

Multi-omics analysis of small extracellular vesicles in osteoarthritis: bridging the gap between molecular insights and clinical applications

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Abstract

Osteoarthritis (OA) is a prevalent degenerative joint disorder with significant socioeconomic impact. Despite advances in understanding its pathophysiology, current therapeutic strategies remain largely palliative. Small extracellular vesicles (sEV) have emerged as crucial mediators of intercellular communication in joint tissues, offering new insights into OA pathogenesis and potential therapeutic targets. This review explores the application of multi-omics approaches to sEV research in OA, assessing how these advanced technologies are contributing to our understanding of the disease and their potential to revolutionize OA management. We discuss the latest findings on the role of sEV in OA, the applications of multi-omics technologies in deciphering sEV cargo, the progress toward clinical translation, and the challenges and opportunities in this field. By synthesizing current knowledge and identifying key research gaps, this review provides a roadmap for leveraging sEV multi-omics to bridge the gap between molecular discoveries and clinical applications in OA.

Keywords: Small extracellular vesicles; Multi-omics; Osteoarthritis

Highlights

- Recent advancements in multi-omics approaches to small extracellular vesicles (sEV) have provided new insights into osteoarthritis (OA) pathogenesis, revealing potential molecular targets for therapeutic intervention.
- The integration of transcriptomics, proteomics, metabolomics, and lipidomics has enabled a comprehensive understanding of sEV cargo, identifying key biomarkers and potential diagnostic tools for early OA detection.
- Multi-omics findings have facilitated the development of engineered sEV for therapeutic use, enhancing cartilage regeneration and offering new approaches for personalized OA treatment.
- Overcoming challenges related to sEV heterogeneity, isolation standardization, and clinical translation is crucial for realizing the full potential of sEV-based diagnostics and therapeutics in OA management.
- Future advances, such as single-vesicle multi-omics and artificial intelligence-driven data integration, are poised to revolutionize our understanding and treatment of OA, paving the way for personalized and precision medicine approaches.

Background

Osteoarthritis (OA) is a prevalent degenerative joint disorder characterized by progressive articular cartilage degradation, subchondral bone remodeling, and synovial inflammation [1, 2]. Despite its significant socioeconomic burden, current therapeutic strategies for OA remain largely palliative, focusing on symptom management rather than disease modification [3, 4]. This limitation stems from an incomplete understanding of OA pathogenesis and the lack of reliable early diagnostic biomarkers and targeted interventions [5, 6].

In recent years, small extracellular vesicles (sEV), including exosomes [30–150 nm, originating from multivesicular bodies (MVBs)], microvesicles (100–1000 nm, budding from the plasma membrane), and apoptotic bodies (500–2000 nm, released during cell death) have emerged as crucial mediators of intercellular communication in joint tissues [7, 8] (Figure 1). These nanoscale, membrane-bound particles through their diverse cargo of proteins, lipids, and nucleic acids can reflect the state of their cell of origin during joint health and disease, thus providing opportunities for

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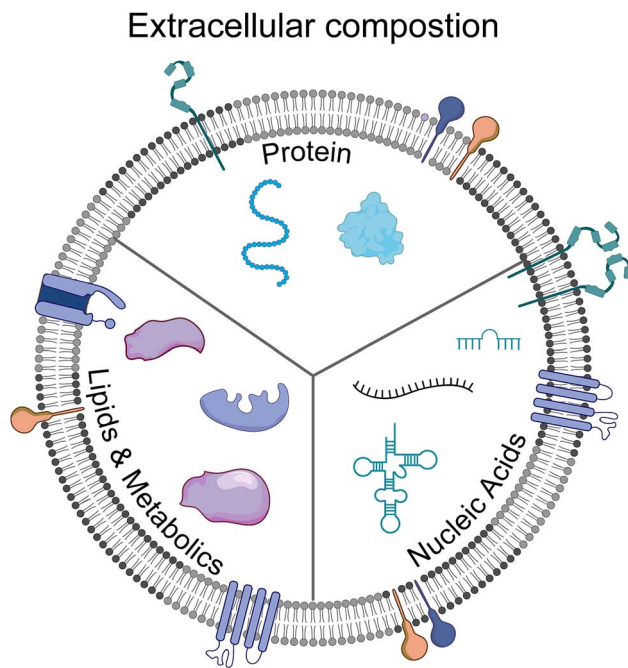


Figure 1. Schematic representation of the bioactive cargo carried by sEV, including proteins, miRNAs, lipids, and metabolites

diagnostic and therapeutic applications [9, 10]. Notably, the role of sEV in OA pathogenesis has garnered significant attention, with evidence suggesting their involvement in cartilage homeostasis, inflammation, and matrix degradation [9, 11, 12].

Concurrently, the advent of high-throughput omics technologies has revolutionized the study of complex biological systems [13]. The integration of multiple omics approaches, including genomics, transcriptomics, proteomics, metabolomics, and lipidomics, offers unprecedented insights into the molecular landscape of diseases [14]. This multi-omics paradigm, when applied to the study of sEV in OA, presents a unique opportunity to elucidate the intricate molecular mechanisms underlying the disease progression and identify novel diagnostic and therapeutic targets [15].

By leveraging these advanced technologies to analyse sEV derived from osteoarthritic tissues, researchers aim to decode the molecular signatures associated with disease onset, progression, and potential therapeutic responses [15–17]. However, translating these molecular insights into clinical applications remains challenging, requiring not only technological advancements but also a deep understanding of the biological context and rigorous validation [18, 19].

This review provides a comprehensive analysis of the current state of sEV multi-omics in OA research and its potential to revolutionize disease management. We evaluate recent findings on the role of sEV in OA pathogenesis, critically assess the applications of various omics technologies in deciphering sEV cargo composition and functionality, and examine the progress toward translating sEV multi-omics findings into clinical tools for OA diagnosis, prognosis, and treatment. We discuss the technical, biological, and translational challenges in the field of sEV multi-omics in OA, to propose potential solutions and future directions. By synthesizing current knowledge and identifying key knowledge gaps, we aim to provide a roadmap for future research efforts in leveraging

sEV multi-omics to bridge the gap between molecular discoveries and clinical applications in OA. This review highlights the transformative potential of sEV multi-omics in advancing our understanding of OA pathogenesis and developing innovative approaches to disease management.

Review

Multi-omics approaches in small extracellular vesicle research

Extracellular vesicles (EV) are phospholipid membrane-bound vesicles released by most cells, playing a crucial role in cell-to-cell communication, with their cargo reflecting the characteristics of the cells of origin [20, 21]. In earlier studies, they were primarily classified based on their biogenesis into exosomes, microvesicles, and apoptotic bodies [22]. Due to the heterogeneity of EV, distinguishing these subclasses can be challenging, leading to the recognition of two types: exosomes and ectosomes [23]. Exosomes are generated through the endosomal pathway and released via the exocytosis of MVBs and amphisomes, whereas ectosomes are produced through plasma membrane budding and blebbing [23]. However, classifying EV subtypes remains difficult due to the lack of specific markers and overlap in size and composition. Therefore, it is recommended to characterize EV by their degree of homogeneity unless the subcellular origin is clearly demonstrated [24]. In this study, we focus on small EV, which are designated as <200 nm in diameter.

The integration of multiple high-throughput omics technologies has revolutionized sEV research, offering unprecedented insights into the molecular intricacies of these complex biological entities. This section provides a comprehensive overview of the various omics techniques applied to sEV research, the sophisticated methods of data integration, and the significant advantages of multi-omics approaches (Figure 2).

Genomics in small extracellular vesicle research

While genomics plays a less prominent role in sEV research compared to other omics approaches, recent studies have revealed intriguing aspects of DNA content in sEV. The presence of mitochondrial DNA (mtDNA) in sEV has been reported in several studies, with significant implications for cellular energetics and signaling [25]. For instance, cancer-associated fibroblasts have been shown to release sEV containing mtDNA, which can be transferred to breast cancer cells, leading to increased oxidative phosphorylation and facilitating the exit from metabolic dormancy [26].

Genomic DNA, although less common, has also been detected in sEV. Studies have shown that tumor-derived sEV contain double-stranded DNA spanning all chromosomes, reflecting the mutational status of parental tumor cells [27]. This finding has been corroborated by subsequent studies, opening up new possibilities for liquid biopsy applications in cancer diagnostics and monitoring [28–30].

A recent study by Lee et al. focused on genomic profiling of extracellular vesicle-derived DNA (EV DNA) from bronchoalveolar lavage fluid (BALF) of patients with lung adenocarcinoma. They found that epidermal growth factor receptor (EGFR) genotyping using BALF EV DNA showed

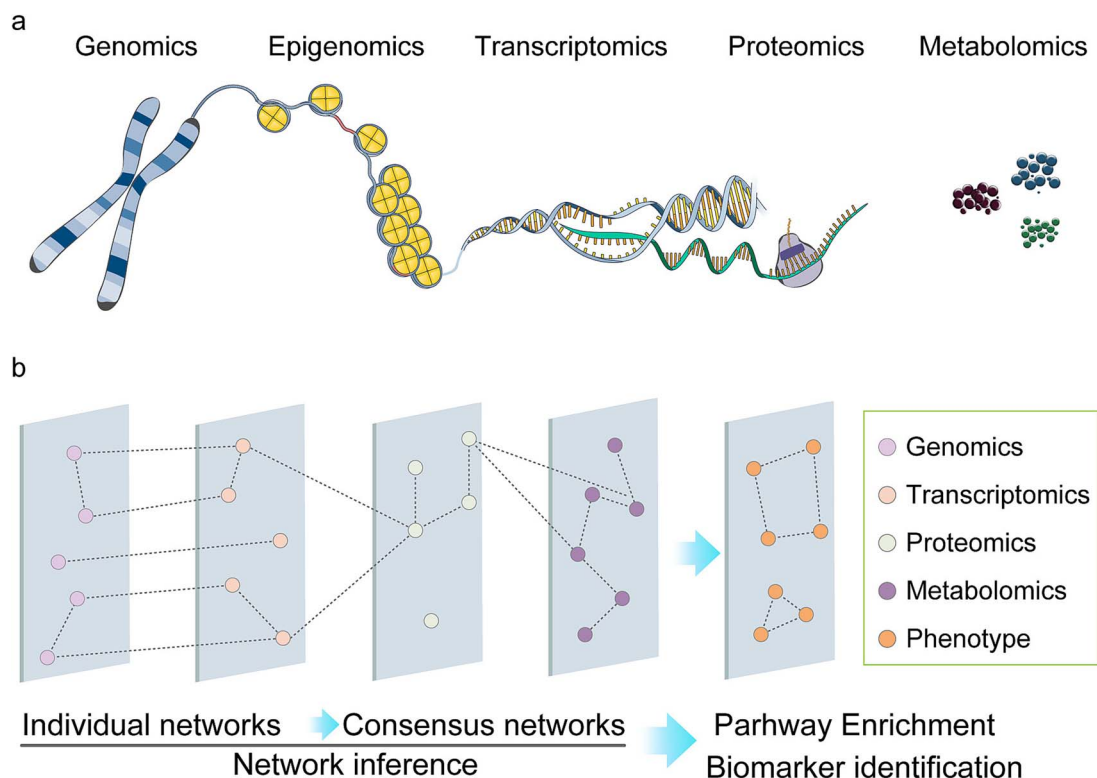


Figure 2. Overview of multi-omics technologies applied in sEV research. (a) Various omics approaches, including genomics, epigenomics, transcriptomics, proteomics, and metabolomics, are utilized to characterize sEV content. (b) The integration of multi-omics data provides insights into the complex interplay of biological pathways involved in OA progression

almost 100% sensitivity in patients with advanced non-small cell lung cancer (NSCLC). Moreover, targeted next-generation sequencing (NGS) of BALF EV DNA showed promising results, with overall mutation concordance between BALF EV DNA and matching tissue DNA being 56% for nonsynonymous somatic mutations and increasing to 81% for clinically significant mutations [31]. These findings demonstrate that BALF EV DNA can be a reliable source for identifying actionable genetic alterations, underscoring the clinical feasibility and utility of sEV-derived DNA in cancer diagnostics.

Epigenetic modifications on sEV-associated DNA are gaining attention in the field. Researchers are actively investigating whether these epigenetic signatures could serve as biomarkers or play functional roles in recipient cells [32]. Additionally, recent research by Huang et al. demonstrated that diabetes impairs the cardioprotective function of endothelial progenitor cell-derived sEV through the inhibition of histone acetylation (H3K9Ac), highlighting how genomic and epigenomic alterations in sEV can affect their therapeutic potential in cardiovascular diseases [33]. This emerging area of study adds another layer of complexity to our understanding of sEV-mediated intercellular communication and their potential impact on recipient cell function.

Transcriptomics in small extracellular vesicle research

Transcriptomics has emerged as a cornerstone in sEV research, revealing the diverse RNA landscape within these vesicles. NGS and microarray technologies have been instrumental in characterizing the RNA content of sEV [34, 35]. The seminal work demonstrating the presence of functional mRNAs in

sEV that can be translated in recipient cells established a new paradigm of horizontal RNA transfer via sEV [20].

MicroRNA (miRNA) analysis has been a particularly fruitful area of sEV research. Small RNA sequencing has revealed the enrichment of miRNAs in sEV, with studies showing that the miRNA content of sEV is modulated by the cells' physiological state and can influence the behavior of recipient cells [36–41]. This findings underscore the dynamic nature of sEV cargo loading and its dependence on cellular state.

Furthermore, Cheng et al. demonstrated that exosomes provide a protected and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood components [42]. Their findings highlight that sEV's miRNA remains intact even after RNaseA treatment, underscoring the value of exosomes as a consistent source for miRNA biomarker detection, which enhances the reliability of disease biomarker assays.

Long non-coding RNAs (lncRNAs) in sEV have also gained attention for their potential regulatory functions. Studies have found that certain lncRNAs are selectively packaged into sEV in response to cellular stress, suggesting a role for these molecules in stress-induced intercellular signaling [43]. The discovery of circular RNAs (circRNAs) in sEV has opened new avenues in understanding RNA-mediated intercellular communication, given the unique stability and regulatory potential of these circular molecules [44].

Moreover, Lässer et al. identified two distinct extracellular RNA (exRNA) signatures released by a single cell type, characterized as high-density (HD) and low-density (LD) exRNA. Both HD and LD exRNA contained mRNA and miRNA, but exhibited different enrichment profiles for other RNA species [45]. This study highlights the complexity of exRNA

release, demonstrating that cells can produce multiple exRNA types with substantial differences in RNA content, which has significant implications for understanding the role of exRNA under various cellular conditions.

Proteomics in small extracellular vesicles research

Proteomic analysis has been instrumental in characterizing the protein cargo of sEV and understanding their biological functions. High-resolution mass spectrometry techniques have enabled the identification of thousands of proteins in sEV [46]. Comprehensive proteome databases of sEV from various sources have been created, providing valuable resources for the field [47].

Recent advances in proteomic technologies have greatly enhanced our ability to study sEVs in detail. Nguyen *et al.* conducted a label-free quantitative proteomic analysis of exosomes enriched from human serum, identifying 329 proteins that differentiated between alcoholic and nonalcoholic fatty liver disease (NAFLD) [48]. This differentiation highlights the potential of sEV as valuable biomarkers for liver disease, providing insight into disease progression and state.

In another study, Doulabi *et al.* employed a combination of high-resolution mass spectrometry and proximity ligation assays to profile surface proteins of sEV in seminal fluid, identifying >1000 surface proteins [49]. Their work underscores the importance of surface proteins for understanding sEV-mediated intercellular communication and their potential as biomarkers for diagnostic applications.

Xu *et al.* utilized quantitative proteomic analysis to study sEV derived from umbilical cord mesenchymal stem cells (UCMSCs) and rat bone marrow stem cells (BMSCs). They identified thousands of proteins and demonstrated differential regulation of proteins involved in the TGF- β signaling pathway between different BMSC passages [50]. These findings suggest that these proteins could serve as potential markers for the therapeutic potency of BMSC-derived sEV.

The potential of mass spectrometry as a tool for rapid and reliable sEV protein detection was further demonstrated by Nguyen *et al.*, who identified platelet factor 4 (PLF4) as a novel sEV marker using MALDI-FTICR-MS analysis of sEV from human serum [51]. This study indicates that MALDI-MS can serve as a reliable, non-invasive tool for detecting sEV biomarkers for liver disease.

Yu *et al.* optimized MALDI-TOF MS protocols for the protein profiling of EVs from human lung carcinoma cells, identifying proteins such as ribosomal protein RPS27A and tumor marker S100A10 [51]. This work highlights the utility of MALDI-TOF MS in rapid screening for cancer biomarkers, contributing to the development of non-invasive diagnostic tools for lung cancer.

Webber *et al.* applied the SOMAscan™ aptamer-based proteomics platform to analyze sEV derived from prostate cancer cells [52]. They identified >300 proteins that had not been previously reported in sEV, demonstrating the versatility of SOMAscan™ for high-throughput analysis and biomarker discovery in clinical settings, particularly for prostate cancer.

Bæk *et al.* investigated the impact of preanalytical treatments on the phenotype of sEV in blood, revealing that pre-analytical conditions such as blood collection tube type, centrifugation protocol, and transportation significantly affect the phenotypic analysis of EVs [53]. Their findings underscore the importance of standardized protocols to ensure reliable and consistent results in sEV research.

Post-translational modifications (PTMs), which are chemical modifications such as phosphorylation and ubiquitination that regulate protein activity and stability, on sEV proteins have also been a focus of proteomic studies. Research has demonstrated that ubiquitination plays a crucial role in protein sorting into sEV, highlighting the importance of PTMs in sEV cargo selection and function [54–56]. Other studies have explored phosphorylation, glycosylation, and other PTMs in sEV proteins [57–59].

Quantitative proteomics approaches, both label-free and labeled (e.g. SILAC, TMT), have been employed to compare protein abundances across different sEV populations or conditions [60]. These studies have revealed distinct protein signatures that reflect the different biogenesis pathways and cellular origins of sEV.

Metabolomics and Lipidomics in small extracellular vesicles research

Metabolomics and lipidomics have provided crucial insights into the small molecule and lipid composition of sEV. Global metabolite profiling using liquid chromatography-mass spectrometry (LC-MS) or nuclear magnetic resonance (NMR) spectroscopy has revealed diverse metabolites in sEV [61, 62]. Studies have demonstrated that sEV contain distinct metabolite profiles that can influence recipient cell metabolism [63–65].

Lipidomic analyses have uncovered the complex lipid composition of sEV membranes. Carayon *et al.* explored the proteolipidic composition of exosomes during reticulocyte maturation, revealing significant changes in the lipid content over the course of a 7-day *in vitro* differentiation process. Their findings indicate that the biogenesis of exosomes in reticulocytes involves multiple pathways for the preferential recruitment of particular proteins and lipids, with changes in the prominence of these pathways during differentiation [66]. This supports the idea that the lipid composition of sEV is dynamically regulated, reflecting their functional roles during cell differentiation.

Additionally, Zhang *et al.* used asymmetric flow field-flow fractionation (AF4) to identify two exosome subpopulations—large exosome vesicles (Exo-L) and small exosome vesicles (Exo-S)—along with a new population of non-membranous nanoparticles called exomeres [67]. The lipidomic profiling of these subpopulations revealed unique lipid signatures for each, suggesting distinct biogenesis pathways and biological functions. These findings underscore the complexity of sEV lipid composition and highlight the functional diversity that may arise from lipid heterogeneity. High-resolution mass spectrometry-based lipidomics has revealed significant enrichment of certain lipid species in sEV compared to their parent cells [68, 69]. These studies have provided insights into the unique lipid signatures that may be crucial for vesicle stability, uptake, and function.

Integration of multi-omics data in small extracellular vesicles research

The integration of data from multiple omics platforms represents both a significant challenge and opportunity in sEV research. Various strategies for data integration have been developed, including vertical integration of different types of molecular data from the same sEV samples, horizontal

integration comparing omics data across different sEV populations or conditions, and temporal integration to understand dynamic changes in sEV composition [70, 71].

Network-based approaches have been particularly powerful in elucidating the functional relationships between different molecular components of tumor-derived. For example, Yan *et al.* used a network-based approach to integrate metabolic profiling and transcriptomics data from cancer cell-secreted sEV, revealing novel insights into how these vesicles reprogram metabolism in recipient cells [72]. This study demonstrated that sEV's miR-105 promotes tumor growth through MYC-dependent metabolic reprogramming of stromal cells.

Machine learning (ML) and artificial intelligence (AI) algorithms are increasingly being applied to extract meaningful patterns from multi-dimensional omics data. For instance, Xu *et al.* developed an ML approach to predict cervical cancer-specific diagnostic biomarkers by integrating DNA methylation and gene expression data [73]. Their model successfully identified several potential biomarkers, including RAB3C, GABRA2, and ZNF257, which could be significant for early diagnosis of cervical cancer. These computational methods hold promise for generating testable hypotheses from complex multi-omics datasets.

Advantages of multi-omics approaches in small extracellular vesicles research

The application of multi-omics approaches in sEV research offers several significant advantages. It provides a comprehensive molecular characterization of sEV, capturing their complexity and heterogeneity in a way that single-omics approaches cannot [74]. This holistic view enhances biomarker discovery, leading to more robust and specific markers for various physiological and pathological states.

Multi-omics data reveal functional relationships between different molecular components of sEV, providing insights into how these vesicles mediate their biological effects. For example, studies integrating small RNA sequencing with proteomics have identified miRNA-protein interactions in tumor-derived sEV, providing insights into sEV-mediated metastasis [75].

The integration of multiple omics datasets enhances the robustness and specificity of biomarker identification. By employing a layered multi-omics approach, including proteomics, lipidomics, and metabolomics, the researchers were able to comprehensively characterize the cargo of pancreatic ductal adenocarcinoma (PDAC)-derived EVs and their functional impact on recipient normal pancreatic epithelial cells [71]. While individual omics techniques provide valuable insights into sEV composition and function, their integration allows for a deeper understanding of OA pathogenesis and the development of targeted therapeutic strategies. The following section explores how multi-omics findings translate into mechanistic insights and clinical applications.

Furthermore, utilizing multi-omics approaches in sEV with therapeutic potential enables the exploration of the sEV biological information and therapeutic mechanisms. After identifying the targets (i.e. HDAC2 and DNMT1) through single-cell RNA sequencing (scRNA-seq) and epigenetic profiling of maladapted sinusoidal endothelial cells associated with liver fibrosis, Zhang *et al.* performed EV transplantation from *shADAMTS1*-infected HUVECs, mitigating liver fibrosis in mice and unveiling an endothelial HDAC2/

DNMT1-IGFBP7/ADAMTS1-T_H17 axis involved in the progression of liver fibrosis in nonalcoholic steatohepatitis [76]. Moreover, using multi-omics approaches, *Clostridium butyricum*-derived EV was identified as a novel therapeutic agent for colitis, by modulating intestinal miRNAs and signaling pathways and regulating microbial tryptophan metabolites [77].

Multi-omics insights into small extracellular vesicles in osteoarthritis pathogenesis

OA is a whole-joint disease that affects all joint tissues, including cartilage, meniscus, ligaments, subchondral bone, and synovium [78]. Progressive cartilage degradation is a hallmark of OA. The breakdown of the cartilage matrix alters the biomechanical environment of chondrocytes, disrupts the regulation between cartilage and subchondral bone, and contributes to synovial inflammation through the release of degradation products [79–81]. The degeneration of articular cartilage, bone remodeling, and synovial inflammation in OA often result in joint structural and functional deterioration, chronic joint pain, and eventually a compromised quality of life [82].

Various mechanical, biochemical, and genetic factors play a role in the complexity of OA [83]. The imbalance between anabolic and catabolic processes, driven by matrix-degrading proteases, inflammatory cytokines, and other factors such as the senescence-associated secretory phenotype (SASP) and damage-associated molecular patterns (DAMPs), contributes to the progression of OA [84–86]. Comprehending the intricate pathophysiological processes and mechanisms of OA is essential for the development of novel therapeutic strategies [87]. Accordingly, employing multi-omics approaches is crucial to gain deeper insights into its pathogenesis.

The cross-talk among cartilage, subchondral bone, synovial tissue, infrapatellar fat pad, and macrophages in OA have highlighted the involvement of sEV in the development and pathological roles of joint tissues. For instance, sEV derived from inflammatory fibroblast-like synoviocytes has been reported to enhance macrophage glycolysis, facilitate M1 polarization of macrophages in the joint, and contribute to synovitis and OA progression [88]. Similarly, inflammatory macrophage-derived sEV induced noncanonical pyroptosis of chondrocytes and enhanced cartilage catabolism [11]. Infrapatellar fat pad-sEV from OA patients have been shown to impair cartilage metabolism and induce chondrocyte senescence [9]. Moreover, calcified EV derived from calcified chondrocytes initiated pathological cartilage calcification in OA [89]. In addition, EV derived from subchondral bone mesenchymal stromal cells (MSCs) possess the potential to modulate cartilage regeneration in OA [90]. The application of multi-omics approaches to the study of sEV in OA has opened new avenues for understanding the pathogenesis, progression, and potential treatment of this debilitating joint disease. This section explores the recent findings on the role of sEV in OA, with a particular focus on insights gained through multi-omics studies (Table 1).

sEV play a crucial role in OA by transferring bioactive molecules that regulate key signaling pathways involved in joint degeneration. The TGF- β pathway, essential for cartilage maintenance, is influenced by sEVs carrying TGF- β 1, which promotes extracellular matrix (ECM) synthesis and inhibits MMP-13-mediated cartilage breakdown [91, 92]. The PI3K/AKT pathway, which supports chondrocyte survival and

Table 1. Multi-omics techniques applied in sEV research

Omics	Technique	Key findings	Biological significance	Specific sEV biomarker identified in OA	Clinical relevance (diagnosis/therapy)	Refs.
Genomics	NGS, qPCR for mtDNA, dsDNA detection	Presence of mtDNA and nuclear DNA in sEV, influencing cellular energetics in fibroblast-derived sEVs; DNA content varies with OA severity.	mtDNA and nuclear DNA in sEVs contribute to inflammation and cellular metabolic states.	mtDNA, dsDNA	Potential for use in liquid biopsy for DNA-based OA biomarkers.	[25–27, 31]
Transcriptomics	NGS, microarray analysis for RNA profiling, small RNA sequencing	Diverse RNA types (miRNA, lncRNA, circRNA) including miR-27b-3p and miR-34a-5p linked to ECM regulation; OA severity alters RNA profiles.	RNA profiles in sEVs impact cartilage degradation, synovial fibrosis, and inflammatory responses.	miR-27b-3p, let-7b-5p, miR-193b-3p	miRNA in sEVs as early detection biomarkers, therapeutic targets for OA.	[20, 34, 42, 100, 105, 115]
Proteomics	Mass spectrometry, SILAC, TMT for protein quantification and post-translational modification (PTM) analysis	Proteomic profiling identifies CD44, lubricin, and fibronectin; PTMs like ubiquitination play key roles in protein sorting in sEVs from synovial fibroblasts.	Proteins in sEVs influence joint lubrication, ECM stability, and inflammatory signaling.	CD44, lubricin, fibronectin	Proteins as joint-specific markers for OA diagnosis and treatment monitoring.	[46, 102, 103, 108]
Metabolomics	Liquid chromatography-mass spectrometry (LC–MS), NMR spectroscopy	Metabolites (prostaglandins, lactate) in sEVs mirror oxidative stress in OA cartilage, impacting chondrocyte metabolism.	Metabolites reflect metabolic stress in joint cells, contributing to inflammation.	Prostaglandins, lactate, leukotrienes	Metabolites as biomarkers for joint inflammation and chondrocyte health.	[61, 63, 64]
Lipidomics	High-resolution mass spectrometry for lipid profiling, lipidomics-specific assays	Fatty acids (palmitic acid, stearic acid) enriched in sEVs, unique lipid composition critical for stability; profiles differ in OA vs. healthy samples.	Lipids in sEVs maintain membrane integrity, stability, and signaling in joint tissues.	Palmitic acid, stearic acid, phosphatidylcholine	Lipids may serve as biomarkers and modulators of membrane function in OA therapies.	[68, 104, 105]

autophagy, is regulated by sEV miRNAs such as miR-140-5p and miR-29a [93, 94]. In OA, a reduction in these miRNAs leads to impaired autophagy and increased chondrocyte apoptosis, accelerating cartilage loss [95]. The Wnt/ β -catenin pathway, critical for joint tissue homeostasis, is modulated by sEVs containing Wnt inhibitors (e.g. DKK-1, sFRP-1) that prevent excessive β -catenin activation [96]. Dysregulation of this pathway in OA promotes chondrocyte hypertrophy and ECM degradation. Inflammation in OA is driven by the NF- κ B pathway, where macrophage-derived sEVs carrying IL-6 and TNF- α trigger NF- κ B activation in synovial fibroblasts and chondrocytes, leading to upregulation of MMPs and ADAMTS5, key enzymes in cartilage degradation [97]. In contrast, sEVs from M2 macrophages contain miR-223 and IL-10, which suppress inflammation and provide joint protection [98]. Finally, the Hippo/YAP pathway, which regulates chondrocyte proliferation and ECM remodeling, is influenced by sEVs from osteoblasts and fibroblasts. Its dysregulation in OA results in abnormal bone remodeling and cartilage

destruction, further contributing to disease progression [99]. These pathways highlight the central role of sEVs in OA pathology and underscore their potential as biomarkers and therapeutic targets.

Transcriptomics insights

Transcriptomics analyses have significantly advanced our understanding of sEV-mediated RNA transfer in OA pathogenesis, particularly highlighting the crucial roles of specific miRNAs. Studies have revealed complex regulatory networks involving miRNAs that affect various aspects of joint pathology, including cartilage degradation, synovial fibrosis, and inflammatory responses.

Tavallae *et al.* demonstrated elevated levels of miR-27b-3p in the synovial fluid of patients with late-stage radiographic knee OA. This miRNA was found to contribute to synovial fibrosis by regulating extracellular matrix (ECM) genes and promoting pro-fibrotic responses in fibroblast-like synovio-cytes. The study uncovered a PPARG/ADAMTS8 signaling

axis regulated by miR-27b-3p, emphasizing its key role in ECM regulation associated with synovial fibrosis during OA progression [100].

Similarly, Endisha *et al.* investigated miR-34a-5p, finding its expression elevated in the plasma, cartilage, and synovium of late-stage OA patients and in mouse models of OA. Their research demonstrated that miR-34a-5p accelerates joints breakdown in OA, with its inhibition offering cartilage-protective effects. This finding suggests potential therapeutic strategies targeting specific miRNAs to mitigate OA progression [101].

A groundbreaking study by Cao *et al.* focused on sEV secreted by the infrapatellar fat pad in OA patients. They found that these IPFP-derived sEV could be delivered into articular chondrocytes, significantly promoting ECM catabolism and inducing cellular senescence. Mechanistically, they identified let-7b-5p and let-7c-5p as highly enriched miRNAs in IPFP-sEV. These miRNAs were found to mediate detrimental effects by directly decreasing the expression of lamin B receptor, a negative regulator of senescence [9]. This study not only uncovered the function and mechanism of IPFP-sEV in OA progression but also suggested a potential therapeutic strategy targeting specific sEV miRNA cargoes.

These transcriptomics studies collectively underscore the complex involvement of miRNAs in OA pathogenesis, affecting various aspects of joint pathology. The identification of key miRNAs such as miR-27b-3p, miR-34a-5p, let-7b-5p, and let-7c-5p, along with their regulatory networks, provides potential targets for therapeutic interventions in OA. Moreover, these findings highlight the potential of sEV-associated miRNAs as both biomarkers for OA progression and targets for novel treatment strategies.

Proteomics insights

While transcriptomics provides insights into the differential expression of sEV-associated RNAs, it does not fully capture the functional impact of sEVs in OA. Proteomics serves as a complementary approach by identifying specific proteins within sEVs that mediate their biological effects, offering a more comprehensive understanding of their molecular mechanisms. Recent proteomic studies have significantly advanced our understanding of the complex protein cargo carried by sEV in the context of OA. These investigations have revealed a diverse array of proteins within OA-associated EVs, shedding light on their potential roles in disease pathogenesis and progression.

Boere *et al.* provided crucial insights into the proteomic profile of synovial fluid-derived EVs [102]. Their analysis uncovered the presence of several joint-relevant proteins, including CD44, a key receptor involved in hyaluronan binding and cell-matrix interactions. Additionally, they identified other important proteins such as lubricin and fibronectin within these EVs. These findings underscore the potential role of EVs in maintaining joint homeostasis and suggest their involvement in OA pathogenesis. The presence of these specific proteins points to the multifaceted functions of EVs in the joint microenvironment, potentially influencing cellular behavior, matrix interactions, and tissue lubrication.

Further elucidating the functional implications of EV-associated proteins in OA, Kato *et al.* conducted a study focusing on sEV derived from IL-1 β -stimulated synovial fibroblasts [103]. Their proteomic analysis revealed that inflammatory cytokines, such as IL-6, the angiogenic factor

VEGF, and MMP-3, showed increased levels in sEV from IL-1 β -stimulated synovial fibroblasts. Importantly, when applied to chondrocytes, these EVs promoted the expression of inflammatory genes and facilitated cartilage degradation. This study provides direct evidence for the role of EV proteins in disease progression, highlighting how EVs can serve as mediators of inflammatory and catabolic signals in the OA joint.

Collectively, these proteomic studies highlight the diverse array of proteins carried by OA-associated EVs and their potential to modulate cellular behavior and tissue homeostasis in affected joints. The presence of matrix-associated proteins, inflammatory mediators, and signaling molecules in these EVs points to their multifaceted involvement in OA pathophysiology. These insights not only enhance our understanding of disease mechanisms but also suggest potential targets for therapeutic interventions and biomarker development in OA.

Metabolomics insights

Beyond transcriptomic and proteomic analyses, metabolomics provides a functional readout of sEV activity by identifying metabolic signatures that may contribute to cartilage degradation and inflammation in OA. While not directly mentioned in the provided text for OA-related sEV, metabolomics is a rapidly developing field that offers new perspectives on the role of sEV in OA pathogenesis. Metabolomic analysis can reveal changes in energy metabolism-related molecules in OA-associated sEV. These changes may reflect the metabolic state of OA chondrocytes and potentially influence energy metabolism in recipient cells through sEV. Metabolomics studies have the potential to identify inflammation-related metabolites, such as prostaglandins and leukotrienes, in OA-associated sEV. These findings can help us understand the role of sEV in inflammatory processes. Additionally, metabolomics analysis can detect oxidative stress-related metabolites in sEV, which may reflect the redox state of the OA cartilage.

Lipidomics insights

While metabolomics sheds light on the biochemical pathways influenced by sEVs, lipidomics further refines our understanding by characterizing the lipid components of sEV membranes, which play a crucial role in sEV stability, cargo loading, and intercellular signaling. Recent lipidomics studies have provided valuable insights into the role of lipids, particularly fatty acids (FAs), in OA pathogenesis. A study by Mustonen *et al.* compared FA profiles in equine synovial fluid (SF) and its sEV-enriched fraction between control and OA joints, revealing distinct FA signatures modified by OA. The EV-enriched pellets from OA joints showed distinct changes in saturated FAs. Palmitic acid (16:0), stearic acid (18:0), and behenic acid (22:0) levels were all indicative of OA, pointing to potential alterations in EV membrane composition and function in the disease state [104]. These FA modifications are potentially detrimental and could contribute to inflammatory processes and cartilage degradation in OA.

Future research should focus on elucidating the functional implications of these lipid alterations and exploring their potential as therapeutic targets in OA. Understanding the complex role of lipids in OA pathogenesis could open up new possibilities for disease management and treatment strategies.

Table 2. Integration of multi-omics for sEV research in OA

Techniques used	Application	sEV source	Sample size	Findings	Multi-omics integration	Ref.
Transcriptomics and proteomics	Identify surface markers of pathogenic SF EVs with confirmed OA joint tissue origins	Human knee cartilage and synovial membrane, plasma, and SF from knee OA patients	Transcriptomics: 3 OA samples (36 832 cells) Proteomics: 16 OA samples	Transcriptomics: 21866 genes identified in chondrocytes (11 579 from damaged sites, 14 613 from intact sites), 21 253 genes identified in synoviocytes Proteomics: 8538 peptides corresponding to 670 proteins identified	Positive correlation between scRNA-seq and proteomic data supports the OA SF EV profile	[105]
Transcriptomics and proteomics	EV profiling for potential biomarkers and understanding early OA pathogenesis	Equine SF from middle carpal joints and plasma	Transcriptomics: 4 equine samples (control and OA, 6 OA induction time points) Proteomics: 4 equine samples (control and OA, 6 OA induction time points)	Transcriptomics: 158 non-coding RNAs from plasma EVs, 216 from control SF EVs, 233 from OA SF EVs Proteomics: 331 proteins in SF EVs and 203 in plasma EVs	Multi-omics factor analysis (MOFA2) revealed temporal expression patterns during OA progression. EV protein cargo appears more important than small non-coding RNA cargo during OA progression	[15]
Proteomics and lipidomics	EV profiling and identify candidate composite diagnostic biomarkers of OA	Equine SF from metacarpophalangeal joint	Proteomics: 7 healthy, 4 mild OA, 3 severe OA Lipidomics: 6 healthy, 4 mild OA, 3 severe OA	Proteomics: 5774 peptides corresponding to 290 proteins identified Lipidomics: 280 phospholipid species identified	Unsupervised sPLS2 regression and PCA revealed strong similarity between the SF-EV phospholipidome and proteome, indicating potential composite biomarkers	[16]

Integrating multi-omics approaches

Recent studies have demonstrated the power of integrating multiple omics approaches to gain comprehensive insights into the role of sEV in OA pathogenesis, including the temporal dynamics of EV cargo changes during disease progression (Table 2).

Zhang *et al.* combined proteomics with single-cell RNA sequencing to characterize pathogenic SF EVs in knee OA. They identified 692 peptides in SF EVs associated with OA severity, with 57.4% of these pathogenic peptides from immune system proteins [105]. By integrating proteomic findings with scRNA-seq data, they traced the cellular origins of key EV surface markers, linking them to specific cell types in damaged cartilage and synovial immune cells.

Clarke *et al.* employed a multi-omics strategy combining proteomics and phospholipidomics to identify composite biomarkers in EVs from equine OA joints [16]. Their integrated analysis revealed correlations between the proteome and phospholipidome, with some phospholipids and related proteins changing in abundance as OA severity progressed. Although the number of EVs was unaffected by OA, proteome profiling identified 40 differentially expressed proteins in osteoarthritic joints, which were associated with 7 significant canonical pathways linked to OA. Phospholipid profiling also showed a relative increase in sphingomyelin and a decrease in phosphatidylcholine, phosphatidylinositol,

and phosphatidylserine in EVs from osteoarthritic joints compared to healthy joints. The integrative multi-omics analysis unveiled potential composite biomarkers for OA, and significantly enriched molecular pathways that are linked to the development and perpetuation of the disease. This exploratory study has relevance due to shared joint biology and OA pathogenesis between horses and humans, offering insights into potential diagnostic biomarkers for OA.

Anderson *et al.* utilized a temporal model of OA to define changes in matched synovial fluid and plasma-derived EV small non-coding RNA and protein cargo using sequencing and mass spectrometry. Their multi-omic approach revealed that EV protein cargo appears to be more important during OA progression than small non-coding RNAs [15].

These studies highlight how integrating multiple omics approaches, including temporal profiling [106], and combining proteomic, transcriptomic, lipidomic, and temporal data can provide a comprehensive understanding of the complex and dynamic role of EVs in OA pathogenesis, not achievable with single-omics and/or single timepoint approaches [15, 16, 105]. These recent multi-omics studies exemplify the power of integrative approaches in unraveling the complex role of sEV in OA. By providing a more holistic view of sEV biology in the context of joint health and disease, these studies are paving the way for novel diagnostic and therapeutic strategies in OA management.

Integrating multi-omics approaches provides a holistic perspective on sEV function in OA, bridging molecular insights with potential clinical applications. The following section explores how these findings translate into biomarker discovery and therapeutic strategies.

Clinical applications and future prospects of small extracellular vesicles multi-omics in osteoarthritis

The insights gained from multi-omics studies of sEV in OA are gradually being translated into clinical applications. This section reviews the current state of clinical translation, emerging therapeutic strategies, and the future prospects of sEV-based approaches in OA management (Figure 3).

Translating multi-omics insights into clinical diagnostics

The integration of multi-omics approaches in the study of sEV has ushered in a new era of possibilities for early detection and precise staging of OA. By synergizing transcriptomics, proteomics, and lipidomics analyses of sEV derived from both plasma and SF, researchers have unveiled a sophisticated network of biomarkers that provide unprecedented insights into OA pathogenesis and progression.

Transcriptomic analysis of plasma-derived sEV has revealed promising miRNA biomarkers for early OA detection. Notably, Meng *et al.* identified a significant downregulation of sEV's miR-193b-3p in the plasma of OA patients, which correlated with similar alterations observed in cartilage tissue [107]. This finding underscores the potential of circulating sEV to serve as surrogate markers reflecting joint-specific molecular alterations, thereby offering a minimally invasive approach to early OA screening.

Complementing these miRNA profiles, proteomic studies of serum-derived sEV have identified differentially expressed proteins between OA patients and healthy controls. The observed elevation in cathepsin F levels and reduction in Ig α 2 chain C region in OA patient sEV demonstrate the potential of protein biomarkers to improve diagnostic accuracy [108]. The integration of miRNA and protein biomarkers within sEV presents a more comprehensive diagnostic approach, potentially augmenting both sensitivity and specificity in early OA detection.

Relative to plasma/serum-derived sEV, SF sEV offer a more direct window into joint pathology, providing a rich landscape of molecular markers that reflect various aspects of OA pathogenesis. Multi-omics analysis of SF sEV has revealed an elevated presence of HLA-DR, -DP, and -DQ-carrying sEV compared to blood, highlighting the importance of studying joint-specific sEV populations [109]. These findings underscore the potential of SF sEV in providing a more precise assessment of local joint inflammation and immune cell activity.

The power of multi-omics approaches is further exemplified in the identification and characterization of OA subtypes and stages. Zhao *et al.* demonstrated that SF sEV's long non-coding RNA PCGEM1 could not only distinguish OA patients from healthy individuals but also differentiate between early and late-stage OA [110]. This study illustrates how transcriptomics analysis of sEV can yield markers for disease staging, potentially guiding more personalized treatment strategies.

The integration of proteomics and transcriptomics in sEV analysis has also shown promise in evaluating treatment responses. Jeon *et al.* observed similarities between the miRNA and protein content of OA SF-derived sEV and those

of senescent chondrocytes [111]. Changes in chondrogenesis-associated miRNAs following senolytic treatment suggest that sEV multi-omics could be invaluable in monitoring treatment response, therapeutic efficacy and elucidating underlying mechanisms at a molecular level.

In conclusion, the application of multi-omics approaches to sEV analysis in OA has revealed a complex interplay of RNA, protein, and lipid biomarkers that can collectively provide a more comprehensive view of disease onset, progression, and stratification. By integrating data from various molecular levels, researchers can develop more accurate and personalized diagnostic tools for early OA detection and staging.

Therapeutic applications of small extracellular vesicles multi-omics in osteoarthritis

Multi-omics studies have not only enhanced our understanding of OA pathogenesis but also revealed potential therapeutic targets and strategies. Several approaches leveraging these insights are currently in various stages of preclinical and clinical development.

Engineered therapeutic sEV, informed by multi-omics insights, represent an exciting frontier in OA treatment. Studies have reported the development of 'designer' sEV enriched with specific miRNAs identified through transcriptomic studies as key regulators of chondrocyte homeostasis. Tao *et al.* engineered sEV overexpressing miR-140-5p, a miRNA known to regulate cartilage development and homeostasis [112]. These engineered sEV demonstrated enhanced chondroprotective effects in a rat model of OA, reducing cartilage degradation and promoting tissue regeneration.

An emerging therapeutic strategy in OA treatment involves modulating the production and content of endogenous sEV through preconditioning approaches. Integrative transcriptomic and proteomic analyses have elucidated key differentially expressed proteins and miRNAs in preconditioned sEV. Wu *et al.* demonstrated that tumor necrosis factor- α (TNF- α) preconditioning enhanced sEV production via the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway and upregulated autophagy-related protein 16 like 1 (ATG16L1). Proteomic profiling revealed elevated levels of low-density lipoprotein receptor-related protein 1 in preconditioned sEV, contributing to their chondroprotective effects [113]. Concurrently, Wang *et al.* employed electromagnetic field stimulation, with multi-omics analysis revealing enrichment of microRNA-99b and altered protein profiles in the resultant sEV [37, 114]. These preconditioning-induced modifications enhanced the capacity of sEV to modulate extracellular matrix homeostasis and macrophage polarization in OA models [37]. These studies underscore how preconditioning strategies, guided by multi-omics insights, can optimize both sEV yield and therapeutic efficacy, potentially revolutionizing OA treatment paradigms.

Challenges in clinical translation

Despite the promising advances in sEV-based diagnostics and therapeutics for OA, several challenges remain in translating these approaches to clinical practice. The heterogeneity of sEV populations and variability in isolation methods pose significant challenges for standardization and reproducibility of sEV-based assays. Ongoing efforts to establish universally accepted protocols for sEV isolation and analysis in clinical settings are crucial for overcoming these hurdles [24].

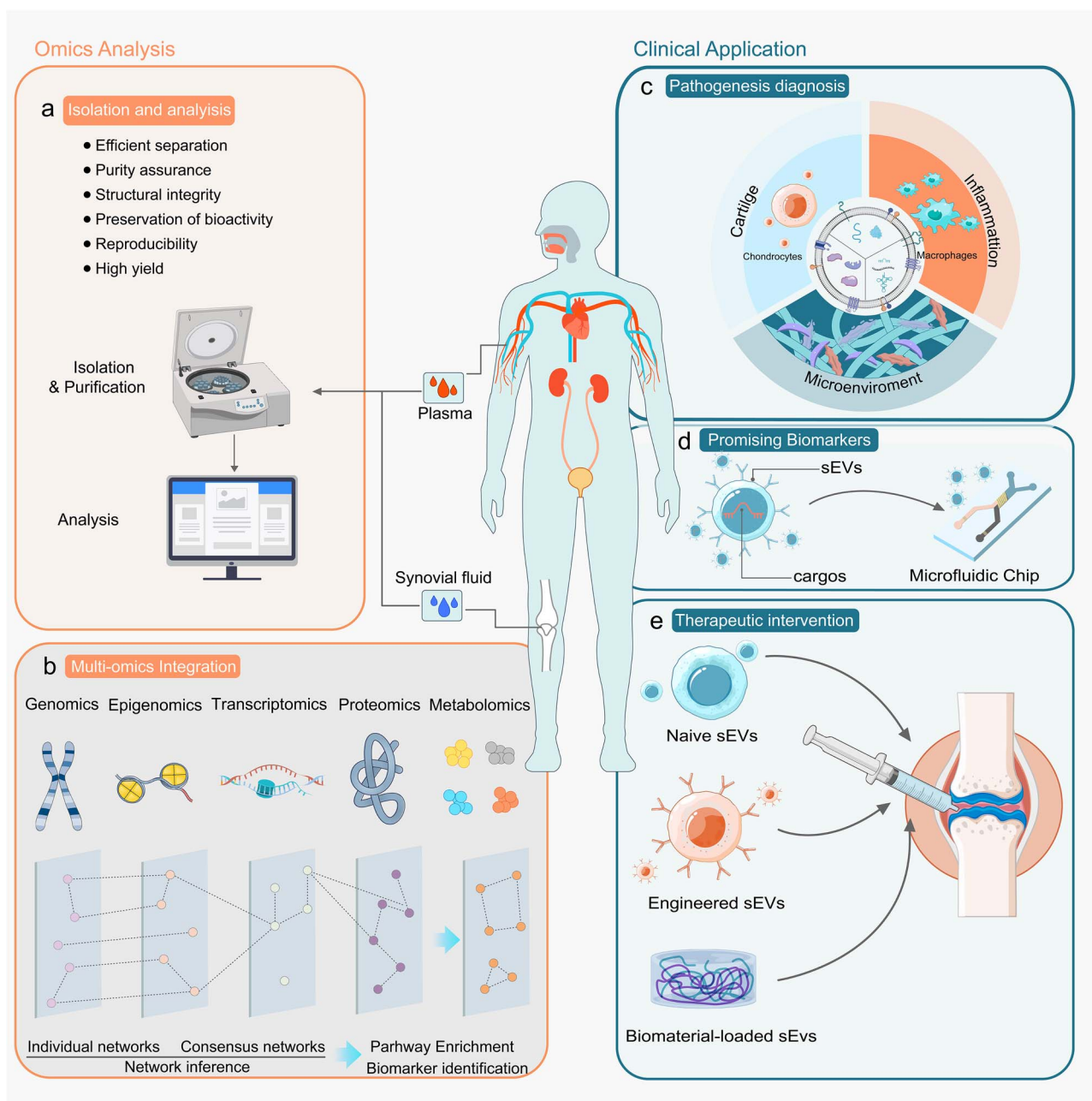


Figure 3. Potential clinical applications of sEVs in OA. (a) sEV isolation and characterization techniques. (b) Multi-omics interactions to decipher sEV function. (c) sEV involvement in OA pathogenesis and diagnosis. (d) sEV-derived non-coding RNAs as potential biomarkers. (e) Therapeutic strategies, including native sEV therapy, engineered sEVs, and biomaterial-assisted sEV delivery for OA treatment. OA osteoarthritis

The complex multi-omics analyses required for comprehensive sEV profiling are currently time-consuming and expensive, limiting their scalability for widespread clinical use. Developing streamlined, cost-effective multi-omics workflows remains an important area of research. Srinivasan *et al.* have made significant strides in this field by optimizing small RNA sequencing protocols for exRNA analysis across diverse biofluids, potentially reducing the cost and complexity of transcriptomic profiling in clinical settings [115].

The regulatory landscape for sEV-based diagnostics and therapeutics is still evolving. The complex and multifaceted nature of sEV poses unique challenges for regulatory agencies in defining appropriate safety and efficacy standards. Collaborative efforts between researchers, clinicians, and regulatory

bodies are ongoing to establish a framework for evaluating sEV-based approaches in OA management. Reiner *et al.* have proposed best-practice models for the therapeutic use of EVs, which could serve as a foundation for developing regulatory guidelines specific to sEV-based therapies in OA [116].

Future prospects and emerging trends

Despite the promising advancements in sEV research for OA, several limitations must be addressed to improve the reliability and translational potential of multi-omics findings. Sample size constraints and patient heterogeneity remain critical challenges, as many studies are conducted on small cohorts with variable OA severity, age, and comorbidities, limiting the

generalizability of identified biomarkers. Model system limitations also pose a concern; while *in vitro* studies using chondrocytes, synovial fibroblasts, and MSC-derived sEVs provide mechanistic insights, they fail to fully replicate the joint microenvironment. Similarly, preclinical animal models (e.g. surgically induced OA in rodents) do not fully mimic human OA, particularly in aging-related or metabolic OA. Technical biases in sEV isolation and multi-omics data integration further complicate reproducibility. Differences in isolation techniques (e.g. ultracentrifugation, size-exclusion chromatography, polymer precipitation) can affect sEV purity and downstream molecular analyses. Additionally, batch effects and inter-study variability in transcriptomic, proteomic, and metabolomic datasets necessitate standardized protocols and computational batch correction methods to ensure consistency. Finally, the clinical translation of sEV-based biomarkers and therapies remains in its early stages, with limited validation in large patient cohorts or clinical trials. Future studies should prioritize multi-center validations, standardized diagnostic criteria, and humanized models to enhance the reproducibility and real-world applicability of sEV-based findings in OA.

In addition to these limitations, potential biases in study design and data interpretation must be considered. Funding sources and conflicts of interest may influence research outcomes, particularly in industry-sponsored studies, where selective reporting of positive findings is more likely. Publication bias also remains a challenge, as studies with negative or non-significant results are often underreported, leading to an overrepresentation of favorable data. Furthermore, many findings are based on single-center studies with limited external validation, underscoring the need for multi-center collaborations and independent replication to enhance reproducibility. Variability in experimental methodologies, omics platforms, and bioinformatics pipelines introduces further inconsistencies, necessitating standardized guidelines for data analysis and interpretation. Finally, ethical and regulatory considerations, including disparities in patient consent protocols and data-sharing policies, highlight the need for greater transparency and adherence to global ethical standards in sEV research [117]. Addressing these challenges will be essential for advancing sEV-based diagnostics and therapeutics from experimental research to clinical application in OA.

Moving forward, several emerging trends and technological advancements are expected to drive significant progress in sEV multi-omics research and its clinical applications in OA. Single-vesicle multi-omics technologies hold great potential in unraveling sEV heterogeneity, enabling the identification of distinct subpopulations with unique molecular signatures and functional properties. Recent reviews by Bordanaba-Florit *et al.* have highlighted various high-resolution single-vesicle analysis techniques, which could be adapted to OA research to provide deeper insights into the functional specialization of sEVs in joint homeostasis and disease progression [118].

The development of real-time tracking technologies for *in vivo* analysis of sEV behavior represents another transformative advancement. High-speed atomic force microscopy (HS-AFM), as demonstrated by Sajidah *et al.*, has enabled spatiotemporal tracking of sEV nanotopology under physicochemical stress [119], providing new opportunities to study sEV-mediated signaling dynamics in the OA joint microenvironment. Adaptation of such techniques to monitor sEV trafficking, uptake, and interactions in cartilage and

synovial tissues could significantly enhance our understanding of their therapeutic and pathological roles.

Additionally, the integration of AI and ML algorithms into multi-omics data interpretation is expected to revolutionize sEV-based diagnostics and personalized OA management. Yin *et al.* successfully applied ML-driven multi-omics integration of serum sEV biomarkers and clinical data to predict colorectal cancer diagnosis, showcasing the potential of AI in improving disease classification and prognosis [120]. As these approaches continue to advance, they could enable precision OA medicine, tailoring interventions based on individual sEV molecular profiles, disease phenotypes, and patient-specific risk factors.

These cutting-edge technologies, combined with ongoing efforts to standardize sEV isolation and characterization, are poised to accelerate the clinical translation of sEV-based biomarkers and therapeutics, ultimately leading to more effective, targeted interventions for OA management.

Conclusions

The field of sEV multi-omics in OA is advancing rapidly, with promising applications in diagnostics, monitoring, and therapeutics. While challenges remain in standardization, scalability, and regulatory approval, emerging technologies such as single-vesicle multi-omics, *in vivo* tracking, and AI-driven data integration are driving progress. The successful clinical implementation of these approaches will require continued interdisciplinary collaboration, investment in research, and careful consideration of ethical and regulatory aspects. With sustained advancements, sEV-based strategies have the potential to revolutionize OA management, offering more precise and effective solutions for improving patient outcomes.

Author contributions

Tianqi Wang (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Investigation [lead], Methodology [lead], Resources [lead], Software [lead], Supervision [equal], Validation [equal], Writing—original draft [lead], Writing—review & editing [equal]), Chiew Yong Ng (Data curation [equal], Investigation [equal], Resources [equal], Validation [equal], Visualization [equal], Writing—review & editing [equal]), Bryan Zheng Jie Ng (Validation [equal], Visualization [equal], Writing—review & editing [equal]), Wei Seong Toh (Conceptualization [equal], Data curation [equal], Methodology [equal], Project administration [equal], Supervision [equal], Validation [equal], Visualization [equal], Writing—review & editing [equal]), and James Hoi Po Hui (Conceptualization [lead], Data curation [equal], Funding acquisition [lead], Investigation [equal], Methodology [lead], Resources [equal], Software [equal], Supervision [equal], Writing—review & editing [lead])

Consent for publication

All authors agreed to publish this review.

Conflict of interest

None declared

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