IMMOBILIZATION AND ISOLATION OF EXOSOMES USING POLYETHYLENE GLYCOL-LIPID-MODIFIED SURFACE IN A MICROCHANNEL AND EVALUATION BY ATOMIC FORCE MICROSCOPY

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ABSTRACT

The immobilization and isolation of exosomes using a polyethylene glycol (PEG)-lipid-modified surface in a microchannel were studied. The immobilized exosomes can be isolated by supplying liquid at a high flow rate using a microchannel. Since a PEG-lipid-modified surface can be fabricated simply and inexpensively compared with an antibody-modified surface, this technique has applicability for the manipulation and analysis of exosomes in clinical applications.

KEYWORDS

Atomic force microscopy in liquid, Exosome, Microfluidic isolation, Polyethylene glycol-lipid

INTRODUCTION

Exosomes, which are lipid-covered nanovesicles (30-100 nm) released from biological cells via exocytosis, have attracted considerable attention as diagnostic and prognostic biomarkers since they contain proteins and nucleic acids derived from their parent cells [1]. However, a direct handling or measurement technique for exosomes has not yet been established for medical applications. In this research, we studied the immobilization and isolation of exosomes in a microfluidic device and their observation using atomic force microscopy (AFM).

EXPERIMENTS

To immobilize exosomes, a glass surface was modified with PEG-lipid by the procedure shown in Fig. 1(a) [2]. Exosomes were immobilized physically on the surface by inserting oleyl chains of PEG-lipid into the lipid bilayer of exosomes, and the nonspecific adsorption was reduced by methoxy-PEG (Fig. 1(b)).

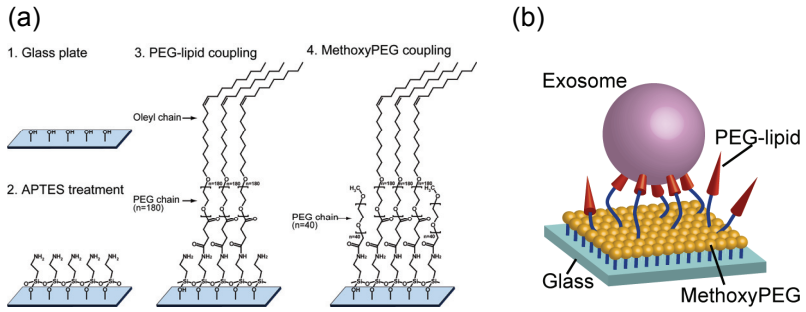

Figure 1 (a) Process scheme for surface modification using PEG-lipid and methoxy-PEG molecules. (b) Conceptual scheme of exosome immobilization. Exosomes are immobilized physically on the surface by inserting an oleyl chain of PEG-lipid into the lipid bilayer of nanoesicles. Methoxy-PEG is used for reducing nonspecific adsorption.

To prepare an exosome fraction, human embryonic kidney 293 (HEK293) cells were cultured in a serum-free medium for 24 h, and the medium was ultracentrifuged at 110,000×*g* for 70 min. Nanovesicles including exosomes (30-100 nm) and microvesicles (100-400 nm) were observed on the surface when the surface was incubated with the exosome fraction for 20 min (Fig. 2). Figure 3 shows that the area number density of nanovesicles on the surface was proportional to the concentration of exosomes at concentrations of less than 2.9×10^9 particles/ml, whereas it saturated above 2.9×10^9 particles/ml.

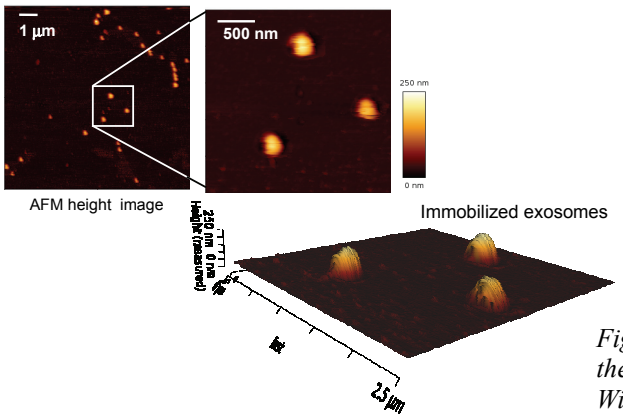

Figure 2 AFM images of exosomes immobilized on the surface. They were evaluated by AFM (Nano Wizard II) by intermittent contact mode in fluid.

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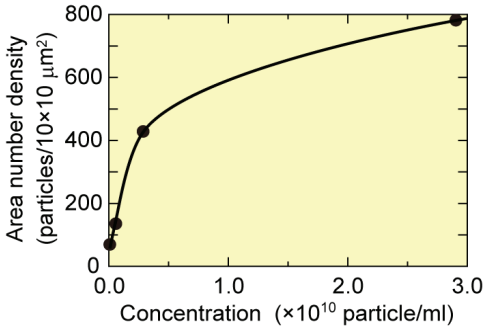


Figure 3 Relationship between concentration and area number density of nanovesicles after incubatopn with 75 μl of exosome fraction and its diluted solutions for 20 min.

For the reproducible immobilization and isolation of exosomes, microchannels (length: 28.3 mm, width: 2 mm, height: 500 μm) were fabricated on PEG-lipid-modified glass (Fig. 4). Figure 5(a) shows three-dimensional AFM images of exosomes immobilized on the surface. To study the relationship between immobilization efficiency and the washing flow rate, the area number density of exosomes was measured after washing with PBS at flow rates of 10, 100 and 1,000 μl/min. Figure 5(b) shows the effect of the washing flow rate on exosome recovery on the microfluidic device. The area number density of nanovesicles after the surface was washed at flow rates of 100 and 1,000 μl/min decreased by 73.5 and 81.3%, respectively, relative to that in the case of washing at a flow rate of 10 μl/min.

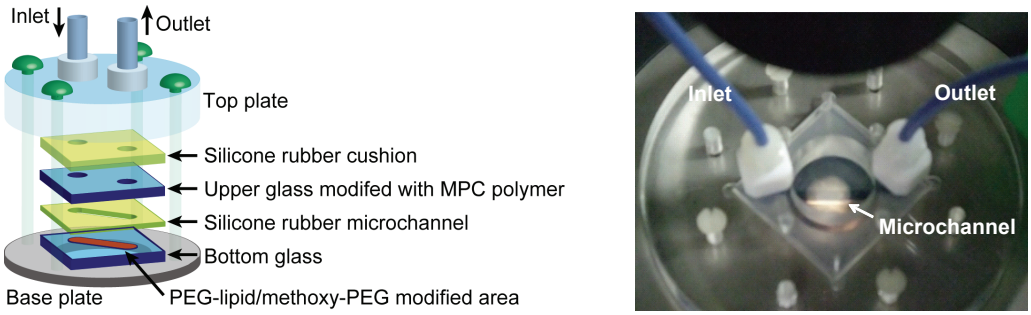
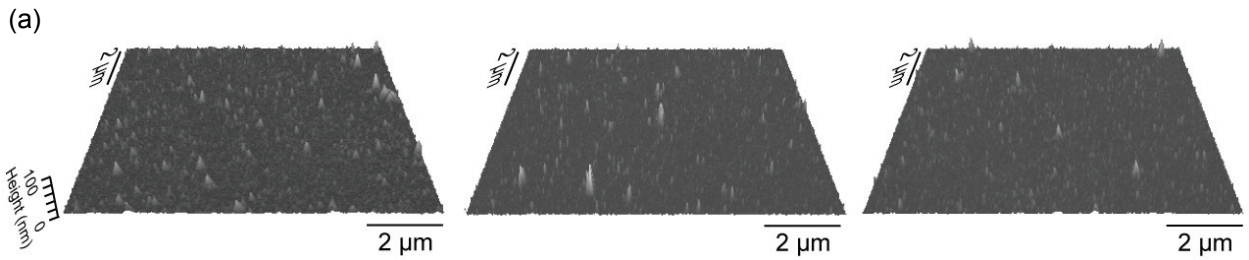


Figure 4 Schematic (left) and photograph (right) of a microfluidic device for immobilization and washing of exosome fraction.



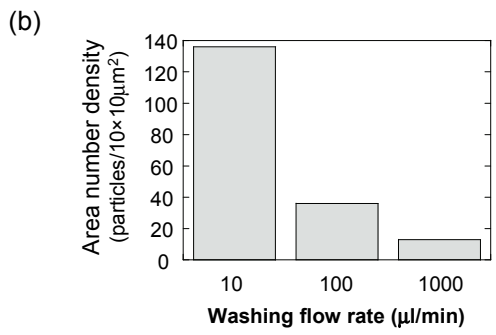


Fig. 5 (a) Three-dimensional AFM images of exosomes on the PEG-lipid-modified glass surface. After a suspension of exosomes in PBS was supplied at a flow rate of 10 μl/min for 20 min for immobilization, the surface was washed with PBS at a flow rate of 10, 100 or 1,000 μl/min. (b) The area number density of exosomes immobilized on the surface after washing.

Furthermore, to investigate the size breakdown of exosomal loss, the height distribution of vesicles on the surface was evaluated (Fig. 6(a)). Vesicles under 80 nm in height were removed when the surface was washed with PBS at a flow rate of 100 µl/min, whereas vesicles remained in the case of washing at a flow rate of 10 µl/min. Figure 6(b) shows one possible explanation for this phenomenon. The Stokes force acting on exosomes is proportional to the exosome radius *r*, while the number of PEG-lipids anchored each exosome is nearly proportional to the square of *r*.

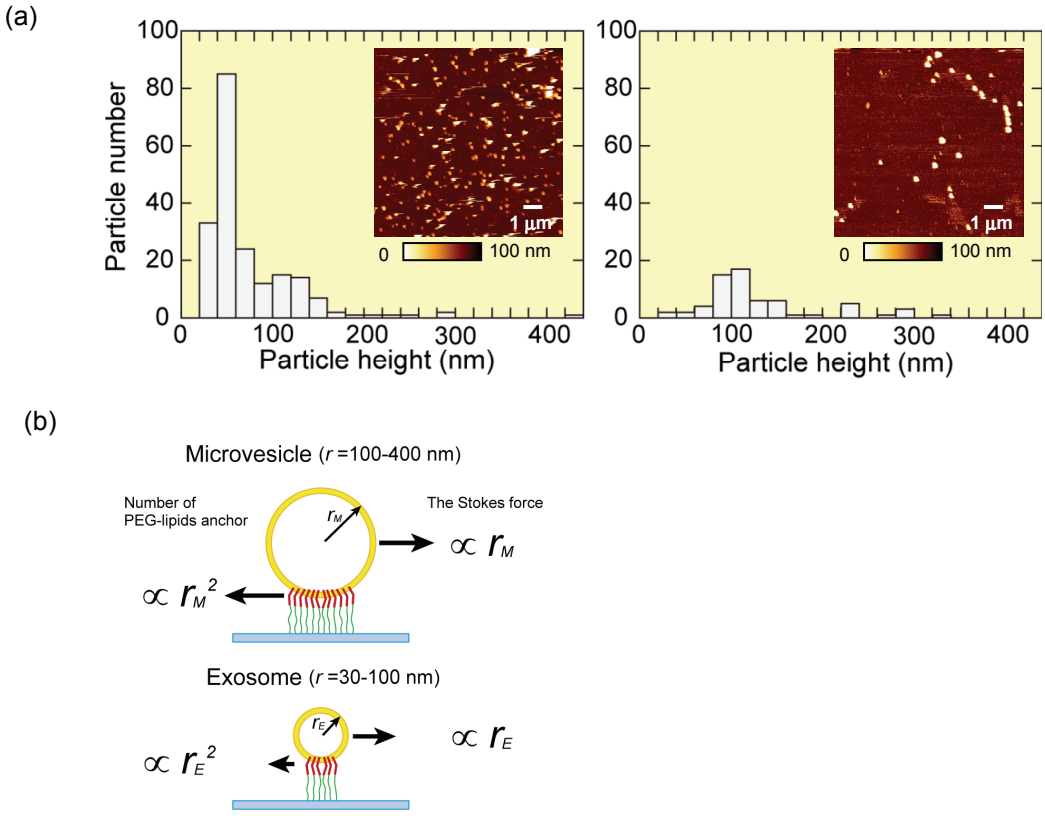


Figure 6 (a) Height distributions and AFM images of vesicles on the PEG-lipid-modified glass surface. After supplying the exosome fractions at a flow rate of 10 ml/min for 20 min for immobilization, the surface was washed with PBS at flow rates of 10 (left) and 100 ml/min (right). (b) Schematic of vesicles on the surface during washing. The Stokes force acting on exosomes is proportional to the exosome radius r, while the number of PEG-lipids anchored each exosome is nearly proportional to the square of r.

CONCLUSION

The immobilization and isolation of exosomes using a polyethylene glycol (PEG)-lipid-modified surface in a microchannel were studied. The immobilized exosomes can be isolated by supplying liquid at a high flow rate using a microchannel. Since a PEG-lipid-modified surface can be fabricated simply and inexpensively compared with an antibody-modified surface, this technique has applicability for the manipulation and analysis of exosomes in clinical applications.

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