



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## Review

## Interaction of cationic carbosilane dendrimers and their complexes with siRNA with erythrocytes and red blood cell ghosts



Dominika Wrobel <sup>a,\*</sup>, Katarzyna Kolanowska <sup>a</sup>, Arkadiusz Gajek <sup>b</sup>, Rafael Gomez-Ramirez <sup>c</sup>, Javier de la Mata <sup>c</sup>, Elżbieta Pedziwiatr-Werbicka <sup>a</sup>, Barbara Klajnert <sup>a</sup>, Iveta Waczulikova <sup>d</sup>, Maria Bryszewska <sup>a</sup>

<sup>a</sup> Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland

<sup>b</sup> Department of Thermobiology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland

<sup>c</sup> Departamento Quimica Inorganica, Universidad de Alcala de Henares, Spain

<sup>d</sup> Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia

## ARTICLE INFO

## Article history:

Received 30 April 2013

Received in revised form 1 November 2013

Accepted 26 November 2013

Available online 5 December 2013

## Keywords:

Carbosilane

Red blood cell ghost

Hemolysis

Membrane fluidity

HIV-1

siRNA

## ABSTRACT

We have investigated the interactions between cationic NN16 and BDBR0011 carbosilane dendrimers with red blood cells or their cell membranes. The carbosilane dendrimers used possess 16 cationic functional groups. Both the dendrimers are made of water-stable carbon–silicon bonds, but NN16 possesses some oxygen–silicon bonds that are unstable in water. The nucleic acid used in the experiments was targeted against GAG-1 gene from the human immunodeficiency virus, HIV-1.

By binding to the outer leaflet of the membrane, carbosilane dendrimers decreased the fluidity of the hydrophilic part of the membrane but increased the fluidity of the hydrophobic interior. They induced hemolysis, but did not change the morphology of the cells. Increasing concentrations of dendrimers induced erythrocyte aggregation. Binding of short interfering ribonucleic acid (siRNA) to a dendrimer molecule decreased the availability of cationic groups and diminished their cytotoxicity. siRNA–dendrimer complexes changed neither the fluidity of biological membranes nor caused cell hemolysis. Addition of dendriplexes to red blood cell suspension induced echinocyte formation.

© 2013 Elsevier B.V. All rights reserved.

## Contents

1.	Introduction . . . . .	882
2.	Materials and methods . . . . .	883
2.1.	Materials . . . . .	883
2.2.	Blood preparation . . . . .	884
2.3.	Membrane preparation . . . . .	884
2.4.	Dendriplex preparation . . . . .	884
2.5.	Fluorescence spectroscopy . . . . .	884
2.6.	Hemolysis . . . . .	885
2.7.	Microscopy method . . . . .	885
2.8.	Statistical analysis . . . . .	885
3.	Results . . . . .	885
3.1.	Fluorescent anisotropy measurements . . . . .	885
3.2.	Erythrocyte hemolysis . . . . .	885
3.3.	Morphology changes . . . . .	885
4.	Discussion . . . . .	886
5.	Conclusions . . . . .	887
	Acknowledgements . . . . .	888
	References . . . . .	888

## 1. Introduction

Medicine in the start of the XX century looks very archaic when compared with current knowledge and technical achievements. Progress

\* Corresponding author at: Department of General Biophysics, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland. Tel.: +48 42 6354144; fax: +48 42 6354474.  
E-mail address: [domwro@biol.uni.lodz.pl](mailto:domwro@biol.uni.lodz.pl) (D. Wrobel).

is truly impressive, but even today there are many diseases that remain impossible to cure or at least to reduce symptoms burdensome for the patient. One of the more promising therapies that can be used for treating cancer, some infectious disease and even genetic diseases, is gene therapy based on ribonucleic acid interference (RNAi). RNAi is a natural defense mechanism that exists in many eukaryotes; it is activated by double-stranded RNAs (dsRNA) with specific sequences. dsRNA causes selective post-transcriptional gene silencing, which means that production of the corresponding protein is blocked. In mammalian cells, dsRNA longer than 30 nucleotides shuts down protein syntheses by non-specific degradation of messenger RNA (mRNA), and activates production of interferon- $\gamma$ -interferon (IFN) pathway 4. This is why 20–25 nucleotide length RNAs are being used, known as short interfering RNA (siRNA). These RNA fragments are short enough to avoid a cellular interferon response, but are long enough to suppress genes [1]. RNAi therapy holds promise for some diseases, such as cancer [2,3], virus infection (HIV, hepatitis, severe acute respiratory syndrome (SARS)) [4–6], respiratory diseases (asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis) [7–9] and parasitic infections (malaria, human amoebiasis) [10,11]. Ribonucleic acid can be used as a short hair-pin RNA transported by viral vectors or as a double-stranded siRNA. The first system is very efficient, but viral vectors can induce immunogenic response, making the use of many of them unsuitable. The other seems to be less problematical, but it is not easy to create an efficient delivery system for siRNA protection and transportation. There are several types of non-viral carriers used for delivery of nucleic acids, including lipids, polymeric amines and dendrimers.

There is confidence that dendrimers may be ideal molecules as drug carriers because of their highly branched structure and unique properties. The dendrimer surface is an excellent site for binding drugs or cell-specific targeting groups through electrostatic forces or covalent bonds. They possess high drug-loading capacity and they can be made relatively non-toxic to cells by appropriate surface modifications [12–14].

To create ideal drug carriers, the mechanism of interaction of dendrimers and dendrimer–drug conjugates with lipid membranes needs to be understood, which can improve the properties of the molecule by increasing its targeting efficiency and protective properties. In basic investigations, liposomes are often used as model membranes because of their simple composition, uncomplicated preparation and high stability [15–30]. Studies on model membranes gives information about their interaction with a relatively simple lipid structure, leading to phenomena that are not disturbed by the proteins associated with them. In addition to model membranes, erythrocytes have been frequently

undertaken. While there are some reports on interactions of PAMAM dendrimers with cells and biomembranes [31,32], this article focuses those involving cationic carbosilane dendrimers (CBS) and their complexes with siRNA (dendriplexes) in terms of their action on red blood cells. CBS dendrimers were synthesized for siRNA and an oligonucleotide delivery system. They possess 16 cationic groups on their surface and can bind negatively charged nucleotide backbones through electrostatic interactions. Two second generation carbosilane dendrimers were used, BDBR0011 and NN16. Second generation CBS dendrimers show good toxicity profiles (low toxicity) up to 5  $\mu\text{M}$  [33]. They can form stable complexes with nucleic acids and protect them from degradation and sequestration by proteins [34,35]. Both these dendrimers are made of water-stable carbon–silicon bonds, but NN16 possesses some oxygen–silicon bonds that are unstable in water (Fig. 1). Slow hydrolyzation in aqueous solutions released cargos from dendrimers gradually, and this degradation takes over 4 to 24 h [36]. Since both appear to be promising candidates as drug carriers, we have tested these two very similar compounds to check which one is superior as a conveyor for siRNA in gene therapy.

## 2. Materials and methods

### 2.1. Materials

Blood from healthy donors was obtained from the Central Blood Bank (Lodz, Poland); fluorescent probes: 1,6-diphenyl-1,3,5-hexatriene (DPH); N,N,N-trimethyl-4-(6-phenyl-1,3,5-hexatriene-1-yl) phenylammonium p-toluenesulfonate (TMA-DPH); Hepes and Tris-HCl buffers were purchased from Sigma Chemical Company; 10 mM Hepes buffer at pH 7.4 was made in distilled water and NaCl was added to 150 mM. Water-soluble carbosilane dendrimers of generation 3 were synthesized in the Departamento de Quimica Inorganica, Universidad de Alcala, Spain [33,36]. The molecular formula and molecular weights of those compounds are: NN16— $\text{C}_{128}\text{H}_{316}\text{I}_{16}\text{N}_{16}\text{O}_8\text{Si}_{13}^{16+}$ , Mw = 4 603.56 g/mol and BDBR0011— $\text{C}_{144}\text{H}_{348}\text{I}_{16}\text{N}_{16}\text{Si}_{13}^{16+}$ , Mw = 4 699.99 g/mol.

Non-labeled siRNA was synthesized by Sigma-Aldrich. Nucleic acid was targeted against the GAG-1 gene from HIV-1 virus, with the sequence:

sense: GAGAACCAAGGGGAAGUGACAdTdT,  
antisense: UGUCACUCCCCUUGGUUCUCdTdT.

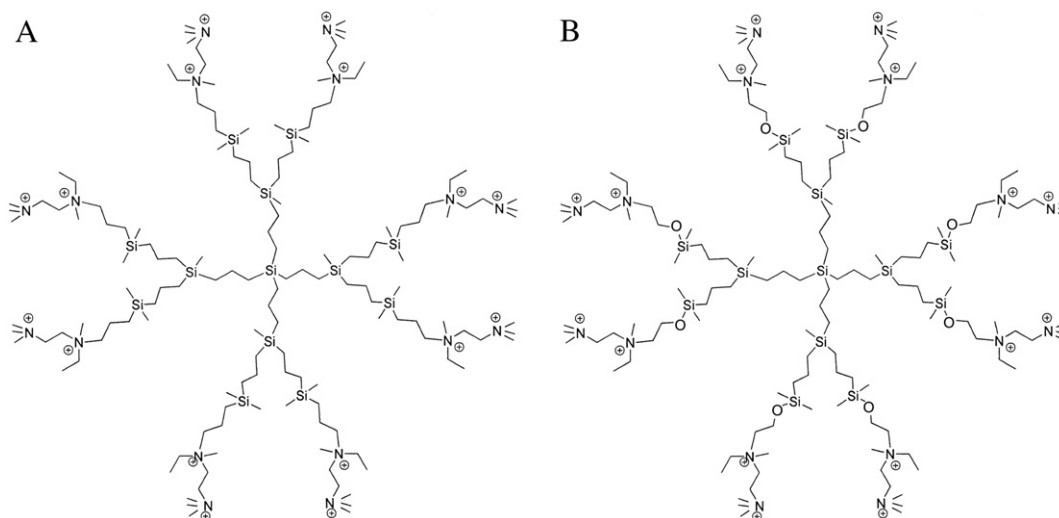
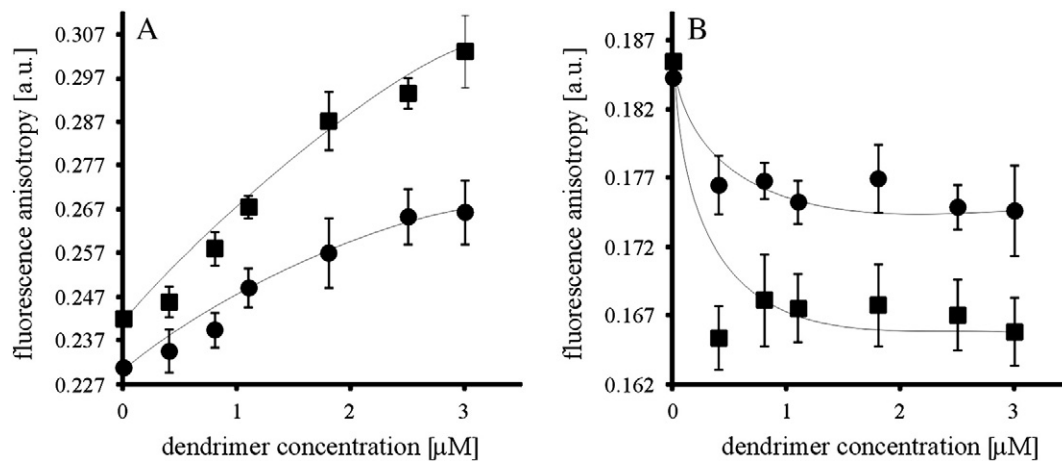


Fig. 1. Structure of carbosilane dendrimers; (A) NN16; (B) BDBR0011.



**Fig. 2.** Fluorescence anisotropy of TMA-DPH (A) and DPH (B) in cell membrane; ■—NN16 dendrimer, ●—BDBR0011 dendrimer. All data were expressed as mean  $\pm$  S.E.M.  $n = 4$ ; \*  $p < 0.05$  for each point vs control.

## 2.2. Blood preparation

Fresh blood was anticoagulated with 3% sodium citrate. Erythrocytes were separated from the plasma and leukocytes by centrifugation (5000 g, 5 min) at 4 °C, washed 3 times with 150 mM NaCl and suspended in 10 mM HEPES buffer with 150 mM NaCl (pH 7.4). They were used immediately after isolation.

## 2.3. Membrane preparation

Erythrocytes were hemolyzed in cold (4 °C) Tris-HCl buffer for 30 min and centrifuged (20 min, 15,000 g, 4 °C). Ghosts were separated from hemoglobin and washed several times with Tris-HCl diluted with water (1:1). Membranes were washed until their color became creamy. Membrane protein concentration was measured by the Lowry method [37]. Erythrocyte membranes were frozen and used within 2 weeks.

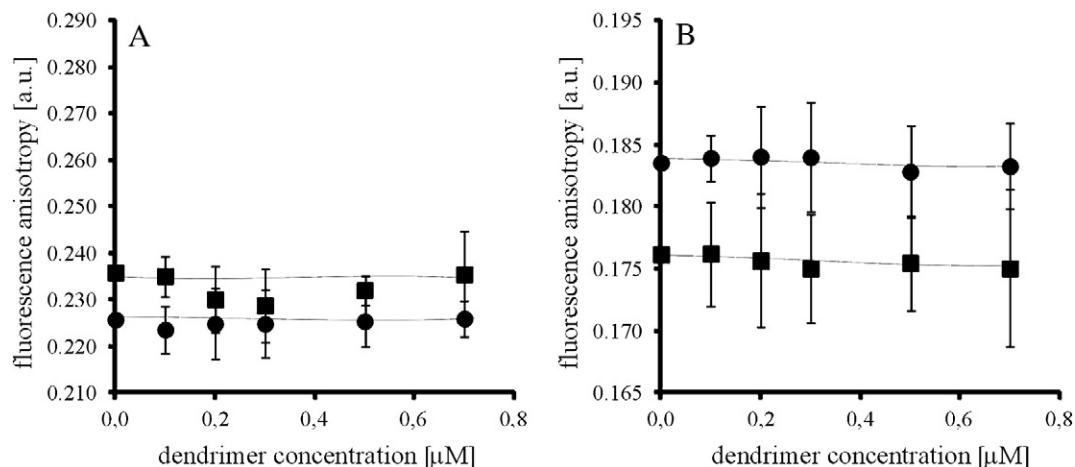
## 2.4. Dendriplex preparation

Carbosilane dendrimers and siRNA were diluted in 10 mM HEPES buffer with 150 mM NaCl at pH 7.4, filtered to remove any particulates (polycarbonate filter of 100 nm pore size). Carbosilane dendrimer/siRNA complexes were formed by mixing appropriate volumes of dendrimers (16 positive charges) and siRNA (46 negative charges) solutions in a molar ratio of 4:1 (dendrimer:siRNA) and stirring for 15 min prior to

analysis. The final concentration of dendrimers was 200  $\mu\text{M}$  and siRNA was 50  $\mu\text{M}$ . The molar ratio 1:4 was chosen based on previous experiments. The process of forming complexes and their characterization have already been described [38]. Complexes were prepared in 10 mM HEPES with 150 mM NaCl buffer, pH 7.4.

## 2.5. Fluorescence spectroscopy

Fluorescence anisotropy was measured with a LS-50B (Perkin-Elmer, U.K.) spectrofluorimeter. To monitor the fluidity of a biomembrane, two fluorescent probes were used; one, DPH (an apolar molecule), was incorporated into the hydrophobic region of the membrane, whereas TMA-DPH was anchored on its surface, exposed to a hydrophilic environment due to its positively charged amino groups. The excitation and emission wavelengths were 348 nm and 426 nm, respectively [39–42]. The slit width of the excitation monochromator was 8 nm and that of the emission monochromator was 6 nm for both labels. The cuvette holder was temperature controlled. Erythrocyte membranes were dissolved at 50  $\mu\text{g}$  proteins/ml and the fluorescent probe added. The concentration of each fluorescent probe was 1 nM. Subsequently the sample was incubated for 15 min and the fluorescence measured. All dendrimers were dissolved in 10 mM HEPES buffer, pH 7.4, with 150 mM NaCl, and added to the sample to reach the appropriate concentration.



**Fig. 3.** Fluorescence anisotropy of TMA-DPH (A) and DPH (B) in cell membrane; ■—NN16 dendrimer/siRNA, ●—BDBR0011 dendrimer/siRNA. All data were expressed as mean  $\pm$  S.E.M.  $n = 4$ ; \*  $p < 0.05$  for each point vs control.

Fluorescence polarization values ( $r$ ) were calculated by the fluorescence data manager program using Jablonski's equation:

$$r = (I_{VV} - GI_{VH}) / (I_{VV} + 2GI_{VH})$$

where  $I_{VV}$  and  $I_{VH}$  are the vertical and horizontal fluorescence intensities, respectively, to the vertical polarization of the excitation light beam. Factor  $G = I_{HV} / I_{HH}$  (the grating correction factor) corrects the polarizing effects of the monochromator.

### 2.6. Hemolysis

The absorbance of released hemoglobin was measured with a Jasco V-650 spectrophotometer. Erythrocytes were suspended in dendrimer solution at a hematocrit of 2% and incubated for 30 min at 37 °C. As a control, erythrocytes were suspended in a 10 mM Hepes buffer, pH 7.4, with 150 mM NaCl; for reference, they were treated with double-distilled water. The percent of hemolysis was measured spectrophotometrically by their absorbance at 540 nm.

### 2.7. Microscopy method

Erythrocytes were suspended in dendrimer solution at a hematocrit of 2% and incubated for 30 min at 37 °C. They were resuspended in a 10 mM Hepes buffer, pH 7.4, with 150 mM NaCl serving as a control. Cell samples were viewed under an Olympus IX70 microscope at a magnification of 400×.

### 2.8. Statistical analysis

Statistical analysis and exponential curve fitting used Statistica 9 (StatSoft) software. The distribution was checked by the Shapiro–Wilk test; if the distribution was normal, student's  $t$ -test was used. Results are expressed as mean  $\pm$  standard error of the mean (S.E.M.).

## 3. Results

### 3.1. Fluorescent anisotropy measurements

Changes in the fluorescence anisotropy show that dendrimers interact with the erythrocyte membranes. This parameter increases when a membrane becomes more rigid, reflecting the fact that dendrimers have probably moved into the membrane. Fluorescence anisotropy of TMA-DPH increased with both dendrimers (Fig. 2A), whereas they reduced that of DPH (Fig. 2B).

Significant changes in the fluorescence anisotropy of DPH and TMA-DPH were seen that indicated that both of the dendrimers affected the lipid order packing of erythrocyte membranes both in the hydrophilic and hydrophobic regions of the bilayer.

The dendrimers are of similar size and possess 16 cationic groups on their surface. For NN16 dendrimer, stronger interactions with membranes labeled with both fluorescent probes were seen compared to BDBR0011. Both caused the hydrophilic part of the membrane to become more rigid (Fig. 2A), whereas the hydrophobic part became more fluid (Fig. 2B).

The dendriplexes did not induce any significant change in fluorescence anisotropy of either fluorescent probe (Fig. 3).

### 3.2. Erythrocyte hemolysis

Hemolysis occurring as hemoglobin is released from erythrocytes when their membranes are destroyed (Fig. 4A). Both the carbosilane dendrimers caused hemolysis; however the BDBR0011 dendrimer had a stronger lytic effect. A 0.4  $\mu$ M concentration induced 19 times higher hemolysis for BDBR0011 than for the other dendrimer. The concentration of NN16 dendrimer that caused 5% hemolysis was 1.8  $\mu$ M, whereas BDBR0011 was as much as 32% at this concentration. The relationship between dendrimer concentration and hemolysis was not non-linear; the shape of both of their hemolysis curves suggests that erythrocytes aggregate.

Hemolysis experiments were also carried out with the dendriplexes. The concentration of dendriplexes was taken as the pure dendrimer concentration and 5 measurement points were chosen to compare hemolysis caused by pure dendrimers. Both kinds of dendriplexes failed to destroy erythrocyte membranes and no hemoglobin was released (Fig. 4B).

### 3.3. Morphology changes

Microscopy showed changes in the erythrocyte shape; the control erythrocytes were discocytes (Fig. 5), whereas increasing dendrimer or dendriplex concentrations led to changed morphology (Figs. 5 and 6). Both dendrimers at 0.4  $\mu$ M induced echinocytic transformation. Many cells were shrunk and had characteristically irregular contours. For higher dendrimer concentrations, aggregates occurred but without echinocytes, the cells having regular shapes. The most striking difference between the effects of the two dendrimers was seen at 50  $\mu$ M, at which erythrocytes with BDBR0011 formed huge aggregates (~140  $\mu$ m) whereas the NN16 dendrimer led to much smaller aggregates (~30  $\mu$ m).

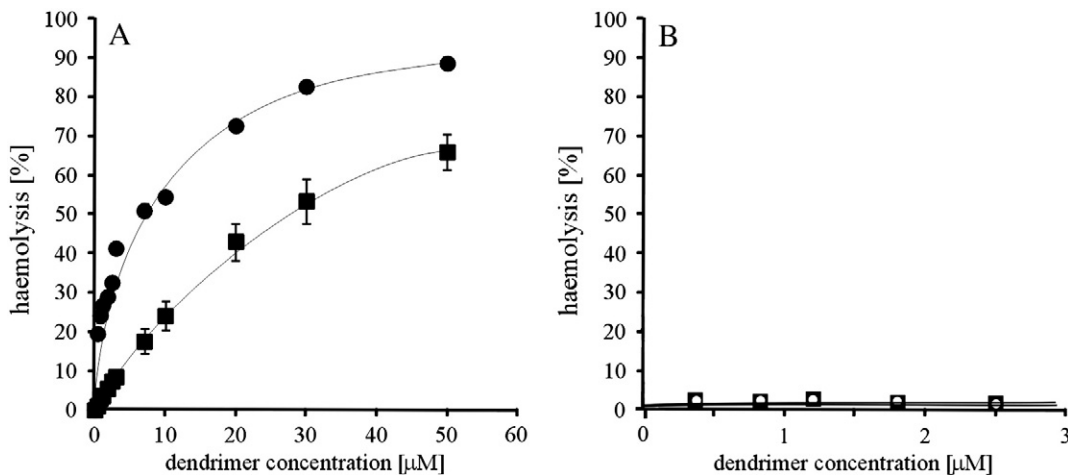


Fig. 4. Red blood cell hemolysis; (A) ■—NN16 dendrimer, ●—BDBR0011 dendrimer; (B) □—NN16 dendrimer/siRNA, ○—BDBR0011 dendrimer/siRNA. All data were expressed as mean  $\pm$  S.E.M.  $n = 4$ ; \*  $p < 0.05$  for each point vs control.

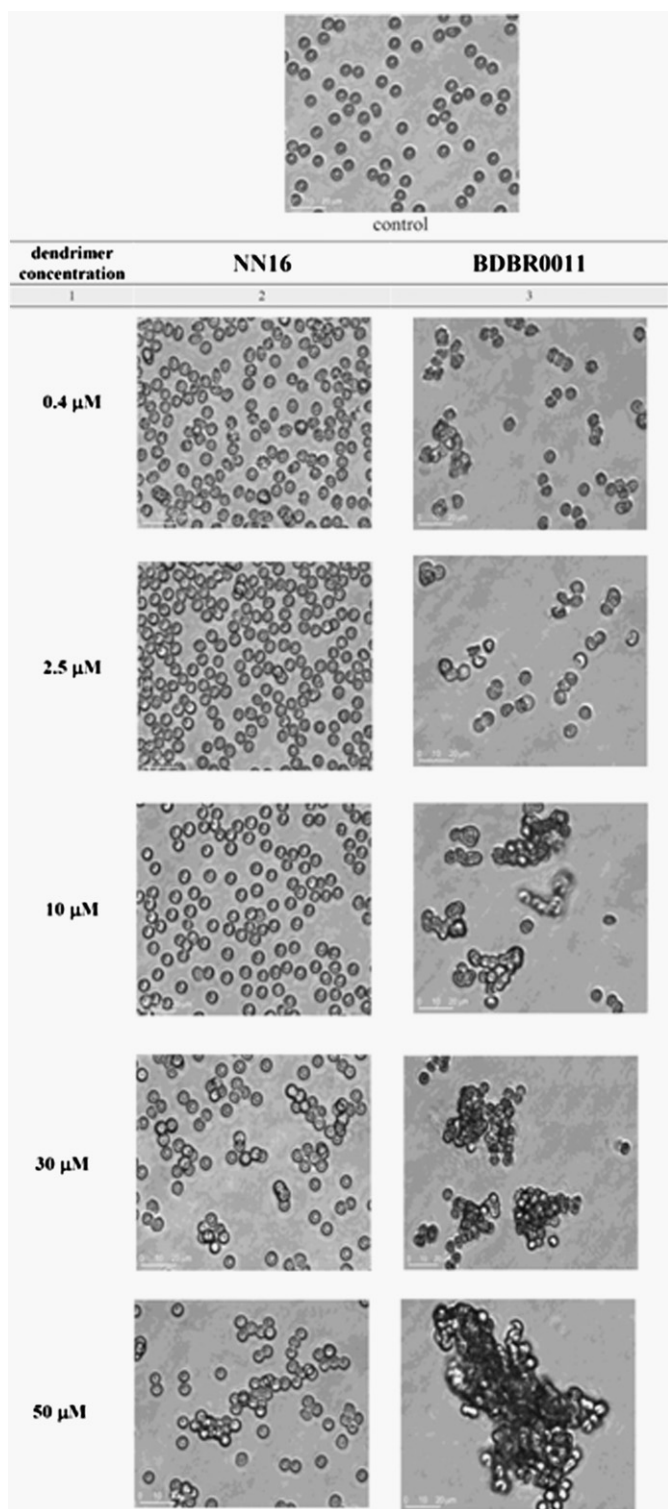


Fig. 5. Erythrocytes morphology changes; NN16 dendrimer, BDBR0011 dendrimer.

The presence of dendriplexes changed erythrocyte morphology (Fig. 6); only the lowest and the highest concentrations of dendriplexes were selected to follow morphological changes. Erythrocytes were created, covered with short, sharply pointed projections.

#### 4. Discussion

Dendrimers are promising molecules in medicine; their structure and properties predispose them as potential drug or nucleic acid carriers.

Because of their chemical and physical properties, they can effectively interact with biological membranes, proteins and organelles [21,22,43], and their molecules are easily subject to chemical transformation.

The positively charged surface groups of cationic dendrimers interact strongly with negatively charged biological membranes, which disturb membrane structure or in severe cases lead to collapse of the structure of the membrane. Numerous plausible models of dendrimers and model lipid membrane interactions have been reported. Globular polymers can remove lipids from the bilayer structure, forming nanoscale holes [16,17,22,23,44–47]. Cationic nanoparticles lyse cells because of their ability to increase permeability and destroy the integrity of the cell membrane [40–42]. Dendrimers can also interact with hydrophobic parts of the lipid bilayer and change its fluidity [18,19,24,26].

The strength of interaction depends on the charge and shape of the molecule. Higher generations of globular polymers have more functional groups and a stronger effect on membrane structure [18,19,28,48–50]. Cationic dendrimers, like PPI or PAMAM, are more cytotoxic than anionic or those with surface modifications [51,52]. But there are not many reports on the interaction of dendrimers with biological membranes. The high complexity of the structure is found with biological membranes, which is important in the regulation of its properties and shape, including protein activity and diffusion pathways.

Our results relate specifically to biological membrane–dendrimer interactions. Changes in the membrane fluidity suggest that dendrimers change the physical properties of the whole membrane. The strongest interactions occurred in the hydrophilic region of the membrane which became more rigid, and thus dendrimers may have been bound to the lipid head groups. NN16 dendrimer which possesses not only Si–C, but also Si–O bonds, structurally stiffened the hydrophilic part of the membrane more than BDBR0011. Differences in the strength of the interaction between the two compounds are probably related to the Si–O bonds associated with affinity to water. Dendrimer properties regarding the back-folding phenomenon were evident; this is a reorganization process due to dendrimer conformation by their folding into the interior of the molecule. Neutral pH induces protonation of surface amines of PAMAM dendrimers; as a consequence hydrogen bonding between surface and interior amines appears [53]. In the two kinds of carbosilane dendrimers investigated, the only real difference between them was in the Si–O bonds of NN16e through which interactions occurred. This can be due to the back-folding process and hydrogen bonding between the positively charged surface amines and oxygen atoms. This phenomenon may explain the higher affinity of the NN16 dendrimer for the hydrophilic part of the bilayer. The less ordered state of the dendrimer molecule and its higher water accessibility makes it preferable to the charged hydrophilic part of a bilayer, which causes greater destabilization of a bilayer.

In the hydrophobic part of the membrane, fluidity increases, with the NN16 dendrimer affecting the membrane more strongly than the other dendrimer. Increasing fluidity of the membrane in the hydrophobic part of bilayer may be due to extraction of lipids from the membrane structure by dendrimers. This may increase the mobility of acyl chains in the membrane tail region. The model used here was that of erythrocyte ghosts. Biological membranes consist of lipids, proteins and sugars, so the observed changes can have also been related to dendrimer–protein interactions [19].

Both dendrimers caused lysis, but the BDBR0011 dendrimer was more lytic than NN16. Hemolysis was concentration dependent. For high concentrations of dendrimers, hemolysis kinetics gave a non-linear shape that may be evidence of aggregation between dendrimers and erythrocytes. High hemolytic behavior of these dendrimers is due to the cationic groups on their surfaces [54,55]. Those molecules, with or without surface modification, are designed as carriers in drug delivery systems [56]. The surface charge of dendrimers has been modified in our experiments, which is a result of interaction between dendrimers and siRNA. The toxicity of dendrimers bound to siRNA (dendriplexes) decreases, which is easily seen when the results for carbosilane

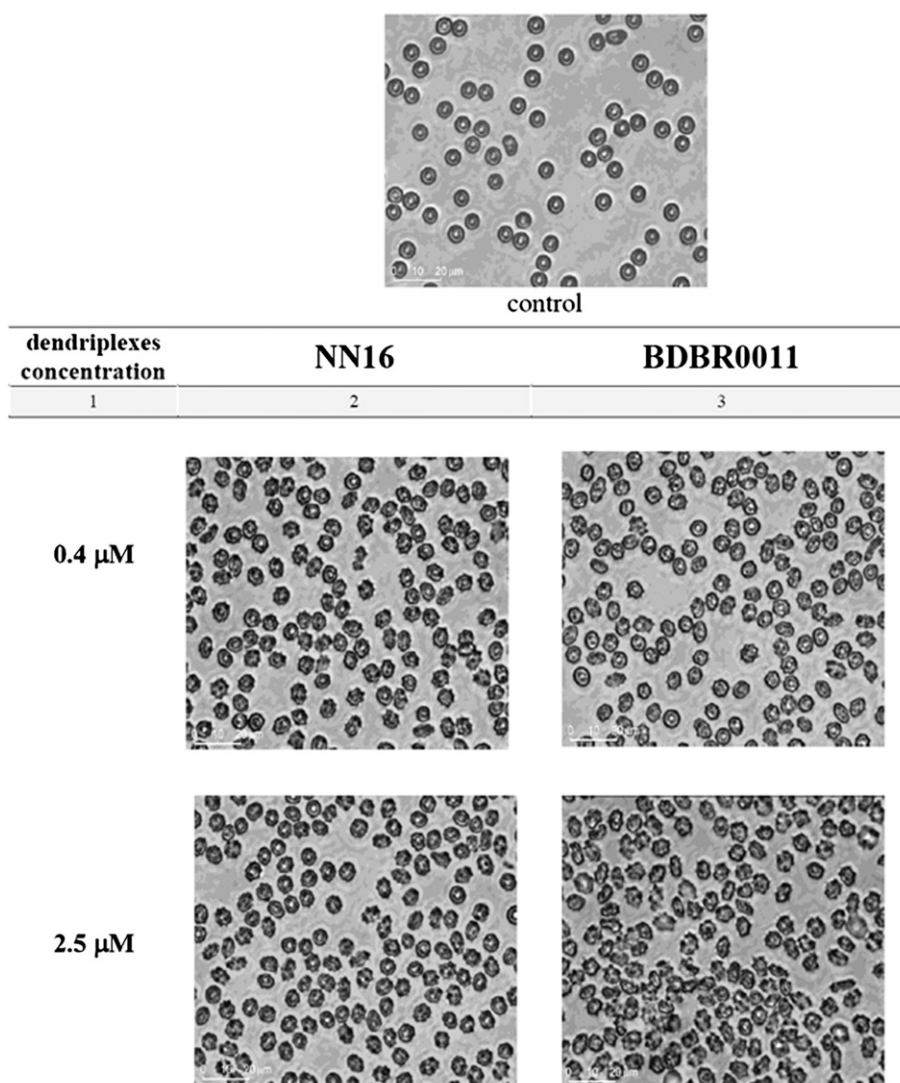


Fig. 6. Erythrocytes morphology changes; NN16 dendrimer/siRNA, BDBR0011 dendrimer/siRNA.

dendrimers alone and dendriplexes are compared. The hemolytic behavior of dendrimers can be reduced by 32% and 7% for the BDBR0011 dendrimer and NN16 dendrimer, respectively, in a concentration of 2.5 μM to zero for both dendriplexes given in an equimolar level to the dendrimer. It is interesting that the BDBR0011 dendrimer was more hemolytic. As already mentioned, its structure is more ordered; thus it could destabilize membranes in a different way and induce mainly fragmentation or pore formation. At neutral pH, all protonated cationic groups are presented on the surface of BDBR0011, which can result in hemolysis and aggregation of erythrocytes even at very low concentrations. For the NN16 dendrimer, toxicity is reduced due to the back-folding phenomenon that hides some cationic groups. However, interactions with membranes are still strong because of remaining positive surface charges, higher affinity to water and hydrogen bonding.

Usually hemolysis is preceded by echinocytic transformation [29,51]. For both dendrimers, erythrocytes only changed their shapes at the lowest concentration (0.4 μM). Some cells had characteristic, irregular, deformable shapes, with many small knoblike projections. Echinocytes were not observed at a higher concentration of dendrimers. Erythrocytes had regular shapes (particularly in the case of NN16), and stuck together (particularly in the case of BDBR0011). Aggregation occurred in hemolysis experiments. Membrane cell degradation was dependent on dendrimer concentration, the number of erythrocytes

decreasing with increasing amounts of globular polymers in the sample. Aggregation was observed above a 10 μM concentration.

Research on the influence of dendrimers on erythrocyte concentrates has mostly focused on commercially available cationic PAMAM dendrimers. Earlier studies showed that increasing concentrations of chemicals with a growing number of functional groups on the surface produces more toxicity in cells [12,13,31,51,57,58]. Addition of protein to such samples reduces their toxicity [16,57].

Carbosilane dendrimers complexed with siRNA neither influenced the fluidity of membranes nor caused hemolysis. Addition of dendriplexes induced echinocyte transformations; the cells became shrunken. siRNA bound to dendrimers decreased their toxicity, which might be due to a reduction in the availability of functional cationic groups. These results confirm the data obtained in other experiments for cationic dendrimers, in which cytotoxicity decreased as a result of the binding of sugars, peptides or drug molecules to the surface groups [12–14].

## 5. Conclusions

Carbosilane dendrimers interact mainly with the hydrophilic part of biological membranes. By binding to the outer leaflet, they decrease its fluidity while increasing that of the hydrophobic interior. They induce hemolysis, but they do not change the morphology of erythrocytes.

Increasing concentrations of dendrimers induced erythrocyte aggregation. Reduction of the number of cationic groups that follows the binding of siRNA decreases the toxicity of cationic dendrimers. siRNA-dendrimer complexes neither change the fluidity of biological membranes nor cause hemolysis. Addition of dendriplexes to erythrocyte suspensions induces echinocyte transformation. Because of lower toxicity and a weaker ability to form aggregates, NN16 dendrimers may be better siRNA carriers than BDBR0011.

## Acknowledgements

Research was supported by the Polish–Slovak Cooperation Project “Dendrimers as carriers for siRNA targeted against HIV-1 virus–interactions with membranes” and the project “Biological Properties and Biomedical Applications of Dendrimers” operated within Foundation for Polish Science TEAM program cofinanced by the European Regional Development Fund, Project TEAM number TEAM/2008-1/5.

## References

- [1] Y. Shi, Mammalian RNAi for the masses, *Trends Genet.* 19 (2003) 9–12.
- [2] T.R. Brummelkamp, R. Bernards, R. Agami, Stable suppression of tumorigenicity by virus-mediated RNA interference, *Cancer Cell* 2 (2002) 243–247.
- [3] S. Freimann, I. Ben Ami, L. Hirsh, A. Dantes, R. Halperin, A. Amsterdam, Drug development for ovarian hyper-stimulation and anti-cancer treatment: blocking of gonadotropin signaling for epiregulin and amphiregulin biosynthesis, *Biochem. Pharmacol.* 68 (2004) 989–996.
- [4] R.S. Dave, R.J. Pomerantz, Antiviral effects of human immunodeficiency virus type 1-specific small interfering RNAs against targets conserved in select neurotropic viral strains, *J. Virol.* 78 (2004) 13687–13696.
- [5] J.A. Wilson, S. Jayasena, A. Khvorova, S. Sabatino, I.G. Rodrigue-Gervais, S. Arya, RNA interference blocks gene expression and RNA synthesis from hepatitis C replicons propagated in human liver cells, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 2783–2788.
- [6] Y. Zhang, T. Li, L. Fu, C. Yu, Y. Li, X. Xu, Silencing SARS-CoV spike protein expression in cultured cells by RNA interference, *FEBS Lett.* 560 (2004) 141–146.
- [7] T.K. Jeffery, P.D. Upton, R.C. Trembath, N.W. Morrell, BMP4 inhibits proliferation and promotes myocyte differentiation of lung fibroblasts via Smad1 and JNK pathways, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 288 (2005) L370–L378.
- [8] J.H. Finigan, S.M. Dudek, P.A. Singleton, E.T. Chiang, J.R. Jacobson, S.M. Camp, Activated protein C mediates novel lung endothelial barrier enhancement: role of sphingosine 1-phosphate receptor transactivation, *J. Biol. Chem.* 280 (2005) 17286–17293.
- [9] M. Ulanova, L. Puttagunta, M. Marcet-Palacios, M. Duszyk, U. Steinhoff, F. Duta, Syk tyrosine kinase participates in beta1-integrin signaling and inflammatory responses in airway epithelial cells, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 288 (2005) L497–L507.
- [10] R. Kumar, B. Adams, A. Oldenburg, A. Musiyenko, S. Barik, Characterisation and expression of a PPI serine/threonine protein phosphatase (PpPPI) from the malaria parasite, *Plasmodium falciparum*: demonstration of its essential role using RNA interference, *Malar. J.* 1 (2002) 5.
- [11] L. Vayssie, M. Vargas, C. Weber, N. Guillen, Double-stranded RNA mediates homology-dependent gene silencing of gamma-tubulin in the human parasite *Entamoeba histolytica*, *Mol. Biochem. Parasitol.* 138 (2004) 21–28.
- [12] A. Asthana, A.S. Chauhan, P. Diwan, N.K. Jain, Poly(amidoamine) (PAMAM) dendritic nanostructures for controlled site-specific delivery of acidic anti-inflammatory active ingredient, *AAPS Pharm. Sci. Technol.* 6 (2005) E536–E542.
- [13] P. Agrawal, U. Gupta, N.K. Jain, Glycoconjugated peptide dendrimers-based nanoparticulate system for the delivery of chloroquine phosphate, *Biomaterials* 28 (2007) 3349–3359.
- [14] A. Janaszewska, K. Maczynska, G. Matuszko, D. Appelhans, B. Voit, B. Klajnert, M. Bryszewska, Cytotoxicity of PAMAM, PPI and maltose modified PPI dendrimers in Chinese hamster ovary (CHO) and human ovarian carcinoma (SKOV3) cells, *New J. Chem.* 36 (2012) 428–437.
- [15] B. Klajnert, R.M. Eppard, PAMAM dendrimers and model membranes: differential scanning calorimetry studies, *Int. J. Farm.* 305 (2005) 154–166.
- [16] D. Shcharbin, M. Jokić, B. Klajnert, M. Bryszewska, Effect of dendrimers on pure acetylcholinesterase activity and structure, *Bioelectrochemistry* 68 (2006) 56–59.
- [17] H. Lee, R.G. Larson, Molecular dynamics simulations of PAMAM dendrimer-induced pore formation in DPPC bilayers with a coarse-grained model, *J. Phys. Chem. B* 110 (2006) 18204–18211.
- [18] D. Wróbel, M. Ionov, K. Gardikis, C. Demetzos, J.P. Majoral, B. Palecz, B. Klajnert, M. Bryszewska, Interactions of phosphorus-containing dendrimers with liposomes, *Biochim. Biophys. Acta* 1811 (2011) 221–226.
- [19] B. Klajnert, J. Janiszewska, Z. Urbanczyk-Lipkowska, M. Bryszewska, D. Shcharbin, M. Labieniec, Biological properties of low molecular mass peptide dendrimers, *Int. J. Pharm.* 309 (2006) 208–217.
- [20] K. Gardikis, S. Hatziantoniou, K. Viras, M. Wagner, C. Demetzos, A DSC and Raman spectroscopy study on the effect of PAMAM dendrimer on DPPC model lipid membranes, *Int. J. Pharm.* 318 (2006) 118–123.
- [21] H. Lee, R.G. Larson, Coarse-grained molecular dynamics studies of the concentration and size dependence of fifth- and seventh-generation PAMAM dendrimers on pore formation in DMPC bilayer, *J. Phys. Chem. B* 112 (2008) 7778–7784.
- [22] H. Lee, R.G. Larson, Lipid bilayer curvature and pore formation induced by charged linear polymers and dendrimers: the effect of molecular shape, *J. Phys. Chem. B* 112 (9) (2008) 12279–12285.
- [23] T. Li Yan, X. Yu, Enhanced permeability of charged dendrimers across tense lipid bilayer membranes, *ACS Nano* 8 (2009) 2171–2176.
- [24] C.V. Kelly, P.R. Leroueil, B.G. Orr, M.M. Banaszak Holl, I. Andricioaei, Poly(amidoamine) dendrimers on lipid bilayers II: effects of bilayer phase and dendrimer termination, *J. Phys. Chem. B* 112 (2008) 9346–9353.
- [25] C.V. Kelly, M.G. Liroff, L.D. Triplett, P.R. Leroueil, D.G. Mullen, J.M. Wallace, S. Meshinchi, J.R. Baker, B.G. Orr, M.M. Banaszak Holl, Stoichiometry and structure of poly(amidoamine) dendrimer lipid complexes, *ACS Nano* 3 (2009) 1886–1896.
- [26] M. Ionov, D. Wróbel, K. Gardikis, S. Hatziantoniou, C. Demetzos, J.P. Majoral, B. Klajnert, M. Bryszewska, Interaction of cationic phosphorus dendrimers with lipid membranes, *Int. J. Nano Dimens.* 1 (2011) 275–278.
- [27] P.E.S. Smith, J.R. Brender, U.H.N. Dürr, J. Xu, D.G. Mullen, M.M. Banaszak Holl, A. Ramamoorthy, Solid-state, NMR reveals the hydrophobic-core location of poly(amidoamine) dendrimers in biomembranes, *J. Am. Chem. Soc.* 132 (2010) 8087–8097.
- [28] V. Tiriveedhi, K.M. Kitchens, K.J. Nevels, H. Ghandehari, P. Butko, Kinetic analysis of the interaction between poly(amidoamine) dendrimers and model lipid membranes, *Biochim. Biophys. Acta* 1808 (2011) 209–218.
- [29] K. Nishikawa, K. Matsuoka, E. Kita, N. Okabe, M. Mizuguchi, K. Hino, S. Miyazawa, C. Yamasaki, J. Aoki, S. Takashima, Y. Yamakawa, M. Nishijima, D. Terunuma, H. Kuzuhara, Y. Natori, A therapeutic agent with oriented carbohydrates for treatment of infections by Shiga toxin-producing *Escherichia coli* O157:H7, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 7669–7674.
- [30] K. Matsuoka, M. Terabatake, Y. Esumi, D. Terunuma, H. Kuzuhara, Synthetic assembly of trisaccharide moieties of globotriaosyl ceramide using carbosilane dendrimers as cores. A new type of functional glyco-materials, *Tetrahedron Lett.* 40 (1999) 7839–7842.
- [31] D.M. Domański, B. Klajnert, M. Bryszewska, Influence of PAMAM dendrimers on human red blood cells, *Bioelectrochemistry* 63 (2004) 189–191.
- [32] B. Klajnert, M. Sadowska, M. Bryszewska, The effect of polyamidoamine dendrimers on human erythrocyte membrane acetylcholinesterase, *Bioelectrochemistry* 65 (2004) 23–26.
- [33] P. Ortega, J.F. Bermejo, L. Chonco, E. de Jesus, F.J. de la Mata, G. Fernández, J.C. Flores, R. Gómez, M.J. Serramia, M.A. Muñoz-Fernandez, Novel water-soluble carbosilane dendrimers: synthesis and biocompatibility, *Eur. J. Inorg. Chem.* 7 (2007) 1388–1396.
- [34] L. Chonco, J.F. Bermejo-Martín, P. Ortega, D. Shcharbin, E. Pedziwiatr, B. Klajnert, F.J. de la Mata, R. Eritja, R. Gómez, M. Bryszewska, M.A. Muñoz-Fernandez, Water-soluble carbosilane dendrimers protect phosphorothioate oligonucleotides from binding to serum proteins, *Org. Biomol. Chem.* 5 (2007) 1886–1893.
- [35] D. Shcharbin, A. Drapeza, V. Loban, A. Lisichenok, M. Bryszewska, The breakdown of bilayer lipid membranes by dendrimers, *Cell. Mol. Biol. Lett.* 11 (2007) 242–248.
- [36] J.F. Bermejo, P. Ortega, L. Chonco, R. Eritja, R. Samaniego, M. Müllner, E. de Jesus, F.J. de la Mata, J.C. Flores, R. Gomez, A. Muñoz-Fernandez, Water-soluble carbosilane dendrimers: synthesis biocompatibility and complexation with oligonucleotides; evaluation for medical applications, *Chemistry* 13 (2007) 483–495.
- [37] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [38] M. Ionov, Z. Garaiova, I. Waczulikova, D. Wróbel, E. Pędziwiatr-Werbicka, R. Gomez-Ramirez, F.J. de la Mata, B. Klajnert, T. Hianik, M. Bryszewska, siRNA carriers based on carbosilane dendrimers affect zeta potential and size of phospholipid vesicles, *Biochem. Biophys. Acta Biomembr.* 1818 (2012) 2209–2216.
- [39] M. Shinitzky, Y. Barenholz, Dynamics of hydrocarbon layer in liposome of lecithin and sphingomyelin containing dicycylphosphate, *J. Biol. Chem.* 249 (1974) 2652–2657.
- [40] M. Shinitzky, Y. Barenholz, Fluidity parameters of lipid regions determined by fluorescence polarization, *BBA Rev. Biomembr.* 515 (1978) 367–394.
- [41] D. Hirsch-Lerner, Y. Barenholz, Probing DNA–cationic lipid interactions with the fluorophore trimethylammonium diphenyl-hexatriene (TMADPH), *Biochim. Biophys. Acta* 1370 (1998) 17–30.
- [42] F. Tanfani, G. Curatola, E. Bertoli, Steady-state fluorescence anisotropy and multifrequency phase fluorometry on oxidized phosphatidylcholine vesicles, *Chem. Phys. Lipids* 50 (1989) 1–9.
- [43] Y.S. Choi, T.S. Cho, J.M. Kim, S.W. Han, S.K. Kim, Amine terminated G-6 PAMAM dendrimer and its interaction with DNA probed by Hoechst 33258, *Biophys. Chem.* 121 (2006) 142–149.
- [44] A. Mecke, S. Uppuluri, T.J. Sassanella, D.K. Lee, A. Ramamoorthy, J.R. Baker, B.G. Orr, M.M. Banaszak Holl, Direct observation of lipid bilayer disruption by poly(amidoamine) dendrimers, *Chem. Phys. Lipids* 132 (2004) 3–14.
- [45] A. Mecke, I.J. Majoros, A.K. Patri, J.R. Baker, M.M. Banaszak Holl, Lipid bilayer disruption by polycationic polymers: the roles of size and chemical functional group, *Langmuir* 21 (2005) 10348–10354.
- [46] A. Mecke, D.K. Lee, A. Ramamoorthy, B.G. Orr, M.M. Banaszak Holl, Membrane thinning due to antimicrobial peptide binding: an atomic force microscopy study of MSI-78 in lipid bilayers, *Biophys. J.* 89 (2005) 4043–4050.
- [47] A. Mecke, D.K. Lee, A. Ramamoorthy, B.G. Orr, M.M. Banaszak Holl, Synthetic and natural polymer nanoparticles interact selectively with fluid-phase domains of DMPC lipid bilayers, *Langmuir* 21 (2005) 8588–8590.
- [48] M.F. Ottaviani, R. Daddi, M. Brustolon, N.J. Turro, D.A. Tomalia, Structural modifications of DMPC vesicles upon interaction with poly(amidoamine) dendrimers studied by CW-electron paramagnetic resonance and electron spin echo techniques, *Langmuir* 15 (1999) 1973–1980.



- [49] M.F. Ottaviani, P. Matteini, M. Brustolon, N.J. Turro, S. Jockusch, D.A. Tomalia, Characterization of starburst dendrimers and vesicle solutions and their interactions by CW- and pulsed-EPR, TEM, and dynamic light scattering, *J. Phys. Chem. B* 102 (1998) 6029–6039.
- [50] G. Purohit, T. Sakthivel, A.T. Florence, Interaction of cationic partial dendrimers with charged and neutral liposomes, *Int. J. Pharm.* 214 (2001) 71–76.
- [51] N. Malik, R. Wiwattanapatapee, R. Klopsch, K. Lorenz, H. Frey, J.W. Weener, E.W. Meijer, W. Paulus, R. Duncan, Dendrimers: relationship between structure and biocompatibility *in vitro*, and preliminary studies on the biodistribution of 125 I-labelled polyamidoamine dendrimers *in vivo*, *J. Control. Release* 65 (2000) 133–148.
- [52] H.T. Chen, M.F. Neerman, A.R. Parrish, E.E. Simanek, Cytotoxicity, hemolysis, and acute *in vivo* toxicity of dendrimers based on melamine, candidate vehicles for drug delivery, *J. Am. Chem. Soc.* 126 (2004) 10044–10048.
- [53] U. Boas, J.B. Christensen, P.M.H. Heegaard, Dendrimers: design, synthesis and chemical properties, *J. Mater. Chem.* 16 (2006) 3785–3798.
- [54] M. Ciolkowski, J.F. Petersen, M. Ficker, A. Janaszewska, J.B. Christensen, B. Klajnert, M. Bryszewska, Surface modification of PAMAM dendrimer improves its biocompatibility, *Nanomedicine* 8 (2012) 815–817.
- [55] G. Thiagarajan, K. Greish, H. Ghandehari, Charge affects the oral toxicity of poly(amidoamine) dendrimers, *Euro. J. Pharm. Biopharm.* 84 (2013) 330–334.
- [56] U. Gupta, H.B. Agashe, A. Asthana, N.K. Jain, A review of *in vitro*–*in vivo* investigations on dendrimers: the novel nanoscopic drug carriers, *Nanomedicine* 2 (2006) 66–73.
- [57] M.H. Han, J. Chen, J. Wang, S.L. Chen, X.T. Wang, Blood compatibility of polyaminoamide dendrimers and erythrocyte protection, *J. Biomed. Nanotechnol.* 6 (2010) 1–11.
- [58] B. Ziemba, A. Janaszewska, K. Ciepluch, M. Krotewicz, W.A. Fogel, D. Appelhans, B. Voit, M. Bryszewska, B. Klajnert, *In vivo* toxicity of poly(propyleneimine) dendrimers, *J. Biomed. Mater. Res. A* 99 (2011) 261–268.