Journal of Experimental and Basic Medical Sciences 2022;3(1):6-12

Review

Exosomes: Classification, Isolation, and Therapeutic Applications in Various Diseases

Yaren Kul[1], Oytun Erbaş[1]

ABSTRACT

Exosomes are rich in nucleic acids, lipids, and proteins, and they are involved in various physiological and pathophysiological processes. Proteins on their membrane surfaces serve as biomarkers for multiple diseases. For this reason, exosomes take part in the diagnosis and prognosis processes. Additionally, they are involved in a variety of biological processes, such as intercellular communication, signal transduction, and immune response control. Moreover, exosomes can originate from various cell groups and their contents and functions vary according to their origin. Innovative treatments are being developed based on the several therapeutic properties of exosomes. Due to their diversity in structure and content, many methods have been developed for exosome isolation and characterization. This review covers the biogenesis of exosomes, their contents, the cell types from which they originate, and their effects on diseases, as well as isolation and characterization methods, diagnosis, and treatment approaches.

Keywords: Biomarkers, characterization, diagnosis, exosomes, isolation, therapeutic effects

In multicellular organisms, billions of cells must communicate in order to fulfill their functions at the same time. Cells communicate with one another by secreting chemical messages that may travel great distances. It is possible to classify this communication as local and long-distance communication. Local communication is the communication of cells with other cells in their surroundings, and local regulators such as neurotransmitters and local regulators are involved, while hormones and extracellular vesicles (EVs) are effectively involved in long-distance communication. EVs are lipid-layered nanovesicles that secrete biomolecules including microRNA (miRNA), messenger RNA (mRNA), deoxyribonucleic acid (DNA), and proteins into the extracellular environment, as well as perform critical functions such as intercellular signal transmission and genetic material transfer. EVs are divided into three types according to their biological formation, size, and cellular origin: apoptotic bodies, microvesicles, and exosomes.[1]

Exosomes ranging in size from 30 to 100 nm are the smallest EVs. Meanwhile, exosomes are distinguished from other EVs by their unique biogenesis, heterogeneous lipid composition, protein and nucleic acid (miRNAs, mRNAs, non-coding RNAs (ncRNA), etc.) contents, and size. They contain specific clusters of differentiation (CD) biomarker molecules such as CD9, CD63, and CD81 on their membrane surfaces.[2]

It has a variety of functions, including intercellular communication, genetic material transfer, and immune response modulation, and many diagnosis and treatment applications have sprung up as a result. Whilst applications for the diagnosis of various diseases are developed based on their role in the pathogenesis of the disease, applications for the treatment of cancer and autoimmune disorders are still being developed and have become widespread rapidly by utilizing their immune regulatory properties.[1,2] The focus of this research is to consolidate data from the literature concerning exosomes' main features, biogenesis, sources, isolation methods, diagnostics, and therapy applications.[1]

[1]ERBAS Institute of Experimental Medicine, Illinois, USA & Gebze, Turkey

Correspondence: Yaren Kul. Institute of Experimental Medicine, 41470 Gebze-Kocaeli, Türkiye

E-mail: yaren.kull@gmail.com

Cite this article as: Kul Y, Erbaş O. Exosomes: Classification, Isolation, and Therapeutic Applications in Various Diseases. JEB Med Sci 2022;3(1):6-12.

doi: 10.5606/jebms.2022.1002

Received : February 12, 2022
Accepted : February 18, 2022
Published online : June 13, 2022

Several methods for isolating and characterization of exosomes are also highlighted.[2,3]

EXOSOME BIOGENESIS

Extracellular vesicles are divided into three types: apoptotic bodies (1000–5000 nm), microvesicles (50–1000 nm), and exosomes (30–100 nm) in diameter. Microvesicles and apoptotic bodies are usually formed by budding outward from the membrane.[4] In exosome biogenesis, early endosomes (EEs) are formed as the main cell membrane folds inward under ceramide control.[1] During this phase, bioactive molecules begin to fill the EEs.[5]

Under the control of the proteins required for transport and endocytosis sorting complex, the EE buds inwards and makes indentations, transforming into the late sorting endosomes (LSEs), while forming many intraluminal vesicles (ILVs).[1,5]

The structures formed at this stage are referred to as multivesicular bodies (MVBs) and comprise numerous nanovesicles. The resulting MVBs can follow two different paths; In the first of these, it decomposes as a result of coming together with the lysosome.[1] These are called degradative MVBs (dMVBs).[4] In a second way, MVBs fuse with the plasma membrane and are released out of the cell as exosomes.[1] These are called secretive MVBs (sMVBs).[4]

Exosomes can be absorbed by the target cell in three ways after they are released into the environment:

Via ligands: Exosomes can be taken into the cell by attaching the ligands on the exosomes to the target cell receptor via receptor interaction.

Via fusion: Exosomes transport the cargo they carry to the cytoplasm of the target cell as a result of the direct fusion of the exosome membrane with the membrane of the target cell.

Via phagocytosis: The pseudopods formed on the target cell membrane can surround the exosomes and allow them to be taken into the cell.[1]

EXOSOME CONTENTS

Exosomes contain lots of macromolecules like lipids, proteins, and nucleic acids and are crucial for intercellular communication. The results of the investigations have revealed the existence of 41,860 exosomal proteins, >7540 exosomal RNAs, and 1116 exosomal lipid molecules.[6]

Exosomes can be utilized as drug delivery systems since they include lipids including those of sphingomyelin, cholesterol, phospholipids (e.g. phosphatidic acid, phosphatidylserine), arachidonic acid (AA), monosialodihexosylganglioside (ganglioside GM3), desaturated lipids, phosphatidylserine, and ceramide. Excess lipids in exosomes increase the stiffness of the cell membrane. Numerous proteins are found scattered throughout the membrane and cytoplasm of exosomes. Some of these are fusion proteins, enzymes, chaperones, heat shock proteins, cytoskeletal proteins, and proteins that enable the formation of MVBs such as CD9, CD63, CD81, and ALG-2-interacting protein X (Alix), tumor susceptibility gene 101 (TSG101).[4,7]

Exosomes also include nucleic acids, which constitute a major component of their composition. Exosomal RNAs comprise mRNAs, miRNAs, and various ncRNA, which are involved in cell cycle progression, cellular migration, angiogenesis, and histone alteration.[7,8]

ORIGIN OF EXOSOMES

Exosomes can be obtained from various sources such as stem cells, foods, immune cells, body fluids, and tumors. Source must be chosen based on the therapy for which the exosome will be utilized. This selection affects both the proteins and lipids on the surface of exosomes and their contents.[7] They generated from stem cells help stem cells with self-renewal, immunomodulation, proliferation, and damage repair. Simultaneously, these exosomes have a great potential for cell-free regenerative medicine. Exosomes derived from embryonic stem cells facilitate signaling between stem cells as well as accelerate tissue repair by enhancing cell proliferation. Exosome isolation is also made from mesenchymal stem cells (MSCs) obtained from various sources such as bone marrow, adipose, umbilical cord, muscle, etc. They are used in the treatment of immune diseases thanks to their immunomodulatory effects.[9]

Additionally, these exosomes are employed in the tissue recovery process due to their therapeutic capabilities.

Exosomes derived from foods, such as fruits, have increasingly gained attention. The results obtained after the discovery of exosomes in animal cells also support the formation of exosome-like vesicles in plant cells.[1] These plant-derived exosome-like nanoparticles have anti-inflammatory properties and contain miRNAs.[7]

Recent studies showed that exosomes have been obtained from fruits such as grapefruit, grapes, and carrots.[10,11] Exosomes derived from bovine milk have shown promising outcomes. These exosomes are used in research on the transport of drugs and bioactive chemicals.[12,13] In another study, it was concluded that exosome-like nanoparticles obtained from garlic play an important role in the apoptosis of cancer cells.[14]

Obtaining exosomes from several immune cells is another exciting topic. The main cell types used to acquire exosomes are monocytes, macrophages, and dendritic cells (DCs).[7] These kinds of exosomes have a therapeutic effect and an important role in developing adaptive immunity. At the same time, immune cell-derived exosomes have a role in cancer progression, thus they can be used in cancer diagnosis and therapeutic studies.[15]

Exosomes obtained from the tumor have immunosuppressive properties. Since they provide cell-cell interaction, they closely affect the tumor progression and metastasis process. These vesicles have an impact on tumor progression and metastasis because of their function in cellular communication.[16] Tumor-based exosomes promote immunosuppression in the tumor microenvironment (TME) by triggering the differentiation of CD4+ T cells into CD39+ regulatory T cells.[17]

ISOLATION TECHNIQUES

Exosomes are structures that vary in terms of function, size, and content. Due to these complicated structures, exosome isolation is difficult and the possibility of obtaining fully isolated exosomes with high purity is low.[5] To solve this problem, several techniques for isolating exosomes utilizing cell culture medium or bodily fluids have been developed.[1]

Many methods have been applied to obtain exosomes, including differential centrifugation, immunoaffinity, ultrafiltration, and polymer-based precipitation. Exosome isolation is generally monitored based on size, morphology, buoyant densities, and the presence of marker proteins like Alix, TSG101, heat shock protein 70 (HSP70), and CD9.[18]

Differential Centrifugation

The most widely used isolation technique is the differential centrifugation method. In this technique, basically, the separation is made according to their physical properties and volumes.[3]

Cells, large particles, macromolecular proteins, and cell debris in the culture media are gradually removed using centrifugal forces ranging from 200 to 100,000 x g in this approach. Sedimentation at 100,000 x g for 70 minutes separates the exosomes from the supernatant. All centrifuges applied during this process are performed at 4°C.[1,3]

The resulting exosomes may contain other molecules in the sample, so this technique may not be efficient enough for purification.[1]

Ultracentrifugation

Ultrafiltration (UC), another method used in the isolation of exosomes, is a kind of membrane filtration method in which isolation is made according to size differences using pressure. The UC method basically consists of two parts. Initially, low-speed centrifugation is performed to remove dead cells, large molecules, and cell debris. In the next step, high-speed centrifugation is done at 100,000 × g force. The resulting exosomes are cleaned with phosphate-buffered saline (PBS) to remove contaminating agents.[5] The UC technique has some disadvantages such as removal of nano-sized membrane-adhering proteins, difficulty in eluting exosomes, and low yield with loss of many exosomes.[1,3] It is therefore a more suitable technique to apply in large amounts of samples.[5]

Immunoaffinity

The ability to separate monoclonal antibody molecules based on their biological activity using chemical or physical characteristics is the basis of affinity chromatography. The basic principle in this method is the separation of the targeted molecule by the interaction between the antibody and the ligand. The ligand is covalently fixed to the solid support called the matrix and the sample in the buffer solution is passed through the column. Meanwhile, the targeted antibodies interact with the ligands contained in the solid support. As a result, the target molecule stays in the column while the undesired ones are eliminated. This approach allows for the qualification and quantification of exosomes.[1,19]

Immunomagnetic Beads

Immunomagnetic beads are a kind of spherical particle coated with monoclonal antibodies. The interaction between antibodies on the coating with receptors on the exosome surface, such as CD9, CD63, and CD81, is the basis of exosome separation in this technique.[20,21] In the first step of the technique, the immunomagnetic beads are coated with antibodies that

will bind to the exosome receptors. The exosomes are separated from the sample by triggering the directed movement of these complexes under the effects of a magnetic field. In addition to being a low-cost technique, it offers some advantages such as being unique to the target and simplicity of application.[22]

Polymer Precipitation

Another frequently preffered technique to exosome isolation is polymer precipitation. This method is mostly performed by using polyethylene glycol (PEG) media. It works with the principle of collecting exosomes whose solubility is reduced by centrifugation. It is used for exosome isolation and purification. The technique is simple and fast to use, making it ideal for processing large volumes of samples. However, there are certain drawbacks, such as the difficulty in removing polymers and the low purity.[5]

CHARACTERIZATION TECHNIQUES

Nanoparticle Tracking Assay

The size distribution and concentration of exosomes can be determined via using nanoparticle tracking analysis (NTA). Based on the optical particle tracking principle, NTA obtains data about particle velocities by measuring exosome motion paths. With this method, it is possible to quickly and easily measure even small vesicles with a diameter of 30 nm. Another benefit is that the particles can be easily obtained in their initial form after the measurements are completed.[5,22]

Dynamic Light Scattering

Exosome sizes are measured by passing monochromatic coherent laser beams through the sample solution. Dynamic light scattering (DLS) measurements can be performed for 1 nm to 6 µm sized molecules. More accurate measurements are obtained in monodisperse systems, which are suspensions containing single particles. Since the solution is not suitable for complex measurements, various problems occur in the diagnosis of small particles when the solution contains large molecules, even in the low concentration range.[5,22]

Flow Cytometry

Flow cytometry, a technique used to determine the biomarker proteins, sizes, and structures of exosomes, is one of the most invariably implemented methods in EV studies. It analyzes molecules with a size of 300 nm and above this by this approach exosomes can not determine directly. The scattering density is determined after laser beams are supplied to the suspension containing the particles to be examined. Fluorescent dyes can be used to stain the particles utilized in this process, and the fluorescence intensity emitted can then be evaluated. Flow cytometry works with low sample concentrations. It has high efficiency and gives fast results.[5,22]

ENZYME-LINKED IMMUNOSORBENT ASSAY

Enzyme-linked immunosorbent assay (ELISA) is widely used for the detection of specific antibodies and antigens in samples. It is applied in qualitative and quantitative analyses of exosome-specific marker proteins due to its great specificity. It is a technique that makes it possible to make fast and highly efficient analyzes.[5]

TRANSMISSION ELECTRON MICROSCOPE

It is a type of microscope used for the characterization of various molecules related to their internal structures, particle size distributions, and morphologies. As a consequence of sending an electron beam to the sample, it collects the scattered electrons and converts them into an image. However, a transmission electron microscope (TEM) has some limitations like having many requirements in sample preparation and complicated operation.[5,22]

DIAGNOSIS OF DISEASES

With the understanding that exosomes have an important role in disease pathologies and that regulatory molecules are transferred between cells, the idea that these nanovesicles can also be used in the diagnosis of the disease has gained importance. Messenger RNA and miRNAs are selectively loaded into exosomes, which can be obtained from all body fluids. It shows that a new generation of unique diagnostic kits can be developed for the diagnosis of the disease by using exosomes obtained from body fluids.[2]

Molecules such as nucleic acid and protein in the content of exosomes are very important for use as biomarkers and these exosomal proteins are crucial in the detection of a variety of disorders, including cancer and renal disease. Biomarkers include proteins like TSG101, CD63, and CD81, as well as lipids like cholesterol and sphingomyelin. The membrane scaffold proteins CD63 and CD81 are employed as cancer indicators.[23] At the same time, the CD151

level is high in lung cancer patients.[5] MicroRNAs are abundant in exosomes, and they are also frequently utilized as markers. Prostate cancer markers include miR21, 107, 141, 181a-2, 301a, 326, 331- 3p, 625, and 2110, whereas breast cancer patients have high levels of miR-1246 and miR-21, and miR-638 acts as a marker for colorectal cancer cells.[2,5,23]

miR-499, 133, 208, 192, 194, and 34a are upregulated in patients with myocardial infarction and heart failure, this makes them markers for cardiovascular disease.[5]

Fetuin-A and epidermal growth factor receptor (EGFR) pathway-related proteins, which are abundant in patients with acute kidney injury, are other molecules used as markers.[23]

TREATMENT APPROACHES

Several characteristic behaviours of exosomes such as cargo carrying capacity, immune regulatory affect and their ability to enhance communication between cells reveal that these nanovesicles can be used as therapeutic purposes. Exosomes are readily available as natural carriers, and have high biodistribution and high stability in plasma, making them suitable candidates for therapeutic applications. Treatment applications are divided into two: using exosomes on their own or designing them to be carriers for an exogenic molecule.[2]

The small size ranges of exosomes prevents them from being phagocytosed by mononuclear macrophages and also allows them to easily pass through various biological barriers such as the blood vessel wall and the blood-brain barrier. The CD55 and CD59 they contain on the membrane faces ensure their stability and dispersion in biological fluids.[5]

Exosomes, which are involved in the physiological and pathophysiological processes of diseases, decrease inflammation, improve neural and motor activities, and allow numerous intravenous dosages without adverse effects. They have a wide range of therapeutic activities and vary according to the cell type from which they originate.[4,5]

Exosomes generated from MSCs diminish inflammation by inhibiting pro-inflammatory cytokines. They support extracellular matrix (ECM) remodeling. Exosomes produced by bone marrow-derived MSCs preserve against conditions like hypoxia-induced pulmonary hypertension, brain injury, and myocardial ischemia-reperfusion injury, while exosomes based on human umbilical cord-MSCs protect against acute renal injury and liver fibrosis. Furthermore, MSC-derived exosomes improved cognition in diabetic mice with decreased cognition by healing oxidative damage in neurons and astrocytes. A cancer-specific T cell response is generated when dendritic cell-derived exosomes are matured with cancer antigens.[23] Injection of DC-originated exosomes which contain tumor peptide-major histocompatibility complexes (MHC) provides tumor rejection.[2] Exosomes have been used as a drug delivery molecule in several studies due to their membrane composition. Thanks to various membrane proteins on the exosome surface, communication between the target cell and the exosome are ensured by receptor-mediated endocytosis. The encapsulated drug's internalization is facilitated by its great transport efficiency.[5]

Drug and genetic material loading studies on various nanoparticles use a number of loading methods. These techniques are divided into two categories: active and passive. Passive loading, also known as incubation, is the most studied method because it does not cause a significant negative effect on the exosome structure and content, is a simple and low-cost procedure and causes only minor damage to exosome integrity.[24]

Incubation can be categorized into two subclasses: incubation of exosomes with a drug solution or isolation and drug-loaded exosomes secreted by incubated cells after incubation of cells with a drug solution.[25]

Nowadays, incubation is applied for a variety of drugs with different properties; nevertheless, it seems that the method is more frequently used for hydrophobic drug molecules because hydrophobic drugs are more stable in the bilayer lipid layer of exosome membranes. Exosome loading can be accomplished by physical mechanisms via generating micropores in the exosomal membrane or membrane recombination. These processes are sonication, electroporation, extrusion, freeze-thawing, surfactant treatment, and dialysis. Electroporation is the most widely utilized method for drug loading of exosomes after incubation. The aforementioned different approaches result in varying loading efficiencies and stability of drugs in exosome vesicles.[25,26]

In conclusion, the release of exosomes with different types and effects depending on their origins and their conditions, the presence of various markers on their membrane, and special biological functions such as providing intercellular communication make

the biomedical use of exosomes possible in both therapeutic and diagnostic applications. However, significant barriers to therapeutic usage of exosomes exist today, such as the fact that they are collected as a heterogeneous population from body fluids, the mechanisms of protein and RNA classification in exosomes are still not well understood, and the absence of sensitive procedures for isolation. A better understanding of exosome biology will help overcome these problems and develop novel therapeutic strategies. At the same time, with the development of techniques that will provide more sensitive miRNA detection, the possible power of exosomal miRNAs in the diagnosis of disease will increase and they will be used more effectively and broadly in the clinic areas.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Kahlert C, Kalluri R. Exosomes in tumor microenvironment influence cancer progression and metastasis. J Mol Med (Berl). 2013 Apr;91:431-7.
2. Willis GR, Kourembanas S, Mitsialis SA. Toward Exosome-Based Therapeutics: Isolation, Heterogeneity, and Fit-for-Purpose Potency. Front Cardiovasc Med. 2017 Oct 9;4:1-13.
3. Yu LL, Zhu J, Liu JX, Jiang F, Ni WK, Qu LS, et al. A Comparison of Traditional and Novel Methods for the Separation of Exosomes from Human Samples. Biomed Res Int. 2018 Jul 26;2018:1-9.
4. Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014 Aug;29:1-144.
5. 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 Sep 22;15:6917-34.
6. Keerthikumar S, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, et al. ExoCarta: A Web-Based Compendium of Exosomal Cargo. J Mol Biol. 2016 Feb 22;428:688-92.
7. Ocansey DKW, Zhang L, Wang Y, Yan Y, Qian H, Zhang X, et al. Exosome-mediated effects and applications in inflammatory bowel disease. Biol Rev Camb Philos Soc. 2020 Oct;95:1287-307.
8. Xia X, Wang Y, Huang Y, Zhang H, Lu H, Zheng JC. Exosomal miRNAs in central nervous system diseases: biomarkers, pathological mediators, protective factors and therapeutic agents. Prog Neurobiol. 2019 Dec;183:101694.
9. Han C, Sun X, Liu L, Jiang H, Shen Y, Xu X, et al. Exosomes and Their Therapeutic Potentials of Stem Cells. Stem Cells Int. 2016; 2016:1-11.
10. Zhang M, Viennois E, Xu C, Merlin D. Plant derived edible nanoparticles as a new therapeutic approach against diseases. Tissue Barriers. 2016 Feb 11;4:e1134415.
11. Rome S. Biological properties of plant-derived extracellular vesicles. Food Funct. 2019 Feb 20;10:529-38.
12. Pérez-Bermúdez P, Blesa J, Soriano JM, Marcilla A. Extracellular vesicles in food: Experimental evidence of their secretion in grape fruits. Eur J Pharm Sci. 2017 Feb 15;98:40-50.
13. Munagala R, Aqil F, Jeyabalan J, Agrawal AK, Mudd AM, Kyakulaga AH, et al. Exosomal formulation of anthocyanidins against multiple cancer types. Cancer Lett. 2017 May 1;393:94-102.
14. Özkan İ, Koçak P, Yıldırım M, Ünsal N, Yılmaz H, Telci D, et al. Garlic (Allium sativum)-derived SEVs inhibit cancer cell proliferation and induce caspase mediated apoptosis. Sci Rep. 2021 Jul 20;11:14773.
15. Yan W, Jiang S. Immune Cell-Derived Exosomes in the Cancer-Immunity Cycle. Trends Cancer. 2020 Jun;6:506-17.
16. Whiteside TL. Tumor-Derived Exosomes and Their Role in Cancer Progression. Adv Clin Chem. 2016;74:103-41.
17. Hejrati A, Hasani B, Esmaili M, Bashash D, Tavakolinia N, Zafari P. Role of exosome in autoimmunity, with a particular emphasis on rheumatoid arthritis. Int J Rheum Dis. 2021 Feb;24:159-69.
18. Tauro BJ, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, et al. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods. 2012 Feb;56:293-304.
19. 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 Sep 22;15:6917-34.
20. İpek Konaklı M, Atasoy Ö, Erbaş O. Intranasal applications in Alzheimer's treatment. D J Med Sci 2020;6:157-65.
21. Konadu KA, Huang MB, Roth W, Armstrong W, Powell M, Villinger F, et al. Isolation of Exosomes from the Plasma of HIV-1 Positive Individuals. J Vis Exp. 2016 Jan 5:53495.
22. Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta. 2012 Jul;1820:940-8.
23. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes. Cells. 2019 Apr 3;8:307.
24. Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharmacol Sin. 2017 Jun;38:754-63.
25. Mehryab F, Rabbani S, Shahhosseini S, Shekari F, Fatahi Y, Baharvand H, et al. Exosomes as a next-generation drug delivery system: An update on drug loading approaches,

characterization, and clinical application challenges. Acta Biomater. 2020 Sep 1;113:42-62.

26. Han Y, Jones TW, Dutta S, Zhu Y, Wang X, Narayanan SP, et al. Overview and Update on Methods for Cargo Loading into Extracellular Vesicles. Processes (Basel). 2021 Feb;9:356.