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Dendrimersomes Exhibit Lamellar-to-Sponge Phase Transitions

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Abstract

Lamellar to nonlamellar membrane shape transitions play essential roles in key cellular processes, such as membrane fusion and fission, and occur in response to external stimuli, including drug treatment and heat. A subset of these transitions can be modeled by means of thermally inducible amphiphile assemblies. We previously reported on mixtures of hydrogenated, fluorinated, and hybrid Janus dendrimers (JDs) that self-assemble into complex dendrimersomes (DMSs), including dumbbells, and serve as promising models for understanding the complexity of biological membranes. Here we show, by means of a variety of complementary techniques, that DMSs formed by single JDs or by mixtures of JDs undergo a thermally induced lamellar-to-sponge transition. Consistent with the formation of a three-dimensional bilayer network, we show that DMSs become more permeable to water-soluble fluorophores after transitioning to the sponge phase. These DMSs may be useful not only in modeling isotropic membrane rearrangements of biological systems but also in drug delivery since nonlamellar delivery vehicles can promote endosomal disruption and cargo release.

Graphical abstract

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Supporting Information

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S.E.W., Q.X., T.B., and V.P., designed the experiments. Q.X. and S.E.S. synthesized the Janus dendrimers. S.E.W. performed all confocal microscopy experiments. Z.T.G. and S.E.W. performed cryo-TEM experiments. The manuscript was written by S.E.W. and T.B. All authors have reviewed the manuscript.

Notes

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INTRODUCTION

Self-assembled amphiphile membranes can serve as valuable models for understanding the complexity of biological membranes because they assemble into a range of structures and phases dictated by (1) intrinsic chemical characteristics such as the hydrocarbon tail length or headgroup structure and (2) externally controllable variables including temperature and water content. Using amphiphiles to model the shape transitions of biological membranes from lamellar to non-lamellar is particularly desirable as these transitions play essential roles in key cellular processes such as membrane fusion and fission, protein activation, and membrane composition regulation.^{1,2} Nonlamellar membrane rearrangements also occur in response to external stimuli such as hypoxia, drug treatment, anesthetics, viral infection, and changes in temperature.^{1–3}

Janus dendrimers (JDs) are one class of synthetic amphiphiles that have been shown to assemble into a diverse range of structures called dendrimersomes (DMSs) with morphologies including cubosomes, disks, tubular vesicles, helical ribbons, and onionlike vesicles.^{4–6} As the word “Janus” describes, JDs are two-faced molecules in that they consist of two structurally and functionally different branches.⁷ Like lipids, the JDs described here are amphipathic, bearing both hydrophobic and hydrophilic branches. The range of structures produced via JD self-assembly, along with the fact that JDs are stable and biocompatible, suggests that they can model membrane rearrangements, including those induced by external stimuli.^{8–10} Understanding how and when these transitions occur also has the potential to better inform the design of drug delivery vehicles that benefit from nonlamellar structures for enhanced cargo release.^{11–13}

We previously reported that hydrogenated (R_H), fluorinated (R_F), and hybrid hydrogenated–fluorinated (R_{HF}) Janus dendrimers self-assemble into a variety of structures dependent on composition.^{14,15} At 20 wt % R_{HF} , maintaining equal weight components of R_H and R_F (40 wt %, respectively), we observed supramolecular JD assemblies that are dumbbell-shaped in which two DMSs, one consisting primarily of R_H and the other of R_F , are surrounded by an exterior shell of R_H dendrimers. Here, we characterize a previously unobserved thermally inducible lamellar-to-sponge transition for these dumbbell-like DMSs and for homogeneous, single-component DMSs.

The sponge (L_3) phase is a macroscopically isotropic multiconnected bilayer that forms a three-dimensional network.¹⁶ The sponge phase has been observed in a range of binary and ternary systems including (1) nonionic surfactant–water, (2) ionic surfactant–water in the

presence of salts, (3) oil–water–surfactant, (4) certain ionized phospholipids, and (5) block copolymers.^{17–19} The phase behavior of these systems is affected by composition, molecular structure, and external stimuli such as temperature.^{18–22} In the case of branched-linear block copolymers, Cho et al. demonstrated that by changing the ratio of two polymers with differing lengths of polystyrene they could dictate the morphology of self-assembled nanostructures in solution, ranging from spheres to sponges to hexasomes. In another example, the binary mixture of pentaerythritol dodecylether (C₁₂E₅) and water, the sponge phase is observed at both high water content and high temperature (>50 °C).^{16,23,24}

The formation of the sponge phase can be explained by considering the spontaneous mean curvature (H_0) of the membrane. At a certain temperature (45–50 °C in the case of C₁₂E₅), the lamellar state is most stable and $H_0 \approx 0$. Increasing the temperature results in the formation of the sponge phase with $H_0 < 0$, i.e., membrane bending toward water. In general, the formation of the L₃ phase can be characterized as one in which the surfactant monolayer of a bilayer exhibits spontaneous curvature toward the bulk solvent and one in which the interbilayer interactions are weak.¹⁶ In fact, the structure of alkyl oligo(ethylene glycol) and that of the linear block copolymers described by Cho et al. are reminiscent of the JDs studied here, supporting our observations. Hydrophobic portions of both molecules are alkyl chains (either fluorinated or hydrogenated in the case of our JDs), and the hydrophilic portions are ethylene glycol repeats.

The sponge phase is typically characterized by small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS), cryo-transmission electron microscopy (cryo-TEM), polarization microscopy, conductivity measurements, NMR, or fluorescence recovery after photobleaching (FRAP).^{23–31} Changes in the permeability of DMSs can also be assessed relaxometrically.^{32,33} DMS heterogeneity in our preparations, in terms of both structure and size, required fluorescently labeled JDs to characterize the lamellar-to-sponge transition using fluorescence polarization, FRAP, and permeability experiments rather than spectroscopic techniques which rely on sample homogeneity. Using these techniques along with cryo-TEM, we demonstrate that the lamellar-to-sponge transition occurs not only for dumbbell-like DMSs of mixed JD composition but also for single-component DMSs made of R_H only or R_F only. Our methods allow for real-time observation of the lamellar-to-sponge transition at specific temperatures using fluorescence confocal microscopy imaging.

EXPERIMENTAL SECTION

Preparation of Dendrimersomes by Film Hydration

A solution of JDs (10 mg/mL, 200 μ L) in THF was deposited on the top surface of a roughened Teflon sheet, with a surface area of 1 cm², in a flat-bottomed vial followed by evaporation of the solvent for 2 h. The Teflon sheet was dried in vacuo for an additional 12 h. PBS (2 mL) was added to submerge the dendrimer film on the Teflon sheet at a final concentration of 1 mg/mL, and the vial was placed in an oven maintained at 60 °C for 12 h for hydration. The sample was then mixed using a vortex mixer for 30 s.

Confocal Fluorescence Microscopy

DMSs were imaged by confocal fluorescence microscopy using the FluoView 3000 scanning system configured on an IX83 inverted microscopy platform (Olympus, Center Valley PA). For images taken at room temperature, a 60× 1.1 NA water-immersion lens was used (Olympus). Imaging chambers (10 μL) containing DMSs were formed between two coverslips (25 × 25 mm², Fisher Scientific) sealed with vacuum grease. For all experiments involving changes in temperature, a 40× 0.75 NA air objective (Olympus) was used since the water immersion lens would act as a heat sink. Imaging chambers (10–15 μL) for heating experiments were made between two circular coverslips of different diameters (22 and 18 mm diameters, respectively) and sealed with vacuum grease and clear nail polish. The imaging chamber was then attached via silicone-based thermal paste to a Peltier device supported between two thin aluminum plates (Figure S1). Temperature was controlled using a PTC-CH series chassis mount temperature controller (PTC5K-CH, Wavelength Electronics, Bozeman, MT) via LabVIEW software (National Instruments, Austin, TX). DMSs containing 7HC were excited at a wavelength of $\lambda = 405$ nm, and emission was monitored at $\lambda = 430$ –470 nm. Those containing NBD were excited at $\lambda = 488$ nm, and emission was monitored at $\lambda = 500$ –540 nm. Those containing RhB were excited at $\lambda = 561$ nm with emission monitored at $\lambda = 570$ –620 nm. Laser intensities were adjusted so that the fluorescence signal was not oversaturated. Image processing and analysis were completed with ImageJ 1.49v software.

Fluorescence Polarization

DMSs (40:40:20 R_H/R_F/R_{HF}) with 1% R_H-RhB and 1% R_F-NBD or with 1% R_H-NBD and 1% R_F-RhB were prepared by film hydration. DMSs were imaged via confocal microscopy as described above with the addition of a linearly polarizing film that was placed beneath the objective lens during imaging. The RhB fluorescence intensity was observed without the polarizing film and in the presence of the film at two angles, differing by 90°. Lamellarity was identified through the observation of alternating bands of RhB fluorescence intensity maxima and minima that shifted by 90° when the polarizing film was rotated by 90°. With a loss of lamellarity, these differences in fluorescence intensity were no longer observed, and rotation of the polarizing film had no effect on the distribution of fluorescence intensity across the DMS.

Fluorescence Recovery after Photobleaching (FRAP)

DMSs consisting of 40:40:20 R_H/R_F/R_{HF} with 1% R_H-RhB and 1% R_F-NBD were prepared by film hydration as described above. Imaging chambers were made with 10 μL of DMS between two glass coverslips (25 × 25 mm²) and sealed with vacuum grease. FRAP experiments were conducted on both onionlike and spongelike DMSs. Onionlike DMSs were identified by confocal fluorescence microscopy as having multiple bilayers that appeared unconnected. The interior layer of the onion was selected as the region of interest for photobleaching (Figure 3B). For experiments involving spongelike DMSs, DMSs were first heated to 50 °C for 10 min in an eppendorf tube and cooled to room temperature before imaging and subsequent FRAP measurements. Photobleaching of RhB was conducted using

both 488 and 561 nm lasers at 100% intensity for 250–350 ms. The recovery of RhB fluorescence intensity was recorded at 3.2 s intervals for 5 min after photobleaching.

Dendrimersome Permeability

DMSs consisting of 40:40:20 $R_H/R_F/R_{HF}$ with 1% R_H -RhB and 1% R_F -NBD were prepared by film hydration as described above. DMSs were mixed with two water-soluble fluorophores of varying molecular weight: CF 405S-amine (CF405S, 1.1 kDa, Millipore Sigma, St. Louis MO) at a final concentration of 0.015 mg/mL in PBS or Cascade Blue-dextran (CB-dex, 10 kDa, Thermo Fisher Scientific, Waltham MA) at a final concentration of 0.1 mg/mL CB-dex in PBS. DMSs were imaged via confocal fluorescence microscopy while heating as described above. CF405S and CB-dex were excited at $\lambda = 405$ nm, and emission was monitored at $\lambda = 430$ –470 nm. The fluorescence intensity was measured both outside and inside each dumbbell-shaped DMS using ImageJ 1.49v software.

Cryo-Transmission Electron Microscopy (Cryo-TEM)

Cryo-TEM images were acquired using a JEOL 2100 TEM microscope at a voltage of 200 kV. A 2 μ L drop of each sample was deposited on a 100 μ m lacey carbon film on a copper mesh grid (Electron Microscopy Sciences, Hatfield, PA). The grid was blotted for 5 s with filter paper to remove excess liquid before plunging the grid into liquified ethane (cooled by liquid nitrogen) to freeze the sample and ensure vitrification of the water in the sample. For heated samples, the sample and grid were kept on a heating block at 60 °C until immediately before blotting and cryo-plunging to prevent significant cooling of the sample before freezing. Vitrified grids were transferred to a cryo-transfer stage immersed in liquid nitrogen for insertion into the TEM microscope. TEM images were acquired at 6–12K magnification. During imaging, the samples were kept below –175 °C to prevent sublimation of the vitrified solvent.

Silanization of Glass Surfaces

Glass coverslips were acid cleaned overnight using a 2% mixture of Nochromix (Godax Laboratories, Cabin John MD) in sulfuric acid. Cleaned slides were washed excessively with water and dried first with nitrogen gas followed by heating to ~150 °C. Dried, cooled slides were then quickly submerged (10 s) in a 4% solution of trichloro(octadecyl)silane (OTS) in toluene. Slides were immediately and rigorously washed with toluene after submersion and dried using nitrogen gas followed by heating for 1 h at 150 °C. Advancing and receding contact angles were imaged using a contact angle goniometer and measured using the contact angle plugin in ImageJ. DMSs were pipetted onto these silanized glass surfaces for subsequent imaging experiments.

RESULTS AND DISCUSSION

Imaging Dendrimersomes at Constant Temperature and during Stepwise Heating

Dumbbell-shaped DMSs were prepared by thin film hydration of a three-component system of JDs made of $R_H/R_F/R_{HF}$ (40:40:20 wt %) from a Teflon sheet. Fluorescently labeled JDs were added to this mixture at 1 wt % each before deposition on the Teflon sheet. JDs with R_H chains were labeled with Rhodamine B (R_H -RhB, red), JDs with R_F chains were labeled

with 7-nitrobenzofurazan (R_F -NBD, green), and JDs with R_{HF} chains were labeled with 7-hydroxycoumarin (R_{HF} -7HC, blue) as previously described (Scheme 1).^{34,35}

These dumbbell-shaped DMSs consisted of two phases, shown as green and red, surrounded by a red shell (Figure 1A). On the basis of previous work, we assumed that the fluorescently labeled molecules accurately report on their environment.^{14,15} Thus, the green phase marked by R_F -NBD primarily consisted of R_F and the red phase marked by R_H -RhB primarily consisted of R_H . R_{HF} -7HC was observed to colocalize preferentially with R_H -RhB (Figure 1). Cryo-TEM and schematic drawings of these dumbbell-shaped DMSs were published previously.¹⁵

Representative dumbbell-shaped DMSs were heated in a stepwise manner and imaged via time-lapse fluorescence confocal microscopy. DMSs prepared at 1 mg/mL were further diluted 1:2 in PBS and then sandwiched between two glass coverslips sealed with vacuum grease and clear nail polish to form the imaging chamber. The imaging chamber was then attached to a Peltier device with thermal paste (Figure S1). DMSs were heated from 25 to 50 °C followed by cooling back to 25 °C in a stepwise manner (Figure 1). By 40 °C, DMSs lost their dumbbell-like shape and transitioned into a core-shell structure in which the core was enriched in R_F -NBD and the shell was enriched in R_H -RhB (Figure 1C). Further heating resulted in the coalescence of features within each phase of the DMS (Figure S2), a transition which appeared to be unstable, followed by another transition in which R_F appeared to wet the R_H core (Figure 1E). Cooling resulted in dewetting and separation of R_F and R_H components (Figure 1F). This transition was irreversible as we did not observe a restoration of the dumbbell shape after cooling, which may be due to the strength of interbilayer interactions. Importantly, these structural transitions were not unique to dumbbell-shaped DMSs. Similar structural transitions were observed for R_H -only and R_F -only DMSs, respectively, implying that the presence of R_{HF} was not a requirement in these transitions (Figure S3).

To elucidate whether this overall transition is unique to single DMSs or to the DMS sample as a whole, we used confocal fluorescence microscopy to characterize the bulk sample preparation before and after heating. Hydration of the $R_H/R_F/R_{HF}$ (40:40:20 wt %) mixture resulted in a distribution of morphologies including dumbbell-shaped, isotropic, and onionlike DMSs (Figure S4). Quantification of three independent DMS preparations via confocal fluorescence microscopy, where uncertainty is expressed as a standard deviation, revealed that $36.1 \pm 1.4\%$ of the observed DMSs were isotropic, with the remaining sample consisting of lamellar, onionlike, core-shell, dumbbell-shaped, or otherwise aggregated or uniquely structured DMSs. Dumbbell-shaped DMSs comprised $2 \pm 1.4\%$ of the sample population; however, their structure remains of interest because of its similarity to biological membranes, representing either a prefusion or prefission stalk depending on the directionality of the pathway.¹⁵ Most interestingly, bulk heating of the $R_H/R_F/R_{HF}$ (40:40:20 wt %) mixture to 60 °C shifted the distribution of DMSs to structures that were isotropic in both shape and composition ($89.3 \pm 11.4\%$ of the total population), suggesting that there is a thermally inducible morphological transition at which the general DMS population loses its morphological heterogeneity. Cooling the bulk sample back to 25 °C after heating did not restore sample heterogeneity, suggesting that the transition is irreversible.

Distinguishing between Lamellar and Nonlamellar Structures Using Fluorescence Polarization Microscopy

We hypothesized that the structural transition observed via confocal fluorescence microscopy imaging is lamellar-to- sponge. Using electron microscopy, we previously showed that $R_H/R_F/R_{HF}$ (40:40:20 wt %) DMSs are lamellar.¹⁵ Here, we used fluorescence polarization measurements to distinguish between lamellar and nonlamellar structures in order to test our hypothesis that both dumbbell-shaped and single-component DMSs lose lamellarity upon heating (Figure 2). As described above, an imaging chamber containing $R_H/R_F/R_{HF}$ (40:40:20 wt %) with 1% R_F -NBD and 1% R_H -RhB DMSs was attached to a Peltier device. Dumbbell-shaped DMSs were imaged during heating in the presence or absence of a linear polarizer. Fluorescent molecules with transition dipole moments oriented parallel to the electric field vector of the linearly polarized light exhibited maximum fluorescence intensities. In the lamellar state, this resulted in alternating bands of fluorescence intensity maxima and minima since molecules are structurally oriented in a lamellar membrane. We observed these bands of fluorescence intensity for R_H -RhB at 25 °C in a representative dumbbell- shaped DMS (Figure 2C, white and gray arrows). Rotating the linear polarizer by 90° resulted in a shift in fluorescence maxima and minima by 90° (Figure 2D, white and gray arrows). During and after heating, we observed that the rotation of the linear polarizer had no effect on fluorescence intensity (Figure 2C,D, columns 2–4), which indicated a loss of lamellarity as fluorescent molecules became randomly distributed across the DMS.

To confirm that the R_F phase of these DMSs also undergoes a lamellar-to-sponge transition upon heating, we synthesized new fluorescent JDs in which R_F was labeled with RhB and R_H was labeled with NBD. These JDs were used to make DMSs composed of $R_H/R_F/R_{HF}$ (40:40:20 wt %) with 1% R_H -NBD and 1% R_F -RhB. NBD is likely not rotationally restricted in the DMS membrane in the lamellar state due to its longest axis being approximately 3 times shorter than RhB, which prevents it from being a useful marker in fluorescence polarization experiments. Therefore, we used the RhB fluorescence intensity as a marker for lamellarity and measured the fluorescence intensity of R_F -RhB during the heating of dumbbell-shaped DMSs. These measurements confirmed that the R_F component of the dumbbell-shaped DMSs transitions from lamellar to sponge during heating (Figure S5). This lamellar-to-sponge transition was also observed for single-component R_H DMSs labeled with 1% R_H -RhB (Figure S6).

Characterizing Lamellar and Spongelike Structures Using FRAP

To confirm the loss of lamellarity upon heating, we compared fluorescence recovery patterns using FRAP. FRAP measurements were conducted on a representative two- layer onion and on a spongelike DMS (Figure 3). A single interior layer of an onion made of $R_H/R_F/R_{HF}$ (40:40:20 wt %) with 1% R_F -NBD and 1% R_H -RhB was photobleached, and fluorescence recovery was monitored over time. No recovery was observed within the bleached layer due to a lack of connection between layers of the onion (Figure 3B). On the other hand, complete recovery was expected after photobleaching a region of a spongelike DMS since the bilayer network is interconnected. DMSs made of $R_H/R_F/R_{HF}$ (40:40:20 wt %) with 1% R_F -NBD and 1% R_H -RhB were heated to 50 °C for 10 min before photobleaching (Figure

3A). The recovery of R_H -RhB was measured over time. Fluorescence recovery was observed in the bleached region, confirming that these structures are not lamellar but are in fact spongelike (Figure 3A).

Monitoring DMS Permeability Changes Using Water- Soluble Dyes

The lamellar-to-sponge transition is expected to coincide with an increase in DMS permeability, dependent on the size of pores formed in the sponge phase. To measure changes in permeability upon heating, DMSs made of $R_H/R_F/R_{HF}$ (40:40:20 wt %) with 1% R_F -NBD and 1% R_H -RhB were mixed with either a small water-soluble dye, CF405S (1.1 kDa) at 0.015 mg/mL, or with Cascade Blue-dextran (CB-dex, 10 kDa) at 0.1 mg/mL. DMSs were then heated, and the fluorescence intensity of either CF405S or CB-dex was measured in the interior of the dumbbell-shaped DMS during heating. Consistent with the loss of lamellarity and the formation of the sponge phase, dumbbell-like DMSs became permeable to both molecules during heating (Figure 4). DMSs became more permeable to the smaller water-soluble fluorophore, CF405S, as compared to the larger CB-dex.

Measuring Structural Transitions of Dumbbell-like DMSs on Silanized Glass Surfaces

To exclude the possibility that the thermally inducible lamellar-to-sponge transition is dependent upon the adherence of DMSs to a glass surface, we conducted additional experiments using silanized glass coverslips. Coverslips were acid cleaned and then incubated with trichloro(octadecyl)silane (OTS) before use. Successful silanization was confirmed by performing contact angle measurements. Advancing and receding contact angles on silanized surfaces were measured to be $101.1 \pm 4.2^\circ$ and $102.4 \pm 3.1^\circ$, respectively, where error is expressed as the standard deviation. These measurements are in good agreement with each other, indicating homogeneous silanization, and are equivalent to published values.³⁶ DMSs prepared with $R_H/R_F/R_{HF}$ (40:40:20 wt %) and 1% R_F -NBD and 1% R_H -RhB were imaged as described above using these silanized glass coverslips. Structural changes were observed via confocal fluorescence microscopy as the dumbbell-shaped DMS was heated and cooled (Figure S7). These structural changes were identical to the changes observed using an untreated glass surface.

Visualizing DMSs Using Cryo-TEM

Visual confirmation of spongelike DMSs was obtained via cryo-TEM. DMSs prepared with $R_H/R_F/R_{HF}$ (40:40:20 wt %) were heated to 60 °C immediately before freezing and imaging. A greater fraction of spongelike DMSs was observed in the heated sample as compared to the unheated sample (Figure 5).

SUMMARY AND CONCLUSIONS

Using confocal fluorescence microscopy, we have demonstrated thermally inducible structural changes in Janus dendrimersomes. We observed these transitions by fluorescence polarization, FRAP, permeability experiments, and cryo-TEM, all of which suggest that this transition is lamellar-to-sponge. Increasing temperature in our system results in membrane bending toward water ($H_0 < 0$) and fusion events between bilayers forming the interconnected three-dimensional network that is characteristic of the sponge phase.^{16,23} As

described in theoretical detail by Anderson et al., the volume fraction of this bilayer network is determined by the product of the spontaneous curvature of the monolayer (H_0) and half the width of the bilayer (L). Furthermore, the sponge phase is favored over the cubic phase when interbilayer interactions are weak.¹⁶ Our experimental evidence supports a lamellar-to-sponge rather than a lamellar-to-cubic transition, but we note that cubosomes have been observed via the self-assembly of other JDs.^{4,6}

The lamellar-to-sponge transition described above was found to be irreversible with respect to temperature cycles. We hypothesize that the morphology of initial DMS assemblies formed during thin film hydration can be explained by a change in the hydration state of the membrane (i.e., swelling). During this swelling stage, the water chemical potential is not constant in the system as water availability is limited, and thus DMSs form due to an initially low hydration state. Subjecting DMSs to cycles of heating and cooling after initial assembly (i.e., high hydration) results in the observed lamellar-to-sponge transition. During these temperature cycles, water is present in excess, and we believe that this difference in water chemical potential explains why we do not recapture the initial composition of assemblies observed immediately after thin film hydration. Although structural transitions involving changes in hydration can be reversible (e.g., water-*n*-decane- $C_{12}E_5$ and glycerol monooleyl ether-propylene glycol-water systems), such transitions are dependent upon temperature, composition, and water content.^{23,29} In our system, the initial swelling process resulting in DMS formation is irreversible.

Despite this irreversibility, changes in membrane curvature observed in this lamellar-to-sponge transition may be caused by entropy-driven dehydration of the oligo(ethylene glycol) units that make up these JDs. At elevated temperatures, the hydration number per monomer of poly(ethylene glycols) (PEGs) is known to decrease, with phase separation occurring above the lower critical solution temperature (LCST).^{37,38} The thermosensitive behavior of PEG and similar polymers has been exploited to control the aggregation state and phase transitions of other microstructures by tuning polymer LCSTs.^{39,40} In the case of our DMSs, partial dehydration of the oligo(ethylene glycol) units as temperature increases may induce curvature changes that promote the lamellar-to-sponge transition. We would expect the membrane to be in a partially dehydrated state during these temperature cycles, and since water remains in excess as described above, we do not expect to observe the initial composition of DMS assemblies.

As our DMSs transitioned from lamellar to sponge, we observed characteristic structural changes including the independent coalescence of hydrogenated and fluorinated dendrimers within single DMSs between 40 and 45 °C and a wetting transition at 50 °C (Figure 6). Ultimately, a dewetting transition occurs upon cooling resulting in the formation of two distinct isotropic spheres. The distribution of dendrimers is similar to that observed in aqueous two-phase systems (ATPS) containing giant vesicles (GVs). In ATPS-containing GV, for example, one in which PEG and dextran are encapsulated within a GV, changing the polymer concentration affects the wetting transition. In our work, increases in temperature induce a wetting transition of two sponge phases, giving rise to the observed structural transitions.^{41–43}

The thermally inducible lamellar-to-sponge transition observed among Janus dendrimersomes is a structural change that has implications in drug delivery and can provide insight into isotropic membrane rearrangements that occur in biological membranes. The potential utility of DMSs in therapeutic applications has recently been established. Importantly, Nummelin et al. demonstrated the ability of DMSs to encapsulate propranolol while Filippi et al. validated the use of DMSs as prednisolone phosphate drug carriers and MRI contrast agents in vivo.^{34,35} DMSs with nonlamellar structures as described here have the potential to serve as delivery vehicles with enhanced endosomal disruption and cargo release, a strategy which has been effective at enhancing gene silencing via siRNA in vitro in other delivery systems.^{11–13} Introducing lipids (e.g., DOPE) into drug delivery vehicles that are prone to form nonlamellar structures has also been used to promote cargo release upon ultrasound exposure.⁴⁴ Furthermore, the development of thermosensitive liposomes suggests that heat can similarly be used to trigger cargo release from other drug delivery vehicles.⁴⁵ In fact, Thermodox, a lysolipid- containing thermosensitive liposome encapsulating doxorubicin, is in phase III clinical trials for the treatment of primary liver cancer.^{46,47} Similarly, we envision that heat may be used to trigger the release of cargo from DMSs.

Not only can we thermally induce a lamellar-to-sponge phase transition, but using these synthetic amphiphiles we also have the potential to control sponge formation by tuning the spontaneous curvature of the membrane in order to generate a range of thermally inducible spongelike structures.⁴⁸ Such control has been possible in analogous block copolymer systems by tuning characteristics such as the branch architecture, polymer length, and block copolymer ratio, suggesting that it would be possible to create a library of DMSs with distinct morphologies and thermally inducible transitions.^{17,49,50}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Dumbbell-like DMSs undergo structural changes upon heating. Representative confocal microscopy images of a single dumbbell-like DMS heated in a stepwise manner to 50 °C (A–E) and cooled to 25 °C (F). The DMS composition was $R_H/R_F/R_{HF}$ (40:40:20 wt %) and 1 wt % each of R_H -RhB (column 1), R_F -NBD (column 2), and R_{HF} -7HC (column 3). $N=20$; scale bars = 5 μm .

Figure 2.

Fluorescence polarization experiments following R_H -RhB confirm the loss of lamellarity during heating and cooling of a representative dumbbell-like DMS. Fluorescence polarization comparison of a single dumbbell-like DMS heated to 50 °C (columns 1–3) and cooled to 25 °C (column 4). Representative confocal microscopy images show the DMS labeled with R_H -RhB and R_F -NBD merged (A) or R_H -RhB only in grayscale (B). Applying a linear polarizer at 0° (C) versus rotated 90° (D) shows bands of higher fluorescence intensity (indicated by white arrows) dependent on the orientation of the polarizer, indicating the lamellar organization of the membrane. Gray arrows represent bands of lower intensity. The orientation of white and gray arrows switches upon rotation of the linear polarizer. The loss of lamellarity was observed as the DMS was heated and cooled. The DMS composition was $R_H/R_F/R_{HF}$ (40:40:20 wt %) and 1 wt % each of R_H -RhB and R_F -NBD. $N = 4$; scale bars = 5 μm .

Figure 3.

Photobleaching recovery occurring in a spongelike region of a heated DMS is consistent with the formation of a sponge phase. Representative photobleaching recovery experiment performed on a heated DMS (A) and on a layer of an onionlike DMS (B). Photobleaching regions are indicated by the white dotted circle (A) or white arrow (B). In the spongelike DMS (A), the fluorescence intensity in the photobleached region recovered to reach the fluorescence intensity of the background whereas the fluorescence intensity of the bleached layer of the two-layer onion did not recover (B). $N = 5$; scale bars = $5 \mu\text{m}$.

Figure 4.

Dumbbell-like DMSs become permeable to dye upon heating, consistent with formation of a sponge phase. Dumbbell-like DMSs consisting of $R_H/R_F/R_{HF}$ (40:40:20 wt %) and 1 wt % each of R_H -RhB and R_F -NBD (A, C) were incubated with either a water-soluble fluorophore (CF405S, 1.1 kDa) or Cascade Blue-labeled dextran (CB-dex, 10 kDa). Initially, CF405S (B) and CB-dex (D) were excluded from the interior of the DMS at 25 °C. As each DMS was heated to 40 °C, it became increasingly permeable, and the fluorescence intensity of CF405S (E, F) and CB-dex (G, H) in the interior of the DMS increased. Error bars represent standard deviations. $N=3$; scale bars = 5 μm .

Figure 5.

Spongelike DMSs observed via cryo-TEM. Cryo-TEM was performed on DMS preparations of $R_H/R_F/R_{HF}$ (40:40:20 wt %) with 1 wt % each of R_H -RhB, R_F -NBD, and R_{HF} -7HC that were either bulk heated to 60 °C or not. Representative image of a spongelike DMS observed in the heated sample (A) as compared to representative lamellar DMSs observed in a nonheated DMS preparation (B). Scale bars = 100 nm.

Figure 6.

Schematic of structural transitions observed during heating and cooling of a dumbbell-like DMS. Schematic of proposed structural transitions occurring while heating a dumbbell-like DMS consisting of $R_H/R_F/R_{HF}$ (40:40:20 wt %) to 50 °C followed by cooling to room temperature. The dumbbell-like DMS changes from a lamellar state to one that is spongelike as the DMS is heated. Cooling does not restore lamellarity.

Scheme 1.

Structure of Janus Dendrimers with Hydrogenated Chains (R_H), Fluorinated Chains (R_F), and Hybrid Hydrogenated-Fluorinated Chains (R_{HF}), Each Labeled with the Fluorophores Rhodamine B (RhB, Red), 7-Nitrobenzofurazan (NBD, Green), or 7-Hydroxycoumarin (7HC, Blue)