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REVIEW ARTICLE

Engineered Exosomes as Next-generation Nanotherapeutics for Glioma: Crossing the Blood-brain Barrier Toward Precision Medicine

Shatrudhan Prajapati[1,2] and Shikha Yadav[1,*]

[1]*Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India;* [2]*School of Pharmacy, Lingaya's Vidyapeeth, Faridabad, Haryana, 121002, India*

Abstract: ***Background*:** Glioma is one of the most aggressive and treatment-resistant malignancies of the central nervous system, characterized by rapid progression and poor prognosis. Conventional treatment approaches, including surgery, radiotherapy, and chemotherapy, often fail to achieve long-term remission due to the restrictive nature of the blood-brain barrier and the inherent heterogeneity of tumor cells.

***Methods*:** This review summarizes and critically analyzes current preclinical and clinical evidence on exosome biology, including their biogenesis, molecular cargo, and therapeutic engineering strategies. It further highlights recent advances in exosome-based delivery of chemotherapeutic agents, microRNAs, and gene-editing systems for glioma management.

***Results*:** Tumor- and stem cell-derived exosomes have demonstrated the ability to cross biological barriers while carrying functional biomolecules and modulating the tumor microenvironment. Their inherent stability, low immunogenicity, and capacity for surface modification make them promising nanocarriers for therapeutic applications. Engineered exosomes loaded with anti-tumor microRNAs, small interfering RNAs, or chemotherapeutic nanoparticles have shown promising results in enhancing drug sensitivity and reducing tumor proliferation in experimental glioma models.

***Discussion*:** The findings support the therapeutic potential of exosome-based platforms while also highlighting major challenges, including inconsistencies in isolation protocols, limited cargo-loading capacity, targeting specificity, and *in vivo* stability. Although exosomes have demonstrated the ability to overcome biological barriers and therapeutic resistance, standardized manufacturing protocols and robust clinical validation remain essential for successful clinical translation.

***Conclusion*:** Exosome-based systems represent a promising approach for the diagnosis and treatment of glioma. Their dual role as biomarkers and therapeutic drug carriers offers significant potential for personalized medicine through non-invasive disease monitoring and targeted therapeutic strategies. However, further optimization of large-scale production, purification methods, and clinical translation is necessary before exosome-based therapeutics can be integrated into standard glioma treatment protocols.

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1. INTRODUCTION

Glioma is one of the most aggressive and treatment-resistant malignant brain tumors, characterized by rapid progression and highly invasive behavior within the brain. Unlike many solid tumors that remain localized, glioma cells diffusely infiltrate the surrounding normal brain tissue, making complete surgical resection extremely difficult and contributing to high recurrence rates. This diffuse infiltration represents a major biological challenge in glioma management and significantly limits the effectiveness of conventional therapeutic approaches.

Brain tumors are abnormal growths of cells that arise within the brain tissue or associated structures and may be classified as either benign or malignant [1, 2]. More than 150 histopathological variants of brain tumors have been identified and classified according to their cellular origin, morphological characteristics, and degree of invasiveness [3]. Gliomas, meningiomas, and medulloblastomas are among the most commonly diagnosed brain tumor types [4]. Gliomas constitute the majority of primary malignant Central Nerv-

*Address correspondence to this author at the Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India; E-mail: shikha21samy@gmail.com

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ous System (CNS) tumors, accounting for approximately 81% of malignant CNS neoplasms [5]. These glial-derived tumors include astrocytomas, oligodendrogliomas, ependymomas, and mixed oligoastrocytomas [6].

The 2021 World Health Organization (WHO) classification integrates histological and molecular characteristics to classify gliomas and improve diagnostic accuracy and prognostic stratification [7]. Grade IV glioma, commonly referred to as Glioblastoma (GBM), represents the most aggressive subtype and is associated with the poorest clinical prognosis [8]. Lower-grade gliomas (grades I-II) generally demonstrate more favorable outcomes, with reported 5-year survival rates ranging from 70% to 97% and 10-year survival rates between 49% and 76% [9, 10]. However, recurrence remains common, occurring in approximately 52-62% of patients within five years, and some tumors may undergo malignant transformation into High-Grade Gliomas (HGG) [11, 12]. The median overall survival for patients with grade III gliomas is approximately three years, whereas patients with grade IV GBM have a markedly lower median survival of nearly 15 months [13].

The clinical manifestations of gliomas depend on their size, anatomical location, and extent of infiltration. Common manifestations include recurrent headaches, nausea, vomiting, seizures, and progressive neurological impairments, such as cognitive deficits, motor dysfunction, speech and visual impairments, and behavioral changes [14]. Despite advances in imaging techniques and surgical procedures, treatment outcomes remain unsatisfactory. Conventional treatment interventions include maximal surgical resection of the tumor followed by radiotherapy and chemotherapy, which are intended to eliminate residual cancer cells [15]. Nevertheless, GBM is associated with poor long-term survival, with 5-year survival rates typically ranging from 3% to 5%, primarily because of therapeutic resistance and the inability of most anticancer agents to effectively penetrate the Blood-Brain Barrier (BBB) or Blood–Brain Tumor Barrier (BBTB) [16].

The BBB serves as a highly selective regulatory interface that maintains CNS homeostasis by regulating molecular and ionic exchange through tightly connected endothelial cells [17, 18]. Although vascular abnormalities surrounding tumors may partially impair BBB integrity, its permeability remains heterogeneous across different tumor regions, resulting in suboptimal drug distribution and incomplete tumor eradication [19]. This has increased the need for advanced drug delivery strategies capable of overcoming BBB-related limitations and enhancing targeted delivery to infiltrative glioma cells.

Exosomes have emerged as promising natural nanocarriers owing to their ability to cross the BBB and their significant therapeutic potential for targeted glioma therapy. These nanoscale extracellular vesicles are secreted by various cell types and can transport diverse therapeutic cargos, including chemotherapeutic agents, RNAs, and proteins, directly to brain tissue [20]. Owing to their biological stability, low immunogenicity, and intrinsic targeting capabilities, exosomes represent attractive platforms for precise drug delivery in glioma treatment. In addition to their role in therapeutic delivery, exosomes actively contribute to tumor progression through intercellular communication, tumor invasion, angiogenesis, immune regulation, and microenvironmental remodeling.

This review critically analyzes the application of engineered exosome-based delivery systems in glioma therapy, with particular emphasis on their ability to overcome the Blood-Brain Barrier (BBB), enhance targeted drug delivery, and improve therapeutic efficacy. It also explores the diagnostic and prognostic utility of exosomal biomarkers for monitoring disease progression and therapeutic response. By integrating mechanistic insights with recent translational advances, this review aims to provide a comprehensive and conceptually structured framework for advancing exosome-mediated approaches in glioma management.

2. METHODOLOGY AND REVIEW STRATEGY

2.1. Literature Search and Study Identification

This review adopted a focused narrative synthesis approach rather than a formal systematic review and was designed to provide mechanistic and translational insights rather than exhaustive coverage of the literature. A systematic literature search was conducted using PubMed, Scopus, and Web of Science to identify peer-reviewed articles published in recent years. The search strategy included combinations of the following keywords: glioma, glioblastoma, extracellular vesicles, exosomes, cell migration, invasion, intercellular communication, vesicle cargo, and drug delivery. Additional relevant studies were identified through backward and forward citation tracking of key publications. Preference was given to original research articles, high-quality review papers, and books with substantial experimental or clinical relevance.

2.2. Study Inclusion Criteria and Thematic Focus

The selection of studies was based on predefined criteria emphasizing biological relevance, mechanistic depth, and translational significance. Studies providing functional evidence linking extracellular vesicles to glioma invasion, migration, tumor-microenvironment communication, therapeutic resistance, or drug delivery were prioritized. Rather than attempting to include all available literature, this review focuses on studies supported by experimental perturbation approaches, *in vivo* validation, patient-derived samples, and clinically relevant data. Investigations reporting only correlative or descriptive findings without mechanistic or methodological validation were assigned lower priority to maintain analytical rigor.

2.3. Rationale for Emphasizing Specific Molecular Pathways

The molecular pathways discussed in this review were selected based on three primary criteria: (i) established involvement in glioma cell migration, invasion, and tumor progression; (ii) demonstrated association with extracellular vesicle cargo or vesicle-mediated signaling; and (iii) reproducibility across independent experimental systems. Priority was given to pathways involved in integrin-mediated adhesion signaling, Rho-family GTPase regulation, cytoskeletal remodeling, ion channel-mediated control of cell migration, and neuron–glioma communication because of their strong

mechanistic relevance and functional validation. Pathways lacking direct functional evidence or supported solely by limited profiling data were intentionally de-emphasized to minimize speculative interpretation.

2.4. Selection Logic for Extracellular Vesicle Cargo **Types**

Extracellular vesicle cargo categories were evaluated based on their demonstrated biological effects rather than their mere presence. MicroRNAs were emphasized because of the consistent evidence supporting their roles in regulating glioma migration, angiogenesis, immune modulation, and therapeutic resistance across multiple cancer models. Protein cargos were prioritized when they were associated with extracellular matrix remodeling, adhesion signaling, activation of oncogenic pathways, or immune regulation, and when their downstream functional effects had been experimentally validated. Lipid and metabolic cargos were discussed selectively, with emphasis placed on mechanistic evidence supporting their involvement in tumor metabolism and survival adaptation. Cargo classes identified solely through high-throughput profiling studies, without functional validation, were not prioritized in order to maintain the review's focus on biologically relevant mechanisms.

2.5. Prioritization of Extracellular Vesicle Subtypes and Methodological Rigor

Exosomes were prioritized among extracellular vesicle populations because of their comparatively well-characterized biogenesis, stability, long-range signaling capabilities, and demonstrated ability to cross the blood–brain barrier. Studies employing standardized protocols for vesicle isolation, characterization, and validation were preferred to ensure methodological reliability. Studies that did not clearly classify vesicle subtypes, lacked adequate purity controls, or exhibited poor reproducibility were interpreted with caution. This selective emphasis reflects not only biological relevance but also methodological rigor.

2.6. Balancing Novelty, Evidence Strength, and Clinical Relevance

To avoid bias toward novelty alone, this review considers both emerging findings and well-established mechanistic evidence. Novel pathways, cargo molecules, or therapeutic strategies were included only when supported by functional validation, *in vivo* relevance, or translational potential. Clinical studies involving patient-derived samples, orthotopic tumor models, or evaluations of therapeutic applications were prioritized to enhance real-world relevance. Where data availability influenced the inclusion or exclusion of specific findings, this limitation is acknowledged to ensure transparency and maintain scientific balance.

2.7. Analytical Framework and Synthesis Strategy

Rather than discussing studies as individual reports, this review organizes the findings within a systematic conceptual framework focused on vesicle-mediated mechanisms involved in glioma invasion, migration, intercellular communication, and therapeutic response. Data were interpreted using a comparative approach to identify shared biological themes, glioma-specific patterns, points of convergence, and existing knowledge gaps. Literature providing only descriptive extracellular vesicle profiling without functional or mechanistic validation was deliberately excluded to avoid redundancy within an already saturated field of research. By applying stringent selection criteria and integrating findings into a coherent biological framework, this review aims to provide an analytical synthesis and a guided perspective rather than a comprehensive catalog of published studies.

3. GLIOMA INVASION AND MIGRATION AS THE CENTRAL BIOLOGICAL DRIVER OF DISEASE PROGRESSION

Glioma-related mortality is driven less by distant metastasis than by the tumor's remarkable ability to invade and diffusely infiltrate surrounding brain tissue. Unlike most solid tumors, glioma cells do not form well-defined masses; instead, they extensively migrate along white matter tracts, blood vessels, and neural pathways, making complete surgical resection virtually impossible. In many cases, even when imaging suggests complete removal of the gross tumor, migratory tumor cells remain beyond the resection margins, leading to inevitable recurrence. Therefore, glioma should be regarded not only as a rapidly growing tumor but also as a highly invasive and migratory disease in which cell migration is a key determinant of clinical outcome.

This invasive behavior is neither passive nor random but is regulated by specific molecular and biophysical mechanisms that actively control cytoskeletal remodeling, cell adhesion, ion flux, and interactions with the extracellular matrix. Experimental studies have demonstrated that glioma cell motility can be regulated independently of tumor cell proliferation, highlighting migration as a distinct and therapeutically targetable biological process. For example, ion channel-associated proteins have been shown to promote glioma cell motility independent of their canonical electrical functions, indicating that invasion is governed by specific molecular mechanisms rather than representing a loosely defined phenotypic state. These findings underscore the importance of investigating glioma migration as a targetable biological process requiring detailed mechanistic understanding.

The connection between glioma progression and vesicle-mediated communication represents another important conceptual framework. Growing evidence indicates that extracellular vesicles, particularly exosomes, function as key signaling mediators that regulate migratory behavior within tumor cells and facilitate communication between glioma cells and their microenvironment. Vesicle-associated cargoes, including microRNAs, growth factors, adhesion molecules, and metabolic enzymes, can reprogram recipient cells to enhance motility, remodel the extracellular matrix, and promote invasive phenotypes. Consequently, vesicle biology should not be viewed as a peripheral topic but rather as a mechanistic extension of the central process of glioma invasion.

By positioning glioma migration as a central biological challenge, this review examines the role of extracellular vesicle-mediated signaling in tumor infiltration, therapeutic resistance, and disease recurrence. This conceptual framework provides a rationale for investigating vesicle cargo, intercellular communication, and targeted therapeutic strategies within an integrated mechanistic context.

4. GLIOMA AS A THERAPEUTIC DELIVERY CHALLENGE

4.1. Infiltrative Growth as a Spatially Unresolvable Disease Constraint

Tumor progression in glioma is driven not only by an increase in tumor size but also by the extensive invasion of surrounding healthy brain tissue. Glioma cells utilize white matter tracts and perivascular spaces as migratory pathways, enabling them to spread beyond regions detectable by conventional imaging techniques. Even when gross total resection is achieved, residual tumor cells often remain after surgery, limiting the effectiveness of treatment. The biological behavior of glioma is characterized by the infiltration of malignant cells into critical regions of the brain, making complete surgical removal extremely challenging and contributing to disease recurrence [21].

4.2. Spatial Escape and Functional Resistance of Migratory Tumor Cells

Glioma-derived cells can undergo phenotypic changes that confer treatment resistance and promote tumor progression. Rather than actively developing resistance to therapeutic agents, these cells often migrate to regions where drugs cannot achieve effective concentrations, a phenomenon referred to as "spatial escape" rather than conventional drug resistance. Studies have shown that invasive glioma cells can acquire stem cell-like characteristics, enhanced metabolic flexibility, and increased survival capacity, enabling them to resist cell death and initiate new tumor growth. Furthermore, these cells may develop enhanced DNA damage response mechanisms and upregulate drug efflux transporter genes, allowing them to withstand therapeutic challenges. The combined effects of spatial escape and intrinsic treatment resistance contribute significantly to continued disease progression [22].

4.3. Mechanistic Control of Migration: Cytoskeletal and Adhesion Dynamics

Glioma cell migration is not a random process but is tightly regulated by specific molecular mechanisms. Members of the Rho-family GTPases, including RhoA, Rac1, and Cdc42, regulate actin cytoskeletal dynamics that drive cellular behaviors such as protrusion, adhesion, and contraction. Integrin-mediated adhesion and Focal Adhesion Kinase (FAK) signaling establish traction forces that facilitate cyclic attachment and detachment, enabling directional migration through the brain microenvironment and its mechanical constraints. Matrix Metalloproteinases (MMPs) further promote invasion by degrading extracellular matrix components, thereby creating pathways through which glioma cells can infiltrate dense neural tissues. Experimental studies have demonstrated that disruption of these pathways impairs cell migration without significantly affecting cellular proliferation. These findings support the concept that motility represents an independent biological phenotype and a potential therapeutic target for anti-invasive drug development [23].

4.4. Non-canonical Regulatory Modules and Therapeutic Targetability

Recent mechanistic studies suggest that glioma invasion should not be viewed as an emergent and poorly defined feature of malignancy. Instead, evidence indicates that cell migration is driven by specific molecular modules that can be experimentally manipulated. For example, the voltage-gated sodium channel β3 subunit has been shown to regulate glioma cell motility through non-conductive mechanisms that influence cytoskeletal organization and adhesion turnover. These findings demonstrate that ion channel-associated proteins perform dual functions, contributing not only to electrophysiological signaling but also to the structural regulation of cell migration. Collectively, this research supports the concept that glioma invasion is a biologically regulated process governed by distinct molecular pathways and regulatory networks. This understanding provides a strong foundation for the development of therapeutic strategies aimed at inhibiting cellular migration and limiting glioma progression [24, 25].

4.5. Blood-brain Barrier as a Determinant of Therapeutic Failure

The Blood-Brain Barrier (BBB), together with the intrinsic biological characteristics of glioma, presents substantial challenges to effective therapeutic intervention. Although tumor-associated vascular abnormalities may partially compromise BBB integrity in certain regions, this disruption is often heterogeneous and does not ensure uniform drug penetration throughout the tumor. Consequently, infiltrative glioma cells residing behind relatively intact BBB regions may be exposed to subtherapeutic drug concentrations, enabling their survival and contributing to disease recurrence. This limitation remains a major obstacle to current treatment strategies, as many therapeutic agents fail to achieve adequate distribution across all tumor-infiltrated areas. Therefore, there is a critical need for advanced drug delivery systems capable of efficiently traversing the BBB and facilitating targeted, sustained, and therapeutically effective drug accumulation within glioma tissues (Table **1**) [26].

5. EXOSOME BIOLOGY RELEVANT TO THERAPEUTIC CARGO DELIVERY

5.1. Biogenesis and Selective Cargo Sorting Mechanisms

Exosome biogenesis originates within the endosomal pathway, where early endosomes mature into late endosomes and undergo inward budding of the endosomal membrane to generate Intraluminal Vesicles (ILVs), leading to the formation of Multivesicular Bodies (MVBs). These MVBs subsequently fuse with the plasma membrane, releasing exosomes into the extracellular space. The biogenesis process involves the selective incorporation of proteins, lipids, and nucleic acids through both ESCRT-dependent and ESCRT-independent mechanisms [39]. The Endosomal Sorting Complex Required For Transport (ESCRT), comprising ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, orchestrates cargo recognition, membrane invagination, and vesicle scission. Accessory proteins, including ALIX and TSG101, further facilitate cargo sorting and exosome formation. In addition, ESCRT-independent pathways mediated by ceramide-rich lipid domains and tetraspanins contribute to membrane remodeling and selective cargo packaging. This highly regulated biogenetic process ensures the efficient

Table 1. Biological, mechanistic, and clinical significance of cell motility in glioma.

Aspect	Biological Dimension	Mechanistic Basis	Experimental/Clinical Evidence	Impact on Disease Progression	Therapeutic Implications	References
Infiltrative Tumor Growth	Glioma spreads diffusely rather than forming confined tumor masses	Migration along white matter tracts, perivascular spaces, and extracellular matrix	Imaging and histopathological studies show tumor cells extending beyond surgical margins	Prevents complete tumor resection and drives recurrence	Targeting migration may reduce tumor dispersal and recurrence	[27]
Therapy Resistance	Migratory cells evade surgery, radiotherapy, and chemotherapy	Spatial escape from treatment zones and activation of survival pathways	Residual invasive cells detected after maximal therapy	Major cause of treatment failure and relapse	Combining cytotoxic and anti-motility therapies may improve outcomes	[28]
Stemness & Tumor Recurrence	Migratory cells often display stem-like properties	Enhanced self-renewal, metabolic flexibility, and resistance to apoptosis	Glioma stem-like cells enriched in invasive tumor regions	Enables tumor repopulation and long-term persistence	Targeting motile stem-like cells may prevent relapse	[29]
Cytoskeletal & Adhesion Remodeling	Cell movement depends on cytoskeletal plasticity	Rho GTPases, focal adhesion kinase, integrins, actin remodeling	*In vitro* and *in vivo* migration assays confirm pathway involvement	Facilitates invasion through dense brain tissue	Pathway inhibitors represent anti-invasive drug targets	[30]
Extracellular Matrix Degradation	Migration requires breakdown of surrounding tissue	Matrix metalloproteinases (MMPs) and proteolytic signaling	Elevated MMP expression in invasive glioma fronts	Promotes deep tissue infiltration	MMP inhibition may slow invasion	[31]
Ion Channel–Associated Regulation	Migration regulated independently of electrical signaling	Voltage-gated sodium channel β3 subunit signaling	C6 glioma motility modulated without ion conductance	Demonstrates migration is mechanistically targetable	Ion channel–related proteins offer novel anti-motility targets	[32]
Microenvironmental Adaptation	Motile cells adapt to oxygen, nutrients, and tissue stiffness	Mechanosensing, hypoxia response pathways, metabolic plasticity	Migratory cells preferentially occupy adaptive niches	Enhances tumor survival in hostile environments	Targeting adaptation pathways may limit invasion	[33]
Tumor–Microenvironment Crosstalk	Migratory cells remodel surrounding brain tissue	Signaling with astrocytes, microglia, endothelial cells	Co-culture and *in vivo* studies show reciprocal activation	Reinforces invasion, angiogenesis, and immune evasion	Disrupting tumor–host signaling may reduce invasion	[34]
Angiogenesis Promotion	Migration linked to vascular remodeling	VEGF signaling and endothelial activation	Increased neovascularization in invasive glioma regions	Supports tumor expansion	Anti-angiogenic therapies may complement anti-motility approaches	[35]
Immune Evasion	Migratory cells avoid immune surveillance	Immunosuppressive signaling and microglial polarization	Invasive fronts enriched in immunosuppressive markers	Facilitates tumor persistence	Immunotherapy combined with anti-migration strategies	[36]
Neural Network Exploitation	Glioma cells migrate along neuronal pathways	Axon guidance molecules and synaptic signaling	Neuron–glioma synaptic interactions observed experimentally	Enhances directional invasion	Targeting neuron–tumor signaling may restrict spread	[37]
Functional Targetability	Migration governed by defined molecular modules	Pharmacological inhibition alters motility	Preclinical anti-migration drug studies	Demonstrates the invasion is not inevitable	Validates motility as a therapeutic vulnerability	[38]

encapsulation and transfer of biologically active molecules, thereby underpinning the physiological functions, intercellular communication capabilities, and therapeutic potential of exosomes [40].

5.2. Molecular Composition and Functional Implications of Exosomal Cargo

Exosomes carry a diverse array of biomolecules, including proteins, lipids, messenger RNAs (mRNAs), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and DNA fragments. The composition of their cargo varies according to the physiological or pathological state of the donor cell, enabling exosomes to function as context-specific carriers of biological information. In glioma, exosomal cargo frequently contains oncogenic microRNAs, such as miR-21, miR-301a, and miR-148a, which modulate gene expression in recipient cells and promote tumor growth, invasion, and therapeutic resistance. In addition to regulatory RNAs, exosomes transport biologically active proteins involved in cellular signaling, adhesion, metabolism, and immune modulation. They may also carry DNA fragments harboring tumor-specific genetic alterations, including EGFRvIII and IDH

mutations. The molecular complexity of exosomal cargo enables these vesicles to influence the phenotype and behavior of recipient cells, remodel the tumor microenvironment, and facilitate disease progression. These properties also highlight their potential as valuable diagnostic biomarkers and promising therapeutic delivery vehicles in glioma management [41].

5.3. Exosome-mediated Intercellular Communication and Tumor Microenvironment Modulation

Exosomes serve as critical mediators of intercellular communication within the glioma microenvironment by transporting a diverse range of regulatory molecules from tumor cells to stromal, endothelial, and immune cells. Through this vesicle-mediated signaling network, exosomes influence multiple aspects of tumor biology, including proliferation, invasion, angiogenesis, immune evasion, and therapeutic resistance. The transfer of bioactive cargo, particularly microRNAs, proteins, and other signaling molecules, can induce phenotypic alterations in recipient cells, promote extracellular matrix remodeling, and enhance migratory and invasive capacities. In addition, exosomes facilitate metabolic reprogramming of both tumor and neighboring cells, enabling adaptation to hypoxic and nutrient-deprived conditions commonly encountered within the tumor microenvironment. By orchestrating complex cellular interactions across the tumor ecosystem, exosomes play a central role in glioma progression and have emerged as promising targets for therapeutic intervention as well as innovative vehicles for targeted drug delivery [41].

5.4. Blood-brain Barrier Translocation and Systemic Distribution

Exosomes possess a unique and clinically significant ability to traverse biological barriers, including the Blood-Brain Barrier (BBB), making them highly attractive candidates for targeted drug delivery. Their natural cellular origin, biocompatibility, and low immunogenicity enable them to evade rapid clearance by the immune system and circulate efficiently within the body. Following their release, exosomes can travel through various biological fluids, including blood, cerebrospinal fluid, saliva, and urine, facilitating communication between distant cells and tissues. Importantly, their capacity to cross the BBB addresses one of the major challenges in glioma therapy, where conventional therapeutic agents often fail to achieve adequate penetration into brain tissue. Furthermore, exosomes can be internalized by recipient cells through receptor-mediated interactions, membrane fusion, or endocytic pathways, enabling the efficient delivery of therapeutic cargo to specific target cells. These properties position exosomes as promising nanocarriers for enhancing drug delivery, improving therapeutic efficacy, and advancing precision medicine approaches for glioma treatment [42].

4.5. Implications for Therapeutic Engineering and Delivery Efficiency

Exosomes possess several intrinsic biological properties that make them highly promising nanocarriers for therapeutic applications. These include their ability to encapsulate diverse biomolecules, maintain stability during systemic circulation, evade immune surveillance, and efficiently deliver cargo to recipient cells. Owing to these advantages, considerable research efforts have focused on engineering exosomes to enhance drug-loading capacity, targeting specificity, and therapeutic efficacy. Various loading strategies, including passive incubation, electroporation, sonication, and microfluidic-assisted approaches, have been investigated to improve encapsulation efficiency and cargo retention. Simultaneously, advances in isolation and purification techniques aim to achieve higher exosome yield, purity, and scalability for clinical applications. A comprehensive understanding of the interplay among exosome biogenesis, cargo composition, biodistribution, cellular uptake, and intracellular trafficking is essential for optimizing their performance as therapeutic nanocarriers. Continued progress in these areas is expected to facilitate the successful translation of exosome-based drug delivery systems for glioma treatment and other neurological disorders [43].

6. ENGINEERING STRATEGIES FOR **THERAPEUTIC CARGO DELIVERY USING EXOSOMES**

Exosome engineering has become the primary method to increase the treatment effectiveness of these vesicles through its capacity to enhance their ability to deliver substances and direct them to specific areas while overcoming multiple body barriers, including the blood–brain barrier. The various engineering methods can be divided into four categories, which include cargo loading techniques, donor cell-based engineering methods, surface-based methods, and hybrid nanobiotechnology systems. [44].

6.1. Passive Cargo Loading: Physicochemical Constraints and Applicability

The process of passive loading depends on two mechanisms, which are concentration gradient-driven diffusion and hydrophobic partitioning, that allow therapeutic agents to move through the exosomal lipid bilayer. Small hydrophobic molecules such as paclitaxel tend to enter lipid membrane domains because their lipid-drug interactions produce better results in this area. This method maintains vesicle structure and native surface protein structure, which allows the vesicles to keep their natural targeting functions.

The process of passive loading faces fundamental restrictions because both membrane permeability and equilibrium partitioning determine its limits. The lipid bilayer prevents hydrophilic drugs, nucleic acids, and large biomolecules from being effectively encapsulated because it creates an impermeable barrier. The process of passive diffusion creates different cargo distribution patterns among vesicles, which leads to insufficient therapeutic effects in diverse glioma tissue areas. The method offers easy and scalable implementation, but it limits pharmacological applications because it needs post-loading purification to eliminate any remaining unincorporated drug [45].

6.2. Active Loading Strategies: Enhancing Encapsulation at the Cost of Structural Stability

Research investigates active loading methods that use exosome membrane disruption as their primary method to

transport cargo. Electroporation creates temporary nanopores that permit the delivery of negatively charged molecules, including siRNA, miRNA, and CRISPR components. The method provides effective treatment to target the oncogenic pathways that exist within glioma. The loading efficiency requires specific electrical settings and buffer specifications because improper conditions will result in RNA aggregation, vesicle fusion, and irreversible membrane damage.

Sonication causes the membrane to experience mechanical changes, which create pathways for drugs to enter the cell through short-term lipid movements. The method increases loading efficiency for chemotherapeutic agents, yet it causes membrane alterations which result in surface protein loss and changes to drug distribution throughout the body. Active loading creates a conflict between two goals which require precise adjustments to meet therapeutic needs. [46].

6.3. Donor Cell Engineering: Endogenous Cargo Sorting and Functional Stability

The process of engineering donor cells creates a biological method to load exosomes by using their built-in cargo sorting systems. Donor cell activation through therapeutic nucleic acid or protein overexpression enables specific exosomal inclusion, which occurs *via* RNA-binding proteins including hnRNPA2B1 and YBX1, and Ago2, which identify specific sequence patterns during multivesicular body development. The method maintains the original vesicle structure to securely load materials, which leads to better delivery inside cells and increased activity for cells that receive these materials. Donor cell-engineered exosomes from donor cells in glioma models showed extended gene silencing effects, which led to stronger tumor growth control and better ability to cross blood-brain barrier systems that mimic natural conditions. The method creates difficulties that stem from its difficult production process, which leads to uneven results and makes it hard to manage and control for regulatory purposes while it carries the danger of mistakenly packaging cancer-causing substances or immune system-stimulating materials [47].

6.4. Surface Functionalization: Targeting Specificity and BBB Transcytosis

Researchers require surface engineering strategies that help them to achieve better tumor targeting and develop methods for effective blood–brain barrier transport. Researchers use chemical conjugation methods such as click chemistry and antibody coupling to create links between targeting ligands and exosomal membrane proteins, which include CD63 and CD81. Genetic engineering methods provide researchers with the ability to produce targeting peptides during the biogenesis process of vesicles [48].

Ligands that target receptors such as transferrin receptors, integrins, and HER2 increase tumor cell uptake and brain accumulation in preclinical models through their capacity for receptor-mediated endocytosis and transcytosis. Surface modification creates benefits for vesicle systems, but it causes changes in their physicochemical characteristics, including surface charge and protein corona development, that may disrupt drug distribution and immune system detection. The research found that high ligand density causes two problems, which include off-target interactions and receptor saturation, so they must optimize their targeting methods according to their research findings [49].

6.5. Hybrid Exosome-based Platforms: Expanding Functional Versatility

The advanced strategy of hybrid systems, which integrate exosomes with synthetic nanomaterials, enables researchers to solve the fundamental problems associated with natural vesicles. The combination of exosomes and liposomes creates hybrid systems that provide two benefits through their improved ability to carry drugs and their extended time in circulation, while polymer-based systems deliver precise drug release through controlled responses to the tumor microenvironment. The use of metallic nanoparticles such as gold nanoparticles and superparamagnetic iron oxide nanoparticles enables theranostic applications which include both imaging and hyperthermia-based therapy. Hybridization produces two effects that reduce the natural biocompatibility of exosomes and their ability to operate undetected, while creating new problems about their safety, their ability to be replicated, and their legal status. Standardizing protocols, together with a complete assessment of safety requirements, will address the problems arising from the intricate nature of these systems that hinder their ability to be produced at scale and used in medical practice (Table **2**) [50].

7. EXTRACELLULAR VESICLES AND METASTASIS IN GLIOMA: A BRAIN-RESTRICTED, INVASION-CENTRIC COMMUNICATION PARADIGM

7.1. Glioma Progression is Driven by Diffuse Local Invasion Rather Than Classical Metastasis

The evolution of glioma contrasts with the evolution of most epithelial solid tumors, in which the pathogenicity of the disease is determined by massive local invasion rather than metastasis to organs. While breast and prostate cancer are dependent on hematogenous dispersal and secondary colonizing the distant organs, glioma cells spread diffusely *via* surrounding brain parenchyma along white matter tracts, perivascular space, and extracellular matrix scaffold. This invasive growth pattern makes it impossible to do a complete surgical resection and is the basis of the almost universal recurrence even with the use of aggressive multimodal therapy. Within this biological context, extracellular vesicles are not so much a long-range host of organ-to-organ communications, but viewed more as local communication platforms for cellular coordination of invasion, survival, and adaptation within the central nervous system. A glioma vesicle biology, which is viewed in terms of a metastasis context, is thus incapable of reflecting the actual functional contribution of EV-based communication in brain tumors [70].

7.2. Blood-brain Barrier Architecture Constrains EV Trafficking and Shapes Functional Signaling Range

The blood-brain barrier is one of the major structural and physiological limitations that redesigns the extracellular vesicle diffusion in glioma. In peripheral cancer tumors, tumor-secreted vesicles can very easily penetrate into the

Table 2. Engineered exosome strategies for glioma therapy.

Engineering Method	Cargo Loaded	Targeting Strategy	Experimental Model	Key Outcome	References
Passive incubation	Paclitaxel	Native tumor tropism	Glioma cell lines	Enhanced cytotoxicity with preserved vesicle integrity	[51]
Freeze–thaw loading	Doxorubicin	Passive uptake	*In vitro* GBM models	Increased drug encapsulation; moderate membrane stress	[52]
Surfactant-assisted loading	Methotrexate	None	Glioma cells	Improved hydrophilic drug incorporation	[53, 54]
Electroporation	siRNA/CRISPR-Cas9	Gene-specific silencing	GBM xenograft mouse model	Efficient gene knockdown; tumor growth suppression	[55, 56]
Sonication	Paclitaxel/Doxorubicin	Passive	*In vitro* glioma models	High loading efficiency; mild membrane perturbation	[57
Parent cell transfection	miR-146b	Natural tumor homing	Mouse glioma model	EGFR downregulation; reduced tumor progression	[58, 59]
Lipid bilayer integration	Hydrophobic anticancer drugs	Membrane insertion	GBM cell culture	Sustained drug release; improved retention	[60]
Size/charge optimization	Large RNAs/siRNA	Physicochemical tuning	*In vitro* models	Increased encapsulation efficiency	[61, 62]
Click chemistry functionalization	Imaging agents/peptides	Peptide-mediated BBB targeting	Glioma-bearing mice	Improved brain accumulation; enhanced tumor selectivity	[63]
Avidin–biotin coupling	Antibody-linked cargo	Receptor-mediated tumor binding	Mouse tumor models	Increased tumor-specific uptake	[64]
Genetic surface engineering	HER2-targeted siRNA	Ligand expression on membrane	HER2+ glioma model	High targeting specificity; strong gene silencing	[65]
Exosome–liposome hybrid	Paclitaxel	Extended circulation	*In vivo* glioma model	Improved pharmacokinetics; enhanced tumor suppression	[66, 67]
Exosome–polymer composite	Doxorubicin	pH-responsive release	Glioma xenografts	Controlled release; reduced systemic toxicity	[68]
Nanoparticle-enriched exosomes	Gold NPs/SPIONs	Theranostic targeting	Mouse GBM model	Combined imaging capability and targeted tumor ablation	[69]

systemic circulation and affect distant endothelial beds, immune compartments, and stromal tissues. Conversely, glioma-derived vesicles can act in the parenchyma, the cerebrospinal fluid, and the areas of the localized BBB disruption. Vesicle exchange in high-grade tumors where the barrier integrity is compromised is still spatially heterogeneous and mostly localized in the CNS. This confined radius of trafficking imposes selective pressure on EV cargo composition toward factors influencing cell migration, cytoskeletal plasticity, tolerance of metabolic stress, immune modulation, and blood vessel permeability, rather than factors that influence niche conditioning in more distant sites. Therefore, BBB architecture not only restricts the diffusion of EVs, but also actively addresses the biological role of vesicle-mediated signaling in glioma [71].

7.3. Integration of EV Signaling with Neuronal and Glial Communication Networks

In contrast to peripheral tumors, glioma grows in an electrically excited tissue environment that is full of neuronal firing, release of neurotransmitters, and neuron-glia interactions. This has been shown to promote neural invasion and metabolic support through neuronal activity and glioma cells' ability to respond to it, as well as to recruit neural signaling pathways. In this context, extracellular vesicles are probably mediators that successfully convert neural and glial signals into tumor-intrinsic migratory and survival responses. Calcium signaling, cytoskeletal dynamics, mitochondrial activity, and energy consumption in recipient tumor and stromal cells can be manipulated by vesicle-mediated transfer of regulatory RNAs, signaling proteins, and metabolic enzymes. This EV signaling-neuron and glia integration is a brain-specific specialization of vesicle biology with no parallel in breast or prostate cancer and the need to interpret contextually [72].

7.4. Local Microenvironmental Reprogramming as a Functional Analog of Pre-metastatic Niches

Extracellular Vesicles (EVs) play a pivotal role in the progression of many metastatic cancers by establishing pre-metastatic niches through the reprogramming of distant stromal and immune cell populations before the arrival of tumor cells. However, this paradigm differs substantially in glioma, where tumor dissemination occurs primarily through local infiltration rather than distant metastasis. In glioma, EV-mediated communication continuously remodels the peritumoral microenvironment, thereby facilitating tumor invasion and progression. Glioma-derived EVs influence multiple components of the tumor ecosystem, including astrocyte activation, microglial polarization, vascular permeability, and extracellular matrix remodeling. Collectively, these alterations create a permissive microenvironment that supports tumor cell migration and infiltration into surrounding brain tissue. This localized microenvironmental repro-

gramming can be considered a functional analogue of pre-metastatic niche formation, adapted specifically to promote sustained local invasion rather than episodic metastatic dissemination. Recognizing this distinction provides a valuable conceptual framework for understanding the diverse biological effects of EVs in glioma progression and highlights their potential as therapeutic targets for limiting tumor infiltration [73].

7.5. Immune Modulation Through Resident Microglia Rather Than Systemic Immune Escape

The extracellular vesicle-mediated immune regulation in glioma has a different reasoning than peripheral tumors. While in breast and prostate cancer, vesicles may be responsible for suppressing systemic immune surveillance or condition several circulating myeloid populations, glioma-derived vesicles act mainly on tissue-resident microglia in a sort of immune-privileged CNS environment. Vesicle-mediated signaling modifies microglial activation states, cytokine profiles, antigen presentation ability, and phagocytic behavior, reinforcing a localized immunosuppressive niche that supports invasive growth. This form of immune modulation is spatially limited and mechanistically different than the systemic immune evasion mechanisms, and this further highlights the brain selectivity of EV-mediated communication [74].

8. HISTOPATHOLOGICAL GRADING AND GENETIC ALTERATIONS IN GLIOMA

According to the World Health Organization (WHO) classification system, gliomas are categorized based on a combination of histopathological and molecular characteristics that reflect their degree of malignancy and clinical behavior. Diffuse gliomas are classified into different grades, ranging from lower-grade tumors with relatively favorable prognoses to highly aggressive malignancies. Grade I tumors, such as pilocytic astrocytoma, are generally well-circumscribed and associated with favorable clinical outcomes. In contrast, grade IV gliomas, particularly glioblastoma, represent the most aggressive subtype and are characterized by rapid growth, extensive invasiveness, therapeutic resistance, and poor patient survival. The incorporation of molecular markers into the WHO classification has further improved diagnostic precision and prognostic stratification, enabling a more accurate assessment of tumor biology and clinical outcomes [74].

Mutations in the isocitrate dehydrogenase (IDH) gene family are among the most extensively studied molecular alterations in gliomas and are frequently observed in younger patients as well as in secondary gliomas that arise from lower-grade precursor lesions [75]. These mutations predominantly affect specific arginine residues, most commonly arginine 132 (R132) in IDH1 and the corresponding arginine 172 (R172) in IDH2. Mutant IDH enzymes acquire a neomorphic catalytic activity that leads to the accumulation of the oncometabolite D-2-hydroxyglutarate (2-HG), resulting in widespread metabolic and epigenetic alterations. These changes influence cellular differentiation, gene expression, and tumor progression, thereby playing a critical role in glioma pathogenesis and serving as important diagnostic, prognostic, and therapeutic biomarkers [76].

9. MOLECULAR CLASSIFICATION OF GLIOMA BASED ON IDH MUTATION STATUS

9.1. IDH-mutant Astrocytic Gliomas

IDH mutations are widely recognized as among the earliest genetic events involved in gliomagenesis. However, studies using murine models have demonstrated that the presence of an IDH mutation alone is insufficient to initiate tumor formation, indicating that additional genetic alterations are required for malignant transformation [77]. In IDH-mutant astrocytomas, mutations in ATRX and TP53 frequently occur as cooperating genetic events. ATRX mutations, which are commonly associated with the loss of nuclear ATRX expression, play a crucial role in chromatin remodeling, genome stability, and telomere maintenance through the Alternative Lengthening of Telomeres (ALT) pathway [78].

As IDH-mutant gliomas progress from diffuse astrocytoma to anaplastic astrocytoma and eventually to IDH-mutant glioblastoma, they acquire additional molecular abnormalities that contribute to increased tumor aggressiveness. These alterations include deletions of chromosome 9p21, which contains the tumor suppressor genes CDKN2A and CDKN2B encoding the cyclin-dependent kinase inhibitors p16INK4A and p15INK4B, respectively. Additional chromosomal losses, gains, and structural rearrangements further promote tumor progression, genomic instability, and resistance to therapy [79]. Collectively, these cumulative genetic events drive the malignant evolution of IDH-mutant gliomas and influence their clinical behavior and prognosis.

9.2. IDH-mutant and 1p/19q-co-deleted Oligodendroglial Tumors

Oligodendrogliomas are molecularly defined by the presence of an isocitrate dehydrogenase (IDH) mutation in combination with the complete co-deletion of chromosome arms 1p and 19q, a hallmark genetic alteration that typically arises from an unbalanced translocation involving chromosomes 1q10 and 19p10 [80]. This distinctive molecular profile is associated with a more favorable prognosis and enhanced responsiveness to therapy compared with other diffuse glioma subtypes. Oligodendrogliomas are also characterized by a high prevalence of activating mutations in the Telomerase Reverse Transcriptase (TERT) promoter, which are detected in more than 95% of cases and contribute to telomere maintenance and cellular immortality. Additionally, mutations in the capicua transcriptional repressor (CIC) gene occur in over two-thirds of tumors, resulting in the loss of its tumor-suppressive transcriptional regulatory functions [81].

Mutations in the Far Upstream Element-Binding Protein 1 (FUBP1) gene, a regulator of MYC expression, are observed in approximately one-third of oligodendrogliomas and are believed to contribute to tumor development and progression [82]. Furthermore, alterations involving NOTCH1, SETD2, and components of the Phosphatidylinositol 3-Kinase (PI3K) signaling pathway, including PIK3CA, have also been reported [83]. As these tumors progress, additional genetic abnormalities may emerge, including deletions at chromosome 9p21, which are associated with a more aggressive phenotype, enhanced MYC signaling, and poorer clinical outcomes. Mutations in transcription factors such as TCF12 have likewise been implicated in tumor progression and increased biological aggressiveness [84]. Collectively,

these molecular alterations contribute to the pathogenesis, progression, and clinical behavior of oligodendrogliomas.

9.3. IDH-wild-type Glioblastoma

IDH-wild-type glioblastomas represent the most common form of glioblastoma and predominantly occur in individuals over 50 years of age. These tumors are generally classified as primary (*de novo*) glioblastomas, arising without evidence of a pre-existing lower-grade lesion. At the molecular level, IDH-wild-type glioblastomas are characterized by several recurrent genetic alterations, including TERT promoter mutations, homozygous deletions of CDKN2A and CDKN2B, loss of chromosome 10, and mutations or deletions of the tumor suppressor gene PTEN [85]. Less frequently observed alterations include mutations in PIK3R1, PIK3CA, TP53, and NF1, which contribute to dysregulation of critical signaling pathways involved in cell proliferation, survival, and tumor progression.

Gene amplifications involving Platelet-Derived Growth Factor Receptor Alpha (PDGFRA), Epidermal Growth Factor Receptor (EGFR), and Mesenchymal–Epithelial Transition factor (MET) are also commonly detected in these tumors. Amplification of CDK4 and CDK6 promotes dysregulation of the G1-S cell-cycle transition, while amplification of MDM2 and MDM4 contributes to the suppression of p53-mediated tumor suppressor functions [86]. EGFR overexpression is observed in approximately 40% of IDH-wild-type glioblastomas, and nearly half of these cases harbor the EGFRvIII variant, a constitutively active receptor generated by an in-frame deletion of exons 2-7. Furthermore, approximately 50% of epithelioid glioblastomas carry the BRAF V600E mutation, which encodes a therapeutically targetable oncoprotein and represents a promising molecular target for precision therapy (Fig. **1**) [87].

9.4. IDH-mutant Glioblastoma

The molecular profile of IDH-mutant glioblastomas closely resembles that of IDH-mutant astrocytomas, with frequent co-occurring mutations in ATRX and TP53, along with the presence of the glioma CpG Island Methylator Phenotype (G-CIMP). This epigenetic signature is characterized by widespread DNA hypermethylation and is generally associated with a more favorable prognosis compared with IDH-wild-type glioblastomas. However, a subset of IDH-mutant glioblastomas exhibits reduced levels of DNA methylation, commonly referred to as the G-CIMP-low phenotype. This hypomethylated state has been associated with increased tumor aggressiveness, enhanced cellular proliferation, and poorer clinical outcomes. The transition from G-CIMP-high to G-CIMP-low status is thought to reflect tumor progression and may serve as an important prognostic indicator in patients with IDH-mutant gliomas (Table **3** and Fig. **2**) [88].

10. PERFORMANCE, FUNCTIONAL VALIDATION, AND TRANSLATIONAL EFFICIENCY OF ENGINEERED EXOSOMES

10.1. Quantitative Performance of Isolation and Cargo Loading Strategies

The successful development of engineered exosomes for therapeutic applications relies on efficient cargo-loading strategies and robust isolation techniques. Differential ultracentrifugation remains one of the most widely used methods for exosome isolation, typically yielding approximately 10^8-10^9 particles per milliliter with moderate purity. Density-gradient ultracentrifugation offers enhanced purity but often results in lower recovery rates. Size-exclusion chromatography provides a favorable balance between yield and purity, whereas immunoaffinity-based approaches enable highly specific exosome isolation but are limited by low recovery efficiency, high cost, and challenges in large-scale production. More recently, microfluidic-based platforms have emerged as promising alternatives, achieving recovery rates of approximately 60-80% and purity levels ranging from 85% to 95%. These systems offer improved reproducibility, reduced processing time, and greater potential for clinical-scale manufacturing.

Table 3. Molecular and genetic characteristics of major glioma subtypes.

Glioma Subtype	Core Genetic Alterations	Key Molecular Pathways Affected	Functional Consequences	Clinical Implication	References
IDH-Mutant Astrocytoma	IDH1/2 mutation; ATRX loss; TP53 mutation; CDKN2A/B deletion	Epigenetic reprogramming; chromatin remodeling; cell cycle dysregulation	G-CIMP phenotype; telomere instability; progressive malignant transformation	Generally better prognosis than IDH-wild-type; risk of progression to higher grade	[77, 78]
IDH-Mutant, 1p/19q Co-Deleted Oligodendroglioma	IDH mutation; whole-arm 1p/19q co-deletion; TERT promoter mutation; CIC and FUBP1 mutations	Telomerase activation; MYC regulation; transcriptional derepression	Genomic stability with defined molecular identity	Favorable response to therapy; improved overall survival	[80-84]
IDH-Wild-Type Glioblastoma	PTEN loss; TERT promoter mutation; EGFR amplification; CDKN2A/B deletion; monosomy 10; PDGFRA/MET amplification	RTK/PI3K/AKT activation; p53 pathway disruption; cell cycle acceleration	Highly aggressive phenotype; enhanced proliferation and invasion	Poor prognosis; median survival ~15 months; limited therapeutic response	[85, 86]
Epithelioid Glioblastoma (subset)	BRAF-V600E mutation	MAPK pathway activation	Increased proliferative signaling	Potential eligibility for BRAF-targeted therapy	[87]
IDH-Mutant Glioblastoma	IDH mutation; ATRX and TP53 co-mutations; partial DNA hypomethylation	Epigenetic alteration; chromatin remodeling	Shares features with IDH-mutant astrocytoma but more aggressive	Intermediate prognosis; hypomethylated subset linked to worse outcome	[78, 88]

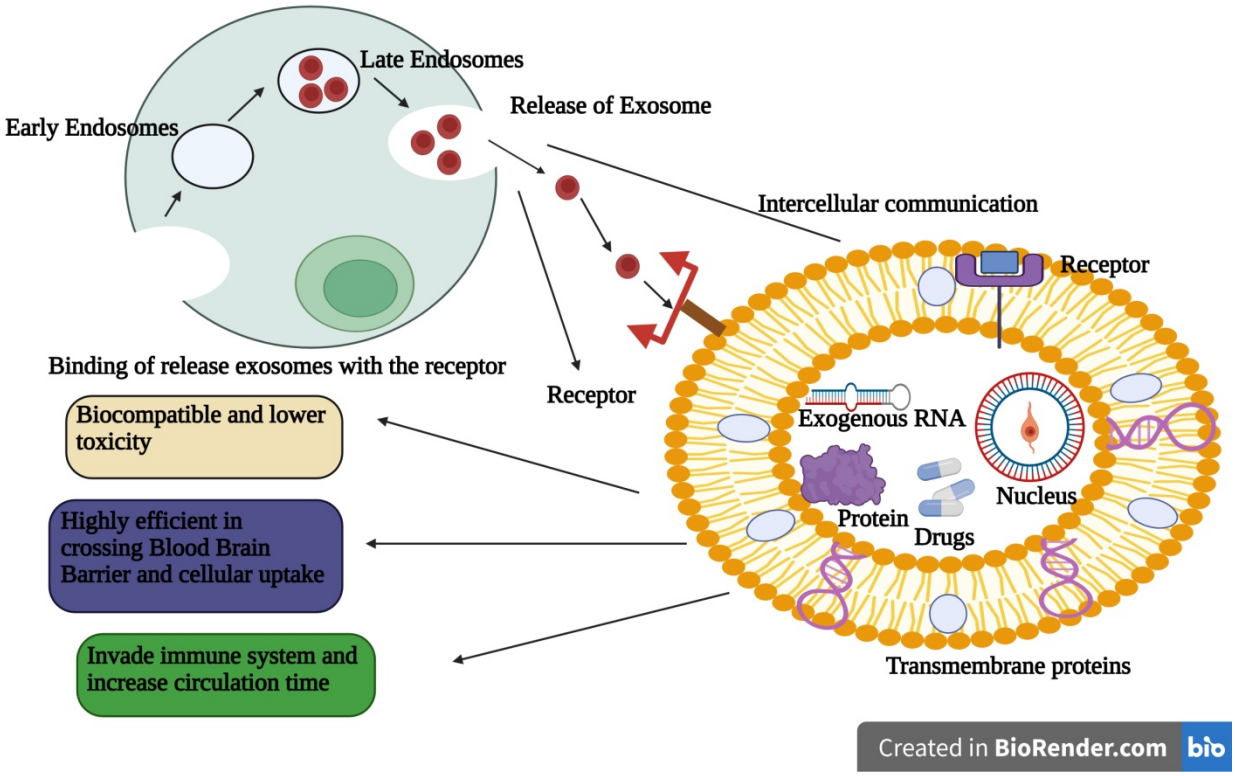

Fig. (1). Schematic representation of exosome biogenesis, release, and intercellular communication. Early and late endosomes form multivesicular bodies that release exosomes into the extracellular space. These vesicles bind to surface receptors on recipient cells, facilitating the delivery of therapeutic cargos such as RNA, proteins, and drugs. Their inherent biocompatibility, ability to evade immune clearance, and efficiency in crossing the blood–brain barrier make them promising vehicles for glioma therapy. "Created with BioRender.com". *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

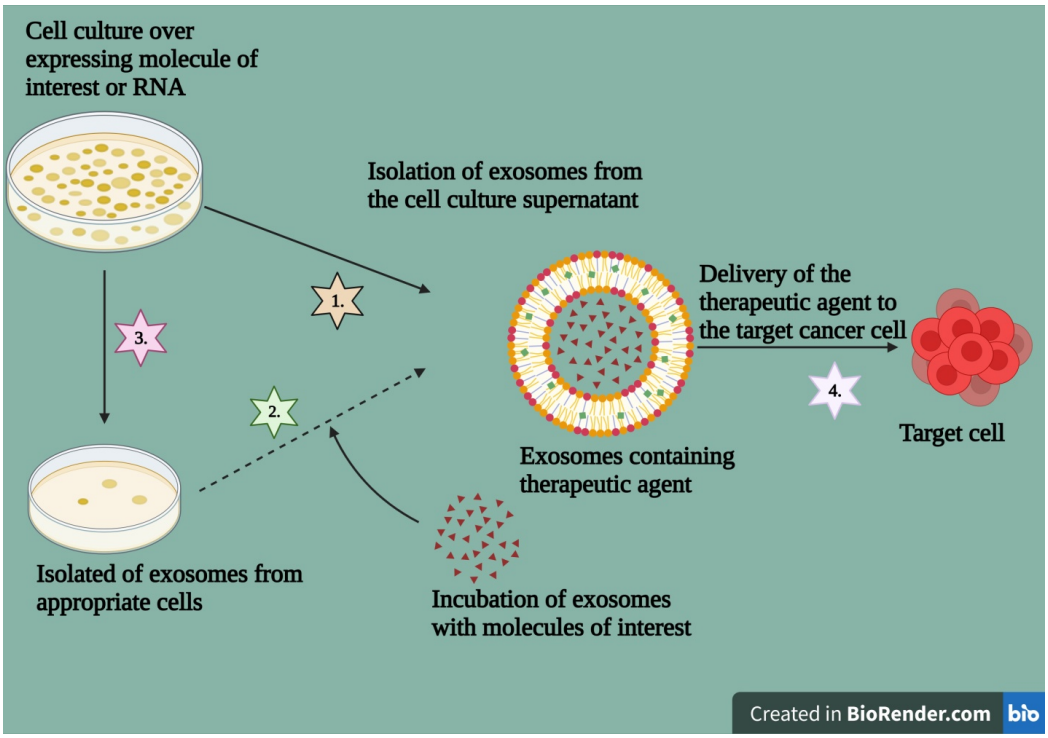

Fig. (2). Glioma therapy: workflow in exosome engineering and targeted delivery. Exosomes are purified from cultured donor cells, filled with therapeutic molecules (drugs, RNA), and then taken up by tumor cells and activated for targeted action. These engineered vesicles can cross the blood–brain barrier, increase drug accumulation in tumor tissue, and reduce systemic toxicity, offering great potential as biocompatible nanocarriers for glioma treatment. "Created with BioRender.com". *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

The efficiency of cargo loading varies considerably among different engineering approaches. Passive incubation generally exhibits low encapsulation efficiency, typically ranging from 5% to 15%, which limits its utility for therapeutic applications. In contrast, electroporation and sonication improve loading efficiency to approximately 20-45% and 25-55%, respectively; however, these methods may compromise exosomal membrane integrity and promote cargo aggregation. Donor cell-based loading strategies and microfluidic-assisted techniques have demonstrated superior performance, achieving loading efficiencies of approximately 40-70% while preserving vesicle stability and biological functionality. Consequently, these advanced approaches are increasingly recognized as promising platforms for the scalable production of engineered exosomes and the development of clinically translatable exosome-based therapeutics [89].

10.2. Structural Stability and Pharmacokinetic Behavior of Engineered Exosomes

Preserving the structural integrity of exosomes is essential for maintaining their biological functionality, biodistribution, and therapeutic efficacy. Intact exosomal membranes protect encapsulated cargo from degradation and facilitate efficient cellular uptake and intercellular communication. Loading approaches such as passive incubation and donor cell engineering generally preserve membrane architecture, surface protein composition, and colloidal stability. In contrast, more aggressive physical techniques, including electroporation and repeated freeze–thaw cycles, may induce membrane disruption, vesicle aggregation, and alterations in exosomal physicochemical properties. Microfluidic-based engineering platforms offer improved control over shear stress, particle size distribution, and processing conditions, thereby enhancing exosome stability and reducing batch-to-batch variability.

Pharmacokinetic studies have demonstrated that engineered exosomes exhibit significantly prolonged systemic circulation compared with free therapeutic agents. Depending on the formulation and route of administration, exosome-mediated delivery can extend the circulation half-life of therapeutic cargo from approximately 0.3-2 hours to 4-10 hours. This improvement is attributed to reduced hepatic clearance, enhanced protection of encapsulated therapeutics from enzymatic degradation, and favorable biodistribution profiles. Consequently, the extended circulation time increases therapeutic exposure, improves bioavailability, and enhances drug accumulation at target sites, thereby contributing to superior therapeutic outcomes [90].

10.3. Blood-brain Barrier Translocation and Tumor Targeting Efficiency

The ability of exosome-based systems to provide efficient delivery through the blood-brain barrier functions as their primary benefit. Native exosomes demonstrate approximately a 1.5-2 fold increase in brain penetration ability relative to free drugs, whereas surface-engineered exosomes achieve 3-7 fold increase in intracranial tumor accumulation through their receptor-mediated transcytosis process. The quantitative biodistribution analyses show that engineered exosomes can transport between 18% and 40% of the injected dose for every gram of tumor tissue, which represents a substantial improvement over traditional nanocarrier systems.

The use of exosome-based delivery systems decreases the liver and spleen off-target accumulation by 45% and 65%, which helps to improve tumor targeting while reducing systemic toxicity. The results of this study demonstrate that engineered exosomes represent the most effective nanocarriers for blood-brain barrier penetration among the current research studies. [91].

10.4. Functional Validation of Therapeutic Cargo in Glioma Models

Both the cellular and molecular effects of exosome-mediated delivery have been demonstrated in functional assays to be more effective. Exosome-based chemotherapeutic agents are shown to inhibit the viability of glioma cells by 40-75% in *in vitro* studies, and to have a significant impact on the clonogenic survival of cells. RNA-based cargo delivery results in strong gene silencing, as exemplified by 60% to 90% silencing of oncogenic targets like EGFR, STAT3, and KRAS.

Migration and invasion assays also show significant decreases (40-70%) in glioma cell motility and in expression of markers of the epithelial-mesenchymal transition and matrix metalloproteinases. Furthermore, the exosome delivery is able to effectively target glioma stem-like cells, where it inhibits the self-renewal capacity and downregulates the transcription factors associated with stemness. [92].

10.5. *In Vivo* Therapeutic Outcomes and Microenvironment Modulation

In vivo experiments in orthotopic models of gliomas show that engineered exosomes have a profound therapeutic effect, causing reductions in tumor volume of 50-80% and markedly increasing survival. The extent of drug accumulation within the tumour (3-6-fold) is directly related to the efficacy of treatment.

In addition to direct cytotoxic effects, exosome cargo can lead to changes in the tumor microenvironment through the induction of apoptosis, anti-angiogenesis, and immune activation. Researchers have documented reduced immunosuppressive cytokine levels and infiltration with increased cytotoxic T cells, suggesting a change in immune phenotype towards an anti-tumor immune response. The multiple levels of effect demonstrate the ability of engineered exosomes to function as therapeutic agents and as regulators of tumor biology [93].

10.6. Safety, Immunocompatibility, and Translational Considerations

Engineered exosomes provide safer treatment results because they show less harmful effects on the body and reduced risk of immune system responses because they originate from natural body processes. Preclinical research studies demonstrated that the treatment significantly decreased markers of cardiotoxicity, hepatotoxicity, and nephrotoxicity while producing only slight inflammatory reactions. Developing large-scale manufacturing operations,

establishing standardized production methods, and verifying long-term safety remain ongoing challenges. The clinical application of the technology faces obstacles because of three factors, which include batch variability, the different methods used for isolation, and the way regulatory authorities classify the technology. The process of successfully implementing exosome-based treatments in medical settings depends on solving these particular problems (Fig. **3**) [94].

11. MECHANISTIC AND TRANSLATIONAL IMPLICATIONS OF EXOSOME-MEDIATED SIGNALING IN GLIOMA

11.1. Exosome-mediated Communication as a Driver of Glioma Progression

The process of extracellular vesicle communication in glioma occurs through restricted spatial channels that enable the communication system to support local invasion and metabolic adaptation and immune modulation but not distant spread of the disease. Exosomes function as integrative signaling units that transfer regulatory RNAs, proteins, and lipids between tumor cells and the surrounding microenvironment, coordinating processes essential for tumor persistence. Glioma-derived exosomes exist as specialized vesicles whose surface systems enable invasive growth throughout the central nervous system, while their structure follows the blood-brain barrier and neural system design [95].

11.2. Functional Role of Exosomes in Tumor Progression and Microenvironment Remodeling

Exosomes originating from gliomas alter the tumor microenvironment by delivering oncogenic proteins, including EGFRvIII, non-coding RNAs, and signaling molecules that control cell growth, blood vessel formation, and mechanisms of immune system evasion. The vesicles lead to changes in nearby stromal and immune cells, which result in tumor-promoting activities that include increased blood vessel development and changes in the extracellular matrix and decreased anti-tumor immune system functions. Exosomes enable cells to develop resistance against therapy through their delivery of drug-resistance molecules, which direct cells to develop survival mechanisms. Hypoxia increases the exosome release process while it changes the composition of exosome cargo, which then heightens pro-invasive signaling and helps tumors adapt to situations of stress. The functions of exosomes establish them as main control elements for glioma development because they act as active components of tumor growth [96].

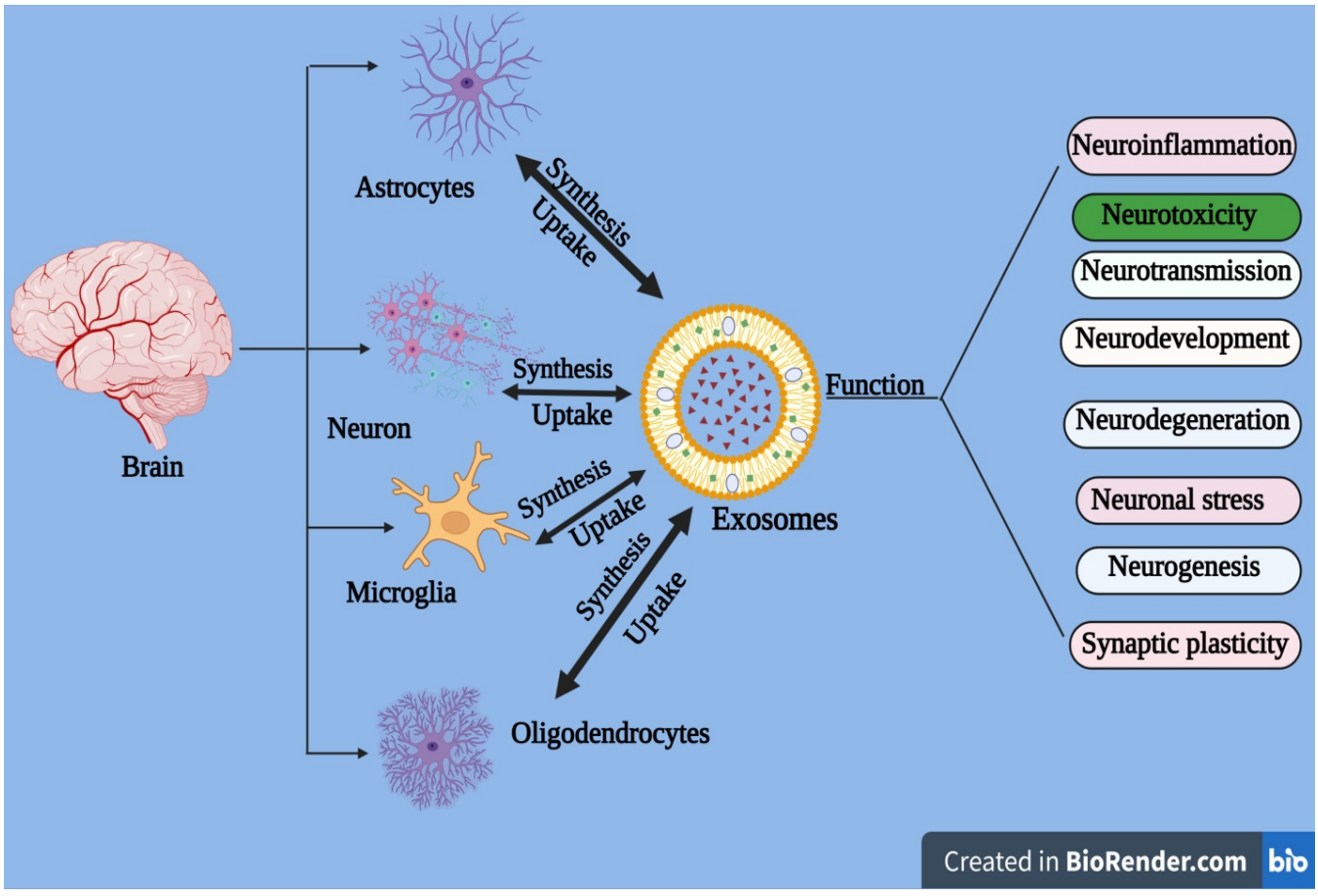

Fig. (3). Exosome-mediated communication among neural and glial cells in the glioma microenvironment. Neurons, astrocytes, microglia, and oligodendrocytes secrete and uptake exosomes that regulate key processes including neuroinflammation, neurotoxicity, synaptic plasticity, neurogenesis, and tumor progression. This bidirectional signaling influences glioma growth, immune evasion, and therapy resistance. "Created with BioRender.com". *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

11.3. Therapeutic Targeting of Exosome-mediated Pathways: Opportunities and Limitations

The development of exosome-based communication systems provides a complex but promising method for medical treatment. Research shows that methods that block vesicle formation, cargo transport, and cell uptake can stop cancer cells from spreading and their ability to adapt to different environments. Translation faces difficulties because researchers cannot distinguish between exosomes that come from tumors and those that exist in the human body, which creates problems for normal brain cell communication. The therapeutic value of exosomal cargo elements, which include specific miRNAs and proteins, remains unproven because their mechanisms require further testing. The proper solution to this problem needs to identify specific biological pathways that have been confirmed through testing instead of using general methods that stop all vesicle functions [97].

11.4. Diagnostic and Therapeutic Integration of Engineered Exosomes

In glioma research, exosomes serve as diagnostic markers and drug carriers. Their molecular cargo is a reflection of the tumor-specific alterations, allowing non-invasive monitoring of the progression of the disease. These products provide the primary therapeutic benefit of engineered delivery of drugs and genetic material across the blood–brain barrier.

Site-specific delivery of chemotherapeutics, siRNA, and miRNA is possible with engineered exosomes, resulting in improved distribution of the drugs throughout the tumor tissue and minimising the side effects on the entire body. The combined diagnostic and therapeutic function of this system creates a foundation for developing complete "theranostic" systems, yet existing clinical applications face obstacles due to difficulties in standard operating procedures, production capacity, and classification by regulatory bodies [98].

11.5. Translational Outlook: From Biological Insight to Clinical Application

The clinical application of exosome-based methods remains undeveloped despite scientists knowing the mechanisms that govern exosome functions. The most effective treatment methods combine precise cargo engineering with targeted delivery systems and established biological pathways. The future progress of this field requires standard manufacturing procedures to be created and researchers to develop methods that improve targeting accuracy and conduct thorough *in vivo* testing. The new treatment approach from tumor debulking to stopping invasion and intercellular contact will improve glioma control methods that work for extended periods [99].

12. TRANSLATIONAL CHALLENGES, COMPARATIVE PERFORMANCE, AND FUTURE CLINICAL INTEGRATION OF ENGINEERED EXOSOMES

12.1. Therapeutic Potential of Exosome-based Delivery Systems in Glioma

The use of exosome-based therapeutic approaches enables researchers to address two major challenges that exist in glioma treatment. Engineered exosomes successfully transmit therapeutic microRNAs and siRNAs, as well as chemotherapeutic agents, to their targets, resulting in better tumor control, restored drug effectiveness, and stopped glioma development in preclinical settings. Exosome delivery through exosome-mediated pathways enables the active regulation of three oncogenic signaling pathways, including STAT3, Wnt/β-catenin, and Notch signaling. Exosome-based delivery systems display cytotoxic properties together with their capacity to change resistant tumor characteristics, which demonstrates that these systems function as both delivery systems and tumor biological controllers [100].

12.2. Comparative Evaluation of Engineering Strategies: Efficiency, Targeting, and Safety

The study compares different methods of exosome engineering, which demonstrate distinct differences when evaluating their ability to deliver therapies. Passive loading methods enable vesicle preservation because they protect vesicle structure, but they struggle with maintaining their contents and delivering therapeutic materials. Active loading methods boost delivery efficiency because they use their full capacity, which can increase the risk of membrane disruptions that lead to aggregation problems and alter how cargo distributes throughout the system.

Donor cell-based engineering methods together with microfluidic-assisted loading methods enable better cargo retention, which ranges from 40 to 80 percent, while providing biological stability and functional delivery capabilities. Exosomes that have undergone surface modification achieve stronger targeting abilities, leading to 2-7 times greater tumor accumulation than unmodified vesicles. The research shows that methods that cause minimal interference preserve exosomal components and produce low immunogenicity, whereas methods that use physical power and hybrid techniques generate structural instability together with higher toxic risk. The research demonstrates that exosome-based therapeutics need three essential elements, which include effective delivery to target areas and safe operation for their development as medical treatment options [101].

12.3. Limitations in Clinical Translation and Biosensing Constraints

Although initial laboratory tests showed positive results, exosome-based technologies face multiple critical obstacles that prevent their successful use in actual medical practice. The main obstacle researchers encounter when trying to extract cancer-specific exosomes from biological samples results from the fact that these exosomes only make up less than 1 percent of all vesicles in the sample. Current detection methods that utilize existing monitoring techniques are limited because these approaches fail to deliver both accurate detection results and reliable performance measurements. The absence of standardized methods for isolating samples, together with differences in cargo composition and missing reliable measurement standards, creates research inconsistencies throughout various studies. The latest biosensing technologies are capable of high performance, but signal interference and biofouling can

hamper their performance, and they are not suitable for routine medical testing protocols because they require multiple tests to be performed simultaneously.

The existing constraints also dictate the need for improved analytical systems as well as standardized operating procedures that scientists must develop reliable systems to detect exosomes, track their development, and apply exosome systems for medical applications [102].

12.4. Manufacturing, Scalability, and Regulatory Barriers

Several issues, including the challenges of scale-up, reproducibility, and regulatory compliance, make the translation of engineered exosomes from the laboratory to the clinic difficult. Because of the combination of different donor cell sources, different culture conditions, and different isolation methods, there are factors that cause batch-to-batch variability that medical-grade therapeutics will not tolerate. The regulatory requirements for establishing complex engineering strategies that involve genetic modification and hybrid nanoparticle systems are strict and consider potential safety hazards. This lack of standardisation of regulatory guidelines for therapeutic exosome delivery further hampers clinical translation. The emerging field of microfluidic technologies, in combination with automated manufacturing platforms, offers solutions to improve process control and reproducibility and to scale up, but further testing is needed before they are adopted as a general practice [103].

12.5. Future Directions: Toward Clinically Viable Exosome-based Therapeutics

For future development of exosome-based therapies, organizations will need to merge their exact engineering approaches, their entire manufacturing processes, and their comprehensive validation protocols. The research focuses on three main areas, which include improving targeting specificity and developing methods to increase cargo loading efficiency while maintaining vesicle integrity and creating standardized protocols for large-scale production. The fusion of the properties of the hybrid exosome platforms and the theranostic systems with the properties of personalized exosome-based therapies opens novel possibilities for the creation of improved methods for the treatment of gliomas. For building strong clinical applications, two key components must be developed: comprehensive *in vivo* testing and safety studies, and meeting regulatory requirements [104].

Although engineered exosomes are a novel approach to glioma therapy, scientists will need to work together to address the current technological challenges for effective use in clinical applications [105].

CONCLUSION

Exosomes have emerged as a transformative platform in glioma research, offering new opportunities for both diagnosis and therapy. Their inherent ability to cross biological barriers, particularly the Blood–Brain Barrier (BBB), provides a significant advantage over conventional drug delivery systems. Owing to their small size and secretion by nearly all cell types, exosomes can efficiently transport a wide range of biomolecules, including microRNAs, long noncoding RNAs, proteins, and therapeutic agents, directly to tumor sites. In glioma, exosomes play critical roles in disease progression and therapeutic resistance while also serving as promising targets for treatment and non-invasive biomarkers for disease monitoring.

Exosomes have demonstrated considerable potential as delivery vehicles for therapeutic agents such as temozolomide, doxorubicin, and curcumin, as well as for the transfer of genetic materials capable of modulating tumor-associated signaling pathways. Furthermore, exosome-based liquid biopsies have the potential to revolutionize glioma diagnosis by enabling early detection and continuous monitoring of treatment response. Despite these promising advances, several challenges hinder their clinical translation, including difficulties associated with large-scale production, standardization, characterization, and safety validation. Additional interdisciplinary research integrating molecular biology, nanotechnology, and bioengineering is essential to overcome these limitations. Overall, exosome-mediated therapeutic strategies have the potential to transform glioma management by enabling personalized, targeted, and minimally invasive interventions, ultimately improving patient survival and quality of life**.**

FUTURE PERSPECTIVES

Exosome-based research is also promising in the field of glioma therapy, and it has the potential to grow in the future, with new innovations bound to break the current limitations. Improved exosome isolation, loading, and delivery accuracy could be perfected by means of the integration of the advanced technologies microfluidics, artificial intelligence, and biocurve analytics. The exosomes with personalized compositions based on patient-specific stem cells can potentially reduce immunity and make the therapeutic selectivity. In addition, linking exosomes and gene-editing technologies, such as CRISPR-Cas9 and RNA interference, provides a chance to silencer specifically oncogenic pathways. Exosome delivery in populations with hard-to-treat immunotherapies, such as checkpoint blockers or CAR-T cells, could also be used to increase anti-tumor immunity in the glioma microenvironment. Another key area is creating multifunctional exosomes that can perform imaging and treatment simultaneously (so-called theranostic vesicles), through which the efficiency of the treatment might be monitored in real time. New developments in biomanufacturing and standardized exosome purification systems will allow the production of clinically grade exosomes. Additionally, considering the hybrid systems that combine natural exosomes with synthetic nanomaterials could be more stable and allow regulating drug release patterns. The next round of clinical trials, with attention to safety, pharmacokinetics, and long-term effectiveness, will play a crucial role in enabling exosome-based strategies to move from labs to beds. Overall, the coming decade promises a lot of potentials on the exosome-based precision medicine, in which these naturally derived nanocarriers would emerge as one of the greatest tools of personalized glioma treatment and monitoring.

CHALLENGES

There are key challenges to successful translation of advanced exosome-based glioma therapies to the clinic. One of the current challenges is the large-scale production of exo-

somes with homogeneous quality and purity. Isolation methods used currently involve ultracentrifugation and precipitation, and yield mixed groups along with low recovery rates, causing problems with standardization of treatment. Drug loading is a challenge due to the fact that various methods such as electroporation and sonication can lead to potential damage to vesicles or reduce their biological activity. Targeting tumor tissues is critical, as researchers aim to maximize targeting with minimal off-target effects. Surface modification of glioma with ligands or peptides is highly complex due to tumor heterogeneity and dynamic changes in the tumor microenvironment. The complete evaluation of engineered exosomes demands testing for their immunogenic potential, clearance processes, and toxic effects. The absence of standardized procedures to assess drug quality, dosage, and storage stability creates ethical and regulatory obstacles for drug implementation. The therapeutic response of exosomes varies among different cell sources because their biological variability results in different therapeutic properties, which makes it difficult to develop standard treatment procedures. Multidisciplinary collaboration through nanotechnology, molecular pharmacology, and clinical oncology shows the required approach to solve the challenges that need to be resolved. The establishment of global standards for exosome characterization and exosome clinical testing needs to proceed through international guidelines development.

AUTHORS' CONTRIBUTIONS

S.P: Conceptualization; literature search; data collection; data interpretation; preparation of original draft; preparation of figures and tables; formatting and organization of the manuscript. S.Y: Conceptualization; supervision; critical revision of the manuscript for intellectual content; validation of scientific accuracy; final editing and approval of the manuscript. Both authors reviewed and approved the final version of the article.

LIST OF ABBREVIATIONS

AFM	=	Atomic Force Microscopy
BBB	=	Blood–Brain Barrier
BBTB	=	Blood–Brain Tumor Barrier
BCSFB	=	Blood–Cerebrospinal Fluid Barrier
BIGH3	=	Transforming Growth Factor-Beta-Induced Gene-Human Protein
BMSC	=	Bone Marrow-Derived Mesenchymal Stem Cell
CA	=	Chromosomal Aberration (used in molecular context)
CADM1	=	Cell Adhesion Molecule 1
CAR-T	=	Chimeric Antigen Receptor T-Cells
CD	=	Cluster of Differentiation
CDKN2A/B	=	Cyclin-Dependent Kinase Inhibitor 2A/2B
CIC	=	Capicua Transcriptional Repressor
CRISPR	=	Clustered Regularly Interspaced Short Palindromic Repeats
CSF	=	Cerebrospinal Fluid
CT	=	Computed Tomography
CTFE	=	Chlorotrifluoroethylene (used in plasmonic sensor context)
DNA	=	Deoxyribonucleic Acid
EGFR	=	Epidermal Growth Factor Receptor
EGFRvIII	=	Epidermal Growth Factor Receptor Variant III
EMT	=	Epithelial–Mesenchymal Transition
ESCRT	=	Endosomal Sorting Complex Required for Transport
EV	=	Extracellular Vesicle
FUBP1	=	Far Upstream Element Binding Protein 1
G-CIMP	=	Glioma-CpG Island Methylator Phenotype
GBM	=	Glioblastoma Multiforme
GSC	=	Glioma Stem Cell
HGG	=	High-Grade Glioma
Hsp	=	Heat Shock Protein
IDH	=	Isocitrate Dehydrogenase
ILV	=	Intraluminal Vesicle
ISEV	=	International Society for Extracellular Vesicles
L1CAM	=	L1 Cell Adhesion Molecule
LGG	=	Low-Grade Glioma
lncRNA	=	Long Non-Coding RNA
LSPR	=	Localized Surface Plasmon Resonance
MCT1	=	Monocarboxylate Transporter 1
MDSC	=	Myeloid-Derived Suppressor Cell
MGMT	=	O-6-Methylguanine-DNA Methyltransferase
miRNA/miR	=	MicroRNA
MISEV	=	Minimal Information for Studies of Extracellular Vesicles
mRNA	=	Messenger RNA
MSC	=	Mesenchymal Stem Cell
MVB	=	Multivesicular Body
NF1	=	Neurofibromin 1
NOTCH	=	Neurogenic Locus Notch Homolog
OS	=	Overall Survival
PAR1	=	Protease-Activated Receptor 1
PC	=	Prostate Cancer (PC-3 line used)

PDGFRA = Platelet-Derived Growth Factor Receptor Alpha

PLGA = Poly(lactic-co-glycolic acid)

PTEN = Phosphatase and Tensin Homolog

RNA = Ribonucleic Acid

RTK = Receptor Tyrosine Kinase

siRNA = Small Interfering RNA

SOX2 = SRY-Box Transcription Factor 2

SPION = Superparamagnetic Iron Oxide Nanoparticle

STAT3 = Signal Transducer and Activator of Transcription 3

TCEAL7 = Transcription Elongation Factor A-Like 7

TCF12 = Transcription Factor 12

TEM = Transmission Electron Microscopy (implied in EV characterization)

TERT = Telomerase Reverse Transcriptase

TGF-β = Transforming Growth Factor Beta

TME = Tumor Microenvironment

TMZ = Temozolomide

TNBC = Triple Negative Breast Cancer (mentioned in comparative MSC context)

Tsg101 = Tumor Susceptibility Gene 101

Vps4 = Vacuolar Protein Sorting-Associated Protein 4

WHO = World Health Organisation

ETHICAL STATEMENT

There are no human subjects in this article, and informed consent is not applicable.

AVAILABILITY OF DATA AND MATERIALS

In this article, we conducted a narrative review and did not generate new data sets. All data referenced in this study are taken from previously published sources and are thoroughly referenced in this manuscript. No further datasets were created or analyzed; therefore, data sharing is not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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BioRender was used under a license permitting publication in academic journals. Grammarly was used solely for language editing, grammar correction, and improvement of manuscript readability.

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