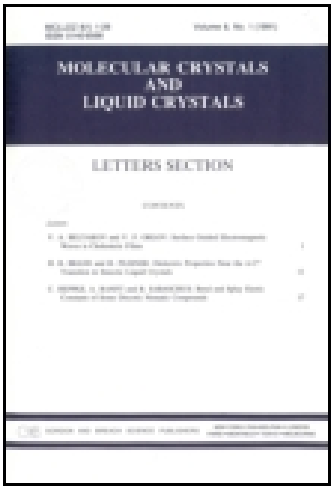

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Coarsening Nature of Liquid-Ordered Domain in Model Membrane

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We present the coarsening dynamics of a liquid-ordered (Lo) domain in a supported lipid membrane under an electric field. In a relatively small nanosmooth region surrounded by geometrical walls of nanocorrugated regions, full coarsening of the Lo domain was produced while in a large nanosmooth region, an intermediate, less-ordered Lo domain was developed so that the diffusion of charged phospholipids in the liquid-disordered (Ld) domain was allowed across the geometrical walls in the presence of an electric field. No appreciable diffusion of the charged lipids into a fully coarsened Lo domain by the electric field implies that the structural ordering of the membrane components plays a significant role on the formation of lipid rafts for biological processes.

Keywords Coarsening dynamics; charged lipids; supported lipid membrane; liquid-ordered; lipid raft

1. Introduction

Cell membranes are supermolecular, two-dimensional fluids consisting of various types of lipids and proteins. The heterogeneous lipids in biological membranes are known to be laterally organized into phase-separated microdomains (called lipid rafts [1–3] *in vivo*) for performing a variety of biochemical functions such as signal transductions and budding. A liquid-ordered (Lo) phase is represented by a microdomain where sphingolipids and cholesterols are enriched while a liquid-disordered (Ld) phase enriched in phospholipids, having unsaturated hydrocarbon tails, serves as a background. The physicochemical properties such as the phase behavior [4], the chemical composition [5], and the physical size [6], and the elasticity [7] of the Lo phase have been extensively studied in model membranes [8–10] prepared from giant unilamellar vesicles (GUVs). For example, on the basis of the reconstitution of the Lo domains in model membranes using the GUVs [11], the relationship between sterol structures and the natural curvature inherent to the Lo phase was investigated to provide an insight into vesicular budding. In addition, the coupling between the local curvature and the phase separation revealed in supported lipid membranes (SLMs)

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[12] suggests a mechanical scheme of spatially controlling the lipid rafts *in vivo*. However, it still remains to be understood how the coarsening nature of the Lo phase on the surface topography with diffusion walls is influenced by the structural ordering of the membrane components such as lipids.

In this work, we show how the coarsening dynamics of a liquid-ordered domain in a patterned SLM can be controlled by an electric field. It was observed that a fully coarsened Lo domain was developed in a relatively small nanosmooth region surrounded by geometrical walls of nanocorrugated regions while an intermediate, less-ordered Lo domain was produced in a large nanosmooth region. As a result, the charged phospholipids were diffused into the less-ordered Lo domain across the geometrical walls from the liquid-disordered domain in the presence of an electric field. However, no appreciable diffusion of the charged lipids into a fully coarsened Lo domain was observed even under the electric field due to the high elasticity of the Lo phase. This is consistent with the previous results for the lipid rafts grown on the nanocorrugated and nanosmooth topographies [13]. Our electric field-driven coarsening approach provides a very useful strategy of manipulating the structural ordering of charged lipids in the Lo domains in model membranes.

2. Experiment

2.1. Materials

The membrane components used in this study were 1,2-dioleoyl-*sn*-glycero-3-phophocholine (DOPC), sphingomyelin (brain, porcine) (SM), and cholesterol purchased from Avanti Polar Lipids (Alabaster, AL). Texas red-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Texas red-DHPE) used for imaging was commercially available from Molecular Probes (Eugene, OR).

2.2. Substrate Fabrication

For the nanocorrugated topography, a SiO_2 layer of 1.5 μm thick was prepared by chemical vapor deposition of TEOS (P-5000, Applied Material Korea) on a glass substrate. The deposition rate was 12.5 nm/sec. For producing binary topographic patterns, the nanosmooth topography was obtained by chemically etching the nanocorrugated region by 1 μm in depth using a buffer solution of HF (7:1 (v/v) NH_4F:HF). The etching rate was approximately 100 nm/min. The photolithographic process using a positive photoresist (AZ1512, AZ Electronic Materials, USA) was used for producing a variety of patterns by selective etching. The substrate was cleaned with a piranha solution (3:1 (v/v) H_2SO_4:H_2O_2) at 120°C for 10 min, followed by ultra-sonication in deionized water for 10 min before the formation of the supported lipid bilayer (SLB) on the patterned substrate.

2.3. Formation of SLBs

A mixture of the membrane components, 1:1:1 (mol/mol/mol) DOPC:SM:cholesterol, was prepared in chloroform and doped with Texas red-DHPE at 1 mol% of the total lipid composition. The rapid solvent exchange method [14] was employed for the evaporation of chloroform and the hydration with Tris buffer (100 mM NaCl and 10 mM Tris at pH 8.0) simultaneously. The total concentration of the mixture was 0.2 mg/ml. Small unilamellar vesicles (SUVs) were produced by extruding (Mini-Extruder, Avanti Polar Lipids) 20 times through a filter with pores of 50 nm in diameter. When the cleaned substrate was incubated in the SUV solution, the SLBs were formed via vesicle adsorption, rupture, and fusion. The

Texas red channel was monitored using an epifluorescence microscopy (Eclipse E600-POL, Nikon) during the coarsening process of the Lo domains.

3. Results and Discussion

Figure 1(a) shows a schematic diagram of the coarsening dynamics of the Lo domains in the SLM on the substrate with the nanocorrugated topography (in the left) and the nanosmooth topography (in the right). The underlying mechanism for the selective growth of the Lo domain where sphingolipids and cholesterols are enriched in the nanosmooth region

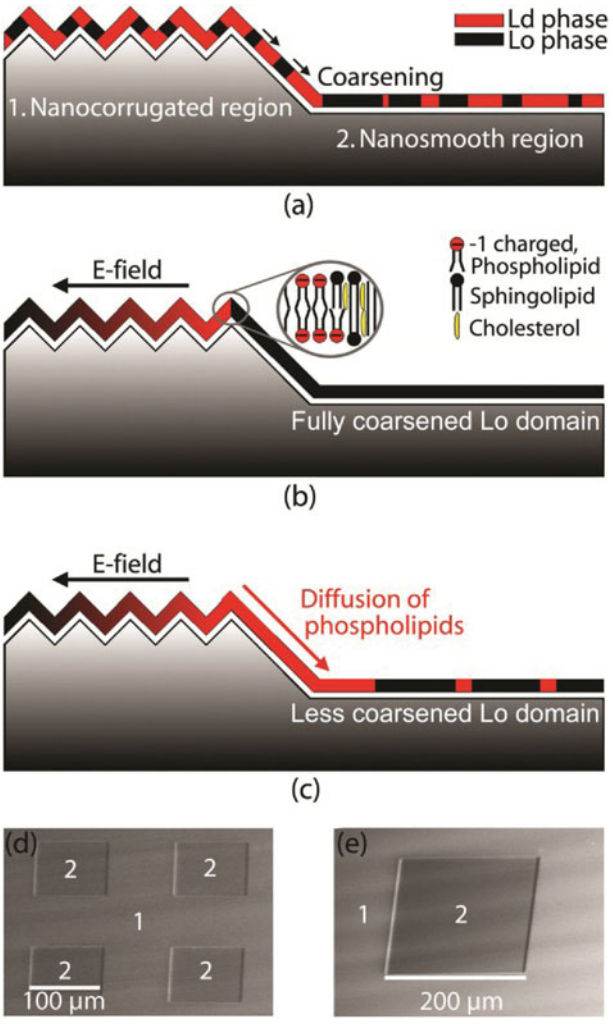

Figure 1. Schematic diagrams showing (a) the coarsening process of the Lo domains where sphingolipids and cholesterols are enriched in the nanosmooth region surrounded by the nanocorrugated regions, (b) a fully coarsened Lo domain which serves as a diffusion barrier for charged phospholipids under an electric field, and (c) a less coarsened Lo domain which allows the diffusion of phospholipids. Microphotographs of two substrates, taken with a scanning electron microscopy, having nanosmooth regions of (d) $100 \times 100\ \mu m^2$ and (e) $200 \times 200\ \mu m^2$. The nanocorrugated topography and the nanosmooth topography is denoted by 1 and 2, respectively.

originates from the high elasticity of the Lo domain experienced in the nanocorrugated region [13]. Note that microdomains of the Lo phase in the nanocorrugated region are allowed to freely diffuse into the nanosmooth region without costing a large elastic energy. In principle, the degree of coarsening or the coverage of the Lo phase depends on the elapsed time for given dimension of the nanosmooth region. In a fully-grown (or coarsened) Lo domain (Fig. 1(b)), the diffusion of charged phospholipids by an electric field (E) from the Ld phase will not be energetically favorable while it will be promoted by E in an intermediate, less-grown Lo domain (Fig. 1(c)). In our study, the area of the nanosmooth region was varied to be 100 $\times$ 100 μm^2 (Fig. 1(d)) and 200 $\times$ 200 μm^2 (Fig. 1(e)) for producing the two types of the Lo domains for given elapsed time. In Figs. 1(d) and 1(e), number 1 and number 2 denote the nanocorrugated topography and the nanosmooth topography, respectively.

We now describe the dynamic coarsening process of the Lo domain in two different sizes of the nanosmooth topography (indicated by 2). For imaging the coarsening process, the SLM was doped with Texas red-DHPE in 1 mol%. The epifluorescence micrographs in Fig. 2 show the temporal evolution of the Lo domains (seen in black) in two different nanosmooth regions, one of which is 100 $\times$ 100 μm^2 and the other is 200 $\times$ 200 μm^2, surrounded by the Ld background (seen in red). At the elapsed time t = 6 h after the SLM formation, the microdomains of the Lo phase were slightly developed inside the nanosmooth regions (Figs. 1(a) and (b)). The intensity profiles in Fig. 1(c) (along the white lines in Figs. 2(a) and 2(c)) indicate that the Lo domains are dense at the edge and are

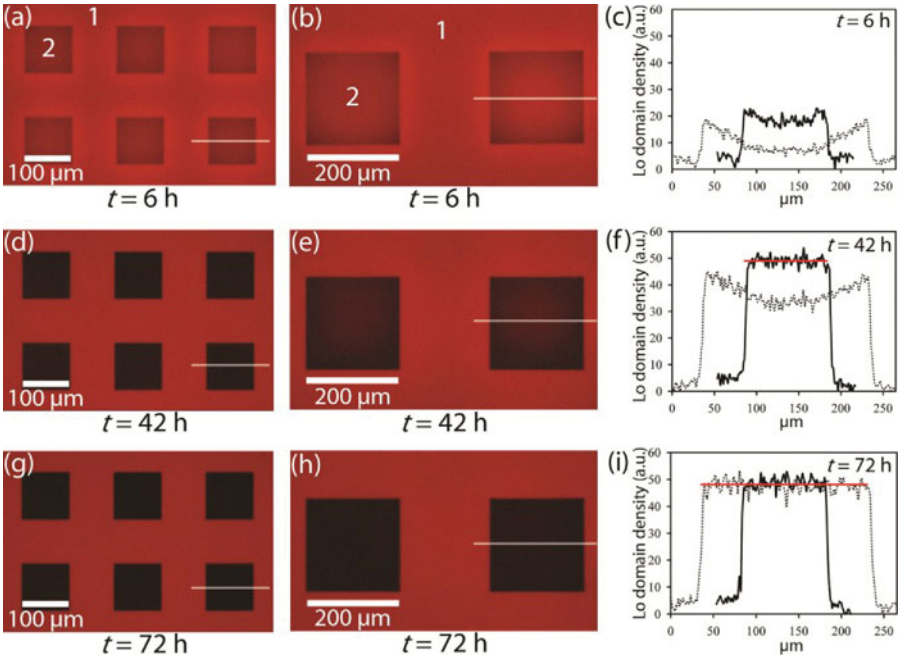

Figure 2. The temporal evolution of the Lo domains in two different nanosmooth regions of 100 $\times$ 100 μm^2 and 200 $\times$ 200 μm^2; at t = 6 h, epifluorescence micrographs in (a) and (b) and the corresponding intensity profiles in (c), at t = 42 h, epifluorescence micrographs in (d) and (e) and the corresponding intensity profiles in (f), and at t = 72 h, epifluorescence micrographs in (g) and (h) and the corresponding intensity profiles in (i).

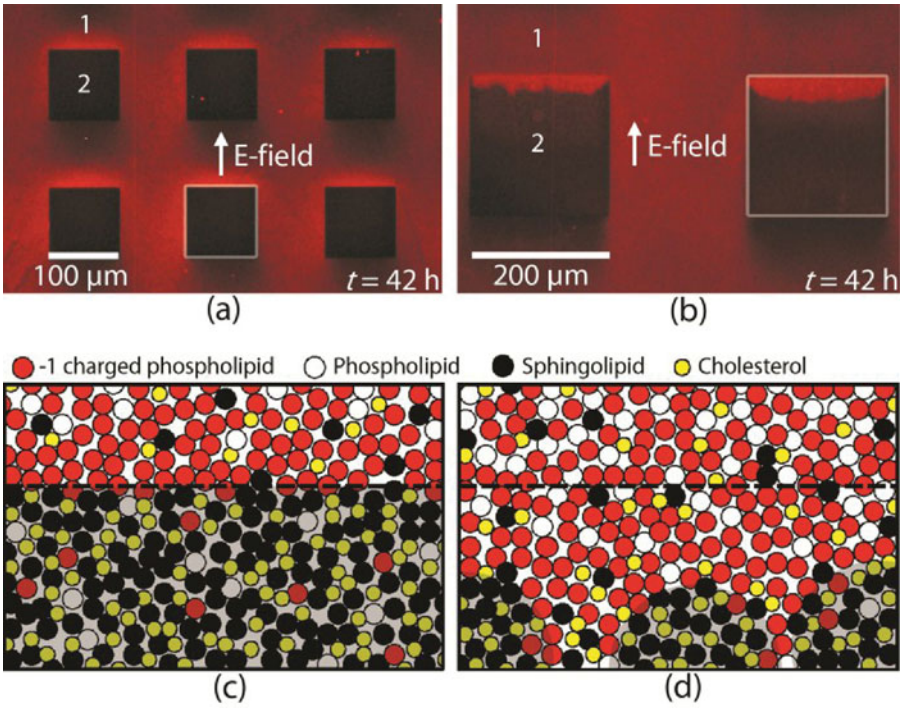

Figure 3. The diffusion behavior of charged phospholipids into (a) a fully coarsened Lo domain in the nanosmooth regions of 100 × 100 μm^2 each and (b) an intermediate, less coarsened Lo domain in the nanosmooth regions of 200 × 200 μm^2 each at $t = 42$ h in the presence of an electric field $E = 25$ V/cm for 1 h. The two cases are schematically illustrated in (c) and (d).

gradually dispersed toward the center of the nanosmooth region. For given elapsed time of 6 h, the density of the Lo domains is higher in the region of 100 × 100 μm^2 than that of 200 × 200 μm^2 as expected. The micrographs in Figs. 2(d) and 2(e), taken at $t = 42$ h, show that the Lo domains seem apparently well coarsened in the two cases. However, as shown from the intensity profiles in Fig. 2(f), the whole nanosmooth region of 100 × 100 μm^2 was uniformly covered with a fully coarsened Lo domain while the coverage of the Lo phase over the region of 200 × 200 μm^2 was incomplete. The micrographs in Figs. 2(g) and 2(h) together with the intensity profiles in Fig. 2(i) confirm that the Lo domains were indeed fully coarsened for the two regions after $t = 72$ h. This suggests that the degree of coarsening of the Lo domain should be taken into account in lipid raft-based bioassays.

In the presence of an electric field $E = 25$ V/cm for 1 h, the diffusion behavior of charged phospholipids into a fully coarsened Lo domain (Fig. 3(a)) and that of an intermediate, less coarsened Lo domain (Fig. 3(b)) at $t = 41$ h are shown in Fig. 3(a) and Fig. 3(b), respectively. The fully coarsened Lo domain in Fig. 3(a) remains essentially unchanged under the electric field E, meaning that no electrophoretic drift [15] of the charged Texas red-DHPEs into the Lo domain is allowed due to the high rigidity. In contrast, as shown in Fig. 3(b), the charged lipids were drifted into the less coarsened Lo domain across the geometrical wall under E. The two cases are schematically illustrated in Figs. 3(c) and 3(d). This difference is related to the packing density and the structural ordering of the neutral lipids in the Lo domain.

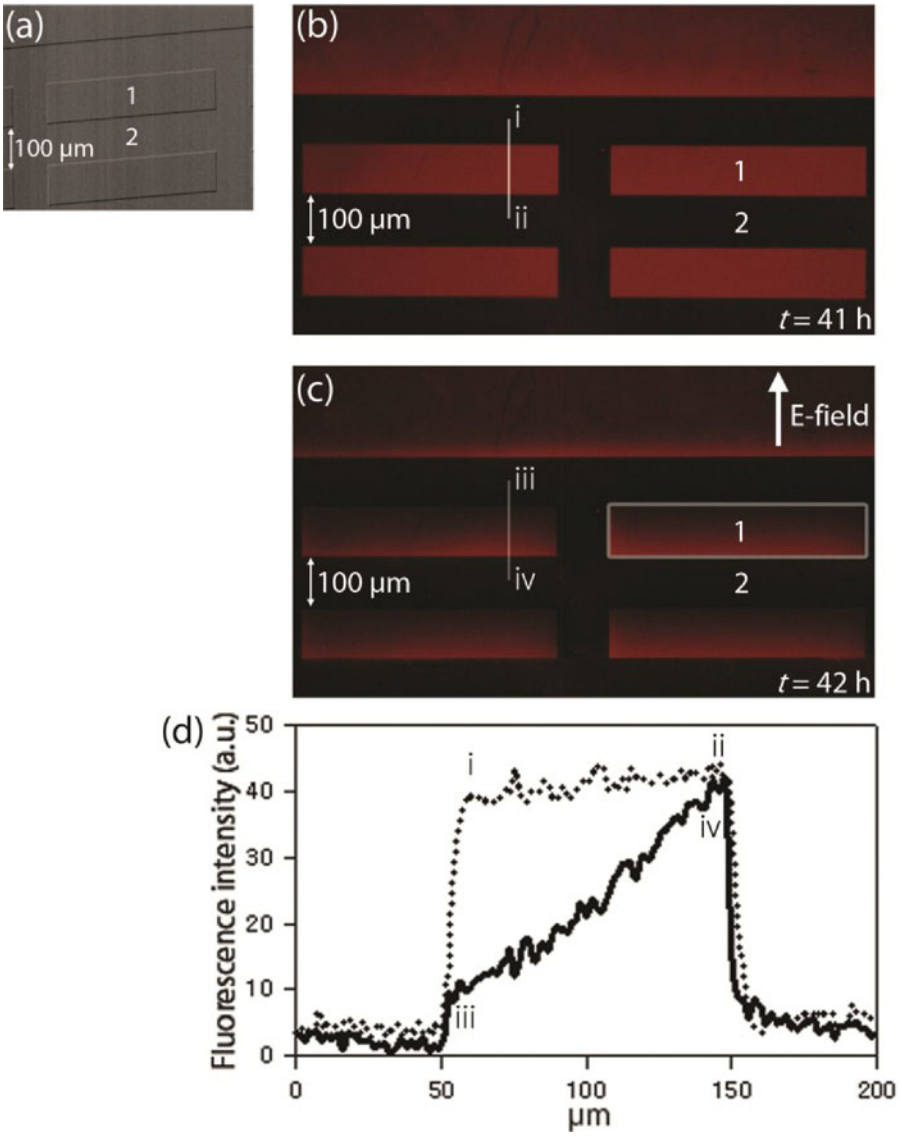

Figure 4. The density gradient of the charged lipids in (a) an isolated geometry with nanocorrugated patterns (denoted by 1) surrounded by nanosmooth background (denoted by 2). The epifluorescence micrographs (b) at $t = 41$ h under no electric field and (c) at $t = 42$ h in the presence of $E = 25$ V/cm for 1 h, and (d) the corresponding intensity profiles along the white lines (i–ii in b and iii–iv in c).

As the final example showing the density gradient of the charged lipids in an isolated geometry, we used a substrate with nanocorrugated patterns (denoted by 1) surrounded by nanosmooth background (denoted by 2) as shown in Fig. 4(a). In this case, the Ld domain consisting mostly of phospholipids (seen in red) was confined by the fully coarsened Lo domain (seen in black) formed at $t = 41$ h in Fig. 4(b). Again, in the presence of the electric field E for 1 h, no electrophoretic drift of Texas red-DHPEs into the Lo domain was observed but the density gradient of the charged lipids appeared in the nanocorrugated

patterns as shown in Fig. 4(c). The corresponding intensity profiles along the white lines (i–ii in Fig. 4(b) and iii–iv in Fig. 4(c)) are shown in Fig. 4(d). Clearly, the lateral distribution of the charged lipids in the background of the fully coarsened Lo phase can be precisely controlled by an electric field. Based on this observation, we strongly speculate that the coarsening of lipid rafts in cellular membranes might play an important role in regulating the diffusion or the isolation of other membrane molecules for the manipulation of the associated biochemical processes.

Conclusions

We presented the coarsening dynamics of a liquid-ordered domain in a patterned SLM using the nanosmooth and nanocorrugated topographies. Depending on the elapsed time for the formation of the Lo domain and the physical dimension of the nanosmooth region, either a fully coarsened Lo domain or an intermediate, less-ordered Lo domain was produced. It was found that no appreciable diffusion of the charged lipids into a fully coarsened Lo domain was observed even under the electric field due to the high elasticity of the Lo phase. This suggests that the degree of coarsening of the Lo domain should be taken into account in lipid raft-based bioassays since the boundary of a fully coarsened Lo domain serves as a diffusion barrier for the transport of other molecules in and out of biological membranes.

Acknowledgment

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