Human Mesenchymal Stem Cell-Derived Small Extracellular Vesicles Post-Loaded with Curcumin Synergistically Inhibit Migration in U87 Glioblastoma Cells

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ABSTRACT:

Glioblastoma (GBM) is an aggressive brain tumor with limited therapeutic options. Recent advances in regenerative medicine have highlighted the therapeutic potential of small Extracellular Vesicles (EVs) from mesenchymal stem cells, which can modify the tumor microenvironment and deliver bioactive compounds that cross the Blood-Brain-Barrier. On the other hand, the recent therapies for cancers are targeting the biotic potential of various phytochemicals, so the adverse side effects of using synthetic drugs could be avoided. Curcumin is one such phytochemical with known anti-glioma, anti-proliferative, and anti-migratory properties, which is hindered by poor solubility and bioavailability.

This study aimed to develop and evaluate a curcumin-loaded EV formulation using EVs derived from human Umbilical Cord Mesenchymal Stem Cells (hUCMSCs) as a delivery vehicle for glioblastoma therapy. EVs were isolated under serum-free conditions and characterized by nanoparticle tracking analysis and zeta potential measurements. Curcumin was solubilized using a natural deep eutectic solvent (NADES - choline chloride: glycerol, 2:1) to increase its bioavailability and was loaded into EVs via a microwave-assisted method. MTT assay reveals a dose-dependent reduction in viability of U87 cells, with 5 µL of curcumin-NADES reducing viability of U-87 to 57.95%. Furthermore, scratch assay results showed that curcumin-loaded EVs achieved the strongest inhibition of cell migration, with only 5.37% wound closure—indicating a potent anti-migratory effect—compared to curcumin or EVs alone.

Our findings demonstrate that the combination of hUCMSCs and curcumin significantly reduces GBM cell growth, triggers apoptosis, and decreases cell migration when compared to individual treatments. The synergistic effect could be linked to the regulation of major signalling pathways involved in cell survival and metastasis. In conclusion, the combination of umbilical cord MSC-derived EVs with curcumin appears to be a viable therapeutic method for glioblastoma treatment

Key words: Glioblastoma, U-87, Curcumin, NADES, EVs, hUCMSCs, MTT assay, Scratch assay

INTRODUCTION:

Glioblastoma (GBM), previously termed glioblastoma multiforme, is the most aggressive and lethal primary brain tumor in adults, accounting for nearly 15% of all brain neoplasms [1]. Originating from astrocytic glial cells, GBM is classified as a WHO grade IV tumor and is characterized by rapid proliferation, diffuse infiltration, and marked resistance to therapy. With a global incidence of 3.19 cases per 100,000 population and a median age of diagnosis at 64 years, GBM imposes a considerable health burden [2]. Clinical manifestations such as seizures, persistent headaches, cognitive decline, and progressive neurological dysfunction underscore the tumor's aggressive nature [3]. Despite centuries of study, beginning with early observations by Berns (1800) and Abernety (1804), and detailed histomorphological descriptions by Virchow (1865) [4], the etiology remains largely idiopathic, with only a few known risk factors such as neurofibromatosis and prior cranial irradiation [5].

Current standard-of-care for GBM comprises maximal surgical resection followed by concurrent chemoradiotherapy, typically with temozolomide, which often fails due to resistance mechanisms such as MGMT promoter methylation. The tumor's highly infiltrative nature hinders complete surgical excision, while microscopic extensions beyond the resection margins are often undetectable on imaging [6]. Recurrence rates exceed 70% within a year, and the five-year survival rate is less than 5% [7]. Contributing factors to this grim prognosis include limited chemotherapy efficacy, radio-resistance, and especially, the Blood-Brain-Barrier (BBB), which impedes the penetration of most therapeutic agents [8]. Furthermore, the tumor's molecular heterogeneity, rapid mutational adaptability, and immunosuppressive microenvironment—characterized by glioma stem cells (GSCs), tumor-associated macrophages (TAMs), and immune checkpoint upregulation (e.g., PD-1/PD-L1)—pose significant obstacles to therapeutic success [9,10].

Given the limitations of conventional synthetic nanoparticle-based delivery systems—such as immunogenicity, requirement for immunosuppression, and inability to cross the BBB—there is a critical need for biologically compatible, effective drug delivery alternatives. In this context, EVs derived from human umbilical cord mesenchymal stem cells (hUCMSCs) were chosen for their high yield, ethical sourcing, and tumor-tropic properties, making them ideal nanocarriers for this application. These natural extracellular vesicles, ranging from 30–200 nm, can cross the BBB and offer high biocompatibility, low immunogenicity, and intrinsic anti-

tumor properties [11,12]. Their non-invasive sourcing and high proliferative yield from umbilical cord MSCs further enhance their clinical viability [13].

EVs possess a unique capacity to encapsulate and deliver bioactive molecules, including phytochemicals with known anti-cancer activity. One such candidate is curcumin, a polyphenolic compound extracted from *Curcuma longa*, renowned for its multi-targeted anti-tumor effects, including induction of apoptosis, inhibition of angiogenesis, and modulation of oncogenic signalling pathways [14,15]. However, curcumin's clinical application has been hindered by poor solubility, low systemic bioavailability, and rapid metabolic degradation [16]. Encapsulating curcumin within hUCMSC-derived EVs offers a synergistic solution—leveraging the EVs' tumor-targeting ability and biological compatibility to enhance curcumin's therapeutic potential [17,18].

This study addresses a critical gap in GBM therapy by exploring the use of hUCMSC-derived EVs as a natural, biocompatible delivery vehicle for curcumin. While synthetic nanoparticles continue to face immune system rejection and limited brain permeability, EVs offer a next-generation solution for targeted drug delivery with minimal systemic toxicity. By harnessing the inherent anti-cancer properties of both hUCMSC-ex and curcumin, this research aims to investigate their combined therapeutic efficacy in inhibiting glioblastoma progression.

MATERIALS AND METHODS:

Cell Lines and Materials

The human glioblastoma cell line U87 was utilized throughout the study. Human umbilical cord mesenchymal stem cells (hUCMSCs) were isolated and cultured at AcaDiCell Innovations International Pvt. Ltd., Chennai. Curcumin (purity ≥ 95%) was procured from Sigma-Aldrich. All culture media, supplements, and reagents were of analytical grade and used as received.

Isolation and Culture of hUCMSCs

Fresh human umbilical cords were obtained from C-section deliveries at Bhaarath Medical College and Hospital, Chennai, to prevent vaginal contamination. Tissues were promptly drained of cord blood, placed in antibiotic-buffered transfer medium, and transported to the laboratory for same-day processing.

In a Class 10,000 cleanroom using a biosafety cabinet, the tissue was minced into small explants and placed in 25 mm Petri dishes containing 5 mL of complete culture medium (α-MEM supplemented with 20% FBS). Explants were spatially separated to avoid contact. Plates were incubated at 37°C in a humidified 5% CO_2 incubator. This explant-based method has been widely used for efficient isolation of mesenchymal stem cells from umbilical cords [22].

Media changes were performed as follows: the first on day 3 to remove contaminants, followed by changes on days 10, 13, 17, and 20. By day 22, the outgrowth of mesenchymal stem cells from the explants formed a monolayer. The primary culture was passaged by gently removing

tissue remnants, washing with PBS, followed by trypsinization and centrifugation. The resulting P1 cells were seeded into fresh dishes. Upon reaching 80–90% confluency, cells were further expanded into T-75 flasks (P2 stage).

Once the P2 flasks reached 80-90% confluency, the complete medium was replaced with serum-free basal α-MEM to induce cellular stress and stimulate EV release [23]. After 24 hours, the conditioned medium was collected and centrifuged at low speed to remove debris. The supernatant containing EVs was retained for isolation.

EV Isolation and Characterization

EVs were isolated from the conditioned medium using differential ultracentrifugation, a widely accepted method for EV purification [19]. The process involved sequential centrifugation steps to remove cells and debris, followed by ultracentrifugation at 100,000×g for 1 hour to pellet the EVs. The resulting pellet was resuspended in 200 µL phosphate-buffered saline (PBS) and cryopreserved for further use. A portion of the sample was characterized via nanoparticle tracking analysis (NTA) and zeta potential measurements to confirm the size distribution and surface charge properties of the EV [20].

Glioblastoma Cell Culture

U87 glioblastoma cells were cultured in high-glucose α-MEM supplemented with 10% fetal bovine serum and 1% Gentamicin. Cells were maintained in a 37°C incubator with 5% CO_2 and passaged upon reaching approximately 80% confluency.

MTT Cytotoxicity Assay

The cytotoxic potential of the curcumin-NADES formulation was evaluated using the MTT assay, based on the method of Mosmann [26]. U87 cells were seeded into 96-well plates at a density of 5,000 cells per well in 100 µL of complete medium. After overnight incubation, cells were treated with curcumin-NADES at various volumes (1, 3, 5, 7, and 9 µL). Control wells included a blank (medium only) and a positive control (cells without drug).

After 24 hours, 10 µL of MTT reagent was added to each well. Plates were incubated in the dark for 2 hours. Then, 100 µL of solubilization solution was added to each well, followed by overnight incubation to ensure complete dissolution of formazan crystals. Absorbance was read at 570 nm using an ELISA plate reader.

Cell viability was calculated as:

Cell Survival Rate (%) = OD Sample – OD Blank / OD Control – OD Blank x 100%

All treatments were performed in triplicate, and results were reported as mean ± standard deviation.

Curcumin Loading into EVs

A natural deep eutectic solvent (NADES) composed of choline chloride and glycerol (2:1 molar ratio) was prepared by heating at 70–100°C to obtain a clear solution. Purified curcumin powder (750 µg) was dissolved in 5 mL of NADES, followed by vortexing and mild heating (35°C), and filtered through a 0.2 µm filter to ensure sterility. This NADES formulation method has proven effective in solubilizing poorly water-soluble bioactive compounds such as curcumin [25].

The curcumin-NADES solution (100 µL) was mixed with 100 µL of EV suspension in a cryovial. This mixture was exposed to microwave radiation (2.45 GHz) for 10 seconds, ensuring the internal temperature did not exceed 40°C. The vial was incubated at room temperature for 10 minutes to allow EV membrane resealing. Microwave-assisted loading is an emerging technique shown to improve drug entrapment efficiency and stability [24]. The resulting curcumin-loaded EVs were applied immediately to U87 cultures for downstream assays.

Scratch Assay and Experimental Design

The wound healing (scratch) assay was used to evaluate the migration-inhibitory potential of curcumin, EVs, and their combination on U87 glioblastoma cells. Cells were seeded in a 12-well tissue culture plate and grown in complete α-MEM medium until approximately 80–90% confluence.

A uniform scratch was made across the center of each well using a sterile 200 µL pipette tip. Detached cells were removed by gently washing with phosphate-buffered saline (PBS), and the following treatments were added to each well:

- **Positive control**: complete medium
- **Negative control**: serum-free basal medium
- **EV treatment**: 5 µL of EV suspension in basal medium
- **Curcumin-NADES treatment**: 5 µL of curcumin solution in basal medium
- **Curcumin-loaded EV treatment**: 10 µL of combined EV-curcumin formulation in basal medium

Plates were incubated at 37°C in a humidified 5% CO_2 incubator for 24 hours. Phase-contrast microscopic images of each scratch were taken immediately after treatment (0 h) and again after 24 hours using an Olympus Inverted Microscope. To ensure reproducibility, three fixed regions per well (top, middle, bottom) were imaged.

Images were analysed using **ImageJ software** to measure the remaining wound area [21]. Wound closure percentage was calculated using the formula:

% of Wound Closure =

{Initial Scratch Wound – Scratch wound at time x / Initial Scratch Wound} X 100 %

RESULTS

Characterization of hUCMSC-Derived EVs

EVs isolated from the conditioned medium of human umbilical cord mesenchymal stem cells (hUCMSCs) were characterized using nanoparticle tracking analysis (NTA) and zeta potential measurements via the ZetaView® platform (Particle Metrix, Germany).

Nanoparticle Tracking Analysis (NTA): The EV population exhibited a heterogeneous size distribution with a mean diameter of 140.0 nm, median (X50) size of 126.0 nm, and a standard deviation of 80.2 nm. The particle concentration was approximately 3.7×10^7 particles/mL, falling within the expected range for MSC-derived EVs (Figure 1A).

Zeta Potential: The zeta potential of the EV suspension was measured at -1.88 ± 1.16 mV, which is lower than the commonly observed range of −10 to −30 mV for colloidally stable EVs. This reduced surface charge is likely attributable to several factors:

1. Ultracentrifugation, the isolation method used, is known to alter vesicle morphology and co-pellet protein aggregates, potentially lowering the apparent zeta potential [27].
2. The use of serum-free medium eliminates stabilizing serum proteins, which normally form a protein corona that influences surface charge and vesicle dispersion [30, 32].
3. Phosphate-buffered saline (PBS), used for storage, is a high-ionic-strength buffer that compresses the electrical double layer and diminishes electrostatic repulsion, reducing colloidal stability [29, 33].

Despite the lower zeta potential, the EVs remained visually stable and were within the expected size range, supporting their successful isolation and usability for downstream applications.

Curcumin Loading into EVs

Direct quantification of curcumin encapsulation was not performed in this study due to technical constraints. However, curcumin loading was carried out via a microwave-assisted method after solubilization in a natural deep eutectic solvent (NADES). Literature supports this approach for enhancing encapsulation efficiency and vesicle compatibility [24,25].

Visual assessment indicated good miscibility and physical integrity of the EVs post-loading. Future studies will involve UV-Vis or fluorescence-based quantification to confirm drug entrapment and efficiency. [31]

Curcumin-Induced Cytotoxicity in U87 Cells - MTT Assay

The cytotoxicity of curcumin-NADES was assessed using the MTT assay in U87 cells. A dose-dependent decrease in cell viability was observed with increasing volumes of curcumin solution. After 24 hours of treatment:

- **1 µL** curcumin: **87.96%** survival
- **3 µL**: **72.55%**
- **5 µL**: **57.95%**
- **7 µL**: **43.04%**
- **9 µL**: **30.68%**

These results confirm curcumin's cytotoxic potential at escalating doses (Table 1).

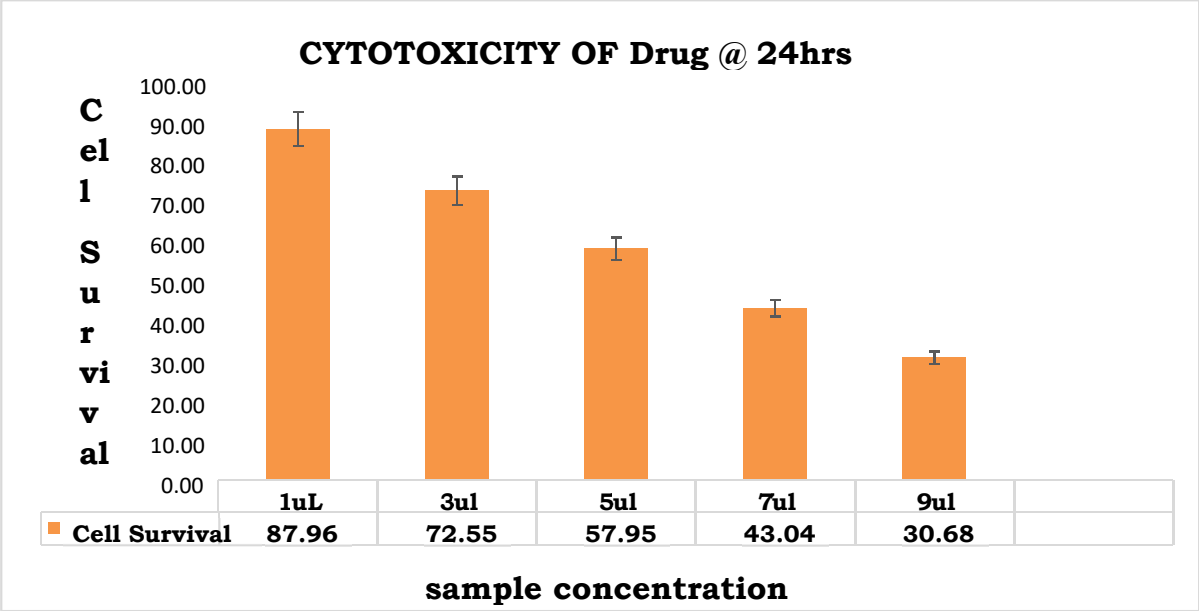


Figure 1

Table 1. U87 Cell Viability (%) After 24 h Treatment with Curcumin-NADES

Sample Volume (µL)	Cell Viability (%)
1	87.96
3	72.55
5	57.95
7	43.04
9	30.68

Inhibition of U87 Cell Migration - Scratch Assay

The anti-migratory effects of various treatments were assessed using a scratch assay. Cells were treated with:

- **EVs (5 µL)**
- **Curcumin-NADES (5 µL)**
- **Curcumin-loaded EVs (10 µL)**
- **Positive control** (complete medium)
- **Negative control** (serum-free medium)

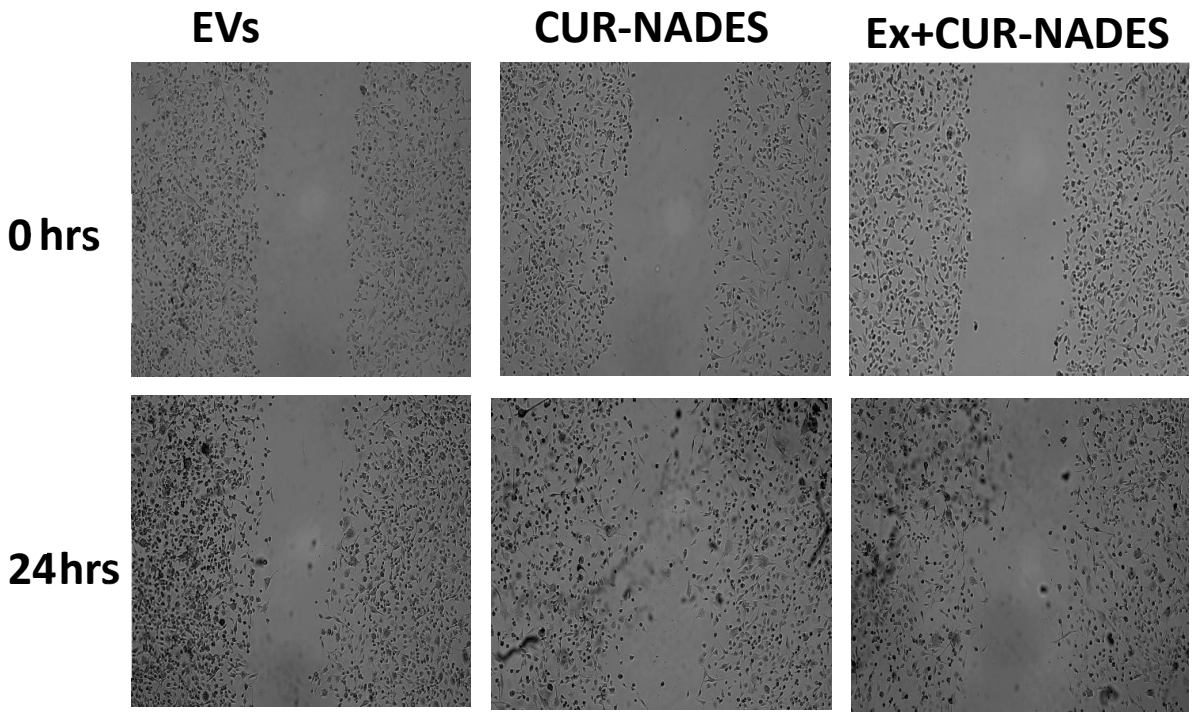
After 24 hours, wound closure percentages were:

- **Positive control**: 27.57%
- **EVs only**: 16.49%
- **Curcumin-NADES**: 12.99%
- **Curcumin-loaded EV**: **5.37%**
- **Negative control**: 8.90%

The lowest migration was observed in the **curcumin-loaded EV group**, even less than the negative control, indicating **synergistic inhibition of glioblastoma cell migration** (Table 2).

Table 2. Scratch Assay Wound Closure (%) After 24 h

Treatment	**Wound Closure (%)**
Positive Control	27.57
Negative Control	8.90
EVs Only	16.49
Curcumin-NADES	12.99
EV + Curcumin-NADES	**5.37**



DISCUSSION

This study presents a successful demonstration of using hUCMSC-derived EVs as a nano-carrier platform for curcumin delivery in glioblastoma treatment. EVs were isolated under serum-free conditions via ultracentrifugation and characterized by nanoparticle tracking analysis (NTA) and zeta potential measurements. The NTA confirmed the vesicles had a mean diameter of 140 nm and a median of 126 nm, well within the expected range for EVs. However, the measured zeta potential (−1.88 ± 1.16 mV) was lower than the conventional stability range (−10 mV to −30 mV), indicating reduced colloidal stability. Such low values are not uncommon and have been attributed to factors such as ultracentrifugation, which may cause vesicle aggregation [27, 28], storage in high-ionic-strength phosphate-buffered saline (PBS) [29], and the absence of serum proteins that otherwise form stabilizing coronas around EVs [30, 32]. Despite this, visual and size stability remained intact, validating their structural integrity.

To encapsulate curcumin into these vesicles, a microwave-assisted loading method was employed following solubilization in a natural deep eutectic solvent (NADES). Although drug loading efficiency was not quantified due to technical limitations, the biological outcomes suggest successful incorporation. This method is supported by literature for its practicality and effectiveness in increasing solubility and entrapment efficiency of hydrophobic compounds [24, 25].

The biological evaluation of this formulation was conducted through MTT and scratch assays in U87 glioblastoma cells. The MTT assay revealed a clear dose-dependent cytotoxicity of curcumin over the U87 cells; cell viability decreased from 87.96% at 1 µL, to 72.55% (3 µL), 57.95% (5 µL), 43.04% (7 µL), and 30.68% at 9 µL. These findings are consistent with earlier studies reporting that curcumin suppresses fascin expression—a key cytoskeletal protein

promoting migration and invasion [34, 35], downregulates PI3K/AKT/mTOR signalling while restoring PTEN tumor suppressor levels [36], and triggers apoptosis through ROS-mediated oxidative stress [37].

The scratch assay further demonstrated that the combination of curcumin and EVs resulted in the most pronounced inhibition of cell migration, with only 5.37% wound closure compared to 16.49% for EVs alone, 12.99% for curcumin alone, and 8.90% in the negative control. These results point toward a synergistic effect between the exosomal carrier and curcumin payload. The enhanced anti-migratory response may be attributed to the ability of EVs to facilitate targeted cellular uptake and sustained intracellular delivery.

hUCMSC-derived EVs were specifically chosen due to their well-documented tumor-tropic nature, immune invisibility, and ability to cross the Blood-Brain-Barrier (BBB) [38, 39]. These vesicles have shown therapeutic potential in modulating glioma pathways, including through miRNAs that suppress Wnt/β-catenin signaling [40], and in delivering anti-angiogenic and apoptotic cues to glioblastoma cells [41]. By integrating a bioavailable form of curcumin into this delivery system, our study bridges a significant translational gap in EV-based phytochemical therapy for neuro-oncology.

Although direct quantification of drug loading and molecular pathway activation was not performed, the robust phenotypic outcomes affirm the potential of this approach. The methodology used here offers a practical, scalable, and biologically relevant solution that avoids the complexities of synthetic nanocarriers. This makes it an attractive avenue for developing cost-effective and patient-friendly glioblastoma therapies. The synergistic effect stems from EVs enhancing curcumin's intracellular delivery, potentially downregulating PI3K/AKT signaling or inducing ROS-mediated apoptosis [36, 37].

In summary, this study establishes the curcumin-loaded hUCMSC-derived EV system as a promising drug delivery strategy capable of suppressing glioblastoma proliferation and migration. This platform could extend to other cancers or neurodegenerative diseases, leveraging EVs' BBB-crossing ability. However, clinical translation will require standardized EV production and regulatory approval.

LIMITATIONS

This study offers meaningful insight into the EV-mediated delivery of curcumin for glioblastoma treatment. However, several limitations should be acknowledged for transparency and to guide future research directions.

First, direct quantification of curcumin encapsulation into EVs was not performed. While this limits precise confirmation of loading efficiency, the robust anti-migratory effect observed in the scratch assay serves as strong functional evidence of successful curcumin incorporation. Future studies should employ quantitative methods such as UV-Vis spectrophotometry or high-

performance liquid chromatography (HPLC), which are widely used in similar research for confirming drug loading in extracellular vesicles [42].

Second, more refined techniques for EV isolation—such as size exclusion chromatography (SEC) and the use of protein-enriched or colloidally stabilizing buffers—were not adopted due to technical and financial limitations. These approaches have been shown to improve vesicle purity, minimize aggregation, and enhance colloidal stability [43, 44]. Their absence may partly explain the relatively low zeta potential observed in this study.

Third, the study did not incorporate in vivo validation or track long-term therapeutic effects. Although the in vitro results are promising, in vivo animal model studies will be essential to confirm the BBB penetration, pharmacokinetics, and systemic biocompatibility of the formulation.

Lastly, limited access to advanced instrumentation restricted the ability to confirm cellular uptake and mechanistic pathways at the molecular level. Techniques such as confocal microscopy, flow cytometry, or Western blotting are commonly employed in EV studies to validate internalization, receptor interactions, and downstream signalling [45].

Despite these limitations, the current study presents a robust proof-of-concept for curcumin delivery via hUCMSC-derived EVs, establishing a promising platform for further translational research in glioblastoma therapeutics.

CONCLUSION

This study presents a promising, accessible strategy for glioblastoma treatment by harnessing the natural delivery potential of hUCMSC-derived EVs to encapsulate and deliver curcumin. EVs were successfully isolated under serum-free conditions and characterized. Curcumin loading was achieved through a microwave-assisted method using a natural deep eutectic solvent system.

Functionally, the curcumin-loaded EVs demonstrated superior anti-migratory and cytotoxic effects on U87 glioblastoma cells compared to curcumin or EVs alone. These outcomes support the hypothesis that delivery through EVs enhances curcumin’s bioactivity, potentially overcoming solubility and delivery barriers commonly associated with phytochemical therapeutics.

While certain technical limitations remain, this work establishes a robust proof-of-concept for EV-based curcumin delivery in neuro-oncology, with potential for combination with immunotherapy or radiotherapy to enhance treatment outcomes. The approach is biologically relevant, ethically sourced, and adaptable to translational applications. Future studies integrating molecular pathway analysis, in vivo validation, and formulation optimization will be essential to advance this system toward clinical relevance.

REFERENCES:

1. Ostrom, Q. T., Bauchet, L., Davis, F. G., Deltour, I., Fisher, J. L., Langer, C. E., ... & Barnholtz-Sloan, J. S. (2014). The epidemiology of glioma in adults: A "state of the science" review. *Neuro-Oncology, 16*(7), 896–913. https://doi.org/10.1093/neuonc/nou087

2. Thakkar, J. P., Dolecek, T. A., Horbinski, C., Ostrom, Q. T., Lightner, D. D., Barnholtz-Sloan, J. S., & Villano, J. L. (2014). Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiology, Biomarkers & Prevention, 23*(10), 1985–1996. https://doi.org/10.1158/1055-9965.EPI-14-0275

3. Ellor, S. V., Pagano-Young, T. A., & Avgeropoulos, N. G. (2014). Glioblastoma: Background, standard treatment paradigms, and supportive care considerations. *Journal of Neurological Sciences, 347*(1–2), 8–19. https://doi.org/10.1111/jlme.12133

4. Virchow, R. (1865). *Die krankhaften Geschwülste* (Vol. 1). August Hirschwald. http://resource.nlm.nih.gov/62231840R

5. Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., ... & Ellison, D. W. (2016). The 2016 World Health Organization classification of tumors of the central nervous system: A summary. *Acta Neuropathologica, 131*(6), 803–820. https://doi.org/10.1007/s00401-016-1545-1

6. Michael Weller, Timothy Cloughesy, James R. Perry, Wolfgang Wick, Standards of care for treatment of recurrent glioblastoma—are we there yet?, Neuro-Oncology, Volume 15, Issue 1, January 2013, Pages 4–27, https://doi.org/10.1093/neuonc/nos273

7. Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J. B., ... & Mirimanoff, R. O. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New England Journal of Medicine, 352*(10), 987–996. https://doi.org/10.1056/NEJMoa043330

8. Starting points for the development of new targeted therapies for glioblastoma multiformehttps://doi.org/10.1016/j.tranon.2024.102187

9. Rong, L., Li, N. & Zhang, Z. Emerging therapies for glioblastoma: current state and future directions. *J Exp Clin Cancer Res* **41**, 142 (2022). https://doi.org/10.1186/s13046-022-02349-7

10. Hambardzumyan, D., Gutmann, D. H., & Kettenmann, H. (2016). The role of microenvironmental cells in glioblastoma. *Nature Reviews Cancer, 16*, 482–495. https://doi.org/10.1038/nrc.2016.60

11. Kalluri, R., & LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science, 367*(6478), eaau6977. https://doi.org/10.1126/science.aau6977

12. Doyle, L. M., & Wang, M. Z. (2019). Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells, 8*(7), 727. https://doi.org/10.3390/cells8070727

13. Reza-Zaldivar, E. E., Hernández-Sapiéns, M. A., Gutiérrez-Mercado, Y. K., Sandoval-Ávila, S., Gómez-Pinedo, U., Márquez-Aguirre, A. L., ... & Márquez-Aguirre, A. L. (2018). Mesenchymal stem cell-derived exosomes promote neurogenesis and cognitive function recovery in a mouse model of Alzheimer's disease. *Stem Cell Research & Therapy, 9*(1), 1–15. DOI: 10.4103/1673-5374.255978

14. Aggarwal, B. B., Sundaram, C., Malani, N., & Ichikawa, H. (2007). Curcumin: The Indian solid gold. *Advances in Experimental Medicine and Biology, 595*, 1–75. https://doi.org/10.1007/978-0-387-46401-5_1

15. Shehzad, A., Wahid, F., & Lee, Y. S. (2013). Curcumin in cancer chemoprevention: Molecular targets, pharmacokinetics, bioavailability, and clinical trials. *BioFactors, 39*(1), 20–28. https://doi.org/10.1002/biof.1041

16. Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics, 4*(6), 807–818. DOI: 10.1021/mp700113r

17. Zhuang, X., Xiang, X., Grizzle, W., Sun, D., Zhang, S., Axtell, R. C., ... & Zhang, H.-G. (2011). Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Biomaterials, 32*(28), 8515–8523. DOI: 10.1038/mt.2011.164

18. Sun, D., Zhuang, X., Xiang, X., Liu, Y., Zhang, S., Liu, C., ... & Zhang, H.-G. (2010). A novel nanoparticle drug delivery system: The anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Molecular Therapy, 18*(9), 1606–1614. DOI: 10.1038/mt.2010.105

19. Théry, C., Amigorena, S., Raposo, G., & Clayton, A. (2006). Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Current Protocols in Cell Biology*, 3.22.1–3.22.29. https://doi.org/10.1002/0471143030.cb0322s30

20. Kalani, A., Tyagi, A., & Tyagi, N. (2015). Exosomes: Mediators of neurodegeneration, neuroprotection and therapeutics. *Molecular Neurobiology*, 52(1), 1–7. https://doi.org/10.1007/s12035-013-8544-1

21. Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089

22. Hendijani, F. (2017). *Explant culture: An advantageous method for isolation of mesenchymal stem cells from human tissues. Cell Proliferation, 50(2), e12334.* https://doi.org/10.1111/cpr.12334

23. Purushothaman, A. (2019). Exosomes from Cell Culture-Conditioned Medium: Isolation by Ultracentrifugation and Characterization. In: Vigetti, D., Theocharis, A.D. (eds) The Extracellular Matrix. Methods in Molecular Biology, vol 1952. Humana Press, New York, NY. https://doi.org/10.1038/srep21933

24. Briones-Márquez LF, Navarro-Partida J, Herrera-González A, García-Bon MA, Martínez-Álvarez IA, Uribe-Rodríguez D, González-Ortiz LJ, López-Naranjo EJ. HPLC-UV evaluation of a microwave assisted method as an active drug loading technique for exosome-based drug delivery system. Heliyon. 2023 Oct 6;9(10):e20742. doi: http://doi.org/10.1016/j.heliyon.2023.e20742

25. Jeliński, T., Przybyłek, M. & Cysewski, P. Natural Deep Eutectic Solvents as Agents for Improving Solubility, Stability and Delivery of Curcumin. *Pharm Res* **36**, 116 (2019). https://doi.org/10.1007/s11095-019-2643-2

26. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

27. Helwa, I., Cai, J., Drewry, M. D., Zimmerman, A., Dinkins, M. B., Khaled, M. L., … Liu, Y. (2017). *A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three commercial reagents*. https://doi.org/10.1371/journal.pone.0170628

28. Mendonca, A., Acharjee, A., Kumar, J.S. *et al.* Comparative analysis of exosomes isolated by ultracentrifugation and total exosome isolation reagent: a biophysical and physicochemical study. *J Nanopart Res* **27**, 28 (2025). https://doi.org/10.1007/s11051-025-06232-2

29. Midekessa, G., Godakumara, K., Ord, J., Viil, J., Lättekivi, F., Dissanayake, K., … Fazeli, A. (2020). *Zeta Potential of Extracellular Vesicles: Toward Understanding the Attributes that determine colloidal stability https://doi.org/10.1021/acsomega.0c01582*

30. Heidarzadeh, M., Zarebkohan, A., Rahbarghazi, R. *et al.* Protein corona and exosomes: new challenges and prospects. *Cell Commun Signal* **21**, 64 (2023). https://doi.org/10.1186/s12964-023-01089-1

31. Kalimuthu S, Gangadaran P, Rajendran RL, Zhu L, Oh JM, Lee HW, Gopal A, Baek SH, Jeong SY, Lee S-W, Lee J and Ahn B-C (2018) A New Approach for Loading Anticancer Drugs Into Mesenchymal Stem Cell-Derived Exosome Mimetics for Cancer Therapy. *Front. Pharmacol.* 9:1116. https://doi.org/10.3389/fphar.2018.01116

32. Gräfe, C., Weidner, A., vd Lühe, M., Bergemann, C., Schacher, F. H., Clement, J. H., & Dutz, S. (2016). Intentional formation of a protein corona on nanoparticles: Serum concentration affects protein corona mass, surface charge, and nanoparticle–cell interaction. *The international journal of biochemistry & cell biology*, *75*, 196-202. https://doi.org/10.1016/j.biocel.2015.11.005

33. Maroto, R., Zhao, Y., Jamaluddin, M., Popov, V. L., Wang, H., Kalubowilage, M., … Brasier, A. R. (2017). Effects of storage temperature on airway exosome integrity for diagnostic and functional analyses. *Journal of Extracellular Vesicles*, *6*(1). https://doi.org/10.1080/20013078.2017.1359478

34. Cheng, G., Zhang, Y., Li, Y., & Han, X. (2020). Curcumin suppresses fascin expression in glioblastoma cells. *Neoplasia*, 21(3), 254–265. https://doi.org/10.1593/neo.07909

35. Lee, Y.-S., Kim, S., Jeon, Y.-K., & Park, J.-H. (2019). Curcumin suppresses glioblastoma cell migration through downregulation of fascin expression. *Brain Tumor Research and Treatment*, 7(1), e28. https://doi.org/10.14791/btrt.2019.7.e28

36. Lee, Y.-S., et al. (2020). Curcumin induces apoptosis in glioblastoma cells via p-AKT/mTOR and PTEN pathways. *Archives of Biochemistry and Biophysics*, 695, 108412. https://doi.org/10.1016/j.abb.2020.108412

37. Wang, H., Chu, H., & Sun, Y. (2017). Curcumin decreases malignant features via ROS in glioblastoma. *BMC Cancer*, 17, 558. https://doi.org/10.1186/s12885-017-3058-2

38. Yang, C., et al. (2023). Exosome-mediated drug delivery across the BBB. *Molecular Neurobiology*. https://doi.org/10.1007/s12035-023-03365-0

39. Mehdizadeh, S., Mamaghani, M., Hassanikia, S. *et al.* Exosome-powered neuropharmaceutics: unlocking the blood-brain barrier for next-gen therapies. *J Nanobiotechnol* **23**, 329 (2025). https://doi.org/10.1186/s12951-025-03352-8

40. Wei, X., et al. (2022). Umbilical MSC exosomal miR-133b inhibits glioma via Wnt/β-catenin. *Stem Cell Research & Therapy*, 13, 214. https://doi.org/10.1186/s13287-019-1446-z

41. Ghasempour, E., Hesami, S., Movahed, E. *et al.* Mesenchymal stem cell-derived exosomes as a new therapeutic strategy in the brain tumors. *Stem Cell Res Ther* **13**, 527 (2022). https://doi.org/10.1186/s13287-022-03212-4

42. Sun, D., Zhuang, X., Xiang, X., et al. (2010). A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Molecular Therapy*, 18(9), 1606–1614. https://doi.org/10.1038/mt.2010.105

43. Böing, A. N., van der Pol, E., Grootemaat, A. E., Coumans, F. A., Sturk, A., & Nieuwland, R. (2014). Single-step isolation of extracellular vesicles by size-exclusion chromatography. *Journal of Extracellular Vesicles*, 3(1), 23430. https://doi.org/10.3402/jev.v3.23430

44. Lobb, R. J., et al. (2015). Optimized exosome isolation protocol for cell culture supernatant and human plasma. *Journal of Extracellular Vesicles*, 4(1), 27031. https://doi.org/10.3402/jev.v4.27031

45. Théry, C., Witwer, K. W., et al. (2018). Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the ISEV. *Journal of Extracellular Vesicles*, 7(1), 1535750. https://doi.org/10.1080/20013078.2018.1535750