Journal of Controlled Release 361 (2023) 280–296

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review article

Extracellular vesicle-embedded materials

Yingchang Ma, Steve Brocchini, Gareth R. Williams *

UCL School of Pharmacy, University College London, 29 – 39 Brunswick Square, London WC1N 1AX, UK

A R T I C L E I N F O

Keywords:
formulation
extracellular vesicle
biomaterial
scaffold
stability

A B S T R A C T

Extracellular vesicles (EVs) are small membrane-bound vesicles released by cells. EVs are emerging as a promising class of therapeutic entity that could be adapted in formulation due to their lack of immunogenicity and targeting capabilities. EVs have been shown to have similar regenerative and therapeutic effects to their parental cells and also have potential in disease diagnosis. To improve the therapeutic potential of EVs, researchers have developed various strategies for modifying them, including genetic engineering and chemical modifications which have been examined to confer target specificity and prevent rapid clearance after systematic injection. Formulation efforts have focused on utilising hydrogel and nano-formulation strategies to increase the persistence of EV localisation in a specific tissue or organ. Researchers have also used biomaterials or bioscaffolds to deliver EVs directly to disease sites and prolong EV release and exposure. This review provides an in-depth examination of the material design of EV delivery systems, highlighting the impact of the material properties on the molecular interactions and the maintenance of EV stability and function. The various characteristics of materials designed to regulate the stability, release rate and biodistribution of EVs are described. Other aspects of material design, including modification methods to improve the targeting of EVs, are also discussed. This review aims to offer an understanding of the strategies for designing EV delivery systems, and how they can be formulated to make the transition from laboratory research to clinical use.

1. Introduction

Extracellular vesicles (EVs) have in recent years gained considerable attention across a wide range of interdisciplinary fields including biomaterials, bioengineering, and biomedicine. EVs are small membrane-bound particles actively released by various cell types into biological fluids, and play a crucial role in intercellular communication [1]. Following release from donor cells, EVs may interact with receptor cells through various mechanisms, primarily classified as EV endocytosis, fusion with the cell membrane, and kiss-and-run interactions without subsequent internalisation [2]. In all cases, transmembrane proteins present on the EV surface may be involved in mediating ligand-receptor interactions with the cell membrane. In the first two mechanisms, the cargo of proteins, lipids, and RNA within EVs is transferred to the recipient cell, triggering a response [3]. This inherent capability of EVs to serve as natural carriers for intercellular cargo transfer has led to investigations into their potential as therapeutics.

A number of studies have revealed that EVs possess similar functions to their parent cells, making them a promising alternative to cell therapies. EV treatment can also mitigate the risk of tumor formation associated with immune rejection and cell transplantation, which is often a concern in cell therapy [4]. On the other hand, EVs possess inherent advantageous characteristics including protecting their bioactive cargos from degradation in vivo, and the ability to efficiently deliver these cargos to specific targets with minimal or no immunogenicity. As a result, EVs are considered more potent candidates for therapeutic delivery than artificial nanocarriers [5]. Despite the proven medical potential of EVs at the laboratory level, there are significant technical challenges in their clinical application. These challenges primarily arise from the lack of standardised protocols for EV isolation, characterisation of heterogeneous EV populations, and challenges with large-scale production of EVs, particularly EVs that remain stable in terms of their physicochemical and biological properties after isolation and formulation into an appropriate form for clinical use [6]. Advancements have been made in scaling up the production of EVs to meet demand, but there remains much to be done [7]. In addition, when administered systemically, EVs tend to undergo rapid clearance by the liver and kidney, resulting in insufficient concentrations in tissues and the circulatory system [8]. Local injections of EVs also face limitations due to their suboptimal retention rates. To optimise the clinical utility of EVs and

* Corresponding author.
E-mail address: g.williams@ucl.ac.uk (G.R. Williams).

https://doi.org/10.1016/j.jconrel.2023.07.059
Received 27 June 2023; Received in revised form 27 July 2023; Accepted 31 July 2023
Available online 9 August 2023
0168-3659/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

expand their applications, formulation strategies can be explored. Two approaches have been explored to enhance the efficiency of EVs. To begin with, various biomaterials and bioscaffolds have been developed to sustain the release of EVs, such as hydrogels, nanofibers, polymeric and inorganic scaffolds. These carriers effectively retain EVs in local tissues for extended periods while preserving their bioactivity [9]. Additionally, efforts are being made to improve EV targeting by engineering vesicles, via both genetic and chemical engineering [10].

To assess and maintain the stability and functionality of EVs before and after formulation is another crucial aspect to consider in ensuring their bioactivity. The Minimal Information for Studies of EVs 2018 (MISEV2018) guidelines recommended several characterisation steps to evaluate the quality of EVs. These assessments involve evaluating factors such as vesicle integrity, protein quantification, surface markers, and functional properties of EVs [11]. However, it is worth noting that comprehensive discussions on the stability of EVs before and after formulation are not always extensively covered in all research papers. It is crucial for researchers to consider and report on the stability of EVs throughout the formulation process, as it directly impacts their therapeutic potential. By ensuring the bioactivity and stability of EVs, researchers can enhance the translation and clinical applicability of EV-based therapies.

In this review, after briefly describing EV biology and their intrinsic therapeutic potential, we summarise the recent advancements in EV formulations, encompassing EV engineering and the combination of EVs with biomaterials. We will also examine the advantages of formulating EVs with a specific emphasis on enhanced stability and therapeutic effects. By elucidating the benefits of EV formulations, we aim to provide insights into their potential for clinical translation and therapeutic applications.

2. Overview of EVs

2.1. Discovery of EVs

Like many scientific discoveries, the discovery of EVs was accidental. Experiments that clearly identify EVs as a biological entity with enzymatic and functional potential began in 1978 [12]. Prior to this, there were numerous studies that hinted at the existence of EVs. The origins of EV research started with Chargaff's coagulation study in 1945 [13]. He had centrifuged blood at high speed to isolate clotting factors from cells, and observed that the high-speed sediments can significantly shorten the clotting time when added to supernatant plasma. Peter Wolf later referred to these sediments from platelet-free plasma as “platelet dust” [14]. These sediments were subsequently explored by Crawford, who published images of the vesicles and named them as 'microparticles'. Crawford also suggested that these microparticles contain lipids and can carry ATP and contractile proteins [15].

Later, Nunez discovered the presence of multivesicular bodies (MVBs) by electron microscopy (EM) and suggested that the fusion of the outer or limiting membrane of the MVB with the apical plasma membrane may result in the release of internal vesicles into the luminal space (Fig. 1) [16]. In 1983, Cliff Harding captured EM images of these internal vesicles as they were released upon fusion of MVBs with the plasma membrane. He further speculated a possible intracellular sorting and transport pathway, currently known as the exosome secretion pathway [17]. The term “extracellular vesicle” was first reported in 1971 [18]. Trams and colleagues [19] introduced the term "exosome" in 1981 to describe EVs shed from the surface of the cell, but the term was later redefined by Rose Johnstone in 1987 to specifically refer to vesicles released from the internal cavity of MVBs following fusion with the plasma membrane [20]. This definition of exosomes became widely accepted and is still used today. A seminal paper published in 1996 by Raposo and colleagues [21] presented evidence for vesicles derived from the endocytic compartments of B lymphocytes, which were capable of presenting major histocompatibility complex class II (MHC II) molecules

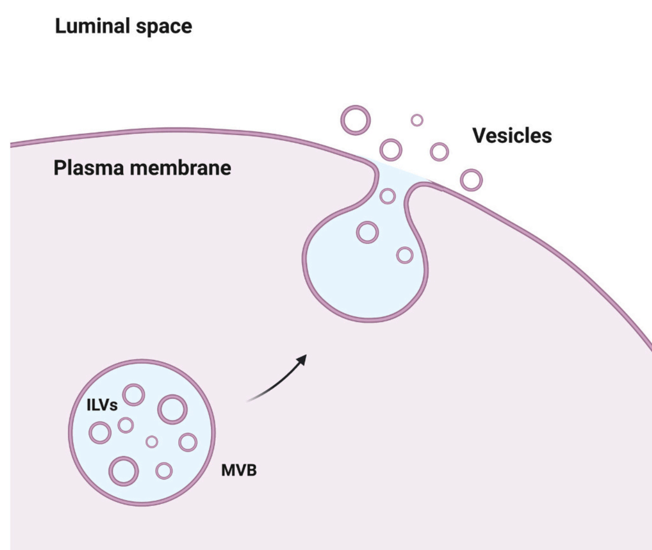


Fig. 1. Schematic representation of the release of intraluminal vesicles (ILVs) into the luminal space by fusion between the multivesicular body (MVB) and the plasma membrane. (Drawn in Biorender)

and inducing an immune response. This discovery challenged the previous understanding of EVs as simply cellular waste and highlighted their potential roles in intercellular communication and immune regulation. This paper and subsequent research in the field led to a paradigm shift in the understanding of the role of EVs in biology and medicine, and sparked significant interest in the potential therapeutic applications of EVs.

2.2. Nomenclature

The size, cargos and membrane compositions of EVs are highly heterogeneous and depend on cell origins, cell states, and environmental conditions [22]. It is difficult to classify them accurately because EVs released by various biogenesis routes may carry common markers, and no specific markers have been identified so far to distinguish EV subtypes [23]. Furthermore, different EV subtypes often have overlapping biophysical properties including size, density and membrane properties, and therefore cannot be completely separated by currently available methods [24]. EV is accepted by the International Society of Extracellular Vesicles (ISEV) as a generic term for particles with lipid bilayers that are naturally released by cells and lack functional nuclei to replicate. To further distinguish them, the MISEV2018 protocol suggests researchers use nomenclature that reflects the physical characteristics of EVs, such as size ($<$100 nm or $<$200 nm for small EVs, $>$200 nm for medium or large EVs), density, specific biochemical compositions (e.g., $CD63^{+}$ EVs), or origin (e.g., podocyte EVs) [11]. Thus, in this review, the term “EV” is used following the MISEV2018 guidance, when there is a lack of EV categorisation or unclear evidence of the type of EV employed for a particular study.

2.3. Biogenesis of EVs

EVs are broadly divided into three subtypes including exosomes, microvesicles (MVs), and apoptotic bodies [23]. Exosomes are small EVs with a mean diameter of 30 to 200 nm, which originate from endosomal membranes, and have a specific density of 1.13-1.19 g/ mL [25]. The biogenesis of EVs is described in Fig. 2. Exosomes are generally generated through the endosomal pathway. During this process, the plasma membrane first invaginates inward to form early endosomes, which can mature into late endosomes. The membranes of some late endosomes then bud inward to generate intraluminal vesicles (ILVs). These ILV-

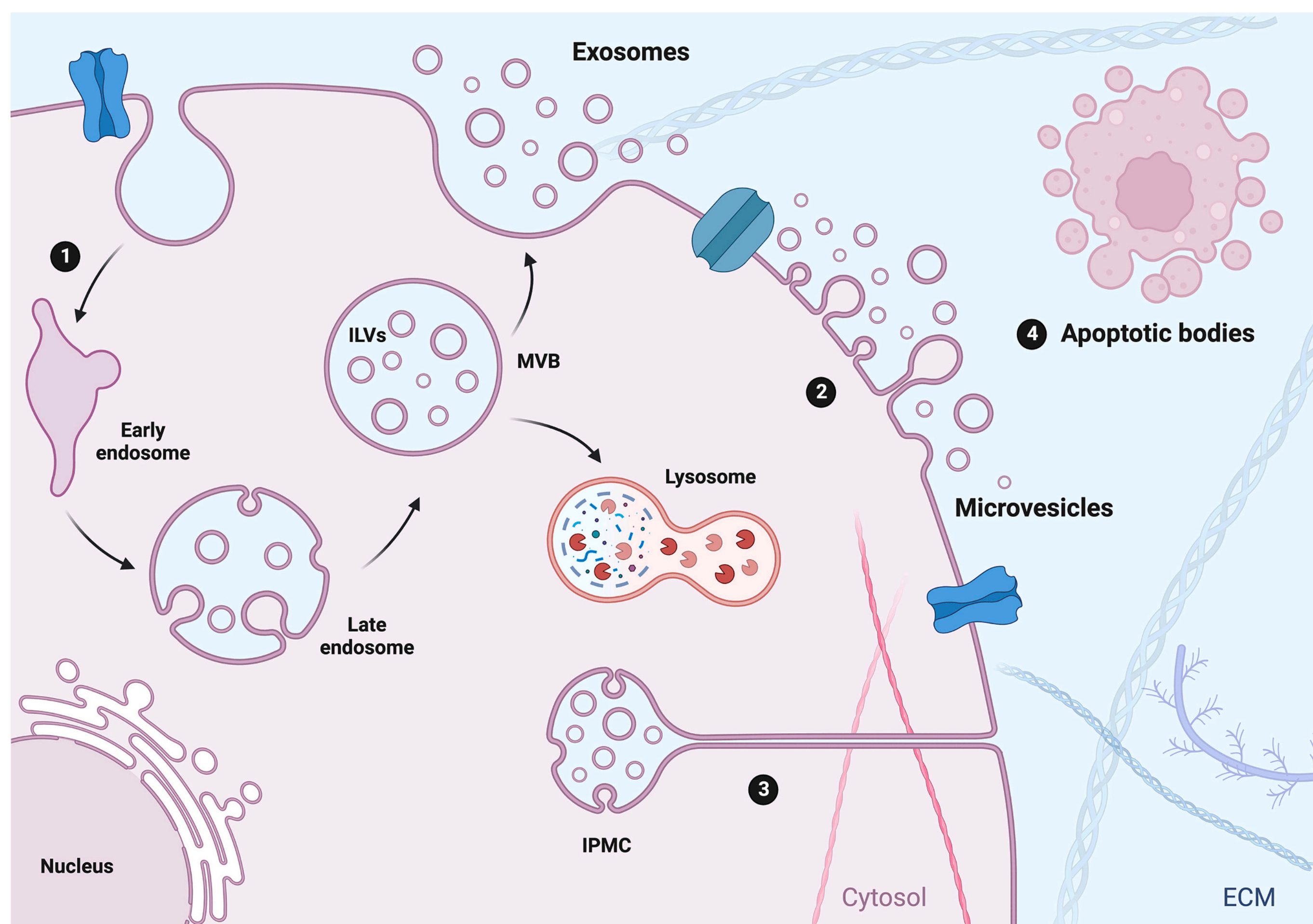


Fig. 2. Extracellular vesicle (EV) biogenesis pathways include: 1. the endosomal pathway, in which vesicles are formed by the inward invagination of the endosome membrane and further mature into ILV-enriched MVBs. These MVBs can either be degraded through autophagy by lysosomes or be released through exocytosis, after which they are named exosomes. 2. The plasma membrane pathway, in which microvesicles (MVs) are formed by the outward budding of the plasma membrane. 3. The intracellular plasma membrane-attached vesicles (IPMCs) pathway, in which small extracellular vesicles (EVs) are stored in the compartment of IPMCs and released in a pulsatile manner. 4. The apoptotic bodies pathway, in which vesicles are released by blebbing of cells undergoing apoptosis. (Drawn in Biorender)

enriched endosomes are also named MVBs [22]. ILVs carried by MVBs are transported to the plasma membrane through the cytoskeleton, where they are released into the extracellular space through exocytosis. ILVs released into the extracellular space are called exosomes [22]. Some MVBs carry ILVs to the plasma membrane, while other MVBs are degraded either by directly fusing with lysosomes, or by fusing with autophagosomes and then lysosomes [26]. MVs, also termed ectosomes, are large EVs with a more heterogeneous size of 50-1000 nm. They are formed and released by outward budding of the plasma membrane [27]. Apoptotic bodies are released by blebbing of cells undergoing apoptosis. Their size tends to be larger (50 to 5000 nm) [28], closer to the upper limit of the EV size range [29,30].

Additionally, some cells can generate small EVs by inward budding from intracellular plasma membrane-attached vesicles (IPMCs) [25,31]. A thin neck is formed by deep invagination of the plasma membrane that connects IPMCs with the extracellular environment and allows the free passage of small molecule probes and extracellular buffer. IPMCs are complex networks of interconnected membranes that can act as reservoirs for vesicle accumulation and pulsatile release. They are indistinguishable from MVBs by conventional transmission electron microscopy [32]. Vesicles derived from IPMCs are similar to a subpopulation of exosomes, which express CD81, CD9, CD53, and CD63 [25,33,34]. This highlights the fact that the EV population is a mixture of various EV subtypes, and the biogenesis of EVs is a complex process that involves multiple pathways and mechanisms.

EVs are commonly described as having a single lipid bilayer, but recent studies have indicated the existence of multilayered EVs [35]. The formation of these multilayered structures is thought to arise via various biogenesis pathways (Fig. 3). One hypothesised mechanism is that smaller ILVs are encapsulated in larger ILVs within the MVB, but there is not strong evidence for this. Another proposed mechanism is based on the formation of lamellar bodies, secreted multilamellar organelles found in certain epithelial cells. In this process, the formation of lamellar bodies drives the generation of multivesicular structures through the flipping and rearrangement of phospholipids within the membranes [36]. Additionally, the presence of ILVs adjacent to the cell membrane may lead to the formation of multilayered EVs during membrane outgrowth. In certain cases of eukaryotes, ILVs have been found to encapsulate entire membrane-bound organelles, such as mitochondria. These organelle-containing vesicles are subsequently transported to the cell surface and shed when budding outwards [37]. Multilayered EVs could also form through several rounds of cellular internalisation, structural preservation, and release via membrane outgrowth. However, the supporting evidence for this is currently inconclusive. Furthermore, gram-negative bacteria can release bilayered EVs through simultaneous outgrowth of the cytoplasmic and outer membranes [38,39]. This sort of EV formation is limited to prokaryotes with two membranes, while eukaryotic membrane-bound organelles in close proximity to the cell surface may experience simultaneous budding (fragmentation) with the cell membrane. Moreover, work has identified

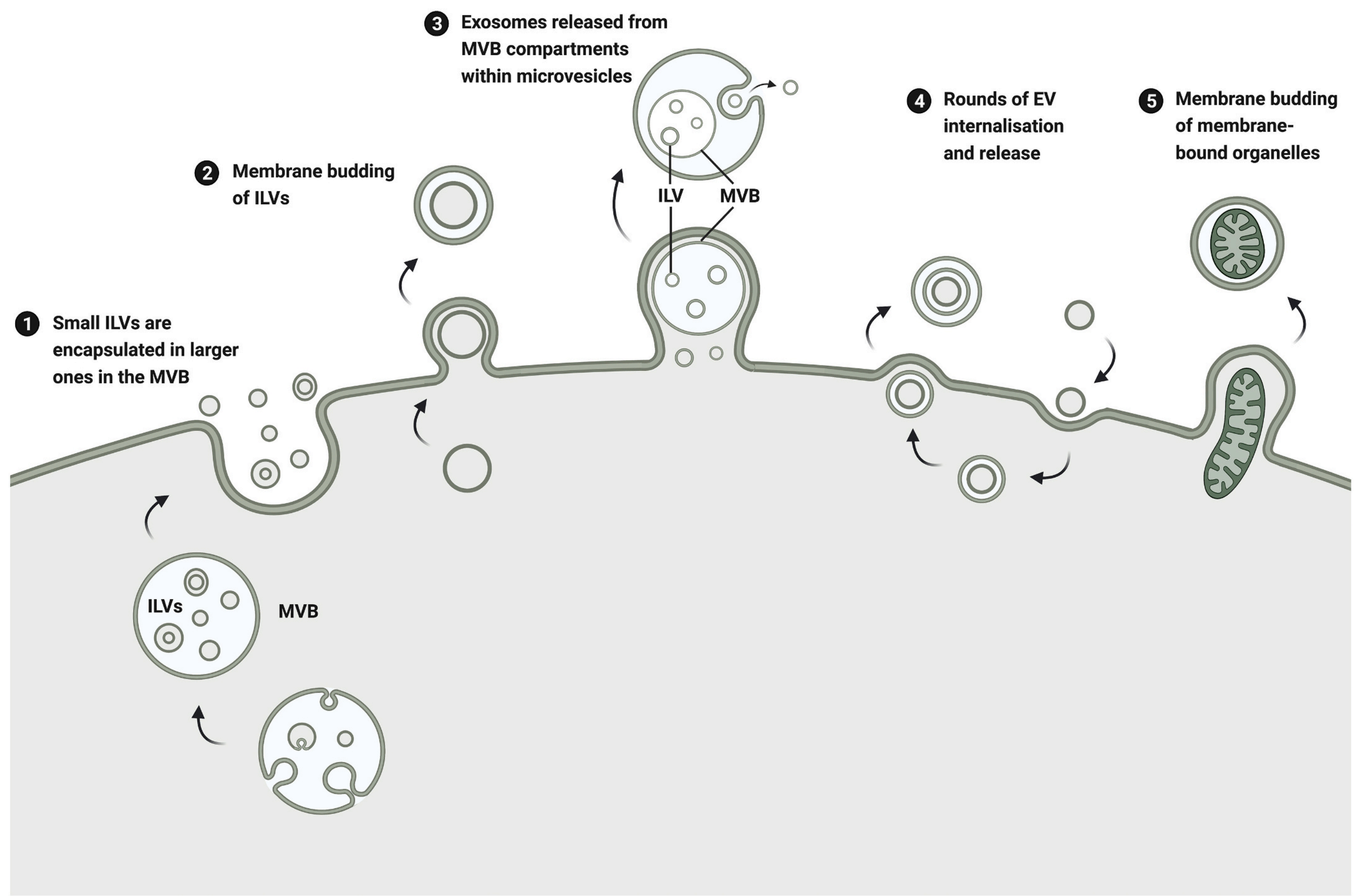


Fig. 3. Schematic representation of the proposed biogenesis mechanisms of multilayered EVs. (Drawn in Biorender)

that cell protrusions on the plasma membrane of endothelial cells can form MVs that contain MVBs. After being released from the parent cells, exosomes can be stored within and subsequently released from these transporter MVs, presenting a novel site for exosome biogenesis in endothelial cells and other potential cell types [40].

2.4. Uptake and function of EVs

EVs have been shown to facilitate intercellular communications by internalisation or attachment to the cell surface. They can deliver a variety of cargos including proteins, lipids, and nucleic acids, which can affect the behaviour and phenotype of target cells [41–43]. EVs can also deliver signalling molecules through surface-expressed ligands [44]. The internalisation of EVs by recipient cells can occur through multiple mechanisms, including macropinocytosis, phagocytosis, clathrin-dependent endocytosis, and clathrin-independent endocytosis [45]. In addition, EV uptake can also be mediated by directly fusion with the plasma membranes of a target cell [46,47].

Due to the broad heterogeneity of EVs, their contents can vary significantly. This heterogeneity is expected due to their limited carrying capacity and the various forces that drive differential distribution of biomolecules, including lipids, carbohydrates, nucleic acids and proteins, along the spectrum of endosome and plasma membranes. The multiple biogenesis pathways necessarily result in distinct compositions that are determined by the local concentration of biomolecules in the vicinity where the EVs are generated [48]. Additionally, differential gene expression is another factor that contributes to the heterogeneity of EVs. For instance, EVs released by antigen-presenting cells contain high levels of MHC class II proteins and costimulatory proteins, while those released from other cell types lack these proteins [25]. Environmental factors such as diet, circadian rhythms, hormones, physical activity, infections, and cell cycle stage can also drive EV heterogeneities by inducing changes in gene expression [48]. This can lead to differential expression of cell surface receptors on recipient cells and can result in either survival or apoptosis [26].

2.5. Different sources of EVs and their therapeutic potential

Over the years, extensive research and clinical trials have focused on cell-based therapies, which aim to replace dysfunctional or deceased cells with functional ones, and modify the microenvironment of damaged tissue to prevent further harm and stimulate repair processes [49,50]. However, increasing evidence suggests that the therapeutic effects of transplanted cells are mediated by their released components, particularly EVs, which have been shown to possess comparable or even superior therapeutic properties compared to the parent cells [51,52]. As a result, EV therapy has emerged as a viable alternative to cell therapy, offering the advantage of not eliciting an immune response and thereby eliminating the need for immunosuppressive treatments [53]. While the cell-free nature of EVs offers advantages in preventing cell-based infections during transport and storage [54], it is crucial to acknowledge that viruses and mycoplasma, which share size similarities with EVs, may inadvertently be included or enriched during the isolation process. Thus, rigorous quality control measures should be implemented to minimise the risk of contamination with these infectious agents.

2.5.1. Therapeutic effects of EVs from mesenchymal stem cells (MSCs)

MSC-derived EVs possess similar properties to MSCs, offering immunomodulation, tissue regeneration, wound healing and anti-tumor effects [55]. In a mouse model of breast cancer, MSC-derived EVs

enriched with the anti-angiogenic molecule miR-16 significantly attenuated the expression of vascular endothelial growth factor (VEGF), thereby inhibiting angiogenesis in breast cancer cells [56]. MSC-derived EVs have also been explored in treating neurodegeneration-related disorders (due to their neuroprotective effects). Intercellular adhesion molecule 1 (ICAM1) can promote angiogenesis in human brain microvascular endothelial cells (HBMECs) through activation of the SMAD3 and P38MAPK signaling pathways. In a mouse model of Parkinson's disease (PD), MSC-derived EVs could promote angiogenesis in HBMECs by increasing the expression of ICAM1, which in turn contribute to PD recovery [57]. In addition, MSC-derived EVs have been indicated to improve cardiac function and reduce infarct size after ischemia-reperfusion (I/R) injury in mice. This study found that these EVs can also promote the survival of cardiomyocytes, and contribute to the recovery of cardiac function [58]. Furthermore, studies have demonstrated that EVs carrying microRNAs (miRNAs) can promote the polarisation of macrophages towards the anti-inflammatory M2 phenotype, which is beneficial for treating inflammatory diseases. In one experiment, interleukin-1β (IL-1β) pretreated human umbilical cord-derived MSCs were found to release EVs with upregulated miR-146a, an anti-inflammatory miRNA that was selectively encapsulated in EVs. This led to the effective induction of M2 polarisation via EVs, which ultimately ameliorated the symptoms of sepsis [59]. Macrophage polarisation to the M2 phenotype can also enhance wound healing. Studies have shown that miR-223 in bone marrow MSC-derived EVs can target the protein Pknox1, leading to the suppression of pro-inflammatory macrophages and the promotion of anti-inflammatory macrophages, which in turn accelerates wound healing [60].

2.5.2. Therapeutic effects of EVs from dendritic cells (DCs)

DC-derived EVs can carry functionally active molecules on their surfaces that participate in the formation of immunological synapses. These molecules include MHC class I and II, as well as adhesion molecules and co-stimulatory markers such as CD40, CD80, and CD86. These molecules are crucial in the induction of anti-tumor T-cell immune responses, activating and recruiting T-cells to target and destroy cancer cells [61]. Given the enormous potential benefits of DC-derived EVs as an immunotherapy, they have been developed as a cell-free cancer vaccine for clinical use [62]. A Phase II clinical trial utilising EVs derived from DCs on patients with end-stage non-small-cell lung cancer (NSCLC) demonstrated significant activation of natural killer cells, but no specific T-cell responses against tumor cells were observed [63]. The finding indicates that immunotherapeutic methods based on DC membrane vesicles hold significant promise for use as an efficient anti-tumor treatment.

2.5.3. Therapeutic effects of EVs from macrophages

Macrophage-derived EVs are beneficial in cancer therapy, immunomodulation, infectious defence and tissue repair, with the precise application depending on their phenotypes and the molecules they carry [64]. Research has revealed intriguingly opposite effects of EVs obtained from macrophages with different activation states. EVs produced by pro-inflammatory macrophages stimulate the production of Th1-promoting cytokines including IL-12 and IFN-γ by both macrophages and DC cell lines. When injected in combination with a peptide vaccine in vivo, they elicit a stronger antigen-specific cytotoxic T cell response. On the other hand, EVs derived from anti-inflammatory macrophages boost the production of anti-inflammatory cytokines IL-4 and IL-10 in macrophages and DCs [65]. Additionally, these EVs carry a large number of costimulatory molecules on their surface, such as CD47, that allow them to escape immunological surveillance [66]. Macrophage-derived EVs also have functions in repairing injured tissues. For instance, EVs from M2-like macrophages carry miR-148a to the site of myocardial injury, and then secrete anti-inflammatory factors as well as suppressing the accumulation of excess calcium ions within the cells of the heart muscle, thereby alleviating I/R injury [67].

2.5.4. Therapeutic effects of EVs from tumor cells

Tumor cell-derived EVs, particularly autologous ones, are thought to carry DNA fragments, tumor antigen repertoires and costimulatory molecules comparable to their parental cells. By generating a robust T cell-dependent anti-tumor immune response, they show therapeutic effects in animal models of colon cancer, melanoma and hepatocellular carcinoma [68]. By utilising intrinsic homo-adhesive properties mediated by membrane surface antigens, EVs from tumor cells have better targeting capabilities compared to EVs from non-tumor cells [69]. However, it should be noted that these EVs can also have effects including the promotion of cell proliferation, inducing angiogenesis, and evading immune surveillance: these attributes can cause an acceleration of cancer progression [70]. For instance, epithelial-mesenchymal transition (EMT) is a critical process in cancer cell metastasis, in which epithelial cells lose their cell-cell adhesion properties and acquire a mesenchymal phenotype, which enables them to migrate and invade surrounding tissues. EVs containing integrin-α2 subunit (ITAG2) can activate EMT in prostate cancer cells, leading to increased metastasis [71]. Therefore, the safety and effectiveness of such EVs as therapeutic agents requires more investigation.

2.5.5. Clinical trials of EV therapies

As a consequence, EVs have attracted significant research interest. They have been explored for stem cell replacement or combination therapy with cells. According to a search on the PubMed database (30-05-2023), more than 90 % of publications published before 2023 and using the keywords “exosome” or “extracellular vesicle” were published within the last decade. Pre-clinical studies, particularly those involving stem cell-derived EV therapies, have shown encouraging results in pre-clinical studies including for cancer and tissue regeneration, which has resulted in several ongoing clinical trials (Table 1). The majority of these trials have used MSC-derived EVs. Other stem cells (e.g. adipose derived stem cells), immune cells (e.g. T cells, monocyte-derived DCs), and even plant cells, have also been employed to produce EVs. In addition, the HEK293 cell line has been used to produce therapeutic EVs.

2.6. EV isolation and characterisation methods

EVs can be obtained from cell culture supernatant or body fluids [74]. There are several common isolation techniques available, including differential ultracentrifugation (DUC), density gradient centrifugation (DGC), size exclusion chromatography (SEC), ultrafiltration (UF), immunoaffinity capture, and polymer-based precipitation. More recently, microfluidic techniques and aptamer affinity-based methods have also been developed [75]. The advantages and disadvantages of each method are summarised in Table 2. The appropriate method should be selected based on the sample source and downstream applications. DUC is often considered as the “gold standard” for EV isolation, but it can lead to the retention of proteins and lipoproteins in the EV pellets [76]. Consequently, utilising a combination of separation techniques is more advantageous than relying on a single method in terms of both the resultant EV purity and yield. For instance, when SEC is employed in conjunction with low-speed centrifugation, it offers the highest level of EV purity while ensuring satisfactory EV yields [77].

Careful selection of appropriate isolation techniques can improve the quality and validity of EV samples by reducing the presence of contaminants and artifacts that may affect the accuracy and reliability of the results. However, the transformation of EVs from academia to the clinic requires compliance with large-scale Good Manufacturing Practices (GMP). Current scale-up methods are described in two recent reviews [74,86]. Scaled processes require standardisation of EV enrichment, purification and quality determination. These processes can be applied to enrich EVs from various types of biofluids, such as cell culture media, plasma, serum, and urine. In the case of EVs produced from cell culture media, both static systems such as flasks and dynamic systems such as bioreactors are applied to collect conditioned media in the upstream cell

Table 1
A list of publicly disclosed clinical trials of EV therapies. Data collected from https://clinicaltrials.gov/ct2/home on 30-May-2023.

Origin of EVs	Condition	Therapeutic cargo	Phase	Identifier/ Reference
Adipose derived stem cells	Periodontitis	None	I	NCT04270006
Plasma	Cutaneous wound healing	None	I	NCT02565264
Placenta MSCs	Resistant perianal fistula	None	I/II	NCT05499156
Placenta MSCs	Complex anal fistula	None	I/II	NCT05402748
Human umbilical cord MSCs	Psoriasis	None	I	NCT03765957 [72]
MSCs	Acute ischemic stroke	miR-124	I/II	NCT03384433 [73]
MSCs	Pancreatic cancer	KrasG12D siRNA	I	NCT03608631
MSCs	Familial hypercholesterolemia	Low Density Lipoprotein Receptor mRNA	I	NCT05043181
DCs	Non-small cell lung cancer	mCTX, tumor antigen	II	NCT01159288
HEK T-REx™-293 cells	COVID-19	overexpressing CD24	II	NCT04969172

Table 2
The advantages and disadvantages of different isolation methods to produce EVs.

Isolation method	Principle	Advantages	Drawbacks	References
Differential ultracentrifugation (DUC)	Separation based on sediment coefficient of vesicles with different size and mass density	• Simple implementation • High-yield	• Low yield, high equipment cost, time consuming, and labour-intensive • Low purity, mixture of vesicles and protein aggregates • Deformation of EV structure	[78,79]
Density gradient centrifugation (DGC)		• Higher purity compared to DUC • Better separation of EV subpopulations	• Low yield, low recovery, high equipment cost, time consuming, and labour-intensive • Lipoprotein contamination	[80]
Size exclusion chromatography (SEC)	Separation of vesicles based on size difference by filtration through a gel column	• High purity and uniform size of samples is possible • High reproducibility • Preservation of the integrity and biological activity of EVs	• Low yield, moderate equipment cost, and time consuming • Hard to scale-up	[81]
Ultrafiltration (UF)	Separation based on size difference, using membranes with specific pore sizes	• Low equipment cost and rapid process	• Moderate yield and purity due to membrane blocking • Fragmentation of larger vesicles	[76]
Immunoaffinity capture	Separation by incubation with magnetic beads or gold-loaded iron oxide nanocubes containing antibodies against specific EV surface markers	• High purity • Separation of specific EV subpopulations	• Low yield and high reagent cost • Challenging to scale-up	[75]
Polymer-based precipitation	Separation based on wrapping EVs with aqueous polyethylene glycol solution to facilitate aggregation and then pelleting by low-speed centrifugation	• Low cost, fast, and high recovery • Preservation of the integrity of EVs	• Contamination with non-EV-bound soluble proteins • Retention of polymers	[82]
Aptamer-based method	Aptamers (short single-stranded DNA or RNA sequences) recognise and bind to specific EV surface markers	• High affinity, specificity and sensitivity • Low immunogenicity and low cost • Low batch-to-batch variability	• Deformation of EV structure by eluents • Hard to fully separate EVs and aptamer ligands	[83,84]
Microfluidic techniques	Separation based on immunoaffinity, size, and density of EVs using microfluidic devices	• High throughput, high purity with easy automation and integration. • Able to process small sample volume	• High equipment cost • Lack of standardisation and large-scale tests on clinical samples. • Lack of method validation.	[85]

culture process. Bioreactors in particular can create a dynamic and continuous environment for cell culture, and the use of hollow fibres with molecular weight cut-off membranes allows for the acquisition of concentrated conditioned media, which is beneficial for the GMP process and downstream purification [87]. Tangential flow filtration (TFF) has emerged as an alternative to DUC for purification, as it requires less time and labor for large-scale production. EVs purified by TFF have been found to exhibit higher immunomodulatory potency compared to those obtained from DUC. TFF can also avoid aggregation or destruction of EVs due to centrifugal forces [88]. Another advantage of TFF is the controllable shear rate and flow rate, which prevents cake formation and subsequent vesicle fragmentation. This is in contrast to conventional UF where larger particles can often clog the membrane pores, leading to compromised separation based on the initial pore size [89].

The downstream characterisation process is critical to evaluate the quality of EVs. Detailed quality control parameters are summarised in Table 3, based on the MISEV2018 guidelines [11]. Identification of EVs typically involves quantification of protein content and particle number, determination of purity, evaluation of protein markers, and characterisation of single vesicles in terms of morphology.

3. Routes of administration of EVs

EVs may have several benefits over other manufactured nanoparticles. They are naturally non-immunogenic when used autologously (i.e. when they are derived from the patient's own cells), while evidence regarding the immunogenicity of allogeneic EVs is currently limited [90]. Plasma transfusions, which involve the transfer of trillions of allogeneic EVs, have been performed in clinical settings for many years without major immunogenic reactions. However, further research is needed to fully understand the potential immunogenicity of allogeneic EVs. Additionally, selected membrane proteins expressed on the surface of EVs, such as integrins and tetraspanins, can fuse with specific cells, allowing them to exhibit natural targeting [91]. Whether they can be selectively retained at the target site or kept away from certain cells is critical to the effectiveness of EV therapy [92]. While EVs have natural

Table 3
The minimum EV characterisation steps required for EV quality control, based on the MISEV2018 guidelines [11].

Quality control parameters	Characterisation steps
Quantification of EV source	Determine the number of cultured cells; total volume of biofluid, or volume/weight/size of collected tissue
Quantification of EV preparation	Measure the total protein amount and total particle number, or total lipid quantification
EV purity	Assess the ratios of proteins to particles, lipids to particles or lipids to proteins
Evaluation of EV protein markers	For all EVs: 1) At least three positive protein markers of EVs must be identified, including at least one transmembrane or lipid-bound protein, and one cytosolic protein 2) At least one negative (exclusion) protein marker must be included For studies focusing on EV subtypes: 1) Subtypes distinguished by EV size: transmembrane/lipid-bound protein markers associated with other intracellular compartments than plasma membrane/endosomes are recommended to be included 2) Subtypes have identified functional soluble factors: secreted soluble proteins with functional activities associated with EVs may be included
Characterisation of single vesicles	1) Techniques providing images of single EVs at high resolution, such as electron and atomic force microscopy to identify the phospholipid bilayer of EVs, which is crucial in distinguishing EVs from other extracellular particles of similar size. 2) Single particle analysis techniques that estimate biophysical features of EVs from other techniques than high-resolution images, such as nanoparticle tracking analysis (NTA) and high-resolution flow cytometry

targeting capabilities, these are not always sufficient for them to be recruited to the specific target site. Therefore, formulating EVs can be necessary to achieve more precise targeting and delivery to the desired location.

3.1. Systemic administration of EVs

Systemic routes like intravenous injection are commonly explored, but require the application of higher doses (as compared to local routes) to compensate for systemic clearance and non-specific uptake. Such high doses can occasionally induce adverse effects [93]. The majority of EVs that are injected systemically are recognised by the mononuclear phagocyte system (MPS) and rapidly cleared from the circulation [94]. Most of the free EVs will accumulate in the liver, lung and spleen in vivo [8]. It is important to highlight that administration of high concentrations of EVs through systemic injection in a short timeframe may not mimic the natural biodistribution pattern of endogenous EVs. To address this limitation, micro-osmotic pumps have emerged to enable the chronic intravenous administration of EVs, mimicking the gradual and continuous manner in which EVs naturally exist and circulate in the body [95].

Recently, a study by Luo and colleagues [96] revealed notable differences in the in vivo distribution pattern of endogenous EVs released from cardiac myocytes compared to injected EVs from the same source. Under physiological conditions, endogenous EVs did not accumulate in significant amounts in the liver and spleen. This observation suggests that endogenous EVs may follow a different distribution pattern compared to injected ones [97]. This difference may be attributed to the requirement to use a higher concentration of exogenous EVs to ensure their detection. To visualise and track EVs in vivo, reporters such as radioisotopes, magnetic nanoparticles or fluorescent dyes are commonly employed. These reporters often require a high EV concentration to be detectable, which may potentially trigger adverse effects in vivo, making the EVs more susceptible to clearance by the MPS [97]. Furthermore, EV isolation, preparation, and storage may have negative impacts on the biological function of EVs. Contamination, low recovery rates, or aggregation may be introduced during these steps. Consequently, the paracrine and autocrine effects exhibited by EVs may be compromised or even lost. Therefore, most studies have observed similar EV distribution patterns. Another study also showed that EVs exhibit different transport patterns when administered orally and intravenously. These findings together suggest the administration method can affect EV transport patterns, and thus to potentially misleading results [98]. These variations in transport patterns further emphasise the importance of considering the administration route when studying EV distribution and targeting.

In addition, the low stability of EVs and their susceptibility to non-specific uptake pose major problems for clinical applications [99]. Therefore, strategies to reduce non-specific uptake are crucial for prolonging circulation duration, minimising the applied dosage, and facilitating the distribution of EVs to target cells. One way to achieve this is through surface modifications, using techniques such as genetic engineering and chemical modifications, which can confer target specificity [10].

3.1.1. Genetic engineering to modify the EV surface

EVs can be genetically engineered to incorporate transmembrane proteins on the surface that can be coupled to ligands or homing peptides. This is done by transfecting donor cells with plasmids or lentiviral vectors encoding the fusion proteins. The cells then release EVs with the targeting ligands on their surface [10]. This allows for the EVs to be directed towards specific cells or tissue environments with improved targeting capabilities. Lysosome-associated membrane protein 2b (Lamp2b) is currently the most extensively used modification site and is abundantly expressed on the surface of EVs. Targeting peptides can fuse with the N-terminus of Lamp2b [100]. For instance, rabies virus glycoprotein (RVG) peptides have been demonstrated to specifically bind to acetylcholine receptors and have been exploited to generate neuro-specific EVs for central nervous system delivery [101]. Another example is to fuse iRGD peptides with Lamp2b, which enabled EVs to interact with αv integrin on the surface of breast cancer cells, and successfully deliver incorporated doxorubicin after intravenous administration [102]. Other potential binding sites for targeting ligands include CD63 (Lamp3) [103] and platelet-derived growth factor receptor (PDGFR) [104].

3.1.2. EV modification by chemical modification

Chemically modifying proteins by covalent conjugation of drugs, polymers and other proteins has been widely examined and, in some cases, translated to the clinic. Chemical modification of EVs has been less studied than genetic engineering. Copper-catalysed azide alkyne cycloaddition (click chemistry) has been used to conjugate both small molecules and large azide-containing model proteins to the EV surface [105]. For example, in an in vivo study, researchers coupled glioma-targeting arginylglycylaspartic acid (RGE) peptides on the surface of EVs via a cycloaddition reaction with sulfonyl azide. The modified EVs were able to penetrate the blood brain barrier (BBB) and accumulate in tumor sites after being administrated intravenously in a mouse model. Meanwhile, unmodified EVs were found to be concentrated in the liver and spleen [106]. Biomolecules can also be conjugated covalently onto the surface of EVs. CD47 is overexpressed on tumor cells and sends a "don't eat me" signal by binding to signal regulatory protein-α (SIRPα) on macrophages. Engineered EVs carrying SIRPα variants can act as immune checkpoint blockage by antagonising the interactions between CD47 and SIRPα. This leads to significantly enhanced tumor phagocytosis, ultimately resulting in the inhibition of tumor growth in mice with tumors [107]. Similarly, EVs with high levels of CD47 isolated from adipose tissue-derived MSCs have been shown to be capable of immune escape in vivo [108]. Furthermore, prior injection of EVs loaded with siRNA against clathrin heavy chain could effectively block MPS endocytosis in the spleen and liver, thereby enhancing the in vivo distribution

of later injected EV doses in other target organs [94].

Other modifications include polyethylene glycol (PEG) functionalisation to improve the retention time of EVs. PEG-conjugated EVs displayed retention times improved from 10 to 60 minutes in the bloodstream of mice [109]. Another study also discovered that paclitaxel-loaded aminoethylanisamide-PEG modified EVs have enhanced accumulation in lung cancer cells in mice after systemic administration [110]. Moreover, modification with magnetic nanoparticles (MNPs) can allow the targeting of EVs. For instance, Li and colleagues found that by culturing superparamagnetic iron oxide nanoparticles (SPIONs) with MSCs, they were able to isolate SPION-loaded EVs. After systemic injection, applying an external magnetic field could cause EVs to accumulate at the injury site, thereby promoting skin wound healing [111]. This approach allows for the targeted delivery of EVs to specific sites in the body using an external magnetic field.

3.1.3. EVs as colloid coating materials

Apart from the modifications of EVs, EVs themselves can also be exploited as coating materials to modify particle-based systems, improve the internalisation efficiency, and sustain the release of EVs and other cargo in the particle. An example is the use of EVs to coat immune-activating nanocomplexes. A core-shell hybrid system was developed with the shell comprising rabies virus glycoprotein (RVG) peptide modified immature dendritic cell (imDC)–derived EVs, and the core a reactive oxygen species (ROS) responsive nanocomplex loaded with hydrophilic genes and a hydrophobic small molecular drug [112]. The EV shell protects the nanocomplex core, and the delivery system can efficiently cross the BBB, target neurons, and release its therapeutic payload in the high ROS environment of diseased dopaminergic neurons [112].

In addition, EVs can be used to coat microspheres. You and colleagues prepared spherical PLGA microparticles coated with polydopamine and successfully attached EVs on the microspheres by covalent linkages [113]. Microfluidic devices can be used to fabricate microspheres with EVs encapsulated internally, and these microspheres can be adsorbed onto polymer scaffolds to control the release of EVs. Specifically, EVs were loaded in triblock polymer microspheres, which were subsequently attached to nanofibrous poly(l-lactic acid) (PLLA) scaffolds [114]. In this system, EVs must undergo two steps to be released, with the microspheres first detached from the scaffold, followed by the hydrolysis of the microspheres to release the EVs, resulting in a more linear release profile [114].

Nanogels have also been explored to deliver EVs through injection. Nanogels are nanoscale 3D-polymer networks composed of hydrophilic polymer chains connected through physical or chemical crosslinking. Like hydrogels, their hydrophilicity allows them to hold a large amount of water, and their nanometer size is suitable for targeted delivery of bioactive molecules like EVs. Self-assembled nanogels composed of cholesterol group-modified pullulan (CHP) have been shown to form complexes with EVs through hydrophobic interactions between the cholesterol groups of CHP and the lipid domains of EVs. This hybrid system could efficiently deliver functional EVs to cells and significantly increase the EV uptake [115]. Mizuta and colleagues utilised this method and developed magnetic nanogels to deliver EVs. This study mixed magnetic iron oxide nanoparticles with CHP to form a composite nanogel with magnetic sensing ability. A hybrid of EVs and magnetic nanoparticles was formed by hydrophobic and noncovalent interactions, which generated magnetically active EVs. EV-nanogel composites were then created by mixing these two components, which can be efficiently delivered and internalised by target cells when applying an external magnetic field [116].

4. Controlled drug delivery systems for local delivery of EVs

Local drug delivery systems allow for the delivery of high doses of drug molecules to specific sites while limiting their distribution in other tissues. Such formulations have been widely explored for conditions including cancer and tissue repair. One example is the Gliadel wafer, which has been approved by the U.S. Food and Drug Administration (FDA) and is made of a biodegradable polymer. The wafer is surgically placed in the brain for local delivery of the chemotherapeutic drug carmustine to treat malignant glioma. It can provide sustained release of the drug to the tumor site, which enables the accumulation of a high concentration of the drug directly at the tumor site while minimising systemic side effects [117]. Recent FDA-approved formulations (Table 4) demonstrate the ongoing efforts to develop localised drug delivery systems for various applications, ranging from ocular diseases to diabetes.

Similarly, there are growing interests in embedding EVs in biomaterials or bioscaffolds, which provide a more localised and concentrated EV dosage distribution. The use of biomaterials as delivery vehicles can provide enhanced stability and sustained therapeutic effects from the EVs [99]. Additionally, it can allow for a controlled release of the EVs in response to changes in the environment of the diseased sites [99]. To evaluate and compare the suitability of different materials for embedding EVs, it is crucial to consider the pros and cons of these materials (as summarised in Table 5) based on specific therapeutic needs and research objectives.

4.1. Hydrogels

Hydrogels are three-dimensional (3D) polymer networks formed by physical non-covalent [122] and/or chemical (covalent) crosslinking of hydrophilic polymer chains in aqueous buffer [123]. Hydrogels are similar in structure to the extracellular matrix (ECM) and biological tissues, which makes them highly compatible with these structures. Hydrogels can also absorb and maintain large amounts of water or physiological fluids. These properties make them useful for creating a hydrated environment in which cellular secretions such as EVs, can be encapsulated [124].

There are several chemical crosslinking methods employed to

Table 4
FDA-approved implantable formulationsregistered since 2018.

Product name	Dosage form	Therapeutic effects	Application number & references
DURYSTA®	Ophthalmic implant	Intracameral sustained release bimatoprost implants for the treatment of open-angle glaucoma or ocular hypertension.	N211911 [118]
XARACOLL®	Implant	Collagen implants that deliver a sustained release of bupivacaine, gradually releasing for up to 24 hours to provide localised analgesia in the surgical area.	N209511 [119]
YUTIQ®	Intravitreal implant	Intravitreal implants that deliver a sustained release of fluocinolone acetonide over an extended period up to three years for the treatment of chronic non-infectious uveitis.	N210331 [120]
Bydureon® BCise®	Subcutaneous injection	An one-week extended release formulation that encapsulates exenatide in polymeric poly(D,L-lactide-co-colide) (PLGA) microspheres to maintain glycaemic control in patients with type 2 diabetes.	N209210 [121]

Table 5
The advantages and disadvantages of materials used for formulating EVs.

Material	Advantages	Drawbacks
Hydrogels	• Adjustable physicochemical properties to customise the loading and release of EVs • High water-retention properties to protect and enhance the stability of EVs • Biocompatible with ECM and biological tissues • Potential for avoiding surgery by using localised injection	• Potential toxicity of the crosslinking agents • Limited control of mechanical properties
Polymer scaffolds	• Adjustable physicochemical properties • Biocompatible and support cell adhesion • Potential for manufacturing complex geometrical scaffolds for specific needs • Extended EV release	• Require additional modifications of the scaffolds for EV loading • Potential toxicity or foreign body response • Surgical implantation required
Inorganic scaffolds	• Adjustable physicochemical properties • High stability and durability • Biocompatible (based on material composition) • Extended EV release	• Require additional modifications of the scaffolds for EV loading • Potential cytotoxicity or immunogenicity • Complex synthesis or fabrication processes • Surgical implantation required

formulate hydrogels, including radical polymerisation, enzyme catalysis, chemical reaction of complementary groups, and application of high energy radiation [125]. The crosslink density of the hydrogel can influence its properties, including swelling, solubility, porosity and mechanical properties. Natural polymers are generally considered to be more biocompatible and bioconstructive, which make them popular choices for delivering EVs [126]. Alternatively, synthetic polymers have additional features such as precise control over mechanical and physical properties, as well as chemical and thermal stability. This ability to tailor their structure and mechanical qualities makes hydrogels compatible with various types of tissues, particularly soft tissues like neural and cerebral tissues [127]. Using EV-hydrogel composites has been shown to significantly improve the stability and extend the release duration of EVs [128]. Therefore, hydrogels are excellent candidates for encapsulating EVs for the treatment of localised diseases.

4.1.1. EV release through diffusion

Several physiochemical mechanisms regulate the release of EVs from a hydrogel. These mechanisms are mainly classified into diffusion, erosion, swelling and mechanical deformation [124]. Diffusion is the main release mechanism of timed delivery systems, and is usually induced by thermal motion of particles in the liquid. The speed of this spontaneous diffusive mass transport is affected by factors such as the chemical potential gradient of the system, the distance EVs have to travel to exit the gel, and the mobility of the EV particles involved in the system [129]. Due to the existence of molecular networks in hydrogels, the release mechanism of trapped EVs is mainly determined by the mesh size of the matrix, polymer nature, and EV-polymer interactions.

EV diffusion in hydrogels is mainly determined by the average mesh size of the gel, which is generally heterogeneous. When EVs are smaller than the mesh size, they can be released by diffusion. The initial release of EVs from hydrogels is typically fast and driven by the high initial concentration of EVs (often in the range of 10^9 to 10^{12} particles/ml) within the gel. This is known as burst release. As the concentration of EVs within the gel decreases, the release rate slows down and becomes more sustained, resulting in a release profile which follows the standard first-order model. EV release can continue for hours to days, depending on the properties of the hydrogel and its rate of degradation. Eventually, the release of EVs will reach equilibrium when the concentration inside and outside the gel becomes equal. The remaining EVs will be released only when the hydrogel is fully degraded.

When the mesh size is similar to the size of the EVs, frictional resistance occurs between the EVs and the polymer chains. Due to the heterogeneous mesh size of the hydrogel, there will be some pores smaller than the EVs, which will increase the length of their transport path [130]. Consequently, the combination of diffusion and grid hindrance can slow down the EV transit out of the gel. When the EVs are larger than the mesh size, they can only diffuse out of the matrix if the network breaks due to polymer breakdown or degradation, or if the mesh expands via swelling. Increasing the crosslink density or polymer concentration can form hydrogels with high porosity and small pore size, which can effectively prolong the retention time of EVs. This has been shown in an in vitro release study in which photo-crosslinked hydrogels made from higher polymer concentrations (4 % w/v alginate) had a significantly prolonged retention time of EVs compared with hydrogels made from lower concentrations (2 % w/v alginate) [131].

Based on these findings, polymers with high swelling ratio or that swell in response to environmental stimuli are potential candidates for EV delivery. Controlling the swelling of the hydrogel can manipulate the diffusion rate of entrapped EVs. Various polymers bearing acidic (e.g. alginic acid) or basic groups have been exploited to formulate pH-responsive swelling hydrogels. In one study, nanoparticles (NPs) with similar diameter to EVs were incorporated into an alginate hydrogel. The hydrogel shrinks and seals the NPs inside under acidic conditions, but under neutral conditions deprotonation of the alginate carboxylic acid groups occurs. This results in a large amount of water ingress, and hence the gel expands to release the NPs [132]. Though no pH-responsive swelling hydrogel has been studied specifically for EV delivery, such materials are promising candidates for the treatment of solid tumors or wound healing applications because the pH of injury and tumor sites is different from that of healthy tissues, and EVs can be released on demand [133,134].

4.1.2. EV release through degradation

Modulating the mesh degradation is a further strategy to control the release of EVs entrapped in hydrogels. The degradation of the network increases the mesh size, allowing EVs to diffuse from the hydrogel [124]. Degradation can occur at crosslinks or polymer backbones as a result of hydrolysis [135], enzymatic activity [136], and other factors. Commonly used hydrolysable crosslinking molecules include anhydrides, esters and amides. Ester linkages with moderate hydrolytic half-lives under physiological conditions have for instance been employed to create PEG hydrogels. By adjusting the crosslinking density and the composition of the system, the in vitro release rate of EVs could be controlled over a range from 6 to 26 days. EVs released maintained their structural and functional integrity. In a mouse model of skin wound healing, a comparison between a subcutaneously injected EV solution and hydrogel EV depot was conducted to compare the biodistribution. On day 6 after administration, the EVs in the hydrogel group were concentrated at the injection site, while free EVs spread out from the injection site within 6 hours and accumulated mostly in the liver and kidneys 2 days after administration [137].

Matrix metalloproteinases (MMPs) are responsible for biodegradation of gelatin, and have been exploited to formulate enzyme-responsive hydrogels loaded with EVs [138]. One example comprises gelatin methacryloyl (GleMA) hydrogels, which can be designed to have MMP-sensitive degradation properties, allowing for controlled release of EVs. Tang and colleagues [139] found that this system can significantly improve the retention of EVs in heart tissue in mice over free EVs. The hydrogel can prevent EVs from washing out, resulting in over 20 times more EVs being identified and distributed around the myocardium and endocardium. EVs remained visible in the cardiac tissue after 48 hours in the GelMA group, while most free EVs were washed out within 24 hours. Degradation of hydrogels can also be triggered by an external

environmental stimulus, such as changes in pH. For example, Wang and colleagues [140] developed a pH-sensitive hydrogel composed of hyaluronic acid (HA), Pluronic F127, and poly-ε-L-lysine that can degrade more rapidly under the acidic environment in the wound area. It was found in an *in vitro* study that at pH 5.5, a higher percentage of EVs (80 %) were released from the hydrogel compared to pH 7.5 (65 %) on day 12. EV release can also be triggered by photodegradation of hydrogels containing ortho-nitrobenzyl ester moieties under ultraviolet (UV) light [141]. By finely tuning the properties of the hydrogel, such as its degradation rate and mechanical properties, it is possible to design hydrogels that release EVs in a manner that closely matches the degradation of the material. This has been demonstrated in studies using HA hydrogel scaffolds [142].

4.1.3. EV release through mechanical deformation

Deformation of the hydrogel network can also affect the release of encapsulated EVs. The application of external forces, such as mechanical stress, magnetic fields, and ultrasound, can cause the deformation of the hydrogels and allow the EVs to escape from the network. Mechanical deformation can also induce convection within the network, resulting in the transient release of EVs in pulses. Hydrogels containing magnetic NPs can be deformed under the action of a magnetic field, resulting in the creation of macropores and rapid deformation of the hydrogel without mechanical damage, which leads to rapid release of loaded particles [143]. Also, ultrasound can transiently disrupt the hydrogel structure. The potential benefits of ultrasound include triggering the release of the contents of hydrogels, such as nanobubbles [144], liposomes [145] and NPs [146], as well as enhancing the deep penetration and uptake of drugs within tissues [147]. However, it is worth noting that external forces can damage or to some extent interfere with the EVs and render them non-functional [148]. To avoid irreversible mechanical damage to hydrogels, self-healing hydrogels can be used. For example, hydrogels with reversible physical crosslinks can repair their networks after mechanical damage [149].

4.1.4. Hydrogel EV loading

There are three strategies for formulating hydrogel-EV composites [99]. One strategy is directly mixing EVs with the hydrogel precursor solution, followed by crosslinking through the addition of crosslinking agents or physical methods. This method utilises an active precursor for covalent crosslinking, resulting in hydrogels with adjustable properties, controllable mechanical properties, and degradation rates, making them attractive materials for EV encapsulation. The use of macromonomers is also beneficial for EV delivery, as these are usually derived from biocompatible polymers, which can minimise the potential harm. Nevertheless, introducing new compounds like crosslinking agents may pose a risk of damaging the EVs [150].

The second strategy is simultaneous mixing EVs with polymers and crosslinking agents, which allows the encapsulation of EVs during the formation of the network. This method is particularly suitable for in-situ gelation, where the formulation can be injected directly at the desired position and then form a gel. Hence, it is beneficial for localised delivery of EVs to fill complex voids [99]. A dual-cavity syringe is required in this approach [150].

The third strategy is called the "breathing" method, also known as the swelling method, which refers to soaking a pre-formed and lyophilised hydrogel in an EV suspension and letting it swell and become loaded with EVs [151]. This method avoids any influence of polymerisation conditions on the integrity of EVs, and the formed composite can be directly lyophilised, which is convenient for transportation. The breathing method also allows for a more homogeneous distribution of EVs within the hydrogel. To employ this method for the loading of EVs into a hydrogel, the gel porosity should be large enough to allow EVs to load. If the EV particle size is bigger than the pores of the hydrogel, it will be challenging to incorporate the EVs. EVs that are loosely attached to the matrices could potentially escape through the larger pores [151].

Beyond direct encapsulation of EVs, creating physical or chemical interactions between EVs and hydrogels can improve their affinity and retention in the gels (Fig. 4). The phospholipid bilayer membranes of EVs are often decorated with various types of proteins, including spanning proteins that cross the membrane and surface markers that are attached to the outer surface of the membrane. Phosphate groups on phospholipids, and amide, amine, hydroxyl, and carboxyl groups on proteins and peptidoglycans, can interact with polar groups of polymers (e.g. carbonyl or hydroxyl groups) through hydrogen bonds or van der Waals forces [152]. In one study, tannic acid (TA) was used to crosslink two components, photo-cross-linkable gelatin methacrylate (GM) and polypyrrole (PPy), to synthesise GMP hydrogels. TA can interact with the amide bonds on the GM backbone, as well as the nitrogen groups on the PPy chains as shown in Fig. 4. The polyphenol groups in TA can also form reversible hydrogen bonds with the phosphate groups on the surface of EVs, thereby effectively retaining EVs in the hydrogel and resulting in sustained in vitro release over 14 days [153].

Charge-mediated attractions between EVs and biomaterials are much stronger and more effective at retaining EVs than weaker hydrogen bonds and van der Waals interactions [154]. EVs are negatively charged due to the presence of anionic phosphatidylserine (PS) and the charged residues of glycocalyx on their surface. This has led to the exploration of positively charged polymers for EV incorporation [155]. For instance, chitosan (CS)-containing hydrogels, which possess a relatively weak cationic charge, can provide a prolonged release of EVs in vitro over 6 days. This study further indicated in a diabetic rat model that wounds treated with the CS-EV composite closed faster than those treated with CS alone or untreated wounds [156]. Similarly, Wang and colleagues [157] loaded adipose MSC-derived EVs into pH-responsive polysaccharide-based fluorinated ethylene propylene (FEP) hydrogels via electrostatic interactions, and observed extended release for up to 21 days in acidic conditions in vitro. Compared with wounds treated by free EVs, FEP hydrogel-treated animals showed faster healing. This was thought to be due to the bioactivity of the free EVs reducing with time, as a result of degradation when they are unprotected in the in vivo environment. The FEP scaffold dressing was found to protect the bioactivity of EVs and efficiently release them to stimulate angiogenesis in both in vitro and in vivo settings.

Forming chemical covalent bonds between EVs and polymers can completely immobilise EVs in the hydrogel, and they will only be released when the network is degraded or the covalent bonds are cleaved. Covalent bonds can in principle be engineered to break down over time or triggered by external environmental stimuli. In one study, an HA hydrogel was prepared by photocleavable linkers that were attached to EVs. HA was initially conjugated with cysteine to have thiol groups. Photocleavable linker-modified EVs were then crosslinked with cysteine-modified HA hydrogels by chemical conjugation. Photocleavable linkers connected EVs with the hydrogel monomers, and the gel could thus release EV upon UV light exposure. This hydrogel system maintained the stability and integrity of EVs in vivo for several days, and provided tissue regeneration activity [141]. Further, the expression of adhesion molecules on the EV surface can also enhance their affinity with biomaterials. EVs can bind to fibronectin and collagen through integrins, and bind to HA through CD44 [158]. Li et al. exploited this interaction by modifying HA hydrogels with laminin-derived peptides, which effectively retained EVs by interacting with integrins on the EV surface [159]. This hydrogel maintained sustained release of EVs for 11 days in vitro. According to an in vivo biodistribution study, EVs released from implanted hydrogels were retained in the injection site, while EVs given through intravenous injection were randomly distributed [159]. Overall, it appears that the hydrogel platform is a promising candidate for local delivery of EVs, offering protection against degradation and clearance and potentially enhancing the therapeutic effects.

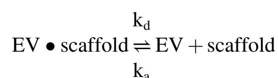
Fig. 4. Methods to improve the affinity between EVs and hydrogels through various physical or chemical interactions. 1. Creating intermolecular forces between surface proteins on EVs and polymers, such as hydrogen bonds, Van der Waals forces and hydrophobic interactions. 2. Utilising electrostatic interactions between negatively charged phosphatidylserine (PS) on the EV surface and positively charged polymers. 3. Establishing covalent bonds between EVs and polymers. 4. Binding of adhesion molecules on the EV surface and polymers such as collagen, fibronectin and hyaluronic acid (HA) via receptor-ligand interactions. (Drawn in Biorender)

4.2. Solid polymer scaffolds

In addition to soft material scaffolds such as hydrogels, there are other solid polymer scaffolds that have been studied to deliver EVs. These involve both 2D (polymer membranes or thin films) and 3D (conventional or printed scaffolds) forms. Polymeric scaffolds serve as microporous matrices that support cell adhesion and bioactivity, and can ultimately lead to tissue regeneration in the implanted area. The presence of porosity and open channels in 2D films and 3D scaffolds are beneficial to deliver bioactive molecules and build complex composite tissue constructs [160,161]. Recently, 3D printing-based additive manufacturing has emerged as a promising technique for preparing more complex geometrical scaffolds, which cannot be prepared through conventional methods [162]. The release kinetics of EVs from these scaffolds can be affected by various factors, including the properties of the scaffold and the manufacturing method. Therefore, it is essential to understand the manufacturing methods and properties of scaffolds in order to optimise the EV release profile.

4.2.1. Release kinetics of EVs from scaffolds

Depending on how the EVs are loaded in the scaffold, there are two main delivery mechanisms: 1. release of EVs from within the scaffold, and 2. release of EVs anchored to the scaffold or their internalisation by cells located adjacent to the scaffold. In the first mechanism, EVs can attach and detach from the scaffold through adsorption and desorption, which is a dynamic reversible process described in the equation below.



$$\text{EV} \bullet \text{scaffold} \underset{k_a}{\overset{k_d}{\rightleftharpoons}} \text{EV} + \text{scaffold}$$

Where EV·scaffold and EV+scaffold denote EVs attached and released from the scaffolds, and k_a and k_d represent the adsorption and desorption coefficients respectively. The value of k_a is related to the diffusion rate of EVs, and is impacted by factors including the EV concentration difference between the scaffold and the exterior environment, and the liquid shear force present in the vicinity. Meanwhile, the value of k_d correlates to the rate of EV binding to the scaffold surface, and is affected by the concentration differences and the nature of the EV-surface interactions [152].

Incubating scaffolds with EV suspensions is the simplest method to formulate EV-loaded scaffolds. Highly concentrated EV suspensions create relatively high chemical potentials, which facilitate the attachment and loading of EVs in the scaffolds. Similarly, due to the difference of chemical potentials between the scaffold and the tissue, EVs are rapidly released into the external environment after the scaffold has been implanted [114,163]. As a result, simply loading EVs by adsorption is unable to maintain a stable release profile and often leads to an initial burst release. It is essential to enhance the interactions between EVs and the scaffold surface to control the release. Polydopamine (PDA)-mediated surface modification is a method to retain EVs on solid scaffolds. In a study of a poly(lactic-co-glycolic acid) (PLGA) scaffold for bone tissue engineering, PDA-coated scaffolds significantly reduced the burst release of EVs from 60 % to 20 % of the total loading on day 1, and led to

an increased retention time from 4 days to over 8 days [164]. PDA coating of porous nanospheres also leads to increased EV absorption and achieved more extended and controlled EV release [165].

4.3. Nanofibers

The porous structure and high surface area to volume ratio of nanofiber mats can mimic the interwoven fibrous structure of the ECM. Nanofibers are also conducive to cell adhesion, proliferation, and differentiation, making them promising scaffolds in local tissue regeneration [166]. Nanofibers can improve the stability and biological activity of EVs as well as giving release over a prolonged period of time. This can be achieved by adhering EVs to the scaffold surface or by incorporating them during the fabrication process [167]. Electrospinning is the most widely explored technique to produce nanofibers with controllable structure and high surface area [168]. Various surface modification strategies can be used to functionalise electrospun nanofibers [169].

The principles of the electrospinning technology are described in detail in recent reviews [170]. Bioactive agents, including EVs, can be incorporated in nanofibers by using single-needle and/or coaxial electrospinning methods. Studies have demonstrated that embedding EVs into nanofibers can improve their stability. Németh and colleagues [167] successfully formulated EV-polyvinylpyrrolidone (PVP) nanofibers by mixing EVs with a PVP solution and then electrospinning. The EV-loaded nanofibers maintained their fibrillar structure for up to 12 weeks, independent of temperature. This can be explained by dint of the fact that PVP can be attached to the EV surface by hydrogen bonding to form a protective shell of macromolecules, thus increasing the stability of the EVs and preventing aggregation. Currently, only single-needle systems have been applied to formulate EV-entrapped nanofibers, but there have been studies of successfully embedding liposomes into the core of coaxial fibres [171].

It is also possible to attach EVs to the nanofiber surface, for instance by electrostatic adsorption. Su and colleagues conjugated PEG molecules to the surface of poly(ε-caprolactone) (PCL) fibres, giving a positive substrate for EV adsorption. Over 40 % of the EVs were still retained after one week in vitro, and the release of EVs was mainly through active uptake by cells in contact with the scaffold rather than passive release [172]. PDA-coating is also effective in loading bioactive factors [173]. Researchers coated PDA on the surface of electrospun fibres to adhere EVs, and found it significantly improved the loading capacity by 1.4-fold compared to regular physical adsorption or conjugation to phospholipids on the EV surface. In an in vitro release study, PDA-coated nanofibers retained nearly 90 % of their EVs over 12 days [174].

4.4. 3D-bioprinted scaffolds

3D printing, also known as additive manufacturing, enables precise fabrication of scaffolds with well-defined structure and shape. EVs can either be loaded into/onto the scaffold after its fabrication or printed directly with the scaffold using a bio-ink composed of EVs, polymer, and potentially other biomaterials. Chen and colleagues [175] developed a 3D-printable bio-ink consisting of MSC-derived EVs, decellularised cartilage ECM, and gelatin methacrylate (GelMA). The bio-ink possessed a photo-crosslinking function and was printed to obtain a radially oriented 3D hydrogel scaffold, which retained EVs in vitro for 2 weeks and facilitated the migration of chondrocytes to promote cartilage regeneration. In another study, a bio-ink composed of EVs, sodium alginate and HA was printed layer by layer by extrusion technology and then crosslinked with $CaCl_2$ solution to form a hydrogel scaffold [176]. The 3D-printed scaffold formed structures with a uniform EV distribution. An in vitro release study exhibited an initial burst release within 24 h, followed by a more gradual and sustained release, with around 80 % of the EVs retained in the gel after a month. The system could enhance tissue repair by modulating the inflammatory response [176]. However, direct attachment of EVs to 3D-printed scaffolds may result in low loadings, so surface modifications of EVs or the scaffolds are often required to improve their adhesion. Zha and colleagues [177] utilised the EV anchoring peptide CP05 to modify a 3D-printed PCL scaffold to specifically bind to CD63 on the surface of the EVs, thereby improving the loading efficiency. Another option is to use polyethyleneimine (PEI), a biocompatible polymer with a “proton sponge” effect: protonation causes the polymer to swell, resulting in enlarged pores that facilitate the release of an internal cargo. PEI has been exploited to modify the surface of 3D-printed porous PLA scaffolds and thereby improve EV adhesion. In one study, PEI-coated scaffolds effectively increased the retention of EVs with enhanced in vitro cellular internalisation. After implantation in vivo for 6 weeks, this system was found to stimulate bone regeneration in rats [178]. However, it is worth noting that cationic polymers such as PEI also have potential drawbacks, including demonstrated toxicity [179].

4.5. Solid inorganic scaffolds

Due to their stiffness and biocompatibility, inorganic scaffolds are widely explored as EV delivery systems for bone tissue regeneration. Commonly used inorganic materials include metals [180], hydroxyapatite [181], tricalcium phosphate (TCP) [163], ceramics [182] and bioactive glass (BAG) [183]. Clinically, metals are the most widely used, especially in bone implants and cardiovascular stents. Titanium alloys are commonly used in orthopaedics due to their good biocompatibility and corrosion resistance [184]. In addition, micro/nano-textured layered titanium can also promote EV release and biogenesis, thereby promoting bone regeneration [185].

EV-modified metal systems have been shown to give enhanced bone regeneration and repair effects over conventional scaffolds, for instance in the case of porous titanium alloy scaffolds and titanium oxide nanotubes [180,186]. Recently, metal-organic framework (MOF) scaffolds for delivering EVs have been proposed to have promise in bone tissue regeneration. MOFs are composed of organic ligands connecting metal ions or metal cluster nodes through coordination bonds, and have useful properties such as high surface area, adjustable porosity and biocompatibility [187]. Kang and colleagues [188] used electrospinning to prepare PLGA-MOF composite scaffolds loaded with magnesium ions and gallic acid, and tethered EVs to the surface through electrostatic interactions. The scaffold showed in vitro release of EVs over up to 10 days. The slow release of magnesium ions created a high-magnesium environment that accelerated cell adhesion and growth. The study observed new bone formation in rat calvarial bone defects at 5 and 10 weeks post-implantation. At the 10-week mark, the EV-scaffold group showed greater bone formation (with the defective areas covered by collagen and new bone tissue) compared to the control group (which primarily contained fibroblasts and proliferating fibrous tissue). These results suggest that the EV scaffold has excellent osteoconductive capacity and potential to promote new in vivo bone growth [188].

Many inorganic scaffolds require high-temperature sintering to burn off a polymer template, which is not compatible with incorporating EVs into the scaffold during initial synthesis. Currently, EVs are often loaded onto inorganic scaffolds by infiltration. Liu and colleagues explored different polymer templates to manufacture a hierarchical mesoporous bioactive glass (MBG) scaffold with macro, micro and meso porosities [189]. This scaffold includes polyurethane sponges forming large pores (200-500 μm), Pluronic F-127 micelles forming mesopores (7.7 nm), and lyophilised EVs packaged in micropores (0.5-2 μm). The high surface area and microporosity of this layered MBG scaffold provided an ideal environment for protecting EVs from disruption during the lyophilisation process, leading to preserved bioactivity. This feature of the scaffold makes it highly promising for use in bone repair applications [189]. Being relatively biologically inert, most inorganic scaffolds are surface-modified with coatings to enhance their interaction with EVs and ensure prolonged release. Studies indicate that layer-by-layer self-assembly technology can alternately deposit positively charged poly-L-lysine

(PLL) polypeptides and negatively charged HA on β-tricalcium phosphate (β-TCP) scaffolds. This biomimetic coating improves the immobilisation of EVs on the scaffold [190]. Additionally, nanocement matrix (NC) was also utilised as a carrier to deliver EVs. Qayoom and colleagues reported a calcium sulfate and nanohydroxyapatite-based NC system that enabled controlled release of bone marrow MSC-derived EVs and other bioactive molecules. The gradual dissolution of calcium sulfate facilitates the formation of pores and the release of encapsulated EVs. This slow dissolution process also provides sufficient space for cell attachment and infiltration, which promoted bone healing [182].

5. Conclusion and future perspectives of EV delivery system

As cell-free therapeutics, EVs possess numerous advantages due to their ability to carry a variety of biomolecules, the lack of immunogenicity and targeting capabilities. In particular, EVs derived from MSCs have shown potential in multiple medical conditions. However, the clinical application of EVs is still in its infancy and there are several challenges that need to be addressed. A lack of standardisation in the EV isolation and processes is a key reason why clinical studies fall short of their goals. Beyond this, there are other variations preventing their clinical use, including differences in purification procedures that can result in potential heterogeneity of the product EV populations. Nonetheless, the accessibility of EVs and their potential to cross physiological barriers make them highly promising for future clinical applications. Even though there have been many studies on EVs in recent years, some crucial parameters still need to be established in order to prevent off-target effects on other organs that may lead to toxicity or tumorgenesis. These parameters include identifying the specific EV subpopulations responsible for therapeutic effects and determining the optimal mode of administration

One potential solution is to formulate EVs with biomaterials or bioscaffolds, which are already known and used with cell therapy. This approach could mitigate the potential risk of undesired EV distribution in the body. Such advanced formulations can also provide sustained release of EVs and protect them from rapid clearance by the immune system. A number of pre-clinical studies have shown the enhanced stability of encapsulated or carried EVs. Additionally, the carrier biomaterials themselves are able to provide supportive or tissue-repairing properties. Various types of EV delivery systems have been described in this review, including hydrogels, nanofibers, polymer-based scaffolds, and inorganic materials. Modification of scaffolds can improve their mechanical properties, biocompatibility, and EV-binding ability. While associating EVs with scaffolds is a potential solution to limit off-target effects, there are also several challenges that need to be addressed. For example, it is necessary to investigate how the scaffolds might affect the EV cargo over extended periods of time. Additionally, it is crucial to develop suitable sterilisation, storage and transportation methods for material-EV composites and determine whether these post-fabrication processes compromise the integrity and therapeutic efficacy of the EVs. Most importantly, in vivo studies of the biodistribution of EVs are required to further evaluate the safety and efficacy of these systems. Once these challenges are addressed, advanced drug delivery systems of EVs have the potential to revolutionise therapeutic interventions and provide new opportunities for the treatment of a variety of diseases, including cancer therapy, regenerative medicine, and gene therapy.

CRediT authorship contribution statement

Yingchang Ma: Writing – original draft, Visualization, Investigation, Conceptualization. **Steve Brocchini:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Gareth R. Williams:** Writing – review & editing, Supervision, Investigation, Conceptualization.

Declaration of Competing Interest

None.

Data availability

No data was used for the research described in the article.

References

[1] E. van der Pol, A.N. Böing, P. Harrison, R. Nieuwland, Classification, functions, and clinical relevance of extracellular vesicles, Pharmacol. Rev. 64 (2012), https://doi.org/10.1124/pr.112.005983.

[2] K.V. Morris, K.W. Witwer, The evolving paradigm of extracellular vesicles in intercellular signaling and delivery of therapeutic RNAs, Mol. Ther. 30 (2022), https://doi.org/10.1016/j.ymthe.2022.05.015.

[3] S. El Andaloussi, I. Mäger, X.O. Breakefield, M.J. Wood, Extracellular vesicles: biology and emerging therapeutic opportunities, Nat. Rev. Drug Discov. 12 (2013), https://doi.org/10.1038/nrd3978.

[4] S. Rani, A.E. Ryan, M.D. Griffin, T. Ritter, Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications, Mol. Ther. 23 (2015), https://doi.org/10.1038/MT.2015.44.

[5] D.E. Murphy, O.G. de Jong, M.J. Evers, M. Nurazizah, R.M. Schiffelers, P. Vader, Natural or synthetic RNA delivery: A stoichiometric comparison of extracellular vesicles and synthetic nanoparticles, Nano Lett. 21 (2021), https://doi.org/10.1021/acs.nanolett.1c00094.

[6] S. Gandham, X. Su, J. Wood, A.L. Nocera, S.C. Alli, L. Milane, A. Zimmerman, M. Amiji, A.R. Ivanov, Technologies and standardization in research on extracellular vesicles, Trends Biotechnol. 38 (2020), https://doi.org/10.1016/j.tibtech.2020.05.012.

[7] D. Buschmann, V. Mussack, J.B. Byrd, Separation, characterization, and standardization of extracellular vesicles for drug delivery applications, Adv. Drug Deliv. Rev. 174 (2021), https://doi.org/10.1016/j.addr.2021.04.027.

[8] I.K. Herrmann, M.J.A. Wood, G. Fuhrmann, Extracellular vesicles as a next-generation drug delivery platform, Nat. Nanotechnol. 16 (2021), https://doi.org/10.1038/s41565-021-00931-2.

[9] C. Gu, J. Feng, A. Waqas, Y. Deng, Y. Zhang, W. Chen, J. Long, S. Huang, L. Chen, Technological advances of 3D scaffold-based stem cell/exosome therapy in tissues and organs, Front. Cell Dev. Biol. 9 (2021), https://doi.org/10.3389/fcell.2021.709204.

[10] Y. Liang, L. Duan, J. Lu, J. Xia, Engineering exosomes for targeted drug delivery, Theranostics 11 (2021), https://doi.org/10.7150/thno.52570.

[11] C. Théry, K.W. Witwer, E. Aikawa, M.J. Alcaraz, J.D. Anderson, R. Andriantsitohaina, A. Antoniou, T. Arab, F. Archer, G.K. Atkin-Smith, D. C. Ayre, Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, J. Extracell. Vesicles 7 (2018), https://doi.org/10.1080/20013078.2018.1535750.

[12] S.E. Kahn, A.M. Jafri, N.J. Lewis, C. Arsenis, Purification of alkaline phosphatase from extracellular vesicles of fracture callus cartilage, Calcif. Tissue Res. 25 (1978), https://doi.org/10.1007/bf02010755.

[13] E. Chargaff, Cell structure and the problem of blood coagulation, J. Biol. Chem. 160 (1945), https://doi.org/10.1016/S0021-9258(18)43131-6.

[14] P. Wolf, The nature and significance of platelet products in human plasma, Br. J. Haematol. 13 (1967), https://doi.org/10.1111/j.1365-2141.1967.tb08741.x.

[15] N. Crawford, The presence of contractile proteins in platelet microparticles isolated from human and animal platelet-free plasma, Br. J. Haematol. 21 (1971), https://doi.org/10.1111/j.1365-2141.1971.tb03416.x.

[16] E.A. Nunez, J. Wallis, M.D. Gershon, Secretory processes in follicular cells of the bat thyroid. III. The occurrence of extracellular vesicles and colloid droplets during arousal from hibernation, Am. J. Anat. 141 (1974), https://doi.org/10.1002/aja.1001410203.

[17] C. Harding, J. Heuser, P. Stahl, Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes, J. Cell Biol. 97 (1983), https://doi.org/10.1083/jcb.97.2.329.

[18] S. Aaronson, U. Behrens, R. Orner, T.H. Haines, Ultrastructure of intracellular and extracellular vesicles, membranes, and myelin fig.s produced by Ochromonas danica, J. Ultrastruct. Res. 35 (1971), https://doi.org/10.1016/s0022-5320(71)80003-5.

[19] E.G. Trams, C.J. Lauter, J.N. Salem, U. Heine, Exfoliation of membrane ecto-enzymes in the form of micro-vesicles, Biochim. Biophys. Acta Biomembr. 645 (1981), https://doi.org/10.1016/0005-2736(81)90512-5.

[20] R.M. Johnstone, A. Mathew, A.B. Mason, K. Teng, Exosome formation during maturation of mammalian and avian reticulocytes: Evidence that exosome release is a major route for externalization of obsolete membrane proteins, J. Cell. Physiol. 147 (1991), https://doi.org/10.1002/jcp.1041470105.

[21] G. Raposo, H.W. Nijman, W. Stoorvogel, R. Liejendekker, C.V. Harding, C. J. Melief, H.J. Geuze, B lymphocytes secrete antigen-presenting vesicles, J. Exp. Med. 183 (1996), https://doi.org/10.1084/jem.183.3.1161.

[22] M. Colombo, G. Raposo, C. Théry, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014), https://doi.org/10.1146/annurev-cellbio-101512-122326.

[23] J. Lötvall, A.F. Hill, F. Hochberg, E.I. Buzás, D. Di Vizio, C. Gardiner, Y.S. Gho, I. V. Kurochkin, S. Mathivanan, P. Quesenberry, S. Sahoo, Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles, J. Extracell. Vesicles 3 (2014), https://doi.org/10.3402/jev.v3.26913.
[24] Y. Jia, L. Yu, T. Ma, W. Xu, H. Qian, Y. Sun, H. Shi, Small extracellular vesicles isolation and separation: Current techniques, pending questions and clinical applications, Theranostics 12 (2022), https://doi.org/10.7150/thno.74305.
[25] D.M. Pegtel, S.J. Gould, Exosomes, Annu. Rev. Biochem. 88 (2019), https://doi.org/10.1146/annurev-biochem-013118-111902.
[26] R. Kalluri, V.S. LeBleu, The biology, function, and biomedical applications of exosomes, Sci. 367 (2020), https://doi.org/10.1126/science.aau6977.
[27] C. Tricarico, J. Clancy, C. D'Souza-Schorey, Biology and biogenesis of shed microvesicles, Small GTPases 8 (2017), https://doi.org/10.1080/21541248.2016.1215283.
[28] L.M. Doyle, M.Z. Wang, Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis, Cells 8 (2019), https://doi.org/10.3390/cells8070727.
[29] R. Kakarla, J. Hur, Y.J. Kim, J. Kim, Y.J. Chwae, Apoptotic cell-derived exosomes: messages from dying cells, Exp. Mol. Med. 52 (2020), https://doi.org/10.1038/s12276-019-0362-8.
[30] Y. Zhang, Y. Liu, H. Liu, W.H. Tang, Exosomes: biogenesis, biologic function and clinical potential, Cell Biosci. 9 (2019), https://doi.org/10.1186/s13578-019-0282-2.
[31] S.J. Gould, A.M. Booth, J.E.K. Hildreth, The Trojan exosome hypothesis, Proc. Natl. Acad. Sci. 100 (2003), https://doi.org/10.1073/pnas.1831413100.
[32] D.O. Nkwe, A. Pelchen-Matthews, J.J. Burden, L.M. Collinson, M. Marsh, The intracellular plasma membrane-connected compartment in the assembly of HIV-1 in human macrophages, BMC Biol. 14 (2016), https://doi.org/10.1186/s12915-016-0272-3.
[33] M. Deneka, A. Pelchen-Matthews, R. Byland, E. Ruiz-Mateos, M. Marsh, In macrophages, HIV-1 assembles into an intracellular plasma membrane domain containing the tetraspanins CD81, CD9, and CD53, J. Cell Biol. 177 (2007), https://doi.org/10.1083/jcb.200609050.
[34] R. Nardacci, A. Amendola, F. Ciccosanti, M. Corazzari, V. Esposito, C. Vlassi, C. Taibi, G.M. Fimia, F. Del Nonno, G. Ippolito, G. D'Offizi, Autophagy plays an important role in the containment of HIV-1 in nonprogressor-infected patients, Autophagy 10 (2014), https://doi.org/10.4161/auto.28678.
[35] K. Broad, S.A. Walker, I. Davidovich, K. Witwer, Y. Talmon, J. Wolfram, Unraveling multilayered extracellular vesicles: speculation on cause, J. Extracell. Vesicles 12 (2023), https://doi.org/10.1002/jev2.12309.
[36] S. Klein, B.H. Wimmer, S.L. Winter, A. Kolovou, V. Laketa, P. Chlanda, Post-correlation on-lamella cryo-CLEM reveals the membrane architecture of lamellar bodies, Commun. Biol. 4 (2021), https://doi.org/10.1101/2020.02.27.966739.
[37] D.G. Phinney, M. Di Giuseppe, J. Njah, E. Sala, S. Shiva, C.M. St Croix, D.B. Stolz, S.C. Watkins, Y.P. Di, G.D. Leikauf, J. Kolls, Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs, Nat. Commun. 6 (2015), https://doi.org/10.1038/ncomms9472.
[38] C. Perez-Cruz, O. Carrion, L. Delgado, G. Martinez, C.L. Pez-Iglesias, E. Mercade, New type of outer membrane vesicle produced by the Gram-negative bacterium Shewanella vesiculosa M7T: implications for DNA content, Appl. Environ. Microbiol. 79 (2013), https://doi.org/10.1128/aem.03657-12.
[39] M. Toyofuku, N. Nomura, L. Eberl, Types and origins of bacterial membrane vesicles, Nat. Rev. Microbiol. 17 (2019), https://doi.org/10.1038/s41579-018-0112-2.
[40] J.D. Petersen, E. Mekhedov, S. Kaur, D.D. Roberts, J. Zimmerberg, Endothelial cells release microvesicles that harbour multivesicular bodies and secrete exosomes, J. Extracell. Vesicles 2 (2023), https://doi.org/10.1002/jex2.79.
[41] G. Rackov, N. Garcia-Romero, S. Esteban-Rubio, J. Carrión-Navarro, C. Belda-Iniesta, A. Ayuso-Sacido, Vesicle-mediated control of cell function: The role of extracellular matrix and microenvironment, Front. Physiol. 9 (2018), https://doi.org/10.3389/fphys.2018.00651.
[42] M. Simons, G. Raposo, Exosomes-vesicular carriers for intercellular communication, Curr. Opin. Cell Biol. 21 (2009), https://doi.org/10.1016/j.ceb.2009.03.007.
[43] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J.J. Lee, J.O. Lötvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (2007), https://doi.org/10.1038/ncb1596.
[44] P. Chen, L. Wang, X. Fan, X. Ning, B. Yu, C. Ou, M. Chen, Targeted delivery of extracellular vesicles in heart injury, Theranostics 11 (2021), https://doi.org/10.7150/thno.51571.
[45] L.A. Mulcahy, R.C. Pink, D.R.F. Carter, Routes and mechanisms of extracellular vesicle uptake, J. Extracell. Vesicles 3 (2014), https://doi.org/10.3402/jev.v3.24641.
[46] C. He, S. Zheng, Y. Luo, B. Wang, Exosome theranostics: Biology and translational medicine, Theranostics 8 (2018), https://doi.org/10.7150/thno.21945.
[47] K.J. McKelvey, K.L. Powell, A.W. Ashton, J.M. Morris, S.A. McCracken, Exosomes: mechanisms of uptake, J. Circ. Biomark. 4 (2015), https://doi.org/10.5772/61186.
[48] F. Fordjour, G. Daaboul, S. Gould, (2019) A shared pathway of exosome biogenesis operates at plasma and endosome membranes, BioRxiv (2019), https://doi.org/10.1101/545228.
[49] V.F.M. Segers, R.T. Lee, Stem-cell therapy for cardiac disease, Nat. 451 (2008), https://doi.org/10.1038/nature06800.
[50] X. Wei, X. Yang, Z.P. Han, F.F. Qu, L. Shao, Y.F. Shi, Mesenchymal stem cells: a new trend for cell therapy, Acta Pharmacol. Sin. 34 (2013), https://doi.org/10.1038/aps.2013.50.
[51] M. Jarrige, E. Frank, E. Herardot, S. Martineau, A. Darle, M. Benabides, S. Domingues, O. Chose, W. Habeler, J. Lorant, C. Baldeschi, The future of regenerative medicine: cell therapy using pluripotent stem cells and acellular therapies based on extracellular vesicles, Cells 10 (2021), https://doi.org/10.3390/cells10020240.
[52] J.H. Jung, X. Fu, P.C. Yang, Exosomes generated from iPSC-derivatives, Circ. Res. 120 (2017), https://doi.org/10.1161/circresaha.116.309307.
[53] G.E. Melling, E. Carollo, R. Conlon, J.C. Simpson, D.R.F. Carter, The challenges and possibilities of extracellular vesicles as therapeutic vehicles, Eur. J. Pharm. Biopharm. 144 (2019), https://doi.org/10.1016/j.ejpb.2019.08.009.
[54] C.K. Das, B.C. Jena, I. Banerjee, S. Das, A. Parekh, S.K. Bhutia, M. Mandal, Exosome as a novel shuttle for delivery of therapeutics across biological barriers, Mol. Pharm. 16 (2019), https://doi.org/10.1021/acs.molpharmaceut.8b00901.
[55] B. Zhang, Y. Yin, R.C. Lai, S.S. Tan, A.B.H. Choo, S.K. Lim, Mesenchymal stem cells secrete immunologically active exosomes, Stem Cells Dev. 23 (2014), https://doi.org/10.1089/scd.2013.0479.
[56] J.K. Lee, S.R. Park, B.K. Jung, Y.K. Jeon, Y.S. Lee, M.K. Kim, Y.G. Kim, J.Y. Jang, C.W. Kim, Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0084256.
[57] C. Xue, X. Li, L. Ba, M. Zhang, Y. Yang, Y. Gao, Z. Sun, Q. Han, R.C. Zhao, Msc-derived exosomes can enhance the angiogenesis of human brain mecs and show therapeutic potential in a mouse model of parkinson's disease, Aging Dis. 12 (2021), https://doi.org/10.14336/AD.2020.1221.
[58] F. Arslan, R.C. Lai, M.B. Smeets, L. Akeroyd, A. Choo, E.N. Aguor, L. Timmers, H. V. van Rijen, P.A. Doevendans, G. Pasterkamp, S.K. Lim, Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury, Stem Cell Res. 10 (2013), https://doi.org/10.1016/j.scr.2013.01.002.
[59] Y. Song, H. Dou, X. Li, X. Zhao, Y. Li, D. Liu, J. Ji, F. Liu, L. Ding, Y. Ni, Y. Hou, Exosomal miR-146a contributes to the enhanced therapeutic efficacy of interleukin-1β-primed mesenchymal stem cells against sepsis, Stem Cells 35 (2017), https://doi.org/10.1002/stem.2564.
[60] X. He, Z. Dong, Y. Cao, H. Wang, S. Liu, L. Liao, Y. Jin, L. Yuan, B. Li, MSC-derived exosome promotes M2 polarization and enhances cutaneous wound healing, Stem Cells Int. (2019), https://doi.org/10.1155/2019/7132708.
[61] S. Munich, A. Sobo-Vujanovic, W.J. Buchser, D. Beer-Stolz, N.L. Vujanovic, Dendritic cell exosomes directly kill tumor cells and activate natural killer cells via TNF superfamily ligands, OncoImmunology 1 (2012), https://doi.org/10.4161/onci.20897.
[62] J.M. Pitt, F. André, S. Amigorena, J.C. Soria, A. Eggermont, G. Kroemer, L. Zitvogel, Dendritic cell-derived exosomes for cancer therapy, J. Clin. Investig. 126 (2016), https://doi.org/10.1172/jci81137.
[63] B. Besse, M. Charrier, V. Lapierre, E. Dansin, O. Lantz, D. Planchard, T. Le Chevalier, A. Livartoski, F. Barlesi, A. Laplanche, S. Ploix, Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC, OncoImmunology 5 (2016), https://doi.org/10.1080/2162402X.2015.1071008.
[64] Y. Wang, M. Zhao, S. Liu, J. Guo, Y. Lu, J. Cheng, J. Liu, Macrophage-derived extracellular vesicles: diverse mediators of pathology and therapeutics in multiple diseases, Cell Death Dis. 11 (2020), https://doi.org/10.1038/s41419-020-03127-z.
[65] L. Cheng, Y. Wang, L. Huang, Exosomes from M1-polarized macrophages potentiate the cancer vaccine by creating a pro-inflammatory microenvironment in the lymph node, Mol. Ther. 25 (2017), https://doi.org/10.1016/j.ymthe.2017.02.007.
[66] S. Kamerkar, V.S. LeBleu, H. Sugimoto, S. Yang, C.F. Ruivo, S.A. Melo, J.J. Lee, Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, Nat. 546 (2017), https://doi.org/10.1038/nature22341.
[67] Y. Dai, S. Wang, S. Chang, D. Ren, S. Shali, C. Li, H. Yang, Z. Huang, J. Ge, M2 macrophage-derived exosomes carry microRNA-148a to alleviate myocardial ischemia/reperfusion injury via inhibiting TXNIP and the TLR4/NF-κB/NLRP3 inflammasome signaling pathway, J. Mol. Cell. Cardiol. 142 (2020), https://doi.org/10.1016/j.yjmcc.2020.02.007.
[68] R. Rahbarghazi, N. Jabbari, N.A. Sani, R. Asghari, L. Salimi, S.A. Kalashani, M. Feghhi, T. Etemadi, E. Akbariazar, M. Mahmoudi, J. Rezaie, Tumor-derived extracellular vesicles: Reliable tools for cancer diagnosis and clinical applications, Cell Commun. Signal. 17 (2019), https://doi.org/10.1186/s12964-019-0390-y.
[69] C. Aslan, S. Maralbashi, F. Salari, H. Kahroba, F. Sigaroodi, T. Kazemi, P. Kharaziha, Tumor-derived exosomes: Implication in angiogenesis and antiangiogenesis cancer therapy, J. Cell. Physiol. 234 (2019), https://doi.org/10.1002/jcp.28374.
[70] W. Meng, Y. Hao, C. He, L. Li, G. Zhu, Exosome-orchestrated hypoxic tumor microenvironment, Mol. Cancer 18 (2019), https://doi.org/10.1186/s12943-019-0982-6.
[71] R. Gaballa, H.E. Ali, M.O. Mahmoud, J.S. Rhim, H.I. Ali, H.F. Salem, M. Saleem, M.A. Kandeil, S. Ambs, Z.Y. Abd Elmageed, Exosomes-mediated transfer of ITGA2 promotes migration and invasion of prostate cancer cells by inducing epithelial-mesenchymal transition, Cancers 12 (2020), https://doi.org/10.3390/cancers12082300.
[72] L. Cheng, S. Wang, C. Peng, X. Zou, C. Yang, H. Mei, C. Li, X. Su, N. Xiao, Human umbilical cord mesenchymal stem cells for psoriasis: a phase 1/2a, single-arm

study, Signal Transduct. Targeted Ther. 7 (2022), https://doi.org/10.1038/s41392-022-01059-y.
[73] J. Yang, X. Zhang, X. Chen, L. Wang, G. Yang, Exosome mediated delivery of miR-124 promotes neurogenesis after ischemia, Mol. Ther. Nucleic Acids 7 (2017), https://doi.org/10.1016/j.omtn.2017.04.010.
[74] S.H. Ahn, S.W. Ryu, H. Choi, S. You, J. Park, C. Choi, Manufacturing therapeutic exosomes: from bench to industry, Mol. Cell 45 (2022), https://doi.org/10.14348/molcells.2022.2033.
[75] W.Z. Liu, Z.J. Ma, X.W. Kang, Current status and outlook of advances in exosome isolation, Anal. Bioanal. Chem. 414 (2022), https://doi.org/10.1007/s00216-022-04253-7.
[76] M.Y. Konoshenko, E.A. Lekchnov, A.V. Vlassov, P.P. Laktionov, Isolation of extracellular vesicles: general methodologies and latest trends, Biomed. Res. Int. (2018), https://doi.org/10.1155/2018/8545347.
[77] J. Stam, S. Bartel, R. Bischoff, J.C. Wolters, Isolation of extracellular vesicles with combined enrichment methods, J. Chromatogr. B 1169 (2021), https://doi.org/10.1016/j.jchromb.2021.122604.
[78] R.J. Lobb, M. Becker, S. Wen Wen, C.S. Wong, A.P. Wiegmans, A. Leimgruber, A. Möller, Optimized exosome isolation protocol for cell culture supernatant and human plasma, J. Extracell. Vesicles 4 (2015), https://doi.org/10.3402/jev.v4.27031.
[79] D.K. Jeppesen, M.L. Hvam, B. Primdahl-Bengtson, A.T. Boysen, B. Whitehead, L. Dyrskjøt, T.F. Ørntoft, K.A. Howard, M.S. Ostenfeld, Comparative analysis of discrete exosome fractions obtained by differential centrifugation, J. Extracell. Vesicles 3 (2014), https://doi.org/10.3402/jev.v3.25011.
[80] M. Rosado, R. Silva, M.G. Bexiga, J.G. Jones, B. Manadas, S.I. Anjo, Advances in biomarker detection: Alternative approaches for blood-based biomarker detection, Adv. Clin. Chem. 92 (2019), https://doi.org/10.1016/bs.acc.2019.04.003.
[81] K. Sidhom, P.O. Obi, A. Saleem, A review of exosomal isolation methods: Is size exclusion chromatography the best option? Int. J. Mol. Sci. 21 (2020) https://doi.org/10.3390/ijms21186466.
[82] Y. Weng, Z. Sui, Y. Shan, Y. Hu, Y. Chen, L. Zhang, Y. Zhang, Effective isolation of exosomes with polyethylene glycol from cell culture supernatant for in-depth proteome profiling, Anal. 141 (2016), https://doi.org/10.1039/c6an00892e.
[83] G. Ströhle, J. Gan, H. Li, Affinity-based isolation of extracellular vesicles and the effects on downstream molecular analysis, Anal. Bioanal. Chem. 414 (2022), https://doi.org/10.1007/s00216-022-04178-1.
[84] X. Yu, L. He, M. Pentok, H. Yang, Y. Yang, Z. Li, N. He, Y. Deng, S. Li, T. Liu, X. Chen, An aptamer-based new method for competitive fluorescence detection of exosomes, Nanoscale 11 (2019), https://doi.org/10.1039/c9nr04050a.
[85] J.C. Contreras-Naranjo, H.J. Wu, V.M. Ugaz, Microfluidics for exosome isolation and analysis: Enabling liquid biopsy for personalized medicine, Lab Chip 17 (2017), https://doi.org/10.1039/c7lc00592j.
[86] S. Estes, K. Konstantinov, J.D. Young, Manufactured extracellular vesicles as human therapeutics: challenges, advances, and opportunities, Curr. Opin. Biotechnol. 77 (2022), https://doi.org/10.1016/j.copbio.2022.102776.
[87] Y.S. Chen, E.Y. Lin, T.W. Chiou, H.J. Harn, Exosomes in clinical trial and their production in compliance with good manufacturing practice, Tzu Chi Med. J. 32 (2020), https://doi.org/10.4103/tcmj.tcmj_182_19.
[88] E. Bari, S. Perteghella, L. Catenacci, M. Sorlini, S. Croce, M. Mantelli, M. A. Avanzini, M. Sorrenti, M.L. Torre, Freeze-dried and GMP-compliant pharmaceuticals containing exosomes for acellular mesenchymal stromal cell immunomodulant therapy, Nanomed. 14 (2019), https://doi.org/10.2217/nnm-2018-0240.
[89] S. Busatto, G. Vilanilam, T. Ticer, W.L. Lin, D.W. Dickson, S. Shapiro, P. Bergese, J. Wolfram, Tangential flow filtration for highly efficient concentration of extracellular vesicles from large volumes of fluid, Cells 7 (2018), https://doi.org/10.3390/cells7120273.
[90] F. Villa, R. Quarto, R. Tasso, Extracellular vesicles as natural, safe and efficient drug delivery systems, Pharmaceutics 11 (2019), https://doi.org/10.3390/pharmaceutics11110557.
[91] P. Vader, E.A. Mol, G. Pasterkamp, R.M. Schiffelers, Extracellular vesicles for drug delivery, Adv. Drug Deliv. Rev. 106 (2016), https://doi.org/10.1016/j.addr.2016.02.006.
[92] J. Song, B. Song, L. Yuan, G. Yang, Multiplexed strategies toward clinical translation of extracellular vesicles, Theranostics 12 (2022), https://doi.org/10.7150/thno.75899.
[93] D. Gupta, A.M. Zickler, S. El Andaloussi, Dosing extracellular vesicles, Adv. Drug Deliv. Rev. 178 (2021), https://doi.org/10.1016/j.addr.2021.113961.
[94] Z. Wan, L. Zhao, F. Lu, X. Gao, Y. Dong, Y. Zhao, M. Wei, G. Yang, C. Xing, L. Liu, Mononuclear phagocyte system blockade improves therapeutic exosome delivery to the myocardium, Theranostics 10 (2020), https://doi.org/10.7150/thno.38198.
[95] L.B. James-Allan, F.J. Rosario, K. Barner, A. Lai, D. Guanzon, H.D. McIntyre, M. Lappas, T.L. Powell, C. Salomon, T. Jansson, Regulation of glucose homeostasis by small extracellular vesicles in normal pregnancy and in gestational diabetes, FASEB J. 34 (2020), https://doi.org/10.1096/fj.201902522rr.
[96] W. Luo, Y. Dai, Z. Chen, X. Yue, K.C. Andrade-Powell, J. Chang, Spatial and temporal tracking of cardiac exosomes in mouse using a nano-luciferase-CD63 fusion protein, Commun. Biol. 3 (2020), https://doi.org/10.1038/s42003-020-0830-7.
[97] O.P. Wiklander, J.Z. Nordin, A. O'Loughlin, Y. Gustafsson, G. Corso, I. Mäger, P. Vader, Y. Lee, H. Sork, Y. Seow, N. Heldring, Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting, J. Extracell. Vesicles 4 (2015), https://doi.org/10.3402/jev.v4.26316.
[98] D. Sun, X. Zhuang, X. Xiang, Y. Liu, S. Zhang, C. Liu, S. Barnes, W. Grizzle, D. Miller, H.G. Zhang, A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes, Mol. Ther. 18 (2010), https://doi.org/10.1038/mt.2010.105.
[99] A.K. Riau, H.S. Ong, G.H. Yam, J.S. Mehta, Sustained delivery system for stem cell-derived exosomes, Front. Pharmacol. 10 (2019), https://doi.org/10.3389/fphar.2019.01368.
[100] L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal, M.J. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, Nat. Biotechnol. 29 (2011), https://doi.org/10.1038/nbt.1807.
[101] S. El-Andaloussi, Y. Lee, S. Lakhal-Littleton, J. Li, Y. Seow, C. Gardiner, L. Alvarez-Erviti, I.L. Sargent, M.J. Wood, Exosome-mediated delivery of siRNA in vitro and in vivo, Nat. Protoc. 7 (2012), https://doi.org/10.1038/nprot.2012.131.
[102] Y. Tian, S. Li, J. Song, T. Ji, M. Zhu, G.J. Anderson, J. Wei, G. Nie, A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy, Biomater. 35 (2014), https://doi.org/10.1016/j.biomaterials.2013.11.083.
[103] G. Liang, S. Kan, Y. Zhu, S. Feng, W. Feng, S. Gao, Engineered exosome-mediated delivery of functionally active miR-26a and its enhanced suppression effect in HepG2 cells, Int. J. Nanomedicine 13 (2018), https://doi.org/10.2147/ijn.s154458.
[104] Q. Cheng, X. Shi, M. Han, G. Smbatyan, H.J. Lenz, Y. Zhang, Reprogramming exosomes as nanoscale controllers of cellular immunity, J. Am. Chem. Soc. 140 (2018), https://doi.org/10.1021/jacs.8b10047.
[105] T. Smyth, K. Petrova, N.M. Payton, I. Persaud, J.S. Redzic, M.W. Graner, P. Smith-Jones, T.J. Anchordoquy, Surface functionalization of exosomes using click chemistry, Bioconjug. Chem. 5 (2014), https://doi.org/10.1021/bc500291r.
[106] G. Jia, Y. Han, Y. An, Y. Ding, C. He, X. Wang, Q. Tang, NRP-1 targeted and cargo-loaded exosomes facilitate simultaneous imaging and therapy of glioma in vitro and in vivo, Biomater. 178 (2018), https://doi.org/10.1016/j.biomaterials.2018.06.029.
[107] E. Koh, E.J. Lee, G.H. Nam, Y. Hong, E. Cho, Y. Yang, I.S. Kim, Exosome-SIRPα, a CD47 blockade increases cancer cell phagocytosis, Biomater. 121 (2017), https://doi.org/10.1016/j.biomaterials.2017.01.004.
[108] X.Y. Ben, Y.R. Wang, H.H. Zheng, D.X. Li, R. Ren, P.L. Ni, H.Y. Zhang, R.J. Feng, Y.Q. Li, Q.F. Li, X.N. Yi, Construction of exosomes that overexpress CD47 and evaluation of their immune escape, Front. Bioeng. Biotechnol. 10 (2022), https://doi.org/10.3389/fbioe.2022.936951.
[109] S.A.A. Kooijmans, L.A.L. Fliervoet, R. van Der Meel, M.H.A.M. Fens, H.F. G. Heijnen, P.V.B. en Henegouwen, P.C.V.V. Vader, R.M., Schiffelers, PEGylated and targeted extracellular vesicles display enhanced cell specificity and circulation time, J. Control. Release 224 (2016), https://doi.org/10.1016/j.jconrel.2016.01.009.
[110] M.S. Kim, M.J. Haney, Y. Zhao, D. Yuan, I. Deygen, N.L. Klyachko, A.V. Kabanov, E.V. Batrakova, Engineering macrophage-derived exosomes for targeted paclitaxel delivery to pulmonary metastases: in vitro and in vivo evaluations, Nanomed. Nanotechnol. Biol. Med. 14 (2018), https://doi.org/10.1016/j.nano.2017.09.011.
[111] X. Li, Y. Wang, L. Shi, B. Li, J. Li, Z. Wei, H. Lv, L. Wu, H. Zhang, B. Yang, X. Xu, Magnetic targeting enhances the cutaneous wound healing effects of human mesenchymal stem cell-derived iron oxide exosomes, J. Nanobiotechnology 18 (2020), https://doi.org/10.1186/s12951-020-00670-x.
[112] L. Liu, Y. Li, H. Peng, R. Liu, W. Ji, Z. Shi, J. Shen, G. Ma, X. Zhang, Targeted exosome coating gene-chem nanocomplex as "nanoscavenger" for clearing α-synuclein and immune activation of Parkinson's disease, Sci. Adv. 6 (2020), https://doi.org/10.1126/sciadv.aba3967.
[113] G. You, Y. Kim, J.H. Lee, J. Song, H. Mok, Exosome-modified PLGA microspheres for improved internalization into dendritic cells and macrophages, Biotechnol. Bioprocess Eng. 25 (2020), https://doi.org/10.1007/s12257-020-0008-7.
[114] W.B. Swanson, Z. Zhang, K. Xiu, T. Gong, M. Eberle, Z. Wang, P.X. Ma, Scaffolds with controlled release of pro-mineralization exosomes to promote craniofacial bone healing without cell transplantation, Acta Biomater. 118 (2020), https://doi.org/10.1016/j.actbio.2020.09.052.
[115] S.I. Sawada, Y.T. Sato, R. Kawasaki, J.I. Yasuoka, R. Mizuta, Y. Sasaki, K. Akiyoshi, Nanogel hybrid assembly for exosome intracellular delivery: effects on endocytosis and fusion by exosome surface polymer engineering, Biomater. Sci. 8 (2020), https://doi.org/10.1039/C9BM01232J.
[116] R. Mizuta, Y. Sasaki, R. Kawasaki, K. Katagiri, S.I. Sawada, S.A. Mukai, K. Akiyoshi, Magnetically navigated intracellular delivery of extracellular vesicles using amphiphilic nanogels, Bioconjug. Chem. 30 (2019), https://doi.org/10.1021/acs.bioconjchem.9b00369.
[117] A. Bregy, A.H. Shah, M.V. Diaz, H.E. Pierce, P.L. Ames, D. Diaz, R.J. Komotar, The role of Gliadel wafers in the treatment of high-grade gliomas, Expert. Rev. Anticancer. Ther. 13 (2013), https://doi.org/10.1586/14737140.2013.840090.
[118] P.E. Sirinek, M.M. Lin, Intracameral sustained release bimatoprost implants (Durysta), Semin. Ophthalmol. 37 (2021), https://doi.org/10.1080/08820538.2021.1985145.
[119] S.L. Cusack, M. Jaros, M. Kuss, H.S. Minkowitz, P. Winkle, L. Hemsen, Clinical evaluation of XaraColl®, a bupivacaine-collagen implant, for postoperative analgesia in two multicenter, randomized, double-blind, placebo-controlled pilot studies, J. Pain Res. (2012), https://doi.org/10.2147/jpr.s33453.
[120] H. Mahmud, T.R. Ahmad, J.A. Gonzales, J.M. Stewart, Efficacy of the Fluocinolone Acetonide (Yutiq) intravitreal implant as monotherapy for uveitis,

Ocul. Immunol. Inflamm. (2022), https://doi.org/10.1080/09273948.2022.2076131.
[121] E.D. Deeks, Exenatide extended-release pre-filled pen (Bydureon® BCise®): a profile of its use', Drugs Ther. Perspect. 35 (2019) https://doi.org/10.1007/s40267-018-00597-2.
[122] J.H. Lee, Injectable hydrogels delivering therapeutic agents for disease treatment and tissue engineering, Biomater. Res. 22 (2018), https://doi.org/10.1186/s40824-018-0138-6.
[123] G. Deng, F. Li, H. Yu, F. Liu, C. Liu, W. Sun, H. Jiang, Y. Chen, Dynamic hydrogels with an environmental adaptive self-healing ability and dual responsive sol–gel transitions, ACS Macro Lett. 1 (2012), https://doi.org/10.1021/mz200195n.
[124] J. Li, D.J. Mooney, Designing hydrogels for controlled drug delivery, Nat. Rev. Mater. 1 (2016), https://doi.org/10.1038/natrevmats.2016.71.
[125] W.E. Hennink, C.F. van Nostrum, Novel crosslinking methods to design hydrogels, Adv. Drug Deliv. Rev. 64 (2012), https://doi.org/10.1016/j.addr.2012.09.009.
[126] Y. Ju, Y. Hu, P. Yang, X. Xie, B. Fang, Extracellular vesicle-loaded hydrogels for tissue repair and regeneration, Mater. Today Bio (2022), https://doi.org/10.1016/j.mtbio.2022.100522.
[127] A. Vedadghavami, F. Minooei, M.H. Mohammadi, S. Khetani, A.R. Kolahchi, S. Mashayekhan, A. Sanati-Nezhad, Manufacturing of hydrogel biomaterials with controlled mechanical properties for tissue engineering applications, Acta Biomater. 62 (2017), https://doi.org/10.1016/j.actbio.2017.07.028.
[128] F. Rizzo, N.S. Kehr, Recent advances in injectable hydrogels for controlled and local drug delivery, Adv. Healthc. Mater. 10 (2021), https://doi.org/10.1002/adhm.202001341.
[129] J. Siepmann, F. Siepmann, Modeling of diffusion controlled drug delivery, J. Control. Release 161 (2012), https://doi.org/10.1016/j.jconrel.2011.10.006.
[130] B. Amsden, Solute diffusion within hydrogels. Mechanisms and models, Macromolecules 31 (1998), https://doi.org/10.1021/ma980765f.
[131] C.C. Huang, M. Kang, S. Shirazi, Y. Lu, L.F. Cooper, P. Gajendrareddy, S. Ravindran, 3D Encapsulation and tethering of functionally engineered extracellular vesicles to hydrogels, Acta Biomater. 126 (2021), https://doi.org/10.1016/j.actbio.2021.03.030.
[132] N. Kaushal, M. Singh, Fabrication and characterization of a bilayered system enabling sustained release of bioflavonoids derived from mandarin biomass, Food Hydrocoll. Health 3 (2023), https://doi.org/10.1016/j.fhfh.2022.100114.
[133] H. Haidari, Z. Kopecki, A.T. Sutton, S. Garg, A.J. Cowin, K. Vasilev, pH-responsive "smart" hydrogel for controlled delivery of silver nanoparticles to infected wounds, Antibiot. 10 (2021), https://doi.org/10.3390/antibiotics10010049.
[134] M. Kanamala, W.R. Wilson, M. Yang, B.D. Palmer, Z. Wu, Mechanisms and biomaterials in pH-responsive tumour targeted drug delivery: A review, Biomater. 85 (2016), https://doi.org/10.1016/j.biomaterials.2016.01.061.
[135] H.J. Kong, D. Kaigler, K. Kim, D.J. Mooney, Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution, Biomacromolecules 5 (2004), https://doi.org/10.1021/bm049879r.
[136] M. Ishihara, K. Obara, T. Ishizuka, M. Fujita, M. Sato, K. Masuoka, Y. Saito, H. Yura, T. Matsui, H. Hattori, M. Kikuchi, Controlled release of fibroblast growth factors and heparin from photocrosslinked chitosan hydrogels and subsequent effect on in vivo vascularization, J. Biomed. Mater. Res. A 64 (2003), https://doi.org/10.1002/jbm.a.10427.
[137] G. Kwak, J. Cheng, H. Kim, S. Song, S.J. Lee, Y. Yang, J.H. Jeong, J.E. Lee, P. B. Messersmith, S.H. Kim, Sustained Exosome-Guided Macrophage Polarization Using Hydrolytically Degradable PEG Hydrogels for Cutaneous Wound Healing: Identification of Key Proteins and MiRNAs, and Sustained Release Formulation, Small 18 (2022), https://doi.org/10.1002/smll.202200060.
[138] M. Toth, R. Fridman, Assessment of gelatinases (MMP-2 and MMP-9) by gelatin zymography, Metastasis Res. Protoc. (2003), https://doi.org/10.1385/1-59259-136-1:163.
[139] J. Tang, X. Cui, Z. Zhang, Y. Xu, J. Guo, B.G. Soliman, Y. Lu, Z. Qin, Q. Wang, H. Zhang, K.S. Lim, Injection-free delivery of MSC-derived extracellular vesicles for myocardial infarction therapeutics, Adv. Healthc. Mater. 11 (2022), https://doi.org/10.1002/adhm.202100312.
[140] C. Wang, M. Wang, T. Xu, X. Zhang, C. Lin, W. Gao, H. Xu, B. Lei, C. Mao, Engineering bioactive self-healing antibacterial exosomes hydrogel for promoting chronic diabetic wound healing and complete skin regeneration, Theranostics 9 (2019), https://doi.org/10.7150/thno.29766.
[141] H. Henriques-Antunes, R.M. Cardoso, A. Zonari, J. Correia, E.C. Leal, A. Jimenez-Balsa, M.M. Lino, A. Barradas, I. Kostic, C. Gomes, J.M. Karp, The kinetics of small extracellular vesicle delivery impacts skin tissue regeneration, ACS Nano 13 (2019), https://doi.org/10.1021/acsnano.9b00376.
[142] Y. Zhang, Y. Xie, Z. Hao, P. Zhou, P. Wang, S. Fang, L. Li, S. Xu, Y. Xia, Umbilical mesenchymal stem cell-derived exosome-encapsulated hydrogels accelerate bone repair by enhancing angiogenesis, ACS Appl. Mater. Interfaces 13 (2021), https://doi.org/10.1021/acsami.0c22671.
[143] W.J. Han, J.H. Lee, J.K. Lee, H.J. Choi, Remote-controllable, tough, ultrastretchable, and magneto-sensitive nanocomposite hydrogels with homogeneous nanoparticle dispersion as biomedical actuators, and their tuned structure, properties, and performances, Compos. B. Eng. 236 (2022), https://doi.org/10.1016/j.cimpositesb.2022.109802.
[144] X. Han, J. Shen, S. Chen, Z. Cai, Y. Zhu, W. Yi, K. Li, W. Cai, B. Tao, W. Cui, D. Bai, Ultrasonic-controlled "explosive" hydrogels to precisely regulate spatiotemporal osteoimmune disturbance, Biomater. 295 (2023), https://doi.org/10.1016/j.biomaterials.2023.122057.
[145] G.J. Crasto, N. Kartner, N. Reznik, M.V. Spatafora, H. Chen, R. Williams, P. N. Burns, C. Clokie, M.F. Manolson, S.A. Peel, Controlled bone formation using ultrasound-triggered release of BMP-2 from liposomes, J. Control. Release 243 (2016), https://doi.org/10.1016/j.jconrel.2016.09.032.
[146] T. Kubota, Y. Kurashina, J. Zhao, K. Ando, H. Onoe, Ultrasound-triggered on-demand drug delivery using hydrogel microbeads with release enhancer, Mater. Des. 203 (2021), https://doi.org/10.1016/j.matdes.2021.109580.
[147] S.R. Sirsi, M.A. Borden, State-of-the-art materials for ultrasound-triggered drug delivery, Adv. Drug Deliv. Rev. 72 (2014), https://doi.org/10.1016/j.addr.2013.12.010.
[148] Q. Yang, G.K. Nanayakkara, C. Drummer, Y. Sun, C. Johnson, R. Cueto, H. Fu, Y. Shao, L. Wang, W.Y. Yang, P. Tang, Low-intensity ultrasound-induced anti-inflammatory effects are mediated by several new mechanisms including gene induction, immunosuppressor cell promotion, and enhancement of exosome biogenesis and docking, Front. Physiol. 8 (2017), https://doi.org/10.3389/fphys.2017.00818.
[149] N. Huebsch, C.J. Kearney, X. Zhao, J. Kim, C.A. Cezar, Z. Suo, D.J. Mooney, Ultrasound-triggered disruption and self-healing of reversibly cross-linked hydrogels for drug delivery and enhanced chemotherapy, Proc. Natl. Acad. Sci. 111 (2014), https://doi.org/10.1073/pnas.1405469111.
[150] P. Khayambashi, J. Iyer, S. Pillai, A. Upadhyay, Y. Zhang, S.D. Tran, Hydrogel encapsulation of mesenchymal stem cells and their derived exosomes for tissue engineering, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22020684.
[151] V. Thomas, M.M. Yallapu, B. Sreedhar, S.K. Bajpai, Breathing-in/breathing-out approach to preparing nanosilver-loaded hydrogels: Highly efficient antibacterial nanocomposites, J. Appl. Polym. Sci. 111 (2009), https://doi.org/10.1002/app.29018.
[152] A. Chen, H. Tian, N. Yang, Z. Zhang, G.Y. Yang, W. Cui, Y. Tang, Towards extracellular vesicle delivery systems for tissue regeneration: material design at the molecular level, Extracell. Vesicles Circ. Nucl. Acids 3 (2022), https://doi.org/10.20517/evcna.2022.37.
[153] L. Fan, C. Liu, X. Chen, L. Zheng, Y. Zou, H. Wen, P. Guan, F. Lu, Y. Luo, G. Tan, P. Yu, Exosomes-loaded Electroconductive hydrogel synergistically promotes tissue repair after spinal cord injury via Immunoregulation and enhancement of myelinated axon growth, Adv. Sci. 9 (2022), https://doi.org/10.1002/advs.202105586.
[154] J.Q. Gerlach, M.D. Griffin, Getting to know the extracellular vesicle glycome, Mol. BioSyst. 12 (2016), https://doi.org/10.1039/c5mb00835b.
[155] N. Seo, J. Nakamura, T. Kaneda, H. Tateno, A. Shimoda, T. Ichiki, K. Furukawa, J. Hirabayashi, K. Akiyoshi, H. Shiku, Distinguishing functional exosomes and other extracellular vesicles as a nucleic acid cargo by the anion-exchange method, J. Extracell. Vesicles 11 (2022), https://doi.org/10.1002/jev2.12205.
[156] S.C. Tao, S.C. Guo, M. Li, Q.F. Ke, Y.P. Guo, C.Q. Zhang, Chitosan wound dressings incorporating exosomes derived from microRNA-126-overexpressing synovium mesenchymal stem cells provide sustained release of exosomes and heal full-thickness skin defects in a diabetic rat model, Stem Cells Transl. Med. 6 (2017), https://doi.org/10.5966/sctm.2016-0275.
[157] M. Wang, C. Wang, M. Chen, Y. Xi, W. Cheng, C. Mao, T. Xu, X. Zhang, C. Lin, W. Gao, Y. Guo, Efficient angiogenesis-based diabetic wound healing/skin reconstruction through bioactive antibacterial adhesive ultraviolet shielding nanodressing with exosome release, ACS Nano 13 (2019), https://doi.org/10.1021/acsnano.9b03656.
[158] C.C. Huang, R. Narayanan, S. Alapati, S. Ravindran, Exosomes as biomimetic tools for stem cell differentiation: applications in dental pulp tissue regeneration, Biomater. 111 (2016), https://doi.org/10.1016/j.biomaterials.2016.09.029.
[159] L. Li, Y. Zhang, J. Mu, J. Chen, C. Zhang, H. Cao, J. Gao, Transplantation of human mesenchymal stem-cell-derived exosomes immobilized in an adhesive hydrogel for effective treatment of spinal cord injury, Nano Lett. 20 (2020), https://doi.org/10.1021/acs.nanolett.0c00929.
[160] B. Dorj, J.E. Won, O. Purevdorj, K.D. Patel, J.H. Kim, E.J. Lee, H.W. Kim, A novel therapeutic design of microporous-structured biopolymer scaffolds for drug loading and delivery, Acta Biomater. 10 (2014), https://doi.org/10.1016/j.actbio.2013.11.002.
[161] H.W. Kang, S.J. Lee, I.K. Ko, C. Kengla, J.J. Yoo, A. Atala, A 3D bioprinting system to produce human-scale tissue constructs with structural integrity, Nat. Biotechnol. 34 (2016), https://doi.org/10.1038/nbt.3413.
[162] T. Weigel, G. Schinkel, A. Lendlein, Design and preparation of polymeric scaffolds for tissue engineering, Expert Rev. Med. Dev. 3 (2006), https://doi.org/10.1586/17434440.3.6.835.
[163] J. Zhang, X. Liu, H. Li, C. Chen, B. Hu, X. Niu, Q. Li, B. Zhao, Z. Xie, Y. Wang, Exosomes/tricalcium phosphate combination scaffolds can enhance bone regeneration by activating the PI3K/Akt signaling pathway, Stem Cell Res Ther 7 (2016), https://doi.org/10.1186/s13287-016-0391-3.
[164] W. Li, Y. Liu, P. Zhang, Y. Tang, M. Zhou, W. Jiang, X. Zhang, G. Wu, G.Y. Zhou, Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration, ACS Appl. Mater. Interfaces 10 (2018), https://doi.org/10.1021/acsami.7b17620.
[165] Y. Gao, Z. Yuan, X. Yuan, Z. Wan, Y. Yu, Q. Zhan, Y. Zhao, J. Han, J. Huang, C. Xiong, Q. Cai, Bioinspired porous microspheres for sustained hypoxic exosomes release and vascularized bone regeneration, Bioact. Mater. 14 (2022), https://doi.org/10.1016/j.bioactmat.2022.01.041.
[166] Z. Ma, M. Kotaki, R. Inai, S. Ramakrishna, Potential of nanofiber matrix as tissue-engineering scaffolds, Tissue Eng. 11 (2005), https://doi.org/10.1089/ten.2005.11.101.
[167] K. Németh, A. Kazsoki, T. Visnovitz, B. Pinke, L. Mészáros, E.I. Buzás, R. Zelkó, Nanofiber formation as a promising technology for preservation and easy storage of extracellular vesicles, Sci. Rep. 12 (2022), https://doi.org/10.1038/s41598-022-25916-6.

[168] A. Eatemadi, H. Daraee, N. Zarghami, H. Melat Yar, A. Akbarzadeh, Nanofiber: Synthesis and biomedical applications, Artif. Cells Nanomed, Biotechnol. 44 (2016), https://doi.org/10.3109/21691401.2014.922568.
[169] Z. Mohammadalizadeh, E. Bahremandi-Toloue, S. Karbasi, Recent advances in modification strategies of pre- and post-electrospinning of nanofiber scaffolds in tissue engineering, React. Funct. Polym. 172 (2022), https://doi.org/10.1016/j.reactfunctpolym.2022.105202.
[170] J. Xue, T. Wu, Y. Dai, Y. Xia, Electrospinning and electrospun nanofibers: methods, materials, and applications, Chem. Rev. 119 (2019), https://doi.org/10.1021/acs.chemrev.8b00593.
[171] A. Mickova, M. Buzgo, O. Benada, M. Rampichova, Z. Fisar, E. Filova, M. Tesarova, D. Lukas, E. Amler, Core/shell nanofibers with embedded liposomes as a drug delivery system, Biomacromolecules 13 (2012), https://doi.org/10.1021/bm2018118.
[172] N. Su, Y. Hao, F. Wang, W. Hou, H. Chen, Y. Luo, Mesenchymal stromal exosome–functionalized scaffolds induce innate and adaptive immunomodulatory responses toward tissue repair, Sci. Adv. 7 (2021), https://doi.org/10.1126/sciadv.abf7207.
[173] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Mussel-inspired surface chemistry for multifunctional coatings, Sci. 318 (2007), https://doi.org/10.1126/science.1147241.
[174] J. Li, S. Yan, W. Han, Z. Dong, J. Li, Q. Wu, X. Fu, Phospholipid-grafted PLLA electrospun micro/nanofibers immobilized with small extracellular vesicles from rat adipose mesenchymal stem cells promote wound healing in diabetic rats, Regen. Biomater. 9 (2022), https://doi.org/10.1093/rb/rbac071.
[175] P. Chen, L. Zheng, Y. Wang, M. Tao, Z. Xie, C. Xia, C. Gu, J. Chen, P. Qiu, S. Mei, L. Ning, Desktop-stereolithography 3D printing of a radially oriented extracellular matrix/mesenchymal stem cell exosome bioink for osteochondral defect regeneration, Theranostics 9 (2019), https://doi.org/10.7150/thno.31017.
[176] Y. Sun, B. Zhang, D. Zhai, C. Wu, Three-dimensional printing of bioceramic-induced macrophage exosomes: immunomodulation and osteogenesis/angiogenesis, NPG Asia Mater. 13 (2021), https://doi.org/10.1038/s41427-021-00340-w.
[177] Y. Zha, Y. Li, T. Lin, J. Chen, S. Zhang, J. Wang, Progenitor cell-derived exosomes endowed with VEGF plasmids enhance osteogenic induction and vascular remodeling in large segmental bone defects, Theranostics 11 (2021), https://doi.org/10.7150/thno.50741.
[178] F. Diomede, A. Gugliandolo, P. Cardelli, I. Merciaro, V. Ettorre, T. Traini, R. Bedini, D. Scionti, A. Bramanti, A. Nanci, S. Caputi, Three-dimensional printed PLA scaffold and human gingival stem cell-derived extracellular vesicles: a new tool for bone defect repair, Stem Cell Res Ther 9 (2018), https://doi.org/10.1186/s13287-018-0850-0.
[179] M. Khansarizadeh, A. Mokhtarzadeh, M. Rashedinia, S.M. Taghdisi, P. Lari, K. H. Abnous, M. Ramezani, Identification of possible cytotoxicity mechanism of polyethylenimine by proteomics analysis, Hum. Exp. Toxicol. 35 (2016), https://doi.org/10.1177/0960327115591371.
[180] F. Wei, M. Li, R. Crawford, Y. Zhou, Y. Xiao, Exosome-integrated titanium oxide nanotubes for targeted bone regeneration, Acta Biomater. 86 (2019), https://doi.org/10.1016/j.actbio.2019.01.006.
[181] A. Zamiri, S. De, Mechanical properties of hydroxyapatite single crystals from nanoindentation data, J. Mech. Behav. Biomed. Mater. 4 (2011), https://doi.org/10.1016/j.jmbbm.2010.11.001.
[182] I. Qayoom, A.K. Teotia, A. Kumar, Nanohydroxyapatite based ceramic carrier promotes bone formation in a femoral neck canal defect in osteoporotic rats, Biomacromolecules 21 (2020), https://doi.org/10.1021/acs.biomac.9b01327.
[183] L. Liu, Y. Liu, C. Feng, J. Chang, R. Fu, T. Wu, F. Yu, X. Wang, L. Xia, C. Wu, B. Fang, Lithium-containing biomaterials stimulate bone marrow stromal cell-derived exosomal miR-130a secretion to promote angiogenesis, Biomater. 192 (2019), https://doi.org/10.1016/j.biomaterials.2018.11.007.
[184] V.S. de Viteri, E. Fuentes, Titanium and titanium alloys as biomaterials, Tribol. Fundam. Adv. 1 (2013), https://doi.org/10.5772/55860.
[185] Z. Zhang, R. Xu, Y. Yang, C. Liang, X. Yu, Y. Liu, T. Wang, Y. Yu, F. Deng, Micro/nano-textured hierarchical titanium topography promotes exosome biogenesis and secretion to improve osseointegration, J. Nanobiotechnology 19 (2021), https://doi.org/10.1186/s12951-021-00826-3.
[186] Z. Wu, P. Pu, Z. Su, X. Zhang, L. Nie, Y. Chang, Schwann Cell-derived exosomes promote bone regeneration and repair by enhancing the biological activity of porous Ti6Al4V scaffolds, Biochem. Biophys. Res. Commun. 531 (2020), https://doi.org/10.1016/j.bbrc.2020.07.094.
[187] S. He, L. Wu, X. Li, H. Sun, T. Xiong, J. Liu, C. Huang, H. Xu, H. Sun, W. Chen, R. Gref, Metal-organic frameworks for advanced drug delivery, Acta Pharm. Sin. B 11 (2021), https://doi.org/10.1016/j.apsb.2021.03.019.
[188] Y. Kang, C. Xu, X. Dong, M. Qi, D. Jiang, Exosome-functionalized magnesium-organic framework-based scaffolds with osteogenic, angiogenic and anti-inflammatory properties for accelerated bone regeneration, Bioact. Mater. 18 (2022), https://doi.org/10.1016/j.bioactmat.2022.02.012.
[189] A. Liu, D. Lin, H. Zhao, L. Chen, B. Cai, K. Lin, S.G. Shen, Optimized BMSC-derived osteoinductive exosomes immobilized in hierarchical scaffold via lyophilization for bone repair through Bmpr2/Acvr2b competitive receptor-activated Smad pathway, Biomater. 272 (2021), https://doi.org/10.1016/j.biomaterials.2021.120718.
[190] S.C. Tao, X.R. Li, W.J. Wei, Z.Y. Wei, C.R. Zhang, F. Wang, H. Dawes, S.C. Guo, Polymeric coating on β-TCP scaffolds provides immobilization of small extracellular vesicles with surface-functionalization and ZEB1-Loading for bone defect repair in diabetes mellitus, Biomater. 283 (2022), https://doi.org/10.1016/j.biomaterials.2022.121465.