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Generation of nanovesicles with sliced cellular membrane fragments for exogenous material delivery

Jaewoong Yoon [a, 1], Wonju Jo [a, 1], Dayeong Jeong [b, 2], Junho Kim [b, 2], Hwapyeong Jeong [a, 1], Jaesung Park [a, b, *]

[a] Department of Mechanical Engineering, POSTECH, 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 790-784, Republic of Korea
[b] School of Interdisciplinary Bioscience and Bioengineering, POSTECH, 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 790-784, Republic of Korea

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ABSTRACT

We propose a microfluidic system that generates nanovesicles (NVs) by slicing living cell membrane with microfabricated 500 nm-thick silicon nitride (Si_xN_y) blades. Living cells were sliced by the blades while flowing through microchannels lined with the blades. Plasma membrane fragments sliced from the cells self-assembled into spherical NVs of ~100–300 nm in diameter. During self-assembly, the plasma membrane fragments enveloped exogenous materials (here, polystyrene latex beads) from the buffer solution. About 30% of beads were encapsulated in NVs, and the generated NVs delivered the encapsulated beads across the plasma membrane of recipient cells, but bare beads could not penetrate the plasma membrane of recipient cells. This result implicates that the NVs generated using the method in this study can encapsulate and deliver exogenous materials to recipient cells, whereas exosomes secreted by cells can deliver only endogenous cellular materials.

1. Introduction

Exogenous material delivery such as drug and gene delivery has wide applications in therapeutics. For these purpose, transmembrane delivery is critical with minimal toxic effect. Recently, exosomes (~30–200 nm vesicles composed of a lipid bilayer) secreted by cells into the extracellular environment, are used as nanocarriers for drugs and nucleic acids due to their low toxicity and nano-scale size [1–8]. Due to these unique characteristics, exosomes have possible therapeutic applications [9–11]. However, utilizing exosomes for research is difficult because they are secreted in extremely small numbers (~0.1 μg from million cells for 24 h) and isolation of exosomes requires a lengthy process that give only a low yield [3,4,12].

Liposomes are synthetic vesicles composed of a lipid bilayer being also used as nanocarriers. Most liposome production methods use organic solvent to form and deposit planar lipid bilayers due to insolubility of lipids in aqueous solution [13]. The planar lipid bilayers are mechanically agitated using extrusion or ultrasound, and broken into small free-standing pieces, which then tend to self-assemble into spherical liposomes to reduce their free energy [14–18]. If the planar lipid bilayers self-assemble in some materials dissolved aqueous solution, the lipid bilayers encapsulate the materials [19–22]. Recently, the liposomes encapsulating materials were demonstrated using microfluidic encapsulation systems [23,24]. Although liposomes have similar morphology to those of cell-secreted exosomes and can encapsulate materials, liposomes have critical drawbacks. The liposome generation procedure requires organic solvents and chemical additives which may not be biocompatible [25–28]. Furthermore, the generated liposomes lack functional membrane proteins which have important functions in initiating signal pathways in recipient cells. Despite these limitations, the liposome generation procedure provides an insight into lipid bilayer self-assembly.

Cellular plasma membranes are also composed of lipid bilayers which have the ability to self-assemble into vesicles. We extruded living cells through micro-sized channels to generate NVs [7,29–31]. The NVs enclose cellular contents from origin cells, such as membrane proteins, cytosolic proteins and RNAs, and can also deliver endogenous RNA into cells [29]. To investigate the effect of NV treatment of cells, we developed a polycarbonate device that

* Corresponding author. Department of Mechanical Engineering, POSTECH, 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 790-784, Republic of Korea. Tel.: +82 54 279 5418, +82 54 279 2188; fax: +82 54 279 5899.
E-mail address: jpark@postech.ac.kr (J. Park).
[1] Tel.: +82 54 279 5418; fax: +82 54 279 5899.
[2] Tel.: +82 54 279 2188; fax: +82 54 279 5899.

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makes large amount of NVs; ES cellular materials in NVs were delivered into skin fibroblasts, and the treated skin fibroblasts proliferated faster than did non-treated cells [8]. Although the NVs generated by extrusion method were effective in deliver endogenous cellular materials, NVs that can be loaded with exogenous materials such as genetic materials are necessary for extensive applications, and the loading efficiency of exogenous materials are closely related to the process of NV generation. In the previous method, the plasma membranes are attached to the wall of microchannels/micropores, elongated, and suddenly broken down without enough opening to the surround buffer, and the NVs generated using this method is not effective to encapsulate exogenous materials.

In this article, we designed a cell-slicing system to generate NVs from living cells; this method exploits the self-assembly of plasma membrane fragments. An array of 500 nm-thick low-stress silicon nitride (Si_xN_y) blades along microchannels was fabricated to slice the plasma membranes of cells flowing through the device. When sliced from a living cell, a free-standing piece of plasma membrane with intact lipid bilayer structure forms a vesicle that contains cellular contents in the origin cells and exogenous materials. This approach to generating exosome-like NVs will provide a promising strategy for delivery of exogenous drugs and genes.

2. Materials & methods

2.1. Device design and fabrication

The cell-slicing device (Fig. 1) consists of two parts: a silicon substrate with Si_xN_y blades and a polydimethylsiloxane (PDMS) block with lithographed microfluidic channel array (Fig. 1a). The Si_xN_y blades on the silicon substrate are 1 μm long and 500 nm thick and are spaced 23 μm apart (Fig. 1b). The microfluidic channels in the PDMS part are 2.5 mm long, 50 μm high and have four different widths (10, 50, 100, 200 μm) with a fixed total cross section (8×10^{-8} m^2); these dimensions were decided because the embryonic stem (ES) cells (~5 μm diameter) clogged the microchannels when the channel height is less than 10 μm. In contrast, the NV generation yield decreased with the elevated height. After aligning and assembling the silicon substrate and the PDMS block, the Si_xN_y blades are perpendicularly aligned in the middle of the PDMS channel array to slice the plasma membranes of incoming cells.

The device was fabricated using conventional bulk silicon fabrication processes and soft lithography. The cantilever-blades were fabricated from Si_xN_y. First, a 100 nm thick silicon oxide (SiO_2) layer was thermally grown and a 500 nm thick Si_xN_y layer was deposited on a (100) silicon wafer by low pressure chemical vapor deposition. For low-stress nitride deposition, SiH_2Cl_2 gas (100 ccm) was used more than NH_3 gas (40 ccm), so the Si/N ratio was estimated as 1.1 [32]. On the wafer, the pattern for Si_xN_y cantilevers was formed by a lithography process using AZ-4330 photoresist. The patterned Si_xN_y layer on the wafer was over-etched using inductively-coupled plasma reaction-ion-etching to develop the pattern on the layer. The SiO_2 layer exposed through the dry-etched Si_xN_y layer was removed by buffered oxide etching to expose single-crystal silicon substrate. The silicon wafer was anisotropically wet-etched using 40% KOH at 70 °C for 1 h.

The pattern for microfluidic channels in the PDMS part was fabricated using soft lithography. SU-8 2100 was spun with 50 μm thickness and lithographically patterned to form the microfluidic channel array, an inlet chamber and an outlet chamber (Fig. 1a). The wafer with the SU-8 pattern was used as a master for the PDMS mold. A mixture of nine parts PDMS silicon elastomer base and one part curing agent was cured at 65 °C for 2 h after bubbles in the mixture were removed. The cured PDMS part was aligned and bonded to the silicon part by oxygen plasma treatment, then baked on a hotplate at 150 °C for 2 h to achieve strong bonding. After connecting tubes to the inlet and outlet port of the device, the assembled apparatus was washed by passing 75% ethanol and deionized water sequentially through the tubes.

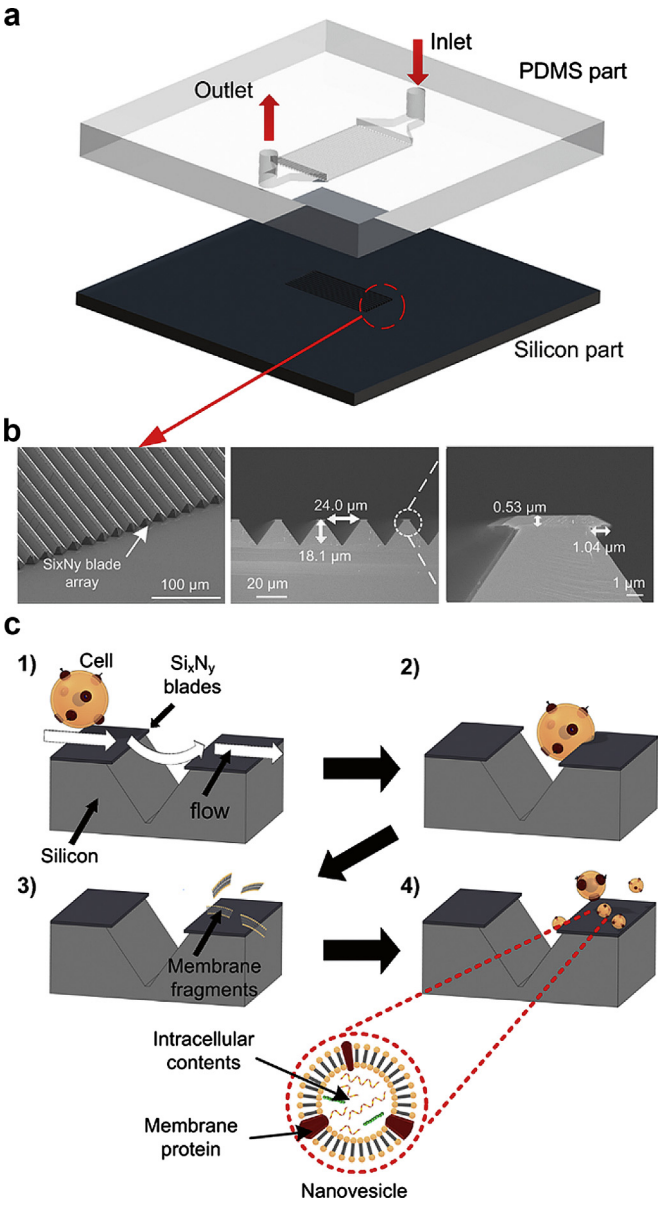

Fig. 1. (a) Schematic structure of cell-slicing device. It is composed of the silicon part with 500 nm-thick Si_xN_y cantilever-blades and the PDMS part with microchannels and an inlet port and an outlet port. (b) The silicon part has parallel Si_xN_y cantilever-blades which have 1 μm-long protrusion and 500 nm-thickness. (c) A schematic of NVs generation by slicing cells. As cells flowed through microchannels, 1) the cells moved down and up due to the flow at the groove and 2) the cells were sliced by Si_xN_y blades 3) into plasma membrane fragments. 4) The plasma membrane fragments self-assembled into small NVs, which were collected along with unsliced cells and cell debris at the outlet.

2.2. Cell slicing in microchannel

Living cells were entrained in the flowing medium and plasma membrane of the cells were sliced when they touched the edge of a cantilever-blade (Fig. 1c). The cross section of a microchannel in