Harnessing exosomes for targeted therapy: strategy and application

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Key Words:
biomedical engineering; exosomes; modification; nanotechnology; targeted therapy

From the Contents

ABSTRACT

Exosomes, nanoscopic extracellular vesicles produced by cells, are pivotal in mediating intracellular communication by transporting nucleic acids, proteins, lipids, and other bioactive molecules, thereby influencing physiological and pathological states. Their endogenous origin and inherent diversity confer distinct advantages over synthetic vehicles like liposomes and nanoparticles in diagnostic and therapeutic applications. Despite their potential, the clinical utility of exosomes is hampered by challenges such as limited storage stability, yield, purity, and targeting efficiency. This review focuses on exosomes as targeted therapeutic agents, examining their biogenesis, classification, isolation, and characterisation, while also addressing the current limitations in yield, purity, and targeting. We delve into the literature to propose optimisation strategies that can enhance their therapeutic efficacy and accelerate the translation of exosome-based therapies into clinical practice.

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http://doi.org/10.12336/biomatertransl.2024.01.005

How to cite this article:
Ren, X.; Xu, R.; Xu, C.; Su, J. Harnessing exosomes for targeted therapy: strategy and application. *Biomater Transl.* **2024**, *5*(1), 46-58.

Introduction

The advancement of nanotechnology in medical science has opened new frontiers in the treatment of various diseases, notably in the realm of targeted therapy.[1] This paradigm shift towards precision medicine emphasises the importance of delivering therapeutic agents directly to diseased tissues while minimising collateral damage to healthy cells. In this context, the development of nanoparticles for targeted delivery has emerged as a critical area of research.[2, 3] While these nanocarriers, including polymers, liposomes, and inorganic nanoparticles, have shown promise, their clinical application is often hampered by challenges such as limited tissue penetration and potential immunogenicity. Exosome therapies are distinct from synthetic nanoparticle treatments in several key areas.

Among the various strategies explored, exosomes, naturally occurring vesicles secreted by cells, stand out due to their unique properties as targeted delivery vehicles.[4] Exosomes are lipid-bound vesicles produced under physiological conditions or in response to specific biological stimuli.[5] Their role as key players in intercellular communication renders them particularly effective for both diagnostic and therapeutic applications. Unlike synthetic nanocarriers, exosomes exhibit an intrinsic ability to interact with specific recipient cells or tissues, facilitated by a plethora of surface adhesion proteins and carrier ligands.[6] This interaction enables the transfer of their cargo, which can include proteins, mRNA, and microRNA (miRNA), to target cells with remarkable specificity. Synthetic nanoparticles, like liposomes or polymeric nanoparticles, are engineered to have specific sizes, shapes, and surface properties, which can be consistently reproduced. They can be designed to release their payload in response to certain stimuli and are often easier to characterise and manufacture at scale. However, these synthetic systems can sometimes elicit an immune response or accumulate in non-target organs, posing a safety concern. In contrast, exosomes are endogenous nanocarriers that inherently possess biocompatibility and are less likely to be recognised as foreign by the immune system.

Their natural targeting ability stems from their surface proteins, which can be exploited to deliver therapeutic agents to specific cell types. However, their production is more complex and less controllable, often resulting in a heterogeneous population that requires rigorous purification and characterisation. Furthermore, the loading capacity of therapeutic agents into exosomes can be limited, and their stability in circulation can be variable, presenting challenges for clinical translation. Despite these challenges, the unique properties of exosomes offer a valuable approach to targeted therapy that complements the capabilities of synthetic nanoparticles.

Research has shown that the origin of exosomes greatly influences their targeting capabilities.[7] For instance, tumour-derived exosomes have been found to possess innate tumour-homing characteristics, mediated by mechanisms such as stroma-derived factor 1 and C-X-C chemokine receptor type 4 axis. This specificity not only enhances the therapeutic efficacy of these vesicles but also opens new avenues for non-invasive diagnostic methods through the analysis of their cargo.[8] Despite their potential, the clinical translation of exosome-based therapies faces significant hurdles, primarily related to their natural properties. In their unmodified state, exosomes tend to exhibit suboptimal targeting and may accumulate in organs such as the liver, kidney, and spleen.[9] To address these limitations, recent advances have focused on the surface modification of exosomes.[10] By displaying targeting molecules such as peptides or antibody fragments on their exterior, exosomes can be engineered for enhanced site-specific drug delivery and improved *in vivo* tracking. This review aims to present the latest developments in the biology of exosomes, elucidate their targeting mechanisms, and highlight innovative strategies for targeted exosome therapy. To ensure a thorough and systematic review, we employed a detailed methodology for our literature search. Using databases such as PubMed, Scopus, and Web of Science, we conducted searches with key terms including "exosomes", "extracellular vesicles", "targeted drug delivery", "exosome therapeutics", and "clinical trials exosomes". The search was limited to studies published within the last 10 years to focus on the most recent advances in the field. Our inclusion criteria targeted peer-reviewed original research articles, reviews, and meta-analyses that provided insights into exosome biology, engineering for targeted therapy, and clinical applications. We excluded non-English articles, conference abstracts without full text, and studies not directly related to exosome-mediated therapy. By integrating these diverse approaches, the field of exosome-based therapy holds the promise of revolutionising drug delivery systems, offering more efficient, targeted, and personalised treatment modalities (**Figure 1**).

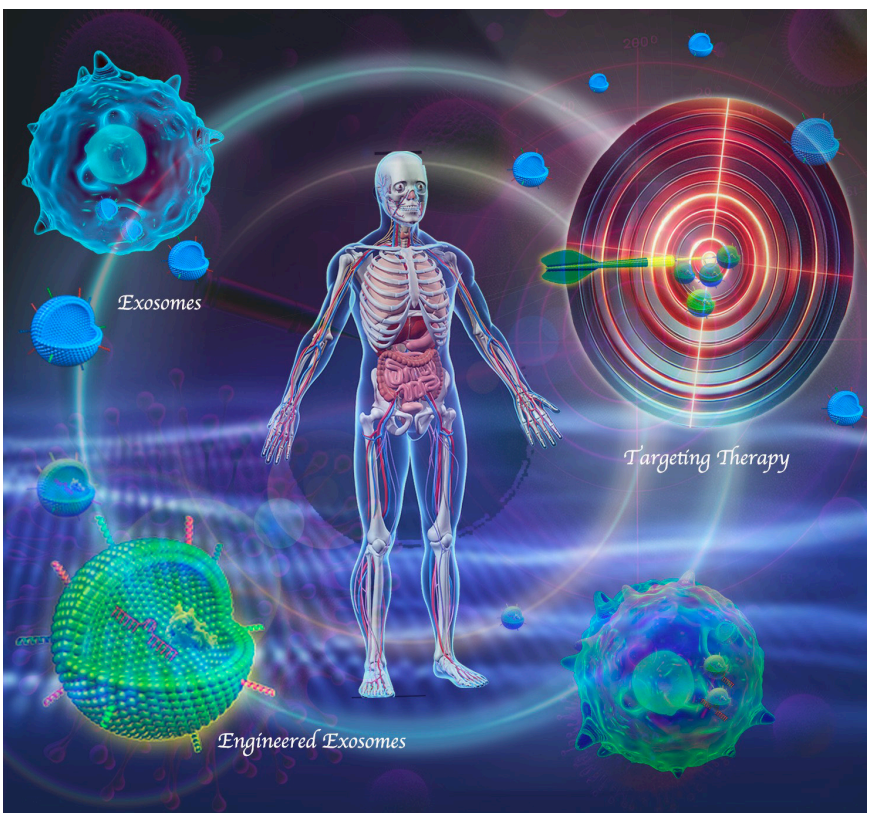

Figure 1. Engineered exosomes offer a targeted therapeutic approach, enhanced by direct or indirect modifications to treat diseases with precision and reduced toxicity. Created with Adobe Photoshop 2024.

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The Biology of Exosomes

Compositions and architecture of exosomes

Exosomes, a distinct subset of extracellular vesicles (EVs) also known as intraluminal vesicles (ILVs), are integral to cellular communication and are secreted by a wide range of cell types (**Figure 2**). These vesicles are ubiquitous present in various body fluids, including plasma, urine, semen, and saliva. Structurally, exosomes are enclosed within a single lipid bilayer membrane and typically range in size from 30 to 150 nm in diameter.[11] In solution, they are spherical in shape, but can assume a double concave or cup shape when subjected to drying during sample preparation processes. The composition of exosomes is remarkably diverse, reflecting the physiological processes of their cell of origin. They are rich in proteins that play critical roles in cell adhesion, membrane fusion, metabolism, and signal transduction. In addition to proteins, exosomes are carriers of various nucleic acids, including miRNAs, mRNAs, DNA fragments, and long non-coding RNAs, which are crucial for intercellular communication and regulation of recipient cell functions.[12]

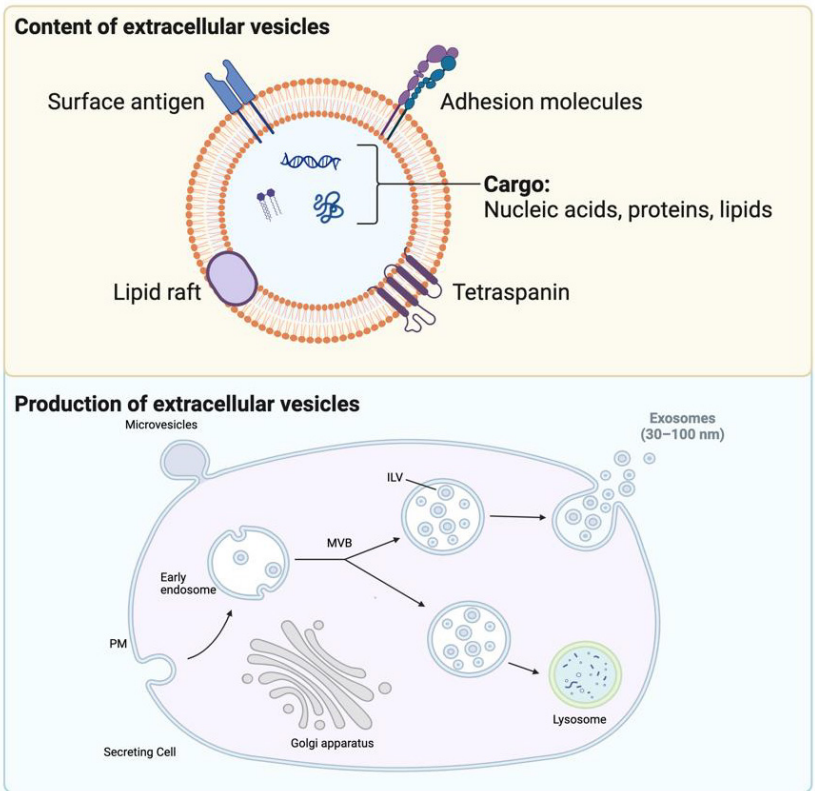

Figure 2. The content and production of exosomes. ILV: intraluminal vesicle; MVB: multivesicular body; PM: plasma membrane. Created with BioRender.com.

Among the proteins embedded in the exosomal membrane are tetraspanins (such as CD9, CD63, and CD81), antigen-presenting molecules such as major histocompatibility complex class I and II, various glycoproteins, and adhesion molecules. The cytoplasmic content of exosomes typically includes proteins associated with the endosomal sorting complex required for transport, heat shock proteins, cytoskeletal proteins, and various proteins involved in membrane transport and fusion.[13] Notably, while certain proteins such as heat shock proteins and endosomal sorting complex required for transport components are commonly found across different exosome types, molecules such as major histocompatibility complex class I and II are specific to the exosomes' cell of origin. The lipid bilayer of exosomes is composed of cholesterol, ceramides, sphingomyelin, phosphatidylinositol, phosphatidylethanolamine, and gangliosides. These lipids play critical roles in defining the exosome's cargo, secretion process, structural integrity, and signalling capabilities.[14]

Regarding their nucleic acid content, miRNAs are one of the most abundant and functionally significant types of RNAs found in exosomes.[15] They are involved in regulating various biological processes, including exocytosis, haematopoiesis, angiogenesis, and are key players in exosome-mediated cell communication.[16] Exosomes also contain other RNA species such as ribosomal RNA, long non-coding RNA, transfer RNA, small nuclear RNA, and small nucleolar RNA, all of which contribute to their role as signalling entities.[17] Given the rich and diverse nature of their content, exosomes have garnered considerable interest in the biomedical field as potential tools for disease diagnosis, treatment strategies, and prognostic evaluations, particularly in the context of cancer and other pathologies.

Production and biofunctions of exosomes

The production of exosomes for therapeutic purposes is a critical aspect of their clinical translation. Extraction and purification are pivotal steps that significantly affect the quality and efficacy of the final exosome preparation. Commonly employed methods for exosome extraction include differential ultracentrifugation, which separates exosomes based on their size and density, and size-exclusion chromatography, which isolates exosomes based on their size. Immunoaffinity capture, which uses antibodies targeting exosome surface markers, is another method that can yield highly specific exosome populations. Each method has its advantages and limitations regarding yield, purity, and scalability. Ultracentrifugation, while widely used, is time-consuming and requires specialised equipment. Size-exclusion chromatography is less labour-intensive but may co-isolate non-exosomal vesicles. Immunoaffinity capture offers specificity but can be costly and is not easily scalable for large volumes. Ongoing research is focused on refining these methods and developing new techniques to improve the efficiency and cost-effectiveness of exosome production.

The biogenesis and functionality of exosomes are areas of burgeoning research, with several mechanisms identified, though much remains to be understood. The formation of exosomes involves a multi-step process encompassing budding, invagination, the formation of multivesicular bodies (MVBs), and eventual secretion.[18] Initially, exosome production begins with the invagination of the cell membrane, leading to the formation of clathrin-coated vesicles. These vesicles enter early endosomes in the cytoplasm. Subsequently, with the aid of the Golgi complex, a selective packaging process occurs where proteins, lipids, and nucleic acids are sorted and incorporated into ILVs, which are the precursors to exosomes.[19] These ILVs accumulate within late endosomes as they mature from the early endosomes. MVBs and late endosomes are distinct endosomal compartments, characterised by their rich content of ILVs, which encapsulate specific proteins, lipids, nucleic acids, and cytoplasmic components.[20] The transport of MVBs and their fusion with the plasma membrane are regulated by various Rab GTPase proteins, with Rab27A and Rab27B playing pivotal roles in guiding MVBs to the cell periphery.[21] The SNARE complex is instrumental in facilitating the fusion of MVBs with the plasma membrane, leading to the release of ILVs, now known as exosomes, into the extracellular environment.[22] While the exact mechanisms governing whether MVBs are routed to lysosomes for degradation or to the plasma membrane for exosome release remain elusive, it is posited that different subsets of MVBs may coexist within cells, each destined for either degradation or exocytosis.[23] This complex interplay of pathways highlights the intricate nature of exosome biogenesis and underscores the need for further research to fully elucidate these processes.

Modification Strategies of Exosomes

Targeting mechanisms and construction strategies for exosome-based systems capitalise on their inherently low immunogenicity, specific targeting properties, and superior tissue/cell penetration capabilities, positioning exosomes as promising vectors for drug delivery (**Figure 3**). However, native exosomes face challenges with respect to their innate targeting capacity, which can impede their practical application and clinical translation. To overcome these limitations, numerous strategies have been devised to augment exosomes with targeting molecules, thereby enhancing their targeting efficiency.

Native targeting function of exosomes

Exosomes, as endogenous carriers originating from tissue cells, possess distinct advantages over traditional nanocarriers, including low immunogenicity and an enhanced ability to traverse biological barriers. Furthermore, exosomes have demonstrated inherent targeting abilities that are advantageous during drug delivery. For instance, docetaxel-loaded exosomes isolated from A549 cancer cells have exhibited increased tumour tissue accumulation and improved targeting relative to free docetaxel.[24] A study has verified that certain molecules and proteins on the exosomal surface membrane are pivotal to their innate targeting transport, facilitating enhanced interactions with target cell membranes.[25] Additionally, exosomes sourced from diverse cell types inherently migrate to different target sites. Specifically, liver metastatic cell-derived exosomes, which express integrin αvβ5, can selectively home to Kupfer cells.[26] Conversely, integrins α6β4 and α6β1, prevalent in exosomes from lung cancer cells, have an affinity for binding specifically to fibroblasts and epithelial cells in the lungs. Consequently, exosomes from various cancer cells inherently possess organ-specific targeting capabilities, which could significantly impact the tissue microenvironment, underscoring the potential of cancer-derived exosomes in targeted tumour therapy. Exosomes from dendritic cells (DCs) generated during DC-T cell interactions are recruited by activated T cells in a lymphocyte function-associated antigen 1-dependent manner.[27] DCs can also engage active T cell exosomes through exosomal lymphocyte function-associated antigen 1, which may have therapeutic implications for autoimmune diseases.[28] B-cell-derived exosomes are known to be assimilated by hepatic and splenic macrophages through α2,3-sialic acid linkages.[29] Furthermore, natural killer cell-derived exosomes can be internalised by tumour cells via micropinocytosis, impeded by blocking CD226.[30] Additionally, miR-155-5p, found in exosomes from M2 macrophages, can target the zinc-finger protein zinc finger E-box-binding homeobox 2 in colon cancer cells.[31] The targeting potential of exosomes in their natural state is considered inadequate for the precise demands of contemporary therapy. Native exosomes' tendency for non-selective distribution throughout the body and their clearance by the body's reticuloendothelial system contribute to a high likelihood of off-target effects, diluting their therapeutic potential. They may not naturally home to the specific tissues or pathological sites that require intervention, posing a challenge in their application as targeted therapeutic vehicles. To enhance their targeting capability, exosomes are bioengineered with surface modifications. The integration of specific ligands or antibodies that recognise and bind to markers unique to target cells improves their homing efficiency. For instance, equipping exosomes with peptides that target receptors overexpressed in tumour cells can transform them into a delivery system with sequential targeting abilities, increasing the delivery precision of therapeutic agents.

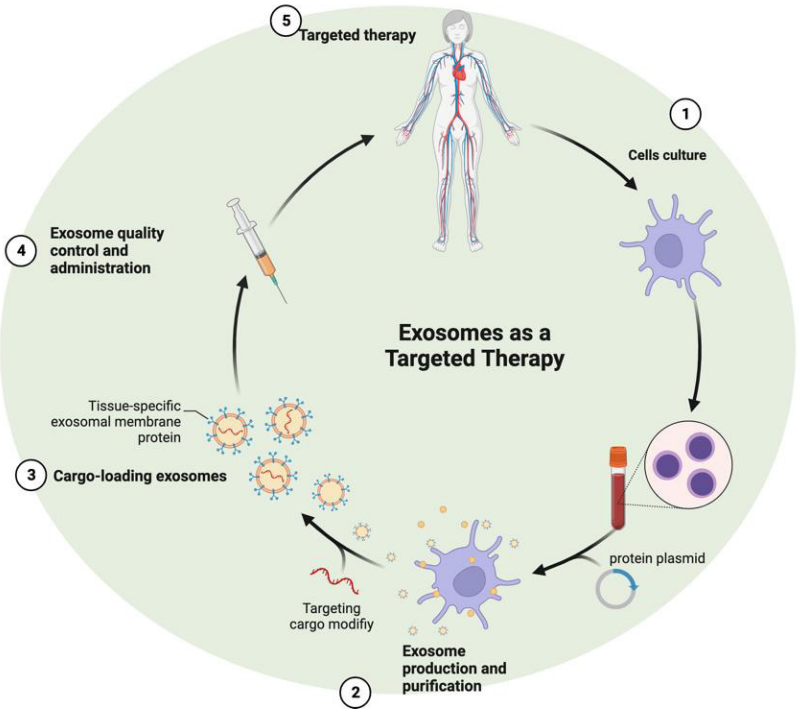

Figure 3. The process of targeted therapy of exosomes. 1. Cells culture: The process begins with the culture of cells, which are the source of exosomes. 2. Exosome production and purification: Following cell culture, exosomes are produced and then isolated and purified from the cell media. 3. Cargo-loading exosomes: The isolated exosomes are then loaded with therapeutic cargo. This step involves incorporating the desired molecules, such as drugs or proteins, into the exosomes. 4. Exosome quality control and administration: After loading the cargo, the exosomes undergo quality control to ensure they meet the necessary standards for therapeutic use. Once approved, they are ready for administration to the patient. 5. Targeted therapy: The final step is the administration of these engineered exosomes to the patient, where they can deliver their therapeutic cargo to the targeted tissues or cells in the body. This sequence represents the full cycle from cell culture to the delivery of targeted therapy using exosomes. Created with BioRender.com.

Modification strategies of exosomes for targeted therapy

Exosomes possess inherent targeting capabilities, but these are not robust enough to satisfy the stringent requirements of precise therapeutic delivery. To bridge this gap, substantial efforts are being made to enhance their innate targeting abilities through various engineering techniques (**Figure 4**). Advanced strategies involve both genetic and chemical modifications that allow for the integration of a diverse array of bioactive ligands into the exosome structure.[32] These modifications not only augment the targeting precision of exosomes but also boost their overall therapeutic efficacy. The incorporation of such ligands is meticulously designed to ensure that the modified exosomes can navigate more effectively to their intended destinations, promising a new level of precision in the delivery of treatments. Through these sophisticated engineering methods, exosomes are being transformed into highly specialised vehicles for targeted therapeutic applications.

Indirect modification: genetically modified exosomes

Genetic engineering is a cornerstone in the modification of exosome surfaces to enhance their targeting ability. By transfection of plasmid vectors, specific proteins can be grafted onto exosome membranes. Key proteins involved in this process include non-specific transmembrane proteins such as lysosome-associated membrane protein 2b (Lamp2b) and members of the tetraspanin family such as CD63, CD9, and CD81.[33, 34]

Lamp2b, which is mainly located in lysosomes and endosomes, is frequently utilised for surface modification due to its structure that includes an amino acid signal peptide, a short-tailed C-terminal transmembrane region, and a large N-terminal extramembrane domain.[35-37] This structure allows Lamp2b to be fused with targeting ligands or cell-specific peptides, enhancing the delivery precision of exosomes. For instance, the rabies virus glycoprotein (RVG) peptide, which binds selectively to acetylcholine receptors, has been used to generate neuro-specific exosomes for delivering drugs to the central nervous system.[38] Modified exosomes with RVG-Lamp2b have shown promise in delivering miRNA to specific sites, such as miR-124 to ischaemic injury sites, and opioid receptor mu small-interfering RNA (siRNA) to treat morphine relapse (**Figure 5A**).[39, 40]

The modification of exosomes extends beyond Lamp2b. For instance, T7 peptides have been attached to Lamp2b to create exosomes with improved efficiency in delivering drugs to glioblastoma cells (**Figure 5B**).[41] The use of receptor for advanced glycation end product-binding peptide linked to Lamp2b has shown higher intracellular delivery efficacy of curcumin into lung tissues.[42] Moreover, genetic modifications using internalising RGD peptide (iRGD) peptide-fused

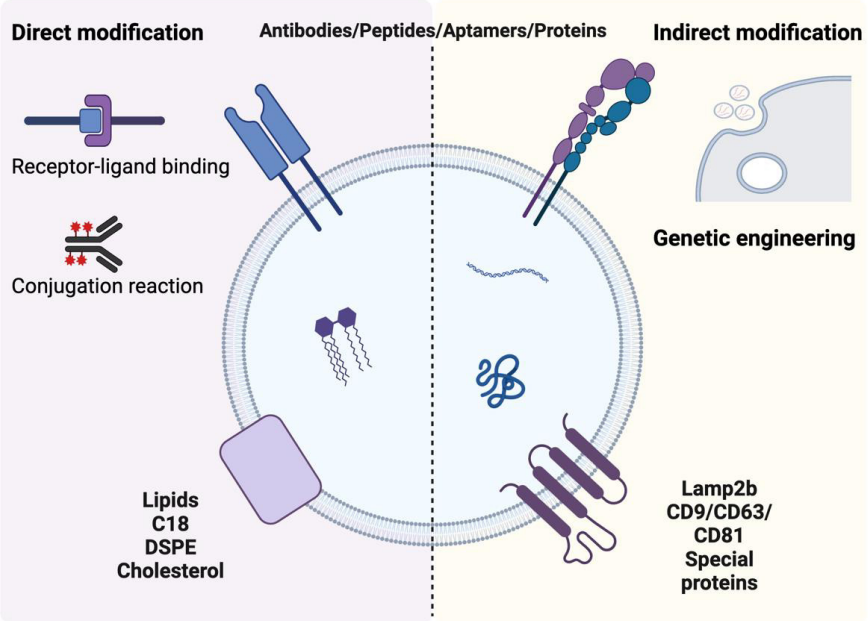

Figure 4. The modification strategy of exosomes. DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; Lamp2b: lysosome-associated membrane protein 2b. Created with BioRender.com.

Lamp2b have successfully targeted doxorubicin to tumours and delivered kirsten rat sarcoma viral oncogene homolog (KRAS) siRNA to inhibit tumour growth (**Figure 5C**).[43] Additionally, engineering Lamp2b with interleukin-3 receptor targets chronic myeloid leukaemia cells to deliver tumour-suppressive drugs effectively.[44]

Similarly, the tetraspanin proteins CD63, CD9, and CD81 are attractive candidates for modification to impart targeting properties to exosomes. These modifications have facilitated the targeted delivery of miRNA and antagonists against specific cellular receptors (**Figure 5D**).[45] Furthermore, receptor proteins such as epidermal growth factor receptor, human epidermal growth factor receptor 2, and platelet-derived growth factor receptor located on exosomes have been utilised as modification sites to enhance targeting for therapeutic and imaging applications, like tumour visualisation in mice using ^{99}mTc-labelled exosomes.[46-48]

Although genetic engineering is potent for protein display, it is limited to genetically encodable peptides and proteins. Therefore, the development of strategies that allow a broader spectrum of ligands to be displayed on the exosomal surface is necessary to widen the therapeutic potential and applicability of exosomes in targeted therapy.

Direct modification

Direct modification of exosomes may offer a more practical alternative to complex genetic engineering, especially when customisation of exosome-producing cells is challenging. Techniques such as click chemistry, hydrophobic insertion, and receptor-ligand binding are standard for direct exosome modification.[49] These methods have been employed effectively; for instance, the peptide CP05 has been identified for its ability to bind to the CD63 protein on exosomes, which facilitates direct conjugation independent of exosomal origin (**Figure 6A**).[50] Moreover, the functionalisation of exosomes with the AS1411 aptamer and the immunomodulatory protein has shown to alter cellular uptake and demonstrate substantial biological activity (**Figure 6B**).[51]

Exosomes can be modified through click chemistry, a suite of highly efficient reactions ideal for bioconjugation. Using copper-catalysed azide-alkyne cycloaddition, often referred to as the quintessential click reaction, we can functionalise exosome surfaces. Here, azide-functionalised molecules are embedded within the exosome lipid bilayer using lipophilic anchors. These azide groups are then covalently linked to alkyne-bearing therapeutic agents in the presence of a copper catalyst, creating a stable triazole ring. Alternatively, strain-promoted azide-alkyne cycloaddition allows for copper-free modification, where strained alkynes like dibenzocyclooctyne are used. This spontaneous reaction with azide-modified molecules circumvents copper's cytotoxicity, making it advantageous for modifying biological materials. Additionally, thiol groups can be added to alkenes in a radical-mediated thiol-ene reaction. By first introducing thiol-reactive moieties such as maleimides or vinyl sulfones on exosome surfaces, thiolated compounds, including peptides or antibodies, can be subsequently conjugated, equipping exosomes with various functionalities for targeted therapy.

Hydrophobic insertion methods have also been employed for targeted delivery. For example, stromal cell-derived exosomes have been modified with a bone marrow-specific aptamer to deliver therapeutic agents directly to bone marrow stromal cells, showing promising results in bone mass enhancement and fracture healing.[52] Such techniques highlight the versatility of polypeptides as targeting ligands due to their small size, low immunogenicity, and specific binding capabilities.

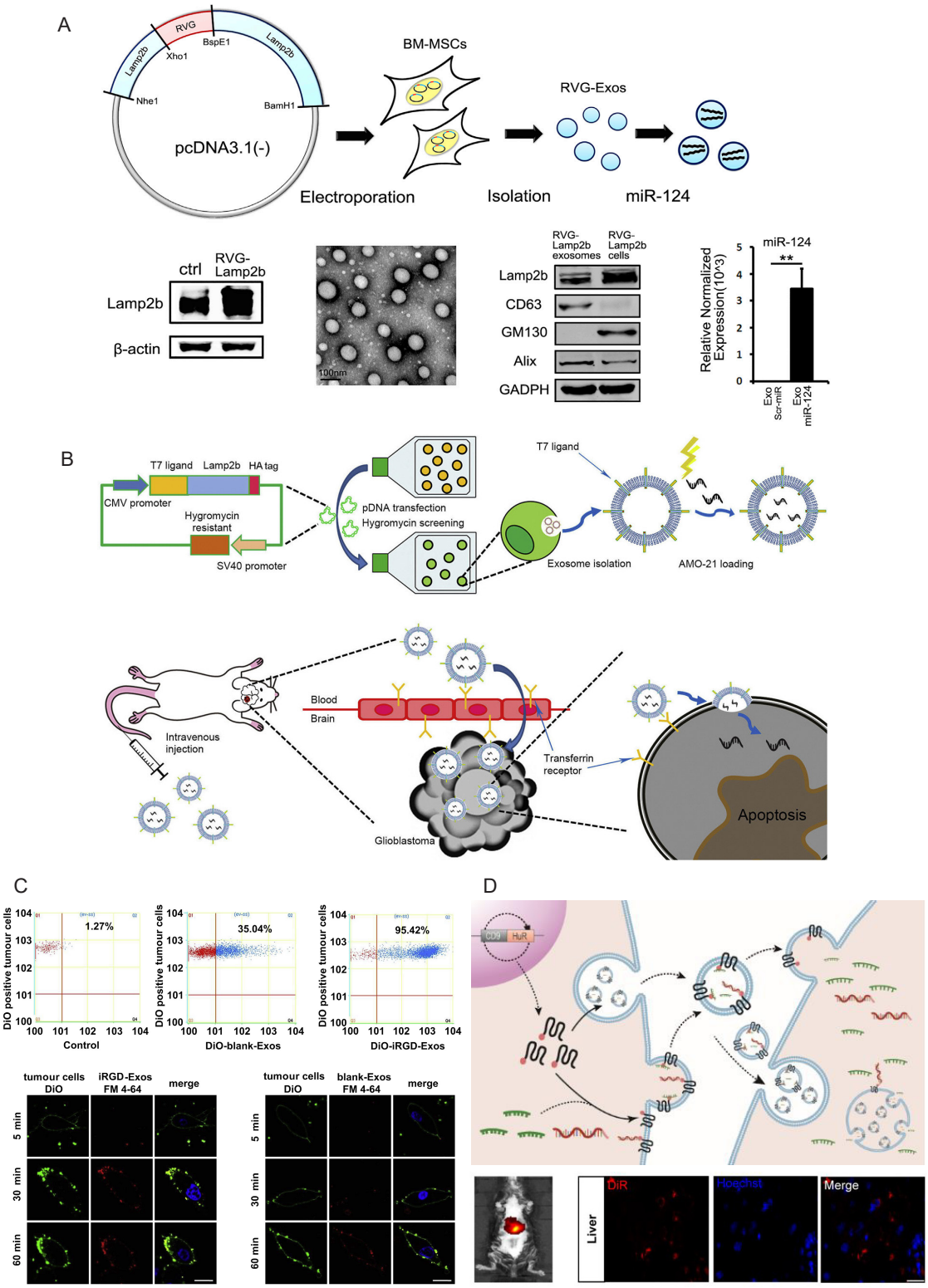

Figure 5. Genetically modified exosomes. (A) Exosomes modified with RVG-Lamp2b promote neurogenesis. Reprinted from Yang et al.[39] (B) T7-peptide decorated exosomes deliver microRNA-21 antisense oligonucleotides to the brain. Reprinted from Kim et al.[41] Copyright 2019, with permission from Elsevier B.V. (C) Binding of iRGD-Exos to a human breast cancer cell line *in vitro*. Reprinted from Tian et al.[43] Copyright 2013, with permission from Elsevier B.V. (D) CD9-HuR functionalized exosomes encapsulated with miRNA or CRISPR/dCas9. Reprinted with permission from Li et al.[45] Copyright 2019, American Chemical Society. Alix: apoptosis linked gene-2-interacting protein X; BM-MSC: bone marrow derived mesenchymal stem cell; CMV: cytomegalovirus; DiO: 3,3′-dioctadecyloxacarbocyanine perchlorate; DiR: 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide; Exos: exosomes; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GM130: Golgi matrix protein 130; HuR: human antigen R; miRNA: microRNA; RVG: rabies virus glycoprotein.

Targeting specificity of exosomes has been further refined by adding peptide ligands to exosome membrane proteins, such as the cyclic peptide iRGD, which has shown to increase the delivery of anticancer drugs to tumours while minimising side effects.[53] Additionally, peptides like c(RgdyK) have demonstrated a high affinity for integrin αvβ3, important for targeting endothelial cells after cerebral ischaemia.[54] These innovative approaches have been used to create exosomes that can navigate to ischaemic brain injuries and potentially inhibit inflammation and apoptosis more effectively than drugs alone.

The use of targeting peptides such as arginine-glycine-glutamic acid (RGE), which binds to neurokinin-1, has allowed the creation of exosomes that can cross the blood-brain barrier, enhancing the imaging and treatment of gliomas.[55] To improve tumour targeting, macrophage-derived exosomes have been modified with peptides to target overexpressed receptors in certain cancer types, such as c-Met in triple negative breast cancer, allowing for the efficient delivery of chemotherapeutic agents.[56]

Furthermore, the engineering of exosomal donor cells to express peptides such as GE11, which targets epidermal growth factor receptor, has facilitated the delivery of tumour-suppressive miRNAs to breast cancer tissues, significantly improving tumour targeting *in vivo* (**Figure 6C**).[57] This strategy demonstrates the potential of using exosomes as platforms for miRNA-based therapies across various cancers. Overall, the development of direct modification strategies for exosomes is vital for translating exosome-based therapeutics from preclinical research to clinical application, ensuring the effective delivery of therapeutic molecules to specific target receptors.

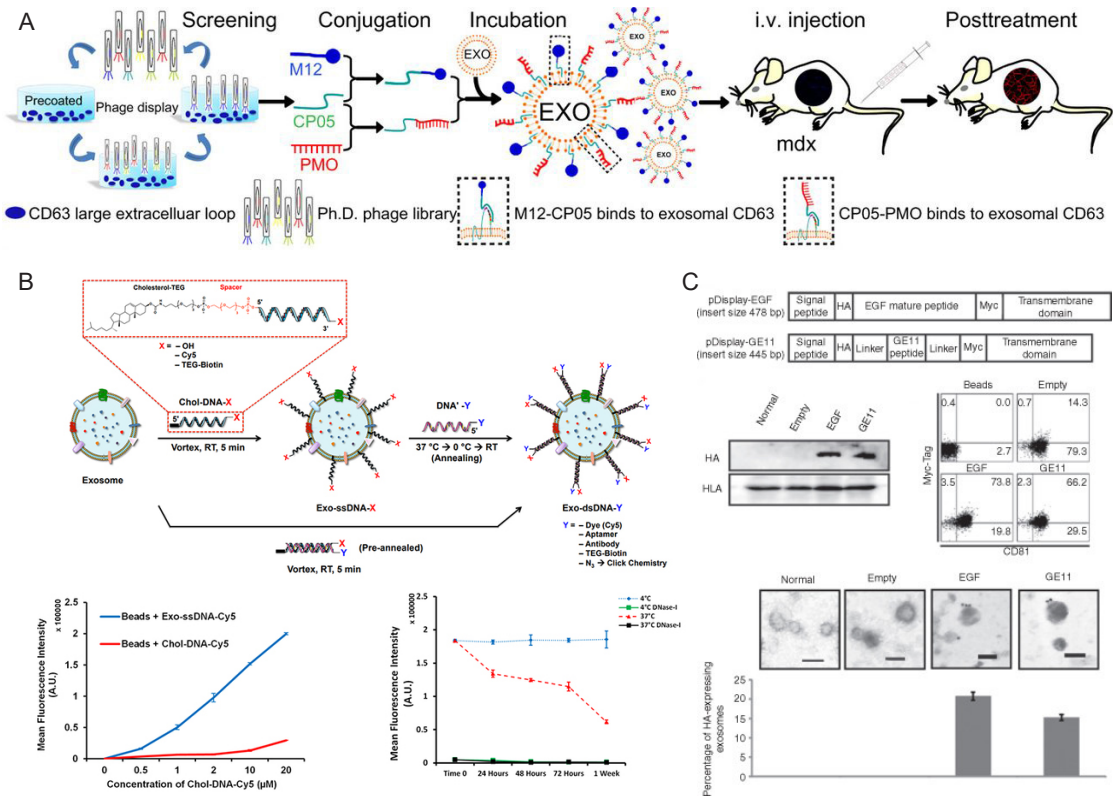

Figure 6. Direct modified exosomes. (A) Screening for exosomal anchor peptides on exosomes. Reprinted from Gao et al. [50] Copyright 2018 Gao et al., some rights reserved; exclusive licensee American Association for the Advancement of Science. (B) Cholesterol-oligonucleotide tethering method on exosome membrane. Reprinted with permission from Yerneni et al.[51] Copyright 2019, American Chemical Society. (C) Epidermal growth factor receptor (EGFR) ligands on the outer surfaces of the exosomes. Reprinted from Ohno et al.[57] Copyright © 2013 The American Society of Gene & Cell Therapy. Published by Elsevier Inc. A.U.: augmentation unit; dsRNA: double-stranded RNA; EGF: epidermal growth factor; EXO: exosome; i.v.: intravenous; Ph.D. phage library: Ph.D.-12 phage display library (New England BioLabs, Ipswich, MA, USA); PMO: phosphorodiamidate morpholino oligomer; RT: room temperature; ssRNA: single-stranded RNA.

The Applications of The Targeted Therapy of Exosomes

Exosomes are emerging as a potent tool for targeted therapy, demonstrating significant potential across various medical fields (**Figure 7**). In the treatment of brain disorders, exosomes can traverse the blood-brain barrier to deliver therapeutic molecules directly to neural tissues, offering a promising approach for conditions that are otherwise difficult to treat.[58] For cardiac diseases, exosomes can be engineered to home to heart tissues, potentially aiding in the repair of damaged myocardium and

delivering cardioprotective agents.[59] In the context of bone regeneration, exosomes can facilitate the delivery of osteogenic factors, thereby enhancing bone healing and remodelling.[60] Lastly, in oncology, tumour-targeting exosomes are being explored for their ability to selectively deliver chemotherapeutic agents and immunomodulatory molecules, aiming to improve treatment efficacy while reducing systemic toxicity.[61] This multifaceted approach underscores the versatility of exosomes as vehicles for precision medicine, targeting complex diseases ranging from neurological disorders to cancer.

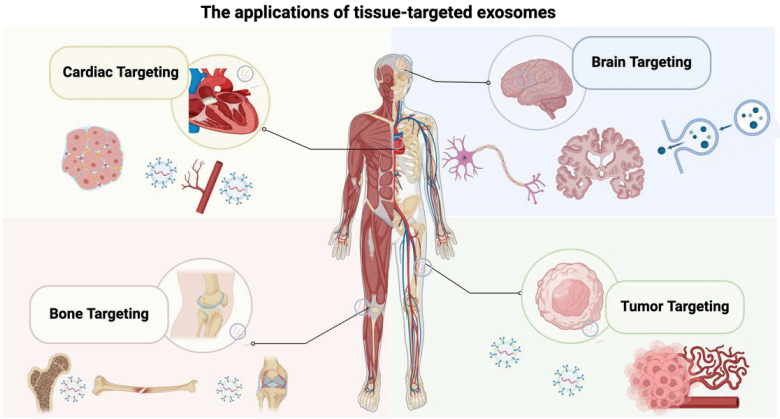

Figure 7. The applications of targeted therapy of exosomes. Created with BioRender.com.

Tumor targeting

Exosome-based systems have been a focal point in the targeted treatment of an array of cancers, including those affecting the brain, colon, liver, lung, and breast. Our cutting-edge research has led to the creation of a dual-functional exosome-based platform that incorporates superparamagnetic nanoparticles.[62,63] This innovation is pivotal, as the resultant drug delivery system harnesses the unique superparamagnetic properties at room temperature, showing an amplified response to external magnetic fields beyond that of standalone superparamagnetic nanoparticles.[64] This allows for more precise navigation and concentration of the therapeutic agents at the tumour site when subjected to a magnetic field, enhancing the efficacy of the drug delivery and providing a notable suppression of tumour growth.[65]

Our novel approach has surmounted significant barriers that previously limited the application of exosomes in oncology. We have developed a system utilising TRAIL-engineered exosomes designed to carry thapsigargin-like molecules that target malignant melanoma.[66] This system has been rigorously tested and the findings are promising: TRAIL-engineered exosomes/thapsigargin-like molecules significantly improve tumour targeting, increase cellular uptake, and hamper tumour cell proliferation, invasion, and migration. Furthermore, it triggers apoptosis in melanoma cells by activating both the extrinsic TRAIL pathway and the intrinsic mitochondrial pathway.[67]

Expanding the utility of exosome-based therapies, Zou et al.[68] have ingeniously developed an aptamer-functionalised exosome platform, which facilitates the cell type-specific delivery of molecular therapeutics. This bespoke delivery method holds the potential to revolutionise how treatments are administered, allowing for personalised therapy regimens tailored to the unique molecular profile of a patient's tumour, thereby minimising side effects and maximising treatment outcomes. In summary, the strategic design of these exosome-based delivery systems could significantly enhance the precision and effectiveness of cancer therapy, paving the way for a new era in the fight against cancer.[69] As we continue to refine these technologies, we anticipate a surge in clinical applications that will lead to more successful patient outcomes.

Cardiac targeting

Cardiac targeting using exosome-based systems is emerging as a pivotal area in regenerative medicine, with significant advances being made to enhance the delivery and efficacy of therapies for heart diseases. Exosomes engineered to express cardiac targeting peptide-Lamp2b on their membranes have shown increased delivery efficiency to cardiac cells and tissues.[70] This is a breakthrough in cardiac therapy, as cardiac targeting peptide-Lamp2b facilitates the natural homing of exosomes to cardiac tissues, thereby improving the delivery of therapeutic agents directly to the affected areas of the heart.[70] This targeted approach is particularly beneficial in treating conditions such as myocardial ischaemia, where precise delivery to the damaged heart tissue is crucial for effective treatment.[71] To further enhance cardiac targeting, exosomes modified with Lam2b have been shown to successfully target heart tissues. This modification increases the affinity of exosomes to heart cells, making the delivery of therapeutic agents more efficient and specific.

A significant advancement in cardiac targeting is the fusion of exosomes with ischaemic myocardium-targeting peptide CSTSMLKAC. This modification has been applied to mesenchymal stem cell-derived exosomes, greatly enhancing their specificity and efficiency in targeting ischaemic myocardium.[72] Ischaemic myocardium-targeting peptide-fused exosomes specifically home to areas of ischaemic injury within the heart, delivering therapeutic agents directly to the site of damage. This targeted delivery is crucial in post-myocardial infarction treatment, as it facilitates the delivery of regenerative factors or drugs to promote healing and prevent further tissue damage.

Brain targeting

The use of exosomes for brain targeting in gene therapy represents a cutting-edge approach in treating neurological disorders. The unique challenges in this field, especially in delivering therapeutic agents across the blood-brain barrier, have prompted innovative strategies involving exosomes.

Exosomes naturally possess the ability to cross biological barriers, including the blood-brain barrier, making them ideal vectors for brain targeting. Their specificity in delivering exogenous genes and the capability to systematically exert gene therapy without eliciting an immune response are pivotal attributes for the clinical application of oligonucleotide-based therapies.[73] The modification of exosomes to improve their targeting to brain tissues and increase the efficiency of gene delivery is a significant advancement. This approach can potentially revolutionise the treatment of various neurodegenerative and neurological disorders.

A breakthrough in this area is the use of DC-derived exosomes engineered to transport Gapdh siRNA specifically to neurons, microglia, and oligodendrocytes in the brain. This targeted delivery has been shown to result in a specific gene knockdown in mice models, providing a new therapeutic strategy for Alzheimer's disease.[74] The ability of these engineered exosomes to specifically target and modify gene expression in brain cells opens new avenues for the treatment of Alzheimer's disease, potentially slowing or halting the progression of the disease. This method offers a more targeted approach than traditional drug therapies, which often have limited effectiveness due to their inability to specifically target affected brain cells or regions.

Bone targeting

The targeting of bone tissue for therapeutic intervention, particularly for conditions like osteoporosis, has been significantly advanced by the use of engineered exosomes.[75, 76] These developments represent a major leap in treating bone-related diseases, given the complexity and dynamic nature of bone as an organ.[77, 78] Song et al.[79] found that exosome rich in miR-155, secreted by vascular endothelial cells, possess an innate ability to target bone tissue. This finding is pivotal as it opens up the possibility of using these endothelial cell-exosomes to deliver therapeutic agents directly to bone sites, enhancing the efficacy of treatments for bone diseases. Hu et al.'s work[80] demonstrated that exosomes expressing C-X-C chemokine receptor type 4 on their surface, when fused with liposomes carrying antagomir-188, tend to accumulate in bone marrow. This accumulation is crucial as it promotes osteogenic differentiation, offering a new avenue for enhancing bone regeneration and treating conditions like osteoporosis. A significant advancement in this field is the development of bone marrow stromal cell-derived exosomes conjugated with a bone marrow-derived mesenchymal stem cell-specific aptamer. This conjugation significantly enhances bone mass and accelerates bone healing, particularly in osteoporosis models such as ovariectomised mice. This targeted approach is a promising strategy for improving bone regeneration. The bone-targeting Exo-siShn3 platform has been engineered to deliver siRNA specifically to osteoblasts.[81] This targeted delivery system could be instrumental in modulating gene expression in osteoblasts, offering a novel approach to treat various bone disorders. In another innovative approach, alendronate has been used to modify mesenchymal stem cell-derived EVs to create alendronate-EVs.[82] These modified EVs have shown a high affinity for bone, making them particularly effective in the treatment of osteoporosis.

Exosome research has progressed considerably, yet its clinical adoption is intricate, shaped by a regulatory framework that is still developing. The use of exosomes in targeted therapies is subject to numerous constraints. The diversity within exosome populations, for instance, can result in inconsistent treatment outcomes. Achieving specific targeting is also complex, given the biological barriers and immune defenses that challenge the delivery to intended cells. Scalability presents another hurdle, with existing isolation and purification techniques not yet adequate for mass production. Ensuring the stability of exosomes, both in storage and post-administration, is essential for maintaining therapeutic activity. Moreover, the development of thorough safety profiles is imperative, as there is a risk of exosomes carrying harmful biomolecules, such as oncogenic proteins or RNAs, potentially leading to deleterious effects. Overcoming these obstacles necessitates a collaborative approach that melds innovations in nanotechnology, molecular biology, and clinical research.

Presently, exosome-based products are regulated as biological drugs, a classification demanding rigorous assessment of their safety, efficacy, and consistent quality prior to clinical application. Regulatory authorities, including the U.S. Food and Drug Administration and European Medicines Agency, are in the process of defining explicit standards for exosome therapies, encompassing isolation, characterisation, and preservation protocols. These guidelines are vital for ensuring the uniformity of therapeutic exosomes across different batches, which is fundamental for patient safety and the predictability of treatment outcomes. Ethical considerations, especially regarding the procurement of exosomal material and the handling of genetic data, are critical. There is a pressing need for ethical policies that cover donor consent, the implications of genetic material transfer, and confidentiality concerns tied to exosome therapies that may contain individual genetic data. As the field progresses, it is imperative that ethical guidelines adapt to meet emerging challenges like fair access to these therapies, particularly in under-resourced settings, and the balancing of intellectual property rights to encourage innovation while safeguarding patient access. An ongoing exchange between scientific progress, regulatory governance, and ethical principles is essential to ensure the responsible integration of exosome research into medical practice.

Perspective and Conclusion

In the rapidly evolving field of exosome research, particularly in targeted therapy, several promising directions are beginning to take shape. Foremost among these is the engineering of exosomes to enhance their innate ability to home in on specific tissues, thus improving the specificity and efficacy of therapeutic delivery. Innovations such as the incorporation of targeting ligands on the exosome surface are being explored to facilitate the precise delivery of drugs, genes, and even CRISPR-Cas9 complexes to diseased cells. Additionally, the emergence of 'designer exosomes' has opened up avenues for the creation of synthetic exosomes, which are expected to offer

improved stability, scalability, and customizable interfaces for diverse therapeutic payloads. On the translational front, the development of robust and scalable exosome purification systems is pivotal for the transition of exosome-based therapies from bench to bedside. These systems are expected to pave the way for large-scale production that meets clinical-grade standards. Lastly, the potential for exosomes to serve as platforms for personalised medicine is being recognised, with research trending towards the utilisation of patient-derived exosomes to tailor treatments to individual molecular profiles, potentially revolutionising the approach to targeted therapy.

Exosome-based therapies have undergone several phase-I and phase-II clinical trials, confirming their safety and effectiveness in anti-tumour and anti-bacterial vaccines. Despite these successes, challenges remain in standardising exosome extraction and maintaining the integrity of exosome content during engineering processes. Furthermore, biosafety concerns necessitate a thorough understanding of exosome biogenesis and donor cell selection, to prevent unwanted genetic information transfer. To harness the full therapeutic potential of exosomes, we must develop uniform, efficient, and effective standardisation methods, and ensure the content and composition of exosomes remain unaltered and safe throughout the engineering process. The rapidly evolving field of nanomedicine holds promise for overcoming these challenges and realising the full clinical potential of exosome-based targeted therapies.

Author contributions

XR conceptualised the review and drafted the manuscript; CX and RX checked and revised figures and language; JS checked and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Financial support

This work was supported by the Shanghai Rising-Star Program Yangfan Project, No. 22YF1414000, and National Natural Science Foundation of China, Nos. 82202335, 82230071, 82172098.

Acknowledgement

None.

Conflicts of interest statement

The authors declare no conflict of interest.

Editor note: Jiacan Su is an Editorial Board member of *Biomaterials Translational*. He was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer review handled independently of this Editorial Board member and his research group.

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Received: January 3, 2024
Revised: January 22, 2024
Accepted: February 6, 2024
Available online: March 28, 2024