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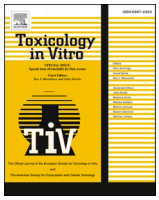

Review Article

Minimum information for studies of extracellular vesicles (MISEV) as toolbox for rigorous, reproducible and homogeneous studies on extracellular vesicles

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ABSTRACT

Studies based on extracellular vesicles (EVs) have been multiplying exponentially for almost two decades, since they were first identified as vectors of cell-cell communication. However, several of these studies display a lack of rigor in EVs characterization and isolation, without discriminating between the different EV populations, thus generating conflicting and unreproducible results. There is therefore a strong need for standardization and guidelines to conduct studies that are rigorous, transparent, reproducible and comply with certain nomenclatures concerning the type of EVs used. The International Society for Extracellular Vesicles (ISEV) published the Minimum Information for Studies of Extracellular Vesicles (MISEV) in 2014, updating it in 2018 and 2023 to reflect different study contexts and technical advancements. The primary objective of this review is to inform future authors about EVs, including their history, nomenclature, and technical recommendations for the for isolation and functionality analysis for conducing EV-based studies according to current standards. Additionally, it aims to inform reviewers about the key parameters required for characterizing EV preparations.

1. A brief history of EV discovery

The history of extracellular vesicles (Fig. 1) traces back to the 1960–1970s when researchers first observed the release of small vesicles from cells and viruses (Sun, 1966; Bonucci, 1967; Anderson, 1969; Nunez et al., 1974; Dalton, 1975). However, these observations were initially considered artifacts or cellular debris rather than functionally significant entities (Hargett and Bauer, 2013; Couch et al., 2021). In 1983, two significant developments paved the way for the recognition of EVs as important biological entities. Both studies, based on monitoring the fate of the transferrin receptor in cells, i.e. its recycling or degradation, have unexpectedly revealed the involvement of extracellular vesicles of endosomal origin in the extracellular release of this receptor. The teams directed by Rose Johnstone and Philip Stahl proposed that they played a role in the removal of obsolete proteins during reticulocyte maturation (Harding et al., 1983; Pan and Johnstone, 1983). These were the two first studies to suggest that vesicles could be involved in cellular processes rather than being mere waste products.

In the 1990s the term “exosome” was introduced to describe a specific subtype of extracellular vesicles. These exosomes were characterized by their small size (30–150 nm) and their endosomal origin. Researchers identified exosomes in various biological fluids, including blood, urine, and saliva, and began to uncover their roles in cell communication and immune modulation. One pivotal study in 1996 by Graça Raposo and colleagues demonstrated that B cells could secrete exosomes carrying major histocompatibility complex (MHC) molecules capable of stimulating T cells (Raposo et al., 1996). This finding highlighted the potential role of exosomes in immune responses and set the stage for further investigations into their functions in health and disease.

By the early 2000s, it was evident that cells emit a variety of vesicles. This led to the adoption of the more inclusive term “extracellular vesicles”, which encompasses various types such as exosomes, microvesicles and apoptotic bodies. The distinctions between these vesicle types were based on their size, biogenesis pathways, and molecular composition. In 2006–2007, the teams of Mariuzs Ratajczak and Jan Lötvall made a groundbreaking discovery by showing that exosomes could transfer RNA between cells, introducing a novel mechanism of genetic exchange (Ratajczak et al., 2006; Valadi et al., 2007). This finding highlighted the role of EVs in horizontal gene transfer and their implications in various physiological and pathological processes.

Given their significant role in cell-cell communication, the number of studies on EVs has increased substancially since 2007. As of January

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2025, there are approximatively 50.140 publications on PubMed with the search term "extracellular vesicles" and 35.750 with "exosomes". With the growing interest in EVs and their potential applications in diagnostics, therapeutics and toxicology, the scientific community recognized the need for standardized methodologies and reporting criteria. Variability in isolation techniques, characterization methods, and nomenclature posed significant challenges in comparing and reproducing findings across studies. In response to these challenges, the International Society for Extracellular Vesicles (ISEV) introduced the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines. First published in 2014 (Lotvall et al., 2014) and updated in 2018 (Thery et al., 2018) and 2023 (Welsh et al., 2024), MISEV provide a comprehensive framework to ensure the quality and reproducibility of EV research.

2. Overview of the MISEV recommendations

MISEV provides valuable recommendations for optimizing the rigor, transparency and reproducibility of EV studies. MISEV are not obligations for authors, especially as not all technologies are necessarily accessible to everyone. However, as their name suggests, they are the minimum required to produce quality studies in the field of EVs. These guidelines provide comprehensive recommendations on the production, separation, and characterization of the different EV types and subpopulations from various sources, including cell culture, body fluids, and solid tissues. They aim to facilitate robust scientific discoveries by offering an updated snapshot of the available methodologies and their respective advantages and limitations. The main argument being to cross-reference techniques, follow standardized protocols depending on the source sample used and report the data in a state-of-the-art manner. MISEV emphasize five main critical aspects detailed below.

2.1. Nomenclature and classification of EVs: the simpler, the better

Precaution is required for EV nomenclature. Indeed, the all-too commonly used term "exosome" only refers to a small extracellular vesicle with a diameter of around 100 nm generated as an intraluminal vesicle (ILV) within the multivesicular body (MVB) and sorted from the cell through an exocytosis pathway. An exosome is per se an EV, however not all EVs are exosomes. The temptation to name small EVs exosomes is certainly strong, but it corresponds to only a small fraction of the EVs generated by a cell and is therefore mistaken. This drift is not recent, since from the pioneering studies in the field, numerous names have been given to EVs, many of them related to their cell or tissue of origin such as prostasomes from the prostate gland (Ronquist et al., 2012), large oncosomes from tumors (Minciacchi et al., 2015) or matrix vesicles for bone and cartilage mineralization (Anderson, 1969).

The MISEV guidelines emphasize clear and consistent communication of concepts related to EVs, addressing the complexity of EV classification and terminology (Welsh et al., 2024). As summarized in Fig. 2, a cell can produce various types of EVs which are defined as lipid-bilayer bound particles that do not contain a nucleus and cannot replicate. MISEV recommend clear definitions and classification of EVs based on their size, biogenesis, and molecular composition to ensure consistent communication. EVs have two main origins: endosomal and derived from plasma membrane (van Niel et al., 2018; van Niel et al., 2022). Considering that EV populations are isolated according to the ultracentrifuge speed or kits, it is more than advisable to refer to the group of isolated EVs - large, medium or small EVs (Table 1) - rather than clumsily trying to give them a more restrictive name. As an example, the small EVs fraction, which is obtained at 100–120000*g* ultracentrifugation speed, contains the so-called exosomes, but also other small EV subtypes such as small ectosomes derived from plasma membrane, autophagosome-derived EVs, small apoptotic bodies or mitochondria-derived small vesicles (Buzas, 2023).

2.2. Isolation and purification of EVs: be mindful of limitations and possible contaminants

The MISEV2023 guidelines provide key methods for the isolation and purification of EVs including differential ultracentrifugation and density gradient ultracentrifugation, size exclusion chromatography (Welsh et al.), immunoaffinity capture and EV precipitation methods such as polyethylene glycol (PEG) precipitation (Table 2).

Differential ultracentrifugation is a traditional method using a series of increasing centrifugal forces to isolate EVs based on size and density (Thery et al., 2006; Kowal et al., 2016). Initially, lower speeds remove cells and debris, followed by higher speeds to pellet large and small EVs. Although considered a "gold standard" it can introduce contaminants and may not be suitable for all applications due to its labor-intensive nature and potential for damaging EVs (Zhang et al., 2023). To help overcome these contaminants, an ultrafiltration step can be included leading to a non-negligible improvement of EV purity. However, loss of material during this stage leads to a reduction in EVs yield (Merchant et al., 2010; Shu et al., 2020). Density gradient ultracentrifugation improves the separation of EVs from proteins and other contaminants based on buoyant density using density gradients (e.g., sucrose or iodixanol). This method is particularly useful for purifying EVs from complex fluids like blood but remains time-consuming and requires significant equipment (Momen-Heravi, 2017; Brennan et al., 2020). Tangential Flow Filtration (TFF) and cyclic TFF allow the concentration and purification of EVs by filtering samples through membranes with specific molecular weight cut-offs. This technique is efficient for processing large volumes and is less damaging to EVs compared to high-speed centrifugation (Busatto et al., 2018; Kim et al., 2021).

Size Exclusion Chromatography (SEC) is a technique used to improve the purification of EVs from contaminants and is based on size by passing a sample through a column packed with a porous matrix. Larger particles elute first, while smaller ones elute later (Witwer et al., 2013; Lobb et al., 2015; Vanderboom et al., 2021). Automated SEC systems can provide high-purity EVs preparations and are scalable, making them suitable for clinical applications and high-throughput settings.

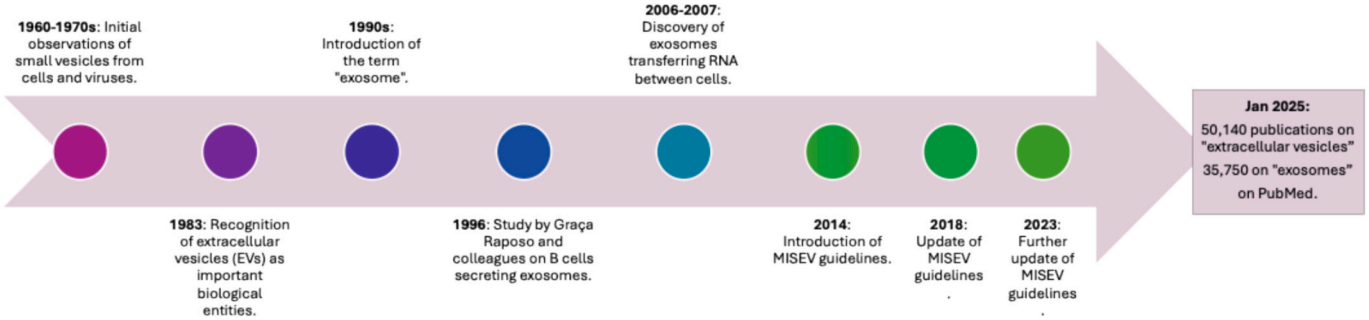

Fig. 1. Timeline and milestones in the research of extracellular vesicles.

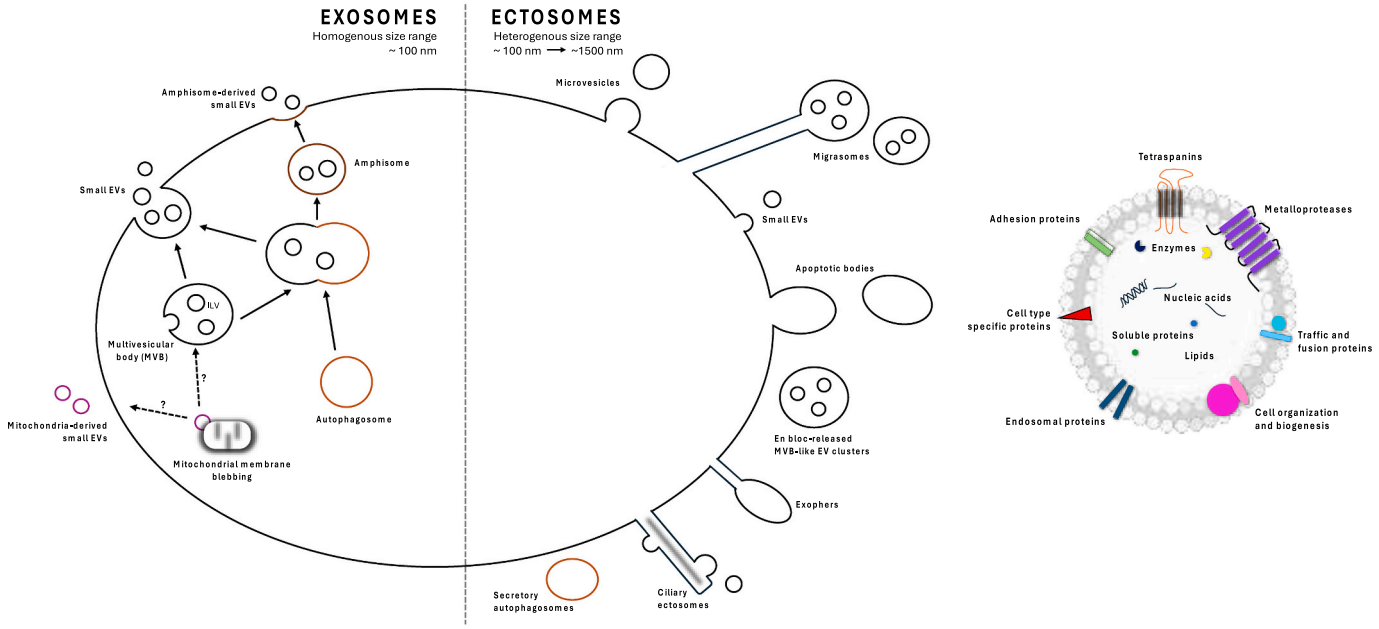

Fig. 2. One cell, various types of extracellular vesicles (EVs). Two main categories of EVs can be produced by a cell: (i) exosomes which are produced endogenously and released into the extracellular medium through exocytosis, and (ii) ectosomes originating from the budding of the plasma membrane. The term "exosome" does not refer to one subtype of small EVs, since intracellular vesicular formations can occur from various origin (autophagosomes, mitochondria, …). A few examples of ectosomes have been illustrated here, but the variety of ectosomes is important and reflect the cell type and the pathophysiological state of the cell (tumor cells, virus-infected cells, migrating cells, immune cells, dividing cells etc.…).

Table 1
The three main EV subpopulations. *hydrodynamic diameter. **real or truncated diameter.

	Small EVs	Medium EVs	Large EVs
Diameter	50–150 nm	200–800 nm	≥ 1000 nm
Size determination	Transmission Electron Microscopy Atomic Force Microscopy Dynamic Light Scattering Nanoparticle Traccking Analysis		
Origin	Endosomal Plasma membrane	Plasma membrane	
Ultracentrifuge speed	≥ 100.000 g	20.000 g	2.000 g < x < 3.000 g
Examples	Exosomes Small ectosomes Autophagosome-derived EVs	Microvesicles Secretory midbody remnants	Apoptotic bodies Migrasomes Exophers Secretory autophagosomes Large oncosomes

Immunoaffinity capture uses antibodies specific to EVs surface markers to capture EVs from a sample (Filipovic et al., 2022). It provides high specificity to sort out specific EVs subtypes expressing the desired surface markers, but may not capture all EV subtypes and can be expensive due to the cost of antibodies. PEG precipitation is a simpler and less costly method that uses PEG to precipitate EVs from solution (Rider et al., 2016; De Sousa et al., 2023). However, it can introduce contaminants and affect downstream applications due to the presence of PEG residues on EV surfaces (Akbar et al., 2022).

These various methods for isolating and purifying EVs have their advantages and disadvantages, but the choice of method must first and foremost reflect the use of EVs in the experimental design. The pros and cons of each method are tabled in recent references (Xu et al., 2016; Gurunathan et al., 2019) as well as in MISEV 2023 (Welsh et al., 2024).

2.3. Characterization of EVs, the art and practice of combining techniques for in-depth clarification

For the characterization of EVs, MISEV2023 emphasize the use of multiple, complementary techniques to sort out the physical properties of EVs and to distinguish EVs from non-EVs vesicles (Table 3).

Table 2
List of methods used for EV purification.

Method to purify EVs	Description	Advantages	Disadvantages
Differential Ultracentrifugation	Uses increasing centrifugal forces to isolate EVs based on size and density.	Considered as the 'gold standard'; widely used.	Labor-intensive; potential contaminants; may damage EVs.
Density Gradient Ultracentrifugation	Separates EVs from contaminants based on buoyant density using gradients (e.g., sucrose, iodixanol).	Improves EV purity; useful for complex fluids like blood.	Time-consuming; requires significant equipment.
Tangential Flow Filtration (TFF)	Filters samples through membranes with specific molecular weight cut-offs for purification.	Efficient for large volumes; less damaging to EVs.	Membrane clogging; loss of small EVs possible.
Size Exclusion Chromatography (SEC)	Separates EVs from contaminants based on size using a porous matrix column.	Provides high purity; scalable and suitable for clinical applications.	Limited yield; requires specialized equipment.
Immunoaffinity Capture	Uses antibodies specific to EV surface markers to capture EVs.	High specificity; captures specific EV subtypes.	Expensive; may not capture all EV subtypes.
PEG Precipitation	Uses polyethylene glycol (PEG) to precipitate EVs from solution.	Simple and cost-effective.	Contaminants and PEG residues may affect downstream applications.

For physical aspects, Transmission Electron Microscopy (Consortium et al.) is a "golden standard" used to visualize the size, shape, and structure of individual EVs (Pan and Johnstone, 1983; Rikkert et al., 2019; Pascucci and Scattini, 2021). TEM involves negative staining or cryo-EM techniques to provide high-resolution images, confirming the presence and morphology of EVs. Moreover, this technique allows the measurement of the real diameter of EVs comparing to other techniques described below. TEM also allow to clearly see the EVs and to distinguish between EVs and non-EV particles. TEM analysis can be completed by Atomic Force Microscopy (AFM, (Parisse, 2017 #41)). AFM provides nanoscale resolution, allowing detailed imaging of EV morphology and surface structures, and this technique does not require extensive sample preparation that could alter the EVs, preserving their native state. AFM can also generate three-dimensional topographical maps, offering insights into the size, shape, surface features of EVs such as mechanical properties, i.e. stiffness and elasticity, providing additional information about the vesicles and distinction between EVs and non-EV particles. However, AFM is relatively slow and labor-intensive, making it less suitable for high-throughput analysis. Moreover, AFM still requires careful sample preparation to ensure accurate measurements, and adsorption of EVs onto the substrate can introduce artifacts, potentially skewing results.

In complement to microscopy approaches, three main techniques can provide physical information of EVs. Dynamic Light Scattering (DLS) measures particle size distribution by analyzing the scattering of light caused by particles in suspension (Khan et al., 2022). This technique is effective for determining the average size of EVs but has limitations in resolving heterogeneous populations and determining the concentration of EVs in solution (Szatanek et al., 2017). Tunable Resistive Pulse Sensing (TRPS) measures not only the size but also the concentration of EVs by detecting changes in electrical resistance as particles pass through a nanopore (Maas et al., 2014; Maas et al., 2017). This technique provides high-resolution data and can distinguish EVs from other particles based on their physical properties, but without sorting out an image of EVs in solution. TRPS can provide zeta potentials (ζ) of EVs, even if this charge does not reflect the real number of charges surrounding the EVs, and ζ is tough to determine for heterogenous populations such as for mEVs or lEVs samples (as discussed in Tamrin et al., 2023). Nanoparticle Tracking Analysis (NTA) appears to be one of the most used techniques to determine to the size distribution and concentration of EVs by tracking the Brownian motion of particles in suspension (Comfort et al., 2021). This method is particularly useful for quantifying EVs and assessing their size profile, although it may not distinguish between EVs and similarly sized particles.

Table 3
List of techniques used for EV characterization.

Technique for EVs characterization	Description	Advantages	Disadvantages
Transmission Electron Microscopy (TEM)	High-resolution imaging technique to visualize the size, shape, and structure of EVs.	High-resolution; confirms presence and morphology of EVs; distinguishes EVs from non-EVs.	Labor-intensive; requires extensive sample preparation.
Atomic Force Microscopy (AFM)	Provides nanoscale resolution imaging of EV morphology, surface features, and mechanical properties.	Preserves native state of EVs; provides 3D topography and mechanical properties.	Relatively slow; adsorption artifacts may affect accuracy.
Dynamic Light Scattering (DLS)	Measures particle size distribution by analyzing light scattering in suspension.	Effective for measuring average EV size.	Limited resolution; struggles with heterogeneous populations.
Tunable Resistive Pulse Sensing (TRPS)	Detects changes in electrical resistance as EVs pass through a nanopore, measuring size and concentration.	Provides high-resolution data; measures both size and concentration.	Cannot image EVs; challenging for heterogeneous samples.
Nanoparticle Tracking Analysis (NTA)	Tracks Brownian motion of EVs to determine size distribution and concentration.	Commonly used; quantifies EVs and size distribution.	Cannot distinguish EVs from similarly sized particles.
Western Blotting	Detects specific EV-associated proteins to confirm EV presence and purity.	Identifies EV-specific markers; confirms sample purity.	Requires high-quality antibodies; potential for non-specific signals.
Enzyme-Linked Immunosorbent Assay (ELISA)	Quantifies specific proteins and biomarkers on or within EVs.	Useful for targeted analysis in research and clinical diagnostics.	Limited to known biomarkers; may not provide a full EV profile.

Some additional biochemistry approaches can further complete the EV characterization and ensure the purity and nature of the vesicles in samples. Western Blotting is commonly employed to detect specific proteins associated with EVs, such as tetraspanins (CD9, CD63 or CD81), heat shock proteins (HSP70), cell-type specific markers if carried by EVs and others. This technique also helps confirm the presence of EV-specific markers and evaluate the purity of EV preparations by identifying potential contaminants (Kowal et al., 2016). As a control, non-EVs samples such as EV-depleted samples of whole cell lysates from the secreting cells are used to check the expression of negative markers such as α-actinin 4 which is only expressed in whole cell lysates. Enzyme-Linked Immunosorbent Assay (ELISA) is used to quantify specific proteins and biomarkers present on or within EVs. This technique is particularly useful for targeted analysis of EVs cargo in research and clinical diagnostics (Hartjes et al., 2019; Logozzi et al., 2020).

More recently, flow cytometry and particularly nano-flow cytometry (nFCM) allows for the analysis of surface markers on individual EVs. This method can quantify and phenotypically characterize EVs based on the expression of specific proteins. It's useful for understanding EVs heterogeneity and identifying subpopulations (Ekstrom et al., 2022).

MISEV2023 emphasizes the importance of detailed and transparent reporting of EV characterization methods summarized into three main objectives: (i) the use of multiple orthogonal techniques with at least two complementary methods to characterize EVs, such as combining TEM or AFM with NTA or flow cytometry; (ii) well-detailed protocols to provide comprehensive descriptions of the protocols used for each characterization method, including controls and calibration procedures, and (iii) the use of quality controls to ensure the reproducibility and reliability of results, including for example EV-depleted samples and the validation of the methods used with known standards.

2.4. Functional studies on EVs

Advancements in technology have significantly contributed to the progress of EV research and for functional assays in particular (Table 4). High-resolution imaging techniques, such as super-resolution microscopy and cryo-electron microscopy, have provided detailed insights not only into the structure and morphology of EVs, but also into uptake studies to assess the internalization of EVs by target cells (Verweij et al., 2021; Bamford et al., 2023). Mass spectrometry-based proteomics, lipidomics, metabolomics and transcriptomics have enabled comprehensive profiling of EVs cargo and composition, revealing their complex molecular composition and their functional roles in terms of cell proliferation, migration and differentiation in cell cultures and animal models (Shaba et al., 2022; Greening et al., 2024). The elucidation of the functional pathways influenced by EVs and their cargo is all the more

Table 4
Functional assays for EV studies.

Functionality assay	Description	Advantages	Disadvantages
High-Resolution Imaging	Super-resolution and cryo-electron microscopy provide detailed insights into EV structure, morphology, and uptake studies.	Provides high-resolution structural insights and helps in uptake studies.	Requires specialized equipment and expertise.
Omics Analysis	Mass spectrometry-based proteomics, lipidomics, metabolomics, and transcriptomics enable comprehensive profiling of EV cargo and function.	Reveals molecular composition and functional roles of EVs.	Complex data interpretation; requires integration of multiple datasets.
Enzyme Activity Tests	Validation of EV functional integrity using enzyme activity tests, including AChE and CD73 activity.	Allows assessment of EV enzymatic functions and sample-specific selection.	AChE not universally present in all EVs; enzyme selection depends on the sample.
Diagnostic Applications	EVs from various cell types and biofluids serve as biomarkers for diseases such as cancer, neurodegenerative disorders, and cardiovascular conditions.	Offers non-invasive disease biomarkers; reflects physiological changes.	Variability in EV release and content may affect diagnostic consistency.
Therapeutic Applications	Engineered EVs explored as delivery vehicles for therapeutic molecules, including small RNAs, proteins, and drugs.	Potential for targeted drug delivery and therapeutic applications.	Challenges in standardization, targeting, and large-scale production.

precise by combining omics data.

It is also possible to validate their functional integrity using enzyme activity tests. One of the enzymes that has been considered as a standard reference is acetylcholine esterase (AChE), but studies have shown that this enzyme is not present in all types of EVs and that its presence may depend on the cell type studied (Liao et al., 2019). In association with proteomic studies enabling the identification of other enzymes present in EVs, the authors are therefore free to choose an enzymatic activity more suited to their samples. Moreover, CD73 activity has been also reported in EV studies in contexts of cell differentiation or polarization as demonstrated for M2 macrophages (Teo et al., 2023).

Encouraged by in vitro and in vivo studies, the potential of EVs as diagnostic biomarkers and therapeutic agents has garnered considerable attention. EVs derived from various cell types and biofluids have shown promise in diagnosing diseases such as cancer (Irmer et al., 2023), neurodegenerative disorders (Raghav et al., 2022), and cardiovascular conditions (Verwer et al., 2023).Several in vitro studies have reported changes in the release, chemical content, and microRNA signatures of EVs secreted by cells when exposed to xenobiotics (e.g., HepG2 liver cells exposed to *Per*- and polyfluoroalkyl substances (PFAS) – Carberry et al., 2023). These findings indicate that EVs could serve as measurable biological endpoints for assessing xenobiotic-induced toxicology. Furthermore, engineered EVs are being explored as delivery vehicles for therapeutic molecules, including small RNAs, proteins, and drugs (Piffoux et al., 2021; Wang et al., 2023; Ziegler and Tian, 2023).

3. An improvement of reporting standards

The most important points updated in MISEV 2023 concern the clarity and the sharing of methodologies and data. Indeed, comprehensive and transparent reporting of methodologies, including pre-analytical variables and technical parameters, is of importance to enhance reproducibility and comparability of EV studies. Moreover, data sharing via public repositories is highly recommended, encouraging researchers to make their data accessible for validation and meta-analyses. This accessibility is also necessary to feed EV databases, some of which will be mentioned below.

3.1. An example of public repository, the Transparent reporting and centralizing knowledge in extracellular vesicle research (EV-TRACK) platform

To limit the inconsistent reporting and variability in experimental methods used in EV-based studies, the EV-TRACK platform (https://evtrack.org) was created to provide a centralized database for researchers to deposit their experiments and methodologies (Consortium et al., 2017; Van Deun et al., 2017; Roux et al., 2020). This initiative aims to foster reproducibility and transparency in the field, ensuring that all reported data meet a standardized set of criteria known as the EV-METRIC. The EV-METRIC evaluates the quality and completeness of experimental details provided by researchers, which is crucial for the interpretation and reproducibility of EV studies. The EV-METRIC is a key feature of the EV-TRACK platform, designed to evaluate the transparency and completeness of experimental reporting in EVs research. It consists of nine essential parameters: (i) the analysis of three or more EV-enriched proteins; (ii) the assessment of at least one non-EV-enriched protein, (iii) the qualitative and quantitative particle analysis methods; (iv) the inclusion of both wide-field and close-up electron microscopy images; (v) the use of a density gradient for EV separation; (vi) the reporting of obtained EV density; (vii) the detailed ultracentrifugation specifics (g-forces, duration, rotor type); (viii) the antibody specifics (clone/reference number and dilution); and (ix) the lysis buffer composition.

Since its inception, EV-TRACK has grown significantly, with thousands of experiments logged and an increasing number of studies each year. For instance, in 2024, the platform recorded data from 62 experiments across 12 studies, with an average EV-METRIC score of 59 %. This is an improvement from previous years, reflecting ongoing efforts to enhance the quality of EV research. The platform also serves as a collaborative hub, bringing together researchers from diverse backgrounds, including basic and applied sciences, to advance the understanding and application of EVs. The EV-TRACK Task Force, endorsed by the ISEV, plays a crucial role in this initiative. This task force includes researchers at various career stages, contributing to data submission, curation, and the development of the platform's website and survey tools. Furthermore, EV-TRACK emphasizes the importance of proper data annotation and encourages researchers to share detailed information about their experimental protocols. This practice not only aids in replicating studies but also enhances the overall quality and reliability of the research. The platform has been instrumental in promoting best practices and has published guidelines and add-ons to support researchers in integrating comprehensive experimental information into their studies.

3.2. Other existing EV databases

Among the existing EV databases, four complementary databases are commonly used and updated regularly. VesiclePedia (http://www.microvesicles.org) and EVPedia (https://evpedia.info/evpedia2_xe/) have both been created in 2012 and provide databases on proteins, lipids and nucleic acids identified in EVs from different cell types and animals models, as well as a Top 100 EV proteins for the most frequently identified proteins in EV studies in human, rodents and prokaryotes (Choi et al., 2013; Kim et al., 2013; Kim et al., 2015; Chitti et al., 2024). Extracellular vesicles associated DNA database (EV-ADD, https://www.evdnadatabase.com) references the studies led on DNA identification in EVs in human biofluids (Tsering et al., 2022). ExoRBase (http://www.exorbase.org) is a repository of RNA-seq data analysis of long RNA

carried by EVs such as long non-coding RNA (lncRNA), messenger RNA (mRNA) and circular RNA (circRNA) identified in human biofluids (Li et al., 2018; Lai et al., 2022).

4. Conclusions

Following the MISEV recommendations is an important way to conduct high-quality EV-based studies and demonstrates a commitment to standardization and transparency for the scientific community. All the techniques described in this review and in MISEV 2023 are not mandatory, but the combination of some of them gives credibility to EVs characterization. Since the techniques are evolving, MISEV will also evolve including updated protocols and new techniques for a better consideration of EVs whatever the scientific objectives pursued. Moreover, some task forces within ISEV are dedicated to specific areas of research or samples (https://www.isev.org/taskforces), and favor the consensual ways to lead transparent and reproducible EV studies in vitro and in vivo.

CRediT authorship contribution statement

Julien Saint-Pol: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Maxime Culot:** Writing – review & editing, Writing – original draft.

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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Data availability

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

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