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Original Article

Characterization and Comparison of Mesenchymal Stem Cell-Derived Exosome Isolation Methods using Culture Supernatant

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Abstract

Exosomes are extracellular endosomal nanoparticles, which are formed under complex processes during the formation of multivesicular bodies. They are also achieved from conditioned media of a variety of cell types, especially mesenchymal stem cells (MSCs). Exosomes can modulate intracellular physiological actions via signaling molecules on the surface or secretion of components to the extracellular spaces. Furthermore, they are potentially used as crucial agents for cell-free therapy; however, their isolation and characterization can be challenging. In the current study, two methods of exosome isolation have been characterized and compared using a culture media of adipose-derived mesenchymal stem cells, namely ultracentrifugation and a commercial kit; moreover, the efficiency of these two methods was highlighted in this study. Two different isolation methods of exosomes from MSCs were used to compare the efficiency of exosomes. For both isolation methods, transmission electron microscopy, dynamic light scattering (DLS), and bicinchoninic acid (BCA) assay have been performed. The electron microscopy and DLS indicated the presence of exosomes. Moreover, the kit and ultracentrifugation isolates contained approximately comparable amounts of protein measured by the BCA. Overall, the two isolation methods had similar performances. Although ultracentrifugation is used as a gold standard for exosome isolation, the commercial kit has some advantages and can be applied alternatively according to its cost-effectiveness and time-saving properties.

Keywords: Commercial kit, Exosome, Isolation method, Transmission electron microscopy, Ultracentrifugation

1. Introduction

Stem cell-based therapies, especially mesenchymal stem cells (MSCs), have been shown to have great potential for the treatment of many diseases and regulation of the immune system. These cells and their derivatives affect the treatment of tumors, as well as the innate and acquired immune systems. The unique feature of these cells is their ability to suppress and modulate immune responses, as well as treat cancer (1, 2). MSCs-derived extracellular vesicles are abundant in different fluids and tissues, such as umbilical cord blood, bone marrow, adipose tissue, amniotic fluid, placenta, fetal liver, and peripheral blood. Exosome nanoparticles are the most effective and significant among them to communicate between cells (3-7). Exosomes have a diameter of 30-150 nm and are double-membrane nanoparticles, which contain lipids, proteins, micro RNAs (miRNAs), nucleic acids, and other bioactive substances. Exosomes have two categorized protein components in which general proteins, namely TSG101, Alix, tetraspanins (CD63, CD81, CD82, and CD9), are used as indices to identify

exosomes. Moreover, specific components, such as CD45 and major histocompatibility complex class II, are related to their progenitor cells (8, 9).

There is some evidence that RNA molecules, such as mRNAs, and particularly miRNAs, have several functions in the recipient cells. It was definitely claimed that exosomal miRNAs play as a biomarker for the diagnosis and treatment of different types of cancers. Indeed, several cancers may inhibit mediated exosomal miRNAs. Prostate cancer was inhibited by derived micro RNA-145 from adipose mesenchymal stem cells. Moreover, MSC-derived exosomes transport microRNA-133b can reduce glioma progression by the Wnt signaling pathway. Another study revealed that enriched MSCs-derived exosomes with microRNA-16 are able to down-regulate VEGF expression in tumor cells and lead to tumor suppression (10-12). It is approved that exosomes as small molecules can act as a vehicle and be transferred from a cell to another in order to deliver the cargo to regulate targets (5).

Exosome isolation is predominantly accomplished on the supernatant of cell cultures and body fluids by different methods. It is important to purify exosomes from a wide range of cellular debris and other components. To this end, numerous procedures and commercially available reagents have been designed to isolate these nanovesicles from biological samples or culture media with complete physical properties. In this regard, the selected method should display high efficiency and high yield of exosomes (13, 14). There are several different methods to isolate exosomes known as ultracentrifugation, filtration, immunoaffinity capturing, and size exclusion chromatography. Recently, different commercial strategies as exosome isolation kits have been available to purify exosomes more simply and non-invasive than traditional methods (13, 15).

In this study, two methods of exosome isolation have been characterized and compared using the culture media of adipose-derived mesenchymal stem cells (ADSCs), namely ultracentrifugation and the commercial kit; moreover, the efficiency of these two methods was highlighted in this study.

2. Materials and Methods

2.1. Animal

In total, two mice were purchased from the Pasteur Institute of Iran. The male BALB/c mice, 6-8 weeks old, were housed and maintained under the standard condition at least 48 h before cell isolation.

2.2. Isolation of Adipose-Derived Mesenchymal Stem Cells

MSCs used in this study were isolated from the BALB/c mice. The mouse was anesthetized with chloroform, and the abdominal adipose tissue was taken and minced out into a centrifugation tube and digested with collagenase type I (Gibco). The digested tissue was then cultured in a culture flask with culture media (DMED-F12; Bioidea) containing 15% fetal bovine serum (FBS; Gibco) and incubated under the standard condition at 37˚C with 5% CO_2 for several days. The medium was changed during these days, and cells were followed until passage three. In the third passage, the FBS had to be removed from the conditioned media for 48 h because of its exosome contents, and MSCs should stay stable without any significant morphological changes. The conditioned media was collected for the exosome isolation.

2.3. Exosome Isolation by Ultracentrifugation

For the ADSCs, 90 mL cell culture media were used for centrifugation. Cell culture media were first centrifuged at 300×g (15 min at 4˚C). The supernatants were then centrifuged at 4,000×g (30 min at 4˚C), and the supernatants were transferred to ultracentrifugation tubes and centrifuged at 150,000×g (150 min at 4˚C). Upon centrifugation, the supernatants were removed (leaving suspension 0.5 cm above the pellets), and the exosome pellets were suspended with PBS.

2.4. Exosome Isolation by Commercial Kit

The isolation of exosomes with the Exocib kit (Cibzist, Tehran, Iran) was performed according to the protocol of the supplier on the collected condition media. Firstly, cell debris and particles were removed by centrifuging at 300×g for 15 min, and the samples were then filtered through a 0.45- or 0.22-μm filter following the manufacturer's instructions

2.5. Characterization of the Isolated Exosomes

2.5.1. Bicinchoninic Acid Assay

The total protein concentration of isolated exosomes was measured by bicinchoninic acid (BCA) protein quantification kit (Thermo Fisher Scientific, Waltham, MA).

2.5.2. Electron Microscopy

The morphological evaluation of the isolated exosomes was assessed by the use of transmission electron microscopy (TEM). Briefly, the samples were embedded on an electron transparent sample support (EM grid), and 1% phosphotungstic acid was then used for negative staining.

2.5.3. Dynamic Light Scattering

For the identification of exosome nanoparticle size, nano Zetasizer (Malvern Instruments, Malvern, UK) was applied.

3. Results

3.1. Mesenchymal Stem Cells Culture

The MSCs were reconciled at FBS free condition to generate exosomes without any change in their morphology (Figure 1).

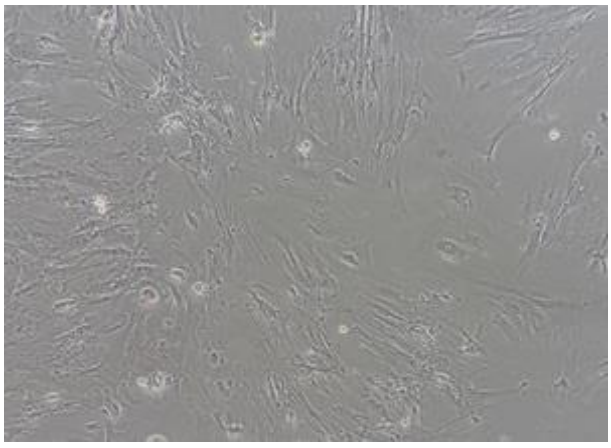

Figure 1. Cellular morphology. Adipose-derived mesenchymal stem cells culture in passage three (×40).

3.2. Characterization of the Exosome Structure by Transmission Electron Microscopy

Electron microscopy revealed the spherical shape of MSC-derived exosomes according to the two mentioned methods (Figures 2A and 2B).

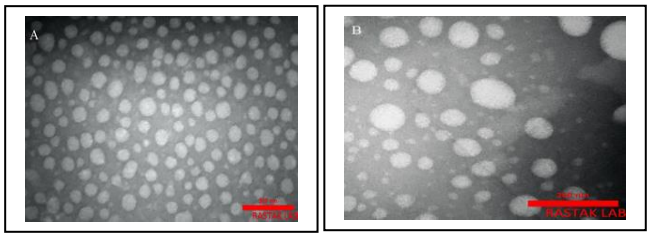

Figure 2. Transmission electron microscopic evaluation. **A:** Ultracentrifugation method (scale bar: 300 nm). **B:** Commercial kit (scale bar: 200 nm). Small vesicles within the expected range of exosomes (30-150 nm) in the sample isolated from the ADMSCs culture medium.

3.3. Comparative Evaluation of the Exosome Size Distribution and Protein Contents

The size distribution of the isolated exosome nanoparticles using two methods was evaluated by a dynamic light scattering (DLS) experiment through Zetasizer software (version 7.11) (Figures 3A and 3B). The DLS-analysis of both isolated exosomes exhibited nanoparticles of approximately similar sizes around 100 nm. Moreover, only one particle diameter was present in the two separate methods.

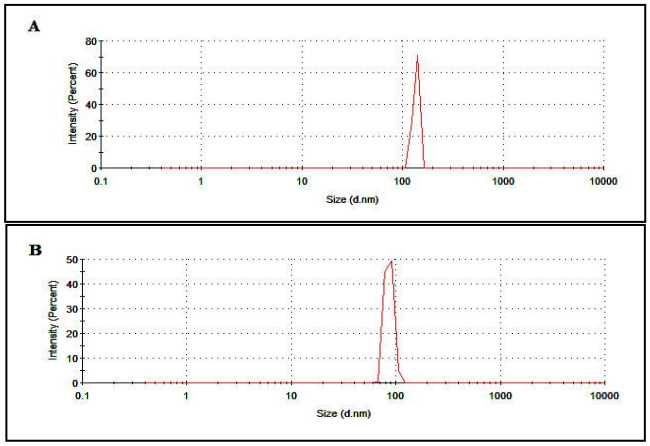

Figure 3. Particle size distribution by DLS analysis of exosomes isolated by **A:** ultracentrifugation and **B:** commercial kit from ADSCs.

According to the BCA assay, the total concentration proteins of the exosomes in two isolation methods, namely ultracentrifugation and the commercial kit, have been measured 1,123 µg/ml and 1,416 µg/ml, respectively (Figure 4).

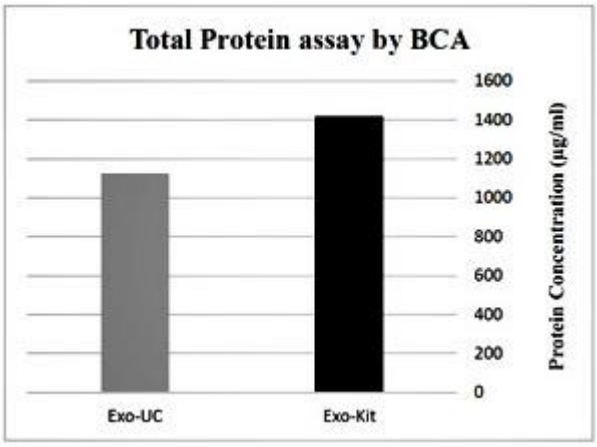

Figure 4. Total protein assay. The measured concentration of protein amount for the isolated through two methods. UC: ultracentrifugation; Exo: Exosome.

4. Discussion

Exosomes were characterized, and two isolation and purification methods (ultracentrifugation and the commercial kit) were compared in this study. Efficient isolation of exosomes as a widely appreciated cell-to-cell communication factor is significantly important. Definitely, a reliable and accessible method for the isolation of these natural nanoparticles is strongly needed to achieve mediators with low immunogenicity to multiple biological functions, such as drug delivery (16, 17). The isolation method should be performed as a time-saving and cost-effective procedure in which isolation would make easier and faster along with purified yield of exosomes (18, 19).

In the current study, three different conformational tests, including TEM, BCA, and DLS, were applied to characterize the presence of exosomes. The measured content of exosomes isolated by ultracentrifugation and the commercial kit was comparatively similar, while the size of the isolated nanoparticles in the two methods was comparable. The electron microscopy images of the two isolation methods revealed that both of them effectively resulted in spherical-shaped exosomes; however, the density of exosomes in the ultracentrifugation method was apparently more than that in the commercial kit isolation method.

In general, the existing techniques to isolate exosomes have advantages and disadvantages. It is notable that the desired method for nanoparticle isolation should not be expensive and requires no complex equipment. On the other hand, the ideal method should be fast and simple and result in a higher end yield (14, 15, 20). Moreover, exosomes' low productivity is a major limitation of exosome isolation. In other words, there should be a large scale of exosome-containing medium and also an improved method to increase the yield of exosomes (21, 22).

Although ultracentrifugation has been used until recently as a gold standard and is considered the most common technique to isolate exosomes, in these recent years, a number of commercial kits have been available for this purpose that has not markedly required specific equipment. On the downside, ultracentrifugation is a time-consuming and multi-step procedure accompanied by the risks of damaging the membrane of exosomes and limiting the accessible methods (23-25).

Taken together, in comparison between ultracentrifugation as a traditional method and the commercial kit to isolate exosomes, using a commercial kit may play as an alternative access method to exosome isolation.

Authors' Contribution

Supervisor of project, conceptualization, writing, review, and editing of the manuscript: H. S.

Conceptualization and writing the original draft of the manuscript: A. H.

Advisor of the project in the field of stem cells, as well as conceptualization and interpretation of data for the work: S. M. H.

Advisor of the project in the field of exosome and data curation: S. B.

Ethics

This investigation followed the "Guide for the Care and Use of Laboratory Animals" approved by the Experimental Animal Ethics Committee of Tarbiat

Modares University, Tehran, Iran (IR.TMU.REC.1396.733).

Conflict of Interest

The authors declare that they have no conflict of interest.

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