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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
damage (12). Distinct pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), (including products from cellular stress and extracellular matrix (ECM) degeneration) are recognised by pattern recognition receptors (PRRs) such as toll like receptors (TLRs) with inflammatory mediators subsequently released in large amount by cells present in the OA joint. Activation of PRRs triggers cell signalling that leads to the production of pro-inflammatory cytokines and chemokines including interleukin 6 (IL-6), IL-8, IL-1, TNF-α along with proteases, such as matrix metalloproteinase (MMP)-1, MMP-3 and MMP-13, which go on to degrade structural components of cartilage ECM (primarily aggrecan and collagen) and cause changes in chondrocyte viability and glycosaminoglycan (GAG) release (5,12–18). Alteration of the subchondral bone also occurs due to an imbalance in remodelling via bone resorption by osteoclasts and bone formation by osteoblasts, resulting in a reduction in tissue mineralisation, loss of stiffness and bone thickening (19).

While roles for these released soluble mediators are well established, a number of recent studies have reported on extracellular vesicles (EVs) and exosomes and their regulatory potential. These vesicles are secreted from a variety of cells and tissues and exosomes released from neutrophils and synovial fibroblasts (SFs) have been detected in both RA and OA synovial fluid (20–22). It is now believed that exosomes play an important role in cellular communication via the transport of biological cargo. Due to difficulty in isolation and characterisation of different groups of extracellular vesicles, most research within the extracellular vesicle (EV) field of OA has to date focused on a mixed population of EVs. However, given recognised differences in cargo associated with distinct vesicle types (23), this review aims to differentiate between exosome and microvesicle (MV) related studies, and encompass the formation and role of extracellular vesicles. Furthermore, it will focus on these emerging pathological players and their potential as therapeutic targets; with particular emphasis on the role of exosomes in OA pathology and the opportunities that may arise from a better understanding of their biology.

Exosome biogenesis & release
Extracellular vesicles (EVs) can be classified and differentiated based on size, biogenesis and release pathways. EVs include multiple subsets, the most researched of which are microvesicles (MV) and exosomes. MVs are membranous extracellular vesicles which range from 100 to 1,000 nm in diameter (24), whereas exosomes range only from 30 to 120 nm (Figure 1A), with a density of between 1.13–1.19 g/ml and a distinguishing cup shaped morphology (25,26). While MVs are shed from the surface membrane by blebbing, exosomes arise via the endocytic pathway, from the endosomal cell compartment, where they are stored in multivesicular bodies (MVBs) of late endosomes and are released in short bursts by exocytosis upon fusion with the cell membrane (27–29). Typical exosome composition relates to the cellular source from which they arise, with a range of biological cargo reported (Figure 1B). The release of exosomes occurs naturally from many tissues and cells but composition and concentration can be regulated, for instance by cellular stressors such as senescence and hypoxia (30–32), both of which have been associated with OA. For example, OA chondrocytes exhibit senescence-associated phenotypes which increase MMP-1 and -13 protein release and degrade ECM collagen (33). It is therefore interesting that EV production from chondrocytes, isolated from human arthritic cartilage, increased with senescence (34). These EVs were also capable of transferring senescence to nonsenescent chondrocytes and
inhibited cartilage and ECM formation by healthy chondrocytes (34). Senolytic treatment also altered expression of miR-34a, -30c, -125a, -24, -92a, -150, and -186 carried in synovial fluid exosomes (34). Furthermore, hypoxia related genes inducible nitric oxide synthetase (iNOS) and hypoxia-inducible factor-1 (HIF-1) are upregulated in OA caused by exacerbated hypoxia seen during progression of the disease (35), which may drive synovial inflammation (33). Importantly, it has been previously reported that hypoxia can drive MV release from human umbilical cord (UC)-derived MSCs (32). Given the hypoxic nature of the OA joint, potentially this could be a regulatory mechanism for exosome production during disease.

Exosome formation (Figure 2) requires multiple pathways, enzymes and mechanisms, and is ATP-dependent (36,37). Notably, exosome formation and release involves the endosomal sorting complex required for transport (ESCRT) and its associated proteins, apoptosis linked gene-2-interacting protein X (ALIX) and tumour susceptibility gene 101 (TSG-101) (38–41). The ESCRT-0, -I, and -II complexes recognise ubiquitinated membrane proteins on endosomes and the ESCRT-III complex is involved in vesicle budding and scission (42). ESCRT machinery is reported to be central to exosome formation and release, as blockage of the complexes impacts upon these processes (43). However, exosome formation does not require ESCRT function in some cells, but is dependent on sphingomyelinase, an enzyme that produces ceramide (44). Syndecan-syntenin interaction with ALIX and activation of protein kinase C, are also thought to be important for the packaging of exosomes into endosomes and their subsequent release respectively (45,46). Finally, two lipid metabolism enzymes and tetraspanins have been seen to promote formation of interluminal vesicles or ILVs (25), (44).

The divergence in formation and sorting mechanisms between extracellular vesicle types is not completely understood, however, some preliminary experiments show differences in the composition of exosomes in comparison to other EVs. Unlike other EVs, exosomes contain an abundance of cholesterol, saccharides, ceramide, sphingolipids and phosphoglycerides, which could play roles in the sorting mechanism (44,47). For example, localisation of cholesterol into MVBs was reported to mark the contents for secretion as exosomes, whereas low cholesterol levels and the presence of lysobisphosphatidic acid marked MVBs for lysosomal degradation (48–50). The fusion and secretion of exosomes from the cellular membrane itself can require the cytoskeleton elements actin and microtubules, molecular motors such as kinesins and myosin and fusion machinery such as SNAREs (51). Importantly, studies of exosome trafficking have shown that the vesicle transport regulators Rab 35, Rab 11, Rab 27a and Rab 27b (all of which are part of the Ras family of monomeric G proteins) are involved with secretion of exosomes (52,53).

**Exosome interaction & function**

Exosomes have been reported to play roles in multiple physiological processes including apoptosis, angiogenesis, inflammation, coagulation and transfer of cargo such as proteins, lipids and RNA to modulate cell communication and epigenetic modifications (24,54,55). Proteomic analysis shows that exosomes contain a common set of membrane and cytosolic proteins (54). Due to their endosomal origin, this includes membrane transport and fusion proteins (Rab GTPases, annexins, flotillin, integrins, fibronectin), tetraspanins (CD9, CD63,
CD81, CD82), heat shock proteins (Hsc70, Hsp 90), proteins involved in multivesicular body biogenesis (ALIX, TSG101), and lipid-related proteins and phospholipases (24,30,56,57).

Observations to date indicate that thymine DNA glycosylase (TDG101), signal-transducing adaptor molecule 1 (STAM1) and heat shock cognate 70 (HSC70) could all play a role in the selective packaging of proteins (51,58). The mechanisms by which miRNA is selectively packaged is currently under investigation, with studies identifying putative pathways relating to miRNA sorting into exosomes, and these include the neutral sphingomyelinase 2 (nSMase2)-dependent pathway, the miRNA motif and sumoylated heterogeneous nuclear ribonucleoproteins (hnRNPs)-dependent pathway and the miRNA induced silencing complex (miRISC)-related pathway (26,59,60). Differences in specific miRNA expression profiles have already been seen between normal and disease states, including in carcinoma patients (61).

Whether exosomes are taken up by specific cell types is still being debated. Some studies indicate cell-specific exosome interactions, where exosomes from parental cells will only interact with particular target cells. For example, activated T and B cells interact with dendritic cell (DC)-derived exosomes that contain major histocompatibility complex (MHC) class II, to down-regulate the immune response during interaction of T cells and DCs. For DC derived exosomes to be taken up, the T cells had to be activated with this process dependent on leukocyte function–associated antigen-1 (LFA-1), indicating a targeted mechanism (62,63).

Once released into the extracellular milieu, exosomes can target and interact with recipient cells in various ways. The exact mechanism responsible for EV uptake is unknown, but numerous mechanisms have been studied, including clathrin mediated endocytosis (CME), phagocytosis, macropinocytosis and plasma or endosomal membrane fusion (as reviewed by Mulcahy et al. (61) and summarised in Figure 3). Current data suggests that the main process for exosome uptake is via endocytosis, which requires clathrin-mediated or caveolin-dependent mechanisms. This involves the specific assembly of clathrin-coated vesicles containing receptors and ligands (often exosomal transmembrane proteins), through caveolae invaginations or micropinocytosis membrane ‘pinching’ (64). Once this receptor-ligand interaction has taken place, the exosomes fuse with the plasma membrane and their contents are released into the cytosol.

**Exosome cargo**

Exosomes contain mRNA transcripts and miRNA as well as small noncoding RNA species, repeat sequences, structural RNAs, tRNA fragments, vault RNA, Y RNA, and small interfering RNAs (65,66). Current studies have found that exosomal small RNAs are differentially distributed, indicating that there is selective incorporation of small RNA species into exosomes (66,67) and that specific proteins and miRNA may be included in EV cargo depending on function (55,68,69). Accordingly, studies differ considerably in terms of reported exosome content; some describe protein and RNA content distinct from that of the parental cell, whereas other exosome populations reflect typical cellular constituents and could potentially be used as biomarkers (68,70). For example, mast cell derived exosomes contained mRNA from genes not present in parental cells (71).

Currently, it has only been confirmed that mRNA and miRNA are horizontally transferred via exosomes (71,72). Studies have shown that transferred mRNAs can be translated into proteins by target cells, altering the target cell phenotype. Multiple studies have shown that
horizontal transfer of mRNA and protein occurs through exosomal machinery, with the genetic material transferred and proteins translated successfully (73). Exosomes from murine and human mast cell lines have been reported to contain mRNA and miRNA, which were interchangeably transferrable (i.e. mouse to human) in in vitro studies where novel murine mast cell proteins were found in human recipient cells, indicating exosomal transfer and mRNA incorporation into the recipient cells (71). Similarly, RNA from exosomes collected from chronic lymphocytic leukemia cells, was observed to modulate the transcriptome of stromal cells (74). These RNA were called exosome shuttle RNA and the study demonstrated that mRNA can be transferred in a functional form. Further studies have confirmed that this horizontal transfer of mRNA requires the presence of RNA in the recipient cell (55,68,71,75).

Of particular interest is the presence of miRNA, with the majority of circulating miRNA believed to be sequestered within exosomes (76). miRNAs are a class of small noncoding RNA of 1-25 nucleotides that regulate mRNA transcription, translation and stability to silence genes (77). miRNAs are extremely diverse and act at multiple sites with diverse effects including proliferation, apoptosis and differentiation (78,79). Microparticle cargo is predominantly pre- and not mature miRNA (80). Packaging in exosomes may offer protection from circulating RNAses, allowing miRNA to retain the ability to control gene expression by regulating target-mRNA turnover (55). Exosomes released by immune cells have been reported to contain specific miRNA cargo, that can be functionally transferred to recipient cells (68,69). This was confirmed by investigation of miRNA expression levels in a variety of cell lines and their derived exosomes, which demonstrated that certain miRNA are selectively and preferentially packed into exosomes including miR-320, miR-150, miR-142-3p and miR-451 (81).

**Exosomes in OA pathophysiology**

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

**Exosomal impact via miRNA cargo**

Exosomal mRNA and microRNA cargo is especially relevant in OA, where epigenetic changes such as histone modifications, DNA methylation and microRNA (miRNA) impact upon multiple transcription and proteinase factors important for pathology such as RUNX2, MMP-13 and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5), which modulate the anabolic/catabolic balance (85,86). The role and subsequent implications of a large number of miRNAs has been investigated in OA (82). In the joint, miRNAs are mainly associated with the regulation of MMPs, ADAMTS, RUNX and collagen type II, as well as various cytokines. In particular, miR-140 has been identified as having a protective role, with its expression found to be decreased in OA and in IL-1β stimulated chondrocytes (79,87,88). In miR-140 deficient mice, proteoglycan loss and fibrillation of cartilage have been reported, whereas mice overexpressing miR-140 were resistant to arthritis (89,90). In human OA tissues, miR-140 expression is reduced and coincided with upregulation of MMP-13 and ADAMTS-5 expression, with proteoglycan loss demonstrated in miR-140 knock out mice (91). Various other miRNA such as miR-139 and miR-9 also cause imbalances between anabolic and
catabolic processes that are seen in OA, and therefore cause cartilage ECM breakdown (90,92).

Findings in the OA literature have only recently begun to focus on the role of exosome miRNA cargo. Studies have found that the miRNA content of EVs differed between OA and non-OA groups and between genders (93,94). Furthermore, profiling of miRNA in synovial fluid exosomes from OA patients has shown miR-200-c to be increased 2.5-fold compared to synovial fluid from healthy subjects, with consequential effects on the zinc finger binding transcription factor (ZEB1), which decreased collagen type II formation (95). SFs obtained from normal joints were stimulated with IL-1β and derived exosomes were found to have 340 distinct miRNAs upregulated and 24 miRNAs downregulated, compared to non-IL-1β-treated SFs. Interestingly, of these, 11 miRNAs were upregulated and 9 downregulated in the exosomes released from IL-1β-treated SFs, confirming selective packaging (87). More recently, it was found that 22 miRNAs were up-regulated and 29 miRNAs (including miR-95-5p) were down-regulated in OA chondrocyte-secreted exosomes compared to normal chondrocytes (96).

**Exosomal Impact via inflammatory regulation**

While the role of EVs in inflammatory disease is currently well researched, for example in tumour biology (97–99), their function in OA is poorly understood. It has been noted that greater numbers of EVs can be isolated from the serum of patients suffering musculoskeletal disorders compared to healthy subjects, some of which contain elevated levels of specific miRNAs, most notably in rheumatoid arthritis (RA) (100,101). However, RA is an overtly inflammatory disease compared to OA were there may indeed be a more complex subtle and nuanced interaction occurring between joint tissues. In recent years it has become widely acknowledged that inflammation and synovitis are common features of OA (9,10,102), suggesting a potential role for OA cell derived exosomes in the pathophysiology of the disease.

Indeed, inflammatory mediators such as TNFα have been seen to affect the protein and RNA concentration of extracellular vesicles (51,103). Similarly, exosomes derived from lipopolysaccharide (LPS) stimulated mouse macrophages (an inflammatory model), also contained increased levels of inflammatory mediators and miRNAs (104). This suggests that exosomes derived under inflammatory conditions may cause further inflammatory changes. In OA joint disease, inflammation of soft tissues, including disruption of normal synovial tissue homeostasis via specific regulated pathways, could potentially lead to an altered secretory exosome profile, which may ultimately impact upon cartilage integrity (Figure 4).

Multiple cell types associated with OA, including chondrocytes and SFs have been shown to be capable of exosome secretion, with the former regulating cartilage catabolic pathways (54,87,105). OA IL-1β stimulated SFs and macrophages have exhibited an increase in exosome production during inflammation, but exosomes prepared from the synovial fluid of OA patients and healthy subjects showed no difference in either concentration or size (87,95). Chondrocyte derived exosomes have been seen to display annexins II, V and VI, which have a role in chondrocyte differentiation (106).
Synovial fluid isolated exosomes were shown to be readily endocytosed (95). Exosomes isolated from OA synovial fluid significantly stimulated the release of several inflammatory cytokines and chemokines and metalloproteinases from M1 macrophages, including increased production of IL-1β, MMP-12, MMP-7, CCL-8, CCL-1, IL-6 and TNF-α, compared to controls (107). Similarly, articular chondrocytes treated with OA synovial fluid derived exosomes decreased cell survival and expression of anabolic genes including COL2A1 and ACAN and increased expression of catabolic genes including IL-6 and TNF-α (82,94).

Chondrocytes treated with SF-derived exosomes exhibited their uptake and endocytosis, raising the possibility that exosome cargo has a role in regulating chondrocyte fate, ECM degradation and OA pathology (95). A mixed population, including exosomes containing TNF-α, have previously been found to be released by SFs, and importantly they have been shown to regulate the release of chemokines and cytokines from synoviocytes and chondrocytes during inflammation (20,108,109). Exosomes isolated from conditioned medium from normal IL-1β stimulated human SFs were cultured with articular chondrocytes resulting in upregulated gene expression of MMP-13 and ADAMTS-5, and decreased expression of COL2A1 and ACAN in comparison to exosomes collected from non stimulated SFs (87). Additionally, exosomes from IL-1β-stimulated fibroblasts have been shown to contain low levels of IL-6, MMP-3 and vascular endothelial growth factor (VEGF) (87).

**Exosomal impact via bone changes**

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

**Mixed populations of extracellular vesicles**

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

**Harnessing the therapeutic potential of exosomes**

Exosomes from different sources can have different impacts on cells. This review has discussed exosomes derived from synovial fibroblasts and other cell types associated with OA, which may potentially modulate joint pathology. A number of studies have focused on the release of exosomes from MSCs due to the extensive proliferative and differentiation abilities of these multipotent cells, presenting opportunities for new approaches in the treatment of joint tissue defects and OA-related damage. Although various MSC cell based therapies are in clinical trials for tissue regeneration of bone and cartilage, it has recently been considered that it is the secretome that exerts this regenerative effect via chemokines, growth factors and derived exosomes (115,116).

MSC derived exosomes have been shown to have a therapeutic effect in various OA models, including collagenase-induced and destabilised medial meniscus models (82). For example, MSC derived exosomes stimulated repair of osteochondral defects in animal models and cartilage damage in chondrocyte cultures, which involved increased cellular proliferation and infiltration (117). MSC exosomes have also been shown to regulate endothelial cell proliferation, migration and angiogenesis through the transfer of various miRNA (31,32,118,119). Exosomes from MSCs also have the capacity to regulate osteoblast differentiation via various miRNA involved in the control of osteoblast activity, e.g. miR-199b and miR-218, and a positive feedback loop was proposed whereby osteoblasts themselves secrete exosomes to increase miRNA levels that can further promote bone growth (110,120). Exosomes from synovial MSCs overexpressing miR-140-5p increased proliferation and migration of chondrocytes *in vitro* and prevented OA in rat models (121). Furthermore, Wnt5a and Wnt5b carried by synovial MSC-derived exosomes activated yes-associated protein 1 (YAP) via the alternative Wnt signalling pathway and increased proliferation and migration of chondrocytes in a rat OA model (121). Synovial MSC-derived exosomes have been reported to reduce bone marrow cell proliferation, apoptosis, bone mineral loss and fat tissue accumulation (122). Furthermore, when co-cultured with OA chondrocytes, BMMSC-EVs upregulated COX2 and pro-inflammatory interleukins, inhibited TNF-alpha-induced collagenase activity and stimulated production of proteoglycans and type II collagen (123). The *in vitro* expansion potential of MSCs, combined with a high yield of derived exosomes with therapeutic potential (compared to other OA cells such as synovial fibroblasts), makes this a very promising research avenue. Further work on how the therapeutic effects of MSC exosomes can be targeted to treat OA is still to be undertaken, but one study has shown that exosomes derived from miR-92a-3p over expressing MSCs promoted chondrogenesis, cartilage proliferation and matrix gene expression (115). Similarly, exosomes from synovial membrane stem cells overexpressing miR1405p increased chondrocyte proliferation and migration, preventing development of OA in a rat model (121).

A comparison of induced mesenchymal stem cell (iMSC) derived exosomes and synovial membrane MSC (SM-MSC) derived exosomes showed that although both reduced pathology in a mouse collagen induced arthritis model and increased chondrocyte proliferation and migration *in vitro*, iMSCs had a superior therapeutic effect (124). Adult stem cell and
embryonic stem cell-derived exosomes increased survival of haematopoietic stem/progenitor cells and expressed transferrable mRNAs (as well as various other RNAs) encoding for several pluripotent transcription factors (125,126). Addition of human embryonic MSCs to chondrocytes showed a therapeutic effect by restoring the balance between synthesis and degradation of chondrocyte ECM (127). Similarly, another study of osteochondral defects in adult rats treated with human embryonic MSC exosomes demonstrated complete restoration of cartilage and subchondral bone by 12 weeks, including ECM and hyaline cartilage formation (128). These findings indicate the potential therapeutic value of MSC derived exosomes from a variety of sources.

**Perspective**

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

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.

Figure 2. Exosome formation and secretion. Early endosomes mature and bud inwards to form late endosomes or multivesicular bodies (MVBs), filled with selectively packaged extracellular-bound vesicles or interluminal vesicles (ILVs). Once matured into MVBs, these are marked for transportation to the plasma membrane for fusion or for degradation by lysosome fusion. Vesicles released in short bursts of exocytosis into the extracellular milieu are then decribed as exosomes. Various proteins such as ESCRT and Rab monomeric G-proteins are required for the packaging, transport and secretion of these exosomes, alongside associated proteins.

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.

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-1β expression. This positive feedback cycle leads to further breakdown of the articular cartilage ECM in OA.
References


60. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez


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40x30mm (300 x 300 DPI)
Figure 2

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