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Advances in Exosomes-Based Drug Delivery Systems

Carmen Gutierrez-Millan, Clara Calvo Díaz, José M. Lanao, and Clara I. Colino*

Exosomes, a subgroup of extracellular vesicles, are important mediators of long-distance intercellular communication and are involved in a diverse range of biological processes such as the transport of lipids, proteins, and nucleic acids. Researchers, seeing the problems caused by the toxic effects and clearance of synthetic nanoparticles, consider exosomes as an interesting alternative to such nanoparticles in the specific and controlled transport of drugs. In recent years, there have been remarkable advances in the use of exosomes in cancer therapeutics or for treating neurological diseases, among other applications. The objective of this work is to analyze studies focused on exosomes used in drug delivery system, present and future applications in this field of research are discussed based on the results obtained.

1. Introduction

It has been said that an ideal drug delivery system should specifically deliver the drug, evade recognition and premature degradation by the immune system and even control the release of the drug depending on specific stimuli.[1] In the last decade, the development of new nanoparticle-based vehicles has become very important, as such systems can improve the therapeutic efficacy of certain drugs by allowing controlled access and administration to target cells. However, there are two main problems associated with synthetic nanoparticles: the cytotoxicity of the material and its rapid elimination by the phagocytic mononuclear system.[2,3] Research on extracellular vesicles has increased in recent years. These vesicles are secreted by almost all cells and are involved in multiple functions of great importance in the clinical field, such as intracellular signaling.[4] There is no precise definition for any of these groups of extracellular vesicles and there is still a certain amount of debate about their exact classification. Nevertheless, distinctions between exosomes and other vesicles can be established based on their endosomal origin, size, content, density, and form of release. Exosomes are small extracellular vesicles with diameters ranging from 30 to 150 nm, and are involved in immune response, coagulation, signaling and cell migration functions.[5]

The use of exosomes as drug carriers has certain advantages over other systems thanks, among other things, to the variety of adhesion proteins they present on their surface.[6] These advantages include specificity, safety, stability and possibility of transport of substances over long distances. Their biological origin reduces the immune response compared to that produced by other synthetic systems and allows them to cross almost all biological membranes including the blood brain barrier.[7] Exosomes confer such remarkable and advantageous properties in this field that different strategies have been explored to obtain synthetic or hybrid exosome-like delivery nanoplatforms. These systems have been proposed with the aim of overcoming some of the inherent drawbacks of exosomes due to their biological origin and lack of knowledge about these novel systems. Nevertheless, this mimicking systems are not yet able to emulate the complexity and functionality of native exosomes.[8,9]

As of now, several small molecules, both hydrophobic and hydrophilic, have already been incorporated into exosomes, presenting an increase in the amount of drug accumulated inside target cells and the time of circulation in the blood, as well as an improvement in molecular stability.[3] However, their use remains limited due to the difficulty of isolating them from culture medium, which involves a long and complex multi-stage process.[1]

This work aims to address exosome applications in the field of drug delivery, analyze the different kinds of exosomes based on their origin and explore the future potential applications of these extracellular vesicles in treatments involving precise and controlled release of drugs.

2. Exosomes: Definition and Concept

Exosomes were first discovered by P. Stahl[10] and R. Johnstone,[11] while observing globules expelled from mature rat and sheep erythrocytes to the extracellular matrix.[12] Exosomes are endocytic vesicles composed of bilayer membranes from their originating cell. The membrane composition of exosomes allows them to fuse with the receptor cells and release their contents inside. They were initially considered to be receptacles of cell waste biomaterial with no particular function. Although important studies on extracellular vesicles were developed before 1990,[13] the main studies demonstrating the immune function of exosomes were developed in the mid-1990's.[14] Since then, numerous studies have demonstrated the role that exosomes play in intercellular communication, being involved

Dr. C. Gutierrez-Millan, C. Calvo Díaz, Prof. J. M. Lanao, Dr. C. I. Colino
Area of Pharmacy and Pharmaceutical Technology
Department of Pharmaceutical Sciences
University of Salamanca
Salamanca 37007, Spain
E-mail: jmlanao@usal.es
Dr. C. Gutierrez-Millan, Prof. J. M. Lanao, Dr. C. I. Colino
The Institute for Biomedical Research of Salamanca (IBSAL)
Salamanca 37007, Spain
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mabi.202000269.

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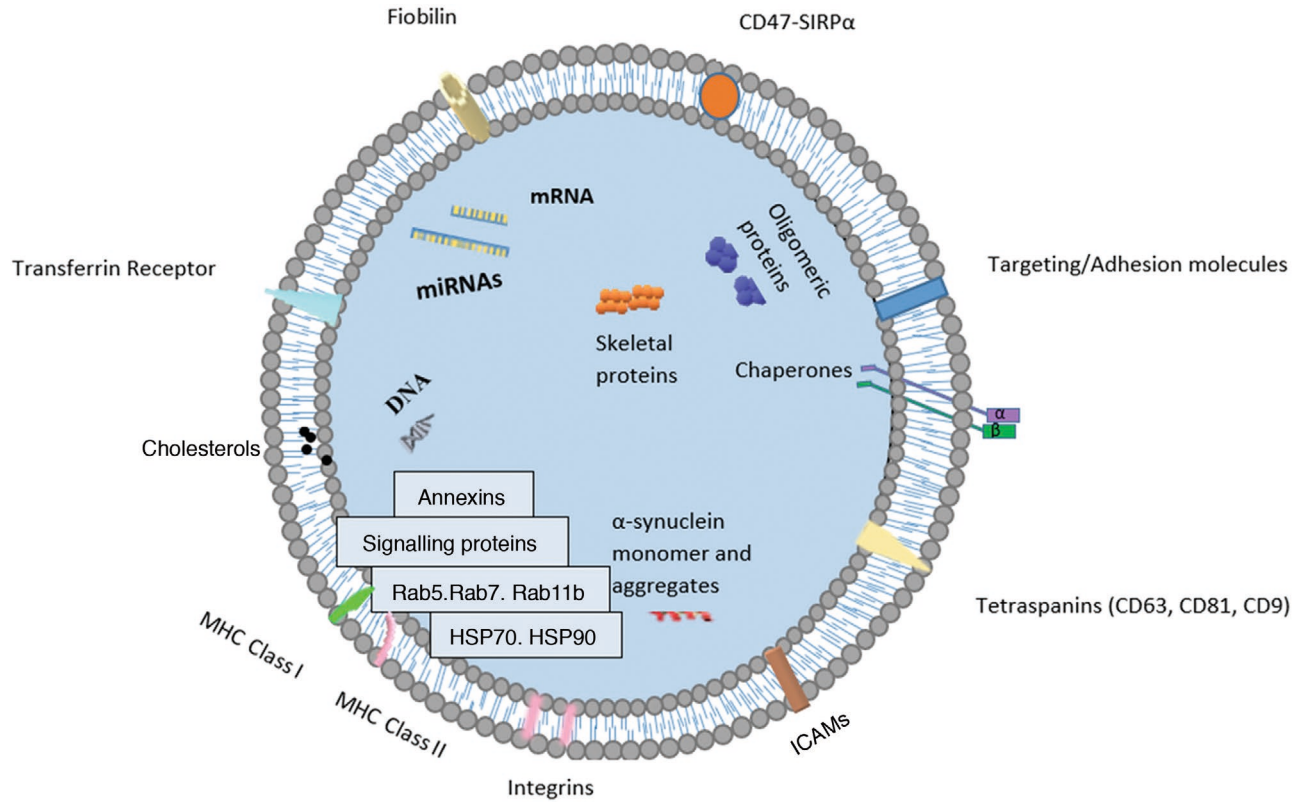

Figure 1. Structure of an exosome. Reproduced under the terms and conditions of the Creative Commons Attribution (CC BY) license 4.0.[19] Copyright 2020, The Authors, published by MDPI.

in important biological processes such as lactation, cell proliferation, inflammation, immune response or neuronal function. In addition, they are involved in certain diseases such as diabetes or atherosclerosis, neurodegenerative processes and cancer.[15–20]

3. Exosome Composition

The composition of exosomes varies depending on their origin, meaning that the cell from which they come is a critical factor in determining the functions they perform in the different biological processes in which they are involved. The ExoCarta database contains information on discoveries that have been made regarding the composition of exosomes. The latest recorded information from Exocarta compiles data from 134 studies that have already isolated exosomes of eight different organisms and 93 different cell types. In addition, 4563 types of proteins, 1639 messenger RNA (mRNA), 764 micro-RNA (miRNA), and 194 different lipids have been found.[21]

There are molecules specific to one or more types of exosomes and their combination gives rise to multiple different kinds of exosomes.[22] **Figure 1** shows several of the variations that can be found amongst exosomes.

Regarding proteins, although they vary depending on the exosomal origin, there are certain groups that are present in all types of exosomes: proteins involved in the biogenesis of multivesicular bodies, cytosolic proteins, proteins of thermal shock, tetraspanins and proteins involved in specific functions, such as transport or intracellular communication mediated by the surface receptors of the target cell. Proteins have even been used as markers for the identification and isolation of exosomes, although the effect that the choice of isolation method can have on the observed protein profile must be also considered.[23]

Lipids are the main component of exosomes and, as with proteins, their composition also depends on the exosome's origin. Even if they do not exactly reproduce the composition of the original cell plasma membrane, the lipidic composition of exosomes includes cholesterol, short-chain saturated fatty acids, waxes and phosphoglycerids.[24,25] Finally, in relation to the genetic material present in exosomes, it has been found that exosomes usually carry a significant load of RNA. Exosomes transfer miRNA to other cells promoting transcriptional regulation of certain proteins involved in numerous biological processes.[18,26–28]

4. Formation of Exosomes

In vivo exosomes are found in many biological fluids such as blood, urine, semen, breast milk, saliva, amniotic fluid, bile, bronchoalveolar lavage fluid, synovial fluid, and cerebrospinal fluid.[23,29]

It has been demonstrated that most cells, including immune cells, hematopoietic cells, epithelial cells, neurons, oligodendrocytes, Schwan cells, tumor cells, platelets, and adipocytes among others, are able to secrete exosomes. It is estimated

that up to 80% in vivo circulating exosomes are derived from platelets.[23,29,30]

The origin of exosomes determines their structure, components, functions and potential, making these vesicles as diverse as the types of cells that secrete them.[30]

Most studies are focused on the effects induced by the delivery of exosome materials to the receptor cell, but it must be also noted that their secretion is a mechanism of material evacuation from producing cell that alters their characteristics and homeostasis, with some authors suggesting that the alteration of this process could be critical in pathogenesis development.[31] It has been demonstrated that the rate of exosome biogenesis is related to the stimuli or stress factors to which the secreting cell is submitted, and therefore to their physiological or pathological status.[23]

This large group of secretory cells not only secrete exosomes but also other vesicles. Extracellular vesicles can be classified into three main groups according to their size, contents and role: microvesicles, apoptotic bodies, and exosomes.

Apoptotic bodies are clearly different from other vesicles since they are only released from cells that undergo the apoptosis process whereas other vesicles are more similar. Exosomes and microvesicles are both extracellular vesicles composed of an aqueous nucleus coated with a lipid bilayer. Despite the similarities between these two types, they differ from each other in the biogenesis, size, surface markers and the biological functions in which they are involved.[13,32]

One of the main differences is that, in contrast to microvesicles, exosomes are preformed vesicles stored inside multivesicular bodies which fuse with the cell membrane for secretion, whereas microvesicles are shed directly from plasma membrane.[33,34]

Exosomes can originate naturally via two pathways, namely, the classical pathway and the direct pathway.

The classical or endocytic pathway of exosome formation is the most widely studied route and consists of three different stages. In a first step, there is an inward budding of the plasma membrane, giving rise to endocytic vesicles (early endosomes that mature into late endosomes). The second stage consists of the formation of multivesicular bodies (MVB) containing many intraluminal endosomic vesicles (ILV). The main mechanism of ILV and MVB formation is dependent on the endosomal sorting complex required for transport (ESCRT). This pathway consists of a series of steps implying four complexes that lead to the recognition, engagement and concentration of transmembrane proteins, cargo sorting, membrane deformation with bud formation and finally vesicle scission.[35,36] Although this is considered the most common pathway, MVB and ILV formation can also take place via ESCRT-independent mechanisms,[35,37] such as ceramide-dependent mechanism[38] or mechanisms involving other molecules such as tetraspanins[39] or lipopolysaccharide induced TNF factor (SIMPLE)[40] among others.[35–37,41,42]

Finally, the formed multivesicular bodies can fuse with lysosomes to degrade their contents or with the plasma membrane, which leads to a release of intraluminal endosome vesicles into the extracellular space in a process of exocytosis, forming the exosomes. The evolution of MVBs to secretory or degradative bodies can be responsive to cellular conditions and the mechanism of transport of the secretory MVBs for docking to the plasma membrane can be specific of the cell type.[43]

These released vesicles constitute the exosomes and can carry out the transport of different molecules to nearby cells or to organs away from the cells of origin,[4,5,15,27,44] although it has also been observed that a certain proportion of exosomes could remain attached to the cell surface in certain types of secreting cells, probably with a signaling function.[43]

This process of exosome biogenesis and some other characteristics allow exosomes to be distinguished from other extracellular vesicles whose exocytosis occurs via outward budding of the plasma membrane.[45]

On the other hand, some exosomes can be produced by the so-called direct pathway. It has been demonstrated that some cells, such as the Jurkat T cells release exosomes directly from their plasma membrane, either spontaneously due to HIV Gag (group-specific antigen) or Nef (negative factor) expression, or after surface receptors binding.[4]

5. The Role of Exosomes

Different functions have been ascribed to exosomes depending on their origin, contents, type and the physiological state of their progenitor cell.[27,46] The first exosome studies pointed out these extracellular vesicles as a mechanism for removing unnecessary excess proteins from reticulocytes.[47] Nevertheless, many studies have shown that they are specialized in intercellular communication at a great distance, being an efficient, robust and economical mechanism for exchanging information among cells.[2]

Exosomes from a cell type can interact with other cells using specific receptors, resulting in direct target cell stimulation. Functional proteins, genetic material or transcription factors can be transferred.[26]

It is also known that exosomes may exert opposing functions in the target cell, depending on the tissue and the composition of their content. Their role in intercellular communication has been especially highlighted in the last decade with the discovery that they are responsible for the control of both normal physiological processes and the progression of certain pathologies such as cancer or neurodegenerative processes.[18]

Thus, highly heterogeneous functions have been attributed to exosomes. Whereas exosomes from antigen-presenting cells can promote both the innate and specific immune response, it has been demonstrated that those from oligodendrocytes favor the remyelination of central nervous system neurons and that exosomes isolated from tumor cells have the ability to promote the oncogenesis of healthy cells.[27,46]

Although the mechanisms by which exosomes control functions in cells are not yet fully understood, it seems clear that vesicular content delivery, exosome-cell interaction or both are necessary. Based on this, the success of exosomal applications requires the effective delivery of the vesicular contents. This can be achieved by receptor-ligand interactions, direct fusion of membranes or via endocytosis:[48]

Communication mediated by membrane receptors can cause the fusion of exosomes and cell membranes (leading to the exchange of proteins and components present in the cytosol) or

allow the interaction to induce the activation of signal transduction molecular pathways that trigger a specific effect.[48]

Endocytosis can be mediated by clathrin, caveoline or phagocytosis, mainly by phosphatidylserine and micropinocytosis.[49] This mechanism is similar to receptor-mediated fusion with the difference that it involves the formation of multivesicular bodies in which the vesicular contents can be released via direct membrane fusion or via the participation of lysosomes. In this route, transcytosis processes that do not involve release of the exosomal contents inside the cell may also take place.[50]

6. Main Types of Exosomes with Applications in Drug Delivery

As previously mentioned, exosomes can be derived from various cell types and be isolated using different methods, and not all the possibilities have been thoroughly investigated. Mesenchymal cells (MSCs) and their exosomes,[51,52] tumor cell exosomes,[53] immune cell derived exosomes[54] and derivatives of bovine milk[55] are some of the most extensively studied fields, the first two because of their promising therapeutic functions and the third type as a possible way to obtain large-scale exosomes economically. This section reviews these three types of exosomes and their current and potential applications in therapeutics.

6.1. Mesenchymal Stem Cell Exosomes

The search for new therapies within the field of cell therapy has led researchers to evaluate the numerous therapeutic applications of mesenchymal stem cells (MSCs). These cells are multipotent and can be found in various tissues in adults, such as adipose tissue, liver periosteum, lung, spleen, muscle connective tissue, placenta, amniotic fluid, and aborted fetal tissues. Regarding the exosomes derived from them, some authors have shown that most of the functions of these cells can be attributed to their exosomes, including their immunomodulatory capacity. What's more, these vesicles can be isolated and purified at a relatively low production cost while maintaining potency and functionality as long as they work with immortalized cell lines.[51,52] Currently, mesenchymal stem cells are the only known cells capable of producing exosomes at a large scale.[51]

Large-scale production can be achieved by obtaining and subsequently culturing mesenchymal stem cells in vitro. Since the ex vivo expansion capacity of this cell line is finite and in view of the fact that the costs of obtaining a new cell line and its validation would be economically unsustainable, their immortalization through the oncogene myc has been approved. It has been demonstrated that, although such immortalization compromises the potential for differentiation of MSCs, it does not affect the production or therapeutic efficacy of the obtained exosomes.[56]

Mesenchymal cells and their exosomes are also promising in the development of new cancer treatments, since the exosomes released influence the progression of the disease by exchanging genetic information among cells and thus acting as paracrine mediators. Exosomes derived from mesenchymal cells are involved in tumor growth, angiogenesis, metastasis and tumor invasion. In addition, new studies suggest that they are responsible for certain drug multiresistance mechanisms.[51]

Figure 2 shows the biogenesis and application in cancer therapy of MSC-derived exosomes.[51]

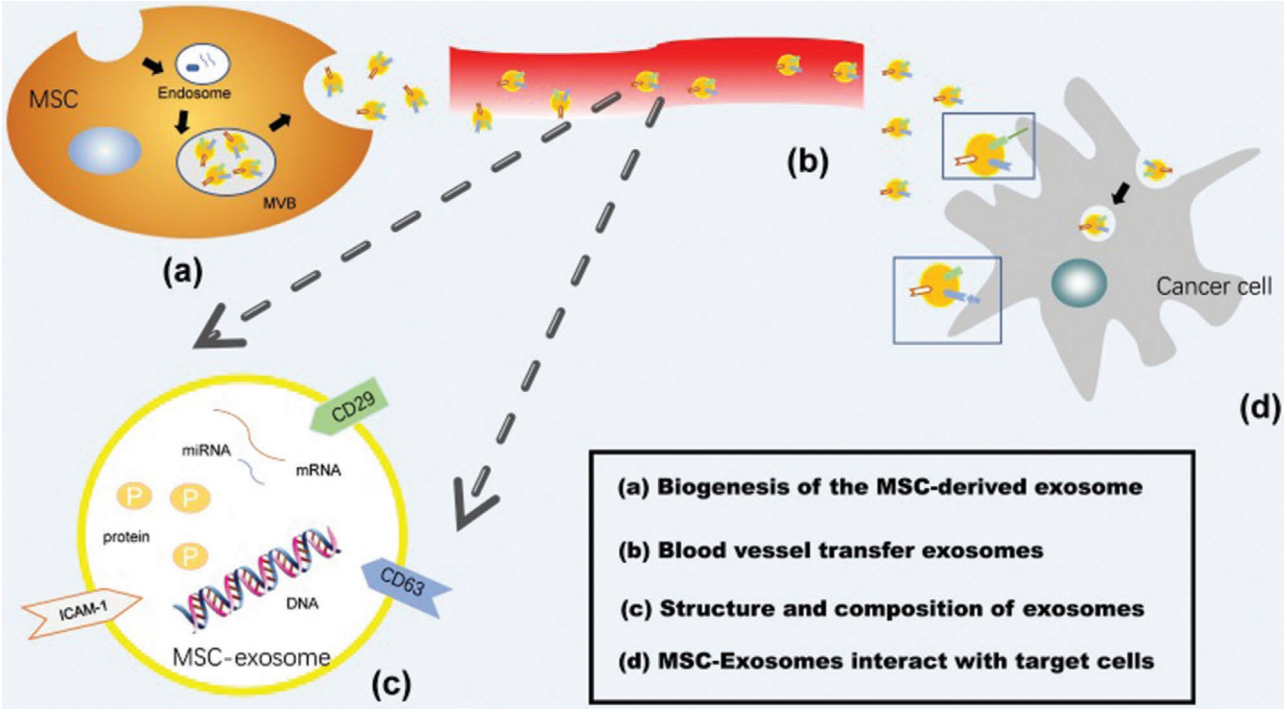

Figure 2. Biogenesis and application for cancer therapy of MSC-derived exosomes. Reproduced with permission.[51] Copyright 2018, Ivyspring International Publisher.

It could be said that basic research on mesenchymal cell-derived exosomes has only just begun, and that the first results are promising, as they demonstrate that these exosomes will be able to be used as transporters of biologically active molecules from progenitor cells to target cells. These cells are in many cases tumor cells or cells from immunological diseases. The functions of mesenchymal stem cells and therefore of the exosomes derived from them are to control the activation/deactivation of various pathways involved in the suppression of tumor growth or the influence on leukocyte proliferation. All this information suggests the evident potential future applications that MSC-derived exosomes could have.[51]

6.2. Exosomes Derived from Tumor Cells

Exosomes releases from tumor cells have an important regulatory role in the progression of metastasis, mediating the entire tumor progression process, from the early stages to colonization of distant organs.[57,58]

Both surface proteins and those found inside exosomes are specific to the type of cell from which they come. Thus, tumor cell derived exosomes contain specific antigens from the tumor cell on their surface, making them ideal candidates in the search for new therapies for the prevention, diagnosis and/or treatment of various cancers.[27] In addition, exosomes derived from tumor cells are involved in modulating growth, metastasis and immunity against certain treatments.[53]

The use of these exosomes as specific transporters of proven drugs already in use has been studied. The advantages of administering drugs via this route instead of the usual one reduced side effects thanks to its more controlled and specific release to tumor cells. Moreover, the exosomes themselves contain biologically active molecules of the tumor cells from which they come. These molecules are involved in routes related to the suppression or progression of tumor proliferation. The exosomes themselves have been studied for disease control by using them to suppress the determining pathways of cell proliferation.[59]

6.3. Exosomes Derived from Immune Cells

Exosomes from a wide range of different immune cells and their potential applications in therapeutics have been explored, with a particular focus on the dendritic cells and macrophages.[54,60,61] Immunotherapy[62] and antitumoral therapeutics are undoubtedly where exosomes derived from immune cells have been studied most thoroughly,[63–65] revealing their great potential and even leading to the development of possible vaccines based on these vesicles.[66,67]

Among the different immune cells from which exosomes are obtained, dendritic cells are one of the most studied cell types, since dendritic cell derived exosomes have been shown to be safe and to have low immunogenicity. Some studies have even alluded to the possibility of using impersonalized exosomes.[68,69] Dendritic cell derived exosomes can be used as immunotherapeutic agents due to their immunomodulatory role, participating in both immune surveillance and tumor promotion processes.[70,71] As drug vehicles, exosomes derived from immature dendritic cells from mice have been studied for doxorubicin loading, demonstrating their capacity to induce inhibition of mice solid tumors growth without apparent toxicity.[68]

In the search for ways to improve antitumor therapeutics, exosomes derived from macrophages have also been explored as carriers of antineoplastic drugs, taking advantage of the important role of macrophages in development of cancer.[72] These systems have been proposed as an alternative for the treatment of multiple drug resistant (MDR) cancers. Thus, macrophage derived exosomes loaded with doxorubicin have demonstrated a greater drug accumulation in MDR cells compared to the free drug while others loaded with paclitaxel have demonstrated, both a higher accumulation than synthetic nanocarriers and an inhibition of pulmonary metastases in a murine model.[73,74]

6.4. Milk-Derived Exosomes

One of the main limitations in the implementation of exosomes for the treatment of diseases is the difficulty of producing them on a large scale in an economically feasible way. In this context, milk-derived exosomes, present in human breast and cow's milk, are presented as a convenient source of exosomes which can be used as drug transporters in humans. Exosomes have been isolated from 2–3 days postpartum colostrum.[55]

It must be taken into consideration that the biological function of the exosomes present in milk is not yet well established, but it is known that they fulfill certain functions, including the transfer of antibodies from the mother to the fetus during the first days of life. Furthermore, it was proved that the miRNA molecules included into milk exosomes promote the development of gastrointestinal tract and immune system of children. Both, human and bovine milk contain plenty of miRNA molecules implicated in DNA methylation and regulation of several via of genes expression. For all these reasons, it has been suggested that they will be applied in the treatment of allergy, cancer and other chronic diseases.[75,76]

MicroRNA-155 (miR-155) is a multifunctional miRNA enriched in cells of the immune system and play a role in the immune response.[77] The **Figure 3** shows the transmission of miR-155 exosomes from cow's milk, breast milk and enhanced prenatal LPS-induction in the stimuli for thymic regulatory T-cells (Tregs) that plays an important role in immune homeostasis and reduces the risk of atopic diseases in a farm environment.

7. Exosomes Isolation

There are numerous methods for the isolation of exosomes from either cells or biological fluids based on their shape, density, size or surface components (**Table 1**). However, this is a difficult task and currently there is no suitable general procedure. In fact, the properties of exosomes depend on their method of isolation.[79,80] Some of the methods implemented achieve high yields but lack specificity while others are very specific but have low yields, as recognized by the MISEV2018 (Minimal

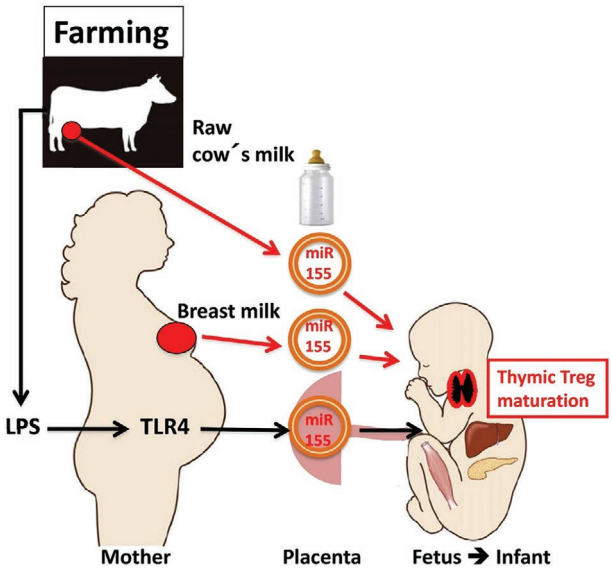

Figure 3. Role of milk exosomes as micro RNA transmitters in the atopy protective effect in a farming environment. Reproduced with permission.[78] Copyright 2014, Springer Nature.

information for studies on extracellular vesicles),which uses these criteria for its classification.[81]

Differential centrifugation with ultracentrifugation allows for separation based on size, density, and shape. It consists of successive steps of centrifugation at 4 °C with increasing speed in order to eliminate dead cells, cell debris and contaminating proteins, after which exosomes are obtained via ultracentrifugation at forces ranging from 100 000 × *g* to 200 000 × *g*.[79,82,83] This is by far the most frequently used exosome isolation method and it is considered the gold standard to which all other procedures are compared. Indeed, many of the other methods include an initial centrifugation-cleaning step. However, it does not work well for highly viscous samples,[84,85] and high centrifugation velocities and times may cause a loss of viable exosomes and some of their components. Moreover, exosomes have low purity,[79] especially when they are obtained from plasma.[84] In order to improve the purification process, additional centrifugation steps[85] or density gradients with iodoxinol o sucrose have been developed.[86] In another variant, the sucrose cushion method separates exosomes via ultracentrifugation in a 30% sucrose solution. An increased yield of exosomes has been claimed for this method in comparison to differential ultracentrifugation. Both methods also show differences in the size distribution and surface markers of exosomes.[79,80]

Sequential filtration methods allow the exosomes to be isolated by size using preliminary steps of filtration through highly porous membranes in order to eliminate cells and debris. This is followed by an ultrafiltration step using tangential flow filtration to discard proteins and other large molecules in the eluate. In a third phase, exosomes and microvesicles are separated via filtration through a pore size ≤0.2 µm[87,88] (**Figure 4**). The scalability is the main advantage of this method. Others are similar exosomes size distribution and a higher yield in comparison to ultracentrifugation which can be attributed to the membrane damaging effect of this last method.[88]

Precipitation with salts[89] or polymers (mainly PEG) are well-implemented exosomes isolating processes and there are several kits that use these as their basis.[90] However, as this method is based on protein precipitating reagents, it leads to exosomes with low purity and high protein contaminating concentrations.[90] By the same token, the polyethylene glycol/dextran aqueous two phase system (ATPS) improves exosome purification while maintaining high recovery yields compared to ultracentrifugation.[91]

Table 1. Main methods for exosomes isolation.

Method	Basis	Advantages	Drawbacks
Centrifugation ultracentrifugation	Density, mass, and size	Cost-effective, large sample capacity, easy to carry out with little technical expertise	Low purity, high speed centrifugation may cause aggregation and loss of exosome components, poor reproducibility, long run time
Density gradient centrifugation	Density, mass, and size	Better purification than ultracentrifugation, difficult procedure	Requires expertise
Sequential filtration	Size	Fast, scalable, automatable, minimal physical and chemical alteration	Requires three steps, lack of specificity in separation, possibility of membrane clogging, and membrane vesicles trapping
Precipitation	Solubility alteration	Easy to use, no specialized equipment required, low cost	High yields, potential for contamination with other sample components and precipitating reagents
SEC Chromatography	SEC:Size	Maintains vesicle integrity, excellent reproducibility	Coelution of impurities, limited scalability
Hydrophobic Chromatography	Hydrophobicity	Good purification, allows for quantification	Complex elution process, limited scalability at the moment
Immunoaffinity	Interactions antibodies-exosome surface components	Very specific isolation, easy to use	High costs, lack of reliable markers
Microfluidics	Microchannels handle of samples coupled to immunoaffinity, density or size	Easy to use, high process control	Low processing capacity

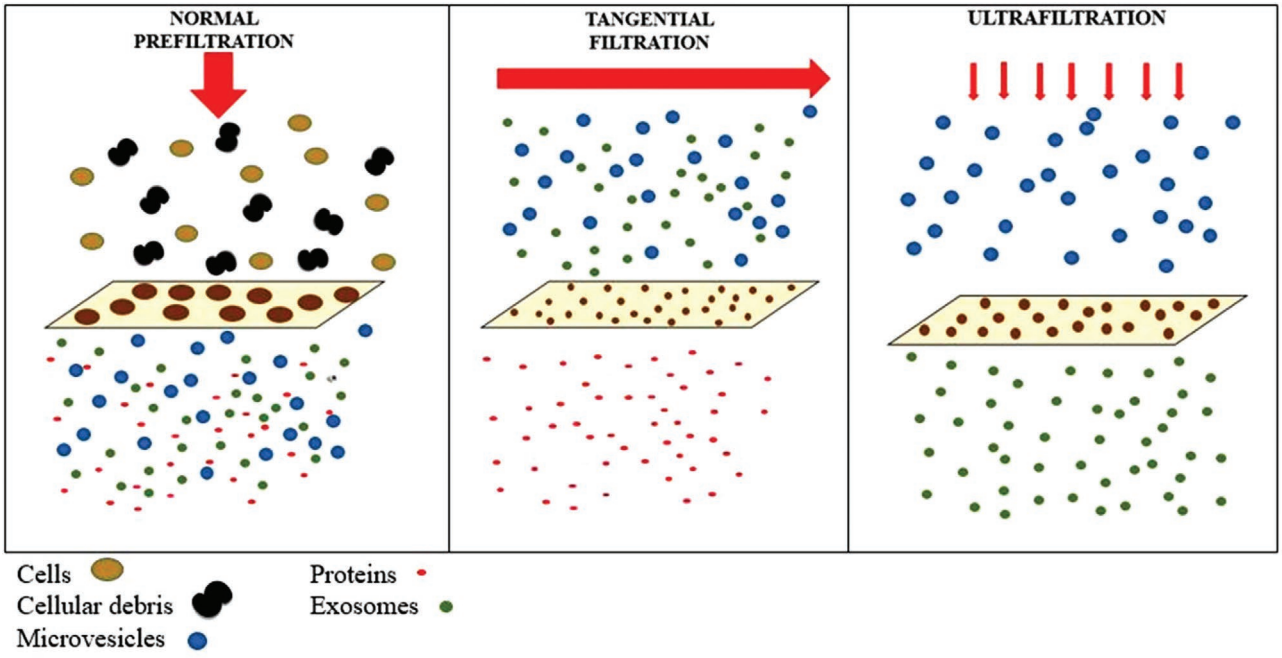

Figure 4. Sequential filtration method for the isolation of exosomes.

The proteins and receptors present on the surface of exosomes allow for the use of specific antibodies or ligands for their isolation in immunoaffinity methods. To implement them, it is necessary to identify common exosome surface components, such as the membrane protein trespanin CD63, and immobilize the corresponding antibodies in an adequate platform.[92,93] Magnetic microbeads facilitate the isolation via magnetoimmnunoaffinity.[94] Furthermore, the use of defined antibodies allows for the isolation of specific exosomes subpopulations.[95] It must be noted that with these methods only exosomes that express the protein complementary to the used antibody are isolated. However, the surface components of exosomes are variable, even when they have the same origin[86] which may complicate the optimization of these methods.

Exclusion chromatography (SEC) also allows exosome isolation using stationary phases of appropriate pore size.[96] This method preserves the structure and contents of the exosomes; however, large proteins or aggregates from serum samples may coelute with exosomes.[49] For this reason, SEC is frequently used as a last step for exosomes purification after ultrafiltration[52,76] or ultracentrifugation.[97] Better results have been claimed for hydrophobic chromatography. The basis of this technique is the interaction of the vesicle with the stationary phase due to its superficial hydrophobicity and has been applied to protein separation. The descendent salt concentration gradient of the mobile phase allows the more hydrophilic proteins to elute before the exosomes that are more hydrophobic. UV detector absorbance shows the amount of light scattering caused by nanoparticles and allows not only for separation but also for the quantification of exosome concentration from different sources with a view to extrapolate to a preparative scale in the future.[98]

Finally antibodies linked to a column allow for exosomes separation by immunoaffinity chromatography.[92]

Recently, microfluidic devices are used in exosome isolation[99,100] (**Figure 5**). Their basis is the controlled handling of the sample through capillary tubes associated to different isolation mechanisms. Size separation is achieved via lateral displacement devices[101] or elastic lift forces in a polyoxyethylene medium[102] or with the application of external forces such as electric (dielectrophoresis)[103] and acoustic fields.[104] Microfluidic technology can also be combined with immunoaffinity by including antibodies for different exosome components in the process.[105] Microfluidic-based systems are proposed in the nanoparticles and liposomes manufacturing and homogenization processes. However, in the exosomes field this technology has mainly evolved to the isolation and identification of exosomes from small samples and the various commercial kits developed are not suitable for large scale exosome isolation.

The type of starting material and the intended use have a major influence on the choice of exosome isolation method.[106] The original sample from which the exosomes are extracted affects the success of vesicle isolation. Ultracentrifugation may be an adequate method for exosomes derived from tissue cultures. Plasma or tissues are very complex samples and contamination with proteins is a drawback. The isolation of exosomes from these matrices could benefit from the combination of various methods such as centrifugation, protein digestion, ultrafiltration, and size exclusion chromatography, which may improve the purity of exosomes.[107] However, additional steps complicate and slow down the process and may lead to low recovery yields.

In summary, despite the important advances of recent years, currently there is no ideal general method for exosome isolation, and it is necessary to select the one that ensures that vesicles will have the adequate characteristics for the application for which they are intended. The use of exosomes for diagnosis requires methods that allow for the isolation of exosomes

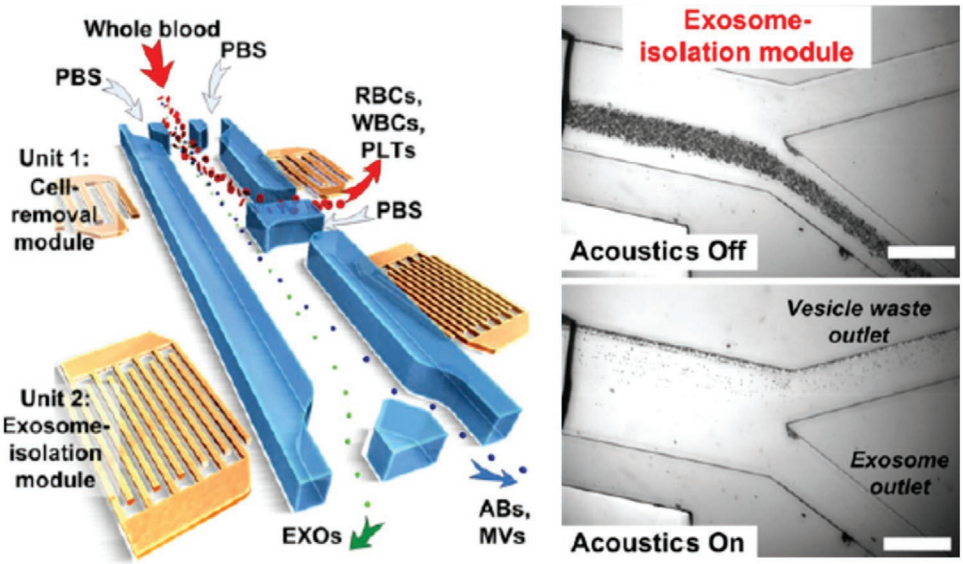

Figure 5. Schematic illustration of integrated acoustofluidic device for isolating exosomes. The cell-removal module filters red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs), and then the exosome-isolation module separates subgroups of extracellular vesicles (ABs: apoptotic bodies; EXOs: exosomes; MVs: microvesicles). Adapted with permission.[104] Copyright 2017, National Academy of Sciences.

of enough purity from very low sample volumes. Various kits based on the above summarized methods have been developed to this end.[108] From the perspective of developing exosomes for drug delivery, size homogeneity is a crucial property as it will determine the biodistribution of the vesicles. However, the behavior of the exosomes also depends on the tropism caused by superficial molecules in the exosome membrane that are responsible for its vectorization, thus this membrane integrity must also be preserved. Moreover, large-scale isolation methods with high yields of recovery are necessary, as well as robust procedures that ensure the reproducibility of the results and GMP compliance. None of the present methods meets these requirements, so there is still work to do to achieve those goals.

Table 1 compiles the most frequently employed methods and their main characteristics.[30,109]

8. Exosomes as Drug Delivery Systems

Several authors have studied the delivery of a wide range of molecules, from drugs to nucleic acids and all kinds of proteins, by exosomes obtained from different sources. To load drugs in this type of delivery systems, different loading methods have been used, mainly incubation, sonication and electroporation among others, while cells from different types of tissues such as tumor, eye or brain tissue, among others, have been used as targets.

Short-term studies have shown that oral administration of both single exosomes and modified exosomes containing various types of drugs, are well tolerated and do not result in adverse effects or inflammatory response.[55]

There are many potential applications of exosomes in the biomedical field as seen in the previous sections. Today, therapeutic applications with exosomes are a reality with basic, pre-clinical and clinical research studies, showing promising results in their use for the diagnosis and treatment of cancer, neurological, immunological or infectious diseases among others, and in general for use in the specific and controlled transmission and release of drugs. This section reviews the existing results and analyses the possibilities that remain open in the light of them.

8.1. Drug Loading Methods

Exosomes can incorporate bioactive compounds inside or associated to their surface molecules. Their amphiphilic nature allows their load with both hydrophobic and hydrophilic molecules. It is possible to obtain exosomes with a cargo by incubating parental cells with the active molecules of interest, which are included in the exosomes secreted by these cells.[110] Overexpression of RNA in exosomes has also been achieved via transfection in parental cells using lipidic vectors.[102,111,112]

However, the process is ordinarily carried out in the isolated exosomes and there are several active and passive processes that aim to improve encapsulation efficiencies (**Table 2**). Simple incubation of exosomes with a drug that penetrates due to a concentration gradient usually leads to low encapsulation efficiencies.[73] The rate of encapsulation with this passive strategy is strongly dependent on the characteristics of the drugs such as their lipophilicity and degree of ionization. This method is adequate for some hydrophobic drugs that diffuse through the membrane.[113] In order to obtain better results with hydrophilic compounds, these are modified with molecules such as cholesterol to make them more lipophilic, thus facilitating their internalization.[114] Creating a pH gradient is another strategy for promoting the internal accumulation of the compound of interest that must be mainly in molecular form in the outer medium and in ionized form in the inner exosomes medium. However, the encapsulation efficiency achieved by this strategy is low.[115]

Table 2. Loading efficiencies achieved with different methods.

Drug	Exosomes origin	Method	Loading efficiency %	Ref.
Triptolide	Ovarian cancer cell	Sonication	76.5±1.8	[124]
Paclitaxel	RAW 264.7 macrophages	Incubation	1.44±0.38	[73]
Paclitaxel	RAW 264.7 macrophages	Electroporation	5.3±0.48	[73]
Paclitaxel	RAW 264.7 macrophages	Sonication	28.29±1.38	[73]
Catalase	RAW 264.7 macrophages	Incubation	4.9±0.5%	[122]
Catalase	RAW 264.7 macrophages	Saponin	18.5±1.3	[122]
Catalase	RAW 264.7 macrophages	Freeze-thawn	14.7±1.1	[122]
Catalase	RAW 264.7 macrophages	Extrusion	22.2±3.1	[122]
Catalase	RAW 264.7 macrophages	Sonication	26.1±1.2	[122]
Tyrosinase	Serum	Electroporation	28.7±4.3	[123]
Tyrosinase	Serum	Saponin	43.2±8.2	[123]
Tyrosinase	Serum	Electroporation-Saponin	32.2±7.3	[123]

Other methods use active mechanisms that physically create transient openings in the membrane in order to increase membrane permeability. One of these procedures involves several cycles of sonication followed by incubation at 37 °C in order to restore the membranes.[74,116] This method improves encapsulation efficiency compared to simple incubation. Amazingly, the sonication increases the size of exosomes, which has been explained to be a result of surface adsorption of the drug through hydrophobic interactions.[74,116] However, exosome size increases even in the absence of drug.[73]

The electroporation of exosomes allows the drug to enter through transient membrane pores generated by electrical pulses. This approach has been applied to drugs[117] though it has mainly been used to RNA incorporation into vesicles.[118–120] This process also leads to exosomes slightly larger than the initial ones.[117] Although this method may cause RNA aggregation it has been used in clinical protocols.[121]

Coextrusion of a mixture of drug and exosomes combined with cycles of freezing and thawing allows for drug incorporation through the reorganization of vesicles as they pass through a membrane under pressure or thanks to the breaks caused by the freezing steps.[122]

When comparing various methods for the encapsulation of an enzyme, sonication and extrusion show the best encapsulation efficiency. However, the size, shape and functionality of the exosomes are different, with all the charged vesicles (especially with the frozen-thaw method that led to aggregates) increasing in size. Better exosomes integrity is associated to the sonicated exosomes.[122] Similarly, sonication leads to the highest encapsulation efficiency when comparing simple incubation, electroporation and sonication.[73]

Membrane permeabilization is also achieved by chemical methods using detergents such as saponin[113,123] and hypotonic dialysis, that improve the encapsulation efficiency in comparison to the simple incubation.[73] Also, lipidic vectors allow incorporation of RNA into exosomes with therapeutic objectives. This "chemical transfection" leads to higher loads in comparison with electroporation,[118] although the toxicity of these cationic vectors must be taken into account.

In summary, hydrophobic compounds enter the exosome membrane more easily, but for hydrophilic cargo it is necessary to introduce changes in the molecule or increase the membrane permeability through mechanical or chemical methods. When comparing the numerous methods that allow the encapsulation of drugs and genetic cargo in the exosomes, sonication shows the best encapsulation efficiencies (Table 2). The consequences of this treatments in the membrane is an issue that needs further research. Also, as happened with isolation methods, standardization, reproducibility and scalability of encapsulation procedure is mandatory for clinical applications.

8.2. Theranostic Applications of Exosomes in Cancer

Many chemotherapeutic drugs have low aqueous solubility and specificity, so it is necessary the use of specialized delivery vehicles for their administration.

Although conventional therapies such as chemotherapy and radiotherapy remain as first option for cancer treatment, they are not fully effective due to the increasing of drug resistance. So new therapies based on extracellular vesicles, immunotherapy and nanotechnology are the new generation of cancer treatment.[125] In this scenario exosomes appear as a promising via of specific drug delivery to tumor cells. Not only exosomes derived from tumor cells but also those released from other immune cells have demonstrated to modulate tumor microenvironment.[20]

Due to their endocytic origin, exosomes contain RNA, DNA, proteins, lipids, metabolites and other molecules of their origin cells. Using these molecules, exosomes carry information among cells which could be implicated in the development of drug resistance, cancer progression and metastasis.[58]

Classical antitumor drugs also have toxic effects and are involved in immune reactions. Based on this premise, the main limitation of cancer treatment is the maximum quantity tolerated, which implies severe side effects and the need to stop the treatment, leading to possible tumor growth.[73] For all these reasons, there are many authors who are involved in testing

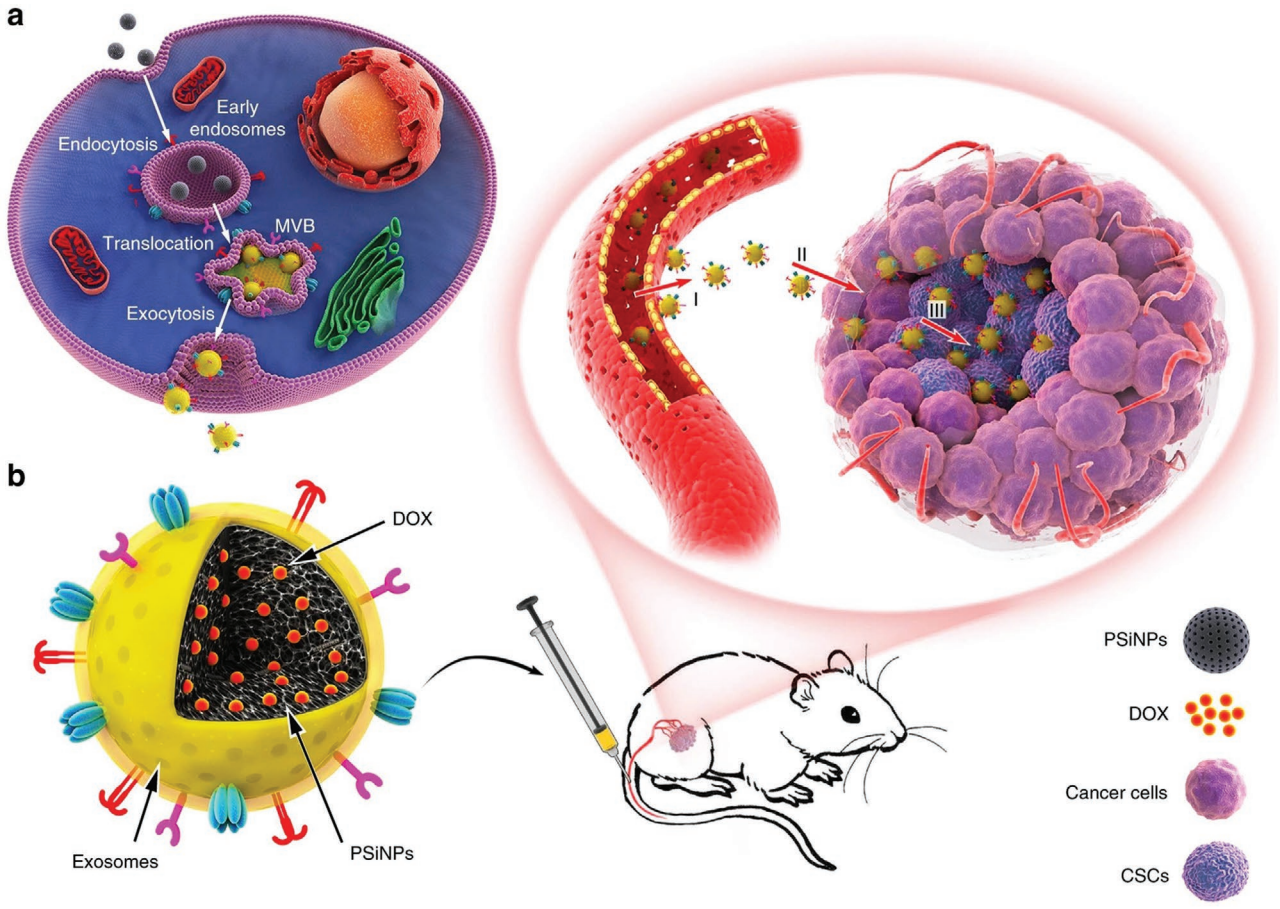

Figure 6. a) Obtention of exosome doxorubicin-loaded silicon nanoparticles DOX@E-PSiNPs). b) Targeting of DOX@E-PSiNPs) to tumor cells in mice. I) DOX@E-PSiNPs accumulate in tumor tissues; II) DOX@E-PSiNPs penetrate into tumor parenchyma; and III) DOX@E-PSiNPs are internalized into bulk cancer cells and cancer stem cells. Reproduced with permission.[127] Copyright 2019, Springer Nature.

the morphology, functionality and efficiency of antitumor drugs when they are encapsulated into exosomes.

One of these drugs is doxorubicin, a chemotherapeutic agent commonly used to treat a broad range of cancers, which also presents significant cardiotoxicity that limits its therapeutic use. Some authors studied the possibility of encapsulating doxorubicin into exosomes to mitigate its side effects and increase its benefits as a therapeutic drug by comparing the results obtained when it is administrated free and when it is encapsulated. In this kind of research, some premises were proved: when doxorubicin was encapsulated in bone marrow MSCs for the treatment of osteosarcoma, there was an increase in tumor accumulation,[100] better cellular uptake and antitumor effects and no cytotoxicity for the osteosarcoma cell line.[126] Moreover, their rapid cellular uptake, redistribution and enhanced potency were proved. No accumulation was shown in the heart, suggesting the potential for limiting the cardiac side effects and improving therapeutic index.[117]

In addition, exosome-biomimetic porous silicon nanoparticles (PSiNPs) have been developed as drug carriers for chemotherapeutic agents such as doxorubicin. First, doxorubicin silicon nanoparticles are obtained by endocytosis into cancer cells after incubation. Later, exosome doxorubicin-loaded PSiNPs (DOX@E-PSiNPs) are obtained via exocytosis of the exosome silicon nanoparticles from tumor cells and used for the delivery of the antineoplastic drug to cancer cells in mice as shown in **Figure 6**.[127]

Another author tested how encapsulating a drug can reduce or prevent its toxicity. In this study, erastin (a low molecular weight chemotherapy drug with poor water solubility and renal toxicity) was encapsulated into exosomes. They showed the drug displayed more efficiency and better inhibitory effect on proliferation and migration.[116] Triptolide loaded exosomes have been tested for the treatment of ovarian cancer. These authors created a triptolide-loaded exosomes delivery system and demonstrated its beneficial effects on proliferation and apoptosis in vitro and in vivo. However, its application in the clinical practice was limited by poor water solubility, hepatoxicity and nephrotoxicity.[124]

Acridine Orange is an acidophilic dye with a strong tumoricidal action which is limited by its potential side effects. The drug was encapsulated into exosomes and the results showed an increase in cytotoxicity as well as an extended drug delivery time to melanoma cells as compared to the free drug.[128] In the same field, some authors have studied the possibility of encapsulating paclitaxel into exosomes. Paclitaxel is a widely used treatment whose effectiveness as a chemotherapy drug is limited by resistance. Other authors studied the consequences of introducing paclitaxel into exosomes. They showed that

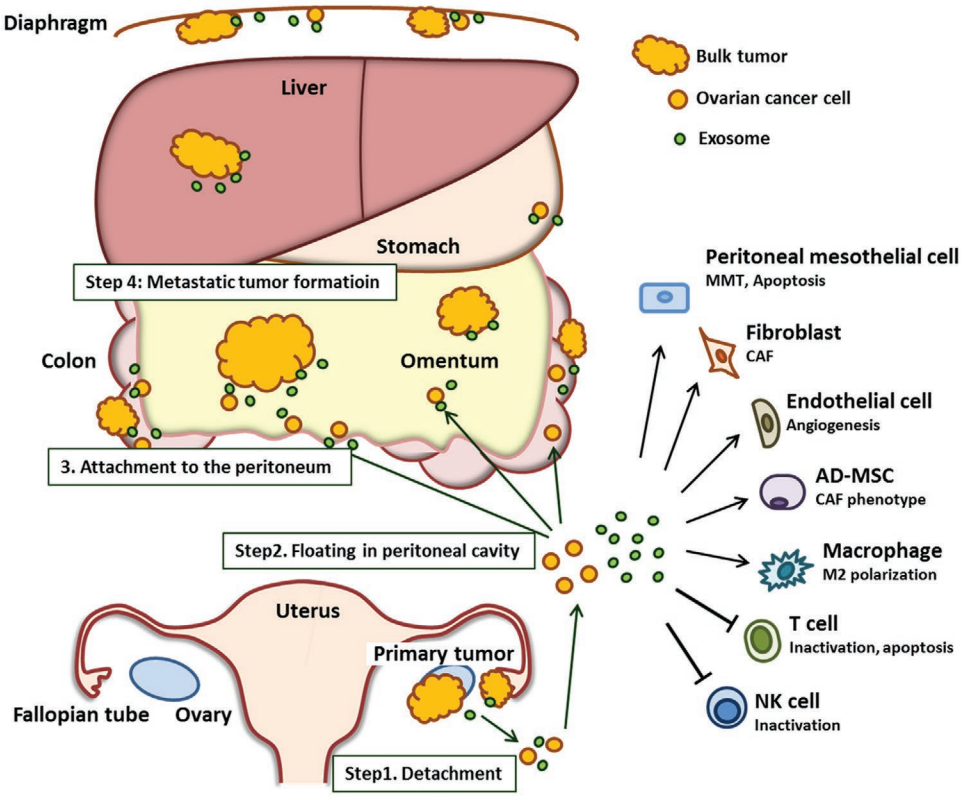

Figure 7. Role of the exosomes secreted by ovarian cancer cells in the microenvironment of the peritoneal cavity that influences the cancer progression. Reproduced under the terms and conditions of the Creative Commons Attribution (CC BY) license.[136]. Copyright 2019, The Authors published by MDPI.

encapsulated paclitaxel reached the cells efficiently and in enough quantity. In addition, when the exosomes used were derived from cancer cells, the cytotoxic effects increased.[73,129] On the other hand, the benefits of encapsulating paclitaxel into exosomes derived from macrophages have been studied. Due to their origin, these exosomes are involved in the suppression of tumor progression, for example, by mediating the release of pro-inflammatory cytokines. In this scenario, the antitumor effects of paclitaxel were increased significantly.[130]

Also, the use of bovine milk derived exosomes for the administration of paclitaxel has been proposed as a strategy for solving problems arising from their low solubility in aqueous components. Results showed the feasibility of this administration system without compromising the drug's properties.[131]

To prevent drug resistance and fight against toxicity, some authors have tried to use exosomes as miRNA transport. This miRNA inhibits releasing compounds involved in tumor progression. MicroRNA is a small, non-coding RNA molecule of about 18–24 nucleotides which functions in the post-transcriptional regulation of gene expression. One of them is miR-128, which is present in many human tumors and is involved in cellular progression, differentiation and apoptosis.[132]

As an example, in prostate cancer, exosomes have demonstrated their usefulness not only in therapeutics but also as biomarkers for diagnosis and prognosis.[19]

Exosomes derived from prostate tumors play a role in cancer progression because they are involved in angiogenesis, metastasis, drug resistance and the regulation of stromal cells, as well as causing dysfunction in cells of the immune system.[19] However, exosomes may be used as potential delivery systems for prostate cancer diagnosis and monitoring. Exosomes functionalized with targeting ligands may be used as delivery systems for chemotherapeutic agents such as doxorubicin to target tumor or as vectors for RNA-based therapy.[19,133,134] Exosomes accumulate large amounts of small RNA, typically miRNA that influences the proliferation, angiogenesis and survival of cancer cells, therefore exosomal miRNA plays an important role as a biomarker in the diagnosis of prostate cancer.[135] miRNA-221 and miR-222 are involved in cancer growth, miR-92a and miR-17-92 in angiogenesis and miR-210 in the induction of metastasis, while exosomal miRNA may be used in RNA-based therapy of prostate cancer.[19]

In addition, miRNA exosomes can be used as biomarkers in ovarian cancer.[136,137] Exosomes secreted by ovarian tumors allow for peritoneal spread and facilitate the interaction between cancer cells and the microenvironment as seen in **Figure 7**.

Ovarian cancer-derived exosomes may be detected in different body fluids and contain many substances such as cell surface antigen and others that play a role in cancer progression.[136,137]

Engineered exosomes may potentially be used for ovarian cancer therapy. Exosomes encapsulating chemotherapeutic drugs have been proposed for the treatment of ovarian cancer that is resistant to conventional chemotherapy. In addition, microRNA replacement therapy with exosomes for ovarian cancer therapy has been explored.[137]

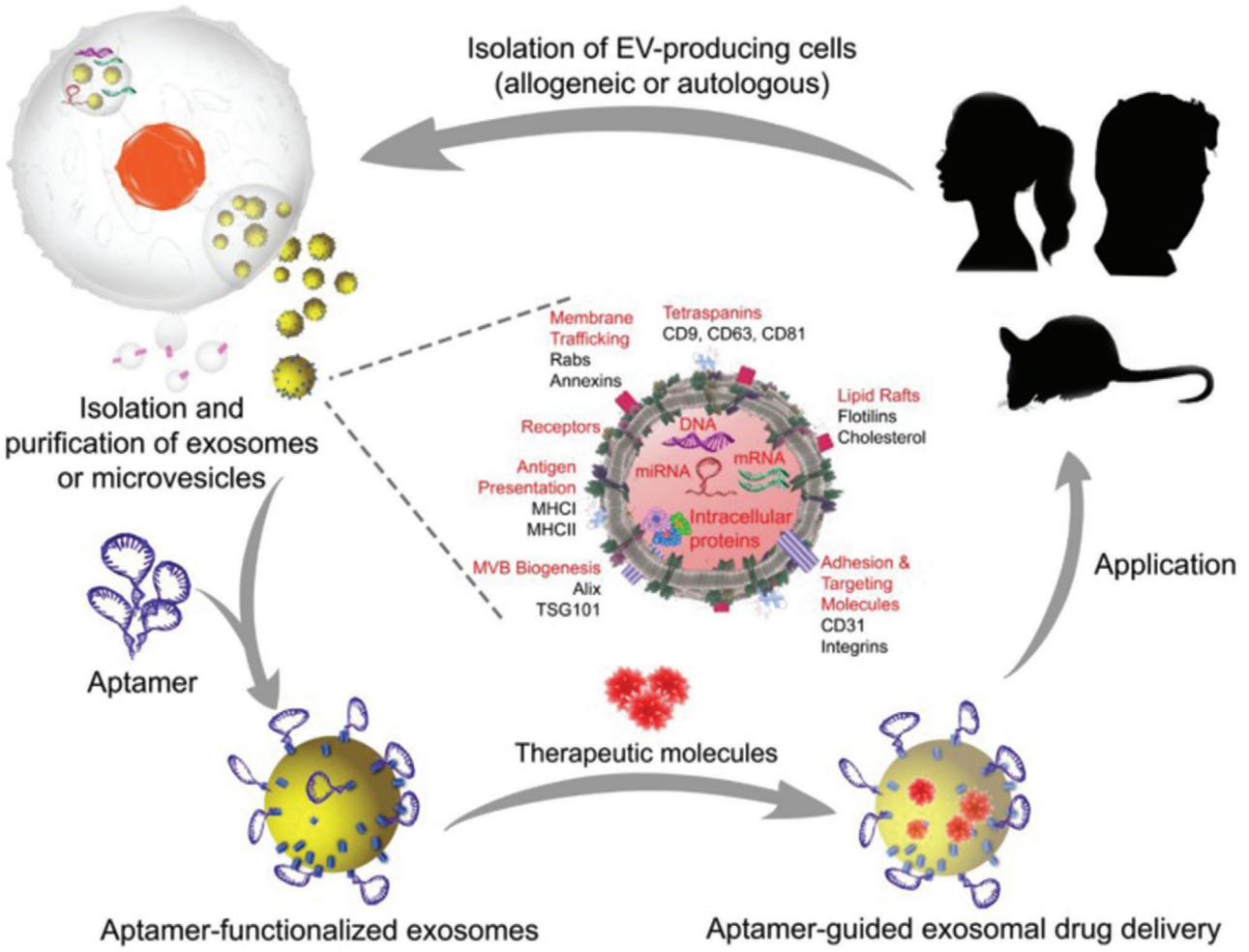

Figure 8. Scheme of engineered-aptamer exosomes for drug delivery. Reproduced under the terms and conditions of the Creative Commons Attribution (CC BY) license 4.0.[139] Copyright 2020, The Authors, published by Ivyspring International Publisher.

Theranostic applications of exosomes have also been proposed for the early detection of cancer through the use of engineered-aptamer exosomes. Aptamers are highly structured single chain nucleic acid molecules that adopt defined 3D structures depending on their sequence, allowing them to bind very specifically to their targets.[138] Aptamer-based technologies are currently applied for early diagnosis of cancer using the aptamers as a probe for the detection of tumor cells. Aptamer functionalized-exosomes play a role in cell to cell communication, transporting diagnostic agents such as RNA and proteins, among others.[139,140]

Aptamers may be developed against a specific overexpressed protein on exorcisms as a diagnostic tool. HSP70 is a heat shock protein (HSP) that is produced by cells and is involved in cellular physiology. HSP70 is overexpressed in many types of human cancer, leading to the development of different HSP70 based therapies for different types of cancer, such as leukemia, colon carcinoma or lung carcinoma, among others. In addition, HSP70 acts as an immune stimulatory factor with potential use in cancer vaccines.[141,142] An approach for targeting tumor-derived exosomes using the HSP70 aptamer has been developed. This aptamer allows for the capturing of HSP70-positive exosomes in blood or urine samples from patients with different types of cancer.[143] In addition, aptasensors have been developed for the detection of aptamers in cancer derived exosomes.[59,144] This kind of aptasensor is based on the detection of exosomes that use different types of sensitive detectors such as Surface plasmon resonance, electrochemical, etc. Another application of aptamer-targeted exosomes is as delivery systems of antineoplastics or other drugs. In this kind of system engineered aptamer exosomes are loaded with active molecules and targeted in vivo with different potential applications as shown in **Figure 8**.[139]

8.3. Exosomes for Neurological Diseases

Based on recent studies, exosomes are increasingly of interest as possible treatments for neurological diseases. These kinds of diseases are difficult to treat due to the blood-brain barrier. There are two main reasons for this: penetrating the blood-brain barrier is a challenge and sufficient drug levels in the brain cannot be achieved via systemic circulation However, exosomes are stable and can pass the blood-brain barrier,

meaning that they appear to be a good choice for diagnosing and treating these types of diseases.[145,146]

In the case of neurodegenerative or other kinds of diseases related to the brain, intranasal delivery is one of the best administration routes because it is practical and noninvasive. However, it presents some limitations that prevent effective concentrations from being achieved in the brain. Some authors tried encapsulating the drugs into exosomes before intranasal administration. Curcumin was encapsulated into exosomes for intranasal administration in mice and their results indicate that curcumin is effectively delivered to the brain without side effects. Also, microglial cells are preferentially targeted by exosomes. However, the mechanism of exosome transport from the olfactory region to the brain has not been clarified.[147] Catalase activity is reduced in the brain of patients with Parkinson's disease. Exosomes obtained from bone marrow macrophages have been used as drug delivery of catalase for the treatment of this disease. The drug encapsulated into exosomes was accumulated in neurons and microglial cells and produce a potent neuroprotective effect in mice with acute brain inflammation.[122]

Another therapeutic application of engineered aptamer exosomes is as delivery systems in the field of Parkinson's disease. Lewy bodies are structures located in neurons and associated with Parkinson's disease. They are composed of α-Synuclein, which is a neural protein associated with Parkinson's disease. A new approach for the treatment of this disease is based on immunotherapy using antibodies that neutralize the aggregates of the protein delaying the clinical progression of the disease. Engineered exosomes containing DNA aptamers that allow the in vivo brain targeting to α-Synuclein aggregates have been developed. Exosomes modified with rabies viral glycoprotein (RVG), which is a viral protein that interacts with the murine p75 neurotrophin receptor, have been used in an experimental mouse model reducing neuropathological disorders associated with Parkinson's disease.[148]

Also, central nervous system (CNS) -derived exosomes can transport amyloidogenic proteins that play an important role in several neurodegenerative diseases, such as Alzheimer's disease. In addition, CNS-derived exosomes can cross the blood brain barrier (BBB) and appear in the blood, meaning that they can be used as biomarkers of neurodegenerative diseases produced in the CNS. The ease with which exosomes cross the BBB can also be used to release drugs and other substances into the brain.[3,149]

Finally the neuroregenerative properties of MCS derived exosomes allow for the enhancement of angiogenesis and axonal density contributing to tissue remodeling in the treatment of strokes and degenerative processes such as Parkinson's.[150–153] The exosomes content and thus their therapeutic effect depend on their origin. From the several vesicle sources that have been assayed such as bone marrow and adipose tissue, dental pulp stands out because of its better neuroregenerative effect even in the spinal cord.[154–156] Similarly, adipose-tissue exosomes show high amounts of the enzyme neprilysin which destroys the amyloid beta-protein and are intended for the treatment of Alzheimer's disease.[157,158] Several exosome microRNAs are involved in the tissue reparations such as miRNA-129-5p in spinal cord injury or miRNA-133-b in stroke. However, further characterization of the biological protective mechanisms is needed for the development of new strategies in the treatment of neuronal diseases.[155,159]

8.4. Exosomes for the Treatment of Ocular Diseases

MSC therapy has been proposed for the treatment of chronic eye diseases such as glaucoma, retinal degeneration, ocular immune-mediated diseases such as uveitis and tissue regeneration in reconstructive surgery.[160,161] Mesenchymal Stem Cell (MSC)-derived extracellular vesicles like exosomes have also been proposed as delivery systems for drugs and genetic material for the treatment of ocular diseases. Mesenchymal stem cell-derived extracellular vesicles can be administered periocularly or by intravenous injection with fewer adverse effects than those produced by mesenchymal stem cells.[162] The paracrine effect produced by exosome-mediated MSCs has an impact on regenerative medicine and can be used to treat ocular diseases and the topical administration of exosomes in ocular surface as sustained delivery systems using biodegradable hydrogels have been suggested.[163] MSC derived exosomes have demonstrated their efficacy in different preclinical studies using animal models of corneal diseases and their use has been suggested for their anti-inflammation, anti-angiogenesis and tissue regeneration properties.[164]

Also, in a rat retinal detachment experimental model, MSC-derived exosomes were sub-retinally injected demonstrating that the expression of inflammatory cytokines such as TNF-α and IL-1β were significantly reduced. Also MSC-derived exosomes help to decrease the photoreceptor cell apoptosis and to maintain the structure of the retina.[165]

8.5. Exosomes for Infectious Diseases

Exosomes are involved in the pathogenesis of many infectious diseases. Different kinds of bacteria and viruses may communicate with infected cells through exosomes and transport cell components such as DNA, miRNA, and proteins.[166] Also, infected cells may use this communication as a mechanism of defense. Strategies that block the exosomes and interrupt the communication may help to control the infection.[167] Exosomes derived from infected cells may also be used for the detection of bacterial infections that cannot be detected by standard methods.[168]

A proposed new application for exosomes as drug delivery systems is in the diagnosis and treatment of infectious diseases mainly in mycobacterial infections. Exosomes secreted by macrophages infected with different types of bacteria may incorporate functional bacterial genetic material such as mRNA or miRNA. In fact, miRNAs have been proposed as biomarkers for the diagnosis of latent and active tuberculosis.[169,170] Also exosomes containing small-RNA inhibits influenza virus replication in infected A549 human lung adenocarcinoma epithelial cells.[171]

Porphyromonas gingivales is a kind of G(–) anaerobic bacteria involved in periodontal diseases. Plant-derived exosomes such as Ginger exosomes like nanoparticles (GELN's) were seen to selectively inhibit the pathogenicity of *P. gingivalis* in a chronic

periodontitis mouse model. The mechanism is based on GELN-derived lipid PA which interact with HBP35 protein expressed on the surface of *P. gingivalis*. In addition the miRNA content of this kind of exosome targets different genes in various types of bacteria and prevents chronic infectious diseases.[172,173]

Antibiotic resistance is a severe and health issue known all around the world. The number of antibiotics without resistance gets lower each day, so finding a solution is a top priority.

Most resistances appear because the bacteria can survival inside phagocytes. This acts as a reservoir of bacteria that are protected from antibiotics. Exosomes derived from culture supernatants of mouse RAW 264.7 macrophages encapsulating the antibiotic linezolid accumulate in macrophages and showed effectiveness against intracellular Methicillin resistant *Staphyloccus aureus* (MRSA) in vitro. This system was also tested in vivo in an experimental infection model of peritonitis in comparison with free antibiotic. The results demonstrate that linezolid incorporated in exosomes significantly inhibited MRSA infection in vivo with a low cytotoxicity to macrophages.[174] Also exosomes have been proposed as vaccines due to their ability to prepare the immune system to kill invading pathogens.[169,175] Exosomes secreted by antibiotic resistant *Pseudomonas* or *Helminth* parasites may potentially be used as vaccine vehicles for the promotion of immunity against these types of organisms.[176,177]

9. Perspectives on Clinical Applications of Exosomes

Currently, the main clinical applications of exosomes in the diagnosis of different types of cancer have been described. In the field of therapeutics, exosome delivery systems for chemotherapeutic agents or for the treatment of inflammatory chronic diseases have been clinically tested.[178,179] In recent years, clinical trials have been carried out in different phases and with different numbers of patients. The main indications for these trials have been the diagnosis and treatment of different types of cancer such as: melanoma, non-small cell lung cancer, colon cancer and metastatic pancreatic cancer among others. Other clinical trials with exosomes have focused on bronchopulmonary dysplasia, type I diabetes, induced oral mucositis, insulin resistance and chronic inflammation in polycystic ovary syndrome. It is also worth noting the excellent properties of exosomes for tissue regeneration, especially in bone and cartilage, skin, cardiac and neural tissues.[180–182] The microRNA of exosomes plays an important role in this activity and their identification allows them to be encapsulated in the vesicles for therapeutic use.[183]

A great future is foreseen for these applications and there are clinical trials underway for the use of mesenchymal stem cell derived exosomes in the repair of macula holes (NCT0347759), as well as the treatment of acute ischemic stroke (NCT 0 338 4433) and Alzheimer's Disease (NCT 0 438 8982).[184]

Other promising potential clinical uses of exosomes include exosome-based cancer vaccines tested in different clinical trials and exosome-based vaccines for emerging infectious diseases such as Ebola and influenza virus among others.[185,186] Good manufacturing practice (GMP) guidelines place limitations on the manufacture and handling of exosomes for use in clinical trials. In most of these clinical trials, the source of exosomes was mesenchymal stem cells (MSCs), dendritic cells (DCs) or plant derived exosomes. The production of exosomes for clinical trials under GMP guidelines involves cell expansion, the isolation of exosomes by different methods such as ultrafiltration or ultracentrifugation and the characterization of exosomes according to particle size and concentration or total protein content.[187]

10. Conclusions

Exosomes are one of the main research topics today in terms of new therapies. The complexity and heterogeneity of the functions that these vesicles perform under physiological and pathological conditions make them interesting candidates for application in therapies for a wide range of diseases. On the other hand, the use of exosomes as vehicles for drug delivery has demonstrated that we can harness their intrinsic advantageous properties to improve drug therapies.

The design of modified exosomes, either to make them carriers of drugs or to express certain molecules on their surface, opens new horizons in the treatment different diseases. Exosomes have outstanding advantages and drugs administered within these systems have the potential to be as effective as free administered drugs but with a higher vectorization. For this reason, it is believed that exosomes will play a key role in the detection, diagnosis and treatment of a large number of diseases including cancer and neurodegenerative diseases.

In cancer applications, exosomes are already being used for the transport of drugs and biological molecules such as mRNA, both in vitro and in vivo studies. The results with regards to tumor cells of various cancers, such as breast, liver tumor or leukemia, are promising in terms of increased efficacy due to inhibition of tumor growth with a reduction in drug toxicity. There have also been promising results in relation to the use of molecules that are inhibitory of cancer progression promoters that are innocuous to individuals, producing effectiveness without adverse effects.

In addition to the important advances in recent years in the use of exosomes, especially for the diagnosis and treatment of cancer, there are other therapeutic areas where research lies little explored and where therapeutic possibilities of exosomes and potential for clinical use may increase in the future. As an example, the ability of exosomes to penetrate the central nervous system (CNS) allows them to be used as delivery systems of different types of drugs for the treatment of neurological diseases. Another important area of expansion in the use of exosomes in the future is in regenerative medicine through mesenchymal stem cells derived exosomes.

Nevertheless, the study and understanding of exosomes is barely in its infancy and there is still much work ahead. The more we can determine about the biology and functions of exosomes, the more we will be able to apply them to therapeutics. Furthermore, the simplification and standardization of isolation and loading methods are still challenging. Although there is a fair amount of evidence of their potential in diagnostic, prognostic and therapeutics, more clinical studies are still necessary.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors have contributed to the manuscript conception and preparation. C.C.D. wrote the original draft of the manuscript. C.G.-M. and C.C.D. contributed to the conception and writing. C.C. and J.L. contributed to the writing and reviewing of the text. All the authors revised and approved the submitted final version.

Keywords

cancer therapy, drug delivery, exosomes, mesenchymal stem cells, neurological diseases

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Carmen Gutierrez-Millan is an associate professor of Pharmacy and the Responsible for Quality Assurance at the Research and Development Laboratory of the University of Salamanca. She holds a Ph.D. degree in Pharmacy and teaches courses in pharmaceutical technology, pharmaceutical regulation, and clinical pharmacy. Her current research focuses on nanosytems derived from cellular membranes and nanoparticles.

Clara Calvo Díaz graduated in Biotechnology by University of Oviedo and Master in Evaluation and Development of drugs by University of Salamanca. Her research interests were already reflected in her Final Degree Project where she studied the potential cytotoxic capacities of microalgae extract and, also, in her Final Master Project dedicated to analyze the applications of exosomes as drug delivery systems. Nowadays, she is working in 3P Biopharmaceuticals, a Biotechnology company located in Navarra, as analyst for the research and development (R&D) department, where she continues working to contribute to biotechnology research.

José M. Lanao holds a Ph.D. in Pharmacy. He is a Professor of Pharmacy and Pharmaceutical Technology. Head of Pharmaceutical R&D Laboratory, University of Salamanca, Spain. His research interests includes nanoparticles for infectious diseases, drug delivery, cellular carriers, hydrophilic matrices, PKPD modelling, and simulation.

Clara I. Colino holds a Ph.D. in Pharmacy and since 2005 has been a full professor in the area of Pharmacy and Pharmaceutical Technology at the University of Salamanca, where she teaches new strategies for drug administration. Her research activity has focused on anti-infectious therapy mainly in drug biodistribution and nanosystems and biological carriers for their vectorization to macrophages.