**Extracellular vesicles and «The minimal information for studies of extracellular vesicles»: mini-review**

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**ABSTRACT**

Extracellular vesicles (EV) are biological microparticles from lipid layers without a nucleus, ranging in size from 100 to 1000 nm. The function of EV is to mediate cellular communication, transport biologically active molecules (cytokines, inflammatory mediators, micrornas), modulate inflammation and immunity, stimulate repair processes, angiogenesis, intercellular interaction, cell survival, inflammatory and immune response, and removal of cellular waste. The International Society for the Study of Extracellular Vesicles (ISEV) constantly updates the "Minimum Information for studies of Extracellular Vesicle". The last updated edition was published in February 2024. The purpose of this mini-review is to provide a brief overview of the content and provide information about MISEV-2023 on the allocation, procurement, identification and functional study of EVs with the possibility of expanding their study and use in Kazakhstan.

**INTRODUCTION**

Extracellular vesicles (EV) are biological microparticles from lipid layers without a nucleus, ranging in size from 100 to 1000 nm, contained in various body fluids such as urine, blood, cerebrospinal fluid, saliva, breast milk, etc. [1,2]. These particles are released by any cells of the body, both under normal physiological conditions and in various pathologies of organs and systems [3,4]. However, the mechanisms of their formation and excretion have not yet been fully studied [4,5,6]. The function of EV is to mediate cellular communication, transport biologically active molecules (cytokines, inflammatory mediators, micrornas), modulate inflammation and immunity, stimulate repair processes, etc. [6,7,8,9]. Yuana Y et al. identifies the 5 main functions of EV: angiogenesis, intercellular interaction, cell survival, inflammatory and immune response, and removal of cellular waste [4]. Despite the identified mechanisms, the functions of the EV are still being investigated.

The possibility of clinical use of EV functions is growing every year and covers a wide range of practical applications: pathology of the cardiovascular system [10, 11], kidney damage, including during transplantation [12, 13], various complications of diabetes mellitus [14], for tissue and wound regeneration [15, 16], and COVID infection [17].

The ability of EV to appear in the pathology of certain organs allows them to be used as markers and criteria for the diagnosis and prediction of diseases. Currently, EV is used as specific markers in lung diseases (including lung cancer) [18,19], diabetes mellitus [20,21], any tumors [22,23], liver diseases [24,25], pathologies of the nervous system [26,27], to assess the condition of organs after their transplantation [28,29] and others. In many cases, the detection of specific EV as markers helps to identify the disease at an early stage.

The number of studies on the clinical use of EV is growing every year. The number of reports on experimental models of the use of EV for therapeutic purposes in vivo is increasing every year too. The purpose of this review is to analyze research in this area with the possibility of expanding the use of EV in Kazakhstan in the light of "Minimal information for studies of extracellular vesicles 2023" [30].

**MISEV-2023: MAIN POINTS**

The published MISEV-2023 includes the following 10 sections:

1. Anintroduction to ISEV and MISEV;
2. Nomenclature;
3. Collection and pre-processing: pre-analytical variables through to storage;
4. EV separation and concentration;
5. [EV](https://pmc.ncbi.nlm.nih.gov/articles/PMC10850029/%22%20%5Cl%20%22jev212404-sec-0300) characterization;
6. Technique-specific reporting considerations for EV characterization;
7. EV release and uptake;
8. Functional studies;
9. EV analysis in VIVO;
10. Conclusions.

The “Introduction” describes the comparisons and differences between MISEV-2023 and previous versions of MISEV-2014 and MISEV-2018. There are also general explanations on the application and application of MISEV-2023 and what it is not.

The second “Nomenclature” section provides a classification of extracellular particles and definitions of all nomenclature concepts. According to MISEV-2023, EV refers to particles that are released from cells, are limited by the lipid bilayer and cannot replicate on their own, i.e. they do not contain a functional nucleus. It is recommended to use the term "Extracellular vesicles" rather than inconsistently defined terms such as "exosomes" and "extracellular bodies", unless such marked definitions have been specifically identified and characterized.

The third section "Collection and pre-processing" consistently describes the methodology for collecting and pre-processing explosives from biological materials. There are also methods for describing the donor material and methods of preliminary collection and purification. There are separate recommendations for the collection of EV, depending on their source: bacteria, blood, urine, cerebrospinal fluid, saliva, synovial fluid, milk, Solid tissue. At the end of the section, the requirements for cell storage and recommendations for describing conditions, both in the short and long term, are described.

The fourth section, "EV separation and concentraion", specifies methods for separation/concentration, even if they cannot be performed. Otherwise, justify why each separation/concentration method was selected in terms of yield and specificity. All methods of separation/concentration are described. At the end, recommendations are given on methodological details; on the need to check EV before and after each stage; and for affinity-based EV separation methods, set the molecular specificity of reagents and EV/EV subtype‐specificity of all targeted markers.

The fifth part, the “EV characterization”, describes quantitative indicators of the sources and the EV themselves (the number of secreting cells, the volume of biological fluid, the mass of tissue; the number of extracellular vesicles, protein and/or lipid content; the presence of components associated with subtypes of EV; the degree of presence of non-vesicular components, etc.).

The next sixth section (“Technique-specific reporting considerations for EV characterization”) is devoted to an overview of existing methods and technologies for reporting identified EVs. The following types of EV identification are described: flow cytometry, genetic protein tagging, mass spectrometry proteomics, seven microscopy‐based methods, nucleic acid characterization (RNA and/or DNA), protein‐ and non‐protein labeling, raman spectroscopy, resistive pulse sensing and western blotting.

The seventh EV “Release and uptake” section describes the possibility of EV activation and release and the ways in which EV interacts with target cells. Possible ways of interaction are binding, internalization, and fusion/content delivery. It is recommended to evaluate the suitability of the labeling/reporting system in terms of effects on normal cellular processes, stability of EV cell association and lifespan in the intracellular environment, if possible, to evaluate the binding, absorption and transfer of EV and to determine the mechanisms of cellular response.

The eighth section (“EV analysis in VIVO”) provides recommendations for the functional study of EV (including negative ones), with an assessment of the factors affecting their activity.

The ninth section contains general recommendations for analysis in conducting EV studies in vivo.

In “Conclusion” (the 10th section), is noted that MISEV-2023 (which was prepared using the most modern technologies and methodologies) contains recommendations for research in the field of EV, and it can serve as a reference material for both specialists and novice researchers in this field.

Every year, the number of studies in the field of EV grows exponentially [31]. Kazakhstan researchers are also involved in the study of this area. There are general reports about the possibility of obtaining and using EV as potential treatment methods [32,33]. There are also studies on EV and extracellular RNAs in neurology [34,35], in COVID-19 [36,37] and in urogynecology [38,39]. Despite the small number of studies presented, there is nevertheless great potential in Kazakhstan for the development of research in the field of stem cells, including EV, based on the best recommendations presented in MISEV-2023.

**CONCLUSIONS**

The rapid growth in the number of extracellular vesicle studies over the past few years has led to the need for MISEV-2023, which is a generalized development of a large number of leading experts in this field. The MISEV-2023 recommendations are important for novice researchers in EV technology in terms of reducing the time that can be spent on preparatory stages and on identifying appropriate and necessary methods for visualizing and interpreting own EV research results. MISEV-2023 is a good reference for specialists to start or continue high-quality research, including in Kazakhstan.

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