IMMOBILIZATION AND LYSIS OF NANOLIPOSOMES IN MICROFLUIDICS BY PHOTOPATTERNING OF BIOCOMPATIBLE ANCHOR FOR MEMBRANE

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ABSTRACT

A technique of micropatterning nanoliposomes on a glass surface was developed by UV photopatterning of biocompatible anchor for membrane (BAM) and polyethylene glycol (PEG). When nanoliposomes (100 nm) were incubated and washed in a microchannel fabricated on the BAM- and PEG-patterned glass, a clear pattern of nanoliposomes on the surface was observed. Subsequently,50% of the nanoliposomes were lysed within 1 min after the initiation of washing with 1% Triton X. Thus, the immobilization and lysis of nanoliposomes on a microchannel were demonstrated successfully. This technique is applicable to the manipulation and analysis of any type of nanovesicle.

KEYWORDS: Biocompatible anchor for membrane, Microchannels, Nanoliposomes, Nanovesicles, Photopatterning

INTRODUCTION

Recently, lipid nanovesicles, ranging from several-dozen nm to hundreds of nm in diameter, have attracted considerable attention in the fields of life science and medicine. In the living body, they are known as microvesicles and exosomes, which are released in the extracellular milieu following the fusion of the vesicular body to plasma membranes, they play a role in intercellular communication by carrying genetic materials [1]. Furthermore, artificial lipid nanovesicles have been recently applied to nanotechnology-based drug delivery systems (nano-DDSs). Unfortunately, however, their characterization is rather difficult and the establishment of biodevice technologies for the direct manipulation and analysis of nanovesicles is required. In this research, we have developed a technique to immobilize nanovesicles anywhere on a glass surface using biocompatible anchor for membrane (BAM), which allows the physical anchoring of lipid bilayer vesicles [2], and demonstrated the capture and lysis of nanoliposomes on a microfluidic device.

EXPERIMENTAL

A conceptual scheme of a nanovesicle immobilizing technology is shown in Fig. 1. Nanovesicles are immobilized physically on a BAM- and PEG-modified glass surface by inserting an oleyl chain of BAM into the lipid bilayer of nanovesicles. PEG is used for reducing nonspecific adsorption. Anionic liposomes (a mixture of phosphatidylcholine (DLPC), phosphatidylserine (PS), cholesterol (Chol), and 100 μM fluorescein in phosphate-buffered saline (PBS)) were prepared by vortexing and subsequently extruding the mixture through a polycarbonate membrane (pore size: 100 nm) to tailor their sizes. To confirm the size distribution of the liposomes, the liposomes were measured by dynamic light scattering and laser Doppler electrophoresis using a Zetasizer analyzer (Malvern Instruments Ltd., U.K.). As shown in Fig. 2, uniformly sized nanoliposomes having a diameter of approximately 100 nm were measured.

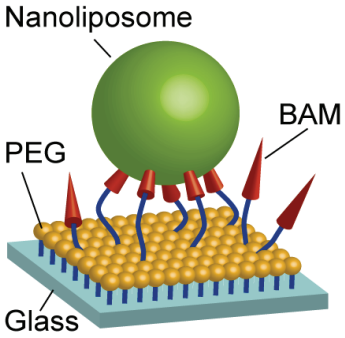

Figure 1: Conceptual scheme of a nanovesicle immobilization technology. Nanovesicles are immobilized physically on the surface by inserting an oleyl chain of BAM into the lipid bilayer of nanovesicles. PEG is used for reducing nonspecific adsorption.

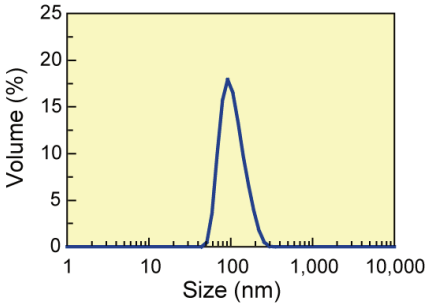

Figure 2: Size distribution of nanoliposomes measured by a Zetasizer.

RESULTS AND DISCUSSION

The BAM- and PEG-modified surface was prepared by the procedures shown in Fig. 3. The glass surface was modified with a self-assembled monolayer (SAM) of 3-aminopropyltriethoxysilane (APTES), followed by photochemical patterning by vacuum ultraviolet (VUV) irradiation and conjugation with BAM and PEG. In order to confirm the BAM and PEG micropattern, floating cells (human promyelocytic leukemia (HL60) cells) and fluorescent nanoliposomes were placed on the surface for 3 and 20 min, respectively. A phase contrast image of the cells' pattern and fluorescence and SEM images of nanoliposomes are shown in Fig. 4.

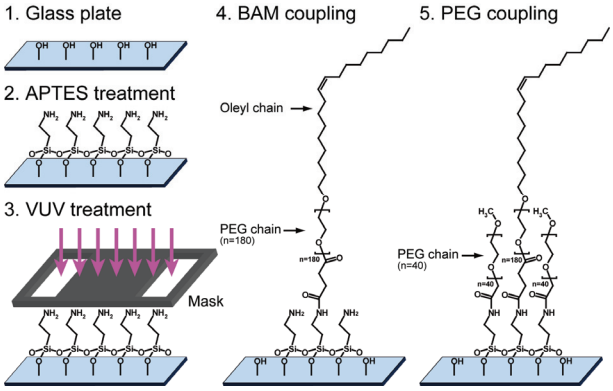

Figure 3: Process scheme for surface modification using BAM and PEG molecules.

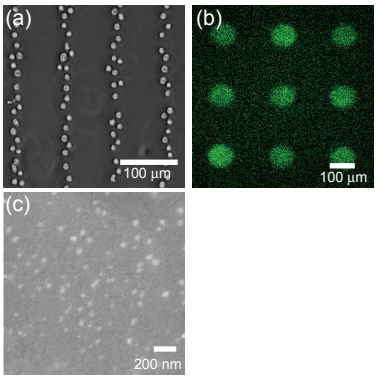

Figure 4: (a) A phase contrast image of HL60 cells, (b) Fluorescence and (c) SEM images of fluorescein-encapsulated nanoliposomes immobilized on the surface.

To examine the capture and lysis of nanoliposomes in the microfluidic device, a rectangular channel (length, 2 cm; width, 2 mm; height, 500 μm) was fabricated on the BAM- and PEG-modified glass (Fig. 5). The immobilized nanoliposomes were observed using a confocal camera. After incubation with 100 μl of suspension of nanoliposomes in PBS, the microchannel was washed with PBS at 10 μl/min for 40 min and 1,000 μl/min for 10 min to remove nonimmobilized nanoliposomes. As shown in Fig. 6, a clear pattern of nanoliposomes on the surface was observed after washing. Subsequently, the channel was washed with 1% Triton X in PBS at 1,000 μl/min to lyse nanoliposomes. The lysis of 50% of nanoliposomes was confirmed within 1 min after the initiation of washing with 1% Triton X.

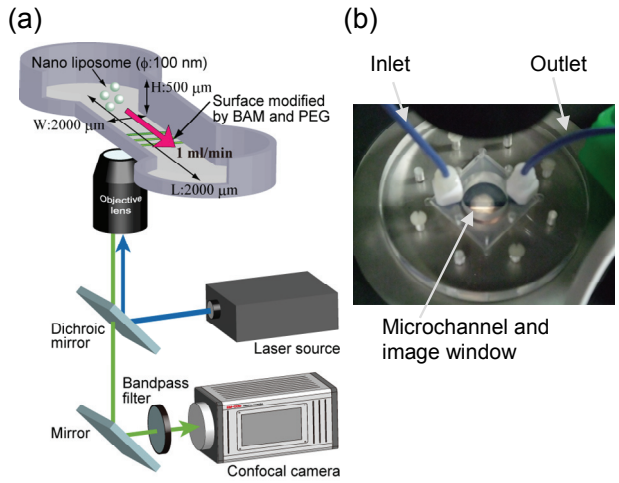

Figure 5: (a) Schematic and (b) photograph of a microfluidic device for immobilization and lysis of nanoliposomes.

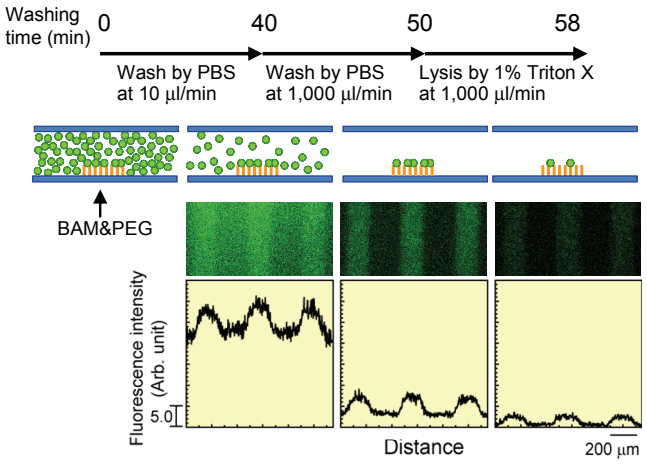

Figure 6: Schematic of a washing process (upper) and fluorescence images and intensites of nanoliposome immobilized on the microchannel (bottom).

CONCLUSION

A technique to immobilize nanovesicles anywhere on a glass surface was developed by UV photopatterning of BAM and PEG and the capture and lysis of nanoliposomes on a microfluidic device were demonstrated. This technique is applicable to the manipulation and analysis of any type of nanovesicle.

ACKNOWLEDGEMENTS

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