Soft Materials

Vesicular Latex**

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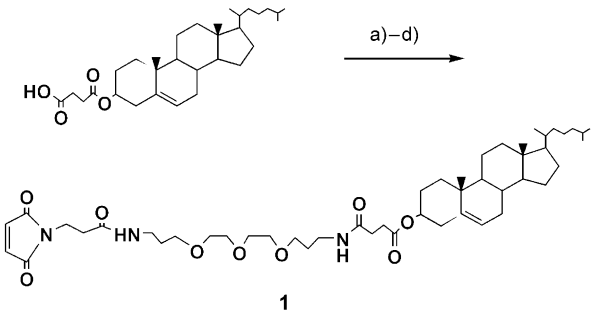
Hydrophobic attraction among projecting lipid chains assembles vesicles into long "strings" capable of gelating water.[1] Exposure to shear stress reversibly disrupts the hydrophobic association, thus breaking the strings and fluidizing the gel. The question arose as to whether vesicles can be joined covalently to render the gels more robust.[2–5] Toward this end we synthesized compound **1** (Scheme 1) in which a vesicle-



Scheme 1. Synthesis of compound **1**. a) $(COCl)_2$; b) $BocNH(CH_2)_3O(CH_2)_2O(CH_2)_3NH_2$; c) TFA, CH_2Cl_2; d) succinimido-3-maleimidopropanoate. Boc = *tert*-butoxycarbonyl, TFA = trifluoroacetic acid.

binding unit (a cholesterol derivative) is attached by a spacer to a maleimide group to which thiols are known to readily undergo Michael addition.[6,7]

When lipid dispersions containing **1** were exposed to dithiothreitol (Cleland's reagent), a room-temperature reaction ensued that interconnected the vesicles [Eq. (1)] to give a material resembling shaving cream in consistency. Its characterization constitutes the basis of this communication.

A more detailed preparation of the new soft material follows. Solutions of DPPC[8] (1.46 mL, 10 mg mL^{-1}), DOPG[8] (88.4 μL, 10 mg mL^{-1}), and **1** (464 μL, 2 mg mL^{-1}) in $CHCl_3$ were added to a 10-mL flask. The solvent was removed by evaporation and vacuum drying, after which degassed phosphate buffer (pH 7; 1 mL) was added and the mixture was either stirred for 10 min (400 rpm) or bath-sonicated for 15 min, to form polydisperse vesicular structures. According to dynamic light-scattering analysis, the vesicles averaged 200 nm in diameter. Finally, dithiothreitol (146 μL, 0.58 mg mL^{-1} in buffer) was added, and the mixture was stirred magnetically under N_2 at high speed (1100 rpm) for 24 h at room temperature to give a thick cream. Only a fluid opalescent suspension of particulate lipid was obtained when dithiothreitol was omitted from the preparation.

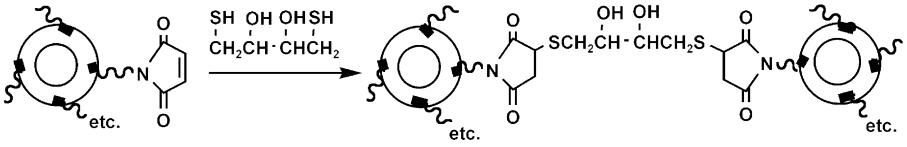
The lipid/**1** ratio was such that, prior to cross-linking, a component vesicle with 10000 lipid molecules would contain 550 cross-linking sites. Sufficient dithiothreitol was added to react with all the maleimide units. If, after the addition of dithiothreitol, stirring was set at slow speeds (250 and 550 rpm), then only fluid suspensions were observed. On the other hand, cross-linking with rapid stirring produced a thick, white, slow-flowing cream, as seen in the inverted vial in Figure 1.

Figure 1. An inverted vial containing cross-linked product.

Examination by phase-contrast microscopy (Nikon Diaphot-TMD inverted microscope) showed that the material is composed of round, densely packed particles ranging from 5–150 μm in diameter (Figure 2). For brevity we call this material a "latex" as a result of its superficial resemblance to colloidal dispersions of polymer particles given that name.

Detailed photos taken by phase-contrast microscopy (Figure 3 A) revealed an unusual "blistered" texture of the particle surface. Confocal microscopy (Figure 3 B, Zeiss 510 Meta laser scanning confocal microscope, $\lambda = 488$ nm) showed more clearly that the blisters (nonremovable by suction with a micropipette) are indeed integrated with the particle surface.

When the sample preparation was carried out at 55 °C (exceeding by 14 °C the gel-to-liquid-crystal transition temperature T_m of DPPC), then only a translucent liquid containing no microscopically visible particles was produced. Absence of latex formation high above the T_m value was also seen with POPC[8] ($T_m = -2.5$ °C) when the synthesis was performed at room temperature with this lipid instead of DPPC.



(1)

Three possibilities presented themselves. The particles could be solid lipid or they could be lipid shells filled either with water or with air (as in a conventional foam). All the evidence points to hollow, water-filled structures: a) No volume increase was observed. Such an increase would be expected if the material was a conventional foam with air-filled "bubbles". b) Since the preparation was carried out in water, the water must logically reside within the particles making up the dense material. c) Dilution with water

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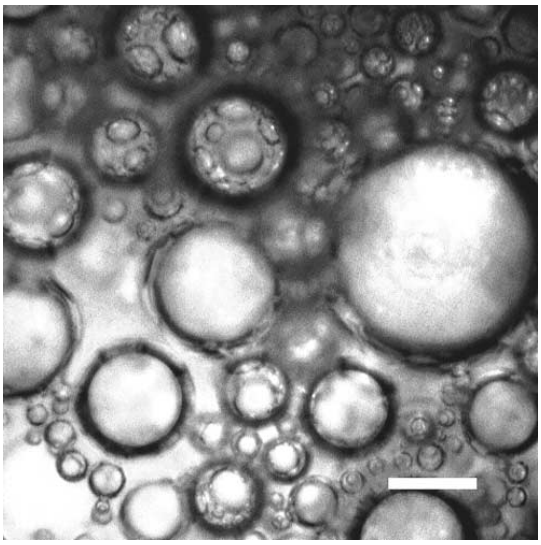


Figure 2. Phase-contrast photomicrograph of a latex smear showing a high particle density. The tops of the larger particles are out of focus. The bar represents 40 μm.

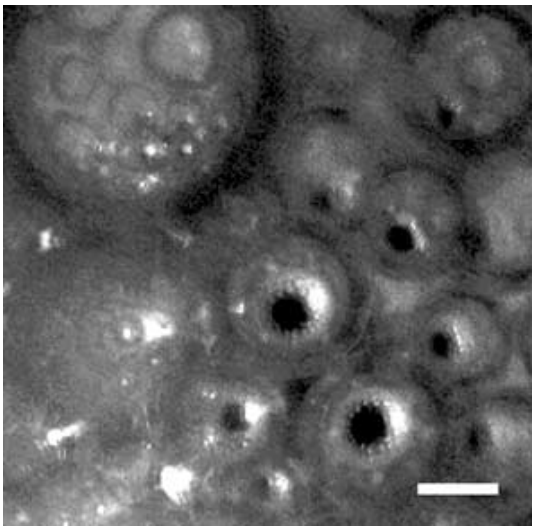
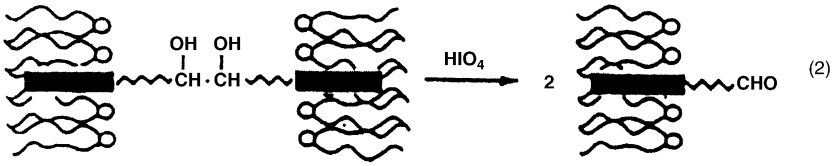


Figure 4. Latex particles showing open pores, as seen by phase-contrast microscopy. The bar represents 30 μm.

produced individual particles that sank rather than floated, a result suggesting the absence of air. d) The particles are tough and, rather than bursting when dragged through water or compressed by a cover slip, merely deform from their spherical shape. e) On occasion, particles are seen with an apparent opening to a hollow sphere (Figure 4). Being hollow, the particle morphology offers the possibility of a useful encapsulation system[9] which is simple, cheap, and endowed with a particularly high capture volume.



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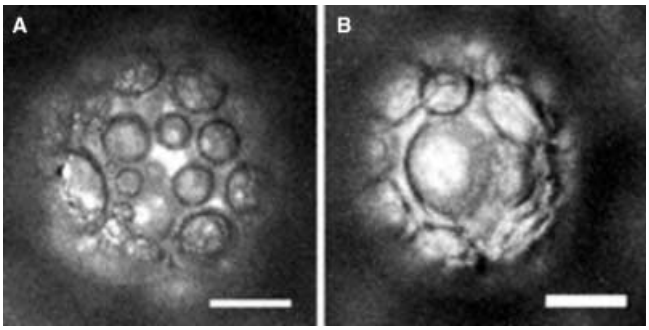

Figure 3. Organic synthesis and self-assembly combine to form the exotic structures seen by A) phase-contrast microscopy and B) confocal microscopy. The bars represent 50 μm.

No change in the material was observed after bath-sonication for 45 min. On the other hand, the vesicular latex particles disintegrate into tiny ill-defined fragments (not shown) upon exposure to periodate (50 μL, 0.1M added to microscope slide). Periodate rapidly cleaves the central C–C bond of the spacer's glycol unit,[10] and this allows the particle constituents to disconnect. The interconnected bilayers are thereby "depolymerized" [Eq. (2)].

The response of the latex particle morphology to drying and freezing was examined next. Slow evaporative drying of a latex cream led to compression and distortion of the spheres (Figure 5A) and ultimately to a dry film (Figure 5B) where small vesicular substructures are visible at the hexagonal peripheries. Freezing of the latex samples causes the particles to divide into small particles that have lost their blistered texture (Figure 5C). As the sample warms up, the particles fuse (Figure 5D) but remain smooth until the original size and mottled appearance are reestablished above the T_m value of 14 °C (DPPC). The blisters are apparently associated with the liquid-crystalline state above the T_m value requiring more space per lipid than the gel state does below the T_m value.

Finally, we carried out high-resolution scanning electron microscopy studies (HRSEM; Figure 6). The photomicrographs showed that the water-filled vesicular latex particles had burst under the high-vacuum conditions. The thickness of the particle shells is < 100 nm. We surmise that the film consists of < 25 lipid bilayers (formed from the shearing of vesicles under the high-speed stirring) in which small vesicles (< 100 nm; visible in Figures 4–6) are embedded. The lamellar/vesicular composite is bonded together by the cross-linking agent.

Experimental Section

1: Oxalyl chloride (3 g, 23.6 mol) was added to cholesteryl hemisuccinate (0.56 g, 1.15 mmol) in CH_2Cl_2 (30 mL), and the mixture was stirred at room temperature for 12 h to give the acid chloride in 98 %

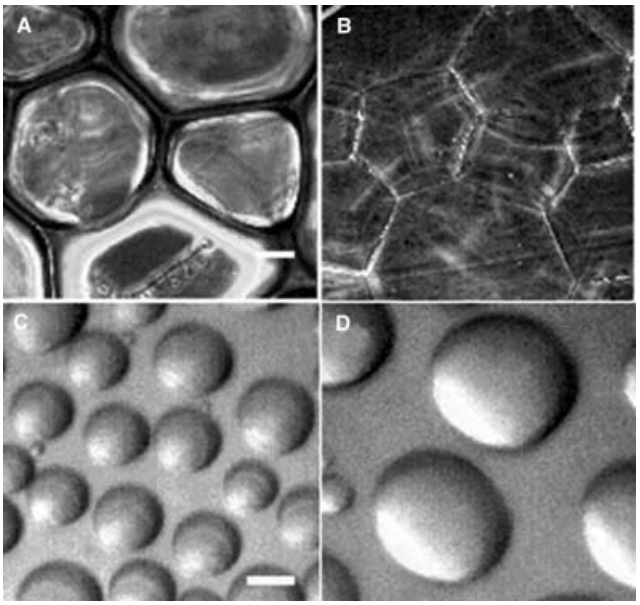

Figure 5. Phase-contrast micrographs of: A) Partial and B) total drying of a vesicular latex; C) a frozen sample of a vesicular latex; D) fused particles of the sample shown in (C) during partial thawing. The bar represents 25 μm. At room temperature the blistered structures of Figure 3 reappear.

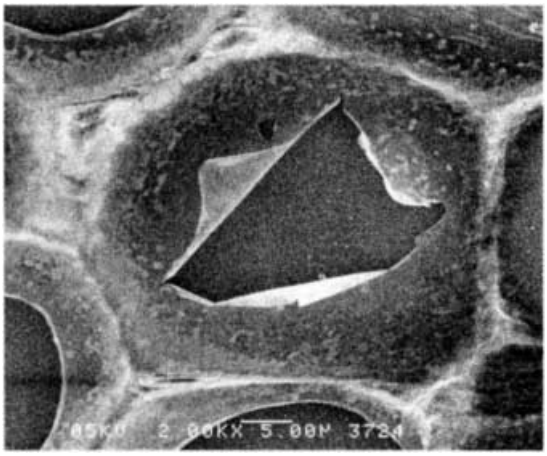

Figure 6. HRSEM photomicrograph of a ruptured latex particle at 2000× enlargement. The bar (7 mm) represents 5 μm. As in Figure 5A, the particles have been forced into proximity by drying.

yield. $(Boc)_2O$ (1.98 g, 9.08 mmol) in $CHCl_3$ (60 mL) was added to 4,7,10-trioxa-1,13-tridecanediamine (10 g, 45.4 mmol) in $CHCl_3$ (100 mL), and the mixture was heated to reflux for 24 h. Purification by chromatography ($MeOH/CH_2Cl_2/NH_4OH$ (5:5:1) over silica) gave 70% of the monoprotected diamine. The acid chloride product of the first reaction (0.4 g, 0.79 mmol) in $CHCl_3$ (20 mL) was added to a mixture of this diamine compound (0.25 g, 0.78 mmol) and Et_3N (0.26 g, 2.34 mmol) in $CHCl_3$ (30 mL). This resulting mixture was then stirred at room temperature for 20 h. Chromatographic purification (CH_2Cl_2/MeOH (10:1)) gave the monoprotected diamine–cholesterol conjugate in 85% yield. Trifluoroacetic acid (1 g, 8.77 mmol) was added to the conjugate (0.2 g, 0.25 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred for 6 h, washed with 5 N $NaHCO_3$, dried over $MgSO_4$, and stripped of solvent to quantitatively remove the Boc group on the terminal amine. The deprotected product (0.63 g, 0.92 mmol) in *N*,*N*-dimethylformamide (DMF; 30 mL) was added to succinimido-3-maleimidopropanoate (0.32 g, 1.2 mmol) plus Et_3N (0.35 g, 3 mmol) in DMF (12 mL), and the resulting mixture was stirred for 12 h. Purification of the final product **1** (following removal of the solvent) was effected by gradient elution chromatography ($MeOH/CHCl_3$, 10→33%). The structures of the protected cholesterol conjugate, the deprotected conjugate, and compound **1** were all affirmed by ^1H NMR spectroscopy, HRMS, and elemental analysis. For example, for **1**: HRMS: calcd for $C_{48}H_{77}O_9N_3$ [M+Li^+]: 846.5820; found 846.5790; elemental analysis: calcd for $C_{48}H_{77}O_9N_3$: C 68.62, H 9.24, N 5.00; found: C 68.47, H 9.36, N 4.81.

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