Exercise-induced exosomal noncoding RNAs: Molecular signaling cascades in bone remodeling and translational applications in sports-related bone injuries (Review)

BIN TIAN, XUESONG CHEN, JIANG ZHENG and XIN KANG

Sports Medicine Center, Honghui Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi 710054, P.R. China

Received April 2, 2026; Accepted June 19, 2026

DOI: 10.3892/ijmm.2026.5928

Abstract. Exercise has profound beneficial effects on bone health, yet the molecular mechanisms that mediate mechanical force transduction remain incompletely understood. Exosomal noncoding RNAs (ncRNAs) have emerged as critical intercellular messengers that translate mechanical stimuli into coordinated signaling cascades within the bone microenvironment. The present review systematically synthesizes evidence demonstrating that exercise dynamically modulates exosomal ncRNA expression in a modality-dependent and temporally regulated manner. These exercise-induced exosomal ncRNAs orchestrate bone remodeling by activating osteogenic pathways such as the Wnt/β-catenin pathway, suppressing osteoclastogenesis via receptor activator of nuclear factor κB (RANK) ligand (RANKL)/RANK/osteoprotegerin axis modulation, and coordinating multicellular interactions. Translational applications for sports-related bone injuries are critically evaluated, including noninvasive biomarkers, personalized exercise prescriptions, and engineered exosome-based therapeutics, alongside current limitations. Collectively, these findings support exercise-induced exosomal ncRNAs as a central paradigm linking physical activity to skeletal adaptation.

Contents

Correspondence to: Dr Xin Kang, Sports Medicine Center, Honghui Hospital, Xi'an Jiaotong University, 555 Friendship East Road, South Gate, Beilin, Xi'an, Shaanxi 710054, P.R. China
E-mail: honghuikangxin@163.com

Abbreviations: BMSC, bone marrow mesenchymal stem cell; BMP, bone morphogenetic protein; circRNA, circular RNA; ERK, extracellular signal-regulated kinase; HIIT, high-intensity interval training; lncRNA, long noncoding RNA; MAPK, mitogen-activated protein kinase; miRNA, microRNA; ncRNA, noncoding RNA; NF-κB, nuclear factor κB; NFATc1, nuclear factor of activated T-cells 1; OPG, osteoprotegerin; ORIF, open reduction and internal fixation; PEG, polyethylene glycol; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; rhBMP-2, recombinant human bone morphogenetic protein-2; Runx2, runt-related transcription factor 2; STAT3, signal transducer and activator of transcription 3

Key words: exercise-induced exosomes, noncoding RNAs, bone remodeling, osteogenesis, signaling pathways, sports-related bone injuries, translational medicine, biomarkers

1. Introduction

Sports-related bone injuries represent a major public health concern across athletic populations and include acute traumatic fractures, stress fractures, and overuse injuries that frequently compromise athletic performance and quality of life. Epidemiological evidence indicates that lower extremity stress fractures are highly prevalent in adolescent and young adult athletes, with distal femoral stress fractures demonstrating particularly elevated surgical intervention rates (1). Similarly, pelvic avulsion fractures in pediatric athletes require specialized management approaches across trauma centers (2). The clinical management of these injuries is associated with substantial challenges, as delayed union and nonunion remain persistent complications that prolong rehabilitation and limit return to sport. A recent meta-analysis revealed that patients with lisfranc injuries treated with different surgical approaches achieved comparable outcomes in terms of return to sport, highlighting the need for optimized treatment strategies (3). Furthermore, the burden of these injuries extends beyond acute management, as long-term functional outcomes and complication rates following surgical

intervention demonstrate considerable variability (4). These persistent clinical challenges underscore the urgent need for novel therapeutic approaches that can accelerate bone healing and improve functional recovery in athletic populations.

Physical activity exerts profound beneficial effects on the skeletal system through mechanical loading that stimulates bone formation and remodeling. The mechanostat concept, originally proposed by Frost, describes how bone tissue adapts to mechanical demands by modulating osteoblast and osteoclast activity (5). Exercise-induced mechanical loading promotes osteogenesis through activation of canonical Wnt/β-catenin signaling and other anabolic pathways, thereby maintaining skeletal integrity (6). Despite the well-established epidemiological evidence linking exercise to improved bone health, the fundamental molecular mechanisms by which mechanical forces are transduced into cellular responses remain incompletely understood. Recent investigations have demonstrated that mechanical loading not only directly affects bone cells but also orchestrates complex intercellular communication networks involving multiple tissue types (7). The muscle-bone axis represents a particularly intriguing paradigm, as skeletal muscle contraction during exercise generates signals that influence bone metabolism (8). However, the identity of the key molecular mediators that convey exercise-induced signals from muscle to bone and the precise signaling cascades activated in recipient bone cells have remained elusive, representing a critical knowledge gap in skeletal physiology.

Exosomes, nano-sized extracellular vesicles ranging from 30 to 150 nm in diameter, have emerged as fundamental mediators of intercellular communication in the musculoskeletal system. These vesicles transport diverse bioactive cargoes, including proteins, lipids, and noncoding RNAs (ncRNAs), that reflect the physiological state of parent cells and modulate the function of recipient cells (9). Within the bone microenvironment, exosomes facilitate critical crosstalk among osteoblasts, osteoclasts, osteocytes, and mesenchymal stem cells, thereby coordinating the tightly regulated processes of bone resorption and formation (10). ncRNAs encapsulated within exosomes, particularly microRNAs (miRNAs or miRs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs), have garnered substantial research interest due to their capacity to simultaneously target multiple signaling pathways (8). Recent studies have demonstrated that exosomal miRNAs such as miR-21-5p, miR-23b-3p, and miR-29a-3p regulate osteoblast differentiation and bone formation through modulation of key transcription factors and signaling cascades (11-13). Specifically, miR-21-5p was shown to promote osteogenesis by targeting sprouty homolog 1 to enhance bone morphogenetic protein (BMP)/Smad signaling (12), miR-23b-3p suppressed osteoblast differentiation by inhibiting runt-related transcription factor 2 (Runx2) expression (13), and miR-29a-3p facilitated bone formation by targeting Dickkopf-related protein (DKK)1 and sclerostin to activate the Wnt/β-catenin pathway (11). The exponential growth of studies in this field reflects the recognition that exosomal ncRNAs represent a previously unappreciated layer of regulatory complexity in bone biology, with implications for understanding both physiological adaptation and pathological conditions.

Although several recent reviews have addressed exosomal ncRNAs in bone metabolism or exercise-induced extracellular vesicles separately, a systematic synthesis integrating these two rapidly evolving fields remains conspicuously absent. Previous reviews have primarily focused on mesenchymal stem cell-derived exosomes for bone regeneration (14,15), while others have examined the muscle-bone axis without specifically addressing the exosomal ncRNA intermediary (8). Furthermore, existing literature has largely concentrated on pathological states such as osteoporosis and osteoarthritis rather than sports-related bone injuries in athletic populations (16,17). A recent review by Zhang *et al* (8) comprehensively summarized exosomal miRNAs in muscle-bone crosstalk with implications for sarcopenia and osteoporosis; however, the study primarily focused on age-related musculoskeletal disorders rather than exercise-induced bone adaptation in the context of sports-related injuries. Critically, the mechanistic link between exercise as a physiological stimulus and exosomal ncRNA-mediated bone adaptation has not been systematically reviewed. Notably, Zhang *et al* (8) did not comprehensively address how different exercise modalities modulate exosomal cargo beyond miRNAs, nor did they focus on the sequential phases of fracture healing or translational applications for sports-related bone injuries. The present review fills this critical gap by providing the first systematic synthesis of exercise-induced exosomal ncRNAs as key mediators of bone remodeling, with specific emphasis on translational applications for sports-related bone injuries.

2. Fundamental biological basis: Exercise, exosomal ncRNAs, and bone remodeling

Bone remodeling is a dynamic process of resorption and formation that maintains skeletal integrity and adapts to mechanical demands. Exercise, a potent physiological stimulus, not only influences bone cell activity but also modulates the release of exosomes-extracellular vesicles that carry bioactive ncRNAs between cells. These exercise-induced exosomal ncRNAs, including miRNAs, lncRNAs, and circRNAs, have emerged as critical mediators that translate mechanical forces into molecular signals regulating osteoblast and osteoclast function. A conceptual overview of these core components is presented (Fig. 1).

Bone remodeling and fracture healing dynamics. Bone remodeling is a continuous process executed by the coordinated actions of osteoclasts, which resorb bone, and osteoblasts, which synthesize new bone matrix. This osteoblast-osteoclast coupling is essential for skeletal homeostasis and is profoundly influenced by mechanical loading (18). The process of fracture healing recapitulates embryonic skeletal development, involving an inflammatory phase, soft and hard callus formation, and remodeling (19). During this cascade, various signaling pathways, including Wnt/β-catenin and BMP/Smad, are sequentially activated to ensure proper repair (20). Recent evidence has highlighted the critical role of skeletal stem cells and their niche interactions in coordinating regenerative responses (21). Pathological conditions, such as stress fractures common in athletes, arise when this delicate balance is disrupted, leading to an imbalance between bone resorption and

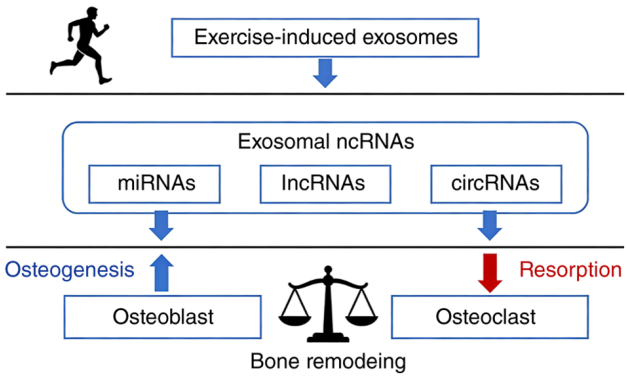

Figure 1. Schematic overview of the core triad: Exercise, exosomal ncRNAs, and bone remodeling. Core framework linking exercise, exosomal ncRNAs, and bone remodeling. Exercise stimulates the release of exosomes containing miRNAs, lncRNAs, and circRNAs, which regulate osteoblast-mediated osteogenesis and osteoclast-mediated resorption to maintain skeletal homeostasis. ncRNAs, noncoding RNAs; miRNAs, microRNAs; lncRNAs, long noncoding RNAs; circRNAs, circular RNAs.

formation (22). Moreover, immune cells and vascular endothelial cells contribute significantly to the regenerative microenvironment, influencing the recruitment and differentiation of skeletal progenitor cells (23).

Exosomes: Biogenesis and intercellular communication in the musculoskeletal system. Exosomes are nano-sized extracellular vesicles (30-150 nm in diameter) that mediate paracrine and endocrine signaling by transferring bioactive molecules, including proteins, lipids, and ncRNAs, between cells (24). Their biogenesis involves the endosomal pathway, beginning with the formation of intraluminal vesicles within multivesicular bodies, which subsequently fuse with the plasma membrane to release exosomes into the extracellular space (25). In the musculoskeletal system, exosomes facilitate critical crosstalk between various cell types, such as between skeletal muscle and bone, a concept central to the muscle-bone axis (26). For instance, exosomes derived from myoblasts can influence the activity of osteoclasts, highlighting their role in systemic skeletal regulation (27). The specific cargo of these vesicles, particularly ncRNAs, dictates their functional impact on recipient cells. Notably, the molecular composition of exosomes reflects the physiological or pathological state of the parent cell, making them attractive targets for biomarker discovery (28).

Classification and functional roles of exosomal ncRNAs in bone metabolism. ncRNAs encapsulated within exosomes are pivotal regulators of gene expression in bone cells. Among these, miRNAs are the most extensively studied, functioning by binding to complementary sequences in target mRNAs to inhibit translation or promote degradation (24). In the context of bone, exosomal miRNAs have been shown to both promote and inhibit osteogenesis. For example, exosomal miR-26a derived from stem cells enhanced bone regeneration by targeting specific inhibitory factors (29), whereas other miRNAs suppressed osteoblast differentiation by modulating key transcription factors (30). LncRNAs and circRNAs add another layer of complexity, acting as competing endogenous RNAs that sponge miRNAs to modulate their activity. For instance, lncRNAs can sequester osteogenic suppressor miRNAs, thereby derepressing osteogenic gene expression (31). This complex regulatory network is now recognized as a fundamental mechanism governing the fate of bone marrow mesenchymal stem cells (BMSCs) and other skeletal progenitors (32). Furthermore, exosomal ncRNAs can simultaneously target multiple signaling nodes, providing a coordinated regulatory mechanism that single soluble factors cannot achieve (8).

Exercise-induced modulation of exosome secretion and ncRNA cargo. Physical activity acts as a potent systemic cue that profoundly influences the exosomal landscape. Exercise, particularly resistance and high-intensity interval training, alters the number of circulating extracellular vesicles and modifies their molecular cargo (33). Garner *et al* (34) demonstrated that acute exercise rapidly increases the expression of genes involved in the multivesicular body and exosome pathways in skeletal muscle. Mechanical strain on skeletal muscle is a potent driver of exosome secretion, with these muscle-derived vesicles carrying specific myomiRs and other factors that can target distant organs, including bone (35). Recent research has shown that mechanically stimulated osteocytes also release exosomes enriched with osteogenic miRNAs that promote osteoblast differentiation (36). Furthermore, the metabolic and mechanical stress associated with exercise can be sensed by various tissues, such as adipose tissue and the vascular endothelium, leading to the secretion of tissue-specific exosomes (37). This exercise-induced 'exosomal signature' is dynamic, with distinct differences observed between acute bouts of exercise and long-term training, as well as being influenced by factors such as age and sex (38). Notably, the dose-effect relationship between exercise parameters, including intensity, duration, and frequency, and the resulting exosomal ncRNA profile remains incompletely characterized, representing a critical knowledge gap (39).

3. Exercise modulation of exosomal ncRNAs: Expression characteristics, regulatory patterns, and tissue sources

Physical activity dynamically reshapes the landscape of circulating exosomal ncRNAs, with exercise modalities, tissue sources, and individual factors collectively determining the specific molecular signatures that mediate systemic adaptation. The regulatory patterns are further influenced by the dominant tissue origins, particularly skeletal muscle-derived exosomes as the primary exercise-responsive carriers, alongside contributions from bone, adipose tissue, and the vascular endothelium. Individual factors such as age, sex, and fitness level introduce substantial heterogeneity in exosomal responses, while the temporal dynamics distinguish acute exercise effects from chronic training adaptations (Fig. 2). A comprehensive overview of these regulatory patterns, summarizing key studies on exercise modalities, tissue sources, dose-effect relationships, individual factors, and temporal dynamics, is presented in Table I.

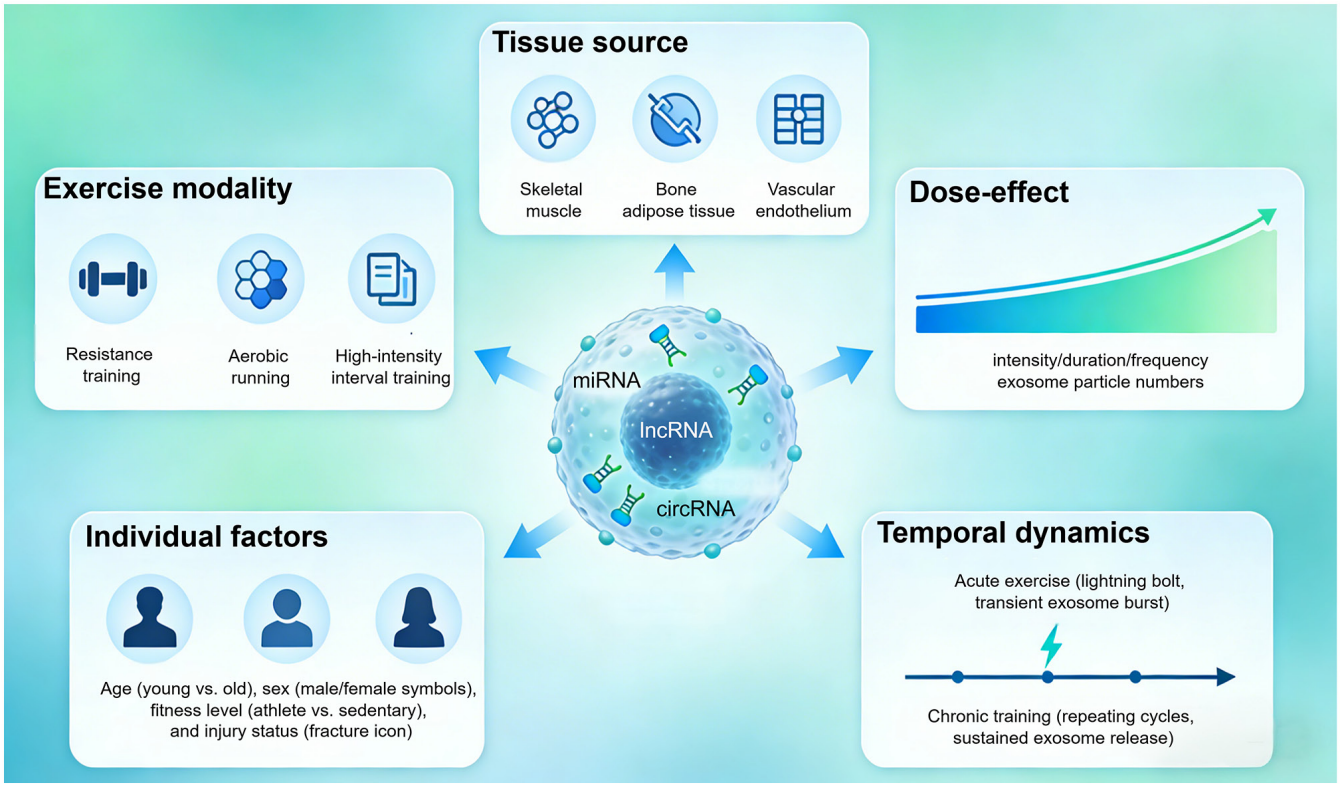

Figure 2. Exercise modulation of exosomal ncRNAs: Regulatory patterns and tissue sources. Exercise modality, tissue origin, dose parameters, individual factors, and temporal dynamics collectively shape exosomal ncRNA profiles. ncRNAs, noncoding RNAs; miRNA, microRNA; lncRNA, long noncoding RNA; circRNA, circular RNA.

Differential modulation by exercise modalities. Different exercise modalities elicit distinct exosomal ncRNA expression profiles, reflecting the unique physiological demands of each training type. As summarized in Table I, resistance exercise has been shown to alter the surface profile and miRNA cargo of circulating extracellular vesicles, with Just *et al* (33) demonstrating that blood flow-restricted resistance exercise modifies the miRNA landscape in a manner distinct from traditional resistance training. Similarly, Garner *et al* (34) reported that a single bout of acute exercise comprising 45 min of cycling followed by resistance exercise rapidly upregulates genes involved in multivesicular body and exosome pathways in skeletal muscle, establishing a mechanistic link between mechanical stress and exosome biogenesis. By contrast, aerobic exercise training regulates serum extracellular vesicle miRNAs linked to metabolic health, as shown by de Mendonça *et al* (40), who observed that aerobic training modulates miRNA profiles associated with obesity-related pathways. High-intensity interval training (HIIT) was shown to induce muscle-derived exosomal miRNAs that improve insulin sensitivity through hepatic forkhead box protein O1 (FoxO1) downregulation, as demonstrated by Castaño *et al* (41). Maggio *et al* (42) further characterized that different exercise regimens, including moderate continuous training and HIIT, produce distinct modulatory effects on circulating extracellular vesicle cargo and their inflammatory properties. Collectively, these findings indicate that exercise modality serves as a critical determinant of exosomal ncRNA composition, with implications for targeted therapeutic applications.

Dominant tissue sources. Skeletal muscle represents the predominant tissue source of exercise-induced exosomal ncRNAs, functioning as a central mediator of the muscle-bone axis. Vechetti *et al* (43) demonstrated that mechanical overload-induced muscle-derived extracellular vesicles carry specific miRNAs that promote adipose tissue lipolysis, highlighting the endocrine capacity of myogenic exosomes. The release of muscle-derived exosomes into the circulation following exercise has been well characterized, with Guescini *et al* (44) providing foundational evidence that skeletal muscle releases α-sarcoglycan-positive extracellular vesicles carrying miRNAs into the bloodstream. Beyond skeletal muscle, adipose tissue contributes significantly to the exercise-responsive exosomal pool. Burke *et al* (37) showed that extracellular vesicle transfer of miR-1 to adipose tissue modifies lipolytic pathways following resistance exercise, establishing a muscle-adipose crosstalk mechanism. Additionally, Zhao *et al* (45) identified that small extracellular vesicles from brown adipose tissue mediate exercise-induced cardioprotection, expanding the understanding of adipose-derived exosomal signaling. Vascular endothelial cells and bone tissue also contribute to the exosomal response, with Lou *et al* (46) demonstrating that liver-derived extracellular vesicles carrying miR-122-5p promote exercise-induced angiogenesis. The multiplicity of tissue sources underscores the complexity of exercise-induced exosomal signaling networks.

Dose-effect relationships. The relationship between exercise parameters, including intensity, duration, frequency, and training cycle, and exosomal ncRNA expression follows

Table I. Summary of exercise-induced exosomal ncRNA expression characteristics, regulatory patterns, and tissue sources.

Authors, year	Exercise modality/parameter	Tissue source	ncRNA cargo	Regulatory pattern	Key finding	(Refs.)
Just *et al*, 2020	Blood flow-restricted resistance exercise	Circulating EVs	miRNAs	Differential modulation	Blood flow-restricted resistance exercise alters surface profile and miRNA cargo of circulating EVs distinct from traditional resistance training	(33)
Garner *et al*, 2020	Acute exercise	Skeletal muscle	Exosome pathway genes	Acute response	Acute exercise rapidly upregulates genes involved in multivesicular body and exosome pathways in skeletal muscle	(34)
Burke *et al*, 2024	Resistance exercise	Muscle-derived EVs	miR-1	Tissue source	Extracellular vesicle transfer of miR-1 to adipose tissue modifies lipolytic pathways following resistance exercise	(37)
Xhuti *et al*, 2023	Resistance training	Circulating exosome-like vesicles, skeletal muscle	miRNAs	Individual factors	Circulating exosome-like vesicle and skeletal muscle miRNAs are altered with age and resistance training	(38)
Lovett *et al*, 2024	Exercise-induced muscle damage	Plasma-derived sEVs	miRNAs	Dose-effect	Time-dependent alterations in miRNA cargo following exercise-induced skeletal muscle damage	(39)
de Mendonça *et al*, 2020	Aerobic exercise training	sEVs	miRNAs	Differential modulation	Aerobic training regulates serum EV miRNAs linked to obesity-related metabolic pathways	(40)
Castaño *et al*, 2020	HIIT	Muscle-derived exosomes	miRNAs	Differential modulation	HIIT induces muscle-derived exosomal miRNAs that improve insulin sensitivity via hepatic FoxO1 downregulation	(41)
Maggio *et al*, 2023	Moderate continuous training vs. HIIT	Circulating EVs	miRNA, protein cargo	Differential modulation	Different exercise regimens produce distinct modulatory effects on circulating EV cargo and inflammatory properties	(42)
Vechetti *et al*, 2021	Mechanical overload	Muscle-derived EVs	miRNAs	Tissue source	Mechanical overload-induced muscle-derived EVs carry specific miRNAs that promote adipose tissue lipolysis	(43)
Guescini *et al*, 2015	Exercise	Skeletal muscle	miRNAs	Tissue source	Skeletal muscle releases α-sarcoglycan-positive EVs carrying miRNAs into the bloodstream	(44)
Zhao *et al*, 2022	Exercise	Brown adipose tissue	miRNAs	Tissue source	Small EVs from brown adipose tissue mediate exercise-induced cardioprotection	(45)
Lou *et al*, 2022	Exercise	Liver-derived EVs	miR-122-5p	Tissue source	Liver-derived EVs carrying miR-122-5p promote exercise-induced angiogenesis	(46)

Table I. Continued.

Authors, year	Exercise modality/parameter	Tissue source	ncRNA cargo	Regulatory pattern	Key finding	(Refs.)
Sapp *et al*, 2019	Moderate vs. high-intensity exercise	Circulating vesicles	Endothelial markers	Dose-effect	Moderate and high-intensity exercise differentially affect vesicle release in an intensity-dependent manner	(47)
Warnier *et al*, 2023	6-Week sprint interval training	Circulating EVs	miRNA, protein cargo	Dose-effect	Sustained training regimens produce cumulative effects on exosomal profiles	(48)
Doncheva *et al*, 2022	Exercise	EVs	miRNAs	Dose-effect	EV and miRNA alterations in response to exercise are modulated by insulin sensitivity and overweight status	(49)
Estébanez *et al*, 2021	Resistance training	Exosomes	CD63 protein	Individual factors	Resistance training diminishes exosome CD63 protein expression in the elderly without modifying plasma miR-146a-5p levels	(50)
Kargl *et al*, 2024	Concurrent exercise training (12 weeks)	Circulating EVs	miRNA, protein cargo	Individual factors	Circulating EV characteristics differ between men and women following concurrent exercise training	(51)
Kargl *et al*, 2026	Resistance exercise	Circulating EVs	miRNA, metabolite	Individual factors	Menstrual cycle phase and hormonal contraceptive use variably influence resistance exercise-induced EV signaling	(52)
Fernandez-Sanjurjo *et al*, 2024	Exercise training	Plasma EVs	miRNAs	Individual factors	Distinct plasma EV miRNA cargo differentiates sedentary young males from athletes	(53)
D'Souza *et al*, 2018	Intense exercise	Circulatory exosomes	miRNAs	Acute vs. chronic	Circulatory exosomal miRNA following intense exercise is unrelated to muscle and plasma miRNA abundances	(54)
Silver *et al*, 2020	Acute moderate-intensity exercise	EVs	miRNAs	Acute vs. chronic	Extracellular vesicular miRNA expression is not a proxy for skeletal muscle miRNA expression following acute exercise	(55)
Hou *et al*, 2019	Long-term exercise	Exosomes	miR-342-5p	Acute vs. chronic	Long-term exercise-derived exosomal miR-342-5p functions as a novel exerkine for cardioprotection	(56)
Di *et al*, 2020	Long-term exercise	EVs	miR-191a-5p	Acute vs. chronic	Long-term exercise-secreted EVs promote browning of white adipocytes by suppressing miR-191a-5p	(57)

ncRNA, noncoding RNA; miRNA, microRNA; EV, extracellular vesicle; sEV, small extracellular vesicle; HIIT, high-intensity interval training.

dose-dependent patterns, although systematic characterization remains incomplete. Sapp *et al* (47) reported that moderate and high-intensity exercise differentially affect circulating markers of endothelial integrity, with intensity-dependent effects on vesicle release. The duration of exercise bouts also influences exosomal cargo, as demonstrated by Lovett *et al* (39), who analyzed plasma-derived small extracellular vesicle characteristics following exercise-induced skeletal muscle damage and observed time-dependent alterations in miRNA cargo. Regarding training frequency and cycle length, Warnier *et al* (48) examined the effects of a 6-week sprint interval training protocol at different altitudes on circulating extracellular vesicles, revealing that sustained training regimens produce cumulative effects on exosomal profiles. Doncheva *et al* (49) further demonstrated that extracellular vesicles and miRNAs are altered in response to exercise in conjunction with insulin sensitivity and overweight status, suggesting that the dose-response relationship is modulated by underlying metabolic conditions. The emerging evidence supports a non-linear, context-dependent relationship between exercise dose and exosomal ncRNA expression.

Modulatory effects of individual factors. As detailed in Table I, individual characteristics substantially modify the exosomal ncRNA response to exercise. Age-related differences have been systematically characterized, with Xhuti *et al* (38) demonstrating that circulating exosome-like vesicle and skeletal muscle miRNAs are altered with age and resistance training, indicating that older adults exhibit distinct exosomal responses compared with younger individuals. Specifically, aging is associated with changes in exosome concentration and altered miRNA cargo, including increased levels of senescence-associated miRNAs such as miR-34a and miR-126-3p, which may contribute to age-related declines in muscle-bone crosstalk. Similarly, Estébanez *et al* (50) reported that resistance training diminishes the expression of exosome CD63 protein in the elderly without modifying plasma miR-146a-5p levels, highlighting age-dependent differences in exosome biogenesis and cargo. Sex differences represent another critical modifying factor, as Kargl *et al* (51) recently demonstrated that circulating extracellular vesicle characteristics differ between men and women following 12 weeks of concurrent exercise training. This includes baseline differences in EV concentration, size distribution, and protein cargo between sexes, as well as sex-specific responses to exercise training. Moreover, Kargl *et al* (52) further elucidated the varying influence of menstrual cycle phase and hormonal contraceptive use on resistance exercise-induced circulating extracellular vesicle signaling, emphasizing the complexity of sex hormone interactions with exosomal responses. Physical fitness level also modulates exosomal profiles, with Fernandez-Sanjurjo *et al* (53) showing that next-generation sequencing reveals distinct plasma extracellular vesicle miRNA cargo differentiating sedentary young males from athletes. Athletes exhibited significantly different levels of miR-16-5p, miR-19a-3p, miR-451a, and miR-25-3p compared with sedentary controls, suggesting that long-term training status induces lasting changes in exosomal miRNA signatures. Injury status further influences exosomal responses, as demonstrated by Lovett *et al* (39), who analyzed exosomal characteristics following exercise-induced skeletal muscle damage.

Dynamic expression patterns: Acute vs. chronic training. The temporal dynamics of exosomal ncRNA expression distinguish acute exercise responses from chronic training adaptations, with distinct molecular signatures characterizing each physiological state. Acute exercise induces rapid but transient changes in exosomal cargo, as demonstrated by Garner *et al* (34), who observed that acute exercise rapidly increases the expression of genes involved in multivesicular body and exosome pathways in skeletal muscle. Similarly, D'Souza *et al* (54) reported that circulatory exosomal miRNA following intense exercise is unrelated to muscle and plasma miRNA abundances, suggesting that acute exercise mobilizes pre-existing exosomal pools rather than reflecting ongoing transcriptional activity. Silver *et al* (55) corroborated these findings, demonstrating that extracellular vesicular miRNA expression is not a proxy for skeletal muscle miRNA expression following acute moderate-intensity exercise, indicating compartmentalized regulation. By contrast, chronic training has been shown to induce sustained alterations in exosomal ncRNA profiles. Hou *et al* (56) demonstrated that long-term exercise-derived exosomal miR-342-5p functions as a novel exerkine for cardioprotection, establishing that sustained training produces durable exosomal signatures. Di *et al* (57) further reported that long-term exercise-secreted extracellular vesicles promote browning of white adipocytes by suppressing miR-191a-5p, confirming that chronic exercise induces persistent changes in exosomal cargo. Beyond these specific miRNAs, profiling studies have identified numerous other exosomal miRNAs altered by chronic exercise training, including miR-16-5p, miR-19a-3p, miR-451a, and miR-25-3p, which differentiate athletes from sedentary individuals (53). The temporal resolution of exosomal responses, ranging from minutes following acute exercise to weeks of training adaptation, reveals a dynamic continuum of molecular regulation.

4. Molecular signaling cascades of exercise-induced exosomal ncRNAs in bone remodeling

Upon release, exercise-induced exosomal ncRNAs exert their effects on recipient bone cells by activating or suppressing specific intracellular signaling cascades. These molecular mechanisms translate mechanical stimuli into coordinated anabolic and anti-catabolic effects. The signaling networks primarily involve canonical pathways that govern osteoblast differentiation, osteoclast formation, and multicellular interactions within the bone microenvironment. A systematic overview of these molecular cascades is presented (Fig. 3).

Osteogenic signaling pathways: Wnt/β-catenin, BMP/Smad, and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK). Exercise-induced exosomal ncRNAs promote osteoblast differentiation and bone formation primarily through activation of the Wnt/β-catenin, BMP/Smad, and MAPK/ERK signaling cascades (58). Mechanical strain-stimulated osteocyte-derived exosomes have been shown to enhance osteogenic differentiation via miRNA-mediated

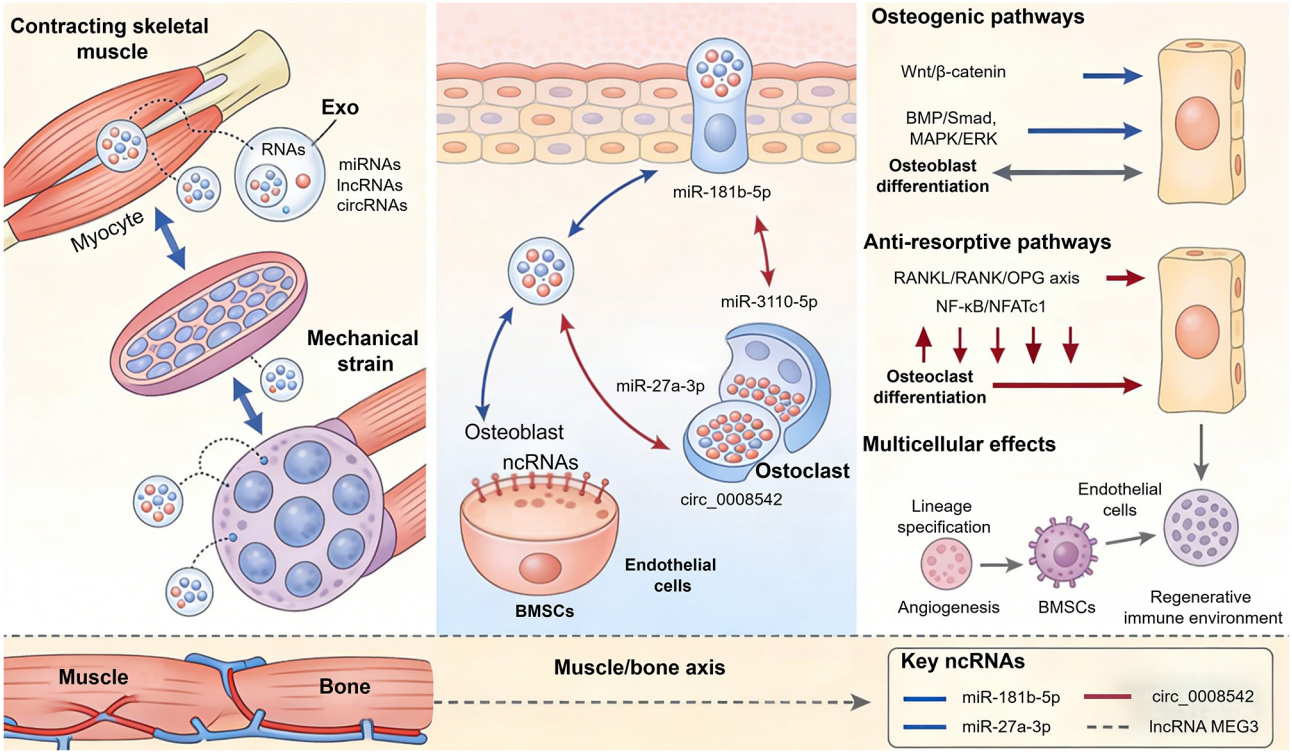

Figure 3. Molecular signaling cascades of exercise-induced exosomal ncRNAs in bone remodeling. Mechanical strain during exercise stimulates release of exosomes from skeletal muscle and osteocytes. These exosomes carry ncRNAs (miRNAs, lncRNAs, circRNAs) that activate osteogenic pathways (Wnt/β-catenin, BMP/Smad, MAPK/ERK) in osteoblasts, suppress osteoclastogenesis via RANKL/RANK/OPG and NF-κB/NFATc1 signaling, and coordinate multi-cellular effects (BMSC lineage commitment, angiogenesis, immune modulation). ncRNAs, noncoding RNAs; miRNAs, microRNAs; lncRNAs, long noncoding RNAs; circRNAs, circular RNAs; BMP, bone morphogenetic protein; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; NF-κB, nuclear factor-κB; NFATc1, nuclear factor of activated T-cells 1; BMSC, bone marrow mesenchymal stem cell.

pathway activation. Lv *et al* (59) demonstrated that mechanically induced osteocyte-derived exosomes promote periodontal ligament stem cell proliferation and osteogenic differentiation through the miR-181b-5p/phosphatase and tensin homolog (PTEN)/protein kinase B (AKT) signaling axis. This finding establishes a direct mechanotransduction link where exosomal miRNAs modulate PTEN expression to relieve AKT pathway inhibition. Similarly, Zhu *et al* (36) reported that mechanically strained osteocyte-derived exosomes promote osteoblastic differentiation, further supporting the concept that mechanical loading generates osteogenic exosomal signals.

The Wnt/β-catenin pathway represents a central convergence point for exosomal ncRNA-mediated osteogenesis. Ibrahim *et al* (60) demonstrated that augmenting canonical Wnt signaling in therapeutically inert cells converts them into potent exosome factories, highlighting the capacity of the pathway to enhance exosomal therapeutic efficacy. In the context of muscle-bone crosstalk, skeletal muscle-derived exosomes have been identified as key mediators of osteogenesis. Chen *et al* (27) showed that histone deacetylase inhibition enhances extracellular vesicles from muscle to promote osteogenesis via miR-873-3p, indicating that muscle-derived exosomes can directly activate osteogenic programs in bone cells. Xu *et al* (61) provided complementary evidence that exosomes from C2C12 myoblasts enhance osteogenic differentiation of MC3T3-E1 pre-osteoblasts by delivering miR-27a-3p. Across these studies, a common regulatory pattern emerges: Exosomal ncRNAs from both osteocytes and myocytes converge on the Wnt/β-catenin pathway as a central effector. Specifically, these ncRNAs function by relieving endogenous inhibitors of the pathway (such as PTEN and DKK2) or by directly targeting transcriptional repressors of osteogenic genes. This pattern suggests that diverse exercise stimuli may converge on a shared exosomal ncRNA-mediated mechanism to activate Wnt/β-catenin signaling, representing a potential therapeutic node for bone regeneration.

Collectively, these studies demonstrate that exosomal miRNAs from both osteocytes and myocytes converge on osteogenic signaling pathways, with the Wnt/β-catenin cascade serving as a primary effector mechanism. It should be noted, however, that the majority of these studies are correlative in nature, reporting associations between exosomal ncRNA levels and osteogenic outcomes. Direct causal evidence through loss-of-function (such as exosomal ncRNA knockdown) or gain-of-function (such as ncRNA overexpression or mimic delivery) approaches remains limited, leaving the necessity and sufficiency of specific exosomal ncRNAs for exercise-induced bone formation incompletely validated.

Anti-resorptive mechanisms: Receptor activator of nuclear factor κB (RANK) ligand (RANKL)/RANK/osteoprotegerin (OPG) axis and nuclear factor-κB (NF-κB) signaling. The inhibitory effects of exercise-induced exosomal ncRNAs on osteoclastogenesis involve targeting the RANKL/RANK/OPG

axis and suppressing NF-κB and nuclear factor of activated T-cells 1 (NFATc1) signaling. Nie *et al* (62) demonstrated that skeletal muscle-derived exosomes regulate endothelial cell functions via reactive oxygen species-activated NF-κB signaling, establishing a mechanistic link between exercise-induced oxidative stress and downstream transcriptional regulation. This pathway also modulates osteoclast precursor sensitivity to RANKL stimulation. Wang *et al* (63) reported that circ_0008542 in osteoblast exosomes promotes osteoclast-induced bone resorption through m6A methylation, revealing that exosomal circRNAs can influence the osteoblast-osteoclast coupling balance. The opposing effects of different exosomal ncRNA species on osteoclastogenesis underscore the complexity of exercise-mediated skeletal regulation.

Muscle-derived exosomes also contribute to anti-resorptive effects through delivery of miRNAs that target osteoclast differentiation factors. Fulzele *et al* (64) demonstrated that muscle-derived miR-34a increases with age in circulating extracellular vesicles and induces senescence of bone marrow stem cells, suggesting that age-related changes in exosomal cargo may compromise bone homeostasis. Conversely, Qu *et al* (65) showed that mechanically stimulated periodontal ligament cell-derived exosomes promote osteoblast differentiation via the miR-181d-5p/tumor necrosis factor (TNF) signaling pathway, indicating that mechanical loading generates exosomal signals that suppress inflammatory osteoclastogenesis.

Synthesizing these findings, a recurrent mechanistic theme is that exosomal ncRNAs modulate osteoclastogenesis by targeting three distinct nodes: i) The RANKL/RANK/OPG ratio, ii) NF-κB transcriptional activity, and iii) NFATc1-mediated downstream gene expression. Notably, different exosomal ncRNA species can exert opposing effects on the same pathway depending on cellular context, suggesting that the net anti-resorptive outcome of exercise is determined by the balance of pro-osteogenic and anti-osteoclastogenic exosomal signals. This balance, rather than the absolute level of any single ncRNA, likely dictates the overall effect on bone mass.

Multicellular regulatory effects in the bone microenvironment. Exercise-induced exosomal ncRNAs exert pleiotropic effects on the bone microenvironment by coordinating mesenchymal stem cell lineage commitment, angiogenesis, and immune homeostasis. Ma *et al* (26) demonstrated that skeletal muscle-derived extracellular vesicles transport glycolytic enzymes to mediate muscle-to-bone crosstalk, revealing a metabolic dimension to exosomal communication beyond ncRNA cargo. This finding expands the understanding of how exercise generates integrated signals that support bone formation. For angiogenesis coupling, Hayashi *et al* (66) reported that exosomal miR-206 secreted from growing muscle promotes angiogenic response in endothelial cells, establishing a direct mechanism by which muscle-derived exosomes support the vascular niche required for bone remodeling. Similarly, Lou *et al* (46) demonstrated that exercise promotes angiogenesis by enhancing endothelial cell fatty acid utilization via liver-derived extracellular vesicle miR-122-5p, highlighting the multi-organ contribution to exercise-induced vascular adaptation.

Mesenchymal stem cell lineage commitment is critically regulated by exosomal ncRNAs from various sources. Xing *et al* (67) showed that skeletal muscle-derived exosomes prevent osteoporosis by promoting osteogenesis, providing direct evidence for the therapeutic potential of muscle-derived exosomes in bone disorders. By contrast, Peruzzi *et al* (68) demonstrated that circulating extracellular vesicles from adolescents with obesity impair mesenchymal stromal cell differentiation, favoring adipogenic rather than osteogenic differentiation, indicating that pathological states alter exosomal cargo to disrupt bone homeostasis. Immune modulation represents another key mechanism, as Qiu *et al* (69) reported that exosomes derived from BMSCs of exercise-trained mice improve wound healing by inhibiting macrophage M1 polarization, suggesting that exercise-conditioned exosomes promote a regenerative immune environment.

Mechanobiological coupling mechanisms. The transduction of mechanical forces into biochemical signals via exosomal ncRNAs constitutes a fundamental mechanobiological coupling mechanism in bone adaptation. Osteocytes serve as primary mechanosensors in bone, and their exosomal response to mechanical loading mediates downstream effects on osteoblasts and osteoclasts. Shen *et al* (70) demonstrated that mechanically activated mesenchymal-derived bone cells drive vessel formation via an extracellular vesicle-mediated mechanism, establishing that mechanical stimulation generates pro-angiogenic exosomal signals that coordinate bone formation with vascular support. Shang *et al* (71) further elucidated that extracellular vesicles allow epigenetic mechanotransduction between chondrocytes and osteoblasts, revealing that mechanical signals can be propagated across cell types through exosomal delivery of regulatory ncRNAs.

The molecular machinery underlying exosomal mechanotransduction involves specific miRNA species that respond to mechanical strain. Zheng *et al* (72) reported the biological characteristics of miRNAs secreted by exosomes of periodontal ligament stem cells due to mechanical force, demonstrating that mechanical loading induces distinct exosomal miRNA profiles. Qu *et al* (65) confirmed that cyclic stretch-induced exosomes from periodontal ligament cells promote osteoblast differentiation via the miR-181d-5p/TNF signaling pathway, providing mechanistic insight into how cyclical mechanical forces are converted into anabolic signals. Collectively, these studies establish that exosomal ncRNAs function as mechanotransducers, converting physical stimuli into molecular signals that orchestrate adaptive bone remodeling.

Synergistic crosstalk in the muscle-bone axis. The muscle-bone axis represents a paradigm of inter-organ communication mediated by exercise-induced exosomal ncRNAs, where synergistic and antagonistic signaling interactions coordinate skeletal adaptation. Multiple recent reviews have synthesized this emerging field. Zhang *et al* (8) comprehensively summarized exosomal miRNAs in muscle-bone crosstalk, detailing mechanistic links and exercise modulation relevant to sarcopenia and osteoporosis. Li *et al* (73) provided a systematic overview of molecular communication from bone to skeletal muscle, establishing that exosomal signaling is bidirectional between these tissues. Kirk *et al* (74) further contextualized

bone and muscle crosstalk in ageing and disease, highlighting the clinical relevance of exosome-mediated inter-organ communication.

Specific molecular mechanisms underlying muscle-bone crosstalk have been elucidated. Qin *et al* (75) demonstrated that myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal miR-218, revealing a novel mechanism by which muscle-derived factors regulate bone formation through exosomal pathways. Li *et al* (76) showed that myoblast-derived exosomal paired related homeobox 2 attenuates osteoporosis via transcriptional regulation of lncRNA-MIR22HG to activate the Hippo pathway, establishing a non-canonical signaling axis in muscle-bone communication. Conversely, Wang *et al* (77) reported that slowpoke homolog 1 (Slo1)-deficient myoblast exosome-derived miR-222-3p inhibits osteogenic differentiation via targeting signal transducer and activator of transcription 3 (STAT3), demonstrating that pathological alterations in muscle exosomal cargo can negatively impact bone. The emerging evidence supports that exercise optimizes muscle-bone crosstalk by enriching exosomal ncRNA cargo with pro-osteogenic and anti-osteoclastogenic signals, while antagonistic signals from pathological muscle states can disrupt skeletal homeostasis.

5. Roles of exercise-induced exosomal ncRNAs in sports-related bone injuries

Exercise-induced exosomal ncRNAs participate in the pathophysiological processes of sports-related bone injuries by modulating inflammation, angiogenesis, and tissue regeneration across different injury types. These molecular mediators exert context-dependent effects that vary according to injury mechanism, healing stage, and the specific tissue microenvironment. Understanding their regulatory roles provides mechanistic insights into how exercise conditioning influences bone injury outcomes and identifies potential therapeutic targets. A systematic overview of these injury-specific roles is presented (Table II).

Traumatic bone fractures in sports. The regulatory roles of exercise-induced exosomal ncRNAs in traumatic fracture healing span the sequential phases of inflammation, soft callus formation, hard callus mineralization, and remodeling. During the inflammatory phase, exosomal miRNAs modulate macrophage polarization and cytokine secretion, creating a pro-regenerative environment. Recent evidence from Lu *et al* (78) demonstrated that an osteoblast/osteoclast and immune cocktail therapy using exosome/drug delivery multifunctional hydrogel accelerates fracture repair, highlighting the potential of exosome-based strategies to coordinate multicellular responses during bone healing. The transition to soft callus formation involves exosomal transfer of osteogenic signals between mesenchymal stem cells and osteoprogenitors. Liu *et al* (79) showed that hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by transferring miR-126, which enhances angiogenesis and osteogenesis simultaneously.

During hard callus mineralization, exosomal ncRNAs regulate osteoblast differentiation and matrix deposition. Jiang *et al* (80) reported that BMSC-derived exosomal miR-25 regulates the ubiquitination and degradation of Runx2 by SMAD specific E3 ubiquitin protein ligase 1 (SMURF1) to promote fracture healing in mice. More recent work by Yang *et al* (81) demonstrated that mesenchymal stem cell-derived extracellular vesicles ameliorate temporomandibular joint osteoarthritis by suppressing osteoclast activity via the let-7a-5p/integrin β3 axis, establishing that exosomal mechanisms are relevant across different joint contexts. In the remodeling phase, exosomal signals coordinate osteoclast-mediated resorption with new bone formation. The stage-dependent effects of exosomal ncRNAs suggest that therapeutic strategies targeting specific healing phases may optimize fracture repair outcomes.

Stress fractures and overuse bone injuries. Stress fractures and overuse bone injuries in athletes represent a distinct pathological entity characterized by repetitive mechanical loading-induced bone fatigue damage, where exercise-induced exosomal ncRNAs may serve as early warning signals and repair mediators. The identification of circulating exosomal miRNA signatures associated with bone fatigue damage offers potential for early detection before radiographic changes appear. Xu *et al* (82) demonstrated that exosomal miR-128-3p from mesenchymal stem cells of aged rats regulates osteogenesis and bone fracture healing by targeting Smad5. The mechanosensitive nature of exosomal release positions these vesicles as ideal candidates for monitoring cumulative bone fatigue. Shang (83) comprehensively reviewed mechanosensitive miRNAs in cartilage and subchondral bone remodeling, identifying them as emerging targets for osteoarthritis therapy, with direct relevance to overuse injuries.

Furthermore, Yang *et al* (84) confirmed that cyclic stretch-induced exosomes from periodontal ligament cells promote osteoblast differentiation via the miR-181d-5p/TNF signaling pathway, indicating that mechanically stimulated exosomes actively participate in the repair of fatigue-damaged bone. Recent work by Tian *et al* (85) demonstrated that miR-877, an exosomal miRNA from mechanical stretch-induced adipose derived stromal cells, enhances fracture healing in nonunion rats with type 2 diabetes mellitus, providing evidence that mechanically conditioned exosomes can overcome healing impairments. The dual role of exercise-induced exosomes, both as sensors of mechanical overload and as mediators of adaptive repair, positions them as critical regulators of the bone fatigue response.

Sports-related osteochondral injury and osteoarthritis. Osteochondral injuries and post-traumatic osteoarthritis represent common sequelae of sports-related joint trauma, with exercise-induced exosomal ncRNAs modulating cartilage-subchondral bone crosstalk and disease progression. The bidirectional communication between cartilage and bone via exosomal ncRNAs influences both acute injury responses and chronic degenerative changes. Shang *et al* (71) elucidated that extracellular vesicles allow epigenetic mechanotransduction between chondrocytes and osteoblasts, revealing that mechanical signals can be propagated across joint tissues through exosomal delivery of regulatory ncRNAs. This mechanism is particularly relevant to sports injuries where aberrant joint loading disrupts normal cartilage-bone homeostasis.

Table II. Roles of exercise-induced exosomal ncRNAs in sports-related bone injuries: A summary of key studies.

Authors, year	Injury type	Cell/Tissue source	Model	Exosomal ncRNA	Target/Pathway	Key findings	(Refs.)
Shang *et al*, 2021	Osteochondral injury	Chondrocytes/ osteoblasts	*In vitro*	Epigenetic regulators	Mechanotransduction	Enabled epigenetic mechanotransduction between chondrocytes and osteoblasts	(71)
Wang *et al*, 2025	Disuse osteopenia	Slo1-deficient myoblasts	*In vitro*	miR-222-3p	STAT3	Inhibited osteogenic differentiation via targeting STAT3, demonstrating quality-dependent effects	(77)
Lu *et al*, 2022	Traumatic fracture	Osteoblast/ osteoclast/ immune cells	Animal (rat)	Exosome/drug delivery hydrogel	Multicellular coordination	Accelerated fracture repair through coordinated multicellular responses	(78)
Liu *et al*, 2020	Traumatic fracture	Hypoxic MSCs	Animal (rat)	miR-126	Angiogenesis and osteogenesis	Promoted bone fracture healing by enhancing angiogenesis and osteogenesis simultaneously	(79)
Jiang *et al*, 2020	Traumatic fracture	BMSCs	Animal (mouse)	miR-25	SMURF1/Runx2	Regulated Runx2 ubiquitination and degradation to promote fracture healing in mice	(80)
Yang *et al*, 2025	Osteochondral injury (TMJ)	MSCs	Animal (rat)	let-7a-5p	Integrin β3	Suppressed osteoclast activity and ameliorated TMJ osteoarthritis	(81)
Xu *et al*, 2020	Stress fracture	Aged rat MSCs	Animal (rat)	miR-128-3p	Smad5	Regulated osteogenesis and bone fracture healing in aged rats	(82)
Tian *et al*, 2025	Nonunion fracture	Mechanical stretch-induced ADSCs	Animal (rat)	miR-877	Not identified	Enhanced fracture healing in nonunion rats with type 2 diabetes mellitus	(85)
Wu *et al*, 2021	Osteoarthritis	Osteoarthritic subchondral bone	Animal (mouse)	miR-210-5p	Cartilage degeneration	Released exosomes promoting cartilage degeneration, establishing pathological feedback loop	(86)
Lin *et al*, 2025	Osteoarthritis	Osteocytes	*In vitro*/ Animal	miR-214-3p	Bone-to-cartilage crosstalk	Mediated bone-to-cartilage crosstalk and promoted osteoarthritis progression	(87)
Wang *et al*, 2022	Osteoporosis	MSCs	Animal (mouse)	miR-27a	DKK2/ Wnt/β-catenin	Inhibited osteoporosis via miR-27a-induced inhibition of DKK2-mediated Wnt/β-catenin pathway	(88)
Van Pelt *et al*, 2020	Disuse osteopenia	Serum EVs	Human	miR-203a-3p	Muscle protein turnover	Associated with skeletal muscle mass and protein turnover during disuse atrophy and regrowth	(90)

Table II. Continued.

Authors, year	Injury type	Cell/Tissue source	Model	Exosomal ncRNA	Target/Pathway	Key findings	(Refs.)
Yang *et al*, 2020	Disuse osteoporosis	hUC-MSCs	Animal (rat)	miR-1263	Mob1/Hippo	Prevented apoptosis in disuse osteoporosis via miR-1263/Mob1/Hippo signaling pathway	(91)
Shao *et al*, 2026	Disuse osteoporosis	Atrophic skeletal muscle	Animal (mouse)	miR-125a-5p	Bone formation	Transferred miR-125a-5p to inhibit bone formation during aging-related osteoporosis	(92)

ncRNAs, noncoding RNAs; Slo-1, slowpoke homolog 1; miR, microRNA; STAT3, signal transducer and activator of transcription 3; MSCs, mesenchymal stem cells; BMSCs, bone marrow mesenchymal stem cells; ADSCs, adipose-derived stromal cells; EVs, extracellular vesicles; SMURF1, SMAD specific E3 ubiquitin protein ligase 1; DKK2, dickkopf-related protein 2; hUC-MSCs, human umbilical cord mesenchymal stem cells; TMJ, temporomandibular joint; Runx2, runt-related transcription factor 2; Mob1, Mps one binder 1.

Wu *et al* (86) reported that osteoarthritic subchondral bone releases exosomes that promote cartilage degeneration, establishing a pathological feedback loop that accelerates osteoarthritis progression. Recent advances by Lin *et al* (87) demonstrated that osteocyte-derived extracellular vesicles mediate the bone-to-cartilage crosstalk and promote osteoarthritis progression, providing mechanistic insight into how bone pathology drives cartilage degeneration.

Conversely, protective exosomal signals from healthy tissues can mitigate injury-induced degeneration. Wang *et al* (88) demonstrated that mesenchymal stem cell-derived extracellular vesicles inhibit osteoporosis via miR-27a-induced inhibition of the DKK2-mediated Wnt/β-catenin pathway. More recent work by Gu *et al* (16) showed that bone remodeling stimulated by Wnt-mediated mitophagy-regulated extracellular vesicles in subchondral bone contributes to osteoarthritis development, highlighting the interconnected nature of bone and cartilage pathology. The identification of osteoarthritis-specific exosomal miRNA signatures provides potential biomarkers for early detection of post-traumatic osteoarthritis following sports injuries. Qiu *et al* (89) comprehensively reviewed the therapeutic potential of exosomal ncRNAs in bone metabolic diseases, focusing on osteoarthritis and rheumatoid arthritis.

Disuse osteopenia after sports injury. Immobilization and disuse following sports injury create a catabolic environment characterized by rapid bone loss, where exercise-induced exosomal ncRNAs may exert protective effects against disuse osteopenia. The absence of mechanical loading during immobilization reduces endogenous exosomal signals that normally maintain bone mass, creating an opportunity for exogenous or exercise-mimetic exosome therapies. Van Pelt *et al* (90) demonstrated that serum extracellular vesicle miR-203a-3p content is associated with skeletal muscle mass and protein turnover during disuse atrophy and regrowth, establishing a link between exosomal cargo and disuse-induced tissue loss. This finding suggests that monitoring exosomal miRNAs could guide rehabilitation strategies following immobilization. Yang *et al* (91) reported that human umbilical cord mesenchymal stem cell-derived exosomes act via the miR-1263/Mps one binder 1 (Mob1)/Hippo signaling pathway to prevent apoptosis in disuse osteoporosis. Recent work by Shao *et al* (92) demonstrated that atrophic skeletal muscle-derived extracellular vesicles transfer miR-125a-5p to inhibit bone formation in osteoporosis during aging, revealing that pathological changes in muscle during disuse can actively promote bone loss. This finding underscores the importance of maintaining muscle activity during immobilization. Bao and He (93) showed that skeletal muscle-derived exosomes prevent osteoporosis by promoting osteogenesis, indicating that delivering muscle-derived exosomes could counteract disuse osteopenia. More recently, Wang *et al* (77) reported that Slo1-deficient myoblast exosomes-derived miR-222-3p inhibits osteogenic differentiation via targeting STAT3, demonstrating that the quality of muscle-derived exosomes critically determines their effects on bone. The concept of using exercise-conditioned exosomes as a replacement therapy during immobilization represents a promising translational strategy for athletes recovering from injury.

6. Translational applications of exercise-induced exosomal ncRNAs in sports-related bone injuries

The translational potential of exercise-induced exosomal noncoding RNAs spans diagnostic, therapeutic, and rehabilitative applications in sports-related bone injuries. These endogenous nanovesicles offer advantages as non-invasive biomarkers for injury assessment and as naturally derived therapeutic agents that recapitulate the beneficial effects of exercise. Their clinical translation requires standardization of isolation protocols, validation of response characteristics for personalized prescription, and integration with existing treatment modalities. A systematic overview of these translational strategies is presented (Table III).

Non-invasive biomarkers. Circulating exosomal ncRNAs have emerged as promising non-invasive biomarkers for early diagnosis, risk stratification, and prognosis evaluation of sports-related bone injuries. Their stability in body fluids and tissue-specific origin make them attractive candidates for liquid biopsy approaches. Shi *et al* (94) identified miRNAs in serum exosomes as circulating biomarkers for postmenopausal osteoporosis, demonstrating the feasibility of exosomal miRNA-based diagnostics for bone disorders. Similarly, Shao *et al* (95) established serum exosomal miRNA expression profiling in menopausal females with osteoporosis using high-throughput sequencing, providing a reference for biomarker discovery. The prognostic value of exosomal ncRNAs has been demonstrated in fracture healing complications. Hou *et al* (96) reported a prospective analysis showing that elevated exosomal miR-21 levels are associated with nonunion in clavicular fractures post-open reduction and internal fixation (ORIF), providing direct clinical evidence linking exosomal cargo to fracture healing outcomes. For osteoarthritis, Zhao and Xu (97) identified synovial fluid-derived exosomal lncRNA PCGEM1 as a biomarker for different stages of osteoarthritis, highlighting the potential for staging sports-related joint injuries. More recently, Sun *et al* (98) identified and evaluated circulating exosomal miRNAs for the diagnosis of postmenopausal osteoporosis, further validating the biomarker potential of exosomal ncRNAs. The specificity of exosomal signatures for distinct injury types and healing stages suggests that multi-panel approaches may achieve higher diagnostic accuracy than single biomarkers.

Therapeutic strategies based on native exercise-derived exosomes. Native exosomes derived from exercise-conditioned tissues offer naturally optimized therapeutic vehicles for bone injury repair. These vesicles inherently carry the molecular cargo that mediates the beneficial effects of exercise, potentially circumventing safety concerns associated with synthetic delivery systems. Deininger *et al* (99) demonstrated that anatomical implant region critically determines the osteogenic potency of small extracellular vesicles, highlighting the importance of delivery site optimization. The extraction and standardization of exercise-modulated exosomes face challenges related to donor variability, exercise protocol standardization, and scalable production. Deluca *et al* (100) showed a synergistic effect of umbilical cord extracellular vesicles and recombinant human bone morphogenetic protein-2 (rhBMP-2) to enhance regeneration of metaphyseal femoral defects in osteoporotic rats, suggesting that exosomes can be combined with established growth factors for enhanced efficacy. Wu *et al* (101) demonstrated that regulatory T cell-derived exosomes mediated macrophage polarization for osteogenic differentiation in fracture repair, revealing that immune cell-derived exosomes may complement exercise-derived vesicles in promoting bone healing. The clinical application potential of exercise-derived exosomes requires rigorous quality control and standardization comparable to pharmaceutical products.

Engineered exosomes and ncRNA-targeted therapy. Engineered exosomes designed to deliver specific ncRNA cargo represent a next-generation therapeutic strategy for refractory bone injuries. These approaches leverage the natural targeting capabilities of exosomes while incorporating synthetic modifications to enhance therapeutic efficacy. Strategies include direct engineering of parent cells to overexpress therapeutic ncRNAs, which are then naturally packaged into secreted exosomes, as well as post-isolation modification of exosomes with targeting ligands or therapeutic cargo. Li *et al* (102) demonstrated the use of engineered stem cells to produce exosomes with enhanced bone regeneration effects, presenting an alternative strategy for gene therapy that avoids direct stem cell transplantation. The delivery of ncRNA therapeutics through engineered exosomes offers advantages including protection from degradation, targeted delivery, and sustained release. For example, exosomes can be surface-modified with targeting peptides to achieve bone-specific delivery, or loaded with therapeutic ncRNAs via electroporation or chemical transfection to enhance their osteogenic or anti-resorptive potency. Huang *et al* (103) developed engineered exosomes as targeted lncRNA MEG3 delivery vehicles for osteosarcoma therapy, demonstrating the versatility of exosome engineering for musculoskeletal applications. Xu *et al* (104) developed osteoclast-targeted delivery of anti-miRNA oligonucleotides by red blood cell extracellular vesicles, showing that cell-specific targeting can be achieved through engineering approaches. For osteoarthritis, Wang *et al* (105) described extracellular vesicle-mediated miR-150-3p delivery in joint homeostasis as a potential treatment, illustrating the therapeutic potential of miRNA-based exosome therapies. More recently, Yan *et al* (106) engineered delta-like ligand 4 (Dll4)-overexpressing osteocyte-derived exosomes to enhance bone regeneration by regulating osteogenesis and angiogenesis, providing a blueprint for tissue-specific exosome engineering.

Beyond these established approaches, recent advances have focused on optimizing the stoichiometry and spatiotemporal control of ncRNA loading to enhance therapeutic efficacy. Comparative studies have shown that electroporation achieves the highest loading efficiency for small ncRNAs (miRNAs and siRNAs) into exosomes, whereas chemical transfection methods (such as Lipofectamine) yield lower encapsulation rates and may introduce residual carrier toxicity that confounds functional outcomes (15). For bone-targeted delivery, surface modification of exosomes with bone-homing peptides, such as the $(DSS)_6$ bone-homing peptide (a six-repeat aspartate-serine-serine sequence) that binds hydroxyapatite, has been successfully employed to concentrate therapeutic ncRNAs at fracture sites while minimizing systemic off-target

Table III. Translational applications of exercise-induced exosomal ncRNAs in sports-related bone injuries.

Authors, year	Study type	ncRNA cargo	Application domain	Key finding	Translational implication	(Refs.)
Shi *et al*, 2022	Human cohort	miRNAs	Non-invasive biomarkers	Identified miRNAs in serum exosomes as circulating biomarkers for postmenopausal osteoporosis	Demonstrates feasibility of exosomal miRNA-based diagnostics for bone disorders	(94)
Shao *et al*, 2020	Human cohort	miRNAs	Non-invasive biomarkers	High-throughput sequencing identified serum exosomal miRNA expression profiles in osteoporotic females	Provides reference for biomarker discovery in bone metabolic conditions	(95)
Hou *et al*, 2024	Human cohort	miR-21	Non-invasive biomarkers	Elevated exosomal miR-21 levels associated with nonunion in clavicular fractures post-ORIF	Supports exosomal miRNAs to predict delayed healing or nonunion following sports-related fractures	(96)
Zhao and Xu, 2018	Human cohort	lncRNA PCGEM1	Non-invasive biomarkers	Synovial fluid-derived exosomal lncRNA PCGEM1 differentiates osteoarthritis stages	Enables staging of sports-related joint injuries	(97)
Sun *et al*, 2023	Human cohort	miRNAs	Non-invasive biomarkers	Circulating exosomal miRNAs identified for postmenopausal osteoporosis diagnosis	Validates multi-panel exosomal miRNA approaches for diagnostic accuracy	(98)
Deininger *et al*, 2025	Preclinical	sEVs	Therapeutic strategy	Anatomical implant region critically determines osteogenic potency of sEVs	Highlights importance of delivery site optimization for exosome-based therapy	(99)
Deluca *et al*, 2024	Preclinical	sEVs	Combination therapy	Umbilical cord sEVs synergize with rhBMP-2 to enhance femoral defect regeneration	Supports combining exosomes with growth factors for enhanced efficacy	(100)
Wu *et al*, 2024	Preclinical	Exosomes	Therapeutic strategy	Regulatory T cell-derived exosomes mediate macrophage polarization for osteogenic differentiation	Reveals immune cell-derived exosomes complement exercise-derived vesicles	(101)
Li *et al*, 2022	Preclinical	Exosomes	Engineered therapy	Engineered stem cells produce exosomes with enhanced bone regeneration effects	Offers alternative gene therapy strategy avoiding direct stem cell transplantation	(102)
Huang *et al*, 2022	Preclinical	lncRNA MEG3	Engineered therapy	Engineered exosomes serve as targeted lncRNA MEG3 delivery vehicles for osteosarcoma	Demonstrates versatility of exosome engineering for musculoskeletal applications	(103)
Xu *et al*, 2023	Preclinical	Anti-miRNA oligonucleotides	Engineered therapy	Osteoclast-targeted delivery achieved via red blood cell EV engineering	Shows cell-specific targeting through engineering approaches	(104)

Table III. Continued.

Authors, year	Study type	ncRNA cargo	Application domain	Key finding	Translational implication	(Refs.)
Wang *et al*, 2022	Preclinical	miR-150-3p	Engineered therapy	EV-mediated miR-150-3p delivery in joint homeostasis as potential osteoarthritis treatment	Illustrates therapeutic potential of miRNA-based exosome therapies	(105)
Yang *et al*, 2023	Preclinical	BMSC-derived exosomes	Combination therapy	Combination therapy with BMSC-exosomes and porous tantalum enhances femoral defect repair	Shows exosomes enhance osteointegration of metallic implants	(107)
Zheng *et al*, 2024	Preclinical	sEVs	Combination therapy	Incorporation of sEVs in PEG/HA-Bio-Oss hydrogel composite scaffold for bone regeneration	Illustrates synergy between exosomes and biomaterial scaffolds	(108)
Yu *et al*, 2024	Preclinical	Shed-derived exosomes	Combination therapy	Synergistic effects with Cu^{2+} and injectable hyaluronic acid hydrogel for periodontal bone regeneration	Showcases multi-component combination approaches	(109)
Zhang *et al*, 2024	Preclinical	Exosome-loaded hydrogel	Combination therapy	Exosome-loaded hyaluronic acid hydrogel with oxygen-producing 3D printed PLA scaffolds	Combines advanced manufacturing with exosome delivery for bone repair	(110)
Frank *et al*, 2025	Human cohort	miRNAs	Non-invasive biomarkers	Circulating miRNAs associated with successful bone regeneration	Provides framework for using exosomal ncRNAs as prognostic markers	(111)
Gao *et al*, 2024	Human cohort	miRNA signature	Non-invasive biomarkers	Plasma-derived exosomal miRNA signature established for early detection of postmenopausal osteoporosis	Demonstrates feasibility of exosomal miRNA-based diagnostics	(113)

ncRNA, noncoding RNA; miRNA or miR, microRNA; ORIF, open reduction and internal fixation; sEV, small extracellular vesicle; rhBMP-2, recombinant human bone morphogenetic protein-2; EV, extracellular vesicle; BMSC, bone marrow mesenchymal stem cell; lncRNA, long noncoding RNA; PEG/HA, polyethylene glycol/hyaluronic acid; PLA, polylactic acid.

effects (17,21). Furthermore, emerging evidence supports the use of sequential loading strategies, where pro-angiogenic ncRNAs (such as miR-126) and osteogenic ncRNAs (such as miR-26a) are packaged into distinct exosome batches and administered according to the temporal phases of bone healing, first promoting vascularization during soft callus formation, followed by enhancing mineralization during hard callus remodeling (23,79). This phased approach mimics the natural time-course of fracture repair and has shown superior outcomes compared with single-cargo or bolus administration in preclinical models (23). However, the clinical translation of such multi-cargo, temporally programmed exosome therapeutics requires rigorous validation of batch-to-batch consistency, long-term storage stability, and scalable manufacturing under Good Manufacturing Practice conditions, barriers that remain incompletely addressed (15,21).

Combination intervention strategies. The integration of exercise-induced exosomal ncRNAs with existing treatment modalities offers synergistic opportunities for enhanced therapeutic outcomes. Combination strategies encompass stem cell therapy, biomaterials, physical therapy, and pharmacotherapy. Yang *et al* (107) demonstrated combination therapy with BMSC-derived exosomes and porous tantalum for the repair of femur supracondylar defects, showing that exosomes can enhance the osteointegration of metallic implants. Similarly, Zheng *et al* (108) incorporated small extracellular vesicles in polyethylene glycol (PEG)/hyaluronic acid (HA)-Bio-Oss hydrogel composite scaffold for bone regeneration, illustrating the synergy between exosomes and biomaterial scaffolds. Yu *et al* (109) demonstrated synergistic effects of Shed-derived exosomes, Cu^{2+}, and an injectable hyaluronic acid hydrogel on antibacterial, anti-inflammatory, and osteogenic activity for periodontal bone regeneration, showcasing multi-component combination approaches. Zhang *et al* (110) developed exosome-loaded hyaluronic acid hydrogel composite with oxygen-producing 3D printed polylactic acid scaffolds, combining advanced manufacturing with exosome delivery for bone tissue repair. For fracture repair, Wu *et al* (101) showed that regulatory T cell-derived exosomes mediated macrophage polarization for osteogenic differentiation, suggesting that immune modulation combined with osteogenic stimulation may optimize healing. The comparative analysis of these combination strategies reveals that scaffold-based delivery provides sustained local release, while systemic administration may benefit multifocal injuries. Future studies should systematically evaluate the relative efficacy of different combination approaches to establish evidence-based guidelines for clinical translation.

Clinical evidence: Human cohort studies. Clinical evidence linking exercise-induced exosomal ncRNAs to the occurrence, severity, and prognosis of sports-related bone injuries is emerging through human cohort studies. Circulating exosomal ncRNAs have been investigated as non-invasive biomarkers for fracture healing outcomes and complication risk. Shi *et al* (94) identified miRNAs in serum exosomes as circulating biomarkers for postmenopausal osteoporosis, establishing a foundation for similar approaches in sports-related bone injuries. The association between exosomal miRNA profiles and fracture healing complications has been demonstrated in clinical populations. Hou *et al* (96) reported a prospective analysis showing that elevated exosomal miR-21 levels are associated with nonunion in clavicular fractures post-ORIF, providing direct clinical evidence linking exosomal cargo to fracture healing outcomes. This finding supports the potential of exosomal miRNAs to predict delayed healing or nonunion following sports-related fractures. Recent advances by Frank *et al* (111) demonstrated that circulating miRNAs are associated with successful bone regeneration, providing a framework for using exosomal ncRNAs as prognostic markers. The prognostic value of exosomal signatures extends to bone metabolic status following injury. Mihanfar *et al* (112) demonstrated that extracellular vesicles and miRNAs are altered in response to exercise, insulin sensitivity and overweight status, indicating that metabolic factors influence exosomal profiles and may confound biomarker interpretation. Gao *et al* (113) recently established a plasma-derived exosomal miRNA signature by small RNA sequencing for early detection of postmenopausal osteoporosis, demonstrating the feasibility of exosomal miRNA-based diagnostics. Zhang *et al* (8) comprehensively reviewed exosomal miRNAs in muscle-bone crosstalk, detailing mechanistic links and exercise modulation with direct implications for sarcopenia, osteoporosis, and osteosarcopenia. Longitudinal studies tracking exosomal ncRNA changes during rehabilitation are needed to establish causality and clinical utility. The integration of exosomal biomarkers into clinical practice for sports-related bone injuries requires standardization of isolation protocols and validation across diverse athlete populations.

7. Current limitations, controversies, and unresolved scientific issues

Despite the rapid advancement in understanding exercise-induced exosomal ncRNAs as key mediators of bone remodeling, the field faces substantial limitations, ongoing controversies, and unresolved scientific issues that critically hinder clinical translation. These challenges span methodological inconsistencies in exosome isolation, insufficient causal evidence linking exosomal ncRNAs to specific skeletal outcomes, significant gaps in clinical study design, and formidable barriers to therapeutic implementation. A systematic overview of these limitations and controversies is presented.

Clinical study limitations. Current clinical evidence on exercise-induced exosomal ncRNAs in bone metabolism is predominantly derived from studies with inherent methodological constraints that limit generalizability. The majority of investigations feature small sample sizes and lack multi-center validation, compromising statistical power and external validity. For instance, studies examining circulating exosomal miRNA signatures in fracture healing typically enroll fewer than 100 participants, precluding robust subgroup analyses (114,115). Population heterogeneity represents another critical concern, as individual characteristics such as age, sex, fitness level, and hormonal status profoundly influence exosomal responses, yet stratification is frequently inadequate (116,117). Furthermore, exercise intervention protocols

vary considerably across studies, with inconsistent parameters regarding intensity, duration, and training modality, impeding direct comparisons and meta-analytic synthesis (118,119). The absence of standardized pre-analytical procedures, including sample collection timing relative to exercise bouts and anticoagulant selection, introduces additional variability (120). Collectively, these limitations underscore the urgent need for prospective, multi-center large-cohort studies employing harmonized protocols to establish clinically actionable exosomal biomarkers.

Mechanistic controversies and gaps. Fundamental mechanistic questions persist regarding whether exercise-induced exosomal ncRNAs exert direct causal effects on bone remodeling or merely represent correlative biomarkers of systemic adaptation. A substantial proportion of published studies relies on descriptive profiling without functional validation, leaving causal inference inadequately supported. Specifically, numerous studies cited in this review (such as those reporting acute exercise-induced changes in circulating exosomal miRNA levels) demonstrate correlation rather than causation. While these findings are valuable for hypothesis generation, they do not constitute molecular mechanism demonstration. Although *in vitro* and animal models have demonstrated that exosomal miRNAs such as miR-181b-5p and miR-27a-3p directly modulate osteogenic pathways (121,122), the translational relevance of these findings to human physiology remains uncertain due to species differences in ncRNA sequences, target repertoires, and exosome biogenesis machinery (123).

A more fundamental issue is that even in animal studies, few have employed rigorous causal validation frameworks. The minimum requirements for establishing causality in this field should include: i) Selective knockout or knockdown of specific exosomal ncRNAs in relevant parent cells (such as skeletal muscle or osteocytes) followed by exercise intervention; ii) rescue experiments using ncRNA mimics or engineered exosomes; and iii) tissue-specific ablation of exosome biogenesis machinery (such as Rab27a or nSMase2 knockout) to determine whether exosomal ncRNAs are necessary for exercise-mediated bone protection. To date, no study has comprehensively met these criteria, representing a critical gap in the literature.

Cell and tissue specificity of exosomal ncRNA actions constitutes another unresolved controversy. Current evidence suggests that exosomes from distinct sources, including skeletal muscle, osteocytes, and adipose tissue, may exert overlapping yet distinct effects on bone cells (124,125); however, the molecular determinants governing recipient cell specificity remain poorly characterized. Additionally, the potential off-target effects of exosomal ncRNAs on non-skeletal tissues raise safety concerns that have not been systematically evaluated. Moreover, most current studies have examined the effect of a given exosomal ncRNA on only one cell type in isolation, without systematically comparing its functional consequences across osteoblasts, osteoclasts, and BMSCs; direct extrapolation of these reductionistic *in vitro* findings to *in vivo* bone adaptation lacks scientific validity. The field therefore requires a paradigm shift from descriptive correlative studies to hypothesis-driven mechanistic investigations using loss-of-function and gain-of-function approaches with cell-type-specific targeting.

Methodological challenges. The absence of standardized protocols for exosome isolation and characterization represents a fundamental barrier to data reproducibility and cross-study comparability. Ultracentrifugation, the most widely employed isolation method, exhibits variable efficiency and co-pellets non-vesicular contaminants, including protein aggregates and lipoproteins (126,127). Size-exclusion chromatography offers improved purity but suffers from lower yield and longer processing times, while polymer-based precipitation methods frequently co-precipitate non-exosomal materials that confound downstream analyses (120,128). The distinction between exosomes and other extracellular vesicle subtypes, as well as lipoprotein particles of similar size, remains technically challenging, with conventional characterization methods lacking sufficient resolution (129). For ncRNA detection, the choice of isolation method profoundly influences miRNA profiles, yet consensus guidelines for method selection are lacking. Functional validation methodologies also exhibit heterogeneity, with limited standardization in exosome labeling, tracking, and uptake assays (128). The development of reference materials and validation protocols, as recently advocated (130), represents a critical priority for advancing the field toward clinical implementation.

Barriers to clinical translation. Translating exercise-induced exosomal ncRNAs into clinical applications for sports-related bone injuries confronts formidable obstacles spanning manufacturing, safety, and regulatory domains. Scalable production of therapeutic exosomes with consistent quality attributes remains technically demanding, as current culture conditions for exercise-mimetic exosome generation lack standardization (131). Good Manufacturing Practice compliance necessitates rigorous quality control measures, including characterization of size distribution, cargo composition, and batch-to-batch consistency, which are not routinely implemented in research settings (132). The optimal route of administration for exosome-based therapies in bone injuries remains undefined, with limited comparative data on local vs. systemic delivery, dosing frequency, and tissue biodistribution (133). Long-term safety evaluations, including immunogenicity, potential for off-target effects, and carcinogenicity, have not been systematically conducted (134). Furthermore, regulatory frameworks for exosome-based therapeutics are still evolving, creating uncertainty regarding approval pathways and quality requirements (132,135). These translational barriers necessitate coordinated efforts between academic researchers, regulatory agencies, and industry partners to establish clear guidelines and accelerate clinical development.

8. Conclusions and future perspectives

The preceding sections have established exercise-induced exosomal ncRNAs as central mediators of bone remodeling, yet the field stands at a critical juncture where fundamental discoveries must be translated into clinical applications. Building upon the limitations and unresolved questions

outlined above, several strategic directions warrant prioritization to advance both mechanistic understanding and therapeutic implementation. Rather than reiterating existing consensus, this section identifies specific unresolved scientific issues that require investigation. Notably, four priority issues emerge from the current literature: i) Elucidating the molecular mechanisms that govern selective ncRNA packaging into exosomes in response to distinct exercise modalities; ii) determining whether exercise-induced exosomal ncRNAs directly cause bone adaptation, or merely serve as correlative biomarkers; iii) overcoming inter-individual variability in exosomal responses for personalized exercise prescription; and iv) addressing the safety barriers that must be addressed before exercise-mimetic exosome therapies can enter clinical trials for sports-related bone injuries.

For basic research, the integration of multi-omics approaches with systems biology represents a transformative opportunity to decipher the complex regulatory networks governing exercise-induced exosomal ncRNA function. Recent advances enable simultaneous profiling of transcriptomic, proteomic, and lipidomic signatures from limited sample volumes, which could elucidate how mechanical stimuli are translated into specific exosomal cargo selection mechanisms (136,137). Single-cell and spatial transcriptomic technologies offer unprecedented resolution to map exosomal ncRNA dynamics within the bone microenvironment, identifying rare cell populations that serve as primary exosomal targets and revealing how exosomal signaling gradients establish regional specialization during fracture healing (138). A fundamental unresolved issue concerns causality. To address whether exosomal ncRNAs are necessary and sufficient for exercise-mediated bone protection, future investigations should employ conditional knockout models targeting exosome biogenesis machinery in tissue-specific manners, combined with *in vivo* tracking technologies and CRISPR-Cas9 functional interrogation (139-141).

The translation of exosomal ncRNA biology into clinical applications for sports-related bone injuries requires substantial investment in large-scale, multi-center cohort studies. Current clinical evidence is constrained by small sample sizes, heterogeneous populations, and inconsistent methodological standards (142). A key clinical question is whether exosomal ncRNA signatures can predict fracture healing complications with sufficient sensitivity and specificity to guide decision-making. Future studies should adopt harmonized protocols for sample collection, processing, and data reporting to enable cross-study comparability and meta-analyses. The development of standardized reference materials for exosome isolation and characterization, as recently advocated by international consortia, represents a critical prerequisite for clinical implementation (137). Such standardization efforts must extend to pre-analytical variables including anticoagulant selection, storage conditions, and timing of sample collection relative to exercise bouts, factors that profoundly influence exosomal cargo composition yet remain inconsistently reported (143).

Personalized exercise prescription guided by exosomal ncRNA response profiles represents an emerging frontier in sports medicine. The substantial inter-individual variability in exosomal responses to standardized exercise protocols suggests that molecular phenotyping could optimize exercise interventions for bone health (8). Future studies should characterize how age, sex, genetic background, and baseline fitness level modulate exosomal ncRNA responses, thereby establishing normative reference ranges against which individual responses can be benchmarked. Machine learning approaches integrating multi-dimensional molecular data with clinical parameters hold promise for developing predictive algorithms that recommend optimal exercise modalities, intensities, and durations for individuals at risk of bone injury (136,141). This precision exercise paradigm represents a shift from population-based recommendations to individualized molecularly guided interventions.

Therapeutic strategies based on engineered exosomes offer unprecedented opportunities for treating refractory bone injuries. Recent proof-of-concept studies have demonstrated that exosomes can be engineered to display targeting ligands, encapsulate therapeutic ncRNA cargo, and achieve controlled release profiles (144,145). However, safety issues remain unanswered, including whether exercise-mimetic exosomes are immunogenic; and what the potential off-target effects on non-skeletal tissues are. Future translational efforts should prioritize the development of exercise-mimetic exosomes that recapitulate the multi-targeted effects of physical activity without requiring patient participation, as this represents a particularly valuable approach for injured athletes undergoing immobilization. Scalable manufacturing processes compliant with Good Manufacturing Practice standards are essential for clinical translation, requiring rigorous quality control measures encompassing size distribution, cargo composition, and batch-to-batch consistency (139,146). Furthermore, combination strategies integrating exosome therapeutics with biomaterial scaffolds or pharmacological agents may achieve synergistic effects that surpass either approach alone. In summary, addressing the four priority issues outlined above, namely cargo selection mechanisms, causality, biomarker utility, and safety, will determine whether exercise-induced exosomal ncRNAs remain a descriptive phenomenon or become a translatable paradigm for sports medicine.

In conclusion, the study of exercise-induced exosomal ncRNAs in bone remodeling represents an emerging paradigm that links physical activity to skeletal adaptation. Current evidence supports a correlative framework in which exercise dynamically modulates exosomal ncRNA cargo and these changes are associated with osteogenic outcomes. However, whether this paradigm is causally valid remains to be established. Direct evidence demonstrating that specific exosomal ncRNAs are necessary and sufficient for exercise-induced bone protection is still lacking. The present review offered several distinctive advantages: It systematically integrated exercise physiology, exosome biology, and bone remodeling into a cohesive framework; detailed specific signaling cascades (Wnt/β-catenin, BMP/Smad, and RANKL/RANK/OPG) modulated by exercise-induced exosomal ncRNAs; and addressed the translational potential for sports medicine applications including biomarker development, personalized exercise prescription, and exosome-based therapeutics. The convergence of multi-omics technologies, single-cell resolution analyses, rigorous causal validation frameworks, and standardized clinical protocols provide a roadmap for addressing current limitations. For sports medicine practitioners, these

advances promise the development of noninvasive diagnostic biomarkers, personalized exercise prescriptions, and novel exosome-based therapeutics that harness the regenerative potential of physical activity. Realizing this potential will require sustained interdisciplinary collaboration among molecular biologists, bioengineers, exercise physiologists, and clinicians, united by the shared goal of translating mechanistic insights into improved outcomes for athletes with bone injuries.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

BT and XK conceived and designed the review framework. XC and JZ performed the literature search, screening, and synthesis of relevant studies. All authors participated in drafting and critically revising the manuscript, and read and approved the final version, and accept accountability for all aspects of the work. Data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. DeFoor MT, Mullin EP, Cuenca RA and Pierrie SN: Distal femoral stress fractures has high operation rates in adolescent and young adult athletes. J Pediatr Orthop 44: e439-e445, 2024.
2. Salášek M, Stančák A, Čepelík M, Pešl T, Havlas V, Pavelka T, Havránek P and Džupa V: Pelvic avulsion fractures in children: A retrospective study from four trauma centers. Acta Chir Orthop Traumatol Cech 92: 7-14, 2025 (In Czech).
3. Feldman MW, Fucaloro S, Krivicich L, Kent S and Salzler MJ: Studies directly comparing Lisfranc injuries treated with primary arthrodesis or open reduction and internal fixation show no significant difference in return to sport and complications: A systematic review and meta-analysis. J Foot Ankle Surg 64: 318-327, 2025.
4. Williams BA, Titus M, Chaclas N, Cardin S, Wells L, Maguire KJ and Lawrence JT: Surgically treated ischial tuberosity avulsion fractures in adolescents: Risks and outcomes of 3 fixation constructs. J Pediatr Orthop 45: e30-e36, 2025.
5. Frost HM: The mechanostat: A proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone Miner 2: 73-85, 1987.
6. Yang J, Pei Q, Wu X, Dai X, Li X, Pan J and Wang B: Stress reduction through cortical bone thickening improves bone mechanical behavior in adult female Beclin-1$^{+/-}$ mice. Front Bioeng Biotechnol 12: 1357686, 2024.
7. Penna F, Garcia-Castillo L and Costelli P: Extracellular vesicles and exosomes in the control of the musculoskeletal health. Curr Osteoporos Rep 22: 257-265, 2024.
8. Zhang B, Chen Y, Chen Q and Zhang H: Exosomal miRNAs in muscle-bone crosstalk: Mechanistic links, exercise modulation and implications for sarcopenia, osteoporosis and osteosarcopenia. Metabolism 170: 156333, 2025.
9. Binet F, Entz L, Amiot M, Tessier S, Véziers J, De Beaurepaire L, André-Grégoire G, Dingli F, Loew D, Weiss P, *et al*: Exploring the molecular cargos associated with extracellular vesicles extracted from bone tissue to identify novel players in osteoblast function. Am J Physiol Cell Physiol 329: C1521-C1538, 2025.
10. Alhazmi AS: Bone remodeling optimization in orthodontics: Harnessing periodontal ligament cell-derived exosomes and anti-inflammatory compounds. J Contemp Dent Pract 26: 77-85, 2025.
11. Zhang F, Cao K, Du G, Zhang Q and Yin Z: miR-29a promotes osteoblast proliferation by downregulating DKK-1 expression and activating Wnt/β-catenin signaling pathway. Adv Clin Exp Med 28: 1293-1300, 2019.
12. Yang N, Wang G, Hu C, Shi Y, Liao L, Shi S, Cai Y, Cheng S, Wang X, Liu Y, *et al*: Tumor necrosis factor α suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. J Bone Miner Res 28: 559-573, 2013.
13. Yu H, Li Y, Tang J, Lu X, Hu W and Cheng L: Long non-coding RNA RP11-84C13.1 promotes osteogenic differentiation of bone mesenchymal stem cells and alleviates osteoporosis progression via the miR-23b-3p/RUNX2 axis. Exp Ther Med 22: 1340, 2021.
14. Jiang Y, Shi J, Di W, Teo KYW and Toh WS: Mesenchymal stem cell-based therapies for temporomandibular joint repair: A systematic review of preclinical studies. Cells 13: 990, 2024.
15. Li X, Wang C, Du K, Wang J, Lin J and Yang C: Mesenchymal stem cell-derived exosomes: A promising therapeutic strategy for spinal cord injury. Int J Nanomedicine 20: 15901-15920, 2025.
16. Gu Y, Zhou Q, Sheng S, Yang H, Huang D, Zhang Q, Zhang H, Cao Z, Zhang Y, Li Z, *et al*: Bone remodeling stimulated by Wnt-mediated mitophagy regulated extracellular vesicles in subchondral bone contributes to osteoarthritis development. Theranostics 15: 10007-10027, 2025.
17. Minami S, Fujii Y, Yoshioka Y, Hatori A, Kaneko K, Ochiya T and Chikazu D: Extracellular vesicles from mouse bone marrow macrophages-derived osteoclasts treated with zoledronic acid contain miR-146a-5p and miR-322-3p, which inhibit osteoclast function. Bone 190: 117323, 2025.
18. Petrousek SR, Kronemberger GS, O'Brien G, Hughes C, O'Rourke SA, Lally C, Dunne A, Kelly DJ and Hoey DA: Mechano-immunomodulation of macrophages influences the regenerative environment of fracture healing through the regulation of angiogenesis and osteogenesis. Acta Biomater 200: 187-201, 2025.
19. Zhong F, Hao W, Chao H, Li Z, Chen S, Li S, Zhang S, Zhang K, Li Z and Liu H: Tenascin-C promotes endochondral ossification and fracture healing through Hedgehog and Hippo signaling. Biochem Biophys Res Commun 703: 149634, 2024.
20. Haffner-Luntzer M, Ragipoglu D, Ahmad M, Schoppa A, Steppe L, Fischer V, Luther J, Yorgan T, Bockamp E, Amling M, *et al*: Wnt1 boosts fracture healing by enhancing bone formation in the fracture callus. J Bone Miner Res 38: 749-764, 2023.
21. Hu Y, Chen Y, Peng X, Li H, Zuo G, Xu H, Wu F, Wang Y, Shao Z and Wei Y: Overactivation of EGFR signaling in skeletal stem/progenitor cells promotes bone formation and repair. Theranostics 15: 8117-8136, 2025.
22. Qiao Z, Wang W, Luo P, Hofman M, Horst K, Müller-Newen G, Greven J and Hildebrand F: The impact of plasma-derived microvesicles from a femoral fracture animal model on osteoblast function. Shock 53: 78-87, 2020.
23. Wu T, Wang L, Jian C, Zhang Z, Zeng R, Mi B, Liu G, Zhang Y and Shi C: A distinct 'repair' role of regulatory T cells in fracture healing. Front Med 18: 516-537, 2024.
24. Garcia-Martin R, Wang G, Brandão BB, Zanotto TM, Shah S, Kumar Patel S, Schilling B and Kahn CR: MicroRNA sequence codes for small extracellular vesicle release and cellular retention. Nature 601: 446-451, 2022.

25. Hu L, Zheng X, Zhou M, Wang J, Tong L, Dong M, Xu T and Li Z: Optimized AF4 combined with density cushion ultracentrifugation enables profiling of high-purity human blood extracellular vesicles. J Extracell Vesicles 13: e12470, 2024.
26. Ma S, Xing X, Huang H, Gao X, Xu X, Yang J, Liao C, Zhang X, Liu J, Tian W and Liao L: Skeletal muscle-derived extracellular vesicles transport glycolytic enzymes to mediate muscle-to-bone crosstalk. Cell Metab 35: 2028-2043.e7, 2023.
27. Chen M, Li Y, Zhang M, Ge S, Feng T, Chen R, Shen J, Li R, Wang Z, Xie Y, *et al*: Histone deacetylase inhibition enhances extracellular vesicles from muscle to promote osteogenesis via miR-873-3p. Signal Transduct Target Ther 9: 256, 2024.
28. Liu Y, Lu S, Yang J, Yang Y, Jiao L, Hu J, Li Y, Yang F, Pang Y, Zhao Y, *et al*: Analysis of the aging-related biomarker in a nonhuman primate model using multilayer omics. BMC Genomics 25: 639, 2024.
29. Zuo R, Kong L, Wang M, Wang W, Xu J, Chai Y, Guan J and Kang Q: Exosomes derived from human $CD34^+$ stem cells transfected with miR-26a prevent glucocorticoid-induced osteonecrosis of the femoral head by promoting angiogenesis and osteogenesis. Stem Cell Res Ther 10: 321, 2019.
30. Kim DK, Bandara G, Cho YE, Komarow HD, Donahue DR, Karim B, Baek MC, Kim HM, Metcalfe DD and Olivera A: Mastocytosis-derived extracellular vesicles deliver miR-23a and miR-30a into pre-osteoblasts and prevent osteoblastogenesis and bone formation. Nat Commun 12: 2527, 2021.
31. Sun Q, Zhang C, Chen B, Liao J, Bao A, Zhao C, Chen H, He D, Huang W and Dai G: Knockdown of LncRNA H19 inhibits vascularization and endochondral ossification via the MiRNA-21a-5p-Smad7/p-Smad2/3 pathway in fracture repair. Sci Rep 15: 26054, 2025.
32. Gatti M, Beretti F, Malenchini M, Bertucci E, Ceneri E, Follo MY and Maraldi T: Mesenchymal stromal cell-derived extracellular vesicles as a therapeutic treatment for osteosarcopenia: Crosstalk among neurons, muscle, and bone. Int J Mol Sci 26: 7875, 2025.
33. Just J, Yan Y, Farup J, Sieljacks P, Sloth M, Venø M, Gu T, de Paoli FV, Nyengaard JR, Bæk R, *et al*: Blood flow-restricted resistance exercise alters the surface profile, miRNA cargo and functional impact of circulating extracellular vesicles. Sci Rep 10: 5835, 2020.
34. Garner RT, Solfest JS, Nie Y, Kuang S, Stout J and Gavin TP: Multivesicular body and exosome pathway responses to acute exercise. Exp Physiol 105: 511-521, 2020.
35. Mullen M, Williams K, LaRocca T, Duke V, Hambright WS, Ravuri SK, Bahney CS, Ehrhart N and Huard J: Mechanical strain drives exosome production, function, and miRNA cargo in C2C12 muscle progenitor cells. J Orthop Res 41: 1186-1197, 2023.
36. Zhu Y, Li Y, Cao Z, Xue J, Wang X, Hu T, Han B and Guo Y: Mechanically strained osteocyte-derived exosomes contained miR-3110-5p and miR-3058-3p and promoted osteoblastic differentiation. Biomed Eng Online 23: 44, 2024.
37. Burke BI, Ismaeel A, Long DE, Depa LA, Coburn PT, Goh J, Saliu TP, Walton BJ, Vechetti IJ, Peck BD, *et al*: Extracellular vesicle transfer of miR-1 to adipose tissue modifies lipolytic pathways following resistance exercise. JCI Insight 9: e182589, 2024.
38. Xhuti D, Nilsson MI, Manta K, Tarnopolsky MA and Nederveen JP: Circulating exosome-like vesicle and skeletal muscle microRNAs are altered with age and resistance training. J Physiol 601: 5051-5073, 2023.
39. Lovett J, McColl RS, Durcan P, Vechetti I and Myburgh KH: Analysis of plasma-derived small extracellular vesicle characteristics and microRNA cargo following exercise-induced skeletal muscle damage in men. Physiol Rep 12: e70056, 2024.
40. de Mendonça M, Rocha KC, de Sousa É, Pereira BMV, Oyama LM and Rodrigues AC: Aerobic exercise training regulates serum extracellular vesicle miRNAs linked to obesity to promote their beneficial effects in mice. Am J Physiol Endocrinol Metab 319: E579-E591, 2020.
41. Castaño C, Mirasierra M, Vallejo M, Novials A and Párrizas M: Delivery of muscle-derived exosomal miRNAs induced by HIIT improves insulin sensitivity through down-regulation of hepatic FoxO1 in mice. Proc Natl Acad Sci USA 117: 30335-30343, 2020.
42. Maggio S, Canonico B, Ceccaroli P, Polidori E, Cioccoloni A, Giacomelli L, Ferri Marini C, Annibalini G, Gervasi M, Benelli P, *et al*: Modulation of the circulating extracellular vesicles in response to different exercise regimens and study of their inflammatory effects. Int J Mol Sci 24: 3039, 2023.
43. Vechetti IJ Jr, Peck BD, Wen Y, Walton RG, Valentino TR, Alimov AP, Dungan CM, Van Pelt DW, von Walden F, Alkner B, *et al*: Mechanical overload-induced muscle-derived extracellular vesicles promote adipose tissue lipolysis. FASEB J 35: e21644, 2021.
44. Guescini M, Canonico B, Lucertini F, Maggio S, Annibalini G, Barbieri E, Luchetti F, Papa S and Stocchi V: Muscle releases alpha-sarcoglycan positive extracellular vesicles carrying miRNAs in the bloodstream. PLoS One 10: e0125094, 2015.
45. Zhao H, Chen X, Hu G, Li C, Guo L, Zhang L, Sun F, Xia Y, Yan W, Cui Z, *et al*: Small extracellular vesicles from brown adipose tissue mediate exercise cardioprotection. Circ Res 130: 1490-1506, 2022.
46. Lou J, Wu J, Feng M, Dang X, Wu G, Yang H, Wang Y, Li J, Zhao Y, Shi C, *et al*: Exercise promotes angiogenesis by enhancing endothelial cell fatty acid utilization via liver-derived extracellular vesicle miR-122-5p. J Sport Health Sci 11: 495-508, 2022.
47. Sapp RM, Evans WS, Eagan LE, Chesney CA, Zietowski EM, Prior SJ, Ranadive SM and Hagberg JM: The effects of moderate and high-intensity exercise on circulating markers of endothelial integrity and activation in young, healthy men. J Appl Physiol (1985) 127: 1245-1256, 2019.
48. Warnier G, DE Groote E, Delcorte O, Nicolas Martinez D, Nederveen JP, Nilsson MI, Francaux M, Pierreux CE and Deldicque L: Effects of a 6-wk sprint interval training protocol at different altitudes on circulating extracellular vesicles. Med Sci Sports Exerc 55: 46-54, 2023.
49. Doncheva AI, Romero S, Ramirez-Garrastacho M, Lee S, Kolnes KJ, Tangen DS, Olsen T, Drevon CA, Llorente A, Dalen KT and Hjorth M: Extracellular vesicles and microRNAs are altered in response to exercise, insulin sensitivity and overweight. Acta Physiol (Oxf) 236: e13862, 2022.
50. Estébanez B, Visavadiya NP, de Paz JA, Whitehurst M, Cuevas MJ, González-Gallego J and Huang CJ: Resistance training diminishes the expression of exosome CD63 protein without modification of plasma miR-146a-5p and cfDNA in the elderly. Nutrients 13: 665, 2021.
51. Kargl CK, Sterczala AJ, Santucci D, Conkright WR, Krajewski KT, Martin BJ, Greeves JP, O'Leary TJ, Wardle SL, Sahu A, *et al*: Circulating extracellular vesicle characteristics differ between men and women following 12 weeks of concurrent exercise training. Physiol Rep 12: e16016, 2024.
52. Kargl CK, Nindl BC, Goulart JB, Feigel ED, Sahu A, Fazeli PK, Lee AL, Rajasundaram D, Ambrosio F, Hubal MJ, *et al*: Varying influence of menstrual cycle phase and hormonal contraceptive use on resistance exercise-induced circulating extracellular vesicle and metabolite signalling. J Extracell Vesicles 15: e70244, 2026.
53. Fernandez-Sanjurjo M, Pinto-Hernandez P, Dávalos A, Díaz-Martínez AE, Martín-Hernández R, Castilla-Silgado J, Toyos-Rodríguez C, Whitham M, Amado-Rodríguez L, Muñiz-Albaiceta G, *et al*: Next-generation sequencing reveals that miR-16-5p, miR-19a-3p, miR-451a, and miR-25-3p cargo in plasma extracellular vesicles differentiates sedentary young males from athletes. Eur J Sport Sci 24: 766-776, 2024.
54. D'Souza RF, Woodhead JST, Zeng N, Blenkiron C, Merry TL, Cameron-Smith D and Mitchell CJ: Circulatory exosomal miRNA following intense exercise is unrelated to muscle and plasma miRNA abundances. Am J Physiol Endocrinol Metab 315: E723-E733, 2018.
55. Silver JL, Alexander SE, Dillon HT, Lamon S and Wadley GD: Extracellular vesicular miRNA expression is not a proxy for skeletal muscle miRNA expression in males and females following acute, moderate intensity exercise. Physiol Rep 8: e14520, 2020.
56. Hou Z, Qin X, Hu Y, Zhang X, Li G, Wu J, Li J, Sha J, Chen J, Xia J, *et al*: Longterm exercise-derived exosomal miR-342-5p: A novel exerkine for cardioprotection. Circ Res 124: 1386-1400, 2019.
57. Di W, Amdanee N, Zhang W and Zhou Y: Long-term exercise-secreted extracellular vesicles promote browning of white adipocytes by suppressing miR-191a-5p. Life Sci 263: 118464, 2020.
58. Chidambaram D, Subashini V, Nanthanalaxmi M and Selvamurugan N: Role of mesenchymal stem cell-derived exosomal non-coding RNAs in bone and bone-related disorders. World J Stem Cells 17: 111241, 2025.
59. Lv PY, Gao PF, Tian GJ, Yang YY, Mo FF, Wang ZH, Sun L, Kuang MJ and Wang YL: Osteocyte-derived exosomes induced by mechanical strain promote human periodontal ligament stem cell proliferation and osteogenic differentiation via the miR-181b-5p/PTEN/AKT signaling pathway. Stem Cell Res Ther 11: 295, 2020.

60. Ibrahim AGE, Li C, Rogers R, Fournier M, Li L, Vaturi SD, Antes T, Sanchez L, Akhmerov A, Moseley JJ, *et al*: Augmenting canonical Wnt signalling in therapeutically inert cells converts them into therapeutically potent exosome factories. Nat Biomed Eng 3: 695-705, 2019.
61. Xu Q, Cui Y, Luan J, Zhou X, Li H and Han J: Exosomes from C2C12 myoblasts enhance osteogenic differentiation of MC3T3-E1 pre-osteoblasts by delivering miR-27a-3p. Biochem Biophys Res Commun 498: 32-37, 2018.
62. Nie Y, Sato Y, Garner RT, Kargl C, Wang C, Kuang S, Gilpin CJ and Gavin TP: Skeletal muscle-derived exosomes regulate endothelial cell functions via reactive oxygen species-activated nuclear factor-κB signalling. Exp Physiol 104: 1262-1273, 2019.
63. Wang W, Qiao SC, Wu XB, Sun B, Yang JG, Li X, Zhang X, Qian SJ, Gu YX and Lai HC: Circ_0008542 in osteoblast exosomes promotes osteoclast-induced bone resorption through m6A methylation. Cell Death Dis 12: 628, 2021.
64. Fulzele S, Mendhe B, Khayrullin A, Johnson M, Kaiser H, Liu Y, Isales CM and Hamrick MW: Muscle-derived miR-34a increases with age in circulating extracellular vesicles and induces senescence of bone marrow stem cells. Aging (Albany NY) 11: 1791-1803, 2019.
65. Qu F, Zhang YF, Wang YY, Cao XM, Shen YY, Wu ZA, Wu YQ and Xu C: Cyclic stretch-induced exosomes from periodontal ligament cells promote osteoblasts osteogenic differentiation via the miR-181d-5p/TNF signaling pathway. Arch Oral Biol 157: 105843, 2024.
66. Hayashi H, Izumiya Y, Ishida T, Arima Y, Hayashi O, Yoshiyama M, Tsujita K and Fukuda D: Exosomal miR206 secreted from growing muscle promotes angiogenic response in endothelial cells. Circ J 88: 425-433, 2024.
67. Xing Z, Guo L, Li S, Huang W, Su J, Chen X, Li Y and Zhang J: Skeletal muscle-derived exosomes prevent osteoporosis by promoting osteogenesis. Life Sci 357: 123079, 2024.
68. Peruzzi B, Urciuoli E, Mariani M, Chioma L, Tomao L, Montano I, Algeri M, Luciano R, Fintini D and Manco M: Circulating extracellular vesicles impair mesenchymal stromal cell differentiation favoring adipogenic rather than osteogenic differentiation in adolescents with obesity. Int J Mol Sci 24: 447, 2022.
69. Qiu J, Zhao Y, Chen Y, Wang Y, Du J, Xu J, Guo L and Liu Y: Exosomes derived from bone marrow-derived mesenchymal stem cells of exercise-trained mice improve wound healing by inhibiting macrophage M1 polarization. Stem Cells 43: sxae081, 2025.
70. Shen N, Maggio M, Woods I, C Lowry M, Almasri R, Gorgun C, Eichholz KF, Stavenschi E, Hokamp K, Roche FM, *et al*: Mechanically activated mesenchymal-derived bone cells drive vessel formation via an extracellular vesicle mediated mechanism. J Tissue Eng 14: 20417314231186918, 2023.
71. Shang X, Böker KO, Taheri S, Lehmann W and Schilling AF: Extracellular vesicles allow epigenetic mechanotransduction between chondrocytes and osteoblasts. Int J Mol Sci 22: 13282, 2021.
72. Zheng X, Zhao N, Peng L, Li Z, Liu C, You Q and Fang B: Biological characteristics of microRNAs secreted by exosomes of periodontal ligament stem cells due to mechanical force. Eur J Orthod 45: 408-417, 2023.
73. Li G, Qi M, Wang Y, Liang S and Xu H: Molecular communication from bone to skeletal muscle: An overview. Front Cell Dev Biol 13: 1715009, 2025.
74. Kirk B, Lombardi G and Duque G: Bone and muscle crosstalk in ageing and disease. Nat Rev Endocrinol 21: 375-390, 2025.
75. Qin Y, Peng Y, Zhao W, Pan J, Ksiezak-Reding H, Cardozo C, Wu Y, Divieti Pajevic P, Bonewald LF, Bauman WA and Qin W: Myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal microRNA-218: A novel mechanism in muscle-bone communication. J Biol Chem 292: 11021-11033, 2017.
76. Li Y, Wang X, Pan C, Yuan H, Li X, Chen Z and He H: Myoblast-derived exosomal Prrx2 attenuates osteoporosis via transcriptional regulation of lncRNA-MIR22HG to activate Hippo pathway. Mol Med 29: 54, 2023.
77. Wang Y, Ma X, Chen Y, Xia C, Lei X, Liu J, Jiang Y, Xu R and Gao Y: Slo1 deficient myoblast exosomes-derived miR-222-3p inhibits osteogenic differentiation via targeting of STAT3. J Cachexia Sarcopenia Muscle 16: e70115, 2025.
78. Lu H, Yang Y, Ou S, Qi Y, Li G, He H, Lu F, Li W and Sun H: miRNA-382-5p carried by extracellular vesicles in osteoarthritis reduces cell viability and proliferation, and promotes cell apoptosis by targeting PTEN. DNA Cell Biol 41: 1012-1025, 2022.
79. Liu W, Li L, Rong Y, Qian D, Chen J, Zhou Z, Luo Y, Jiang D, Cheng L, Zhao S, *et al*: Hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by the transfer of miR-126. Acta Biomater 103: 196-212, 2020.
80. Jiang Y, Zhang J, Li Z and Jia G: Bone marrow mesenchymal stem cell-derived exosomal miR-25 regulates the ubiquitination and degradation of Runx2 by SMURF1 to promote fracture healing in mice. Front Med (Lausanne) 7: 577578, 2020.
81. Yang Z, Zhang S, Faqeer A, Anjum S, Deng Y, Cao J, Yu T and Zhang Y: Mesenchymal Stem cell-derived extracellular vesicles ameliorate temporomandibular joint osteoarthritis by suppressing osteoclast activity via let-7a-5p/integrin β3 axis. ACS Nano 19: 34928-34941, 2025.
82. Xu T, Luo Y, Wang J, Zhang N, Gu C, Li L, Qian D, Cai W, Fan J and Yin G: Exosomal miRNA-128-3p from mesenchymal stem cells of aged rats regulates osteogenesis and bone fracture healing by targeting Smad5. J Nanobiotechnology 18: 47, 2020.
83. Shang X: Mechanosensitive miRNAs in cartilage and subchondral bone remodeling: emerging targets for osteoarthritis therapy. J Inflamm Res 18: 9609-9625, 2025.
84. Yang S, Guo S, Tong S and Sun X: Exosomal miR-130a-3p regulates osteogenic differentiation of human adipose-derived stem cells through mediating SIRT7/Wnt/β-catenin axis. Cell Prolif 53: e12890, 2020.
85. Tian L, Zhang D, Wang Z, Su J, Liu C, Jian C and Yu A: MiR-877, an exosomal miRNA from mechanical stretch induced adipose derived stromal cells, enhances fracture healing in nonunion rats with type 2 diabetes mellitus. Stem Cell Res Ther 16: 575, 2025.
86. Wu X, Crawford R, Xiao Y, Mao X and Prasadam I: Osteoarthritic subchondral bone release exosomes that promote cartilage degeneration. Cells 10: 251, 2021.
87. Lin S, Xia J, Ding J and Wan J: Exosomal miR-214-3p reprograms BMSC fate: A novel intercellular mechanism linking vascular insufficiency to impaired bone regeneration in nontraumatic ONFH. Mol Immunol 186: 147-160, 2025.
88. Wang Y, Zhou X and Wang D: Mesenchymal stem cell-derived extracellular vesicles inhibit osteoporosis via MicroRNA-27a-induced inhibition of DKK2-mediated Wnt/β-catenin pathway. Inflammation 45: 780-799, 2022.
89. Qiu D, Yan B, Xue H, Xu Z, Tan G and Liu Y: Perspectives of exosomal ncRNAs in the treatment of bone metabolic diseases: Focusing on osteoporosis, osteoarthritis, and rheumatoid arthritis. Exp Cell Res 446: 114457, 2025.
90. Van Pelt DW, Vechetti IJ Jr, Lawrence MM, Van Pelt KL, Patel P, Miller BF, Butterfield TA and Dupont-Versteegden EE: Serum extracellular vesicle miR-203a-3p content is associated with skeletal muscle mass and protein turnover during disuse atrophy and regrowth. Am J Physiol Cell Physiol 319: C419-C431, 2020.
91. Yang BC, Kuang MJ, Kang JY, Zhao J, Ma JX and Ma XL: Human umbilical cord mesenchymal stem cell-derived exosomes act via the miR-1263/Mob1/Hippo signaling pathway to prevent apoptosis in disuse osteoporosis. Biochem Biophys Res Commun 524: 883-889, 2020.
92. Shao X, Zhang P, Fan Z, Lin J, Chen X, Liu N, Gong W, He Y, Zhou Y, Shi T, *et al*: Atrophic skeletal muscle-derived extracellular vesicles transfer miR-125a-5p to inhibit bone formation in osteoporosis during aging. Adv Sci (Weinh) 13: e15362, 2026.
93. Bao C and He C: The role and therapeutic potential of MSC-derived exosomes in osteoarthritis. Arch Biochem Biophys 710: 109002, 2021.
94. Shi H, Jiang X, Xu C and Cheng Q: MicroRNAs in serum exosomes as circulating biomarkers for postmenopausal osteoporosis. Front Endocrinol (Lausanne) 13: 819056, 2022.
95. Shao JL, Li H, Zhang XR, Zhang X, Li ZZ, Jiao GL and Sun GD: Identification of serum exosomal MicroRNA expression profiling in menopausal females with osteoporosis by high-throughput sequencing. Curr Med Sci 40: 1161-1169, 2020.
96. Hou X, Peng G, Zhao W, Cheng X, Wang Q, Gan T, Yang Q and Zhang J: Association of elevated exosomal miR-21 levels with nonunion in clavicular fractures post-ORIF: A prospective analysis. Injury 55: 111963, 2024.
97. Zhao Y and Xu J: Synovial fluid-derived exosomal lncRNA PCGEM1 as biomarker for the different stages of osteoarthritis. Int Orthop 42: 2865-2872, 2018.
98. Sun Z, Shi J, Yang C, Chen X, Chu J, Chen J, Wang Y, Zhu C, Xu J, Tang G and Shao S: Identification and evaluation of circulating exosomal miRNAs for the diagnosis of postmenopausal osteoporosis. J Orthop Surg Res 18: 533, 2023.

99. Deininger C, Wagner A, Wichlas F, Rohde E, Freude T, Deluca A, Fierlbeck J, Holzleitner M, Senck S, Tempfer H, *et al*: Anatomical implant region-a critical determinant for the osteogenic potency of small extracellular vesicles and rhBMP-2. Eur J Trauma Emerg Surg 51: 359, 2025.
100. Deluca A, Wagner A, Heimel P, Deininger C, Wichlas F, Redl H, Rohde E, Tempfer H, Gimona M and Traweger A: Synergistic effect of umbilical cord extracellular vesicles and rhBMP-2 to enhance the regeneration of a metaphyseal femoral defect in osteoporotic rats. Stem Cell Res Ther 15: 144, 2024.
101. Wu T, Wang L, Jian C, Gao C, Liu Y, Fu Z and Shi C: Regulatory T cell-derived exosome mediated macrophages polarization for osteogenic differentiation in fracture repair. J Control Release 369: 266-282, 2024.
102. Li F, Wu J, Li D, Hao L, Li Y, Yi D, Yeung KWK, Chen D, Lu WW, Pan H, *et al*: Engineering stem cells to produce exosomes with enhanced bone regeneration effects: An alternative strategy for gene therapy. J Nanobiotechnology 20: 135, 2022.
103. Huang X, Wu W, Jing D, Yang L, Guo H, Wang L, Zhang W, Pu F and Shao Z: Engineered exosome as targeted lncRNA MEG3 delivery vehicles for osteosarcoma therapy. J Control Release 343: 107-117, 2022.
104. Xu L, Xu X, Liang Y, Wen C, Ouyang K, Huang J, Xiao Y, Deng X, Xia J and Duan L: Osteoclast-targeted delivery of anti-miRNA oligonucleotides by red blood cell extracellular vesicles. J Control Release 358: 259-272, 2023.
105. Wang H, Shu J, Zhang C, Wang Y, Shi R, Yang F and Tang X: Extracellular vesicle-mediated miR-150-3p delivery in joint homeostasis: A potential treatment for osteoarthritis? Cells 11: 2766, 2022.
106. Yan Y, Wang P, Tang X, Wang Y, Xiao M, Liu Z, Tu X and Li X: Engineered Dll4-overexpressing osteocyte-derived exosomes enhanced bone regeneration by regulating osteogenesis and angiogenesis. Theranostics 16: 2780-2797, 2026.
107. Yang F, Wu M, Chen H, Ma S, Liu J, Li C, Li Y, Yang J, Liu B and Zhao D: Combination therapy with BMSCs-exosomes and porous tantalum for the repair of femur supracondylar defects. Mol Med Rep 28: 130, 2023.
108. Zheng W, Zhu Z, Hong J, Wang H, Cui L, Zhai Y, Li J, Wang C, Wang Z, Xu L, *et al*: Incorporation of small extracellular vesicles in PEG/HA-Bio-Oss hydrogel composite scaffold for bone regeneration. Biomed Mater 19: 065014, 2024.
109. Yu Y, Li X, Ying Q, Zhang Z, Liu W and Su J: Synergistic effects of shed-derived exosomes, Cu^{2+}, and an injectable hyaluronic acid hydrogel on antibacterial, anti-inflammatory, and osteogenic activity for periodontal bone regeneration. ACS Appl Mater Interfaces 16: 33053-33069, 2024.
110. Zhang Y, Fang M, Zhu J, Li T, Li N, Su B, Sun GD, Li L and Zhou C: Exosome-loaded hyaluronic acid hydrogel composite with oxygen-producing 3D printed polylactic acid scaffolds for bone tissue repair and regeneration. Int J Biol Macromol 274: 132970, 2024.
111. Frank JK, Kampleitner C, Heimel P, Leinfellner G, Hanetseder D, Sperger S, Frischer A, Schädl B, Tangl S, Lindner C, *et al*: Circulating miRNAs are associated with successful bone regeneration. Front Bioeng Biotechnol 13: 1527493, 2025.
112. Mihanfar A, Shakouri SK, Khadem-Ansari MH, Fattahi A, Latifi Z, Nejabati HR and Nouri M: Exosomal miRNAs in osteoarthritis. Mol Biol Rep 47: 4737-4748, 2020.
113. Gao P, Li S, Dong Z, Luo Y, Zhang X, Han L, Peng S, Shen J, Xu F and Deng Z: A plasma-derived exosomal microRNA signature by small RNA sequencing for early detection of postmenopausal osteoporosis. Clin Transl Med 14: e1637, 2024.
114. Lozano N, Prescilla-Ledezma A, Calabuig E, Trelis M, Arce JMS, López Hontangas JL, de Pablos LM, Gomez-Samblas M and Osuna A: Circulating extracellular vesicles in sera of chronic patients as a method for determining active parasitism in Chagas disease. PLoS Negl Trop Dis 18: e0012356, 2024.
115. Marassi V, La Rocca G, Placci A, Muntiu A, Vincenzoni F, Vitali A, Desiderio C, Maraldi T, Beretti F, Russo E, *et al*: Native characterization and QC profiling of human amniotic mesenchymal stromal cell vesicular fractions for secretome-based therapy. Talanta 276: 126216, 2024.
116. Yuan R, Zhou Y, Arias GF and Dittmer DP: Extracellular vesicle isolation by a tangential-flow filtration-based large-scale purification method. Methods Mol Biol 2668: 45-55, 2023.
117. Zhang Q, Jeppesen DK, Higginbotham JN, Franklin JL and Coffey RJ: Comprehensive isolation of extracellular vesicles and nanoparticles. Nat Protoc 18: 1462-1487, 2023.
118. Takizawa K, Nishimura T and Harita Y: Enzyme-linked immunosorbent assay to detect surface marker proteins of extracellular vesicles purified from human urine. STAR Protoc 4: 102415, 2023.
119. Wang Y, Zhang Y, Li Z, Wei S, Chi X, Yan X, Lv H, Zhao L and Zhao L: Combination of size-exclusion chromatography and ion exchange adsorption for improving the proteomic analysis of plasma-derived extracellular vesicles. Proteomics 23: e2200364, 2023.
120. Taha HB: Plasma versus serum for extracellular vesicle (EV) isolation: A duel for reproducibility and accuracy for CNS-originating EVs biomarker analysis. J Neurosci Res 101: 1677-1686, 2023.
121. Batool SM, Escobedo AK, Hsia T, Ekanayake E, Khanna SK, Gamblin AS, Zheng H, Skog J, Miller JJ, Stemmer-Rachamimov AO, *et al*: Clinical utility of a blood based assay for the detection of IDH1.R132H-mutant gliomas. Nat Commun 15: 7074, 2024.
122. Bockorny B, Muthuswamy L, Huang L, Hadisurya M, Maria Lim C, Tsai LL, Gill RR, Wei JL, Bullock AJ, Grossman JE, *et al*: A large-scale proteomics resource of circulating extracellular vesicles for biomarker discovery in pancreatic cancer. Elife 12: RP87369, 2024.
123. García-Fernández J and Fuente Freire M: Exosome-like systems: Nanotechnology to overcome challenges for targeted cancer therapies. Cancer Lett 561: 216151, 2023.
124. Moccia V, Sammarco A, Ferro S, Cavicchioli L and Zappulli V: Characterization and function of extracellular vesicles in a canine mammary tumour cell line: Ultracentrifugation versus size exclusion chromatography. Vet Comp Oncol 21: 36-44, 2023.
125. Wu H, Chen G, Zhang G, Lv Q, Gu D and Dai M: Mechanism of vascular endothelial cell-derived exosomes modified with vascular endothelial growth factor in steroid-induced femoral head necrosis. Biomed Mater 18: 025017, 2023.
126. André-Grégoire G, Roux Q and Gavard J: Isolating plasma extracellular vesicles from mouse blood using size-exclusion chromatography, density gradient, and ultracentrifugation. STAR Protoc 4: 102740, 2023.
127. Chin AR: Challenges for studying and isolating extracellular vesicles from cell-conditioned media. Methods Mol Biol 2666: 299-315, 2023.
128. Soares M, Pinto MM, Nobre RJ, de Almeida LP, da Graça Rasteiro M, Almeida-Santos T, Ramalho-Santos J and Sousa AP: Isolation of extracellular vesicles from human follicular fluid: Size-exclusion chromatography versus ultracentrifugation. Biomolecules 13: 278, 2023.
129. Welsh JA, Arkesteijn GJA, Bremer M, Cimorelli M, Dignat-George F, Giebel B, Görgens A, Hendrix A, Kuiper M, Lacroix R, *et al*: A compendium of single extracellular vesicle flow cytometry. J Extracell Vesicles 12: e12299, 2023.
130. Poudel S, Nelson DL, Yen JH, Wang Y, Zhang H, He Z, Green AB, Veerland WN, Cleveland Iv TE IV, Lehman SE, *et al*: Intermethod characterization of commercially available extracellular vesicles as reference materials. Biomolecules 16: 66, 2025.
131. Zhou Y, Ren C, Zhang Q, Xie P, Chen M, Zhang H, Wang Y, Niu Q, Bianbaduojie, Liu Y and Bian B: Isolation of small extracellular vesicles derived from mesenchymal stem cells from large-volume samples with GMP compliance for clinical trials. J Vis Exp, 2025.
132. Primorac D, Brlek P, Bulić L, Hrvatin N, Škaro V, Projić P, Glavan M, Oleru I, Rocheteau P, Tremolada C, *et al*: Therapeutic potential of extracellular vesicles: From biogenesis, isolation and molecular characterization to addressing translational gaps and regulatory barriers. Int J Mol Sci 27: 1676, 2026.
133. Chuah BH, Law JX, Leong XF, Pang KL, Farm YR, Razali M and Ng SL: Safety assessment of extracellular vesicle-based therapy in regenerative dentistry. Int J Mol Sci 27: 798, 2026.
134. Lee KWA, Chan LKW, Hung LC, Phoebe LKW, Park Y and Yi KH: Clinical applications of exosomes: A critical review. Int J Mol Sci 25: 7794, 2024.
135. Zhang J, Pan Y, She P and Rao L: From bench to bedside: The promise and roadblocks of extracellular vesicle therapeutics. Theranostics 16: 5044-5064, 2026.
136. Ragni E: Extracellular vesicles: Recent advances and perspectives. Front Biosci (Landmark Ed) 30: 36405, 2025.
137. Zhang J, Tian X, Li Y, Fang C, Yang F, Dong L, Shen Y, Pu S, Li J, Chang D, *et al*: Stem Cell-derived exosomes: A comprehensive review of biomedical applications, challenges, and future directions. Int J Nanomedicine 20: 10857-10905, 2025.

138. Dalle Carbonare L, Minoia A, Braggio M, Piritore FC, Vareschi A, Cominacini M, Gandini A, Antoniazzi F, Cui D, Romanelli MG and Valenti MT: Extracellular vesicles in osteogenesis: Comparative analysis of stem cell sources, conditioning strategies, and in vitro models toward advanced bone regeneration. Cells 15: 27, 2025.
139. Yoo D, Jung SY, Go D, Park JY, You DG, Jung WK, Li Y, Ding J, Park JH and Um W: Functionalized extracellular vesicles of mesenchymal stem cells for regenerative medicine. J Nanobiotechnology 23: 219, 2025.
140. Kong H, Luo J, Zou Z, Li Y, Tang X, Han J, Meng X, Wang X, Fu Y, He M and Zhang XA: Exercise-derived exosomes: Molecular mediators of systemic health and disease therapy. J Nanobiotechnology 24: 232, 2026.
141. Zhong Y, Liu X, Ma L, Fu P, Chen X and Zhang C: MicroRNAs as key paracrine factors in stem cell therapy: Molecular mechanisms, therapeutic applications, and future perspectives. Eur J Pharmacol 1008: 178333, 2025.
142. Sung SE, Seo MS, Park WT, Lim YJ, Park S and Lee GW: Extracellular vesicles: Their challenges and benefits as potential biomarkers for musculoskeletal disorders. J Int Med Res 53: 3000605251317476, 2025.
143. Wang Y, Wang H, Tan J, Cao Z, Wang Q, Wang H, Yue S, Li W and Wang D: Therapeutic effect of mesenchymal stem cells and their derived exosomes in diseases. Mol Biomed 6: 34, 2025.
144. Emami A and Oskouie IM: Engineered exosomes for targeted bone regeneration: Design, delivery, and functionalization. Cell Tissue Bank 27: 9, 2026.
145. Staubli F, Zhou Y, Vader P, Hofmann S, Bergsma JE, Gawlitta D and Man K: Harnessing extracellular vesicles for endochondral bone regeneration: Mechanisms and applications. Acta Biomater 212: 168-186, 2026.
146. Aziziyan F, Asl SS, Mahdipour M, Fard RN and Sheykhhasan M: Mesenchymal stem cell-derived extracellular vesicles in musculoskeletal regeneration: Mechanisms, applications, and future prospects. Stem Cell Res Ther 17: 66, 2026.