ULTRATHIN, HYPERELASTIC PDMS NANO MEMBRANE : FABRICATION AND CHARACTERIZATION

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ABSTRACT
In this study, we fabricated ultrathin and hyperelastic polydimethylsiloxane(PDMS) nano membranes with various thicknesses and sizes by using PDMS diluted with hexane, sacrificial layer and PDMS block. We used these membranes to study the characteristics and possibilities of applying as a cell device such as lung on a chip. [1] We discovered that this free standing PDMS nano membrane is so elastic and thin that it is sufficiently permeable to air and methanol. We also investigated the usage as a cell device by culturing lung cells on the membrane.[2]

KEYWORDS: polydimethylsiloxane nano membrane, ultrathin nano membrane, hyperelastic

INTRODUCTION
In nature, ultrathin, hyperelastic and selectively-permeable membrane, such as plasma membrane, is widely common and employed. Many researchers have been intrigued in bio mimicking thin membrane which demonstrates possibilities of application for filters of biomolecules, highly sensitive biosensor, and physiological study of cells and organs. Although such thin membranes have been intensively studied, these studies had limitations such as small size and low elasticity. [3,4]

Here, we fabricated a large scale (more than 2 cm in diameter), ultrathin (thickness < 70nm ) and free-standing PDMS nano membrane. The membrane is very soft and has extremely high elasticity so that it can sustain a water droplet up to 760μl. This hyperelasticity enabled us to be aware of very slight movements such as ant’s walking on the membrane. Using the air permeability characteristics, we cultured a lung cell line A549 on the membrane for up to 3 days. The cells were clearly well-cultured on the membrane.

EXPERIMENTAL
To generate PDMS nano membranes of various thicknesses, we varied the ratio of the hexane to control the thickness. Fabrication process is shown schematically in Figure 1. At first, PDMS diluted with hexane was prepared for spin coating. AZ1512 as a sacrificial layer was spin coated onto the wafer to ensure easy detachment of the membrane from the wafer surface. Secondly, the PDMS-hexane (wt%) solution was spin-coated onto sacrificial layer at a high speed rpm. After curing the solution on a hotplate for at least 2 hours, PDMS block, which is for making the membrane free-standable and for handling it, was attached on the membrane by sticky PDMS. After bonding the PDMS block, PDMS nano membrane was detached in the methanol. In methanol, membrane is shaken even by small movements, so it is hard to treat the membrane as it gets thinner. Fig 1 iv) shows high degree of transparency and the large size of the PDMS nano membrane.

We measured various thicknesses of the PDMS nano membranes made by solution of different dilution ratios with atomic force microscopy (AFM) and scanning electron microscope (SEM).(Fig 2)
RESULTS AND DISCUSSION

Figure 2.a is an AFM image of the membrane fabricated by a dilution ratio of 1:120(PDMS:Hexane), indicating that thickness at two different points is both 62nm. Membranes made by 4 types of dilution ratio were measured by AFM. One by 1:40(PDMS:hexane) is 373nm, 1:50 is 207.5nm, 1:90 is 114nm, and 1:120 is 77nm. The optimized ratio for capability of detachment and ultrathin thickness of membrane was 1:120. Membranes made by ratio higher than 1:120 could also form the membrane, but it was easily torn during the detachment process. Fig 2.c is a SEM image illustrating the side view of the membrane on the Anodisc, which is made by a dilution ratio of 1:30 and has thickness of 534.3nm (Fig 2.c.)

Figure 2: Character of the PDMS nano membrane (a) AFM image of PDMS membrane (b) A graph of the thickness depend on mixing ratio (c) The side view of the PDMS membrane on the Anodisc by SEM

Figure 3.a indicates that membrane is permeable to methanol drop. While water drop kept its own shape, methanol spread on the membrane surface. Transmission electron microscope (TEM) image of Cu grid on the membrane (Thickness: 100nm) shows the nano porous structure on the membrane surface, which may have been formed during the spin-coating process. (Fig 3.c) We assume that this porous structure enables air or methanol to permeate through the membrane.

100nm thickness membrane was used for water droplet test (Figure 3.b). Each of the water droplets showed the hyperelastic characteristics, and the shape of the membrane is similar to that of water droplet, depicting effects by the surface tension.

Figure 3: (a) Permeable test using the water droplet and methanol droplet measured CCD camera (b) Water droplet test on the membrane (c) TEM image of the membrane on the Cu grid (d) Ant (Lasius Niger, weight: 1.4mg, length: ≈ 2.5 mm) is walking on the membrane

Figure 3.d shows that the membrane, with its hyperelasticity, responses even to tiny movements such as ant’s walking. We placed an ant (Lasius Niger, weight: 1.4mg, length: ≈ 2.5 mm) on the membrane, and the membrane shaked
longitudinally (in a up and down motion) when the ant walked on the membrane. Ant could also walk beneath the membrane without falling down.

To identify air permeability, we cultured lung cell line A549 on the membrane (thickness: 100nm). Control device was made of bulk PDMS (thickness: 5mm). Figure 4 shows the side view of the cell device (left) and image of the cell on the membrane by microscopy (right). Membrane is attached on the PDMS block which has 7.5mm hole. Cell devices were sterilized using EO gas prior to cell seeding. The Live/Dead® Viability assay for mammalian cells assay (Invitrogen CA) was used for analyzing the viability of the lung epithelial cells cultured on the PDMS nano membrane.

After culturing for 3 days, live and dead assay was used to analyze the cell viability. The viability of A549 on the 100nm membrane was 68%. For the 5mm bulk PDMS, it was 20%. Compared to bulk PDMS, 100nm PDMS membrane has 3 times higher viability, indicating that the culture media was supplied through the permeable ultrathin PDMS membrane.

Figure 4: A549 3day-culture on the PDMS nano membrane

Further study, this membrane may be applied in culture of human stem cells. Currently human stem cells are co-cultured with murine embryo fibroblasts, so it is inevitable that human stem cells are not affected by murine cells. If we use the nano membrane, which is porous and permeable to air, we can prevent the direct contact between human stem cells and murine cells, and thus membrane could keep the unique characteristics of human cells.

CONCLUSION
In summary, we proposed this optimized method of fabricating and characterizing large area thin PDMS membrane. We investigated the characteristics of ultrathin and highly elastic properties of the membrane by SEM, TEM, and AFM. The permeability of air and liquid expands possibilities to research and development in cell culture. Since PDMS nano membrane is biocompatible and hyperelastic, it will be useful for improvements in various bio-MEMS(micro electro mechanical system) applications including ultra-sensitive chemical sensors to measure pressure, and it will enable further studies of cell and organ physiology.

ACKNOWLEDGEMENTS
This study was supported by a grant of the NRL (National Research Lab) program, the Korea Science and Engineering Foundation (KOSEF), Republic of Korea (no R0A-20110020455)

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