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# Exosomes and Disused Osteoporosis: A Survey

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## Abstract

This survey explores the multifaceted roles of exosomes in intercellular communication and bone metabolism, emphasizing their potential as biomarkers and therapeutic agents in conditions such as disused osteoporosis. Exosomes, nano-sized extracellular vesicles, facilitate the transfer of proteins, lipids, and nucleic acids, influencing cellular signaling and maintaining bone homeostasis. The survey details the mechanisms of exosome biogenesis and cargo selection, highlighting their significance in bone cell communication and the modulation of bone remodeling processes. Challenges in exosome research, including heterogeneity and standardization issues, are addressed, alongside recent technological advances that enhance exosome detection and characterization. The potential of exosomes as biomarkers for osteoporosis is underscored by their stable presence in biofluids, enabling non-invasive diagnostics. Innovative methodologies, such as label-free detection and colorimetric assays, are enhancing the clinical applicability of exosomes. Future research directions include the development of standardized protocols for exosome production and characterization, exploration of therapeutic applications in immunotherapy and drug delivery, and integration of multi-omics approaches to uncover disease mechanisms. As the understanding of exosome-mediated communication advances, their role in diagnostics and therapeutics, particularly for bone-related disorders, becomes increasingly promising. Continued research is essential to fully leverage the capabilities of exosomes in clinical applications, offering novel insights into disease mechanisms and potential therapeutic interventions.

## 1 Introduction

### 1.1 Structure of the Survey

This survey provides a thorough exploration of the multifaceted roles of exosomes in disused osteoporosis. It begins with an introduction to exosomes, emphasizing their critical functions in intercellular communication and bone metabolism. Subsequent sections clarify essential concepts, including extracellular vesicles (EVs) and their relevance to bone health, while examining how exosomes mediate communication among bone cells and their mechanisms of signaling.

The survey further investigates the mechanisms through which exosomes influence bone metabolism, focusing on aspects such as biogenesis, cargo composition, and the modulation of cellular signaling pathways. A dedicated section evaluates the potential of exosomes as biomarkers, discussing their clinical relevance and methodologies in diagnostics [1]. Current research and applications are reviewed to showcase advancements in this domain.

Challenges and future directions in exosome research are also addressed, identifying key obstacles and highlighting recent technological innovations that may enhance detection and analysis. The paper concludes by underscoring the significance of exosomes in understanding and potentially treating disused osteoporosis, providing a roadmap for future research. Each section is designed to build cohesively upon the previous one, ensuring a unified narrative throughout the survey. The following sections are organized as shown in Figure 1.

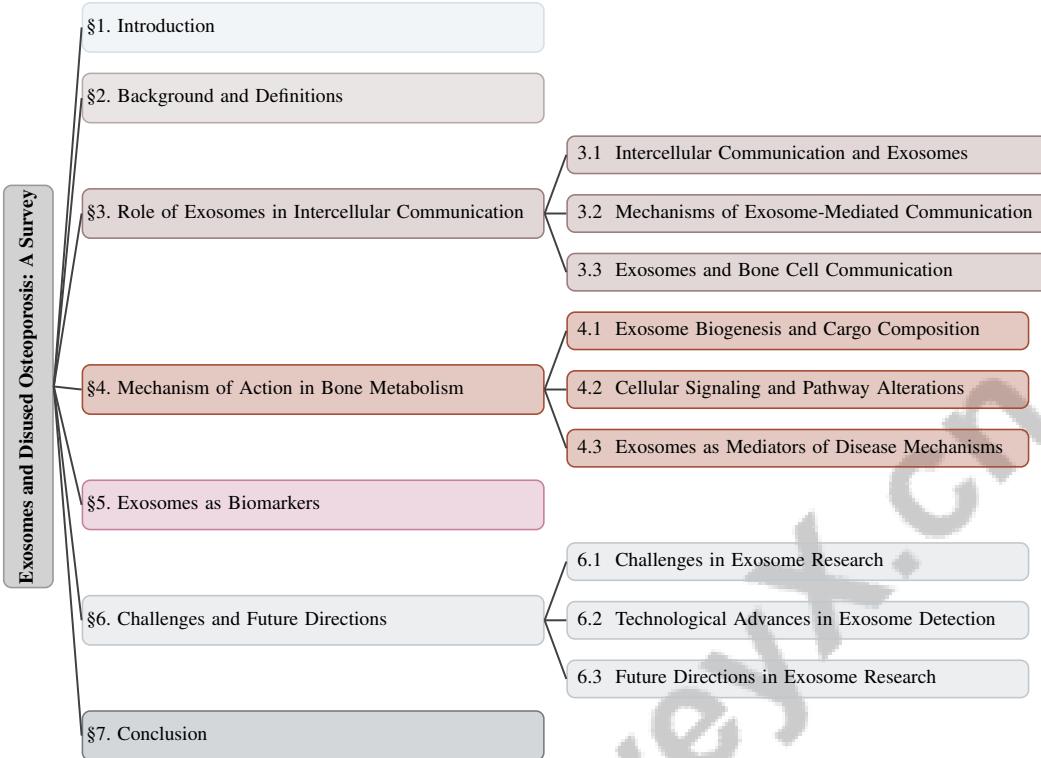


Figure 1: chapter structure

## 2 Background and Definitions

### 2.1 Overview of Exosomes

Exosomes are nanosized extracellular vesicles (EVs), typically 30 to 150 nm, originating from the endosomal compartment of cells. Enclosed by a lipid bilayer, they facilitate intercellular communication by delivering proteins, lipids, RNAs, and DNAs, significantly affecting recipient cell physiology locally and distally. Beyond basic cellular functions, exosomes are integral to various physiological processes and diseases, notably cancer, thus serving as promising biomarkers and therapeutic agents [2, 3, 4]. Released into the extracellular space via multivesicular body fusion with the plasma membrane, exosomes differ from microvesicles and apoptotic bodies in biogenesis. Their roles in mediating cell-cell interactions, altering signaling pathways, and participating in regulatory mechanisms underscore their importance in disease diagnostics and therapeutic strategies [5].

Their presence in biofluids enhances their potential as non-invasive biomarkers [6]. Recent developments highlight their role as natural drug delivery messengers, leveraging biocompatibility and targeting capabilities. Exosome-mimetic nanovesicles further augment this potential by providing stability and replicating natural exosome functions [7]. The biophysical properties of exosomes, including lipid bilayer fluidity and composition, are crucial for interactions with recipient cells, affecting adsorption and fusion [3].

Emerging insights into exosomes as regulatory hubs in cellular communication underscore the need for continued research. Frameworks like ICELLNET, integrating ligand-receptor interactions to infer cell-to-cell communication from transcriptomic data, enhance our understanding of exosome-mediated signaling [8]. Standardizing EV characterization platforms, as discussed by Welsh et al., aims to improve measurement reliability and reproducibility, facilitating model comparisons [9]. The therapeutic potential of exosomes, classified by effects, production methods, and clinical applications, presents them as viable alternatives to direct stem cell therapy [10]. Exosome-mediated communication mechanisms between tumor cells and various tumor microenvironment cell types, including fibroblasts, endothelial cells, leukocytes, and stem cells, illustrate their diverse roles in cellular interactions [11].

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## 2.2 Exosomes and Extracellular Vesicles (EVs)

Exosomes, a specialized subset of extracellular vesicles (EVs), are characterized by their size of 40 to 150 nm and unique biogenesis involving endosomal membrane inward budding to form multivesicular bodies. These bodies release exosomes upon plasma membrane fusion, distinguishing them from microvesicles and apoptotic bodies with different origins. Exosomes play a critical role in intercellular communication by transporting proteins, lipids, and nucleic acids, serving as potential biomarkers for various diseases and providing insights into cellular physiology [12, 3, 13, 14, 2].

Despite the shared role of exosomes and other EVs in mediating intercellular communication, exosomes are generally smaller, measuring between 30 to 150 nm, compared to microvesicles, which can reach sizes up to 1,000 nm [15]. The heterogeneity of EV populations poses significant challenges in isolation and characterization, necessitating advanced analytical techniques for precise classification. Accurate measurements of size distribution, concentration, and epitope expression are vital, given the diverse and overlapping nature of particles in biological fluids [9]. Furthermore, the biophysical properties of EVs, including membrane composition and phase behavior, are crucial for their function, highlighting the need for standardized definitions and classifications to enhance their application in diagnostics and therapeutics [16].

## 2.3 Disused Osteoporosis

Disused osteoporosis is marked by reduced bone mass and structural integrity due to insufficient mechanical loading and physical activity, often seen after prolonged immobilization, bed rest, or microgravity exposure. The lack of mechanical stress disrupts bone remodeling balance, increasing bone resorption over formation, leading to decreased bone density and fracture risk [17].

The pathogenesis involves complex cellular signaling networks within the skeletal system, where connexin proteins, particularly Cx43, are pivotal in non-junctional signaling essential for bone cell function and survival, including osteocytes, osteoblasts, and osteoclasts [18]. These proteins facilitate communication necessary for responding to mechanical stimuli and maintaining bone homeostasis [19].

Recent advancements emphasize the role of extracellular vesicles (EVs), especially exosomes, in mediating communication within the bone microenvironment and modulating cellular responses to mechanical unloading. Exosomes carry signaling molecules, such as microRNAs, proteins, and lipids, influencing bone cell activity and serving as potential biomarkers for bone health and disease states. However, challenges persist in isolating and characterizing these vesicles due to the lack of standardized methods and variability in exosomal content based on cellular and environmental contexts [11].

Understanding the molecular and cellular mechanisms underlying disused osteoporosis is crucial for developing therapeutic strategies to mitigate bone loss and enhance regeneration. This includes leveraging the therapeutic potential of exosomes as drug delivery vehicles and their role in facilitating intercellular communication within the bone microenvironment. Despite the promising properties of EVs as drug carriers, translating these therapies from research to clinical application remains challenging [16]. Identifying osteoporosis-related biomarkers from multi-omics datasets presents additional challenges, particularly in accounting for outliers and deviations from expected data distributions [20]. As research continues to elucidate the complexities of bone metabolism and the effects of mechanical unloading, the significance of disused osteoporosis in bone health remains a critical area of investigation.

## 3 Role of Exosomes in Intercellular Communication

Recent advancements highlight extracellular vesicles (EVs), especially exosomes, as crucial mediators in intercellular communication. These membrane-bound particles, released by various cell types, facilitate the exchange of proteins, lipids, and genetic material, influencing numerous physiological and pathological processes. Despite progress, aspects of EV biology, including biogenesis, secretion, and interactions with recipient cells, remain inadequately understood, necessitating further study [13, 21]. Exosomes not only transfer molecular information but also regulate biological processes. This section examines their fundamental role in intercellular communication, focusing on mechanisms

through which they exert effects, laying the groundwork for understanding exosome-mediated interactions in bone health and disease.

To illustrate these concepts, Figure 2 depicts the hierarchical structure of exosome roles in intercellular communication, detailing their functions, mechanisms, and impact on bone cell communication. This figure highlights exosomes as carriers of molecular cargo, emphasizing their biogenesis, interaction mechanisms, and therapeutic potential in promoting bone health. Such visual representation serves to enhance our understanding of the critical functions of exosomes within the broader context of cellular communication and their implications for bone-related conditions.

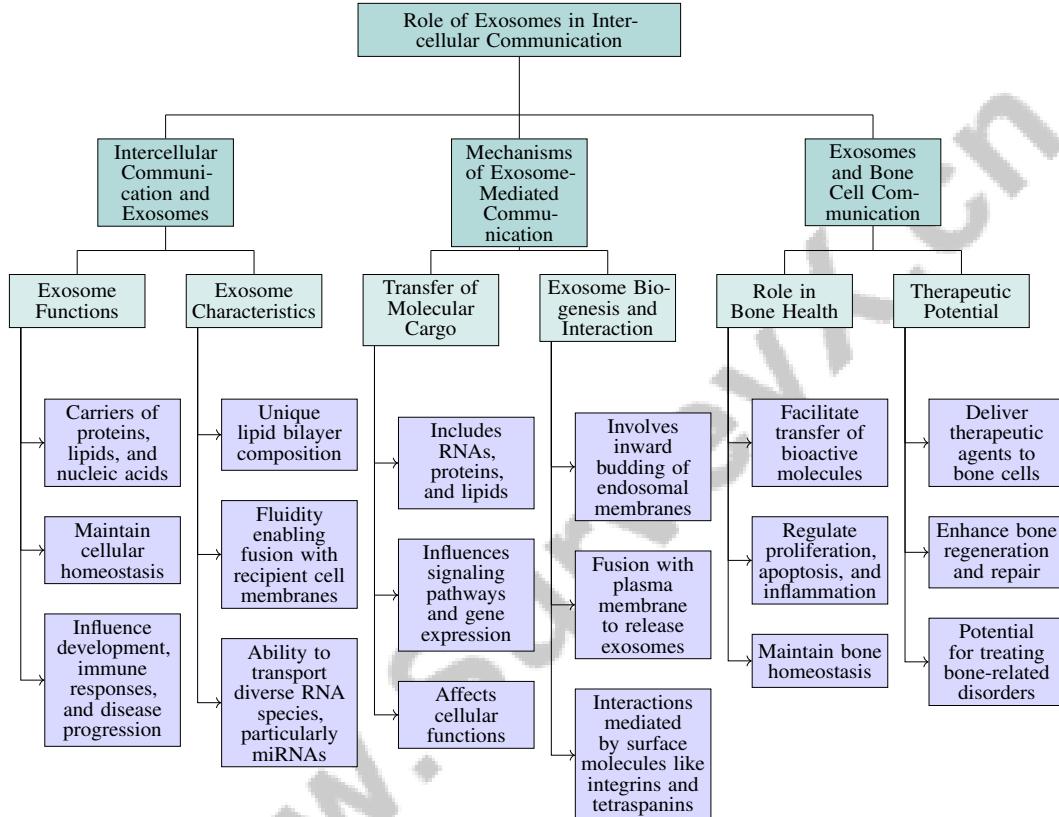


Figure 2: This figure illustrates the hierarchical structure of exosome roles in intercellular communication, detailing their functions, mechanisms, and impact on bone cell communication. It highlights exosomes as carriers of molecular cargo, their biogenesis and interaction mechanisms, and their therapeutic potential in bone health.

### 3.1 Intercellular Communication and Exosomes

Exosomes are vital for intercellular communication, acting as carriers of proteins, lipids, and nucleic acids that transmit signals between cells. This process maintains cellular homeostasis and influences biological activities, including development, immune responses, and disease progression [22]. Their unique lipid bilayer composition and fluidity enable fusion with recipient cell membranes, modulating interactions and signaling pathways.

The ability of exosomes to transport diverse RNA species, particularly miRNAs, underscores their potential to alter gene expression in recipient cells, significant for therapeutic applications [15]. Understanding the molecular cargo of exosomes is vital within cellular communication networks. Tools like ICELLNET quantitatively assess communication scores between cell types based on transcriptomic profiles, highlighting the complexity of exosome-mediated signaling [8].

Innovations like Geometry-induced Electrohydrodynamic Tweezers (GET) allow rapid, non-invasive trapping of single EVs, facilitating detailed studies of individual exosome interactions. The low

immunogenicity and ability of exosomes to traverse biological barriers enhance their potential as therapeutic delivery vehicles, positioning them as promising candidates for clinical applications [23].

Investigating the surface interactome of EVs, encompassing proteins, lipids, and nucleic acids, is crucial for elucidating their roles in cellular communication and function. These interactions are pivotal for modulating biological processes and developing diagnostic and therapeutic strategies. As research progresses into the diverse roles of exosomes, their importance in both healthy and diseased states becomes increasingly evident, with applications in regenerative medicine and disease management, including as biomarkers, therapeutic agents, and drug delivery systems. Exosomes from sources such as mesenchymal stem cells and dendritic cells are actively explored in clinical trials, highlighting their potential as innovative treatment strategies [15, 24].

Figure 3 illustrates the hierarchical structure of exosome-mediated intercellular communication, highlighting their biological roles, therapeutic potential, and technological innovations. This visual representation reinforces the multifaceted nature of exosomes and their significant contributions to cellular interactions and therapeutic advancements.

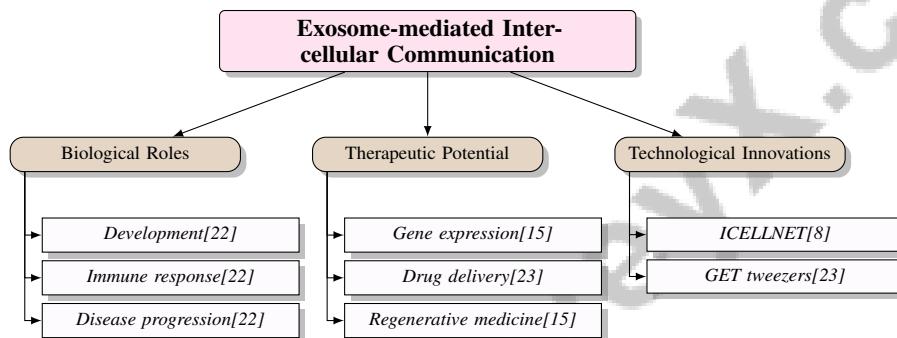


Figure 3: This figure illustrates the hierarchical structure of exosome-mediated intercellular communication, highlighting their biological roles, therapeutic potential, and technological innovations.

### 3.2 Mechanisms of Exosome-Mediated Communication

Exosome-mediated communication involves transferring molecular cargo, such as RNAs, proteins, and lipids, from donor to recipient cells, facilitating direct interactions and influencing signaling pathways, potentially altering gene expression and cellular functions. The stability of EVs in circulation and their biocompatibility make them promising therapeutic candidates, though their heterogeneous composition and biogenesis mechanisms remain active research areas [13, 19, 4, 25]. This process is integral to maintaining cellular homeostasis and modulating responses to environmental stimuli. Exosome biogenesis involves the inward budding of endosomal membranes, forming multivesicular bodies (MVBs) that fuse with the plasma membrane to release exosomes.

The cargo carried by exosomes, including diverse proteins, lipids, and nucleic acids such as mRNA and miRNA, plays a crucial role in modulating the physiological state of recipient cells. This cargo facilitates intercellular communication and influences signaling pathways, affecting gene expression and cellular function in local and distant environments. Exosomes, released by nearly all cell types, have emerged as significant players in normal physiological processes and pathological conditions, making them potential biomarkers and therapeutic vehicles [13, 19, 2, 4]. For instance, miRNA delivery by exosomes can suppress target gene expression, modulating cellular processes like proliferation, differentiation, and apoptosis, underscoring their significance in physiological and pathological contexts.

Interactions between exosomes and recipient cells are mediated by surface molecules, including integrins and tetraspanins, facilitating binding to specific cell types [26]. This specificity is crucial for selectively modulating signaling pathways in recipient cells. Once internalized, exosomal cargo can influence intracellular signaling cascades, such as the MAPK and PI3K/Akt pathways, critical for cell survival and proliferation [15].

Advanced methodologies like ICELLNET provide quantitative assessments of cell-cell communication scores based on transcriptomic data, offering insights into complex signaling networks facilitated

by exosomes [8]. The development of single-vesicle analysis techniques, such as GET, enables detailed studies of individual exosome interactions, enhancing our understanding of their roles in cellular communication [23].

The ability of exosomes to traverse biological barriers and their low immunogenicity further enhance their potential as therapeutic delivery vehicles, making them promising candidates for drug delivery and regenerative medicine [23]. As research elucidates the mechanisms underlying exosome-mediated communication, their applications in therapeutic and diagnostic contexts become increasingly apparent, providing novel insights into their roles in health and disease.

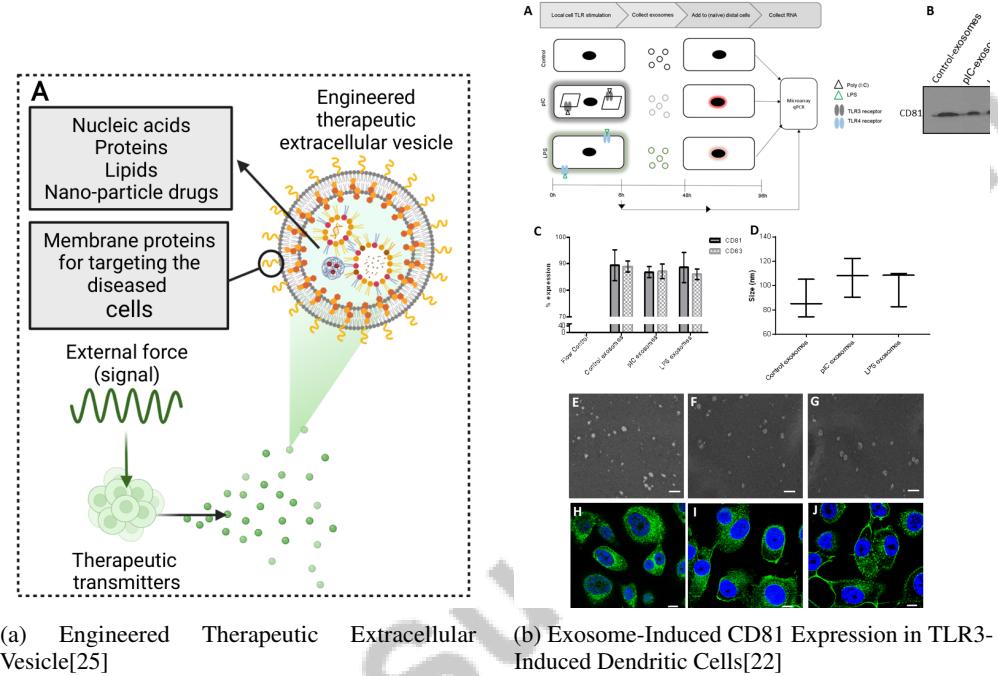


Figure 4: Examples of Mechanisms of Exosome-Mediated Communication

As shown in Figure 4, exosomes play a pivotal role in mediating intercellular interactions, serving as vehicles for transferring molecules such as proteins, lipids, and nucleic acids. This section explores two distinct scenarios of exosome-mediated communication. The first scenario presents an engineered therapeutic extracellular vesicle (ETEV), designed to target diseased cells with precision, utilizing membrane proteins for targeting and an external force for disintegration. The second scenario focuses on exosomes' biological effects in immune responses, particularly their role in modulating CD81 expression in TLR3-induced dendritic cells. Flow cytometry analysis reveals how exosomes influence immune cell behavior, highlighting their therapeutic potential. Together, these examples underscore the diverse functionalities of exosomes in intercellular communication, emphasizing their significance in therapeutic engineering and immune modulation [25, 22].

### 3.3 Exosomes and Bone Cell Communication

Exosomes are crucial mediators of intercellular communication among bone cells, including osteoblasts, osteoclasts, and osteocytes. They facilitate the transfer of bioactive molecules, such as noncoding RNAs and proteins, vital for regulating cellular processes like proliferation, apoptosis, and inflammation. This communication is essential for maintaining bone homeostasis and responding to mechanical stimuli, influencing bone health and disease progression [27, 15]. These vesicles carry bioactive molecules that modulate signaling pathways and cellular functions in recipient bone cells. The transfer of miRNAs via exosomes is particularly critical, as they regulate gene expression in osteoblasts and osteoclasts, influencing bone formation and resorption processes.

In bone remodeling, exosomes from osteoblasts promote osteoclast differentiation by delivering RANKL, a key regulator of osteoclastogenesis, enhancing bone resorption [8]. Conversely, osteoclast-

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derived exosomes can influence osteoblast activity through miRNAs that suppress osteogenic differentiation, highlighting the bidirectional communication mediated by exosomes in bone metabolism. This intricate exchange of molecular signals underscores the importance of exosomes in coordinating the balance between bone formation and resorption.

Moreover, mechanical unloading associated with disused osteoporosis can alter the production and cargo composition of exosomes released by bone cells, potentially disrupting normal communication and contributing to bone loss [17]. The ability of exosomes to mediate intercellular communication in the bone microenvironment suggests their potential as therapeutic targets for modulating bone cell activity and mitigating the effects of mechanical unloading on bone health.

Recent studies demonstrate that exosomes can serve as vehicles for delivering therapeutic agents, such as osteogenic factors or miRNAs, directly to bone cells, enhancing bone regeneration and repair. The development of exosome-based therapies holds promise for treating bone-related disorders, including disused osteoporosis, by leveraging their natural targeting capabilities and low immunogenicity [16]. As research continues to unravel the complex interactions mediated by exosomes in the bone microenvironment, their potential applications in bone disease diagnostics and therapeutics are becoming increasingly clear.

## 4 Mechanism of Action in Bone Metabolism

Exploring the interaction between exosomes and bone metabolism necessitates a comprehensive understanding of the mechanisms driving their formation and function. This section delves into exosome biogenesis and cargo composition, elucidating how these vesicles regulate bone metabolism and contribute to skeletal health.

### 4.1 Exosome Biogenesis and Cargo Composition

Exosome biogenesis involves the inward budding of endosomal membranes, resulting in multivesicular bodies (MVBs) that release exosomes upon fusing with the plasma membrane, distinct from microvesicle formation [16]. This process, along with the selective packaging of proteins, lipids, and nucleic acids, underscores their role in intercellular communication and bone metabolism [28]. Exosomes transport critical signaling molecules, particularly miRNAs, which regulate gene expression in bone cells, affecting bone formation and resorption [27].

The biophysical properties of exosomal membranes, such as lipid composition and fluidity, are crucial for their interaction with recipient cells, affecting vesicle uptake and cellular responses [7]. Techniques like coarse-grained molecular dynamics provide insights into exosome cargo delivery dynamics [29]. Advanced analytical methods, including Dynamic Light Scattering and Atomic Force Microscopy, enhance our ability to characterize exosome cargo, demonstrating greater stability in physiological exosomes compared to synthetic vesicles [5]. The NICE framework offers a normalized measure of protein expression within EV subpopulations [30].

Understanding exosome biogenesis and cargo composition is vital for their role in bone metabolism. As research advances, engineering exosomes for targeted therapy in bone disorders becomes increasingly viable, highlighting their significance in regenerative medicine and disease management [31].

### 4.2 Cellular Signaling and Pathway Alterations

Exosomes significantly influence cellular signaling pathways in bone metabolism by transferring proteins, lipids, and nucleic acids between bone cells, affecting pathways that regulate bone formation and resorption [31]. The biogenesis and selective cargo packaging of exosomes are pivotal in determining the nature of transmitted signals [16]. Integrin-mediated pathways, such as ITGB3, facilitate exosome uptake by recipient cells, activating downstream signaling cascades [26].

Advanced techniques like surface-enhanced Raman spectroscopy, combined with Principal Component Analysis, enhance our understanding of exosomal populations and their role in cellular signaling [32]. This approach identifies biophysical signatures of exosomal membranes and their influence on cellular communication [33]. The alteration of cellular signaling by exosomes can be likened to dynamics within citation networks, emphasizing their role in modulating complex signaling pathways

[28]. The NICE framework provides insights into protein interactions driving signaling changes in bone cells [30].

Coarse-grained molecular simulations offer potential for optimizing exosome-mediated drug delivery systems [34]. These insights are crucial for designing therapeutic strategies that utilize exosomes' capabilities to modulate cellular signaling pathways in bone metabolism. Multi-omics approaches are necessary to accurately infer the mechanisms underlying exosome-mediated signaling alterations [35]. As research progresses, exosome-based therapies hold promise for addressing bone-related disorders by modulating these pathways.

### 4.3 Exosomes as Mediators of Disease Mechanisms

Exosomes are pivotal in the pathogenesis of bone-related diseases, transporting bioactive molecules that influence disease mechanisms by altering signaling pathways and modulating immune responses [22]. In bone health, exosomes from bone cells impact remodeling processes, affecting conditions like osteoporosis [17].

Their therapeutic potential is highlighted by their role in enhancing immune responses and applications in cancer and infectious disease treatments. However, challenges in translating exosome-based therapies into clinical practice include scalability, immunogenicity, and exosome modification complexity [36]. Standardization in exosome isolation and characterization remains a hurdle [37].

Technological advancements, such as the Geometry-induced Electrohydrodynamic Tweezers method, have improved the analysis of nanosized EVs, crucial for understanding bone health-related disease mechanisms [38]. Despite innovations, challenges persist in distinguishing EVs from other microvesicles, especially at smaller sizes [39]. Effective separation enhances the potential for purifying exosomes, vital for understanding their role in bone metabolism [5].

Engineering exosomes for targeted drug delivery is promising, offering solutions to overcome existing technological hurdles [23]. Integrating emerging technologies like microfluidics improves diagnostics' sensitivity and specificity, particularly in cancer research where exosomes are crucial [40]. Effective drug delivery requires countermeasures to mitigate agglomeration, ensuring stability and efficacy [41].

Exosomes also play roles in viral pathogenesis, offering new avenues for treating infections and complications [42]. Challenges remain in understanding non-classical communications compared to classical pathways [15]. Robust methodologies like RobKMR effectively identify biomarkers related to bone mineral density, outperforming existing methods [20]. As research continues, the multifaceted roles of exosomes in disease mechanisms become increasingly evident, enhancing their potential applications in bone health and beyond.

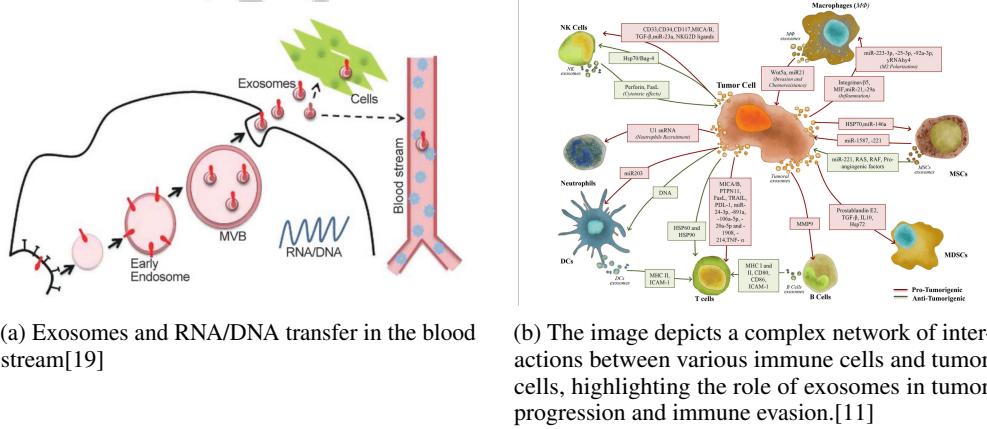


Figure 5: Examples of Exosomes as Mediators of Disease Mechanisms

As shown in Figure 5, the examples illustrate the intricate role of exosomes in bone metabolism and their broader implications in disease mechanisms, particularly in cancer. Exosomes facilitate RNA and DNA transfer through the bloodstream, crucial for cellular communication and influencing bone

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metabolism. The second image explores exosomes' role in the tumor microenvironment, highlighting interactions between immune and tumor cells, and their contribution to tumor progression and immune evasion, underscoring their potential as therapeutic targets in diseases like cancer [19, 11].

## 5 Exosomes as Biomarkers

The investigation into exosomes as biomarkers necessitates an understanding of their diverse roles and molecular composition across clinical contexts. This section examines their clinical relevance, particularly in osteoporosis, and their potential as indicators of physiological and pathological changes. By studying exosome characteristics and stability in biofluids, we can appreciate their utility in non-invasive diagnostics and personalized medicine. The following subsections elucidate the significance of exosomes in clinical diagnostics and innovative methodologies that enhance their detection and characterization.

### 5.1 Biomarkers and Clinical Relevance

Exosomes have garnered attention as biomarkers for osteoporosis due to their role in intercellular communication and their capacity to encapsulate bioactive molecules, such as proteins, lipids, and nucleic acids, integral to cellular signaling and gene regulation [21]. Their lipid bilayer composition, reflecting that of their originating cells, underscores their potential as biomarkers, indicating changes associated with diseases like cancer and osteoporosis [43].

Research highlights small extracellular vesicles (sEVs) as potential biomarkers, with their molecular composition reflecting the state of originating cells [43]. Integrating sub-diffusive effects into nanoparticle tracking analysis (NTA) has improved size estimates for extracellular vesicles (EVs), aligning them with atomic force microscopy (AFM) results, enhancing exosome characterization accuracy and their application as biomarkers [29].

The clinical relevance of exosomes in osteoporosis is further supported by their stable presence in biofluids, facilitating non-invasive diagnostics. The NICE framework, analyzing protein co-enrichment in exosomes, offers insights into variations serving as indicators of physiological changes and potential disease biomarkers, enhancing predictive modeling and clinical decision-making [20].

Continued research into exosomes promises to enhance their integration into clinical diagnostics and therapeutics, improving osteoporosis understanding and management. Advancements in isolating and analyzing extracellular vesicles, particularly exosomes, are expected to solidify their role as crucial biomarkers, facilitating accurate disease diagnosis and prognosis while unlocking new avenues for personalized medicine [14, 2].

### 5.2 Exosomes in Clinical Diagnostics

The integration of exosomes into clinical diagnostics has advanced significantly due to methodologies enabling rapid and accurate detection of these nanosized vesicles. These advancements leverage the diagnostic potential of exosomes, which carry biomolecules reflecting the physiological and pathological states of their cells of origin. A notable contribution is the label-free detection method, developed to identify exosomes from various cellular sources with high precision, providing a robust platform for clinical diagnostics [32].

Additionally, a newly proposed colorimetric method allows rapid detection of extracellular vesicles at concentrations as low as  $10^7 EVs/ml$ , demonstrating potential for clinical applications by facilitating early detection of disease-related changes in exosome populations [44]. The sensitivity and specificity of this technique underscore its utility in clinical diagnostics.

Exosomes' stable presence in biofluids, such as blood and urine, enables non-invasive sampling and repeated measurements over time, advantageous for monitoring disease progression and therapy response. Ongoing refinement of detection methodologies and advancements in exosome isolation and characterization enhance their feasibility in clinical diagnostics, paving the way for routine medical practice integration. As research progresses, methodologies for evaluating biomarkers are becoming increasingly sophisticated, improving diagnostic precision and effectiveness. This evolution is expected to lead to better patient outcomes through more personalized healthcare

strategies, integrating new biomarkers into predictive models that account for individual biological variations and disease mechanisms [14, 45, 46].

### 5.3 Current Research and Applications

Recent advancements in exosome research emphasize their potential as biomarkers for various clinical applications, particularly in disease diagnostics and therapeutic monitoring. Innovative sensing mechanisms, such as the Q-switched technique, have significantly enhanced exosome detection sensitivity, achieving a detection limit as low as 129 aM for lung cancer exosomes, showcasing substantial progress in biosensing technology [7]. Such advancements highlight exosomes' reliability as biomarkers for early disease detection and monitoring.

In addition to technological innovations, there is a growing emphasis on establishing standardized protocols for evaluating exosomal biomarkers. Future research should prioritize developing these protocols, focusing on external validation and cost-benefit analyses to ensure clinical utility and integration into routine healthcare practices [45]. These efforts are crucial for addressing current challenges in exosome research, such as variability in isolation and characterization methods, impacting the reproducibility and accuracy of diagnostic results.

Moreover, the proposal of fast virus detection systems has implications for exosome research, particularly concerning public health challenges. These systems could facilitate the rapid detection of exosomes, enabling timely diagnosis and intervention in various diseases [47]. As research continues to explore exosomes' multifaceted roles, their integration into clinical diagnostics and therapeutic strategies promises to enhance patient care and improve outcomes across a range of medical conditions. The ongoing development of advanced analytical techniques and refinement of detection methodologies will further solidify exosomes' role as pivotal biomarkers in the clinical landscape.

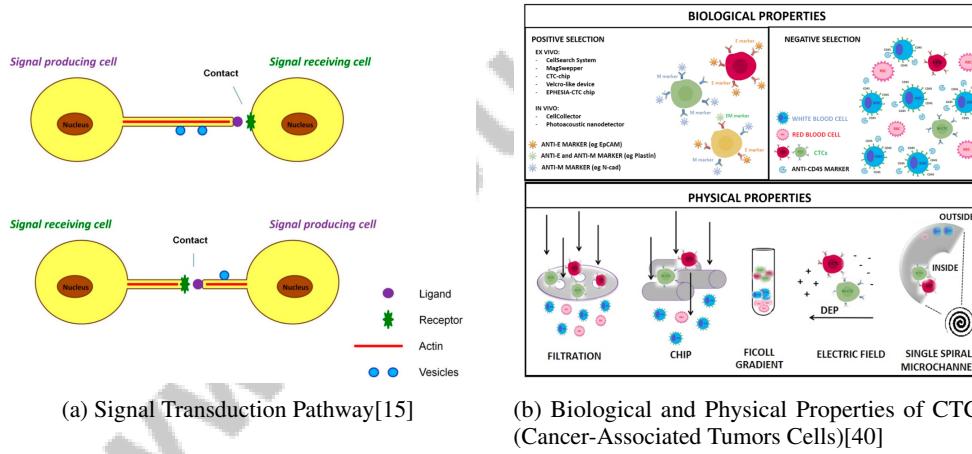


Figure 6: Examples of Current Research and Applications

As shown in Figure 6, exosomes, small extracellular vesicles secreted by cells, have emerged as promising biomarkers in various medical research fields due to their role in intercellular communication and potential as diagnostic tools. The current research and applications of exosomes as biomarkers are vividly illustrated in the provided figures, which highlight key aspects of cellular processes and cancer research. The first image depicts the signal transduction pathway, emphasizing the intricate communication between cells through signaling molecules, or ligands, which mediate critical biological responses. This pathway is fundamental to understanding how cells interact and respond to their environment, a process in which exosomes play a significant role. The second image focuses on the biological and physical properties of cancer-associated tumor cells (CTCs), presenting a comparative analysis under positive and negative selection scenarios. This diagram underscores the complexity of cancer biology and the importance of advanced technologies like the CellSearch System in identifying and analyzing CTCs. Together, these examples underscore the potential of exosomes as invaluable biomarkers in advancing our understanding of cellular processes and improving cancer diagnostics and treatment strategies [15, 40].

## 6 Challenges and Future Directions

### 6.1 Challenges in Exosome Research

Exosome research faces several challenges that hinder their comprehensive understanding and clinical application. The inherent heterogeneity of exosomes complicates their characterization and functional analysis [16]. This is exacerbated by a lack of standardized protocols for extracellular vesicle (EV) isolation and characterization, leading to inconsistencies across studies [9]. The absence of certified reference materials and variability in measurement techniques further impede standardization efforts [9]. Technical difficulties also arise from the small size of exosomes, complicating detection and analysis, with nanoparticle tracking analysis (NTA) often yielding inflated size estimates compared to direct imaging methods like Atomic Force Microscopy (AFM) [29]. Additionally, the complexity of exosome biogenesis and cargo selection remains poorly understood, necessitating sophisticated analytical techniques for deeper insights [24].

The rapid clearance of exosomes from circulation and challenges in achieving targeted delivery to specific tissues complicate the design of exosome-based therapies [23]. The similarity in biophysical properties between EVs and viral particles complicates their study and therapeutic potential, demanding advanced separation techniques [5]. Variability in production and the need for rigorous testing to establish safety and efficacy are compounded by the complexity of EVs, underscoring the necessity for standardized characterization methods [16]. The development of robust methodologies, such as RobKMR, may encounter challenges with noisy datasets or when biological assumptions are not met, affecting result accuracy [20]. Moreover, prolonged immobilization due to injuries, particularly in wartime, increases the risk of fractures and complications, highlighting the need for effective therapeutic strategies [31]. Advancing exosome research necessitates the establishment of standardized methods and innovative technologies, including optimized isolation and purification techniques, improved storage conditions, and a greater understanding of pharmacokinetics or biodistribution patterns. Continued research is essential to overcome these challenges and enable the effective use of exosomes in diagnostics and therapeutics.

### 6.2 Technological Advances in Exosome Detection

Method Name	Detection Methods	Technological Innovations	Clinical Applications
GET[38]	Get Method	Plasmonic Cavities	Molecular Diagnostics
LIFC[47]	Laser Interferometry	Measurement Accuracy	Virus Detection Systems
k-scope[39]	K-space Holography	Dynamic Range	Clinical Applications

Table 1: Summary of recent technological advancements in exosome detection methods, highlighting their detection techniques, technological innovations, and clinical applications. The table provides a comparative overview of the GET, LIFC, and k-scope methods, illustrating their contributions to enhancing the precision and applicability of exosome research.

Recent technological advancements have significantly improved the detection and analysis of exosomes, enhancing their clinical diagnostic and therapeutic applications. Table 1 presents a comprehensive comparison of recent technological advancements in exosome detection, focusing on their methodologies, innovations, and clinical relevance. The Geometry-induced Electrohydrodynamic Tweezers (GET) method enables high-throughput, tether-free trapping of EVs without photothermal damage, advancing EV analysis and allowing detailed studies of their properties and interactions [38]. Laser interferometry in flow cytometry has emerged as a cutting-edge approach for detecting small bioparticles, including exosomes, offering precise measurement capabilities that enhance the identification and characterization of exosomes in complex biological samples [47]. Integrating such advanced methodologies into exosome research promises to improve sensitivity and specificity, leading to more accurate analyses.

Emerging techniques, including microfluidics and nanoparticle tracking, present promising tools for future EV research, providing enhanced control over exosome manipulation and analysis [48]. Innovations in nanosizing methods have achieved a dynamic range of up to 110 dB, surpassing conventional systems and allowing for more precise characterization of exosomes, thereby improving our understanding of their size distribution and heterogeneity [39]. The ongoing development of these advanced detection technologies is crucial for addressing existing challenges in exosome research, such as heterogeneity and characterization. As methodologies for analyzing EVs, including exosomes,

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evolve, they are set to transform exosome research significantly. These innovations enhance our understanding of EVs as mediators of intercellular communication and potential biomarkers for various diseases, facilitating the development of effective diagnostic tools and therapeutic strategies. By addressing barriers in sensitivity and standardization of EV analysis, these evolving technologies could lead to more reliable clinical applications and improved patient outcomes in diagnostics and therapeutics [14, 24, 2, 3].

### 6.3 Future Directions in Exosome Research

The future of exosome research is poised to advance significantly through the establishment of standardized methodologies and exploration of both physiological and therapeutic applications. Developing certified reference materials and comprehensive guidelines for EV characterization is essential for enhancing standardization across the field [9]. This will address current challenges related to exosome heterogeneity and ensure consistency in research outcomes. Future research should prioritize optimizing exosome production and developing standardized protocols, which are crucial for exploring novel applications in immunotherapy and targeted drug delivery [24]. Refining robust manufacturing processes and improving characterization methods are necessary steps to enhance the therapeutic efficacy of engineered EVs [16]. Additionally, exploring exosomes in the context of disuse osteoporosis, particularly for developing targeted therapies for post-immobilization osteoporosis, presents a promising research avenue [31].

Investigating the integration of physical rehabilitation strategies to enhance recovery post-immobilization, alongside developing targeted therapies, will be vital. The exploration of novel applications in immunotherapy and targeted drug delivery will benefit from optimizing exosome production and establishing standardized protocols [24]. Furthermore, examining integrin interactions and signaling pathways involved in EV uptake could unveil new therapeutic targets, enhancing the potential of exosomes in clinical applications. Advancements in precise and minimally invasive imaging techniques, combined with innovative labeling strategies, are expected to enhance the investigation of EVs in diverse disease contexts. These improvements will enable researchers to visualize and characterize the spatiotemporal dynamics of EVs, crucial for understanding their roles in intercellular communication and their potential as biomarkers for various diseases, including cancer. As the field evolves, developing standardized methods for EV analysis will further facilitate their application in both basic and translational research [14, 3, 48]. Integrating multi-omics approaches to understand complex interactions facilitated by exosomes is another promising area, offering insights into disease mechanisms and potential therapeutic interventions.

Future research should also focus on refining methodologies such as robust kernel machine regression (RobKMR), exploring its application to other diseases, and validating identified biomarkers through additional biological experiments [20]. These efforts will drive the field forward, offering novel insights into disease mechanisms and potential therapeutic interventions. As research progresses, these initiatives will continue to propel the field forward, offering new insights into disease mechanisms and therapeutic possibilities.

## 7 Conclusion

This survey highlights the critical function of exosomes in mediating intercellular communication and their potential utility in diagnosing and treating disuse osteoporosis. By transporting biomolecules that modulate disease progression and therapeutic responses, exosomes emerge as promising candidates for novel therapeutic approaches. Recent research has advanced our understanding of cell-cell communication mechanisms, facilitating the identification of disease-specific biomarkers and therapeutic targets. The role of extracellular vesicles as both biomarkers and therapeutic carriers necessitates the development of refined methodologies to delve deeper into their intricate biological functions.

In the domain of bone health, osteocytes serve as pivotal regulators of mechanotransduction, underscoring the importance of targeted interventions to address bone loss due to disuse. Advances in frameworks such as ICELLNET have expanded our comprehension of exosome-mediated interactions, particularly within cancer and immune system contexts. These insights collectively underscore the potential of exosomes in enhancing our understanding and treatment of disuse osteoporosis, advocating for sustained research efforts to unlock their full clinical potential. Additionally, the

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therapeutic promise of stem cell-derived exosomes, which mimic the benefits of stem cells while mitigating associated risks, offers an exciting prospect for future surgical applications.

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