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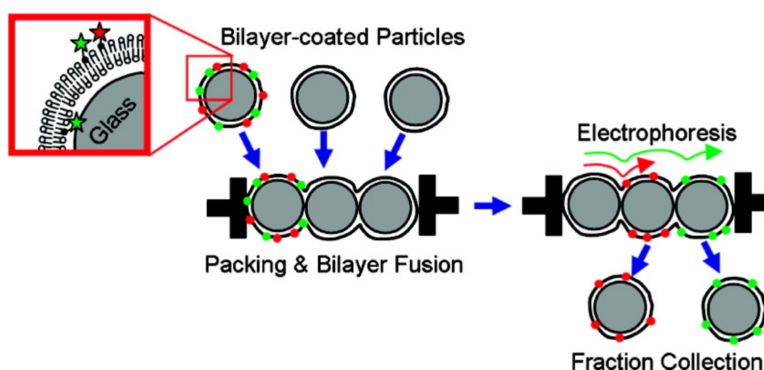

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Electrophoresis of Membrane-Associated Molecules in Packed Beds of Bilayer-Coated Particles

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Lipid bilayers in the liquid-crystalline phase are two-dimensional fluids which form the structural basis for all biological membranes. Electrophoresis in a fluid lipid bilayer is an ideal strategy for separation of membrane-associated biomolecules (e.g., lipids and membrane proteins) in their native environment,[1] because it does not require their extraction from the membranes by solvents or detergents in contrast to conventional methods using chromatography or gel electrophoresis. So far, electrophoretic accumulation and separation of charged lipids,[2] peripheral-types of membrane proteins,[3] and tethered vesicles[4] have already been accomplished through the use of supported planar bilayers (SPBs) on solid substrates such as glass, for analytical purposes. However, the planar substrates are not suitable for preparative purposes because of the difficulty in large-scale separation as well as fraction collection.

Herein, we describe a new method for large-scale separation of membrane-associated biomolecules using a packed bed of bilayer-coated particles as an electrophoretic medium. Our strategy is illustrated in Figure 1. First, solid particles coated with fluid lipid bilayers and those coated with fluid lipid bilayers containing the sample to be separated (lipids or membrane proteins) are packed in a column so as to form a "sample plug". Next, electrophoretic separation of the sample components is carried out by applying a dc electric field along the column. Finally, the separated fractions are collected by taking the particles out from the column. A major assumption in the above strategy is continuity of the bilayer throughout the column—the bilayers on adjacent particles are fused together under a densely packed condition. The inherent large surface-to-volume ratio and redispersibility of the particle bed allow large-scale separation and easy manual collection of the fractions, respectively.

We carried out proof-of-concept experiments by utilizing Texas Red-DHPE[5] and NBD-PS[6] as the samples for separation. Texas Red-DHPE and NBD-PS have net molecular charges of −1 and −2, respectively. The detailed experimental procedure is described in the Supporting Information (SI). Briefly, small unilamellar vesicles (SUVs) of some mixtures of neutral phospholipids (e.g., DOPC,[7] DOPE,[8] DPPC,[9] and Egg-PC)[10] were prepared according to the Barenholz procedure[11] with minor modifications. SUVs containing the fluorescently labeled lipids were also prepared in the same way. Bilayer-coated particles were prepared by the vesicle fusion method[12] using polydisperse glass beads in the size range of 1−13 μm. The bilayer-coated glass beads were thoroughly washed with a buffer solution to eliminate an excess of SUVs. The amount of phospholipids finally left on 1 mg of the glass beads was estimated as ~2 μg. The bilayer-coated particles were packed into a glass tube column with an inner diameter of 1.2 mm. The packed particles were sandwiched between two pieces of filter paper. The final consolidation of the bead bed was realized by inserting a glass capillary with an outer diameter of 1.1 mm from the entrance of the column. The pressure exerted on the bead bed through the capillary was kept constant (6.5×10^5 N/m^2) by a steel spring. The length of the bead bed was ~1 cm. Each end of the column was then connected to a reservoir through a silicone rubber tube which is filled with the same buffer solution. Platinum electrodes were inserted into the reservoirs.

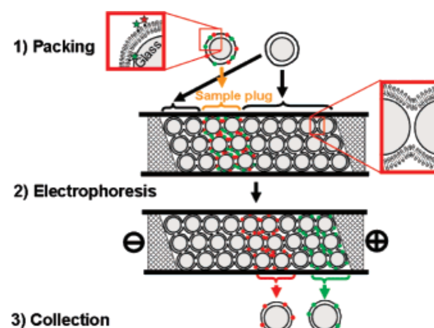

Figure 1. Schematic illustration of the strategy for electrophoretic separation and collection of membrane-associated biomolecules on bilayer-coated particles. In this figure, fluorescently labeled lipids are drawn as the sample molecules for separation.

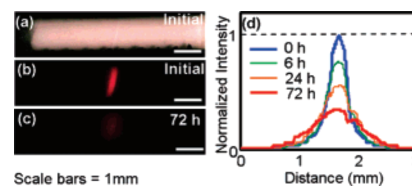

Figure 2. Diffusive migration of Texas Red-DHPE in a packed bed of bilayer-coated glass beads without an electric field: (a) a glass tube column after packing of the beads; (b, c) fluorescence images of the column (b) immediately after preparation and (c) after 72 h; (d) fluorescence intensity profiles measured along the center of the column. Conditions: bilayer composition, DOPC:DOPE = 65:35 in mol; sample, 0.1 mol % of Texas Red-DHPE; buffer, 10 mM Tris-HCl, pH8, 1 mM $MgCl_2$.

First, we confirmed the continuity of the bilayer in the packed column without applying an electric field. As shown in Figure 2, the band of Texas Red-DHPE was broadened with the course of time. The band broadening was probably caused by molecular diffusion propagating from one particle surface to another, because Texas-Red DHPE is insoluble in the surrounding buffer. Apparent diffusion coefficient of Texas-Red DHPE along the column axis was 0.36 ± 0.04 μm^2/s at 20 °C (see SI). This value is smaller than the lateral diffusion coefficient of lipids in SPBs[13] by about an order of magnitude, probably due to the complicated geometry of the bilayers in the packed column. These results support the assumption of bilayer fusion between the particles.

Next, electrophoretic separation of the fluorescently labeled lipids was carried out by applying a dc voltage of 600 V between the platinum electrodes. Figure 3 shows separation of Texas Red-DHPE and NBD-PS followed by collection of their fractions. Before electrophoresis, the sample plug containing both of the fluorescently labeled lipids appeared as a single band (Figure 3b). After 30 min

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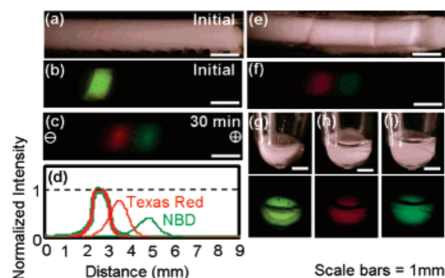

Figure 3. Electrophoretic separation and collection of Texas Red-DHPE and NBD-PS: (a) a glass tube column after packing of the beads; (b, c) merged fluorescence images of the packed column (b) before electrophoresis and (c) after 30 min of electrophoresis; (d) fluorescence intensity profile measured along the center of the column before electrophoresis (bold lines) and after 30 min of electrophoresis (thin lines); (e) the bead bed pushed out from the column; (f) a merged fluorescence image of the pushed-out bead bed; (g) a microtube containing the sample beads before electrophoresis (up) and its merged fluorescence image (down); (h, i) microtubes containing the beads collected after electrophoresis (up) and their merged fluorescence images (down); (h) the fraction of Texas Red-DHPE and (i) the fraction of NBD-PS. Conditions: bilayer composition, DOPC:DOPE = 65:35 in mol; sample, 0.1 mol % of Texas Red-DHPE and 1 mol % of NBD-PS; buffer, 10 mM Tris-HCl, pH 8, 1 mM $MgCl_2$.

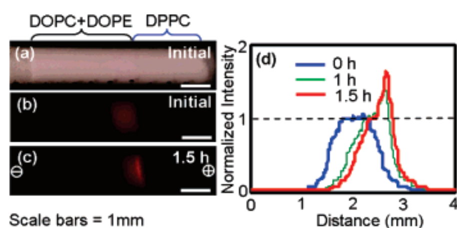

Figure 4. Electrophoretic accumulation of Texas Red-DHPE at the boundary between a fluidic (DOPC + DOPE) and a nonfluidic (DPPC) regions of a packed column: (a) a glass tube column after packing of the beads; (b, c) fluorescence images of the packed column (b) before electrophoresis and (c) after 1.5 h of electrophoresis; (d) fluorescence intensity profiles measured along the center of the column. Conditions: bilayer composition of the fluidic region, DOPC:DOPE = 50:50 in mol; bilayer compositions of the nonfluidic region, 100% of DPPC; sample, 0.1 mol % of Texas Red-DHPE; buffer, 10 mM Tris-HCl, pH 8.

of electrophoresis, the band was separated into a red and a green bands as shown in Figure 3c. They correspond to Texas Red-DHPE and NBD-PS, respectively. Figure 3d shows intensity profiles along the column before and after the electrophoresis. To collect the separated fractions, the bead bed was pushed out from the column with a metal wire (Figure 3 panels e and f) and cut with a knife. Both of the separated fractions were successfully collected into microtubes using a conventional pipet, as shown in Figure 3 panels h and i. ζ-Potential measurements of the collected beads were also successful; this implies the possibility of label-free characterization (see SI). In addition, we confirmed that similar electrophoretic separations are also possible with different bilayer compositions as well as a different buffer solution without Mg^{2+} (see SI). We speculate that the band broadening was mainly caused by the polydispersity of the beads and would be improved by using monodisperse beads.

Finally, we confirmed that the migration of the charged lipids is restricted within the fluid lipid bilayer even with the application of the dc voltage of 600 V. We employed DPPC-bilayer-coated beads as one of the packing materials. Generally, DPPC bialyers are in the gel phase with negligible fluidity at room temperature because of their higher phase transition temperature (∼41 °C). As shown in Figure 4a, we constructed a packed bed composed of a fluidic (DOPC + DOPE) and a nonfluidic (DPPC) regions. The fluidic region contained a sample plug of Texas Red-DHPE. A dc voltage of 600 V was applied so that the sample plug was electrophoresed toward the nonfluidic region. Joule heating was small enough to keep the DPPC bilayers nonfluidic (see SI). As a result, the band was stopped at the boundary and was significantly compacted there (Figure 4b−d). These results indicate that the major pathway of Texas Red-DHPE is not in the bulk solution but in the fluid lipid bilayer on the particles.

In conclusion, we have developed a new separation method for membrane-associated biomolecules in fluid lipid bilayers. The use of a packed bed of bilayer-coated particles facilitates large-scale separation and subsequent collection of the fractions. This method could be extended to the separation of membrane proteins by introducing an appropriate polymer cushion[14] into the bilayer-coated particle to ensure two-dimensional mobility of integral membrane proteins on the particles. Recently, bilayer-coated particles containing purified lipids or membrane proteins have been used for various purposes including a highly selective purification of proteins,[15] detection of protein−ligand interactions,[16] and local stimulation of cell surfaces.[17] Our method could offer an attractive shortcut to make such functional particles.

Supporting Information Available: Experimental section, electrophoretic separation under other conditions, calculation of apparent diffusion coefficient, ζ-potential measurements, and influence of Joule heating. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) diammonium salt (NBD-PS).
(7) 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC).
(8) 1,2-dioleoyl-*sn*-glycero-3-phosphoetanolamine (DOPE).
(9) 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC).
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