EXOSOME-MIMETIC NANOVESICLE GENERATION SYSTEM

W. Jo[1*], J. Yoon[1], J. Kim[1], D. Jeong[1] and J. Park[1]
[1]POSTECH, Republic of Korea

ABSTRACT

This paper introduces a developed simple device for generating exosome-mimetic nanovesicles by using centrifuge that can apply force to extrude cells through micro pore filters. Using a simple polycarbonate structure, we generate large quantity of nanovesicles which is successfully mimicked exosomes. Also, we verify that the generated exosome mimetic nanovesicles have cellular materials of source cell and can deliver these materials to target cells.

KEYWORDS: Exosome, Nanovesicle, Biomimetic

INTRODUCTION

Exosomes released from various cells have known as important factors in cell to cell communications, however, their characteristics are not fully understood [1]. The exosomes are nanoparticles which is enclosed by phospholipid bilayer and contain biological contents of cells. When they were generated from cell, the bilayer of the exosomes are engraved with membrane proteins which activate signaling pathway. The enclosed vesicles contains the cellular materials such as mRNA and cytosolic proteins which can be delivered to other cells. From these critical abilities to deliver the biological contents to recipient cells, exosomes are considered to have high potential to be applicable in many biomedical applications, such as drug delivery system (DDS).

Despite those potential applications, researches of exosome suffer from the extremely small amount of exosomes [2]. To isolate enough exosomes from cells, significant efforts are required. Moreover, the whole sequential processes of exosome purification also require long time and complex steps. To overcome this shortage for further applications, fabricating exosome-mimetic nanovesicles in large scale was introduced. Alternative exosome-mimetic nanovesicles derived from cells were proposed by using microchannels [3]. Although this method are successful in mimicking exosomes, still there are concerns about delivery efficiency and scaling-up for applications.

In this work, we have developed a scale-up exosome-mimetic nanovesicles generation system by using a simple polycarbonate structure and common centrifuge that can apply force to extrude cells through micro pore filters.

EXPERIMENTAL

A simple device was designed to generate nanovesicles (Figure 1). The device has a pair of syringes, pistons and caps, a filter holder and a micro pore filter. The size of device is same as a 50 ml tube to use the centrifuge bucket. Also, a pair of syringes, pistons and caps are assembled across the filter holder for repeating processes. Micro pore filter is located on side of filter holder. Syringe can loaded buffer up to 5 ml. Cell-suspending buffer is loaded into one syringe and pass through the micro pore filter by centrifugal force. When cell pass through the micro pore, the cell was crushed and generated nanovesicles. The symmetric structure across the filter holder allow cell pass through the pore repeatedly.

The murine embryonic stem (ES) cell line D3 was used as source cells. ES D3 cells (1×10^8) were diluted at 1ml phosphate buffered saline buffer. They were pass through 10 μm polycarbonate filter (Whatman) 3 times and 5 μm polycarbonate filter 3 times at 1320 × g at 95 sec. Generated nanovesicles were separated by density gradient method (50 %/10 % optiprep) at 100,000 × g for 1 hr. Exsosomes from ES D3 cells were collected by conventional serial centrifugation method [2].

Dynamic laser scattering (DLS) and Transmission electron microscopy (TEM) was used to observe size and morphology. For quantitative comparison, Bradford assay was used. For qualitative comparison, Reverse transcription-polymerase chain reaction (PCR) and western blotting was performed.

Confocal image was taken to observe delivery ability of nanovesicles. Mouse embryonic fibroblast (MEF)-GFP was used as target cells. Nanovesicles dyed by DiO was treated for 1 and 6 h.

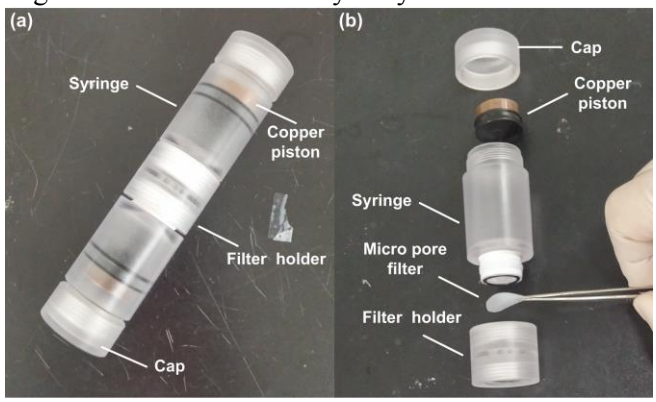

Figure 1: (a)Assembly and (b)exploded image of device.

RESULTS AND DISCUSSION

The size of generated nanovesicles ranges from 50 nm to 150 nm (Figure 2a). Most nanovesicles observed in TEM image (Figure 2b) shows spherical shape enclosed by lipid bilayer. This result proved that they were nano-sized vesicles that have similar shape with exosomes not nano-particles or debris. Also, we compared yield of nanovesicles and exosomes. The device generated about 250 times more nanovesicles than natural exosomes from 1×10^8 of ES D3 cells (Figure 2c). After extrusion, it is hard to find cell sized particles. This result suggests that large portion of cells change to nanovesicle during extrusion.

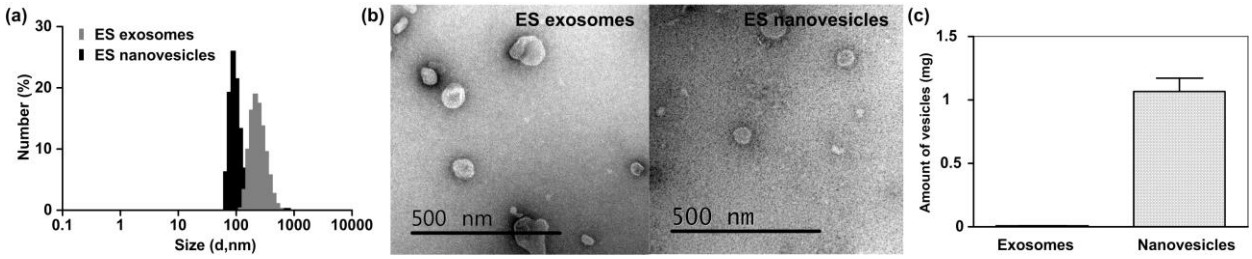

Figure 2: (a)Size and (b)TEM image of exosome and nanovesicle from ES D3 cells. Both have similar size(~100 nm) and structure. (c) amount of exosomes and nanovesicles from the same number of ES D3 cells (1×10^8).

Nanovesicles were expected to have cellular materials because they were directly made of cells. ES D3 expresses specific pluripotent marker (Oct3/4) as well as beta actin. Figure 3a shows that nanovesicles expressed same markers as ES D3 cells and exosomes. Also, nanovesicles have membrane proteins (ICAM-1) of source cells. This results suggest that nanovesicles can encapsulate the intracellular materials like exosomes.

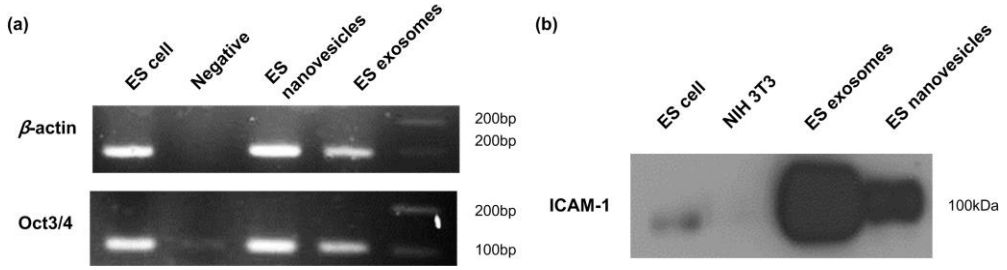

Figure 3: (a) Reverse transcription-PCR of ES D3 cells, Negative control, ES D3 exosomes, and ES D3 nanovesicles. (b) Western blot results for ICAM-1 which is membrane protein of ES D3 cells.

Exosomes were well known as intracellular communicator and can deliver cellular materials. Therefore, exosome-mimetic nanovesicles were also expected to deliver cellular materials to other cells. To verify that nanovesicles can be internalized to target cells, ES D3 nanovesicles dyed with red were treated to MEF-GFP cells. MEF-GFP cells with nanovesicles have a lot of red nanovesicles. Specific RNAs of ES D3 cells, Oct3/4 and Nanog, were expressed at NIH-3T3 treated with ES D3 nanovesicles and exosomes but not expressed at untreated NIH-3T3 (Figure 4b). Two results suggest that nanovesicles can deliver cellular contents to the target cells.

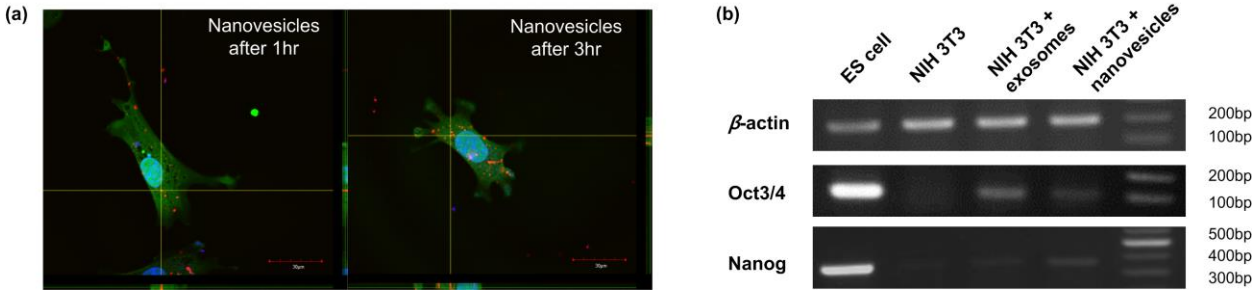

Figure 4: (a) Confocal images of MEF-GFP (green) treated with 15 μg of ES nanovesicles(red) for 1 and 6h. (b) Reverse transcription-PCR of ES D3 cells, NIH 3T3 fibroblast, and NIH 3T3 fibroblast treated with 50 μg of ES D3 exosomes and ES D3 nanovesicles.

CONCLUSION

In this work, the simple device was fabricated to generate large amount of exosome-mimetic nanovesicles from cells. The system is easily applied to research fields, using a common centrifuge. The generated nanovesicles have cellular materials of source cells and can deliver their contents to target cells like exosomes. Thus, the device can be used to easily generate the exosome-mimetic nanovesicles for delivery of cellular materials.

ACKNOWLEDGEMENTS

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CONTACT

* Wonju Jo; phone: +82-10-9369-4065; tofldkfl@postech.ac.kr