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SMART EXOSOMES IN ACTION: STIMULI-RESPONSIVE STRATEGIES FOR CONTROLLED DRUG DELIVERY

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ABSTRACT

Exosomes are considered to be very promising nanocarriers in innovative approaches to drug delivery owing to their biocompatibility, low immunogenicity, and inherent capacity to facilitate intercellular communication. In recent years, increasing attention has been given to the development of construction of stimuli-responsive exosome-based drug delivery systems, whereby therapeutic delivery can be targeted and controlled in response to a particular physiological or external stimulus. This review provides a comprehensive overview of exosomes biology, their formation, structure and main aspects that affect their formation and functional characteristics. Special focus is given to the incorporation of stimuli-responsive approaches into exosome-based systems. The internal stimuli, including pH changes, enzyme overexpression and redox imbalances that exist in the pathological conditions are investigated in their ability to trigger site-specific drug release. Moreover, external stimuli like light irradiation provide possibilities of remote and spatiotemporal regulation of the therapeutic delivery. This review also discusses dual and multi-responsive systems, which can enhance targeting efficiency and eliminate constraints of single-trigger methods. In addition, the review discusses the various biomedical uses of exosomes such as targeted cancer therapy, gene delivery, neurological applications, regenerative medicine, and diagnostic biomarker development. Although these developments are promising, a number of issues still persist, especially the ability to produce in large quantities, standardization, efficiency in loading cargo and the long-term safety testing. In general, stimuli-responsive exosome-based systems

are a fast-growing and highly adaptable platform that can play a major role in precision medicine. Their translation may be accelerated by continued advancements in engineering strategies and manufacturing technologies.

KEYWORDS: Exosomes, Extracellular vesicles, Drug delivery, Nanocarriers.

INTRODUCTION

The Drug delivery systems face challenges in achieving systemic drug distribution. These systems require controlled release of encapsulated medications. These limitations comprise treatment outcomes and reduce therapeutic efficacy. Scientists made drug delivery systems that use outside triggers to solve these problems. The body uses responses to stimuli to deliver drugs to the places through these systems.[1]

Stimuli-responsive drug delivery systems address three major challenges in pharmaceutical therapy. It provides solutions for delivering drugs until the body starts processing each part. The system lets the body get ready to decide when to release the content, which may results in off-target side effects. The medical field needs treatment methods. Researchers study response systems to create advanced systems. These systems treat diabetes, cancer and neurodegenerative diseases because standard treatments do not work well.[2]

Stimulus-based drug delivery systems make the way drugs interact with cells. They provide drug delivery and controlled release through special delivery systems. These systems deliver drugs at specific times. The drug delivery system enables controlled medication release through external triggers. Internal triggers include changes in levels reactive oxygen species (ROS) and specific enzymes. External triggers include light, heat, ultrasonic sound and magnetic influence. These triggers lead to drug release, at targeted locations. Drug delivery systems work because they can identify physical, chemical and biological stimuli.[3]

The overall concept of stimuli-responsive exosome-based drug delivery system is illustrated in Fig. 1.

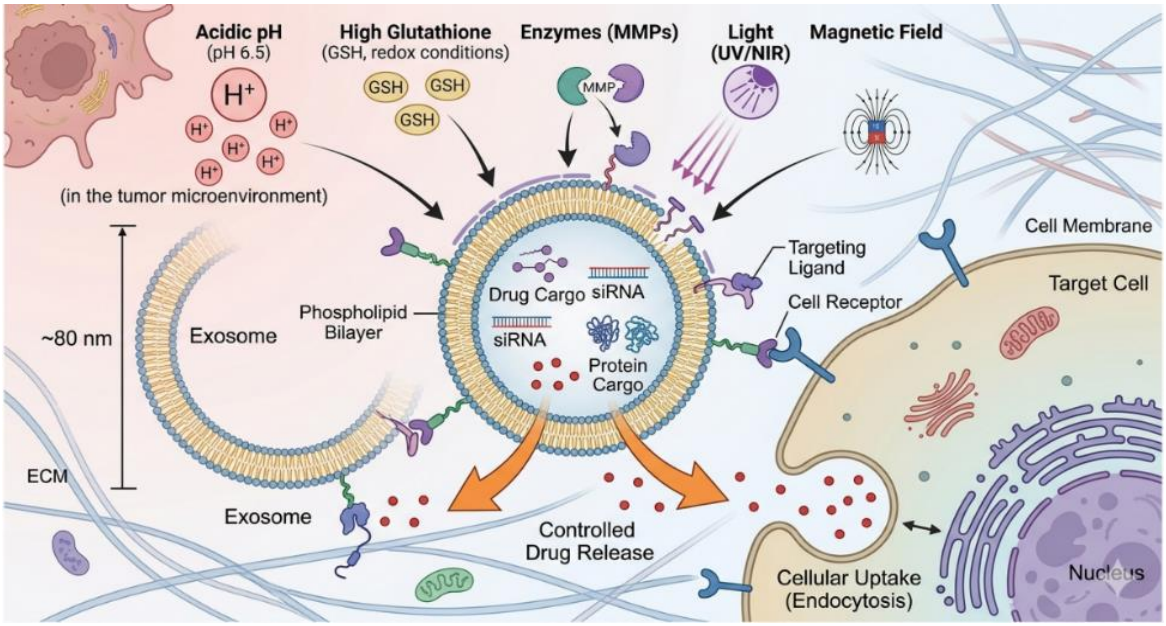

Fig. 1: Schematic representation of an exosome-based stimuli-responsive drug delivery system showing targeted delivery and controlled release triggered by pH, redox conditions, enzymes, and external stimuli.

Abbreviations: pH, hydrogen ion concentration; NIR, near- infrared light.

Various stimuli-responsive vesicular systems have been developed to improve targeted drug delivery and controlled drug release to ensure the medicine reaches the appropriate location in the body and the release of the medicine is also controlled.[4,5]

Vesicles such as liposomes, niosomes, transfersomes and ethosomes, have been extensively investigated as such vesicles have the ability to carry both water-loving and fat-loving drugs and can be modified to release the medicine when they come into contact with factors such as changes in pH, temperature, enzymes or light.[6]

Natural vesicles are being investigated as promising carriers for therapeutic delivery since they are not dangerous to the body, do not provoke many immune reactions and have the ability to get to the target organ on their own. Types of vesicular systems exist, demonstrating that the systems may be modified to react to various things and enhance drug delivery. Exosomes are just fascinating at the moment since they are natural and can be very specific in delivering medicine.[7]

First exosomes are highly stable and non-toxic to the body. They do not elicit high levels of immunity thus making them effective in delivering therapeutic factors to the appropriate location.[8]

Secondly, they are capable of going through the walls that divide body parts and are able to target certain cells or tissues to attack, which makes them superior in delivering the medicine and minimizing the effects, on other parts of the body. The exosomes also contain a layer that ensures that the medicine within is not broken down and is made more stable.[9]

Literature search methodology

A literature search was carried out to identify relevant research published between 2013 and 2025 on exosome-based stimuli-responsive drug delivery systems using electronic databases such as PubMed, Scopus, Web of Science, and Science Direct. The search strategy employed combinations of keywords and Boolean operators (AND/OR), including "exosomes," "extracellular vesicles," "small extracellular vesicles," "drug delivery," "therapeutic delivery," "stimuli-responsive." Duplicate records and publications that were unrelated to exosome-based drug delivery or did not provide information relevant to the scope of the present review were excluded. In addition, the reference lists of relevant articles were manually screened to identify additional studies. The retrieved literature was critically evaluated and organized according to exosome biogenesis and composition, drug-loading techniques, stimuli-responsive mechanisms, biomedical applications, challenges, clinical translation, and future perspectives.

Biology and Characteristic of exosome

- **Biogenesis of exosome**

The initial description of exosomes was made by Rose M. Johnstone and others in 1987, who refer to such small extracellular vesicles (EVs) that carry out the process of eliminating transferrin receptors in the maturation of reticulocytes. Exosomes have since been known to play a role in intercellular communication and are now known to have an endocytic origin.[13]

Exosome biogenesis is a complex, tightly regulated, multiphase process that starts with endocytosis and results in early endosomes. These primitive endosomes are then developed into late endosomes where the endosomal membrane inwardly bulges to form intraluminal vesicles (ILVs). The ILVs which accumulate in the endosome cause the formation of multivesicular bodies (MVBs). The endosomal sorting complex required for transport

(ESCRT) machinery mainly controls this process, but ESCRT-independent lipid (e.g. ceramide) and protein (e.g. Tetraspanin) pathways have also been described.[13]

MVBs then take two routes: they either merge with lysosomes to be degraded or they merge with the plasma membrane to release ILVs to the extracellular environment as exosomes. The membrane fusion proteins that mediate the release process include SNAREs. Upon release, exosomes are able to communicate with recipient cells via endocytosis, direct membrane fusion or via receptor ligand interactions and transfer proteins, lipids and nucleic acids and regulate cellular signaling pathways.

Extracellular vesicles are mainly categorized into exosomes (30-150 nm), microvesicles (100-1000 nm), and apoptotic bodies (>1000 nm) depending on their size and biogenesis. Exosomes among them stand out due to their endosomal origin and are essential in physiological and pathological processes as they aid in intercellular communication.[14]

The sequential stages of exosome biogenesis, including ILV formation, MVB maturation, exosomes release, and uptake by recipient cells, are illustrated in Fig. 2.

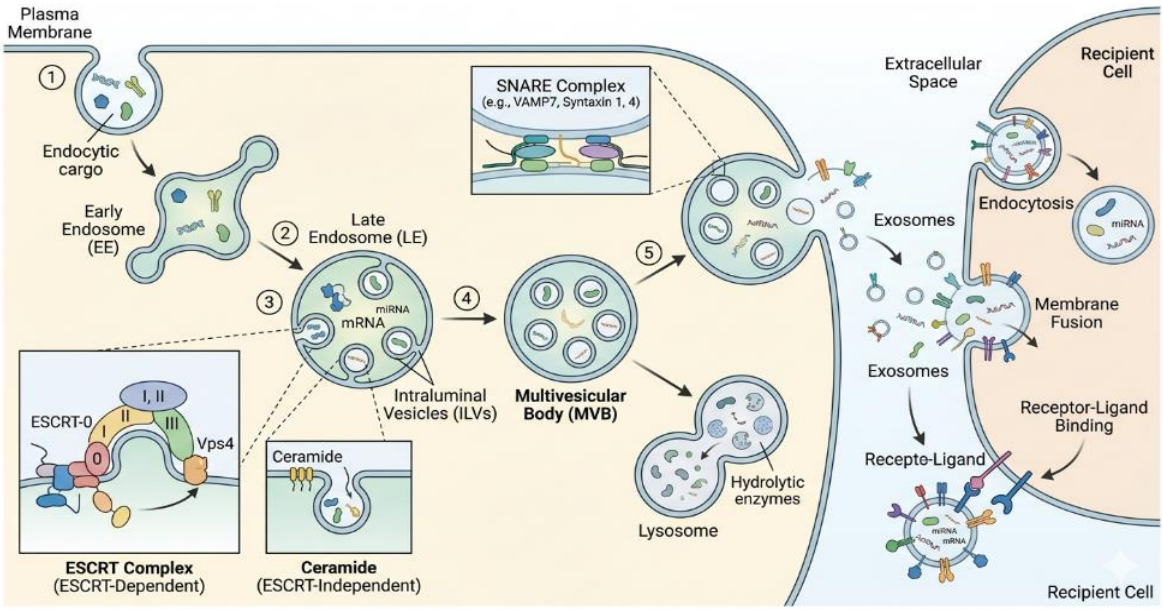

Fig. 2: Biogenesis and cellular uptake pathways of exosomes showing endosomal formation, multivesicular body development, exosome release, and interaction with recipient cells.

Abbreviations: EE, early endosome; LE, late endosome; MVB, multivesicular body; ILV, intraluminal vesicle; ESCRT, endosomal sorting complex required for transport; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor.

Besides proteins and lipids, exosomes also carry a diverse range of nucleic acids, especially RNA species, including messenger RNA (mRNA), microRNA (miRNA) and other non-coding RNAs. Such nucleic acids may be delivered to the recipient cell and they may regulate the expression of genes and alter a number of physiological and pathological processes. Exosomes are released into the target cell when they interact with the cells (through endocytosis, membrane fusion, and receptor-mediated endocytosis) and this release can affect cellular signaling pathways. Since the molecular content of exosomes echoes the physiological and pathological condition of their cells of origin, they are becoming important biomarkers of disease diagnosis and prognosis, and are especially useful in diseases like cancer or neurodegenerative conditions. Their carrying and transferring functional biomolecule capabilities also indicate their usage in therapeutic and drug delivery systems.[18]

Composition of Exosome

Exosomes are endosomal vesicles which are nanosized and have a wide variety of biomolecules which are indicative of their parent cells. They are composed primarily of lipids, proteins and nucleic acids, which all contribute to their structure and biological functions.

Cholesterol, sphingomyelin, diacylglycerol, and glycosphingolipids including GM3 enrich the lipid membrane of exosomes to give structural stability and rigidity to the membrane. A phosphatidylserine is usually found on the outer surface and it is significant in the uptake and recognition of the cell. Besides, lipids are also implicated in membrane fusion, protein anchoring and intracellular signaling pathways, and bioactive lipids like prostaglandins are involved in inflammatory and immune responses.[18]

Exosomal proteins play a crucial role in vesicle construction, transportation, and targeting of cells. Heat shock proteins (Hsp70, Hsp90, Hsc70) aid in the folding and intracellular trafficking of proteins. Such characteristic markers include the Tetraspanins, which are CD9, CD63, CD81, and CD82, which regulate cell adhesion, migration and membrane fusion. Also, there is selective binding of integrins and adhesion molecules to recipient cells. Additional cargo transport proteins include transporter proteins (e.g., ATP-binding cassette proteins, solute carrier proteins), and receptor proteins (e.g., CD46, CD55).[17]

Another type of nucleic acid that is found in exosomes is messenger RNA (mRNA), microRNA (miRNA), and other non-coding RNAs. These molecules facilitate the exchange

of genetic information between cells and the expression of genes in target cells. MicroRNAs are especially significant in targeting and silencing of genes, and long non-coding RNAs are linked to disease advancement, particularly cancer. Moreover, exosomes can include the circulating DNA, such as genomic and mitochondrial DNA, which demonstrates the genetic changes, such as mutations and rearrangements.[16]

Table 1: Composition and Molecular components of exosomes.

Category	Subcategory	Component	Reference
Nucleic Acids	DNA	Double-stranded DNA (ssDNA), Single-stranded DNA, Mitochondrial DNA (mtDNA)	[16][19]
	RNA	Messenger RNA (mRNA), Long non-coding RNA (lncRNA), MicroRNA (miRNA), Circular RNA (circRNA)	[17]
Protein	Tetraspanins	CD9, CD63, CD81, CD82	[18]
	Other Proteins	Integrins, Annexins, Flotillins, Syntenin-1, RAB family proteins	[16][17]
	Cytoskeletal proteins	Actin, Tubulin, Moesin	[17]
	Heat Shock Proteins	Hsp70, Hsp90	[18]
	Functional Proteins	GTPases, MHC molecules	[16][19]
	Biogenesis related Protein	ESCRT-associated proteins, Clathrin, ALIX, TSG101	[16][17][20]
	Other Components	Enzymes, Histones	[16][19]
Lipids	Membrane Lipids	Cholesterol, Ceramide, Sphingomyelin, Phosphatidylcholine	[17][18][19]

Abbreviations: dsDNA = double-stranded DNA; mtDNA = mitochondrial DNA; mRNA = messenger RNA; miRNA = microRNA; lncRNA = long non-coding RNA; circRNA = circular RNA; MHC = major histocompatibility complex; ESCRT = endosomal sorting complex required for transport; ALIX = ALG-2-interacting protein X; TSG101 = tumor susceptibility gene 101.

Exosome as a Drug Delivery System

Exosomes are extracellular membrane vesicles that are released by many cells in the body, and are vital in intercellular communication and eliciting physiological reactions. Their applications are also as cellular membrane-based drug delivery systems. Exosomes and exosome mimetics are considered promising delivery systems because of their peculiarities such as biocompatibility, absence (or low) level of toxicity, high circulation capacity, specificity, and so on. They are more precise and effective carriers compared to those they

have utilized previously such as liposome-mediated, polymeric nanoparticles, metal nanomaterials, etc.[21,22]

Drug loading technique

Drugs with small molecules. The lipid bi-layer structure does not allow the other enzymes and substances to degrade the cargo of the exosomes. Exosomes are also a promising delivery platform due to its excellent characteristics using small-molecule drugs. Incidentally, they are capable of targeting with precision natural payloads (proteins, DNA, siRNA and miRNA) to their target locations.[23] Small-molecule drug exosomes have demonstrated good clinical results and the delivery system can be used to reduce side effects without any killing drugs. The poor exosome drug loading efficacy is however, one of the greatest weaknesses and more research should be carried out to enhance the efficacy of such loading technology.[24,25]

Sonication method

Ultrasonic treatment was applied with the help of a homogenizer probe to mixture of exosomes and drugs. Mechanical shear force disrupted the integrity of the exosome membrane in the initial phase of ultrasound treatment, and the drug got into the exosome in the membrane deformation phase. The exosomes were incubated after ultrasound to repair the membrane integrity.[26,27] Ultrasound yielded the greatest efficiency and exosomes were optimally taken up by target cells, although their shapes and sizes could not but change. For example, sonication has been used to load therapeutic enzymes such as catalase or peroxidase into exosomes. This approach protects the enzyme cargo from degradation, enables sustained release, and enhances delivery to target cells. Ultrasound has the advantages of a high drug loading efficiency and continuous drug release. In contrast to electroporation, ultrasound does not cause nucleic acid aggregation and thus may lead to exosome aggregation and affect the surface protein structure.[28]

Electroporation

Electroporation creates small holes in the exosome membrane, due to the influence of electric field, enhancing the membrane permeability.[29] The drugs or nucleotides are then loaded into the exosome by diffusion and the exosome membrane rebounds to its normal state in a short period after drug loading. In this approach, big nucleotides, including siRNAs or miRNAs, are favored to be loaded into exosomes. A high electric field intensity may increase the accumulation of exosomes, but the appropriate concentration of trehalose can alleviate this situation. In addition, the accumulation of nucleic acids has been reduced by lipid

complexation and increases the drug loading rate. Electroporation is simple to apply and has been extensively employed to encapsulate siRNA, microRNA or miRNA, although it can cause RNA precipitation or exosome aggregation, thus decreasing drug loading capacity.[30]

Saponin-assisted loading

One of the surfactants that are widely used and are membrane permeabilizers is Saponin. The saponification process, which facilitates the creation of small pore in lipid membranes, is the saponin method that causes the entry of hydrophilic and non-permeable molecules into exosomes with enhanced drug loading capacity. The procedure of the saponin method in particular involves exosome purification, followed by 10 minutes of incubation with saponin and drugs. Once more low permeability dialysis is used to perform the purification process to retrieve drug loaded exosomes.[31]

Incubation

Passive loading, also known as the incubation method is a simple technique of loading drugs into exosomes. This incubation technique allows the loading of drugs to exosomes to take place using the concentration gradient which occurs when the concentration of the external drug is high. Though this approach has the benefits of being simple to use and having no destructive impacts on the exosome integrity, it is mainly limited to select classes of drugs. This is especially beneficial to hydrophobic drugs that disengage with the lipid surfaces of exosomes. As a result, the loading capacity depends on the hydrophobicity of the cargo and time of incubation.[32,33]

Freeze Thaw

Freeze thaw process destroys plasma membrane by putting mechanical stress on the membrane due to the presence and dissolution of ice crystals during freezing and thawing. Disruption of the plasma membrane through the transient formation of ice crystals allows water-soluble molecules to be incorporated into the aqueous phase inside vesicles.[33] Cargo is incubated on exosomes at room temperature during an asset time and frozen at −80 °C or liquid nitrogen. The mixture is then thawed at room temperature. The freeze–thaw technique provides an intermediate loading efficiency for cargo into exosomes compared with sonication and extrusion. Three or more freeze thaw cycles are normally done to enhance the drug encapsulation. Thus, it is vital to determine the right temperature and cycling parameters that will result in the best outcomes. Furthermore, the freeze–thaw method can be used to generate hybrid exosomes via the fusion of exosomes and liposomes. Through lipid

composition manipulation of the membrane lipids by manipulating the liposomes through manipulating the freeze-thaw method.[34,35]

Table 2: Comparison of Different Drug-Loading Techniques for Exosomes.

Loading technique	Principle	Suitable cargo	Loading efficiency	Advantages	Limitations
Passive incubation	Diffusion/ concentration gradient	Small lipophilic molecules such as catalase	Low–medium molecular weight	Simple, minimal membrane disruption	Relatively low loading efficiency
Sonication	Mechanical membrane deformation	Small molecules/ proteins	High	High loading efficiency & continuous drug release	Adverse effect on exosome structure, exosomal aggregation
Electroporation	Temporary membrane pores	siRNA/ miRNA/nucleic acids	Moderate–high	Simple, superior loading efficiency	Aggregation/RNA precipitation
Saponin-assisted loading	Membrane permeabilization	Hydrophilic cargo	Moderate–high	Improved cargo entry	Possible membrane damage
Freeze–thaw	Temporary membrane disruption	Proteins/ hydrophilic cargo	Moderate	Simple; can facilitate hybridization	Repeated cycle may affect vesicle integrity

Stimuli responsive Exosome based system

pH responsive Exosome

Internal stimuli-responsive mechanismsexploit the distinct biochemical microenvironment that are characteristic of pathological sites. As an example, pH-responsive mechanisms have been a topic of extensive research in cancer therapy because of acidic microenvironment of solid tumors and intracellular compartments, including endosomes and lysosomes. Acid-labile linkers can be functionalized on drug-loaded exosomes, degrading under low pH conditions, which allows local release in tumor tissues or after endocytic uptake. pH conditions vary considerably depending on the physiological and pathological environment.[36] For example, the extracellular environment of tumors tends to be more acidic (pH ≈ 6.5) than that in normal tissues (pH 7.4), and the pH of endosome/lysosome is even lowered to 5. Thus, the pH heterogeneity has been widely exploited to promote cellular absorption, and regulate drug delivery, etc.[37,38]

Enzyme response exosomes

High concentrations of matrix metalloproteinases (MMPs), cathepsins, and other disease-specific enzymes are common in tumours, inflammatory tissues and sites of infection.[39] Such enzymes have the ability to disintegrate peptide linkers within carriers, so that they do not release into the body unless they are in damaged tissues. A third internal modality is enzyme-responsive systems. Tumors, inflamed tissues and infected sites often express increased levels of matrix metalloproteinases (MMPs), cathepsins and other disease-relevant enzymes. Peptide linkers that are cleaved by these enzymes can be engineered into carriers allowing release in tissues that are affected.[40]

Redox responsive exosomes

The redox potential has emerged as a strong and attractive active intracellular drug release stimulus. It is well known that the cellular redox environment is mainly regulated by the amount of glutathione (GSH). GSH 1 -glutamyl-cysteinyl-glycine, the reducing agent of lowest molecular weight present in the cytoplasm of mammalian cells is necessary to maintain the cellular oxidative balance.[41] It is well-known that the concentration of glutathione (GSH) is the main regulator of the redox state of cells. The most common low-molecular weight reducing agent in the mammalian cell cytoplasm is GSH, or, to be more precise, γ-glutamyl-cysteinyl-glycine, which is needed to maintain the oxidative balance of the cell.[42,43]

Dual or Multi Responsive Drug Release

Rational combinations of two or more of the aforementioned stimuli, e.g. pH/redox and pH/ROS are possible. Moreover, induced drug release under dual or multiple stimuli can occur in a cascade in multiple intracellular compartments or simultaneously in a diseased cell.[44] The various stimuli-responsive nanocarriers can result in long circulation, high tumour accumulation, deep penetration in tumour tissues, internalisation with cancer cells, endosome escape, etc. are all possible. Therefore, a number of stimuli-responsive nanocarriers have been developed to transport payload to tumours.[45,46]

Light/External Stimuli Responsive Exosomes

Since light is an alluring stimulus with the ability to modify the irradiation wavelength, power, and impacting region, nanocarriers that might respond to light have also been extensively created.[40] In biological systems, e.g. cancer cells or tumours, light-sensitive nanocarriers can, in general, be remotely influenced by light irradiation e.g. UV-Visible light

and near-infrared light (NIR). Meanwhile, light-based tumour therapy might also be performed with accuracy, by regulating the range of the irradiation in order to avert or minimize potential harm to the normal tissues and organs.[47,48]

Various stimuli-responsive exosomes systems, including pH-, enzyme-, redox-, dual-, and light-responsive platforms are summarized in Fig. 3.

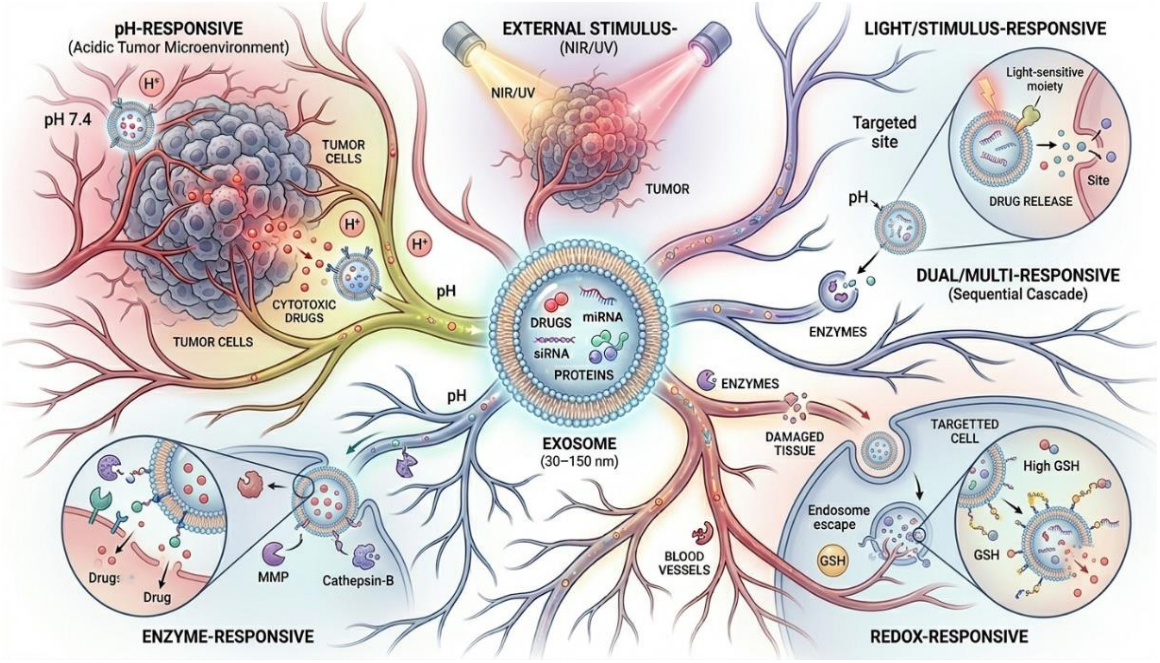

Fig. 3: Schematic illustration of stimuli-responsive exosome-based drug delivery systems enabling targeted and controlled therapeutic release.

(A)pH-responsive exosomes; (B) enzyme-responsive exosomes; (C) redox-responsive exosomes; (D) dual/multi-responsive exosomes; (E) light-responsive exosomes.

Abbreviations: pH, hydrogen ion concentration; GSH, glutathione; MMP, matrix metalloproteinase; NIR, near-infrared light; UV, ultraviolet. Exosome membranes are represented by vesicular bilayers, therapeutic cargos are represented by encapsulated particles, and arrows indicate stimulus-triggered cargo release.

Applications

The exosomes have been under overwhelming attention as the best nanocarriers in modern therapeutics due to their natural origin, high biocompatibility, and inherent ability to transfer bioactive molecules between cells. One of the most significant applications of exosomes is targeted cancer therapy where they carry chemotherapeutic agents, nucleic acids, and even gene-editing tools directly to the tumor tissues. Targeting ligands can be incorporated onto

their surface, allowing them to be more tumor-specific and decreasing off-target toxicity. Also, there are promising studies on exosome-mediated delivery of small interfering RNA (siRNA) and microRNA (miRNA) in regulating of oncogenic pathways and inhibition of tumor growth.[49,50]

Exosomes are also effective vectors to transport nucleic acid-based therapeutics including mRNA, siRNA, and CRISPR/Cas components, in genetic therapy. Exosomes have a better safety profile and low immunogenicity and toxicity in comparison with viral vectors. Being able to protect nucleic acids to prevent their degradation by enzymes enhances their application in gene therapy and precision medicine even further.[51,52]

Exosomes are also being extensively explored in neurological drug delivery, as they can efficiently cross the blood–brain barrier. This special quality enables them to deliver a therapeutic agent to the central nervous system and they can be useful in the treatment of neurodegenerative diseases like Alzheimer, Parkinson and brain tumors. Engineered exosomes have also been shown to be able to transport neuroprotective drugs and RNA molecules, which in turn has enhanced the therapeutic outcomes in preclinical models.[50,53]

Stem cell exosomes have been shown to possess a lot of potential in the field of regenerative medicine, as regards to inducing tissue repair and regeneration. These exosomes are loaded with growth factors, cytokines and regulatory RNAs which promote angiogenesis, decrease inflammation and promote cell proliferation. They are therefore being investigated to be applied in wound healing, cardiovascular repair and musculoskeletal regeneration.[52,53]

The other fast-evolving use of exosomes lies in diagnostics and the discovery of biomarkers. Exosomes can be applied to early disease detection and treatment because they can be non-invasively applied, as they can reflect the molecular picture of the parent cells and, therefore, they can be used as biomarkers of disease. Exosomal RNA, DNA and proteins-based liquid biopsy techniques are fast becoming used to diagnose cancers and other chronic diseases and offer an alternative to traditional biopsy techniques, which are more invasive.[49]

Overall, exosomes represent versatile platforms that can be employed in future drug delivery systems and targeted therapies because of the range of their applications, and engineering and loading technologies.

The major biochemical application of exosome-based drug delivery systems are summarized in Fig.4.

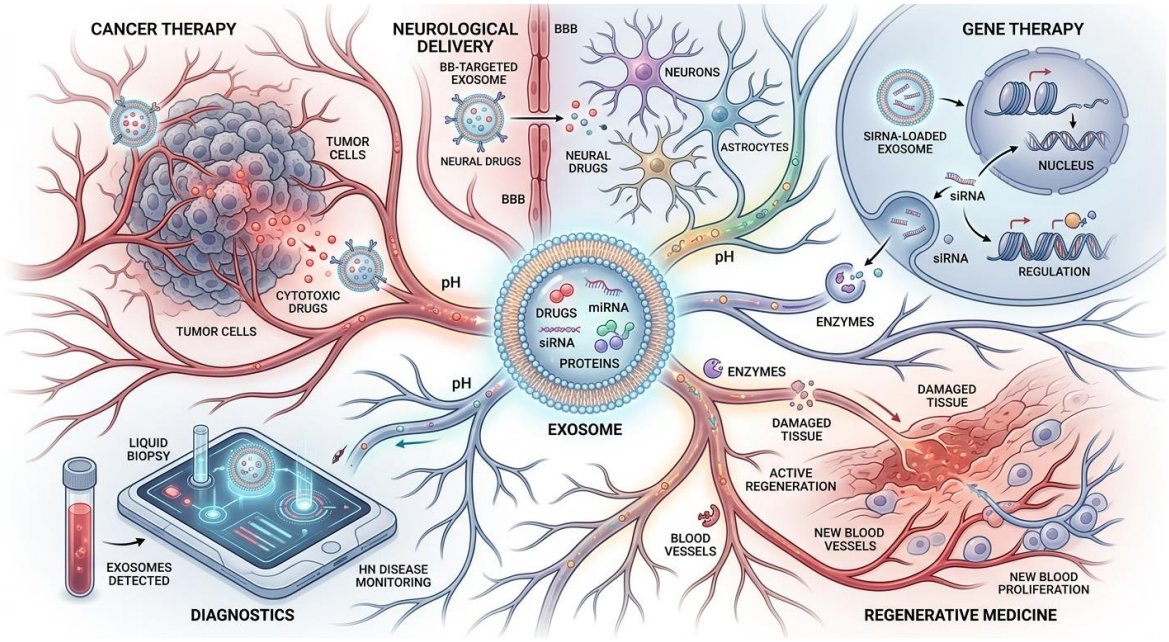

Fig. 4: Network-based representation of exosome-mediated drug delivery applications, The central node shows exosome platform: peripheral nodes represent therapeutic and diagnostic applications. Solid arrows indicate established applications and dashed arrows indicate emerging applications.

Clinical Translation of exosome based therapeutics

Although exosome-based drug delivery has demonstrated promising therapeutic outcomes in numerous preclinical studies, translation into clinical applications remains at an early stage. Clinical investigations have increasingly evaluated exosomes as therapeutic carriers, cell-free therapeutics, cancer vaccines, and diagnostic biomarkers. The clinical development of these systems is supported by their biocompatibility, biological stability, intrinsic cell-interactive properties, and capacity to transport proteins, nucleic acids, and small-molecule therapeutics. However, clinical translation remains challenging because of variability in exosome source, isolation and purification procedures, cargo loading, physicochemical characterization, biodistribution, pharmacokinetics, manufacturing scalability, and regulatory requirements.

Clinical investigations have also explored exosomes as vehicles for therapeutic compounds. Plant-derived extracellular vesicles/exosome-like vesicles loaded with curcumin have been investigated for gastrointestinal and oncological applications, demonstrating the potential of naturally derived vesicles as orally administered therapeutic carriers.

Dendritic-cell-derived exosomes represent one of the earlier clinically investigated exosome platforms, particularly in cancer immunotherapy. Early clinical studies have primarily focused on autologous exosome preparations as cancer vaccines and have provided evidence supporting the feasibility and short-term safety of exosome administration. Nevertheless, further investigation is required to establish pharmacokinetics, biodistribution, long-term safety, and therapeutic efficacy.[60,61]

Challenges

Although exosomes have a significant potential as high-tech drug delivery vehicles, they cannot be successfully translated into clinical use due to a number of challenges. Among the key constraints is that it is linked to the large-scale production and standardization. Exosomes are commonly purified using cell culture supernatants or biological fluids, which usually fails to give high yields, very expensive to produce and there is a high degree of variability between batches. In addition, the absence of universal protocols to follow in isolation and purification also makes reproducibility and scalability to the clinical level more difficult.[54,56]

Another significant challenge is the lack of standardized regulatory and manufacturing frameworks for exosome-based therapeutics. Batch-to-batch variability, differences in donor cell sources, limited isolation purity, uncertain biodistribution profiles, and insufficient understanding of pharmacokinetics continue to hinder clinical translation. Furthermore, long-term storage stability, potential immunogenicity, and large-scale manufacturing costs remain major concerns. Addressing these issues will require standardized characterization methods, robust quality-control procedures, and harmonized regulatory guidelines to ensure product consistency, safety, and efficacy.

The other critical issue is associated with the *in vivo* behavior, biodistribution and long-term safety of exosome-based systems that are yet to be realized. This is especially in the case of tumor-derived exosomes, which can include immunomodulatory factors, like programmed death-ligand 1 (PD-L1), that might result in the suppression of immune responses by T-cell exhaustion. Moreover, these exosomes can be used to carry oncogenic biomolecules that promote tumor growth, metastasis and cancer recurrence. Exosomes that are extracellularly secreted by tumor-associated stromal cells such as cancer-associated fibroblasts have also been involved in facilitating drug resistance by transferring regulatory microRNAs and proteins that modify critical signaling pathways in target cells.[54,55]

To enhance therapeutic performance, several cargo loading methods have been investigated among which are passive loading, transfection, electroporation and sonication. Despite their potential, these approaches have not been as efficient to be used in clinical practice. Specifically, passive incubation has low loading efficiency and only a limited range of compatibility with various therapeutic cargo, thus limiting its practical applicability in targeted drug delivery systems.[55,56]

Moreover, there are still serious technical challenges in the purification and mass production of exosomes. The type of donor cells source is critical in the determination of the yield and functional quality of the exosomes. The existing methods of isolation which include ultracentrifugation, tangential flow filtration and size exclusion chromatography are popular but they all possess certain constraints regarding scalability, purity and reproducibility. These limitations highlight why standardized, efficient, and scalable manufacturing methods need to be developed to support the clinical use of exosome-based therapeutics.[54,56]

Future Perspectives

Precision engineering, scalable production, and clinical translation strategies have the potential to create the future of stimuli-responsive exosome-based drug delivery systems. New strategies are being pursued to enhance the specificity of targeting and therapeutic performance by incorporating ligands, antibodies and stimuli-responsive units in exosomes, including genetic modification and surface functionalization of exosomes.[57-60] Simultaneously, artificial intelligence and machine learning are being considered to optimize exosome design, cargo selection, and patient-specific therapeutic approaches, thus aiding the creation of personalized medicine.[58,59]

Several exosome-based therapeutics have entered early-phase clinical trials, particularly in oncology, regenerative medicine, and inflammatory disorders. These studies primarily evaluate safety, tolerability, biodistribution, and therapeutic efficacy, highlighting the growing translational potential of engineered exosome platforms.[59]

There is also a major advancement in large-scale production technologies such as bioreactor-based production systems and microfluidic isolation methods that would address the existing constraints of low yield, poor reproducibility and lack of standardization.[1,3] In addition, exosome-based hybrid systems with synthetic nanomaterials are under study to improve

stability, drug loading capacity, and multi-functional responsiveness to increase their versatility in the treatment of complex diseases.[59,60]

Translationally, a high safety profile and regulatory acceptability of exosome-based systems are a priority. This involves setting up of standardized characterization procedures, quality assurance and effective regulatory frameworks that will facilitate consistency and clinical reliability.[56,57] Moreover, next-generation multi-stimuli-responsive exosomes, which are expected to react to various internal and external stimuli with high precision, will be developed, which is expected to further optimize targeted delivery and reduce off-target effects.[58]

All in all, the presented developments are likely to speed-up the process of transforming exosome-based drug delivery systems out of the experimental research phase into the clinically-viable therapeutic platform, and promise substantial potential to treat complex and chronic diseases.[56-59]

CONCLUSION

Exosome-based drug delivery system represent a significant advancement in targeted drug delivery, providing a versatile and biocompatible drug delivery platform for precision medicine. Their biological nature, capacity to carry a range of biomolecules, and inherent targeting ability make them an improvement over many existing nanocarriers. The incorporation of stimuli-responsive systems (such as pH, enzymes, redox potential, and external stimuli such as light) further expands their potential to control and target drug release in a controlled manner, thus increasing therapeutic effectiveness and reducing systemic toxicity.

Beyond drug delivery, exosomes have been shown to have great potential for various biomedical applications such as cancer therapy, gene delivery, treatment for nervous system disorders, regenerative medicine, and disease diagnosis. Their capacity to traverse biological barriers and represent the biological characteristics of their parent cells make them potential therapeutic and diagnostic vehicles.

Despite these exciting properties, there are still a number of challenges to widespread clinical applications. Problems with scalable manufacturing, standardization, cargo delivery and long-term safety need to be resolved for them to be reproducible and acceptable for clinical use. In

addition, exploring their *in vivo* fate and reducing risks associated with their natural origin are key for future development.

In summary, ongoing research into exosome modification, loading, and manufacturing techniques are likely to address these current challenges. Through ongoing research, stimuli-responsive exosome systems will undoubtedly become an important part of the next generation of drug delivery and therapeutic strategies.

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest.

AI Use Statement: No artificial intelligence, AI, or AI-assisted tools were used in the writing, data analysis, grammar checking. The author used Chat-GPT for creation of figures for this manuscript and sentence clarity. The author reviewed and edited the output and takes full responsibility for the final content.

REFERENCES

1. Kumbhar R, Jangme C. Stimuli-responsive (smart) drug delivery systems: An in-depth review. International Journal of Pharmaceutical Sciences, 2025; 3(8): 1534–1542.
2. Subramanian J, Padhy R, Arun J, Murthannagari VR, Gnk G. STIMULI-RESPONSIVE DRUG DELIVERY SYSTEMS: EXTENSIVE OVERVIEW. International Journal Of Applied Pharmaceutics, Sep. 7, 2025; 94–106. Doi:10.22159/Ijap.2025v17i5.54389
3. Najjari Z, Sadri F, Varshosaz J. Smart stimuli-responsive drug delivery systems in spotlight of COVID-19. Asian J Pharm Sci., 2023; 18(6): 100873.
4. Zhang Y, Wu BM. Current advances in stimuli-responsive hydrogels as smart drug delivery carriers. Pharmaceutics, 2021; 13(11): 1792. doi:10.3390/pharmaceutics13111792.
5. Ding C, Tong L, Feng J, Fu J. Recent advances in stimuli-responsive release function drug delivery systems for tumor treatment. Pharmaceutics, 2021; 13(3): 373. doi:10.3390/pharmaceutics13030373.
6. Gomathi R, Bharathiraja A, Sabitha V, Snekha K, Susmitha R, Gokulram P, et al. Vesicular nanocarriers: An innovative targeted and sustained drug delivery system. Next Bioengineering, Jun. 2026; 2: 100024. doi:10.1016/j.nxbio.2026.100024
7. Pires PC, Paiva-Santos AC, Veiga F. Liposome-derived nanosystems for the treatment of behavioral and neurodegenerative diseases: The promise of niosomes, transfersomes, and ethosomes. Pharmaceuticals, 2023; 16(10): 1424. doi:10.3390/ph16101424.

8. Upadhyay P, Singh D, Upadhyay S. Vesicular approach on nanocarriers and their applications. Recent Advances in Drug Delivery and Formulations, 2022; 16(4): 256–269.
9. Ge X, Wei M, He S, Yuan WE. Lipid-based nanovesicular drug delivery systems. Pharmaceutics, 2022; 14(1): 1–25. doi:10.3390/pharmaceutics14010001.
10. Kim HI, Kim H, Lee J, et al. Recent advances in extracellular vesicles for therapeutic cargo delivery. Experimental and Molecular Medicines, 2024; 56: 836–849. doi:10.1038/s12276-024-01063-5.
11. Shafiei M, et al. Applications of exosomes and liposomes in regenerative medicine. Polymers, 2021; 13(15): 2529. doi:10.3390/polym13152529.
12. Kim H, Jang H, Cho H, et al. Recent advances in exosome-based drug delivery for cancer therapy. Cancers (Basel), 2021; 13(17): 4435. doi:10.3390/cancers13174435.
13. Akuma P, Okagu OD, Udenigwe CC. Naturally occurring exosome vesicles as potential delivery vehicle for bioactive compounds. Front Sustain Food Syst., 2019; 3: 23. doi:10.3389/fsufs.2019.00023.
14. Li Q, Fu X, Kou Y, Han N. Engineering strategies and optimized delivery of exosomes for theranostic application in nerve tissue. Theranostics, 2023; 13(12): 4266–4286. doi:10.7150/thno.84971.
15. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cells, 2019; 08(4): 307. doi:10.3390/ijms20092239.
16. Mathivanan S, Simpson RJ. ExoCarta: A compendium of exosomal proteins and RNA. Proteomics, 2009; 9(21): 4997–5000. doi:10.1002/pmic.200900351.
17. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science, 2020; 367(6478): eaau6977. doi:10.1126/science.aau6977.
18. Van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. NatureReviews Molecular Cell Biology, 2018; 19(4): 213–228. doi:10.1038/nrm.2017.125.
19. Yáñez-Mó M, Siljander PRM, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles, 2015; 14; 4: 27066 doi: 10.3402/jev.v4.27066.
20. Keerthikumar S, Chisanga D, Ariyaratne D, et al. ExoCarta: A web-based compendium of exosomal cargo. J Mol Biol., 2016; 428(4): 688–692. doi:10.1016/j.jmb.2015.09.019.

21. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles. Journal of ExtracellularVesicles, 2018; 7(1): 1535750.
22. Kučuk N, Primožič M, Knez Ž, Leitgeb M. Exosomes engineering and their roles as therapy delivery tools, therapeutic targets, and biomarkers. International Journal of Molecular Sciences, 2021; 22(17): 9543.
23. Sharma V, Das Mukhopadhyay C. Exosome as drug delivery system: Current advancements. Extracellular Vesicles, 2024; 3: 100032.
24. Cully M. Exosome-based candidates move into the clinic. Nature Reviews Drug Discov, 2020.
25. Guo J, Wang F, Hu Y, et al. Exosome-based bone-targeting drug delivery alleviates impaired osteoblastic bone formation and bone loss in inflammatory bowel diseases. Cell Reports Medicine, 2023; 4: 100881.
26. Zhan C, Ma X, Wang W, et al. Immune exosomes loading self-assembled nanomicelles traverse the blood–brain barrier for chemo-immunotherapy against glioblastoma. ACS Nano, 2021; 15(3): 4566–4580.
27. Liu LN, Zhang HR, Mao HY, Li XH, Hu YM. Exosomal miR-320d derived from adipose tissue-derived MSCs inhibits apoptosis in cardiomyocytes with atrial fibrillation (AF). ArtificialCells, Nanomedicine, and Biotechnology, 2019; 47: 3976–3984.
28. Wang N, Ma J, Ren Y, Xiang S, Jia R. Secreted klotho from exosomes alleviates inflammation and apoptosis in acute pancreatitis. American Journal of Translational Research, 2019; 11: 3375–338.
29. Haney MJ, Klyachko NL, Harrison EB, et al. TPP1 delivery to lysosomes with extracellular vesicles and their enhanced brain distribution in the animal model of Batten disease. Advanced Healthcare Materials, 2019; 8(11): 1801271.
30. Pan S, Pei L, Zhang A, et al. Passion fruit-like exosome nanovehicles for enhanced targeted photodynamic therapy. Biomaterials, 2020; 230: 119606.
31. Xi XM, Chen M, Xia SJ, Lu R. Drug loading techniques for exosome-based drug delivery systems. Pharmazie, 2021; 76: 61–67.
32. Tian J, Han Z, Song D, et al. Engineered exosome for drug delivery: Recent development and clinical applications. International Journal of Nanomedicine, 2023; 18: 7923–7940.
33. Zeng H, Guo S, Ren X, Wu Z, Liu S, Yao X. Current Strategies for Exosome Cargo Loading and Targeting Delivery. Cells., May 17, 2023; 12(10): 1416. doi:

10.3390/cells12101416. Costa AP, Xu X, Burgess DJ. Freeze-anneal-thaw cycling of unilamellar liposomes: Effect on encapsulation efficiency. Pharm Res., 2014; 31: 97–103.

34. Cheng Y, Zeng Q, Han Q, Xia W. Effect of pH, temperature and freezing-thawing on exosomes. Protein Cell., 2019; 10: 295–299.
35. Koh HB, Kim HJ, Kang SW, Yoo TH. Exosome-based drug delivery: Translation from bench to clinic. Pharmaceutics, 2023; 15(8): 2042.
36. Pierigè F, Magnani M. Drug-loaded erythrocytes: Targeted delivery in clinical use. Transfus Med Rev., 2023; 37: 150–164.
37. Chen PP, Qiu M, Deng C, et al. pH-responsive nanoparticles for intracellular delivery. Biomacromolecules, 2015; 16: 1322–1330.
38. Wu W, Luo L, Wang Y, et al. Endogenous pH-responsive nanoparticles for tumor therapy. Theranostics, 2018; 8: 3038–3058.
39. Ruan S, Zhou Y, Jiang X, Gao H. Rethinking CRITID procedure of Brain targeting drug delivery. Advanced Science (Weinh), 2021; 8: 2004025.
40. Jain A, Xu Z, Liu Y, et al. Steering CAR T cell therapy with lipid nanoparticles. J Nanobiotechnol, 2024; 22: 380.
41. Meng F, Hennink WE, Zhong Z. Reduction-sensitive polymers. Biomaterials, 2009; 30: 2180–2198.
42. Zhong YN, Yang WJ, Sun HL, et al. Reduction-sensitive micelles for drug delivery. Biomacromolecules, 2013; 14: 3723–3730.
43. Liang Y, Li S, Wang X, et al. Reduction-triggered drug delivery nanosystem. Theranostics, 2017; 7: 3306–3318.
44. Cheng R, Meng F, Deng C, Klok HA, Zhong Z. Dual stimuli-responsive nanoparticles. Biomaterials, 2013; 34: 3647–3657.
45. Zhao X, Yang CX, Chen LG, Yan XP. Dual-responsive nanoprobe for tumor targeting. NatureCommunications, 2017; 8: 14998.
46. Wu MX, Gao J, Wang F, et al. Multistimuli responsive nanoplatform. Small, 2018; 14: 1704440.
47. Nomoto T, Fukushima S, Kumagai M, et al. Light-induced gene transfer nanocarrier. Nature Communications, 2014; 5: 3545.
48. Nishiyama N, Iriyama A, Jang WD, et al. Light-induced gene transfer system. Nat Materials, 2005; 4: 934–941.
49. Pegtel DM, Gould SJ. Exosomes. Annual Review of Biochemistry, 2019; 88: 487–514.

50. Kamerkar S, LeBleu VS, Sugimoto H, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS. Nature, 2017; 546: 498–503.
51. O'Brien K, Breyne K, Ughetto S, Laurent LC, Breakefield XO. RNA delivery by extracellular vesicles. Nature Reviews Molecular Cell Biology, 2020; 21(10): 585–606.
52. Vader P, Mol EA, Pasterkamp G, Schiffelers RM. Extracellular vesicles for drug delivery. Adv Drug Deliv Rev., 2016; 106: 148–156.
53. Lässer C. Exosomal RNA as biomarkers and therapeutic potential of exosome vectors. Expert Opinion on Biological Therapy, 2012; 12: S189–197. doi:10.1517/14712598.2012.680018
54. Zhang H, Wang S, Sun M, et al. Exosomes as drug delivery vehicles for cancer immunotherapy. Front Immunol, 2023; 13: 1093607.
55. Rezaie J, Feghhi M, Etemadi T. Exosomes in clinical trials: challenges. Cell Commun Signal, 2022; 20: 145.
56. El Andaloussi S, Mäger I, Breakefield XO, Wood MJA. Extracellular vesicles: Biology and emerging therapeutic opportunities. *Nature Reviews Drug Discovery*, 2013; 12: 347–357. doi:10.1038/nrd3978
57. Wiklander OPB, Brennan MA, Lötvall J, Breakefield XO, El Andaloussi S. Advances in therapeutic applications of extracellular vesicles. *SciTransl Med.*, 2019; 11(492): eaav8521. doi:10.1126/scitranslmed.aav8521
58. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. *Nat Nanotechnol*, 2021; 16(7): 748–759. doi:10.1038/s41565-021-00931-2
59. Armstrong JPK, Holme MN, Stevens MM. Re-engineering extracellular vesicles as smart nanoscale therapeutics. *ACS Nano.*, 2017; 11(1): 69–83. doi:10.1021/acsnano.6b07607
60. Sonar S. Clinical trial status of exosomes-based cancer theranostics. Clinical and Translational Dis., Aug. 2024; 4(4): e327. doi:10.1002/ctd2.327
61. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. Cell Commun Signal, Sep. 19, 2022; 20(1): 145. doi:10.1186/s12964-022-00959-4