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RESEARCH ARTICLE

Paclitaxel incorporated exosomes derived from glioblastoma cells: comparative study of two loading techniques

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Abstract

Background Exosomes are natural nanoparticles that are involved in intercellular communication via transferring molecular information between cells. Recently, exosomes have been considered for exploitation as novel drug delivery systems due to their specific properties for carrying specific molecules and surface proteins.

Methods In this study, U-87 cell derived exosomes have been investigated for delivery of a potent chemotherapeutic agent, paclitaxel (PTX). Two methods of loading were utilized to incorporate PTX in exosomes and the exosomes pharmaceutical and cytotoxic characterizations were determined.

Results The drug loaded and empty exosomes were found to have particle size of 50–100 nm and zeta potential of ≈ − 20 mV. Loading capacity of 7.4 ng and 9.2 ng PTX into 1 μg of exosome total protein were also measured for incubation and sonication methods, respectively. Incorporation of PTX into exosomes significantly increased its cytotoxicity against U-87 cell line (59.92% cell viability) while it was found that the empty exosomes exhibited cell viability of 91.98%.

Conclusions Loading method could affect the loading capacity of exosomes and their encapsulated chemotherapeutic molecule showed higher cytotoxicity into exosomes. These results promise exosomes as appropriate drug delivery system for glioblastoma multiform (GBM) treatment.

Keywords Exosome · Paclitaxel · Glioblastoma cell line · Sonication; loading

High lights Exosomes were isolated from glioblastoma cells and paclitaxel was loaded in exosomes through two methods of loading: incubation and sonication; cell viability of U-87 cells was significantly decreased by exosomes containing paclitaxel.

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Introduction

New drug delivery systems (NDDS) such as lipid and polymeric particles, have been used for cancer treatment because of targeted therapeutic release and reducing side effects of chemotherapeutic drugs. Reducing the size of NDDS into nano-scale range, increases the tumor accumulation of particles, but immunogenic and toxicity effects are still important challenges [1]. Exosome containing pharmaceuticals is one of the most novel NDDS which widely has been applied for theragnostic approaches [2].

Exosomes are extracellular vesicles formed in multivesicular bodies and fused out of the cell membrane. Exosomes can be found in abundance in all body fluids including urine, blood, and cerebrospinal fluid as well as conditional cell culture media [3]. As vesicles transport intercellular messages, they have been suggested to be used as drug delivery systems [3–5]. In some studies, exosomes were used for gene delivery [6] and transferring

of hydrophilic and lipophilic drugs such as doxorubicin [1, 7] and curcumin [8].

Due to nano-scale size of exosomes and their surface proteins which are producer cell-type dependent, passing through blood brain barrier (BBB) is facilitated [9]. These specific characteristics making exosomes beneficial for treatment of central nervous system diseases especially brain cancer where BBB permeability is a serious challenge for chemotherapeutic drug delivery [10, 11]. Among the brain malignancies, glioblastoma multiforme (GBM) is the most aggressive and lethal one. The standard treatment for this malignancy is surgery followed by radiotherapy with concurrent chemotherapy [12]. GBM tumors are diverse in cellular phenotypes, so their treatments are complicated as a result of their heterogeneity. This diversity has driven the attention to suggest more specific and targeted treatment of GBM [13].

Paclitaxel (PTX) is a mitotic inhibitor (microtubule stabilizing agents) anti-cancer drug that has been used in treatment of GBM. Because of its chemical nature, it couldn't pass through BBB and has low bioavailability despite of its potency [14, 15]. Many NDDS such as magnetic poly (lactic-co-glycolic acid)-based nanoparticles [16], human serum albumin [17, 18], and positive-charged solid lipid nanoparticles [19] have been used for transport of PTX through BBB.

As mentioned before, due to superiority of exosomes for cancer treatment [10], in this study we tried to formulate exosome containing PTX, in comparison to free PTX against U87, a glioblastom cell line. Two methods were investigated to load PTX into exosomes and nano-vesicles were characterized as particle size and zeta potential determination, morphology study, encapsulation efficiency measurement, and surface protein analysis.

Materials and methods

Materials

Dulbecco's modified Eagle's medium (DMEM) and exosome-depleted fetal bovine serum (FBS) were purchased from GIBCO (Invitrogen Inc. Gibco BRL, USA). Penicillin plus streptomycin solution and 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Exo-spin™ (EX01) and CD9 TRIFic™ exosome assay (EX101) both were obtained from Cell Guidance Systems (Cambridge, UK). Bicinchoninic acid (BCA) protein assay kit was provided by Santa Cruz Biotechnology (Texas, USA). PTX was kindly donated by NanoAlvand pharmaceutical company (Tehran, Iran). Other solvents and reagents were of analytical grade and obtained from Merck (Darmstadt, Germany).

Cell culture

Human brain neuronal glioblastoma-astrocytoma U-87 MG cells (Pasture institute, Tehran, Iran) were cultured in high glucose DMEM supplemented with 10% FBS, 1% penicillin and streptomycin at 37 °C and 5% CO_2. Exosomes were extracted when 80% of cell culture confluence was achieved.

Exosome isolation and purification

Exosomes were isolated from the media of U-87 cells in exosome-depleted media using the Exo-spin™ exosome purification kit (exosome purification kit for cell culture media/urine/saliva and other low-protein biological fluids) according to the manufacturer protocols. In brief, cell media were firstly collected and centrifuged at 300×g for 10 min by Hettich® Universal 320R centrifuge (Tuttlingen, Germany) to remove cellular debris. Thereafter, the resulting supernatant centrifuged for 30 min at 16000×g and the obtained supernatant of the previous step were mixed with Exo-spin™ buffer (2:1 *v*/v) and incubated overnight at 4 °C followed by centrifugation for 1 h at 16000×g. The supernatants were discarded and the collected pellet resuspended in 100 μL phosphate buffered saline (PBS). For purification of exosomes, the collected samples from previous step were applied to Exo-spin™ columns. Eventually, the product stored at −80 °C for next phases of the study.

Drug entrapment into exosomes

A stock solution of PTX (50 mg/ml in dimethyl sulfoxide, DMSO) was prepared and diluted to desired concentration in PBS, pH 6.8. The exosome isolated pellet was resuspended with 100 μL of 40 μg/ml PTX in PBS [5]. Two methods were investigated for drug loading including incubation at 37 °C and sonication [10, 20]. At the first method, the admixture was incubated for 1 h at 37 °C. In the second method, the mixture was sonicated using an ultrasonic probe (UP100H Ultrasonicator Hielscher, Teltow, Germany) with 20% amplitude for 6 cycles that each cycle was 3 min covering 6 periods of 30 s on/off with a 2 min cooling period between each cycle. For recovery of the exosomal membrane after sonication, the obtained solution was incubated at 37 °C for 1 h.

Exosome characterization

Size analysis of exosomes was carried out by photon correlation spectroscopy (PCS) by using Cordouan, VASCO Nano-Particle size analyzer (Pessac-Bordeaux, France). In order to further size analysis and morphology observation of these nanoparticles, field emission scanning electron microscopy (FESEM, Hitachi S-4160, Tokyo, Japan) and transmission electron microscopy (TEM, Philips, Germany) were used.

The exosome suspension was dried at room temperature, then coated with a thin layer of gold to increase the electric conductivity before FESEM imaging. For the TEM, exosomes were added on carbon coated copper grid (200 mesh) and dried grids were examined using an accelerator voltage of 80 KeV. Surface charge on the exosomes was determined in terms of zeta potential using WALLIS Zeta potential analyzer (Pessac-Bordeaux, France).

Exosome protein determination

The total protein content of exosomes was measured by BCA protein assay kit. The exosome prepared samples were diluted in PBS in ratios of 1:10 (by 10-fold) and mixed with BCA reagent and incubated at 60 °C for 15 min, followed by recording the related absorbance at 562 nm using NanoDrop™ spectrophotometer (ND-1000, Thermo Fisher Scientific, Wilmington, DE, USA). The standard curve was drawn by performing the same procedure for different concentrations (50–250 μg/ml) of bovine serum albumin.

Exosome marker detection

To measure the surface protein of exosomes, CD9 TRIFic™ exosome assay kit (europium time-resolved immunofluorescence based for detection of exosome antigens) was applied according to manufacturing protocol. This kit consists of a monoclonal antibody (labeled with biotin) bound to an streptavidin coated plate that capture CD9 in the surface of exosome which then detect by an identical monoclonal antibody labeled with Europium. The wavelength of excitation and emission were 340 nm and 615 nm, respectively and the assay was carried out by multimode microplate reader (Synergy 2, BioTek, Winooski, VT, USA) [21].

PTX loading determination in exosomes

High performance liquid chromatography (HPLC) technique was utilized to measure PTX loaded into exosomes. Pre-analysis treatment of samples were carried out based on Kim et al. [20] protocol with some modification in which an equal volume of acetonitrile (MeCN) was added to exosomes suspension (1:1) and vortexed, sonicated (for destruction of exosome structure), and then centrifuged at 14,000 rpm (Hettich® Universal 320R centrifuge, Tuttlingen, Germany) for 10 min. Thereafter, about 150 μl of the obtained supernatant was transferred into an HPLC vial (containing glass insert) and finally 50 μl aliquots of the samples were injected into a Smartline® HPLC system (Knauer®, Berlin, Germany) consisted of a quaternary pump (model 1000), PDA-UV detector (model 2600), manager (model 5000), autosampler (model 3950), membrane vacuum degasser, and column temperature compartment. The HPLC analysis was performed on a Nucleodur C18ec column (4.6 mm × 150 mm, 3.0 μm) (Macherey-Nagel, Düren, Germany) kept at 35 °C using a UV detector set at 227 nm. Water and MeCN were used as mobile phases at flow rate of 1.2 ml/min in a gradient program. The elution program was started at 30% MeCN which was continued for 3 min followed by a 10% per min increase to 80% MeCN. At the end of elution, MeCN was decreased to 30% in one min and continued for 3 min in order to re-equilibrate the column (totally 12 min run time). The retention time of PTX was about 8.8 min and the area under the curve (AUC) was used for its quantification using the calibration curve (range of 0.5–20 μg/ml). PTX loading capacity was expressed as ng PTX per μg protein of exosomes.

Cytotoxity of exosomal formulations on U-87 cell line

The cytotoxic effects of empty exosomes, PTX-loaded exosomes, and free PTX were determined against the U-87 cell line by the MTT based colorimeteric protocol. In brief, a density of 10^4 cells per well were seeded in flat-bottom 96-well tissue culture microplates and incubated for 24 h (37 °C, 5% CO_2 humidified air) to adhere. Then, each formulation was added to the desired well in triplicate and incubated for 24 h in the same conditions. Cells cultured in medium, served as the control. To evaluate cell survival, 100 μl of MTT solution (1 mg/ml in culture medium) was added to each well and incubated for 2 h. The unreduced MTT and medium were replaced by 100 μl of DMSO and pipetted to dissolve any formed formazan crystals. Absorbance was then measured at 570 nm by an ELISA plate reader (Synergy 2, Biotek, Winooski, Vermont, USA). The percentage of cell viability was determined in compare with control.

Statistical analysis

All data were expressed in the form of the mean ± standard deviation (SD). Data were compared by one-way analysis of variance (ANOVA) with post-hoc test, using SPSS 21 (IBM); Significance was defined as $p < 0.05$.

Results

Isolation and characterization of exosomes

Exosomes collected from the culture media of U-87 were characterized by size, zeta potential, and morphology. Empty exosomes were 61.02 nm in diameter, whereas size of drug loaded exosomes were 62.73 and 85.95, respectively for 37 °C incubation and sonication loading methods (Table 1). The TEM and FESEM results of exosomes showed that all exosomes exhibited spherical shape and size of 50–150 nm (Figs. 1 and 2). Zeta potential of U-87 exosomes in three form

Table 1 Size, zeta potential and loading capacity of exosomes (mean ± SD)

Sample	Size(nm)	Zeta potential (mV)	loading capacity (ng/μg)
Empty exosome	61.02 ± 3.53	−22.18 ± 8.73	–
Incubation at 37 °C	62.73 ± 4.63	−18.22 ± 1.23	7.40 ± 0.37
Sonication	85.95 ± 4.74	−22.46 ± 0.63	9.21 ± 0.41

of empty and PTX loaded (either by incubation at 37 °C or sonication) was measured and represented in Table 1.

Detection of CD9 bio-marker

A tetraspanin surface protein (CD9) was detected by CD9 TRIFic™ exosome assay with ELISA method through excitation and emission of fluorescent identifier-fluorophore-(Europium). The results (Fig. 3) were shown no significant difference between 3 groups ($p > 0.05$).

Determination of PTX loaded in exosomes

In order to load PTX into exosomes, 40 μg/ml PTX was used. PTX was incorporated into exosomes using two methods of incubating at 37 °C and sonication. After removal of the unbound PTX by size-exclusion chromatography (Exo-spin™ column) during exosome purification, the amount of PTX encapsulated into the exosomes was measured by HPLC. To determine the loading capacity, PTX concentration loaded in exosomes was divided by exosome total protein concentration. Loading efficiency in sonication method was 0.92% in comparison to 0.74% for incubation at 37 °C.

Cytotoxicity of PTX and exosomal formulations

Cytotoxicity results of exosomes containing PTX, free PTX, and empty exosomes were exhibited in Fig. 4 where PTX-loaded exosome significantly inhibited the growth of U-87 cell line by 59.92% compared to that of free PTX (80.70%).

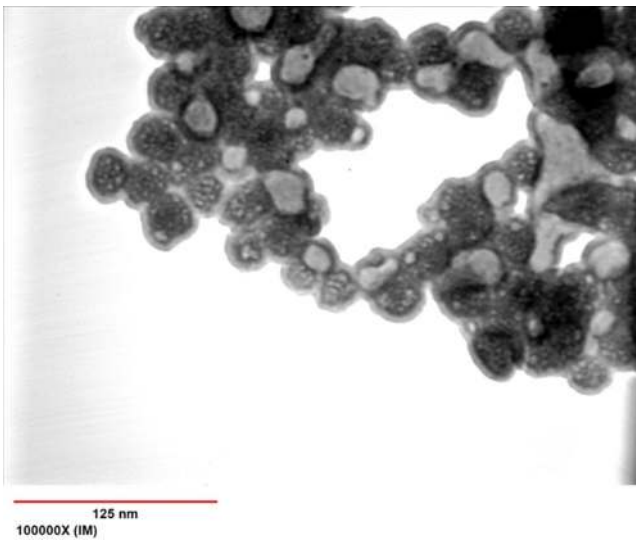

Fig. 1 TEM image of empty exosomes

Discussion

Exosomes are endogenous carriers that provide efficient drug transportation due to their display nano-sized morphology and expression of parent cell-derived markers. These nano-vesicles have been purified by various isolation methods such as ultracentrifugation, density-based separation (sucrose and iodixanol), precipitation, ultrafiltration, and immunoaffinity [22]. Lane et al. [23] described that precipitation techniques possessed more recovery and yield compared to ultracentrifugation and density gradient method which are more user-intensive methods so, it could cause an increased sample loss during handling. In the present study, Exo-spin™ based on precipitation method followed by a column-based cleanup was applied for exosome purification. This kit rely on polymer co-precipitation of exosome and its column (size-exclusion chromatography) provides a convenient, reproducible, and highly effective elimination of non-vesicular proteins [24].

Generally, exosome size may differ on the basis of preparation method, storage conditions or exosomes' cell sources [9, 10, 25, 26]. However, in the study conducted by Marimpietri et al. [27], exosomes isolated from different neuroblastoma cells (HTLA-230, IMR-32, SH-SY5Y, and GI-LI-N) were found to be similar in size. In another investigation Haraszti et al. [28] explained that Huh7 (hepatocellular carcinoma cells), U-87, and MSC (human bone marrow-derived mesenchymal stem cells) have no significant size differences. In the present work, PCS, TEM and FESEM were employed to analyze size and shape of the isolated exosomes. Although, agglomeration of exosomes occurred due to drying process of electronic microscopic analysis, the size scale provided by TEM and FESEM indicated that exosomes were in the range of 50–150 nm (Figs. 1 and 2) which confirmed by PCS results (Table 1). The attained size and spherical morphology of exosomes in the present study were similar to the previous studies [28, 29]. As presented in Table 1, the related size of PTX loaded exosome by sonication method was somehow larger ($p < 0.05$) than PTX loaded exosome by incubation technique which might be ascribed to the probable

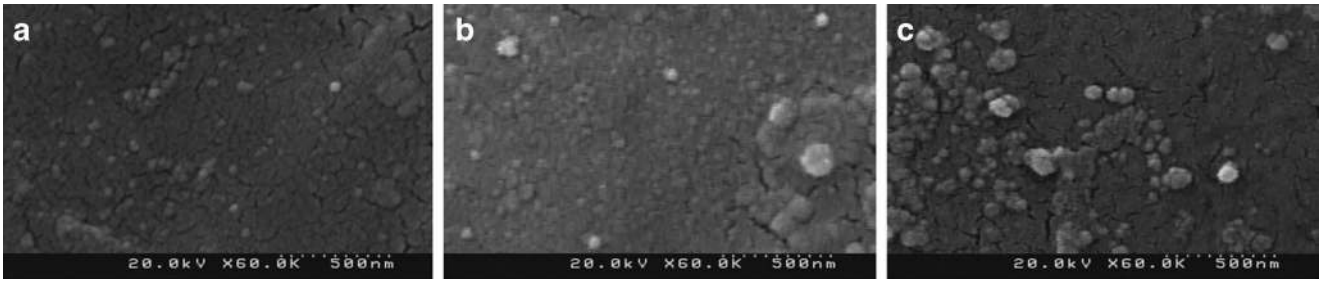

Fig. 2 FESEM image of Empty exosome (**a**), PTX-loaded exosomes by incubation at 37 °C (**b**) and PTX-loaded exosome by sonication (**c**)

reformation of exosomes. The same results was reported by Kim et al. [20] who suggested that sonication might increase exosomes size (217.9 nm) due to reformation of exosomes compared to incubation method (178.7 nm).

Encapsulation of pharmaceutical active ingredients (APIs) could affect the mean diameter of lipid vesicles such as niosomes [30] and exosomes [20] due to surface adsorption and bilayer stabilization [31]. However, in the present study, no significant change in exosome diameter was observed following PTX loading (Table 1, $p > 0.05$).

In various researches for characterization of exosomes derived from different cell lines, large differences of zeta potential (electrokinetic mobility) have been reported [32]. Kato et al. [33] investigated six types of human cells and found strong correlation between the zeta potential of exosomes and their cells of origin. In this study, colloidal stability was measured by assessing isolated exosomes' zeta potential which was ranged from −18 to −22 mV (Table 1). This zeta potential range is near to critical zeta potential need for high colloidal dispersion stability [34]. On the other hand, two methods of drug loading did not significantly affect on zeta potential of exosomes (Table 1, $p > 0.05$), similar to Kim et al. [20] finding. They found a non-significant increase in zeta potential of exosomes containing PTX which might be attributed to PTX interaction with carboxyl groups of surface-adhered proteins present over the surface of exosomes [31]. Similarly, in the present study, PTX encapsulation had negligible effect on exosomes' zeta potential (Table 1).

Obtaining efficient drug loading in exosomes is an important matter especially for hydrophobic or large molecules such as PTX. In this study, sonication method caused more PTX loading compared to incubation method (Table 1, $p < 0.05$). Similar results were reported by Kim et al. [20] who loaded PTX in exosome derived from RAW 264.7 macrophages cell. It was suggested that PTX molecules could better diffuse across relatively tight and highly structured lipid bilayer during exosome membrane reorganization caused by sonication. However, Haney et al. [9] hypothesized that sonication could increase drug loading and accumulation of exosomes in target cells but this reorganization and disruption of exosome membrane uniformity alter their visibility for immune system.

Cytotoxicity of free PTX solution on U-87 cells was less than PTX containing exosome formulation (Fig. 4, $p < 0.05$). This may be consequence of the route of exosomes' internalization to cells that return to endocytosis and fuse to cell membrane. Cell-targeting adhesion molecules (tetraspanins, integrins and etc.) on the surface of exosomes cause superior uptake of these nano-vesicles by target cells such as brain tumor cells [4, 9]. Increase in intracellular uptake and cytotoxicity of chemotherapeutic agents through exosome drug delivery was observed by other researchers. In one of these studies which was done by Parolini et al. [35], they showed acidic condition of tissue microenvironmental, increased uptake of exosomes so it was indicated exosomes may be taken up preferentially by hypoxic cancerous cells.

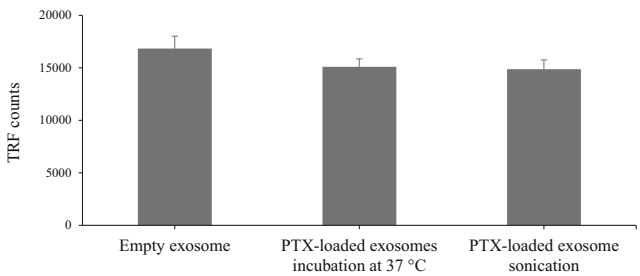

Fig. 3 CD9 level of exosomes: Empty exosome, PTX-loaded exosomes by incubation at 37 °C and PTX-loaded exosome by sonication

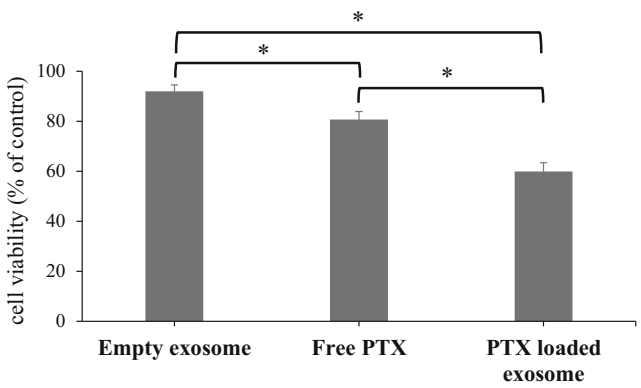

Fig. 4 Cytotoxicity on U-87 cells treated by empty exosomes, free PTX and PTX-loaded exosome (* Results are significantly different, $p < 0.05$)

Conclusions

In summary, PTX loaded exosomes isolated from U-87 cell line were utilized as drug delivery system. Utilizing exosomes as drug delivery devices can overcome the limitations of other drug delivery systems due to their biocompatibility, high cell penetration and targeting functions. However, some obstacles in exosomal formulations exist such as choice of exosomes extraction protocols, drug loading procedures, and so on; for example the method of preparation could affect the encapsulation efficiency and exosome diameter. In this study, it was shown toxicity effect of PTX loaded exosomes against GBM cells was more than free PTX solution. Further studies are planned to investigate in vivo tumor targeting capability of PTX loaded exosomes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

1. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014;35(7):2383–90.
2. Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharm Sin B. 2017;38(6):754–63.
3. Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles—endogenous nanocarriers for targeted cancer therapy. BBA-Rev Cancer. 2014;1846(1):75–87.
4. Batrakova EV, Kim MS. Using exosomes, naturally-equipped nanocarriers, for drug delivery. J Control Release. 2015;219:396–405.
5. Saari H, Lazaro-Ibanez E, Viitala T, Vuorimaa-Laukkanen E, Siljander P, Yliperttula M. Microvesicle-and exosome-mediated drug delivery enhances the cytotoxicity of paclitaxel in autologous prostate cancer cells. J Control Release. 2015;220:727–37.
6. Jiang X-C, Gao J-Q. Exosomes as novel bio-carriers for gene and drug delivery. Int J Pharm. 2017;521(1–2):167–75.
7. Jang SC, Kim OY, Yoon CM, Choi D-S, Roh T-Y, Park J, et al. Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. ACS Nano. 2013;7(9):7698–710.
8. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther. 2010;18(9):1606–14.
9. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release. 2015;207:18–30.
10. Yang T, Martin P, Fogarty B, Brown A, Schurman K, Phipps R, et al. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharm Res. 2015;32(6):2003–14.
11. Katakowski M, Chopp M. Exosomes as tools to suppress primary brain tumor. Cell Mol Neurobiol. 2016;36(3):343–52.
12. Ganipineni LP, Danhier F, Préat V. Drug delivery challenges and future of chemotherapeutic nanomedicine for glioblastoma treatment. J Control Release. 2018;281:42–57.
13. Gourlay J, Morokoff A, Luwor R, Zhu H-J, Kaye A, Stylli S. The emergent role of exosomes in glioma. J Clin Neurosci. 2017;35:13–23.
14. Xin H, Sha X, Jiang X, Zhang W, Chen L, Fang X. Anti-glioblastoma efficacy and safety of paclitaxel-loading Angiopep-conjugated dual targeting PEG-PCL nanoparticles. Biomaterials. 2012;33(32):8167–76.
15. Mu Q, Jeon M, Hsiao MH, Patton VK, Wang K, Press OW, et al. Stable and efficient paclitaxel nanoparticles for targeted glioblastoma therapy. Adv Healthc Mater. 2015;4(8):1236–45.
16. Ganipineni LP, Ucakar B, Joudiou N, Bianco J, Danhier P, Zhao M, et al. Magnetic targeting of paclitaxel-loaded poly (lactic-co-glycolic acid)-based nanoparticles for the treatment of glioblastoma. Int J Nanomedicine. 2018;13:4509–21.
17. Ruan C, Liu L, Lu Y, Zhang Y, He X, Chen X, et al. Substance P-modified human serum albumin nanoparticles loaded with paclitaxel for targeted therapy of glioma. Acta Pharm Sin B. 2018;8(1):85–96.
18. Esfandyari-Manesh M, Mostafavi SH, Majidi RF, Koopaei MN, Ravari NS, Amini M, et al. Improved anticancer delivery of paclitaxel by albumin surface modification of PLGA nanoparticles. Daru. 2015;23(1):28.
19. Chirio D, Gallarate M, Peira E, Battaglia L, Muntoni E, Riganti C, et al. Positive-charged solid lipid nanoparticles as paclitaxel drug delivery system in glioblastoma treatment. Eur J Pharm Biopharm. 2014;88(3):746–58.
20. Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL, et al. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. Nanomed-Nanotechnol. 2016;12(3):655–64.
21. Kamińska A, Platt M, Kasprzyk J, Kuśnierz-Cabala B, Gala-Błądzińska A, Woźnicka O, et al. Urinary extracellular vesicles: potential biomarkers of renal function in diabetic patients. J Diabetes Res. 2016;2016:1–12.
22. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. Cancer Lett. 2016;371(1):48–61.
23. Lane RE, Korbie D, Anderson W, Vaidyanathan R, Trau M. Analysis of exosome purification methods using a model liposome system and tunable-resistive pulse sensing. Sci Rep. 2015;5:7639.
24. Welton JL, Webber JP, Botos L-A, Jones M, Clayton A. Ready-made chromatography columns for extracellular vesicle isolation from plasma. J Extracell Vesicles. 2015;4(1):27269.
25. Helwa I, Cai J, Drewry MD, Zimmerman A, Dinkins MB, Khaled ML, et al. A comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLoS One. 2017;12(1):e0170628.
26. Sokolova V, Ludwig A-K, Hornung S, Rotan O, Horn PA, Epple M, et al. Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy. Colloid Surface B. 2011;87(1):146–50.
27. Marimpietri D, Petretto A, Raffaghello L, Pezzolo A, Gagliani C, Tacchetti C, et al. Proteome profiling of neuroblastoma-derived exosomes reveal the expression of proteins potentially involved in tumor progression. PLoS One. 2013;8(9):e75054.
28. Haraszti RA, Didiot M-C, Sapp E, Leszyk J, Shaffer SA, Rockwell HE, et al. High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources. J Extracell Vesicles. 2016;5(1):32570.
29. De Vrij J, Maas SL, Van Nispen M, Sena-Esteves M, Limpens RW, Koster AJ, et al. Quantification of nanosized extracellular

membrane vesicles with scanning ion occlusion sensing. Nanomedicine-UK. 2013;8(9):1443–58.
30. Pardakhty A, Varshosaz J, Rouholamini A. In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin. Int J Pharm. 2007;328(2):130–41.
31. Agrawal AK, Aqil F, Jeyabalan J, Spencer WA, Beck J, Gachuki BW, et al. Milk-derived exosomes for oral delivery of paclitaxel. Nanomed-Nanotechnol. 2017;13(5):1627–36.
32. Beit-Yannai E, Tabak S, Stamer WD. Physical exosome: exosome interactions. J Cell Mol Med. 2018;22(3):2001–6.
33. Kato K, Kobayashi M, Hanamura N, Akagi T, Kosaka N, Ochiya T, et al. Electrokinetic evaluation of individual exosomes by on-chip microcapillary electrophoresis with laser dark-field microscopy. Jpn of Appl Phys. 2013;52(6S):06GK10.
34. Hood JL, Scott MJ, Wickline SA. Maximizing exosome colloidal stability following electroporation. Anal Biochem. 2014;448:41–9.
35. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem 2009:jbc. M109. 041152.