Materials Today Advances 28 (2025) 100623

Contents lists available at ScienceDirect

Materials Today Advances

journal homepage: www.journals.elsevier.com/materials-today-advances/

Bringing exosomes into the game: Current situation, opportunities, limitations and future perspectives

Emrah Dikici [a,b], Burcu Önal Acet [b,c], Désirée Gül [c,*], Nina Kummer [c], Roland H. Stauber [c], Mehmet Odabaşı [b], Ömür Acet [c,d,**]

[a] Scientific and Technological Application and Research Centre, Aksaray University, Aksaray, 68100, Türkiye
[b] Faculty of Arts and Science, Chemistry Department, Aksaray University, Aksaray, Türkiye
[c] Department of Otorhinolaryngology Head and Neck Surgery, Molecular and Cellular Oncology, University Medical Center, 55131, Mainz, Germany
[d] Vocational School of Health Science, Pharmacy Services Program, Tarsus, 33400, Türkiye

ARTICLE INFO

Keywords:
Exosome
Extracellular vesicles
Therapy
Characterization
Biomarkers

ABSTRACT

Exosomes are microscopic vesicles secreted by cells, serving as carriers of diverse biological substances and playing an essential role in the communication between cells. When meticulously engineered, these small extracellular vesicles transform into highly effective delivery systems for therapeutic agents, enabling the targeted administration of active pharmaceutical ingredients to specific organs, tissues, and cells. Exosomes play an indispensable role in a myriad of biological processes, including intercellular communication, the regulation of gene expression, apoptosis, inflammation, immunity, as well as cell maturation and differentiation. The versatile role of exosomes is largely attributed to their intricate cargoes and composition, which encompasses nucleic acids, proteins, and lipids. In this review, we present a comprehensive overview of state-of-the-art characterization and isolation techniques used for the study of exosomes, especially for exosome-based biomedical applications. We will discuss the potential use of exosomes in personalized treatments, their interactions with other nanostructures focusing on the biomolecule corona, as well as the challenges and future expectations. In conclusion, this review provides evidence that we will witness extremely important functions and advances with innovative therapeutic and diagnostic applications of exosomes in the biomedical field.

1. Introduction

The investigations that distinctly recognized extracellular vesicles (EVs) as biological entities, endowed with enzymatic and functional capabilities, commenced in the 1980s and 1990s. Before this era, a multitude of studies suggested the existence of potential structures that would later be characterized as EVs, or detailed experiments that we can now retrospectively theorize may have involved the functions of EVs. In this regard, the narrative of EV research's inception arguably traces back to the studies surrounding coagulation. The early 1980s started the dawn of a period marked by growth and a more nuanced comprehension of EV research. Although the substantial surge of publications, theories, debates regarding nomenclature, and EV-related societies would not emerge for another two decades, the foundation was laid during this time. Two pivotal and complementary papers from the Johnstone and Stahl laboratories established a compelling argument for the release of intraluminal vesicles from the cell, thereby defining them as exosomes [1].

Exosomes are nanometer-scale vesicles characterized by a spherical shape and a lipid bilayer with a diameter of approximately 40–100 nm. These biological entities are secreted by cells and are known to exhibit a density in a specific range when analyzed in sucrose density gradient solution (1.13–1.19 g/mL) [2]. Both prokaryotic and eukaryotic cells secrete EVs as a fundamental aspect of their physiological processes, as well as in response to various acquired abnormalities. EVs can be broadly divided into two main types: ectosomes and exosomes. Ectosomes are membrane-bound vesicles that are formed through the process of outward budding from the plasma membrane. They encompass a variety of structures, including microvesicles, microparticles, and larger vesicles, which typically range in size from approximately 50 nm to 1 μm in diameter (Fig. 1A). Exosomes are EVs of endosomal origin releases out of multivesicular bodies (MVB) [3].

* Corresponding author. Proteases in Disease Group, University Medical Center, Mainz/ENT Building 102, Langenbeckstraße 1, 55131, Mainz, Germany.
** Corresponding author. Vocational School of Health Science, Pharmacy Services Program, Tarsus, 33400, Türkiye.
E-mail addresses: guel@uni-mainz.de (D. Gül), omuracet@tarsus.edu.tr (Ö. Acet).

https://doi.org/10.1016/j.mtadv.2025.100623
Received 24 July 2025; Received in revised form 10 September 2025; Accepted 10 September 2025
Available online 18 September 2025

Exosomes play a significant role in various biological processes, including immune responses, the pathogenicity of viruses, pregnancy, cardiovascular disorders, diseases of the central nervous system, and the advancement of cancer [3]. Exosomes can transfer their cargo between cells, playing an important role in both physiological and pathological processes along with cell-to-cell communication. Exosomes, which are characterized by their origin, encapsulate various components, including proteins and nucleic acids, within a lipid bilayer membrane. This membrane is enriched with specific membrane proteins, such as tetraspanins, growth factor receptors, cell adhesion molecules, and integrins (Fig. 1B) [4–7]. The diversity of surface proteins contributes to the tissue-specific targeting characteristics exhibited by exosomes [5].

In this review, we discussed the latest potential of exosomes, which have been the subject of very important research in recent years, in the biomedical field, their characterization, isolation and purification, as well as their interactions with possible nanostructures, and personalized exosome corona. The potential to evaluate exosome corona and exosome interactions with nanostructures is one of the important unique aspects of this review article.

2. Relevance of exosomes in diseases

In clinical trials, a significant emphasis is placed on the early diagnosis and prognostic biomarkers of cancer. In therapeutic clinical trials, priority is given to plants, stem cells, and exosomes derived from dendritic cells. It is hoped that future exosome-based clinical trials will yield superior solutions for cancer [8]. Both human- and plant-derived exosomes are currently undergoing clinical trials, although more comprehensive reports are accessible for those derived from humans. Given their role as vesicles that transport cellular secretions, exosomes have been effectively utilized as vehicles for drugs or peptides aimed at treating various diseases. Among the most favored sources for exosome preparation are dendritic cells (DCs) and mesenchymal stem cells (MSCs). Exosomes derived from DCs possess the capability to trigger inflammation in patients, particularly those afflicted with cancer, due to their content of tumor antigens that provoke a specific inflammatory response. A well-established cell bank of MSCs exists, providing an alternative source for exosome preparation. The primary application of MSC-derived exosomes lies in the treatment of inflammation. It is imperative that exosomes involved in clinical trials adhere to good manufacturing practices (GMP). Three critical issues are commonly encountered in GMP concerning exosomes: the upstream processes of cell cultivation, the downstream purification processes, and the quality control of the exosomes themselves [9].

Exosomes, the exquisite extracellular vesicles originating from the intricate process of cell endocytosis, serve as vital communicators between cells. Their significance is profound, influencing both health and disease states. For example, exosomes exhibit a dual regulatory capacity in the context of pathogen infections by delivering their diverse contents. On one hand, exosomes enriched with pathogen-derived proteins and RNAs can exacerbate infections through three primary mechanisms. First, exosomes can facilitating the spread of infection by facilitating the spread of pathogen-associated molecules. Furthermore, exosomes aid in the immune evasion strategies of pathogens, and can suppress immune responses by inducing apoptosis in immune cells [10].

Conversely, exosomes also assume a protective role against infections by directly thwarting pathogen proliferation and invasion or stimulating immune responses, particularly those involving monocyte-macrophages, NK cells, T cells, and B cells [10]. Thus, exosomes are elegantly positioned as “bridges” in the landscape of pathogen infections, orchestrating complex interactions through the aforementioned mechanisms [10].

Exosomes are integral to the process of antigen presentation, which is essential for the activation of immune cells, and they promote the release of inflammatory mediators and the expression of immune-related molecules, thereby fine-tuning the immune responses of host cells. In the context of microbial infections, exosomes enhance both innate and adaptive immune responses, bolstering the host’s defenses against invading pathogens through the engagement of natural killer cells, macrophages, and activated T cells by presenting viral and bacterial antigens. However, it is noteworthy that exosomes can also exert immunosuppressive effects during such infections. Their significant biological activities in intercellular transport, information relay, and modulation of cell-mediated immunity render exosomes invaluable in both research and clinical strategies aimed at combating microbial infections [11]. In conclusion, exosomes have received significant attention in the context of infectious disease management and have the potential to serve as potential diagnostic markers, vaccine candidates,

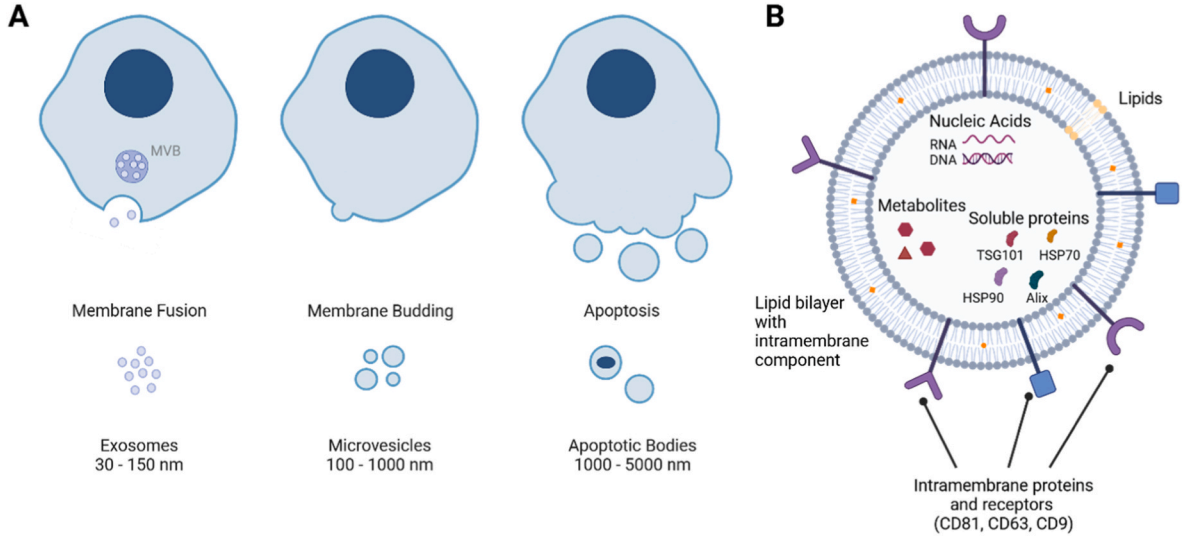

Fig. 1. Schematic overview of different classes of extracellular vesicles (A) and their structure (B). (A) Extracellular vesicles can be divided into three classes depending on indicated sizes and cellular origin. Due to their biogenesis via the endosomal pathway, regulated release out of multivesicular bodies (MVB) and functional relevance, especially exosomes are interesting for biomedical applications. (B) Exosomes can carry various biomolecules, including soluble proteins (such as TSG101, HSP70, HSP90, Alix), metabolites, and nucleic acids (RNA, DNA) within a lipid bilayer membrane. The lipid bilayer is enriched with specific intramembrane proteins, such as tetraspanins (CD9, CD63, CD81), growth factor receptors, cell adhesion molecules, and integrins which can serve as exosomal marker proteins. Created with BioRender.com.

novel drug development targets, and drug delivery vehicles.

2.1. Exosomes as potential diagnostic biomarkers

In the context of diagnostics, exosomes may function as biomarkers for tracking disease progression, as they provide insights into the pathophysiological conditions of both healthy and affected individuals. Exosomes circulate stably in a wide range of body fluids, including blood, urine, saliva, and cerebrospinal fluid, where their lipid bilayer protects enclosed proteins and nucleic acids (DNA, mRNA, miRNA, lncRNA) from enzymatic degradation, ensuring the preservation of biologically relevant information. This stability allows reliable sampling even under conditions where free biomolecules would be rapidly degraded.

Importantly, exosomes can cross biological barriers, such as the blood–brain barrier, making them particularly valuable for detecting central nervous system pathologies through peripheral biofluids [12]. Their molecular cargo reflects the physiological and pathological state of the originating cells, meaning that tumor-derived exosomes often carry oncogenic proteins, altered miRNA profiles, or specific surface markers distinct from normal exosomes. Similarly, in neurodegenerative diseases, neuron- or glia-derived exosomes contain disease-associated proteins such as amyloid-β, tau, or α-synuclein [13].

In cardiovascular diseases, circulating exosomes may carry cardiomyocyte- or endothelial-specific molecules, enabling assessment of tissue stress or damage [14]. Together, these features enable exosomal profiling to yield disease-specific molecular signatures with potential applications in early diagnosis, prognosis, and longitudinal disease monitoring through minimally invasive liquid biopsies.

2.2. Exosomes as nanoscale vehicles for drug delivery

Besides a potential application as diagnostic biomarkers, exosomes have been exploited as nano-sized vehicels for delivery of therapuetic agents, such as drugs and RNA. Exosomes present numerous advantages compared to synthetic nanoparticles (NPs), including their inherent targeting capabilities, reduced immunogenic response, stability in biological fluids, natural capacity to carry nucleic acids and proteins, and proficiency in traversing biological barriers. These characteristics enhance their potential as therapeutic agents and vehicles for drug delivery (DD) [4,7,15].

Naturally, exosomes serve as a sophisticated mechanism for intercellular transport, facilitating the precise delivery of inclusions to both proximal and distant cells or tissues, while also playing a pivotal role in the communication of cellular information that influences physiological functions and characteristics. Exosomes mediate intercellular communication through a cascade of mechanistic interactions beginning with surface recognition via integrin-tetraspanin complexes or Eph receptors that bind cognate receptors or ligands on recipient cells to confer targeting specificity and may initiate signaling cascades even without internalization [16]. Once bound, exosomes can be internalized via distinct endocytic pathways. The well-characterized clathrin-mediated endocytosis involves adaptor protein (AP2) and clathrin coat formation followed by dynamin-mediated vesicle scission. Caveolin-dependent and lipid raft-mediated routes leverage caveolin-1–rich microdomains for uptake. Macropinocytosis captures extracellular milieu in actin-driven membrane ruffles, and, especially in phagocytes, phagocytosis engulfs larger vesicles into phagosomes, often targeting them for lysosomal degradation [1]. Alternatively, direct membrane fusion can occur, whereby lipid bilayers of the exosome and target cell approach to form a hemifusion stalk and expand to a fusion pore, releasing cargo directly into the cytosol. This fusion process is thought to involve SNARE and Rab protein families, and may be favored in specialized contexts such as acidic microenvironments [15]. Collectively, these mechanisms enable exosomes to deliver proteins, lipids, and RNAs that can recalibrate recipient-cell signaling, gene expression, or phenotype based on cargo content and uptake route.

Upon interaction with biological fluids (BFs), nano-scale entities experience a transformation that results in the loss of their initial identity and the assumption of a novel biological identity. This process, referred to as protein corona (PC) formation, significantly modifies several physicochemical properties of NPs, such as their surface charge, dimensions, and aggregation behavior. These alterations significantly influence the biological consequences of NPs, shaping their biodistribution, pharmacokinetics, and overall therapeutic efficacy. It is widely recognized that even subtle variations in the composition of protein-rich fluids, such as plasma and serum, can profoundly influence the architecture of the PC that develops on the surfaces of NPs [17].

Also exosomes representing natural NPs are likely to acquire a specific protein corona. Exosomes are distinguished by their remarkable ability to convey signaling biomolecules, offering numerous benefits over traditional cell-based therapies. Considering the extensive adsorption potential of a large number of molecules to the surfaces of exosomes and the natural variability in serum protein composition among individuals, it is likely that there will be significant differences in the thickness of the protein corona. Such differences are expected to significantly affect the biological activities of exosomes. Since the primary components of the PC are typically serum proteins, it seems reasonable to suggest that incorporation of a PC into allogeneic immunogens may provide some protection from immune system recognition of administered exosomes [17].

However, the precise mechanisms that govern the formation of protein complexes on exosomes are not yet fully elucidated [17]. Research into exosomes remains a dynamic field, with continuous technological and experimental progress poised to yield significant understanding of their diversity and biological roles, thereby enhancing the prospects for utilizing their therapeutic and diagnostic capabilities.

3. Exosome isolation and purification

For large-scale use of exosomes in clinical applications, there is a need for methods that are inexpensive, simple, rapid, and provide high yields. Traditional methods face challenges in meeting this demand. Table 1 and Fig. 2 provide an overview of current methods used for the isolation and purification of exosomes, also evaluating their advantages and disadvantages.

With the growing interest in this field, a review of the literature reveals that exosome isolation and characterization primarily rely on techniques such as ultracentrifugation, density gradient ultracentrifugation steps, ultrafiltration, size exclusion chromatography, immunoaffinity capture, precipitation-based methods, and microfluidics (acoustic) techniques [18,19]. In summary, the downstream application of the isolated exosomes is the decisive factor when selecting the most suitable isolation method. While an exosome sample with low purity may be sufficient for qualitative analysis, such as proteomics or western blot, exosmes of high quality are needed for the analysis of exosome-interactions with e.g. cells.

3.1. Differential ultracentrifugation (DUC) and density gradient ultracentrifugation step (dgUC)

Differential ultracentrifugation method is based on the stepwise sedimentation of particles with different sizes and densities by increasing centrifugation speeds and durations. While large cellular fragments are separated at lower speeds, exosomes are sedimented at higher speeds (up to 100,000×*g*) [18,42].

The ultracentrifugation method, whose advantages and disadvantages are presented in Tables 1, is the most fundamental technique for isolating exosomes from cells (yeast, bacteria, human, and plant cells) using strong centrifugal forces. It is frequently preferred for exosome isolation due to its cost-effectiveness and the lack of extensive expertise required. Two techniques, differential ultracentrifugation (DUC) and

Table 1
Advantages and disadvantages of common methods used in exosome isolation.

Methods	Advantages	Disadvantages
Ultracentrifugation (UC)	• Easy to implement, widely available [20,21]	•Lower purity; contamination with proteins and lipoproteins likely [20,21]
Differential Ultracentrifugation (DUC)	•Cost-effective; widely applied [20,21].	•Contamination with proteins/lipoproteins; lower reproducibility [20,21].
Density Gradient Ultracentrifugation (dgUC)	•Higher purity; better separation from contaminants [20–22].	•Time-consuming; requires technical expertise [20–22].
Ultrafiltration (UF)	•Quick, easy and cost-effective without requiring any special equipment [23,24]	•Risk of disruption of vesicles •Exosome structural distortion •Extrusion [23,24]
Nano-filtration	•Cleaning the dead and filtering their remains [25] •It allows the removal of small-scale foreign substances [25]	•Risk of contamination by non-exosomal free-floating humoral peptides [26] •Risk of aggregate formation, pore clogging and reduced exsosome purity [27,28]
Size exclusion chromatography (SEC)	•Easy-to-produce method that preserves exosome integrity •Low levels of contaminants and exosome homogeneity [29]	•A time-consuming method with problems with sample dilution •Risk of contamination [30]
Immunoaffinity capture	•Fast and efficient [31]	•Requires educated individuals •High cost of reagents •Not applicable for large sample volumes [31,32]
Precipitation-based methods	•Easy to use and adaptable to high sample volumes •Preferred in clinical applications •High exosome yield •processing of high number of samples at the same time [33]	•Low quality and purity •Presence of polymer, positively charged molecules and contamination of the final exosome pellet [34,35]
Microfluidics	•Effective, fast and high purity [36,37]	•Expensive and complex devices are used [36,37]
Microfluidics (Size-based approaches)	•Effective, fast, high purity; label-free separation; suitable for small sample volumes; minimal sample consumption [30,38].	•Expensive and complex devices; may require specialized expertise; throughput limited by chip design [30,38].
Microfluidics (Affinity-based approaches)	•High specificity due to antibody/aptamer capture (CD9, CD63, CD81); suitable for biomarker discovery; allows downstream RNA/protein analysis [39–41].	•High reagent cost; risk of non-specific binding; limited to subpopulations depending on target marker; scalability issues [39–41].

density gradient ultracentrifugation (DGUC), are employed for this purpose [43,44]. DGUC method is based on the separation of particles within a sucrose or iodixanol gradient according to their density differences. In this way, exosomes with high purity are obtained [19].

Differential ultracentrifugation or DUC is the most commonly used method, involving sequential centrifugation steps with increasing force and duration (Fig. 2A). Under these varying centrifugal forces, the separation of extracellular components (based on their density, size, and shape) is achieved. In this method, large particles are first sedimented and removed from the medium using a low centrifugal force of approximately 300 g. Then, sequential centrifugal forces are applied to eliminate cell debris, apoptotic bodies, and protein aggregates. Finally, the supernatant is removed after the last centrifugation step, and exosome pellets are obtained [45,46]. These sequential centrifugation steps, although not requiring specialized expertise, demand additional time and effort. Furthermore, there is a risk of contamination with extracellular vesicles of similar sizes (such as contamination with lipoprotein particles unrelated to exosomes) [24].

Robinson et al. [47] compared the isolation of small extracellular vesicles from 1 mL of human plasma using different methods and reported that dUC yielded EVs within the size range of 113–123 nm, although washing steps were required to reduce lipoprotein contamination. In the same study, it was also noted that SEC provided superior purity. Additionally, Kapoor et al. [48] obtained highly pure EV fractions by performing ultracentrifugation at ~120,000×*g* for ~15 h using the OptiPrep® density gradient, demonstrating that lipoprotein contamination was substantially reduced.

3.2. Ultrafiltration (UF)

In the ultrafiltration (UF) method used for isolating exosomes, membranes with pore sizes defined by size-exclusion limits or molecular weight are utilized (Fig. 2C). In this method, one or more filters can be used for exosome isolation (e.g., 0.22 and 0.1 μm filters separate smaller particles, while 0.8 and 0.45 μm filters separate larger particles) [44]. The advantages of the UF method include ease of application, no requirement for specialized equipment, reusability of filters, and high efficiency. However, disadvantages include reduced efficiency due to filter clogging, structural damage to exosomes, and extrusion. To overcome these disadvantages, tangential flow filtration (TFF) has been developed. In TFF, pressure is applied perpendicular to the flow direction to prevent clogging. Moreover, it enables more efficient separation at high liquid volumes by manipulating hydrodynamic flow forces [49]. Commercial kits often use the ultrafiltration method to isolate exosomes (e.g., the ExoMir™ Kit) [50]. Bae et al. [51] developed a TFF-based system named EVics, enabling EV isolation from sample volumes ranging from 15 μL to 2 L, and demonstrated that it achieved purity comparable to or even higher than that obtained with dUC and SEC in cancer cell lines. Similarly, Visan et al. [52] compared TFF + SEC with UC + SEC combinations and showed that yield and purity parameters varied depending on the chosen method.

3.3. Size exclusion chromatography (SEC)

The method developed by Grant H.L. and Colin R.R. in 1955 was first used to separate solvents with different molecular weights. Size exclusion chromatography method is based on the principle that particles are eluted at different rates according to their hydrodynamic diameters during passage through a porous stationary phase. Exosomes are generally collected in homogeneous fractions [53]. In this method, starch was used as the stationary phase, and water was used as the mobile phase. As the liquid sample passes through the stationary phase with pores of a specific size, molecules with different hydrodynamic radii are retained for varying amounts of time and elute from the column at different intervals. Thus, the molecules in the liquid sample are eluted in fractions [54,55]. In recent years, significant advancements have been made in this method by utilizing fillers such as sephadex, sepharose (agarose), and polyacrylamide to improve the efficiency of separation processes [56]. For exosome purification, the SEC method, developed by using different column fillers (such as dextrans, agarose, polyacrylamide, or allyldextran), is employed to separate particles based on their size [57]. The advantages of the SEC method for exosome isolation include preserving biological activity, achieving high purity, efficient use of time and labor, reproducibility, and suitability for small sample volumes [58]. However, to overcome the disadvantages of distinguishing exosomes from microvesicles of the same size, SEC must be combined with other methods such as membrane affinity. Additionally, high

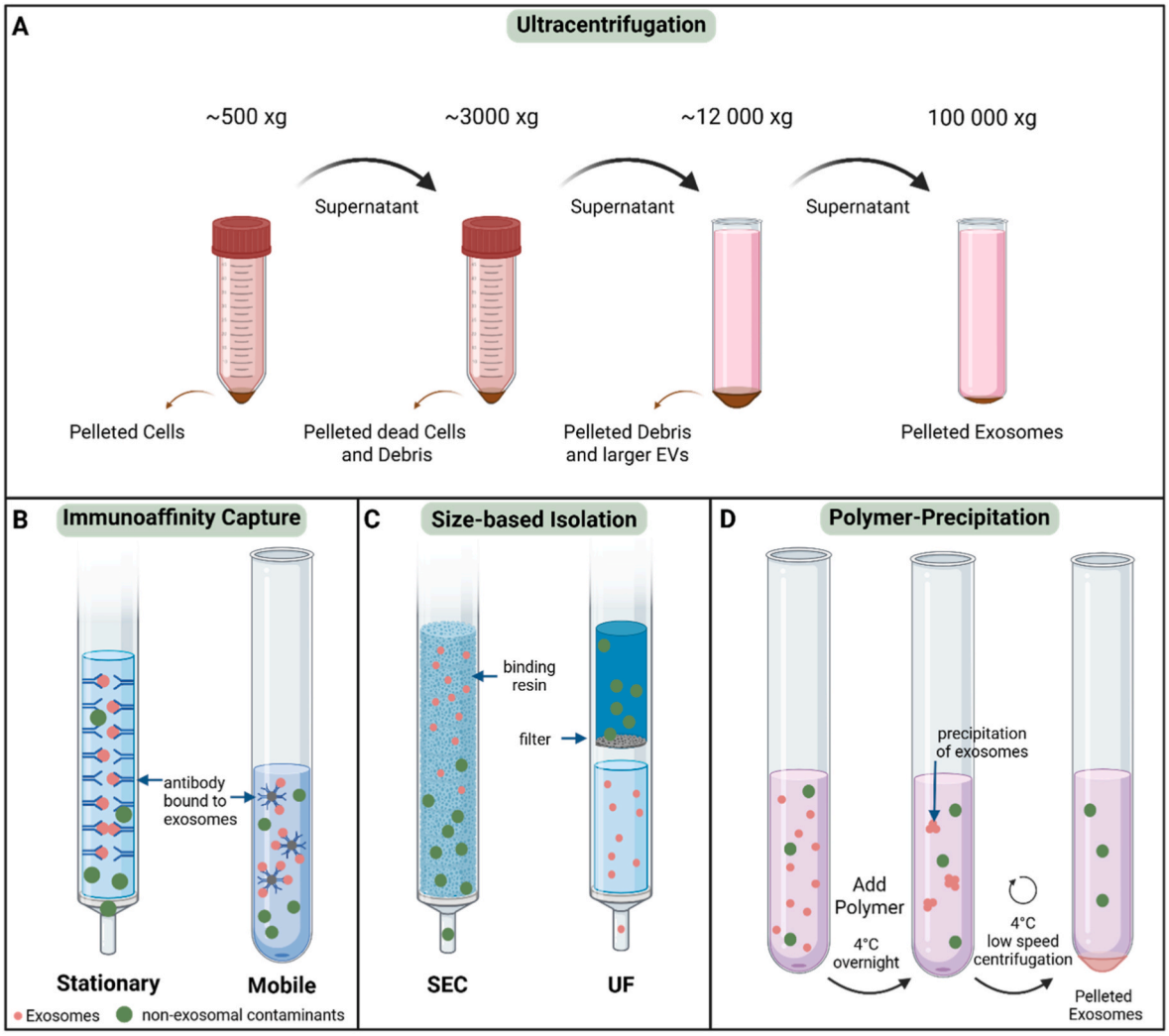

Fig. 2. Overview of state-of-the-art techniques for exosome isolation including ultracentrifugation (A), immunoaffinity capture (B), size-based isolation methods (C), and polymer-based precipitation (D). SEC, size exclusion chromatography; UF, ultrafiltration. Created with BioRender.com.

costs are among the drawbacks of this method [56]. Robinson et al. [47] reported that the SEC method yielded EVs with high purity from 1 mL of plasma in less than 90 min, with a tetraspanin profile most closely resembling that of plasma.

3.4. *Immunoaffinity capture*

This method is essentially an isolation process based on specific interactions between antigens and antibodies. In the isolation of exosomes using this method, similar specific interactions are utilized [21]. In the immunoaffinity method, antibody-coated beads, known as immunomagnetic beads, are commonly used to separate exosomes from contaminants using magnetic force (Fig. 2B) [59]. The exosomal surface contains specific proteins and receptors. Thanks to exosomal surface proteins such as annexins and tetraspanins, exosomes can bind to specific antibodies, enabling their selective isolation [60]. Proteins located on the surface of exosomes, such as tetraspanins (CD9, CD63, CD81), specifically bind to antibody-coated magnetic beads. In this way, high-purity isolation is achieved [31]. Recently, membrane affinity has started to be used in combination with differential ultracentrifugation (DUC) to achieve better separation of exosomes. Commercial kits based on this principle for high-purity exosome isolation are available on the market (e.g., Abcam, Cambridge, UK; Miltenyi Biotec, Bergisch-Gladbach, Germany; Thermo Fisher, CA, USA) [61–63]. The primary disadvantage of this method is that it is an expensive technique due to the large quantities of antibody-conjugated beads required for isolating exosomes from large volumes. Additionally, it allows for the isolation of only specific exosome subpopulations depending on the targeted exosome marker, which is considered a limitation. Another drawback is that antibodies cannot be removed from the vesicles after precipitation, which can compromise the integrity of the exosomes. These disadvantages limit the applicability of the method. Therefore, for researchers who do not require high purity or a specific exosome subpopulation, an isolation method based on this technique may not be a cost-effective or suitable option [21,56,64]. Robinson et al. [47] reported that TIM4-based immunomagnetic capture enriched EV subpopulations from 1 mL of plasma but introduced differences in size and representativeness. Furthermore, Dunlop et al. [65] enriched L1CAM-positive EV subpopulations from the blood of ALS patients using immunoaffinity capture, demonstrating that these subgroups exhibited distinct proteomic profiles.

Immunoaffinity capture approaches are not restricted to antibody–antigen interactions. In addition to antibodies, other ligands such as peptides, aptamers, and phosphatidylserine (PS)-binding proteins have been widely investigated for exosome isolation. Antibodies most commonly target tetraspanins (CD63, CD9, CD81), or tumor-associated markers such as EpCAM, PSMA, and HER2 [20,21]. Peptide ligands include the CD63-binding CP05 peptide, which enables efficient capture

and functional loading of exosomes [66], and membrane-sensing peptides that recognize highly curved lipid bilayers, thereby binding to a broad range of vesicles [67,68]. Aptamer-based platforms targeting CD63, PTK7, or EpCAM have also been developed, offering high specificity and the possibility of gentle elution strategies [69]. In addition, PS-binding proteins such as TIM4 can capture EVs in a calcium-dependent manner and release them under chelating conditions, although this may also co-isolate other PS-enriched vesicles [70]. A key limitation of immunoaffinity capture is marker heterogeneity: not all exosomes express tetraspanins such as CD63, CD9, or CD81. Single-vesicle analyses have shown uneven distribution of these markers, meaning that single-epitope targeting may result in the loss of subpopulations [71–73]. Thus, multiplexed or complementary ligand strategies are recommended to reduce this bias.

3.5. Precipitation-based methods

Hydrophilic polymers such as polyethylene glycol (PEG) reduce the solubility of exosomes, leading to their precipitation. This method is rapid and provides high yield, but the purity may be reduced [34]. This method is a precipitation-based technique in which a more hydrophilic polymeric precipitating agent (PEG), which is non-toxic and reshapes the solubility of materials in the medium) is used to competitively bind to exosomes, displacing the water molecules they are attached to, thereby promoting the precipitation of exosomes (Fig. 2D). The high hydrophilicity of the polymer reduces the solubility of exosomes in the medium, causing them to precipitate at the bottom of the tube during a low-speed centrifugation step [74]. The principle of this approach is that PEG and other polymers create a hydrophilic environment that reduces exosome solubility, leading to their aggregation and sedimentation under mild centrifugation. This allows rapid enrichment from small sample volumes. However, precipitation agents also non-specifically trap proteins, lipoproteins, and other macromolecular complexes, which may compromise exosome purity and reproducibility [24,75]. The advantages of this method include not requiring specialized equipment, being easy, fast, and highly efficient. However, a disadvantage is that the exosomes isolated using this technique may contain contaminants such as protein aggregates, immunoglobulins, lipoproteins, and viral particles, which can affect exosome quantification [76]. Additionally, another alternative method for exosome purification involves neutralizing negatively charged EVs with acetate to precipitate exosomes for charge neutralization [77]. Another important limitation is that precipitation-based approaches are not cost-effective for large-volume samples such as cell culture media, because they require large amounts of polymeric reagents. Thus, while suitable for clinical plasma or serum samples, they are less practical for preparative-scale isolation [20].

Currently, there are many commercial kits available for PEG-based exosome isolation. The most commonly used ones are the ExoQuick Plus (System Biosciences, Palo Alto, CA, USA) and ExoEasy (Qiagen, Venlo, Netherlands) kits. These kits can produce exosomes with high yield and purity, meeting the required needs [78]. Guerrero-Alba et al. [79] introduced the NTI-EXO precipitation method, which requires only 100 μL of blood sample, and reported that it increased serum EV protein recovery by 3–5 fold compared to commercial kits, successfully enabling biomarker detection in lung transplant recipients.

3.6. Microfluidics

These systems integrate separation principles based on size, density, or antigen–antibody interactions into microchannels. They offer high purity and short analysis time [80]. Recently, with the discovery of bio-markers in biological applications and their use in clinical practices, the microfluidics method has been developed to achieve high-yield exosome isolation from very small amounts of fluids. This method uses various isolation principles, including immunoaffinity, size-based separation, and density. In the immuno-microfluidics-based method, specific exosomes are captured by antibodies immobilized on microfluidic chips, and after the addition of buffer to the medium, the exosomes are eluted [38,75]. As the use of this method has become more widespread, improvements in the immobilization surface area using nano-porous structures have minimized the binding of non-exosomal vesicles. This method not only allows for exosome isolation but also enables RNA extraction using immuno-microfluidics, which has increased interest in its application [81]. Polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), silicone, and metals can be used in the manufacturing of microfluidic devices. Among these, PDMS is the most commonly used due to its notable characteristics such as biocompatibility, cost-effectiveness, and flexibility [77].

The advantages of this method include ease of use, minimal sample consumption, benefits in surface-to-volume ratio, and short analysis time. However, high costs and the complexity of the equipment used are considered disadvantages [82,83]. Meng et al. developed a viscoelastic microfluidic chip that enabled label-free, direct isolation of EVs from human blood, significantly reducing processing time. Likewise, Rufo et al. [84] reported that their acoustofluidic method, termed FLOAT, achieved over 90 % EV recovery in less than 10 min from as little as 10 μL of sample volume.

As an alternative method, Hu et al. [85] combined asymmetric flow field-flow fractionation (AF4) with an iodixanol cushion to isolate EVs of high purity and low lipoprotein contamination from human plasma, enabling the identification of more than 1000 EV proteins in proteomic analyses. These examples demonstrate that SEC is superior in terms of purity and representativeness for clinical volumes, TFF-based systems offer scalability and speed, precipitation methods are suitable for small sample volumes and ease of application, immunoaffinity techniques selectively enrich specific subpopulations, microfluidic platforms provide advantages in speed and low sample volume requirements, and AF4 is appropriate for high-purity isolation and subpopulation separation [38,47,51,52,79,84,85].

4. Characterization of isolated exosomes

Another important step after exosome isolation is the characterization of the isolated and purified exosomes. For this purpose, it is necessary to determine properties such as particle size, morphology, and concentration [19].

To determine particle size and concentration, Nanoparticle Tracking Analysis (NTA) is performed (Fig. 3A). NTA analysis allows for the quantification of particles with sizes ranging from approximately 40 to 1000 nm. For the analysis, 500 μL of suspension fluid is required [86]. In this method, a laser beam is directed at the nanoparticle suspension solution. When the laser beam hits the nanoparticles, the scattered light is captured by a camera attached to the device, and several images are taken. Since smaller particles in the suspended liquid move faster than larger ones, the motion of each particle is tracked and analyzed through video analysis. The diffusion coefficient is then determined from this analysis. Using the determined diffusion coefficient, the hydrodynamic diameter of the particles is calculated using the Stokes-Einstein equation [87]. The results of the NTA analysis provide information on size distribution, particle diameter, concentration, particle number, and size. Additionally, since it also provides the intensity of the scattered light, the collected data can be combined to visualize the results. NTA offers the advantage of allowing for easy and rapid quantification of exosomes, while its sensitivity to aggregable structures and larger particles stands out as a disadvantage of the technique [86] (See Figs. 4 and 5.

Alternatively, Dynamic Light Scattering (DLS) analysis is also performed for exosome size analysis (Fig. 3B). DLS analysis works on a similar principle as NTA. It determines the particle size by calculating changes in the intensity of scattered light. A disadvantage of DLS analysis is that the presence of larger particles in the medium makes it difficult to detect smaller particles. This issue is considered a greater

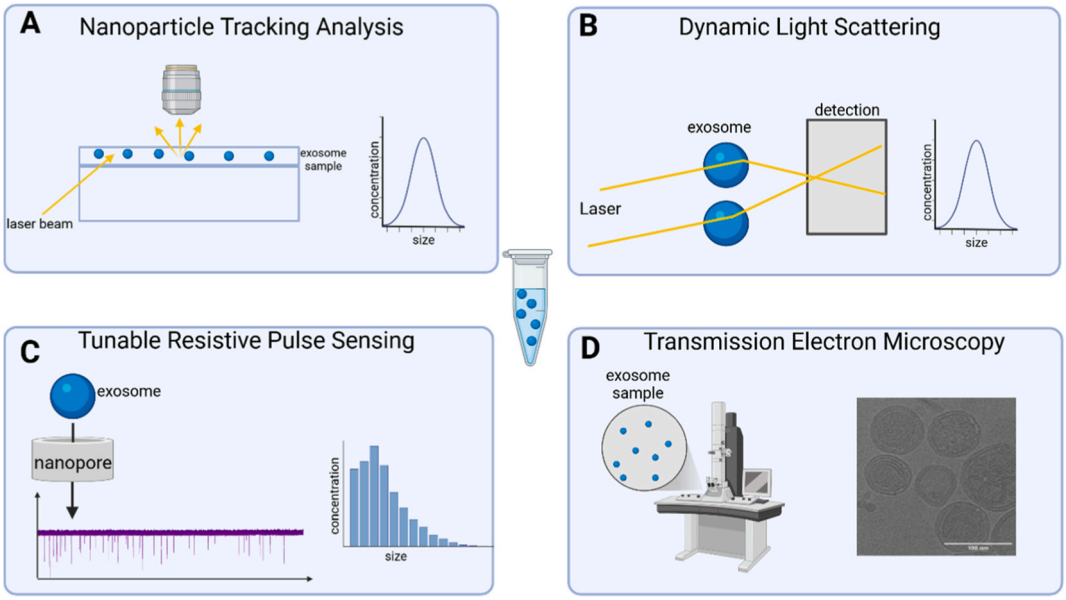

Fig. 3. Principles of common exosome characterization methods. (A) Nanoparticle Tracking Analysis (NTA) allows for quantification of exosomes by scattering of a laser beam which gives information about size and concentration. (B) Dynamic light scattering can be used to determine size and concentration of exosomes by calculating changes in the intensity of scattered light. (C) Tunable Resistive Pulse Sensing (TRPS) is an alternative method used for determining particle size, concentration, and charge. (D) Transmission Electron Microscopy (TEM) is recommended to analyze the morphology of exosomes. Created with BioRender.com.

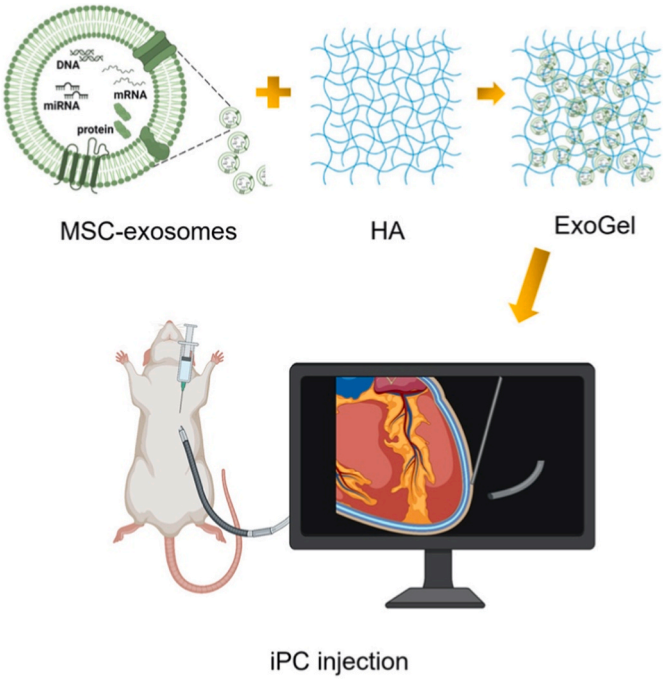

Fig. 4. Illustration of the minimally invasive delivery of a hydrogel-based exosome patch to prevent heart failure. Copyright 2022 Elsevier, Reproduced with permission from Ref. [143].

disadvantage in DLS analysis compared to NTA for size determination [19,88].

Recently, Tunable Resistive Pulse Sensing (TRPS), an experimental technique used to examine and characterize colloidal particles (such as determining particle size, concentration, and charge) [89], has been used to measure the number and diameter of exosomes (Fig. 3C). For this purpose, particles in volumes of 20–30 μL are subjected to a voltage and passed through a nanopore to measure their number and diameter. Changes in electrical current proportional to the particle volume are observed, allowing for concentration calculation. A disadvantage of TRPS is that measurement needs training and experimental experience since there are many factors that have to be adjusted for the measurement, like the pore size, current, and choice of calibration particles. Furthermore, there is the risk for nanopores to be blocked during measurement [90].

Another method used for characterization is Transmission Electron Microscopy (TEM, Fig. 3D). TEM analysis is a commonly used technique for exosome characterization, as it is in many scientific fields. The dehydration conditions of the samples, the use of metal contrast agents, and the drying conditions can affect the shape of exosomes, so these factors should be considered when performing TEM analysis [91]. For Cryo-Electron Microscopy (Cryo-EM) samples are vitrified, preserving their natural hydrated state. As a result, the spherical shape of exosomes can be better preserved and visualized. Cryo-EM also allows for the observation of the lipid bilayer of exosomes [92].

Another technique commonly used as an alternative to electron microscopy for exosome analysis is Atomic Force Microscopy (AFM). Due to its ability to determine the structure, biomechanics, and biomolecular content of exosomes in heterogeneous environments (such as tumors), AFM analysis of isolated exosomes has become a strong alternative to other imaging methods and is increasingly preferred by researchers in recent years [93]. In this technique, topographical and mechanical information of the sample is obtained. For this purpose, the sample is scanned line by line. The height of the tip used during the analysis is adjusted based on feedback (which comes from the tip's oscillation frequency, the force applied to the console, and their combination) [94].

Another commonly used method is Enzyme-Linked Immunosorbent Assay (ELISA), which is used for detecting and measuring analytes such as proteins, peptides, and antibodies. It is used to characterize and determine the quantity of exosomes [95,96].

5. Specially designed exosomes

Over the past two decades, remarkable progress has been achieved in our comprehension of the biology, chemistry, and physiology of exosomes. However, an FDA-approved therapeutic or diagnostic platform based on exosomes has yet to be realized. This delay can be attributed to

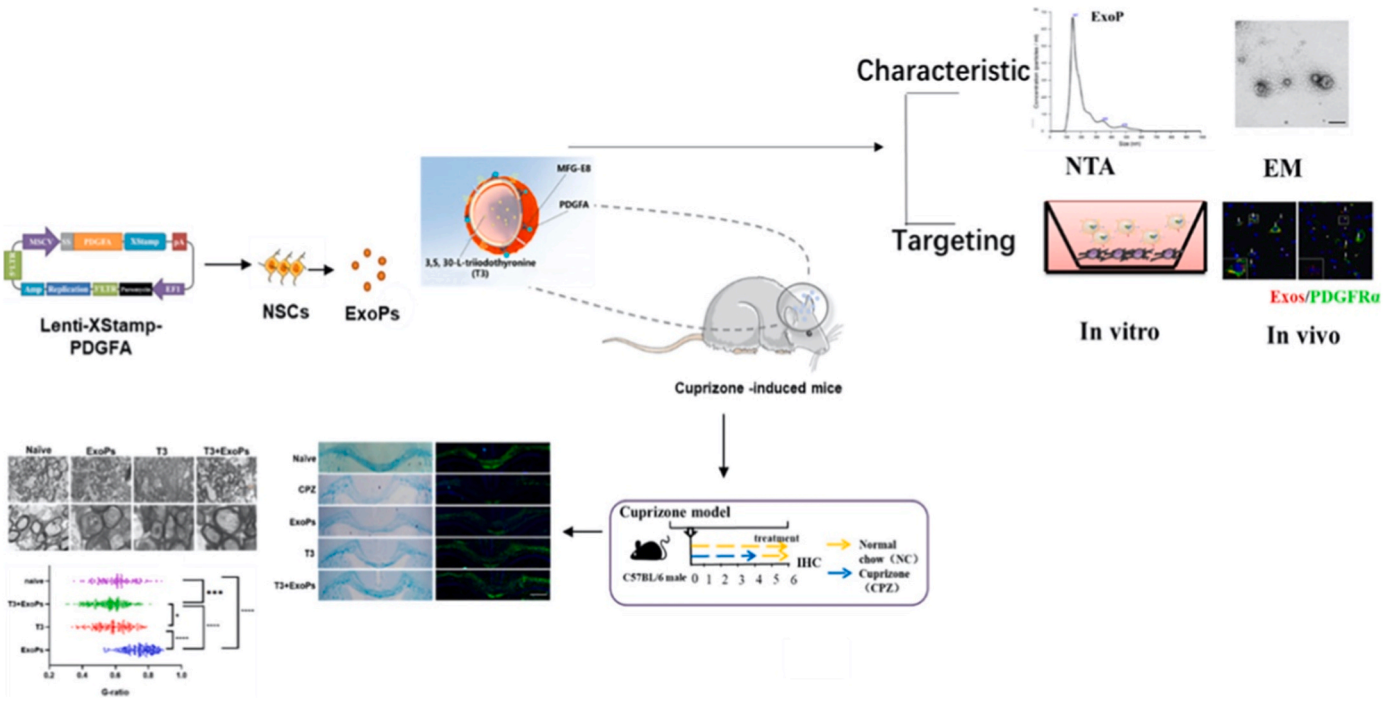

Fig. 5. T3 delivery of engineered PDGFA-ligand-modified exosomes for the treatment of demyelinating disease. Copyright 2024 Elsevier, Reproduced with permission from Ref. [170].

the inherent limitations of exosomes as natural vesicles, which pose challenges for therapeutic applications. For example, in the context of cancer treatment, it is crucial to ensure that therapeutic exosomes are specifically directed towards cancer cells. Furthermore, researchers often need to introduce entirely new drugs, proteins, or RNA into exosomes to achieve the desired therapeutic outcomes. In addition to the complexities associated with the large-scale production of exosomes, these constraints have hindered the translational progress of exosome-based therapies. Nevertheless, the advent of various biotechnological strategies has given rise to the innovative field of exosome engineering [97].

Nevertheless, the emergence of various biotechnological strategies has facilitated the development of a new discipline, exosome engineering. This field focuses on achieving several key goals:

- Targeting exosomes to specific tissues or cell types,
- Incorporating exogenous substances such as drugs, proteins, or nucleic acids into exosomes or adhering them to their external surfaces,
- Increasing the concentration of endogenous molecules within the exosomal lumen or on their surfaces.

Recent studies have increasingly integrated a number of exosome engineering techniques to create highly specialized entities known as designer exosomes [97].

Exosomes have become a prominent subject of fascination in the field of biomedicine. Enhancing our understanding of exosomes is critical for their effective clinical application. Presently, both fundamental and applied research concerning exosomes encounters two significant challenges. The first challenge is the absence of a universally accepted protocol for the isolation of exosomes that ensures both high yield and purity. The second challenge lies in the difficulty of differentiating exosomes from other extracellular vesicles (EVs), particularly functional microvesicles. Addressing these challenges is essential to substantiate the promising results associated with exosomes in various clinical contexts. Furthermore, additional research should concentrate on the nanoengineering of exosomes to optimize drug delivery systems (DDSs) and improve clinical outcomes [98].

In general, engineering of exosomes comes to the fore as biological, immunological, physical and chemical modification [99]. Biological modification represents the predominant approach for altering membranes, encompassing both targeted peptide modifications and biomimetic alterations of exosomes. Biological modification utilizes the predominant approach for altering membranes, encompassing both targeted peptide modifications and biomimetic alterations of exosomes [99].

5.1. *Exosome interactions with nanoparticles*

5.1.1. *Exosome corona and personalized exosome coronas*

Recent studies have revealed that the formation of protein complexes surrounding exosomes occurs in aqueous environments, mostly as a result of electrostatic interactions and aggregation of proteins [100]. However, the comprehension of the elements that govern the formation of protein complexes in biofluids is still in a nascent stage, both in theoretical frameworks and experimental validation. Prior investigations have produced inconsistent findings concerning the presence of albumin on the exosomal surface [17,100,101].

When nanomedicine is introduced into the human body, biomolecules present in biological fluids, especially proteins, create a layer on the nanoparticle's surface referred to as a **"personalized protein corona"**. Gaining insights into the development and dynamics of this personalized protein corona is crucial, as it enhances the effectiveness of nanotherapy and can also contribute to disease diagnosis [102].

Regarding artificial NPs, the development of a unique protein corona specific to each individual has been described in the literature. The composition of protein coronas is influenced by the characteristics of NPs, such as their physicochemical properties, as well as by various environmental factors, including temperature, the source of protein, and the specific disease context. The impact of the physicochemical properties of NPs on the formation of protein coronas has been thoroughly investigated and has been the subject of comprehensive reviews by our research group and others in the field [103–109].

Numerous environmental factors, such as protein and ionic

concentrations [110], the source of origin (human or murine) [111], the type of protein source (fetal bovine serum, serum, or plasma) [112], the selection of anticoagulant [111], and the flow conditions (dynamic versus static) [113], have only recently been incorporated into research. Additionally, certain factors, including personalized protein corona and disease-specific protein corona [114], remain poorly understood.

The concept of personalized protein corona can be broadened to encompass protein coronas that develop around nanoparticles when incubated with the plasma of human patients suffering from particular diseases. Additionally, this term can refer to protein coronas derived from the plasma of healthy individuals, taking into account variations in age, gender, lifestyle, habits, temporal factors, and geographical backgrounds. The personalized protein corona, often neglected in previous research, significantly influences the biological behavior of nanoparticles and may elucidate the unsatisfactory clinical outcomes observed with certain nanoproducts in clinical trials [115].

It is increasingly recognized that interactions between biological entities such as tissues and cells and NPs are significantly affected by the protein composition of the "corona," which refers to proteins attached to the surface of NPs. The specific structure of this corona is largely influenced by the source of the proteins, such as human plasma. The nature of the protein source plays a pivotal role in shaping the NP corona, leading to the intriguing possibility that individuals afflicted with particular diseases may present with distinct NP coronas. Hajipour et al. [114] conducted experiments by incubating two types of hydrophobic/hydrophilic NPs (polystyrene and silica) with plasma samples from human subjects diagnosed with various diseases and medical conditions, including breast cancer, diabetes, hypercholesterolemia, rheumatism, fauvism, smoking, hemodialysis, thalassemia, hemophilia A and B, pregnancy, the common cold, and hypofibrinogenemia. The findings showed that the nature of the disease significantly affects the protein composition of the NP corona.

Exosomes are naturally occurring nanoparticles distinguished by their nanometer dimensions and negative charge in physiological settings. Although it is generally acknowledged that proteins and biological substances attach to various nanomaterials, creating an outer layer termed the biomolecular corona, our comprehension of the elements influencing the formation of this corona and its biological implications remains somewhat superficial. Research has indicated that the formation of the biomolecular corona can influence the physicochemical characteristics of both synthetic and natural nanoparticles when they come into contact with biological fluids. In a study conducted by our team [116] in 2025, we synthesized and characterized peptide-based Fmoc-Lysine (Fmoc-Lys) nanomaterials and performed interaction studies with exosomes derived from cancer cells. Measurements of size, zeta potential, and colloidal stability revealed the establishment of an exosomal corona. Additionally, experiments assessing cell viability showed that the interaction between exosomes and nanomaterials diminishes the nanotoxicity profile of these materials, which holds significant practical relevance for their biological applications. In conclusion, we have provided the inaugural evidence supporting the notion of exosomal corona formation around nanomaterials, which should be integral to the assessment of interactions at nano-bio-interfaces.

Ren et al. [102] employed $Gd@C_{82}(OH)_{22}$ nanoparticles, a form of nanomedicine with efficacy against various cancer types, as a model to explore the natural protein fingerprint of the personalized protein corona in ten patients with lung squamous cell carcinoma. Their investigation identified a distinct biomarker, complement component C1q, present in the personalized protein coronas associated with lung cancer, which exhibited a significant affinity for $Gd@C_{82}(OH)_{22}$ nanoparticles. This interaction resulted in modifications to the secondary structure of the C1q protein and triggered an innate immune response, presenting potential avenues for cancer immunotherapy. Based on these findings, we propose a novel strategy for the advancement of precision nanomedicine, leveraging the opsonization of a unique protein fingerprint specific to individual patients. This methodology addresses the prevalent challenge of protein corona formation while utilizing the associated proteins to develop a precision nanomedicine and diagnostic instrument.

Proteomic alterations are frequently observed in cancer patients, leading to changes in the biological identity of nanoparticles (NPs) found in the bloodstream of cancer patients compared to those found in healthy individuals after administration. Colapicchioni and colleagues [117] investigated the relationship between changes in plasma proteins associated with breast, gastric, and pancreatic cancers and the biological identity of clinically approved AmBisome-like liposomes. This assessment was conducted using a combination of dynamic light scattering, zeta potential analysis, one-dimensional sodium dodeacyl sulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE), and semiquantitative densitometry. Although the size of liposome-protein complexes did not differ significantly across cancer types, the hard corona obtained from pancreatic cancer patients exhibited a significantly reduced negative charge. Furthermore, this hard corona was found to be more enriched in pancreatic cancer patients compared to those with other cancer types; This is probably due to the presence of immunoglobulin A (IgA) and immunoglobulin G (IgG), which may be related to autoantibody production in cancer. Considering the established link between tumor antigen-specific autoantibodies and early diagnosis of cancer, these findings may pave the way for the development of innovative screening tests for cancer based on nanoparticle-corona interactions.

The characterization of the personalized protein corona (PC) that develops around nanomaterials when exposed to human plasma is increasingly recognized as a promising technology for the early detection of cancer. Nevertheless, challenges such as low material stability and variability between batches have hindered its clinical implementation to date [118].

Digiacomo et al. [118] introduced a nanoparticle-enabled blood (NEB) assay that employs 120 nm gold nanoparticles (NPs) for the accumulation of blood plasma proteins. The characterization of the personalized protein corona surrounding the gold NPs is conducted using sodium dodecyl sulfate polyacrylamide gel electrophoresis. As a significant case study, pancreatic ductal adenocarcinoma (PDAC) was selected, given the absence of effective detection methods that contribute to a dismal survival rate post-diagnosis (less than one year) and an exceedingly low five-year survival rate (15–20 %). Through densitometric analysis of 75 protein profiles (comprising 28 from healthy individuals and 47 from PDAC patients), the study successfully differentiated between non-oncological and PDAC patients, achieving a sensitivity of 78.6 % and specificity of 85.3 %. The gold NEB assay aligns with the World Health Organization's criteria for cancer screening and detection, which emphasize affordability, sensitivity, specificity, user-friendliness, rapidity, robustness, and equipment-free delivery to end users. Consequently, this test holds significant potential for clinical application in initial investigations to determine the necessity for more invasive procedures.

The burgeoning realm of exosomes has emerged as a focal point of fascination within various therapeutic approaches and cancer treatments, alongside biomarker research. These exquisite entities are increasingly recognized as an essential conduit for intercellular communication, underscoring their pivotal role in advancing medical science. Tumor cells release substantial quantities of exosomes that are abundant in proteins, mRNAs, and microRNAs, all of which play vital roles in various phases of cancer development and metastasis. The integrins found on tumor exosomes may influence organ-specific metastasis, suggesting that the evaluation of cell surface receptors, such as integrins, could serve as a predictive tool for identifying potential metastasis sites in cancer patients. Extensive research has underscored the role of exosomes in fostering resistance to chemotherapy, demonstrating their ability to encapsulate chemotherapeutic agents in cases such as melanoma and ovarian cancer. The analysis of exosomes and their associated biomarkers is made easier by their

minimally invasive extraction from bodily fluids, which can aid in the stratification of patients and the prediction of therapeutic responses for personalized treatment approaches. Some research is increasingly focused on exploiting the unique properties of exosomes, particularly their extraordinary capacity to protect their contents from the destructive effects of proteolytic enzymes and ribonucleases. This innovative approach aims to create sophisticated targeted drug delivery systems for cancer therapy by effectively overcoming the challenges posed by traditional drug delivery techniques [119].

6. Examples for exosome-mediated biomedical applications

Ideal DDSs are characterized by their ability to deliver minimal concentrations of therapeutic agents directly to the affected area while preserving the integrity of adjacent healthy tissues. [120]. Over the past years, a wide variety of synthetic nanocarriers have been developed to increase therapeutic efficacy [121–125].

Exosomes are small vesicles that are released by nearly all types of cells and serve a crucial function in facilitating communication between cells. Beyond their involvement in normal physiological processes, these extracellular vesicles appear to be significantly implicated in inflammatory responses. This perspective provides a valuable avenue for investigating exosomes as potential biomarkers and therapeutic agents for chronic respiratory diseases [126]. Exosomes are integral to the dynamics of infectious diseases, influencing them through two primary mechanisms: facilitating intercellular communication and modulating the immune responses of the host. Both pathogen and host cells are capable of producing exosomes. The exosomes derived from pathogens contribute to the infectious process by delivering pathogen-associated materials, aiding in immune evasion, and promoting apoptosis in immune cells. In contrast, exosomes secreted by host cells support an anti-infective environment by directly inhibiting the proliferation of pathogens and enhancing immune functions. Consequently, exosomes have received significant attention for infectious disease management, serving as potential diagnostic markers, vaccine candidates, targets for novel drug development, and vehicles for drug delivery [98]. Exosomes have become a rapidly expanding area of investigation, holding significant promise for advancements in cancer detection [127,128]. Initially regarded as mere cellular debris, these nanoscale particles have gained recognition for their essential function in intercellular communication and their ability to carry molecular cargo, such as nucleic acids and proteins [129,130].

For cancer research, exosomes play an important role by carrying tumor-specific molecules that can serve as biomarkers in early diagnosis [131]. The presence of these substances in body fluids such as blood and urine makes them an attractive option for non-invasive and easily accessible diagnostic evaluations [132,133]. In addition, the wide variety of information transmitted through exosomes presents numerous potential biomarkers, facilitating not only the detection of cancer but also the differentiation of specific cancer types along with their unique molecular features [134]. This methodology presents considerable potential for the formulation of tailored therapeutic strategies for individual patients, which in turn enhances treatment efficacy and alleviates the strain of superfluous interventions [135–137]. The remarkable promise it embodies could revolutionize the landscape of early cancer diagnosis, ongoing monitoring, and treatment strategies [136].

Wang et al. [137] developed a “dual-targeted” platform for cancer drug delivery by integrating superparamagnetic iron oxide nanoparticles (SPIONs) with exosomes derived from lung cancer cells. This innovative approach successfully combined the targeted delivery capabilities of exosomes with the magnetic targeting potential of SPIONs, facilitating the cellular delivery of the chemotherapeutic agent doxorubicin (DOX). Additionally, non-small cell lung cancer (NSCLC) tumor models using the NCI-H1299 cell line have been established. The study demonstrated that exosome-SPION conjugates (Exo-SPIONs) loaded with DOX exhibited optimal tumor tissue delivery and tumor suppression in the presence of an external magnetic field, while simultaneously reducing the toxicity of DOX to normal tissues. The developed “dual-targeted” cancer drug delivery platform showed promising approach for targeted chemotherapy in NSCLC.

In the research conducted by Hua et al., [138] a novel drug delivery system utilizing exosomes was developed, integrating synergistic miRNA-mediated autophagy inhibition with 7-coumarin-based chemotherapy for the treatment of ovarian cancer. The inherent biocompatibility and ability to evade the immune system made exosomes ideal carriers for targeted drug delivery. To enhance the separation efficiency of the exosomes, magnetic nanoparticles (MNPs) were incorporated. The drugs, miRNA-FAM and 7-coumarin, were introduced into the EXOs-MNPs through electroporation, resulting in the formation of the miRNA-FAM/7-coumarin@EXOs-MNPs drug delivery system. This engineered exosome-based system demonstrated improved drug uptake and targeted delivery to cancer cells. The concurrent application of both drugs significantly increased the therapeutic efficacy against cancer cells, suggesting a synergistic interaction between the two agents. Consequently, this study presents an innovative drug delivery approach and a synergistic therapeutic strategy, highlighting its considerable potential for future clinical applications.

The presence of the blood-brain barrier (BBB) significantly restricts the effectiveness of chemical drugs in treating glioblastoma. Recently, exosomes have emerged as a promising method for delivering drugs to the brain. Nonetheless, variations in the brain-targeting efficiency of exosomes from different cellular origins, along with the issue of premature drug release during circulation, continue to hinder therapeutic outcomes. In response to these challenges, Wang et al. [139] developed a functional exosome modified with an oligopeptide and loaded with doxorubicin (Pep2-Exos-DOX) specifically for glioblastoma therapy. The BV2 mouse microglial cell line was chosen as the source of exosomes due to its advantageous BBB penetration capabilities. To prevent drug leakage during circulation, a redox-responsive oligopeptide was integrated into the exosomal membranes, effectively securing the drug. The accumulation of the drug within glioblastoma cells was validated. Pharmacodynamic assessments indicated that Pep2-Exos-DOX exhibited notable anti-cancer properties against glioblastoma while maintaining a relative level of biosafety. This innovative exosome-based drug delivery system, enhanced with redox-responsive oligopeptides, presents a novel approach for the treatment of brain disorders.

Neuroprotection represents a fundamental approach in the management of brain injuries, particularly in cases of traumatic brain injury (TBI). The neuroprotective peptide NR2B9c shows considerable promise; however, its clinical application is hindered by inadequate penetration into the brain. Exos, extraordinary naturally occurring nanovesicles, have remarkable therapeutic potential for traumatic brain injuries, serving as sophisticated vehicles for delivering drugs directly to the brain. Haroon et al. [140] designed engineered exosomes by incorporating the neuron-targeting peptide rabies virus glycoprotein (RVG29) through a bio-orthogonal click chemistry method and loaded them with NR2B9c, resulting in the creation of RVG-Exo^{NR2B9c}. The Exos modified with RVG29 demonstrated enhanced targeting efficiency towards neurons compared to unmodified exosomes, both in vivo and in vitro. Furthermore, RVG-Exo^{NR2B9c} exhibited significant cytoprotective effects against Neuro2a cells subjected to oxygen-glucose deprivation. Administration of RVG-Exo^{NR2B9c} via intravenous injection markedly improved behavioral outcomes and decreased lesion volume following TBI in a mouse model of controlled cortical impact. Given their multifunctional properties and notable efficacy, they propose that RVG-Exo^{NR2B9c} holds substantial potential for translation as both a therapeutic agent and a delivery system for brain-targeted treatment of TBI.

Breast cancer currently ranks as the most common cancer globally. Research indicates that the hyperactivation and dysregulation of critical signaling pathways, particularly the PI3K/AKT/mTOR (PAM) pathway, play significant roles in tumorigenesis and the development of resistance to existing treatments. Silva et al. [141] aimed to investigate the effects

of downregulating PAM in various breast cancer cell lines exhibiting distinct phenotypes, utilizing PIK3CA silencing as a strategy. This oncogene was specifically targeted using a pre-designed small interfering RNA (siRNA), which was transfected into both PIK3CA wild-type MDA-MB-231 cells and PIK3CA mutated MDA-MB-453 cells. The findings indicate that the siRNA effectively targeted PIK3CA, resulting in successful gene silencing and a reduction in both cellular viability and migratory ability. Additionally, exosome-like nanovesicles were successfully isolated, characterized, and introduced into the cells, demonstrating their potential as efficient siRNA nanocarriers that enhance the delivery and onset of therapeutic effects. The findings collectively indicate that the incorporation of the validated siRNA with these nanocarriers could signify a highly promising strategy for achieving targeted drug delivery in innovative breast cancer treatments.

The effective delivery of mRNA holds significant promise for clinical applications. Prior research has demonstrated that mRNAs can be successfully transported to cells both in vitro and in vivo using RNA-loaded lipid nanoparticles (LNPs). Jui Tsai et al. [142] presented an alternative method utilizing exosomes, which are the only naturally occurring nanovesicles. Unlike LNPs, which have been associated with notable cellular toxicity, exosomes exhibited no harmful effects in either in vitro or in vivo settings at any tested dosage. Additionally, mRNA-loaded exosomes displayed remarkable characteristics, including approximately 90 % mRNA encapsulation efficiency, substantial mRNA content, uniform size, and a polydispersity index below 0.2. When employing an mRNA that encodes the red light-emitting luciferase Antares2, we found that mRNA-loaded exosomes outperformed mRNA-loaded LNPs in delivering functional mRNA into human cells in vitro. Furthermore, the injection of Antares2 mRNA-loaded exosomes resulted in significant light emission when administered into the vitreous fluid of the eye or into skeletal muscle tissue in mice. Notably, this investigation revealed that repeated injections of Antares2 mRNA-loaded exosomes led to sustained luciferase expression over six injections spanning at least 10 weeks, with no signs of signal attenuation or adverse reactions at the injection sites. In alignment with these observations, exosomes containing mRNAs that encode immunogenic variants of the SARS-CoV-2 Spike and Nucleocapsid proteins were found to elicit long-lasting cellular and humoral immune responses. Collectively, these findings underscore the potential of exosomes as a viable means for delivering functional mRNA to cells in vivo.

Coronary heart disease (CHD) has consistently ranked as the leading cause of mortality in the United States for many years, resulting in millions of fatalities annually. While clinical interventions for heart ischemic injury can alleviate symptoms during the acute phase of CHD, individuals with damaged myocardial tissue may experience heart failure (HF) due to ongoing maladaptive remodeling processes. Regenerative therapies have emerged as promising treatment avenues for myocardial infarction (MI) and HF. Specifically, cardiac patches have been developed and evaluated to enhance therapeutic retention and integration within the heart. Nonetheless, the administration of these patches typically necessitates invasive surgical procedures, such as open-chest surgeries, which can lead to chronic adhesions between the anterior wall of the heart and the thoracic wall. In one study study [143], an injectable ExoGel was formulated by incorporating exosomes derived from mesenchymal stem cells (MSCs) into a hyaluronic acid (HA) hydrogel. The ExoGel was subsequently injected into the pericardial cavity of rats subjected to transverse aortic constriction (TAC) to induce heart failure. The application of ExoGel therapy resulted in a reduction of left ventricular (LV) chamber size and preservation of wall thickness. Additionally, the feasibility and safety of ExoGel injection were validated in a porcine model.

Intracellular methicillin-resistant *Staphylococcus aureus* (MRSA) poses significant challenges for treatment with conventional antibiotics, often resulting in recurrent infections and increased resistance. Yang et al. [144] introduced an innovative exosome-based platform for antibiotic delivery aimed at eliminating intracellular MRSA. This approach utilizes mannosylated exosomes (MExos) as carriers that are preferentially internalized by macrophages, facilitating the delivery of lysostaphin (MExoL) and vancomycin (MExoV) to the intracellular pathogens. The combination of MExoL and MExoV effectively eradicated dormant intracellular MRSA. Furthermore, following intravenous (IV) administration, MExos demonstrated rapid accumulation in the liver and spleen of mice, which are key target organs for intracellular MRSA. Consequently, the MExos antibiotic delivery system represents a promising avenue for addressing intracellular infections.

Some current exosome-based treatment methods are given below in Table 2.

Transplantation of mesenchymal stem cells derived from synovial fluid (SF-MSCs) represents a promising therapeutic approach for addressing cartilage degeneration associated with osteoarthritis (OA). However, the regulation of chondrogenic differentiation of these transplanted SF-MSCs within the joint environment poses significant challenges. Kartogenin (KGN), a small molecule identified for its ability to promote the differentiation of SF-MSCs into chondrocytes both in vitro and in vivo, faces limitations in clinical use due to its poor solubility in water. This low solubility leads to the formation of precipitates

Table 2
Some exosome-based biomedical applications.

Exosome Source	Application/Effect	Reference
Exosomes in plasma	Cancer diagnosis	[145]
Urinary exosomes	Biomarkers of acute myocardial infarction patients	[146]
Stem cell-derived exosome	Alleviation of cognitive impairment against subarachnoid hemorrhage	[147]
Microglia-derived exosomes	Reducing spinal cord injury	[148]
Exosomes derived from colorectal cancer cells	Suppressing B cell-mediated antitumor immunity	[149]
Uterine-derived exosomes	Inducing the M2 polarization of macrophages	[150]
Exosomes-derived M1 macrophages	Inducing macrophage polarization	[151]
Mesenchymal stem cell extracted exosomes	Cardiac tissue regeneration	[152]
Exosomes derived from human placental mesenchymal stem cells	Accelerating diabetic wound healing	[153]
Exosomes derived from bone marrow mesenchymal stem cells	Hemostatic efficacy, antioxidant activity, modulating the inflammatory microenvironment	[154]
Mesenchymal stem cell-derived exosomes	Hepatic ischemia–reperfusion injury	[155]
Endothelial-derived exosomes	For use in LPS-induced myocardial damage	[156]
Exosomes derived from blood serum	Enhancing the wound healing	[157]
Exosomes isolated from non-small cell lung cancer serum (NSCLC)	Decreasing the effects of NSCLC-Exos on NSCLC malignant progression	[158]
Cortical neuron-derived exosomes	Enhancing tissue repair following traumatic spinal cord injury	[159]
Skeletal muscle-derived exosomes	Preventing osteoporosis by promoting osteogenesis	[160]
Exosomes derived from non-small cell lung cancer	Drug delivery system for lung cancer therapy	[161]
Exosomes derived from bone marrow stem cells	For use in holistic repair of hemophilic articular cartilage defects	[162]
Exosome derived from BV2 mouse microglial cell line	Enhancing stability and delivery efficiency for glioblastoma therapy	[163]
Trophoblast stem cells derived-exosomes	Promoting cardiac repair after myocardial infarction	[164]
Exosomes derived from stromal cells of normal tissues adjacent to tumors	Effective cellular internalization, drug loading efficiency, pancreatic tumor targeting, and delivery of payloads for target pancreatic tumors	[165]
Adipose mesenchymal stem cells-derived exosomes	The treatment of peritoneal adhesions	[166]
Vascular endothelial cells-derived exosomes	Preventing osteoporosis development	[167]

within cells, resulting in suboptimal effective concentrations and consequently hindering its chondrogenesis-enhancing properties. Research by Xu et al. [168] has demonstrated that the targeted delivery of KGN to SF-MSCs via engineered exosomes facilitates a more uniform distribution of KGN within the cytosol, thereby increasing its effective concentration and significantly enhancing the chondrogenesis of SF-MSCs in both in vitro and in vivo settings. By fusing an MSC-binding peptide, E7, with the exosomal membrane protein Lamp 2b, exosomes displaying the E7 peptide on their surface (E7-Exo) are created, which possess the ability to specifically target SF-MSCs. The delivery of KGN through E7-Exo allows for efficient internalization by SF-MSCs, resulting in a greater extent of cartilage differentiation compared to KGN alone or KGN delivered via exosomes lacking the E7 peptide. Furthermore, the co-administration of SF-MSCs with E7-Exo/KGN through intra-articular injection in a rat model of OA demonstrates significantly enhanced therapeutic outcomes compared to KGN alone or KGN delivered by exosomes without E7. Collectively, the transplantation of SF-MSCs combined with in situ chondrogenesis facilitated by KGN delivered through E7-Exo presents a promising advancement in stem cell therapy for osteoarthritis.

Exosomes are increasingly being acknowledged as a promising medium for the delivery of pharmaceuticals; however, their potential applications are limited by the low yields obtained from cell culture media. Investigating methods to enhance these yields is therefore of considerable significance. Zhao et al. [169] conducted a study to determine whether low-intensity ultrasound (LIUS) irradiation could increase exosome production without adversely affecting their properties. The application of LIUS at a power density of 0.5 w/cm^2 for 60 min resulted in a marked increase in exosome secretion from A2780 cells, a model for ovarian cancer used in exosome production. Furthermore, the characteristics of the exosomes, including morphology, size, and in vivo distribution, remained largely unchanged. Mechanistic investigations suggest that LIUS may enhance exosome secretion by upregulating the expression of genes associated with exosome biosynthesis and secretion. Collectively, these findings indicate that LIUS has the potential to enhance exosome production, presenting a promising approach to increase exosome yields for drug delivery applications.

Demyelination represents a significant syndrome in numerous central nervous system (CNS) disorders, posing a major barrier to recovery and currently lacking effective therapeutic options. To address the challenges associated with the blood-brain barrier's impact on drug permeability, Wang et al. [170] modified exosomes derived from neural stem cells (NSCs) that had been transfected with a lentivirus carrying platelet-derived growth factor A (PDGFA)-ligand. In both in vivo and in vitro experiments, the modified exosomes demonstrated enhanced migratory capabilities toward lesion sites and oligodendrocyte precursor cells (OPCs). Additionally, the targeted exosomes, which were loaded with 3,5,30-L-triiodothyronine (T3), exhibited significant myelination potential during CNS development when administered to a cuprizone animal model. The findings indicated that this innovative drug delivery system, which incorporated T3, markedly facilitated remyelination compared to T3 alone. Concurrently, it improved the CNS microenvironment by mitigating astrogliosis, suppressing pro-inflammatory microglia, and reducing axonal damage. This research offers a clear strategy for generating targeted exosomes and suggests a promising therapeutic approach for demyelinating diseases.

Lipid-coated microbubbles are extensively utilized as ultrasound contrast agents and as carriers for drug delivery. Nonetheless, two significant challenges persist in the application of microbubbles for ultrasound diagnostics and therapeutic delivery: their brief half-life within the circulatory system and the complexities associated with modifying their surfaces for targeted delivery. Exosomes, a form of extracellular vesicle, exhibit a natural affinity for their originating cells, which presents an opportunity for enhanced targeting. Jang et al. [171] engineered exosome-fused microbubbles (Exo-MBs) by incorporating exosomal membrane proteins into the microbubble structure. This modification significantly enhances the stability of Exo-MBs compared to traditional microbubbles. Following the established principle that ultrasound exposure induces cavitation in microbubbles, leading to the formation of nano-sized particles, Exo-MBs were observed to self-assemble into nanoparticles embedded with exosomal membrane proteins (Exo-NPs). These Exo-NPs demonstrated improved targeting capabilities towards their source cells. Additionally, a photosensitizer, chlorin e6, was encapsulated within Exo-MBs to assess its therapeutic potential as a drug carrier. The results indicated a markedly enhanced therapeutic efficacy in photodynamic therapy, which was further complemented by cancer immunotherapy through the induction of immunogenic cell death. Consequently, they established an innovative ultrasound image-guided drug delivery system that addresses the limitations of conventional ultrasound contrast agents while facilitating concurrent photodynamic therapy and cancer immunotherapy.

Each example described above addresses current and important biomedical applications of exosomes. Based on this, it is an undeniable fact that exosomes will play a larger role and emerge as key players, especially in the biomedical field, in the coming years.

7. Limitations

Exosome samples present significant benefits owing to their straightforward nature, affordability, non-invasive collection methods, and ease of access. Nevertheless, there is a distinct requirement for improvements in the techniques employed for their isolation and purification [172]. A deeper comprehension of exosome biology unveils the intricate challenge of effectively transporting these entities to designated sites while preserving their therapeutic potency. The regularity of exosome administration, coupled with their ability to accurately identify target locations, remains paramount. Given their naturally brief half-life, the task of ensuring successful delivery to sites of injury and sustaining their presence at these critical locations becomes increasingly complex [173].

Exosomes exhibit a low zeta potential, signifying their propensity for aggregation and inherent instability [174]. This characteristic poses significant challenges in the effective delivery of exosomes, achieving targeted locations, and determining appropriate dosing. While aggregation may be evident, the underlying causes of this phenomenon have yet to be explored in depth. It has been noted that aggregation is linked to high-speed centrifugation during standard isolation procedures, and existing literature suggests that various factors—including temperature, buffer composition, detergent presence, and pH—can influence this occurrence. The aggregation of exosome structures may present certain drawbacks if their assembly takes place prior to delivery, potentially leading to a diminished quantity of exosomes that effectively arrive at their intended destinations. In the realm of drug and biological products, the quest for establishing an effective dosage is fraught with numerous critical considerations. However, when it comes to exosomes, one must acknowledge the myriad of unknown variables that complicate the determination of an optimal dose, rendering this task particularly challenging.

The preservation of exosome stability is crucial for tackling the challenges associated with their storage and transportation, serving not only the needs of consistent research but also the demands of clinical applications. Exosomes possess a naturally brief half-life [175–177] and a propensity to aggregate, rendering them inherently unstable due to their poor zeta potential [174]. This combination of biological characteristics contributes to their overall fragility. While there is a consensus in the scientific community regarding the advantages of long-term storage at −80 °C, a definitive standard for the preservation and storage of exosomes remains elusive. An additional crucial aspect to consider is the balance between cost and efficiency. The promise of exosome therapies, particularly within the realm of personalized medicine, necessitates a thorough evaluation of the current financial and operational efficiencies tied to these treatments. At present, the

application of autologous exosomes in clinical settings remains prohibitively expensive, primarily due to the complexities involved in establishing extensive banks of autologous exosomes for individual patients. While many clinical trials are exploring the use of allogeneic exosomes, there is an urgent demand for innovative nanotechnologies that can facilitate the large-scale production of clinical-grade exosomes in a manner that is both cost-effective and timely [173].

Despite the potential, the advancement of exosomal delivery systems remains in its early stages, facing three significant challenges that necessitate further exploration:

1) the isolation and purification of exosomes,
2) the incorporation of drugs and antigens into exosomes, and
3) the transportation of cargo to target cells.

The absence of an effective and standardized method for isolation and purification poses a substantial barrier to the clinical application of exosomal technology [178].

With the advancement of medical technology, exosomes are attracting significant attention due to their promising therapeutic potential. However, the regulatory environment surrounding them is complex and varies from country to country due to their unique intracellular mechanisms of action. Various production methods complicate the standardization process, leading to a fragmented regulatory framework. The current global regulatory framework for exosomes can be broadly divided into two approaches: one focuses on elucidating the constituent components of exosomes, while the other examines the physiological effects of their secretion. When used as therapeutic agents, exosomes should be regulated similarly to biological medicinal products. Like biological drugs, exosomes have undergone investigations to assess their particle size and protein composition. A therapeutic agent derived from exosomes should receive clinical approval only after a thorough understanding of their molecular composition and structure, and evidence of their pharmacokinetics and therapeutic efficacy is provided. However, proving the pharmacokinetics and therapeutic efficacy of exosomes poses significant challenges for regulatory agencies [179].

8. Future perspectives

Personalized cancer medicine, in particular, represents an ambitious endeavor rooted in the groundbreaking human genome project. Exosomes are of utmost importance, particularly for personalized cancer medicine [45]. However, sometimes the advancement of exosome-based personalized and precision medicine encounters a formidable obstacle known as exosome heterogeneity, which is affected by variations in the size, origin, and complex molecular diversity of exosomes. This complex scientific challenge has been addressed by an interdisciplinary approach combined with advanced nanotechnology and multiomics for exome profiling. The discovery of exosomes also requires extensive toxicological studies to create effective and innovative solutions for cancer treatment [180,181]. There is a possibility that exosomes may play a significant role in overcoming the global health crisis caused by cancer through the development of a revolutionary cancer vaccine [182]. The ongoing research on exosomes paves the way for a transformative breakthrough in the field of cancer treatment [183].

Recent breakthroughs in the identification and separation of EVs have paved the way for groundbreaking therapeutic approaches. The diverse adsorption properties of various molecules on exosome surfaces, coupled with the inherent differences in serum proteins across individuals, suggest significant variability in corona thickness. Such discrepancies are poised to greatly affect the kinetics, biodistribution, docking, and cellular uptake of exosomes. Given that the primary components of the protein corona (PC) are standard serum proteins, one might reasonably hypothesize that incorporating PC into allogeneic immunogens could offer a protective barrier for the administered exosomes against immune detection. Nevertheless, it is essential to account for the influence of additional pro-inflammatory cytokines. Unlike synthetic nanoparticles, the intricate nature of the exosomal membrane, along with its array of ligands, results in a pronounced heterogeneity of the PC and other associated factors on the exosome surface. In conclusion, the formation of a PC around exosomes holds the promise of transforming their physicochemical properties and potentially enhancing their targeting efficacy. The composition and functionality of the PC may vary considerably due to genetic differences among individuals, variations in exosomes from distinct donors, and the protein profile present in the recipient's serum [17,100].

Exosomes can serve as indicators of exposure and response, as toxicants can alter the composition of their bioactive cargo and influence exosome production. Research has identified exosome biomarkers indicative of organ toxicity in both human and animal studies; however, in vitro and in vivo models are best suited to investigate the fundamental principles of exosome functionality. Assessing toxicity is an important first step in preclinical drug development to validate the protective benefits of exosome therapies. Furthermore, exosomes hold great potential as effective medical carriers for transporting their cargo between cells [184].

As we delve into the theme of "Future Perspectives," it is undeniable that we are poised to witness remarkable advancements, particularly in the realm of innovative therapeutic and diagnostic applications of Exosomes. Let us remain vigilant for the exciting developments that lie ahead!

9. Conclusion

The world of exosomes is rapidly advancing, with the variety of functions attributed to them increasing daily. Numerous factors contribute to this growing interest. The idea that these tiny messengers can transport cargo from one cell to another for functional application is undeniably fascinating. Findings from numerous studies have further fueled this fascination, highlighting their role in biological processes and the mechanisms behind them. The detection of exosomes in every biological fluid examined so far, their capacity to carry therapeutic cargo for various disorders, their promise of diagnosis and treatment, their ability to retain cargo during circulation, and their role as natural carriers of a variety of complex biological materials make them exceptional.

It would be appropriate to predict that the increasing research interest in exosomes, which has become evident in recent years, will continue in the coming years. Our understanding of exosomes' capabilities will continue to evolve, leading to translational advantages. This review stands out as one of the pioneering studies investigating the interactions between exosomes and nanoparticles, particularly in the context of potential exosome corona research. The aim of this study is to provide researchers with a comprehensive overview of the latest characterization and isolation methodologies used in exosome research, particularly for exosome-based biomedical applications. We examine the potential applications of exosomes in personalized therapies, their interactions with other nanostructures, with an emphasis on the biomolecule corona, and future challenges and opportunities.

Future research trajectories in exosome-centered regenerative medicine are diverse and interconnected, requiring a comprehensive approach that integrates basic science, translational research, and clinical development. Overcoming these complex challenges could further unlock the potential of exosomes, particularly in regenerative medicine, and herald a new era of innovative therapies.

CRediT authorship contribution statement

Emrah Dikici: Writing – review & editing, Writing – original draft, Investigation. **Burcu Önal Acet:** Writing – review & editing, Writing – original draft, Investigation. **Désirée Gül:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project

administration, Funding acquisition, Conceptualization. **Nina Kummer:** Writing – review & editing, Visualization. **Roland H. Stauber:** Writing – review & editing, Writing – original draft, Investigation. **Mehmet Odabaşı:** Writing – review & editing, Writing – original draft, Investigation. **Ömür Acet:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Funding

The work of Désirée Gül, Roland H. Stauber and Nina Kummer was funded by the Brigitte and Dr. Konstanze Wegener Stiftung (project #125).

Declaration of competing interest

On behalf of all authors, the corresponding authors state that there is no conflict of interest.

Acknowledgements

The authors would like to thank The Scientific and Technological Research Council of Türkiye (TÜBİTAK), 2214-A—International Research Fellowship Program for PhD Students and the 2219 International Postdoctoral Research Fellowship Program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtadv.2025.100623.

Data availability

No data was used for the research described in the article.

References

[1] S. Maacha, A.A. Bhat, L. Jimenez, A. Raza, M. Haris, S. Uddin, J.-C. Grivel, Extracellular vesicles-mediated intercellular communication: roles in the tumor microenvironment and anti-cancer drug resistance, Mol. Cancer 18 (2019) 55, https://doi.org/10.1186/s12943-019-0965-7.

[2] Y. Zhang, J. Bi, J. Huang, Y. Tang, S. Du, P. Li, Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications, Int. J. Nanomed. 15 (2020) 6917–6934, https://doi.org/10.2147/IJN.S264498.

[3] R. Kalluri, V.S. LeBleu, The biology , function , and biomedical applications of exosomes, Science (1979) (2020) 367, https://doi.org/10.1126/science.aau6977.

[4] F. Mehryab, F. Taghizadeh, N. Goshtasbi, F. Merati, S. Rabbani, A. Haeri, Exosomes as cutting-edge therapeutics in various biomedical applications: an update on engineering, delivery, and preclinical studies, Biochimie 213 (2023) 139–167, https://doi.org/10.1016/j.biochi.2023.05.010.

[5] K. Popowski, H. Lutz, S. Hu, A. George, P. Dinh, K. Cheng, Exosome therapeutics for lung regenerative medicine, J. Extracell. Vesicles 9 (2020), https://doi.org/10.1080/20013078.2020.1785161.

[6] M. Panigaj, M.B. Johnson, W. Ke, J. McMillan, E.A. Goncharova, M. Chandler, K. A. Afonin, Aptamers as modular components of therapeutic nucleic acid nanotechnology, ACS Nano 13 (2019) 12301–12321, https://doi.org/10.1021/acsnano.9b06522.

[7] M. Lu, Y. Huang, Bioinspired exosome-like therapeutics and delivery nanoplatforms, Biomaterials 242 (2020) 119925, https://doi.org/10.1016/j.biomaterials.2020.119925.

[8] S. Sonar, Clinical trial status of exosomes-based cancer theranostics, Clin. Translat. Discov. 4 (2024), https://doi.org/10.1002/ctd2.327.

[9] Y.-S. Chen, E.-Y. Lin, T.-W. Chiou, H.-J. Harn, Exosomes in clinical trial and their production in compliance with good manufacturing practice, Tzu Chi Med. J. 32 (2020) 113, https://doi.org/10.4103/tcmj.tcmj_182_19.

[10] W. Zhang, X. Jiang, J. Bao, Y. Wang, H. Liu, L. Tang, Exosomes in pathogen infections: a bridge to deliver molecules and link functions, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.00090.

[11] J. Wang, Y. Yao, X. Chen, J. Wu, T. Gu, X. Tang, Host derived exosomes-pathogens interactions: potential functions of exosomes in pathogen infection, Biomed. Pharmacother. 108 (2018) 1451–1459, https://doi.org/10.1016/j.biopha.2018.09.174.

[12] M.N. Huda, M. Nafiujjaman, I.G. Deaguero, J. Okonkwo, M.L. Hill, T. Kim, M. Nurunnabi, Potential use of exosomes as diagnostic biomarkers and in targeted drug delivery: progress in clinical and preclinical applications, ACS Biomater. Sci. Eng. 7 (2021) 2106–2149, https://doi.org/10.1021/acsbiomaterials.1c00217.

[13] S. Dehghani, O. Ocakcı, P.T. Hatipoglu, V.C. Özalp, A. Tevlek, Exosomes as biomarkers and therapeutic agents in neurodegenerative diseases: current insights and future directions, Mol. Neurobiol. 62 (2025) 9190–9215, https://doi.org/10.1007/s12035-025-04825-5.

[14] A.B. Reiss, S. Ahmed, M. Johnson, U. Saeedullah, J. De Leon, Exosomes in cardiovascular disease: from mechanism to therapeutic target, Metabolites 13 (2023) 479, https://doi.org/10.3390/metabo13040479.

[15] F. Mehryab, S. Rabbani, S. Shahhosseini, F. Shekari, Y. Fatahi, H. Baharvand, A. Haeri, Exosomes as a next-generation drug delivery system: an update on drug loading approaches, characterization, and clinical application challenges, Acta Biomater. 113 (2020) 42–62, https://doi.org/10.1016/j.actbio.2020.06.036.

[16] R. Isaac, F.C.G. Reis, W. Ying, J.M. Olefsky, Exosomes as mediators of intercellular crosstalk in metabolism, Cell Metab. 33 (2021) 1744–1762, https://doi.org/10.1016/j.cmet.2021.08.006.

[17] B. Önal Acet, D. Gül, R.H. Stauber, M. Odabaşı, Ö. Acet, A review for uncovering the "Protein-Nanoparticle alliance": implications of the protein Corona for biomedical applications, Nanomaterials 14 (2024) 823, https://doi.org/10.3390/nano14100823.

[18] C. Théry, S. Amigorena, G. Raposo, A. Clayton, Isolation and characterization of exosomes from cell culture supernatants and biological fluids, Curr. Protoc. Cell Biol. 30 (2006), https://doi.org/10.1002/0471143030.cb0322s30.

[19] T.S. Martins, M. Vaz, A.G. Henriques, A review on comparative studies addressing exosome isolation methods from body fluids, Anal. Bioanal. Chem. 415 (2023) 1239–1263, https://doi.org/10.1007/s00216-022-04174-5.

[20] N. Dilsiz, A comprehensive review on recent advances in exosome isolation and characterization: toward clinical applications, Transl. Oncol. 50 (2024) 102121, https://doi.org/10.1016/j.tranon.2024.102121.

[21] J. Chen, P. Li, T. Zhang, Z. Xu, X. Huang, R. Wang, L. Du, Review on strategies and technologies for exosome isolation and purification, Front. Bioeng. Biotechnol. 9 (2022), https://doi.org/10.3389/fbioe.2021.811971.

[22] K. Kumar, E. Kim, M. Alhammadi, U. Reddicherla, S. Aliya, J.N. Tiwari, H.S. Park, J.H. Choi, C.Y. Son, A.T.E. Vilian, Y.-K. Han, J. Bu, Y.S. Huh, Recent advances in microfluidic approaches for the isolation and detection of exosomes, TrAC, Trends Anal. Chem. 159 (2023) 116912, https://doi.org/10.1016/j.trac.2022.116912.

[23] Y. Liu, X. Zhang, X. Cheng, Q. Luo, M. Yu, K. Long, W. Qu, Y. Tang, M. Gong, L. Liang, X. Ke, Y. Song, Characterization of fatty acid metabolism-related lncRNAs in lung adenocarcinoma identifying potential novel prognostic targets, Front. Genet. 13 (2022), https://doi.org/10.3389/fgene.2022.990153.

[24] K. Brennan, K. Martin, S.P. FitzGerald, J. O'Sullivan, Y. Wu, A. Blanco, C. Richardson, M.M. Mc Gee, A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum, Sci. Rep. 10 (2020) 1039, https://doi.org/10.1038/s41598-020-57497-7.

[25] R.M.A. Vergauwe, J. George, T. Chervy, J.A. Hutchison, A. Shalabney, V. Y. Torbeev, T.W. Ebbesen, Quantum strong coupling with protein vibrational modes, J. Phys. Chem. Lett. 7 (2016) 4159–4164, https://doi.org/10.1021/acs.jpclett.6b01869.

[26] M.L. Alvarez, M. Khosroheidari, R. Kanchi Ravi, J.K. DiStefano, Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers, Kidney Int. 82 (2012) 1024–1032, https://doi.org/10.1038/ki.2012.256.

[27] A. Abramowicz, P. Widlak, M. Pietrowska, Proteomic analysis of exosomal cargo: the challenge of high purity vesicle isolation, Mol. Biosyst. 12 (2016) 1407–1419, https://doi.org/10.1039/C6MB00082G.

[28] Y. Li, X. Zhu, M. Zhang, H. Tong, L. Su, Heatstroke-induced hepatocyte exosomes promote liver injury by activating the NOD-like receptor signaling pathway in mice, PeerJ 7 (2019) e8216, https://doi.org/10.7717/peerj.8216.

[29] A.N. Böing, E. van der Pol, A.E. Grootemaat, F.A.W. Coumans, A. Sturk, R. Nieuwland, Single-step isolation of extracellular vesicles by size-exclusion chromatography, J. Extracell. Vesicles 3 (2014), https://doi.org/10.3402/jev.v3.23430.

[30] H. Zhang, D. Lyden, Asymmetric-flow field-flow fractionation technology for exomere and small extracellular vesicle separation and characterization, Nat. Protoc. 14 (2019) 1027–1053, https://doi.org/10.1038/s41596-019-0126-x.

[31] D. Enderle, A. Spiel, C.M. Coticchia, E. Berghoff, R. Mueller, M. Schlumpberger, M. Sprenger-Haussels, J.M. Shaffer, E. Lader, J. Skog, M. Noerholm, Characterization of RNA from exosomes and other extracellular vesicles isolated by a novel spin column-based method, PLoS One 10 (2015) e0136133, https://doi.org/10.1371/journal.pone.0136133.

[32] R. Stranska, L. Gysbrechts, J. Wouters, P. Vermeersch, K. Bloch, D. Dierickx, G. Andrei, R. Snoeck, Comparison of membrane affinity-based method with size-exclusion chromatography for isolation of exosome-like vesicles from human

plasma, J. Transl. Med. 16 (2018) 1, https://doi.org/10.1186/s12967-017-1374-6.
[33] P. Lohmann, N. Galldiks, M. Kocher, A. Heinzel, C.P. Filss, C. Stegmayr, F. M. Mottaghy, G.R. Fink, N. Jon Shah, K.-J. Langen, Radiomics in neuro-oncology: basics, workflow, and applications, Methods 188 (2021) 112–121, https://doi.org/10.1016/j.ymeth.2020.06.003.
[34] J. Wang, K. Yang, W. Yuan, Z. Gao, Determination of serum exosomal H19 as a noninvasive biomarker for bladder cancer diagnosis and prognosis, Med. Sci. Monit. 24 (2018) 9307–9316, https://doi.org/10.12659/MSM.912018.
[35] J.Z. Nordin, Y. Lee, P. Vader, I. Mäger, H.J. Johansson, W. Heusermann, O.P. B. Wiklander, M. Hällbrink, Y. Seow, J.J. Bultema, J. Gilthorpe, T. Davies, P. J. Fairchild, S. Gabrielsson, N.C. Meisner-Kober, J. Lehtiö, C.I.E. Smith, M.J. A. Wood, S. El Andaloussi, Ultrafiltration with size-exclusion liquid chromatography for high yield isolation of extracellular vesicles preserving intact biophysical and functional properties, Nanomedicine 11 (2015) 879–883, https://doi.org/10.1016/j.nano.2015.01.003.
[36] F. Iliescu, D. Vrtačnik, P. Neuzil, C. Iliescu, Microfluidic technology for clinical applications of exosomes, Micromachines 10 (2019) 392, https://doi.org/10.3390/mi10060392.
[37] H. Wijerathne, M.A. Witek, J.M. Jackson, V. Brown, M.L. Hupert, K. Herrera, C. Kramer, A.E. Davidow, Y. Li, A.E. Baird, M.C. Murphy, S.A. Soper, Affinity enrichment of extracellular vesicles from plasma reveals mRNA changes associated with acute ischemic stroke, Commun. Biol. 3 (2020) 613, https://doi.org/10.1038/s42003-020-01336-y.
[38] Y. Meng, Y. Zhang, M. Bühler, S. Wang, M. Asghari, A. Stürchler, B. Mateescu, T. Weiss, S. Stavrakis, A.J. deMello, Direct isolation of small extracellular vesicles from human blood using viscoelastic microfluidics, Sci. Adv. 9 (2023), https://doi.org/10.1126/sciadv.adi5296.
[39] J.C. Contreras-Naranjo, H.-J. Wu, V.M. Ugaz, Microfluidics for exosome isolation and analysis: enabling liquid biopsy for personalized medicine, Lab Chip 17 (2017) 3558–3577, https://doi.org/10.1039/C7LC00592J.
[40] J. Shen, Z. Ma, J. Xu, T. Xue, X. Lv, G. Zhu, B. Huang, Exosome isolation and detection: from microfluidic chips to nanoplasmonic biosensor, ACS Appl. Mater. Interfaces (2024), https://doi.org/10.1021/acsami.3c19396.
[41] J. Hu, D. Gao, Recent advances in aptamer-based microfluidic biosensors for the isolation, signal amplification and detection of exosomes, Sensors 25 (2025) 848, https://doi.org/10.3390/s25030848.
[42] J. Li, Y. Zhang, P.-Y. Dong, G.-M. Yang, S. Gurunathan, A comprehensive review on the composition, biogenesis, purification, and multifunctional role of exosome as delivery vehicles for cancer therapy, Biomed. Pharmacother. 165 (2023) 115087, https://doi.org/10.1016/j.biopha.2023.115087.
[43] S. Gurunathan, M.-H. Kang, J.-H. Kim, A comprehensive review on factors influences biogenesis, functions, therapeutic and clinical implications of exosomes, Int. J. Nanomed. 16 (2021) 1281–1312, https://doi.org/10.2147/IJN.S291956.
[44] M.Yu Konoshenko, E.A. Lekchnov, A.V. Vlassov, P.P. Laktionov, Isolation of extracellular vesicles: general methodologies and latest trends, BioMed Res. Int. (2018) 1–27, https://doi.org/10.1155/2018/8545347.
[45] R. Tenchov, J.M. Sasso, X. Wang, W.-S. Liaw, C.-A. Chen, Q.A. Zhou, Exosomes—Nature's lipid nanoparticles, a rising star in drug delivery and diagnostics, ACS Nano 16 (2022) 17802–17846, https://doi.org/10.1021/acsnano.2c08774.
[46] R. Szatanek, J. Baran, M. Siedlar, M. Baj-Krzyworzeka, Isolation of extracellular vesicles: determining the correct approach, Int. J. Mol. Med. 36 (2015) 11–17, https://doi.org/10.3892/ijmm.2015.2194 (Review).
[47] S.D. Robinson, M. Samuels, W. Jones, N. Stewart, M. Eravci, N.K. Mazarakis, D. Gilbert, G. Critchley, G. Giamas, Confirming size-exclusion chromatography as a clinically relevant extracellular vesicles separation method from 1mL plasma through a comprehensive comparison of methods, BMC Method. 1 (2024) 7, https://doi.org/10.1186/s44330-024-00007-2.
[48] K.S. Kapoor, K. Harris, K.A. Arian, L. Ma, B. Schueng Zancanela, K.A. Church, K. M. McAndrews, R. Kalluri, High throughput and rapid isolation of extracellular vesicles and exosomes with purity using size exclusion liquid chromatography, Bioact. Mater. 40 (2024) 683–695, https://doi.org/10.1016/j.bioactmat.2024.08.002.
[49] S. Busatto, G. Vilanilam, T. Ticer, W.-L. Lin, D.W. Dickson, S. Shapiro, P. Bergese, J. Wolfram, Tangential flow filtration for highly efficient concentration of extracellular vesicles from large volumes of fluid, Cells 7 (2018) 273, https://doi.org/10.3390/cells7120273.
[50] A. Cheruvanky, H. Zhou, T. Pisitkun, J.B. Kopp, M.A. Knepper, P.S.T. Yuen, R. A. Star, Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator, Am. J. Physiol. Ren. Physiol. 292 (2007) F1657–F1661, https://doi.org/10.1152/ajprenal.00434.2006.
[51] J. Bae, C. Lee, D. Jung, K. Yea, B. Song, H. Lee, M. Baek, Extracellular vesicle isolation and counting system (EVics) based on simultaneous tandem tangential flow filtration and large field-of-view light scattering, J. Extracell. Vesicles 13 (2024), https://doi.org/10.1002/jev2.12479.
[52] K.S. Visan, R.J. Lobb, S. Ham, L.G. Lima, C. Palma, C.P.Z. Edna, L. Wu, H. Gowda, K.K. Datta, G. Hartel, C. Salomon, A. Möller, Comparative analysis of tangential flow filtration and ultracentrifugation, both combined with subsequent size exclusion chromatography, for the isolation of small extracellular vesicles, J. Extracell. Vesicles 11 (2022), https://doi.org/10.1002/jev2.12266.
[53] A.N. Böing, E. van der Pol, A.E. Grootemaat, F.A.W. Coumans, A. Sturk, R. Nieuwland, Single-step isolation of extracellular vesicles by size-exclusion chromatography, J. Extracell. Vesicles 3 (2014), https://doi.org/10.3402/jev.v3.23430.
[54] K. Brezinski, B. Gorczyca, An overview of the uses of high performance size exclusion chromatography (HPSEC) in the characterization of natural organic matter (NOM) in potable water, and ion-exchange applications, Chemosphere 217 (2019) 122–139, https://doi.org/10.1016/j.chemosphere.2018.10.028.
[55] A. Gorgzadeh, A. Nazari, A. Ali Ehsan Ismaeel, D. Safarzadeh, J.A.K. Hassan, S. Mohammadzadehsaliani, H. Kheradjoo, P. Yasamineh, S. Yasamineh, A state-of-the-art review of the recent advances in exosome isolation and detection methods in viral infection, Virol. J. 21 (2024) 34, https://doi.org/10.1186/s12985-024-02301-5.
[56] K. Sidhom, P.O. Obi, A. Saleem, A review of exosomal isolation methods: is size exclusion chromatography the best option? Int. J. Mol. Sci. 21 (2020) 6466, https://doi.org/10.3390/ijms21186466.
[57] D. Yang, W. Zhang, H. Zhang, F. Zhang, L. Chen, L. Ma, L.M. Larcher, S. Chen, N. Liu, Q. Zhao, P.H.L. Tran, C. Chen, R.N. Veedu, T. Wang, Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics, Theranostics 10 (2020) 3684–3707, https://doi.org/10.7150/thno.41580.
[58] C. Hu, W. Jiang, M. Lv, S. Fan, Y. Lu, Q. Wu, J. Pi, Potentiality of exosomal proteins as novel cancer biomarkers for liquid biopsy, Front. Immunol. 13 (2022), https://doi.org/10.3389/fimmu.2022.792046.
[59] A. Clayton, J. Court, H. Navabi, M. Adams, M.D. Mason, J.A. Hobot, G. R. Newman, B. Jasani, Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry, J. Immunol. Methods 247 (2001) 163–174, https://doi.org/10.1016/S0022-1759(00)00321-5.
[60] C.F. Ruivo, B. Adem, M. Silva, S.A. Melo, The biology of cancer exosomes: insights and new perspectives, Cancer Res. 77 (2017) 6480–6488, https://doi.org/10.1158/0008-5472.CAN-17-0994.
[61] L. Gangoda, S. Boukouris, M. Liem, H. Kalra, S. Mathivanan, Extracellular vesicles including exosomes are mediators of signal transduction: are they protective or pathogenic? Proteomics 15 (2015) 260–271, https://doi.org/10.1002/pmic.201400234.
[62] M.P. Oksvold, A. Neurauter, K.W. Pedersen, Magnetic Bead-based Isolation of Exosomes, 2015, pp. 465–481, https://doi.org/10.1007/978-1-4939-1538-5_27.
[63] J. Woo, S. Sharma, J. Gimzewski, The role of isolation methods on a nanoscale surface structure and its effect on the size of exosomes, J. Circ. Biomark 5 (2016) 11, https://doi.org/10.5772/64148.
[64] S. Cai, B. Luo, P. Jiang, X. Zhou, F. Lan, Q. Yi, Y. Wu, Immuno-modified superparamagnetic nanoparticles *via* host–guest interactions for high-purity capture and mild release of exosomes, Nanoscale 10 (2018) 14280–14289, https://doi.org/10.1039/C8NR02871K.
[65] R.A. Dunlop, S.A. Banack, P.A. Cox, L1CAM immunocapture generates a unique extracellular vesicle population with a reproducible miRNA fingerprint, RNA Biol. 20 (2023) 140–148, https://doi.org/10.1080/15476286.2023.2198805.
[66] X. Gao, N. Ran, X. Dong, B. Zuo, R. Yang, Q. Zhou, H.M. Moulton, Y. Seow, H. Yin, Anchor peptide captures, targets, and loads exosomes of diverse origins for diagnostics and therapy, Sci. Transl. Med. 10 (2018), https://doi.org/10.1126/scitranslmed.aat0195.
[67] A. Gori, R. Frigerio, P. Gagni, J. Burrello, S. Panella, A. Raimondi, G. Bergamaschi, G. Lodigiani, M. Romano, A. Zendrini, A. Radeghieri, L. Barile, M. Cretich, Addressing heterogeneity in direct analysis of extracellular vesicles and their analogs by membrane sensing peptides as pan-vesicular affinity probes, Adv. Sci. 11 (2024), https://doi.org/10.1002/advs.202400533.
[68] A. Gori, A. Romanato, G. Bergamaschi, A. Strada, P. Gagni, R. Frigerio, D. Brambilla, R. Vago, S. Galbiati, S. Picciolini, M. Bedoni, G.G. Daaboul, M. Chiari, M. Cretich, Membrane-binding peptides for extracellular vesicles on-chip analysis, J. Extracell. Vesicles 9 (2020), https://doi.org/10.1080/20013078.2020.1751428.
[69] Z. Zhou, Y. Chen, X. Qian, Target-specific exosome isolation through aptamer-based microfluidics, Biosensors (Basel) 12 (2022) 257, https://doi.org/10.3390/bios12040257.
[70] W. Nakai, T. Yoshida, D. Diez, Y. Miyatake, T. Nishibu, N. Imawaka, K. Naruse, Y. Sadamura, R. Hanayama, A novel affinity-based method for the isolation of highly purified extracellular vesicles, Sci. Rep. 6 (2016) 33935, https://doi.org/10.1038/srep33935.
[71] J.A. Welsh, D.C.I. Goberdhan, L. O'Driscoll, E.I. Buzas, C. Blenkiron, B. Bussolati, H. Cai, D. Di Vizio, T.A.P. Driedonks, U. Erdbrügger, J.M. Falcon-Perez, Q. Fu, A. F. Hill, M. Lenassi, S.K. Lim, M.G. Mahoney, S. Mohanty, A. Möller, R. Nieuwland, T. Ochiya, S. Sahoo, A.C. Torrecilhas, L. Zheng, A. Zijlstra, S. Abuelreich, R. Bagabas, P. Bergese, E.M. Bridges, M. Brucale, D. Burger, R.P. Carney, E. Cocucci, R. Crescitelli, E. Hanser, A.L. Harris, N.J. Haughey, A. Hendrix, A. R. Ivanov, T. Jovanovic-Talisman, N.A. Kruh-Garcia, V. Ku'ulei-Lyn Faustino, D. Kyburz, C. Lässer, K.M. Lennon, J. Lötvall, A.L. Maddox, E.S. Martens-Uzunova, R.R. Mizenko, L.A. Newman, A. Ridolfi, E. Rohde, T. Rojalin, A. Rowland, A. Saftics, U.S. Sandau, J.A. Saugstad, F. Shekari, S. Swift, D. Ter-Ovanesyan, J.P. Tosar, Z. Useckaite, F. Valle, Z. Varga, E. van der Pol, M.J.C. van Herwijnen, M.H.M. Wauben, A.M. Wehman, S. Williams, A. Zendrini, A. J. Zimmerman, C. Théry, K.W. Witwer, Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches, J. Extracell. Vesicles 13 (2024), https://doi.org/10.1002/jev2.12404.
[72] M. Okada-Tsuchioka, N. Kajitani, W. Omori, T. Kurashige, S. Boku, M. Takebayashi, Tetraspanin heterogeneity of small extracellular vesicles in human biofluids and brain tissue, Biochem. Biophys. Res. Commun. 627 (2022) 146–151, https://doi.org/10.1016/j.bbrc.2022.08.025.
[73] R.R. Mizenko, T. Brostoff, T. Rojalin, H.J. Koster, H.S. Swindell, G.S. Leiserowitz, A. Wang, R.P. Carney, Tetraspanins are unevenly distributed across single extracellular vesicles and bias sensitivity to multiplexed cancer biomarkers,

J. Nanobiotechnol. 19 (2021) 250, https://doi.org/10.1186/s12951-021-00987-1.
[74] D. Yu, Y. Li, M. Wang, J. Gu, W. Xu, H. Cai, X. Fang, X. Zhang, Exosomes as a new frontier of cancer liquid biopsy, Mol. Cancer 21 (2022) 56, https://doi.org/10.1186/s12943-022-01509-9.
[75] P. Li, M. Kaslan, S.H. Lee, J. Yao, Z. Gao, Progress in exosome isolation techniques, Theranostics 7 (2017) 789–804, https://doi.org/10.7150/thno.18133.
[76] P. Li, J. Feng, Y. Liu, Q. Liu, L. Fan, Q. Liu, X. She, C. Liu, T. Liu, C. Zhao, W. Wang, G. Li, M. Wu, Novel therapy for glioblastoma multiforme by restoring LRRC4 in tumor cells: LRRC4 inhibits tumor-infiltrating regulatory T cells by cytokine and programmed cell death 1-Containing exosomes, Front. Immunol. 8 (2017), https://doi.org/10.3389/fimmu.2017.01748.
[77] S.Z. Shirejini, F. Inci, The Yin and Yang of exosome isolation methods: conventional practice, microfluidics, and commercial kits, Biotechnol. Adv. 54 (2022) 107814, https://doi.org/10.1016/j.biotechadv.2021.107814.
[78] M. Macías, V. Rebmann, B. Mateos, N. Varo, J.L. Perez-Gracia, E. Alegre, Á. González, Comparison of six commercial serum exosome isolation methods suitable for clinical laboratories. Effect in cytokine analysis, Clin. Chem. Lab. Med. 57 (2019) 1539–1545, https://doi.org/10.1515/cclm-2018-1297.
[79] A. Guerrero-Alba, S. Bansal, A.N. Sankpal, G. Mitra, M. Rahman, R. Ravichandran, C. Poulson, T.P. Fleming, M.A. Smith, R.M. Bremner, T. Mohanakumar, N.V. Sankpal, Enhanced enrichment of extracellular vesicles for laboratory and clinical research from drop-sized blood samples, Front. Mol. Biosci. 11 (2024), https://doi.org/10.3389/fmolb.2024.1365783.
[80] X. Fan, Y. Zhang, W. Liu, M. Shao, Y. Gong, T. Wang, S. Xue, R. Nian, A comprehensive review of engineered exosomes from the preparation strategy to therapeutic applications, Biomater. Sci. 12 (2024) 3500–3521, https://doi.org/10.1039/D4BM00558A.
[81] C. Chen, J. Skog, C.-H. Hsu, R.T. Lessard, L. Balaj, T. Wurdinger, B.S. Carter, X. O. Breakefield, M. Toner, D. Irimia, Microfluidic isolation and transcriptome analysis of serum microvesicles, Lab Chip 10 (2010) 505–511, https://doi.org/10.1039/B916199F.
[82] W. Zhao, W. Sun, S. Li, Y. Jiao, Z. Wang, T. Wu, P. Liu, L. Tan, C. Yin, Exosomal miRNA-223-3p as potential biomarkers in patients with cerebral small vessel disease cognitive impairment, Ann. Transl. Med. 9 (2021), https://doi.org/10.21037/atm-21-6086, 1781–1781.
[83] H. Wijerathne, M.A. Witek, J.M. Jackson, V. Brown, M.L. Hupert, K. Herrera, C. Kramer, A.E. Davidow, Y. Li, A.E. Baird, M.C. Murphy, S.A. Soper, Affinity enrichment of extracellular vesicles from plasma reveals mRNA changes associated with acute ischemic stroke, Commun. Biol. 3 (2020) 613, https://doi.org/10.1038/s42003-020-01336-y.
[84] J. Rufo, P. Zhang, Z. Wang, Y. Gu, K. Yang, J. Rich, C. Chen, R. Zhong, K. Jin, Y. He, J. Xia, K. Li, J. Wu, Y. Ouyang, Y. Sadovsky, L.P. Lee, T.J. Huang, High-yield and rapid isolation of extracellular vesicles by flocculation via orbital acoustic trapping: FLOAT, Microsyst. Nanoeng. 10 (2024) 23, https://doi.org/10.1038/s41378-023-00648-3.
[85] L. Hu, X. Zheng, M. Zhou, J. Wang, L. Tong, M. Dong, T. Xu, Z. Li, Optimized AF4 combined with density cushion ultracentrifugation enables profiling of high-purity human blood extracellular vesicles, J. Extracell. Vesicles 13 (2024), https://doi.org/10.1002/jev2.12470.
[86] V. Filipe, A. Hawe, W. Jiskoot, Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates, Pharm. Res. 27 (2010) 796–810, https://doi.org/10.1007/s11095-010-0073-2.
[87] C. Gardiner, Y.J. Ferreira, R.A. Dragovic, C.W.G. Redman, I.L. Sargent, Extracellular vesicle sizing and enumeration by nanoparticle tracking analysis, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.19671.
[88] R. Pecora, Dynamic light scattering measurement of nanometer particles in liquids, J. Nanoparticle Res. 2 (2000) 123–131, https://doi.org/10.1023/A:1010067107182.
[89] E. Weatherall, G.R. Willmott, Applications of tunable resistive pulse sensing, Analyst 140 (2015) 3318–3334, https://doi.org/10.1039/C4AN02270J.
[90] T.K. Kurian, S. Banik, D. Gopal, S. Chakrabarti, N. Mazumder, Elucidating methods for isolation and quantification of exosomes: a review, Mol. Biotechnol. 63 (2021) 249–266, https://doi.org/10.1007/s12033-021-00300-3.
[91] A. Emelyanov, T. Shtam, R. Kamyshinsky, L. Garaeva, N. Verlov, I. Miliukhina, A. Kudrevatykh, G. Gavrilov, Y. Zabrodskaya, S. Pchelina, A. Konevega, Cryo-electron microscopy of extracellular vesicles from cerebrospinal fluid, PLoS One 15 (2020) e0227949, https://doi.org/10.1371/journal.pone.0227949.
[92] S.T.-Y. Chuo, J.C.-Y. Chien, C.P.-K. Lai, Imaging extracellular vesicles: current and emerging methods, J. Biomed. Sci. 25 (2018) 91, https://doi.org/10.1186/s12929-018-0494-5.
[93] J. Hardij, F. Cecchet, A. Berquand, D. Gheldof, C. Chatelain, F. Mullier, B. Chatelain, J. Dogné, Characterisation of tissue factor-bearing extracellular vesicles with AFM: comparison of air-tapping-mode AFM and liquid peak force AFM, J. Extracell. Vesicles 2 (2013), https://doi.org/10.3402/jev.v2i0.21045.
[94] Y.F. Dufrêne, T. Ando, R. Garcia, D. Alsteens, D. Martinez-Martin, A. Engel, C. Gerber, D.J. Müller, Imaging modes of atomic force microscopy for application in molecular and cell biology, Nat. Nanotechnol. 12 (2017) 295–307, https://doi.org/10.1038/nnano.2017.45.
[95] R. Reverberi, L. Reverberi, Factors affecting the antigen-antibody reaction, Blood Transfus 5 (2007) 227–240, https://doi.org/10.2450/2007.0047-07.
[96] D. Ter-Ovanesyan, M. Norman, R. Lazarovits, W. Trieu, J.-H. Lee, G.M. Church, D. R. Walt, Framework for rapid comparison of extracellular vesicle isolation methods, eLife 10 (2021), https://doi.org/10.7554/eLife.70725.
[97] D. Jafari, S. Shajari, R. Jafari, N. Mardi, H. Gomari, F. Ganji, M. Forouzandeh Moghadam, A. Samadikuchaksaraei, Designer exosomes: a new platform for biotechnology therapeutics, BioDrugs 34 (2020) 567–586, https://doi.org/10.1007/s40259-020-00434-x.
[98] G.N. Alzhrani, S.T. Alanazi, S.Y. Alsharif, A.M. Albalawi, A.A. Alsharif, M. S. Abdel-Maksoud, N. Elsherbiny, Exosomes: isolation, characterization, and biomedical applications, Cell Biol. Int. 45 (2021) 1807–1831, https://doi.org/10.1002/cbin.11620.
[99] M. Zhang, S. Hu, L. Liu, P. Dang, Y. Liu, Z. Sun, B. Qiao, C. Wang, Engineered exosomes from different sources for cancer-targeted therapy, Signal Transduct. Targeted Ther. 8 (2023) 124, https://doi.org/10.1038/s41392-023-01382-y.
[100] M. Heidarzadeh, A. Zarebkohan, R. Rahbarghazi, E. Sokullu, Protein corona and exosomes: new challenges and prospects, Cell Commun. Signal. 21 (2023) 64, https://doi.org/10.1186/s12964-023-01089-1.
[101] E.Á. Tóth, L. Turiák, T. Visnovitz, C. Cserép, A. Mázló, B.W. Sódar, A.I. Försönits, G. Petővári, A. Sebestyén, Z. Komlósi, L. Drahos, Á. Kittel, G. Nagy, A. Bácsi, Á. Dénes, Y.S. Gho, K.É. Szabó-Taylor, E.I. Buzás, Formation of a protein corona on the surface of extracellular vesicles in blood plasma, J. Extracell. Vesicles 10 (2021), https://doi.org/10.1002/jev2.12140.
[102] J. Ren, R. Cai, J. Wang, M. Daniyal, D. Baimanov, Y. Liu, D. Yin, Y. Liu, Q. Miao, Y. Zhao, C. Chen, Precision nanomedicine development based on specific opsonization of human cancer patient-personalized protein coronas, Nano Lett. 19 (2019) 4692–4701, https://doi.org/10.1021/acs.nanolett.9b01774.
[103] A. Bigdeli, S. Palchetti, D. Pozzi, M.R. Hormozi-Nezhad, F. Baldelli Bombelli, G. Caracciolo, M. Mahmoudi, Exploring cellular interactions of liposomes using protein Corona fingerprints and physicochemical properties, ACS Nano 10 (2016) 3723–3737, https://doi.org/10.1021/acsnano.6b00261.
[104] K. Saha, M. Rahimi, M. Yazdani, S.T. Kim, D.F. Moyano, S. Hou, R. Das, R. Mout, F. Rezaee, M. Mahmoudi, V.M. Rotello, Regulation of macrophage recognition through the interplay of nanoparticle surface functionality and protein Corona, ACS Nano 10 (2016) 4421–4430, https://doi.org/10.1021/acsnano.6b00053.
[105] M. Mahmoudi, I. Lynch, M.R. Ejtehadi, M.P. Monopoli, F.B. Bombelli, S. Laurent, Protein−Nanoparticle interactions: opportunities and challenges, Chem. Rev. 111 (2011) 5610–5637, https://doi.org/10.1021/cr100440g.
[106] C. Corbo, R. Molinaro, A. Parodi, N.E. Toledano Furman, F. Salvatore, E. Tasciotti, The impact of nanoparticle protein Corona on cytotoxicity, immunotoxicity and target drug delivery, Nanomedicine 11 (2016) 81–100, https://doi.org/10.2217/nnm.15.188.
[107] E. Tasciotti, R. Molinaro, F. Taraballi, N. Toledano Furman, M. Sherman, A. Parodi, F. Salvatore, C. Corbo, Effects of the protein corona on liposome–liposome and liposome–cell interactions, Int. J. Nanomed. 11 (2016) 3049–3063, https://doi.org/10.2147/IJN.S109059.
[108] M.P. Monopoli, D. Walczyk, A. Campbell, G. Elia, I. Lynch, F. Baldelli Bombelli, K. A. Dawson, Physical−Chemical aspects of protein Corona: relevance to *in Vitro* and *in Vivo* biological impacts of nanoparticles, J. Am. Chem. Soc. 133 (2011) 2525–2534, https://doi.org/10.1021/ja107583h.
[109] M.P. Monopoli, C. Åberg, A. Salvati, K.A. Dawson, Biomolecular coronas provide the biological identity of nanosized materials, Nat. Nanotechnol. 7 (2012) 779–786, https://doi.org/10.1038/nnano.2012.207.
[110] M. Mahmoudi, A.M. Abdelmonem, S. Behzadi, J.H. Clement, S. Dutz, M. R. Ejtehadi, R. Hartmann, K. Kantner, U. Linne, P. Maffre, S. Metzler, M. K. Moghadam, C. Pfeiffer, M. Rezaei, P. Ruiz-Lozano, V. Serpooshan, M. A. Shokrgozar, G.U. Nienhaus, W.J. Parak, Temperature: the “Ignored” factor at the NanoBio interface, ACS Nano 7 (2013) 6555–6562, https://doi.org/10.1021/nn305337c.
[111] S. Schöttler, K. Klein, K. Landfester, V. Mailänder, Protein source and choice of anticoagulant decisively affect nanoparticle protein corona and cellular uptake, Nanoscale 8 (2016) 5526–5536, https://doi.org/10.1039/C5NR08196C.
[112] D. Pozzi, G. Caracciolo, A.L. Capriotti, C. Cavaliere, G. La Barbera, T. J. Anchordoquy, A. Laganà, Surface chemistry and serum type both determine the nanoparticle–protein corona, J. Proteonomics 119 (2015) 209–217, https://doi.org/10.1016/j.jprot.2015.02.009.
[113] D. Pozzi, G. Caracciolo, L. Digiacomo, V. Colapicchioni, S. Palchetti, A. L. Capriotti, C. Cavaliere, R. Zenezini Chiozzi, A. Puglisi, A. Laganà, The biomolecular corona of nanoparticles in circulating biological media, Nanoscale 7 (2015) 13958–13966, https://doi.org/10.1039/C5NR03701H.
[114] M.J. Hajipour, S. Laurent, A. Aghaie, F. Rezaee, M. Mahmoudi, Personalized protein coronas: a “key” factor at the nanobiointerface, Biomater. Sci. 2 (2014) 1210, https://doi.org/10.1039/C4BM00131A.
[115] C. Corbo, R. Molinaro, M. Tabatabaei, O.C. Farokhzad, M. Mahmoudi, Personalized protein corona on nanoparticles and its clinical implications, Biomater. Sci. 5 (2017) 378–387, https://doi.org/10.1039/C6BM00921B.
[116] B. Önal Acet, Ö. Acet, M. Wandrey, R.H. Stauber, D. Gül, M. Odabaşı, Synthesis, characterization, and exosomal corona formation of self-assembled dipeptide nanomaterials, Sci. Rep. 15 (2025) 13607, https://doi.org/10.1038/s41598-025-98706-5.
[117] V. Colapicchioni, M. Tilio, L. Digiacomo, V. Gambini, S. Palchetti, C. Marchini, D. Pozzi, S. Occhipinti, A. Amici, G. Caracciolo, Personalized liposome–protein corona in the blood of breast, gastric and pancreatic cancer patients, Int. J. Biochem. Cell Biol. 75 (2016) 180–187, https://doi.org/10.1016/j.biocel.2015.09.002.
[118] L. Digiacomo, D. Caputo, R. Coppola, C. Cascone, F. Giulimondi, S. Palchetti, D. Pozzi, G. Caracciolo, Efficient pancreatic cancer detection through personalized protein corona of gold nanoparticles, Biointerphases 16 (2021), https://doi.org/10.1116/6.0000540.

[119] S. Atkinson, Z. Andreu, M. Vicent, Polymer therapeutics: biomarkers and new approaches for personalized cancer treatment, J. Personalized Med. 8 (2018) 6, https://doi.org/10.3390/jpm8010006.
[120] S.P. Dunuweera, R.M.S.I. Rajapakse, R.B.S.D. Rajapakshe, S.H.D.P. Wijekoon, M. G.G.S. Nirodha Thilakarathna, R.M.G. Rajapakse, Review on targeted drug delivery carriers used in nanobiomedical applications, Curr. Nanosci. 15 (2019) 382–397, https://doi.org/10.2174/1573413714666181106114247.
[121] Ö. Acet, Design of enhanced smart delivery systems for therapeutic enzymes: kinetic and release performance of dual effected enzyme-loaded nanopolymers, Catal. Lett. 153 (2023) 3174–3184, https://doi.org/10.1007/s10562-023-04418-8.
[122] M. Zhan, H. Sun, J. Rodrigues, D. Shcharbin, M. Shen, X. Shi, Dendrimer-mediated gene delivery to boost cancer immunotherapy, Nanomedicine 18 (2023) 705–708, https://doi.org/10.2217/nnm-2023-0124.
[123] M. Yıldırım, Ö. Acet, B. Önal Acet, V. Karakoç, M. Odabaşı, Innovative approach against cancer: thymoquinone-loaded PHEMA-based magnetic nanoparticles and their effects on MCF-7 breast cancer, Biochem. Biophys. Res. Commun. 734 (2024) 150464, https://doi.org/10.1016/j.bbrc.2024.150464.
[124] P. Liu, G. Chen, J. Zhang, A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives, Molecules 27 (2022) 1372, https://doi.org/10.3390/molecules27041372.
[125] M. Yıldırım, B.Ö. Acet, E. Dikici, M. Odabaşı, Ö. Acet, Things to know and latest trends in the design and application of nanoplatforms in cancer treatment, BioNanoScience 14 (2024) 4167–4188, https://doi.org/10.1007/s12668-024-01582-y.
[126] B. Purghè, M. Manfredi, B. Ragnoli, G. Baldanzi, M. Malerba, Exosomes in chronic respiratory diseases, Biomed. Pharmacother. 144 (2021) 112270, https://doi.org/10.1016/j.biopha.2021.112270.
[127] K.-A. Hyun, H. Gwak, J. Lee, B. Kwak, H.-I. Jung, Salivary exosome and cell-free DNA for cancer detection, Micromachines 9 (2018) 340, https://doi.org/10.3390/mi9070340.
[128] L. Xiao, S. Hareendran, Y.P. Loh, Function of exosomes in neurological disorders and brain tumors, Extracell Vesicl. Circ. Nucl. Acid. (2021), https://doi.org/10.20517/evcna.2021.04.
[129] J. Maia, S. Caja, M.C. Strano Moraes, N. Couto, B. Costa-Silva, Exosome-based cell-cell communication in the tumor microenvironment, Front. Cell Dev. Biol. 6 (2018), https://doi.org/10.3389/fcell.2018.00018.
[130] W. Zhang, T.H. Pham, D.H. Wang, A "Clue" to improving liquid biopsies for cancer: microfluidic multiparametric exosome analysis, Clin. Chem. 67 (2021) 335–337, https://doi.org/10.1093/clinchem/hvaa209.
[131] N. Regev-Rudzki, D.W. Wilson, T.G. Carvalho, X. Sisquella, B.M. Coleman, M. Rug, D. Bursac, F. Angrisano, M. Gee, A.F. Hill, J. Baum, A.F. Cowman, Cell-cell communication between malaria-infected red blood cells via exosome-like vesicles, Cell 153 (2013) 1120–1133, https://doi.org/10.1016/j.cell.2013.04.029.
[132] C. Agnoletto, Y. Pignochino, C. Caruso, C. Garofalo, Exosome-based liquid biopsy approaches in bone and soft tissue sarcomas: review of the literature, prospectives, and hopes for clinical application, Int. J. Mol. Sci. 24 (2023) 5159, https://doi.org/10.3390/ijms24065159.
[133] M. Wang, Y. Wang, X. Tian, Q. Wang, H. Huang, X. Lu, M. Qi, X. Cao, J. Lei, Diagnostic and predictive value of liquid biopsy-derived exosome miR-21 for breast cancer: a systematic review and meta-analysis, Expert Rev. Mol. Diagn 23 (2023) 315–324, https://doi.org/10.1080/14737159.2023.2195552.
[134] S. Sharma, C. Salomon, Techniques Associated with Exosome Isolation for Biomarker Development: Liquid Biopsies for Ovarian Cancer Detection, 2020, pp. 181–199, https://doi.org/10.1007/978-1-4939-9773-2_8.
[135] Y. Kumata, H. Iinuma, Y. Suzuki, D. Tsukahara, H. Midorikawa, Y. Igarashi, N. Soeda, T. Kiyokawa, M. Horikawa, R. Fukushima, Exosome-encapsulated microRNA-23b as a minimally invasive liquid biomarker for the prediction of recurrence and prognosis of gastric cancer patients in each tumor stage, Oncol. Rep. (2018), https://doi.org/10.3892/or.2018.6418.
[136] Z. Wang, Q. Wang, F. Qin, J. Chen, Exosomes: a promising avenue for cancer diagnosis beyond treatment, Front. Cell Dev. Biol. 12 (2024), https://doi.org/10.3389/fcell.2024.1344705.
[137] J. Wang, X. Zhu, H. Jiang, M. Ji, Y. Wu, J. Chen, Cancer cell-derived exosome based dual-targeted drug delivery system for non-small cell lung cancer therapy, Colloids Surf. B Biointerfaces 244 (2024) 114141, https://doi.org/10.1016/j.colsurfb.2024.114141.
[138] X. Hua, Y. Liu, X. Liu, Q. Zhu, S. Zhou, Q. Li, S. Liu, Engineered exosome-based drug delivery system for synergistic cancer therapy via autophagy inhibition and chemotherapy, Biomed. Analy. 1 (2024) 103–110, https://doi.org/10.1016/j.bioana.2024.05.002.
[139] Y. Wang, Y. Huo, C. Zhao, H. Liu, Y. Shao, C. Zhu, L. An, X. Chen, Z. Chen, Engineered exosomes with enhanced stability and delivery efficiency for glioblastoma therapy, J. Contr. Release 368 (2024) 170–183, https://doi.org/10.1016/j.jconrel.2024.02.015.
[140] K. Haroon, H. Zheng, S. Wu, Z. Liu, Y. Tang, G.-Y. Yang, Y. Liu, Z. Zhang, Engineered exosomes mediated targeted delivery of neuroprotective peptide NR2B9c for the treatment of traumatic brain injury, Int. J. Pharm. 649 (2024) 123656, https://doi.org/10.1016/j.ijpharm.2023.123656.
[141] R. Silva, D. Ferreira, L.R. Rodrigues, Exosome-based delivery of RNAi leads to breast cancer inhibition, J. Drug Deliv. Sci. Technol. 78 (2022) 103931, https://doi.org/10.1016/j.jddst.2022.103931.
[142] S.J. Tsai, N.A. Atai, M. Cacciottolo, J. Nice, A. Salehi, C. Guo, A. Sedgwick, S. Kanagavelu, S.J. Gould, Exosome-mediated mRNA delivery in vivo is safe and can be used to induce SARS-CoV-2 immunity, J. Biol. Chem. 297 (2021) 101266, https://doi.org/10.1016/j.jbc.2021.101266.
[143] G. Cheng, D. Zhu, K. Huang, T.G. Caranasos, Minimally invasive delivery of a hydrogel-based exosome patch to prevent heart failure, J. Mol. Cell. Cardiol. 169 (2022) 113–121, https://doi.org/10.1016/j.yjmcc.2022.04.020.
[144] X. Yang, B. Xie, H. Peng, G. Shi, B. Sreenivas, J. Guo, C. Wang, Y. He, Eradicating intracellular MRSA via targeted delivery of lysostaphin and vancomycin with mannose-modified exosomes, J. Contr. Release 329 (2021) 454–467, https://doi.org/10.1016/j.jconrel.2020.11.045.
[145] N. Li, C. Cheng, D. Wu, Z. Song, B. Wang, G. Li, F. Yang, Immunofluorescent analysis of exosomes using a microchip filled with transparent antibody-conjugated beads for breast cancer liquid biopsy, Anal. Chim. Acta 1345 (2025) 343743, https://doi.org/10.1016/j.aca.2025.343743.
[146] X. Mao, H. Xu, X. Liu, J. Guan, J. Shi, S. Yang, Proteomics of urinary exosomes for discovering novel non-invasive biomarkers of acute myocardial infarction patients, Int. J. Biol. Macromol. 302 (2025) 140427, https://doi.org/10.1016/j.ijbiomac.2025.140427.
[147] S. Gotoh, M. Kawabori, S. Yamaguchi, Y. Nakahara, E. Yoshie, K. Konno, Y. Mizuno, Y. Fujioka, Y. Ohba, Y. Kuge, M. Watanabe, M. Fujimura, Intranasal administration of stem cell-derived exosome alleviates cognitive impairment against subarachnoid hemorrhage, Exp. Neurol. 386 (2025) 115143, https://doi.org/10.1016/j.expneurol.2025.115143.
[148] J. Cui, S. Lin, M. Zhang, Resveratrol loaded microglia-derived exosomes attenuate astrogliasis by restoring mitochondrial function to reduce spinal cord injury, Chem. Biol. Interact. 408 (2025) 111407, https://doi.org/10.1016/j.cbi.2025.111407.
[149] Y. Zhang, Y. Yu, X. Gu, Z. Li, Y. Zhou, J. Xiang, Exosomes derived from colorectal cancer cells suppress B-cell mediated anti-tumor immunity, Int. Immunopharmacol. 148 (2025) 114176, https://doi.org/10.1016/j.intimp.2025.114176.
[150] S. Chen, Y. Jiang, X. Chai, Z. Chen, H. Tian, M. Liu, T. Zhu, W. ShangGuan, X. Wu, Uterine-derived exosomes induce the M2 polarization of macrophages via miR-210-3p/ATP5D to promote endometriosis progression, Life Sci. 363 (2025) 123383, https://doi.org/10.1016/j.lfs.2025.123383.
[151] M. Mahmoudi, M. Taghavi-Farahabadi, S.M. Hashemi, K. Mousavizadeh, N. Rezaei, N. Mojtabavi, Reprogramming tumor-associated macrophages using exosomes from M1 macrophages, Biochem. Biophys. Res. Commun. 733 (2024) 150697, https://doi.org/10.1016/j.bbrc.2024.150697.
[152] S.P. Banikarimi, A. Mellati, M. Abasi, M. Soleimani, M.A. Ghiass, S.H. Ahmadi Tafti, S. Boroumand, E. Hasanzadeh, Cardiac tissue regeneration by microfluidic generated cardiac cell-laden calcium alginate microgels and mesenchymal stem cell extracted exosomes on myocardial infarction model, Int. J. Biol. Macromol. 292 (2025) 139247, https://doi.org/10.1016/j.ijbiomac.2024.139247.
[153] L. Varyani, N. Ahmadpanah, R. Kasiri, S. Shahzamani, S. Tomraee, A. Jafari, H. Mirjalili, N.S. Asl, Human amniotic membrane hydrogel loaded with exosomes derived from human placental mesenchymal stem cells accelerate diabetic wound healing, Tissue Cell 91 (2024) 102590, https://doi.org/10.1016/j.tice.2024.102590.
[154] Y. Tu, W. Zheng, Z. Ding, J. Xiang, Q. Yang, Y. Liu, J. Cao, Y. Shen, Z. Tang, S. Lin, L. Fan, Y. Xu, B. Chen, Exosome-loaded tannic acid–thioctic acid hydrogel enhances wound healing in coagulation disorders, Mater. Today Bio 31 (2025) 101496, https://doi.org/10.1016/j.mtbio.2025.101496.
[155] X. Lu, H. Hu, Y. Zhou, H. Zhang, C. Xie, Y. Sun, Z. Shao, L. Tang, Y. Ren, J. Chen, X. Xu, N. Qiu, H. Guo, One-step engineered mesenchymal stem cell-derived exosomes against hepatic ischemia–reperfusion injury, Int. J. Pharm. (2025) 125292, https://doi.org/10.1016/j.ijpharm.2025.125292.
[156] F. Tang, J.-N. Zhang, L.-Y. Xu, X.-L. Zhao, F. Wan, H. Ao, C. Peng, Endothelial-derived exosomes: a novel therapeutic strategy for LPS-induced myocardial damage with anisodamine, Int. J. Biol. Macromol. 282 (2024) 136993, https://doi.org/10.1016/j.ijbiomac.2024.136993.
[157] M. Rasti, A.H. Parniaei, L. Dehghani, S. Nasr Esfahani, H. Mirhendi, V. Yazdani, V. Azimian Zavareh, Enhancing the wound healing process through local injection of exosomes derived from blood serum: an in vitro and in vivo assessment, Regen. Ther. 26 (2024) 281–289, https://doi.org/10.1016/j.reth.2024.06.004.
[158] B. Liu, Y. Rui, M. Li, L. Huang, Cancer cell-derived exosomes promote NSCLC progression via the miR-199b-5p/HIF1AN axis, Mol. Immunol. 174 (2024) 32–40, https://doi.org/10.1016/j.molimm.2024.08.001.
[159] G. Wang, Q. Li, S. Liu, M. Li, B. Liu, T. Zhao, B. Liu, Z. Chen, An injectable decellularized extracellular matrix hydrogel with cortical neuron-derived exosomes enhances tissue repair following traumatic spinal cord injury, Mater. Today Bio 28 (2024) 101250, https://doi.org/10.1016/j.mtbio.2024.101250.
[160] Z. Xing, L. Guo, S. Li, W. Huang, J. Su, X. Chen, Y. Li, J. Zhang, Skeletal muscle-derived exosomes prevent osteoporosis by promoting osteogenesis, Life Sci. 357 (2024) 123079, https://doi.org/10.1016/j.lfs.2024.123079.
[161] J. Wang, X. Zhu, H. Jiang, M. Ji, Y. Wu, J. Chen, Cancer cell-derived exosome based dual-targeted drug delivery system for non-small cell lung cancer therapy, Colloids Surf. B Biointerfaces 244 (2024) 114141, https://doi.org/10.1016/j.colsurfb.2024.114141.
[162] Q. Yang, G. Liu, G. Chen, G. Chen, K. Chen, L. Fan, Y. Tu, J. Chen, Z. Shi, C. Chen, S. Liu, G. Deng, X. Deng, C. Sun, X. Li, S. Yang, S. Zheng, B. Chen, Novel injectable adhesive hydrogel loaded with exosomes for holistic repair of hemophilic articular cartilage defect, Bioact. Mater. 42 (2024) 85–111, https://doi.org/10.1016/j.bioactmat.2024.08.018.
[163] Y. Wang, Y. Huo, C. Zhao, H. Liu, Y. Shao, C. Zhu, L. An, X. Chen, Z. Chen, Engineered exosomes with enhanced stability and delivery efficiency for

glioblastoma therapy, J. Contr. Release 368 (2024) 170–183, https://doi.org/10.1016/j.jconrel.2024.02.015.
[164] X. Tan, J. Zhang, Y. Heng, L. Chen, Y. Wang, S. Wu, X. Liu, B. Xu, Z. Yu, R. Gu, Locally delivered hydrogels with controlled release of nanoscale exosomes promote cardiac repair after myocardial infarction, J. Contr. Release 368 (2024) 303–317, https://doi.org/10.1016/j.jconrel.2024.02.035.
[165] S. Setua, S. Shabir, P. Shaji, A.M. Bulnes, A. Dhasmana, S. Holla, N.K. Mittal, N. Sahoo, T. Saini, F. Giorgianni, M. Sikander, A.E. Massey, B.B. Hafeez, M. K. Tripathi, V.P. Diego, M. Jaggi, J. Yue, N. Zafar, M.M. Yallapu, S.W. Behrman, S. Khan, S.C. Chauhan, Exosomes derived from tumor adjacent fibroblasts efficiently target pancreatic tumors, Acta Pharm. Sin. B 14 (2024) 3009–3026, https://doi.org/10.1016/j.apsb.2024.04.003.
[166] W. Wang, Y. Ren, Q. Yu, L. Jiang, C. Yu, Z. Yue, Y. Wang, J. Lu, P. Che, J. Li, H. Sun, Biodegradable exosome-engineered hydrogels for the prevention of peritoneal adhesions via anti-oxidation and anti-inflammation, Mater. Today Bio 29 (2024) 101312, https://doi.org/10.1016/j.mtbio.2024.101312.
[167] J. Wang, X. Xie, H. Li, Q. Zheng, Y. Chen, W. Chen, Y. Chen, J. He, Q. Lu, Vascular endothelial cells-derived exosomes synergize with curcumin to prevent osteoporosis development, iScience 27 (2024) 109608, https://doi.org/10.1016/j.isci.2024.109608.
[168] X. Xu, Y. Liang, X. Li, K. Ouyang, M. Wang, T. Cao, W. Li, J. Liu, J. Xiong, B. Li, J. Xia, D. Wang, L. Duan, Exosome-mediated delivery of kartogenin for chondrogenesis of synovial fluid-derived mesenchymal stem cells and cartilage regeneration, Biomaterials 269 (2021) 120539, https://doi.org/10.1016/j.biomaterials.2020.120539.
[169] Z. Zhao, L. Qu, T. Shuang, S. Wu, Y. Su, F. Lu, D. Wang, B. Chen, Q. Hao, Low-intensity ultrasound radiation increases exosome yield for efficient drug delivery, J. Drug Deliv. Sci. Technol. 57 (2020) 101713, https://doi.org/10.1016/j.jddst.2020.101713.
[170] L.-B. Wang, B.-Y. Liao, Y.-J. Li, Z.-H. Wang, Y. Yu, X. Li, Q.-H. Zhang, Engineered PDGFA-ligand-modified exosomes delivery T3 for demyelinating disease targeted therapy, Exp. Neurol. 375 (2024) 114730, https://doi.org/10.1016/j.expneurol.2024.114730.
[171] Y. Jang, J. Park, P. Kim, E.-J. Park, H. Sun, Y. Baek, J. Jung, T. Song, J. Doh, H. Kim, Development of exosome membrane materials-fused microbubbles for enhanced stability and efficient drug delivery of ultrasound contrast agent, Acta Pharm. Sin. B 13 (2023) 4983–4998, https://doi.org/10.1016/j.apsb.2023.08.022.
[172] G. Shanmugam, Insights into the role of exosomes in oral cancer: current challenges and future perspectives, Oral Oncol. Rep. 10 (2024) 100450, https://doi.org/10.1016/j.oor.2024.100450.
[173] E. Tzng, N. Bayardo, P.C. Yang, Current challenges surrounding exosome treatments, Extracellu. Vesicl. 2 (2023) 100023, https://doi.org/10.1016/j.vesic.2023.100023.
[174] Y. Wang, L. Zhang, Y. Li, L. Chen, X. Wang, W. Guo, X. Zhang, G. Qin, S. He, A. Zimmerman, Y. Liu, I. Kim, N.L. Weintraub, Y. Tang, Exosomes/microvesicles from induced pluripotent stem cells deliver cardioprotective miRNAs and prevent cardiomyocyte apoptosis in the ischemic myocardium, Int. J. Cardiol. 192 (2015) 61–69, https://doi.org/10.1016/j.ijcard.2015.05.020.
[175] C.P. Lai, O. Mardini, M. Ericsson, S. Prabhakar, C.A. Maguire, J.W. Chen, B. A. Tannous, X.O. Breakefield, Dynamic biodistribution of extracellular vesicles *in Vivo* using a multimodal imaging reporter, ACS Nano 8 (2014) 483–494, https://doi.org/10.1021/nn404945r.
[176] Y. Takahashi, M. Nishikawa, H. Shinotsuka, Y. Matsui, S. Ohara, T. Imai, Y. Takakura, Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection, J. Biotechnol. 165 (2013) 77–84, https://doi.org/10.1016/j.jbiotec.2013.03.013.
[177] A. Rank, R. Nieuwland, A. Crispin, S. Grützner, M. Iberer, B. Toth, R. Pihusch, Clearance of platelet microparticles in vivo, Platelets 22 (2011) 111–116, https://doi.org/10.3109/09537104.2010.520373.
[178] M. Yang, S.Y. Wu, The advances and challenges in utilizing exosomes for delivering cancer therapeutics, Front. Pharmacol. 9 (2018), https://doi.org/10.3389/fphar.2018.00735.
[179] C. Wang, T. Tsai, C. Lee, Regulation of exosomes as biologic medicines: regulatory challenges faced in exosome development and manufacturing processes, Clin. Transl. Sci. 17 (2024), https://doi.org/10.1111/cts.13904.
[180] D.S. Harischandra, S. Ghaisas, D. Rokad, A.G. Kanthasamy, Exosomes in toxicology: relevance to chemical exposure and pathogenesis of environmentally linked diseases, Toxicol. Sci. 158 (2017) 3–13, https://doi.org/10.1093/toxsci/kfx074.
[181] E.C. Bowers, A.A.I. Hassanin, K.S. Ramos, In vitro models of exosome biology and toxicology: new frontiers in biomedical research, Toxicol. Vitro 64 (2020) 104462, https://doi.org/10.1016/j.tiv.2019.02.016.
[182] R. Dhar, B. Bhattacharya, D. Mandal, A. Devi, N.D. Thorat, Exosome-based cancer vaccine: a cutting-edge approach – correspondence, Int. J. Surg. 108 (2022) 106993, https://doi.org/10.1016/j.ijsu.2022.106993.
[183] R. Dhar, S. Gorai, A. Devi, S.K. Jha, MdA. Rahman, A. Alexiou, M. Papadakis, Exosome: a megastar of future cancer personalized and precision medicine, Clin. Translat. Discov. 3 (2023), https://doi.org/10.1002/ctd2.208.
[184] P. Chandra, Z. Ali, N. Fatma, N. Sachan, Toxicity studies of exosomes and potential overcome approaches, in: Exosomes Based Drug Delivery Strategies for Brain Disorders, Springer Nature Singapore, Singapore, 2024, pp. 425–451, https://doi.org/10.1007/978-981-99-8373-5_15.