OPINION

Extracellular vesicles — new tool for joint repair and regeneration

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Abstract | Cell-derived extracellular vesicles (EVs), present in synovial fluid and cartilage extracellular matrix (ECM), are involved in joint development and in the regulation of joint homeostasis. Although the exact function of EVs in these processes remains incompletely defined, the knowledge already acquired in this field suggests a role for these EVs as biomarkers of joint disease, and as a new tool to restore joint homeostasis and enhance articular tissue regeneration. In addition to direct injection of therapeutic EVs into the target site, surface coating of scaffolds and embedding of EVs in hydrogels might also lead to novel therapeutic possibilities. Based on the existing literature of EVs in synovial fluid and articular tissues, and investigation of the molecular factors (including microRNAs) active in joint homeostasis (or during its disturbance), we postulate novel perspectives for the implementation of EVs as a regenerative medicine approach in joint repair.

Extracellular vesicles (EVs) are small membrane-enclosed particles actively released by cells (BOX 1). These structures are found in all tissue types and body fluids studied to date, including synovial fluid and blood[1,2]. This heterogeneous group of particles (varying in size from ~40 nm to 5 μm) can be formed by direct budding off the cell membrane, or released after fusion of endosomal multivesicular bodies with the plasma membrane[3]. Among other functions, EVs act as protective carriers for biologically active signalling molecules (such as proteins, enzymes, mRNAs, microRNAs (miRNAs), DNA fragments and lipids) and contain ligands for receptors on the cell membrane of recipient cells. Upon binding to the target cell, EVs can signal from the plasma membrane or, alternatively, fuse with the cell membrane and deliver their cargo into the cytosol, thereby activating or inhibiting specific cellular processes. EVs can also be internalized via endocytosis, subsequently releasing their cargo in endosomal compartments[4]. Hence, EVs are efficient shuttling vehicles that participate in intercellular communication in a variety of biological processes *in vivo*[5,6], even over long distances[7,8].

As early as 1969, matrix vesicles — a specialized type of EVs present in the growth plate of developing bone — were shown to initiate cartilage calcification during endochondral ossification[9]. During this process, matrix vesicles derived from chondroblasts and osteoblasts collect inorganic phosphate and calcium from the extracellular matrix (ECM), contributing to mineralization by the formation of hydroxyapatite crystals in the vesicle lumen. Eventually, the deposition of these crystals in the ECM results in further mineralization of cartilage[10]. With the discovery of a range of growth factors and proteins, including bone morphogenetic proteins (BMPs) and vascular endothelial growth factor (VEGF), in matrix vesicles, these structures were thought to be also involved in the regulation of neovascularization and the differentiation of chondrocytes and osteoblasts in the growth plate[11]. Despite the fact that these mineralizing matrix vesicles have not been directly implicated in joint repair and regeneration, they are an example of EVs that facilitate tissue development and regulate homeostasis.

Synovial-fluid-derived EVs, first isolated 2 decades ago[12], were found in patients with rheumatoid arthritis (RA)[2,12] and were suggested to play a part in this inflammation-driven disorder. Nowadays, EV-mediated communication in the joint has become a new field of interest, especially in the context of inflammatory joint diseases[13].

Based on the existing knowledge of EVs derived from synovial fluid and articular tissue, as well as from *in vitro* studies, we evaluate the role of EVs in the maintenance and restoration of joint homeostasis, and discuss their potential for use in tissue regeneration. Taking into account findings concerning molecular factors (including miRNAs) in joint biology and pathology, we define a concept for clinical application of EVs in the field of joint repair and regeneration.

EVs in the joint

Inflammatory joint disease

All joint diseases, including RA and osteoarthritis (OA) as the most prevalent, are characterized by a disturbance of the fine balance between anabolic and catabolic cues[14]. Any disturbance of joint homeostasis is reflected in the levels of soluble factors (such as cytokines, enzymes and growth factors) in the synovial fluid, and possibly also in the numbers and content of EVs. In theory, EVs in the synovial fluid can be derived from two sources: directly from cells in the synovial lining (fibroblast-like synoviocytes (FLSs), macrophage-like synoviocytes and leukocytes recruited from the circulation) or from resting chondrocytes in the cartilage; or indirectly from the blood plasma, of which synovial fluid is an ultrafiltrate. Therefore, various mechanisms of EV-mediated communication in action during deregulation of joint homeostasis could result in inflammatory joint disease (FIG. 1).

Evidence in support of a role for EVs during synovial inflammation is provided by studies showing that synovial-fluid-derived EVs from patients with RA or OA can induce cytokine and growth factor release by synoviocytes *in vitro*[15–17]. Furthermore, the catabolic interaction of EVs, derived from IL-1β-stimulated synoviocytes, with chondrocytes *in vitro* also provides important insights into EV-mediated tissue degradation

Box 1 | **EV types and nomenclature**

Extracellular vesicle (EV) is a generic name for all secreted lipid-bilayer-enclosed, cell-derived vesicles, defined in 2011 by the International Society for Extracellular Vesicles. However, other terms are often used to refer to EVs, including exosomes, microvesicles, apoptotic bodies, matrix vesicles, ectosomes, prostasomes, membrane particles and shedding vesicles. The different names are mainly based on particular research fields, physiological or pathological condition, vesicle morphology (mainly size), cell-type of origin and route of biogenesis (membrane-shed or endosomal-derived). As several different EV types share overlapping characteristics, and often no unique markers are available to clearly distinguish between EV types, the classification of EVs is difficult, and comparison of research data based on terminology alone can lead to inconsistencies and is not recommended[81]. In this Perspectives article, we use the generic term 'extracellular vesicles' for all EVs, with the exception of matrix vesicles, which are a well-defined EV type.

during joint inflammation[18]. Leukocyte and erythrocyte-derived EVs, found at high concentrations in the synovial fluid of patients with RA, expose platelet tissue-factor (also known as coagulation factor III or CD142) and support thrombin generation *in vitro*, suggesting their involvement in hypercoagulation and fibrin deposition[2]. Furthermore, EVs derived from T cells or monocytes increased the synthesis of matrix metalloproteinases (MMPs), prostaglandin G/H synthase 2 (PTGS2, also known as cyclooxygenase-2 (COX-2)), microsomal prostaglandin E synthase 1 (PTGES) and prostaglandin E_2 (PGE_2) in FLSs, and resulted in the activation of nuclear factor κB (NFκB), activator protein 1 (AP-1) and JNK (c-Jun N-terminal kinases) signalling pathways in these FLSs[19,20]. These findings demonstrate an EV-mediated, catabolic effect of immune cells on the cartilage and the synovial membrane.

Studies have suggested the existence of specific EV types and functionalities in different joint diseases, and disease-dependent, morphological EV signatures have been found[21]. For example, Zhang and colleagues described a membrane-bound form of TNF in EVs shed by RA, but not OA, FLSs[22]. In addition, platelet-derived EVs were found in synovial fluid of patients with RA, but not in patients with OA[23]. In this study, Boilard and colleagues identified platelet glycoprotein VI, a collagen receptor, as a trigger for EV production in arthritis[23], and found that collagen-induced EVs co-localized with leukocytes in the synovial fluid, where they were able to stimulate FLSs by activating the IL-1 receptor[23]. Importantly, this was the first observation that suggested a role for ECM molecules (specifically collagen) in cell-activation and EV production. The authors proposed that the synovial membrane would be the ideal location in the joint for interaction between ECM of the synovial membrane and cells from the circulation, theoretically enabling collagen-receptor-mediated platelet activation (and subsequent EV production) via fenestrations in the microvasculature[23]. In addition, a 2015 study has suggested that platelet-derived EVs in synovial fluid are internalized by neutrophils[24], a process that has been suggested to enhance inflammation. Interestingly, conversion of arachidonic acid by EV-associated arachidonate 12(S)-lipoxygenase (12S-LOX) to 12(S)-hydroxyeicosatetraenoic acid (12[S]-HETE) was found to be necessary for platelet-derived EV internalization by immune cells. This conversion seems to be driven by the catalytic activity of secreted phospholipase A_2 IIA ($sPLA_2$-IIA) from the inflamed synovial fluid, which can release arachidonic acid from phospholipids in the EV membrane.

Several EV-related mechanisms have been suggested to act during inflammation, including recognition of pathogen-shed EVs by immune cells, EV-mediated shuttling of inflammation-related cytokines and lipid mediators, and the transportation by EVs of proteolytic enzymes that cause tissue destruction[4,13]. Immune complexes formed after recognition of EV-associated citrullinated proteins (such as vimentin and fibrinogen) by the immune system have also been found[25]; these processes might propagate inflammation in autoimmune diseases. Indeed, citrullinated proteins were detected in EVs from patients with RA, OA and reactive arthritis[26], and EV-associated factors have been discovered that could trigger joint inflammation, including DEK, an autoantigen known to attract neutrophils, $CD8^+$ T cells and natural killer (NK) cells[27]. EVs carrying these antigens have been proposed to facilitate their efficient uptake by dendritic cells (DCs) for antigen presentation[27].

Finally, the expression of Toll-like receptors (TLRs) in RA and OA is upregulated in chondrocytes[28] and FLSs[29], resulting in increased cytokine, chemokine and enzyme production. Consequently, targeting of TLR signalling, specifically of TLR4, has been suggested as a therapy for these diseases[30]. The indication that EVs are able to activate monocytes in a TLR2 and TLR4-dependent manner[31] — two TLR family members previously associated with inflammatory joint diseases[32,33] — suggests that TLR activation in joint inflammation might be mediated by EVs.

ECM–EV interactions

EVs produced by RA FLSs might have the ability to infiltrate aggrecan-rich ECM, as was suggested by Lo Cicero and colleagues after finding aggrecanase activity in these EVs[34]. Similarly, hexosaminidase D and β-glucuronidase activity detected in EVs derived from synovial fluid and FLSs from patients with RA and OA was linked to cartilage degradation[35,36]. Although direct evidence is still lacking, EVs are now thought to have a role in the catabolic cascade during inflammatory joint diseases by contributing to the primary degradation of the ECM.

In the context of cancer, EVs positive for the hyaluronic acid (HA) receptor CD44 have been associated with binding of HA in the extracellular environment[37]. HA, a nonsulfated glycosaminoglycan, is also abundant in the joint, in both synovial fluid and cartilage ECM. Production of HA by FLSs and chondrocytes is regulated by the HA concentration in the synovial fluid, which is affected, in turn, by the disease state in the joint[38]. Interestingly, CD44 was detected on EVs isolated from healthy synovial fluid, and from chondrocyte or synoviocyte-derived culture media (J.B., unpublished data), indicating that HA can also serve as a matrix for EV binding in synovial fluid and cartilage ECM. Differences in HA concentration during inflammatory events might, therefore, be associated with changes in EV binding and diffusion in the joint.

EV-associated miRNAs

EV-mediated transfer of miRNAs — silencing molecules that target specific mRNAs during post-transcriptional regulation of gene expression — has been described in the past decade[5]. Nowadays, EVs are acknowledged as vehicles that efficiently protect fast-degrading RNA molecules, enabling their safe transport within the extracellular space. Given the crucial role of miRNAs in cell function, the transport of these molecules might be one the most important mechanisms by which EVs facilitate intercellular communication.

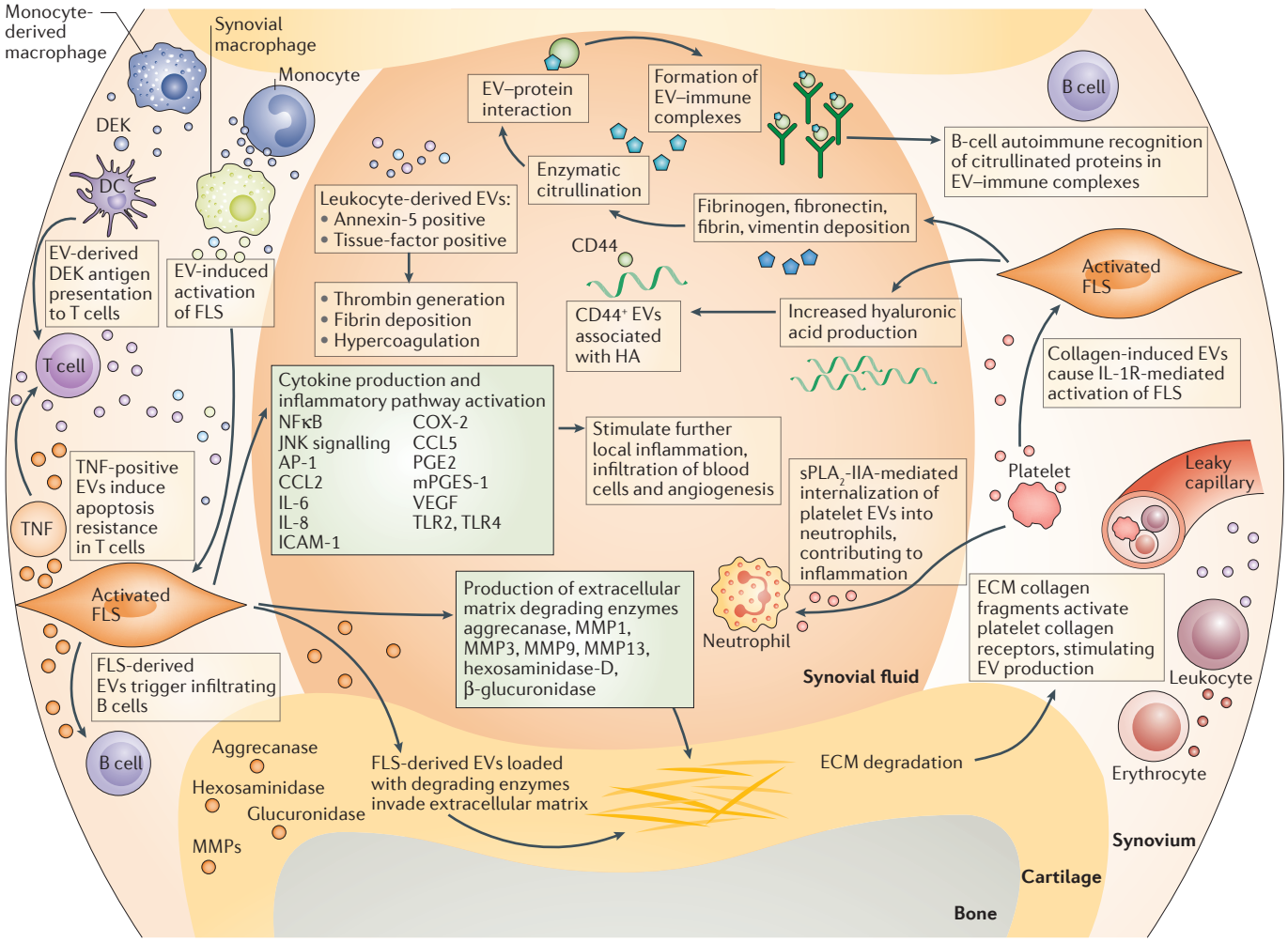

Figure 1 | **Proposed mechanisms of EV-mediated communication in inflammatory joint disease.** During local inflammation, infiltrating leukocytes (T cells, B cells, monocytes, monocyte-derived macrophages) and resident synovial macrophages activate fibroblast-like synoviocytes (FLSs) in the synovial membrane by extracellular vesicle (EV)-mediated cell-to-cell communication. Activated FLSs further maintain inflammation by production of cytokines and enzymes. By the release of their own EVs, FLSs signal back to immune cells and enzyme-loaded, FLS-derived EVs can invade aggrecan-rich extracellular matrix (ECM). Leukocyte-derived EVs can carry factors that cause local hypercoagulation. Activated-platelet-derived EVs cause IL-1 receptor-mediated activation of FLSs and can be internalized by activated neutrophils, maintaining the inflammatory phenotype of the two cell types. B cells recognize citrullinated proteins in EV-immune complexes, which might be part of the autoimmune process in rheumatoid arthritis. AP1, activator protein 1; CCL2, C–C motif chemokine 2; CCL5, C–C motif chemokine 5; COX-2, cyclooxygenase 2 (prostaglandin G/H synthase 2); DC, dendritic cell; HA, hyaluronic acid; ICAM, intercellular adhesion molecule; JNK, c-Jun N-terminal kinase; MMP, matrix metalloproteinase; PGE, prostaglandin E; mPGES-1, microsomal prostaglandin E synthase 1; $sPLA_2$-IIA, secreted phospholipase A_2-IIA; TLR, Toll-like receptor; VEGF, vascular endothelial growth factor.

Two interesting examples of this functional link between EVs and miRNAs involve miR-140 and miR-146a. Although these miRNAs have not been associated with EVs in the joint, they are involved in joint homeostasis and disease, and have been associated with EVs in other tissues[39–41]. MiRNA-140, one of the few miRNAs highly expressed in chondrocytes, is an important regulator of cartilage homeostasis[42]. Expression levels of miR-140 were found to be lower in human articular cartilage from patients with OA than from healthy controls, and IL-1β could suppress miR-140 expression in articular chondrocytes[43]. In addition, mRNA expression of a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) and aggrecan core protein were regulated by miR-140, suggesting a role for this miRNA in regulating the balance between ECM formation and degradation[43]. These effects were only present in IL-1β-stimulated cells and, therefore, seem to be specific for catabolic processes — an important observation in relation to potential treatment options. In addition to its function as a general immune-regulatory factor, miRNA-146a, differentially expressed in both RA and OA[44], also has a role during osteoclastogenesis in RA[45]. Given these observations, EV-mediated transfer of miRNAs is likely to be a mechanism in pathological processes in the joint and, hence, a promising target deserving future research.

EV applications in the joint

Biomarkers

The special features of EVs, including their particular composition, mechanisms of regulated release and cargo-protective properties, have led to hope that these structures can be used as biomarkers to monitor physiological and pathological

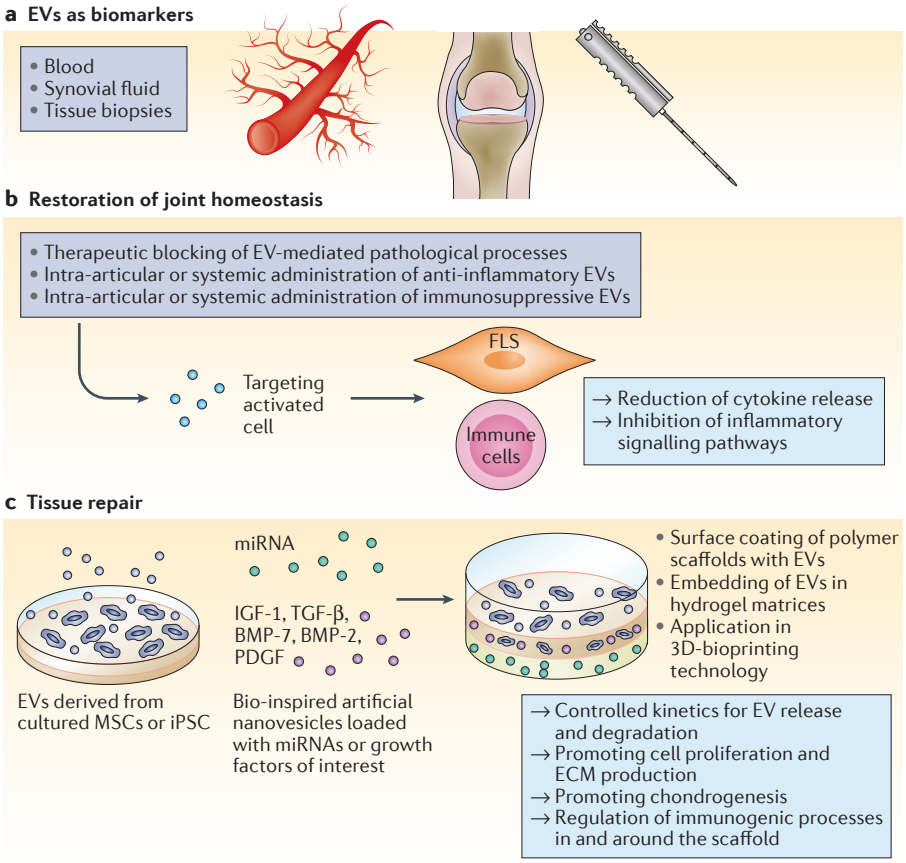

Figure 2 | **Proposed applications of EVs in joint disease. a** | Extracellular vesicles (EVs) have biomarker potential for joint diseases, both to predict disease development in healthy individuals and to monitor disease progression in patients. In the blood, circulating inflammatory EVs can be an alert for early onset of inflammatory joint diseases. In synovial fluid, EVs from patients can provide information about inflammation type, disease state and disease progression. In the context of tissue biopsies, EVs derived from cultured synovial tissue could indicate predisposition for development of autoimmune disease and cartilage disorders. **b**,**c** | Regulatory EVs can be exploited for intra-articular injection or for application in biomaterials designed for implantation purposes to restore joint homeostasis and improve tissue repair in patients. BMP, bone morphogenetic protein; IGF-1, insulin-like growth factor 1; iPSC, induced pluripotent stem cell; miRNA, microRNA; MSC, mesenchymal stem/stromal cell; PDGF, platelet derived growth factor; TGF-β, transforming growth factor-β.

processes[46–48]. For example, in cancer research, the potential of circulating tumour-derived EVs as biomarkers in liquid biopsies is currently being investigated for use in early tumour detection, in defining disease stage and in prediction of treatment outcome[49]. Joint disease-specific EV signatures might also be identified in the circulation or synovial fluid.

Based on the reports of EVs from synovial fluid and blood of patients with RA and OA discussed in this Perspectives, we hypothesize that at least three possible EV biomarker types could be defined for joint diseases (FIG. 2): immune-cell-derived inflammatory EVs in the circulation could be a sign of early-onset joint disease; EVs in the synovial fluid of patients could provide information about inflammation type, disease state and progression; and EVs derived from FLSs and chondrocytes, isolated from healthy individuals, could provide information about predisposition for autoimmune disease or cartilage disorders. Furthermore, considering the possible role of EV-associated miRNAs in inflammatory joint disease, evaluation of miRNA levels in synovial-fluid-derived EVs from patients with RA or OA would further substantiate the involvement of EVs in deregulated joint homeostasis, and might lead to the development of novel diagnostic tools.

Restoration of joint homeostasis

In addition to containing information related to the disease state of an individual, EVs could also be applied as therapy to counteract inflammatory events at the tissue level (FIG. 2). Previous successful attempts to target synovial inflammation in experimental arthritis with liposomes loaded with glucocorticoids or with an immunosuppressive peptide showed the potential of targeted therapies[50,51]. Furthermore, EVs derived from IL-10-stimulated or IL-10-expressing DCs have shown their potential as modulators of the inflammatory immune response during experimentally induced joint disease[52]. Interestingly, besides EV periarticular injection, systemic injection also had a therapeutic effect in the same study[52].

EVs derived from multipotent mesenchymal stem/stromal cells (MSCs) have also been demonstrated to exert immunosuppressive and anti-inflammatory effects[53]; for example, in a clinical trial using MSC-derived EVs as treatment, symptoms were considerably mitigated in a patient with therapy-refractory graft-versus-host disease[54]. Therefore, the role of MSCs as modulators of joint homeostasis, which includes previously described anti-inflammatory properties[55], suggests these cells as interesting therapeutic alternatives in the restoration of homeostasis in the inflamed joint (relevant, for example, for patients with RA). Based on the current knowledge of EV-mediated immune regulation[56], MSC-derived EVs could become a part of these therapeutic efforts for joint diseases[53].

Tissue repair

EVs also hold potential as inducers of tissue regeneration, given that they affect regulatory processes including cell recruitment, proliferation and differentiation[57]. Current regenerative treatments for articular cartilage often rely on the use of biomaterials combined with autologous chondrocytes expanded *in vitro*[58]. In this context, the use of MSCs as a cellular therapy is increasingly gaining attention despite difficulties in controlling undesired ossification of the newly formed tissue[59]. New advances in cocultures of primary chondrocytes with allogeneic MSCs have simplified the treatment[60], and resulted in a shift from *in vitro* expansion and subsequent reimplantation to a single-stage procedure[61]. As MSCs establish a regenerative microenvironment by secreting bioactive molecules[62], the supposed underlying mechanism by which these cells enhance chondrogenic differentiation is thought to be based on trophic factors. Consequently, soluble biomolecules — and, possibly, EVs — are

likely to be the main effectors in the MSC-driven regenerative pathway[53]. In line with this assumption, in a scenario of tissue injury, mRNA and miRNA-laden EVs from local MSCs in the tissue could lead to genetic reprogramming and induction of cell dedifferentiation, instructing a cell-cycle 'reboot' and starting the regeneration process[63,64]. These regenerative approaches could be simplified, potentially, by the replacement of cultured MSCs with the MSC secretome, or even by specific MSC-derived EVs. This is an exciting prospect and might lead to an off-the-shelf regenerative treatment with fewer regulatory constraints than the present strategies, as no living cells would be injected into patients[65]. Moreover, the efficacy of MSC-derived EVs (or of EVs derived from other cell types, such as induced pluripotent stem cells) might be further enhanced by the incorporation of selected biological molecules, such as miRNAs and proteins, which support chondrogenesis[66,67] (FIG. 2).

Challenges and translation

EV preparation and selection

Although ultracentrifugation and density gradient flotation are recommended techniques for EV isolation for analytical and experimental purposes, these procedures are not feasible for clinical applications (BOX 2). One of the biggest challenges for the future is the large-scale production and isolation of (engineered) EVs for clinical application. Therefore, the development of robust, scalable biological production procedures and of appropriate EV isolation methods is urgently needed. Furthermore, detailed knowledge of the complex lipid, protein and nucleic acid composition of EVs could be used to design bio-inspired synthetic carriers with lower variability than that of EVs produced biologically. For example, EV lipid membrane selection or modification could be used to improve the delivery of EVs and their interaction with target cells. Some EV lipids can interact with specific lipid receptor proteins on target cells (for instance, phosphatidylserine binding to TIMD-4, or glycosphingolipids binding to sialoadhesin), and EVs have been shown to carry bioactive lipids (such as eicosanoids and lysophospholipids) and enzymes that convert structural lipids into bioactive molecules (examples of these enzymes include $sPLA_2$-IIA, 12S-LOX and prostaglandin synthases)[24,68]. Hence, targeted delivery of these bioactive compounds to particular cells might enhance their effect and specificity of this therapeutic approach.

Box 2 | **Isolation and purification of EVs**

Isolation of extracellular vesicles (EVs), particularly from body fluids, is challenging, and the quality of the EV isolation procedure determines the validity of the claim of EV-mediated functional effects[1,81]. Lack of sufficient information about EV isolation procedures and EV characteristics complicates the interpretation of data and hampers the comparability of studies. Moreover, limited centrifugation steps, omission of density gradient centrifugation and lack of EV-depleted controls preclude conclusions that observed functional effects are caused solely via EVs[81]. Skriner and colleagues[26] were among the first to isolate EVs from synovial fluid using a multistep centrifugation protocol followed by sucrose density gradient flotation and size determination by electron microscopy. These steps justify their conclusion that the effects observed can be fully attributed to small EVs (<300 nm). In previous reports of EVs derived from synovial fluid, EV characteristics were not specified and the EV fraction isolated most likely contained several EV subtypes as well as nonvesicular particulates. Immune complexes are also known to be present in synovial fluid and can hamper EV analysis, providing an additional reason to purify EVs using ultracentrifugation and density gradient flotation[82]. Finally, to provide researchers with guidelines, the International Society for Extracellular Vesicles published the minimal experimental requirements for definition of EVs and their function in 2014 (REF. 81).

EV delivery

Achieving effective and controlled delivery of EVs to disease sites is challenging, but is paramount to the efficient restoration of joint homeostasis and sustained positive effect on the regenerative process. Different modes of EV delivery could include intravenous (systemic) EV injection, intra-articular EV injection, and surface coating of scaffolds or embedding in hydrogel matrices[57]. Systemic EV injection can result in passive targeting to inflamed joints owing to increased synovial vascular permeability, as has been observed in arthritic joints[51,69]. Conversely, intra-articular injection of EVs, possibly in combination with other therapeutics or regenerative cells, is a direct application at the affected site.

The use of EV-mediated delivery is expected to be more efficient than using soluble proteins or RNA molecules, which are usually prone to fast degradation after injection. However, in view of the gradual clearance of EVs from the joint space, a more sustained effect might be achieved by temporarily immobilizing EVs on or within a biomaterial scaffold. A wide range of scaffolds for musculoskeletal regenerative applications is currently being evaluated, generally in combination with cells and bioactive molecules[70]. Coating with, or incorporation of, EVs is an additional opportunity to optimize the functionality of these scaffolds (FIG. 2). More specifically, EVs could be bound on the surface of polymer scaffolds by specific linkers, antibodies or ECM components such as HA[37].

Alternatively, EVs could also be incorporated within a hydrogel matrix. Hydrogels recapitulate several features of the natural ECM and enable the encapsulation of cells and EVs. Polymer network density and degradation kinetics of hydrogels can be adjusted and tailored to the specific release kinetics of EVs. For this purpose, the field can take particular advantage of the extensive experience available in the embedding of liposomes for drug delivery applications[71]. Moreover, it has been suggested that EV surface modification with functional polymers can be used to modulate their release kinetics further[72]. Nevertheless, how specific biomaterials interact with embedded EVs and preserve their activity needs further research. For example, combinatorial use of different hydrogels loaded with distinct EV populations within a single construct could further facilitate the controlled and sequential release of the different fractions of embedded EVs. 3D printing technologies now have the ability to generate these multimaterial constructs with precisely defined anatomical architectures[73,74], and cell or EV-laden hydrogels can be used as potential building blocks for the production of tissue analogues.

Experimental considerations

In previous clinical trials of EV therapy, these structures were shown to be generally well-tolerated and to have a low risk of immunogenicity and toxicity[75]. However, important questions remain about the kinetics and biodistribution of EVs. The distribution of systemically administered EVs has been demonstrated to be dependent on dose, route of administration and cellular origin of the EVs[76]. No biodistribution data exists for articular tissues yet, and detailed investigations of EV distribution and clearance from the synovial fluid and articular tissues will be necessary for each EV therapy. Directing EVs to the target site and decreasing the risk of fast clearance from the tissues of interest, as has been achieved in cancer research[77], might be a way to increase treatment efficiency.

The future development of EV-mediated therapeutic approaches for joint diseases requires further elucidation of the potential roles of EVs in joint homeostasis and regeneration. Advanced bioreactor systems and *ex vivo* culture models can, to a certain extent, be used for this purpose, but animal models will be indispensable for translation to the clinic. For translational studies in musculoskeletal diseases, the horse is generally seen as the best animal model for joint disease, whereas the dog is better suited for intervertebral disk disease (IVDD) studies[78,79]. The prevalence of OA and IVDD in horses and dogs, respectively, is considerable, enabling the design of clinical trials that use them as experimental animals and therapeutic target animals simultaneously, an important factor from an ethical perspective. A practical advantage of the equine model is the large size of the joints, which enables sequential sampling of relevant quantities of synovial fluid for the monitoring of EV profiles. Furthermore, comparative studies of human and equine articular cartilage have shown great similarities in thickness and composition between the two species[80].

Conclusions

EVs have a prominent role in joint development and in the regulation of intra-articular homeostasis. Although their composition and function in the joint are not yet clearly understood, EVs are envisaged as next-generation biomarkers of the pathophysiological state of the joint. Further, an important role for EVs in future therapies for the treatment of joint disorders is also foreseen, in particular as they provide a simpler and safer alternative to current cell-based therapeutic options. EVs can also be combined with scaffolds, either bound on their surface or embedded within scaffold matrices, enabling the controlled release of specific populations of EVs. Bioengineering approaches could further assist in defining the location of EV delivery by 3D generation of organized tissue constructs. Research on biomaterial-based release of well-defined EV populations is still in its infancy, but holds considerable promise. However, the therapeutic use of EVs demands high-quality standardization of isolation and analysis techniques to yield reproducible EV preparations. In this regard, synthetic alternatives to cell-derived EVs might simplify production and scalability of EV-based therapeutic delivery systems, further enhancing their therapeutic potential.

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doi:10.1038/nrrheum.2015.170
Published online 5 Jan 2016

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Acknowledgements

The authors' research work is supported by the Dutch Arthritis Foundation (grant numbers LLP-12 and LLP-22; J.M., P.R.W.), the EU Seventh Framework Programme (FP7/2007–2013, grant agreement 309962 [HydroZONES]) (J.M.), the European Research Council (grant agreement 647426 [3D-JOINT]) (J.M., P.R.W.), and a grant from the Dutch government to the Netherlands Institute for Regenerative Medicine (NIRM, grant number FES0908) (J.B.).

Author contributions

J.M. and J.B. contributed equally to researching data for the article and writing the manuscript. All authors made substantial contributions to discussion of content and to review and edit the manuscript before submission.

Competing interests statement

The authors declare no competing interests.

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