Spontaneous Alternating Copolymer Vesicles of Alkylmaleimides and Vinyl Gluconamide

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ABSTRACT: Alternating copolymers of *N*-*n*-alkylmaleimides and vinyl gluconamide spontaneously form ultrasmall- and medium-sized (10−20 and 50−300 nm, respectively) vesicles when dissolved in water at room temperature. These materials have molecular weights that are approximately 100 times higher than those of previously reported alternating oligomers of alkylmaleate and vinyl ether monomers and conclusively demonstrate that alternating copolymers can form vesicles. The size and shape of the vesicles are thoroughly characterized by cryogenic transmission electron microscopy (cryo-TEM), dynamic light scattering (DLS), and small-angle neutron scattering (SANS). The copolymer vesicles exhibit alkyl-chain-length-dependent release characteristics and bilayer thickness (1.7, 2.0, and 2.6 nm for alkyl chains of 10, 12, and 14 carbons, respectively).

Introduction

Liquid-core capsules have drawn significant attention from biomedical, pharmaceutical, cosmetic, flavoring, and agriculture industries for encapsulation and release applications. Fabrication methods have been well studied, and common techniques for obtaining such capsules include emulsion polymerization,[1−5] intrabilayer polymerization of vesicles,[6,7] self-assembly of colloidal particles[8] and amphiphilic macromolecules,[9] and cross-linking of polymers absorbed at oil−water interfaces.[10] Lipid- and surfactant-based vesicles, whose flexible shells can be advantageous compared with the often rigid capsules obtained through interfacial polymerization, have also been widely investigated throughout scientific literature. However, polymer chemistry offers an extensive library of materials compared with lipids, and with the initial report of block copolymer vesicles by Discher et al.,[11] polymeric vesicles, dubbed polymersomes, have elicited broad interest ranging from fundamental research of bilayer physics[12] to practical controlled delivery applications using a diverse collection of hydrophilic and hydrophobic polymer blocks.[13−19]

Last year, a new class of vesicle-forming polymers was reported wherein alternating sequences of hydrophilic and hydrophobic monomers yield copolymers with laterally derived amphiphilicity that self-assemble into vesicles.[20] In particular, alternating copolymers of alkylmaleates and vinyl gluconamide were shown to form vesicles upon dissolution in water, and ultrasmall sizes of 10−20 nm in diameter could be obtained through horn sonication. However, the molecular weights of these alternating copolymers, or rather, co-oligomers, were on the order of a few kilodaltons, suggesting only three to nine repeat units. The influence of the charged initiator, tethered on one or both ends of the oligomers, could also be significant. Therefore, it was uncertain whether alternating copolymers of significant molecular weight could form vesicles.

This question is addressed here by studying the vesicle-forming properties of alternating copolymers made from alkylmaleimides and vinyl ethers. Systems of this monomer pair have been reported to polymerize rapidly and alternately when the initial monomer ratio is equimolar[21,22] or when the vinyl ether is in excess.[23,24] Assuming comparable termination rates, we expected that higher-molecular-weight alternating polymers could be obtained using monomers with these functional groups. Moreover, because maleimides absorb UV light to self-induce free radical polymerization,[22−24] no additional initiator is necessary, and this eliminates potential end-group contributions to the capacity of these alternating polymers to form vesicles.

Monomers of *N*-*n*-alkylmaleimides (MI) and sugar-derived vinyl gluconamide (VG) were synthesized and polymerized to high molecular weight, and the resulting copolymers spontaneously formed vesicles when dissolved in water. The size, shape, and bilayer thickness of the vesicles were investigated by cryo-TEM, DLS, and SANS, whereas we examined potential applications of these vesicles by studying the controlled release of encapsulated dye and the transfection of mammalian cells with a green fluorescent protein (GFP) vector.

Experimental Section

Materials. All chemicals and solvents were purchased from either Sigma-Aldrich or Acros Organics/Fisher Scientific and used without further purification. Reactions were performed under an argon atmosphere in oven-dried glassware. Acetylene gas (99.6%) was obtained from Wright Brothers.

Synthesis. 2-(Vinyloxy)ethylamine (**1**) was synthesized by the addition of ethanolamine to acetylene gas.[25] A three-necked round-bottomed flask was fitted with a jacketed short path distillation unit and charged with ethanolamine (40 g) and sodium hydroxide (2 g). The system was purged with argon for 1 h and heated to 140 °C, upon which sodium hydroxide dissolved and the solution became clear. Acetylene gas was bubbled through the liquid with rapid stirring, and the crude product was collected via simultaneous distillation over 19 h and was distilled twice under reduced pressure to yield (**1**) (10.7 g, 19%). ^{1}H NMR (D_2O, 250 MHz, δ): 2.79 (t, J = 5.3 Hz, 2H), 3.72 (t, J = 5.3 Hz, 2H), 4.06 (d, J = 6.6 Hz, 1H), 4.27 (d, J = 14.4 Hz, 1H), 6.45 (dd, J = 14.4, 6.6 Hz, 1H). HRMS (ESI, +): calcd for $C_4H_{10}NO$ (M + 1), 88.0757; found, 88.0757.

2,3,4,5,6-Pentahydroxy-*N*-[2-(vinyloxy)ethyl]hexanamide (vinyl gluconamide, VG) (**2**) was synthesized from δ-gluconolactone and (**1**), as previously described.[20,26] Briefly, (**1**) (8 mL, 83.4 mmol) was added to a suspension of δ-gluconolactone (13.5 g, 75.8 mmol) in methanol (50 mL) and refluxed for 1 h. Upon cooling, a white

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solid precipitated, was collected by suction filtration, and was recrystallized from methanol to give (**2**) (10.5 g, 51%).

N-*n*-Alkylmaleimides (**4a**−**c**) were synthesized by ring-opening addition to maleic anhydride, followed by ring-closing amide condensation.[27] In a 50 mL three-necked round-bottomed flask fitted with a condenser, maleic anhydride (4.59 g, 46.9 mmol) was suspended in 10 mL of anhydrous dichloromethane. A suspension of *n*-tetradecylamine (10.0 g, 46.9 mmol) in 20 mL of anhydrous dichloromethane was added incrementally, and the reaction mixture was refluxed for 30 min, where it partially solidified. The mixture was chilled, and *N*-*n*-tetradecylmaleamic acid (**3c**) was collected by suction filtration, washed with cold dichloromethane, and dried (13.3 g, 91%). ^{1}H NMR ($CDCl_3$, 250 MHz, δ): 0.90 (t, $J = 6.6$, 3H), 1.28 (m, 22H), 1.60 (app quintet, $J = 6.8$, 2H), 3.38 (q, $J = 6.8$, 2H), 6.28 (d, $J = 12.9$, 1H), 6.36 (d, $J = 12.9$, 1H), 7.10 (br s, 1H). HRMS (ESI, +): calcd for (M + 1), 312.2539; found, 312.2531.

The maleamic acid product (10 g, 32 mmol) was added to a 100 mL round-bottomed flask along with sodium acetate (1.8 g, 22 mmol) and 20 mL of acetic anhydride. The mixture was heated to 100 °C for 2 h. After cooling, 50 mL water was added, and the contents were transferred to a separation funnel where the product was extracted with diethyl ether (2 × 30 mL) and washed with 2% KOH (2 × 30 mL) and water (1 × 30 mL). The organic phase was concentrated by rotary evaporation and dried overnight. The solid residue was dissolved in 20 mL of dichloromethane, dried with $MgSO_4$, cold filtered, and again concentrated by rotary evaporation and vacuum drying. The crude product was recrystallized from ethanol to yield *N*-*n*-tetradecylmaleimide (**4c**) (7.18 g, 76%). ^{1}H NMR ($CDCl_3$, 250 MHz, δ): 0.90 (t, $J = 6.6$, 3H), 1.28 (m, 22H), 1.59 (m, 2H), 3.52 (t, $J = 7.3$, 2H), 6.7 (s, 2H). HRMS (ESI, +): calcd for (M + 1), 294.2428; found, 294.2457.

N-*n*-Dodecylmaleimide and *N*-*n*-decylmaleimide were obtained in an analogous fashion to *N*-*n*-tetradecylmaleimide, except the latter was recrystallized from petroleum ether. *N*-*n*-Dodecylmaleimide (**4b**) (46%): ^{1}H NMR ($CDCl_3$, 250 MHz, δ): 0.90 (t, $J = 6.6$, 3H), 1.28 (m, 18H), 1.58 (m, 2H), 3.52 (t, $J = 7.3$, 2H), 6.70 (s, 2H). HRMS (ESI, +): calcd for (M + 1), 266.2115; found, 266.2113. *N*-*n*-decylmaleimide (**4a**) (63%): ^{1}H NMR ($CDCl_3$, 250 MHz, δ): 0.90 (t, $J = 6.6$, 3H), 1.28 (m, 14H), 1.59 (m, 2H), 3.52 (t, $J = 7.3$, 2H), 6.70 (s, 2H). HRMS (ESI, +): calcd for (M + 1), 238.1802; found, 238.1806.

Free Radical Polymerization. All polymerization reactions were performed without additional photoinitiators. MI and VG monomers were dissolved in methanol in equimolar amounts, purged with argon, and immersed in a water bath at 60 °C. Polymerization was induced by UV irradiation (365 nm, output 900 μW/cm^2) at a distance of 1 cm for 20 h. The polymer product was dried under vacuum, dissolved in a small amount of methanol, precipitated with diethyl ether, and dried. The partially purified copolymer was dissolved in deionized water (Milli-Q, 18.2 MΩ cm) by magnetic stirring at room temperature and centrifuged at 2400*g* for 15 min to remove dust, debris, and insoluble material. The clear supernatant was carefully removed and fully dried to yield the copolymer product. Losses from centrifugation were between 0.8 and 3.6 wt %, and overall yields of the copolymers were about 40%.

We verified the conversion of MI groups by measuring absorption at 300 nm before and after polymerization using a Cary 50 Bio UV−vis spectrophotometer (Varian). Values are reported as a percentage of initial absorption. Molecular weights were determined by gel permeation chromatography (GPC) using a Viskotek HPLC system with three single-pore columns (PolyAnalytik, Canada) and DMF with 0.2 M LiBr at 55 °C as eluant. Samples were injected at 0.6 mL/min and analyzed by triple detection (light scattering, differential refractive index, and viscometry) to obtain absolute molecular weight distributions. Samples were analyzed twice to confirm reproducibility.

Vesicle Preparation. Aqueous vesicle solutions were prepared by bulk hydration of copolymer powder in deionized water. Solutions were stirred overnight, although they dissolved completely within 1 h to yield clear, blue-tinted solutions.

Characterization. SANS spectra were acquired at Argonne National Laboratory's Intense Pulsed Neutron Source using a small-angle neutron diffractometer with wavelengths between 1 and 14 Å (q range 0.0035 to 0.6 Å^{-1}) and 2 mm quartz cells. Samples were prepared in D_2O (1 wt % polymer) to minimize incoherent scattering and enhance the scattering contrast between the polymer and solvent. Background correction and data reduction were performed using the onsite instrument software. DLS measurements were performed on an ALV/LSE-5003 CGS-3 instrument at 90° angle using a HeNe laser. Intensity correlation was acquired by ALV Correlator ver. 3.0A, and number-weighted distributions were calculated through CONTIN analysis for vesicles using bilayer thickness information obtained from SANS.

We obtained cryo-TEM images by preparing samples in the controlled environment vitrification system (CEVS)[28] at 24 °C and at water saturation to avoid evaporation. A 7.0 μL drop of each solution was placed on a TEM copper grid covered with a perforated carbon film (Pelco International) and blotted with a filter paper to form a thin liquid film of the sample (100−200 nm thick). Vitrification was achieved by plunging the thinned sample into liquid ethane at its freezing temperature (−183 °C). The vitrified specimen was transferred to liquid nitrogen (−196 °C) for storage until examination. Specimens were studied in a Philips CM120 transmission electron microscope (Philips, The Netherlands) operating at an accelerating voltage of 120 kV with an Oxford CT3500 (Oxford Instruments, U.K.) cryo-holder operated below −175 °C. Digital images were recorded on a cooled Gatan MultiScan 791 CCD camera (Gatan, U.K.) using the DigitalMicrograph 3.1 software (Gatan, U.K.). Imaging was done in the low-dose mode to minimize beam exposure and electron-beam radiation damage.[29]

Controlled Release. The release behavior of the vesicle systems was examined by encapsulation and release of the fluorescent dye rhodamine B (λ_{ex} 554 nm, λ_{em} 575 nm). We encapsulated the dye by hydrating the copolymer with an aqueous solution of rhodamine B (5.2×10^{-6} g/mL) and stirring the mixture overnight. After preparation, 2 mL was transferred to 25 000 MWCO regenerated cellulose dialysis tubing (Spectrum Laboratories) and immersed in 80 mL of fresh deionized water. The bulk solution was assayed for rhodamine B content at periodic intervals using a Cary Eclipse fluorescent spectrophotometer (Varian). The control experiment was done in an identical manner but without copolymer encapsulation. Release of rhodamine B from the vesicles is reported as a percentage of the total amount released from the control. Error bars represent one standard deviation of triplicate independent experiments.

Transfection and Cytotoxicity. For transfection of mammalian cells, we cationically modified C10 copolymer by substituting 25 mol % of VG with *N*-ethyl-*N*,*N*-dimethyl-2-(vinyloxy)ethylammonium chloride[20] (cat-VE) during polymerization. This modification introduced positive charge to promote complex formation with the negatively charged phosphate groups in the backbone of DNA.

Solutions of this cationic copolymer (C10 25%M+) were prepared at 0.2 wt % in sterile, DNase- and RNase-free water (Fisher Scientific) by stirring at room temperature. GFP mammalian expression vector gWiz-GFP (Genlantis) was amplified by *Escherichia coli* and purified using a GenoPure Plasmid Maxi Kit (Roche Applied Science). The transfection reagent was prepared by diluting 50 μL of vesicle solution with 50 μL of serum-free Opti-MEM media (Invitrogen) and incubated for 5 min at room temperature. The solutions were then mixed with 1.5 μg pDNA in 100 μL of serum-free media and incubated for 20 min at room temperature to form polyplexes. NIH 3T3 fibroblasts were seeded 1 day before transfection at 1×10^5 cells/well in 12-well plates and incubated at 37 °C and 5% CO_2 in media supplemented with 10% newborn calf serum (NCS) to achieve 80−90% confluency. On the day of transfection, cells were washed with PBS and supplemented with 200 μL of serum-free media and 200 μL of the polyplexes. The cells were incubated at 37 °C and 5% CO_2 for 7 h, after which the media was replaced with media containing 10% NCS and incubated for 24 h. GFP expression was observed by fluorescence microscopy (λ_{ex} 470−480 nm, λ_{em} 510 nm) and quantified by flow cytometry 24 h after transfection, acquiring 20 000 events for each experiment.

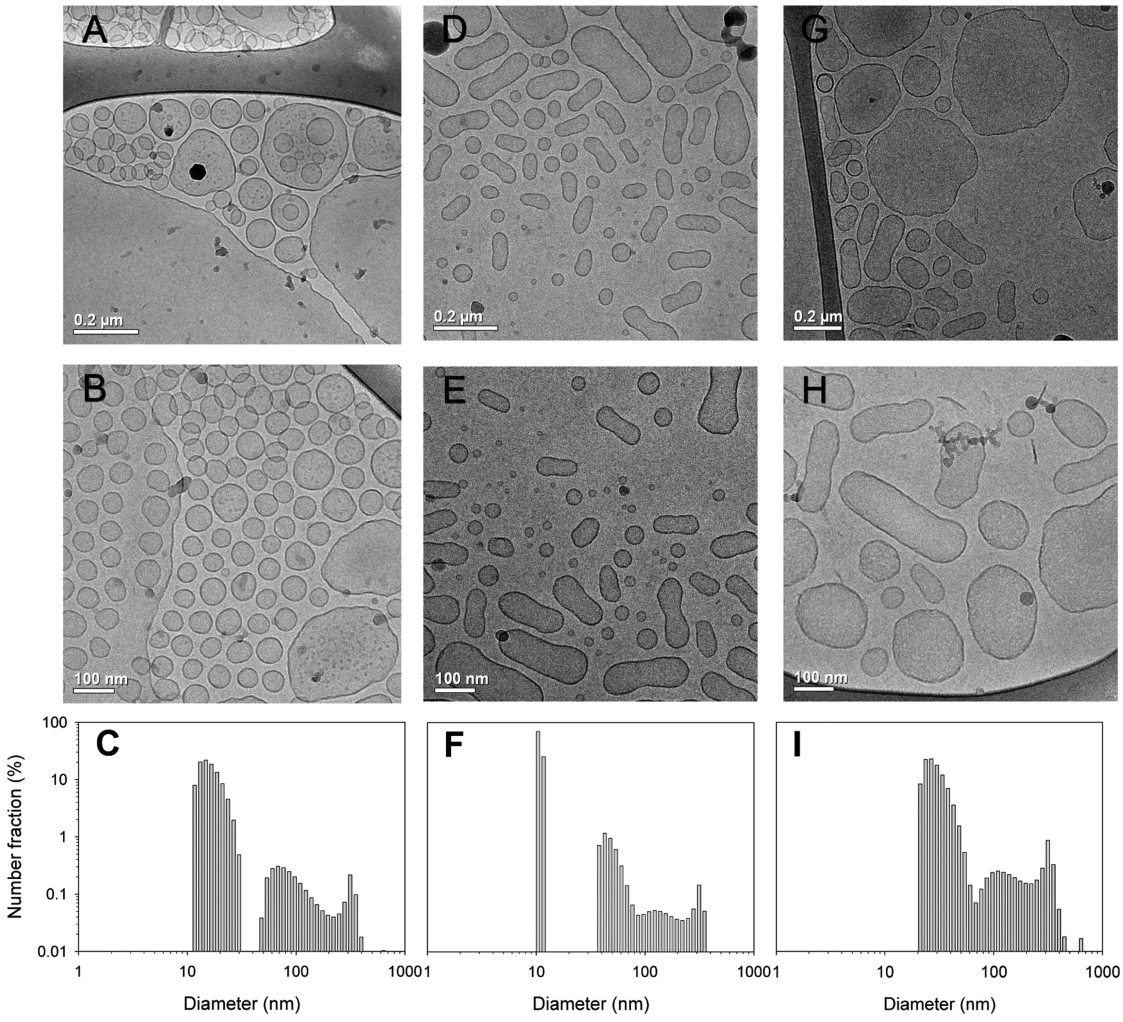


Figure 1. Cryo-TEM images and DLS measurements of vesicle size distributions of (A, B, C) C10, (D, E, F) C12, and (G, H, I) C14 alternating copolymers.

Lipofectamine 2000 (Invitrogen) was used as a positive control following the manufacturer's instructions with an identical amount of pDNA. Untreated cells and cells treated with "naked" pDNA served as negative controls. Cells receiving naked pDNA were treated analogously to cells treated with the polyplexes. All experiments were performed in triplicate.

Cytotoxicity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[30] Briefly, 24 h after transfection 100 μL of 0.5% MTT in PBS was added to each well and incubated for 3 h. The medium was removed, and the purple formazan crystals were dissolved in 3 mL of DMSO. Solutions were centrifuged at 2400g for 5 min, and absorption was measured at 545 nm. Cell survival is reported as a mean percentage of the control group, and error bars represent one standard deviation of triplicate independent experiments.

Results and Discussion

Polymerization. The copolymers recently prepared from the alkylmaleate−VG system had low molecular weights, bringing into question whether vesicle formation is limited to oligomers with alternating side-chain functionality.[20] Listed in Table 1, the molecular weights of the MI−VG copolymers are approximately 100 times higher than their maleate counterparts, as expected from their rapid polymerization kinetics and presumably comparable termination rates. UV−vis measurements confirmed that the reactions went to high conversion of MI groups in 20 h. The polydispersity index, M_w/M_n, however, was found to be unusually low (less than 1.1 in all cases) for a free radical polymerization mechanism.[31] To remove unreacted monomers and contaminants, the copolymer product was precipitated from methanol and dissolved in water, followed by centrifugation. This narrow distribution may be partly due to these purification steps, which resulted in low overall yields of 35−40%. Nonetheless, the polydispersity was unusually low following just two liquid fractionations, which prompted us to verify that the polymerization is nonliving; polymerization of the monomers ceases when the UV light source is removed.

Table 1. Maleimide Conversion, Overall Yield, And Molecular Weight of The Alkylmaleimide-*co*-vinyl Gluconamide Polymers

copolymer	maleimide	conversion	yield	M_w, kDa
C10	*N*-*n*-decylmaleimide	92%	35%	127
C12	*N*-*n*-dodecylmaleimide	91%	38%	353
C14	*N*-*n*-tetradecylmaleimide	88%	37%	290

Vesicle Size and Structure. Alkylmaleimide−VG copolymer vesicles form readily at room temperature with magnetic stirring, whereas vesicles from the previously reported alkylmaleate−VG system required elevated temperatures and the inclusion of a small amount of cationic monomer to promote solubility.[20] We suspect that the single hydrocarbon tail of the alkylmaleimides, in comparison with the two hydrocarbon tails of alkylmaleates, results in greater flexibility of the polymer chain, enabling water to access the hydroxyl groups of VG more readily and promote hydration and self-assembly of the bilayer at room temperature.

The size distributions of the vesicle systems were characterized by DLS and cryo-TEM. Shown in Figure 1, DLS

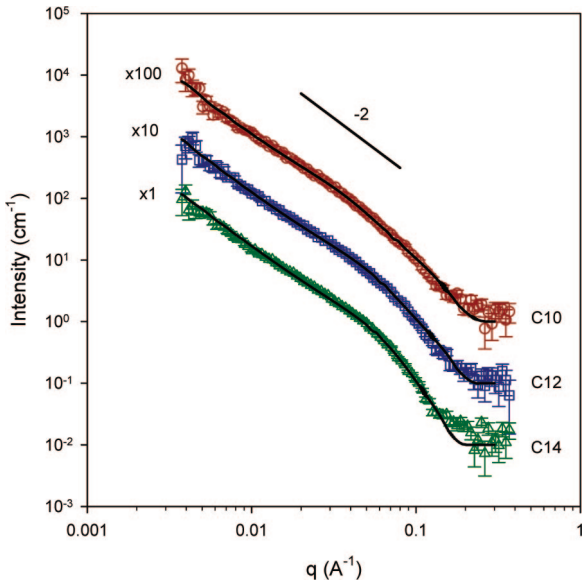


Figure 2. Symbols: SANS data for the copolymer vesicles in D_2O, exhibiting $I \propto q^{-2}$ behavior characteristic of bilayer structures. Lines: model calculations using a bimodal distribution of polydisperse spheres from DLS measurements and a core−shell form factor. Data sets vertically scaled for clarity.

measurements yield a bimodal distribution of ultrasmall- and medium-sized (10−20 and 50−300 nm, respectively) vesicles. Cryo-TEM images are in good agreement with the DLS results, and two representative images for each sample are shown to illustrate the bimodal size distributions. Ultrasmall vesicles are clearly observed in Figure 1D,E, whereas numerous vesicles with diameters between 50 and 100 nm can be observed in Figure 1B. Overall, the size distributions of the spontaneously formed vesicles are rather insensitive to the chain length of the alkyl hydrophobe present. Although limited by imaging resolution, it is evident that the bilayer thicknesses of the vesicles are <5 nm. A more precise determination of the bilayer thickness comes from the analysis of the SANS spectra of the vesicles.

SANS data for the three copolymer vesicle solutions in D_2O are plotted in Figure 2. All samples exhibit $I \propto q^{-2}$ behavior, which is characteristic of thin sheets and vesicular structures.[7,32−34] The thickness of the vesicle bilayer may be calculated from the high q turnover of the $I \propto q^{-2}$ region using a Kratky−Porod plot and the Guinier approximation,[34−36] although it has been suggested that this simplified method may be useful in calculating only relative changes in thickness rather than absolute values.[37] To obtain the vesicle bilayer thickness, we developed a model based on Vrij's equations for scattering from mixtures of hard spheres[38] using core−shell form factors and two Schulz distributions adopted from DLS measurements. Model parameters were constrained by the known concentration of polymer and the relative fraction of medium-sized vesicles to ultrasmall vesicles, as determined from DLS. The scattering length density of the bilayer was assumed to be homogeneous over the entire thickness.

The average diameters, standard deviations, and bilayer thicknesses calculated from the SANS model are summarized in Table 2, and the corresponding model SANS spectra are shown as the solid lines in Figure 2. Departures from $I \propto q^{-2}$ at both low and high q due to the finite size of the vesicles are accurately captured by the model. The average vesicle diameters determined from SANS and DLS are in agreement to within their expected accuracy. Also, the bilayer thicknesses of 1.7, 2.0, and 2.6 nm for C10, C12 and C14 copolymer vesicles, respectively, are consistent with the increasing alkyl-chain length and are in good agreement with those of unilamellar surfactant

Table 2. Results from SANS Model Calculations[a]

copolymer	$\langle D_1 \rangle$ (nm)	$\langle D_2 \rangle$ (nm)	t (nm)
C10	7.0 ± 1.1	62.2 ± 22.7	1.7
C12	6.7 ± 0.7	75.0 ± 27.4	2.0
C14	7.2 ± 0.6	84.4 ± 30.5	2.6

[a] Average shell diameters, $\langle D_1 \rangle$ and $\langle D_2 \rangle$, and standard deviations of the bimodal distributions and bilayer thickness, t.

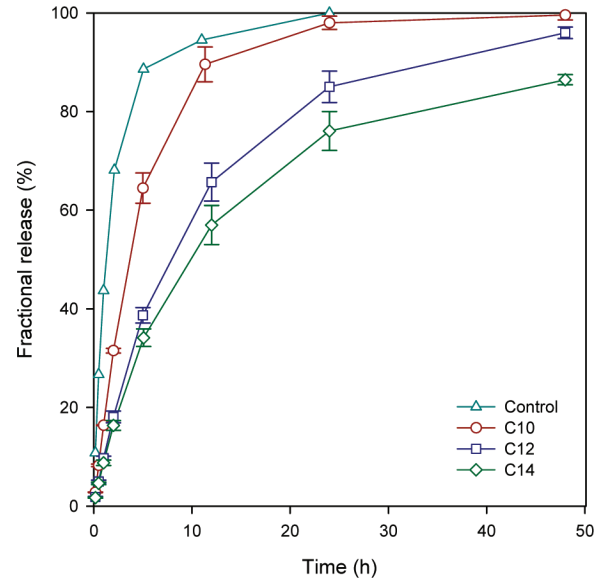


Figure 3. Release of rhodamine B dye from vesicles contained within dialysis tubing. Fractional release was calculated as a percentage of the total amount released from the control. Error bars represent one standard deviation of triplicate independent experiments.

vesicles.[33] These measured thicknesses also confirm that the copolymer chains are oriented such that all hydrophobic tails extend toward each other and the polyhydroxy side chains of VG extend into the aqueous phase.

Release Kinetics. The rate of release of rhodamine B dye from copolymer vesicles was found to be dependent on the length of the alkyl group on the maleimide, indicating decreasing permeability with increasing bilayer thickness, shown in Figure 3. Release of the dye reached completion after 50 h, which is comparable to the release kinetics of liposomes[39−41] and alkylmaleate−VG copolymer vesicles[20] but much faster than release from block copolymer vesicles,[42,43] which typically have thicker walls.

Transfection and Cytotoxicity. Synthetic gene delivery vectors have been studied quite extensively, and many novel systems and materials have been developed including liposomes, dendrimers, and cationic polymers of polyethylenimine (PEI), poly-L-lysine (PLL), and poly(ethylene glycol) (PEG).[44−48] Sugar and polyhydroxy functional groups have been demonstrated to reduce cytotoxicity in polymeric gene delivery vehicles[49] and to be suitable head groups for vesicle-forming materials.[18] We found that VG has low toxicity in mammalian fibroblasts, and we hypothesized that vesicles containing VG would also have low toxicity. Figure 4 shows that C10 25%M+ is moderately nontoxic (>80% survival) and slightly less toxic than commercially available Lipofectamine 2000. Optical and fluorescent microscopy images of cells expressing GFP by C10 25%M+-mediated transfection are also shown in Figure 4. However, transfection efficacy was low for polyplexes of C10 25%M+, and quantification of GFP positive cells by flow cytometry revealed that Lipofectamine was approximately 10 times more efficacious.

Many studies have indicated that the efficacy of a synthetic transfection agent is related to the ratio of available nitrogens

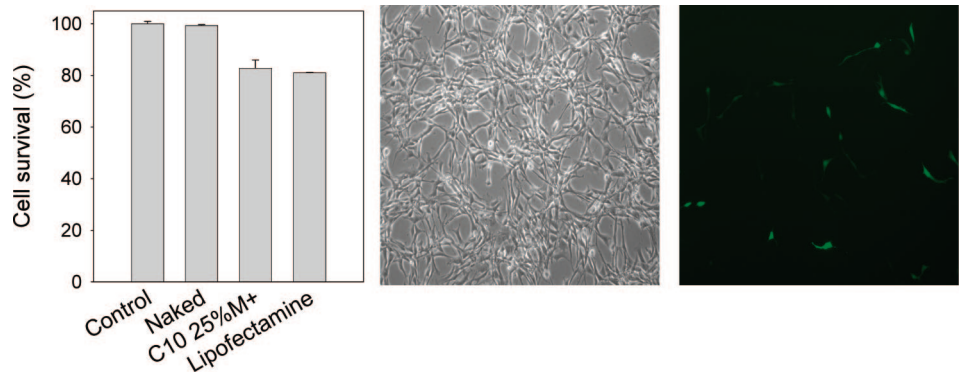
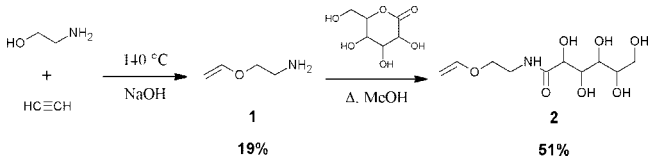
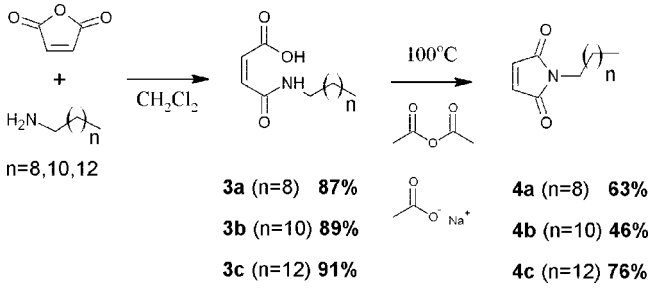


Figure 4. Survival of NIH 3T3 fibroblasts after C10 25%M+ copolymer-mediated transfection compared with cells treated with naked pDNA, cells transfected using Lipofectamine 2000, and a nontransfected control group. Middle and far right images are of the copolymer-transfected cells under optical and fluorescence microscopy, respectively. Cells positive for GFP expression are seen as bright green.

Scheme 1. Synthesis of Sugar-Derived Vinyl Gluconamide



Scheme 2. Synthesis of Hydrophobic *N*-*n*-Alkylmaleimides



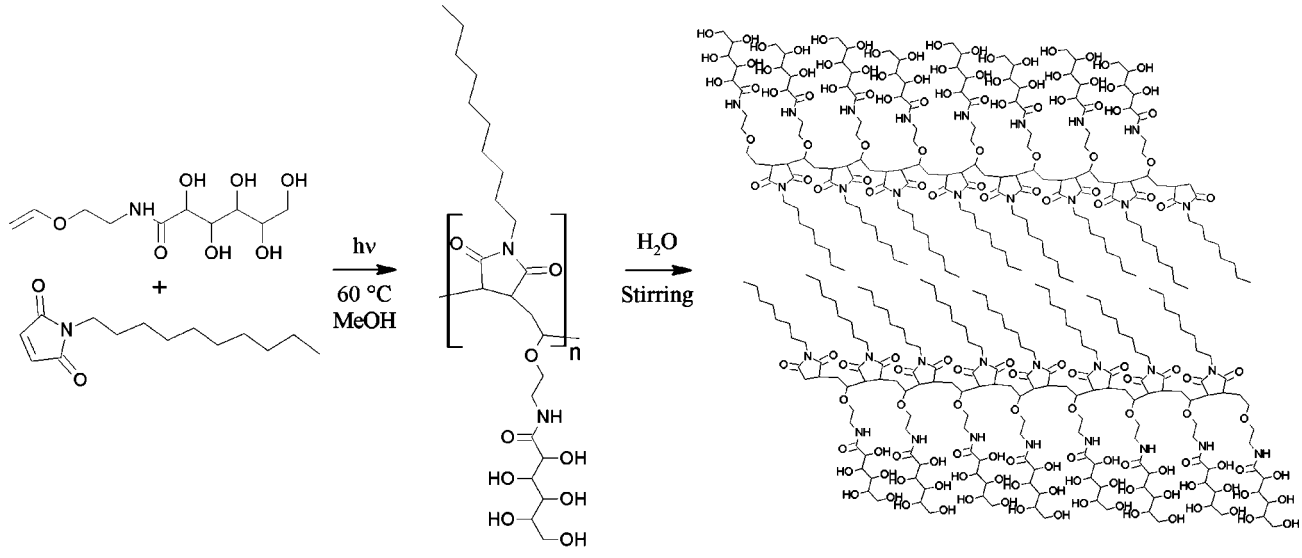
in the polymer to phosphates in the DNA backbone, denoted as *N*/*P*.[44,45,50,51] *N*/*P* for C10 25%M+ as used here was approximately 10, whereas values of 20−50 have been reported as optimum conditions for synthetic vectors. It may be reasonable to conclude that cationically modified C10 copolymer-mediated transfection was not efficacious because *N*/*P* was too low. However, simply increasing the amount of copolymer used in transfection did not improve GFP expression. Also, increasing the loading of cationic monomer during polymerization failed to yield a vesicle-forming material, presumably because the increased number of charged species disrupted the free radical polymerization mechanism.

The low efficacy may also be attributed to the instability of the polyplexes in media. Aggregates of solid material were clearly visible during transfection and may have entrapped a majority of the plasmid, preventing delivery into the cells. Investigations are underway to study how the incorporation of PEG and other functional groups affect the stability of these complexes and promote targeting to various cell types. Following the flexible chemistry of VG synthesis shown in Scheme 1, the lactone may be replaced by other groups susceptible to nucleophilic attack by primary amines, providing access to a library of vinyl ether monomers that may be incorporated into alternating copolymers. Such materials may also be well suited for catalytic reactions where the proximity of specific functional groups is important.

Conclusions

Free radical polymerization of *N*-*n*-alkylmaleimides and vinyl gluconamide yields alternating copolymers that spontaneously form vesicles when dissolved in water at room temperature. These vesicles can be as small as 10−20 nm in diameter without sonication or other high-shear procedures. Cryo-TEM and DLS

Scheme 3. Copolymerization of the Maleimide−Vinyl Ether System and Hypothetical Structure of the Vesicle Wall



data show a bimodal distribution of very small- and medium-sized vesicles, and the analysis of SANS data confirmed vesicular structure and alkyl-side-chain-dependent bilayer thicknesses ranging from 1.7 to 2.6 nm. The inclusion of cationic vinyl ethers yields alternating copolymers that complex with plasmid DNA, and although these materials exhibit poor transfection efficacy, they have low toxicity in mammalian fibroblasts.

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