IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Review Article
February 2024 Vol.:27, Issue:2

Exosomes: Tiny Vesicles Have, Big Impact in Intercellular Communication

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Tondlekar B.M*[1], Bundhada V. L[2], Saigaonkar A.A[3], Malgundkar H.K[4], Hingmire R.S[5].

[1,2,3]Studant, Govindrao Nikam College of Pharmacy, Sawarde 415606. India.

[4,5]Assistant Professor, Govindrao Nikam College of Pharmacy, Sawarde 415606. India.

Submitted: 25 January 2024
Accepted: 31 January 2024
Published: 29 February 2024

Keywords: Exosomes, Chemical Manipulation, Genetic Engineering, Targeted Delivery, Exosome Membrane Proteins

ABSTRACT

Exosomes are cell-derived nanovesicles that are involved in the intercellular transportation of materials. Therapeutics, such as small molecules or nucleic acid drugs, can be incorporated into exosomes and then delivered to specific types of cells or tissues to realize targeted drug delivery. Targeted delivery increases the local concentration of therapeutics and minimizes side effects. Here, we present a detailed review of exosome engineering through genetic and chemical methods for targeted drug delivery. Although still in its infancy, exosome-mediated drug delivery boasts low toxicity, low immunogenicity, and high engineerability, and holds promise for cell-free therapies for a wide range of diseases.

INTRODUCTION

When it comes to delivering bio-functional chemicals for biological applications including imaging, therapy, and diagnosis, nanomaterials provide fresh perspectives. Exosome-based targeted delivery methods, which enclose compounds in a phospholipid-bilayer membrane, are inspired by liposomes(1).

Chemical messengers, mainly in the form of extracellular vehicles (EVs), are used by cells to communicate. Because of their distinct shape, which enables them to incorporate certain proteins, genetic lipids, and genetic materials, EVs have been a focus for the development of therapeutic medicine. Exosomes are interesting for medication delivery since EVs can be further classified as apoptotic bodies, microvesicles, and exosomes(2).

The goal of nanomedicine is to deliver drugs to tumor cells precisely while avoiding damage to healthy tissues. Immune system resistance, site-specific treatments, and controlled drug release are all results of advancements in nanocarriers and vehicles(3).

Exosomes are effective carriers of a variety of chemicals, and content engineering can further develop their therapeutic potential. Numerous approaches have been devised for exosome-based therapeutics of clinical quality(3).

Exosomes are nanosized membrane vesicles (30–100 nm) that show promise for stabilizing and protecting their contents in enhanced medication delivery. According to research, they can be liberated from cells by pathological or physiological processes, which enables them to go to target areas in an active state(4).

Exosome-based therapy allows for the separation and manipulation of exosomes from the patient's own cells and is less immunogenic. Three animal sources—tumor cells, mesenchymal stem cells, and dendritic cells—are the subjects of clinical research. GMP uses five cell types to produce exosomes(3).

Exosomes are membrane vesicles generated from cells that are being studied as potential therapeutic delivery vehicles and biomarkers for conditions like brain and cancer. They are appealing because of their distinct structure, stability, biocompatibility, and effective targeting. Their poor productivity and variability, however, make it difficult to use them therapeutically.

Exosomes have demonstrated promise in the treatment of cancer, autoimmune disorders, and disorders of the central nervous system notwithstanding these obstacles. Enhancing their therapeutic efficacy and potential is essential for their upcoming clinical uses(5).

COMPOSITIONS OF EXOSOMES

Exosomes are formed from endosomes and are made up of different lipids and proteins that are indicative of their physiological roles. By expressing functional proteins on exosomes, the Exosome Display Technology seeks to improve the physicochemical qualities as well as the therapeutic and diagnostic capabilities of exosomes. By enhancing solubility, stability, and prolonged half-lives, this technique makes it possible to produce numerous antigen copies and may even result in the generation of antibodies directed against therapeutic targets and tumor biomarkers. The composition of exosome proteins has advanced significantly with the use of this technique(6).

BIOLOGICAL FUNCTION OF EXOSOMES

Cells release subcellular particles called exosomes, which serve as vehicles for the exchange of information and genetic material between cells. They have the ability to attach to target cells by endocytosis, fusion with cells, membrane or internal proteins, or ligands. Because of their small size, good biocompatibility, and lower toxicity as compared to manufactured nano formulations, exosomes are thought to be promising drug delivery vehicles. Additionally, they show increased anticancer activity in vivo along with better pharmacokinetic and pharmacodynamic characteristics. Exosomes can traverse the blood-brain barrier and permeate deep tissues with greater efficacy(7).

METHOD OF LOADING APPROACHES

Incubation:

For drug delivery, two incubation techniques have been developed: the first involves directly combining medicines and exosomes and is dependent on the drug's hydrophobicity; the second involves combining exosome donor cells with medications and isolating the exosomes that transport the drugs afterward. The loading efficiency is low for both techniques. HGNs, or follow-gold nanoparticles, are essential for cell labeling, imaging, and diagnostics. Nevertheless,

sonication, passive loading, electroporation, and saponin-assisted loading have not proven effective. A loading rate of roughly 50% has been used to isolate and purify heterozygous exosomes. The near-infrared "surface plasmon resonance" for hyperthermia in vivo relies on HGNs in exosomes(8).

Methicillin-resistant S. aureus infections of macrophages have been treated with drug-carrying exosomes. They are efficient in killing bacteria that are concealed in macrophages and in transporting medicinal antibiotics. A greater production of drug-carrying vesicles with characteristics akin to exosomes was achieved. Drug efficacy is increased by exosome-loaded nucleic acid medications since they avoid the nuclear lysosomal route. But there isn't a reliable way to put RNA into exosomes. To create an exosome-based hydrophobically modified small motor RNA (hsiRNA) delivery system for the treatment of Huntington's disease, a U87 glioblastoma cell line source coincubation approach was devised. This technique offers a dependable, effective, and highly repeatable way to add chemically generated oligonucleotides to exosomes(8).

In order to load cargo, desired cargos must be incubated with exosomes or exosome-secreting cells and allowed to diffuse into exosomes via concentration gradients. Most exosomes and plasma membrane are hydrophobic and lipid-enriched, allowing hydrophobic cargos, including curcumin and paclitaxel, to interface and be integrated spontaneously. Hydrophilic cargoes can be loaded into the hydrophilic core of exosomes. The easiest and most direct way to load cargo is with this procedure(9).

Drugs are incorporated into exosomes via diffusion along a gradient of concentration. The drug's hydrophobicity affects loading efficiency because it can interact with the lipid layers of vesicle membranes. Research has indicated that exosomes produced from mice with lymphomas can be loaded with either catalase or curcumin; however, the loading capacity of this approach is poor(10).

Electroporation

Through the process of electroporation, the phospholipid bilayer of exosomes is broken, resulting in the formation of tiny pores. The protocols with the highest efficiency use 10 pulses at 750 V.

These pores allow for the diffusion or sorting of drugs while the membrane is being recovered from loading. Nevertheless, its loading capacity is modest(11).

Through the process of electroporation, the exosome membrane is stimulated by an external electrical pulse, creating tiny pores that allow molecules to flow through. The parameters are controlled by applying different potentials, which makes it a popular technique for loading different compounds into exosomes. For instance, Zhang et al. inhibited apoptosis and proliferation by loading miR-665 into exosomes derived from osteosarcoma cells at 400 V potential. Using exosomes from three different cell lines, Rodriguez et al. assessed the efficiency of insulin loading and discovered that 200 V electroporation produced the highest efficiency. However, adding alginate disaccharide, citric acid, and EDTA can boost encapsulation efficiency because cargos like siRNA, proteins, and DNA have a tendency to agglomerate(12).

By introducing an electric field into a cell culture environment, a process known as electroporation makes it possible for tiny molecules to enter exosomes. Wang et al. used exosomes modified with the tumor-targeting ligand AS1411, which effectively transported siRNA and miRNA to breast cancer tissue, thereby suppressing the proliferation of tumor cells. However, transporting macromolecular nucleic acids is a challenge for conventional electroporation techniques. In 2019, Yang et al. developed a novel kind of cellular nonoperation biochip (CNP) that transcribes plasmid DNA into mRNA by incubating it with donor cells, damaging the cell membrane. By using this technique, the difficulty of inserting macromolecular mRNA into exosomes is resolved, leading to a 1,000-fold increase in exosome production(13).

Transfection

Transfection is a technique that uses chemicals like calcium phosphate or Lipofectamine to load proteins, peptides, and nucleic acids into exosomes. It can enhance the results of tumor treatment, disorders involving the motor nerves, and bone differentiation. According to Choi et al., miRNA inhibitors and osteogenic precursor cells rich in let-7 miRNAs can influence the development of bone. Kojima et al. employed catalase mRNA-loaded exosomes to cure Parkinson's disease in HEK293 cells. Exosomes can also be directly transfected with nucleic acids through chemical treatment(7).

Using this technique, siRNAs and miRNAs are transfected into parental cells and subsequently released into exosomes. Exosomes derived from fat stem cells that overexpress miR126 have the ability to restore cisplatin resistance in stomach malignancies and alleviate acute myocardial ischemia damage. Exosomes loaded with siRNA have been demonstrated to inhibit the migration and proliferation of cancerous and vascular cells. Nevertheless, there are drawbacks to this approach, including poor packing, low specificity, and cytotoxicity. Notwithstanding these drawbacks, the technique has demonstrated promise in reducing the growth of breast cancer cells(14).

The purpose of this thesis was to investigate the cytotoxicity and transfection potential of exosomes, which are employed to temporarily transfect mRNA into cells. Because exosomes can only carry RNA, they are appropriate for short-term transfection. The study concentrated on cell reprogramming, a gene therapy strategy in which brief transfection is adequate. The goal of the study was to comprehend exosomes' potential in this situation(15).

Ultrasound

Because ultrasound-based imaging is inexpensive, portable, and non-invasive, it has proven effective in the diagnosis and treatment of a wide range of illnesses. However, it is not suitable for imaging the lungs, brain, or abdomen because to inadequate penetration into air-filled tissues or bones. Research has also employed ultrasound to eliminate food allergies from milk products and to rid food goods of pesticide contamination. In order to extract bioactive chemicals, it has also been included into food processing operations and medicinal plant extraction techniques. Cereal germination has been improved, crop resilience to environmental challenges has been increased, and safe and effective fertilizers for crop growth have all been prepared using ultrasound-based procedures. Additionally, the removal of both organic and inorganic pollutants from water has proven to be successful with the use of ultrasound-based techniques. The acoustic characteristic of ultrasound has also been used the process of creating active pharmaceutical ingredients, which includes targeted drug delivery, stable nano/micro formulations, enhanced pharmacokinetic qualities, and decreased adverse effects of medications. Significant adjustments must be made to the experimental setup for the therapeutic use of ultrasonography, with diagnostic imaging intensities kept between 0.05 and 0.5 W/cm2. Therapeutic ultrasound uses high intensities ranging from 0.2 W/cm2 to 10,000 W/cm2, concentrating on the thermal and

mechanical impacts of acoustic waves. While therapeutic ultrasound concentrates on the biophysical effects, imaging records acoustic waves as echo(16).

Extrusion

The magnetic extrusion technique makes use of the endosome-specific build-up of magnetic IONPs via internalization of the cell by endocytosis. Parental cells release IONP-encapsulating endosomes, which are then gathered by magnetic separation and extruded as EMs. Free IONPs and IONP-encapsulating EMs are separated to create IONP-free EMs. A more effective methodology for EM synthesis is provided by this method(17).

Exosome research use the extrusion technique to study liposome-based medication delivery. Exosomes and payloads are pushed through polycarbonate membranes with pores ranging from 100 to 400 nm, enabling cargo to permeate into exosomes at regulated temperatures. Extrusion offers a consistent size distribution and good packing efficiency. However, extensive extrusion and severe shear stress can affect exosome membrane properties, such as zeta potential and surface protein structure. After comparing several loading techniques, researchers discovered notable variations in the shape, zeta potential, and size distribution of EVs(1).

By loading a combination of pharmaceuticals and exosomes into a lipid extruder with a porous membrane, this technique produces consistent exosome size and good drug loading efficiency. Researchers are unsure, though, if the mechanical stress affects the zeta potential and membrane protein structure of the exosome membrane(8).

Sonication

Sonication is a technique that facilitates the loading of medicines, proteins, and nanomaterials by weakening the exosome membrane. Drug loading into exosomes using sonication has been demonstrated in studies to be feasible, however membrane damage still poses a significant obstacle to widespread use. For instance, adding gemcitabine to exosomes generated from pancreatic cancer cells and subjecting them to sonication increased their loading capacity. Exosomes can also include gold nanoparticles, catalase, and other medications(9).

Sonication is a physical external process that facilitates drug loading by weakening the structure of exosomes. Although it can load more, the integrity of the exosome may be harmed. According

to studies, DOX can be entrapped by sonication and loaded into mouse macrophage RAW 264.7-based exosomes, with a weight percentage ranging from 7.3 to 9.9 wt%. Purified exosomes were combined with the drugs PTX and DOX by Kim M. et al., producing stable exosomes with a loading capacity of 28.29 ± 1.38%(8).

Freeze Thaw Cycle

The freeze-thaw approach, which involves recurrent lipid bilayer fusing, provides a gentle way to load miRNA and proteins. Exosomes undergo multiple cycles of freezing and thawing, with a minimum of three cycles necessary. Although encapsulation efficiency does not increase, the freeze-thaw loading approach has no discernible effect on exosome structure. It has been altered recently using sonication and incubation techniques(1).

For improved loading efficiency, the freeze and thaw cycles approach use multiple cycles of alternating temperature shocks. Haney and colleagues utilized this technique to introduce the catalase enzyme into exosomes produced from macrophages, with a loading efficiency of 14.7%. While the extrusion and sonication techniques produced more encouraging results, room temperature incubation produced a 4.7% yield. Another study used 34 amino acid peptides to load EVs, and the results were good for a range of sizes(18).

Ultra Centrifugation

UC is considered the gold standard for EV separation because of their unique settling coefficients. Exosomes are precipitated, dead cells, apoptotic vesicles, and biopolymers are removed, and centrifugal force is gradually increased (19). Although ultracentrifugation is a widely used technique for sample separation, it has drawbacks including a lengthy separation time, low reproducibility, and precipitate contaminants. It has cheap running costs and works well with most samples, although it could have an impact on later outcomes(20). Using protease inhibitors to stop protein degradation and cleaning the sample to get rid of big bioparticles are two steps in the pelleting process, also called the simple ultracentrifugation method. For additional analysis, the supernatant is gathered, resuspended in PBS, and kept at -80°C(21). Differential centrifugation is a multi-round ultracentrifugation method used to extract proteins and cell detritus from exosomes. Exosome loss may occur and regular user intervention is necessary. It provides a reasonable yield and purity consistently in spite of these problems(22).

Immunoaffinity Chromatography

Using exosome membranes rich in proteins and receptors to isolate certain particles, immunoaffinity is a technique for sorting and purifying biological particles through antigen-antibody specific interactions(20). Combining immunoaffinity with dUC improves the exosome purity that is isolated. However, if antibodies are difficult to remove after precipitation, it can compromise exosome integrity and diminish exosomal yield(23). Microplates are used in the enzyme-linked immunosorbent analysis method to enrich exosomes in fluids such as serum, plasma, and urine. It uses fewer samples yet produces exosomes that are comparable to those obtained using ultracentrifugation. It is challenging to popularize immunoaffinity chromatography because of its rigorous storage conditions, which make it unsuitable for large-scale separation(24). Magnetic Dynabeads' huge size, poor mobility, and surface-area-to-volume ratio necessitate long incubation times. With their greater surface-area-to-volume ratio and magnetophoretic mobility, temperature- or pH-responsive magnetic nanoparticles can shorten the duration of incubation and separation to just a few minutes, thereby expediting the procedure(22).

ROLES OF EXOSOMES

Role in Inter epithelial Transfer

The transfer of cargo between cells, known as intercellular communication, can occur through extracellular vesicles or more complex mechanisms such as autocrine, paracrine, and endocrine connections(25).

Roles in immunorespnse

It has been reported that CRSwNP and CRSsNP endotypes have Th1- and Th2-responses; CRSwNP is linked to Th2-based endotypes, while CRSsNP has a Th1-response, indicating complicated immunological responses(25).

APPLICATION OF EXOSOMES

Cancer treatment:

By enclosing different medications within them, exosomes have been generated to increase the effectiveness of anti-cancer therapeutic therapy. A DOX-encapsulated exosome with a 35% encapsulation efficiency was created by Bagheri et al. using MSCs. The MUC1 aptamer was used to functionalize the exosome and increase the accumulation of ligands that target cancer. This combination effectively reduced cancer volume and enhanced survival ratio in a mouse model carrying C26 carcinoma(26).

Brain Targeting Exosomes

Drugs cannot cross the blood-brain barrier in the human brain, but tumours and different types of brain cells' exosomes can target the brain in different ways. Creating tailored exosomes with targeted peptides changed on the surface is a useful tactic(27).

Lung Targeting Exosome

As one of the leading causes of cancer worldwide, lung cancer requires precision medicine, which includes tailored therapy. Drugs could be delivered to lung tissue using targeted ligands and engineered exosomes(27).

Exosomes are essential for communication between cells because they use endocytosis to transfer genetic material and proteins. They have functions related to apoptosis, coagulation, angiogenesis, and inflammation. Additionally, they support uteroplacental angiogenesis and reduce the immunological response of the mother during gestation(28).

Biomedical applications:

Studies indicate that exosomes are a safe medication delivery method with potential applications in chemotherapy preventive care. They are able to restore joints, stop the progression of diseases, and anticipate joint problems early. It has been discovered that exosomes contribute to the interaction between synovial fibroblasts (SFB) and articular chondrocytes, which aids in the downregulation of nucleus pulpous cells. Only RA patients have exosomes containing membrane-bound TNF-α; patients with osteoarthritis do not have these exosomes. Men and women produce different

amounts of miRNA in their exosomes, and this difference in exosome production is related to gender. In models of OA produced by collagenase, exosomes have demonstrated a therapeutic value in treating joint disorders(15).

Biomarker: Exosomes are cargo carriers that transport nucleic acids and proteins from cells to act as markers in pathological situations. They maintain stability in biological fluids like urine, blood, and saliva, and are suitable biomarkers for diseases like ovarian cancer, melanoma, glioblastoma, prostate, and colon cancers(15).

Sources

Exosomes are secreted by several cell types and are used in cell-free therapy for a number of conditions, including as drug addiction, status epilepticus, and myocardial infarction. The majority of in vivo-detected circulating exosomes originate from platelets. Exosomes produced from MSCs may help enhance the therapy of cancer and liver damage(29).

Stem cell-derived exosomes: In regenerative medicine, stem cells (SCs) have been employed to restore human tissue. Extracellular vesicles, cytokines, and growth factors—known as exosomes—are released by SCs. These exosomes may be used in cell-free regenerative therapy to control cell functions through the use of miRNAs. Given that miRNA molecules are critical for both the prevention and progression of disorders, this may be advantageous for EMVs-based therapeutic treatments in musculoskeletal ailments(13).

Plant cell derived exosomes: Exosomes derived from non-animal species have not received enough attention because there is no workable method for their isolation, purification, or imaging. Using agarose gel electrophoresis and differential centrifugation, exosomes were separated from Viscum album L., Vinca minor L., and Nicotiana tabacum L. Exosomes can be distinguished from tiny charged contaminants and excess dye(13).

FUTURE PROSPECTIVES

Although exosome research has advanced significantly, its biosynthesis is still unknown. To comprehend their cargo sorting and secretion methods, more research is required. High-throughput and high-purity screening is lacking from current isolation approaches; protocol modification could hasten exosome research for therapeutic uses.

Exosomes have potential applications as drug carriers, biomarkers, and tools for understanding how diseases progress. Certain miRNAs and proteins produced from exosomes are more valuable in the tumor diagnostic process. Glycopican-1 (GPC1), a cell surface proteoglycan abundant on exosomes generated from pancreatic cancer cells, is crucial for the early identification of pancreatic cancer because of its high false positive rate.

CONCLUSION

Exosomes are an essential component of cell-to-cell communication and disease diagnostics, offering a distinct and natural advantage over artificial drug loading methods. Nevertheless, exosome content-based illness detection systems are still in the early stages of development. Solving current exosome issues, like yield and purity, is necessary for using exosomes in therapeutic settings. To fully comprehend how exosome heterogeneity, fusion mechanism, and secretion mechanism affect drug loading efficiency, more investigation is required. This will aid in our understanding of the body and the diagnosis and treatment of diseases in the future.

REFERENCES

1. Chen H, Wang L, Zeng X, Schwarz H, Nanda HS, Peng X, et al. Exosomes, a New Star for Targeted Delivery. Front Cell Dev Biol. 2021;9(October).
2. Bunggulawa EJ, Wang W, Yin T, Wang N, Durkan C, Wang Y, et al. Recent advancements in the use of exosomes as drug delivery systems 06 Biological Sciences 0601 Biochemistry and Cell Biology. J Nanobiotechnology [Internet]. 2018;16(1):1–13. Available from: https://doi.org/10.1186/s12951-018-0403-9
3. Ferreira D, Moreira JN, Rodrigues LR. New advances in exosome-based targeted drug delivery systems. Crit Rev Oncol Hematol. 2022;172(December 2021).
4. Sen S, Xavier J, Kumar N, Ahmad MZ, Ranjan OP. Exosomes as natural nanocarrier-based drug delivery system: recent insights and future perspectives. 3 Biotech [Internet]. 2023;13(3):1–25. Available from: https://doi.org/10.1007/s13205-023-03521-2
5. Huda MN, Zicari JE, Nurunnabi M. Exosome-mediated bioinspired drug delivery. Bioinspired Biomim Mater Drug Deliv. 2021 Jan 1;219–40.
6. Fu W, Li T, Chen H, Zhu S, Zhou C. Research Progress in Exosome-Based Nanoscale Drug Carriers in Tumor Therapies. Front Oncol. 2022;12(June):1–9.
7. Xi XM, Chen-Meng, Xia SJ, Lu R. Drug loading techniques for exosome-based drug delivery systems. Pharmazie. 2021;76(2–3):61–7.
8. Chen Q, Wu D, Wang Y, Chen Z. Exosomes as Novel Delivery Systems for Application in Traditional Chinese Medicine. Molecules. 2022;27(22).
9. Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharmacol Sin [Internet]. 2017;38(6):754–63. Available from: http://dx.doi.org/10.1038/aps.2017.12
10. Gebeyehu A, Kommineni N, Meckes DG, Sachdeva MS. Role of Exosomes for Delivery of Chemotherapeutic Drugs. Crit Rev Ther Drug Carrier Syst. 2021;38(5):53–97.
11. Huang L, Wu E, Liao J, Wei Z, Wang J, Chen Z. Research Advances of Engineered Exosomes as Drug

Delivery Carrier. ACS Omega. 2023;8(46):43374–87.
12. Yin Y, Han X, Li C, Sun T, Li K, Liu X, et al. The status of industrialization and development of exosomes as a drug delivery system: A review. Front Pharmacol. 2022;13(October):1–13.
13. Amiri A, Bagherifar R, Ansari Dezfouli E, Kiaie SH, Jafari R, Ramezani R. Exosomes as bio-inspired nanocarriers for RNA delivery: preparation and applications. J Transl Med [Internet]. 2022;20(1):1–16. Available from: https://doi.org/10.1186/s12967-022-03325-7
14. Homsy M. Exosome-Based Transfection. 2016;(May).
15. Sridharan B, Lim HG. Exosomes and ultrasound: The future of theranostic applications. Mater Today Bio [Internet]. 2023;19(November 2022):100556. Available from: https://doi.org/10.1016/j.mtbio.2023.100556
16. Guo P, Busatto S, Huang J, Morad G, Moses MA. A Facile Magnetic Extrusion Method for Preparing Endosome-Derived Vesicles for Cancer Drug Delivery. Adv Funct Mater. 2021;31(44).
17. Fu S, Wang Y, Xia X, Zheng JC. Exosome engineering: Current progress in cargo loading and targeted delivery. NanoImpact [Internet]. 2020;20(September):100261. Available from: https://doi.org/10.1016/j.impact.2020.100261
18. Nowak M, Górczyńska J, Kołodzińska K, Rubin J, Choromańska A. Extracellular Vesicles as Drug Transporters. Int J Mol Sci. 2023;24(12).
19. Du S, Guan Y, Xie A, Yan Z, Gao S, Li W, et al. Extracellular vesicles: a rising star for therapeutics and drug delivery. J Nanobiotechnology [Internet]. 2023;21(1). Available from: https://doi.org/10.1186/s12951-023-01973-5
20. Chen J, Li P, Zhang T, Xu Z, Huang X, Wang R, et al. Review on Strategies and Technologies for Exosome Isolation and Purification. Front Bioeng Biotechnol. 2022;9(January):1–18.
21. Cgbmri C. Exosomes : A brief review of Biology and Medical applications Author : Katha Sanyal and Abhijit G . Banerjee Running title : Exosomes in Biology and Medicine. :1–13.
22. Lai JJ, Chau ZL, Chen SY, Hill JJ, Korpany K V., Liang NW, et al. Exosome Processing and Characterization Approaches for Research and Technology Development. Adv Sci. 2022;9(15):1–29.
23. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: Is size exclusion chromatography the best option? Int J Mol Sci. 2020;21(18):1–19.
24. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: A review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. Int J Nanomedicine. 2020;15:6917–34.
25. Mueller SK. The Role of Exosomes in the Pathophysiology of Chronic Rhinosinusitis. Front Cell Infect Microbiol. 2022;11(January):1–6.
26. Lee J, Lee JH, Chakraborty K, Hwang J, Lee YK. Exosome-based drug delivery systems and their therapeutic applications. RSC Adv. 2022;12(29):18475–92.
27. Zeng H, Guo S, Ren X, Wu Z, Liu S, Yao X. Current Strategies for Exosome Cargo Loading and Targeting Delivery. Cells. 2023;12(10).
28. Kurian TK, Banik S, Gopal D, Chakrabarti S, Mazumder N. Elucidating Methods for Isolation and Quantification of Exosomes: A Review. Mol Biotechnol [Internet]. 2021;63(4):249–66. Available from: https://doi.org/10.1007/s12033-021-00300-3
29. Antimisiaris SG, Mourtas S, Marazioti A. Exosomes and exosome-inspired vesicles for targeted drug delivery. Pharmaceutics. 2018;10(4).