CURRENT APPLICATION OF EXOSOMES IN MEDICINE

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Abstract

Exosomes belong to structures called extracellular vesicles (EVs). These spherical units, secreted by most eukaryotic cells, attracted significant interest among researchers in recent years. Exosomes undergo secretion from almost all types of mammalian cells, including dendritic cells, B cells, epithelial cells, mastocytes, reticulocytes, platelets, T cells, mesenchymal stem cells, adipocytes, bone marrow-derived stem cells, embryonic stem cells, fibroblasts, cardiac myocytes, endothelial cells, oligodendrocytes, astrocytes, microglia, neurons, neural stem cells, hepatocytes, lung spheroid cells, as well as tumor cells. Exosomes have several features that enable many methods of their isolation from biological material. Furthermore, physicochemical properties such as size, mass, density, or the ability to interact with specific proteins allowed for the development and advance of several effective methods. Work on exosomes' recovery and purity made it possible to most effectively determine their isolation methods' efficiency and accuracy. A common ground for the researchers' interest in exosomal analyses is the role of exosomes as carriers of disease biomarkers. It has been suggested that exosomes can be used in vaccine development and other immunological-related purposes, as one of their characteristics is the ability to present antigens. Moreover, exosomes have a long half-life. As the human body does not perceive them as foreign bodies, they can penetrate cell membranes and target specific cells, making them even better candidates for the applications mentioned above. Therefore, the following review deals with the nature of exosomes, as well as various methods of their isolation and use in medicine.

Running title: Current application of exosomes in medicine

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Introduction

Exosomes belong to a group of structures called extracellular vesicles (EVs). These spherical units, secreted by most eukaryotic cells, have attracted increasing interest among researchers in recent years. The first mentions of exosomes appeared in 1983. Two publications on exosomes were then published in the journals *Cell* and *Journal of Cell Biology* just a week apart, though they had not yet been named at the time. In this study, it was then confirmed that the transferrin receptors in reticulocytes are associated with small vesicles, which are then secreted from the maturing reticulocytes into the extracellular space. The name “exosome” only was clarified a few years later, by Rose Johnstone from the Department of Biochemistry at the Medical Faculty of McGill University in Canada [1]. Fourteen years later, a structure called the exosome complex or – often wrongly – the exosome was also discovered. These two concepts should not be confused as the exosome complex (or PM / Scl complex) is a nucleolar macromolecular complex with ribonuclease properties, playing a role in mRNA degradation and ribosomal RNA processing [2]. Exosome complexes are found in both eukaryotic cells and archaea. In bacteria, on the other hand, there is a simpler complex with similar functions, called the degradosome [3,4]. It is therefore incorrect to use the names “exosome” and “exosome complex” interchangeably. The initial confusion around exosomes contributed to a sharp expansion of interest among researchers, which continues to the present day. In recent years, exosomes have gained enormous popularity, although about 30 years ago no one heard of them. We can be sure that all their known features will be used to improve various areas of science and medicine. The following work discusses the nature of exosomes, i.e. their characterization, source of origin, mechanisms of biogenesis, secretion and uptake and their variable content (cargo). We also present their most popular isolation methods, such as size-based isolation, immunoaffinity, density-based isolation and co-precipitation. Finally, we discuss some of the uses of exosomes in human therapies based on their best-known features.

The source of exosomes

Exosomes undergo secretion from almost all types of mammalian cells, including dendritic cells, B cells, epithelial cells, mastocytes, reticulocytes, platelets, T cells [5], mesenchymal stem cells [6], adipocytes [7], bone marrow-derived stem cells and embryonic stem cells [8], fibroblasts, cardiac myocytes and endothelial cells [9,10], oligodendrocytes, astrocytes, microglia, neurons [11,12], neural stem cells [13], hepatocytes [14], lung spheroid cells [15] as well as tumour cells [16]. Their presence in various body fluids such as plasma, saliva, urine, amniotic fluid [17], serum [18], cerebrospinal fluid [19],

lymph [20], sperm [21], breast milk [22], bile [23], bronchoalveolar lavage fluid [24], synovial fluid [25], gastric juice [26], tears [27] and malignant effusions [28] has also been demonstrated.

For the first time, exosomes were described as vesicles exhibiting 5'-nucleotidase activity released by neoplastic cell lines [29]. Subsequently, their presence was observed in studies of reticulocyte maturation process. It was shown that they localize in multivesicular endosomes and contain transferrin receptors. Initially, exosome secretion was proposed as a means of elimination of cellular contents, such as unnecessary proteins [30,31]. However, in the late 90s, their role in intercellular communication, with implications in immunological response was proved [32]. The 2007 study of Valadi et al. reported that exosomes contain mRNAs and miRNAs, which can participate in cell-cell communication and exert functions in recipient cells [33]. These results evoked increasing interest in the biology and application of these extracellular vesicles.

Exosome biogenesis, release and uptake

The biogenesis of exosomes involves double invagination of the plasma membrane. In the first step, a cup-shaped structure is formed, comprised of cell-surface proteins, as well as soluble proteins associated with the extracellular environment. This, in turn, leads to generation of an early endosome. Early endosome can undergo maturation into late endosome to give rise to multivesicular body (MVB). MVBs formation occurs through inward invagination of endosomal limiting membrane, which produces intraluminal vesicles (ILVs) in the lumen of these organelles. Mature multivesicular bodies can either be degraded via fusion with lysosomes or autophagosomes. Furthermore, if fused with plasma membrane, contained ILVs can be released into the extracellular space to generate a subtype of EVs termed ‘exosomes’ [5,34–36]. It is worth mentioning that, unlike other intracellular budding events, endosome ILVs bud away from the cytosol, which means that their membrane orientation is exactly the same as that of the cell surface – extracellular domains of transmembrane proteins are exposed to the environment, while cytosolic components are enclosed [36]. Numerous molecules have been attributed to the exosome biogenesis and/or release, most notably using ESCRT (The endosomal sorting complex required for transport), TSG101 (tumour susceptibility gene 101 protein), ALIX (apoptosis-linked gene 2-interacting protein X), VPS4 (vacuolar protein sorting-associated protein 4), nSMase-2 (neutral sphingomyelinase), ceramide, syntenin-1, syndecan-1, Rab GTPases, SNAREs (soluble N-ethylmaleimide-sensitive fusion attachment protein receptor), and tetraspanins [37,38]. In addition, it was postulated that pH/acidification may play a role in determining the level of exosome production [39].

The mechanisms underlying exosome uptake are still largely unknown and lack consensus, with their uptake mechanisms, possibly through micropinocytosis, macropinocytosis or in a receptor-dependent manner, remain to be elucidated. Exosome internalization can rely on clathrin-mediated endocytosis (CME) or occur in a clathrin-independent process [40]. Important classes of proteins involved in exosome uptake include: tetraspanins, integrins, lectins, proteoglycans and immunoglobulins [41]. As illustrated by growing evidence, it is also uncertain whether the process is target (acceptor cell)-specific or rather random. For instance, in a study by Fitzner et al., it was demonstrated that, when Oli-neu cell line-derived exosomes containing PLP were applied to neuronal cells in primary cortical culture, microglia, oligodendrocytes and astrocytes, they were preferentially internalized by microglia [42]. However, human carcinoma cell lines, precisely A549 (lung), HCT116 and COLO205 (colon) take up donor-derived exosomes non-selectively [43]. In addition, it should be noted that information from exosomes may be passed to the cell surface, without the necessity of cargo delivery, as in some cases of EVs expressing major histocompatibility complex (MHC)-peptide complexes [36].

Exosome content

Exosomes are a heterogeneous family of lipid-bilayer enclosed extracellular vesicles, with a typical density on sucrose gradients ranging from 1.07 to 1.22 mg/L [44–46] and 30–150 nm in size [47–49]. Their biological content consists of various proteins, lipids and nucleic acids. According to ExoCarta (www.exocarta.org), a database depositing information on exosomal proteins, RNAs and lipids from various organisms and cell types, 41,860 proteins, >7,540 RNA and 1,116 lipids have been identified until now [50]. The content of these EVs is highly dependent on their cellular origin, status quo of the cell and the microenvironment. For instance, the proteomic analysis of breast cancer cells-derived exosomes revealed that exosomal protein content reflects epithelial- or mesenchymal-like phenotype of the cell-of-origin [51]. Exosomes from antigen presenting cells (APCs) carry MHC II molecules, which enable activation of specific T cells [52]. In turn, microglia-derived exosomes express aminopeptidase CD13, involved in neuropeptide catabolism and the lactate transporter MCT – 1 [53]. It was also observed that oncogenic alteration EGFRvIII (epidermal growth factor receptor variant III) in glioblastoma cells influences the cargo of EVs leading to enrichment in proinvasive proteins [54]. Despite their heterogeneity, a subset of molecules can be commonly found in majority of exosomes. These include proteins involved in MVB biogenesis (ALIX, TSG110, VPS, clathrin) and membrane fusion and transport (annexins, Rab proteins, ARF

GTPases), as well as cytoskeletal components (actin, myosin, tubulin, vimentin), tetraspanins (CD9, CD63, CD81, CD82), heat shock proteins (HSP90, HSP70), adhesion proteins (lactadherin, integrins, ICAM-1), metabolic enzymes (GAPDH, PKM, glucose-6-phosphate isomerase) and signal transduction proteins (Ras-related protein, flotillin, syntenin-1) [5,35,55,56].

Another class of molecules considered as essential components of exosomes are lipids. Studies demonstrated that specific lipids, namely cholesterol (CHOL), sphingomyelin (SM), glycosphingolipids, ceramides and phosphatidylserine (PS) are two to four times enriched in exosomes compared to their parent cells, while a similar mole percent in cells and exosomes was observed for phosphatidylethanolamine (PE), with a lower mole percent of phosphatidylcholine (PC) and phosphatidylinositol (PI) [57,58]. Quantitative lipidomic data for PC-3 cells-derived exosomes suggests an asymmetric distribution of lipids in the two membrane leaflets – with very-long-chain sphingolipids predominant in the outer and PS in the inner leaflet [58]. In addition, bioactive lipids, including prostaglandins and leukotriens, as well as lipolytic enzymes (phospholipases), were identified in exosomes [59]. For example, prostaglandin E2 (PGE2) encapsulated in tumor-derived exosomes is involved in MDSC (myeloid-derived suppressor cells) – mediated promotion of tumor progression [60].

Aside from proteins and lipids, it has been reported that exosomes comprise a variety of RNA species, i.e. mRNA, miRNA, lncRNA, circRNA, tRNA, rRNA and other non-coding RNAs, as well as exosomal DNA (exoDNA) [61–64]. RNA-seq analysis of human plasma-derived exosomal RNA has revealed that miRNAs were the most abundant, making up over 42.32% of all raw reads and 76.20% of all mappable reads, while other species detected included ribosomal RNA (9.16% of all mappable counts), long non-coding RNA (3.36%), piwi-interacting RNA (1.31%), transfer RNA (1.24%), small nuclear RNA (0.18%) and small nucleolar RNA (0.01%) [65]. As mentioned above, Valadi et al. have reported that miRNA and mRNA are transported by EVs and that the latter species can undergo translation, thus indicating horizontal transfer of nucleic acids between cells [33]. Another study utilizing the reporter system under the control of the CRE recombinase, provided evidence for the transfer of EV-encapsulated CRE mRNA between hematopoietic system and the brain in response to inflammation [66]. Moreover, a growing body of research demonstrates that exosomes are enriched in small non-coding RNAs and thereby possibly participate in modulation of gene expression via transfer of these molecules [37,61,67,68]. Exosomal miRNAs such as miR-200 [69], miR-92a [70], miR-21 [71], miR-124a [72] are indicated to be involved in phys-

iological and pathological processes: cancer metastasis, tumor angiogenesis, myocardial protection and modulation of synaptic activation, respectively. Curiously, lncRNAs and circRNAs have been detected in exosomes and reported to participate in carcinogenesis. For instance, lncRNA-UCA1 enclosed in exosomes secreted by hypoxic bladder cancer cells promoted tumor growth and epithelial-mesenchymal transition (EMT) [73], while circ-KLDHC10, present in serum-derived exosomes of patients with colorectal cancer was proposed as a new, potential biomarker of this disease [74]. It was demonstrated that sorting of RNAs into exosomes is selective as exosomal RNA profiles do not reflect these observed in parent cells [33,75,76], however, the mechanism behind this process remains to be elucidated [67]. Several RNA-binding proteins (RBP) including HuR (ELAV-like protein 1; Hu-antigen R) [77], MEX3C (RNA-binding E3 ubiquitin-protein ligase) [78] and hnRNP A2B1 (heterogeneous nuclear ribonucleoprotein A2B1) [79] were proposed to be involved in RNA sorting.

Studies have also demonstrated that exosomes can carry DNA, namely fragmented gDNA [80], mtDNA [64], as well as parasitic DNA [81]. The mechanism of exoDNA incorporation is still unknown, however, random sorting has been suggested [37]. Excitingly, Takahashi et al. have indicated that exosome release is crucial for cellular homeostasis maintenance, as a mechanism of harmful cytoplasmic DNA removal [82].

Selected origins

Nervous System

In 2006, Faure et al. have for the first time demonstrated exosome secretion from astrocytes and neurons in primary cortical culture in response to depolarization. The authors further suggested a lysosome-independent mechanism of receptor disposal in synapses [83]. Moving to glial cells, exosomes from Schwann cells play a role in axonal regeneration [84], while exosomes derived from oligodendrocytes contain proteolipid protein (PLP), myelin proteins and proteins associated with protection against oxidative stress [85]. In addition, oligodendrocytes release exosomes which can inhibit the processes of myelin sheath formation and oligodendrocyte differentiation [86]. It was also demonstrated that microglia-derived exosome release can be regulated by neurotransmitters – Glebov et al. highlighted a role for serotonin in this process – stimulation of 5-HT receptors present in microglia results in secretion of exosomes containing insulin-degrading enzyme which can degrade amyloid- β (A β) [87]. As mentioned above, exosomes are also assigned a function as intracellular communication mediators. Exosomes derived from neuroblastoma cells are mainly internalized by astrocytes, while exosomes released from cortical neurons are captured

by other neurons [88]. It was also reported that glutamate can influence oligodendrocyte-derived exosome internalization by neurons [89].

Cardiovascular System

As vesicles rich in various active components, exosomes can play their roles in intercellular communication essential for cardiovascular and arterial physiology. For instance, miR-21* encapsulated in exosomes transferred between cardiac fibroblasts and cardiac myocytes induces cardiomyocyte hypertrophy [90]. Chen et al. have reported upregulation of exosomal miR-21 in conditioned medium derived from OGD (oxygen-glucose deprivation)-treated cardiomyocytes and suggested its function in protection of cardiomyocytes against oxidative stress, fibroblast activation and promotion of angiogenesis in endothelial cells [91]. miR-126 released by endothelial-derived exosomes participates in vascular endothelial repair [92]. It was also demonstrated that, when in glucose-deprived conditions, cardiomyocytes (CMs) increase the production and release of exosomes, which comprise functional glucose transporters and glycolytic enzymes. These vesicles influenced the glucose uptake, glycolytic activity and pyruvate production when internalized by recipient endothelial cells (ECs) [93]. Exosomes secreted by cardiomyocytes, cardiac fibroblasts, platelets, endothelial cells, smooth muscle cells, macrophages and cardiac progenitor cells have been implicated in coronary artery disease, hypertrophy, cardiac regeneration, as well as atherosclerosis [10,94,95].

Reproductive system and development

The female and male reproductive tracts, as well as placenta, secrete exosomes [96]. These EVs were detected in semen, amniotic fluid and breast milk, as mentioned above. The syncytiotrophoblast (STB) of human placenta produces and releases extracellular vesicles to the maternal blood, due to programmed cell death and blebbing. These MVs have mainly immunostimulatory and pro-inflammatory features. Simultaneously, STB synthesizes and secretes exosomes which are reported to have immunosuppressive functions. As it was demonstrated that STBMs (syncytiotrophoblast-derived microvesicles) levels are elevated in the blood of patients with preeclampsia, the most common cause of maternal-fetal illness, it is suggested that the imbalance between these microvesicles and exosomes may contribute to the development of the disease [96–98]. Moreover, it was shown that miRNAs of the chromosome 19 miRNA cluster are packed into trophoblast-derived exosomes and regulate immunity to viral infections [99]. Breast milk contains exosomes with immunomodulatory properties crucial for infant's health. For instance, incubation of milk exosomes with PBMC (peripheral blood mon-

onuclear cells) resulted in an increase in the number of T-regulatory cells [22]. RNA-seq of seminal plasma-derived exosomes revealed an abundance of miRNAs such as miR-148a and let-7 family members, which are implicated in the reduction of cytokine secretion and expression of interleukins – IL-10 and IL-13, respectively, thus possibly play a role in immunophysiology of the genital tract [21].

Methods of exosome isolation

Exosomes have a number of features that are used in numerous methods of their isolation from biological material. Physicochemical properties such as size, mass, density or the ability to interact with specific proteins allowed for the development and advance of several effective methods [100], with the recovery and purity of exosomes making it possible to most effectively determine their efficiency and accuracy. The purity of exosomes can be characterized through the analysis of the intensity of exosome markers using Western blot [101]. Purity is also defined by the ratio of the number of exosomal vesicles and the amount of proteins (particles per microgram) [102–104]. This information can be obtained using the bicinchoninic acid and NTA (Nanoparticle Tracing Analysis) assay. Regarding the recovery of exosomes, we can define it by the ratio of the treated exosome particles and the original exosome particles in the samples (which is also determined by NTA) [105]. Methods for isolating exosomes are listed below according to their properties from different categories.

Size based isolation

The size of the exosomes varies between 20 and 140 nm and is used in the size-based isolation method. Currently, size-based isolation mainly involves three different methods: size exclusion chromatography (SEC), sequence filtration and size dependent microfluidics [106–108]. These methods are relatively fast and do not require a lot of special equipment. However, it is sometimes difficult to separate exosomes of different sizes because of low resolution. SEC is based on the filtration of particles through a porous stationary phase. This phase consists of small, gel balls, the pores of which are specifically sized. As the sample passes through the stationary phase, large particles elute and small particles remain in the pores. When using sequential filtration mechanisms, procedures similar to normal filtration are used. The size and molecular weight of the isolated particles determine the filtration parameters. In separating exosomes, membrane filters with different molecular weight exclusion limits are used. The exosomes separated by such filtration are of high purity, taking into account the low manipulating forces [106]. The size-dependent microfluids are a separate methods, recently increasing in popularity [109]. In 2017, a viscoelastic microflow

was used to separate exosomes from other extracellular vesicles [110]. The same system was also used to isolate exosomes obtained from foetal bovine serum. Due to its wide-ranging exosome separation, the size-dependent microfluidic system holds great promise for the future of exosome analysis. In turn, another method-asymmetric flow field-flow fractionation (AF4), could increase the above-mentioned resolution [111,112]. Thanks to AF4, small exosome vesicles and exomeres (very small non-membranous nanoparticles) could be separated from large exosomes [113]. Furthermore, for even more accurate isolation, AF4 optimizations were conducted, combined with a multi-detection system based on UV and multiangle light scattering. Thanks to this combination, it became possible to distinguish new subsequent groups of exosome sizes (average size 113 nm and 23 nm) [113]. While relatively accurate, these methods are not highly specific. While exosome recovery can reach over 80%, the procedures certainly require further improvement and refinement.

Immunoaffinity

As mentioned above, a number of specific membrane proteins are found on the surface of exosomes. These include CD82, CD81, CD61 and CD9, annexin, programmed cell death 6 interacting protein, RAB5 and epithelial cellular adhesion molecule (EPCAM). These proteins can be used as specific markers for the isolation of exosomes. Hence, several isolation techniques are based on immunoaffinity [114–116]. The construction of superparamagnetic immunoaffinity nanoparticles turned out to be an interesting method, combining antibodies with superparamagnetic particles and confocal laser scanning microscopy to confirm structural and functional integrity [117]. The results showed that the immunoaffinity superparamagnetic nanoparticle method performed excellently in maintaining the functional and structural integrity of exosomes. A rather large downside to the use of immunoaffinity is the availability and high cost of antibodies. In addition, the number of exosomes isolated may be underestimated as some antibodies may not be expressed on the exosome surface [117].

Density-based isolation

UC or ultracentrifugation is the most common method of exosome isolation based on their density. This most widely used technique is often considered the “gold standard” [118]. It was first used in 1987 and has been continuously improved ever since [119]. After more than 20 years, it has been proven that it takes at least five UC cycles to remove non-exosomal proteins [120]. The most universal UC scheme is as follows. Cells, large EVs and cellular debris are separated in the first place by not very high centrifugal forces ($\leq 10,000 \times g$). Next,

exosomes are selected due to the long centrifugation time (~ 70 min) and high centrifugal forces (100,000-200,000 x g) needed for their separation. The exosome pellet is washed with phosphate buffered saline (PBS) to remove residual proteins. The purified exosomes are stored at -80 °C, suspended in a fresh portion of PBS [76]. The sucrose density gradient centrifugation method is also used quite often but is generally considered outdated. It is a density-based method of isolating exosomes that = have a density of 1.15 to 1.19 g / ml [121]. While it is relatively easy to isolate exosomes with these methods, they can only achieve a relatively low purity of 10⁸-10⁹ particles per microgram. It is also quite time consuming and has a recovery of 10% to 80% due to the likely damage to the vesicle. Besides, the coexistence of other large biomolecules, protein aggregates and other particles of similar density is inevitable and may cause bias in the analysis.

Co-precipitation method

Every year, polymers are gaining more and more popularity in the scientific community. Taking advantage of their many properties, they can be used to co-precipitate lipid molecules and hydrophobic proteins. In 1960, it was discovered that polyethylene glycol (PEG) could be used to purify and isolate viruses [122]. Due to some biophysical similarities of viruses to exosomes, PEG can also be used in their isolation. This procedure can be performed with easy-to-use commercial kits such as ExoQuick™. Many scientists have chosen this method in their research because it does not require long ultracentrifugation, as well as is relatively fast and low-effort [123,124].

While not as popular, other innovative sorting methods have also been developed. These are, for example, electromagnetic, electrophoretic or acoustic methods [125–127]. Some time ago, a novel chip was also designed, allowing for the use of the phenomenon of alternating current electrokinetics (ACE) for exosome isolation [128].

As can be seen above, a number of methods have been developed to isolate exosomes since the beginning of their rise to popularity. However, there is no single method that covers all the expectations of researchers, so it is important to know and skilfully combine them, to obtain repeatable and expected outcomes.

Application of exosomes in human therapies

A common ground for the researchers' interest in exosomal analyses is the role of exosomes as carriers of disease biomarkers. For example, exosomes in both plasma and cerebrospinal fluid (CSF) have been found to contain alpha-synuclein, a protein directly related to Parkinson's disease [129,130]. As

an example, Ilario Giusti and his team focused on exosomes as markers of glioma [131]. Furthermore, exosomes isolated from urine have shown the ability to reflect the state of acute renal failure [132]. Markers of pancreatic cancer and lung cancer were also found in exosomes [18,133]. Hence, the use of exosomes as potential biomarkers is very promising as these vesicles are found in most of the parts of human organism. Their presence in body fluids, such as blood and urine, allows the use of minimally invasive methods, e.g. liquid biopsy, to diagnose and monitor the patient's response to treatment in real time [134]. The ability of exosomes to monitor the patient's response is another potential clinical application of these vesicles, as confirmed in previous studies [135]. If a marker is directly correlated with a disease state, and treatment of the patient produces the expected, visible results, the change in the presence of biomarkers (exosomes) should be monitored during treatment. Additionally, it has been suggested that exosomes can be used in vaccine development and for other immunological-related purposes [136,137]. Since one of the characteristics of exosomes is the ability to present antigens, it could also be put to good use. Exosomes have a long half-life, the human body does not perceive them as foreign bodies, they can not only penetrate cell membranes, but also target specific types of cells, which makes them even better candidates for the above-mentioned applications [138]. Furthermore, due to their characteristics, they are also ideal for the design of drug delivery systems aimed at specific targets [139]. While the development of a method for introducing RNA and proteins into exosomes and targeting them to a specific region of the body is still ongoing, the ability to load both protein and genetic material into the exosomes is yet another strength of these vesicles. It has also been proven that mesenchymal stem cell exosomes can act alone as a therapeutic unit to help reduce and regenerate tissue damage [140–142]. Exosomes have been also implicated in the development of neurodegenerative diseases. Reports proposing exosomes' participation in protein aggregates clearance, which would be indicative of neuroprotective function, as well as those describing their role in spreading of unfolded or misfolded proteins in proteinopathies, can both be found. As an example, positive relations between exosomes' release and intercellular transfer of infective prions in Creutzfeldt-Jakob disease has been demonstrated [143]. As for Alzheimer's disease (AD), exosomes containing tau protein have been detected in CSF of early onset cases. It was demonstrated, both *in vitro* and *in vivo*, that exosome secretion by microglia serves as means of tau propagation [144]. Rajendran et al. reported the presence of Aβ in exosomes derived from the N2a cell line and demonstrated the accumulation of exosomal proteins in plaques of AD patients' brains

[145]. Furthermore, Yuyama et al. highlighted the role of neuronal exosomes in A β clearance. They detected A β in CSF-derived exosomes fraction and observed decrease of its level in relation to aging, as well as performed an intracerebral infusion of neuronal exosomes which resulted in decrease in A β and amyloid deposition in the brains of APP transgenic mice. Their results implicated possible role of neuronal exosomes in A β clearance and provides a suggestion that their deregulation might contribute to A β accumulation and therefore to AD [146]. Exosomes containing α -synuclein and Lewy bodies were isolated from CSF of patients with Parkinson's disease (PD) and dementia, respectively [147]. Emmanouilidou et al. demonstrated that α -synuclein can be released by neuronal cells in exosomes in a Ca²⁺-dependent manner, as well as that these exosomes induced neuronal recipient cell death [148]. Moreover, a study by Fraser et al. demonstrated elevated levels of autophosphorylated Ser(P)-1292 LRRK2 in urine of patients with idiopathic Parkinson's disease and its correlation with cognitive impairment severity and difficulty in daily task performance [149]. SOD1 and TDP-43, which are thought to contribute to ALS pathogenesis by adopting abnormal conformation are also contained in exosomes. In turn, null mutations in *GRN* gene, associated with certain cases of frontotemporal dementia, lead to significant reduction in exosome release and consequently to a decrease in progranulin levels, implicated in the pathogenesis of FTD [150]. The role of exosomes in wound healing is also worth elaborating, as it is already known that the exosome, an important paracrine factor in intercellular communication, modulates the molecular activity of recipient cells [151]. The role of stem cell-derived exosomes in promoting tissue repair has also been described in several sources. According to the available information, exosomes derived from human umbilical mesenchymal stem cells alleviated acute ischemic damage to the heart muscle [152], while exosomes derived from human adipose tissue mesenchymal stem cells accelerated the healing of skin wounds by optimizing certain features of fibroblasts [153]. In turn, exosomes derived from human umbilical cord mesenchymal stem cells attenuated liver fibrosis [154], while exosomal microRNAs isolated from umbilical mesenchymal stem cells inhibited myofibroblast differentiation by inhibiting the transforming growth factor- β / Smad2 pathway [155]. However, most interestingly, it was also found that local injection of exosomes promotes the regeneration of damaged tissue [153]. Exosomes also carry functional proteins, mRNAs and microRNAs to neighboring cells, and thus undoubtedly serve as mediators of intercellular communication [156].

As demonstrated above, there is a wide range of potential uses of exosomes in a clinical setting. However, more standardized methods of isolating

and analysing exosomes are needed to meet the regulatory requirements of the FDA (U.S. Food and Drug Administration) and other regulatory agencies regarding their use as biomarkers, vaccines, drug delivery vehicles, and therapeutic tools in general.

Ethical approval

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

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Conflict of interest statement

The authors declare they have no conflict of interest.

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