

# The Reason for the Size Differences of the Isolated Exosomes and the Importance of these Differences

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## Abstract

Exosomes secrete complex structures surrounded by membrane vesicles out of the cell. These complex structures contain lipids, proteins, and nucleic acids. These secreted structures can also affect other cells. Therefore, exosomes are important for the diagnosis and treatment of various diseases. Exosome sizes can vary. Although this situation is seen in exosomes isolated from different sources, it is also seen in exosomes isolated from the same source. The isolation technique, location of the cell, storage conditions, and storage time are effective. It is important for the accuracy of the data to be obtained and that all isolations are made and applied under the same conditions in the studies.

**Keywords:** Exosomes, Extracellular Vesicle, Isolation Technique, Exosome Size, Exosome Source, Storage Conditions and Time

## 1. Exosomes

Exosomes are vesicles with a diameter of 50-150 nm, covered with a lipid bilayer [1]. Containing nucleic acid and protein. Such as dendritic cells, macrophages, lymphocytes, epithelial, and tumor cells are released from different cell sources [2,3,6]. They are found in all body secretions such as urine, blood, oral secretions, and breast milk. Exosomes have intra-endosomal origins [7-11]. The main factor that distinguishes exosomes from other vesicles is that they carry mRNA and miRNA [12,13]. Because when exosomes reach the target cell and are transferred, they can change the function of the cell with the RNA cargo they contain with these features, it can be used in the diagnosis and treatment of diseases [14,15].

## 2. Size Differences and Causes of Isolated Exosomes

It has been shown that exosomes isolated from human bone marrow-derived MSCs average 145 nm [16]. While exosomes isolated from human umbilical cord-derived MSCs average 139 nm [17]. In a study, it was reported that the size of exosomes isolated from the INS-1 cell line was  $145.4 \pm 4.3$  nm [18]. In a study with exosomes isolated from the MiaPaca-2 cell line, it was reported that the average exosome sizes were 107 nm [19]. Although the isolated exosomes are obtained from the same source, it has been shown by different studies that they can be of different sizes. While Ding et al. measured the size of human umbilical cord-derived exosomes as 72.3-206.2 nm, Tan et al. measured the size of exosomes isolated from the same source as 50-150 nm [17,20]. In addition, while the

sizes of exosomes isolated from bone marrow-derived MSCs were measured as 80-200 nm [16]. It was noted in another study that the sizes of exosomes isolated from the same source were  $75.5 \pm 1.4$  [21]. There are many reasons why the exosomes isolated from the same sources may differ in size. One of these reasons is how exosomes are isolated. In a study, while exosomes were isolated from the MiPaca-2 cell line, a total of 5 isolation results with 4 different commercial kits, one of which was ultracentrifuge, were compared and the size analysis of the exosomes was performed. While the exosome size was  $120.07 \pm 8.26$  nm as a result of isolation by ultracentrifuge, the sizes of exosomes measured with 4 different kits were found to be  $182.23 \pm 13.92$ ,  $114.93 \pm 11.92$ ,  $132.7 \pm 2.65$ ,  $134.13 \pm 6.46$  nm, respectively [22]. In another similar study, exosomes from Human Wharton's jelly-derived MSCs were isolated in three different ways. One of them is ultracentrifuge, the other is the ultracentrifuge method with sucrose gradient and the third is an isolation kit. The isolated exosomes were analyzed with an average size of 202.2, 170.9, and 186.8 nm, respectively [23]. In addition, these studies are a different example of the fact that different sizes of exosomes can be obtained, as we mentioned above, even though they are the same cell line. Although exosomes are isolated from the same cell line with the same method, exosome sizes may differ. In the study of Dang et al., mouse cortical collecting duct mother cells (mpkCCD) were used and exosomes were isolated separately from apical cells and basolateral cells by ultracentrifugation method. Size analysis of exosomes showed that exosomes isolated from mpkCCD apical cells were  $147.9 \pm 55.9$

nm, and exosomes isolated from mpkCCD basolateral cells were  $100 \pm 39.2$  nm [24]. Although exosomes were isolated from the same cell line with the same method, the location of the cell also affected the exosome size. Another reason for the size difference of the isolated exosomes is the storage conditions and durations of the exosomes.

In a study, exosomes were isolated from the bronchoalveolar of male mice. To determine the effect of the storage temperature of exosomes on exosome size, isolated exosomes were stored at different temperatures. Exosomes, which were immediately isolated and analyzed for size, were divided into three groups as exosomes were kept at  $+4^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  for 4 days, and size analysis was performed. The size of the exosomes, which were immediately isolated and analyzed for size, was  $94.5 \pm 1.7$  nm on average,  $104 \pm 1.15$  nm on average after 4 days at  $+4^{\circ}\text{C}$ , and  $125 \pm 1.15$  nm for 4 days at  $-80^{\circ}\text{C}$  [25]. As a result, the differences in the sizes of the exosomes show that the source of the exosome, the method of isolation, the storage conditions and times of the isolated exosome, and even the location of the cell where the exosome is released are effective. It is likely that the passage numbers of the cell lines used as the source of the isolated exosomes may also have an effect. Considering all the differences, it is important to complete the experiment process without isolating exosomes from cells with different passage numbers at different times and storing them under different conditions.

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