Adsorption Phenomena of Anionic and Cationic Nanoliposomes on the Surface of Poly(dimethylsiloxane) Microchannel

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The poly(dimethylsiloxane) (PDMS)-based microchannel of a microcapillary chip is attractive for characterization of nanoparticles (NPs). The surface potential of PDMS is negative in nature, and in the presence of charged NPs, an interaction exists between the NPs and microchannel surface. In this study, the adsorption phenomena of nanoliposomes in the microchannel was investigated using a microfluidic chip designed for the autonomous compensation of hydrostatic pressure flow in the presence of neutral or cationic polymer coating. The Brownian motion of the nanoliposomes in the region of interest was stably observed without any fluid flow. The adsorption phenomena of the nanoliposomes on the microchannel surface arose from hydrophobic and electrostatic interaction, and can be attenuated based on electrostatic repulsion and the excluded volume effect.

Keywords: Microcapillary chip, Adsorption, Nanoliposome, Polymer surfactant

1. Introduction

The introduction of liposomes to the research world, more than 50 years ago [1], led to the development of numerous lipid vesicles with diverse shapes and sizes [2,3]. A liposome is composed of an outer lipid bilayer encapsulating the internal environment. It can be uni- or multilamellar, depending on the number of bilayers, and anionic, cationic, or neutral in nature based on the charged surface. Nanoliposomes represent the lipid bilayer in nanoscale dimension, exhibiting high surface area and solubility [4,5]. They are composed of a phospholipid and cholesterol moiety. A phospholipid is amphiphilic in nature and displays hydrophilic (phosphate ester) and hydrophobic (acyl) groups forming the bilayer, whereas the cholesterol plays a strategic role in nanoliposome composition by modulating the membrane fluidity and permeability, lipid vesicle size, and its stability [6].

A microcapillary chip which contains poly(dimethylsiloxane) (PDMS)-based sub-millimeter-sized microchannels is highly attractive for characterizing nanoparticles (NPs) [7–9]. The merits of the microcapillary chip for NP characterization include rapid analysis with short reaction time, low sample and reagent usage, potential of complex analytical procedure integration on a chip, and high portability. In addition, the low Reynolds number and laminar flow give a significant advantage to this technology [10–12]. However, the nonspecific adsorption of biomolecules and NPs onto the surface of a microchannel, owing to the interaction between the particle and microchannel surface, poses a significant challenge to this technology. These interactions can be divided into hydrophobic interaction, electrostatic with Coulombic attraction or repulsion interaction, and van der Waals interaction [13,14].

Recently, the microcapillary chip technology has shown great research potential, especially for the
electrophoretic mobility (EPM) giving the information of the surface charge of nanoparticles as one of the parameters for characterization. A new analytical tool has been developed for characterizing individual extracellular vesicles (EVs) including exosomes with the diameter (nm) and zeta potential (mV) ranging from 30 to 200 nm and -50 to 0 mV, respectively [15,16]. Here, the visualization and measurement of individual NPs were achieved in a dark field within the microchannel of the microcapillary chip. NP detection takes place through the Rayleigh scattering of light of individual NPs in the microchannel [17,18]. This chip technology has been recognized as an important fundamental tool for NP characterization and multiparametric profiling of NPs, such as structure, size distribution, and zeta potential, within a single instrument [19,20]. Moreover, the potential difference between the medium and stationary layers of the fluid attached to the particle disperse in the medium represents the zeta potential, whereas the electroosmotic flow (EOF) is the electrolyte movement in an applied electric field in the fluid relative to a stationary charged surface [21,22].

Microcapillary chips, in many cases, are fabricated by soft lithography using PDMS and a glass substrate [23−25]. However, the surface of native PDMS in the microchannels is negatively charged. Thus, when considering a PDMS-based microcapillary chip for practical use, this negative surface potential causes (i) NP adsorption due to hydrophobic and/or electrostatic interaction and (ii) EOF arising within the microchannel. Hence, the surface properties of a PDMS-based microchannel demand further study to determine the adsorption behavior of charged NPs in the presence of different (negative, positive, and neutral) surface zeta potentials in the microchannel.

In this study, the adsorption phenomenon of nanoliposomes is investigated by observing the neutral, cationic, and anionic nanoliposomes in the microchannels with different surface zeta potentials. The surface zeta potential of the PDMS microchannel is changed using the neutral polyethylene-block-poly(ethylene glycol) (PEG) and a cationic polyethyleneimine (PEI) polymer coated on the microchannel.

2. Materials and methods

2.1. Materials
1,2-Di-O-octadecenyl-3-trimethylammonium propane (chloride salt) (DOTMA), 1,2-dilauroyl-

2.2. Preparation of nanoliposomes
The nanoliposomes were prepared using the thin-film hydration method, where an appropriate molar ratio of lipids was dissolved in chloroform on a vial [26]. The lipid film was deposited by evaporating the organic solvent, and subsequently, hydrated with PBS buffer with pH 7.4 and ionic strength 165 mM for 24 h. The neutral liposome was prepared with DLPC and cholesterol at 4:1 molar ratio, which helps to maintain a similar cholesterol composition within the two monolayers of the liposome [6]. The cationic liposome was prepared with DOTMA and cholesterol at 1:1 molar ratio [27,28], whereas the anionic liposome was prepared using DLPC, PS, and cholesterol at 5.55:0.45:4 molar ratio to avoid the saturation of the absolute value of the average zeta potential and maintaining the stability of the liposome [26]. Later, the original liposomes were extruded through a polycarbonate membrane with 100 nm pore size using an Avanti® mini-extruder to form nanoliposomes with the size distribution to study the adsorption phenomenon on the microchannel.

2.3. PDMS coating and characterization
A PDMS-based microchannel of a microcapillary chip with the bypass channel was coated with the following polymers: neutral polyethylene-block-PEG and cationic PEI. The PEG solution was prepared with 100 mg/mL concentration, whereas for the PEI solution, 126 mg/mL concentration was used. Later, the chip was filled with a polymer solution to coat the PDMS surface, for 4 h. In addition, to characterize the coated PDMS microchannel, 400-nm, noncharged NPs were used
to estimate the zeta potential of the coated microchannel.

2.4. Imaging of nanoliposomes in microcapillary chips

Recently, researchers have reported a microcapillary chip with the bypass channel designed for the autonomous compensation of hydrostatic pressure flow in the microchannel [29]. Here, the chip is modified to compensate for the unstable hydrostatic pressure flow acting in the microchannel. The technological advantage of this chip is the visualization of individual NPs and a stable observation of Brownian motion of NPs in a dark-field image. Figure 1a shows the microcapillary chip comprising a microchannel (10 mm length, 200 μm width, and 200 μm height), reservoirs (3 mm diameter), and a bypass channel (10 mm length, 2 mm width, and 2 mm height), which is open at the top.

The detection of individual nanoliposomes in the microchannel of a microcapillary chip with the bypass channel was based on dark-field imaging of the laser light scattered by the nanoliposomes exhibiting the Brownian motion under its vicinity. Here, the concentration range from $10^7$ to $10^9$ particles/mL are reliably detectable using the micro-capillary chip system [26]. In addition, the adsorption of nanoliposomes was estimated by image acquisition of the nanoliposome-filled microchannel with 1 min interval, starting from 4 min after liposome solution introduction in the microcapillary chip (total experiment time: 15 min). The number of nanoliposomes in the region of interest (ROI) of the microchannel was counted using the vesicle analyzer software [15] to evaluate their adsorption on the microchannel surface, as shown in Fig. 1b.

3. Results and discussion

3.1. Characterization of nanoliposomes

The prepared nanoliposomes were characterized for average size and zeta potential using Malvern® Zeta-Sizer Nano. Figures 2a–c show the measured average size of DLPC, DOTMA, and PS nanoliposomes, whose values were 117.0, 117.3, and 111.7 nm, respectively, whereas the polydispersity index was obtained as 0.10, 0.08, and 0.08.

The net charge accumulated on the liposome surface determined the magnitude and sign of zeta potential. Table 1 shows the average zeta potential of DLPC, DOTMA, and PS nanoliposomes. The phospholipid DLPC molecules are zwitter-ionic in nature. According to Makino et al. [30], the temperature and ionic strength of solution induces conformational changes in the lipid polar head of

![Fig. 1.](image1) (a) Schematic of a microcapillary chip with the bypass channel. (b) Schematics of counting nanoliposomes in the PDMS microchannel of microcapillary chip with the bypass channel (200 μm width and 200 μm height).

![Fig. 2.](image2) Measured average and distribution of size (nm) of nanoliposomes: (a) DLPC, (b) DOTMA, and (c) PS.
Table 1. Average zeta potential (mV) of DLPC, DOTMA, and PS nanoliposomes.

<table>
<thead>
<tr>
<th>Nanoliposomes</th>
<th>Average zeta potential (mV)</th>
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<tr>
<td>DLPC (neutral)</td>
<td>-1.51 ± 0.19</td>
</tr>
<tr>
<td>DOTMA (cationic)</td>
<td>42.17 ± 1.71</td>
</tr>
<tr>
<td>PS (anionic)</td>
<td>-12.50 ± 0.50</td>
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The hydrophobic groups between the PDMS and polymers, namely polyethylene-block-PEG and PEI, form a weak interaction bond during the coating process, resulted in the neutral and cationic surface zeta potential in the microchannel of the microcapillary chip. Table 2 shows the average zeta potential of the coated microchannel.

3.3. Adsorption dynamics of nanoliposomes

Figures 3a−c show the adsorption phenomena of DLPC, DOTMA, and PS nanoliposomes in the PDMS microchannel of the microcapillary chip without coating. The chemical structure of PDMS comprises repeating units of −OSi(CH₃)₂− groups, leading to a hydrophobic surface within the microchannel of a microcapillary chip [32]. The particle number of neutral DLPC nanoliposomes in the ROI of the microchannel decrease gradually owing to the hydrophobic interaction with the hydrophobic surface of PDMS, and subsequently, indicate nanoliposome adsorption on the microchannel surface. In addition, the negative surface potential of PDMS results in electrostatic interaction with cationic DOTMA and anionic PS nanoliposomes arising from the Coulombic attraction or repulsion between the charged groups. Here, the decrease in the particle number of cationic DOTMA nanoliposomes represents the electrostatic attraction, whereas the fairly constant particle number of anionic PS nanoliposomes in the ROI of the microchannel show electrostatic repulsion with PDMS.

Figures 4a−c show the adsorption phenomena of DLPC, DOTMA, and PS nanoliposomes in the PDMS microchannel of the microcapillary chip with PEI coating. PEI is a polycation polymer forming a cationic coating on the PDMS surface.

Table 2. Average zeta potential (mV) of the surface of the PDMS microchannels with and without coating.

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<th>Microcapillary chip</th>
<th>Average zeta potential (mV)</th>
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<tr>
<td>PDMS microchannel without coating</td>
<td>-13.82 ± 2.31</td>
</tr>
<tr>
<td>PDMS microchannel with PEI coating</td>
<td>7.79 ± 0.91</td>
</tr>
<tr>
<td>PDMS microchannel with polyethylene-block-PEG coating</td>
<td>0.00 ± 0.01</td>
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resulting in positive zeta potential. Owing to this, neutral DLPC nanoliposomes and cationic DOTMA nanoliposomes show the excluded volume effect and electrostatic repulsion, respectively, with the PEI-coated microchannel, as indicated by the fairly constant particle number in the ROI of the microchannel throughout the experiment. However, anionic PS nanoliposomes show a decrease in the particle number in the ROI, indicating the electrostatic interaction involving Coulombic attraction with the positively charged PEI-coated microchannel. Hence, the excluded volume effect and electrostatic repulsion of the nanoliposomes help to attenuate the adsorption in the microchannel.

Figures 5a–c show the adsorption phenomena of DLPC, DOTMA, and PS nanoliposomes in the PDMS microchannel of the microcapillary chip with the polyethylene-block-PEG coating. This coating on the PDMS leads to a neutral surface. Hence, all nanoliposomes, namely neutral DLPC, cationic DOTMA, and anionic PS, display a fairly constant particle number in the ROI, indicating the excluded volume effect with a neutral PEG-coated microchannel. Here, the PEG-derived surface modification of the microchannel leads to a significant reduction in the nonspecific interaction of nanoliposomes, causing an effective exclusion owing to the high degree of hydrophilicity and chain flexibility [33]. Therefore, the excluded volume effect of the nanoliposomes and neutral PEG-coated microchannel help to diminish the adsorption of nanoliposomes in the microchannel.

4. Conclusion
A microcapillary chip with a bypass channel was used to observe the adsorption behavior of nanoliposomes in microchannels. The Brownian motion and decreasing number of nanoliposomes in the microchannel, due to adsorption on the microchannel surface, were stably observed. The adsorption phenomena were investigated by observing neutral DLPC, cationic DOTMA, and
Fig. 5. Adsorption phenomena of DLPC, DOTMA, and PS nanoliposomes in PDMS microchannel with the polyethylene-block-PEG coating.

anionic PS nanoliposomes in the microchannels with different surface zeta potentials. In addition, the adsorption of nanoliposomes on the microchannel surface was attenuated based on electrostatic repulsion and the excluded volume effect. The microcapillary chip with a bypass channel combined with dark-field imaging can be used for evaluating the adsorption phenomena of NPs in a liquid environment.

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