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COMPARISON OF THE EFFECTIVENESS OF THE EXPERIMENTAL KIT DEVELOPED FOR EXOSOME ISOLATION COMPARED WITH A COMMERCIAL KIT IN RELEVANT MODELS

A.Ye. Sekenova¹, Ye.A. Sharapatov², M.Ye. Shakhatbayev¹, V.B. Ogay¹, N.M. Mussin³¹Stem Cell Laboratory, LLP "National Center for Biotechnology", Astana, 010000, Republic of Kazakhstan²Medical University Astana, Astana, 010000, Republic of Kazakhstan³West Kazakhstan Medical University named after Marat Ospanov, Aktobe, 030019, Kazakhstan

*Corresponding author: a.sekenova@biocenter.kz

Background: Exosomes derived from mesenchymal stem cells (MSCs) are of considerable interest for regenerative medicine; however, their application is limited by the lack of standardized and reproducible isolation methods.

Objective: To compare the efficiency of an experimental kit and a commercial kit for exosome isolation from the conditioned medium of human umbilical cord-derived MSCs.

Methods: Exosomes were isolated from conditioned medium collected from MSCs at passages 4 and 10 and from different medium volumes (1, 5, and 9 mL). Particle size and zeta potential were determined by particle tracking analysis, while total protein concentration was measured using the Bradford assay.

Results: Both kits enabled the isolation of vesicles of comparable size characteristic of exosomes (161.4–177.9 nm). The commercial kit provided a higher protein yield, whereas the experimental kit differed in the magnitude and polarity of the zeta potential. For both methods, the yield decreased at later MSC passages and increased with larger volumes of conditioned medium.

Conclusion: The experimental kit provides isolation of exosome-like extracellular vesicles comparable to that obtained with the commercial kit and may be considered a promising alternative, provided that additional assessment of preparation purity and molecular markers is performed.

Keywords: mesenchymal stem cell, isolation kit, exosome, size, zeta potential.

INTRODUCTION

Exosomes are extracellular nanovesicles that play an important role in intercellular communication and participate in a wide range of physiological and pathological processes [1]. They are formed within the endosomal compartment, secreted by most cell types, and detected in various biological fluids, including blood, urine, saliva, and cerebrospinal fluid [2–4]. By transferring proteins, lipids, and nucleic acids between cells, exosomes contribute to the regulation of immune responses, angiogenesis, tissue repair, and tumor progression [5].

Exosomes represent one of the smallest subpopulations of extracellular vesicles (EVs). Although particles with similar characteristics were described as early as the 1940s, the term “exosome” was introduced later. Trams et al. used this term in 1981 to describe vesicles released from the cell surface, whereas the modern concept of exosomes was established in 1987 through studies by Johnstone et al. [6,7]. According to the International Society for Extracellular Vesicles (ISEV), EVs are commonly classified into several major categories, including small EVs, microvesicles, and apoptotic bodies [8]. However, because currently available isolation techniques do not allow complete separation of vesicle subtypes, considerable overlap in their size and molecular markers remains [9].

Growing interest in exosomes is associated with their potential applications in diagnostics, drug delivery, and regenerative medicine. Their ability to protect and transport biologically active molecules makes them promising natural delivery systems. In particular, exosomes derived from mesenchymal stem/stromal cells (MSCs) have attracted

considerable attention due to their immunomodulatory, anti-inflammatory, and regenerative effects [10,11].

The biological activity of MSC-derived exosomes largely reflects the functional properties of their parental cells. MSCs are known for their immunoregulatory and tissue-regenerative activities, and exosomes isolated from MSC-conditioned medium may retain many of these effects. Through the transfer of proteins, lipids, messenger RNAs, and microRNAs, MSC-derived exosomes can modulate inflammatory responses, inhibit apoptosis, stimulate angiogenesis, and promote tissue repair [12,13]. Therefore, they are increasingly considered as a promising cell-free alternative to conventional MSC-based therapy, potentially reducing some of the risks associated with direct cell administration.

Exosomes are also being actively investigated as biomarkers for liquid biopsy and disease monitoring [14]. Changes in circulating extracellular vesicle profiles have been reported in cardiovascular, inflammatory, and malignant diseases [15,16]. In oncology, exosomes may participate in tumor-associated angiogenesis, metastasis, immune modulation, and the development of drug resistance, while their molecular cargo may provide valuable diagnostic and prognostic information [17].

The regenerative potential of exosome-based therapies has been demonstrated in numerous experimental studies. A meta-analysis by Liu et al., which included 43 publications and 530 animals with experimental wounds, showed that small EV therapy significantly improved wound closure, reduced scar formation, and increased vascular density compared with placebo treatment [18]. These findings support the potential application of MSC-derived EVs in wound healing and skin regeneration.

Another promising area is regenerative therapy for reproductive disorders. Experimental data suggest that MSCs and their secretome may improve impaired spermatogenesis through several mechanisms, including reduction of oxidative stress, modulation of immune responses, stimulation of testosterone production, regulation of sperm motility-related signaling pathways, and transfer of specific microRNAs involved in spermatogenesis [19,20]. Mahiddine et al. demonstrated that exosomes of amniotic origin improved spermatogenic indices and semen parameters in an experimental rat model of azoospermia [21]. These observations further support the broader therapeutic potential of exosome-based, cell-free strategies.

Reliable isolation and characterization of EVs remain critical for both experimental and translational studies. The Minimal Information for Studies of Extracellular Vesicles 2023 (MISEV2023) guidelines emphasize the importance of standardized particle characterization and transparent reporting of analytical conditions [22]. Particle tracking analysis is widely used to determine vesicle size distribution and concentration. In the present study, particle characteristics were evaluated using a ZetaView analyzer, with measurement conditions and appropriate buffer controls described in accordance with current recommendations.

Despite rapid progress in the field, there is still no universally accepted method for isolating MSC-derived exosomes. Commercial precipitation-based kits are convenient and require limited equipment, but they may vary in efficiency, purity, reproducibility, and cost. They can also result in the co-isolation of proteins, lipoproteins, and other non-vesicular components. Ultracentrifugation, although widely used as a reference approach, is labor-intensive, time-consuming, difficult to scale, and may adversely affect vesicle integrity and recovery.

These limitations highlight the need for comparative evaluation and optimization of exosome isolation methods. An effective isolation strategy should provide sufficient vesicle yield and purity, maintain their physicochemical and biological properties, demonstrate reproducibility, and remain suitable for scaling under translational and potentially GMP-compatible conditions.

Therefore, the development of standardized, reproducible, and economically feasible approaches for isolating MSC-derived exosomes is an important prerequisite for their broader application in regenerative medicine. The present study was designed to compare an experimental isolation kit with a commercially available method for obtaining extracellular vesicles from conditioned medium of human umbilical cord-derived MSCs and to evaluate key characteristics of the resulting preparations.

METHODS

Isolation of UC-MSCs

The sample of umbilical cord (UC) was obtained from a healthy pregnant woman during cesarean section at the Center for Maternal and Child Health (Astana) after approval by the ethics committee of “Marat Ospanov named WKMU” and written informed consent from all participants. Isolation of MSCs was conducted according to Todtenhaupt et al. [23].

The UC biomaterial was taken in phosphate-buffered saline (PBS) (Gibco, USA) and delivered to the laboratory, where they were washed with PBS containing 1% Antibiotic-Antimycotic (100x) (Gibco, USA) and cut into segments of 5 cm², and the blood vessels were removed. The segments were placed in T25 cm² flasks and cultured in α -MEM medium supplemented with 1% Antibiotic-Antimycotic (100x) (Gibco, USA), and 10% fetal bovine serum (Gibco, USA). The flasks with cells were incubated at 37 °C, 5% CO₂, and left undisturbed for 7 days. The first medium change was performed on day 5, and thereafter every 3–4 days. After 2 weeks, the UC explants were removed, and the attached cells were cultured for up to 21 days. The cells were then passaged using 0.05% TrypLE Express Enzyme (1x) (Gibco, USA). The cells were subsequently quantified using the trypan blue method and subcultured. The morphology of MSCs was assessed using an Axio Observer A1 inverted microscope (Carl Zeiss, Germany). For the collection of CM for the isolation of exosomes, UC-MSCs were cultured for 2 days in medium free of animal serum (StemMACS™ XF) (Miltenyi Biotec, UK).

Obtaining of exosomes from UC-MSCs using the experimental kit

Exosomes were isolated from CM of UC-MSC using Experimental kit, developed by “Marat Ospanov named WKMU”, consisting of component A and component B. The CM after UC-MSCs culturing for 2 days, were collected and centrifuged at 3000 rpm for 10 minutes to remove debris and large particles. The obtained supernatant of CM was filtered through a 0.22 μ m or 0.45 μ m filter. Reagent A, pre-warmed at 37°C for 15–20 minutes (as in a water bath), was then vortexed and mixed with the filtered CM at a ratio of 1:5 (5 parts sample: 1-part reagent). The mixture was vortexed for 5 minutes for complete mixing (*Turbidity is observed at this stage.*) The mixture was left at 4°C for approximately 12 hours (with periodic shaking). Next, the tube was vortexed for 1 minute. The mixture was centrifuged at 3000 rpm for 40 minutes. After complete removal of the supernatant, the pellet was washed by adding 3 mL of filtered PBS and centrifuging at 3000 rpm for 40 minutes. The obtained pellet was resuspended in 50–200 μ l of filtered PBS and stored at –20 °C/–80 °C until further use.

Obtaining of exosomes from UC-MSCs by commercial kit

Exosomes were isolated from the CM of UC-MSCs using the commercial kit — Total Exosome Isolation reagent (Invitrogen). The CM after UC-MSCs culturing was collected and centrifuged at 2000 $\times$ g for 30 minutes to remove cells and cell debris. The supernatant was transferred to new tubes, after which Total Exosome Isolation reagent was added at a volume equal to 0.5 of the medium volume (e.g., 3.5 mL of reagent per 7 mL of medium). The mixture was thoroughly homogenized by repeated inversion and brief vortexing, then incubated at 4 °C overnight to precipitate the exosomes. After incubation, samples were centrifuged at 10 000 $\times$ g for 1 hour at 4 °C to obtain the exosome pellet. The supernatant was carefully removed, and the resulting exosome pellet was resuspended in 300 μ l of phosphate-buffered saline (PBS). The resuspended exosomes were aliquoted and stored at –20 °C/–80 °C until further use.

Evaluation of exosomes on ZetaView analyzer

Two exosome preparations were assessed for particle size and zeta potential (surface charge): exosomes from CM of UC-MSCs that were obtained with the commercial kit (Invitrogen, USA) and those obtained with the Experimental kit. A 5-10 μl aliquot of each sample was diluted in PBS to a defined final volume prior to analysis. Particle size and surface charge were determined using a ZetaView instrument (Particle Metrix, Germany) at the Core Facility of Nazarbayev University (Astana). The optimal dilution for measurement was established beforehand by testing for a target particle density of 140-200 particles per frame. Each sample was scanned at 11 distinct positions, with two measurement cycles performed at each. Following injection of the diluted particle suspension into the instrument, data acquisition and analysis were carried out with the built-in ZetaView software (v8.02.31), using the following settings: maximum particle size of 1000, minimum particle size of 2, and minimum particle brightness of 25. The instrument was equipped with a built-in 488 nm, 40 mW laser and a CMOS camera. For every sample, the number of completed particle tracks exceeded the recommended minimum of 1000, ensuring that occasional large particles did not distort the measurements [17].

Protein determination by the Bradford method

Total protein content of the exosome preparations from both isolation kits was quantified spectrophotometrically using the Bradford assay. A bovine serum albumin (BSA) stock solution was diluted in PBS to generate calibration standards at 0.25, 0.5, 1.0, 1.5, and 2.0 mg/ml, alongside a PBS-only control. Aliquots of 20 μl from each standard, and separately 20 μl of exosomes isolated with the commercial kit, were dispensed into labeled tubes, followed by the addition of 1 ml of Bradford reagent (Sigma, USA) to each and thorough mixing by vortexing. Samples were incubated for 10 minutes at room temperature, after which absorbance was read on a spectrophotometer at 595 nm, with PBS serving as the negative control. Protein concentrations were derived from a calibration curve generated by plotting the blank-corrected mean absorbance of each BSA standard against its known concentration in $\mu\text{g/ml}$ [18, 19].

Statistical analysis

Values of two or more groups were compared using an independent two-sample t-test. Statistical analysis was performed using GraphPad Prism 9.1.0 software (GraphPad Software, USA). $P \leq 0.05$ was considered significant.

RESULTS

Kits for isolation of exosomes

For exosome isolation, 10 mL of CM collected from the cultured UC-MSCs was used. Exosomes from CM of UC-MSCs that were cultured to 4-th and 10-th passages, were isolated using commercial kit and Experimental kit. Isolated EVs/exosomes were divided on aliquots and stored at $-20^\circ\text{C}/-80^\circ\text{C}$ until further use.

Analysis of the size of obtained exosomes using ZetaView

Sizes' measurement on the ZetaView analyzer showed

that exosomes isolated from CM of UC-MSCs with the commercial kit had the size of 177.9 nm. Exosomes isolated from CM of UC-MSCs using the Experimental kit had the size of 161.4 nm (Figure 1).

Measurement of the zeta potential of exosomes by ZetaView

The results of the zeta potential (surface charge) study of exosomes from UC-MSCs by dynamic light scattering analysis on the ZetaView analyzer are presented in Figure 2. Zeta potential' measurements showed that exosomes isolated with the commercial kit had zeta potential equal to 3.5 mV, while exosomes isolated using the Experimental kit had the zeta potential equal to 4.7 mV (Figure 2).

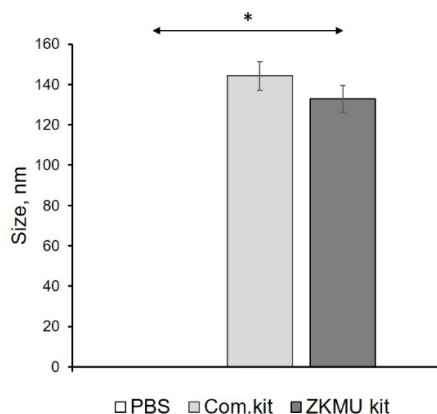


Figure 1. Measurement of the size of exosomes isolated with commercial kit and Experimental kit using ZetaView, particle size (nm), * $p < 0.05$

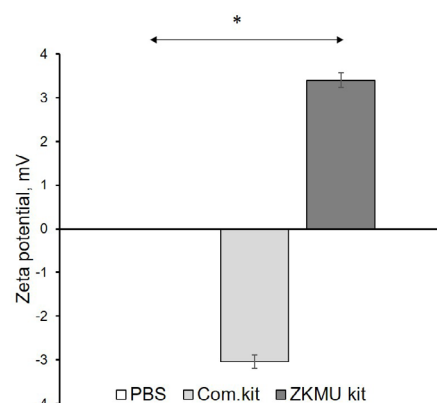


Figure 2. Measurement of the zeta potential of exosomes isolated with the commercial kit and the Experimental kit using ZetaView, (mV), * $p < 0.05$

Protein concentration in exosomes from CM of UC-MSCs

Measurement of protein concentration in exosomes isolated from CM of UC-MSCs using the Bradford method was carried out according to the manufacturer's protocol, using Bradford reagent (Sigma, USA). Figure 3 shows the results of protein concentration measurement in exosomes isolated from 10 mL of CM of UC-MSCs cultured at passage 4. The protein concentration in exosomes isolated from CM of UC-MSCs using the Experimental kit was 149.2 $\mu\text{g/ml}$.

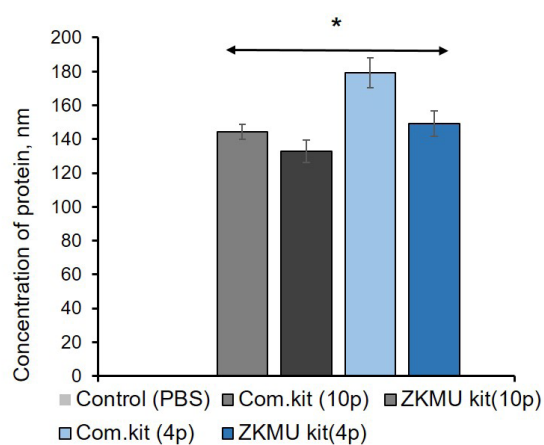


Figure 3. Measurement of protein concentration in exosomes isolated from UC-MSC conditioned medium (passage 4) with the commercial and Experimental kits, * $p < 0.05$.

Exosomes isolated from 10 mL of CM of UC-MSCs using the commercial kit had the protein concentration of 179.3 $\mu\text{g}/\text{mL}$. No protein was detected in the control (PBS).

Figure 3 shows the results of protein concentration measurement in exosomes isolated from 10 mL of UC-MSC conditioned medium cultured at passage 10. The protein concentration in exosomes isolated from CM of UC-MSCs using the Experimental kit was 132.9 $\mu\text{g}/\text{mL}$. Exosomes isolated from 10 mL of CM of UC-MSCs using the commercial kit had the protein concentration of 144.2 $\mu\text{g}/\text{mL}$. In the control (PBS), the protein concentration was equal to 0.

The results of the zeta potential (surface charge) analysis of exosomes from CM of UC-MSCs using hydrodynamic light scattering on the ZetaView analyzer were presented in Figure 6. Zeta potential measurements showed that exosomes isolated from CM of UC-MSCs cultured at passage 4 using the commercial kit had the zeta potential of -3.8 mV, whereas exosomes isolated from the CM of UC-MSCs cultured at passage 4 using the Experimental kit had the zeta potential of 4.7 mV (Fig. 6). In the negative control (PBS), the zeta potential was 0.

The results of the zeta potential (surface charge) analysis of exosomes isolated from cells cultured at passage 10 using hydrodynamic light scattering on the ZetaView analyzer are presented in Figure 6. Zeta potential measurements showed that exosomes isolated from the CM of UC-MSCs using the commercial kit had the zeta potential of -3.04 mV, whereas exosomes isolated from the CM of UC-MSCs using the Experimental kit had the zeta potential of 3.4 mV (Fig. 4). In the negative control (PBS), the zeta potential was 0.

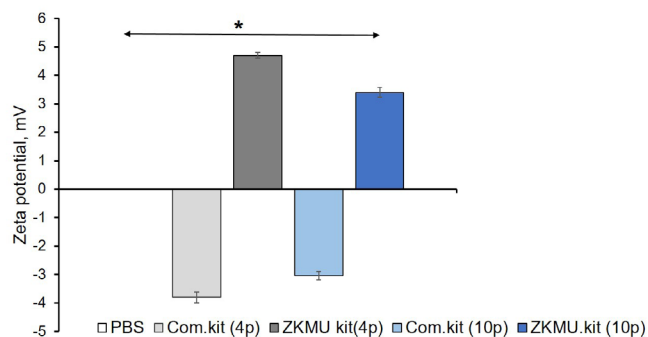


Figure 4. Zeta potential measurement of exosomes isolated by the commercial kit and the Experimental kit, using ZetaView (mV), $p < 0.05$.

Comparative analysis of exosome isolation efficiency using the commercial kit and Experimental kit with varying volumes of CM of UC-MSCs

To determine the comparative efficiency of exosome isolation using the commercial kit and the Experimental kit, the following volumes of CM collected from culturing of UC-MSCs were used: 9 mL, 5 mL, and 1 mL. Data on exosome size, zeta potential, and concentration, that were measured using the ZetaView analyzer, are presented in Table 1.

DISCUSSION

In this study, exosomes from CM of UC-MSCs were isolated using two independent approaches, using of Experimental kit and the commercial exosome kit, which were compared across several biological and physicochemical parameters, including particle size, zeta potential, and total protein content, at early (passage 4) and late (passage 10) passages.

Particle size analysis by ZetaView showed broadly similar exosome diameters between the two isolation methods, with the commercial kit yielding slightly larger particles (177.9 nm) than the Experimental kit (161.4 nm). Both values fall within the broader size range reported for small-to-medium EVs, though they exceed the classical 30-150 nm exosome range typically defined by ISEV/MISEV criteria, as NTA-based instruments such as ZetaView are known to detect larger particles and can co-detect protein aggregates or larger microvesicles alongside exosomes. Modest inter-kit differences of this kind are consistent with findings reported in the study by Afrisham et al. they similarly observed somewhat smaller particle sizes with kit-based isolation of circulating small extracellular vesicles, despite comparable vesicle

Table 1. Comparative analysis of two kits for exosome isolation from varying volumes using the commercial kit and the Experimental kit.

Volume of CM of UC-MSCs	Kit for isolation	Size of exosome (nm)	Zeta potential (mV)	Concentration
9 ml	Com.kit	170,9	-4,2	3,7E+8 particles/ml
	Experimental kit	181,0	-3,4	2.6E+8 particles/ml
5 ml	Com.kit	174,3	1,51	2,3 E+8 particles/ml
	Experimental kit	143,6	-2,1	1.7E+8 particles/ml
1 ml	Com.kit	143,9	-5,1	1,3 E+8 particles/ml
	Experimental kit	148,6	3,9	1,1E+8 particles/ml

morphology by transmission electron microscopy (TEM) [23], suggesting that small shifts in particle size between commercial isolation reagents are a recurring feature of the field rather than an artifact specific to the present comparison. The slightly smaller average diameter obtained with the Experimental kit may reflect a narrower size-selection profile or reduced co-isolation of larger vesicle/aggregate species relative to the commercial reagent.

The zeta potential measurements revealed a more notable difference between the two methods. At the passage 4, exosomes isolated with the commercial kit carried a slightly negative surface charge (-3.8 mV), consistent with the net-negative charge typically attributed to the phospholipid bilayer (phosphatidylserine, sialylated glycoproteins) of native EVs whereas exosomes isolated with the Experimental kit showed a slightly positive charge (+4.7 mV). The same polarity pattern held at passage 10, with the commercial kit yielding -3.04 mV and the Experimental kit +3.4 mV (PBS control = 0 mV at both passages). Both values, however, lie close to the neutral point, and particles with zeta potential below roughly ± 10 mV are generally considered to be at increased risk of aggregation due to insufficient electrostatic repulsion, a limitation already noted in earlier quarterly reports for both kits and reaffirmed here [24]. This suggests that, regardless of isolation method, the resulting preparations may benefit from additional stabilization strategies (e.g., optimized buffer composition, avoidance of repeated freeze-thaw cycles) to preserve colloidal stability during storage. The charge reversal observed with the Experimental kit could plausibly reflect a difference in the isolation mechanism itself for example, differential co-precipitation of medium-derived proteins or residual reagent adsorbed to the vesicle surface, rather than a genuine difference in native membrane composition and would benefit from further characterization (e.g., zeta potential across varying ionic-strength buffers, or surface protein profiling).

Total protein content, quantified by the Bradford method, was slightly higher in preparations isolated with the commercial kit than with the Experimental kit, both at passage 4 (179.3 vs. 149.2 $\mu\text{g/mL}$) and at passage 10 (144.2 vs. 132.9 $\mu\text{g/mL}$). A higher protein yield with the commercial kit could indicate either genuinely higher exosome recovery or lower isolation specificity, with greater co-precipitation of non-vesicular soluble protein from the CM. Interestingly, for MSCs-derived exosomes specifically, this kind of divergence between kits does not always appear; Habibian et al. reported broadly comparable protein content and vesicle presence across isolation methods applied to the CM of adipose-MSCs [25], which contrasts with the modest but consistent gap we observed between the commercial and Experimental kits and raises the possibility that isolation-method effects on protein yield are not uniform across source tissues or CM matrices. Because Bradford-based total protein quantification, as recommended by MISEV2023 §5.3 [22], does not itself distinguish EV-associated protein from co-isolated contaminants, the present data alone cannot determine which explanation predominates. Complementary purity metrics - for example, a particle-to-protein ratio, or tetraspanin marker analysis (CD63/CD81 Western blotting) - would help clarify whether the additional protein recovered by the commercial

kit represents genuine exosomes' cargo or co-isolated impurities [26].

A consistent passage-dependent trend was also observed: for both kits, protein concentration declined from passage 4 to passage 10 (commercial: 179.3 $\rightarrow$ 144.2 $\mu\text{g/mL}$; ZKMU: 149.2 $\rightarrow$ 132.9 $\mu\text{g/mL}$), in line with the previously reported passage-dependent decrease in exosome size and zeta potential. This is consistent with the established phenomenon of replicative senescence in MSCs, where prolonged *in vitro* expansion is associated with reduced secretory activity, including the decline in EVs output and altered vesicle composition. Together with the earlier finding that MSCs at passages 3-6 yielded exosomes with more favorable size and zeta-potential characteristics than later passages, these results support restricting exosome production to earlier-passage cultures wherever feasible, both to maximize yield and to minimize senescence-associated changes in vesicle quality.

Finally, practical considerations relating to kit performance over time and at scale are worth noting. As observed previously, Experimental kit preparations after approximately nine months of reagent use showed larger particle size and higher zeta potential than preparations obtained after only one month of use, suggesting a degree of reagent instability or batch-to-batch variability that should be monitored, particularly for studies spanning long time periods or requiring high reproducibility. Likewise, isolation from larger CM volumes (8-10 mL) consistently yielded higher exosome/protein recovery than smaller volumes, supporting the scalability of both kits for larger-batch preparations - an important consideration for future GMP-oriented work.

Overall, both the commercial and Experimental kits reliably isolated exosome populations from CM of UC-MSCs with broadly comparable particle size, but they differed in surface charge behavior and protein yield. The commercial kit produced higher protein recovery, which may reflect either greater exosome yield or lower isolation purity, while the Experimental kit yielded somewhat smaller particles with the shift toward positive surface charge at a lower protein yield. The choice between the two methods may depend on the intended downstream application: where maximal protein/vesicle yield is the priority (e.g., for functional assays requiring higher exosome quantities), the commercial kit may be preferable, whereas where cost, kit longevity, or the possibility of lower non-EVs protein contamination is more important, the Experimental kit warrants further evaluation - ideally supplemented by orthogonal purity assessments to more definitively establish comparative isolation efficiency and purity.

CONCLUSION

The Experimental and commercial kits both reliably isolate EVs with characteristic exosomes' size from CM of UC-MSCs but differ in surface charge and protein yield. Early-passage (2-5) cultures of UC-MSCs and larger volumes of CM are recommended to maximize exosome yield and stability, and the Experimental kit represents a viable alternative to commercial isolation kits pending further purity validation.

AUTHOR CONTRIBUTIONS

Conception and design: A. Sekenova, V. Ogay; (Collection and assembly of data: A. Sekenova, Ye. Sharapatov, M. Shakhatbayev; Data analysis and interpretation: A. Sekenova, Ye. Sharapatov, M. Shakhatbayev; Manuscript writing: A. Sekenova, Ye. Sharapatov, M. Shakhatbayev; Final approval of manuscript: A. Sekenova, V. Ogay.

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CONFLICT OF INTERESTS

All authors have completed the ICMJE uniform disclosure form. All authors have no conflict of interest to declare.

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**ЭКЗОСОМАЛАРДЫ АЛУ ҮШІН ӘЗІРЛЕНГЕН ЭКСПЕРИМЕНТТІК ЖИНАҒЫНЫҢ ТИІМДІЛІГІН
СӘЙКЕС МОДЕЛЬДЕРДЕГІ КОММЕРЦИЯЛЫҚ ЖИНАҚПЕН САЛЫСТЫРУ**

А.Е. Секенова^{*1}, Е.А. Шарапатов², М.Е. Шахатбаев¹, В.Б. Огай¹, Н.М. Мусин³

¹Діңгек жасушалар зертханасы, ЖШС «Ұлттық биотехнология орталығы»
Астана, Қазақстан Республикасы

²Астана медицина университеті, Астана, Қазақстан Республикасы

³Марат Оспанов атындағы Батыс Қазақстан медицина университеті, Ақтобе,
030019, Қазақстан Республикасы

*Корреспондент авторы: a.sekenova@biocenter.kz

Өзектілігі: Мезенхималық дің жасушаларынан (МДЖ) алынған экзосомалар регенеративті медицина үшін үлкен қызығушылық тудырады, алайда оларды қолдану стандартталған және қайталанымды бөліп алу әдістерінің жеткіліксіздігімен шектеледі.

Мақсаты: Адамның кіндік тінінен алынған МДЖ кондицияланған ортасынан экзосомаларды бөліп алуға арналған эксперименттік және коммерциялық жиынтықтардың тиімділігін салыстыру.

Әдістері: Экзосомалар МДЖ-ның 4 және 10-пассаждарындағы кондицияланған ортасынан, сондай-ақ әртүрлі көлемдегі ортадан (1, 5 және 9 мл) бөлініп алынды. Бөлшектердің өлшемі мен дзета-потенциалы бөлшектер траекториясын талдау әдісімен анықталды, ал жалпы ақуыз мөлшері Брэдфорд әдісімен бағаланды.

Нәтижелері: Екі жиынтық та экзосомаларға тән, өлшемдері салыстырмалы везикулаларды (161,4–177,9 нм) бөліп алуға мүмкіндік берді. Коммерциялық жиынтық ақуыздың жоғары шығымын қамтамасыз етті, ал эксперименттік жиынтық дзета-потенциалдың шамасы мен полярлығы бойынша ерекшеленді. Екі әдісте де МДЖ пассажинының ұлғаюымен шығым төмендеп, кондицияланған орта көлемінің артуымен жоғарылады.

Қорытынды: Эксперименттік жиынтық коммерциялық жиынтықпен салыстырмалы деңгейде экзосома тәрізді жасушадан тыс везикулаларды бөліп алуға мүмкіндік береді және препараттардың тазалығы мен молекулалық маркерлерін қосымша бағалау жағдайында перспективалы балама ретінде қарастырылуы мүмкін.

Түйін сөздері: мезенхималды бағаналы жасушалар, бөлу жинағы, экзосомалар, өлшемі, диаметр, дзета-потенциал.

**СРАВНЕНИЕ ЭФФЕКТИВНОСТИ ЭКСПЕРИМЕНТАЛЬНОГО НАБОРА, РАЗРАБОТАННОГО ДЛЯ
ВЫДЕЛЕНИЯ ЭКЗОСОМ, В СРАВНЕНИИ С КОММЕРЧЕСКИМ НАБОРОМ НА СООТВЕТСТВУЮЩИХ
МОДЕЛЯХ**

А.Е. Секенова^{*1}, Е. А. Шарапатов², М. Е. Шахатбаев¹, В.Б. Огай¹, Н.М. Мусин³

¹Лаборатория стволовых клеток, ТОО «Национальный центр биотехнологии», Астана, Республика Казахстан

²Медицинский университет Астана, Астана, Республика Казахстан

³Западно-Казахстанский медицинский университет имени Марата Оспанова, Ақтобе, 030019, Казахстан

*Автор для корреспонденции: a.sekenova@biocenter.kz

Актуальность: Экзосомы мезенхимальных стволовых клеток (МСК) представляют значительный интерес для регенеративной медицины, однако их применение ограничено отсутствием стандартизированных и воспроизводимых методов выделения.

Цель: Сравнить эффективность экспериментального и коммерческого наборов для выделения экзосом из кондиционированной среды МСК пуповины человека.

Методы: Экзосомы выделяли из кондиционированной среды МСК на пассажах 4 и 10, а также из различных объемов среды (1, 5 и 9 мл). Размер частиц и дзета-потенциал определяли методом анализа траекторий частиц, содержание белка - методом Брэдфорда.

Результаты: Оба набора обеспечивали выделение везикул сопоставимого размера, характерного для экзосом (161,4–177,9 нм). Коммерческий набор обеспечивал более высокий выход белка, тогда как экспериментальный набор отличался по значению и полярности дзета-потенциала. Для обоих методов выход снижался при позднем пассаже МСК и увеличивался при использовании большего объема кондиционированной среды.

Закключение: Экспериментальный набор обеспечивает сопоставимое с коммерческим набором выделение экзосомоподобных внеклеточных везикул и может рассматриваться как перспективная альтернатива при условии дополнительной оценки чистоты и молекулярных маркеров препаратов.

Ключевые слова: мезенхимальные стволовые клетки, набор для выделения, экзосомы, размер, диаметр, дзета-потенциал.