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Review article

Exomeres and supermeres: Current advances and perspectives

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ABSTRACT

Recent studies have revealed a great diversity and complexity in extracellular vesicles and particles (EVPs). The developments in techniques and the growing awareness of the particle heterogeneity have spurred active research on new particle subsets. Latest discoveries highlighted unique features and roles of non-vesicular extracellular nanoparticles (NVEPs) as promising biomarkers and targets for diseases. These nanoparticles are distinct from extracellular vesicles (EVs) in terms of their smaller particle sizes and lack of a bilayer membrane structure and they are enriched with diverse bioactive molecules particularly proteins and RNAs, which are widely reported to be delivered and packaged in exosomes. This review is focused on the two recently identified membraneless NVEPs, exomeres and supermeres, to provide an overview of their biogenesis and contents, particularly those bioactive substances linked to their bio-properties. This review also explains the concepts and characteristics of these nanoparticles, to compare them with other EVPs, especially EVs, as well as to discuss their isolation and identification methods, research interests, potential clinical applications and open questions.

1. Introduction

Extracellular vesicles (EVs) are membranous structures secreted by various cell types, carrying cargos such as proteins, nucleic acids, and lipids [1]. They are ubiquitously present across diverse organisms, from bacteria to humans, and have been conserved to perform distinct biological functions [2,3]. EVs are discovered to serve as an extra means for cells to communicate with each other, thus helping cells to transfer and exchange proteins, lipids, and genetic materials, thereby participating in the regulation of physiological and pathological processes [1]. The recent development and advances in isolation, separation and analytical techniques prompt the identification of various extracellular particle types, which include vesicular, including extracellular vesicles (EVs), synthetic vesicles (SVs), artificial cell-derived vesicles (ACDVs) and non-vesicular extracellular particles (NVEPs) [4]. NVEPs have been proved to be nanoparticles without lipid-bilayer membranes and full-length transmembrane proteins, such as lipoproteins, nucleosomes, vaults, exomeres and supermeres [5–7] (Fig. 1 and Table 1).

NVEPs usually vary in terms of size, structure, and composition. They are enriched with diverse lipids, proteins, and nucleic acids that related to their biological functions. For example, lipoprotein particles (LPs) are composed of a lipid monolayer and range in size from 5 to 1200 nm, playing a crucial role in complex lipid metabolism. They interact with cells through membrane receptors or surface-bound lipoprotein lipase. Their stability, biocompatibility, and efficient transport capabilities make them promising vehicles for drug delivery [8–10]. Nucleosomes, another type of NVEPs, are dynamic assemblies composed of DNA and proteins. The precise positioning of nucleosomes can determine their transcriptional level and intercellular dynamics [11–13]. Vaults, usually within 100 nm in size, are large ribonucleoprotein particles that have a

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hollow barrel-like characteristic structure. Such nanoparticles found in eukaryotic cells are involved in cellular activities, including immune response, signaling and bio-transportation. Vaults are now considered as prognostic markers for multiple cancers and new surrogates for drug-targeted transport [14–16]. Supramolecular attack particles (SMAPs) are multi-protein particles typically around 120 nm in size, enriching perforin and precipitating enzymes, and have glycoprotein-rich shells. They can enter the target cells to maintain the killing effect for a longer period, which indicates a promising future in cancers treatment [17–19]. A recent focus of interest is the discovery of exomeres and supermeres as the newest members of the NVEP family. They are derived from a larger overlapping population of extracellular particles using ultracentrifugation when using different centrifugal speed. Both exomeres and supermeres appear to be broadly released by many cell types and contain components that were previously attributed to exosomes and other EVs [9].

Exomeres and supermeres, as non-membranous extracellular particles, are believed to have the potential to reflect cellular physiological states and undergo dynamic changes in response to disease conditions, including cancer, neurodegenerative disorders, and infectious diseases. Compared to other extracellular vesicles such as exosomes, exomeres and supermeres are smaller in size and more capable of crossing biological barriers such as the blood-brain barrier (BBB) [21]. Their non-membranous nature may also reduce immune recognition by the host, potentially making them safer carriers for therapeutic applications. By further investigating their biogenesis and functional properties, exomeres and supermeres hold promise for precision medicine, offering new attempts for advancements in early disease diagnosis, targeted therapy, and personalized treatment strategies.

This review provides an overview of the most recent reports on the conception, biogenesis, contents, heterogeneity and potential application of exomeres and supermeres. A comparative analysis of EVP isolation and identification methods are also included. Additionally, we have evaluated the open questions in exomere and supermere research. This review is aimed to provide new insights for researchers interested in NVEPs, inspiring further engagement in extracellular vesicle research and broadening perspectives on extracellular particles.

2. Exomeres and supermeres

2.1. Exomeres

With the development of separation techniques, exomeres and supermeres are discovered as new members of NVEPs (Fig. 2). Exomeres are first discovered in 2018 by using asymmetric flow field-flow fractionation (AF4) [6,22]. Researchers found that exomeres showed an enrichment of metabolic enzymes and signature proteins involved in glycolysis and mTORC1 signaling [23]. Furthermore, nucleic acids and lipids are purposefully secreted in the exomere, highlighting the special nature of the nanoparticles [6].

Many works has been conducted to demonstrate that exomeres are a distinct category of nanoparticles secreted by cells, rather than a byproduct of the isolation process. The corresponding fluid medium was extracted together as a blank control, and very few nanoparticles were

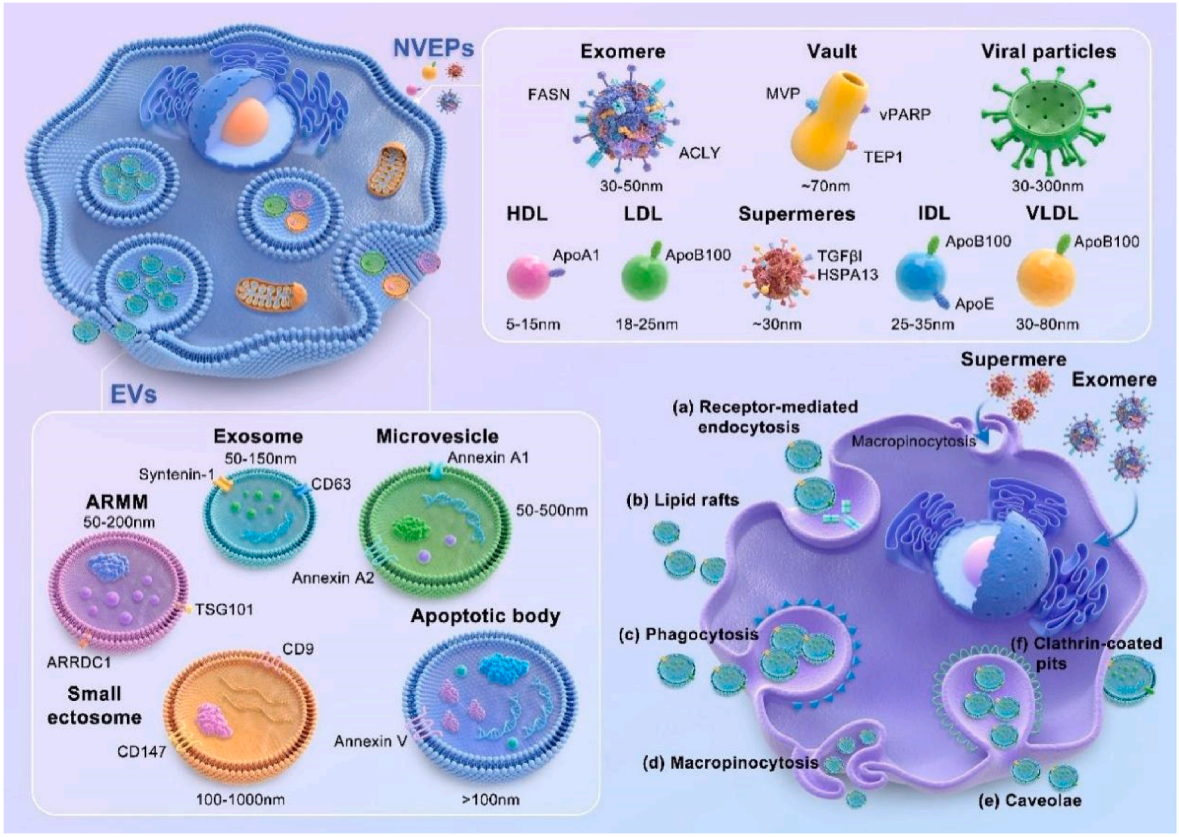

Fig. 1. Extracellular vesicles and nanoparticles secreted by cells. The schematic provides a rough estimate of the size of various substances. While the entry mechanisms of EVs into cells have been extensively studied, those of exomeres and supermeres remain less understood. The caption discusses exosomes as a case study to elucidate the different modalities through which EVs are internalized by cells [18,20]. Recent studies suggest macropinocytosis as a potential pathway for the cellular uptake of supermeres, highlighting ongoing research in this area [21].

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; VLDL, very low-density lipoprotein; ARMM, arrestin domain-containing protein 1-mediated microvesicle; TGFBI, transforming growth factor beta induced; TSP1, thrombospondin 1; HSPA13, heat shock protein family A (Hsp70) member 13; FASN, fatty acid synthase; ACLY, ATP citrate lyase.

found in the fluid medium compared to the cell supernatant, partly indicating that exomeres are secreted by cells. A comparison between exomeres and previously characterized sEVs revealed that exomeres obtained from different cell lines contained 165–483 proteins, of which 38–107 proteins were specific to exomeres, further suggesting that exomeres are unique entities released by cells rather than debris or fragments of exosomes [6,7,24,25].This finding was also verified by another study on exomeres, which analyzed proteins from exomeres and sEVs by LC-MS/MS and identified at least 40 proteins expressed in exomeres that were distinct from those in EVs [7,26]. Principal component analysis (PCA) confirmed exomeres as a featured population. Based on both PCA and consensus clustering analysis, the similarity between exomeres from different cell types was found to be greater than that between EVs within the same cell type. Additionally, a thorough analysis of the protein composition and morphology/structure of these nanoparticles confirmed that exomeres are free from contamination by lipoproteins or other types of protein complexes with high molecular weights, a common concern in nanoparticle research. These findings emphasize the unique biological and functional profiles of exomeres, further distinguishing them from other extracellular vesicles [7].

Exomeres contained a variety of proteins that take part in the metabolism including hexokinase 1 (HK1) [24], glucose 6-phosphate isomerase (GPI) [25], aldolase A (ALDOA) [33], glutamic-oxaloacetic trans-aminase 1 (GOT1), GOT2 [34], fumarate hydrotase (FH) and glycan processing including hexoaminidase A (HEXA), HEXB, glycogen phosphorylase L (PYGL) [35], glucuronidase beta (GUSB) [36], fructose-bisphosphatase 1 (FBP1) [37], and galactosamine-6-sulfatase (GALNS) [38]. This suggests that this particular kind of nanoparticles may be involved in the metabolism and protein modification of recipient cells, and may mediate the recipient cell targeting and regulates glycosylation through glycan recognition. Further studies revealed that Galectin-3 binding protein (LGALS3BP) is specifically enriched in exomeres. Previously, LGALS3BP was thought to mediate glycosylation in recipient cells through glycan recognition [39]. This discovery has the potential to enhance our understanding of the role of extracellular secretions in cellular communication and the mechanisms underlying disease. This will inspire researchers to probe deeper into the target specificity of exomeres.

Furthermore, exomeres are highly enriched with proteins such as amyloid precursor protein (APP) [40], β-site APP-cleaving enzyme (BACE1) [41,42], calsyntenin family members (CLSTN1, 2, and 3) [43] and argonaute proteins (AGO1, AGO2, and AGO3) [44,45]. Beyond these proteins, recent studies have identified shared components between newly discovered exomeres and small extracellular vesicles (sEVs), such as the adaptor-related protein complex 1(AP1, AP2, and AP3) [46], coatomer protein complex subunits G1 and G2 [47,48], and vacuolar protein-associated proteins associated with the retromer complex (VPS26, 29, and 35) [49]. The presence of these proteins suggests that they may have a similar and conservative role in the biogenesis and transport of materials. Zhang and the research group compared exomeres to conventional exosomal markers and discovered that among flotillins, CD9, CD63, CD81, Alix1, TSG101, HSC70 (HSPA8) and HSP90, HSP90 has the strongest correlation with exomeres [6].

Exomeres and sEVs differ significantly in their composition such as RNA, lipid and glycosylation. It was discovered that the RNA content in exomeres is lower than that in sEVs, however, small RNAs are the predominant content in exomeres. Comparison of lipid composition using electrospray ionization-mass spectrometry (ESI-MS) showed that exomeres contained much less lipids than sEVs [7]. Abnormal glycosylation plays a critical role in the pathology of various diseases, including cancer. Exomeres are highly enriched in N-glycans, as evidenced by lectin blotting analysis and glycomic mass spectrometry. The analysis demonstrated that exomeres may contain a high level of sialylated complex N-glycans and these N-glycans varies in exomeres from different cell origin. For example, a high molecular weight of 240 kDa N-glycans was detected in exomeres of breast cancer cells while a molecular weight of 150 kDa N-glycans was detected in exomeres of pancreatic cancer cells [5]. The variation in the N-glycan profiles of exomeres in different cell lines presents a promising avenue for further exploration. It was proved that both exomeres and exosomes can

Table 1
Classification of EVs and NVEPs.

Name	Category	Size	Marker	Origin/release mechanism	References
Lipoprotein	NVEP	5–1200 nm	ApoA1, ApoE, ApoB100, ApoB48	Exocytosis, Plasma membrane (HDL)	[22, 73–75]
Exosome	sEV	50–150 nm	CD9, CD81, CD63, flotillin, TSG101, ceramide, Alix, Rab27a	Endosome	[5,20,76]
Supermere	NVEP	~30 nm	TGF-βl, AGO2, ACE2, PCSK9, VPS35/29/26A, HSPA13, HSP90	Unkown	[21,70],
Exomere	NVEP	<50 nm	HSP90-β, HSPA13, ENO1, GANAB, CD9/63/81, HSPA8/1A, ACT, TUB, GAPDH	Unkown	[5,7,51, 77]
Vault	NVEP	~70 nm	MVP, TEP1, vPARP	Unknown	[14,78,79]
Arrestin domain-containing protein 1-mediated microvesicles (ARMM)	sEV	50–200 nm	ARRDC1, TSG101	Plasma membrane	[22,80,81]
Ectosome	sEV	100–1000 nm	Annexin V, CD147, CD9, CD40, RhoA, PS exposure dependent	Plasma membrane	[82,83]
Supramolecular attack particles (SMAP)	NVEP	120 nm	Thrombospondin 1, Perforin 1, Granzyme B	Secretory granules	[17,84,85]
Viral particle	NVEP (some can also be inside EVs)	30–300 nm	Env, Gag (HIV-1), Spike protein S1 (SARS-CoV-2), Hexon (Adenovirus), VP1 (Polyomavirus)	Plasma membrane	[86,87]
Microvesicle	sEV/lEV	50–500 nm (up to 1 μm)	ARF6, VAMP3, AnnexinA1, AnnexinA2, α-Actin4	Plasma membrane	[88,89]
Apoptotic body or vesicle	sEV/lEV	>100 nm	AnnexinV	Apoptosis	[9,90]
Migrasome	lEV	500-3,000 nm	Integrin-b1, Integrin alpha 5, damaged mitochondria, WGA, Integrinα5β1,TSPAN4, TSPAN7, PH Domain, NDST1, PIGK, CPQ, EOGT	Breakage of retraction fibers during migration	[91–93]
Large oncosome	lEV	>1000 nm	CK18, GOT1, GAPDH, Glutaminase, Cav-1, ARF6,	Plasma membrane during non-apoptotic blebbing	[5,10,91]
Exopher	lEV	50–4000 nm	Huntingtin, Tau protein, LC3, AnnexinV, damaged mitochondria	Plasma membrane, unknown	[9,94]

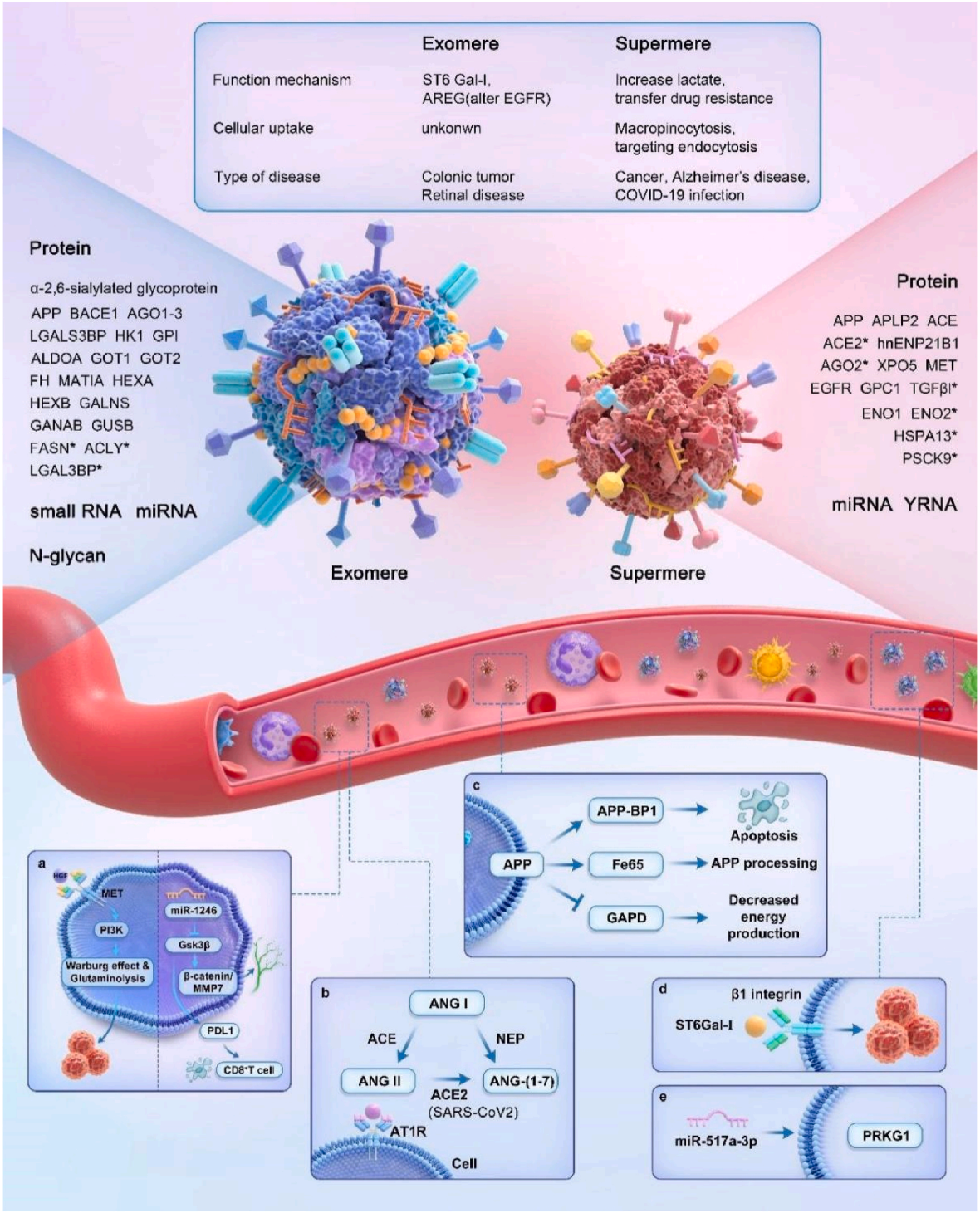

Fig. 2. The composition, function and related pathways of exomeres and supermeres. Exomeres (left) and supermeres (right) precent distinct particle size and components, reflecting their unique particle populations. Key markers are highlighted with an asterisk (*). a. MET and miR-1246 in supermere are related to cancer. HGF-MET pathway is closely related many solid tumors [27]. MiR-1246 is involved in lymphangiogenesis and $CD8^+$ T cell apoptosis during cancer [28]. b. Supermeres are also reported to be featured by ACE2 enrichment, which make them possible targets in cardiovascular diseases and coronavirus related diseases [29]. c. Supermeres convey adequate level of APP, which promote the Alzheimer's disease development [30]. d. ST6Gal-I in exomere proved to promote the metastasis of colorectal tumors [31]. e. Exomere derived miR-517a-3p significantly reduced the expression of PRKG1 in miR-517a-3p-inhibitor (−) Jurkat cells [32].

transfer sialic acid, with exomeres exhibiting higher protease activity. Moreover, this activity can be produced by exosomes through direct delivery to receptor cells [7,21].This encourages researchers to consider these nanoparticles as potential tools or targets in disease diagnosis and treatment strategies.

2.2. *Supermeres*

Supermeres are the most recently discovered member of NVEPs, found in the supernatant of exomeres (Fig. 3a). The nanoparticles, with diameters of 22–32 nm, exhibited a high enrichment of extracellular RNAs and proteins associated with refractory diseases such as cancer,

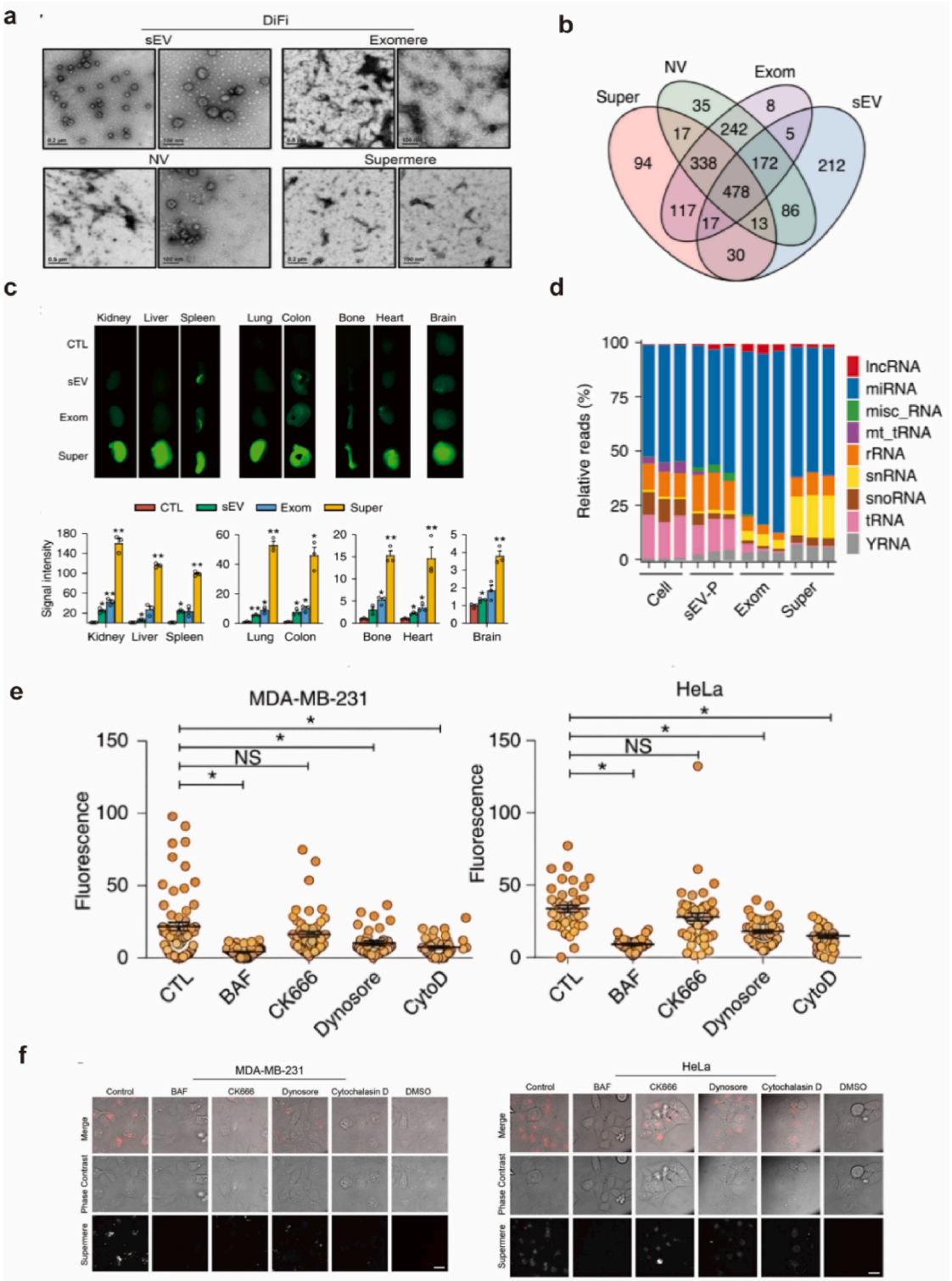

Fig. 3. Exomeres and supermeres are distinct extracellular nanoparticles. a. Negative stain transmission electron microscopy of DiFi-derived sEVs, NV, exomeres and supermeres. b. Venn diagram of unique and common proteins identified in DiFi-derived sEVs, NV fractions, exomeres and supermeres. c. Whole-organ imaging (top). Male C57BL/6 mice were intraperitoneally injected with labelled sEVs, exomeres or supermeres derived from DiFi cells. Their organs were harvested and analyzed after 24 h. Data are the mean ± s.e.m. of n = 3 animals (bottom). The sEVs and extracellular nanoparticles derived from DiFi cells were labelled with Alexa Fluor-647 (Invitrogen, A20173) according to the manufacturer's instructions. d. Percentage of small-RNA reads mapped small noncoding RNA for DiFi cells, the sEV-P, exomeres and supermeres following RNA-seq. Misc RNA, miscellaneous RNA; mt tRNA, mitochondrial tRNA; rRNA, ribosomal RNA; snoRNA, small nucleolar RNA; n = 3 independent samples. e-f. Inhibition of cellular supermere uptake. Cells were pre-incubated with uptake inhibitors for 30 min before the addition of labelled supermeres. After a 24 h incubation, images were acquired using an iSIM imaging system (bottom). scale bar, 20 μm. Data are the mean ± s.e.m. of n = 30 (MDA-MB-231) and 27 (HeLa) cells (top). Images are representative of three independent experiments. Adapted with permission from Ref. [21].*Copyright © 2021, Qin zhang* et al…

cardiovascular diseases, and neurological diseases. Their yield also exceeded that of EVs and exomeres during the isolation process [21].

In order to demonstrate the existence of supermeres as a type of nanoparticle, researchers have conducted many studies. When collecting supernatants for supermeres extraction, Jeppesen et al. used taipan blue dye to detect the activity of cells. They only selected cell cultures with over 95 % cell activity for the test, indicating that the nanoparticles under study are not released passively due to cell death [50,51]. It has also been demonstrated that supermeres are not fragmented EVs since they do not contain full-length transmembrane proteins with EV properties. Additionally, some researchers have successfully isolated supermeres using fast protein liquid chromatography (FPLC), obtaining identical nanoparticles. This finding confirms that supermeres are not artifacts of the separation method but are distinct biological entities [9].

Supermeres show differential selective enrichment of proteins and RNAs compared to exomeres and EVs (Table 2, Fig. 3b and d). Upon analyzing the composition, it was discovered that this kind of nanoparticle contains many disease-associated proteins, including APP [40], APLP2 [52], ACE and ACE2 [53,54], α-enolase [55,56] and glypican-1 [57,58](cancer), and miRNA-related proteins such as hnRNPA2B1 [59–61], AGO2 [62] and exportin 5 (XPO5) [21,51,63]. Previously thought to be an exomere-carrying protein, APP was found to be strongly associated with supermeres in the latest study [9]. In another study, extracellular cleavage products of some proteins has also been found in supermeres, including ectodomain cleavage products of APP, glypican 1, MET [64], amphiregulin [65,66], and EGFR [67,68], GPC1 [57,69]. Supermeres contain various types of RNAs, such as extracellular microRNAs (miRNAs), YRNAs, and small nuclear RNAs (Fig. 3d). Interestingly, transfer RNAs (tRNAs) shows a stronger association with EVs compared to nanoparticles such as supermeres and exomeres, since the concentration of tRNAs in EVs is higher than that in other particles. Tosar et al. also found that some extracellular RNAs (exRNAs) could be modified fragments of cellular RNAs [51]. One example is miRNA-1246, which was identified as being highly concentrated in supermeres. It is believed that miRNA-1246 might be a fragment of U2 snRNA. U2 is wrapped around and protected by the Sm protein heptameric center, which corresponds to the miRNA-1246 sequence. MiRNA-1246 was highly differentially expressed in normal and tumor cells, may suggesting that supermeres could be used as potential exRNA biomarkers [21,51].

Supermeres are enriched with enzymes involved in metabolism, particularly glycolysis and fatty acid metabolism [70]. Supermeres are found to regulate the metabolic profile of tumor cells by increasing lactate secretion. This finding suggests that the release of these nanoparticles influences the tumor microenvironment (TME), offering valuable insights for future TME studies. It is noted that increased lactate secretion has been linked to drug resistance associated with epidermal growth factor receptor (EGFR) and cellular mesenchymal epithelial transition factor (MET) [71]. Therefore, it is possible that supermeres may induce altered drug resistance. Inspired by certain metabolism-related proteins, researchers have been investigating the effects of supermeres on hepatic lipid levels and glycogen levels. Through in vivo experiments on mice and gene set enrichment analysis (GSEA), it has been demonstrated that exomeres and supermeres may have unique roles in regulating AKT and ERK 1/2 signaling, which can impact hepatic glucose and lipid metabolism [72]. Male C57BL/6 mice were injected with equal amounts of exomeres and supermeres via the tail vein, resulting in significant changes in the number and size of hepatic lipid droplets, as well as triglyceride and glycogen levels [21].

2.3. Heterogeneity of exomeres and supermeres

Compared with traditional extracellular vesicles and nanoparticles, exomeres and supermeres may have distinct formation pathways, compositions, sizes, life span, cell-entry mode and correlations with various diseases. To clarify the characteristics and heterogeneity of particles is of great significance for extracellular particle research.

2.3.1. Biogenesis

The mechanisms behind exomeres and supermeres formation are not well-elaborated currently, however some clues can be obtained when compared with other extracellualr vesicles and particles. Exomeres are enriched in some specific proteins, but there is almost none of typical exosome markers found such as endosomal membrane proteins and ESCRT complex components. This distinct and specific composition suggests that exomeres are possibly not generated by classical endosomal pathway (e.g., the multivesicular body pathway). In fact, studies have shown that endosomal and membrane-related proteins that are typically enriched in exosomes are rarely found in exomeres [6]. Therefore, it is speculated that exomeres may be formed via non-classical secretion pathways, where cells release certain cytoplasmic contents directly into the extracellular space instead of packaging them into vesicles. One study supporting this speculation is that there is a relatively high level of galectin-3 binding protein (LGALS3BP) in exomeres [6]. LGALS3BP is a secretory glycoprotein for self-assemble into a cyclic decamer of approximately the same size as exomeres. Some researchers consider that LGALS3BP oligomers may serve as scaffolds for exomeres, binding other proteins and nucleic acids. However, the protein profile of exomeres also includes ribosomal proteins and extracellular domains of membrane receptors like ACE2, suggesting that a single protein scaffold cannot explain their entire composition. Thus, the biogenesis of exomeres may be complicated, and it is not yet clear whether there are specific organelles or pathways within cells contributed to exomeres formation. There are also viewpoints indicating that exomeres may not represent a single particle population or type but a collection of small protein complexes, which may be classified into the same category due to the ultracentrifugation separation conditions [9]. Exomeres may represent a mixture of non-vesicular particles from multiple sources, rather than having a unified biological generation path.

Supermeres also lack a lipid membrane and contain very low lipid content but are rich in proteins and RNAs. Since supermeres lack

Table 2
Protein concentrations and ratios from cell lines in equal volumes Adapted with permission from Ref. [21]. *Copyright © 2021, Qin zhang* et al.

Cell line	Cell origin	Concentration (μg/μl)			Ratio		
		sEV-P	Exomere	Supermere	sEV-P	Exomere	Supermere
DiFi	Colorectal cancer	8.7	4.5	13.8	1	0.52	1.59
LIM1215	Colorectal cancer	14.1	8.9	36.4	1	0.63	2.58
LS174T	Colorectal cancer	10.1	7.5	20.8	1	0.74	2.06
CC	Colorectal cancer	4.5	2.2	15.4	1	0.49	3.42
CC-CR	Colorectal cancer	7.2	3.1	21.5	1	0.43	2.99
SC	Colorectal cancer	7.3	3.7	21.1	1	0.51	2.89
MDA-MB-231	Breast cancer	16.0	9.6	36.0	1	0.60	2.25
PANC-1	Pancreas cancer	17.7	12.8	33.8	1	0.72	1.91
Calu-3	Lung cancer	1.3	1.2	2.1	1	0.92	1.62
HREC	Human renal proximal tubule	3.2	1.8	6.7	1	0.56	2.09

membrane structures, their formation process cannot be explained by vesicular budding. Some researchers hypothesize that supermeres may originate from the shedding of cell surface molecular fragments or the active release of intracellular macromolecular complexes [9,50]. For instance, membrane proteins, when cleaved by proteases, release soluble extracellular domains (such as APP, ACE2), which may aggregate in the extracellular space, forming nanosized complexes and contributing to the formation of supermeres. Furthermore, supermeres are enriched in small RNAs and RNA-binding proteins, suggesting that cells may release cytoplasmic RNP complexes by non-classical pathways like autophagy, thus contributing to the RNA cargo-loading of supermeres. However, it is worth mentioning that there is currently no direct evidence for the existence of specialized organelles or secretory vesicles for the production of supermeres. There is also the viewpoint that supermeres may be a collection of various tiny secreted components, with their formation depending more on the separation method than on a single biological process.

Collectively, existing studies generally indicated that the biogenesis mechanisms of exomeres and supermeres are yet to be fully elucidated. They are not products of the classical exosome pathway but are more likely to involve specialized non-membrane secretion pathways. Current speculations include the self-assembly of specific secretory proteins carrying other cargo to form particles, and the release of cytoplasmic contents via unconventional secretion mechanisms. With the advancement of new technologies (such as more refined separation methods and imaging techniques), researchers are working to track the origins and pathways of exomeres and supermeres within cells, which can deepening our understanding of intercellular communication mechanisms and laying the foundation for the application of these particles in diseases.

2.3.2. Sizes

The size of supermeres is nearly 22–32 nm in diameter, and the volume may be only about half that of exomeres (Fig. 3a) [9,21,50]. By atomic force microscopy, it can be observed that exomeres and supermeres are two types of nanoparticles with different sizes. Researches on EVs has shown that size is a critical factor influencing their composition. For instance, a vesicle with a diameter of 150 nm has a surface area 25 times greater than a 30 nm one, and its volume is 125 times larger, suggesting it could contain more surface proteins and cargo [95]. Even a medium-sized 50 nm vesicle has a surface area three times greater than a 30 nm vesicle. This implies that the size of particles within the NVEP group is likely significant in determining their content and function. Moreover, when these particles are used for therapeutic and regenerative purposes, their effects often depend on the dosage. Therefore, the size differences among EVs or NVEPs can have a substantial impact on their efficacy in treatment and repair processes.

Currently, many detection methods used for analyzing particle sizes need to be further confirmed in terms of detection limitation and accuracy. The heterogeneity of small particle sizes poses a challenge for detecting due to limitations in detection principles and techniques (Table 3). For example, the widely used nanoparticle tracking analysis (NTA) has a lower detection limit of around 70–90 nm, making it difficult to distinguish EVs and NVEPs from various heterogeneous populations. At present, the gold standard for particle size detection remains electron microscopy. It is recommended, based on the latest expert guidelines, to employ a combination of detection methods to improve accuracy and resolve the limitations of individual techniques [4,95].

2.3.3. Morphology and zeta potential

Exomeres differ significantly from sEVs in their biophysical characteristics. While sEVs typically display a cup-shaped morphology and range from 50 to 150 nm in diameter, detected by transmission electron microscopy (TEM), exomeres have a dot-like morphology and are generally smaller than 50 nm, with an average size of approximately 35 nm (Fig. 3a). Atomic force microscopy (AFM) analysis reveals their height to be between 5.9 nm and 7.0 nm. In terms of zeta potential, both exomeres and sEVs carry negative charges, but exomeres (−2.7 mV to −9.7 mV) exhibit weaker potentials compared to sEVs (−9.0 mV to −16 mV). Regarding particle stiffness, exomeres are stiffer (145–816 mPa) than sEVs, which show greater variability (20–420 mPa) [6]. These distinct physical properties may influence the stability, transport, and cellular uptake of these nanoparticles. While research on the biophysical characteristics of supermeres is still limited, AFM confirms that exomeres and supermeres belong to distinct groups [21].

2.3.4. Cargos

Exomeres and supermeres have distinct compositions compared to other types of extracellular secretions. Exomeres are more likely to convey metabolic enzymes associated with glycolysis and proteins involved in mTORC1 signaling, and they are capable of transferring functional cargo [7]. In contrast, supermeres are enriched in exRNAs and enzymes related to glucose and fatty acid metabolism. Additionally, nanoparticles from different sample sources may exhibit unique molecular contents. Analysis of individual EVs reveals that the substances within each vesicle represent only a small fraction of the overall molecular content, suggesting a similar trend in nanoparticles. Unlike extracellular vesicles, exomeres and supermeres lack a bilayer lipid membrane and contain fewer lipid components.

EVs, exomeres, and supermeres are recognized as crucial exRNA carriers involved in various forms of intercellular communication. ExRNAs are either encapsulated within membrane structures or bound to non-membrane proteins to protect them from degradation. Notably, supermeres (~65 %) contain a significantly higher exRNA content compared to exomeres (~10 %) [96]. Current research on EVs highlights considerable variability in the transcriptional profiles of different EVs, even within the same type but derived from different sources or groups, reflecting a high degree of heterogeneity [97]. This variability may arise from differences in sample characteristics, EV sub-populations, detection methods, or experimental errors. Similarly, it is reasonable to expect that exomeres and supermeres also exhibit substantial

Table 3
Comparison of single-particle characterization methodologies.

Characterization method	Single-vesicle resolution	Operational difficulty	Approximate lower limit of detection (nm)	Phenotyping	Able to be calibrated
NTA	Normal	Easy	50	Yes	Yes
RPS	Normal	Easy	30	No	Yes
RPS with immunofluorescence (via ARC platform)	Normal	Easy	65	Yes	Yes
Dynamic light scattering	Normal	Easy	>10	No	Yes
Single-particle FCM	Normal	Easy	90	High	Yes
Laser trapping Raman spectroscopy	Normal	Difficulty	90	Yes	Yes
Hybrid interferometric reflectance imaging–fluorescence microscopy	Normal	Difficulty	50	Yes	Yes
SRM	High	Difficulty	20	High	Yes
AFM	Normal	Difficulty	50	Yes	Yes
EM	High	Difficulty	<30	Yes	Yes

heterogeneity in their transcriptional profiles. Furthermore, many proteins once thought to be specific to EVs are also found in exomeres and supermeres, making it challenging to pinpoint their exact origin. It is possible that these proteins are secreted by multiple types of EVs or nanoparticles simultaneously [9].

2.3.5. Markers

Exomeres and supermeres differ from sEVs in terms of their surface markers. Common surface markers of EVs include tetrapeptides (CD9, CD63, CD81, CD82), heat shock proteins (HSP70, HSP90), TSG101, and Alix. Exomeres are primarily identified by surface markers such as FASN, ACLY, and LGALS3BP, while supermeres are characterized by markers including TGF-βI, HSPA13, ENO2, AGO2, ACE2, and PSCK [70,98]. Similar to EVs, different sub-populations of nanoparticles (NPs) may also carry specific markers, with NPs derived from particular diseases potentially exhibiting unique signatures. There is a pressing need to identify not only general markers specific to EVs or NPs but also those that are unique to particular subclasses, such as exomeres and supermeres. Such markers could significantly improve our understanding of extracellular secretions. Furthermore, identifying specific markers for early disease diagnosis and for tracking disease progression is essential for enhancing diagnostic accuracy and developing tailored treatment strategies.

2.3.6. Life span

The life span of EVs and NVEPs is an important issue worthy of exploration and discussion. On one hand, lifespan concerns how these extracellular secretions are preserved after extraction and processing. On the other hand, it affects the working time after entering cells. Several studies have focused on the storage of EVs under commonly used experimental conditions (such as 4 °C, −20 °C, and −80 °C). These studies found that EVs tend to aggregate regardless of the storage conditions. Additionally, both the particle numbers and protein concentraions of EVs decrease with prolonged storage time. For short-term storage of up to 7 days, EVs can be stored at 4 °C, but they should be placed at −80 °C for a long-term storage and should avoid repeated freezing and thawing [99,100]. EVs with phospholipid bilayers structure, may have the longer lifespan than exomeres and supermeres. However, it is possible that RNA and proteins, which are crucial components of EVs, may undergo similar degradation processes as those seen in exomeres and supermeres, regardless of the presence of a membrane structure. This is a point that deserves to be explored by more researchers, as these components play a significant role in disease development and treatment. Additionally, this will provide valuable guidance for future studies on EVs and NVEPs. The therapeutic effect of diseases have a specific quantitative relationship with the therapeutic substances. Investigating the amount of extracellular secretions entering cells, as well as their lifespan after entry, can provide valuable insights for disease treatment. The content tends to be significantly related to the volume of the substance. While exomeres and supermeres have a smaller volume compared to EVs, which may allow less material to enter cells, they can compensate for this by having a greater particle count per unit volume. Unlike EVs, these NVEP without a membrane structure may reach their target sites more quickly, because they do not require a process of content release after entering the cell. Moreover, the difference among particles are also studied in inter-subpopulation (compositional and functional differences among EVs from various sources) and intra-subpopulation (differences in EVs within subpopulations derived from the same source). These heterogeneities are particularly relevant to particle half-life. For instance, some studies have reported that the overall half-life of EVs is less than 10 min, while noting that a specific subpopulation of EVs expressing CD47 exhibits a longer half-life [95].

2.3.7. Cellular entry and uptake modes

According to available studies, it is believed that EVs can enter cells through receptor-mediated endocytosis, clathrin-coated pits, lipid rafts, phagocytosis, caveolae, and macropinocytosis. These entry ways for exomeres and supermeres into cells have not been fully clarified. The cellular uptake mechanisms of supermeres was investigated by using various cellular uptake blockers, and the findings suggest that inhibiting macromolecular autophagy with bafilomycin A could represent a potential pathway for supermere uptake (Fig. 3e and f). NPs originating from different tissues with distinct biological roles may have varying modes of entering cells. In addition to the ways described above, it is noted is that NVEPs differ from EVs in that they are not wrapped in a membrane structure. Maybe not all exomeres and supermeres enter the cell to perform their functions. Exomeres and supermeres may be able to interact directly with receptors on the cell surface to produce a series of downstream reactions.

As for the cellular uptake mode, exosomes, exomeres and supermeres are distributed differently in different organs. It is proved that, similar to other nanoparticles, exomeres can be effectively taken by hematopoietic organs such as liver (~84 %), spleen (~14 %), and bone marrow (~1.6 %), and are uniformly distributed within these organs (Fig. 3c). Lungs and lymph nodes, where nanoparticles exhibit a punctate distribution, also contained these particles [5]. What is exciting is that supermeres were a type of substance that can cross the blood-brain barrier. In a study using mice injected with labelled sEVs, exomeres and supermeres, it is worth noting that supermeres have a higher uptake in various organs, including brain, bone, kidney, spleen, liver, lung, colon, and heart. The strongest signals were observed in the kidney, spleen, and liver (Fig. 3c). Furthermore, supermeres are more effective in the lungs and lymph nodes than sEVs and exomeres. They can be prioritized to treat these parts of the body. This helps researchers in the treatment of diseases because they can choose different extracellular particles depending on the distribution of them in the body. For instance, sEVs are more effective in hematopoietic organs such as the spleen and colon [21]. When selecting diagnostic and treatment protocols for various diseases, researchers can consider the heterogeneity of tissue distribution of these nanoparticles, as well as the cellular metabolism perspective. In vitro uptake experiments, exomeres and supermeres showed a slower uptake rate than sEVs. However, they have a greater uptake amount, especially supermeres. A deeper exploration of these NPs and sEVs can make up for the shortcomings and achieve better uptake performance in terms of uptake rate, uptake amount, and uptake site [21].

3. Separation and identification of exomeres and supermeres

3.1. Separation methods

Multifarious methods are discovered and available for separating particles and vesicles based on their distinct characteristics, most of which have been developed for sEVs [111](Table 4). An easy-to-operate method for extracting large amounts of particles based on their size and density differences is differential ultracentrifugation [112–114]. The density gradient centrifugation, which modified on the original differential ultracentrifugation, provides higher purity but more procedures [115–117]. Size exclusion chromatography (SEC) separates particles based on their size as well, with the advantage of preserving sEV integrity but requiring specialized equipment [118–120]. The AF4 [121–123], AF4/UV-MALS [124,125], and IAC-AF4 systems [126] can sort particles based on their size while maintaining particle integrity and purity. However, these systems are not ideal for mass production due to their high demands on operators and equipment. In addition, immunoaffinity capture technology is on the strength of different surface markers of EVs, and this method shows the high specificity while unsatisfactory in terms of yield and cost [127,128]. Affinity chromatography techniques are used to separate sEVs by different EVs marker [129,130]. For instance, antibodies against CD9, CD63, and CD81 are employed to capture, purify, and detect EVs in a microarray format, known as the EV micro-array, which allows for multiple phenotyping [131]. Label-free microfluidics is a kind of chip technology developed

Table 4
Commonly used methods for particles separation.

Method	Principle	Advantages	Disadvantages	Purity	Whether for separation of exomeres/ supermeres	References
Differential ultracentrifugation	Based on particle density, size and shape	Low cost, can be used for large samples and high yield, simple operation	Low repeatability, long time-consuming, destroying the integrity and function	Medium	Yes	[162–164]
Gradient density ultracentrifugation	Based on buoyant density using a discontinuous gradient of a sucrose solution or less-viscous iodoxinol	Improved purity	Complex operation	High	No	[116,165, 166]
Size-exclusion chromatography	Based on particle size	High yield, guaranteed integrity, simple operation	Lack specifity, time consuming, high cost	High	No	[153,167]
Polyethylene glycol precipitation	Based on precipitation	Simple operation, fast	Lack specificity, difficulty inscaling	Low	No	[144,168]
Commercial kits	Based on precipitation	Simple operation, fast	High cost, lack specificity, difficulty inscaling	Low	No	[169,170]
Antibody-modifed magnetic beads	Based on an affinity tag or proteoglycan affinity regents covalently fused to either magnetic or agarose breads	Convenient, high effciency	The yield is up to the Ab selection/availability	High	No	[171–174]
Nanoscale lateral displacement	Based on physical feature	Guaranteed integrity, reduced membrane blockage	Lack specifity, contamination	Medium	No	[175]
Membrane flter	Based on physical feature	Guaranteed integrity	Lack specifity, contamination	Medium	No	[176]
HPLC	Go through readily made chromatography columns operated under gravity or pumps	High yield, can be used for large samples	Time-consuming, specialized equipment	Medium	No	[177–179]
Microfluidic devices	Three categories:(a) sieving by nanoporous membranes; (b) trapping nanoparticles on porous structures; (c) trapping with an immune-affinity approach	Guaranteed integrity, high repeatability, simple operation	Still in exploration, high cost	High	No	[180]
Lipid nanoprobe/TiO2	Lipid Mediated-Separation	Guaranteed integrity, minimal damage, efficient	Contamination, lack specificity	Low	No	[181,182]
Thermophoresis	Based on thermophoretic tnrichment	Simple operation, fast, efficient	Contamination, lack specificity	Low	No	[183]
AF4 based(AF4, AF4/ UV-MALS)	Cross flow perpendicular to the parabolic flow pattern, separated by particle size	Guaranteed integrity, high repeatability	High requirements for equipment, can only be used for small samples	High	Yes	[122,124, 184]
Synthetic peptide (Vn96) based isolation method	Based on specific affinity of Vn96 and HSP	Low cost, high efficiency	Still in exploration	High	No	[137]
Optical tweezers	Based on the enhanced near field generated by the anapole antenna	Small sample size and poor operationalization	The diffraction limit of light,	High	Yes	[146]

based on the acoustic, optical, and other physical properties of EVs. It will ensure the integrity of EVs, which is now attracting more and more attention from researchers [132–136]. According to the specific affinity of Vn96, there are also some separation techniques successively [137, 138]. Researchers have developed a protocol that isolates EVs and cell-free DNA in biological samples, which does not require specialized laboratory equipment and can sort out samples suitable for marker analysis. This kit (New England Peptide, Gardner, MA, USA) utilizes the affinity of the synthetic peptide Vn96 for EVs, EVs-related proteins and nucleic acids. The product can be obtained by simple high speed centrifugation. Benefit from the chimeric nanocomposites [139], SAP [140] and other materials, there are also new separation techniques developed continuously. Chimeric nanocomposites known as lactoferrin conjugated 2,2-bis (methylol) propionic acid dendrimer-modified magnetic nanoparticles (LF-bis-MPA-MNPs) have been created. These are used to efficiently isolate EVs from various biological samples such as cells and body excreta. The isolation process is achieved through a combination of electrostatic interaction, physical absorption, and bio-recognition between the surfaces of the EVs and the LF-bis-MPA-MNPs. SAP beads absorb small molecules via nano-sized channels but expel and concentrate EVs. Consequently, the beads drastically enrich EVs by reducing the solution volume in a single step, without affecting EV characteristics. Some researchers currently use a cocktail strategy of combining multiple isolation methods for their own purposes. This allows them to combine several commonly used isolation methods to meet experimental requirements for higher purity or yield. An example of this strategy is the combination of TFF and ultracentrifugation for isolating NK cell-derived exosomes (NK-Exo) [141].

Currently, the commonly used methods for separating exomeres and supermeres are ultracentrifugation (UC), high-resolution gradient fractionation, asymmetric flow field-flow fractionation (AF4), and optical tweezers method(Figs. 4 and 5). Ultracentrifugation is considered as the gold standard to separate substances based on their different densities or sizes [142]. It is particularly useful for extracting large-volume-sample while ensuring high purity levels. It is also widely used for being easy-to-operate and low costs [116,142–144]. The ultracentrifugation and high-resolution gradient fractionation were reported to be used for isolating different populations of extracellular secretions [143]. This protocol provided the method for extracting exomeres and supermeres as well as optimizing EVs isolation. After extracting the sEVs, the remaining liquid is kept for further processing. It is then subjected to ultracentrifugation for 16 h at 167,000g to obtain exomeres. These can be washed by mixing with PBS and then subjected to ultracentrifugation again at 167,000g for 16 h. The remaining liquid after the extraction of exomeres can be subjected to ultracentrifugation again for 16 h at 367, 000g to obtain supermerses. Asymmetric flow field flow fractionation (AsFIFFF4 or AF4) is a separation technique based on the different diffusion coefficients of particles in Brownian motion with varying sizes.

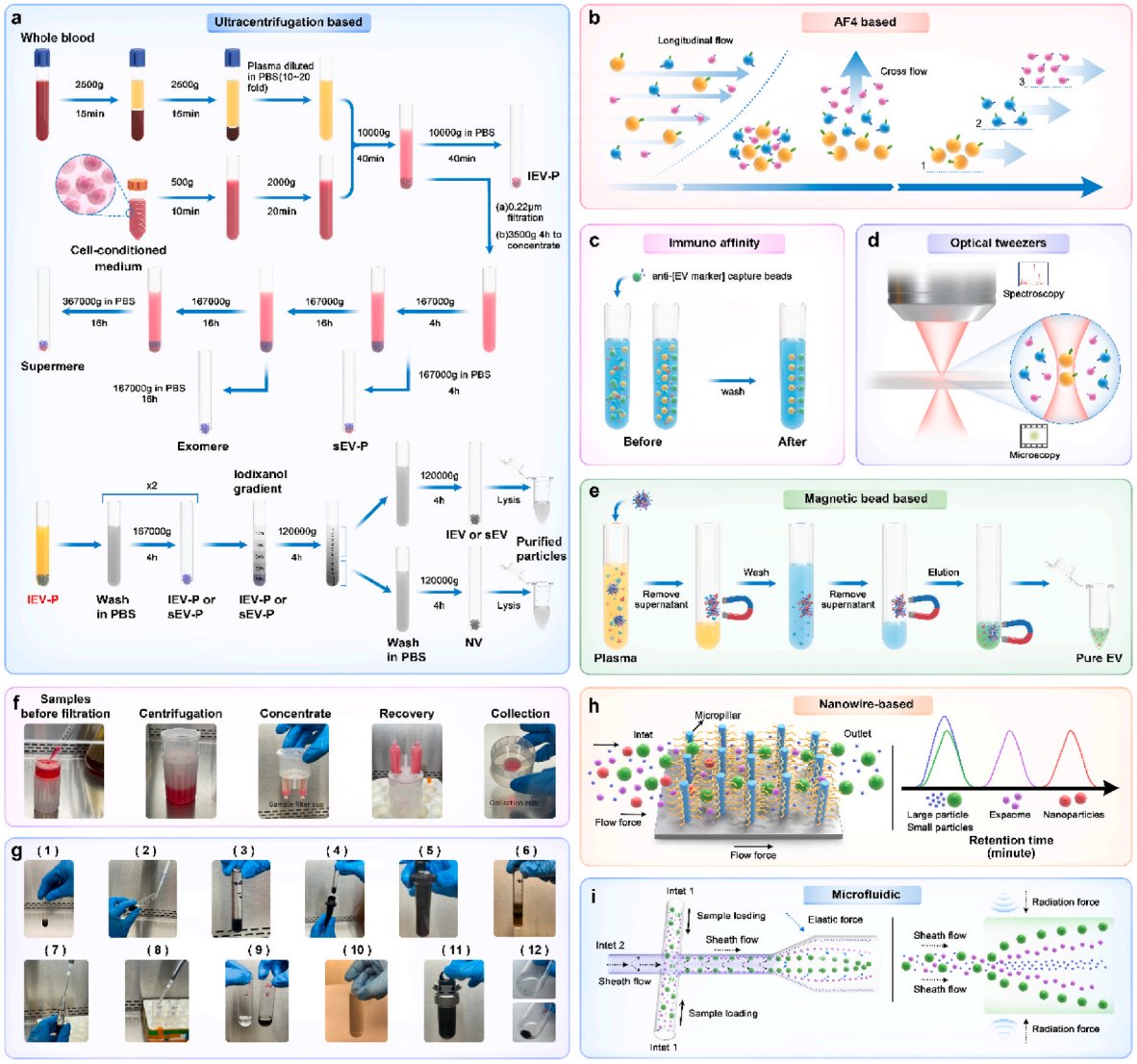

Fig. 4. Isolation methods for EVs and NVEPs from whole blood and cell-conditioned medium. a. Ultracentrifugation is one of the most common methods. It is a method that can separate EVs, exomeres and supermeres. The separated samples can be washed by resuspension in PBS and centrifugation. There are some representative photographs of the most important steps during concentrator procedure(f) and the high-resolution gradient fractionation procedure(g). Adapted with permission from Ref. [143]. *Copyright © 2023, Springer Nature Limited* (1) Transfer the 36 %(wt/vol) iodixanol EV-P suspension to the centrifuge tube. (2) Tilt the centrifugation tube sideways and carefully dispense 2.4 ml of the 30 % and 24 %(wt/vol) iodixanol solution to the centrifuge tube. (3–4) Weigh the loaded centrifugation tube containing 12 ml of 12–36 %(wt/vol) iodixanol gradient on a balance. (5) Centrifuge at 120,000g for 15h at 4 °C. (6–9) Carefully collect fractions of 1 ml each from the top, not to disturb the underlying fractions. (10) Add 11 ml of PBS and mix until the solution appears to be homogenous. (11) Centrifuge at 120,000g for ≥4h at 4 °C. (12) Collect lEVs/sEVs. b. AF4-based methods have been used to separate EVs and exomeres. c and e. Immuno-affinity and magnetic bead are two commonly used methods for separating EVs. d. Optical tweezers has been able to successfully capture supermere, and may be a promising tool for single molecule analysis in the future. h and i. Nanowire-based and microfluidic are two relatively new methods for separating EVs.

The separation occurs when the particles to be analyzed are passed through a flat channel with special material and design, causing the particles of different sizes to diffuse and separate into different laminar flows [77,121,145]. Exomeres, non-membrane-bound nanovesicles measuring around 35 nm, were isolated and characterized using the AF4 method in 2018 [6]. Anapole-Assisted Low-Power Optical Trapping is a new method for isolation of ultra-small particles such as supermeres and other nanoscale extracellular vesicles(Fig. 5). A Bragg reflector was employed to control the reflection phase of the incident light. It optimizes the anapole structure to reduce the heat generated during the capture process. This all-dielectric nanoantenna systems generates strong optical gradient forces that allow it to capture nanoscale particles and ensure the integrity of the captured bioparticles as well.

These methods for isolating extracellular particles, including exosomes, exomeres, and supermeres, each has its own advantages and limitations. UC relies on high-speed centrifugal force to concentrate exosomes, making it suitable for large-scale preparation due to its high throughput and ease of operation. However, it has limited ability to separate different types of particles, and exomeres and supermeres are often difficult to recover efficiently. Additionally, the high shear force during UC may damage particle structures and affect their biological functions. AF4 separates particles based on differences in diffusion coefficients, enabling high-resolution fractionation and providing high-purity exosomes and exomeres in a short time while avoiding structural damage caused by UC. However, it is limited by sample loading capacity, making it less suitable for large-scale separation. Anapole-assisted low-power optical trapping uses nanophotonic antennas to precisely capture individual nanoparticles without applying mechanical stress, preserving particle integrity and functionality to the greatest extent. It is especially useful for isolating very small particles such as

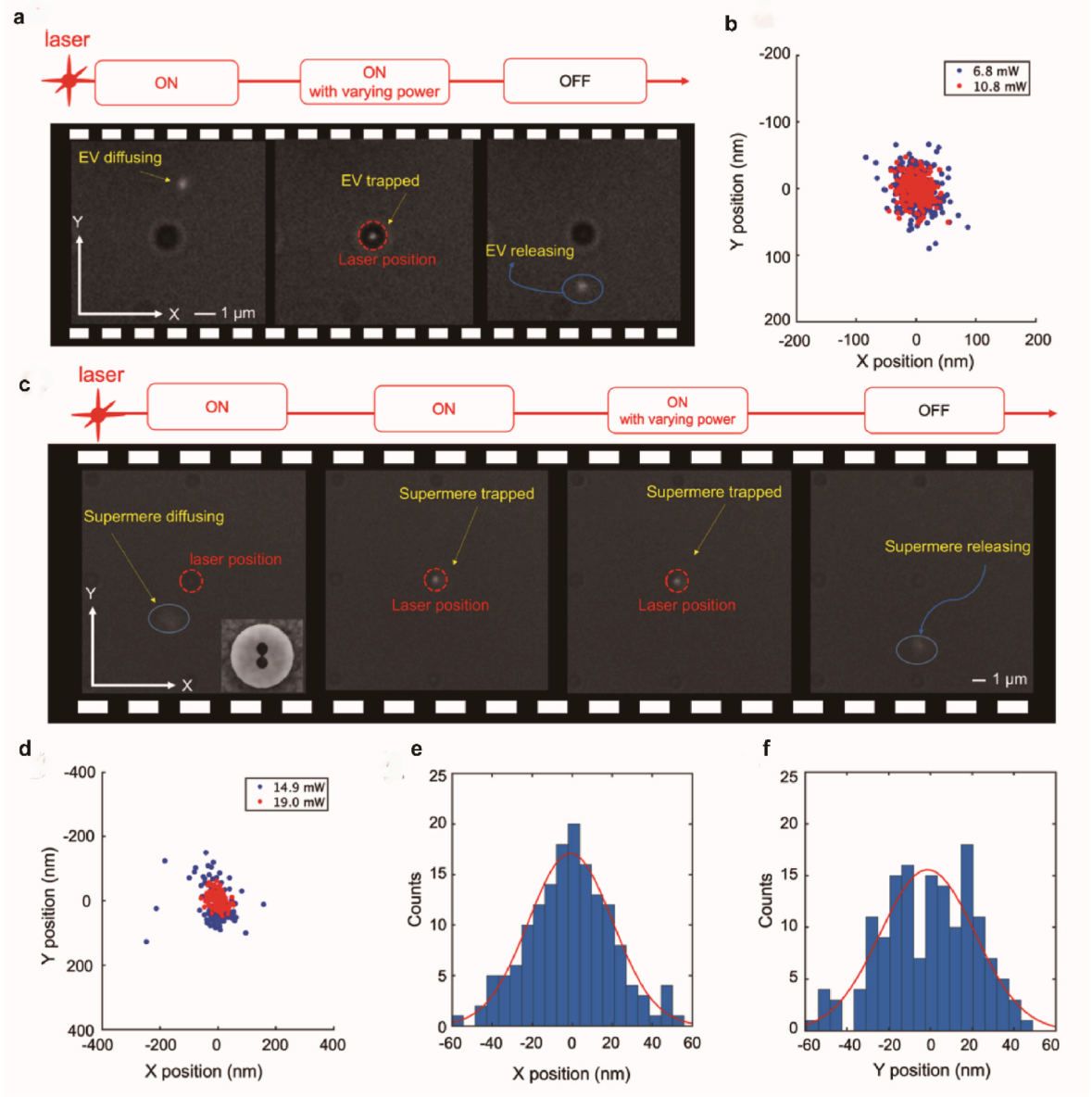

Fig. 5. Trapping mechanism of EVs and supermeres. a. The EV trapping experiment shows that the diffusing EV is trapped on the anapole nanoantenna, and then the EV is released when the 973 nm laser is turned off. b. The scatter plot shows the EV's trajectory when the EV is trapped on the anapole nanoantenna. The highest estimated stiffness along the x- and y-axis is 0.347 fN/nm and 0.329 fN/nm under 10.8 mW incident laser power, respectively. c. The frame sequence of supermere trapping experiment shows how the diffusing supermere is trapped near the anapole nanoantenna until the laser is off. d. The scatter plot shows the supermere's trajectory when the supermere is trapped at the anapole structure. The estimated stiffness for the trapped supermere under 19 mW incidence along the x- and y-axis are 0.215 fN/nm and 0.205 fN/nm, respectively. e,f. Histogram of the trapped supermere particle's position under 19 mW incident laser power along x and y directions, respectively. The red curve is the Gaussian fitting to estimate the trap stiffness. Reprinted with permission from Ref. [146]. *Copyright 2023 American Chemical Society.*

supermeres but is currently restricted to single-particle analysis and is not practical for large-scale preparation. Therefore, when choosing an isolation method, it is essential to balance extraction efficiency, purity, yield, and particle stability based on specific experimental needs. Combining multiple techniques may be necessary to optimize the separation process.

One challenging problem is that non-vesicular particles (such as exomeres and supermeres) and vesicular particles (such as exosomes) may interact with each other. A common method for separating extracellular vesicles (EVs) is multiple rounds of ultracentrifugation. While this method effectively removes most large EVs (lEVs) and small EVs (sEVs), a small number of sEVs and their fragments may still remain, which can be fragments of EVs or EVs with smaller sizes. This is an issue that researchers should be aware of. Exomeres and supermeres, being much smaller in size, challenge the limits of current separation and detection technologies. Not all methods used to isolate EVs are suitable for these smaller extracellular particles. This problem of incomplete separation was also appeared when EVs were first discovered, as there were few techniques available to support their detailed separation. However, as research progresses, scientists began to recognize the heterogeneity and important functions of EVs, leading to the development of new separation technologies tailored to different sub-classes of EVs.

The issue of EVs interfering with NVEPs is possible to be addressed by combining various separation techniques. Researchers have developed several efficient methods for separating EVs by targeting specific markers on their membranes or using their membrane structural properties. These techniques include immunoaffinity separation, aptamer-based separation methods, innovative nanomaterial capture

techniques, phosphatidylserine affinity-based methods, and membrane fusion characterization methods [147]. For example, an EV capture platform based on lipid labeling uses trans-cyclooctene (TCO) to label EVs for capture through click chemistry, allowing for quick retrieval using simple centrifugation [148]. Extracellular secretions with membrane structures, such as EVs, can be removed using these separation methods. Afterward, nanoparticles of different sizes, like exomeres and supermeres, can be separated using a combination of ultracentrifugation and other techniques. Additionally, identifying specific markers associated with NVEPs, as well as unique markers for particles like exomeres, will help develop more efficient separation methods.

However, this combined-approach may create some challenges. For example, immunoaffinity methods rely on antibodies and aptamers, which require highly specific and sensitive surface markers. Moreover, these methods can be costly and may introduce impurities while separating EVs. Lastly, these techniques often have low throughput and are not scalable for large production. Therefore, researchers must carefully select separation technologies based on their experimental goals.

3.2. Identification and characterization

Identification and characterization are the necessary steps for quality guarantee of the obtained particles [6,7,21,149](Fig. 6). Transmission electron microscope (TEM) and fluid-phase atomic force microscopy (AFM) are the most widely used methods. TEM is a technique for studying the image of a sample by collecting secondary electrons generated by an electron beam passing through the sample. By analyzing the collected electrons at a magnified level, the diameter of extracellular secretions can be determined [150,151]. Nanoparticle tracking analysis (NTA) is a more multifunctional method that involves tracking the movement of nanoparticles in a liquid stream and analyzing their Brownian motion process. Particles are exposed to a beam of light by a hydraulic pump at a certain flow rate, and their movements are recorded using a highly sensitive camera. The observed data is then converted to

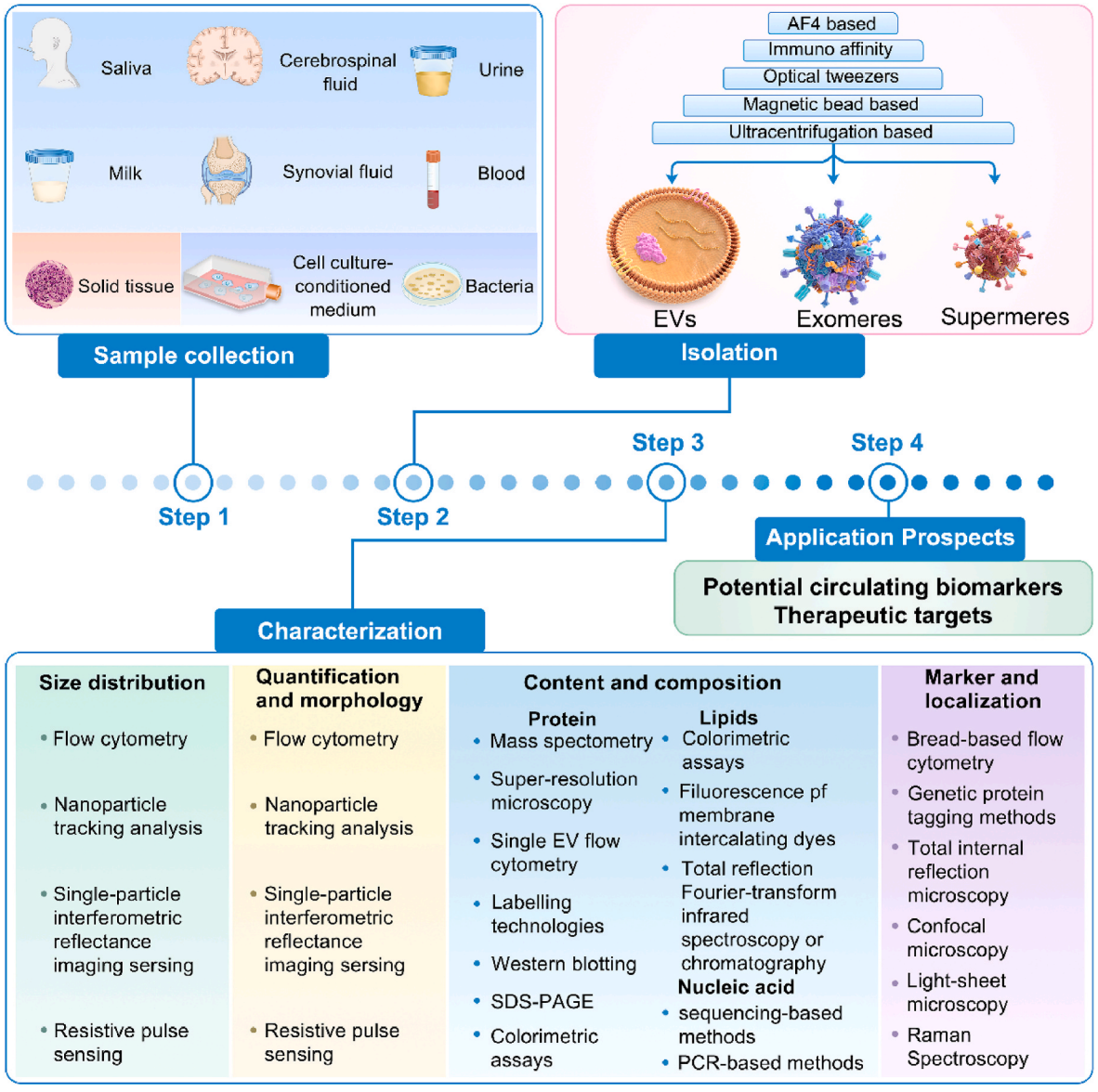

Fig. 6. Workflow of exomeres and supermeres isolation and characterization. The primary sample types include body fluids, tissues, and cell cultures. These samples undergo processes to isolate sEVs, exomeres, and supermeres, which are then subjected to further characterization. This characterization primarily focuses on assessing size distribution, quantification, morphology, content and composition, as well as identifying specific markers and localizations of these particles. The EVs and NPs extracted through this workflow are utilized for subsequent research purposes. It is important to note that while the schematic provides a structured overview, it does not encompass all possible methodologies and variations in the field, as indicated in Ref. [4].

obtain information on the particle size distribution, concentration, and modal value [152–154]. NTA analyzation defines sEVs as particles with 94–173 nm in diameter, while exomeres are 39–71 nm in diameter [7]. These commonly used identification methods, each shows its own advantages and limitations. For size measurement, NTA calculates the hydrodynamic diameter of particles based on Brownian motion, making it suitable for analyzing particle size distribution and concentration in solution. However, results may be affected by particle aggregation and the surrounding environment and currently NTA has not been used to detect the size of supermeres, because of the minimum detection limitation. AFM provides high-resolution three-dimensional topographical imaging and can measure surface roughness and mechanical properties, but the measured size may be overestimated due to the shape of the probe. TEM offers the highest resolution and allows clear visualization of ultrastructural details, such as lipid bilayers and internal vesicles, making it the gold standard for morphological analysis. However, its complex sample preparation process may cause particle shrinkage or shape alterations. In terms of concentration measurement, NTA is the only method that can quantitatively analyze particle concentration in solution, while AFM and TEM are limited to observing localized samples. For surface characterization, AFM provides high-resolution surface morphology and mechanical properties, whereas TEM can reveal external structures but requires staining, which may alter the original surface characteristics. NTA requires the simplest sample preparation and can analyze liquid-phase samples directly, whereas AFM and TEM require sample fixation and drying, with TEM having the most complex preparation process. Therefore, the choice of method should be based on specific experimental needs and it is recommended to combine these techniques can provide a more comprehensive analysis.

EVPs can also be identified immunologically based on their surface markers. Western blotting can identify proteins that are associated or co-isolated with sEVs, exomeres and supermeres [21,115,116,155]. Flow cytometry-based methods also utilize immunomarkers for detection, including bead-based flow cytometry and single-EV flow cytometry. Bead-based flow cytometry is widely used to detect EV markers. This method captures small particles with beads and identifies them using fluorescently labelled affinity reagents (or mixtures of reagents). Single-EV flow cytometry detects vesicle particle size, epitope abundance, epitope density, effective refractive index, and vesicle count through light scattering and fluorescence.This technique requires higher calibration of fluorescence and light scattering parameters [4]. When identifying markers for EVs and NVEPs, it is important to consider not only protein markers but also nucleic acid markers, which warrant significant attention. These can often be analyzed using reverse transcription real-time quantitative PCR (RT-qPCR) and RNA sequencing (RNA-Seq). More sensitive methods such as ELISA and capillary electrophoresis immunoassays also can be used as options [156]. Notably, DELFIA (dissociation-enhanced lanthanide fluorescence immunoassay) is a method based on time-resolved fluorescence. It is able to detect samples with decent sensitivity. However, this method is more demanding and the antibodies used need to have to be experimentally validated [157,158]. The use of mass spectrometry allows for more comprehensive proteomic analysis that independent of gene-specific antibodies. The problem is the low sensitivity and it is recommended to be used in combination with antibody-based techniques [21,159,160]. By integrating total protein measurements with particle counting assays, we can obtain a more accurate assessment of the concentration and purity of the particles in samples [113]. Combination of various functional activity assays help to identify a particular functional nanoparticle, but the standardized metrics and specific guidelines needs to be considered. It is crucial to establish a convincing mechanism to confirm that the effect is indeed mediated by a particular secretion instead of other heteroproteins.

Collectively, compared to EVs, the separation and detection techniques for exomeres and supermeres are less-developed due to the much smaller size and non-membrane structure. Detection limits pose a challenge for techniques like flow cytometry, which cannot detect particles smaller than 300 nm in size [161]. Many ways currently used for separation and detection are not completely suitable for exomeres and supermeres (Table 4).

4. Applications, unknown issues and future perspectives

4.1. Promising applications

Extracellular vesicles and particles (EVPs) are secretions found in the intercellular spaces and tissues. Their composition varies in response to the physiological and pathological state (Table 5). Studies have shown that exomeres and supermeres carry unique proteins and nucleic acids, which are closely associated with various diseases. The detection of these extracellular substances can be used to understand slight changes occurring in the body, and serve the early detection and diagnosis of intractable diseases [185,186]. Additionally, these particles can be used in drug delivery and disease treatment [187], since they can help transport substances in the organs and even cross the blood-brain barrier [21](Fig. 7, Table 6).

4.1.1. Potential roles in tumors

Potential tumor biomarkers: Exomeres and supermeres are enriched in various cancer-related molecules and can serve as novel biomarkers for tumor detection and diagnosis. For example, supermeres are highly enriched with tumor-associated proteins and RNAs, including glycolytic enzymes, TGFBI protein, miR-1246, c-MET receptor, GPC1 protein, and AGO2, all of which play roles in various cancers [21,22,51,96,206]. Many tumor biomarkers traditionally considered to be present in exosomes are actually more concentrated in these nanoparticles. For instance, the glycoprotein GPC1, previously regarded as an early detection marker for pancreatic cancer exosomes, is found in high concentrations in supermeres [9]. This discovery suggests that detecting specific molecules carried by exomeres and supermeres in blood can improve the sensitivity and specificity of cancer liquid biopsy. Some studies have pointed out that the levels of these particles in the blood and the biomarkers they carry vary with disease states [22,205,207]. For example, the levels of EV/particles in the pulmonary circulation of lung cancer patients are closely related to clinical staging. Therefore, using tumor molecules in exomeres and supermeres as biomarkers holds promise for early detection and diagnosis of cancer. More detailed candidate molecules are listed in Table 6.

Table 5
Small secretions and their associated diseases.

Name	Category	Associated diseases	References
Supermere	NVEPs	Multiple cancers, Alzheimer's disease, cardiovascular disease, coronavirus disease 2019 (COVID-19) infection	[9,21]
Exomere	NVEPs	Intestinal organoid tumors, neurodegenerative diseases like Alzheimer's disease and Parkinson's disease.	[7,51]
Lipoprotein	NVEPs	Lipid metabolism-related diseases like hypercholesterolemia, cardiovascular disease	[101,102]
Nucleosome	NVEPs	Regulating various epigenetic signaling pathways	[98,103]
Vault	NVEPs	Cancer, metabolic diseases	[104,105]
Apoptotic EV	EVs	Cancer, acute immune response, cardiovascular disease, tuberculosis, Bone-related diseases,	[106,107]
Ectosome	EVs	Acute coronary syndromes, atherosclerosis, stroke, cancer, metabolic disease	[108,109]
Exosome	EVs	Immune responses, viral pathogenicity, pregnancy, cardiovascular diseases, central nervous system-related diseases, and cancers	[20,110]

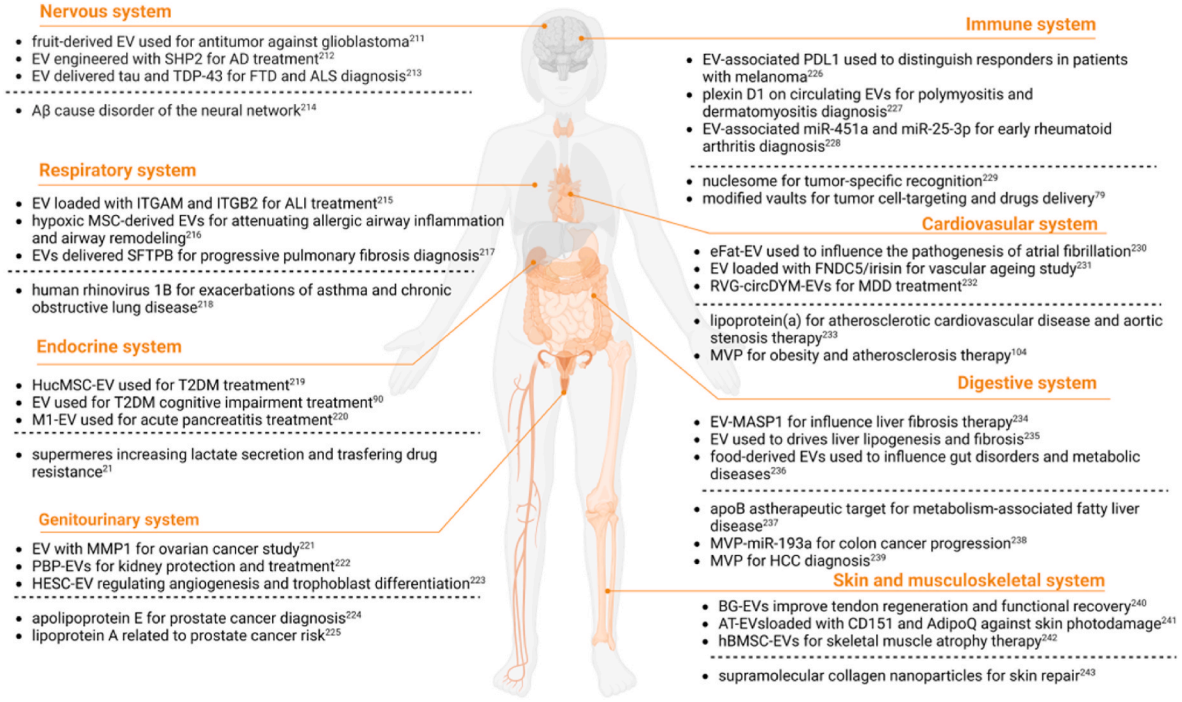

Fig. 7. Extracellular particles and their associated diseases. There is an ever-increasing researches on EVs and NVEPs in various diseases, including nervous system, respiratory system, endocrine system, skin and musculoskeletal system, immune system, cardiovascular system, digestive system and genitourinary system. Summarization in the schematic is not exhaustive [21,79,90,104,211–243].

Prognostic evaluation and disease monitoring: Some proteins contained in exomeres and supermeres indicate tumor prognosis. A typical example is TGFBI (TGF-β induced protein), which has been found to be one of the most abundant proteins in supermeres [21]. In colorectal cancer patients, the levels of TGFBI in tumor tissue and plasma particles are significantly elevated. Clinical cohort analysis shows that high TGFBI expression in colorectal cancer tumors is associated with poor overall survival and progression-free survival. More importantly, TGFBI levels in exomeres and supermeres separated from cancer patients' plasma are consistently higher than those in healthy controls [21]. One study separated plasma from three colorectal cancer patients and healthy controls by gradient centrifugation, and the results showed that the levels of TGFBI in all fractions, including small exosomes, non-vesicular components, exomeres, and supermeres, were significantly higher in the patient plasma than in the healthy controls [21]. This suggests that TGFBI can serve as a potential liquid biopsy biomarker for colorectal cancer, used for disease detection and treatment monitoring. Researchers have also confirmed the prognostic value of TGFBI through immunohistochemistry and survival analysis. Therefore, in colorectal cancer and other tumors, detecting proteins such as TGFBI carried by exomeres and supermeres is promising for evaluating patient prognosis. Similarly, miR-1246, which is enriched in supermeres and upregulated in cancer tissues while expressed at low levels in healthy tissues, is also a potential tumor-related nucleic acid marker [21,51]. These findings suggest that exomeres and supermeres can aid in tumor risk stratification and recurrence monitoring.

Treatment response and drug resistance monitoring: Exomeres and supermeres also have potential applications in tumor treatment. On one hand, the tumor signaling molecules they carry reflect treatment response; on the other hand, they themselves are involved in mediating resistance processes, providing targets for monitoring and intervention. Studies show that supermeres derived from drug-resistant cancer cells can transfer drug resistance to sensitive cells. For instance, supermeres secreted by colorectal cancer cells resistant to the anti-EGFR monoclonal antibody cetuximab can transfer resistance traits to otherwise sensitive cells [21]. This process may be related to membrane proteins such as c-MET, which is a known pathway for cancer cells to acquire resistance to anti-EGFR therapy [21]. Therefore, in treated cancer patients, an increase in the levels of drug resistance-related proteins (such as MET) carried by supermeres in the blood may indicate the development of resistance. By dynamically monitoring the contents of exomeres and supermeres in patient circulation, early resistance signals can be detected, guiding treatment adjustments. In addition, these nanoparticles' impact on receptor cell function also suggests new treatment strategies: if tumor cells secrete exomeres and supermeres to induce malignant phenotypes in surrounding cells, blocking the production or uptake of these particles could slow tumor growth and metastasis. Thus, exomeres and supermeres can not only be used for efficacy monitoring but also as new therapeutic targets for anti-tumor treatments. For example, developing intervention strategies to inhibit the secretion of these particles by tumors or clearing pathogenic supermeres in circulation may help enhance the efficacy of cancer therapies.

4.1.2. Potential roles in neurological diseases

Alzheimer's disease (AD): The research on exomeres and supermeres in neurodegenerative diseases is still in its early stage, but there is evidence suggesting that they carry molecules closely related to the pathogenesis of AD and may serve as early diagnostic biomarkers. Supermeres are enriched with proteins associated with AD pathology, such as amyloid precursor protein (APP). Studies have confirmed that exomeres and supermeres released from cell cultures are significantly enriched with extracellular fragments of APP, while the full membrane-bound APP is primarily found in cells and traditional exosomes [21]. Detection using specific antibodies and analytical techniques has revealed that non-membranous particles (exomeres and supermeres) contain large amounts of APP fragments cleaved by β and γ-secretase. These fragments (such as soluble APPα/β) are key molecules in the pathogenesis of AD and are typically released into body fluids after being generated in the brain [208]. Therefore, these findings suggest that brain-derived APP metabolic products in AD patients may enter the peripheral blood through exomeres and supermeres. This provides a new avenue for developing blood biomarkers for AD: using high-sensitivity

Table 6
Potential applications of EVPs.

Function	Source	Component	Associated diseases	References
Therapeutic potential functions	supermere and exomere	ACE and ACE2	Alzheimer's diseases, cardiovascular disease and COVID-19	[21,188]
	supermere and exomere	APP and APLP1	Alzheimer's disease and cancers	[7]
	exomere	ACLY	NAFLD, cancers, liver fibrosis and dyslipidemia	[189,190]
	exomere	ALDOA	glucose metabolism-related diseases and liver cancer	[33,191]
	exomere	BACE1	Alzheimer's disease, vascular-related diseases, aging, and immune	[192,193]
	exosome	CD55	inflammatory and infectious diseases	[194]
Diagnostic potential functions	exosome, exomere and supermere	DPEP1	colorectal cancer, renal inflammation	[195,196]
	exosome, exomere and supermere	CD73	cancers,	[197,198]
	exosome, exomere and supermere	CEACAM5	cancers especially in colorectal cancer	[199]
	exosome, exomere and supermere	CD59	complement-related diseases like PNH and aHUS	[200]
	exosome	PLAUR	atherosclerosis and cardiovascular disease	[201,202]
	exosome, exomere and supermere	CEACAM6	pancreatic cancer and COPD	[203,204]
	exosome	GPC1	pancreatic cancer	[57]
	exosome	miRNAs (e.g., miR-1277, miR-194–5p/miR-16–5p ratio), lncRNAs (e.g., SChLAP1, PCA3, MALAT1), mRNAs (e.g., ETV1, FASN, BRN4, BRN2), circRNAs (e.g., circHIPK3), DNA (e.g., PTEN, CK-8), proteins (e.g., HSPD1, CK18, MMP2, PSA), and metabolites (e.g., cholesteryl oleate, PA, PS, LPE, PC)	prostate cancer	[205]

methods to capture exomeres and supermeres in the blood and detect APP fragments or related proteins, which may enable the detection of AD pathology in an early stage. Currently, scientists are exploring the use of exosomes and nanoparticles derived from the central nervous system in blood as diagnostic tools for AD. For example, some reviews point out that elevated levels of Aβ42, total Tau, and phosphorylated Tau (such as p-T181 or p-S396) in blood-derived neurogenic exosomes could be used for early AD diagnosis [209]. As exomeres and supermeres have been identified, we realize that many AD-related proteins and RNAs are primarily enriched in these smaller particles. Therefore, targeting exomeres and supermeres for detection may further improve the accuracy of blood-based AD diagnosis. This field is currently under active investigation, with the NIH's Extracellular RNA program highlighting the biomarker potential of supermeres in AD and other diseases (https://commonfund.nih.gov/Exrna/highlights/supermeres-new-extracellular-vesicle). More detailed candidate molecules are listed in Table 6.

Other neurodegenerative diseases: Currently, there is relatively little evidence regarding exomeres and supermeres in Parkinson's disease (PD) and other neurological disorders. However, given that these nanoparticles can cross biological barriers and distribute widely throughout the body, their role in neurological diseases is worth attention. Animal experiments have shown that supermeres injected intravenously are more easily taken up by various organs, particularly the brain, compared to traditional exosomes. This suggests that supermeres may cross the blood-brain barrier and enter the central nervous system. Similarly, supermeres or exomeres produced by the central nervous system may escape into peripheral circulation. Therefore, in diseases like Parkinson's, abnormal aggregation of proteins (such as α-synuclein) or other pathogenic molecules in the brain may be released through these particles into the bloodstream. Studies are exploring the use of blood EVs containing α-synuclein, DJ-1 protein, and others as diagnostic markers for Parkinson's disease. In the future, as research on exomeres and supermeres deepens, we may discover that specific miRNAs or proteins they carry are related to the development of Parkinson's disease and can be used for early screening. Furthermore, the excellent blood-brain barrier-crossing ability of supermeres makes them potential drug delivery carriers: scientists envision using engineered supermeres to deliver therapeutic molecules into the brain, overcoming the bottleneck of traditional drugs being unable to enter the central nervous system. In conclusion, although research on exomeres and supermeres in neurological diseases is still in its early stages, their ability to carry brain pathology molecules makes them promising early diagnostic biomarkers and new therapeutic tools.

4.1.3. Potential clinical research and translational applications

Clinical trial progress: Given that exomeres and supermeres are newly recognized concepts, clinical trials focusing on them are still limited. According to a systematic review of clinical trial registries in 2024, there are no direct clinical trials specifically named after "exomere" or "supermere". However, many related efforts are underway to prepare these novel particles for clinical use. For example, research teams have filed patents on exomeres and are exploring their application in cancer detection and treatment [22]. Researchers at Weill Cornell Medical College have established a standard procedure for isolating exomeres and propose using exomeres as tools for cancer diagnosis, prognosis evaluation, and treatment decision-making. This technology is already in the translational pipeline, aiming to develop rapid, highly reproducible detection methods. Similarly, the NIH's Extracellular RNA Common Fund has listed supermeres as an important scientific breakthrough and highly praises their potential as circulating biomarkers and therapeutic targets. Some preclinical studies have already validated the utility of exomeres and supermeres in patient samples, such as successfully detecting tumor markers like TGFBI in supermeres isolated from colorectal cancer patients' plasma [21]. These findings lay the foundation for subsequent clinical research. As understanding of

exomeres and supermeres deepens, clinical trials are expected to assess their sensitivity for early detection and recurrence monitoring in cancer. In the neurological field, research may attempt to use blood-derived brain-specific exomeres as early screening tools for Alzheimer's disease [192,193]. Overall, although large-scale clinical trial results have not yet been published, translational research on exomeres and supermeres is actively progressing, and there is optimism in the scientific and industrial communities about their future applications.

Therapeutic potential: In addition to diagnostic applications, the potential therapeutic value of exomeres and supermeres has also garnered attention. On one hand, some therapeutic targets they carry can be intervened by drugs, such as PCSK9, which is an important target in cardiovascular diseases, and ACE2, which is related to COVID-19 and heart failure; these particles offer new pathways for targeted delivery or inhibition [210]. On the other hand, as previously mentioned, interfering with the release of supermeres from tumors may reduce the tumor cells' resistance to treatment. Therefore, some scholars suggest developing neutralizing strategies against exomeres/supermeres or drugs to inhibit their biogenesis, which could serve as adjuncts to cancer therapy [21]. Additionally, since supermeres can efficiently enter tissues [21], they may be used as natural nanocarriers to load drugs or siRNA for targeted delivery to tumors or the brain, thereby improving therapeutic outcomes and reducing side effects. These therapeutic directions are still at the concept validation and animal experiment stage and require further research and clinical verification.

4.2. Unknown issues and future perspectives

4.2.1. What can we learn more about exomeres and supermeres?

Exomeres and supermeres are still less studied with many problems to be solved. Exomeres and supermeres refer to a group of extracellular complexes with comparable sedimentation coefficients, rather than a distinct set of nanoparticles. Researchers have posed inquiries about the possibility that exomeres and supermeres may form a coherent category of extracellular nanoparticles, and whether there might be additional types of particles that have not yet to be detected is unknown. As for the source and biogenesis mechanism of exomeres and supermere, are they produced by all types of cells or certain cell types? The current findings suggest that exomeres and supermeres should not be considered as waste in cells. How are these NVEPs produced? Some researchers have linked protein phase separation to these NVEPs, a process in which specific substances within a cell rapidly aggregate to form small droplets. Are cargoes of exomeres and supermeres selectively loaded or unconsciously and randomly? There are many proteins present in exomeres and supermeres that were previously thought to enriched and work in sEVs (Table 7), why are some of the protein and nucleic acid components present in both EVs and NVEPs? Do cargoes coexisting act differently on receptor cells depending on the carrier?

4.2.2. What are the exact working mechanism of exomeres and supermeres?

Exomeres and supermeres can be uptaken into the cells by macropinocytosis. Previous studies have shown that the uptake of exosomes is influenced by their sizes, cell types, and surface proteins [244,245]. Do exomeres and supermeres show other potential uptake mechanism in different cells and different subtypes? Moreover, since they lack a bilayer lipid membrane structure compared to EVs, will this influence preferential uptake by cells? Supermeres and exomeres show slower cellular uptake compared with sEVs within 24 h [21]. What are the causes of this?

When exomeres and supermeres are taken up by cells, how do they exert their biological effects? Do they function through ligand-receptor binding at the cell membrane, or activate downstream signaling pathways through transporter-active molecules? In addition, it is unclear whether the primary biological function of these NVEPs is carried out by RNA, protein, or both working together.

Table 7
Select proteins previously associated with EVs that are highly enriched in supermeres. With permission from Ref. [9]. *Copyright 2024 Elsevier.*

Protein	Gene symbol	Category
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	Metabolic enzyme
Enolase 1	ENO1	Metabolic enzyme
Enolase 2	ENO2	Metabolic enzyme
Pyruvate kinase M1	PKM1	Metabolic enzyme
Pyruvate kinase M2	PKM2	Metabolic enzyme
Aldolase A	ALDOA	Metabolic enzyme
Glucose-6-phosphate isomerase	GPI	Metabolic enzyme
Lactate dehydrogenase A	LDHA	Metabolic enzyme
Lactate dehydrogenase B	LDHB	Metabolic enzyme
Triosephosphate isomerase 1	TPI1	Metabolic enzyme
Hexokinase 1	HK1	Metabolic enzyme
Hexokinase 2	HK2	Metabolic enzyme
Glypican 1	GPC1	Cell surface heparan sulfate proteoglycan
Amyloid beta precursor protein	APP	Cell surface receptor, myeloid plaques
MET proto-oncogene	MET	Receptor tyrosine kinase
Heterogeneous nuclear ribonucleoprotein A2/B1	HNRNPA2B1	miRNA binding
Argonaute 1	AGO1	miRNA binding
Argonaute 2	AGO2	miRNA binding

4.2.3. How to improve the handling of nanoparticles?

Numerous clinical ectodomain structures of transmembrane receptors, such as MET and GPC1, have been discovered in supermere. It remains unclear whether these structures are inherently abundant in the supermere and serve specific functions, or if they are enriched in the final products during the extraction process due to methodological limitations. Perhaps exploring more optimised methods of isolation and purification and identification, and making standardized and well-documented methodological comparisons of these methods, could be beneficial to a better understanding of this population.

During the process of extracting supermeres, plasma-derived supermere samples contained high levels of proteins, such as albumin. These proteins had the potential to interfere with subsequent analysis and detection. To improve downstream analysis, an albumin depletion kit (Abcam) was used to effectively remove the interference caused by albumin [136]. This raises the question of whether albumin interference is present in other sources of supermeres. It is possible that during the extraction of sEVs and supermeres, albumin proteins could be enriched and have a deeper impact on the samples due to the extraction method (such as ultracentrifugation). Therefore, continual optimization of the extraction method may be necessary to address this problem.

The International Society for Extracellular Vesicles (ISEV) has recently released a set of recommendations that can be useful for researchers in regulating separation methods. For instance, during the extraction of EVs and NPs, it is advised to eliminate cells and their debris as much as possible, to avoid their influence on the target isolates. Moreover, in the characterization and analysis of NPs, experts highlighted the significance of atomic force microscopy, and proposed that the subcellular localization of particles could be studied before studying cellular functions to enhance the understanding of cellular mechanisms [4].There is a great amount of RNA in exomeres and supermeres. The ISEV has made recommendations that could standardize the description of these extracellular secretions, which includes paying attention to the large canopy of EVPs, focusing on a variety of transcriptomics methods and techniques, focusing on the various RNA subclasses in EVPs, revisiting established bioinformatics pipelines and novel strategies for reproducible EV transcriptomics analyses and some other points of attention. There are still many problems in RNA studies of EVPs. For exmaple, recovering small amounts of EV-RNA from biofluids can result in a lack of reproducibility. The quality control for EV-RNA has not yet been established [206].

Exomeres and supermeres have captured the attention of researchers due to their potential value in basic biology, clinical diagnostics, and therapeutics. However, the complexity of these nanoparticles poses several issues that need to be addressed. These issues include their classification, definition, pre-processing variables, isolation, identification, and characterization, as well as in vitro and in vivo analysis of their release, uptake, functions, engineering, and clinical applications. Therefore, there is a need for widely accepted standards to regulate these nanoparticles. The aim of these activities and recommendations is to increase the rigor, reproducibility, and transparency of future research. They will also assist practitioners in implementing or developing best practices for each individual source and application.

4.2.4. How can exomeres and supermeres be translated into clinical applications in the future?

The clinical translation and application pathways of EVs hold significant reference value. The diverse cargo carried by EVs enables a multi-component diagnostic approach for disease detection and monitoring. Additionally, their ability to deliver functional cargo to diseased cells makes them promising candidates as therapeutic vehicles. Several clinical trials are already underway, exploring the therapeutic potential of EVs in clinical settings. (Table 8). However, NPs and EVs may face the similar challenges in clinical translation. In disease observation and detection, there is an urgent need for more standardized and precise separation and characterization methods to analyze the slight differences within these heterogeneous populations. Establishing a complete and reliable data network is crucial for more comprehensive analysis. In disease treatment, the first priority is to assess the efficacy and biosafety of these extracellular secretions. The next challenge is to address their heterogeneity to ensure the reproducibility and rigor of the tests. Once standardized protocols are established, further steps will include determining the appropriate dosage, mode of administration, half-life in the body, and other critical parameters.

Table 8
Ongoing clinical trials.

Research purpose	Research title
Disease observation and diagnosis	Circulating Extracellular Vesicles Released by Human Islets of Langerhans
	Deciphering the Role of Dietary Fatty Acids on Extracellular Vesicles-mediated Intercellular Communication
	Characterization of Exosomes Platelets-released
	Differential Regulation of miRNA and Protein in Human Plasma Extracellular Vesicles by Different Types of Exercise.
	Extracellular Vesicles as Stroke Biomarkers
	ncRNAs in Exosomes of Cholangiocarcinoma
	Multicenter Clinical Research for Early Diagnosis of Lung Cancer Using Blood Plasma Derived Exosome
	Extracellular Vesicle Surface Markers In Acute Cerebrovascular Syndromes.
	Comparision of Various Biomarkers Between Peripheral and Pulmonary Blood
	A Prospective Feasibility Study Evaluating Extracellular Vesicles Obtained by Liquid Biopsy for Neoadjuvant Treatment
	Contents of Circulating Extracellular Vesicles: Biomarkers in Colorectal Cancer Patients
	Rheo-Erythrocrine Dysfunction as a Biomarker for RIC Treatment in Acute Ischemic Stroke
Disease treatment	Mesenchymal Stem Cells Derived Exosomes in Osteoarthritis Patients
	MSC EVs in Dystrophic Epidermolysis Bullosa
	The Pilot Experimental Study of the Neuroprotective Effects of Exosomes in Extremely Low Birth Weight Infants
	Safety and Efficacy of Umbilical Cord Mesenchymal Stem Cell Exosomes in Treating Chronic Cough After COVID-19
	Extracellular Vesicle Treatment for Acute Respiratory Distress Syndrome (ARDS)

4.2.5. Are exomeres/supermeres complementary or alternative pathways to EVs?

The biological significance of EVs has been revealed through the long-term collaborative efforts, which include their role in intercellular communication during disease development, their potential for disease diagnosis, and their use as therapeutic carriers or raw materials in treatment. Moreover, their additional effects are still being explored. Similarly, NVEPs, which also originate from various types of cells, is possess to share the similar biological values as EVs. However, their biogenesis, biological properties, and potential diagnostic and therapeutic uses have yet to be thoroughly investigated. The biological origin and significance of exomeres and supermeres were explored by researchers and they demonstrated that these NVEPs are objective entities, rather than mere fragments resulting from cell death or by-products of the isolation methods, as previously described. Furthermore, comparisons were made between the composition of EVs, exomeres and supermeres using various techniques, revealing that exomeres and supermeres possess unique expressions in several aspects, including proteins, nucleic acids, and polysaccharides. This indicates that NVEPs possess distinctive characteristics that may offer research value similar to that of other EVs. Both types of secretions respond to crucial signals involved in intercellular communication, which may partially overlap or complement each other.

It is also hard to tell which of the two types of particles, the EVs and NEVPs is more economical and practical currently, since the proofs still lack. If exomeres and/or supermeres would serve as complementary way to EVs or even to be more enriched extracelluar RNA or proteins origins, they should also be considered as promising and valuable candidates. Latest research has shown that there might be a misunderstanding regarding the roles that were previously attributed to EVs, possibly due to isolation technologies. Although the current process of separating exomeres and supermeres, is relatively cumbersome, it is believed that with the development of new technologies (advanced separation methods and imaging techniques), our understanding of intercellular communication mechanisms and the value of extracellular particle-based research will certainly be broadened.

5. Concluding remarks

Research on how to define and generate nano-scaled extracellular vesicles and particles are hitting up for that these nano-sized particles are showing great potential to be new biomarkers and targets for diseases and precision medicine. The improvements in isolation methodology and the awareness of the particle heterogeneity make our understanding of the extracellular particles and their subsets, as well as the intercellular communication mediated by these particles, in a state of flux. It is lately appreciated that NVEPs without a lipid bilayer membrane are also exist and even abundant in cell culture medium and human bodily fluids. Exomeres and supermeres are new members of NVEPs family. It is interesting that many bioactive substances, such as proteins and exRNAs are found to be enriched several times more than those in exosomes. Whether these informational biomolecules could be released by different carriers or the result is limited by the current isolation and purification techniques remains unclear, but it can be determined that exomeres and supermeres are responsible and functional in disease progression and treatment, particularly in cancers and neurodegenerative diseases. Further research is necessary to elaborate the basic cell biology of secretion used by cells or tissues to release these particles. The advanced separation and purification method is also critical for particle classification and the accuracy of subsequent research.

To understand and optimize their efficacy and safety, efforts should also be dedicated to addressing the challenges associated with their functions and mechanisms, pharmaceutical manufacturing, scalability

and the batch-to-batch consistency. Additionally, formulation and storage of exomeres and supermeres, along with the comparison to EVs and quality controls should be considered. In conclusion, there is still a long but promising way to go for both basic and translational research on exomeres and supermeres.

CRediT authorship contribution statement

Li Yu: Writing – review & editing, Writing – original draft. **Hui Shi:** Writing – review & editing, Supervision, Conceptualization. **Tingxin Gao:** Writing – review & editing, Visualization. **Wenrong Xu:** Writing – review & editing. **Hui Qian:** Writing – review & editing, Validation. **Jiajia Jiang:** Writing – review & editing, Supervision, Conceptualization. **Xiao Yang:** Writing – review & editing, Supervision, Conceptualization. **Xingdong Zhang:** Supervision.

Ethics approval and consent to participate

This study did not involve human or animal subjects. Thus, no ethical approval was required. The study protocol adhered to the guidelines established by the journal.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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