Jankowski et al.
Medical Journal of Cell Biology 2022
DOI: 10.2478/acb-2022-0003
Received: 18.02.2022
Accepted: 21.03.2022

Current application of exosomes in medicine

Maurycy Jankowski[1], Ana Angelova Volponi[2], Rafael Shinoske Siroma[3], Nelson Pinto[3], Marcelo A. Melo[3], Kornelia Krajnik[4], Jamil A. Shibli[3], Paul Mozdziak[5], Mariusz T. Skowroński[6], Marta Dyszkiewicz-Konwińska[1,7]

Abstract

Exosomes are a distinct type of extracellular vesicles that play a major role in intracellular transport and communication. Depending on the cell of origin, exosomes can contain diverse constituents of a cell, including DNA, RNA, lipids, metabolites, cytosolic and cell-surface proteins, playing important roles in a wide range of physiological and pathological processes. Due to these facts, they are subject of extensive research aiming at translating the knowledge into clinical approaches that are at the interface between nanomedicine and biopharmaceuticals.

Their potential clinical use mostly revolves around the fields of diagnostics and drug delivery, especially important in treatment of cancer. The conventional and emerging methods of exosome isolation are either based on their physical properties (such as density and/or size) or their functions. However, the isolation approaches are still characterised by significant downsides, lacking standardisation, and ensuring purity. The review gives a critical overview on exosomes characteristics, isolation approaches and the potential that exosomes hold in developing new clinical approaches of modern medicine, highlighting the need for further research to fully grasp their potential and translate the knowledge into future therapeutic solutions.

Running title: Exosomes in medicine

Keywords: exosomes, applications, medicine

[1]Department of Anatomy, Poznań University of Medical Sciences, Poznan, Poland
[2]Centre for Craniofacial and Regenerative Biology, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, United Kingdom
[3]Department of Periodontology and Oral Implantology, Dental Research Division, Guarulhos University, Guarulhos, Brazil
[4]Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Toruń, Poland
[5]Physiology Graduate Program, North Carolina State University, Raleigh, NC, USA
[6]Department of Basic and Preclinical Sciences, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Toruń, Poland
[7]Department of Biomaterials and Experimental Dentistry, Poznan University of Medical Sciences, Poznań, Poland
***Correspondence:** mjankowski@ump.edu.pl
Full list of author information is available at the end of article

sciendo

Introduction

Exosomes are extracellular vesicles, 30-200nm in diameter, secreted by most cell types through the process of endocytosis. First discovered in 1981, they have been described as pivotal in wide range of cell product transport and intracellular communication [1].

In the initial stage of exosome research, the exosomes attracted attention as carriers of protein, RNA, DNA, and lipid material, important for malignant tumours, highlighting the potential of their usage in disease diagnostics [2]. However, their potential is often overshadowed by the difficulties in obtaining the exosomes using reliable and standardised techniques. Therefore, many current studies focus on the improvement of the technologies used for exosome derivation, particularly establishing standardised, cost-effective techniques, as well as novel nano-based chip approaches, to enable their widespread clinical use [3].

There is a significant interest in the application of exosomes in therapy of various diseases [4]. The exosomes obtained from specific cell types, such as mesenchymal stem cells, have been indicated as potential therapeutics for conditions such as stroke and cardiovascular disease [5,6]. Furthermore, due to their unique transport capabilities, there is a significant number of studies focusing on their use in drug delivery.

These characteristics could be particularly useful in targeted cancer therapies, which often require transport of highly specialised compounds to a particular isolated location in the organism [7].

However, all the above applications are still in a relatively early stage of development and require further research aiming at safe translation of the knowledge into diagnostic and therapeutic methods [4]. Hence, this review summarises the current knowledge about exosomes, their characteristics, sources for isolation, methods of extraction and potential applications in clinical therapies.

The characteristics and sources of exosomes

Exosomes are a subcategory of extracellular vesicles, secreted into the extracellular space by a wide range of cell types [8]. They are surrounded by a single membrane and can be obtained from a range of samples such as: plasma, urine, saliva, cerebrospinal fluid, and blood serum. Exosomes are highly heterogenous since they closely reflect the nature of cells of their origin [3]. Their sizes highly vary and fall in a wide range of 30-200 nm, even when they are expressed by cells from a singular cell line. When it comes to density, this parameter varies depending on the protein to lipid ratio, generally accepted to be in the ~1.1-1.2 g/mL range [9]. These high variabilities in size and density are due to the extreme variability of their content [10]. Exosome formation starts from invagination of cellular membrane, resulting in creation of early sorting endosomes. There, the molecules that will constitute the final exosome content are accumulated and later sorted in a structure known as a multivesicular body. Then, the latter merge with cell membranes to release the contained substances into the extracellular space as vesicles. While the process of exosome formation and secretion are relatively well described, the exact mechanisms governing these processes remain subject of scientific debate [11]. The heterogeneity of exosomes may be conceptualized on the basis of: their size, content (cargo), functional impact on recipient cells or cell of origin (source). Diverse combinations of these characteristics define the complex heterogeneity of exosomes.

There is a wide range of contents that can be transported in exosomes. These include proteins, nucleic acids such as DNA, RNA or lipids. Moreover, there is a wide range of transmembrane proteins that can be expressed on their surface. These serve multiple functions, including modification of exosome properties to enable their transport through specific extracellular environments, as well as mediate their uptake through receptor-mediated endocytosis [12], important in physiology and disease. During physiological processes of homeostasis, all cells of the organism can express exosomes. Stem cells, such as mesenchymal stem cells (MSCs), have been of particular interest for the researchers, due to their widely studied role in repair and regeneration, therefore exosomes derived from these cells have been in the focus of many researchers. Furthermore, pathological tissues, such as tumours, also express this type of extracellular vesicles, attracting the attention of further studies to understand the behaviour and communication of these cells and the underlying mechanisms that drive the processes, with a prospect to clinical use [13].

Methods of exosome isolation

Currently used methods of exosome-isolation are based on their physical properties (such as density and/or size) or their functions. Most commonly used methods include: ultracentrifugation, density-gradient centrifugation, ultrafiltration and immunoisolation. The success of exosome isolation is confirmed by transmission electron microscopy (TEM) or flow cytometry [14] and further molecular profiling can be done by using proteomics, lipidomics and genomics. Detailed molecular profiling contributes to further understanding of exosome functions, as well as reveals clues in exosome biogenesis.

The most used methods of exosome purification from liquid samples, such as blood, saliva, or cell culture medium, are based on their high-speed differential centrifugation. While this method is relatively cost- and time- efficient, as well as yields a large

amount of extracellular vesicle material, there are several downsides to its use [15]. The ultracentrifugation speed can cause significant damage to the preparations, often resulting in low efficiency to obtain material of high integrity. The large amount of initial sample needed prevents the use of this method for smaller samples, e.g. needle biopsies, which also presents limitation. Finally, extracellular vesicle heterogeneity and the presence of protein aggregates in the initial samples might cause low purity of the isolated fraction [16]. Hence, while differential centrifugation is the most used method of exosome isolation, it does have several shortcomings described above. Therefore, other available methods, as well as those that are under development, might offer solution to these shortcomings and be better fitted to some applications [14].

Density gradient centrifugation is a method also based on the initial step of ultracentrifugation, often as fast as 100 000 x g and for periods of time up to 16h, followed by enrichment of exosomes in a gradient of medium such as sucrose. This allows to extract exosomes from a particular gradient (1.10 to 1.18 g/mL), while protein aggregates accumulate at the bottom of the tube. Therefore this method offers isolation of exosome samples with significantly higher purity, due to the isolation based on a specific range of weights [14]. Studies comparing different methods of differential centrifugation-based methods of exosome isolation from patient serum proved that those based on medium density gradients resulted in a highest yield and purity. Nevertheless, this technique doesn't permit isolation of viral exosomes or microvesicles of similar size to exosomes, as well as requires special ultracentrifugation equipment and trained personnel [17].

Another method based on physical properties, such as size of the exosomes, is ultrafiltration. This process is based on the application of ultrafiltration membrane, which allows for selective size or molecular weight-based sorting in a principle like conventional filtration. The liquid sample, such as e.g. blood or cell culture medium, is passed through a series of nanomembranes of varying sizes, or molecular-weight cut-off membranes, allowing to intercept exosomes. This process facilitates their separation from macromolecules of different molecular mass [18]. Ultrafiltration allows to shorten the centrifugation time, as well as eliminates the need for specialised, expensive equipment. Nevertheless, this method is not without its flaws, as problems such as membrane clogging might result in exosome loss or damage, which often necessitates multiple washing steps to mitigate such occurrences [19].

Exosomes can be isolated by methods other than those based on their physical properties, but based on their affinity and interaction with surface biomarkers and immobilised antibodies, such as CD82, CD81, CD9, CD63, EpCAM, annexin, and Rab5. These immunoaffinity-based approaches allow precise isolation of exosomes from liquid samples, using magnetic beads, plates, microfluidic devices, and chromatography matrices [14]. Magnetic beads, often used in combination with flow cytometry cell sorting or sorting columns, are among the most used, cost-effective, and well-established approaches. In turn, microfluidic methods are a relative novelty, having been developed in the last decade. While they allow for unprecedented purity of the obtained material, the yields associated with their nanoscale are rather low. Moreover, the equipment and reagents required are associated with a relatively large costs, making most labs omit this method, especially if high purity or specificity of obtained exosomes is not the highest priority [20]. These isolation methods, based on the principle of immunoaffinity, enable higher recovery rate and purity of the isolated exosomes, but large quantities of biological samples cannot be processed and have high reagents costs.

Another group of methods used in exosome isolation are based on the principle of precipitation. In these approaches, a water-binding polymer, such as polyethylene glycol, is added to the sample, causing precipitation of particles, including macrovesicles [14]. This method can be effectively used with larger volume liquid samples, as well as is characterised by a relative ease of use and cost effectiveness. However, its biggest downside is low selectivity, requiring application of other pre-treatment steps to ensure high specificity of isolation [21].

Many of the above methods are commercially available as isolation kits, allowing to efficiently isolate exosomes from a range of sample sources. However, these commercially available kits have high costs and could yield protein contaminated samples [22].

Application of exosomes in clinical therapies

Due to the physical properties and the ability to transport wide range of contents, exosomes can be defined as natural nanoparticles, that are promising tool for clinical nanomedicine. They can potentially play an important role in the diagnosis, treatment, and prevention of diseases and have wide range of clinical applications [14]. Their presence in easily obtainable physiological liquids, such as blood and saliva, makes the exosomes an attractive tool for diagnostic purposes. [3]. Multiple exosomal contents have been associated with conditions such as cancer, cardiovascular disease, and central nervous system defects [14]. These properties fit in the concept of a proposed diagnostic practice known as "liquid biopsy". The approach is based on detection of a range of biomarkers in patients' blood to determine the presence or progression of a disease [23]. Exosomes serve as carriers of such markers, and

their relevance has been highlighted in occurrence of cancer. The presence of miRNAs contained inside were indicated as good candidate molecules for determining the presence of tumours or other diseases [24]. Such miRNAs, (miR-133, miR-194 and miR-34a) were found to be upregulated in patients with myocardial infraction [25]. In turn, exosomes containing miR-21 and miR-373 could be an indicator of the presence of colorectal and receptor-negative breast cancer, respectively [26,27]. Furthermore, the fact that upregulation of miR-1290 and miR-375 have been correlated with poor survival of prostate cancer patients, while levels of miR-19a corresponded with colorectal cancer recurrence, exosomes containing these molecules could potentially be used to determine prognosis and tailor specific therapies in patients affected by these malignancies [28,29]. While there are still several limitations of liquid biopsy, as well as those associated with exosome isolation methods, exosomes' application in disease diagnostics remains with huge potential.

A further potential application of exosomes in the field of therapeutics revolves around their use as natural nanocarriers for drug loading and delivery. Several properties of these extracellular vesicles, namely their size, allowing them to avoid phagocytosis, their ability to freely cross extracellular matrix and blood vessel walls, and the excellent biocompatibility and relatively low immunogenicity, make them a viable alternative to synthetic drug delivery carriers [7]. Moreover, due to their significant heterogeneity, exosomes can express a wide range of extracellular proteins. Through the process of surface modification, they can be induced to host a range of ligands compatible with specific receptors. This property allows to exploit internalization through receptor-mediated endocytosis for delivery of their contents to the intracellular space, which can be translated into targeted cancer therapy approaches [30]. A wide range of advanced and traditionally used molecules have already been loaded into exosomes and used in research aiming to design new methods of treatment for cancer and other diseases. These included chemotherapeutic drugs such as doxorubicin, paclitaxel, as well as medicines deriving from traditional practices, including curcumin or anthocyanins derived from berries [14]. Furthermore, exosomes have also been implicated as vesicles for the delivery of DNA and RNA for the purpose of gene therapy. This approach opens wide possibilities for their future use in advanced treatments based on this approach, potentially providing the tool needed to tackle a range of currently untreatable diseases [31]. There are essentially two ways of loading therapeutic agents into exosomes. The first one, known as pre-secretary loading, bases on engineering of specially modified cells, facilitating packing and, in some cases, production of desired molecules. While this method is relatively simple, it does not allow for a control of the rate and efficiency of loading, as well as is associated with all the burdens of cell engineering, including possible structural damage to the resulting cells [32]. Alternatively, loading can be conducted after exosome membrane assembly, in a process known as post-secretory loading. In this approach, methods such as electroporation or ultrasonic treatment are used to move therapeutic molecules into the exosomes. Although simple and effective, this method is not applicable for loading all types of compounds and there is a need for a tailored choice in the methodology depending on the characteristics of therapeutics that need to be loaded into the exosomes [33]. Molecule loaded exosomes can be administered in a variety of ways, including intravenous or subcutaneous injection, as well as nasal and oral administration.

Finally, there is a number of other innovative approaches to exosome application, based on their origin from stem cells of high plasticity and regenerative ability. As an example, vesicles obtained from MSCs can be used to evoke effects similar to stem cell therapy, mitigating the risks of immunogenicity and tumorigenicity usually associated with their use [34]. Such approaches allow for the use of exosomes in fields such as cell engineering, promoting regeneration of tissue damage. These properties were proven to be mostly associated with activation of stem cell specific renewal abilities via factors and signalling pathways regulated by the content of MSC-derived exosomes. Due to the above, there is a large amount of research focusing on stem cell vesicles, as development of therapies based on them could potentially lead to treatment of damage resulting from a range of conditions, e.g. cardiac ischemia or neurodegenerative disorders [35].

Conclusions

Exosomes undoubtedly play a major role in cell-cell transport, playing a pivotal role in diverse physiological and pathological processes. Their physical properties, as well as accessibility make them highly promising tool in the development of new methods, as part of future nanomedicine. Their potential to offer solutions for daily monitoring of disease indicators and adjuvant medication, provide us with perspective to achieve personalized and precise treatment for diverse diseases, including cancer. Recent studies highlight new information on exosome biogenesis and functions, opening opportunities to manipulate their composition, properties, and cell interactions to further advance their therapeutic application.

However, while the methods of exosome isolation and identification are well-defined, high standardisation and purity needs to be established before translating the usage of exosomes as new diagnostics and therapeutic approaches.

Ethical approval
The conducted research is not related to either human or animal use.

Acknowledgments
Not applicable.

Corresponding author
Maurycy Jankowski, Department of Anatomy, Poznań University of Medical Sciences, 6 Święcickiego St., 60-781 Poznań, Poland, Tel./ Fax: +48 61 8546565, e-mail: mjankowski@ump.edu.pl.

Conflict of interest statement:
The authors declare they have no conflict of interest.

References

1. Ståhl A-L, Johansson K, Mossberg M, Kahn R, Karpman D. Exosomes and microvesicles in normal physiology, pathophysiology, and renal diseases. Pediatr Nephrol. 2019;34(1):11–30; DOI:10.1007/S00467-017-3816-Z/TABLES/3.
2. Shehzad A, Islam SU, Shahzad R, Khan S, Lee YS. Extracellular vesicles in cancer diagnostics and therapeutics. Pharmacol Ther. 2021;223:107806; DOI:10.1016/J.PHARMTHERA.2021.107806.
3. Pegtel DM, Gould SJ. Exosomes. Ann Rev Bioch. 2019;88:487–514; DOI:10.1146/ANNUREV-BIOCHEM-013118-111902.
4. Wang J, Chen D, Ho EA. Challenges in the development and establishment of exosome-based drug delivery systems. J Control Release. 2021;329:894–906; DOI:10.1016/J.JCONREL.2020.10.020.
5. Lai RC, Chen TS, Lim SK. Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease. Regen Med. 2011;6(4):481–92; DOI:10.2217/RME.11.35/ASSET/IMAGES/LARGE/FIGURE2.JPEG.
6. Chen J, Chopp M. Exosome therapy for stroke. Stroke. 2018;49(5):1083–90; DOI:10.1161/STROKEAHA.117.018292.
7. Moon B, Chang S. Exosome as a delivery vehicle for cancer therapy. Cells 2022;11(3):316; DOI:10.3390/CELLS11030316.
8. Arraud N, Linares R, Tan S, Gounou C, Pasquet JM, Mornet S, Brisson AR. Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration. J Thromb Haemost. 2014;12(5):614–27; DOI:10.1111/JTH.12554.
9. Raposo G, Nijman HW, Stoorvogel W, Leijendekker R, Harding C V., Melief CJM, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. J Exp Med. 1996;183(3):1161–72; DOI:10.1084/JEM.183.3.1161.
10. Smith ZJ, Lee C, Rojalin T, Carney RP, Hazari S, Knudson A, Lam K, Saari H, Ibañez EL, Viitala T, Laaksonen T, Yliperttula M, Wachsmann-Hogiu S. Single exosome study reveals subpopulations distributed among cell lines with variability related to membrane content. J Extracell Vesicles. 2015;4(1):28533; DOI:10.3402/JEV.V4.28533/SUPPL_FILE/ZJEV_A_11815546_SM0001.PDF.
11. Bobrie A, Colombo M, Raposo G, Théry C. Exosome Secretion: Molecular Mechanisms and Roles in Immune Responses. Traffic. 2011;12(12):1659–68; DOI:10.1111/J.1600-0854.2011.01225.X.
12. Li X, Corbett AL, Taatizadeh E, Tasnim N, Little JP, Garnis C, Daugaard M, Guns E, Hoorfar M, Li ITS. Challenges and opportunities in exosome research - perspectives from biology, engineering, and cancer therapy. APL Bioeng. 2019;3(1):011503; DOI:10.1063/1.5087122.
13. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science 2020;367(6478); DOI:10.1126/SCIENCE.AAU6977.
14. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. Int J Nanomedicine. 2020;15:6917; DOI:10.2147/IJN.S264498.
15. Livshts MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov E V., Govorun VM. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. Sci Rep. 2015;5; DOI:10.1038/SREP17319.
16. Cvjetkovic A, Lötvall J, Lässer C. The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. J Extracell Vesicles. 2014;3(1); DOI:10.3402/JEV.V3.23111.
17. Ford T, Graham J, Rickwood D. Iodixanol: a nonionic iso-osmotic centrifugation medium for the formation of self-generated gradients. Anal Biochem. 1994;220(2):360–6; DOI:10.1006/ABIO.1994.1350.
18. Van Deun J, Mestdagh P, Agostinis P, Akay Ö, Anand S, Anckaert J, Martinez ZA, Baetens T, Beghein E, Bertier L, Berx G, Boere J, Boukouris S, Bremer M, Buschmann D, Byrd JB, Casert C, Cheng L, Cmoch A, Daveloose D, De Smedt E, Demirsoy S, Depoorter V, Dhondt B, Driedonks TAP, Dudek A, Elsharawy A, Floris I, Foers AD, Gärtner K, Garg AD, Geeurickx E, Gettemans J, Ghazavi F, Giebel B, Kormelink TG, Hancock G, Helsmoortel H, Hill AF, Hyenne V, Kalra H, Kim D, Kowal J, Kraemer S, Leidinger P, Leonelli C, Liang Y, Lippens L, Liu S, Lo Cicero A, Martin S, Mathivanan S, Mathiyalagan P, Matusek T, Milani G, Monguió-Tortajada M, Mus LM, Muth DC, Németh A, Nolte-'T Hoen ENM, O'Driscoll L, Palmulli R, Pfaffl MW, Primdal-Bengtson B, Romano E, Rousseau Q, Sahoo S, Sampaio N, Samuel M, Scicluna B, Soen B, Steels A, Swinnen J V., Takatalo M, Thamiy S, Théry C, Tulkens J, Van Audenhove I, Van Der Grein S, Van Goethem A, Van Herwijnen MJ, Van Niel G, Van Roy N, Van Vliet AR, Vandamme N, Vanhauwaert S, Vergauwen G, Verweij F, Wallaert A, Wauben M, Witwer KW, Zonneveld MI, De Wever O, Vandesompele J, Hendrix A. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. Nat Methods. 2017;14(3):228–32; DOI:10.1038/NMETH.4185.
19. Vergauwen G, Dhondt B, Van Deun J, De Smedt E, Berx G, Timmerman E, Gevaert K, Miinalainen I, Cocquyt V, Braems G, Van Den Broecke R, Denys H, De Wever O, Hendrix A. Confounding factors of ultrafiltration and protein analysis in extracellular vesicle research. Sci Rep. 2017;7(1); DOI:10.1038/S41598-017-02599-Y.
20. Yang F, Liao X, Tian Y, Li G. Exosome separation using microfluidic systems: size-based, immunoaffinity-based and dynamic methodologies. Biotechnol J. 2017;12(4):1600699; DOI:10.1002/BIOT.201600699.
21. Rider MA, Hurwitz SN, Meckes DG. ExtraPEG: A Polyethylene Glycol-Based Method for Enrichment of Extracellular Vesicles. Sci Rep. 2016;6; DOI:10.1038/SREP23978.
22. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation Techniques. Theranostics. 2017;7(3):789; DOI:10.7150/THNO.18133.
23. Hirahata T, Quraish R, Quraish A, Quraish S, Naz M, Razzaq MA. Liquid biopsy: a distinctive approach to the diagnosis and prognosis of cancer: Cancer Inform. 2022;21: 11769351221076062; DOI:10.1177/11769351221076062.
24. Sun Z, Shi K, Yang S, Liu J, Zhou Q, Wang G, Song J, Li Z, Zhang Z, Yuan W. Effect of exosomal miRNA on cancer biology and clinical applications. Mol Cancer 2018 171. 2018;17(1):1–19; DOI:10.1186/S12943-018-0897-7.
25. Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, Qin YW, Jing Q. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. Eur Heart J. 2010;31(6):659–66; DOI:10.1093/EURHEARTJ/EHQ013.
26. Eichelser C, Stückrath I, Müller V, Milde-Langosch K, Wikman H, Pantel K, Schwarzenbach H. Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients. Oncotarget. 2014;5(20):9650–63; DOI:10.18632/ONCOTARGET.2520.
27. Rotelli MT, Di Lena M, Cavallini A, Lippolis C, Bonfrate L, Chetta N, Portincasa P, Altomare DF. Fecal microRNA profile in patients with colorectal carcinoma before and after curative surgery. Int J Color Dis. 2015;30(7):891–8; DOI:10.1007/S00384-015-2248-0.
28. Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, Ueda M, Uchi R, Ueo H, Takano Y, Shinden Y, Eguchi H, Yamamoto H, Doki Y, Mori M, Ochiya T, Mimori K. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. Br J Cancer. 2015;113(2):275–81; DOI:10.1038/BJC.2015.201.
29. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, Wang D, See W, Costello BA, Quevedo F, Tan W, Nandy D, Bevan GH, Longenbach S, Sun Z, Lu Y, Wang T, Thibodeau SN, Boardman L, Kohli M, Wang L. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol. 2015;67(1):33–41; DOI:10.1016/J.EURURO.2014.07.035.
30. Tatischeff I, Alfsen A. A New Biological Strategy for Drug Delivery: Eucaryotic Cell-Derived Nanovesicles. J Biomater Nanobiotechnol. 2011;02(05):494–9; DOI:10.4236/JBNB.2011.225060.
31. Didiot MC, Hall LM, Coles AH, Haraszti RA, Godinho BMDC, Chase K, Sapp E, Ly S, Alterman JF, Hassler MR, Echeverria D, Raj L, Morrissey D V., Di Figlia M, Aronin N, Khvorova A. Exosome-mediated delivery of hydrophobically modified siRNA for Huntingtin mRNA silencing. Mol Ther. 2016;24(10):1836–47; DOI:10.1038/MT.2016.126.
32. Lee J, Lee H, Goh U, Kim J, Jeong M, Lee J, Park JH. Cellular engineering with membrane fusogenic liposomes to produce functionalized extracellular vesicles. ACS Appl Mater Interfaces. 2016;8(11):6790–5; DOI:10.1021/ACSAMI.6B01315.
33. Fuhrmann G, Serio A, Mazo M, Nair R, Stevens MM. Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. J Control Release. 2015;205:35–44; DOI:10.1016/J.JCONREL.2014.11.029.
34. Wei W, Ao Q, Wang X, Cao Y, Liu Y, Zheng SG, Tian X. Mesenchymal stem cell - derived exosomes: a promising biological tool in nanomedicine. Front Pharmacol. 2021;11:1954; DOI:10.3389/FPHAR.2020.590470/BIBTEX.
35. Rani S, Ritter T. The exosome - a naturally secreted nanoparticle and its application to wound healing. Adv Mater. 2016;28(27):5542–52; DOI:10.1002/ADMA.201504009.