PREPARATION OF CELL-SIZED LIPOSOMES OF CONTROLLED SIZE CONTAINING LONG GENOMIC DNA

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Abstract

This article describes a method to prepare cell-sized liposomes of controlled size and containing long genomic DNA. Liposomes were obtained by the electroswelling of micro-printed lipid films of various motif sizes while duplex giant DNA (T4 DNA) was inserted during the swelling procedure. We found that the motif size of the micro-patterned lipid film controlled the final size of liposomes and that reversibly compacted DNA molecules could be inserted in the liposomes with a yield of about 10%.

Keywords: Artificial cell, micro-contact printing, liposome, DNA

1. Introduction

In nature, up to 2 m of genomic DNA materials is packaged to fit within narrow micrometric spaces, such as the nucleus of eukaryotic cells or the capsid of viruses. The preparation of artificial cell-sized liposomes with a controlled size and containing genetic materials is thus of great interest to investigate and understand the hierarchical self-organization and gene regulation in living complex systems. However, the double challenge of i) preparation of monodisperse liposomes and ii) insertion of long genomic DNA molecules inside liposomes has never been achieved up to now. Here, we describe a novel method that combines micro-contact printing technology, electroswelling formation of liposomes and DNA compaction methodology.

2. Experimental

Figure 1 shows a schematic representation of our method to prepare cell-sized liposomes of controlled size and containing long genomic DNA. First, a L-α-phosphatidylcholine (EPC) lipid solution (10 mg/mL in a 10:1 chloroform/methanol mixture) was spread on a temporary substrate (e.g., ITO) and dried under vacuum for one hour to obtain a well-organized dry and thin lipid film. Then, a PDMS stamp was used to transfer the lipid film with a desired pattern onto ITO-coated glass slides. Next, liposomes were obtained by the electroswelling (2 V, 10 Hz, 10 hours) of the micro-patterned lipid film in a 0.1 M sucrose solution. We used PDMS stamps with two motif sizes (dots of 15 and 30 μm in diameter, respectively).

For DNA insertion experiments, molecules of genomic duplex T4 DNA (166,000 base pairs) were first compacted by spermine, a tetravalent cationic polyamine, (figure 3) and added to the solution for liposome swelling. This was obtained by the successive addition of 10 mM Tris-HCl buffer (pH = 7.4), 10 μM spermine, 0.1 μM fluorescent

dye DAPI, 0.1 µM DNA in the 0.1 M sucrose swelling solution. For the observation and after the swelling, the solution containing the liposomes was extracted from the cell for electroswelling and mixed (1:2 v/v) with a 0.1 M glucose solution adjusted in osmolarity (110 mOsmol/L). After 2 hours, almost all liposomes had fallen down to the bottom of the observation cell by gravity and were observed using an Axiovert 200 inverted microscope (Zeiss) equipped with a 100× oil-immersion objective lens.

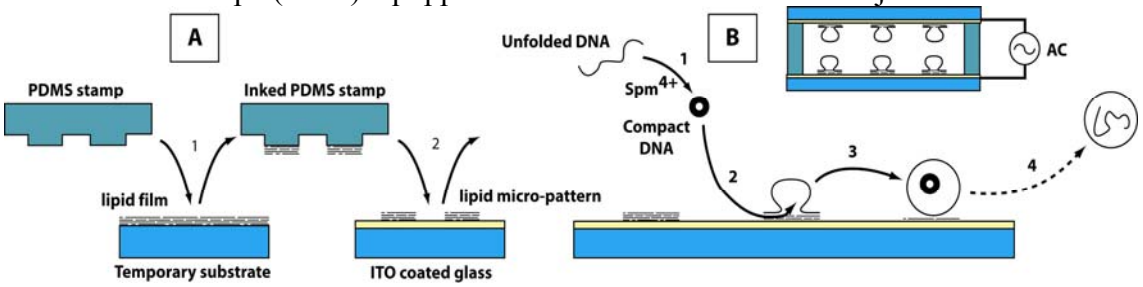

Figure 1. Schematic procedure of the preparation of liposomes of controlled size and containing long genomic DNA. A. Transfer of a lipid micro-pattern on an ITO-coated glass: 1) the PDMS stamp is inked by transferring a dry lipid film from a temporary substrate to the motifs of the stamp; 2) the lipid is stamped onto an ITO-coated glass slide. B. Electroswelling of liposomes from the lipid micro-pattern and simultaneous DNA insertion: 1) individual molecules of genomic T4 DNA (166,000 base pairs) are compacted in the presence of spermine (Spm^{4+}), a tetravalent cationic polyamine; 2) Toroidal condensates of such compacted DNA (approx. 90 nm in outer diameter) enter the liposomes while they are swelling under AC electric field. 3) When the electroswelling has been completed, liposomes detach from the surface. 4) Under appropriate conditions, compacted DNA may be unfolded due to the reversible nature of DNA compaction phase transition.

3. Results and discussion

Our micro-printing technique combines i) the simplicity of microcontact printing [1] and ii) a control deposition of the lipid film (thickness, organization) [2]. We found that the size of final liposomes is directly controlled by the motif size of the PDMS stamp, in agreement with [1-2]. Figure 2 shows two arrays of liposomes obtained after the swelling for 2 hours of micro-patterned lipid films with a motif size of 30 (left) and 15 µm (right), respectively. There is a clear increase in the mean diameter of obtained liposomes with an increase in the lipid motif size.

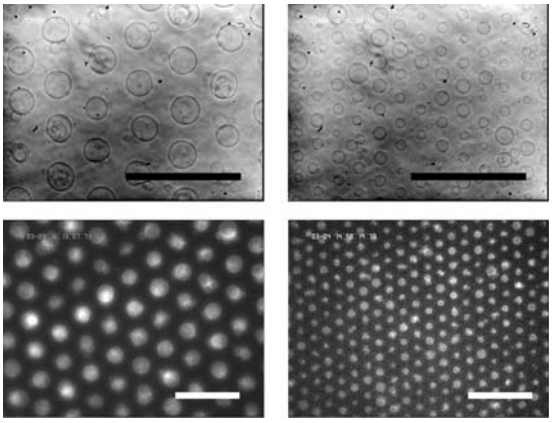

Figure 2. Phase-contrast (top) and Fluorescence (bottom) microscopy images of liposomes formed on the lipid pattern after 2 hours of electroswelling (2V, 10 Hz). The lipid patterns consisted of hexagonal arrays of lipid dots having a diameter of 30 µm (left) and 15 µm (right), respectively. Liposomes are made fluorescent by the use Rhodamine-Phosphatidylethanolamine, a fluorescent lipid. For each picture, scale bar is 200 µm.

Up to now, usual techniques to include DNA inside a liposome (electroporation, swelling) have been limited to short DNA fragments (up to a few thousands base pairs). To insert very long molecules of genomic DNA, we used the fact that single molecules of long duplex DNA fold reversibly in the presence of multivalent counter-

ions into a very dense compact state [3]. Figure 3 shows the single-chain compaction of T4 DNA by spermine (Spm^{4+}) prior to the swelling of liposomes. Thus obtained DNA condensates have a toroidal shape with an outer diameter of ca. 90 nm (Fig. 3c).

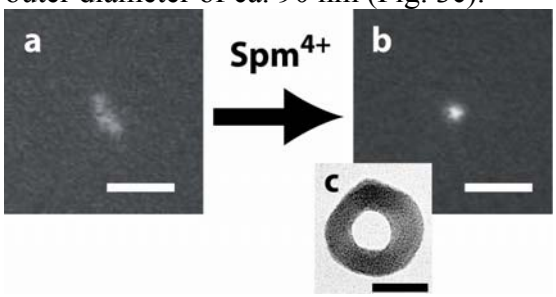

Figure 3. Single-molecule compaction of genomic T4 DNA (166,00 base pairs, 0.1 μM in 10 mM Tris-HCl buffer, *pH* =7.4) by spermine (Spm^{4+}). Without spermine, all DNA molecules are in the unfolded elongated coil state. After sufficient addition of spermine, all DNA molecules fold individually into a dense compact state. a) Fluorescent microscopy (FM) image of an unfolded DNA molecule in the absence of spermine. Scale bar is 5 μm. b) FM image of compacted DNA molecule after addition of 10 μM spermine. Scale bar is 5 μm. c) Transmission Electron Microscopy (TEM) image of the compacted DNA molecule. Scale bar is 50 nm.

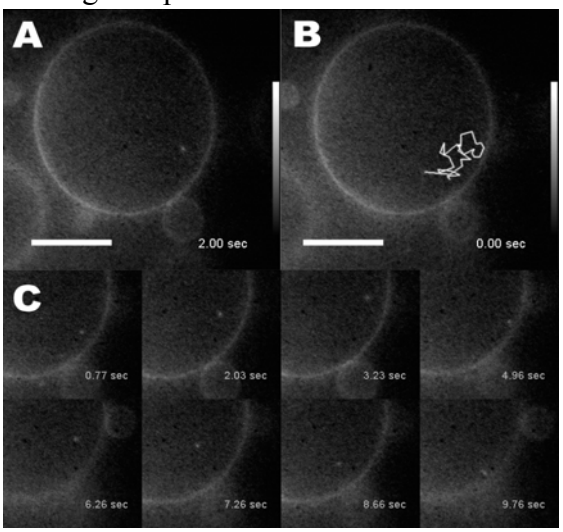

Figure 4. Fluorescence microscopy images of a liposome containing a freely moving single T4 DNA molecule. A) DNA (0.1 μM) is labeled with DAPI (0.1μM) and appears as a fluorescent blue spot in the fluorescent yellow liposome, which contains 4% PEA fluorescein labeled phospholipid. B) The path of the DNA molecule is enlightened to trace the Brownian motion of DNA during 10 s. C) 8 images taken at different time. Scale bar is 20 μm.

Figure 4 shows typical fluorescence microscopic images of a liposome obtained after swelling in a sucrose solution containing the compacted DNA molecules. In this figure, a compact DNA molecule can be seen as a fluorescent blue spot (fluorescence from DAPI-labeled DNA) freely moving within the fluorescent yellow liposome (fluorescence from fluorescein-labeled phospholipids composing the membrane of the liposome). The yield of such DNA insertion in cell-sized liposomes was estimated to be about 10%.

4. Conclusion

The method presented here, based on the electroswelling of micro-patterned lipid films and DNA compaction methodology, allows for the formation of cell-sized liposomes of controlled size (in the range 10-50 μm) and containing long genomic DNA. With a better yield in DNA insertion, it should pave the way for the controlled bottom-up preparation of artificial cells.

References

1. P. Taylor, C. Xu, P. D. I. Fletcher, and V. N. Paunov, A novel technique for preparation of monodisperse giant liposomes, Chem. Commun., vol. 14, pp. 1732-1733, (2003)
2. K. Kuribayashi and S. Takeuchi, Formation of monodisperse giant liposomes using micro-patterned lipid films, Proc. Micro Total Analysis Systems 2005, pp. 1455-1457, (2005)
3. D. Baigl and K. Yoshikawa, Dielectric control of counterion-induced single-chain folding transition of DNA, Biophys. J., vol. 88, pp. 3486-3493, (2005)