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Review

Emerging Role of Exosomes in the Joint Diseases

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Key Words
Exosome • Joint • Osteoarthritis • Rheumatoid arthritis • Osteonecrosis of femoral head

Abstract
Exosomes are a subset of small, membrane-bound extracellular vesicles that are important for communication among cells. They originate from the cell membrane during endocytic internalization, and are stable in biological fluids, including blood and synovial fluids. Increasing knowledge is emerging about exosomes in joint diseases, including osteoarthritis, rheumatoid arthritis, osteonecrosis of the femoral head, and others. Exosomes in synovial fluid can lead to inflammation, degeneration of cartilage, and destruction of joints. Exosomes in blood have diagnostic value in the early disease stage or for complicated conditions of joint diseases. Exosomes from stem cells could delay diseases and repair joints. For a comprehensive understanding about the emerging role of exosomes in joint diseases, we introduced the isolation and verification of exosomes from synovial fluid, reviewed the physiological and pathological effects of exosomes on joints, and discussed the diagnostic value and therapeutic potential of exosomes in joint diseases. In the future, immunologically active exosomes and engineered exosomes will of interest in the joint diseases. Challenges in the field of exosomes in joint-disease research include complex and expensive isolation, detection of contributing molecular, effectiveness and safety evaluation. In summary, challenges remain, but the field of exosomes in joint diseases has potential, including in mechanisms, diagnoses and therapies.

Introduction

Exosomes are a subset of small, membrane-bound extracellular vesicles (EVs) with diameters ranging from 30 to 100 nm [1]. They were first described in the early 1980s by Pan and Johnstone and were believed to be a way to eliminate cellular waste during sheep reticulocyte maturation [2]. After being neglected for many years, researchers began to realize that exosomes were more complex and they became popular research topics [3,

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4]. Within a decade, more than 1800 studies about exosomes appeared and the number increases each year [5].

Characteristics of exosomes

In mammals, exosomes can be released by almost all cell types, from normal to pathological. Exosomes are stable in biological fluids, including blood, urine, saliva, synovial fluids, breast milk, amniotic fluids, nasal secretions, bronchoalveolar lavage, pleura effusions and malignant ascites [4]. *In vitro*, they are also present in conditioned medium from cell cultures, especially from stem cells [6]. Exosomes originate from the cell membrane during endocytic internalization. The most well-established mechanism for exosome biogenesis has three stages: endosomes, multivesicular bodies (MVBs), and exosomes [1]. First, endocytic vessels are transferred to early endosomes, which are tube-like and located near the outer edge of the cytoplasm. Early endosomes mature into late endosomes, which are spherical and located closer to the nucleus. Late endosomes that carry intraluminal vesicles with a variety of information are MVBs. In the second stage, MVBs are either degraded by fusing with lysosomes or released to the extracellular space as exosomes [7]. In the extracellular space, recipient cells take up exosomes by three modes: paracrine (exosomal fusion), juxtacrine or endocytosis [4].

Functionally, exosomes have mainly two types. Immunologically active exosomes are from dendritic cells, macrophages, lymphocytes and various tumor cells. They participate in antigen presentation, immune activation, immune suppression, and immune surveillance [8, 9]. Cargo-containing exosomes mediate communication between cells. The cargo of exosomes includes factors such as proteins, DNAs, RNAs, and lipids, and is loaded into exosomes in MVB stage [1, 10, 11]. It reflects the biological state of parent cells and can be released into the extracellular space, and transferred to recipient cells, for tissue-tissue and cell-cell communication in homeostasis and disease.

Exosomes in joint diseases

Knowledge is increasing about the role of exosomes in joint diseases. A literature search was performed in March 2018 using the PubMed electronic database and terms about connective tissue disease (rheumatoid arthritis, RA; systemic lupus erythematosus, SLE), spondyloarthritis (ankylosing spondylitis, AS), osteoarthritis (OA) and osteonecrosis around joints (osteonecrosis of femoral head, ONFH) (Fig. 1). We included 19 original studies on exosomes and joint diseases before 2018. Most research on exosomes and joint diseases focused on three topics: OA, RA and ONFH. In the included 19 articles, nine (47.4%) were studies on OA, five (26.3%) on ONFH, and four (21.1%) on RA (Fig. 2). These diseases are all highly prevalent worldwide, seriously affect the quality of life of patients and cause significant social burden. OA is the most common joint disease. It is characterized by progressive degradation of articular cartilage, synovial hyperplasia, bone remodeling and angiogenesis [12]. ONFH is a joint disease with femoral head ischemia that may be caused by hip trauma, alcohol abuse, corticosteroids overuse or some hematological diseases. ONFH involves the necrosis of cancellous bone and ultimately

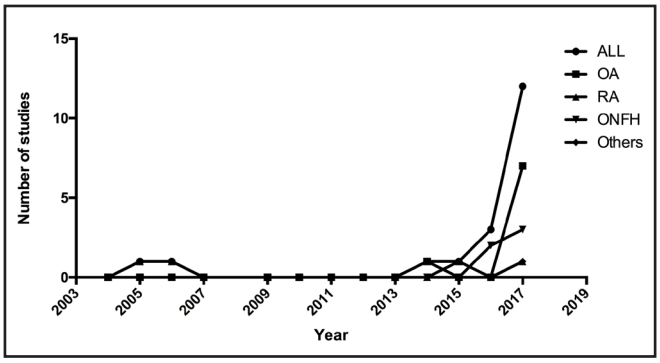

Fig. 1. Number of exosome studies in joint-disease field by year. This review covered 19 articles. Among these, most of them (16/19, 84.2%) were published in the last 3 years and more than 60% were published in 2017.The chart shows the rapid increase in number of studies in 2017. OA: osteoarthritis. RA: rheumatoid arthritis. ONFH: osteonecrosis of femoral head.

the collapse of the femoral head [13]. RA is a chronic autoimmune joint disease with inflammatory synovitis, degradation of articular cartilage, erosion of the marginal bone, systemic immune and inflammatory manifestations [14]. However, none of these diseases have a clear mechanism, and lack effective diagnosis and treatment strategies in the early stage. In this review, we focus on common joint diseases and summarize existing knowledge about using exosomes in joint-disease research.

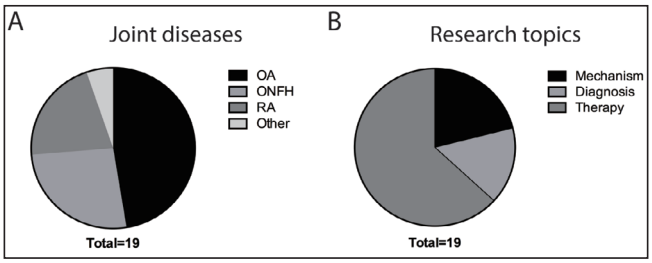

Fig. 2. Diseases and research aspects of exosome studies in joint-disease field. A. We found nine studies (47.4%) for OA, five (26.3%) for ONFH, four (21.1%) for RA, and one (5.3%) for synovitis. B. Research topics were four studies (21.1%) on mechanisms, three (15.8%) on diagnoses, and 12 (63.2%) on therapies.

Isolation and verification of exosomes

Isolation is the first step for all exosome studies. Identifying an optimal method to purify exosomes for downstream functional, biomarker or therapeutic studies is important. Domenis et al. compared two methods for exosome isolation from synovial fluid (SF), Exoquick polymer precipitation and immunoaffinity purification [15]. Although the concentration of exosomes purified by immunoaffinity ($1.5 \pm 1.2 \times 10^{11}$ particles/ml) was significantly lower than the concentration from polymer precipitation ($7.6 \pm 3.1 \times 10^{11}$ particles/ml), the size distribution of the immunoaffinity method (diameter: 88.01 ± 25.5 nm, range: 55.5–125.9 nm) was significantly more restricted, at 30 to 100 nm, than from the polymer precipitation method (diameter: 144.4 ± 22.2 nm, range 124–187.9 nm). After isolation with Exoquick polymer precipitation, contamination by immune complexes was detected, requiring magnetic bead-based purification [15]. This finding indicated that the immunoaffinity purification method has higher accuracy and less contamination than the polymer precipitation method. Although differential centrifugation coupled with ultracentrifugation is one of the most widely used methods to isolate exosomes, it has some disadvantages, such as being time-consuming and requiring large amounts of cells and biological fluid. Ultracentrifugation, however, may damage isolated vesicles, reduce their quality and influence functional studies [16]. After isolation, verification of exosomes is necessary because of the many other types of EVs such as microvesicles, membrane fragments, and apoptotic bodies that may still be evident in the mixture. Verification has three aspects. First, the size distribution should be between 30 and 100 nm. The second is cup-shaped or round morphology by electron microscopy. The third is western blots of biomarkers including CD9, CD63, CD81, and Tsg101.

Physiological and pathological effects of exosomes in joint diseases

Exosome content includes significant amounts of proteins, mRNA, miRNA, and some small noncoding RNAs (lncRNA, cirRNA). Through communication among cells, the biologically active content of exosomes may modulate the gene expression and change the downstream function of recipient cells, as well as physiological or pathological processes [6]. Some studies focused on the effects of specific exosomes on different cells in the joint disease process.

Cell-derived EVs released in the SF of inflamed joints of patients with OA and RA might have substantial effects on disease progression, including propagation of inflammation and degeneration of cartilage. Therefore, most studies about mechanisms of joint diseases focused on exosomes derived from synovial fibroblasts or SF. Domenis et al. investigated the immune regulatory properties of SF-derived exosomes of end-stage OA patients on macrophages differentiated from human peripheral blood mononuclear cells (PBMCs) [15]. After treatment with exosomes, macrophages were found to produce a spectrum of pro-inflammatory cytokines and chemokines, including MMP12, MMP7, IL-1β, CCL8, CCL15,

CCL20, and CXCL1, which lead to inflammation and degradation of cartilage in joints [15]. Kolhe et al. did similar experiments and found articular chondrocytes treated with SF exosomes from OA significantly decreased cell survival and expression of anabolic genes (COL2A1, ACAN), and elevated expression of catabolic and inflammatory genes (IL-6, TNF-α) [13]. Kato et al. tested the function of exosomes in communication between inflammatory synovial fibroblasts (SFBs) and articular chondrocytes. Normal articular chondrocytes were treated with exosomes from SFBs and stimulated with IL-1β. Kato et al. observed that exosomes from IL-1β stimulated SFBs significantly upregulated MMP-13 and ADAMTS-5 expression in articular chondrocytes, and downregulated COL2A1 and ACAN compared with SFB-derived exosomes. In addition, exosomes from IL-1β stimulated SFBs to induce OA-like changes in *in vitro* and *in vivo* models [17].

Two studies compared differences between exosomes from different sources to explore disease mechanisms. Zhang et al. compared SF exosomes from patients with OA and RA. They found a membrane-bound form of TNF-α that was detected on exosomes produced in SF of patients with RA, but not in OA. The study also showed that this type of TNF-α enhanced SFB exosome production in RA and formed a positive amplification loop in RA pathogenesis [18]. To investigate gender differences in OA prevalence, Kolhe et al. compared SF exosomes between women and men with OA and non-OA. They found the miRNA content of EVs differed between OA and non-OA groups and between genders [13]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation analysis for downregulated miRNAs in OA for both genders showed glycan degradation, cell adhesion molecules and mucin type O-glycan biosynthesis were involved. For upregulated miRNAs, thyroid hormone synthesis, biotin metabolism, and amphetamine addiction signaling were involved. The KEGG annotation analysis for female OA patients showed involvement of ovarian steroidogenesis signaling and estrogen signaling pathways.

Diagnostic value of exosomes in joint diseases

Exosomes produced by cells can be released into extracellular space, including into blood and body fluids. The content carried by exosomes may reflect the physiological or pathological conditions of parental cells, including different diseases or stages of a disease [19, 20]. Therefore, exosomes from the extracellular space can act as diagnostic biomarkers. Current RA clinical biomarkers are not sufficient and can be influenced by age, anemia, and the presence of immunoglobulins. Thus, there is a great need to identify more effective biomarkers [21]. Song et al. compared exosomes from blood samples of RA and non-RA patients. They found that expression of Hotair, an lncRNA that can lead to migration of active macrophages, was significantly increased by an average of about four-fold in all exosomes isolated from ten patients with RA. The highest increase by five-fold. Hotair decreased in exosomes isolated from non-RA patients with high C-reactive protein (CRP), unlike the results observed in exosomes from patients with RA. This result suggested that Hotair could be a potential biomarker for diagnosing RA [22]. To evaluate disease activity in patients with RA, Yoo et al. extracted exosomes from serum samples from 60 randomly selected female patients with RA: 30 were in a clinical remission (CR) group with a disease activity score in 28 joints based on erythrocyte sedimentation rate (DAS28-ESR) ≤2.6 and 30 were in a nonclinical remission (non-CR) group with ESR >2.6 [23]. The study found that exosomal levels of amyloid A (AA) and lymphatic vessel endothelial hyaluronic acid receptor-1 (LYVE-1) differed between the CR and non-CR groups. Serum and exosomal AA levels were higher in the non-CR group than the CR group ($p = 0.001$). Although serum levels of LYVE-1 did not differ between the groups, exosomal levels of LYVE-1 were lower in the non-CR group than the CR group ($p = 0.01$). This result indicated that AA and LYVE-1 were potential markers of RA disease activity.

Early-stage ONFH is usually asymptomatic and confirmed with magnetic resonance imaging (MRI), which is expensive and inconvenient. Zhu et al. assessed serum exosome levels of 85 patients with steroid-induced ONFH and 115 healthy donors. Their results showed that the level of circulating exosomes was lower in patients with steroid-induced

ONFH than in healthy donors. The area under the receiver operating characteristic (ROC) curve was 0.72, suggesting the level of serum exosomes had moderate diagnostic accuracy for steroid-induced ONFH. In addition, serological examinations are more convenient and economical than traditional MRI scanning [24].

Therapeutic potential of exosomes in joint diseases

Exosomes are a promising therapy because of their small size, stability, biologically active content and specific targeting make them a natural delivery system. Unlike typical delivery systems such as liposomes or polymeric nanoparticles, exosomes can potentially avoid degradation, go through barriers and deliver cargo directly into the cytoplasm [25, 26]. Transfer of exosomes into recipient cells is an important clinical translation research area.

Most studies on joints focus on the therapeutic potential of stem cell-derived exosomes, especially from mesenchymal stem cells (MSCs) (Table 1, Fig. 3). Clinical and animal studies demonstrated the efficacy of MSC therapies and the importance of MSC paracrine secretion in various diseases [27, 28]. Cosenza et al. injected MSC-derived exosomes into a collagenase-induced OA model and found that it protected mice from joint damage [29]. Zhang et al. investigated if intra-articular injection of human embryonic MSC-derived exosomes repaired and regenerated osteochondral defects in a rat model [30]. Generally, exosome-treated defects showed enhanced gross appearance and improved histological scores compared to contralateral nontreated defects. These studies demonstrated the efficacy of exosomes for cartilage repair, and the utility of MSC exosomes as a cell-free therapeutic alternative therapy. Wang et al. did a similar study on exosomes from human embryonic MSCs in a mouse OA

Table 1. Studies of Exosomes in the joint diseases SF: synovial fluid, SFB: synovial fibroblasts, CM: culture medium, MSC: mesenchymal stem cells, C: dendritic cells, PRP: Platelet-rich plasma

Studies	Type	Disease	Source
Domenis 2017 [15]	Mechanism	OA	SF
Kolhe 2017 [13]	Mechanism	OA	SF
Kato 2014 [17]	Mechanism	OA	CM (SFB)
Zhang 2006 [18]	Mechanism	RA	CM (SFB)
Song 2015 [22]	Diagnosis	RA	Plasma
Yoo 2017 [23]	Diagnosis	RA	Serum
Zhu 2016 [24]	Diagnosis	ONFH	Serum
Zhang 2016 [30]	Therapy	OA	CM (MSC)
Wang 2017 [31]	Therapy	OA	CM (MSC)
Zhu 2017 [32]	Therapy	OA	CM (MSC)
Tao 2017 [36]	Therapy	OA	CM (MSC)
Liu 2017 [40]	Therapy	OA	CM (MSC)
Cosenza 2017 [29]	Therapy	OA	CM (MSC)
Guo 2016 [33]	Therapy	ONFH	CM (MSC)
Liu 2017 [34]	Therapy	ONFH	CM (MSC)
Li 2017 [39]	Therapy	ONFH	CM (MSC)
Tao 2017 [41]	Therapy	ONFH	Plasma (PRP)
Kim 2005 [38]	Therapy	RA	CM (DC)
Casado 2017 [35]	Therapy	Synovitis	CM (MSC)

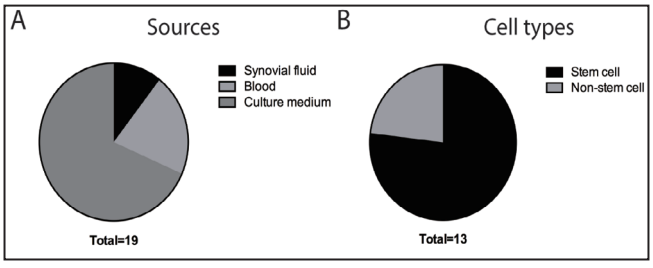

Fig. 3. Sources of exosomes in joint-disease field. A. Resource used were: four studies (21.1%) using blood (plasma, serum and PRP), two (10.5%) using synovial fluid and 13 (68.4%) using culture medium. B. Among these 13 studies, ten (76.9%) used exosomes from stem cells.

model with destabilized medial meniscus [31]. Intra-articular injection of exosomes from embryonic MSCs alleviated cartilage destruction and matrix degradation in the OA model. Zhu et al. compared the effectiveness of exosomes secreted by synovial membrane MSCs (SMMSC-Exos) and exosomes secreted by induced pluripotent stem cell-derived MSCs (iMSC-Exos) for OA treatment [32]. Injection of iMSC-Exos and SMMSC-Exos both attenuated OA in a collagenase-induced mouse OA model, but iMSC-Exos had a superior therapeutic effect. Similarly, chondrocyte migration and proliferation were stimulated by both iMSC-Exos and SMMSC-Exos, with iMSC-Exos exerting a stronger effect. For ONFH, Guo et al. demonstrated that early treatment with exosomes secreted by human synovial-derived mesenchymal stem cells (SMSC-Exos) prevented glucocorticoid-induced ONFH in a rat model. SMSC-Exos were internalized into bone marrow derived stromal cells (BMSCs), which enhanced their proliferation and prevented apoptosis [33]. Liu et al. found that administration of induced pluripotent stem cell-derived exosomes significantly prevented bone loss, and increased microvessel density in the femoral head through the PI3K/Akt signaling pathway in endothelial cells [34]. A report studied synovitis, which is the inflammation of the synovium around a joint. Synovitis is frequently observed in the early phase of OA and in patients with clinically active RA. Casado et al. evaluated the anti-inflammatory effect of MSC exosomes in an antigen-induced pig model of synovitis. Synovial lymphocytes decreased, with downregulation of TNF-α transcripts in exosome-treated joints [35].

Modified exosomes, especially genetically engineered, are a popular therapy. MiR-140-5p is essential for chondrogenic differentiation of MSCs, shown by analysis of genomes and genes, Tao et al. overexpressed miR-140-5p in SMSCs and generated an improved version of exosomes (SMSC-140-Exos) that promoted chondrocyte proliferation and migration with less influence on the secretion of extracellular matrix. In an OA rat model, SMSC-140-Exos prevented OA [36, 37]. Kim et al. also used modified exosomes for RA [38]. They previously found that local, adenoviral-mediated gene transfer of viral IL-10 can suppressed disease. They periarticularly administrated exosomes purified from either bone marrow-derived dendritic cells (DCs) transduced ex vivo with an adenovirus expressing viral IL-10 or from bone marrow-derived DCs treated with recombinant murine IL-10. Exosomes suppressed delayed-type hypersensitivity responses in injected and untreated contralateral joints. In addition, systemic injection of IL-10-treated DC-derived exosomes suppressed the onset of murine collagen-induced arthritis and reduced severity of established arthritis. For ONFH, Li et al. transfected BMSCs with adenovirus carrying mutant HIF-1α, which is essential for bone development and made the BMSCs more stable in the normoxic condition than wild-type. Exosomes of transfected BMSCs facilitated the repair of steroid-induced ONFH [39].

Exosomes can be combined with specific scaffolds for cell-free methods for tissue-engineering of cartilage. Liu et al. used a photo-induced imine crosslinking hydrogel glue with excellent maneuverability, biocompatibility and cartilage integration as an exosome scaffold to prepare an acellular tissue patch (EHG) for cartilage regeneration [40]. EHG retained stem cell-derived exosomes and positively regulated both chondrocytes and BMSCs *in vitro*. Furthermore, EHG integrated with native cartilage matrix and promoted cell deposition at cartilage defect sites, promoting of cartilage defect repair.

Platelet-rich plasma (PRP), an autologous derivative of whole blood that contains a supraphysiological concentration of platelets, can enhance bone regeneration, cartilage repair and tissue repair. PRP is also a source of exosomes. Tao et al. found that exosomes derived from human PRP prevents apoptosis induced by glucocorticoid-associated endoplasmic reticulum stress in rat osteonecrosis of the femoral head via the Akt/Bad/Bcl-2 signaling pathway [41].

Emerging roles of exosomes in joint diseases

Exosomes are an intercellular information exchange that has generated research interest in various fields, including joint diseases. This review included 19 articles (Table 1). Of these studies, most (16/19, 84.2%) were published in the last 3 years and more than 60% were published in 2017 (Fig. 1). We found nine studies (47.4%) on OA, five (26.3%) on ONFH, four

(21.1%) on RA, and one (5.3%) on synovitis. In research area, four studies (21.1%) were on mechanism, three (15.8%) on diagnosis and 12 (63.2%) on therapy (Fig. 2). As exosome sources, four studies (21.1%) used exosomes from blood (include plasma, serum and PRP), two (10.5%) from synovial fluid, and 13 (68.4%) from culture medium. Among these 13 studies, ten (76.9%) used exosomes from stem cells (Fig. 3).

The field of joint diseases has several future directions. For mechanisms, almost all studies focused on cargo-containing exosomes from SF, but not immunologically active exosomes in blood. Some diseases that can also influence joints, such as AS and SLE are related to immune disorders. However, little research focused on the joint aspects of these diseases [42, 43]. MSC-derived exosomes can suppress secretion of pro-inflammatory factors and increase anti-inflammatory factors. In addition, exosomes may influence the function of T cells [44]. These results suggest that MSC-derived exosomes have immunomodulatory properties. The immunological function of exosomes in these diseases should be the topic of future studies.

Biomarkers can help improve diagnosis and prognosis, as well as assess treatment response. Progress should continue in discovery and development of biomarkers for joint diseases, especially those that are hard to diagnose in the early stage [45]. Only a few studies focus on diagnosis (Fig. 2), probably because of the few exosomes in blood or synovial fluid, making them hard to collect. In all exosome research, only about 30% of studies collected the exosomes from blood or synovial fluid (Fig. 3). Therefore, more effectively and efficiently collecting exosomes from samples remains a problem.

Therapy is the most popular research topic on exosomes in joint diseases. More than 60% of studies focused on this area and among these, most exosomes were from stem cells (Fig. 2, 3). Studies suggest that the therapeutic effect of stem cells largely depends on their paracrine actions [27, 28]. Exosomes secreted from stem cells carry critical, biologically active contents that have disease intervention potential [46, 47]. In addition, stem cells can excrete more exosomes than other cells and these exosomes are easier to culture and collect *in vitro* [27]. Therefore, stem cell-derived exosomes will be popular parental cells in the future. Another popular therapeutic method is genetically engineered exosomes. The functions of exosomes and their biologic effects depend on the origin and functional status of their parental cells, which to a large degree affects the contents of exosome cargos to be delivered to recipient or target cells [48]. Taking advantage of the highly efficient delivery system of exosomes, genetically engineered exosomes from parental cells with biologically active content, such as miRNA and small molecules for therapy are currently under investigation. Enriching exosomes with genetic materials such as therapeutically functional miRNA has drawn particular attention. MiRNA is commonly carried by exosomes and miRNA mimics are easily loaded into exosomes [49]. In many other fields, genetically engineered exosomes have been used, especially in the cancer therapy [47, 50-52]. Engineered exosomes in cancer therapy including drug-loading exosomes, surface-modifying exosomes, and content-modifying exosomes [51] that may provide novel therapeutic strategies for joint diseases.

Summary

Joint diseases have a wide range and can influence the cartilage, subchondral bone, and synovium, destroying joint function. Exosomes, which are important for communication among cells, are involved in joint diseases. Exosomes in synovial fluid or released from synovial fibroblasts could induce joint disease progression. Exosomes in blood could be helpful in diagnosing joint diseases. Exosomes from stem cells could delay diseases and repair joints. The emerging role of exosomes will improve the development of mechanisms, diagnoses and therapeutic research. However, some challenges exist. First, the isolation of exosomes is complicated and expensive. If we find an excellent exosome biomarker, costs or time will limit its application. Second, because of the varied content of exosomes that includes proteins, miRNA, lncRNA, and cirRNA, accurately finding the contributing molecules is difficult. Third, although modified exosomes, especially genetically engineered exosomes are available, their effectiveness and safety *in vivo* are under investigation. The therapeutic

tools might not be ready for use in clinical medicine for years. In conclusion, challenges remain but the field of exosomes in joint diseases has potential, including in mechanisms, diagnoses and therapies. These small vesicles may have a big impact in the future.

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Disclosure Statement

The authors declare no competing interests.

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