Synopsis of Original Research Paper

Fabrication of microfluidic devices for the production of monodisperse liposomes and the fusion of cells

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This report describes a method for preparation of monodisperse giant liposomes from patterned lipid films using electroformation. The lipid films were patterned successfully on an indium tin oxide (ITO) electrode by a Parylene lift-off process. We found that the coefficient of variation (CV) of diameters of liposomes improved from 34 % to 7.6 % by using the patterned lipid films for liposome formation.

Giant liposomes (10 - 100 μm in diameter) are cell-sized spherical lipid membranes and can enclose bio-functional materials (e.g DNA, proteins); they can be used as an artificial cell or biological reactor. In our previous work, we have succeeded in enclosing different types of nano/micro materials in giant liposomes efficiently using microfluidic channels with electroformation method. In order to control the volume of materials enclosed in the liposomes, it is important to prepare uniform-sized liposomes. The size is also an important factor determining the success for cell-liposome fusions. Micro-contact printing of lipids using PDMS stamp to achieve size control of liposomes was reported. However, in general, PDMS tends to swell when in contact with organic solvents. Here, we have used a Parylene sheet instead of PDMS for preparing lipid patterns in order to avoid such a swelling problem. Also, the Parylene sheet has advantages that it can be patterned by standard photolithography and peeled off from the substrate easily.

Two indium tin oxide (ITO) glasses were used as electrodes and separated by a silicone spacer chamber filled with degassed water. The lipid films were patterned on the bottom ITO glass using a Parylene sheet. When AC signal (10 Hz, 0.5-2.5 Vpp) was applied to the top and bottom ITO electrodes, the giant liposomes were formed from the patterned lipid films. Fluorescent images of the lipids with squares of 20 μm showed clear edges of the patterned lipids on the ITO glass. Patterned lipids of 50 x 50 μm^2 square swelled and became round after applying electric field.

The size of the liposomes became lager as the size of patterned lipid films increased. When the liposomes were produced without the patterned lipid films, the size of the liposomes were widely distributed (CV = 34 %). In contrast, our preliminary experiment shows that the CV became narrow to 7.6 % when the patterned lipid films were used. These results imply that this method — electroformation of giant liposomes from lipid patterns — is useful to produce uniform-sized giant liposomes.

Finally we fabricate a device that combines microfluidic and electrical manipulation of cells or artificial vesicles in order to perform well controlled electrofusion. The actual device allows immobilizing cells or vesicles by suction onto an array of microholes and aligning different vesicles over the holes by dielectrophoresis.