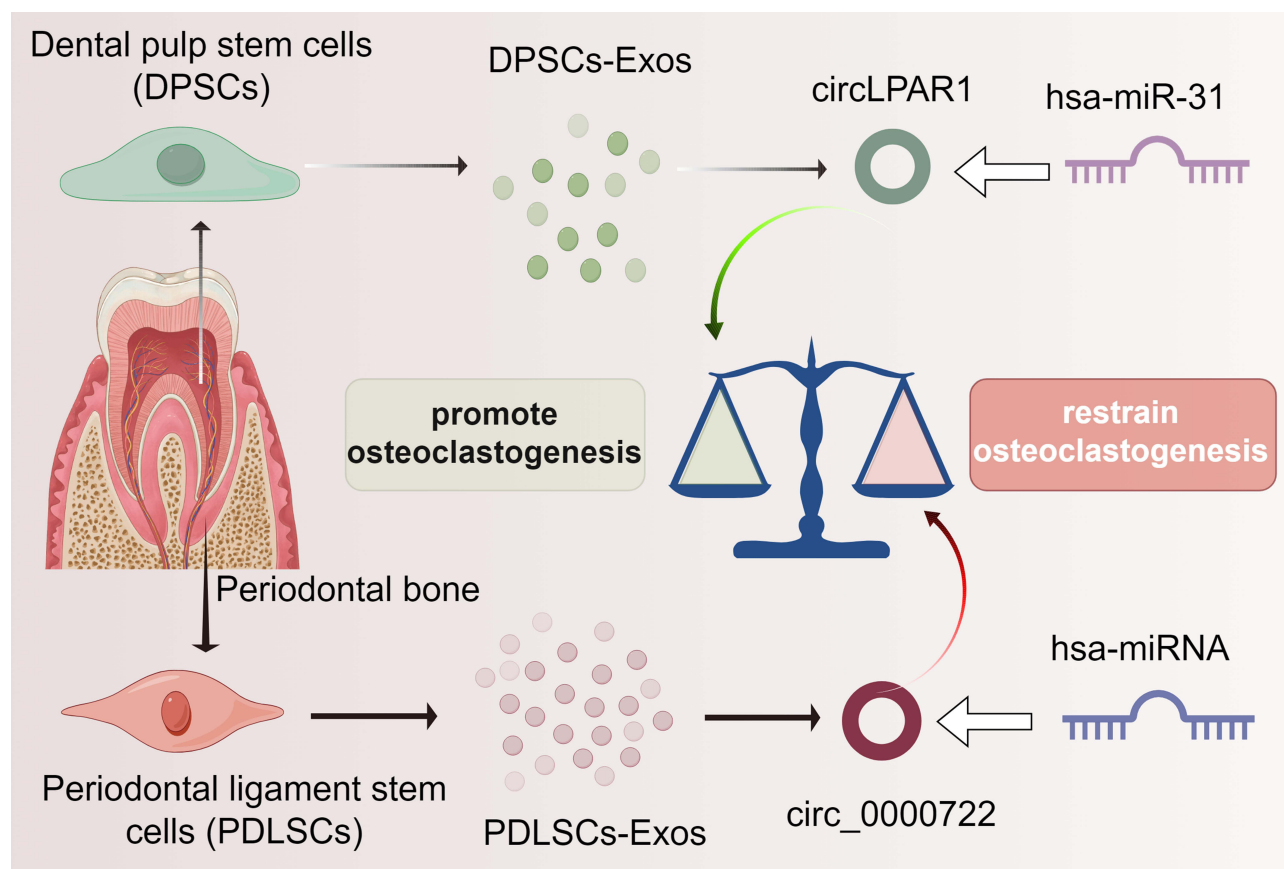


Figure 2 Odontogenic cells derived exosomes and their associated circRNAs on osteogenesis. This figure shows how exosomes (Exos) from dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs) influence osteogenesis. DPSCs-derived circLPAR1 promotes osteogenesis by interacting with hsa-miR-31, while PDLSCs-derived circ_0000722 enhances osteoclastogenesis via the TRAF6, AKT, and NF- κ B pathways. These exosomal circRNAs regulate bone formation and remodeling. The figure was created by Figdraw (www.figdraw.com).

notable changes in expression during the initial stages of osteogenic differentiation in periodontal ligament stem cells (PDLSCs).⁵⁷ Their further investigation revealed that exosomes containing circ_0000722 from PDLSCs undergoing osteogenic differentiation may increase osteoclastogenesis by increasing the expression of TRAF6 and activating the AKT and NF- κ B pathways (Figure 2).⁵⁸ Moreover, recent studies have revealed the significant role that exosomal circRNAs play in bone growth evolution. Exosome-derived hsa_circ_0063476 inhibits the expression of endochondral ossification markers and disrupts longitudinal bone growth through miR-518c-3p/DDX6,⁵⁹ whereas exosomal circRNA_0001236 overexpression increases the expression of cartilage-related genes and proteins via miR-3677-3p/Sox9.⁶⁰ These studies provide important new perspectives on how exosomal circRNAs regulate bone generation and remodeling through both positive and negative regulatory mechanisms. The relevant circRNAs are summarized in Table 2, and targeting these circRNAs may represent a novel therapeutic approach in the future.

Exosomes in the Bone Formulation Network

Exosomes and Matrix Vesicles: Counterparts or Interacting Partners for Initial Calcification in Bone?

Investigations into the calcification mechanism of skeletal structures have been conducted over an extended period. Studies have revealed that a characteristic shared by nearly all normal mineralization processes is the production of extracellular vesicles (EVs)—small membrane-bound particles in the nanometer size range. Both matrix vesicles (MVs) and exosomes participate in bone mineralization, yet accumulating evidence indicates that they originate from distinct biogenetic pathways and different functions in bone formation, despite their partial overlap in size and occasional sharing of tetraspanins.^{61–63} Biochemical and ultrastructural studies show that MVs are matrix-anchored vesicles enriched in phosphatidylserine, annexins, TNAP, and PHOSPHO1, enabling them to concentrate Ca^{2+} and PO_4^{3-} and initiate

Table 2 CircRNA Related to Osteogenesis in the Exosomes

Exosome Source	RNA	Axis	Biological Effect	Ref
Exosomes derived from BMSCs	CircHIPK3	CircHIPK3/miR-29a-5p/PINK1 axis	Exosomal circHIPK3 promotes osteogenic differentiation of MC3T3-E1 cells through regulating PINK1 expression by sponging miR-29a-5p	[52]
Exosomes were separately collected from MC3T3-E1 cells	Circ_0008542	Circ_0008542 /miR-185-5p/RANK axis	Circ_0008542 acts as a miRNA sponge for miR-185-5p and promotes osteoclast-induced bone resorption through the miR-185-5p/RANK axis	[53]
Exosomes-derived from circ-Rtn4-modified BMSCs (Rtn4-Exos)	Circ-Rtn4	Circ-Rtn4/ miR-146a	Rtn4-Exos attenuated TNF- α -induced cytotoxicity and apoptosis in murine MC3T3-E1 cells by sponging miR-146a	[54]
Exosomes from osteogenic-induced DPSCs	CircLPAR1	CircLPAR1/ hsa-miR-31/ SATB2	Exosomes had the osteogenic effect on the recipient homotypic DPSCs via exosomal circLPAR1 that upregulated SATB2 expression by competitively binding to hsa-miR-31.	[55]
HBMSCs exosomes	CircFAM63B	Circ_0008542/miR-578/HMGA2 axis	Exosomal circFAM63B suppresses the bone regeneration of PMOP by regulating the miR-578/HMGA2 axis.	[56]
Periodontal ligament stem cells exosomes	Circ_0000722	NF- κ B and AKT signaling pathways	Exosomal circ_0000722 might promote osteoclastogenesis by upregulating TRAF6 expression and activating downstream NF- κ B and AKT signaling pathways, which further induced transcription of NFATc1 and c-Fos.	[58]
Plasma exosomes	Hsa_circ_0063476	Hsa_circ_0063476/miR-518c-3p /DDX6 axis.	Exosome-derived hsa_circ_0063476 retards the expression of markers of endochondral ossification and impairs longitudinal bone growth via miR-518c-3p/DDX6 axis	[59]
Exosomes derived from bone marrow mesenchymal stem cells	CircRNA_0001236	CircRNA_0001236/miR-3677-3p/Sox9	Overexpression of exosomal circRNA_0001236 promoted cartilage-specific gene and protein expression through the miR-3677-3p/Sox9 axis.	[60]

intravesicular apatite nucleation, often incorporating mitochondrial Ca-P granules to form early “calcifying globules”.^{64,65} Their firm attachment to collagen fibrils and the presence of characteristic “crystal ghosts” further indicate that MVs act as microreactors for the onset and spatial patterning of mineral deposition.⁶⁶ In contrast, exosomes are nonadherent, freely diffusible vesicles that mediate long-range communication by delivering osteogenic miRNAs, proteins, and matrix-modifying factors that regulate osteoblast differentiation, angiogenesis, immune balance, and neuroskeletal signaling.⁶⁷ Thus, despite phenotypic overlap, current evidence supports the view that MVs and exosomes represent functionally distinct but complementary vesicle populations, with MVs initiating primary mineral nucleation and exosomes orchestrating broader regulatory signaling within the osteogenic microenvironment.

MVs act as the primary initiators of bone mineralization. During early mineral deposition, the rupture of MVs leaves behind characteristic “crystal ghosts” that help direct the outward expansion of nascent mineral clusters. Their stable anchorage within the extracellular matrix also places them at sites where mineral deposition must be spatially regulated during endochondral ossification. Through this combination of localized enzymatic activity and matrix-bound positioning, MVs function as microreactors that define both the onset and spatial patterning of mineral formation, distinguishing their role from the broader signaling functions attributed to exosomes.^{66,68} Exosomes exert broad regulatory functions throughout osteogenesis. Exosomes released by osteoblasts, osteocytes, and MSCs carry osteogenic microRNAs, signaling mediators, and ECM-modulating proteins that promote osteoblast differentiation, enhance matrix deposition, and coordinate extracellular matrix remodeling.^{69,70} Their capacity to move freely within the extracellular space enables them to distribute regulatory cues beyond the immediate mineralization front, supporting long-range intercellular communication and integrating osteogenic, angiogenic, and immunomodulatory signals essential for organized tissue development. Through this multifaceted signaling network, exosomes help fine-tune matrix organization and sustain the cellular interactions that drive progressive bone formation. Although exosomes and MVs exhibit distinct functional tendencies, current evidence remains insufficient to determine whether they undergo coordinated changes in secretion dynamics, cargo composition, or spatial distribution across osteogenic stages. Existing studies instead indicate that the two vesicle populations participate in osteogenesis through partially overlapping yet functionally biased roles: MVs mainly initiate and spatially anchor early bone mineralization, whereas exosomes predominantly modulate matrix remodeling and intercellular signaling as bone formation progresses. These patterns suggest a complementary, stage-associated mode of action, while still allowing for potential convergence or shared pathways that have not yet been fully elucidated. Future studies integrating spatial transcriptomics or EV-tracking tools may help elucidate whether these populations interact cooperatively within the bone microenvironment.

Exosome-Extracellular Matrix Interactions and Dynamic Remodeling

The extracellular matrix (ECM) constitutes an essential regulatory unit within the bone microenvironment, functioning far beyond a passive structural scaffold. Through biochemical cues (eg, integrins, matricellular proteins, proteases) and biomechanical properties (eg, stiffness, topography), the ECM orchestrates cell adhesion, migration, proliferation, and lineage commitment. Increasing evidence indicates that exosomes dynamically interact with the ECM, serving both as modulators and responders within this regulatory network.^{26,71}

Exosomes released into the bone extracellular matrix (ECM) actively modulate matrix architecture through both proteolytic and non-proteolytic pathways. Beyond functioning as passive vesicular carriers, exosomes serve as targeted delivery vehicles for matrix-remodeling enzymes and regulatory molecules. They have been shown to contain proteases such as MMP2, MMP9, MMP14, ADAM10/17, and the glycosidase hyaluronidase, together with upstream activators (eg, BMP).^{72–74} Acting synergistically, these vesicle-associated factors can modulate extracellular matrix organization and turnover, thereby contributing to the formation of microenvironments that facilitate osteoblast recruitment and mineralization processes. Once released, exosomes can also become embedded within the extracellular matrix (ECM), where they act as structural and biochemical modulators. Notably, the phosphatidylserine-rich surface of exosomes can promote hydroxyapatite nucleation, and this mineralization process is further supported by calcium bound to exosomal annexins and phosphate generated by exosomal ATPases, nucleotidases, phosphatases, and related transporters.^{75,76} In parallel, exosomal microRNAs such as miR-21, miR-375 and miR-140-5p regulate expression of osteogenic and ECM-related genes, thereby contributing to matrix maturation and stabilization of nascent osteoid tissue.^{77–79} Through these

reciprocal degradation-and-reconstruction activities, exosomes fine-tune ECM renewal dynamics, the formation of mineralization-competent niches, and the maintenance of microenvironmental integrity throughout bone modeling and remodeling.

At the same time, the ECM actively governs extracellular vesicle mobility, retention, and uptake: the densely crosslinked collagen network can serve as a three-dimensional vesicle depot, while size-selective filtering, electrostatic interactions and ligand–receptor anchorage collectively dictate their sequestration, lateral diffusion, and spatial distribution.^{80–82} Integrins expressed on exosome membranes—including $\alpha 5\beta 1$, $\alpha v\beta 3$, and $\alpha 2\beta 1$ —bind specific ECM ligands such as collagen, fibronectin, and vitronectin, enabling haptotactic positioning and guided paracrine signaling within confined bone niches.⁸³ Although direct evidence in bone is limited, studies showed that ECM mechanics regulate exosome behavior—for example, stiff matrices enhance secretion via the Akt–Rab8 pathway—implying that matrix stiffness and mineral architecture may similarly shape exosome distribution in osteogenic niches.⁸⁴ In addition, ECM structure and hydration influence extracellular vesicle diffusion and retention, generating localized vesicle-enriched zones that may help sustain paracrine signaling during regeneration.^{80,85} Together, exosome-ECM interactions represent a dynamic bidirectional axis within the bone microenvironment, linking biochemical signaling, matrix remodeling, and biomechanical regulation. Recognizing this interplay is also crucial for the rational design of biomaterials, as engineered scaffolds increasingly aim to mimic the natural ECM to optimize exosome retention, stability, and therapeutic efficacy.

Exosomes Modulate the Osteogenesis–Angiogenesis Interaction and Coupling

Bone formation is an intricate process that requires exquisite coordination among various cellular functions. Previous studies have focused primarily on stimulating the individual functions of cells. However, bone is recognized as a complex structure that is highly vascularized.⁸⁶ The blood vessels within bone deliver vital elements, including oxygen, nutrients, and growth factors, and cells, such as immune, stem, or precursor cells, to the surrounding microenvironment. This finding raises an important question: how does cell-to-cell communication and crosstalk between different functions occur? As research progresses, osteogenesis and angiogenesis have been shown to exhibit close spatiotemporal coordination during bone formation.⁸⁷ Exosomes have been identified as key players in paracrine signaling, serving as crucial carriers for intercellular communication. Notably, in a rat model of stabilized fracture, a previous study demonstrated that exosomes derived from human umbilical cord mesenchymal stem cells accelerate fracture healing via HIF-1 α -driven angiogenesis.⁸⁸ Furthermore, the role of exosomes in osteogenesis–angiogenesis coupling has been revealed to be more complex. Studies have shown that exosomes derived from endothelial cells (EC-Exos) initiate a positive feedback mechanism in the interplay between osteogenesis and angiogenesis.⁸⁷ From a mechanistic perspective, EC-Exos increase the expression of BTB domain-containing 16 in BMSCs, facilitating the transformation of the osteoprogenitor phenotype. Osteoprogenitors facilitate the development of type H endothelial cells (H-ECs) by triggering HIF-1 α signaling, thereby enhancing the osteogenic differentiation of BMSCs. Furthermore, the use of BMSC-Exos during the osteogenesis–angiogenesis coupling process is of significant interest. Molecular biology experiments have provided detailed insights indicating that BMSC-derived exosomes effectively stimulate the osteogenic differentiation of BMSCs and angiogenesis in ECs. These effects are associated with an increased presence of lncRNA-19 in BMSC-derived exosomes, which facilitates the interaction between BMSCs and ECs during bone homeostasis through the lnc-H19-Angpt1/Tie2 signaling pathway.⁸⁹ The significance of osteogenic–angiogenic coupling in bone has been hypothesized by N. Shen et al,⁹⁰ who further illustrated a novel mechanism whereby mechanical loading influences blood vessel formation through the release of extracellular vesicles from mature bone cells, such as osteoblasts and osteocytes.

Exosomes in the Cross-Talk Between the Immune System and Bone

Bone is a living organ characterized by a substantial presence of both bone and immune cells within its niche. Discussions of the activity of bone cells in isolation from their ecological context lack practical significance, particularly in pathological states. Factors such as infection, trauma, transplantation, and autoimmunity can contribute to alterations in the bone–immune microenvironment, leading to an imbalance in bone homeostasis. For example, macrophages (Ms),

the most important defensive cells of innate immunity, tend to polarize to the M1 phenotype in patients with periodontitis, osteoporosis, or diabetes mellitus.^{91–93} M1 macrophages play a proinflammatory role, which is critical for the removal of bacteria and necrotic tissue; however, an excessively sustained inflammatory response can inhibit the functions of MSCs and ECs and can even lead to increased bone resorption. Conversely, M2 macrophages mediate the anti-inflammatory response and regulate bone repair.^{9,94–97} In this context, researchers have hypothesized that M2-derived exosomes (M2-Exos), which are important communication substances, would promote the osteogenic differentiation of MSCs. This conjecture has been further validated. Pathway-specific investigations have demonstrated that the characteristics of exosomes originating from M2 macrophages enhance the luciferase reporter activity stimulated by BMP2, which is regulated by a promoter specific to SMAD1/5/8. In contrast, M1 macrophage-derived exosomes exert negative regulatory effects on MSCs, particularly affecting the expression of BMP2 and BMP9.⁹⁸ In vitro experiments have shown that M2-Exos facilitate the osteogenesis of BMSCs and inhibit adipogenesis through the miR-690/IRS-1/TAZ axis.¹⁵ A recent study consistently demonstrated that M2-Exos stimulate the osteogenic differentiation of BMSCs, reduce their adipogenic differentiation capacity, and increase the expression of SOX and aggrecan. Furthermore, M2-Exos accelerate extracellular matrix (ECM) remodeling by decreasing the levels of matrix metalloproteinase 13.⁹⁹ Notably, a recently published study indicated that M2-Exos reduced osteonecrotic alterations in a rat model of steroid-induced osteonecrosis of the femoral head (ONFH).¹⁰⁰ M2-Exo administration ameliorated inflammatory responses by increasing the expression levels of proinflammatory factors such as TNF- α and IL-6 while inhibiting the expression of the anti-inflammatory factor IL-10. Moreover, M2-Exos promoted osteogenesis and angiogenesis in vivo. Consistent with findings from previous studies, M2-Exos also reduced adipogenesis or even osteoclastogenesis in vivo.

The polarization of M1 and M2 macrophages indicates that the tissue is primarily in a state of damage or repair. The question of whether a conversion between these different cellular phenotypes can create a beneficial bone immune microenvironment therefore arises. In vivo investigations have revealed that M2-Exos can act as immunomodulators, facilitating the conversion of macrophages from the M1 phenotype to the M2 phenotype by influencing the PI3K/AKT pathway.¹⁰¹ Research on a rat model of ONFH showed that treatment with M2-Exos suppressed M1 macrophage infiltration and enhanced M2 macrophage polarization in the femoral head, which may accelerate tissue repair.¹⁰⁰ Given that excessive M1 macrophages are detrimental to bone regeneration,^{9,94–97} the question of whether the adverse effects can be mitigated by directly improving M1 function remains. Interestingly, research has indicated that exosomes derived from human serum (Serum-Exos) can modulate macrophage inflammation, enhancing VCAM1-mediated processes of angiogenesis and bone regeneration.¹⁰² Serum-Exos reduced lipopolysaccharide (LPS)-induced expression of IL-1 β , IL-6, iNOS, and the inflammatory macrophage marker CD86. When M1 macrophages were treated with Serum-Exos, their conditioned medium (CM) promoted proliferation, migration, and angiogenic differentiation in human umbilical vein endothelial cells (HUVECs), along with elevated levels of the H-type blood vessel markers CD31 and endomucin. Additionally, increased VCAM1 expression was observed in HUVECs. The local application of Serum-Exos in mandibular bone defect repair resulted in increased angiogenesis and osteogenesis. Exosome-based communication between the immune and bone microenvironments is bidirectional. These findings suggest that exosomes from BMSCs support osteogenic differentiation by regulating M2 macrophage polarization through the ubiquitination and degradation of myeloid cells mediated by tripartite motif 25.¹⁰³ Overall, this research may provide valuable insights for therapies targeting delayed bone healing due to the imbalance between M1 and M2 macrophages.

Exosomes Mediate Osteoclast–Osteoblast Communication and Balance

Bone formation is ultimately achieved through the precise coordination of two fundamental processes: osteoclast-mediated bone resorption and osteoblast-mediated bone formation. These processes do not occur randomly on the bone surface; instead, they occur at particular anatomical locations and adhere to a clearly defined series of events that are supported by intercellular communication.^{104,105} The interaction of these two fundamental processes across time and space within the bone's environmental substrates raises an important question: How do they influence each other? The interaction between RANKL and receptor activator of nuclear factor-kappa B (RANK) lies at the core of bone biology.¹⁰⁶ An article published in *Nature* suggested that small extracellular vesicles containing RANK, which are released by maturing osteoclasts, bind to RANKL on osteoblasts. These vesicles serve as “coupling factors” that may

promote new bone formation in areas of osteoclastic bone resorption by initiating RANKL reverse signaling, which in turn activates Runt-related transcription factor 2 (Runx2).¹⁰⁷ Investigations using electron microscopy have revealed that RANK in EVs is concentrated in a small fraction of the total EVs released by osteoclasts, with a diameter of approximately 40 nm, which is consistent with exosome characteristics, as the markers CD63 and EpCAM are abundant, whereas Golgi and endoplasmic reticulum markers are absent.^{108,109} Furthermore, the data suggest that exosomes enriched with RANK may interact with RANKL on osteoblasts, thereby acting as competitive inhibitors of the RANKL–RANK interaction via osteoprotegerin (Figure 3).¹⁰⁸ Notably, RANK within small extracellular vesicles is proposed to interact with RANKL as a trimer, forming the high-affinity heterohexameric RANKL–RANK complex. Thus, osteoclast-derived Exo-RANK may target RANKL, and these exosomes may transfer their luminal cargo, including proteins, mRNAs, and microRNAs, into the cytosol of target cells to potentially “instruct” osteocytes, osteoblasts, or other cells expressing RANKL, thereby exerting regulatory effects. Conversely, a notable upregulation of miR-214 and ephrinA2 has been detected in serum exosomes from both osteoporotic patients and mice. Further studies

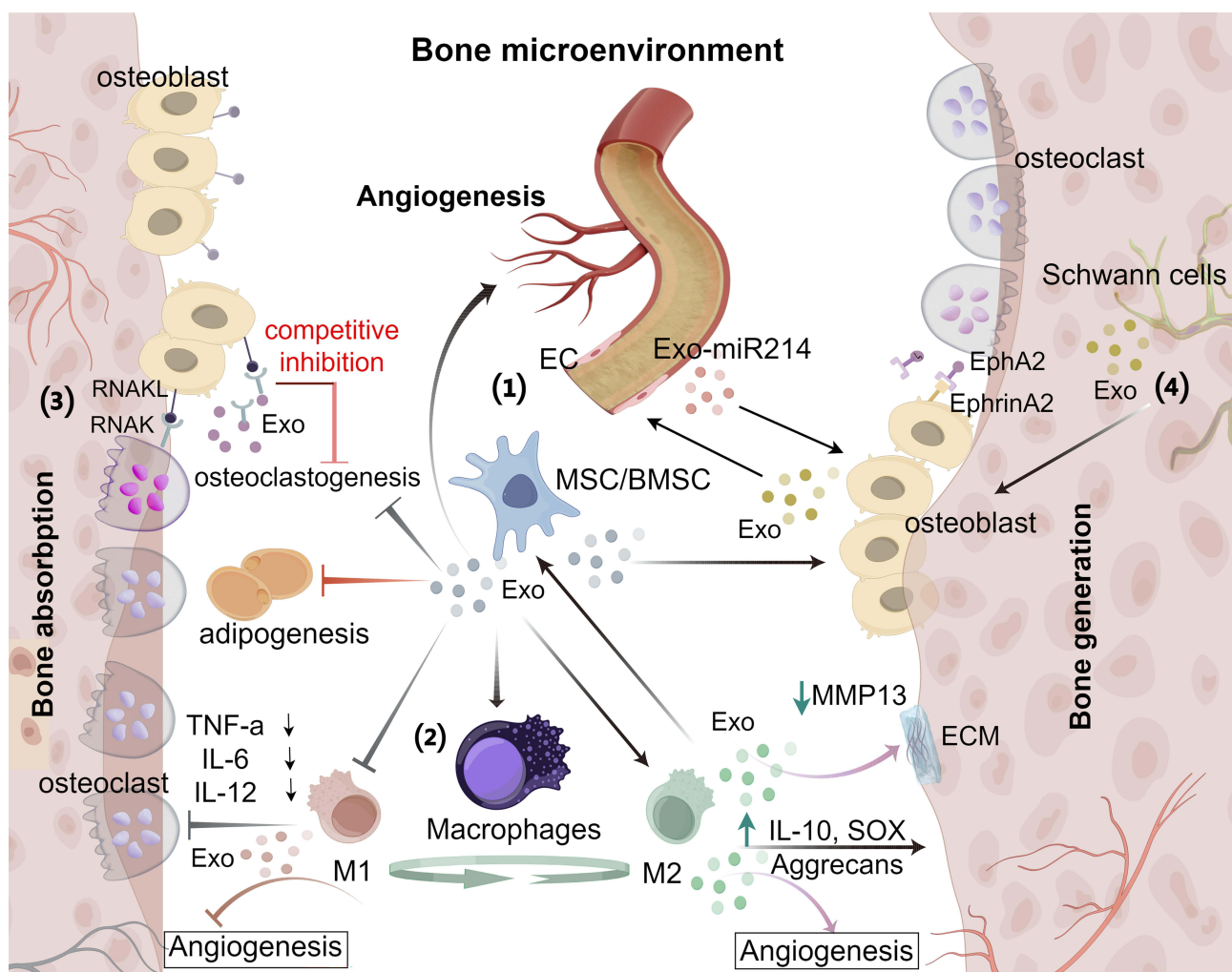


Figure 3 Exosomes act as versatile messengers fine-tuned for complex intercellular crosstalk in bone microenvironment. This process encompasses multiple functional units: (1) Vascular unit: endothelial cell-derived exosomes (EC-Exo) initiate a positive feedback loop in the osteogenesis-angiogenesis process. Bone marrow stem cell-derived exosomes (BMS-Exo) effectively stimulate osteogenic differentiation and angiogenesis while inhibiting adipogenesis; (2) Immune units: Exosomes secreted by M2 macrophages (anti-inflammatory) promote osteogenesis and reduce adipogenesis, and may even inhibit osteoclastogenesis. In contrast, exosomes secreted by M1 macrophages (pro-inflammatory) inhibit osteogenesis. M2-derived exosomes can act as immunomodulators, facilitating the conversion of macrophages from M1 to M2. Furthermore, exosomes from BMSCs support osteogenic differentiation by regulating M2 macrophage polarization; (3) Bone resorption and formation units: Exosomes from osteoclasts, enriched with RANK, may interact with RANKL on osteoblasts, acting as competitive inhibitors of the RANKL-RANK interaction, similar to osteoprotegerin. However, osteoclasts also release exosomes carrying miR-214, which suppress osteoblast activity; (4) Neuro unit: The initial nerve supply in the bone defect region is favorable for the bone healing process, with exosomes mediating neuro-skeletal crosstalk. The figure was created by Figdraw (www.figdraw.com).

have suggested that osteoclasts release exosomes rich in miRNAs, primarily delivering miR-214 to osteoblasts through ephrinA2/EphA2 recognition, and thus suppressing osteoblast activity. Additionally, miR-214-containing exosomes can be released into the bloodstream, where circulating miR-214 may serve as a biomarker for bone loss.¹¹⁰ These results appear to contradict those previously published, which suggested that osteoclast-derived Exos improve bone formation. However, the exosomal contents vary at different stages of osteoclast development and between different physiological and pathological conditions. We speculate that Exo regulation is a dynamic process; these vesicles may be released or function at distinct temporal phases or, alternatively, act at different levels of the regulatory hierarchy during bone generation. After RANKL stimulation during osteoclastogenesis, osteoclasts release exosomes containing information that inhibits osteogenesis. Conversely, during the new bone formation phase, osteoclasts release exosomes that provide “brake” signals during osteoclastogenesis while simultaneously activating osteogenesis. This process may ensure that the bone resorption center is also the center of bone generation. Nonetheless, the exact mechanisms underlying this process require further investigation.

Increasing Attention to Neural Platforms Utilizing Exos in the Bone Microenvironment

Research has underscored the involvement of neuroskeletal, neurovascular, and neuroimmune interactions during various phases of bone healing.¹¹¹ Notably, a disruption of the inferior alveolar nerve (IAN) may lead to a reduction in the number of Schwann cells (SCs), which in turn results in inadequate bone healing due to functional deficits in skeletal stem cells.¹¹² Regenerative bone exhibits a degree of nerve dependency. However, the mechanisms by which regulatory signals are transmitted from neurons to the bone microenvironment remain poorly understood. Despite the close proximity of osteoblasts, immune cells, and vascular endothelial cells to free nerve endings, direct connections are infrequent, challenging the hypothesis of synaptic interactions. Recent evidence indicates that SC-derived exosomes induce the transformation of M1 macrophages into a reparative M2 phenotype and suppress inflammation during the inflammatory stage (Figure 4).¹¹³ The initial nerve supply in the bone defect region is essential for transitioning the bone healing process from inflammation to the fibrovascular stage. During the subsequent fibrovascular phase, these exosomes promote angiogenesis, providing both material support and a cellular source for bone remodeling. Finally, SC-derived

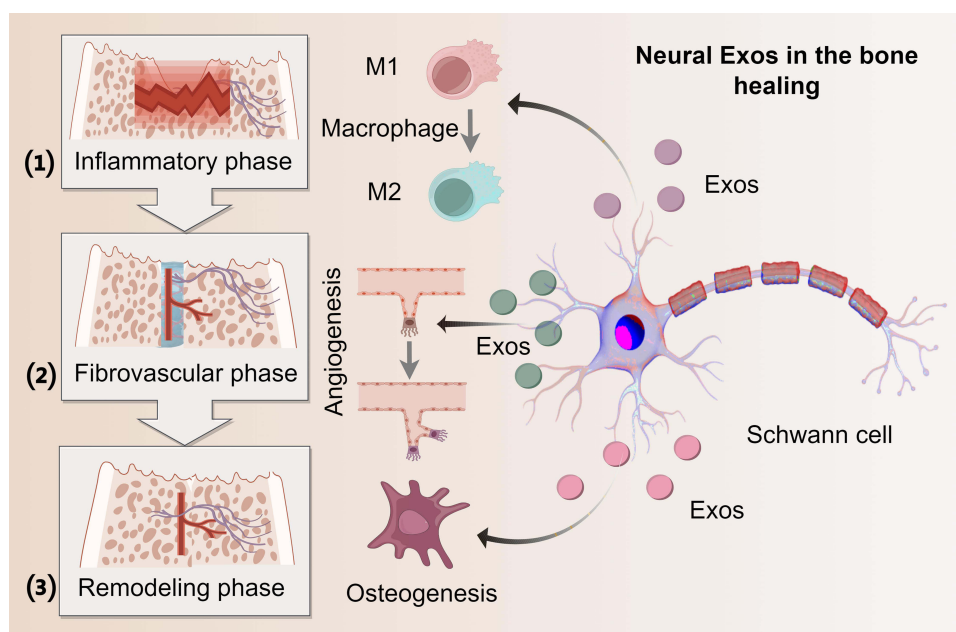


Figure 4 The neural platform utilizing exosomes in the bone microenvironment primarily regulates three major stages of bone repair: (1) the inflammatory phase, during which schwann cell-derived exosomes (SC-Exos) facilitate the conversion of M1 macrophages to a reparative M2 phenotype and attenuate inflammatory responses; (2) the fibrovascular phase, wherein SC-Exos promote angiogenesis, providing both material support and a cellular source for bone formation; (3) the bone remodeling phase, in which SC-Exos enhance osteogenesis by modulating the proliferation, migration, and differentiation of bone marrow-derived mesenchymal stem cells. The figure was created by Figdraw (www.figdraw.com).

Abbreviations: SC, Schwann cell; Exos, Exosomes.

exosomes facilitate bone formation by regulating the proliferation, migration, and differentiation of BMSCs through the activation of the TGF- β 1/SMAD2/3 pathway during the remodeling stage.¹¹³ Severe bone injuries are often accompanied by damage to the surrounding nerves, which results in a slow repair process, and SC-derived exosomes may compensate for the deficiency in neurological functions. Exosomes derived from MSCs stimulated with nerve growth factor (NGF) have been shown to enhance neurotrophic signaling and thereby potentiate osteogenic differentiation through PI3K–Akt and MAPK pathways.¹¹⁴

Recent studies further expand the understanding of how neural cues and neural-derived exosomes regulate bone regeneration. Bioprinted nerve–bone co-culture constructs demonstrated that peripheral nerves significantly enhance osteogenesis by modulating the bone microenvironment via secreted vesicular factors, including exosomes.¹¹⁵ SC-derived Exos have also been shown to accelerate periodontal bone regeneration by simultaneously promoting osteogenesis, angiogenesis, and neurogenesis, highlighting their ability to coordinate multiple regenerative axes within bone defects.¹¹⁶ Engineered SC-Exos can also be therapeutically enhanced, as acousto-electric fiber networks locally boost SC-exosomal miRNAs (eg, miR-494-3p, miR-381-3p, miR-369-3p) to activate PI3K–Akt and Wnt signaling in BMSCs, thereby promoting neurogenic bone repair.¹¹⁷ In addition, SC-derived exosomes have demonstrated the capacity to enhance the bioactivity of biomaterials, as evidenced by their ability to improve the osteogenic performance of porous Ti6Al4V scaffolds in vivo.¹¹⁸ The role of SCs exosomal signaling in pathological conditions is also being clarified. Under hyperglycemic conditions, SC-Exos carrying reduced levels of miR-15b-5p were shown to impair peri-implant osteogenesis through the TXNIP pathway; conversely, normal SC-derived exosomes restored osteogenic capacity in type 2 diabetic models.¹¹⁹ Together, these findings reinforce the concept that bone regeneration is highly dependent on neuro-skeletal interactions. SC-derived exosomes function not only as inflammatory modulators and angiogenic stimulators but also as potent osteoinductive vesicles. Their ability to traverse the bone microenvironment, deliver miRNA cargo, and coordinate multicellular communication suggests that neural–exosomal pathways represent an emerging and under-explored regulatory axis in bone tissue homeostasis and repair.

Designing Exosomal Biomaterials for Bone Generation

Exosome contents provide informative advantages over single growth factor strategies, which often fail to replicate the natural complexity of bone formation. Emerging evidence has provided profound insights into the fundamental biology of exosomes in osteogenesis and the interactions among different systems. Based on this biological background, how can an exosomal biomaterial be designed to enhance bone regeneration?

Exosome Optimization Design/Exosome Modification

Site-specific targeted exosomes generally fulfill the requirements for effective bone regeneration therapy. However, not all exosomes exhibit optimal bone-targeting capabilities. Moreover, unmodified exosomes may be rapidly sequestered and eliminated by the reticuloendothelial system when administered systemically. M2-Exos have been shown to attenuate inflammation and promote bone tissue repair; however, their therapeutic application is limited by a low response rate and unpredictable efficacy due to a lack of specific targeting properties. As a method to address this issue, M2-Exos functionalized with aptamers were developed and administered using 3-way junction RNA nanoparticles to precisely target bone fractures. Studies have shown that these aptamer-functionalized M2-Exos can effectively target BMSCs in vitro and localize to the fracture site following systemic intravenous injection. Further investigations have confirmed that the targeted action of these M2-Exos enhances bone formation.¹²⁰ Several additional stimuli can enhance the functions of exosomes. For example, macrophage-derived exosomes enhance the activity of endothelial cells and osteoblasts upon stimulation with Zn ions.¹²¹ Conversely, magnesium ion (Mg²⁺) stimulation may decrease the expression of macrophage-derived exosomes containing miR-381 through autophagy, thereby promoting the osteogenic differentiation of BMSCs.¹²² Interestingly, research has shown that engineered exosomes derived from BMSCs stimulated with lithium ions tend to polarize toward the M2 phenotype, thus improving the bone healing microenvironment.^{123,124} Furthermore, the mechanisms by which lithium promotes osteogenesis have been elucidated, highlighting the role of Rab11a-facilitated exosomal Wnt10a secretion and the activation of β -catenin signaling.¹²⁵ Additionally, the combination of shed-derived exosomes (Shed-exosomes) incorporated within a hyaluronic acid (HA)

hydrogel with copper ions (Cu^{2+}) displays synergistic osteogenic activity.¹²⁶ Another intriguing stimulation method for BMSCs involves the use of magnetic nanoparticles in conjunction with a static magnetic field, which leads to the release of exosomal miR-1260a, enhancing bone formation and promoting blood vessel growth.⁴¹ The conversion of mechanical stimuli into biological signals, known as mechanotransduction, is a widespread phenomenon. Notably, exosomes derived from BMSCs subjected to cyclic mechanical stretching have been shown to inhibit RANKL-induced osteoclastogenesis.¹²⁷ Further research has shown that exosomes derived from myoblasts stimulated by mechanical strain can be transferred to BMSCs, thereby facilitating osteogenic differentiation and positively impacting mice undergoing hormone-induced bone loss.¹²⁸ The osteogenic potential of Yoda1, an agonist of the mechanically sensitive ion channel Piezo1, has been confirmed. However, the hydrophobic nature of Yoda1 poses challenges for its effective incorporation into hydrogel matrices. Researchers have addressed this issue by utilizing exosomes derived from BMSCs pretreated with Yoda1 (Exo-Yoda1). These exosomes can rapidly and robustly transmit mechanical signals, thereby stimulating osteogenesis through the engagement of phospho-ERK signaling mediated by Yoda1.¹²⁹

Among the osteoinductive molecules identified, bone morphogenetic proteins, particularly BMP-2, have proven essential for bone regeneration. However, the clinical application of such agents is complicated by dosage challenges and potential immunological and neurological complications, as well as the risk of ectopic bone formation. Recent studies have demonstrated that exosomes derived from BMP2-treated macrophages significantly increase the expression of early osteoblastic differentiation markers, such as alkaline phosphatase (ALP) and BMP-2, in MSCs.¹³⁰ Furthermore, exosomes with enhanced osteoinductive capabilities can be engineered through the genetic modification of parental MSCs to overexpress BMP-2.^{131,132} Additionally, the selective inhibition of translation in donor cells allows the enrichment of Bmp2 mRNA in exosomes.¹³³ These strategies may represent novel approaches with advantages over the application of single BMP-2 factors for bone healing in the future. Hypoxia-inducible factor-1 α (HIF-1 α) performs dual functions by promoting both blood vessel regeneration and osteogenesis; however, its application is limited by rapid degradation (within minutes) by the body under normoxic conditions. Engineered BMSC-derived exosomes carrying mutant HIF-1 α facilitate faster bone healing by enhancing new bone formation and neovascularization.¹³⁴ Progenitor cell-derived exosomes endowed with VEGF plasmids exhibit a similar positive dual function.¹³⁵ Dendritic cell-derived exosomes (DC-Exos) exhibit a high affinity for inflamed areas and are absorbed by both DCs and T cells at the site of inflammation. When loaded with immunoregulatory cargo such as TGF β 1 and IL-10, these exosomes inhibit the maturation of recipient DCs and the induction of Th17 effectors while enhancing the recruitment of regulatory T cells. This process results in the suppressed expression of cytokines that promote bone resorption and a decrease in the loss of bone due to osteoclasts.¹³⁶ Under hyperglycemic conditions, the endoplasmic reticulum (ER) experiences significant stress and dysfunction, which can potentially result in osteogenic disorders. Sephin1, a specific small molecule designed to assist in protein folding,¹³⁷ was incorporated into MSC-Exos with the objective of mitigating ER stress by preserving ER proteostasis. Studies have revealed that these exosomes deliver Sep and provide SHP2 to recipient cells, activating mitophagy and removing mitochondrial reactive oxygen species (mtROS), which are direct causes of ER dysfunction.¹³⁸ Exosomal miRNAs significantly influence osteogenesis,¹³⁹ suggesting that modifying miRNA expression may enhance the functional capacity of exosomes. For example, engineered exosomes overexpressing miR-181b,¹⁴⁰ miR-375,⁷⁸ miR-29a,¹⁴¹ or miR-26a¹⁴² have been shown to improve osteointegration. Notably, the RNA content within exosomes does not reflect the RNA composition of their parent cells. However, microRNAs and transfer RNAs, which indicate the origin of the parent cells, are selectively packaged into exosomes.¹⁴³ Therefore, targeting genetic modifications of the parent cells from which the exosomes originate may be more appropriate, although the underlying mechanisms require further exploration.

Importantly, exosomal cargo is highly dependent on the tissue origin of the parent cells; thus, the current engineering of exosomes has focused primarily on the treatment of these parent cells. Direct encapsulation of exosomes often necessitates supplementary techniques to increase the cargo loading efficiency. For example, Mahmoud Elashiry et al¹³⁶ employed sonication to facilitate loading, whereas Zha et al¹³⁵ utilized electrical pulses to induce pore formation on the exosome surface, thereby increasing membrane permeability. Liu Y et al¹³⁸ conducted a comparative analysis of drug loading efficiencies between direct incubation and intermittent ultrasound methods, revealing that the latter method

exhibited significantly greater efficiency. Additionally, other strategies, such as incubation, extrusion, freeze–thaw cycles, and saponin assistance, are also employed to load cargo into exosomes.^{144,145}

It is worth noting that engineering strategies designed to enhance the therapeutic performance of exosomes differ substantially in their mechanisms, controllability, and application contexts. Surface modification approaches enable precise tissue homing and receptor-specific interaction but may require chemical manipulation that affects membrane integrity or scalability.^{146,147} Cargo-loading techniques, such as electroporation, sonication, extrusion, incubation, or endogenous genetic loading, allow the incorporation of exogenous nucleic acids, proteins, and small molecules to augment the biological activity of exosomes. Although these methods provide considerable functional flexibility, they face challenges such as variable loading efficiency, potential vesicle deformation, and protocol-dependent heterogeneity.^{148,149} By contrast, stimulation-based pretreatment of donor cells (eg, hypoxia, cytokine exposure, small-molecule priming) induces natural enrichment of therapeutic cargos and may enhance exosome yield and physiological relevance.^{150,151} However, this biological approach also offers less precise control over cargo composition and may introduce batch-to-batch variability.^{151,152} A comparative summary of these engineering approaches, including their mechanisms, advantages, limitations, and applicable scenarios, is presented in Table 3. Collectively, engineering strategies for exosome optimization address complementary aspects of therapeutic enhancement. Surface modification improves targeting precision, cargo-loading techniques expand functional versatility, and stimulation-based pretreatment enriches biologically relevant cargos while preserving vesicle integrity. Each strategy therefore offers distinct advantages and trade-offs, and their selection should be guided by specific therapeutic objectives, required delivery precision, and acceptable levels of engineering complexity. In bone regeneration applications, the optimal approach may involve combining strategies to balance targeting, bioactivity, and stability in the mineralized microenvironment.

Exosome-Functionalized Scaffolds/Bone Implants

Exosomes have gained recognition as potential agents for cell-free therapy in bone regeneration. However, their fluid-state form often falls short in addressing critical-sized bone defects, particularly in the context of structural reconstruction. Scaffolds play a crucial role in tissue engineering, as they are designed to replicate the architecture of the native tissue and frequently deliver mechanical, topographical, and bioinstructive signals to facilitate regeneration. Recent progress in 3D printing has greatly expanded the design flexibility, spatiotemporal control, and functional integration of exosome-based scaffolds for bone regeneration. 3D-printed architectures allow precise tuning of pore geometry, mechanical strength, and degradation kinetics, enabling stable immobilization of exosomes and their sustained release within defect sites. Attempts have been made to fabricate a bioactive 3D porous polylactic acid (PLA) scaffold modified with MSC-Exos for tissue defect repair (Figure 5A–C).¹⁵³ 3D-printed PCL scaffold provides an optimal porous framework for cell adhesion and supports efficient internalization and retention of progenitor cell–derived exosomes carrying VEGF plasmids, thereby enhancing osteogenic induction and vascular remodeling in large segmental bone defects (Figure 5D–I).¹³⁵ In another study, a laser-melted 3D-printed porous zinc scaffold filled with serum exosomes and poloxamer 407 thermosensitive hydrogel mimics trabecular bone architecture and achieves enhanced osteogenesis, inhibition of bone resorption, and improved angiogenesis during repair of rabbit radial defects.¹⁵⁴ More recently, an exosome-loaded hyaluronic acid hydrogel composite integrated with an oxygen-producing 3D-printed PLA scaffold

Table 3 Comparison of Major Engineering Strategies for Exosome Modification

Engineering Strategy	Key Mechanisms	Advantages	Limitations	Applicable Scenarios	References
Surface modification (ligand conjugation, PEGylation, click chemistry)	Chemical/biomimetic membrane modification	High targeting specificity; Improved distribution	Membrane disruption risk; Limited scalability	Tissue-specific targeting; Precision therapy	[146, 147]
Cargo loading (electroporation, sonication, extrusion, incubation, endogenous loading)	Incorporation of exogenous nucleic acids/proteins/drugs	Flexible cargo design; Functional enhancement	Variable loading efficiency; Vesicle deformation	Gene therapy; miRNA/siRNA delivery; Drug-EV systems	[148, 149]
Stimulation-based pretreatment (hypoxia, cytokines, biomaterial cues)	Parent-cell priming to enrich endogenous cargo	Maintains vesicle integrity; Increases bioactivity; Boosts yield	Limited cargo control; Batch variability	Immunomodulation; Angiogenesis	[150, 151]

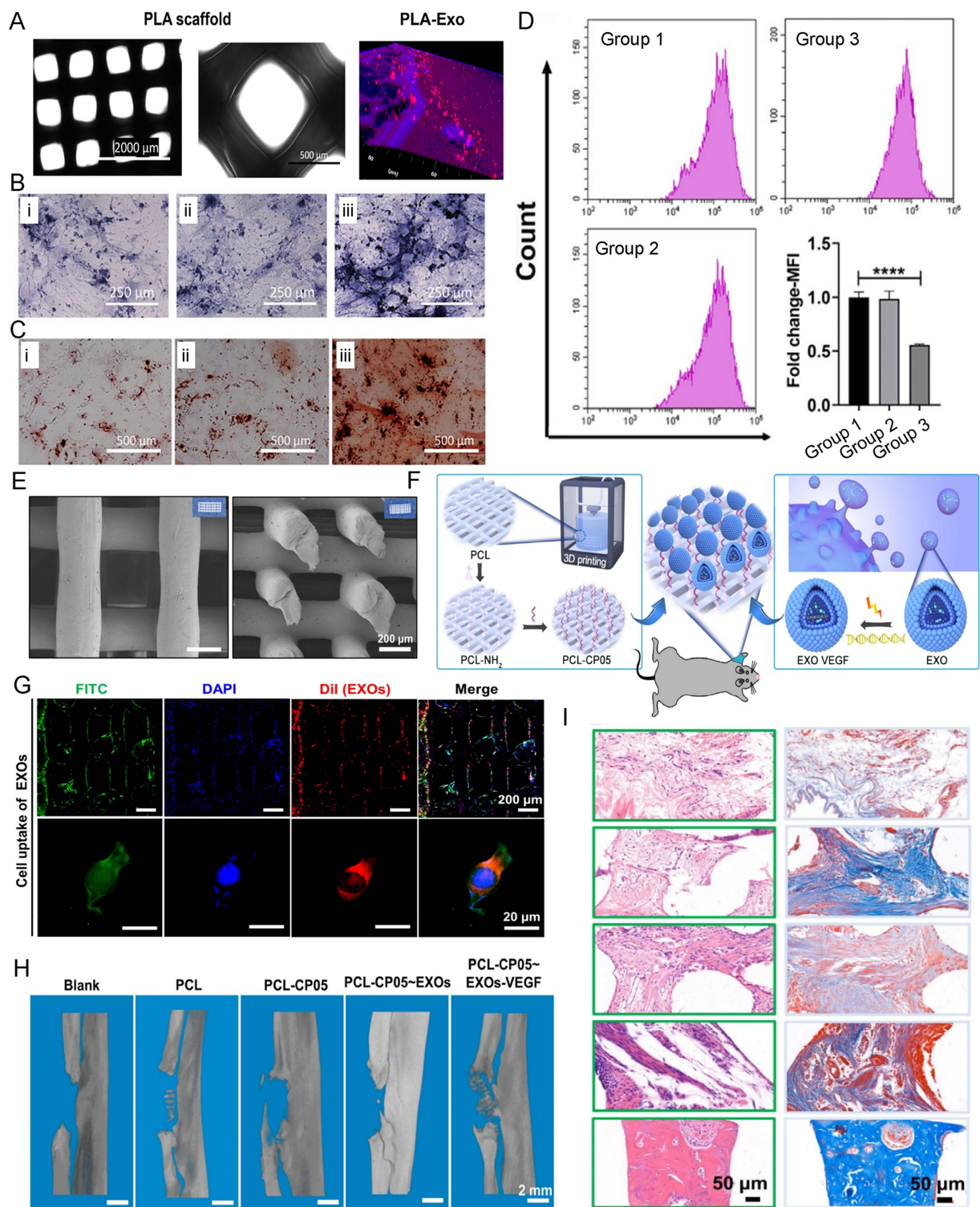


Figure 5 Poly(lactic acid) (PLA) and polycaprolactone (PCL) scaffold of modified with exosomes (Exos) for enhanced bone regeneration. **(A–C)** The 3D-printed PLA scaffold modified with PKH26-labeled Exos significantly decrease reactive oxygen species (ROS) in inflammatory macrophages, and improved ALP expression (Bi, Bii, Biii) and mineralization (Ci, Cii, Ciii) in hBMSCs. Group 1=control, group 2=PLA scaffold, group 3=PLA-Exo scaffold. Copyright 2022 The Author(s). Published by Springer Nature; **(D and E)** 3D-printed porous PCL scaffolds were functionalized with 1,6-hexanediamine and modified with a CP05 peptide linker, combined with ATDC5-derived exosomes encapsulating VEGF; **(F)** EXOs were internalized into 3D-printed scaffolds based on cell uptake; **(G)** Micro-CT images at 12 weeks showed the PCL-CP05-EXOs-VEGF group achieved the best repair and new bone formation in rat radial defect mo **(H)** HE and Masson staining at 12 weeks revealed new bone formation in the PCL-CP05-EXOs-VEGF group. Copyright Ivyspring International Publisher. **Note:** **** $P < 0.0001$.

(CaO₂-containing CPS) has been shown to alleviate hypoxia, modulate local inflammation, promote BMSC proliferation and ALP activity, and significantly improve new bone formation *in vivo*.¹⁵⁵ Collectively, these advances underline that 3D-printing-based strategies not only provide structural support but also offer precise control over exosome loading, release, and microenvironmental modulation, representing a rapidly evolving platform with considerable potential for personalized and biologically instructive bone regeneration.

Besides, the addition of MSC-Exos considerably improved the biofunctionality of the initial PLA scaffold, leading to significant decreases in the expression of proinflammatory markers and the production of reactive oxygen species (ROS) in inflammatory macrophages and high expression of osteoblastic markers and increased mineralization in hBMSCs. Recently, an exosome-based modification strategy has been employed to create dual biofunctional PLA scaffolds using a microdroplet (MD)-guided delivery system for nuclear factor of activated T cells cytoplasmic 1 (NFATc1). Subsequent cellular experiments have shown that MD-NFATc1/PLA-Exo scaffolds exhibit excellent biocompatibility, significantly reducing osteoclast formation while promoting the differentiation of osteogenic cells and altering cytokine expression.¹⁵⁶ Similarly, the immunoregulatory and osteogenic potential of polycaprolactone (PCL), a bioresorbable polymer that lacks bioactivity, can be noticeably improved when simply modified with Exos.^{135,157} Recently, researchers have also developed a bone implant functionalized with BMSC-derived exosomes, which incorporate exosomes into tannic acid (TA)-modified sulfonated polyetheretherketone (SPEEK) (Figure 6).¹⁵⁸ Western blot analysis revealed that Exo-coated SPEEK promoted M2 macrophage polarization, as iNOS expression was inhibited while Arg-1 expression was promoted. Mechanistically, this effect may be related to the NF- κ B signaling pathway. The results of new bone formation *in vivo* revealed obvious bone tissue healing in the Exo-TA-SPEEK group.

Biologically inert titanium (Ti) is widely used in dental and bone implants; however, the issue of slow and insufficient osseointegration has not been fully addressed, particularly in patients with chronic inflammatory conditions. Research has revealed that titanium surfaces functionalized with exosomes derived from licensed TNF- α can modulate macrophage immune responses and accelerate osseointegration in subjects with type 2 diabetes.¹⁵⁹ Notably, the porous structure of the Ti surfaces was more conducive to exosome loading. The use of Exos in adorning anodized (AO) Ti scaffolds resulted in superior bone tissue regeneration. This scaffold surface exhibited a greater adsorption capacity but a slower release profile of Exos than did the pure Ti surface.¹⁶⁰ Researchers have also demonstrated a novel approach of using exosomes from BMP2-stimulated macrophages to increase the biofunctionality of nanotube titanium (NT) (Figure 7A).¹³⁰ They revealed that this combination (BMP2-exo/NT) increased the expression of early markers of osteoblastic differentiation, such as ALP and BMP-2. BMP2-exo/NT may alter cytokine secretion and activate autophagy, thus creating a favorable milieu for osteogenesis. Interestingly, a recent dual biomimetic strategy combines a 3D-printed biomimetic trabecular porous Ti-6Al-4V scaffold (BTPS) with exosome-loaded PEGDA/GelMA hydrogel microspheres (PGHExos) (Figure 7B–E).¹⁶¹ *In vivo* assays indicated that this scaffold significantly enhanced new bone formation and angiogenesis.

Among the various scaffold materials, hydrogel systems are three-dimensional structures with high moisture contents that resemble the extracellular matrix and exhibit favorable shaping properties. This technique is particularly effective for filling critical-sized defects with complex shapes while ensuring the viability of the incorporated biomolecules.^{162,163} Furthermore, it enables minimally invasive treatment through needle injection. Engineered exosomes are frequently utilized in conjunction with hydrogel scaffolds to achieve prolonged exosome release and protect them from degradation, thereby maintaining the stability of the exosomal contents, including proteins and miRNAs.^{23,130,141,142,164,165} A natural polymer hyaluronic acid-based hydrogel (HA hydrogel) has been designed as a carrier for engineered EC-ExosmiR-26a-5p and APY29, which augments M2 polarization (Figure 8).²³ The hydrogels were injectable and possessed an interconnected porous structure. The storage modulus of the HA hydrogel increased with increasing solid concentration; 4% HA was suitable, and the delivery of Exos did not affect the mechanical properties of the hydrogel. The shear-thinning, self-healing and tissue-adhesive properties of this HA hydrogel were also verified. The study also demonstrated that this combination facilitated the sustained release of Exos and specific agents to enhance bone healing. Similarly, engineered BMSC-Exo-loaded hydrogel microparticles (HMPs) exerted long-term effects during new bone formation.¹⁴¹ A significant decrease in HDAC4 expression and a significant increase in RUNX2 and VEGF expression in HUVECs were observed in the HMP@Agomir-29a-Exo group compared with those in the HMP@Exo and HMP groups. Recently, another novel hydrogel delivery system was constructed with Smpd3-

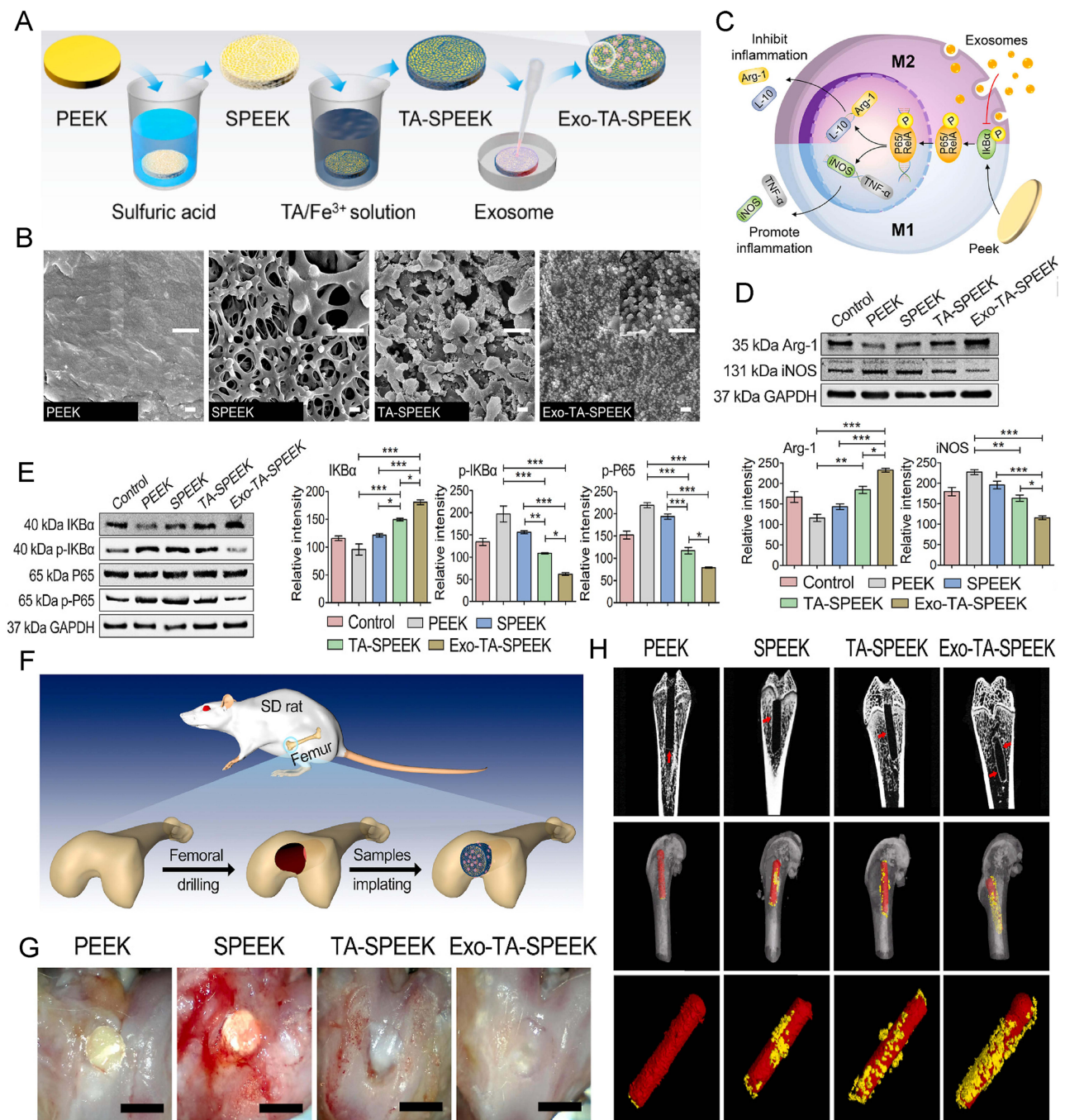


Figure 6 BMSC-Derived Exosomes (Exos) Functionalized on TA-SPEEK Scaffolds Modulate Macrophage Polarization and Promote Bone Regeneration in Rat Femur Implantation. **(A–C)** BMSC-derived Exos bound to TA-SPEEK through hydrogen bonds between Exos’ phosphate groups and TA’s polyphenol groups modulating macrophage polarization. Scale bar represents 500 nm for FE-SEM images; **(D and E)** Effect of Exos loaded TA-SPEEK on Arg-1, iNOS, IKB- α , p-IKB- α , P65, and p-P65 expressions in RAW264.7 cells polarization through Western blot analysis; **(F)** Diagram of the rat femur surgery procedure; **(G)** Images of the implantation site on the rat femur, with a 2 mm scale bar; **(H)** Micro-CT images exhibiting bone regeneration around the implants, with red arrows indicating new bone. Copyright 2021 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd.
Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

reprogrammed BMSC-Exos and nanosilver ions.¹⁶⁵ In the dog bone defect DM model, after Exos-Smpd3@N treatment, significant new bone formation was observed by staining techniques and a micro-CT evaluation.

Therefore, optimization strategies aimed at enhancing the osteogenic functions of exosomes are analogous to commonly employed exosome modification techniques, primarily involving surface modifications and various

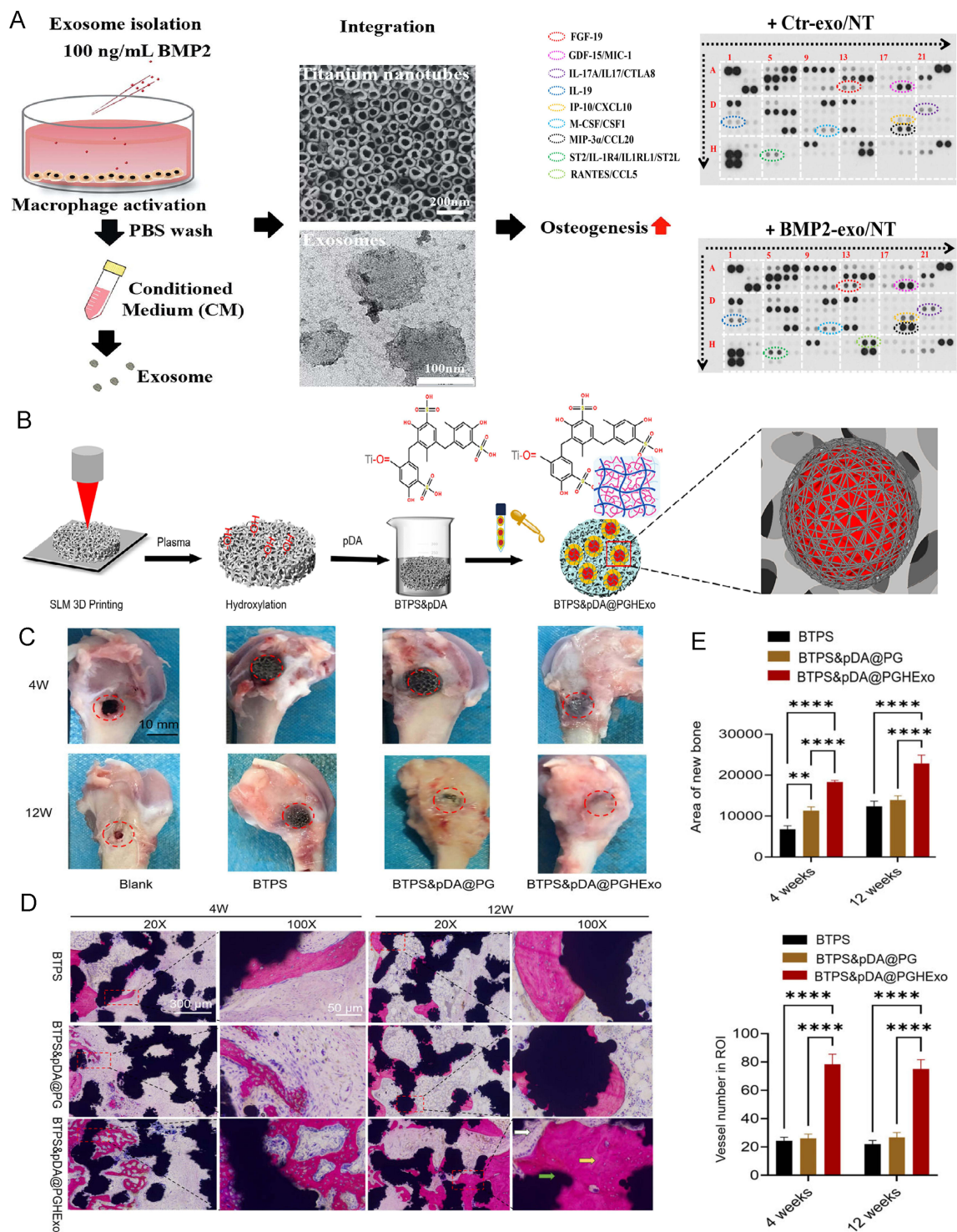


Figure 7 Exosome-enhanced osteogenesis on titanium nanotubes and 3D-printed biomimetic Ti-6Al-4V scaffolds. **(A)** Schematic depicts the modification of titanium nanotube implants with BMP2/macrophage-derived exosomes to promote osteogenesis. hBMSCs were treated with Ctr-exo or BMP2-exo encapsulated nanotubes for 4 days under osteogenic conditions, and the conditioned medium was analyzed using a cytokine array; *Copyright* 2019 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved. The red arrows indicate a promoting effect; **(B)** The construction process of a 3D-printed biomimetic trabecular Ti-6Al-4V scaffold (BTSP) with exosome-loaded PEGDA/GelMA hydrogel microspheres (PGHExo); **(C and D)** Representative macroscopic and methylene blue/fuchsin-stained images of femoral defect sites at 4 and 12 weeks after implantation in different groups in a rabbit femoral defect model. The red dotted circles indicate femoral defect sites; Yellow arrows denote new bone formation, green arrows indicate scaffold material and white arrows point to newly formed blood vessels; **(E)** The BTSP&pDA@PGHExo group showed enhanced bone formation and increased vessel density in the region of interest (ROI) at 4 and 12 weeks, indicating improved bone healing and neovascularization. *Copyright* 2025 The Author(s). Advanced Science published by Wiley-VCH GmbH. **Note:** ** $P < 0.01$; **** $P < 0.0001$.

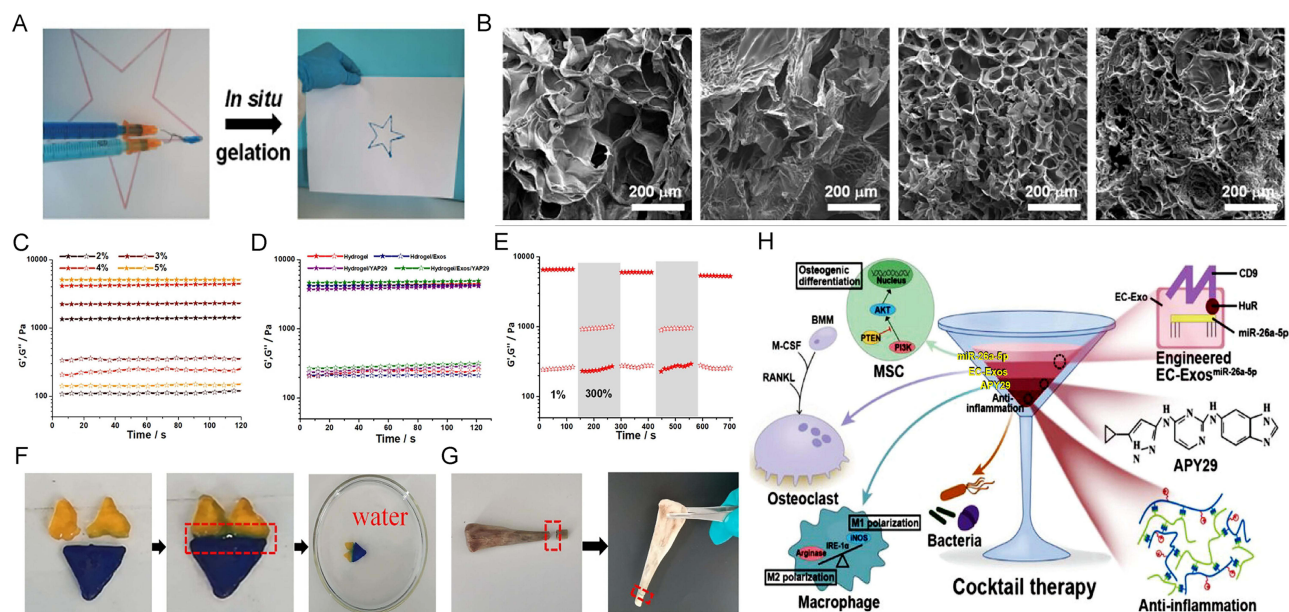


Figure 8 Hyaluronic acid-based hydrogel for sustained delivery of exosomes to enhance bone healing. (A) In situ hydrogel formation via dual-syringe injection; (B) SEM images of HA hydrogel at varying solid concentrations (left to right: 2%, 3%, 4%, and 5%); (C and D) Rheological analysis of HA hydrogel at varying solid concentrations, as well as HA hydrogel with or without Exos; (E) Rheological behavior of 4% solid concentration HA hydrogel under alternating low (1%) and high (300%) strain; (F and G) Illustration of the self-healing ability and tissue-adhesive properties of the HA hydrogel. The red dotted square indicates the effect of self-healing and tissue adhesion; (H) A cocktail therapy using a hyaluronic acid-based hydrogel loaded with engineered endothelial cell-derived exosomes (EC-ExosmiR-26a-5p) and the IRE-1 α inhibitor APY29, was developed to regulate osteoblast/osteoclast and M1/M2 macrophage balance, effectively promoting fracture repair. Copyright 2022 The Authors. Published by American Chemical Society.

interventions that alter their contents. Engineered exosomes are frequently utilized alongside scaffold materials to improve mechanical support and the biological properties. The fundamental design rationale is based on the biological regulatory functions of exosomes within the bone microenvironment, which are critical for creating a favorable osteogenic milieu that includes improving inflammation and immunity, promoting angiogenesis, balancing osteoclast activity and osteogenesis, facilitating innervation, and enhancing biological utilization efficiency (Table 4 and Figure 9).

Registered Clinical Trials, Markets and Challenges

Clinical Landscape and Market Prospects of Exosome-Based Therapies

With recent technological advancements, exosome therapy has garnered increasing attention in clinical studies. To date, eight clinical trials have been registered in the field of bone regeneration medicine (Table 5). The dates of registration ranged from 2019 to 2024, likely due to the introduction of various isolation strategies and the availability of commercial kits in recent years. Among the registered clinical trials, mesenchymal stem cells were the most commonly utilized exosome-producing cells. These trials predominantly involved patients suffering from osteoarthritis, degenerative diseases, or periodontitis, a chronic progressive condition associated with inflammation and bone resorption. Bone regeneration in these patients remains a significant clinical challenge, and no specific treatment exists. Exosomes offer new promise in this regard. Market research has indicated that the exosome therapeutic sector is expected to grow from \$33.1 million in 2021 to \$169.2 million by 2026.^{166,167}

Potential Impact of Methodological Heterogeneity on Research Findings

Substantial methodological heterogeneity across published studies remains a major source of inconsistency in the exosome field. Isolation techniques, such as differential ultracentrifugation, density-gradient centrifugation, size-exclusion chromatography, polymer-based precipitation and microfluidic isolation, differ markedly in yield, purity, and vesicle subset recovery. These methodological differences significantly influence the detected protein, lipid, and RNA cargo profiles, thereby altering the apparent biological activities of exosomes and complicating cross-study

Table 4 Designing Exosomal Biomaterials for Bone Formulation

Exosome Derive	Design	Biological Effect	Application Areas	Strategy Comparison	Ref
M2-Exos	M2-Exos fabricated by 3-way junction RNA nanoparticles	Target BMSCs to promote the healing of bone fractures	Bone fractures, Critical-sized bone defects, Fracture non-union	A: Target BMSCs, enhance healing. D: Limited in large defects, need precise delivery.	[120]
M-Exos	Parental cells stimulated by Zn ion	Promote osteoblast and endothelial cell functions	Bone tissue regeneration, Bone fractures, Osteogenesis	A: Stimulates osteoblasts and angiogenesis. D: Zn ion concentration needs management.	[121]
M-Exos	Parental cells stimulated by Mg ion	Facilitated the osteogenic differentiation of BMSCs	Bone tissue regeneration, Osteoporosis, Bone loss repair	A: Facilitates osteogenesis in BMSCs. D: Mechanism unclear, requires further study.	[122]
BMSCs-Exos	Lightgel-Li-Exo hydrogel	Enhancing macrophage M2 polarization, osteogenesis, and angiogenesis to promote bone repair	Glucocorticoid-induced osteonecrosis of the femoral head, Osteoarthritis, Bone fractures	A: Promotes osteogenesis and angiogenesis. D: Lithium release control is a challenge.	[123]
BMSCs-Exos	BMSCs stimulated by Li ion	Promotes osteogenesis and the great promise of lithium for engineering BMSCs and their exosomes for bone regeneration	Bone tissue regeneration, Critical bone defects, Bone fractures	A: Enhances osteogenesis. D: Lithium toxicity risks.	[125]
Shed-derived Exo	Shed-Cu-HA	Facilitated regeneration of periodontal bone tissues and collagen	Periodontal bone tissue repair, Small bone defects	A: Aids collagen formation. D: Limited for large bone defects.	[126]
BMSCs-Exos	BMSCs stimulation by magnetic nanoparticles and a static magnetic field	Release of exosomal miR-1260a osteogenesis and angiogenesis	Bone tissue regeneration, Fracture healing, Osteogenesis	A: Magnetic targeting enhances delivery. D: Requires complex setup.	[41]
BMSCs-Exos	BMSCs stimulation by cyclic mechanical stretch	Inhibited osteoclast differentiation by attenuating the NF- κ B signaling pathway in vitro and rescued osteoporosis caused by mechanical unloading in an HU mouse model in vivo.	Osteoporosis, Bone loss repair, Bone regeneration	A: Inhibits osteoclasts, promotes osteogenesis. D: Requires mechanical stimulation, limiting applicability in some clinical settings.	[127]
Myocytes-Exos	Mechanically stimulated myocytes	Promote the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells through miR-92a-3p /PTEN/AKT signaling pathway, and can have a therapeutic effect on glucocorticoid-induced osteoporosis in mice in vivo	Glucocorticoid-induced osteoporosis, Bone fractures, Osteoporosis treatment	A: Enhances osteoblast proliferation and osteogenesis. D: Requires mechanical stimulation, which complicates delivery and clinical implementation.	[128]
BMSCs-Exos	Yoda1-pretreated BMSCs	Accelerate osteogenesis by activating phospho-ERK signaling via Yoda1-mediated signal transmission	Bone defect reconstruction, Bone fracture healing, Osteoporosis	A: Accelerates osteogenesis. D: Yoda1 delivery challenges.	[129]
M-Exos	BMP2-stimulated macrophages + titanium oxide nanotubes	Dramatically increased the expression of early osteoblastic differentiation markers, alkaline phosphatase and BMP2	Bone tissue regeneration, Bone fractures, Bone repair	A: BMP2 accelerates osteogenesis. D: Risk of ectopic bone formation.	[130]
MSCs-Exos	Genetically modified MSCs were generated by constitutively expressing BMP2	Triggered an increase in the gene expression levels of osteoinductive growth factors (BMP2, BMP9) and transcription factors (RUNX2, OSX) in naive HMSC	Bone regeneration, Fracture healing, Osteogenesis	A: Enhances bone repair. D: Risk of unwanted bone formation.	[131]
293T cells -Exos	Engineered exosomes enriched in BMP2 mRNA-loaded GelMA	Engineered exosomes could be slowly released and thus ensure prolonged effect of BMP2 when endocytosed by the recipient cells. In the in vivo calvarial defect model, ExoBMP2 + NoBody-loaded GelMA displayed great capacity in promoting bone regeneration.	Bone regeneration, Fracture healing, Critical-sized bone defects	A: Slow-release BMP2 provides sustained osteogenesis. D: Challenges in BMP2 release control.	[133]

BMSC-Exos	BMSC-Exos-HIF1 α combined with TCP scaffold	Engineered exosomes promoted new bone regeneration and neovascularization. Exosome functionalized scaffolds could repair critical sized bone defects	Critical-sized bone defects, Bone regeneration, Neovascularization	A: Enhances osteogenesis and angiogenesis. D: HIF1 α instability limits effectiveness.	[134]
ATDC-Exos	ATDC-Exos endowed with VEGF plasmids + 3D-printed porous bone scaffolds	Engineered exosomes enhance osteogenic induction and vascular remodeling. Engineered exosome-mediated bone scaffolds could effectively induce the bulk of vascularized bone regeneration	Large segmental bone defects, Bone tissue regeneration, Fracture healing	A: Promotes bone regeneration and vascularization. D: Requires advanced 3D printing technology.	[135]
DC-Exos	DC EXO-encapsulated immunoregulatory cargo (TGFB1 and IL10)	Engineered exosomes inhibition of bone resorptive cytokines and reduction in osteoclastic bone loss	Osteoporosis, Periodontal bone regeneration	A: Reduces bone resorption. D: Limited to inflammatory bone diseases.	[136]
MSC-Exos	MSC-Exos loaded with Sep + hydrogel	Excellent self-healing properties, and provides sustained release of Exo-Sep to maintain ER homeostasis	Diabetic bone defect repair, Osteogenesis	A: Sustained release improves healing. D: Need more studies to confirm long-term effect.	[138]
BMSC-Exos	BMSC-Exos carrying microRNA-122-5p	Promote proliferation of osteoblasts	Osteonecrosis of the femoral head, Bone regeneration	A: Enhances osteoblast proliferation. D: Requires efficient microRNA delivery.	[139]
MSC-Exos	MSC-Exo +3D PLA scaffold	This bioactive 3D-printed PLA scaffolds with MSC-Exo modification holds immunoregulatory potential and favor osteogenic differentiation	Bone tissue regeneration, Fracture healing	A: Supports osteogenesis and immune response. D: 3D printing limitations.	[153]
HBMSCs-Exos	MDs-NFATc1/PLA-Exo scaffold	Significantly reducing osteoclast formation while enhancing the osteogenic differentiation and modulating cytokine expression	Osteoporosis-related fractures, Bone remodeling	A: Inhibits osteoclasts, enhances osteogenesis. D: Needs further in vivo validation.	[156]
MSC-Exos	PCL/PDA + MSC-Exos; PCL/PDA + GSNO + MSC-Exos scaffolds.	Incorporating exosomes onto PCL/PDA bio-membrane scaffolds have profound pro-osteogenic effects.	Bone tissue regeneration, Fracture healing	A: Enhances osteogenesis, aids in bone healing. D: Biocompatibility of PCL/PDA may need optimization.	[157]
BMSCs-Exos	BMSC-Exos + TA modified SPEEK	Promoted macrophage M2 polarization via the NF- κ B pathway to enhance BMSCs osteogenic differentiation.	Bone tissue regeneration	A: Promotes M2 polarization, supports osteogenic differentiation. D: Need for better understanding of pathway modulation in vivo.	[158]
MSC-Exos	TNF- α -treated MSC-Exos on micro/nano-network titanium (Ti) surfaces.	Effectively enhanced M2 macrophage polarization in hyperglycemic conditions, enhanced angiogenesis and osteogenesis of ECs and BMSCs. More importantly, TNF-exo-Ti markedly promoted osseointegration in T2D mice	Implant treatments in T2D condition	A: Enhances osseointegration in diabetic conditions. D: Limited to T2D conditions, needs further clinical validation.	[159]
hDPSCs-Exos	Ti scaffolds loaded with hDPSCs-Exos	Superior bone tissue regeneration	Implantation	A: Promotes bone tissue regeneration. D: Limited data on long-term outcomes.	[160]
M-Exos	Exo from the BMP2-stimulated macrophages + Ti scaffolds	Dramatically increased the expression of early osteoblastic differentiation markers, alkaline phosphatase (ALP) and BMP2.	Implantation	A: Enhances bone regeneration on Ti scaffolds. D: Potential BMP2 overexpression effects.	[130]
HUVEC-Exos	3D-printed biomimetic trabecular porous Ti-6Al-4V scaffold with hypoxia-induced exos-loaded PEGDA/GelMA hydrogel	Significantly enhanced osteogenesis and angiogenesis.	Bone defect repair	A: Promotes both osteogenesis and angiogenesis. D: 3D printing may limit scalability.	[161]
EC-Exos	Engineered EC-Exos + hydrgel	Pro-inflammatory cytokines inhibition, M2 polarization and osteogenic differentiation promotion, and osteoclast differentiation suppression, thereby exerting a synergistic effect on fracture repair acceleration.	Bone fractures	A: Enhances fracture healing and immune modulation. D: Needs clinical validation in diverse fracture types.	[23]

Abbreviations: A, advantage; D, disadvantage; M, macrophages; M2, type 2 macrophages; MSC, marrow stem cells; HBMSCs, human bone marrow stem cells; BMSCs, bone marrow stem cells; PLA, polylactic acid; NFATc1, nuclear factor of activated T cells cytoplasmic 1; TA, tannic acid; SPEEK, sulfonated polyetheretherketone; PCL, polycaprolactone; GSNO, S-nitrosoglutathione; TNF-Exo-Ti, TNF- α -licensed exosome-integrated titanium; ECs, endothelial cells; T2D, type 2 diabetes; HA, hyaluronic acid hydrogel; Shed-Cu-HA, incorporating copper ions and Shed-derived exosomes inside the hyaluronic acid hydrogel; Li-Exo, exosomes derived from bone marrow stem cells (BMSCs) stimulated by lithium ions; BMSC-Exos-HIF1 α , bone marrow mesenchymal stem cell carry mutant HIF-1 α ; ATDC; DC, Dendritic cell.

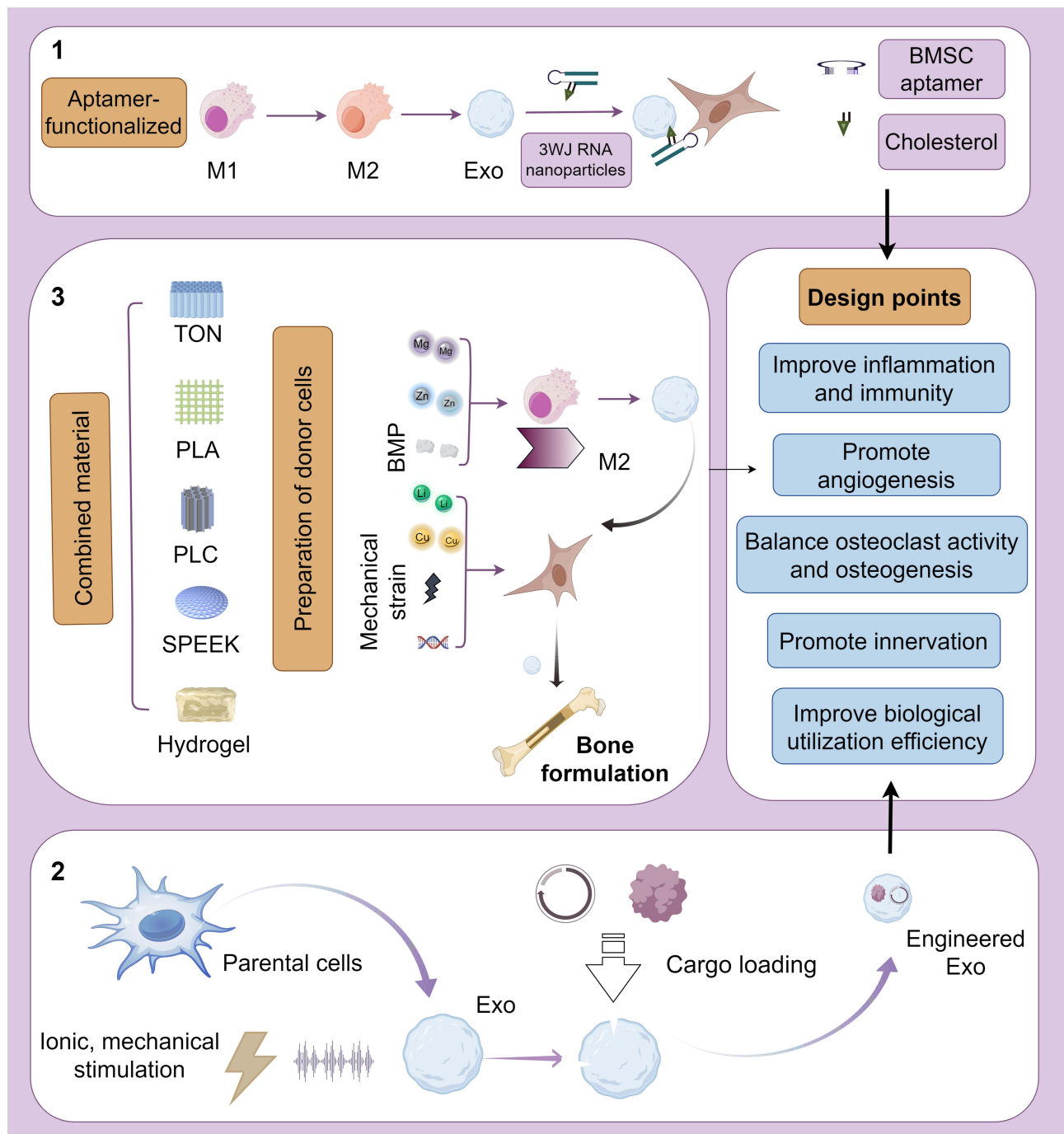


Figure 9 Overview diagram of designing exosomal biomaterials for bone generation. The enhancement of osteogenesis efficiency through engineered exosomes is primarily achieved via two fundamental approaches: (1) parental cell-based exosome engineering, which includes ionic stimulation, mechanical stimulation, transfection, or conditioned culture;^{150,151} (2) direct exosome engineering, which involves surface targeting modification or cargo loading into exosomes;^{148,149} (3) engineered exosomes are frequently utilized in conjunction with scaffolds such as polylactic acid (PLA), sulfonated polyetheretherketone (SPEEK), polycaprolactone (PCL), hyaluronic acid hydrogel (HA), and titanium dioxide nanotubes (TON).^{23,130,135,153,158} The rationale behind material design is largely informed by the biological context of exosomes within the bone microenvironment, which encompasses: 1) the improvement of inflammation and immunity; 2) the promotion of angiogenesis; 3) the balance of osteoclast activity and osteogenesis; 4) the facilitation of innervation; and 5) the enhancement of biological utilization efficiency. The figure was created by Figdraw (www.figdraw.com).

comparisons.^{168,169} For example, polymer precipitation tends to co-isolate protein aggregates and lipoproteins, whereas density gradients enrich for higher-purity vesicles but may preferentially recover specific EV subpopulations.^{169,170} Such variability is particularly relevant in bone-related EV research, where small shifts in isolation purity can alter interpretations of osteogenic potency, ECM-remodeling activity, or mechanosensitive cargo loading.

Table 5 Registered Clinical Trials

ID	Date of Registration	Patient	Intervention	Study Design	Phase	Primary Sponsor	Linkage to Basic Research Mechanism
IRCT20241008063300N1 ChiCTR2300069120	2024.10.19 2023.03.07	Osteoarthritis Periodontitis/Dental defect	MSC-Exos Erythrocyte-Exo	RCT Parallel	Recruiting Recruiting	Tabriz University of Medical Sciences The First Affiliated Hospital of Jinan University	NR Erythrocyte-derived exosomes are linked to studies showing their potential in modulating inflammation and supporting bone regeneration through targeted exosome-based therapies.
ChiCTR1900027140	2019.11.01	Chronic periodontitis with bone defect	Dental pulp stem cell + exosome complex + Bio-Oss bone meal	Parallel	Pending	College of Stomatology, Xi'an Jiaotong University	This trial focuses on the use of dental pulp stem cells combined with exosomes, which links to the exosome-mediated regulation of osteogenesis and osteoclastogenesis as discussed in recent studies.
NCT05060107	2021.09.28	Knee Osteoarthritis	MSC-Exos	Interventional	NR	Universidad de los Andes, Chile	This study evaluates MSC-derived exosomes in knee osteoarthritis, supporting basic research that shows MSC-exosomes promote osteogenesis, angiogenesis, and immune modulation.
NCT04849429	2021.05.31	Chronic low back pain, degenerative disc disease	Platelet-rich plasma with exosomes	RCT	Completed	Dr. Himanshu Bansal Foundation	The trial explores the synergistic effects of platelet-rich plasma combined with exosomes, aligning with studies on how exosomes and growth factors interact to enhance tissue regeneration.
NCT06466850	2024.06.20	Osteoarthritis	MSC-Exos	Single Group Assignment	Recruiting	Isfahan University of Medical Sciences	The study evaluates the effects of MSC-derived exosomes on osteoarthritis, supporting findings from basic research that MSC-exosomes facilitate osteogenesis, angiogenesis, and immune modulation.
NCT05261360 NCT04270006	2022.03.04 2020.02.08	Degenerative meniscal injury Advanced Periodontitis (Stage III or IV)	MSC-Exos Adipose derived stem cells exosomes	NR Interventional	NR NR	Eskisehir Osmangazi University Beni-Suef University	NR This trial examines the potential of adipose-derived stem cell exosomes, reflecting research on their ability to modulate osteogenesis and inflammation in bone regeneration.

Moreover, heterogeneity in characterization standards—markers used, quantification methods, and functional read-outs—further contributes to discrepancies across studies. These issues underscore the importance of adopting harmonized practices, such as the MISEV2018 recommendations, and reporting isolation conditions, characterization criteria, and functional assay protocols in detail to improve transparency, reproducibility, and interpretability.¹⁷¹ Addressing methodological variability will be essential for strengthening mechanistic conclusions and enabling meaningful comparison across studies investigating bone-derived exosomes.

Technical Bottlenecks in Clinical Translation and Potential Solutions

Despite the promising market landscape and an increasing number of registered clinical trials, the clinical translation of exosome-based therapies continues to face several unresolved technical bottlenecks. Large-scale production remains difficult, as conventional culture and purification methods yield low quantities and inconsistent vesicle quality.^{172,173} Standardization is another major obstacle: different isolation techniques produce exosomes with variable purity, cargo composition, and biological potency, complicating batch-to-batch reproducibility. Heterogeneity linked to donor cell source, culture conditions, and dynamic cargo loading further limits the predictability of therapeutic outcomes. In addition, safety and regulatory frameworks are not yet well defined, with uncertainties surrounding biodistribution, immunogenicity, long-term effects, and validated criteria for storage and formulation.¹⁷⁴ Multiple strategies are being explored to overcome these limitations. Scalable biomanufacturing systems, such as bioreactor expansion combined with automated downstream processing (eg, tangential-flow filtration, microfluidic isolation), offer pathways toward higher yield and consistency. Standardization may be improved through adherence to ISEV's MISEV guidelines, harmonized characterization panels, and development of reference materials.¹⁷¹ Addressing heterogeneity will require integrated multi-omics profiling together with robust potency assays. To strengthen safety and regulatory readiness, comprehensive biodistribution studies, selection of well-characterized donor cell sources, and early evaluation of off-target risks are essential.^{174,175} Finally, advances in formulation and storage, including lyophilization and optimized stabilizing excipients, will facilitate the development of stable, clinically deployable exosome therapeutics.¹⁷⁶

Conclusions and Future Directions

Over the past decade, the use of exosomes has emerged as a promising therapeutic approach for potential applications in regenerative orthopedics, garnering significant attention. Our study describes the capacity of exosomes to activate key osteogenic signaling pathways and mediate cross-talk between different cell types, which are critical for bone formation. Osteogenesis is not only associated with angiogenesis and osteoclastogenesis but also involves immunomodulation and neuromodulation. Instead of merely listing the functions of exosomes derived from various cells, we emphasize the exosome-mediated interactions among different components of the bone microenvironment, thereby providing a more holistic perspective on the regulatory mechanisms of exosomes. Exosomes provide several significant advantages in bone regeneration therapy, with potential for further enhancement in terms of production, content, and specificity through advanced engineering techniques. Additionally, insights into the design logic of exosomal biomaterials are summarized based on the biological context. Despite these advances, critical gaps in exosome mechanisms and scalable biomaterial integration remain, and resolving them is essential for predictable clinical translation.

Future progress in exosome-based bone regeneration will hinge on a deeper mechanistic understanding of how exosomes coordinate osteogenic, immune, vascular, and neural interactions within the bone microenvironment, as well as on the development of more controllable and clinically scalable biomaterial platforms. Emerging tools such as single-vesicle analytics, spatial transcriptomics, and *in vivo* EV-tracking technologies may help unravel the spatiotemporal rules governing exosome release, cargo sorting, and target-cell specificity during different phases of bone repair.^{177,178} On the biomaterials side, next-generation scaffolds should aim for programmable exosome loading, microenvironment-responsive release, and patient-specific architectural design enabled by advanced 3D printing and microfluidics. Translation will also require breakthroughs in standardized large-scale EV manufacturing, quality control, and mechanistic potency assays to ensure reproducibility and regulatory compliance. Collectively, integrating mechanistic discovery with engineered delivery systems represents a critical path toward transforming exosome-based therapeutics into predictable, customizable, and clinically applicable strategies for bone regeneration.

Abbreviations

TNF- α , Tumor necrosis factor-alpha; RANKL, Nuclear factor kappa-B ligand; M2, Type 2 macrophages; BMSCs, Bone marrow-derived stem cells; EIF2, Eukaryotic initiation factor 2; miRNAs, microRNAs; circRNA, Circular RNA; DPSCs, Dental pulp stem cells; PDLSCs, Periodontal ligament stem cells; MVs, Matrix vesicles; EVs, extracellular vesicles; ALP, Alkaline phosphatase; EC-Exo, Exosomes derived from endothelial cells; ONFH, Osteonecrosis of femoral head; LPS, Lipopolysaccharide; HUVEC, Human umbilical vein endothelial cells; Runx2, Runt-related transcription factor 2; SC, Schwann cells; IAN, Inferior alveolar nerve; NGF, Nerve growth factor; HA, Hyaluronic acid; HIF-1 α , Hypoxia-inducible factor-1 α ; DC-Exo, Dendritic cell-derived exosomes; ER, Endoplasmic reticulum; mtROS, mitochondrial reactive oxygen species; PLA, Polylactic acid; ROS, Reactive oxygen species; NFATc1, Nuclear factor of activated T cells cytoplasmic 1; PCL, Polycaprolactone.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests in this work.

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