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Review

EXOSOMES IN TISSUE ENGINEERING AND CELL-FREE THERAPY: A COMPREHENSIVE REVIEW

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Abstract

As a critical subclass of extracellular vesicles, exosomes have emerged as a research focus in tissue regeneration and precision therapy because of their unique molecular delivery and intercellular communication capabilities. This article systematically reviews the biogenesis, isolation, and characterization of exosomes and their role in advancing tissue engineering applications. Their multifaceted regulatory roles in bone/cartilage repair, neural regeneration, wound healing, and cardiovascular regeneration, including antiapoptotic, proangiogenic, immunomodulatory, and antifibrotic mechanisms, are highlighted. The innovations of this work lie in (1) the comprehensive analysis of engineered exosome strategies—such as surface modification, cargo-loading optimization, and synergistic integration with biomaterials—to overcome the limitations of traditional delivery systems; (2) the proposal of the dual regulatory potential of exosomes in cancer immunotherapy and autoimmune diseases, offering novel insights for clinical translation; and (3) the envisioning of future directions by integrating artificial intelligence (AI) and three-dimensional (3D) bioprinting to advance scalable production and precision design of exosome-based therapies. This article further addresses current challenges (e.g., heterogeneity, standardization, and safety) and emphasizes interdisciplinary collaboration to bridge the gap between fundamental research and clinical translation. This review provides a theoretical framework and technical foresight for advancing regenerative medicine and precision therapeutics.

Keywords: Exosomes, tissue regeneration, cell-free therapy.

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Introduction

The secretion of extracellular vesicles (EVs) was originally conceptualized as a cellular mechanism for eliminating obsolete molecules [1]. Based on their biogenic mechanisms, EVs can be systematically categorized into three major subtypes: exosomes derived from multivesicular bodies (MVBs), microvesicles directly shed from the plasma membrane, and apoptotic bodies released during programmed cell death [2]. A comparative visualization of the three subtypes is shown in Fig. 1, highlighting their key differences. Owing to the lack of universally established criteria for the absolute separation and characterization of EV subpopulations—based on size, biogenic pathways, or postrelease surface markers—we collectively refer to both exosomes and microvesicles as "EVs" in the context of drug delivery. Readers should note that the field of exosome research is rapidly evolving, and current definitions remain under refinement. Accordingly, this article uses the terms "exosomes" or "EVs" as contextually appropriate, acknowledging ongoing developments in the field [3]. Exosomes are an important subclass of EVs and are nanoscale particles that are encapsulated by lipid bilayers with diameters ranging from 30 to 150 nm [4]. The field of exosome research was inaugurated in 1983 when seminal studies first demonstrated that reticulocytes release transferrin receptor-enriched vesicles during erythroid differentiation—a discovery that later became recognized as the earliest documented evidence of exosome biogenesis [5–7]. The process of biogenesis begins with the inward budding of endosomal membranes, resulting in the formation of multi-

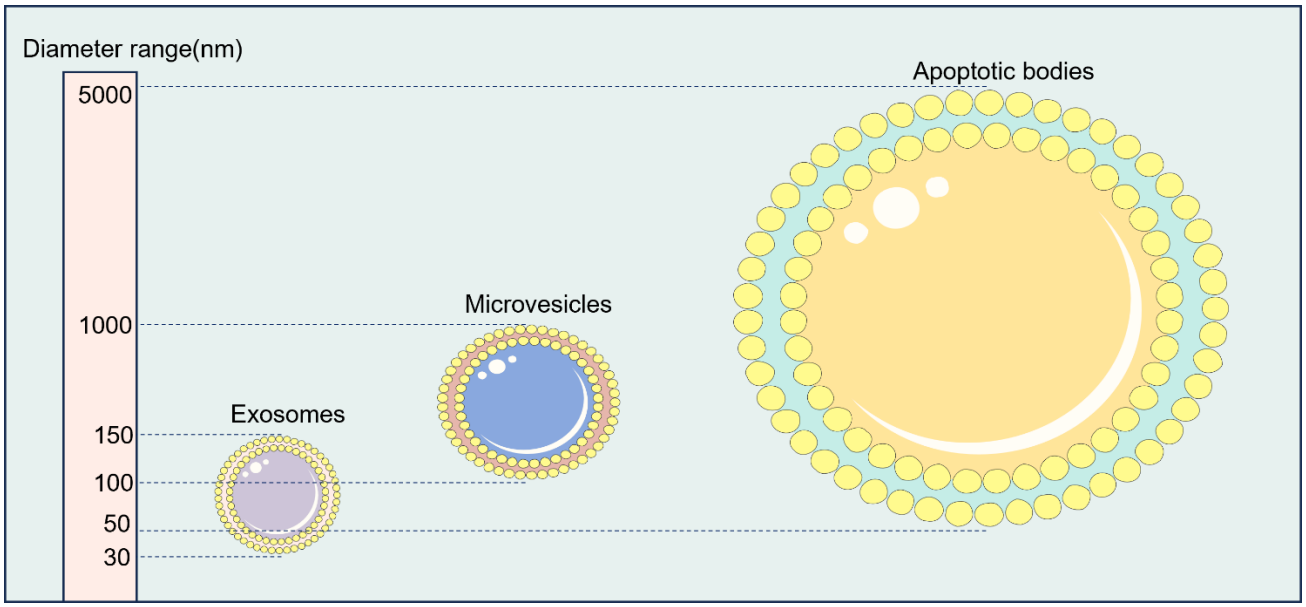

Fig. 1. EV subtypes. EV subtypes have been conventionally classified based on their physical dimensions. Currently, there is a lack of definitive molecular markers that can reliably discriminate between these EV subpopulations. Therefore, comprehensive characterization of isolated exosomes through multiple analytical approaches is strongly recommended prior to reporting any research findings about exosomes. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

vesicular bodies (MVBs) [8,9]. These MVBs subsequently fuse with the plasma membrane, releasing exosomes into the extracellular milieu [10]. During this process, exosomes are selectively loaded with a diverse array of molecular cargos, including proteins and nucleic acids (messenger RNAs (mRNAs), microRNAs (miRNAs), and long non-coding RNAs) [11]. These molecular constituents confer upon exosomes their fundamental ability to act as molecular ambassadors in cellular cross-talk and signalling transmission. Their specific biomolecular payload assembly is precisely coordinated through dynamic interactions between intracellular regulatory systems and signalling cascades [12,13]. This sophisticated regulation enables exosomes to execute targeted functional programs that orchestrate intercellular signalling networks and maintain physiological homeostasis.

The nanoscale dimensions and biomimetic properties of exosomes position these vesicles as potent mediators in regenerative processes. They demonstrate a remarkable ability to modulate cellular activities and enhance tissue regeneration through sophisticated paracrine signaling mechanisms, establishing them as frontrunners in next-generation therapeutic platforms. In contrast to conventional whole-cell-based therapies, exosome-based therapies present superior therapeutic profiles characterized by three distinct advantages: (1) minimal host immune rejection responses, (2) improved biostability under physiological conditions, and (3) enhanced biodistribution capabilities enabling effective barrier penetration [14]. These distinctive attributes allow exosomes to surmount the inherent constraints of conventional cellular interventions while establishing a robust conceptual framework for their evolution as precision-engineered therapeutic agents. This paradigm shift not only addresses longstanding challenges in cell-based therapies—particularly regarding immunological risks and scalability constraints—but also reveals novel translational potential across diverse regenerative applications, from neural reconstruction to musculoskeletal rehabilitation.

Biology of Exosomes

Biogenesis and Cargo Loading

The biogenesis of exosomes is governed by four critical sequential phases: the selective sorting of molecular cargo, the biogenesis and maturation of MVBs, the intracellular trafficking of MVBs, and the subsequent fusion of MVBs with the plasma membrane. After recycling a subset of proteins back to the plasma membrane, early endosomes subsequently encapsulate diverse cargos into intraluminal vesicles (ILVs) to form MVBs [15]. This process is crucial for sorting and trafficking cellular components, ensuring the targeted segregation of diverse biomolecular constituents into distinct exosomal subpopulations. Mature MVBs have one of two distinct fates: they may fuse with lysosomes, leading to the degradation of their ILVs and their cargo, or they may merge with the plasma membrane, releasing ILVs into the extracellular space as exosomes, as shown in Fig. 2. This dual pathway plays a pivotal role in cellular waste management and intercellu-

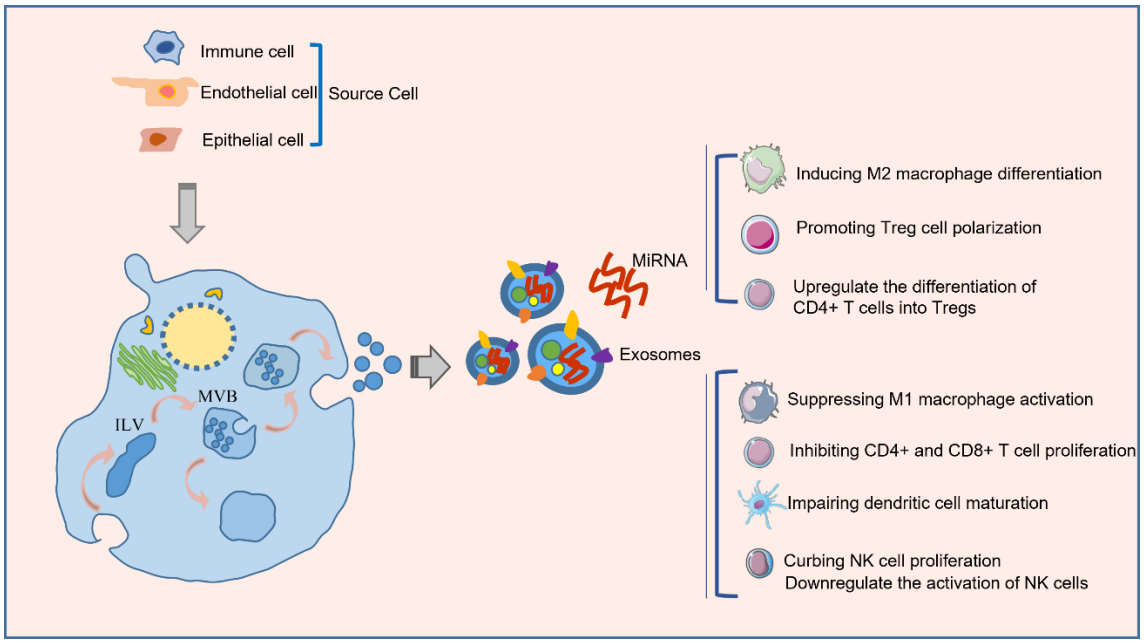

Fig. 2. The biogenesis of exosomes and their immunomodulatory activities. Exosome biogenesis initiates with endosomal maturation, whereby early endosomes encapsulate cargo into ILVs to form MVBs. The fate of mature MVBs is dichotomous: fusion with the plasma membrane releases ILVs as exosomes into the extracellular space, while lysosomal fusion leads to cargo degradation. The therapeutic efficacy of exosomes in tissue repair is largely attributed to their immunomodulatory activities, which orchestrate inflammatory responses and modulate immune cells to foster a pro-regenerative microenvironment. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

lar communication, highlighting the functional versatility of MVBs in cellular physiology. This sophisticated regulatory network fundamentally underpins the extensive heterogeneity that characterizes exosome populations at the molecular and functional levels [16].

The regulation of cargo expression, which can be either cell type-specific or induced by external stimuli, constitutes the primary regulatory element influencing exosome biogenesis [17]. Notably, deficiencies in exosome secretion may reflect impaired expression of exosomal cargo [18]. The abundant RNA content in exosomes represents a significant source of signalling diversity. Numerous studies have demonstrated that exosomes contain a variety of RNA species, including mRNAs, miRNAs, and other noncoding RNAs, which play crucial roles in intercellular communication and regulatory processes [19,20]. During circulation, exosomes can be taken up by recipient cells, resulting in the regulation of gene expression, the modification of signalling pathways, and broader effects on the cellular functions of target cells [21]. Previous studies have demonstrated that the levels of miRNAs, a prominent class of small RNAs, are significantly greater in exosomes than in their host cells [22,23]. These studies demonstrate that host cells possess a selective sorting mechanism for guiding specific miRNAs into exosomes. A recent study employed the systematic evolution of ligands by exponential enrichment (SELEX) to screen a single-stranded DNA aptamer library against CP05-immobilized murine myotube-derived exosomes [24]. Through this approach, an orthogonal DNA aptamer exhibiting specific binding to exosomes was successfully identified, which was designated the exosome-anchoring aptamer (EAA). Characterization study has demonstrated that EAA has high binding affinity for exosomal membranes and enables the efficient loading of nucleic acid therapeutics onto exosomes [24]. The specific sorting of proteins into exosomes is orchestrated through multiple distinct mechanisms, notably involving the endosomal sorting complex required for transport (ESCRT) machinery, tetraspanin family proteins, and lipid-dependent pathways. Emerging evidence indicates that noncanonical mechanisms are also involved in the regulation of exosomal protein sorting. A recent study revealed that proteins containing KFERQ pentapeptide motifs can be sorted into exosomes through a lysosome-associated membrane protein 2A (LAMP2A)-mediated process, representing an ESCRT-independent mechanism for cargo loading [25,26]. The presence of both ESCRT-mediated and ESCRT-independent pathways for protein incorporation into exosomes should not be viewed as mutually exclusive but rather as a reflection of the inherent heterogeneity of MVB populations and their corresponding exosomal derivatives [27]. Other RNA-based loading approaches include the EXOtic system, which utilizes the interaction between C/D box RNA motifs and the L7Ae ribosomal pro-

tein fused to the tetraspanin CD63 to facilitate exosomal RNA transfer both intra- and extracellularly; and a strategy involving the binding of mRNA transcripts to transactivation response (TAR) RNA elements via the Tat protein (along with ARRDC1-mediated microvesicles (ARMM)-associated membrane proteins) for selective mRNA loading into EVs [28]. Comparative lipidomic analysis has revealed distinct lipid signatures in exosomes, particularly manifested by the substantial enrichment of cholesterol, sphingomyelin, and saturated molecular species of phosphatidylcholine and phosphatidylethanolamine relative to the plasma membrane of their parental cells, which provides compelling evidence for the existence of specific lipid-sorting mechanisms [29].

Isolation and Characterization Techniques

The field of exosome isolation currently employs five main techniques: ultracentrifugation, ultrafiltration, precipitation, immunoaffinity capture, and size-exclusion chromatography. Ultracentrifugation separates sample components based on density gradients through differential centrifugation [30]. As the current gold standard for exosome isolation, this technique offers moderate sample purity with relatively low operational costs. However, it is time-consuming and may lead to non-negligible sample loss during processing [31,32]. Ultrafiltration is a pressure-driven membrane separation technique that uses nanoporous filters (typically with a 100-nm pore size) to achieve the size-based isolation of exosomes [33]. This approach demonstrates significantly higher processing efficiency than conventional ultracentrifugation methods do. However, the application of hydraulic pressure during filtration may induce two notable technical challenges: (1) potential structural deformation of exosomal membranes due to mechanical shear forces; and (2) progressive membrane fouling that can lead to substantial vesicle retention and subsequent yield reduction [33,34]. The precipitation technique exploits volume-excluding polymers to preferentially hydrate water molecules, thereby excluding biomaterials from the polymer-occupied solvent domain. This exclusion effect induces progressive biomolecular concentration until supersaturation conditions are achieved, culminating in the selective precipitation of less soluble components [35]. While this approach results in enhanced yield profiles, it is frequently associated with compromised product purity because of coprecipitation phenomena [33]. This approach enables robust RNA recovery while maintaining structural integrity, making it particularly suitable for exosomal RNA analysis [36]. Immunoaffinity capture enables the highly specific isolation of exosomes through antibody recognition of their unique surface markers. In this technique, antibodies are immobilized onto solid substrates such as magnetic beads and microfluidic devices [37]. The ExoCarta database (http://www.exocarta.org) serves as a valuable resource for identifying exosomal protein markers [38,39]. Compared with ultracentrifugation methods, immunoaffinity capture preserves exosomal bioactivity through its gentle isolation process. However, several limitations should be noted: (1) the technique is restricted by antibody availability and specificity; (2) the relatively small processing volume results in low yield; and (3) sample pretreatment and prolonged incubation times (typically 2–4 hours) may limit its applicability [37]. Size-exclusion chromatography is a liquid chromatographic technique that separates biomolecules based on their hydrodynamic radii as they migrate through porous, nonreactive, low-adsorption, resin-packed columns. The separation mechanism relies on differential access to the stationary phase pores: (1) molecules larger than the pore size are completely excluded and elute first in the void volume, while (2) smaller molecules penetrate the pores to varying extents depending on their molecular dimensions, resulting in longer retention times for progressively smaller species [33]. Compared with precipitation-based methods, size-exclusion chromatography, which is a gentle isolation method, is superior for preserving vesicle integrity and biological activity and significantly enhances vesicle purity. However, several technical limitations should be acknowledged: (i) potential protein contamination due to nonspecific interactions; (ii) relatively low recovery yields; (iii) the requirement for specialized instrumentation with substantial capital investment; (iv) multistep operational complexity requiring skilled personnel; and (v) the need for sample dilution when processing viscous biological matrices to avoid column overpressure [30,32,33,40,41].

Each methodology has distinct advantages and limitations in terms of exosomal purity, recovery yield, and processing efficiency, and combined approaches are frequently employed to optimize isolation outcomes [33]. The isolation of exosomes with high purity and concentration remains technically challenging, primarily because of their inherent biological heterogeneity in terms of cellular origin, molecular composition, functional diversity, and size variability [42]. Current exosome isolation techniques encounter limitations in achieving adequate discrimination between exosomes and other extracellular components with similar physical characteristics, particularly lipoproteins and nonendosomal EVs. This frequently leads to compromised sample purity and potential misinterpretation of experimental results [43]. Consistent and reproducible isolation protocols, coupled with efficient enrichment strategies, are crucial for accurately assessing the biological functions and therapeutic potential of exosomes.

The characterization of exosomes from different cellular sources is specific and identical; therefore, multiple characterization metrics are needed to determine whether the extracted components are exosomes [43]. Exosomes for use in clinical trials should meet the minimum standards for the characterization of EVs, as specified in the Minimal Information for Studies of EVs 2018 (MISEV2018) [44]

guidelines, which include labelling and physical characterization [45]. Exosome characterization methods can be divided into two main types: external characterization, which primarily encompasses morphological analysis and particle size determination, and internal characterization, which involves the identification of specific molecular components such as membrane proteins and lipid rafts [43]. Nanoparticle tracking analysis (NTA) can provide quantitative size and concentration data, transmission electron microscopy (TEM) can provide high-resolution imaging of exosome morphology, and flow cytometry and Western blotting can help detect specific surface biomarkers [17]. To precisely measure exosome size and concentration, alternative methods such as dynamic light scattering (DLS) [46] and tuneable resistive pulse sensing (TRPS) [47] are also available. The distinguishing features of TRPS are *in situ* single-particle characterization and exosome concentration quantification, although TRPS-based measures are vulnerable to system stability issues [48]. Atomic force microscopy (AFM), as a nanoscale tool for characterizing the abundance, morphology, biomechanics, and biomolecular structure of exosomes, has revealed new levels of complexity in exosomes [49–51]. Recent investigations into the refinement of exosome isolation techniques have employed biotinylated antibodies that specifically target four transmembrane proteins and tumour-associated antigens to directly capture exosomes from both urine and cellular supernatants. This innovative approach eliminates the need for extensive preprocessing of exosomes before measurement, thereby streamlining the separation process and yielding a significant reduction in time relative to traditional methods such as Western blotting and flow cytometry [52].

Exosomes in Tissue Engineering

Mechanistic Roles of Exosomes in Tissue Regeneration

Exosomes, which act as natural nanocarriers, exert profound biological effects through their diverse protein and nucleic acid cargos. These membrane-bound vesicles mediate a cascade of critical processes, including (1) inhibition of apoptosis and enhancement of cell survival to mitigate extensive cellular loss; (2) promotion of angiogenesis; (3) regulation of cell proliferation and extracellular matrix (ECM) remodelling; and (4) establishment of an anti-inflammatory microenvironment that facilitates tissue regeneration [53]. The integral lipid bilayer membrane of exosomes ensures structural integrity and provides remarkable protection against enzymatic degradation, thereby enhancing their stability and biological efficacy in intercellular communication [54,55]. Tissue injury invariably precipitates a cascade of cellular demise, predominantly mediated through apoptosis, necrosis, and other cell death pathways. Notably, apoptotic cells do not merely undergo programmed cell death in isolation; rather, they exert a paracrine effect, inadvertently triggering an amplification of cell death in adjacent viable cells. This deleterious propagation mechanism significantly exacerbates the initial tissue damage, leading to an expanded lesion area with profound pathological implications [56,57]. Compelling evidence from systematic *in vivo* and *in vitro* investigations has revealed that exosomes exert their therapeutic effects by orchestrating the regulation of antiapoptotic proteins via their miRNA cargo, leading to a substantial reduction in apoptosis and an increase in cellular viability. This multifaceted mechanism of action has been consistently demonstrated to facilitate functional recovery across multiple injury models, including acute kidney injury (AKI) [58,59], heart failure [60], and spinal cord injury [61].

Angiogenesis, defined as the sprouting and formation of new blood vessels from preexisting vascular networks, represents a critical biological mechanism essential for the re-establishment of oxygen, nutrient, and growth factor supplies to injured tissues [62]. In various tissue injury models, the administration of exosomes derived from either stem cell progenitor cells or differentiated cells has been demonstrated to significantly increase tissue regeneration through the promotion of neovascularization [63, 64]. Specifically, exosomes originating from differentiated cell types, including endothelial, epithelial, and immune cells, exhibit profound proangiogenic properties [63,65]. These exosomes facilitate angiogenesis primarily through the protein-mediated activation of intracellular signalling cascades within endothelial cells, thereby contributing to the restoration of vascular integrity and tissue homeostasis. An important intracellular mechanism mediating angiogenesis is the initial upregulation and release of vascular endothelial growth factor-A (VEGF-A) signalling upon vascular injury, which activates endothelial cells in the peripheral vasculature by binding to vascular endothelial growth factor receptor 2 (VEGFR2). The upregulation of endothelial cell VEGF signalling and VEGFR2 expression can be activated by the overexpression of several proteins in exosomes. VEGF-A has dual transport mechanisms; it can be released independently of exosomes, enabling direct activation of VEGFR2 and subsequent downstream signalling cascades while also participating in exosome-mediated transport pathways [66].

In contrast, numerous growth factors predominantly utilize exosomal transport mechanisms, requiring vesicular attachment for subsequent receptor activation on target cell membranes [67]. Emerging evidence supports a molecular mechanism involving specific binding interactions between growth factors and transmembrane proteins displayed on exosomal membranes [68]. This vesicular transport system effectively serves as an efficient delivery platform for secreted proteins, facilitating their systemic distribution and long-range intercellular communication. Additional proangiogenic mechanisms are orchestrated via exosome-dependent processes, including the intracellular delivery of transcriptional regulators [69] and Wnt signalling molecules [64], as well as the extracellular enzy-

matic activity mediated by membrane-associated proteins [70]. These molecular interactions contribute to the transcriptional activation of genes encoding proteins critical for cellular migration and proliferation, thereby establishing a multifaceted regulatory network in angiogenic signalling. The ECM, a complex and dynamic network predominantly composed of collagen, fibronectin, elastin, proteoglycans, and myriad other biomolecules, not only provides critical structural integrity to tissue architecture but also functions as a pivotal substrate for cellular migration while acting as an essential reservoir for an array of signalling molecules that play crucial roles in maintaining tissue architecture and functionality [71]. During the regenerative process, after the formation of an initial provisional fibrin clot, a diverse array of cellular entities—including but not limited to immune cells, fibroblasts, and myofibroblasts—initiate a meticulously orchestrated sequence of events. These cells facilitate the degradation of select ECM components through the secretion of matrix metalloproteinases (MMPs) and other proteolytic enzymes. Concurrently, they deposit nascent ECM constituents, thereby orchestrating progressive and sequential remodelling of the matrix architecture as the repair process advances. This intricate and dynamic interplay ensures the precise restoration of tissue integrity and functionality, albeit with notable variability in the specific combinations of cell types and matrix molecules involved, which are contingent upon the unique compositional and functional demands of each tissue type [71,72]. During the regenerative repair of mouse and human liver tissues, ECM modification is typically executed with remarkable coordination and precision [73].

However, in the context of skin trauma, spinal cord injury, ischaemic cardiac and renal injury, and particularly chronic inflammation, the regenerative repair process often deviates from its optimal trajectory, leading to detrimental outcomes such as tissue necrosis and scarring, characterized by the aberrant deposition of necrotic ECM substrates [74–78]. Exosomes, leveraging their unique ability to modulate the differentiation and functional activity of ECM-producing cells, effectively attenuate fibrotic processes in diverse injury models, thereby highlighting their remarkable therapeutic potential in mitigating pathological fibrosis and promoting tissue repair [79]. Beyond their direct regenerative ability, exosomes serve as pivotal mediators in the orchestration of inflammatory responses and the modulation of immune cell activity, both of which are indispensable for fostering a microenvironment that facilitates effective tissue repair and regeneration. In investigations of the therapeutic properties of stem cell-derived exosomes in tissue repair, their therapeutic efficacy has largely been attributed to their immunomodulatory activities. Stem cell-derived exosomes regulate inflammatory responses through multiple mechanisms, including inducing M2 macrophage differentiation and suppressing M1 macrophage activation [80,81], inhibiting $CD4^+$ and $CD8^+$ T-cell proliferation while promoting regulatory T-cell (Treg) polarization [82–85], impairing dendritic cell (DC) maturation [86], and curbing natural killer (NK) cell proliferation [87]. Previous investigations have demonstrated that mesenchymal stem cell (MSC)-derived exosomes (MSC-Exos) can upregulate the differentiation of $CD4^+$ T cells into Tregs and concurrently downregulate the activation of natural killer (NK) cells, thereby suggesting a dual immunomodulatory role of these exosomes in cellular immune responses [88,89]. Notably, one of the immunosuppressive mechanisms mediated by MSC-derived exosomes involves the expression of CD73 on their surface, which converts adenosine monophosphate (AMP) to adenosine, thereby suppressing T cells [90]. This intriguing mechanism is also utilized by exosomes derived from cancer cells [91] and Tregs [82], highlighting the complex interplay in immune regulation. The roles of exosomes and their released miRNAs in immune cells are shown in Fig. 2.

Applications in Specific Tissues

Bone and Cartilage Repair

Bone is a highly dynamic organ system that undergoes continuous regeneration by replacing aged tissue with a newly formed matrix. This intricate biological process, termed bone remodelling, is precisely orchestrated through the coupled actions of osteoclast-mediated bone resorption and osteoblast-driven bone formation [92,93]. The remodelling process serves critical physiological functions, including replacing damaged bone tissue, maintaining calcium homeostasis, and preserving biomechanical integrity. The equilibrium between bone formation and resorption is tightly regulated by a complex interplay of systemic factors (e.g., hormones, cytokines, and chemokines) and local mechanical stimuli [94]. However, with advancing age or the progression of certain pathological conditions, this homeostatic balance may become disrupted, leading to an excessive predominance of bone resorption over formation. Such an imbalance in bone remodelling can result in structural deterioration and the subsequent development of debilitating skeletal disorders, including osteoporosis, osteoarthritis, and pathological fractures. Exosomes derived from MSCs have emerged as promising therapeutic agents for bone and cartilage regeneration because of their dual ability to enhance osteoblast-mediated bone formation through facilitating osteogenic differentiation while concomitantly suppressing osteoclast activity to mitigate bone resorption [95,96].

In addition, exosomes can promote chondrocyte proliferation and migration by activating protein kinase B (AKT) and extracellular regulated protein kinase (ERK) signalling. One study demonstrated that human embryonic stem cell-derived mesenchymal stem cell (hESC-MSC)-derived exosomes mediate the conversion of extracellular AMP to adenosine via CD73/ecto-5′-nucleotidase expression. Subsequently, the generated adenosine activates

pro-survival AKT/ERK signalling through adenosine receptor interactions [97]. Exosome-based treatment has been shown to have significant immunomodulatory effects through the regulation of macrophage polarization, which is accompanied by a marked reduction in proinflammatory cytokine levels within the synovial microenvironment [97]. Liu *et al*. [98] developed an innovative acellular tissue patch composed of hydrogel-encapsulated human induced pluripotent stem cells (hiPSC)-MSC-derived exosomes for the treatment of rabbit cartilage defects, which demonstrated superior repair efficacy compared with direct exosome application because of the sustained *in vivo* vesicle release from the hydrogel. Recent advancements in exosome-based therapeutic approaches have demonstrated that three-dimensional (3D)-printed radially oriented scaffolds composed of ECM/gelatine methacrylate hydrogels incorporating exosomes exhibit significant chondroprotective effects. These biocompatible scaffolds effectively ameliorate mitochondrial dysfunction and oxidative stress in cartilage tissue, increase chondrocyte migration efficiency, and induce the M2 polarization of synovial macrophages, thereby promoting cartilage tissue regeneration [99]. Mitochondrial proteins represent a significant fraction (10.3 %) of the exosomal proteome, exhibiting complex interaction networks and metabolic involvement. Notably, 3.6 % of these exosomal proteins participate in metabolic processes, and functional study has confirmed that MSC-derived exosomes can transfer mitochondrial proteins to chondrocytes, effectively restoring mitochondrial function in recipient cells [99].

Neural Tissue Engineering

In the field of neural tissue engineering, exosomes have garnered significant attention because of their multifaceted roles in neuroprotection and axon regeneration [100]. These nanoscale vesicles exert therapeutic effects by enhancing the survival of neurons, a critical factor in preventing cell death in neurodegenerative and injury contexts [101]. Additionally, exosomes play a pivotal role in modulating neuroinflammation through the precise regulation of microglial activity [102] and astrocytic activity [103,104], thereby establishing a microenvironment conducive to neural repair and regeneration. This regulatory mechanism underscores the therapeutic potential of exosomes in ameliorating neuroinflammatory responses and promoting neural tissue homeostasis [105]. Moreover, exosomes facilitate axonal regeneration by transporting bioactive agents that trigger cytoskeletal reorganization and increase synaptic plasticity, highlighting their substantial promise as therapeutic tools for managing intricate neurological conditions, such as neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease, as well as traumatic neural injuries, particularly those affecting the spinal cord [106–111]. Their ability to modulate neural repair processes and cross the blood-brain barrier [112–114] highlights the use of exosomes as a promising method for advancing treatments in neuroregeneration and functional recovery, underscoring their utility as a strategic tool for central nervous system (CNS) repair and regeneration.

In general, nanoparticles can be specifically engineered to enhance their ability to penetrate elastic mucin fibres through deliberate electrostatic modifications. The application of a hydrophilic polyethylene glycol (PEG) coating to the surface of nanoparticles not only markedly enhances their penetration efficacy and diffusion capacity across the mucosal barrier but also optimizes the physical interactions between the nanoparticles and mucin fibres, potentially increasing the bioavailability and therapeutic efficacy of the encapsulated agents [115]. Translating these advanced bioengineering principles into the development of cellular nanoparticles, specifically MSC-derived exosomes, holds significant promise for enhancing their bioavailability through intravenous administration. This innovative approach offers a novel and potentially transformative strategy for the treatment of neurodegenerative diseases, leveraging the inherent therapeutic properties of exosomes combined with optimized delivery mechanisms.

Skin and Wound Healing

The cellular and biochemical processes involved in wound healing are systematically organized into four distinct phases: haemostasis, inflammation, proliferation, and remodelling [116]. Exosomes have emerged as a promising therapeutic modality for accelerating skin wound healing because of their multifaceted biological activities. EV-based interventions have demonstrated significant efficacy in promoting skin regeneration in both diabetic and nondiabetic animal models. A comprehensive meta-analysis revealed that small EVs (sEVs) modulate multiple aspects of the intricate wound healing process, enhancing regenerative capacity and reducing fibrotic responses [117]. Numerous studies have consistently demonstrated that the treatment of wound beds with exosomes significantly increases cellular viability [118,119]. However, subsequent longitudinal investigations revealed that this enhanced biological activity progressively attenuated during the later phases of the observation period [120]. A significant increase in immature collagen fibres was detected in close proximity to the wound bed immediately following exosome treatment. Histological analysis revealed markedly improved fibre alignment and enhanced collagen maturation during the healing process compared with those in the control groups [121].

Through comprehensive evaluation of scar formation in exosome-treated tissues, experimental evidence has demonstrated the therapeutic efficacy of exosomes in attenuating scar development [120,122–124]. Scar reduction was quantitatively analysed using a multidimensional assessment protocol, including precise measurements of the scar width, depth, axial length, and vertical elevation, with subsequent calculation of the total scar area [120,125–127]. Exosomes facilitate the phenotypic shift of macrophages

from the M1 (proinflammatory) state to the M2 (anti-inflammatory) state, a transformation that is substantiated by a significant increase in the M2:M1 ratio [128,129]. A marked decrease in the infiltration of inflammatory cells, predominantly macrophages and neutrophils, was observed in the group treated with exosomes [129–132]. Moreover, studies have demonstrated the upregulation of VEGF expression, indicating that exosomes may exert a proangiogenic effect, ensuring an adequate oxygen and nutrient supply during the process of wound healing [121,133,134]. These synergistic characteristics establish exosomes as a highly efficacious and adaptable biotherapeutic tool, offering remarkable potential for revolutionizing wound management and skin tissue regeneration paradigms.

Cardiovascular Tissue Engineering

Exosomes serve as pivotal components in cardiovascular tissue engineering, offering transformative potential for cardiac repair and vascularization [135]. These EVs exert their therapeutic effects through multiple mechanisms critical to cardiovascular regeneration [136]. Specifically, exosomes increase cardiomyocyte survival by delivering protective factors that mitigate apoptosis and oxidative stress, preserving myocardial function [60]. Studies have demonstrated that MSC-derived exosomes (MSC-Exos) exert cardioprotective effects by mitigating apoptosis and oxidative stress, modulating inflammatory responses, which collectively contributes to the preservation of cardiomyocyte viability and the maintenance of left ventricular function following ischaemia-reperfusion injury [137,138]. A recent study demonstrated that the administration of MSC-Exos in a mouse model of ischaemia/reperfusion (I/R) injury significantly increased adenosine triphosphate (ATP) levels, mitigated oxidative stress, and promoted cardiomyocyte survival [139]. Cardiac regeneration and extracellular collagen deposition constitute two concurrent processes during the repair phase. The equilibrium between scar formation and cardiac regeneration is pivotal for effective cardiac repair.

While scar formation imparts mechanical stability, prevents ventricular rupture, and supports short-term cardiac recovery, it is inversely related to long-term cardiac regeneration. Consequently, antifibrotic therapies should be tailored to address reactive fibrosis occurring in the context of ventricular remodelling. In support of this notion, a prior study [140] demonstrated that systemic suppression of fibrogenic signalling exacerbates cardiac injury during the inflammatory phase following myocardial infarction. Conversely, inhibiting systemic fibrosis during the postinfarction repair phase enhances cardiac remodelling and ameliorates functional impairment. MicroRNAs (miRNAs) encapsulated within MSC-Exos have been shown to ameliorate collagen deposition during cardiac remodelling [141]. Additionally, they play a key role in reducing fibrosis by modulating ECM remodelling and suppressing the activation of profibrotic signalling pathways, which is essential for maintaining tissue elasticity and contractility [142–144]. Thus, exosomes have the potential to establish an equilibrium between collagen deposition and tissue fibrosis, facilitating the restoration of cardiac structure and function. Furthermore, exosomes stimulate angiogenesis by promoting endothelial cell proliferation, migration, and tube formation, facilitating the development of new blood vessels to restore adequate perfusion to damaged tissues [145]. These multifaceted actions highlight the immense potential of exosomes as a therapeutic strategy for addressing cardiovascular diseases, including myocardial infarction and ischaemic heart disease, by fostering the structural and functional recovery of cardiac tissues. The use of exosomes in the treatment of different tissue injuries is summarized in Fig. 3. The mechanisms by which exosomes contribute to tissue regeneration are summarized in Fig. 4.

Exosomes in Therapeutics

Exosomes as Cell-Free Therapeutics

Cell-free therapy has emerged from conventional cell-based therapy as an evolutionary advancement. While cell therapy holds therapeutic promise, its clinical application faces inherent limitations: (1) dose-dependent safety concerns such as potential pulmonary embolism risk; (2) phenotypic instability during prolonged *in vitro* cultivation; and (3) inefficient biodistribution of administered cells to target organs [14]. Such concerns have prompted extensive exploration of alternative therapeutic strategies, and cell-free therapies may overcome certain limitations associated with cell-based treatments. One study demonstrated that peripherally administered, fluorescently labelled exosomes resulted in significantly stronger detectable fluorescence signals in injured liver tissue than in normal liver parenchyma within 6 hours after administration [146]. Previous studies have indicated that intravenously administered exosomes are predominantly internalized by hepatic macrophages upon entering the liver [147,148]. These findings suggest that exosomes do not accumulate in the lungs but instead exhibit targeted enrichment at pathological sites, such as injured liver tissue. The “cell-free approach” has been conceptually described by some researchers as the isolation of bioactive contents (e.g., stem cell-derived exosomes) from conditioned media [149]. Accordingly, we propose the following standardized definition for “cell-free therapeutics”: therapeutic strategies employing acellular components—including extracellular vesicles (e.g., exosomes), secreted proteins, nucleic acids (mRNAs/small interfering RNAs (siRNAs)), or synthetic bioinspired nanoparticles—to achieve pharmacological effects without the direct administration of intact living cells.

Exosomes offer significant advantages over MSCs in terms of storage and transport efficiency for biological molecules while simultaneously mitigating the inherent risks of direct cell-based therapies [135]. This char-

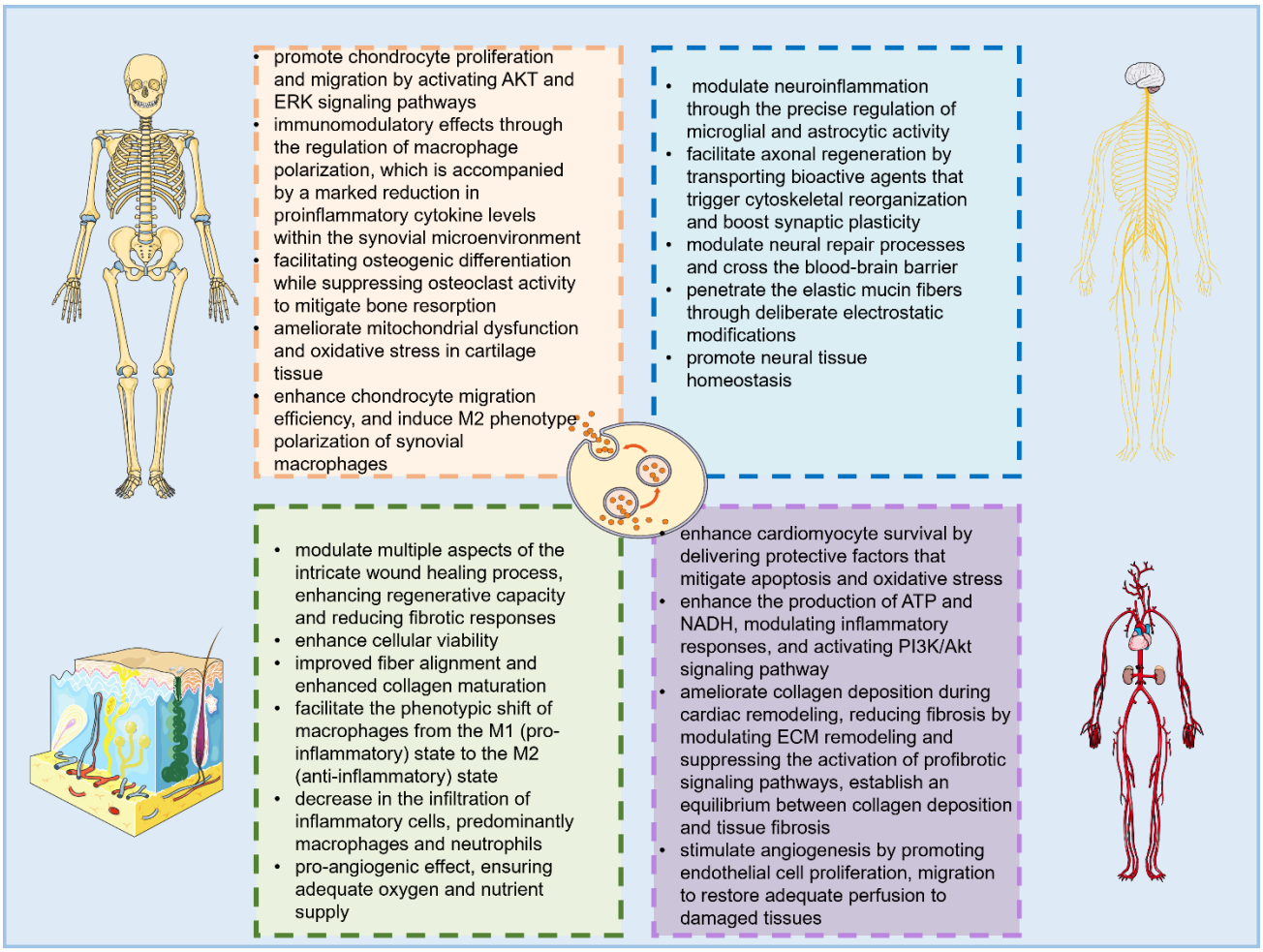

Fig. 3. Representative examples of exosome-mediated tissue regeneration and damage repair in the skeletal, nervous, integumentary, and cardiovascular systems. Acting as innate nanocarriers, exosomes execute their functions via the delivery of protein and nucleic acid cargoes. Their key roles encompass the inhibition of apoptosis, induction of angiogenesis, modulation of cell proliferation and ECM remodeling, and the facilitation of tissue regeneration by fostering an anti-inflammatory milieu. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

acteristic establishes exosome-based approaches as safer and more efficient therapeutic strategies in clinical applications. Exosomes utilize multiple distinct mechanisms for cellular internalization and cargo delivery. The primary entry pathways include (1) receptor-mediated endocytosis and (2) direct membrane fusion with the plasma membrane of the target cell. Alternatively, exosomes can facilitate surface-level signalling through specific molecular interactions, wherein membrane-associated lipid ligands engage with complementary cellular receptors, enabling the targeted delivery of bioactive molecules without complete internalization [55]. This strategy has many significant advantages that render it particularly suitable for therapeutic applications [90]. These include the following: (i) straightforward isolation and storage procedures ensure the long-term preservation of biological activity without the loss of cargo potency; (ii) the inherent lipid bilayer structure confers remarkable stability, protecting encapsulated compounds from enzymatic degradation or environmental disturbances; (iii) the excellent dose scalability enables effective therapeutic concentrations to be precisely delivered to target organs; (iv) the capability for intravenous administration facilitates systemic distribution, including access to capillaries with diameters as small as 5–10 μm; (v) the unique ability to traverse the blood-brain barrier makes it applicable for CNS therapeutics; (vi) critical safety concerns associated with conventional therapies, including oncogenic potential, cellular dedifferentiation, thrombotic embolism, and immunological rejection, are inherently eliminated; (vii) similar to standard pharmaceutical formulations, exosomal formulations can be systematically evaluated for pharmacokinetic parameters, safety profiles, dosage optimization, and therapeutic efficacy through established clinical protocols; and (viii) the structural flexibil-

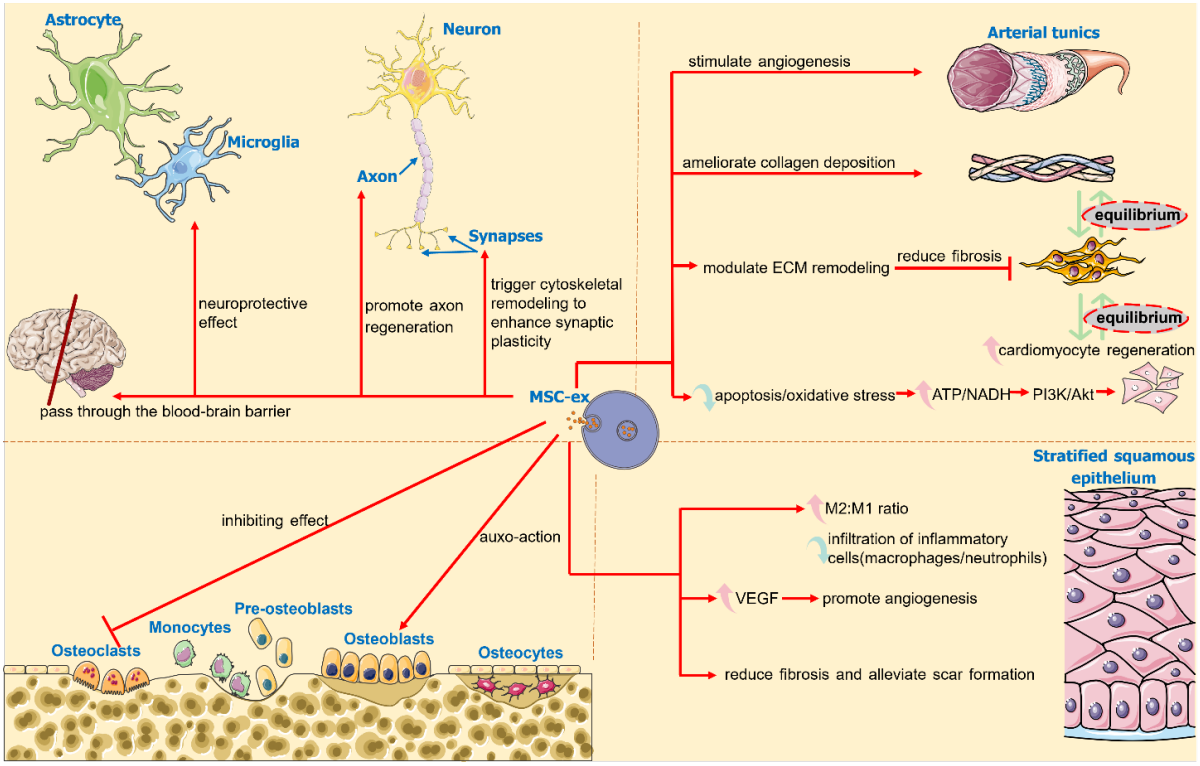

Fig. 4. Mechanisms by which exosomes contribute to tissue regeneration. Exosomes traverse biological barriers to coordinate reparative processes. In the nervous system, they cross the blood-brain barrier to exert neuroprotective effects and promote neurite outgrowth. In cardiovascular tissue, exosomes stimulate angiogenesis and inhibit ECM fibrosis to aid cardiac repair. In bone, they concurrently enhance osteoblast-mediated bone formation and suppress osteoclast activity. During skin wound healing, exosomes facilitate a pro-reparative microenvironment by shifting macrophage polarization from M1 to M2, enhancing vascularization, and reducing fibrotic scarring. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

ity of exosomes permits precise engineering modifications to optimize performance parameters for diverse therapeutic applications.

Applications in Therapeutics

MSCs and DCs represent two prominent cellular sources for exosome production. Owing to their immunomodulatory properties and superior biocompatibility, MSC-derived exosomes exhibit remarkable therapeutic potential for attenuating inflammation and serving as effective nanocarriers for drug delivery systems. According to the ClinicalTrials.gov database (https://clinicaltrials.gov/ct2/home), a total of 33 clinical trials investigating exosome-based therapies are currently registered. Notably, 20 of these trials (60.6 %) have utilized exosomes derived from MSCs, reflecting their predominant role in current therapeutic development [150]. In contrast, DC-derived exosomes are predominantly employed in oncological immunotherapy, where their ability to potentiate proinflammatory responses plays a critical role in eliciting antitumour immunity in cancer patients [150]. Furthermore, compared with other immunotherapies, DC-derived exosomes exhibit superior biostability and enhanced bioavailability while demonstrating significant cost-effectiveness [151]. In the initial phase I clinical trial (NCT01159288), researchers demonstrated the safety profile of autologous DC-derived exosome vaccination in patients with metastatic melanoma. In the phase II immunotherapy trial, researchers evaluated the ability of interferon-γ (IFN$-\gamma$) DC-exosomes to enhance T-cell- and NK-cell-mediated immune responses in patients with advanced non-small cell lung cancer (NSCLC). The results demonstrated that these exosomes could potentiate NK-cell-mediated antitumour immunity *in vivo* [152]. Macrophage-derived EVs have emerged as promising nanocarriers for the targeted delivery of chemotherapeutic agents, such as doxorubicin (Dox), to tumour tissues [153]. NK-cell-derived exosomes exhibit potent cytotoxicity against cancer cells through their constitutive expression of cytotoxic proteins and cytokines, including FasL and cytokines tumour necrosis factor α (TNF-α) [154,155]. EVs derived from human embryonic kidney 293 (HEK293) cells represent another promising platform for cancer therapeutic applications. Programmed death receptor 1 (PD-1)-expressing membrane vesicles derived from HEK293 cells were engineered to simultaneously block tumour programmed cell death ligand 1 (PD-L1) and

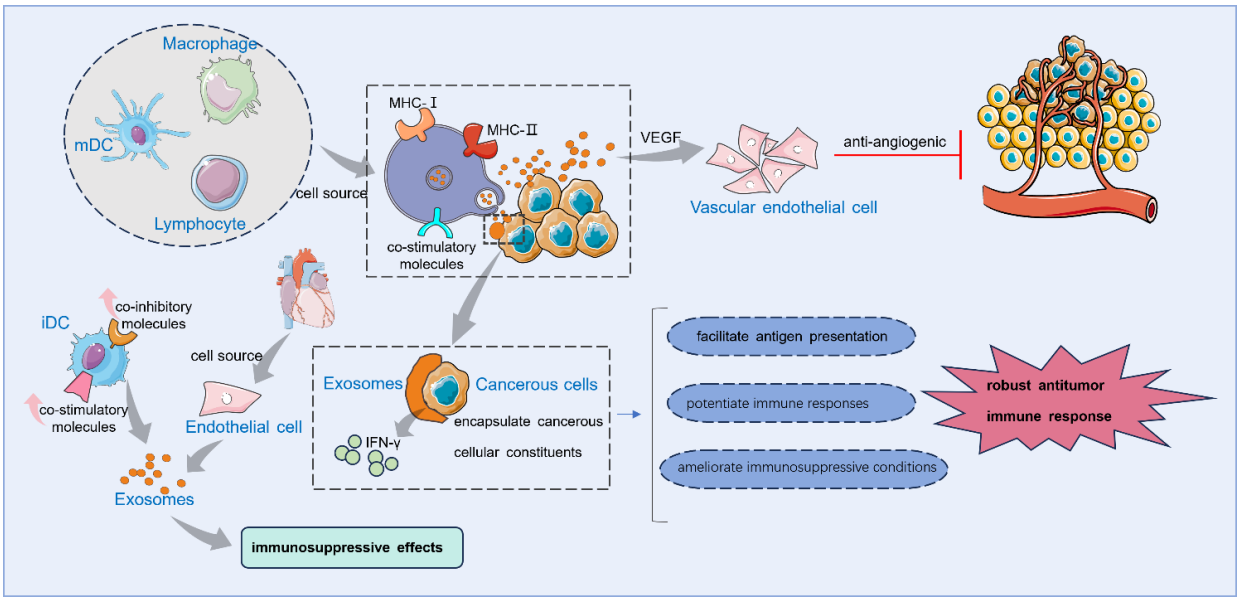

Fig. 5. Immune responses elicited by exosomes against tumour cells and the cell types involved in immunosuppression. Exosomes derived from activated APCs, such as dendritic cells, macrophages, T lymphocytes, and B cells, are widely utilized in immunological research. These exosomes express both MHC class I and II molecules along with T-cell costimulatory molecules on their surface, which is essential for efficient antigen presentation and immune activation. Experimental evidence indicates that immune-responsive cells can efficiently encapsulate tumor-derived components into exosomes upon exposure to cancer cells. Beyond their immunostimulatory roles, such exosomes modulate endothelial cell responses to VEGF, altering angiogenic processes through effects on tube formation and VEGF-associated gene expression. This supports their potential application in antiangiogenic cancer therapy. Moreover, iDCs display a higher ratio of coregulatory to costimulatory molecules, conferring immunosuppressive activity. Additionally, exosomes from cardiac endothelial cells have been shown to specifically induce the differentiation of Bregs, which exhibit potent immunosuppressive functions. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

deliver an indoleamine 2,3-dioxygenase-1 inhibitor to the tumour microenvironment, resulting in a significant reduction in Treg infiltration and enhanced antitumour immune responses [156]. Engineered and synthetic vesicles have emerged as promising platforms for drug delivery because of their enhanced structural stability and precise targeting capabilities. Viral encapsulation within tumour-derived exosomes enables systemic delivery and tumour-specific targeting, thereby achieving precise treatment of metastatic cancer [157]. This biomimetic strategy demonstrates enhanced tropism to disseminated tumour sites through inherent homing properties. The therapeutic applications of exosomes from different cellular origins are presented in Table 1 (Ref. [150,152–157]).

Cancer Immunotherapy

Exosomes, which are enriched in immunomodulatory molecules, including cytokines, antigens, and noncoding RNAs, are critical mediators of immune regulation and have profound effects on innate and adaptive immunity; thus, they offer significant potential in cancer immunotherapy and autoimmune disease management. Exosomes derived from activated antigen-presenting cells (APCs), including DCs, macrophages, T lymphocytes, and B cells, are predominantly employed in immunological studies. The surface expression of both major histocompatibility complex (MHC) class I and II molecules, coupled with T-cell costimulatory molecules, constitutes a critical mechanism for efficient antigen presentation and immune activation [158]. In the context of cancer therapy, exosomes have demonstrated a remarkable ability to facilitate antigen presentation, potentiate antitumour immune responses, and ameliorate immunosuppressive conditions within the tumour microenvironment. In multiple experimental investigation, immune-responsive cells demonstrated a remarkable capacity to encapsulate cancerous cellular constituents within exosomes upon exposure to cancer cells [159–163]. This sophisticated cellular mechanism facilitates the presentation of tumour-associated antigens to the immune system, consequently eliciting a robust antitumour immune response with significant therapeutic implications in cancer immunotherapy; however, the precise molecular mechanisms underlying this novel immunogenic pathway warrant further systematic investigation to elucidate its full potential in oncological interventions.

Mouse B lymphoma cells release exosomes that carry several heat shock proteins (HSPs) that can induce significant antitumour immune responses in T cells [164]. Emerg-

Table 1. Summary of clinical applications of exosomes derived from various cell sources.

Source of exosomes	Area of application	Reference
MSCs	Attenuate inflammation and serve as effective nanocarriers for drug delivery systems	[150]
DCs	Antitumour immunity (metastatic melanoma, NSCLC)	[150,152]
Macrophages	Targeted nanocarriers for chemotherapeutics	[153]
NK cells	Potent cytotoxicity against cancer cells through constitutive expression of cytotoxic proteins and cytokines, including FasL and TNF-α	[154,155]
HEK293 cells	Simultaneously block tumour PD-L1 and deliver indoleamine 2,3-dioxygenase-1 inhibitor to the tumour microenvironment, resulting in a significant reduction of Treg infiltration and enhanced antitumour immune responses	[156]
Viral encapsulation within tumour-derived exosomes	Systemic delivery and tumour-specific targeting, precise treatment of metastatic cancer	[157]

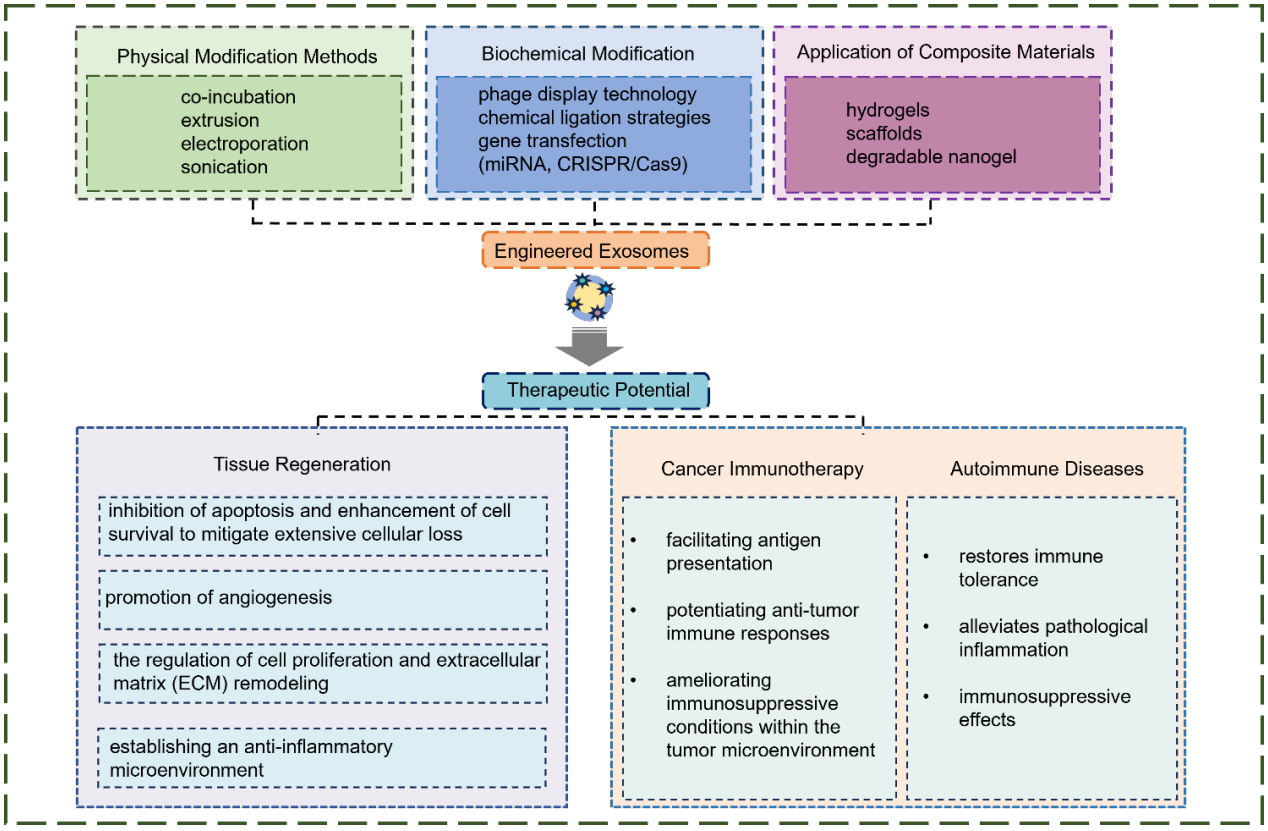

Fig. 6. The therapeutic potential of engineered exosomes in tissue regeneration and immunological disease. Engineered exosomes, modified through physicochemical and biomaterial-based approaches, function as a double-edged sword in regenerative medicine and immunomodulation. These customized vesicles carry distinct molecular cargo, such as gene-regulating miRNAs and signal-transducing cytokines, that enable them to precisely target damaged tissues to stimulate regeneration while simultaneously triggering intricate immune responses. For instance, such bioengineered exosomes can help prevent infection and improve antitumour defences by activating immune cells to present pathogen-derived markers via MHC-mediated antigen presentation. Conversely, they may inadvertently induce immune tolerance or exacerbate chronic inflammatory conditions. This paradoxical behaviour stems from the specific molecular composition of their cargo and the biological context in which they operate. This figure was created with Power Point.

Table 2. Strategies and biomedical applications of engineered exosomes.

Applied technology	Area of application	Reference
Exosome-laden scaffold for fabricating engineered hydrogel-based cellular tissue patches (EHGs)	Targeted cellular deposition at defect sites, promoting cartilage defect repair	[98]
Genetic engineering of DCs to coexpress Lamp2b fusion proteins and rabies virus glycoprotein (RVG) peptide ligands	Exosome-based delivery for efficient targeting of central nervous system (CNS) tissues	[112]
Incorporation of the chemotherapeutic agent doxorubicin into exosomes utilizing electroporation methodology	Antitumour effects in breast carcinoma models	[169]
Stable integration of functionalized polyethylene lipid chains into exosomes derived from bovine milk exosome (mExo) membranes	Transmit the tumour-targeting drug doxorubicin (Dox) to FR-positive tumour cells; induce marked tumour cell death	[171]
Conjugation of CP05-muscle-targeting peptide with the exosomal surface protein CD63	Upregulate dystrophin expression in muscle tissues	[172]
Covalent conjugation of tumour-targeting cyclic peptide c(RGDyK) to exosomal surfaces through chemical crosslinking strategies	Therapeutic agent for glioblastoma	[173]
Loading of exosomes with rifampicin (RIF) through electroporation and chemical conjugation with angiopoietin (ANG)	Promising therapeutic strategy for the effective management of central nervous system tuberculosis	[174]
Electrostatic interactions to facilitate the assembly of exosome-nanocomplexes, incorporating pH-sensitive fusogenic peptides and cationic lipid components	Improving targeted cellular delivery efficiency	[175]
Engineering of exosomes with superparamagnetic iron oxide nanoparticles (SPIONs)	Enhancing the efficacy and specificity of drug delivery	[176]
Hybridization of exosomes with neutral or anionic liposomes	Enhanced uptake by cancer cells; reduced in-body retention	[177,178]
PEG-mediated hybridization to generate a protective hydration layer	Enhanced stability and circulation duration of engineered exosomes	[178]
Integration of ultrasonic incubation and membrane extrusion techniques to produce a novel hyaluronic acid-liposome -exosome transdermal delivery system (HL@Exo)	Enhanced therapeutic efficacy of exosomes in the treatment of acute skin injuries	[179]
Synergistic combination of incubation procedures and ultrasonic treatment to introduce paclitaxel into exosomes derived from RAW 264.7 cells	Inhibition of drug resistance mechanisms in cancerous cell populations	[180]
Incorporation of let-7a microRNA, a tumour-suppressing molecule, into HEK293-derived exosomes via transfection techniques	Targeted breast carcinoma treatment	[181]

ing evidence has demonstrated that human macrophages selectively transfer EV-associated miRNAs to hepatocellular carcinoma cells (HCCs), effectively suppressing their proliferative capacity [165]. Notably, exosomes derived from DCs were shown to robustly stimulate tumour-sensitized T cells to secrete elevated levels of interferon-γ (IFN-γ) upon incubation with human breast adenocarcinoma (SK-BR-3) cells [162]. Furthermore, T-cell-derived exosomes exhibit

Table 2. Continued.

Applied technology	Area of application	Reference
CRISPR/Cas9-loaded exosomes, delivered via transfection kits	Reduced pancreatic cell proliferation and tumour growth *in vitro* and *in vivo*	[182]
CD19-engineered exosomes, encapsulating CRISPR/Cas9 via electroporation	Specific targeting of the *MYC* oncogene in B-cell malignancies	[183]
Efficient delivery of engineered exosomes, modified with an aptamer and loaded with CRISPR/Cas9, via ultrasound to target *WNT10B*	Markedly suppressed tumour growth	[184]
Encapsulation of uMSC-Exos within HA-Gel, subsequently integrated with a tailored 3D-printed nanocomposite scaffold composed of nHP	Cranial reconstruction	[185]
Loading of an exosome-sheathed degradable NG, known as HA NG@exosomes, with pituitary adenylate cyclase-activating polypeptide (PACAP) and oestradiol (E2) for precise peptide/drug delivery	Improved therapeutic outcomes for perimenopausal depression through attenuating oxidative and inflammatory conditions and improving synaptic plasticity	[186]
Application of MSC-EVs onto a decalcified bone matrix	Improved pro-angiogenic and osteogenic properties	[187]

multifaceted antitumour potential, demonstrating the ability to not only disrupt the tumour stroma and inhibit tumour invasion and metastasis but also to exert immunomodulatory effects. In addition to their immune-enhancing properties, these exosomes have been shown to significantly modulate endothelial cell responsiveness to VEGF. This regulatory function extends to the alteration of angiogenic processes, as evidenced by modified tube formation dynamics and VEGF-related gene expression profiles in target endothelial cells, suggesting a potentially novel therapeutic avenue for antiangiogenic cancer therapies [3,166].

Autoimmune Diseases

Conversely, the exosome-mediated modulation of immune cell activity in autoimmune diseases restores immune tolerance and alleviates pathological inflammation. Immature DCs (iDCs) exhibit an increased ratio of coregulatory to costimulatory molecules on their surface, thereby exerting immunosuppressive effects. Compared with parental cells, exosomes harvested from *in vitro*-generated immunosuppressive DCs more effectively reversed early-onset collagen-induced arthritis, underscoring their enhanced therapeutic potential [167]. Furthermore, exosomes derived from cardiac endothelial cells have been demonstrated to specifically induce the differentiation of regulatory B cells (Bregs), which are endowed with potent immunosuppressive capabilities [168].

The dual role of exosomes in promoting or suppressing immune responses highlights their versatility and underscores their potential as next-generation therapeutic agents. Nevertheless, exosome isolation, characterization, and targeted delivery challenges must be addressed to fully harness their clinical potential. The immune responses elicited by exosomes against tumour cells and the cell types involved in immunosuppression are illustrated in Fig. 5.

Engineered Exosomes as Advanced Nanocarriers for Targeted Therapeutic Delivery

Engineered exosomes have emerged as highly versatile and efficient nanovehicles for the targeted delivery of diverse therapeutic payloads, including small interfering RNAs (siRNAs), clustered regularly interspaced short palindromic repeats (CRI
SPR)-Cas components, and small-molecule drugs [11,159,169,170]. By leveraging their inherent biocompatibility, low immunogenicity, and natural ability to traverse biological barriers, exosomes can be precisely tailored to deliver therapeutic agents to specific cells or tissues [4]. Table 2 (Ref. [98,112,169,171–187]) summarizes the techniques used to engineer exosomes and the areas of application.

Surface Engineering of Exosomes: Advancing Targeting for Precision Therapeutics

Exosomes exhibit distinct advantages, including low toxicity, nonimmunogenicity, enhanced bioavailability, excellent permeability, and specific tissue tropism. As naturally occurring vesicles derived from living tissues, exosomes minimize drug leakage before they reach target cells. Their ability to efficiently deliver therapeutic payloads is facilitated by multiple cellular uptake mechanisms, such as membrane fusion, receptor-mediated endocytosis, clathrin-

dependent or clathrin-independent pathways, phagocytosis, macropinocytosis, lipid raft-dependent uptake, and caveolin-mediated internalization. These properties collectively contribute to their potential for increased clinical efficacy [188,189]. Jang *et al*. [171] demonstrated that functionalized polyethylene lipid chains could be stably integrated into exosomes derived from bovine milk exosome (mExo) membranes through optimization of the postinsertion technique, significantly enhancing their surface functionality. Specifically, folic acid (FA)-modified mExos (mExo-FA) exhibited enhanced cellular uptake in cancer cells via FA receptor (FR)-mediated endocytosis, and both *in vitro* and *in vivo* analyses revealed that the engineered mExo-FA effectively delivered the tumour-targeting drug doxorubicin (Dox) to FR-positive tumour cells, resulting in a marked induction of tumour cell death [171]. The surface characteristics of exosomes fundamentally determine their biodistribution, cellular targeting specificity, and therapeutic efficacy; consequently, surface engineering techniques can be strategically employed to tailor these properties for optimized biomedical applications [178]. The surface of exosomes can be strategically engineered by conjugating specific targeting ligands, such as peptides, antibodies, or aptamers, to achieve precise tissue- and cell-specific delivery of therapeutic cargo [177]. Peptides, as therapeutic biomolecules, possess distinct advantages over monoclonal antibodies, particularly because of their lower molecular weight and enhanced tissue penetration.

While research on extracellular vesicle (EV)-mediated peptide delivery remains relatively limited, notable progress has been made in functionalizing EV surfaces with specific targeting peptides. For instance, the successful incorporation of arginylglycylaspartic acid (RGD) and receptor for advanced glycation end products (RAGE)-binding peptide (RBP) onto EV membranes has led to significant improvements in targeted delivery capabilities, opening new avenues for precision medicine applications [190]. Recent studies have revealed that a peptide (CP05), identified via phage display technology, has a specific binding affinity for the exosomal surface protein CD63. This interaction facilitates precise targeting, efficient cargo loading, and effective capture of exosomes derived from diverse sources, including those isolated from patient samples. Furthermore, the conjugation of the CP05-muscle-targeting peptide with exosome-loaded phosphorodiamidate morpholino oligomers (EXOPMO) has been shown to significantly upregulate dystrophin expression in muscle tissues, concomitantly improving functional outcomes. Notably, these therapeutic interventions are accomplished without inducing any detectable toxicity, underscoring their potential for clinical applications [172].

Through advanced surface engineering techniques, exosomes can be precisely functionalized for targeted therapeutic applications via two primary approaches: molecular coupling strategies, where gene transfection and chemical modification enable the precise attachment of targeting ligands to exosomal surfaces; and physical modification methods, including coincubation, extrusion, electroporation, and sonication, which effectively facilitate the incorporation of targeting molecules and collectively enhance the specificity of exosomes for designated tissues or cells, thereby optimizing their therapeutic potential in precision medicine applications [191,192]. Genetic modification can be employed by transfecting genes encoding targeted moieties fused to various exosomal membrane proteins [193]. The general strategy involves the transfection of cells with expression vectors (e.g., plasmids or viral vectors) containing target genes that are engineered to encode fusion proteins with exosomal membrane-associated domains. Cells that undergo successful transfection subsequently produce exosomes that exhibit targeting peptides localized on their surface membranes [194]. Notably, in a pioneering study conducted by Alvarez-Erviti *et al*. [112], DCs were genetically engineered to coexpress Lamp2b fusion proteins and rabies virus glycoprotein (RVG) peptide ligands, establishing a novel exosome-based delivery system capable of efficiently targeting CNS tissues.

Chemical ligation strategies have been developed by leveraging reactive functional groups inherent to vesicle membrane lipids and proteins, including amino (-NH2), carboxyl (-COOH), and thiol (-SH) moieties [195]. The targeting peptides are conjugated to the extracellular vesicle (EV) surface through biorthogonal reactions between these functional groups and reactive fragment-tagged peptides. Currently, the tumour-targeting cyclic peptide c(RGDyK) has gained significant recognition as a novel therapeutic agent for glioblastoma, with recent studies demonstrating its successful covalent conjugation to exosomal surfaces through well-established chemical crosslinking strategies [173,196]. Exosomes are successfully loaded with rifampicin (RIF) through electroporation and chemically conjugated with angiopoietin (ANG), demonstrating remarkable targeting efficiency, potent antituberculosis activity, and superior biocompatibility; hence, this approach represents a promising therapeutic strategy for the effective management of CNS tuberculosis [174]. However, challenges related to ligand stability, conjugation efficiency, and *in vivo* validation must be addressed to fully understand the clinical potential of these engineered exosomes.

Furthermore, innovative approaches leveraging electrostatic interactions have been successfully employed to facilitate the assembly of exosome-nanocomplexes, incorporating pH-sensitive fusogenic peptides and cationic lipid components, and significantly improving targeted cellular delivery efficiency [175]. By employing physical strategies, targeted delivery can be effectively realized through the utilization of an external magnetic field, wherein exosomes functionalized with superparamagnetic nanoparticles are precisely guided to specific locations via the application of an external magnetic force, facilitating efficient

and site-specific drug delivery [197]. Through the application of an external magnetic field to specific regions, exosomes engineered with magnetic components, particularly superparamagnetic iron oxide nanoparticles (SPIONs), can be administered into a patient's bloodstream and precisely directed to the desired target site, thereby enhancing the efficacy and specificity of drug delivery [176]. Furthermore, lipid components are crucial for target cell uptake; exosome hybridization alters the plasma membrane, enhancing its delivery to target cells [191]. Studies have demonstrated that exosomes hybridized with neutral or anionic liposomes exhibit enhanced uptake by cancer cells; however, this hybridization process increases the size of exosomes, thereby reducing in-body retention. Conversely, it enhances the encapsulation efficiency of large carriers or drugs, a capability that native exosomes inherently lack because of their limited size [177,178]. A novel hyaluronic acid-liposome-exosome transdermal delivery system (HL@Exos) was successfully developed through the integration of ultrasonic incubation and membrane extrusion techniques. This innovative system markedly enhanced the therapeutic efficacy of exosomes in the treatment of acute skin injury [179]. Furthermore, PEG-mediated hybridization can generate a protective hydration layer that shields the hybrid system from immune recognition, enhancing the stability and circulation duration of engineered exosomes [178].

Exosome Therapeutic Cargo Loading Techniques

Advanced methodologies have been developed to facilitate the efficient encapsulation of therapeutic agents into exosomes, ensuring their optimal delivery and functionality. Key techniques include electroporation, which uses electrical pulses to create temporary pores in the exosomal membrane for cargo entry; sonication, which employs ultrasonic waves to increase membrane permeability and promote cargo loading; and transfection, which uses chemical reagents or viral vectors to introduce therapeutic molecules into exosomes. Viral transduction strategies employing retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses serve as foundational delivery systems because of their stable and well-characterized transfection efficiency. After infection, specific genes or transcriptional regulators overexpressed in infected cells can be incorporated into exosomes, which subsequently transport biologically active viral components to distant noninfected cells [198]. Recent advancements in exosome-mediated drug delivery have yielded promising results in cancer treatment. A study conducted by Tian *et al*. [169] demonstrated the effective incorporation of the chemotherapeutic agent doxorubicin into exosomes using electroporation methodology, which resulted in notable antitumour effects in breast carcinoma models. Similarly, researchers led by Kim [180] established a novel method for drug encapsulation, employing a synergistic combination of incubation procedures and ultrasonic treatment to introduce paclitaxel into exosomes derived from RAW 264.7 cells, demonstrating successful inhibition of drug resistance mechanisms in cancerous cell populations.

A groundbreaking study revealed, for the first time, that the combined application of SonoVue^{TM} microbubbles and ultrasound-targeted microbubble destruction (UTMD) technology significantly enhances the infiltration and endocytosis of exosomes in recalcitrant tissues, with minimal UTMD exposure sufficient to promote transient, yet efficient, exosome delivery, demonstrating the safety and efficacy of this innovative strategy [199]. The structural integrity and molecular composition of exosome membranes can be effectively preserved through mild sonication-induced remodelling processes, an innovative approach that not only maintains the essential characteristics of exosome membranes but also significantly enhances drug encapsulation efficiency, leading to improved therapeutic payload delivery, while modified exosomes simultaneously exhibit desirable sustained release profiles, making them particularly suitable for controlled drug delivery applications [199]. Viruses can participate in exosome biogenesis, whereby viral RNA genomes, miRNAs, and proteins can be efficiently encapsulated within exosomes [198]. In another study, Ohno *et al*. [181] engineered a sophisticated drug delivery platform using exosomes for therapeutic applications; their innovative approach successfully incorporated let-7a microRNA, a tumour-suppressing molecule, into HEK293-derived exosomes via transfection techniques, representing a significant advancement in targeted breast carcinoma treatment strategies. Pegtel *et al*. [200] reported functional miRNA transfer via exosomes as a possible mechanism for intercellular communication and immunomodulation. These methods enable the precise incorporation of diverse payloads, such as nucleic acids (e.g., siRNA and mRNA), proteins, and small-molecule drugs, while preserving the structural integrity and biological activity of both the cargo and the exosome. Despite their advantages, each technique has limitations, such as potential membrane damage, cargo degradation, and low loading efficiency [201], which underscore the need for continued optimization and innovation in exosomal engineering. Exosome-mediated drug delivery can be optimized through the strategic selection of specific donor cells or the application of advanced bioengineering approaches [202].

The CRISPR/Cas9 system comprises two essential components: the Cas9 protein, an RNA-guided endonuclease capable of precise double-stranded DNA cleavage, and a synthetic guide RNA (sgRNA) 20 nucleotides in length, which determines the sequence specificity of Cas9-mediated DNA targeting and cleavage [203]. An ideal vector must be consistent, safe, nonimmunogenic, and effective while minimizing off-target effects and maintaining high target specificity. While viral and nonviral vectors face limitations in gene therapy, exosomes have emerged as a promising alternative for efficient CRISPR/Cas9 de-

livery [204,205]. CRISPR/Cas9-loaded exosomes, delivered via transfection kits, can specifically target the mutant KrasG12D oncogene in pancreatic cancer cells, effectively reducing proliferation and inhibiting tumour growth *in vitro* and *in vivo*. These findings highlight the therapeutic potential of exosome-mediated CRISPR/Cas9 delivery for treating pancreatic cancer [182]. CD19-engineered exosomes encapsulating CRISPR/Cas9 via electroporation can specifically target the *MYC* oncogene in B-cell malignancies, demonstrating efficient tumour site accumulation and effective eradication of malignant cells in both *in vitro* and *in vivo* models [183]. The engineered exosomes, modified with an aptamer and loaded with CRISPR/Cas9, were efficiently delivered via ultrasound to target *WNT10B*, resulting in marked suppression of tumour growth. This strategy significantly enhanced the accumulation of the modified exosomes at the tumour site *in vitro, in vivo*, and *ex vivo* [184].

Synergistic Integration of Exosomes with Biomaterials for Sustained Therapeutic Delivery

The strategic incorporation of exosomes into biomaterial matrices, including hydrogels and scaffolds, has emerged as a promising approach to achieve the controlled and sustained release of therapeutic agents. This synergy enhances the temporal and spatial distribution of bioactive molecules, thereby prolonging their therapeutic efficacy and maximizing their biological impact. Hydrogels, with their tuneable mechanical properties and high biocompatibility, provide an ideal environment for encapsulating exosomes while maintaining their structural and functional integrity. Photoimprinted imide cross-linked hydrogels, which exhibit exceptional manipulability, biocompatibility, and superior cartilage matrix integration properties, have been demonstrated in a recent study as exosome-laden scaffolds for the fabrication of engineered hydrogel-based cellular tissue patches (EHGs) specifically designed for cartilage regeneration. The developed EHG system not only preserves synovium-derived exosomes (SC-Exos) but also actively modulates the biological behaviour of chondrocytes and human bone marrow-derived mesenchymal stem cells (hBMSCs) *in vitro*. Furthermore, EHGs demonstrate excellent integration capacity with native cartilage ECM, facilitating targeted cellular deposition at defect sites and ultimately promoting cartilage defect repair through synergistic effects on cellular regulation and matrix integration [98].

Moreover, a study demonstrated the encapsulation of umbilical cord mesenchymal stem cell-derived exosomes (uMSC-Exos) within a hyaluronic acid-based hydrogel (HA-Gel), which was subsequently integrated with a tailored 3D-printed nanocomposite scaffold composed of nanohydroxyapatite/poly(ε-caprolactone) (nHP) for cranial reconstruction. This innovative composite system was designed to increase angiogenesis through upregulation of the NOTCH1/DLL4 signalling pathway, thus facilitating the repair of cranial defects in an experimental rat model [185]. The encapsulation of exosomes within natural and synthetic hydrogels represents a critical strategy for preserving their structural integrity and achieving controlled release at targeted sites. Current and emerging methodologies for the application of hydrogel-encapsulated exosomes in the field of CNS tissue engineering have been well summarized by Zakeri *et al*. [206]. In addition, an innovative approach involving the synthesis of an exosome-sheathed degradable nanogel (NG) as a targeted drug delivery system to enhance brain-specific accumulation and controlled pharmaceutical release has been developed. Specifically, an exosome-sheathed degradable nanogel (NG), known as hyaluronic acid (HA) NG@exosomes, loaded with pituitary adenylate cyclase-activating polypeptide (PACAP) and oestradiol (E2), was synthesized for precise peptide/drug delivery. This platform has demonstrated significant potential for improving therapeutic outcomes in perimenopausal depression through attenuating oxidative and inflammatory conditions and improving synaptic plasticity [186].

Similarly, scaffolds designed with precise porosity and degradation profiles offer a three-dimensional framework that supports the localized delivery and prolonged retention of exosomes at target sites. In a cutting-edge investigation, Xie *et al*. [187] successfully engineered an extracellular vesicle (EV)-functionalized scaffold that exhibited improved proangiogenic and osteogenic properties. This innovative methodology involves the application of MSC-EVs onto a decalcified bone matrix (DBM). This pioneering fabrication technique not only harnesses the therapeutic potential of exosomes but also establishes a robust foundation for their application in bone tissue engineering. The compelling therapeutic strategy underscores the efficacy of the scaffold in promoting vascularization and bone regeneration. The meticulous integration of MSC-EVs with DBM represents a significant advancement in the field, offering a promising avenue for future regenerative therapies [187]. The synergistic utilization of exosomes in conjunction with this bioscaffold for the treatment of neurological injuries, including traumatic brain injury (TBI), has been comprehensively reviewed [101,207]. This integrated approach leverages the regenerative potential of exosomes to enhance the therapeutic efficacy of the bioscaffold, addressing critical aspects of neural repair and functional recovery. Such studies underscore the promising outcomes of this combined strategy in mitigating neuroinflammation, promoting neurogenesis, and facilitating tissue regeneration. The physicobiochemical methods used to engineer exosomes and the applications of these composites are summarized in Fig. 6, and the roles of exosomes in both tissue regeneration and immune disorders are highlighted.

Challenges and Future Directions

Key Challenges in Exosome Research and Development: Scalability, Stability, and Standardization

In terms of the current regulatory landscape, the United States Food and Drug Administration (FDA) has approved 23 nanomedicinal products. Among these authorized nanomedicines, the predominant formulations are essentially composed of three principal nanocarrier systems: liposomal structures, polymeric micellar assemblies, and nanocrystalline matrices [208]. As a class of natural biologics distinct from synthetic compounds, exosomes have emerged as pivotal components in the development of advanced smart drug delivery platforms. However, one of the foremost challenges in advancing exosome-based therapies lies in achieving scalable production of high-purity exosomes, a critical prerequisite for clinical translation and industrial applications. Current production methods often face limitations in yield and scalability, underscoring the need for innovative approaches to optimize exosome isolation and purification [209]. Equally pressing is the challenge of ensuring stable long-term storage, as exosomes are susceptible to degradation or aggregation under suboptimal conditions, which can compromise their therapeutic efficacy.

Furthermore, the lack of standardized protocols for isolation, purification, and characterization poses a significant barrier to the reproducibility and consistency of experimental and clinical outcomes [210]. Efforts to establish universal guidelines and develop robust analytical tools are essential for enhancing the reliability and comparability of exosome research across laboratories and applications [211]. Addressing these technical challenges is imperative to unlock the full potential of exosome-based technologies in precision medicine and beyond.

Advancing Exosome Research: Addressing Heterogeneity, Biodistribution, and Safety for Clinical Translation

Substantial knowledge gaps persist regarding the molecular mechanisms governing exosome biogenesis. Furthermore, the current lack of reliable methodologies for manipulating cargo loading processes or vesicular release dynamics continues to impede the comprehensive elucidation of their physiological functions *in vivo* [3]. A thorough understanding of the inherent heterogeneity of exosomes and their biodistribution profiles is paramount for optimizing their therapeutic potential and ensuring targeted delivery in clinical settings. Exosomal heterogeneity, driven by variations in size, cargo composition, and surface markers, presents both opportunities and challenges in tailoring exosome-based therapies to specific diseases [100]. Similarly, elucidating the biodistribution patterns of exosomes is essential for enhancing their ability to reach target tissues while minimizing off-target effects [212–214]. Equally critical is addressing safety concerns, including the potential for tumorigenic effects and immune activation, which could pose risks in therapeutic applications. Rigorous preclinical evaluation and the development of robust strategies to mitigate these risks are indispensable for advancing exosome-based technologies towards clinical approval.

Emerging Trends in Exosome Therapy

Emerging trends include the integration of artificial intelligence (AI)-driven engineering methods, the application of advanced 3D bioprinting technologies incorporating exosomes, and the progression of clinical translation strategies to expedite the development of exosome-based therapeutic interventions [99,215]. These innovations collectively represent a paradigm shift in the field, offering unprecedented opportunities for precision medicine and regenerative therapies. AI-driven engineering approaches enable sophisticated data analysis and predictive modelling [216,217], whereas 3D bioprinting techniques facilitate the precise spatial arrangement of exosomes to mimic native tissue architectures [218–220]. Microfluidic technology enables the high-throughput collection of both natural and synthetic exosomes, which can be effectively incorporated into bioinks [221]. Consequently, the synergistic integration of microfluidic systems with 3D printing techniques holds significant promise as a pivotal strategy for advancing exosome-based therapeutics into clinical applications. These comprehensive approaches underscore the interdisciplinary nature of contemporary biomedical research and its potential to revolutionize therapeutic landscapes. However, the clinical translation of therapeutic exosomes requires overcoming challenges such as bioink optimization, high-resolution patterning, spatiotemporal release control, and standardized stability protocols under clinical conditions [219].

Conclusions

As biological couriers redefine regenerative paradigms, exosomes epitomize the convergence of molecular ingenuity and clinical innovation in tissue engineering. Their paradigm-shifting capabilities transcend the constraints of conventional cellular therapeutics, providing targeted intervention modalities for biological challenges spanning regenerative tissue engineering to inflammatory tumour microenvironment reprogramming. To actualize this biomedical revolution, the field demands the (1) systematic integration of omic-driven biodesign platforms; (2) resolution of critical challenges in cargo-loading specificity and pharmacokinetic profiling; and (3) establishment of standardized clinical translation protocols. Such multidimensional advancements will catalyse the metamorphosis of exosome research into clinically actionable solutions, ultimately reconfiguring the therapeutic landscape of precision medicine.

List of Abbreviations

AI, artificial intelligence; EVs, extracellular vesicles; MVBs, multivesicular bodies; miRNAs, microRNAs; ILVs, intraluminal vesicles; ESCRT, endosomal sorting complex required for transport; TEM, transmission electron microscopy; DLS, dynamic light scattering; TRPS, tuneable resistive pulse sensing; AFM, atomic force microscopy; ECM, extracellular matrix; AKI, acute kidney injury; VEGF-A, vascular endothelial growth factor-A; VEGFR2, vascular endothelial growth factor receptor 2; MMPs, matrix metalloproteinases; NK, natural killer; MSC-Exos, mesenchymal stem cell-derived exosomes; AKT, protein kinase B; ERK, extracellular regulated protein kinase; CNS, central nervous system; PEG, polyethylene glycol; APCs, antigen-presenting cells; HSP, heat shock protein; Bregs, regulatory B cells; siRNA, small interfering RNA; Dox, doxorubicin; NSCLC, non-small cell lung cancer; PI3K/Akt, phosphatidylinositol 3-kinase/protein kinase B; EAA, exosome-anchoring aptamer; TAR, transactivation response; NTA, nanoparticle tracking analysis; Treg, regulatory T-cell; DC, dendritic cell; IFN-γ, interferon-γ; TNF-α, tumour necrosis factor α; HEK293, human embryonic kidney 293; iDCs, immature DCs; FA, folic acid; FR, FA receptor; RVG, rabies virus glycoprotein; RIF, rifampicin; ANG, angiopoietin; SPIONs, superparamagnetic iron oxide nanoparticles; UTMD, ultrasound-targeted microbubble destruction; EHGs, engineered hydrogel-based cellular tissue patches; HA-Gel, hyaluronic acid-based hydrogel; uMSC-Exos, umbilical cord mesenchymal stem cell-derived exosomes; nHP, nanohydroxyapatite/poly(ε-caprolactone); NG, nanogel; PACAP, pituitary adenylate cyclase-activating polypeptide; DBM, decalcified bone matrix; mExo, milk exosome; E2, oestradiol; 3D, three-dimensional; mRNAs, messenger RNAs; AMP, adenosine monophosphate; PD-L1, programmed cell death ligand 1; MHC, major histocompatibility complex.

Availability of Data and Materials

Not applicable.

Author Contributions

LZ contributed to the design of this work and drafted the work. YBW contributed to the interpretation of data. YBW and FM analyzed the data. FFY, JBL, ML, FM, DKWO, and YBW revised critically for important intellectual content. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

As this manuscript pertains to a review article, so conventional requirements of ethical approval and participant consent are not applicable in this context.

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Conflict of Interest

The author(s) declare no conflict of interest.

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