Journal of Experimental Nanoscience
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t716100757

Structural characterization of novel gemini non-viral DNA delivery systems for cutaneous gene therapy

Marianna Foldvari[a]; Ildiko Badea[a]; Shawn Wettig[a]; Ronald Verrall[b]; Mukasa Bagonluri[a]
[a] College of Pharmacy and Nutrition, Drug Delivery and Pharmaceutical Nanotechnology Laboratory, University of Saskatchewan, Saskatoon, SK [b] Department of Chemistry, University of Saskatchewan, Saskatoon, SK

To cite this Article Foldvari, Marianna , Badea, Ildiko , Wettig, Shawn , Verrall, Ronald and Bagonluri, Mukasa(2006) 'Structural characterization of novel gemini non-viral DNA delivery systems for cutaneous gene therapy', Journal of Experimental Nanoscience, 1: 2, 165 — 176
To link to this Article: DOI: 10.1080/17458080500411965
URL: http://dx.doi.org/10.1080/17458080500411965

Journal of Experimental Nanoscience,
Vol. 1, No. 2, June 2006, 165–176

Taylor & Francis
Taylor & Francis Group

Structural characterization of novel gemini non-viral DNA delivery systems for cutaneous gene therapy

MARIANNA FOLDVARI*†, ILDIKO BADEA†, SHAWN WETTIG†, RONALD VERRALL‡ and MUKASA BAGONLURI†

†College of Pharmacy and Nutrition, Drug Delivery and Pharmaceutical Nanotechnology Laboratory, University of Saskatchewan, Saskatoon, SK, S7N 5C9
‡Department of Chemistry, University of Saskatchewan, Saskatoon, SK, S7N 5C9

(*Received August 2005; in final form October 2005*)

The structural and physicochemical properties of novel cationic lipid-based DNA complexes have been investigated for the purpose of designing micro/nano-scale self-assembling delivery systems for cutaneous gene therapy. DNA/gemini surfactant (spacer $n = 3$–16; chain $m = 12$ or 16) complexes (1 : 10 charge ratio), with or without dioleoylphosphatidyl-ethanolamine (DOPE), designed for cellular transfection, were generally in the range of 100–200 nm as demonstrated by atomic force microscopy and particle size analysis. Small-angle X-ray scattering measurements indicated that the DNA/gemini complexes lacked long-range order, whereas DNA/gemini/DOPE complexes exhibited lamellar and polymorphic phases other than hexagonal. Correlation studies using transfection efficiency data in PAM 212 keratinocytes and *in vitro* skin absorption indicated that formulations containing gemini surfactants having the ability to induce structures other than lamellar in the resulting complexes, generally exhibited greater transfection activity and cutaneous absorption.

Keywords: Gene therapy; Gemini surfactant; Transfection; SAXS

1. Introduction

The objective in gene therapy is the introduction of a missing or defective gene into the cell nucleus where the encoded protein can be expressed. During the transfection process the DNA crosses several barriers such as the cellular, lysosomal, and nuclear membranes prior to expression. In the absence of a suitable carrier/vector/delivery system DNA is not taken up into cells and the protein is not expressed. Viral vectors have been shown to increase DNA delivery; however, due to safety and production concerns better systems are needed [1]. Non-viral approaches offer many advantages including absence of viral components and lack of immunogenicity; they are less expensive, easily manufactured and can be readily altered to form different combinations depending on the intended treatment. Various types of cationic

*Corresponding author. Email: foldvari@duke.usask.ca

Journal of Experimental Nanoscience
ISSN 1745-8080 print/ISSN 1745-8099 online
http://www.tandf.co.uk/journals
DOI: 10.1080/17458080500411965

supramolecular assemblies of DNA have been developed; however, further improvements are still needed to enhance efficiency.

Cationic lipids have been used as successful non-viral transfection agents in cell culture. Our group and others [2–4] have shown that cationic formulations also increase interaction with the skin, resulting in greater fluxes of drugs into the skin. However, most cationic lipids (including monocationic and polycationic) in general have failed to provide the necessary degree of cationic charge density and complexation with DNA. In order to find a more suitable cationic agent that would be effective *in vivo*, not just *in vitro*, gemini surfactant derivatives were selected for investigation due to their several unique characteristics. Gemini surfactants are a class of surfactants in which two traditional surfactant moieties have been chemically linked at or near the surfactant head groups, resulting in molecules that contain two cationic head groups and two long alkyl chains. Among their advantageous properties are their low critical micellar concentration (cmc), which is orders of magnitude smaller, and surface activity (interfacial tension lowering capacity), which is orders of magnitude greater than those of single-chain surfactants and other cationic lipids [5–7]. Gemini surfactants, especially the ones with long (16C) spacers, were shown to promote cubic phase formation [6]. Moreover, the cationic head groups can be positioned at different distances from each other [8], which provides flexibility for designing optimum spacer characteristics to achieve the required charge density and DNA complexing efficiency.

The objective of this study was to characterize the structural and physicochemical properties of novel gemini surfactant-based DNA complexes by atomic force microscopy (AFM), small-angle X-ray scattering (SAXS), zeta potential and particle size analysis in order to determine the optimum parameters required for cellular transfection and provide data for the development of micro/nano-scale self-assembling delivery vehicles for cutaneous gene therapy.

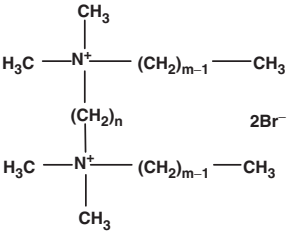
2. Materials and methods

2.1. *Formulation of DNA complexes*

A series of cationic DNA complexes based on the dicationic (gemini) surfactant series (12-*n*-12 with $n = 2$-16, see structures in table 1) and the gemini compound 16-3-16 and other lipids of various compositions were constructed. Transfection mixtures consisting

Table 1. Structure of gemini surfactant derivatives.

Spacer (*n*)	Alkyl chain length (*m*)	Symbol
2	12	12-2-12
3	12	12-3-12
4	12	12-4-12
6	12	12-6-12
8	12	12-8-12
10	12	12-10-12
12	12	12-12-12
16	12	12-16-12
3	16	16-3-16



of plasmid/gemini surfactant complexes (PG complexes – 1 : 10 plasmid/gemini surfactant charge ratio) and plasmid/gemini surfactant/helper lipid vesicles (PGL vesicles – with 1,2 dioleyl-*sn*-glycero-phosphatidylethanolamine (DOPE) as helper lipid) were prepared. Aqueous solutions (3 mM) of the gemini cationic surfactants were prepared and filtered through 0.2 μm Acrodisc® filters (Pall Gelman, Ann Arbor, MI). The pGTmCMV.IFN-GFP plasmid was used at a concentration of 0.2 μg/well for transfection. Lipid vesicles were prepared by sonication techniques. DOPE (Avanti Polar Lipids, Alabaster, AL) and α-tocopherol (Spectrum, Gardena CA) in 1 : 0.2 weight ratios were dissolved in 100% ethanol (Commercial Alcohols Inc., Brampton, ON) and deposited as a thin film on a round bottom flask. The lipid was lyophilized overnight, to remove traces of solvent, and resuspended at 1 mM concentration in 9.25% isotonic sucrose (Spectrum, Gardena, CA) solution (pH 9) by sonication, then filtered through 0.45 μm Acrodisc® filters. The PG systems were prepared as follows: 0.2 μg of plasmid was mixed with aliquots of gemini surfactant solution to obtain plasmid DNA/gemini surfactant charge ratios of 1 : 10 and incubated at room temperature for 15 minutes. The PGL vesicles were prepared by adding 25 μL of DOPE liposomes to the PG complexes. The PGL systems were incubated for 30 minutes at room temperature prior to transfection.

2.2. *In vitro transfection*

Murine keratinocytes (PAM212 cell line, kindly provided by Dr. S Yuspa, NCI, Bethesda, MA) were grown in antibiotic and 10% fetal bovine serum supplemented MEM. The day before transfection, 5×10^4 cells/well were seeded in 24-well plates (Greiner Labortechnik GmbH, Germany) and grown to 60–70% confluency. The supplemented MEM was changed to MEM one hour prior to transfection. The cells were transfected with PG or PGL systems containing 0.2 μg plasmid/well. The supernatants were collected 24 hours after transfection and stored at –20 °C.

The expressed protein (murine interferon γ) was determined by enzyme-linked immunosorbent assay (ELISA). ELISAs were performed using round bottom 96-well plates (Immulon 2, Dynatech Laboratories, Chantilly, VA). The plates were coated with 50 μL/well of capture rat antimouse IFNγ antibody (Pharmingen, Mississauga, ON), 2 μg/mL, and incubated for 24 hours at 4°C. The wells were blocked with 1% bovine serum albumin (BSA) (New England Biolabs, Mississauga, ON) solution in PBS at room temperature for one hour. The supernatants from cell cultures were added onto the plates and incubated overnight at 4°C. Biotinylated rat antimouse IFNγ (Pharmingen) was added at 0.5 ng/mL concentration in 1% BSA solution. The plates were incubated for a further 2 hours at room temperature. The streptavidin-alkaline phosphatase conjugate (Jackson Immuno Research Laboratories, Inc., West Grove, PA) was added in 1 : 5000 dilution and incubated for 1 hour at room temperature, followed by addition of 4-nitrophenyl phosphate di(tris) salt (PNPP) 1 mg/mL in PNPP buffer (1% diethanolamine, 0.5mM $MgCl_2$, pH 9.8) (Sigma). The optical density of the samples was measured at 405 nm using a Benchmark Microplate Reader (BioRad, Mississauga, ON). The concentration of the IFNγ was calculated from the standard curve, using recombinant murine IFNγ (Pharmingen).

2.3. *Physicochemical characterization by atomic force microscopy and zetasizer*

AFM measurements were made using a PicoSPM instrument (Molecular Imaging Inc., Tempe, AZ), in MAC-mode, using MI MAC cantilever Type II ($K = 1.2$–5.5 N/m). The DNA, PG and PGL systems, 10 µl each, were spread on the surface of freshly cleaved mica and incubated for 30 seconds to 15 minutes at room temperature. The excess formulation was removed with lint free absorbent tissue and the mica surface dried with a stream of N_2. A 4×4 µm or 35×35 µm surface area was scanned.

Particle size and zeta potential (ζ) measurements were made using a Malvern Zetasizer NanoZS instrument (Malvern Instruments, Worchestershire, UK) and data were processed using the Malvern DTS software.

2.4. *Small-angle X-ray scattering*

SAXS measurements were made using beamline X21 at the National Synchrotron Light Source at Brookhaven National Laboratory, Long Island, NY. The measurements were performed with 12 KeV X-rays and the data covered a q-range from 0.008 $Å^{-1}$ to 0.5 $Å^{-1}$. Samples were loaded into 1.5 mm capillaries (Charles Supper #15-BG) and the scattering pattern was recorded using a 13 cm Mar CCD detector (Mar USA, Evanston, IL), at 1.26 m (calibrated with the scattering pattern of silver behenate) downstream of the sample. All spectra were processed to remove background contributions by subtracting the scattering profile obtained for a water-filled capillary.

2.5. *Statistics*

Statistical analysis was carried out using the Prism (Graphpad version 4) software package. Results were processed using the ANOVA and Tukey's multiple comparison test. Correlations were determined using the Pearson method.

3. Results and discussion

We have tested eight different gemini surfactants to determine the effect of head group spacer length and alkyl chain length on their transfection ability *in vitro* [9]. These studies indicated that the transfection efficiency (TE) and cutaneous absorption was dependent on the length of the spacer between the two positively charged head groups, with a C3 spacer showing the highest activity, and that the transfection efficiencies for the gemini surfactants are correlated to other physical properties (such as the head group area at the air/water interface, critical micelle concentration, etc.) that depend upon the size and/or nature of the spacer group [9].

In this work, the AFM and light scattering analysis indicated that the PG particles were generally in the range of 100–200 nm (table 2), regardless of the spacer length of the gemini surfactant. This particle size is similar to other DNA-gemini surfactant complexes prepared for physicochemical analysis by Chen *et al.* [10]. The formation of the PG complex was a time-dependent process as shown on the AFM images (figure 1). The gemini surfactant (16-3-16) dication was observed to bind to the naked DNA plasmid (figure 1a) and within 30 seconds compaction of the large plasmid 'loops'

Table 2. Properties of transfection formulations for *in vitro* studies.

System	AFM size (nm)	Light scattering	
		size* (nm)	ζ (mV)
DNA	100–1000	50–100	–47 ± 15
PG complexes			
12-2-12/DNA	–	115 ± 7	24 ± 1
12-3-12/DNA	100–200	137 ± 31	15 ± 2
12-4-12/DNA	–	146 ± 44	16 ± 2
12-6-12/DNA	–	153 ± 49	23 ± 6
12-8-12/DNA	–	125 ± 27	21 ± 3
12-10-12/DNA	–	85 ± 7	26 ± 3
12-12-12/DNA	–	103 ±17	30 ± 8
12-16-12/DNA	–	119 ± 16	55 ± 5
16-3-16/DNA	100–200	220 ± 70	58 ± 1
PGL vesicles			
12-2-12/DNA/DOPE	–	175 ± 5	63 ± 2
12-3-12/DNA/DOPE	100–300	143 ± 21	41 ± 5
12-4-12/DNA/DOPE	–	154 ± 15	42 ± 3
12-6-12/DNA/DOPE	–	155 ± 16	39 ± 7
12-8-12/DNA/DOPE	–	153 ± 15	38 ± 3
12-10-12/DNA/DOPE	–	158 ± 14	43 ± 7
12-12-12/DNA/DOPE	–	158 ± 13	42 ± 1
12-16-12/DNA/DOPE	–	161 ± 11	37 ± 9
16-3-16/DNA/DOPE	100–400	210 ± 30	44 ± 4

(*average of three trials of three repeats)

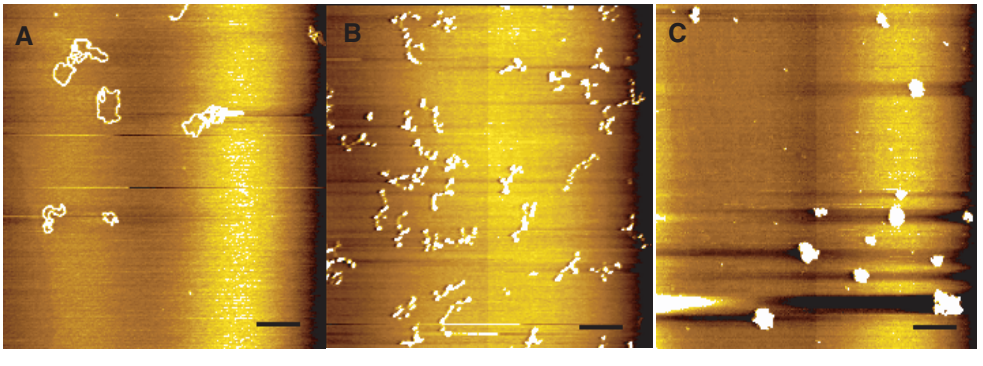


Figure 1. AFM images showing plasmid DNA compaction by the gemini surfactants on freshly cleaved mica surface (bar represents 400nm). (a) plasmid alone; (b) plasmid-gemini complex after a 30-second incubation; (c) plasmid-gemini complex after a 15-minute incubation.

(figure 1b) occurred. More compact particles formed within 15 minutes (figure 1c). Table 1 shows the particle size, measured by AFM and light scattering, and zeta potential of both PG and PGL systems. Particle sizes measured by the two methods are in good agreement for the DNA complexes. The apparent discrepancy in size for the uncomplexed DNA can be attributed to the assumed model in the light scattering analysis. A 'globular' model is generally assumed in solution; however, on a mica

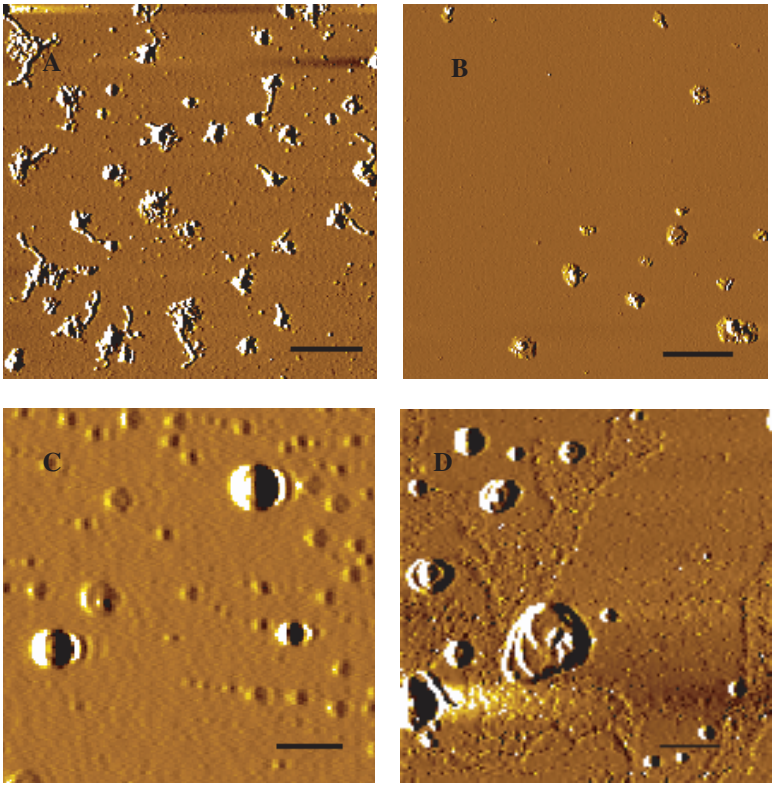


Figure 2. AFM images of DNA complex structures (bar represents 400 nm). (a) PG complexes with the 12-3-12 surfactant; (b) PG complexes with the 16-3-16 surfactant; (c) PGL vesicles with the 12-3-12 surfactant; (d) PGL vesicles with the 16-3-16 surfactant.

surface during an AFM experiment (see figure 1a) this is clearly not the case. Figure 2 shows the morphology of selected PG complexes (a and b) and PGL vesicles (c and d).

In vitro transfection experiments indicated that only PGL vesicles, and not PG complexes, had the ability to transfect PAM 212 keratinocytes. Within the PGL group, while there was a small variation in the size as a function of spacer length, specific correlation was not observed between particle size and transfection efficiency (figure 3a). Interestingly, as can be seen in table 2 and figure 3 by comparing data for the 12-3-12 and 16-3-16 surfactants, smaller particle size was not a requirement for increased transfection efficiency. Similarly no correlation was observed between the ζ potential and transfection efficiency (figure 3b); however, it can be seen that ζ in all cases is >30 mV, indicating that not only do the complexes possess the necessary positive surface charge needed for transfection, but also that the complexes have sufficient surface charge to remain stable in solution.

The effect of variation in the spacer group, at a fixed alkyl tail length, on the structure of the PGL systems determined from SAXS is more complex. Figure 4 illustrates the scattering profiles for the PG (figure 4a) and PGL (figure 4b) systems. For the PG

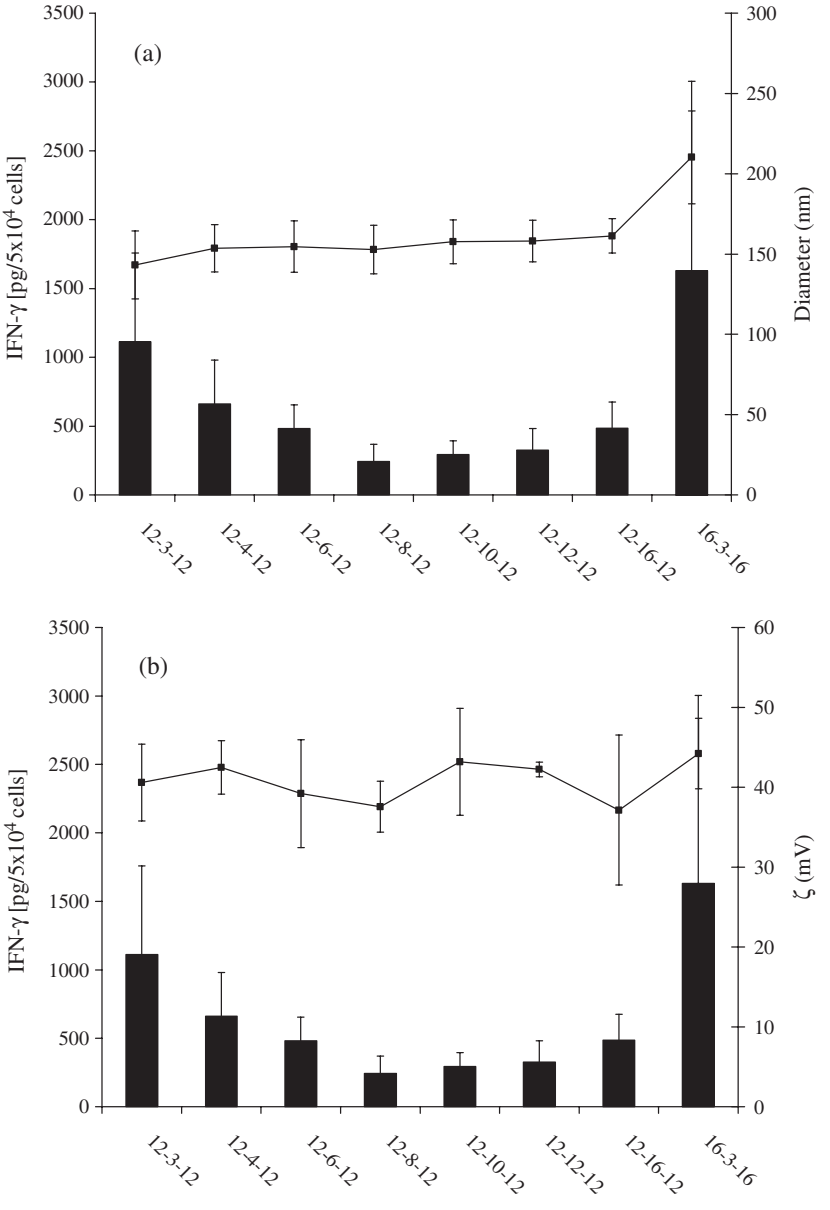


Figure 3. Correlation of transfection efficiency shown as IFNγ expressed (bars) with (a) particle size and (b) zeta potential of the DNA/gemini/DOPE (PGL) vesicles.

complexes, there was no long-range order and a specific polymorphic structure could not be assigned. The calculated *d*-spacing for the main scattering peak in both PG and PGL systems (tables 3 and 4) inversely correlated ($p = 0.01$) with the head group areas [7] of the gemini surfactants (figure 5), and, generally, with other properties of the

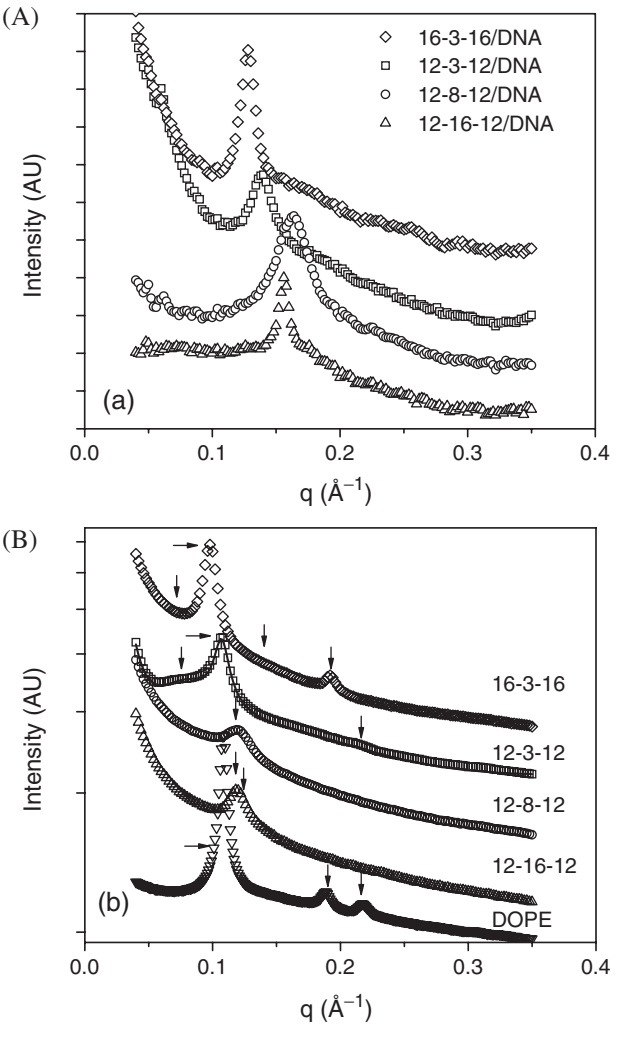


Figure 4. SAXS profiles for (a) DNA-gemini, and (b) DNA/gemini/DOPE (PGL) systems: 16-3-16 (◇); 12-3-12 (□); 12-8-12 (○); 12-16-12 (△); DOPE (▽). A fit of the experimental data, assuming multiple Lorentzian peaks, is shown for the DNA/12-3-12/DOPE system as a solid line.

surfactant specifically related to the size of the surfactant head group. The *d*-spacings for the PGL systems also correlate ($p = 0.05$) to observed transfection efficiencies.

For the PGL vesicles, the 12-3-12 surfactant exhibits a lamellar morphology ($d = 58.7$ Å) and, additionally, a weak scattering peak is also evident at $q = 0.080$ Å^{-1}. This is indicative of the presence of additional phases; however, the identities of these phases are not yet known. Similar lamellar phases and the presence of additional phases were obtained for the 16-3-16 surfactant (table 4, figure 4b). The presence of additional phases is also observed for the 12-2-12, 12-4-12 and 12-6-12 PGL systems (table 4). Since both PG and PGL systems have similar particle size and zeta potential, the ability

Table 3. Structural parameters for the DNA/gemini surfactant (PG) systems determined from small-angle X-ray scattering measurements.

PG systems	q (Å^{-1})	d (Å)
12-2-12/DNA	0.136	46.0
	0.184	34.1
12-3-12/DNA	0.139	45.2
	0.188	33.3
12-4-12/DNA	0.151	41.7
	0.152	41.4
12-6-12/DNA	0.124	50.7
	0.160	39.3
12-8-12/DNA	0.163	38.6
12-10-12/DNA	0.157	40.0
	0.172	36.5
12-12-12/DNA	0.157	40.1
	0.172	36.6
12-16-12/DNA	0.156	40.2
	0.160	39.4
16-3-16/DNA	0.128	49.2
	0.169	37.1

Table 4. Structural parameters for the DNA/gemini surfactant/DOPE (PGL) systems determined from small-angle X-ray scattering measurements. Complexes **in bold** exhibit a lamellar phase with a possible second phase.

PGL systems	q (Å^{-1})	d (Å)	σ_m[a] (e/Å^2)
12-2-12/DNA/DOPE	0.080	79.0	1.14×10^{-2}
	0.106	59.0	
12-3-12/DNA/DOPE	**0.080**	**78.7**	1.03×10^{-2}
	0.107	**58.7**	
	0.215	**29.3**	
12-4-12/DNA/DOPE	0.110	57.2	9.65×10^{-3}
	0.122	51.4	
12-6-12/DNA/DOPE	0.053	118.5	8.38×10^{-3}
	0.116	51.4	
12-8-12/DNA/DOPE	0.120	52.3	7.19×10^{-3}
12-10-12/DNA/DOPE	0.120	52.4	6.30×10^{-3}
12-12-12/DNA/DOPE	0.124	50.7	6.32×10^{-3}
12-16-12/DNA/DOPE	0.113	55.4	8.06×10^{-3}
	0.120	52.5	
16-3-16/DNA/DOPE	**0.070**	**89.5**	9.22×10^{-3}
	0.098	**64.1**	
	0.138	**45.5**	
	0.193	**32.6**	
DOPE[b]	0.109	57.9[c]	–
	0.189	33.3	
	0.218	28.8	

[a] average membrane charge density (σ_m) was calculated based on the equation: $\sigma_m = eZN_{cl}/(N_{nl}A_{nl} + N_{cl}A_{cl}) = [1 - \Phi_{nl}/\Phi_{nl} + r\Phi_{cl})]\ \sigma_{cl}$ where the ratio of the headgroup area of the cationic to neutral lipid is $r = A_{cl}/N_{cl}$; $\sigma_{cl} = eZ/A_{cl}$ is the charge density of the cationic lipid with valence Z; $\Phi_{nl} = N_{nl}/(N_{nl} + N_{cl})$ and $\Phi_{cl} = N_{cl}/(N_{nl} + N_{cl})$ are the mole fractions of the neutral and cationic lipids, respectively, from Ewert *et al.* [13].

[b] exhibit hexagonal phase.

[c] a, the lattice spacing $(= 4\pi/(\sqrt{3}q)) = 66.9$ Å.

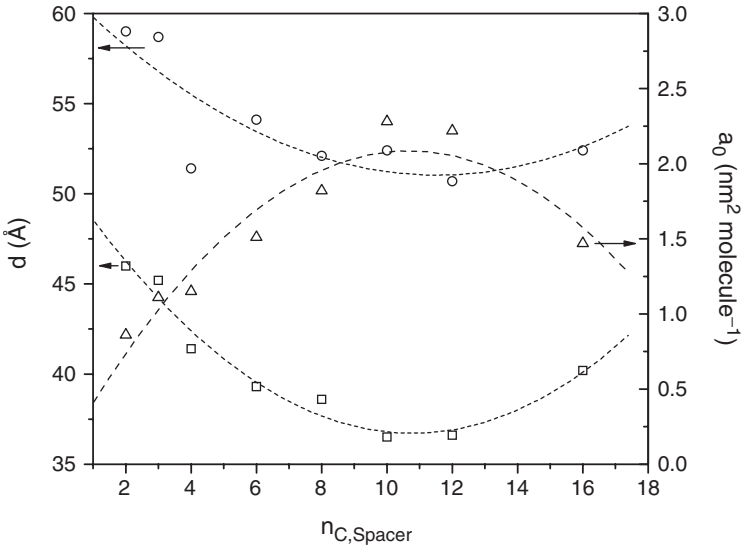


Figure 5. *d*-spacings as a function of spacer chain length for the 12-s-12 series of surfactants; DNA-gemini (○), DNA/gemini/DOPE systems (PGL) (□). The *d*-spacings for the DNA/gemini (PG) systems correlate ($p = 0.01$) to the head group areas (△) [7] of the gemini surfactants.

to transfect cells can be attributed to the structural differences between these two groups. PG complexes showed particle-like morphology (figures 1 and 2), no specific polymorphic arrangement and no transfection. On the other hand, the PGL systems appeared vesicle-like and the gemini surfactants formed mixed polymorphic systems in the presence of DNA and DOPE and have the ability to induce polymorphic structures other than hexagonal (H_{II}) in the predominantly lamellar PGL systems. This may facilitate the eventual release of the DNA, resulting in increased transfection, which was specifically observed for the surfactants having short spacer groups. This is an interesting observation in the light of previous reports where a hexagonal structure is thought by many to be required for transferring DNA to cells [11–13]. Plasmid-DOPE complexes without any gemini surfactant show a typical hexagonal profile ($q = 0.109$, 0.188 and $0.218\,\text{Å}^{-1}$; figure 4b) with a lattice constant $a = 66.9\,\text{Å}$; however, these complexes do not have the ability to transfect PAM212 cells. Recently a model has been proposed to explain high transfection efficiencies observed for those systems having a lamellar morphology, based upon the average membrane charge density (σ_M) for the resulting cationic liposomes [13, 14]. In such systems efficient transfection is observed, provided that σ_M is greater than a critical value ($1.04 \times 10^{-2}\,\text{e/Å}^2$). Interestingly, for our systems, only the 12-2-12 and 12-3-12 DNA-gemini-DOPE systems have a calculated membrane charge density equivalent to or greater than this critical value (table 3). Although the PGL system with 12-3-12 gemini surfactant has the highest transfection efficiency compared to the other PGL systems with longer spacers in this series, the PGL system with the 16-3-16 surfactant, exhibits the greatest transfection even though it has a lower σ_M value ($9.22 \times 10^{-3}\,\text{e/Å}^2$). These results provide additional support for the hypothesis that it is the ability of the DNA complexes (gemini surfactants and other

helper lipids) to adopt polymorphic arrangements that may facilitate DNA transfection.

4. Conclusions

Successful transfection is a complex process, dependent on many factors, which are still not fully understood. We have demonstrated here that particle size variation of the DNA-gemini-DOPE complexes may not be a significant factor in *in vitro* transfection below a certain limit, possibly below 200 nm. However, we found that an important factor is the ability of the complexes to form polymorphic structures, which are not necessarily hexagonal.

Acknowledgements

We thank Dr Lin Yang for his assistance in carrying out the SAXS experiments at the National Synchrotron Light Source, Brookhaven National Laboratory, which is supported by the US Department of Energy, Division of Materials Sciences and Division of Chemical Sciences, under Contract No. DE-AC02-98CH10886. The research in this paper was supported in part by the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Council (NSERC) of Canada and the Saskatchewan Synchrotron Institute. AFM experiments were carried out at the Saskatchewan Structural Sciences Center.

References

[1] Y. Kaneda. Gene therapy: a battle against biological barriers. *Curr. Mol. Med.*, **1**, 493 (2001).
[2] J. Hadgraft. Recent developments in topical and transdermal delivery. *Eur. J. Drug Metab. Pharmacokinet.*, **21**, 165 (1996).
[3] M. Foldvari, M.E. Baca-Estrada, Z. He, J. Hu, S. Attah-Poku, M. King. Dermal and transdermal delivery of protein pharmaceuticals: lipid-based delivery systems for interferon alpha. *Biotechnol. Appl. Biochem.*, **30(Pt 2)**, 129 (1999).
[4] M.J. Pikal. The role of electroosmotic flow in transdermal iontophoresis. *Adv. Drug. Deliv. Rev.*, **46**, 281 (2001).
[5] S. Karaborni. Order–disorder transition during approach and separation of two parallel surfaces. *Phys. Rev. Lett.*, **73**, 1668 (1994).
[6] F.M. Menger, J.S. Keiper. Gemini surfactants. *Angew Chem. Int. Ed. Engl.*, **39**, 1906 (2000).
[7] S.D. Wettig, R.E. Verrall. Thermodynamic studies of Aqueous m-s-m gemini surfactant systems. *J. Colloid Interface Sci.*, **235**, 310 (2001).
[8] R. Zana. Dimeric (gemini) surfactants: Effect of the spacer group on the association behavior in aqueous solution. *J. Colloid Interface Sci.*, **248**, 203 (2002).
[9] I. Badea, R. Verrall, M. Baca-Estrada, S. Tikoo, A. Rosenberg, P. Kumar, M. Foldvari. In vivo cutaneous interferon-gamma gene delivery using novel dicationic (gemini) surfactant-plasmid complexes. *J. Gene Med.*, **7**, 1200 (2005).
[10] X.D. Chen, J.B. Wang, N. Shen, Y.H. Luo, L. Li, M.H. Liu, R.K. Thomas. Gemini surfactant/DNA complex monolayers at the air–water interface: effect of surfactant structure on the assembly, stability, and topography of monolayers. *Langmuir*, **18**, 6222 (2002).
[11] I. Koltover, T. Salditt, J.O. Radler, C.R. Safinya. An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery. *Science*, **281**, 78 (1998).
[12] M.T. Kennedy, E.V. Pozharski, V.A. Rakhmanova, R.C. MacDonald. Factors governing the assembly of cationic phospholipid-DNA complexes. *Biophys. J.*, **78**, 1620 (2000).

[13] K. Ewert, N.L. Slack, A. Ahmad, H.M. Evans, A.J. Lin, C.E. Samuel, C.R. Safinya. Cationic lipid-DNA complexes for gene therapy: understanding the relationship between complex structure and gene delivery pathways at the molecular level. *Curr. Med. Chem.*, **11**, 133 (2004).
[14] A.J. Lin, N.L. Slack, A. Ahmad, C.X. George, C.E. Samuel, C.R. Safinya. Three-dimensional imaging of lipid gene-carriers: membrane charge density controls universal transfection behavior in lamellar cationic liposome-DNA complexes. *Biophys. J.*, **84**, 3307 (2003).