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Exosome-Inspired Nanovesicles for Drug Delivery: Emerging Strategies, Applications, and Future Perspectives

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ABSTRACT

Exosomes are naturally secreted extracellular vesicles (30–150 nm) with unique potential as drug delivery vehicles due to their biocompatibility, stability, and innate targeting ability. However, clinical application of natural exosomes remains limited by challenges in scalability, heterogeneity, and regulatory approval. To overcome these limitations, researchers have developed exosome-inspired nanovesicles (EINVs), which replicate exosomal structure and functionality while offering controllability, reproducibility, and scalability. EINVs can be fabricated using top-down (cell-derived), bottom-up (synthetic), or hybrid approaches, and can be engineered to deliver therapeutic cargo such as small molecules, nucleic acids, proteins, and vaccines. This review provides a detailed overview of EINVs, including their types, fabrication methods, cargo-loading strategies, therapeutic applications, and comparative advantages over natural exosomes. We also discuss challenges in reproducibility, immunogenicity, and regulatory pathways, before highlighting future research directions needed for clinical translation.

KEYWORDS: Exosome-inspired nanovesicles, Extracellular vesicles, Biomimetic drug delivery, Hybrid nanocarriers, Precision nanomedicine

INTRODUCTION

Nanomedicine has transformed drug delivery by offering nanoscale platforms that enhance drug stability, bioavailability, and targeting.(1) Conventional delivery systems such as liposomes, polymeric nanoparticles, and micelles have demonstrated clinical impact, yet limitations such as immunogenicity, toxicity, and poor targeting persist. This has fuelled interest in biomimetic carriers that harness biological structures for safer and more effective delivery.(2)

Exosomes, small extracellular vesicles secreted by almost all mammalian cells, have emerged as highly attractive candidates.(3) Their natural role in intercellular communication, combined with their ability to cross biological barriers such as the blood–brain barrier, makes them ideal therapeutic carriers.(4) Exosomes transport proteins, lipids, and nucleic acids between cells and have been implicated in immune regulation, angiogenesis, and cancer progression. Importantly, their lipid bilayer protects encapsulated molecules from enzymatic degradation, increasing therapeutic stability in circulation.(5)

Despite these advantages, exosomes face substantial barriers to clinical use. They are secreted in low amounts, making large-scale production impractical. Their molecular composition is heterogeneous, depending on the donor cell type, state, and environment, making standardization difficult. Isolation techniques such as ultracentrifugation, precipitation, and size-exclusion chromatography are labor-intensive and not scalable. Furthermore, the presence of unwanted biomolecules raises safety and regulatory concerns.(6,7)

To address these issues, exosome-inspired nanovesicles (EINVs) have been developed. EINVs are engineered systems designed to mimic the structural, functional, and biological properties of natural exosomes while overcoming their limitations. EINVs may be derived from cells (top-down), assembled synthetically from defined lipids or polymers (bottom-up), or designed as hybrid vesicles that integrate natural and synthetic components. Compared to natural exosomes, EINVs are more reproducible, scalable, and amenable to engineering, making them strong candidates for translation into clinical nanomedicine.(8,9)

This review provides a comprehensive overview of EINVs, focusing on their types, fabrication strategies, drug loading approaches, therapeutic applications, advantages, challenges, and future perspectives.

TYPES OF EXOSOME-INSPIRED NANOVESICLES

Cell-Derived Nanovesicles (Top-Down Approach)

Cell-derived nanovesicles are produced by mechanical disruption of cells via serial extrusion, sonication, or microfluidic shear followed by reassembly into nanosized vesicles.(10) These nanovesicles retain lipids and membrane proteins of the donor cell, endowing them with biomimetic features such as recognition by specific cell types. For example, macrophage-derived nanovesicles can preferentially target inflamed tissues, while stem-cell-derived nanovesicles promote tissue regeneration.(11)

The major strength of this approach is yield, as cell disruption produces nanovesicles in quantities orders of magnitude higher than natural secretion. However, these vesicles may also contain undesired cytoplasmic proteins or nucleic acids, raising concerns about safety and consistency. Their composition is less controlled compared to fully synthetic systems, creating challenges for batch reproducibility.(12)

Synthetic Nanovesicles (Bottom-Up Approach)

Synthetic EINVs are fabricated by assembling defined lipids, cholesterol, or polymers into bilayer vesicles that structurally resemble exosomes. Techniques such as lipid film hydration, ethanol injection, or microfluidic self-assembly allow precise control of vesicle size, charge, and cargo encapsulation. Unlike cell-derived nanovesicles, synthetic EINVs do not carry unwanted cellular material, enabling greater purity and reproducibility.(13)

Synthetic systems also allow extensive engineering flexibility, such as incorporating ligands, PEGylation, or responsive elements (pH-sensitive lipids, redox-sensitive linkers). However, synthetic EINVs may lack the natural signaling proteins present in exosomes, which could reduce uptake and targeting efficiency unless compensated by artificial modifications.(14)

Hybrid Nanovesicles

Hybrid EINVs integrate natural and synthetic elements, often by fusing exosomal membranes with liposomes or polymeric nanoparticles. These hybrids combine the biological specificity of exosomes with the scalability and versatility of synthetic nanocarriers. For example, tumor-derived exosomal membranes fused with drug-loaded liposomes have shown enhanced targeting to tumor sites.(15,16)

The hybrid strategy is particularly attractive but technically complex. Achieving uniform membrane fusion, retaining bioactivity, and ensuring reproducibil-

ity are ongoing challenges. Moreover, hybrid EINVs present unique regulatory classification issues, as they straddle the line between biologics and synthetic nanomedicines.

FABRICATION TECHNIQUES

A variety of methods are employed to fabricate EINVs:

- **Serial Extrusion:** Cells are forced through nanoporous membranes, fragmenting them into nanosized vesicles. The resulting nanovesicles mimic cell membranes but may contain cytoplasmic material.
- **Microfluidics:** Provides precise control of vesicle formation by mixing lipids, proteins, and drugs in microchannels. This method yields highly uniform vesicles and is compatible with GMP-scale production.
- **Sonication & Electroporation:** These techniques can assist both vesicle fabrication and drug loading, but overuse may damage membrane integrity.

Self-Assembly: Amphiphilic molecules such as lipids spontaneously form bilayer vesicles under controlled conditions, enabling bottom-up construction with precise composition.(13,15,16)

CARGO LOADING STRATEGIES

Cargo loading is central to the therapeutic function of EINVs and can be performed either during or after fabrication. Pre-loading involves engineering donor cells to express or uptake therapeutic molecules before vesicle generation. For example, transfected cells can produce nanovesicles enriched with siRNA or microRNA. This approach allows physiological incorporation but is limited by cellular processing capacity. Post-loading methods introduce cargo after vesicle formation. Passive incubation allows hydrophobic drugs to diffuse into vesicles, while electroporation facilitates nucleic acid loading by temporarily disrupting membranes. Sonication, freeze–thaw cycles, and chemical transfection reagents can further enhance loading efficiency, though they risk altering vesicle properties. To improve targeting, EINVs are often functionalized with ligands such as antibodies, peptides, aptamers, or polyethylene glycol (PEG). These modifications enhance tissue specificity, circulation half-life, and therapeutic index.(17,18)

THERAPEUTIC APPLICATIONS

Cancer Therapy

EINVs have been widely explored in oncology. By encapsulating chemotherapeutics such as doxorubicin or paclitaxel, EINVs improve tumor accumulation through enhanced permeability and retention (EPR) and ligand-mediated targeting. Nucleic acid therapies, including siRNA and CRISPR/Cas9, have also been successfully delivered using EINVs, enabling gene silencing or editing within tumors while minimizing systemic toxicity.(8)

Neurological Disorders

The ability to cross the blood–brain barrier is a major advantage of EINVs. Ligand-decorated EINVs carrying siRNA or neuroprotective agents have shown efficacy in preclinical models of glioblastoma, Parkinson's disease, and Alzheimer's disease.

Regenerative Medicine

Stem-cell-derived EINVs retain regenerative signaling functions, delivering microRNAs and growth factors that stimulate angiogenesis and tissue repair. Applications are being investigated in cardiac regeneration, bone healing, and chronic wound treatment.

Immunotherapy and Vaccines

EINVs are also promising in immunotherapy. By presenting tumor antigens or delivering immune-stimulatory nucleic acids, EINVs can activate dendritic cells and enhance T-cell responses. They are also being investigated as carriers for **mRNA vaccines**, combining the stability of lipid nanoparticles with the biomimetic properties of exosomes.(8,19)

ADVANTAGES OVER NATURAL EXOSOMES

Exosome-inspired nanovesicles (EINVs) present several significant advantages when compared to natural exosomes, particularly in terms of yield, reproducibility, composition, scalability, and engineering flexibility. Yield is perhaps the most immediate advantage; while natural exosomes are secreted in small quantities over extended culture times, EINVs can be fabricated in bulk within hours using top-down or bottom-up techniques. This dramatic increase in yield addresses one of the greatest bottlenecks in exosome-based drug delivery, enabling the preparation of clinically relevant doses.

Reproducibility is another critical advantage. Natural exosomes vary in composition depending on donor cell type, environmental conditions, and isolation method, leading to batch-to-batch variability. In contrast, synthetic EINVs are fabricated using controlled assembly processes that produce highly uniform vesicles with defined physicochemical properties. Similarly, composition can be fine-tuned in EINVs; researchers can selectively incorporate specific lipids, proteins, or ligands to optimize biodistribution, cargo stability, and cellular uptake.

Scalability is also a decisive factor for clinical translation. Isolation of natural exosomes typically relies on ultracentrifugation or size-exclusion chromatography, which are not practical at industrial scales. Fabrication of EINVs via extrusion, microfluidics, or self-assembly, however, can be scaled to large volumes while maintaining consistency. Finally, EINVs offer unparalleled engineering flexibility. They can be surface-functionalized with antibodies, aptamers, or peptides to achieve targeted delivery; embedded with responsive lipids for stimuli-triggered release; or hybridized with polymers to enhance stability. Collectively, these features make EINVs much more suitable than natural exosomes for regulatory approval and eventual clinical adoption.(8,9)

CHALLENGES AND LIMITATIONS

While EINVs address many limitations of natural exosomes, they also introduce their own challenges. A major concern is reproducibility of biological function. Synthetic EINVs, while structurally similar to exosomes, often lack the full spectrum of membrane proteins and signaling molecules that mediate natural exosome-cell interactions. This may limit their bioactivity unless compensated through advanced engineering strategies. On the other hand, cell-derived EINVs, although more biologically representative, may inadvertently encapsulate unwanted cytoplasmic proteins, DNA fragments, or immunogenic components during fabrication, raising safety concerns.

Immunogenicity represents another key limitation. While exosomes are naturally tolerated by the immune system, EINVs—especially synthetic ones with artificial polymers or ligands—may trigger immune responses. The long-term consequences of repeated EINV administration are not yet well understood, highlighting the need for comprehensive immunotoxicology studies.

Another critical issue is stability. Natural exosomes are relatively stable in circulation, aided by their natural lipid composition. EINVs, however, may face challenges with aggregation, cargo leakage, or loss of structural integrity during storage and transport. Stabilization techniques such as lyophilization and PEGylation are under investigation but require optimization.

Finally, regulatory uncertainty poses significant hurdles. Because EINVs fall between biologics and synthetic nanomedicines, regulatory agencies have not yet established clear frameworks for their approval. This uncertainty compli-

cates clinical translation, as companies face difficulties in aligning production, quality control, and safety standards with regulatory expectations.(9)

FUTURE PERSPECTIVES

Future research on EINVs should prioritize the development of standardized protocols and nomenclature to clearly distinguish them from natural exosomes and traditional nanoparticles. Standardized characterization methods—covering size distribution, cargo encapsulation efficiency, surface chemistry, and bioactivity—will be essential to ensure reproducibility and comparability across studies.

Scaling up fabrication remains a central priority. GMP-compatible workflows leveraging microfluidics, continuous-flow extrusion, or automated self-assembly systems are promising solutions to produce EINVs at industrial scales with high reproducibility. At the same time, advanced targeting strategies should be pursued, including site-specific ligands, aptamers, or CRISPR-based engineering of donor cells to enrich EINVs with desired proteins or nucleic acids.

Rigorous safety validation is indispensable for clinical translation. Studies must move beyond small animal models to large-animal systems that better predict human pharmacokinetics and biodistribution. Long-term evaluations of immune responses, toxicity, and potential accumulation in organs are required before regulatory approval.

Finally, early and proactive regulatory engagement will be crucial. Dialogue between researchers, industry stakeholders, and agencies like the FDA and EMA can help establish quality standards, safety parameters, and classification criteria for EINVs. In the long run, EINVs could become an integral component of precision nanomedicine, enabling patient-specific therapies optimized through computational modeling and AI-driven design.(19–21)

CONCLUSION

Exosome-inspired nanovesicles represent a paradigm shift in drug delivery, bridging the gap between natural exosomes and synthetic nanoparticles. By combining scalability, reproducibility, and engineering flexibility with biomimetic design, EINVs address many of the shortcomings of natural exosomes while opening new possibilities for targeted therapy. Their applications span oncology, neurology, regenerative medicine, and immunotherapy, making them versatile tools in modern nanomedicine. However, their success will depend on addressing key challenges, including bioactivity optimization, immunogenicity, stability, and regulatory acceptance. With ongoing advances in fabrication technologies, standardization efforts, and clinical validation, EINVs are poised to play a transformative role in the future of drug delivery and personalized medicine. As the field matures, they may become not just an alternative to exosomes but a superior and clinically preferred platform for precision therapeutics.

REFERENCES

1. Singh S. Nanomedicine–nanoscale drugs and delivery systems. J Nanosci Nanotechnol. 2010;10(12):7906–18. https://doi.org/10.1166/jnn.2010.3617
2. Tran S, DeGiovanni PJ, Piel B, Rai P. Cancer nanomedicine: a review of recent success in drug delivery. Clin Transl Med. 2017;6(1):44. https://doi.org/10.1186/s40169-017-0175-0
3. Witwer KW, Théry C. Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. Vol. 8, Journal of extracellular vesicles. Taylor & Francis; 2019. p. 1648167. https://doi.org/10.1080/20013078.2019.1648167
4. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science (80-). 2020;367(6478):eaau6977. https://doi.org/10.1126/science.aau6977
5. Negahdaripour M, Owji H, Eskandari S, Zamani M, Vakili B, Nezafat N. Small extracellular vesicles (sEVs): Discovery, functions, applications, detection methods and various engineered forms. Expert Opin Biol Ther. 2021;21(3):371–94. https://doi.org/10.1080/14712598.2021.1825677
6. Meng W, He C, Hao Y, Wang L, Li L, Zhu G. Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source. Drug Deliv. 2020;27(1):585–98. https://doi.org/10.1080/10717544.2020.1748758
7. Zhang M, Zang X, Wang M, Li Z, Qiao M, Hu H, et al. Exosome-based nanocarriers as bio-inspired and versatile vehicles for drug delivery: recent advances and challenges. J Mater Chem B. 2019;7(15):2421–33. https://doi.org/10.1039/C9TB00170K
8. Sunkara SP, Kar NR, Kareemulla S, Sarma KN, Thool KU, Katual MK, et al. Recent Advancement in Exosome-Inspired Lipid Nanovesicles for Cell-Specific Drug Delivery. Int J Pharm Investig. 2024;14(4). https://doi.org/10.5530/ijpi.14.4.119
9. Antimisiaris SG, Mourtas S, Marazioti A. Exosomes and exosome-inspired vesicles for targeted drug delivery. Pharmaceutics. 2018;10(4):218. https://doi.org/10.3390/pharmaceutics10040218
10. Jang HJ, Shim KS, Lee J, Park JH, Kang SJ, Shin YM, et al. Engineering of cell derived-nanovesicle as an alternative to exosome therapy. Tissue Eng Regen Med. 2024;21(1):1–19. https://doi.org/10.1007/s13770-023-00610-4
11. Khalilzad MA, Mohammadi J, Amirsaadat S, Najafi S, Zare S, Nilforoushzadeh MA, et al. Therapeutic potential of apoptotic vesicles in modulating inflammation, immune responses, and tissue regeneration. J Nanobiotechnology. 2025;23(1):260. https://doi.org/10.1186/s12951-025-03278-1
12. Lee JR, Kyung JW, Kumar H, Kwon SP, Song SY, Han IB, et al. Targeted delivery of mesenchymal stem cell-derived nanovesicles for spinal cord injury treatment. Int J Mol Sci. 2020;21(11):4185. https://doi.org/10.3390/ijms21114185
13. Mougenot MF, Pereira VS, Costa ALR, Lancellotti M, Porcionatto MA, Da Silveira JC, et al. Biomimetic nanovesicles—sources, design, production methods, and applications. Pharmaceutics. 2022;14(10):2008. https://doi.org/10.3390/pharmaceutics14102008
14. Karimi M, M. Moosavi Basri S, Vossoughi M, S. Pakchin P, Mirshekari H, R. Hamblin M. Redox-sensitive smart nanosystems for drug and gene delivery. Curr Org Chem. 2016;20(28):2949–59. https://doi.org/10.2174/1385272820666160510154557
15. Sun M, Yang J, Fan Y, Zhang Y, Sun J, Hu M, et al. Beyond extracellular vesicles: hybrid membrane nanovesicles as emerging advanced tools for biomedical applications. Adv Sci. 2023;10(32):2303617. https://doi.org/10.1002/advs.202303617
16. Mukherjee A, Bisht B, Dutta S, Paul MK. Current advances in the use of exosomes, liposomes, and bioengineered hybrid nanovesicles in cancer

detection and therapy. Acta Pharmacol Sin. 2022;43(11):2759–76. https://doi.org/10.1038/s41401-022-00902-w

17. Tay A, Melosh N. Nanostructured materials for intracellular cargo delivery. Acc Chem Res. 2019;52(9):2462–71. https://doi.org/10.1021/acs.accounts.9b00272

18. Erana-Perez Z, Igartua M, Santos-Vizcaino E, Hernandez RM. Genetically engineered loaded extracellular vesicles for drug delivery. Trends Pharmacol Sci. 2024;45(4):350–65. https://doi.org/10.1016/j.tips.2024.02.006

19. Shinge SAU, Xiao Y, Xia J, Liang Y, Duan L. New insights of engineering plant exosome-like nanovesicles as a nanoplatform for therapeutics and drug delivery. Extracell Vesicles Circ Nucleic Acids. 2022;3(2):150. https://doi.org/10.20517/evcna.2021.25

20. Preetam S, Lata S, Bora J, Thapliyal S, Mehta S, Ramniwas S, et al. Exosome-driven biohybrid nanorobots: bridging nature and nanotechnology in biomedical innovation. RSC Adv. 2025;15(40):33390–409. https://doi.org/10.1039/D5RA05296C

21. 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. 2023;13(3):101. https://doi.org/10.1007/s13205-023-03521-2