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## Exosomes in intercellular communication and implications for osteoarthritis (OA)

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## Abstract

Osteoarthritis (OA) is the most prevalent of the musculoskeletal conditions and represents a significant public health burden. While degeneration of articular cartilage is a key feature, it is now increasingly recognised as a complex condition affecting the whole joint, with synovial inflammation present in a significant proportion of patients. As a secretory tissue, the OA synovium is a rich source of both soluble inflammatory mediators and extracellular vesicles (EV), including exosomes, which have been implicated in cell-cell communication. Exosome cargo has been found to include proteins, lipids and various RNA subtypes such as mRNA and miRNA, potentially capable of regulating gene expression in target cells and tissues. Profiling of exosome cargo and understanding effects on cartilage could elucidate novel regulatory mechanisms within the joint, providing insight for targeted treatment. The aim of this article is to review current literature on exosome biology, highlighting the relevance and application for OA pathogenesis.

## Keywords

Osteoarthritis (OA), exosomes, inflammation, cartilage, synovium, synovitis, microvesicles, mRNA, microRNA.

## Key Messages

1. Multiple cell types within the osteoarthritic joint are capable of releasing exosomes
2. Exosomes carry bioactive material with the potential to regulate osteoarthritis joint pathogenesis
3. Profiling of exosome content may identify novel biomarkers and inform targeted therapy for osteoarthritis

## Introduction

Osteoarthritis (OA) is the most prevalent musculoskeletal condition in the UK, affecting 9 million people and costing the NHS an estimated £5 billion p.a. (1). Approximately 80% of those with OA suffer significantly limited mobility and 25% cannot perform major daily activities (2). There is presently no gold standard treatment available for OA, and current approaches aim largely to manage the pain associated with the disease, rather than modifying the underlying cause. Elucidating pathogenic mechanisms underpinning OA provides a pathway for future therapeutic interventions that can address this unmet clinical need.

OA is a low grade inflammatory disease, which results in cartilage degeneration, inflammation of the synovium, changes to the subchondral bone, formation of osteophytes, degeneration of ligaments, hypertrophy of the joint capsule and pro-angiogenic phenotypes (3,4). Inflammatory changes to the synovium are collectively referred to as synovitis and include synovial lining hyperplasia, infiltration of inflammatory cells, neoangiogenesis and fibrosis (5–7). Some 70% of OA patients present with synovitis, the degree of which correlates with pain and cartilage damage (8,9). Acute synovitis is thought to be one of the first joint changes to occur, with synovial tissue from early OA patients showing increased expression of pro-inflammatory mediators (10).

Joint tissue homeostasis is regulated by numerous pathways and molecules that are disrupted during OA including transcription factors, epigenetic modifications, cytokines and proteases (5). This disruption causes widespread changes and stops the synovial joint from performing its function of allowing smooth and frictionless movement. Not only does this disturbance cause inflammation and thickening of synovial tissue (11), but proinflammatory mediators released by OA immune cells from synovium and infrapatellar fat pad contribute to cartilage

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3 damage (12). Distinct pathogen associated molecular patterns (PAMPs) and damage-  
4 associated molecular patterns (DAMPs), (including products from cellular stress and  
5 extracellular matrix (ECM) degeneration) are recognised by pattern recognition receptors  
6 (PRRs) such as toll like receptors (TLRs) with inflammatory mediators subsequently released  
7 in large amount by cells present in the OA joint. Activation of PRRs triggers cell signalling that  
8 leads to the production of pro-inflammatory cytokines and chemokines including interleukin  
9 6 (IL-6), IL-8, IL-1, TNF- $\alpha$  along with proteases, such as matrix metalloproteinase (MMP)-1,  
10 MMP-3 and MMP-13, which go on to degrade structural components of cartilage ECM  
11 (primarily aggrecan and collagen) and cause changes in chondrocyte viability and  
12 glycosaminoglycan (GAG) release (5,12–18). Alteration of the subchondral bone also occurs  
13 due to an imbalance in remodelling via bone resorption by osteoclasts and bone formation  
14 by osteoblasts, resulting in a reduction in tissue mineralisation, loss of stiffness and bone  
15 thickening (19).  
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20 While roles for these released soluble mediators are well established, a number of recent  
21 studies have reported on extracellular vesicles (EVs) and exosomes and their regulatory  
22 potential. These vesicles are secreted from a variety of cells and tissues and exosomes  
23 released from neutrophils and synovial fibroblasts (SFs) have been detected in both RA and  
24 OA synovial fluid (20–22). It is now believed that exosomes play an important role in cellular  
25 communication via the transport of biological cargo. Due to difficulty in isolation and  
26 characterisation of different groups of extracellular vesicles, most research within the  
27 extracellular vesicle (EV) field of OA has to date focused on a mixed population of EVs.  
28 However, given recognised differences in cargo associated with distinct vesicle types (23), this  
29 review aims to differentiate between exosome and microvesicle (MV) related studies, and  
30 encompass the formation and role of extracellular vesicles. Furthermore, it will focus on these  
31 emerging pathological players and their potential as therapeutic targets; with particular  
32 emphasis on the role of exosomes in OA pathology and the opportunities that may arise from  
33 a better understanding of their biology.  
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### 39 **Exosome biogenesis & release**

40 Extracellular vesicles (EVs) can be classified and differentiated based on size, biogenesis and  
41 release pathways. EVs include multiple subsets, the most researched of which are  
42 microvesicles (MV) and exosomes. MVs are membranous extracellular vesicles which range  
43 from 100 to 1,000 nm in diameter (24), whereas exosomes range only from 30 to 120 nm  
44 (Figure 1A), with a density of between 1.13–1.19 g/ml and a distinguishing cup shaped  
45 morphology (25,26). While MVs are shed from the surface membrane by blebbing, exosomes  
46 arise via the endocytic pathway, from the endosomal cell compartment, where they are  
47 stored in multivesicular bodies (MVBs) of late endosomes and are released in short bursts by  
48 exocytosis upon fusion with the cell membrane (27–29). Typical exosome composition relates  
49 to the cellular source from which they arise, with a range of biological cargo reported (Figure  
50 1B). The release of exosomes occurs naturally from many tissues and cells but composition  
51 and concentration can be regulated, for instance by cellular stressors such as senescence and  
52 hypoxia (30–32), both of which have been associated with OA. For example, OA chondrocytes  
53 exhibit senescence-associated phenotypes which increase MMP-1 and -13 protein release  
54 and degrade ECM collagen (33). It is therefore interesting that EV production from  
55 chondrocytes, isolated from human arthritic cartilage, increased with senescence (34). These  
56 EVs were also capable of transferring senescence to nonsenescent chondrocytes and  
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3 inhibited cartilage and ECM formation by healthy chondrocytes (34). Senolytic treatment also  
4 altered expression of miR-34a, -30c, -125a, -24, -92a, -150, and -186 carried in synovial fluid  
5 exosomes (34). Furthermore, hypoxia related genes inducible nitric oxide synthetase (iNOS)  
6 and hypoxia-inducible factor-1 (HIF-1) are upregulated in OA caused by exacerbated hypoxia  
7 seen during progression of the disease (35), which may drive synovial inflammation (33).  
8 Importantly, it has been previously reported that hypoxia can drive MV release from human  
9 umbilical cord (UC)-derived MSCs (32). Given the hypoxic nature of the OA joint, potentially  
10 this could be a regulatory mechanism for exosome production during disease.  
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14 Exosome formation (Figure 2) requires multiple pathways, enzymes and mechanisms, and is  
15 ATP-dependent (36,37). Notably, exosome formation and release involves the endosomal  
16 sorting complex required for transport (ESCRT) and its associated proteins, apoptosis linked  
17 gene-2-interacting protein X (ALIX) and tumour susceptibility gene 101 (TSG-101) (38–41).  
18 The ESCRT-0, -I, and -II complexes recognise ubiquitinated membrane proteins on  
19 endosomes and the ESCRT-III complex is involved in vesicle budding and scission (42). ESCRT  
20 machinery is reported to be central to exosome formation and release, as blockage of the  
21 complexes impacts upon these processes (43). However, exosome formation does not  
22 require ESCRT function in some cells, but is dependent on sphingomyelinase, an enzyme  
23 that produces ceramide (44). Syndecan-syntenin interaction with ALIX and activation of  
24 protein kinase C, are also thought to be important for the packaging of exosomes into  
25 endosomes and their subsequent release respectively (45,46). Finally, two lipid metabolism  
26 enzymes and tetraspanins have been seen to promote formation of interluminal vesicles or  
27 ILVs (25), (44).  
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35 The divergence in formation and sorting mechanisms between extracellular vesicle types is  
36 not completely understood, however, some preliminary experiments show differences in the  
37 composition of exosomes in comparison to other EVs. Unlike other EVs, exosomes contain an  
38 abundance of cholesterol, saccharides, ceramide, sphingolipids and phosphoglycerides,  
39 which could play roles in the sorting mechanism (44,47). For example, localisation of  
40 cholesterol into MVBs was reported to mark the contents for secretion as exosomes, whereas  
41 low cholesterol levels and the presence of lysobisphosphatidic acid marked MVBs for  
42 lysosomal degradation (48–50). The fusion and secretion of exosomes from the cellular  
43 membrane itself can require the cytoskeleton elements actin and microtubules, molecular  
44 motors such as kinesins and myosin and fusion machinery such as SNAREs (51). Importantly,  
45 studies of exosome trafficking have shown that the vesicle transport regulators Rab 35, Rab  
46 11, Rab 27a and Rab 27b (all of which are part of the Ras family of monomeric G proteins) are  
47 involved with secretion of exosomes (52,53).  
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### 52 **Exosome interaction & function**

53 Exosomes have been reported to play roles in multiple physiological processes including  
54 apoptosis, angiogenesis, inflammation, coagulation and transfer of cargo such as proteins,  
55 lipids and RNA to modulate cell communication and epigenetic modifications (24,54,55).  
56 Proteomic analysis shows that exosomes contain a common set of membrane and cytosolic  
57 proteins (54). Due to their endosomal origin, this includes membrane transport and fusion  
58 proteins (Rab GTPases, annexins, flotillin, integrins, fibronectin), tetraspanins (CD9, CD63,  
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3 CD81, CD82), heat shock proteins (Hsc70, Hsp 90), proteins involved in multivesicular body  
4 biogenesis (ALIX, TSG101), and lipid-related proteins and phospholipases (24,30,56,57).  
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7 Observations to date indicate that thymine DNA glycosylase (TDG101), signal-transducing  
8 adaptor molecule 1 (STAM1) and heat shock cognate 70 (HSC70) could all play a role in the  
9 selective packaging of proteins (51,58). The mechanisms by which miRNA is selectively  
10 packaged is currently under investigation, with studies identifying putative pathways relating  
11 to miRNA sorting into exosomes, and these include the neutral sphingomyelinase 2  
12 (nSMase2)-dependent pathway, the miRNA motif and sumoylated heterogeneous nuclear  
13 ribonucleoproteins (hnRNPs)-dependent pathway and the miRNA induced silencing complex  
14 (miRISC)-related pathway (26,59,60). Differences in specific miRNA expression profiles have  
15 already been seen between normal and disease states, including in carcinoma patients (61).  
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18 Whether exosomes are taken up by specific cell types is still being debated. Some studies  
19 indicate cell-specific exosome interactions, where exosomes from parental cells will only  
20 interact with particular target cells. For example, activated T and B cells interact with dendritic  
21 cell (DC)-derived exosomes that contain major histocompatibility complex (MHC) class II, to  
22 down-regulate the immune response during interaction of T cells and DCs. For DC derived  
23 exosomes to be taken up, the T cells had to be activated with this process dependent on  
24 leukocyte function-associated antigen-1 (LFA-1), indicating a targeted mechanism (62,63).  
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28 Once released into the extracellular milieu, exosomes can target and interact with recipient  
29 cells in various ways. The exact mechanism responsible for EV uptake is unknown, but  
30 numerous mechanisms have been studied, including clathrin mediated endocytosis (CME),  
31 phagocytosis, macropinocytosis and plasma or endosomal membrane fusion (as reviewed by  
32 Mulcahy et al. (61) and summarised in Figure 3). Current data suggests that the main process  
33 for exosome uptake is via endocytosis, which requires clathrin-mediated or caveolin-  
34 dependent mechanisms. This involves the specific assembly of clathrin-coated vesicles  
35 containing receptors and ligands (often exosomal transmembrane proteins), through  
36 caveolae invaginations or micropinocytosis membrane 'pinching' (64). Once this receptor-  
37 ligand interaction has taken place, the exosomes fuse with the plasma membrane and their  
38 contents are released into the cytosol.  
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### 43 **Exosome cargo**

44 Exosomes contain mRNA transcripts and miRNA as well as small noncoding RNA species,  
45 repeat sequences, structural RNAs, tRNA fragments, vault RNA, Y RNA, and small interfering  
46 RNAs (65,66). Current studies have found that exosomal small RNAs are differentially  
47 distributed, indicating that there is selective incorporation of small RNA species into  
48 exosomes (66,67) and that specific proteins and miRNA may be included in EV cargo  
49 depending on function (55,68,69). Accordingly, studies differ considerably in terms of  
50 reported exosome content; some describe protein and RNA content distinct from that of the  
51 parental cell, whereas other exosome populations reflect typical cellular constituents and  
52 could potentially be used as biomarkers (68,70). For example, mast cell derived exosomes  
53 contained mRNA from genes not present in parental cells (71).  
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57 Currently, it has only been confirmed that mRNA and miRNA are horizontally transferred via  
58 exosomes (71,72). Studies have shown that transferred mRNAs can be translated into  
59 proteins by target cells, altering the target cell phenotype. Multiple studies have shown that  
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3 horizontal transfer of mRNA and protein occurs through exosomal machinery, with the  
4 genetic material transferred and proteins translated successfully (73). Exosomes from murine  
5 and human mast cell lines have been reported to contain mRNA and miRNA, which were  
6 interchangeably transferrable (i.e. mouse to human) in *in vitro* studies where novel murine  
7 mast cell proteins were found in human recipient cells, indicating exosomal transfer and  
8 mRNA incorporation into the recipient cells (71). Similarly, RNA from exosomes collected from  
9 chronic lymphocytic leukemia cells, was observed to modulate the transcriptome of stromal  
10 cells (74). These RNA were called *exosome shuttle RNA* and the study demonstrated that  
11 mRNA can be transferred in a functional form. Further studies have confirmed that this  
12 horizontal transfer of mRNA requires the presence of RNA in the recipient cell (55,68,71,75).  
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16 Of particular interest is the presence of miRNA, with the majority of circulating miRNA  
17 believed to be sequestered within exosomes (76). miRNAs are a class of small noncoding RNA  
18 of 1-25 nucleotides that regulate mRNA transcription, translation and stability to silence  
19 genes (77). miRNAs are extremely diverse and act at multiple sites with diverse effects  
20 including proliferation, apoptosis and differentiation (78,79). Microparticle cargo is  
21 predominantly pre- and not mature miRNA (80). Packaging in exosomes may offer protection  
22 from circulating RNAses, allowing miRNA to retain the ability to control gene expression by  
23 regulating target-mRNA turnover (55). Exosomes released by immune cells have been  
24 reported to contain specific miRNA cargo, that can be functionally transferred to recipient  
25 cells (68,69). This was confirmed by investigation of miRNA expression levels in a variety of  
26 cell lines and their derived exosomes, which demonstrated that certain miRNA are selectively  
27 and preferentially packed into exosomes including miR-320, miR-150, miR-142-3p and miR-  
28 451 (81).  
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### 33 **Exosomes in OA pathophysiology**

34 Once taken up by recipient cells, exosomes can be categorised functionally based on their  
35 ability to impact cellular biology. They can be immunologically active and be involved in  
36 antigen presentation to interact with the immune system, or immunologically inactive and  
37 use packaged small RNA cargo to cause epigenetic changes (82,83). Alternatively, exosomes  
38 are able to fuse with endosomes to be degraded or re-released (84). The processes by which  
39 exosomes may impact OA pathophysiology are discussed below.  
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### 43 **Exosomal impact via miRNA cargo**

44 Exosomal mRNA and microRNA cargo is especially relevant in OA, where epigenetic changes  
45 such as histone modifications, DNA methylation and microRNA (miRNA) impact upon multiple  
46 transcription and proteinase factors important for pathology such as RUNX2, MMP-13 and a  
47 disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5), which  
48 modulate the anabolic/catabolic balance (85,86). The role and subsequent implications of a  
49 large number of miRNAs has been investigated in OA (82). In the joint, miRNAs are mainly  
50 associated with the regulation of MMPs, ADAMTS, RUNX and collagen type II, as well as  
51 various cytokines. In particular, miR-140 has been identified as having a protective role, with  
52 its expression found to be decreased in OA and in IL-1 $\beta$  stimulated chondrocytes (79,87,88).  
53 In miR-140 deficient mice, proteoglycan loss and fibrillation of cartilage have been reported,  
54 whereas mice overexpressing miR-140 were resistant to arthritis (89,90). In human OA  
55 tissues, miR-140 expression is reduced and coincided with upregulation of *MMP-13* and  
56 *ADAMTS-5* expression, with proteoglycan loss demonstrated in miR-140 knock out mice (91).  
57 Various other miRNA such as miR-139 and miR-9 also cause imbalances between anabolic and  
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3 catabolic processes that are seen in OA, and therefore cause cartilage ECM breakdown  
4 (90,92).  
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7 Findings in the OA literature have only recently begun to focus on the role of exosome miRNA  
8 cargo. Studies have found that the miRNA content of EVs differed between OA and non-OA  
9 groups and between genders (93,94). Furthermore, profiling of miRNA in synovial fluid  
10 exosomes from OA patients has shown miR-200-c to be increased 2.5-fold compared to  
11 synovial fluid from healthy subjects, with consequential effects on the zinc finger binding  
12 transcription factor (ZEB1), which decreased collagen type II formation (95). SFs obtained  
13 from normal joints were stimulated with IL-1 $\beta$  and derived exosomes were found to have  
14 340 distinct miRNAs upregulated and 24 miRNAs downregulated, compared to non-IL-1 $\beta$ -  
15 treated SFs. Interestingly, of these, 11 miRNAs were upregulated and 9 downregulated in the  
16 exosomes released from IL-1 $\beta$ -treated SFs, confirming selective packaging (87). More  
17 recently, it was found that 22 miRNAs were up-regulated and 29 miRNAs (including  
18 miR-95-5p) were down-regulated in OA chondrocyte-secreted exosomes compared to normal  
19 chondrocytes (96).  
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#### 24 **Exosomal Impact via inflammatory regulation**

25 While the role of EVs in inflammatory disease is currently well researched, for example in  
26 tumour biology (97–99), their function in OA is poorly understood. It has been noted that  
27 greater numbers of EVs can be isolated from the serum of patients suffering musculoskeletal  
28 disorders compared to healthy subjects, some of which contain elevated levels of specific  
29 miRNAs, most notably in rheumatoid arthritis (RA) (100,101). However, RA is an overtly  
30 inflammatory disease compared to OA where there may indeed be a more complex subtle and  
31 nuanced interaction occurring between joint tissues. In recent years it has become widely  
32 acknowledged that inflammation and synovitis are common features of OA (9,10,102),  
33 suggesting a potential role for OA cell derived exosomes in the pathophysiology of the  
34 disease.  
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39 Indeed, inflammatory mediators such as TNF $\alpha$  have been seen to affect the protein and RNA  
40 concentration of extracellular vesicles (51,103). Similarly, exosomes derived from  
41 lipopolysaccharide (LPS) stimulated mouse macrophages (an inflammatory model), also  
42 contained increased levels of inflammatory mediators and miRNAs (104). This suggests that  
43 exosomes derived under inflammatory conditions may cause further inflammatory changes.  
44 In OA joint disease, inflammation of soft tissues, including disruption of normal synovial tissue  
45 homeostasis via specific regulated pathways, could potentially lead to an altered secretory  
46 exosome profile, which may ultimately impact upon cartilage integrity (Figure 4).  
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50 Multiple cell types associated with OA, including chondrocytes and SFs have been shown to  
51 be capable of exosome secretion, with the former regulating cartilage catabolic pathways  
52 (54,87,105). OA IL-1 $\beta$  stimulated SFs and macrophages have exhibited an increase in exosome  
53 production during inflammation, but exosomes prepared from the synovial fluid of OA  
54 patients and healthy subjects showed no difference in either concentration or size (87,95).  
55 Chondrocyte derived exosomes have been seen to display annexins II, V and VI, which have a  
56 role in chondrocyte differentiation (106).  
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3 Synovial fluid isolated exosomes were shown to be readily endocytosed (95). Exosomes  
4 isolated from OA synovial fluid significantly stimulated the release of several inflammatory  
5 cytokines and chemokines and metalloproteinases from M1 macrophages, including  
6 increased production of IL-1 $\beta$ , MMP-12, MMP-7, CCL-8, CCL-1, IL-6 and TNF- $\alpha$ , compared to  
7 controls (107). Similarly, articular chondrocytes treated with OA synovial fluid derived  
8 exosomes decreased cell survival and expression of anabolic genes including *COL2A1* and  
9 *ACAN* and increased expression of catabolic genes including *IL-6* and *TNF- $\alpha$*  (82,94).  
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12  
13 Chondrocytes treated with SF-derived exosomes exhibited their uptake and endocytosis,  
14 raising the possibility that exosome cargo has a role in regulating chondrocyte fate, ECM  
15 degradation and OA pathology (95). A mixed population, including exosomes containing TNF-  
16  $\alpha$ , have previously been found to be released by SFs, and importantly they have been shown  
17 to regulate the release of chemokines and cytokines from synoviocytes and chondrocytes  
18 during inflammation (20,108,109). Exosomes isolated from conditioned medium from normal  
19 IL-1 $\beta$  stimulated human SFs were cultured with articular chondrocytes resulting in  
20 upregulated gene expression of *MMP-13* and *ADAMTS-5*, and decreased expression of  
21 *COL2A1* and *ACAN* in comparison to exosomes collected from non stimulated SFs (87).  
22 Additionally, exosomes from IL-1 $\beta$ -stimulated fibroblasts have been shown to contain low  
23 levels of IL-6, MMP-3 and vascular endothelial growth factor (VEGF) (87).  
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### 28 **Exosomal impact via bone changes**

29 Exosomes also affect other factors associated with OA pathogenesis, such as bone growth  
30 and angiogenesis (32,110). Synovial derived exosomes were shown to exhibit increased *VEGF*,  
31 which stimulates angiogenesis and contributes towards OA pathology (87). Exosomes were  
32 also seen to increase angiogenic activity when incubated with human umbilical vein  
33 endothelial cells (*HUVECs*), with migration and tube formation activity found to be  
34 significantly higher when cultured with conditioned media containing exosomes from SFs  
35 (87). Chondrocyte derived exosomes have also been implicated in mineral formation in OA  
36 (106). Importantly, matrix vesicles, which share many exosomal characteristics are now  
37 regarded as “anchored exosomes”; these are small (20-200nm) spherical bodies in the pre-  
38 mineralised matrix of cartilage and bone. Notably, these vesicles contain mediators such as  
39 bone morphogenetic proteins (BMP), which are required for calcification (111,112).  
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### 44 **Mixed populations of extracellular vesicles**

45 Considering the difficulties in isolation and yield of exosomes, it is interesting to note that  
46 other forms and mixed populations of extracellular vesicles are also reported to exhibit similar  
47 effects to isolated exosomes, although research has focused mainly on inflammatory arthritis.  
48 For example, in early inflammation, T cell and monocyte derived MVs cause inflammatory  
49 changes, with increased IL-6 and IL-8 release observed later (113). Furthermore, neutrophil  
50 derived MVs were increased in RA synovial fluid and in human chondrocyte models. These  
51 MVs stimulated increased ECM production and cartilage protection, by reducing *IL-8* and  
52 prostaglandin E2 (*PGE2*) expression and increasing production of TGF $\beta$ , type II collagen and  
53 soluble sulphated glycosaminoglycan (sGAG) (22). ECVs from T cells and monocytes, isolated  
54 from normal blood, increased MMP-1, MMP-3, MMP-9, MMP13, IL-6 and IL-8 protein  
55 synthesis in human RA SFs (113). EVs isolated from the blood of patients with RA were found  
56 to inhibit osteoclast differentiation, which could indicate an impact in OA bone remodelling  
57 (114). Despite this body of evidence, further research needs to be undertaken looking at the  
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3 differences and similarities in functionality of exosomes, MVs and mixed populations isolated  
4 from different cell types and disease conditions, including OA.  
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### 6 **Harnessing the therapeutic potential of exosomes**

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8 Exosomes from different sources can have different impacts on cells. This review has  
9 discussed exosomes derived from synovial fibroblasts and other cell types associated with  
10 OA, which may potentially modulate joint pathology. A number of studies have focused on  
11 the release of exosomes from MSCs due to the extensive proliferative and differentiation  
12 abilities of these multipotent cells, presenting opportunities for new approaches in the  
13 treatment of joint tissue defects and OA-related damage. Although various MSC cell based  
14 therapies are in clinical trials for tissue regeneration of bone and cartilage, it has recently  
15 been considered that it is the secretome that exerts this regenerative effect via chemokines,  
16 growth factors and derived exosomes (115,116).  
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21 MSC derived exosomes have been shown to have a therapeutic effect in various OA models,  
22 including collagenase-induced and destabilised medial meniscus models (82). For example,  
23 MSC derived exosomes stimulated repair of osteochondral defects in animal models and  
24 cartilage damage in chondrocyte cultures, which involved increased cellular proliferation and  
25 infiltration (117). MSC exosomes have also been shown to regulate endothelial cell  
26 proliferation, migration and angiogenesis through the transfer of various miRNA  
27 (31,32,118,119). Exosomes from MSCs also have the capacity to regulate osteoblast  
28 differentiation via various miRNA involved in the control of osteoblast activity, e.g. miR-199b  
29 and miR-218, and a positive feedback loop was proposed whereby osteoblasts themselves  
30 secrete exosomes to increase miRNA levels that can further promote bone growth (110,120).  
31 Exosomes from synovial MSCs overexpressing miR-140-5p increased proliferation and  
32 migration of chondrocytes *in vitro* and prevented OA in rat models (121). Furthermore, Wnt5a  
33 and Wnt5b carried by synovial MSC-derived exosomes activated yes-associated protein 1  
34 (YAP) via the alternative Wnt signalling pathway and increased proliferation and migration of  
35 chondrocytes in a rat OA model (121). Synovial MSC-derived exosomes have been reported  
36 to reduce bone marrow cell proliferation, apoptosis, bone mineral loss and fat tissue  
37 accumulation (122). Furthermore, when co-cultured with OA chondrocytes, BMMSC-EVs  
38 upregulated COX2 and pro-inflammatory interleukins, inhibited TNF-alpha-induced  
39 collagenase activity and stimulated production of proteoglycans and type II collagen (123).  
40 The *in vitro* expansion potential of MSCs, combined with a high yield of derived exosomes  
41 with therapeutic potential (compared to other OA cells such as synovial fibroblasts), makes  
42 this a very promising research avenue. Further work on how the therapeutic effects of MSC  
43 exosomes can be targeted to treat OA is still to be undertaken, but one study has shown that  
44 exosomes derived from miR-92a-3p over expressing MSCs promoted chondrogenesis,  
45 cartilage proliferation and matrix gene expression (115). Similarly, exosomes from synovial  
46 membrane stem cells overexpressing miR1405p increased chondrocyte proliferation and  
47 migration, preventing development of OA in a rat model (121).  
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55 A comparison of induced mesenchymal stem cell (iMSC) derived exosomes and synovial  
56 membrane MSC (SM-MSC) derived exosomes showed that although both reduced pathology  
57 in a mouse collagen induced arthritis model and increased chondrocyte proliferation and  
58 migration *in vitro*, iMSCs had a superior therapeutic effect (124). Adult stem cell and  
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3 embryonic stem cell-derived exosomes increased survival of haematopoietic stem/progenitor  
4 cells and expressed transferrable mRNAs (as well as various other RNAs) encoding for several  
5 pluripotent transcription factors (125,126). Addition of human embryonic MSCs to  
6 chondrocytes showed a therapeutic effect by restoring the balance between synthesis and  
7 degradation of chondrocyte ECM (127). Similarly, another study of osteochondral defects in  
8 adult rats treated with human embryonic MSC exosomes demonstrated complete restoration  
9 of cartilage and subchondral bone by 12 weeks, including ECM and hyaline cartilage formation  
10 (128). These findings indicate the potential therapeutic value of MSC derived exosomes from  
11 a variety of sources.  
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### 15 16 **Perspective**

17 With OA synovial fluid containing a plethora of extracellular vesicles including exosomes, as  
18 well as inflammatory proteins, the synovial derived secretome makes a significant  
19 contribution to synovial phenotype and ultimately impacts on joint pathology. The presence  
20 of exosomes in synovial fluid (through release from joint cells), with modified cargos and  
21 target effects, may be particularly pertinent to mechanisms of disease progression. With  
22 emergent 'omics' approaches, we are now able to sequence exosome content to identify  
23 specific cargo (e.g. miRNA) and investigate how this affects OA pathogenesis. Therapeutic  
24 approaches could include attempting to modulate these exosomes, or target down-stream  
25 cellular pathways that are activated upon exosome uptake. Importantly, miRNA expression  
26 profiles can be indicative of disease, and potentially used as diagnostic biomarkers. For  
27 example, exosomes in peripheral blood could help in early stage OA diagnosis (82). Reports  
28 that synovial fluid microRNA content is altered in patients with osteoarthritis, and that these  
29 changes are gender-specific, highlights the possible utilisation of exosome profiling for  
30 identification of novel biomarkers in early OA (94). Therefore, as well as determining points  
31 of interest for therapeutic intervention, the deciphering of cargo also allows them to be used  
32 as biomarkers (54). The fact that exosomes are available in large quantities in synovial fluid  
33 means they can be easily isolated and analysed (129). The idea that exosome cargo could  
34 potentially be manufactured and engineered to carry specific proteins and surface markers,  
35 for instance using nanoparticle based technology, could ultimately allow targeted regulation  
36 of intercellular communication (130,131). For example, exosomes derived from miR92a3p  
37 overexpressing tumour MSCs increased chondrogenesis and decreased cartilage damage via  
38 interaction with Wnt, a critical protein involved in bone and cartilage development (115).  
39 Despite the recent resurgence of interest in exosomes in disease states, obstacles to  
40 understanding and exploitation still remain, not least in terms of standardising isolation  
41 techniques. Additional research is also needed to fully elucidate the mechanisms of exosome  
42 formation and sorting. Given that effective treatment remains an urgent unmet clinical need  
43 for many OA patients, further studies looking at the role of exosomes in the pathobiology of  
44 the disease is necessary and holds therapeutic promise for the future management of the  
45 condition.  
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## 16 17 **Figure Legends**

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19 **Figure 1. Structure and composition of exosomes.** (A) Cargo including RNA, lipids and  
20 proteins, such as cytoskeletal and ESCRT (endosomal sorting complexes required for  
21 transport) proteins, are packaged into membranous vesicles which contain various proteins  
22 for transport and adhesion as well as communication with cell receptors [adapted from  
23 Colombo et al.(54)]. (B) Exosomes as seen in scanning electron microscopy (SEM) imaging  
24 analysis. Vesicles are within the size range of 50-120nm and have a cup shaped morphology.  
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28 **Figure 2. Exosome formation and secretion.** Early endosomes mature and bud inwards to  
29 form late endosomes or multivesicular bodies (MVBs), filled with selectively packaged  
30 extracellular-bound vesicles or interluminal vesicles (ILVs). Once matured into MVBs, these  
31 are marked for transportation to the plasma membrane for fusion or for degradation by  
32 lysosome fusion. Vesicles released in short bursts of exocytosis into the extracellular milieu  
33 are then described as exosomes. Various proteins such as ESCRT and Rab monomeric G-  
34 proteins are required for the packaging, transport and secretion of these exosomes, alongside  
35 associated proteins.  
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39 **Figure 3. Exosomes are able to selectively target recipient cells.** (1) by direct fusion with the  
40 plasma membrane and release of exosomal content into the cytosol (2) by interaction with  
41 receptors initiating signalling within the cell (3) via endocytosis of exosomes, where fusion  
42 with endosomes is required for cellular effect. Exosomes are able to transfer contents,  
43 including genetic material, which is translated by the recipient cell.  
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46 **Figure 4. Exosomal communication between OA cells.** Inflammatory cytokines, secreted in  
47 response to danger-associated molecular patterns (DAMPs) engaging with toll-like receptors  
48 (TLRs) expressed by various cells within the joint, stimulate exosome release from synovial  
49 fibroblasts (SFs). SF derived exosomes act on chondrocytes to increase MMP-13 and ADAMTS-  
50 5. Similarly, exosomes released from chondrocytes stimulated with inflammatory cytokines  
51 are released into the joint space and can act on cartilage and chondrocytes to increase e.g.  
52 MMP-13, COX-2, IL-1 $\beta$  expression. This positive feedback cycle leads to further breakdown of  
53 the articular cartilage ECM in OA.  
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For Peer Review



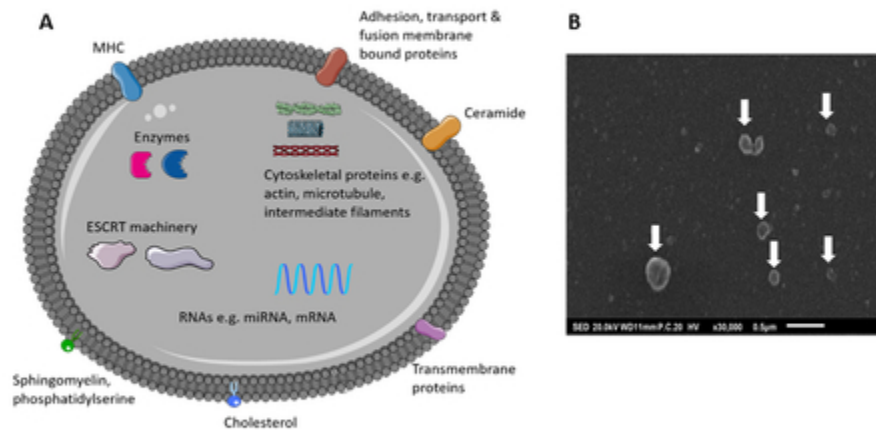


Figure 1. Structure and composition of exosomes. (A) Cargo including RNA, lipids and proteins, such as cytoskeletal and ESCRT (endosomal sorting complexes required for transport) proteins, are packaged into membranous vesicles which contain various proteins for transport and adhesion as well as communication with cell receptors [adapted from Colombo et al.(54)]. (B) Exosomes as seen in scanning electron microscopy (SEM) imaging analysis. Vesicles are within the size range of 50-120nm and have a cup shaped morphology.

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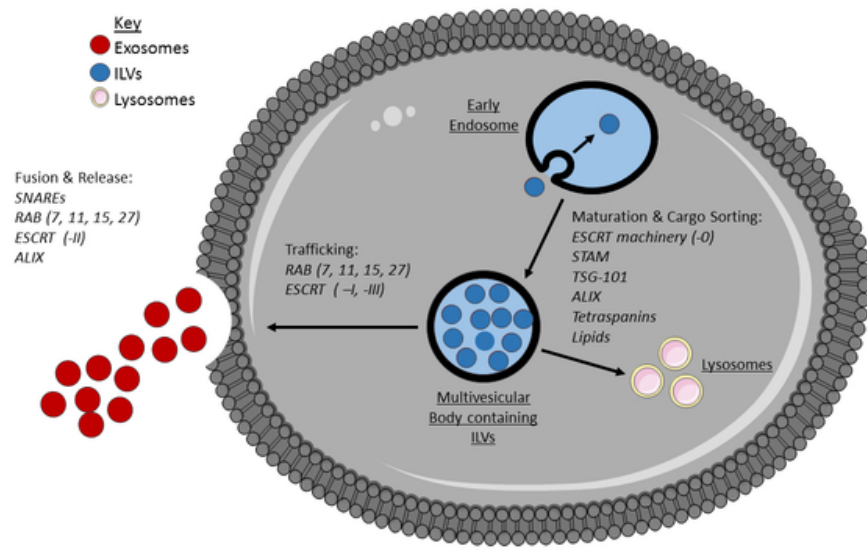


Figure 2

54x30mm (300 x 300 DPI)

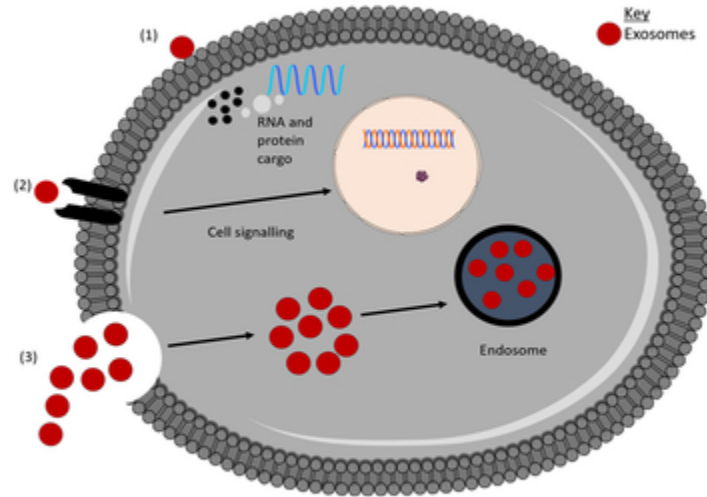


Figure 3. Exosomes are able to selectively target recipient cells. (1) by direct fusion with the plasma membrane and release of exosomal content into the cytosol (2) by interaction with receptors initiating signalling within the cell (3) via endocytosis of exosomes, where fusion with endosomes is required for cellular effect. Exosomes are able to transfer contents, including genetic material, which is translated by the recipient cell.

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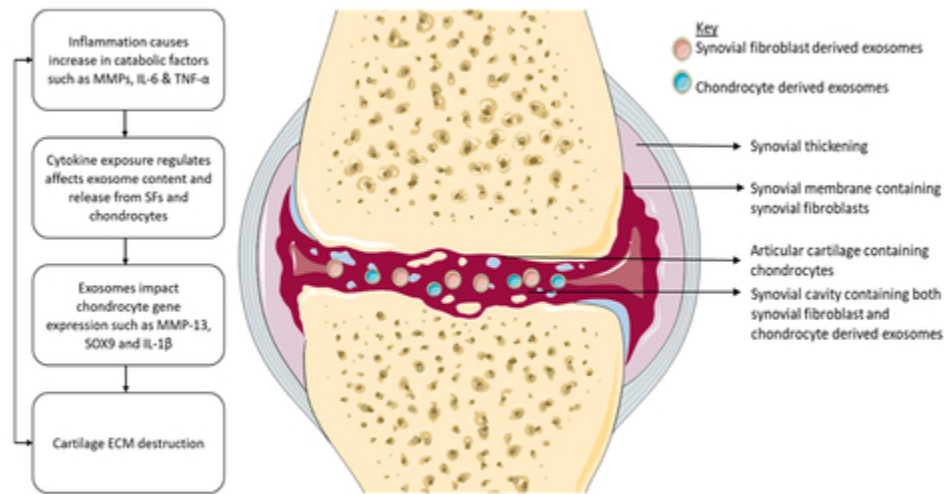


Figure 4. Exosomal communication between OA cells. Inflammatory cytokines, secreted in response to danger-associated molecular patterns (DAMPs) engaging with toll-like receptors (TLRs) expressed by various cells within the joint, stimulate exosome release from synovial fibroblasts (SFs). SF derived exosomes act on chondrocytes to increase MMP-13 and ADAMTS-5. Similarly, exosomes released from chondrocytes stimulated with inflammatory cytokines are released into the joint space and can act on cartilage and chondrocytes to increase e.g. MMP-13, COX-2, IL-1 $\beta$  expression. This positive feedback cycle leads to further breakdown of the articular cartilage ECM in OA.

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