Protective Effect of PEGylation Against Poly(amidoamine) Dendrimer-Induced Hemolysis of Human Red Blood Cells

Wei Wang,[1] Wei Xiong,[1] Yanhong Zhu,[1] Huibi Xu,[2] Xiangliang Yang[1]

[1] College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, People's Republic of China

[2] Department of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China

Received 19 April 2009; revised 26 July 2009; accepted 12 October 2009
Published online 22 February 2010 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.31558

Abstract: Poly(amidoamine) (PAMAM) dendrimers are widely used in medical applications. However, dendrimers bearing positively charged surface groups are prone to destabilize cell membrane and cause cell lysis. The lytic effect of dendrimers on red blood cells (RBCs) namely hemolysis is extremely dangerous when administered *in vivo*. To diminish the hematologic toxicity, we modified PAMAM dendrimers with poly(ethylene glycol) (PEG) of three molecular weights (2k, 5k, and 20k). The protective effect of PEGylation against PAMAM dendrimer-induced hemolysis was studied. RBCs morphology and surface structure were analyzed by optical microscopy (OM) and atomic force microscopy (AFM). The results indicated that PAMAM and PEG-2k modified dendrimers induced hemolysis at 0.1 and 0.5 mg/mL respectively, whereas PEG-5k and PEG-20k modified dendrimers showed no significant difference in hemolysis compared with control even at 5 mg/mL. OM and AFM investigation indicated PAMAM and PEG-2k modified dendrimers caused RBCs aggregation and lysis. However, no changes were observed in the overall shape of RBCs treated with PEG-5k and PEG-20k modified dendrimers. The surface roughness of RBCs treated with PEGylated dendrimers were far lower than that of RBCs treated with PAMAM dendrimers. This study demonstrated that hemocompatibility of PAMAM dendrimers could be greatly enhanced by PEGylation. © 2010 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 93B: 59–64, 2010

Keywords: poly(amidoamine) dendrimers; poly(ethylene glycol); RBC; hemolysis; atomic force microscopy

INTRODUCTION

Dendrimers are globular, nanoscaled macromolecules with a particular architecture constituted of core, branches, and terminal groups. Poly(amidoamine) (PAMAM) dendrimers are a specific family of dendrimers, based on an ethylene diamine core, an amidoamine repeat branching structure, and amine terminals.[1] With each repeat branching (generation) added, the number of amine terminals exactly double. The presence of these terminal groups in dendrimers facilitates multiple simultaneous interactions with other molecules.[2] As one of the most popular dendrimers, generation 5 (G5) PAMAM dendrimers with 128 amine terminals have been successfully used in field of biomedicine such as drug delivery, gene transfection, imaging, and anti-infection.[3–15]

Despite the extensive interest in pharmaceutical applications of dendrimers, there are conflicting reports regarding their biological safety.[16,17] It has been reported that G2–G4 PAMAM dendrimers would cause concentration- and generation-dependent damage to red blood cells (RBCs).[18] Because the circulatory system is the most convenient way of drug administration, detrimental interaction of PAMAM dendrimers with RBCs, the most abundant cells in blood, must be avoided.[19] One way to reduce the toxicity of PAMAM dendrimers may reside in partial surface derivatization with chemically inert functionalities such as poly (ethylene glycol) (PEG). It has been reported that partial derivatization with as few as 13 PEG chains on a G5 PAMAM was sufficient to lower the cytotoxicity.[20] However, to the best of our knowledge, there are no data in the literature about the impact of PEGylation on dendrimers hemocompatibility. With this respect, it is important to

Correspondence to: X. Yang (e-mail: yangxl@mail.hust.edu.cn)
Contract grant sponsor: MOST 973 program of China; Contract grant number: 2006CB705600

investigate the interaction between PEGylated dendrimers and RBCs.

RBCs are among the first cells that come into contact with foreign materials in the blood system.[21] They take the shape of distinctive flexible biconcave disks. Detrimental interaction with erythrocytes such as aggregation, crenation, and hemolysis are indicators of incompatibility of these materials with RBCs.[22–24] RBCs lack nuclei and major organelles because they are constructed by a membrane that contains the cytoplasm mainly composed of hemoglobin. It is generally believed that the plasma membrane is responsible for maintaining the shape of RBCs.[25] Hemolysis test is a simple method widely used to study polymer–membrane interaction. The release of hemoglobin from erythrocyte was used to quantify the membrane damaging effect of foreign materials. Although less specialized than other cell membranes, RBC membranes exhibit many basic functions such as active and passive transport and the production of ionic and electric gradients[26,27]; for this reason, they could be used as a model of the plasma membrane in general.

Atomic force microscopy (AFM) has been widely used as an intrinsically three-dimensional, high-resolution, nondestructive surface characterizing technique. The detailed investigation of the overall shape morphology and specific surface characteristics of RBCs can be performed by AFM at nanometer level. The roughness of RBCs is a morphology-related parameter that has been shown to be characteristic of the single cells composing a sample, but independent of the overall geometric shape (discocyte or spherocyte) of the erythrocytes, thus providing extra information with respect to a conventional morphology study.[28]

In this study, we modified G5 PAMAM dendrimers with three different molecular weight (MW) PEG chains (2k, 5k, and 20k). The protective effect of PEGylation against PAMAM-induced RBCs damage was investigated; meanwhile, the role of MW of PEG on the protective effect was studied. RBCs morphology and surface structure were further analyzed by using optical microscopy (OM) and AFM.

MATERIALS AND METHODS

Materials

PAMAM dendrimers of G5 (MW 28,826) were purchased from Dendritech (Midland, MI). Methoxy polyethylene glycol succinimidyl carbonates (mPEG-SC) with an average MW of 2k, 5k, and 20k were obtained from Kaizheng Biotech Development (Beijing, China). Sodium chloride for injection (0.9% w/v) was purchased from a local pharmacy. Standard glass microscope slides (Corning, NY) were used for preparation of blood films. All other reagents were of analytical grade.

Synthesis and characterization of PEGylated dendrimers

PEGylated dendrimers were synthesized as described in the literature.[29] PEG 2k, 5k, and 20k were conjugated to PAMAM dendrimers; three conjugates were produced: PEG-2k-PAMAM, PEG-5k-PAMAM, and PEG-20k-PAMAM. G5 PAMAM dendrimers have 128 amino terminals. The number of attached PEG chains of every conjugate was preset to 13, which was about 10% of the amino terminals of G5 PAMAM. The starting materials were shown in Table I. The characterization of PEGylated dendrimers was described previously, which confirmed that the practical value coincided with the preset value.[20]

TABLE I. Starting Materials of PEG-PAMAM

Sample Code	PAMAM	PEG
G5 PAMAM	1.6 μM, 45 mg	–
PEG-2k-PAMAM	1.6 μM, 45 mg	20 μM, 40.5 mg
PEG-5k-PAMAM	1.6 μM, 45 mg	20 μM, 103 mg
PEG-20k-PAMAM	1.6 μM, 45 mg	20 μM, 405 mg

Preparation of RBCs

Human RBCs were separated from heparinized blood that was drawn from a healthy donor. Erythrocytes were separated from blood plasma and leukocytes by centrifugation (5000 rpm, 5 min) at 4°C. Then, RBCs were washed three times with physiological saline and finally suspended in physiological saline to obtain an RBC suspension at 2% (v/v) hematocrit.

Hemolysis Assay

RBC suspensions were incubated with PEGylated and PAMAM dendrimers at different concentrations (0.005–5 mg/mL). The incubation of the RBC suspensions was performed at 37°C for 4 h under gentle shaking. RBC suspensions incubated with physiological saline were subjected to the exact same process for comparison. Another suspension was treated with Triton X-100 (1% v/v) to get complete hemolysis.

After 4 h, the RBC suspensions were centrifuged at 1500 rpm for 10 min. The supernatants were assayed spectrophotometrically for the released hemoglobin from the absorbance at 540 nm.[30] The degree of hemolysis was determined by the following equation:

$$\text{Hemolysis } (\%) = \frac{\text{Abs} - \text{Abs}_0}{\text{Abs}_{100} - \text{Abs}_0} \times 100. \qquad (1)$$

Abs, Abs_0, and Abs_{100} are the absorbance of test samples, the suspension treated with physiological saline, and the suspension of complete hemolysis, respectively.

OM and AFM Investigation

OM technique enables the observations of the overall shape morphology of RBCs. The OM images were obtained by

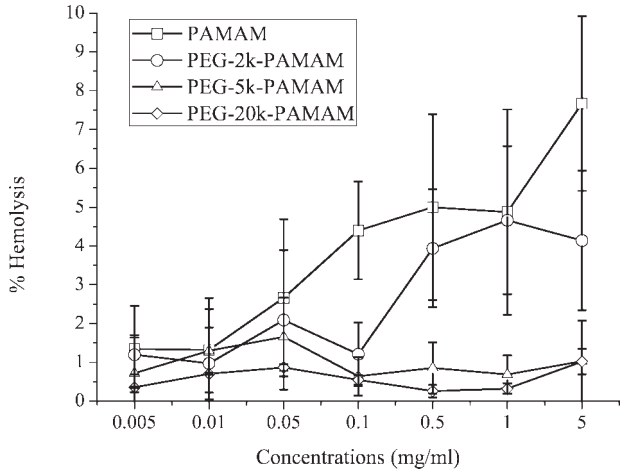

Figure 1. Hemolysis induced by PAMAM and three PEGylated dendrimers.

means of an Axiovert 200MAT metallurgical microscope (Zeiss, Germany) using magnification of 200× and 1000×.

AFM technique enables the study of the detailed morphological changes of RBCs membrane. For AFM investigation, RBCs were fixed by addition of 0.25% glutaraldehyde and 25 μL of each sample was applied to an ethanol-cleaned standard microscope slides. After air drying, the samples were gently rinsed with deionized water to remove salt crystals and then air dried again before analysis.

All AFM images were obtained by means of a Veeco Multimode scanning probe microscope (Vecco, USA). Three independently produced samples were analyzed, and several different areas were investigated on each sample. Characteristic images of individual RBCs are shown. The mean surface roughness of the obtained images from RBCs surfaces was analyzed.

Statistical Analysis

Data are expressed as means ± standard deviations of at least three different experiments. Calculations were performed using Excel. Statistical analysis was performed using SPSS 11.5 with Student's *t* test.

RESULTS

Hemolysis Testing

Effects of PAMAM and three PEGylated dendrimers on the erythrocytes are shown in Figure 1. The results indicated that PAMAM dendrimers caused hemolysis in a concentration-dependent manner. The hemolysis effect was insignificant when the PAMAM concentration was <0.01 mg/mL, whereas it was significant, the percentage of hemolysis exceeded 5%, when the concentration reached 0.1 mg/mL. It was supposed that PAMAM would cause the RBCs membrane disrupting. The hemolysis level of PEG-2k-PAMAM was slightly lower than that of PAMAM. Moreover, the hemolysis levels of high-MW PEG modified dendrimers namely PEG-5k-PAMAM and PEG-20k-PAMAM were much lower than that of PAMAM. No appreciable hemolysis was observed in PEG-5k-PAMAM and PEG-20k-PAMAM groups even at high concentration. Especially, in PEG-20k-PAMAM group, the percentage of hemolysis kept below 1.5%, that is, only minimal hemoglobin released after 4 h incubation with this polymer.

Investigation of the Shape Morphology of RBCs with OM

Changes in the RBCs shape in response to interactions with PAMAM and three PEGylated dendrimers were studied by metallurgical microscope, and representative images of RBCs were shown in Figure 2. Figure 2(a–c) showed the shape changes of RBCs with increasing PAMAM dendrimer concentration. The presence of 0.05 mg/mL PAMAM dendrimers induced partial erythrocyte echinocytic transformation. At a higher PAMAM concentration (0.5 mg/mL), erythrocytes aggregated and formed clusters. The agglutinated RBCs were difficult to disperse. The presence of 5 mg/mL PAMAM dendrimers caused considerable hemolysis, crenation, and aggregation. Similar shape transitions were observed in RBCs suspensions treated with PEG-2k-PAMAM. However, the degrees of aggregation and hemolysis were lower [Figure 2(d–f)]. On the other hand, the high-MW PEG modified dendrimers namely PEG-5k-PAMAM and PEG-20k-PAMAM did not induce any measurable hemolysis even at high concentration (5 mg/mL). The erythrocytes were morphologically normal [Figure 2(g,h)] and were similar to that of saline controls [Figure 2(i)].

Investigation of the Surface Structure of RBCs with AFM

The morphological investigation of the RBC in saline solution showed typically biconcave erythrocyte of 7.0–8.0 μm in diameter with smooth surface [Figure 3(a,b)]. Figure 3(c–j) shows representative AFM images of RBC incubated with the same concentration (5 mg/mL) of PAMAM [Figure 3(c,d)], PEG-2k-PAMAM [Figure 3(e,f)], PEG-5k-PAMAM [Figure 3(g,h)], and PEG-20k-PAMAM [Figure 3(i,j)]. The panels on the left in Figure 3 present surface images of RBCs at the micrometer level; the panels on the right are high-resolution images at a scanned area of 1×1 μm^2 of the left. The intact cell structure was not seen in Figure 3(c). Spherocyte and collapsed erythrocyte with irregular contour were observed in PEG-2k-PAMAM-treated group [Figure 3(d)]. RBCs displayed the irregular contour because of the collapse of the membrane. It was notable that RBCs incubated with PEG-5k-PAMAM and PEG-20k-PAMAM kept the typically biconcave shape with smooth surfaces, except for two or three fissures [Figure 3(g,i)]. From the right panels in Figure 3, we could clearly see the ultrastructure changes of erythrocyte membrane. Overall, the PEGylated dendrimers caused much less

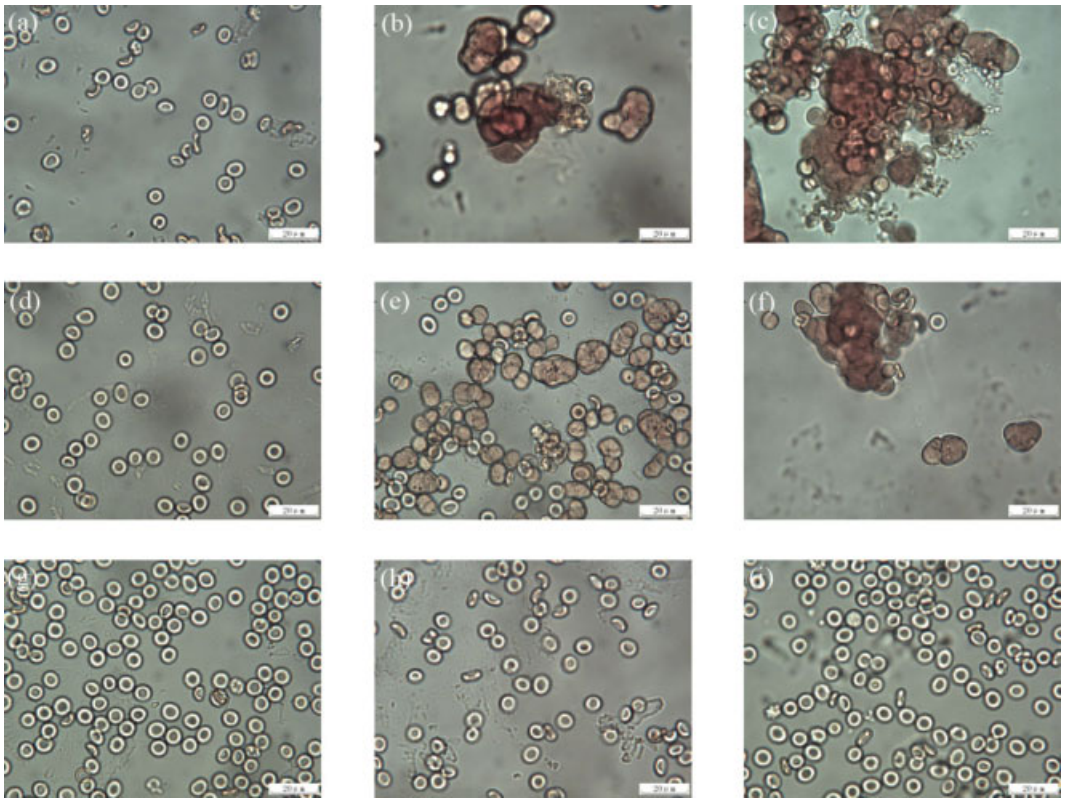

Figure 2. Optical micrographs of human RBCs incubated with dendrimers at different concentrations. All images are at 1000× magnification. (a) PAMAM (0.05 mg/mL); (b) PAMAM (0.5 mg/mL); (c) PAMAM (5 mg/mL); (d) PEG-2k-PAMAM (0.05 mg/mL); (e) PEG-2k-PAMAM (0.5 mg/mL); (f) PEG-2k-PAMAM (5 mg/mL); (g) PEG-5k-PAMAM (5 mg/mL); (h) PEG-20k-PAMAM (5 mg/mL); and (i) saline control. Bar = 20 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

damage to the surface structure than did PAMAM. Moreover, RBCs treated with high-MW PEG-modified dendrimers had more smooth surfaces.

Surface Roughness Analysis

To quantitative comparatively analyze the roughness of RBCs with different polymers, the surface roughness were measured from high-resolution images at a scanned area of 1×1 μm^2. Figure 4 presents mean surface roughness of RBCs incubated with saline solution, PAMAM, and PEGylated dendrimers at 5 mg/mL. Roughness values (Rms) accordant with the above images were attained; that is, a decrease in Rms was observed in PEGylated groups compared with PAMAM group; furthermore, the lower Rms attained in the higher MW PEG modified groups.

DISCUSSION

Hemolysis experiment was performed to investigate the interactions of PAMAM and PEGylated dendrimers with RBCs. The leakage of hemoglobin was used to quantitatively compare the membrane-damaging properties of these dendrimers. The surface of a normal erythrocyte is negatively charged because of the presence of glycolipids and some glycated integral and peripheral proteins. Electrostatic repulsion among RBCs prevents their self-aggregation and adhesion to the walls of blood vessels.[31] Cationic polymers such as PAMAM dendrimers come close to the RBCs as a result of electrostatic attraction. The hemolysis caused by PAMAM dendrimers is thought to be the result of interaction between positively charged dendrimers and negatively charged cell surfaces.[18]

A greater surface coverage with biocompatible terminal groups like PEG was widely used to create less cytotoxic dendrimers.[20,32] PEG is a highly hydrated polymer and has a high degree of segmental flexibility in aqueous solutions.[33] The hemolysis caused by PEGylated dendrimers was much lower compared with the parent dendrimers. Moreover, dendrimers conjugated with high-MW PEG were more effective at reducing toxicity.[20] The OM images (Figure 2) corroborated the hemolysis study. In PEG-5k-PAMAM and PEG-20k-PAMAM groups, the integrity of cell membrane was preserved. Neither cell aggregation nor cell lysis occurred. The results of hemolysis test and morphology investigation for high-MW PEG modified groups were identical to those for normal RBCs. It was thought to be the result of reduction/shielding of the positive charge. Flexibility of longer PEG chain was supposed to make it cover more surface areas. Consequently, dendrimers conjugated with higher MW PEG showed much lower hematological toxicity.

AFM has emerged in ultrastructural biology as a novel tool to quantitative description of morphological details of cell surface. A typical biconcave erythrocyte with smooth surface was observed in physiological group [Figure 3(a,b)]. Treatment with PAMAM generated RBC membrane disintegrating completely, which resulted in leakage of hemoglobin from cells and cell lysis. PEGylation could effectively reduce PAMAM-induced damage.

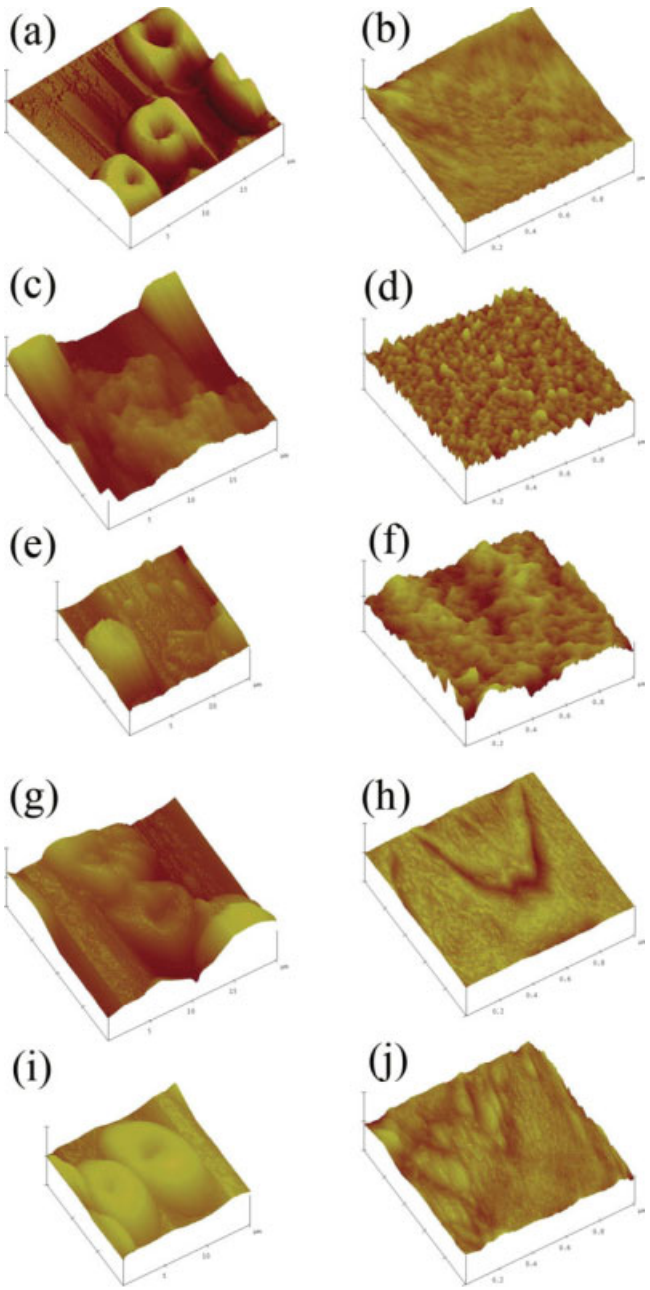

Figure 3. Representative AFM images of RBCs treated with saline control (a and b), PAMAM (c and d), PEG-2k-PAMAM (e and f), PEG-5k-PAMAM (g and h), and PEG-20k-PAMAM (i and j). The concentration of PAMAM and PEGylated dendrimers was 5 mg/mL. The panels on the right are high-resolution images at a scanned area of 1×1 μm^2 of the left. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

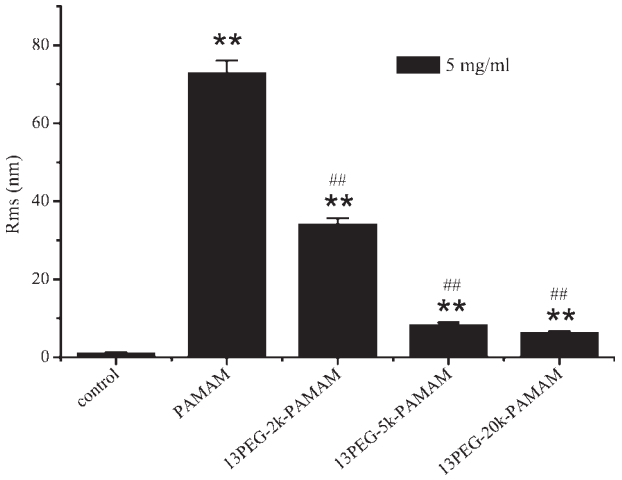

Figure 4. Mean surface roughness of RBCs treated with PAMAM and PEGylated dendrimers at 5 mg/mL. $^{**}p < 0.01$ represents the comparison with the control group. $^{\#\#}p < 0.01$ represents the comparison with the PAMAM group.

RBCs treated with high-MW PEG-modified dendrimers were almost identical to those of reference. Rms values obtained by AFM provided qualitative information for the surface characteristics. The roughness results showed a decreasing tendency with the increasing of MW of PEG and gradually approached the Rms value of the untreated erythrocyte (1.154 ± 0.12 nm). These results confirmed that PEGylation could effectively decrease the PAMAM-induced RBC damage. On the other hand, PEGylated dendrimers with many residual amino terminals would maintain the efficacy on their various applications. Moreover, the efficacy would be improved because the PEG chains could provide steric stabilization to the primary PAMAM–DNA or PAMAM–drug association.[34]

CONCLUSIONS

Cationic PAMAM dendrimers caused RBC membrane disruption, morphology change, and hemolysis. Grafted hydrophilic PEG corona to PAMAM prevented the amine groups from interacting with RBCs, which could effectively protect RBCs from hemolysis. Moreover, hemocompatibility of PAMAM dendrimers could be greatly enhanced by conjugated with longer PEG chains. PEGylated dendrimers were more suitable and valuable for practical application compared with the parent dendrimers because they presented a much lower hematologic toxicity.

REFERENCES

1. Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, Ryder J, Smith P. A new class of polymers: Starburst-dendritic macromolecules. Polym J 1985;17:117–132.
2. Mammen M, Choi SK, Whitesides GM. Polyvalent interactionsin biological systems: Implications for design and use of multivalent ligands and inhibitors. Angew Chem Int Ed Engl 1998;37:2754–2794.

3. Yang W, Cheng Y, Xu T, Wang X, Wen LP. Targeting cancer cells with biotin-dendrimer conjugates. Eur J Med Chem 2009;44:862–868.
4. Dribek M, Le Potier I, Rodrigues A, Pallandre A, Fattal E, Taverna M. Determination of binding constants of vasoactive intestinal peptide to poly(amidoamine) dendrimers designed for drug delivery using ACE. Electrophoresis 2007;28:2191–2200.
5. Majoros IJ, Myc A, Thomas T, Mehta CB, Baker JR Jr. PAMAM dendrimer-based multifunctional conjugate for cancer therapy: Synthesis, characterization, and functionality. Biomacromolecules 2006;7:572–579.
6. Wu G, Barth RF, Yang W, Kawabata S, Zhang L, Green-Church K. Targeted delivery of methotrexate to epidermal growth factor receptor-positive brain tumors by means of cetuximab (IMC-C225) dendrimer bioconjugates. Mol Cancer Ther 2006;5:52–59.
7. Kubát P, Lang K, Janda P, Anzenbacher P Jr. Interaction of porphyrins with a dendrimer template: Self-aggregation controlled by pH. Langmuir 2005;21:9714–9720.
8. Majoros IJ, Thomas TP, Mehta CB, Baker JR Jr. Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy. J Med Chem 2005;48:5892–5899.
9. Dung TH, Kim J, Kim MS, Kim JS, Yoo H. Preparation and biophysical characterization of pluronic F127-dendrimer conjugate as a delivery agent of antisense oligonucleotides. J Nanosci Nanotechnol 2008;8:5326–5330.
10. Kang H, DeLong R, Fisher MH, Juliano RL. Tat-conjugated PAMAM dendrimers as delivery agents for antisense and siRNA oligonucleotides. Pharm Res 2005;22:2099–2106.
11. Swanson SD, Kukowska-Latallo JF, Patri AK, Chen C, Ge S, Cao Z, Kotlyar A, East AT, Baker JR. Targeted gadolinium-loaded dendrimer nanoparticles for tumor-specific magnetic resonance contrast enhancement. Int J Nanomed 2008;3:201–210.
12. Lebdusková P, Sour A, Helm L, Tóth E, Kotek J, Lukes I, Merbach AE. Phosphinic derivative of DTPA conjugated to a G5 PAMAM dendrimer: An 17O and 1H relaxation study of its Gd(III) complex. Dalton Trans 2006;28:3399–3406.
13. Wang W, Guo Z, Chen Y, Liu T, Jiang L. Influence of generation 2–5 of PAMAM dendrimer on the inhibition of Tat peptide/TAR RNA binding in HIV-1 transcription. Chem Biol Drug Des 2006;68:314–318.
14. Kobayashi H, Kawamoto S, Bernardo M, Brechbiel MW, Knopp MV, Choyke PL. Delivery of gadolinium-labeled nanoparticles to the sentinel lymph node: Comparison of the sentinel node visualization and estimations of intra-nodal gadolinium concentration by the magnetic resonance imaging. J Control Release 2006;111:343–351.
15. Rekas A, Lo V, Gadd GE, Cappai R, Yun SI. PAMAM dendrimers as potential agents against fibrillation of alpha-synuclein, a Parkinson's disease-related protein. Macromol Biosci 2009;9:230–238.
16. Roberts JC, Bhalgat MK, Zera RT. Preliminary biological evaluation of polyamidoamine (PAMAM) Starburst dendrimers. J Biomed Mater Res 1996;30:53–65.
17. Malik N, Wiwattanapatapee R, Klopsch R, Lorenz K, Frey H, Weener JW, Meijer EW, Paulus W, Duncan R. 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 2000;65:133–148.
18. Domański DM, Klajnert B, Bryszewska M. Influence of PAMAM dendrimers on human red blood cells. Bioelectrochemistry 2004;63:189–191.
19. Mendiratta S, Qu ZC, May JM. Erythrocyte ascorbate recycling: Antioxidant effects in blood. Free Radic Biol Med 1998;24:789–797.
20. Wang W, Xiong W, Wan J, Sun X, Xu H, Yang X. The decrease of PAMAM dendrimer-induced cytotoxicity by PEGylation via attenuation of oxidative stress. Nanotechnology 2009;20:105103.
21. Zobel HP, Stieneker F, Atmaca-Abdel Aziz S, Gilbert M, Werner D, Noe CR, Kreuter J, Zimmer A. Evaluation of aminoalkylmethacrylate nanoparticles as colloidal drug carrier systems. II. Characterization of antisense oligonucleotides loaded copolymer nanoparticles. Eur J Pharm Biopharm 1999;48:1–12.
22. Duncan R, Izzo L. Dendrimer biocompatibility and toxicity. Adv Drug Deliv Rev 2005;57:2215–2237.
23. Li J, Xiao H, Li J, Zhong Y. Drug carrier systems based on water-soluble cationic beta-cyclodextrin polymers. Int J Pharm 2004;278:329–342.
24. Fischer D, von Harpe A, Kunath K, Petersen H, Li Y, Kissel T. Copolymers of ethylene imine and *N*-(2-hydroxyethyl)-ethylene imine as tools to study effects of polymer structure on physicochemical and biological properties of DNA complexes. Bioconjugate Chem 2002;13:1124–1133.
25. Elgsaeter A, Mikkelsen A. Shapes and shape changes in vitro in normal red blood cells. Biochim Biophys Acta 1991;1071:273–290.
26. Bennett V. The membrane skeleton of human erythrocytes and its implications for more complex cells. Ann Rev Biochem 1985;54:273–304.
27. Suwalsky M, Fierro P, Villena F, Sotomayor C. Effects of lithium on the human erythrocyte membrane and molecular models. Biophys Chem 2007;129:36–42.
28. Girasole M, Pompeo G, Cricenti A, Congiu-Castellano A, Andreola F, Serafino A, Frazer BH, Boumis G, Amiconi G. Roughness of the plasma membrane as an independent morphological parameter to study RBCs: A quantitative atomic force microscopy investigation. Biochim Biophys Acta 2007;1768:1268–1276.
29. Belcheva N, Woodrow-Mumford K, Mahoney MJ, Saltzman WM. Synthesis and biological activity of polyethylene glycol-mouse nerve growth factor conjugate. Bioconjugate Chem 1999;10:932–937.
30. Yoshida Y, Kashiba K, Niki E. Free radical-mediated oxidation of lipids induced by hemoglobin in aqueous dispersions Biochim Biophys Acta 1994;1201:165–172.
31. Zhang TL, Gao YX, Lu JF, Wang K. Arsenite, arsenate and vanadate affect human erythrocyte membrane. J Inorg Biochem 2000;79:195–203.
32. Jevprasesphant R, Penny J, Jalal R, Attwood D, McKeown NB, D'Emanuele A. The influence of surface modification on the cytotoxicity of PAMAM dendrimers. Int J Pharm 2003;252:263–266.
33. Mao S, Neu M, Germershaus O, Merkel O, Sitterberg J, Bakowsky U, Kissel T. Influence of polyethylene glycol chain length on the physicochemical and biological properties of poly(ethylene imine)-graft-poly(ethylene glycol) block copolymer/SiRNA polyplexes. Bioconjugate Chem 2006;17:1209–1218.
34. Luo D, Haverstick K, Belcheva N, Han E, Saltzman WM. Poly(ethylene glycol)-conjugated PAMAM dendrimer for biocompatible, high-efficiency DNA delivery. Macromolecules 2002;35:3456–3462.