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Novel Aldehyde-Terminated Dendrimers; Synthesis and Cytotoxicity Assay

Aliasghar Hamidi[1,2,3], Simin Sharifi[1,3], Soodabeh Davaran[1,2], Saeed Ghasemi[2,3], Yadollah Omidi[1,4], Mohammad-Reza Rashidi[1,2*]

[1]*Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Department of Medicinal Chemistry, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
[4]*Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*

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ABSTRACT

Introduction: Polyamidoamine (PAMAM) dendrimers are a unique family of dendritic polymers with numerous pharmaceutical and biomedical applications. One major problem with these polymers is their cytotoxicity. The purpose of this study was to synthesize novel dendrimers with aldehyde terminal groups and compare their cytotoxicity with that of dendrimers containing amine-terminated groups. ***Methods:*** G1(first generation) and G2 (second generation) dendrimers with amine-terminated groups were synthesized by divergent method and then the amine-terminated groups were converted to the aldehyde groups using surface modification of the functional group inversion (FGI) method. The cytotoxicity of the novel G1 and G2 polyamidoaldehyde (PAMAL) dendrimers together with that of G1 and G2 PAMAM-NH2 dendrimers was investigated by MTT assay using MCF-7 cell line. ***Results:*** The results showed that cytotoxicity of dendrimers with aldehyde-terminated groups is much lower than that of G1 and G2 PAMAM-NH2 dendrimers. ***Conclusion:*** Dendrimers with aldehyde-terminated groups could be used as novel and convenient carriers for drug delivery with low cytotoxic effect compared with the amine-terminated dendrimers. The results revealed that the same generations of the dendrimers with aldehyde-terminated groups are far less toxic than the corresponding amine-terminated dendrimers.

Introduction

Controlled drug delivery systems are one of the most important fields of pharmaceutical sciences which have seen rapid advances in recent decades. Due to their great potential to improve the quality of life, these systems have been used by pharmaceutical scientists.

Controlled drug delivery systems have various advantages compared to conventional treatment ways, such as high efficacy, reduced toxicity, and improved quality of life of patient. In addition, maintaining a drug concentration in the therapeutic level for adequate time in the circulation system and overcoming drug-resistance are reasons to use of using polymers in drug delivery systems (Majoros and Jr 2008).

In order to control drug release, avoid harmful side-effects, and increase drug bioavailability, several drug delivery systems are under development at present. One of the important components of the controlled drug delivery systems are drug carriers. Drug carriers contain microparticles, biodegradable natural polymers, microcapsules, lipoproteins, liposomes, micelles and dendritic polymers (dendrimers). To date, several types of synthetic polymers are used as drug carriers including dendrimers. Dendritic polymers are highly branched, mono-disperse macromolecules of nanometer dimensions and each specified generation has a specified size (Fre´chet and Tomalia 2001). Dendrimers have wide ranges of potential applications in a large number of different areas. Properties associated with these dendrimers such as regular size, high degree of branching, water solubility, modifiable surface, functionality and available internal cavities make them interesting for biological and drug-delivery applications (Uhrich *et al* 1999, Kaparissides *et al* 2005). Known cationic polymers containing polyamidoamine (PAMAM),

***Corresponding author:** Mohammad-Reza Rashidi (PhD), Tel.: +98-411-3367914, Fax: +98-411-3367929, E-mail: rashidi@tbzmed.ac.ir
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polyethylenimine (PEI) and polypropyleneimine (PPI) are commonly used for in vitro gene delivery which generation is depended on degree of branching (Kafil and Omidi 2011)

PAMAM dendrimers are a novel class of branched polymers which possess a number of interesting and useful properties with several pharmaceutical and bio-medical applications. In addition, the solubility of low soluble drugs can be increased by these dendrimers. They are able ability to cross the cell membrane at high rates to act as potential delivery systems (Boas *et al* 2006). These unique nonlinear polymers have many functional end groups that can be converted to other groups by functional group inversion (FGI) method.

In spite of these interesting properties and multiple applications, the use of dendrimers in biological system is limited because of their inherent toxicity (Jain *et al* 2010). The studies have shown that PAMAM dendrimers are hemolytic and cytotoxic, with toxicity tending to be higher for cationic PAMAM dendrimers and to increase with generation (Kitchens *et al* 2005). The PAMAM toxicity has been attributed to the interaction of surface cationic charge of dendrimers with negatively charged biological membranes in vivo (Jain *et al* 2010) and the cytotoxicity could be reduced by surface engineering (D'Emanuele *et al* 2004).

Cytotoxicity of PAMAM dendrimers depends on the generation, size, their surfaces, concentration, type of cells and condition of reaction. PAMAM dendrimers with anionic surface groups like carboxylates or sulfonates have low or no cytotoxicity (Boas *et al* 2006). Lack of toxicity in anionic dendrimers may also arise from the non-adhesive nature of these dendrimers towards the cellular membranes being negatively charged (Boas *et al* 2006).

If these dendrimers are used for gene delivery, interaction of positive charge on the dendrimers surface with the negative charge on phosphate groups of DNA helps to reduce their cytotoxicity. But, when using these polymers in drug delivery, positive charge on the surface of amine dendrimers at physiological pH can interact with negative charge on cell membrane leading omitted cell lysis and consequently cytotoxicity. Therefore, to prevent this interaction, the surface charge can be modified by biocompatible polymers for example PEG or aldehyde-terminated groups (neutralized at physiological pH) which can be generated from amine-terminated groups by functional group inversion (FGI) method (Hamidi *et al* 2012).

The aldehyde-terminated dendrimers are usually prepared through the activation of amine-terminated dendrimers by glutaraldehyde. This method has two disadvantages of glutaraldehyde toxicity and cross-linking due to existence of two aldehyde functional groups on its both ends. To overcome these problems, in the present study, the glutaraldehyde was omitted from the reaction and the aldehyde-terminated dendrimers were synthesized using aminoacetaldehyde dimethylacetal (AADA) in a shorter synthetic sequence for the first time.

Therefore, two new PAMAL dendrimers with aldehyde-terminated groups (G1-CHO and G2-CHO) were synthesized using a novel synthetic method and their cytotoxicity was evaluated in comparison with that of G1 and G2 amine dendrimers.

Materials and methods

Methanol, ethylenediamine (EDA), methylacrylate, hydrochloric acid, trifluoroacetic acid (TFA), aminoacetaldehyde dimethyl acetal (AADA), dimethylesulfoxide (DMSO), DMSO-d_6, deuterated chloroform ($CDCl_3$) and fetal bovine serum (FBS) were purchased from Merck (Darmstadt, Germany). Streptomycin, penicillin G and 3-(4,5-dimethylthiazolyl-2)-2,5- diphenyltetrazolium bromide (MTT) were obtained from Sigma Aldrich Co, Germany. The human breast cancer MCF-7 cells was purchased from National Cell Bank of Iran was supplied by Pasteur Institute, Iran. RPMI 1640 medium and fetal bovine serum (FBS) was purchased from Gibco, UK.

Trypsin was prepared from Gibco-Invitrogen, Canada. All chemicals were analytical grade and used without further purification except for ethylenediamine which was distilled before using. The optical density (OD) of formazan (as an indicator of cell viability) was measured at 570 nm using a spectrophotometric plate reader (Sunrise plate reader; Tecan, Austria).

The FT-IR spectra were recorded on a Shimadzu 4300 spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Varian unity 400 spectrometer and chemical shifts (δ) are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.

Synthesis of zero generation (G0.5) PAMAM dendrimer with 8 ester-terminated groups

The solution of G0 PAMAM dendrimer (12 g, 0.0225 mol) in methanol (30 ml) under nitrogen was added dropwise to a stirred solution of methylacrylate (19.4 g, 0.225mol) in methanol (30 ml) over a period of 3 h. The resulted mixture was stirred for 80 min at 0 °C and then allowed to warm to room temperature and stirred for a further 24 h. The solvent was removed under reduced pressure at 40 °C with a rotary evaporator and the colorless oily product was dried under vacuum (2 mm Hg, 50 °C) for 12 h to give the final product (25.4 g; 89% yield) (Fig. 1).

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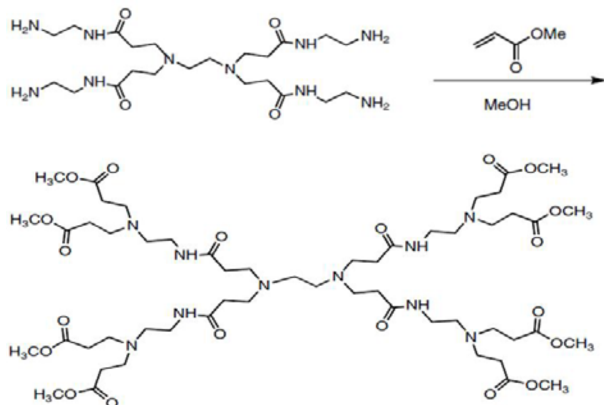

Fig. 1. The synthetic scheme of G0.5 PAMAM Ester-terminated dendrimer: dropwise addition of G0 PAMAM dendrimer to a stirred solution of methylacrylate at 0 °C for 3 h then at 25 °C for 24 h under nitrogen in methanol.

Acetalization of G0.5 dendrimer with 8 ester-terminated groups

The solution of G0.5 PAMAM dendrimer with 8 Ester-terminated groups (2 g, 1.65 mmol) and AADA (200 µL) in methanol (30 mL) was stirred at room temperature for 3 days. Then, removal of the solvent under vacuum (2 mm Hg, 40 °C, 14 h) gave the PAMAM dendrimer with acetal-terminated groups as a viscous and adhesive product (60% yield) (Fig. 2).

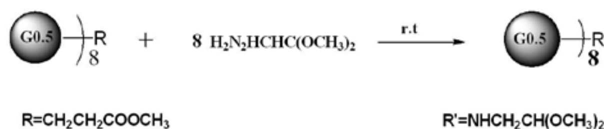

Fig. 2. The synthetic scheme of G0.5 PAMAM acetal-terminated dendrimer: dropwise addition of aminoacetaldehyde dimethyl acetal vigorously stirred at 0 °C to 25 °C under nitrogen in methanol, 72 h.

Hydrolysis of G0.5 acetal-terminated PAMAM dendrimer

Hydrolysis of acetal-terminated PAMAM was carried out according to the literature with slight modification (Lutz and Borner 2008). The solution of G0.5 PAMAM dendrimer with acetal-terminated groups (0.5 g, 0.28 mmol) and trifluoroacetic acid (200 µL) in dichloro-methane (15 ml) was stirred at room temperature for 48 h. Then, solvent was removed under vacuum (10 mmHg, 40 °C, 8 h). Final product is first generation of polyamidoaldehyde (PAMAL) with aldehyde-terminal groups.

Synthesis of G1.5 PAMAM dendrimer with ester-terminated groups

The solution of G1 (9.75 g, 6.8 mmol) in methanol (30 ml) under nitrogen was added to a stirred solution of methylacrylate (10.05 g, 0.116 mol) in methanol (30 ml), during 3 h. The final mixture was stirred at 0 °C for 1 h and then allowed to reach to room temperature and stirred for 3 days. The solvent was removed under reduced pressure (2 mm Hg, 40 °C) and the resultant pale yellow oil was dried under vacuum (2 mm Hg, 50 °C, 24 h) to give the final product (Fre´chet and Tomalia 2001).

Synthesis of (G1.5) PAMAM dendrimer with acetal-terminated groups

The solution of G1.5 PAMAM dendrimer with ester-terminated groups (2 g, 0.71 mmol) and AADA (200 µL) in methanol (30 mL) was stirred at room temperature for 4 days. Then, the solvent was removed under vacuum (2 mm Hg, 45 °C, 12 h) yielding the PAMAM dendrimer with acetal-terminated groups as a viscous product (55% yield) (Fig. 3).

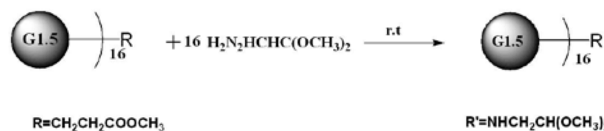

Fig. 3. The synthetic scheme of G2 PAMAM acetal-terminated dendrimer: dropwise addition of aminoacetaldehyde-dimethyl-acetal vigorously stirred at 0 °C to 25 °C under nitrogen in methanol, 96 h.

Synthesis of aldehyde-terminated dendrimer (G2)

Conversion of terminal acetal groups of PAMAM A to aldehyde was carried out in the presence of TFA in Methanol. Fig. 4 shows synthetic pathway of final product G2 PAMAL from PMAM dendrimer A. The formation of the aldehyde group was confirmed by both FTIR and NMR spectroscopy. Formation of a peak at 1725 cm^{-1} in FTIR spectrum (Fig. 5.), a singlet peak at 10.82 in ^{1}HNMR spectrum (Fig. 6.), and a peak in 203.319 ppm in ^{13}CNMR spectrum (Fig. 7.) confirm the production of the G2 PAMAL dendrimer B in Fig. 4.

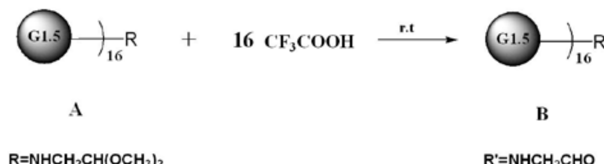

Fig. 4. The synthetic scheme of aldehyde-terminated dendrimer (G2): Stirring at room temperature in methanol, 6 h.

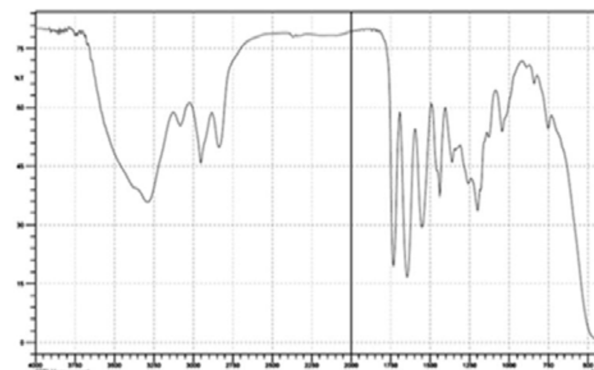

Fig. 5. FT-IR spectrum of G2 PAMAL dendrimer with aldehyde-terminal groups.

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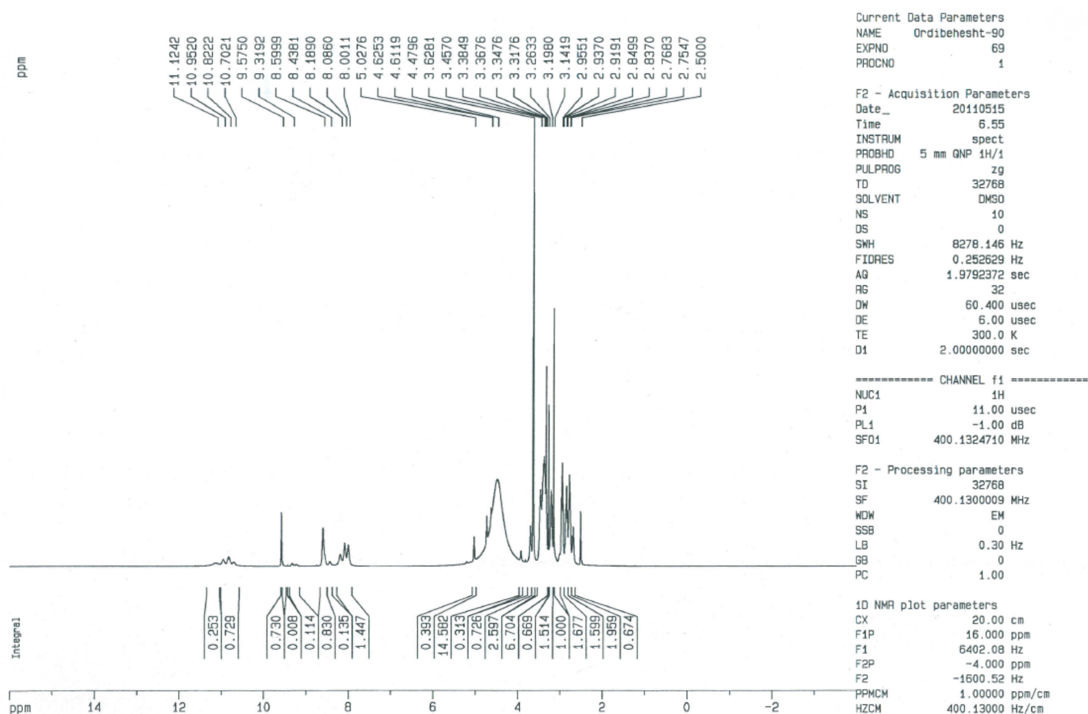

Fig. 6. ^{1}HNMR spectrum of G2 PAMAL dendrimer with aldehyde-terminal groups.

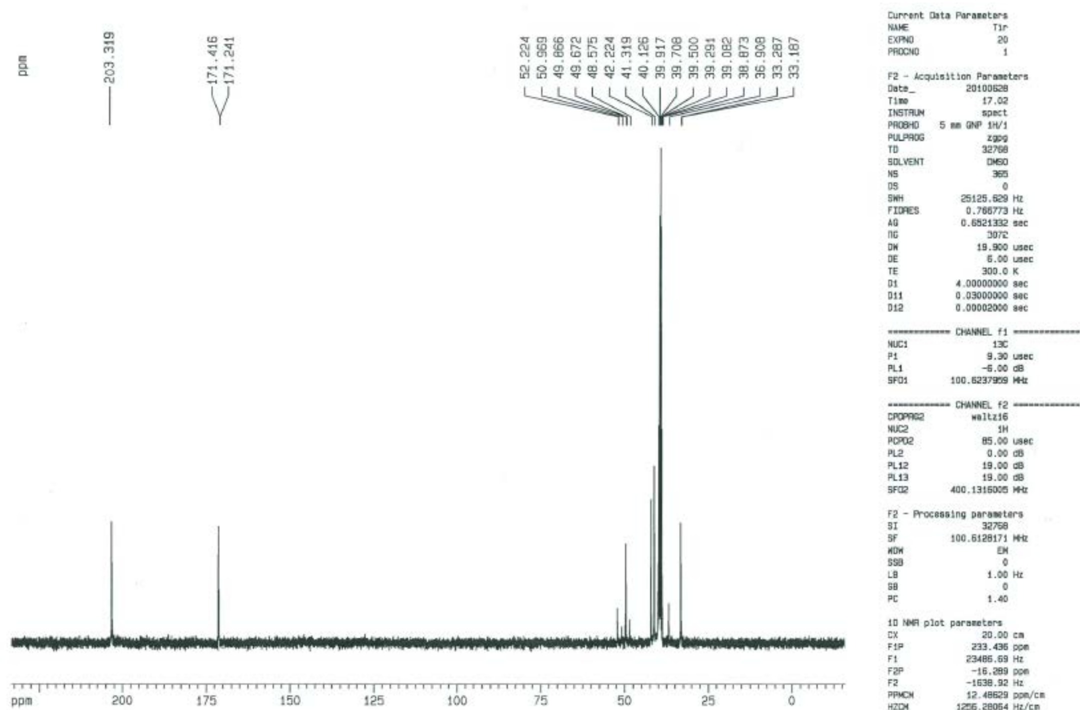

Fig. 7. ^{13}CNMR spectrum of G2 PAMAL dendrimer with aldehyde-terminal groups.

MTT assay

Cytotoxicity of the novel synthesized G1 and G2PAMAL nanoparticles together with that of G1 and G2PAMAM dendrimers were evaluated by widely established 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-tetrazolium bromide (MTT) assay using MCF-7 cell line. MCF-7 cells were cultured in RPMI-1640 medium (Gibco, UK) supplemented with 10% heat inactivated

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FBS (Gibco, UK), penicillin and streptomycin (Sigma Aldrich, Germany). MCF-7 cells were maintained at 37 ºC with 5% CO_2 in a 95% humidity incubator. The cells were seeded in a 96-well plate in 200 µl media per well at a density of 15,000 cells/well and incubated for 24 h. The media was then replaced with 200 µl of medium-containing nanoparticles at different concentrations (400, 200, 100, 50, 10 and 5 µM) and incubated for 24 and 48 h. All nanoparticle concentrations were tested at least in triplicate wells. About 150 µl of RPMI-1640 medium and 50 µl MTT solution (5 mg/ml in PBS) were added to each well, followed by incubation at 37 °C for 4 h to allow formazan formation then the medium and MTT were removed. To the resulting purple crystals, 200 µl of DMSO was added to dissolve the formazan crystals and was incubated for 5 min at 37 ºC. The optical densities (OD) at 570 nm were determined using a microplate reader (Sunrise Tecan, Austria). Optical densities measured for wells containing cells that received no nanoparticle were considered to represent 100% viability. The assays were performed in duplicate, (replicated at least once) (Cory *et al* 1991, Freshney 1982).

Results

Synthesis and characterization of G0.5 PAMAM dendrimer with 8 ester-terminated groups

$({}^{a}CH_2{}^{a}CH_2)[N({}^{b}CH_2{}^{c}CH_2{}^{d}CO{}^{e}NH{}^{f}CH_2{}^{g}CH_2N({}^{h}CH_2{}^{i}CH_2{}^{j}CO_2{}^{k}CH3)_2)_2]_2$

FTIR ν (cm^{-1}):1745-1730;(C═O); ^{1}H NMR ($CDCl_3$) δ_H(ppm) : 3.62(12H, s, CH_3), (2.72 8H, t, N-CH2), 2.45 (4H, s,CH_2-N,CH2- CH2), 2.40 (8H, t, CH2-CO); ^{13}C NMR ($CDCl_3$): (171.88 (d), 51.14(e), 50.45 (b), 48.65 (a), 31.52 (c);

The reaction of G0 PAMAM dendrimer with methyl-acrylate resulted in G0.5 PAMAM ester-terminated dendrimer (yield 89%). The ^{1}HNMR spectrum showed a single peak at 3.625 ppm, a triplet peak at 2.72 ppm, a singlet peak at 2.44 ppm and a triplet peak at 2.39 ppm corresponding to methoxy proton, 8 protons in *b* position, 4 protons in *a* position, and 8 protons in *c* position, respectively (Hamidi *et al* 2012). A peak was observed at 1740 cm^{-1} in the FTIR spectrum of the compound which is related to the ester carbonyl.

Synthesis and characterization of (G1.5) PAMAM dendrimer with ester-terminated groups

$({}^{a}CH_2{}^{a}CH_2)[N({}^{b}CH_2{}^{c}CH_2{}^{d}CO{}^{e}NH{}^{f}CH_2{}^{g}CH_2N({}^{h}CH_2{}^{i}CH_2{}^{j}CO{}^{k}NH{}^{l}CH_2{}^{m}CH_2N({}^{n}CH_2{}^{o}CH_2{}^{p}CO_2{}^{q}CH_3)_2)_2)_2]_2$

IR(ν /cm^{-1}):1730(ester C=O), 1650(amide C=O) 3300(NH); ^{1}H NMR ($CDCl_3$) δ_H(ppm):7.04 (12H,bt,e ,k), 3.62 (48H,s,q),3.23(24H,bm,f,l), 2.83(8H,bt,g),2.69-2.76 (52H,m,a,b,c,h,i) , 2.47(32H,bt, o) ; 2.38(32H,bt, n); 2.30(16H,bt, m)

In studying of FTIR spectrum of G1.5 with acetal terminal group, the peak in 1725 cm^{-1} confirms the existence of carbonyl group, the study of ^{1}HNMR spectrum presents a single peak in 3.670ppm related to methoxy protons (Hamidi *et al* 2012).

Synthesis and characterization of aldehyde-terminated dendrimer (G2)

$({}^{a}CH_2{}^{a}CH_2)[N({}^{b}CH_2{}^{c}CH_2{}^{d}CO{}^{e}NH{}^{f}CH_2{}^{g}CH_2N({}^{h}CH_2{}^{i}CH_2{}^{j}CO{}^{k}NH{}^{l}CH_2{}^{m}CH_2N({}^{n}CH_2{}^{o}CH_2{}^{p}CO{}^{q}NH{}^{r}CH_2{}^{s}CHO)_2)_2)_2]_2$

IR(ν /cm^{-1}):1725Aldehyde C=O), 1630(amide C=O) 3300(NH); ^{1}H NMR ($CDCl_3$) δ_H (ppm): 10.82 (16H, s, s),5.02 (16H,bt,q), 4.72 (8H,bt,k), 4.62(4H, bt, e) 3.69 (32H,bs, r), 3.34-3.45(24H, bt, f,l), 2.93 (24H,bt,g,m) , 2.67-2.83(116H,bt, a,b,c,h,i,o,n)

Conversion of acetal moiety of terminated PAMAM to aldehyde through hydrolysis reaction afforded G2 PAMAL. The formation of the aldehyde group was confirmed using both NMR and FTIR. There was a singlet peak at 10.82 ppm ^{1}HNMR spectrum and a peak at 203.319 ppm in the^{13}CNMR spectrum related to the aldehyde group (Hamidi *et al.*, 2012). The FTIR analysis of the compound showed a peak in 1725 cm^{-1} further confirming the formation of the aldehyde carbonyl group in FTIR spectrum.

MTT assay

The cytotoxicity of the synthesized dendrimers was examined on MCF-7 cell lines by MTT assay. As shown in Fig. 8, MCF-7 cell viability after treating with G1 amine-terminated dendrimer was 83% at 200 µM and decreased to 76% at 400 µM of concentration, while treating with G1 aldehyde-terminated dendrimer showed 97-96% cell viability in the same concentration range. Although both G2 dendrimers reduced the number of viable cells in a concentration-dependent manner, compared with G2 amine dendrimer, the G2 aldehyde-terminated dendrimer showed very low toxic effects on the cells even at 600 µM concentration in which the amine-terminated dendrimer was highly cytotoxic (28% *vs.* 93% loss of the viability) (Fig. 9).

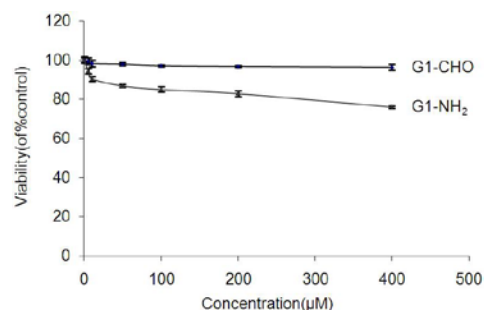

Fig. 8. Effects of surface functional groups and concentration of G1 dendrimers on the viability of MCF-7. Cell viability was measured by the MTT assay (in G1-NH_2; mean±SD for concentration(µM) of 0,5,10,0,100,200,400 are 100±1.69, 94.32±1.63, 90.27±1.69, 86.77±0.73 ,84.93±0.35,82.84±0.42, 75.95± 1.62 Respectively and in G1-CHO; mean±SD for concentration(µM) of , 0,5,10,0,100,200,400 are 100±1.19, 99.2±1.31, 8.22±1.13, 7.84±1.71, 97.06±1.36, 96.69±1.59, 96.28±0.69 Respectively.

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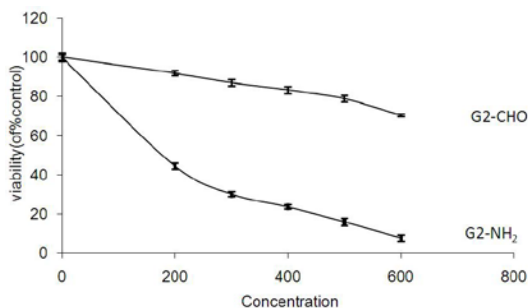

Fig. 9. Effects type of surface functional groups and concentration of G2dendrimers on the viability of MCF-7 measured by the MTT assay (in G2-NH_2; mean±SD for concentration(μM) of 0,200,300,400,500,600 are 100±1.92, 44.48±1.50, 30.13±1.32, 23.72±1.21 ,15.93±1.92,7.77±1.54 Respectively and in G2-CHO; mean±SD for concentration(μM) of ,200,300,400,500,600 are 100±1.44, 91.91±1.31, 87.07±1.71, 83.23±1.77, 78.92±1.61, 70.28±0.64 Respectively.

Discussion

In spite of the extensive interest in the pharmaceutical and biomedical applications of dendrimers, their clinical use is constrained due to the cytotoxicity associated with dendritic polymers and the results regarding their biological safety are conflicting (Jevprasesphant *et al* 2003, Malik *et al* 2000). PAMAM dendrimers are the most common class of dendritic polymers with many materials science and biotechnology applications. As a dendrimer, they exhibit toxicity and this has limited their application. The toxicity of PAMAM dendrimers are more significant for the cationic form and it has been shown that this cytotoxic effect of the dendrimers arises mostly from the interaction between positively charged dendrimers and negatively charged cell surfaces (Jain *et al* 2010, Malik *et al* 2000). Therefore, it is likely that the modification of cationic dendrimers can decrease or shield the positive charge on the dendrimer surface and lead to a decrease in the cytotoxicity (Jevprasesphant *et al* 2003, D'Emanuele *et al* 2004).

In the present study, the surface charge of the PAMAM dendrimer was modified by conversion of the amine-terminated groups of the dendrimer to aldehyde groups using AADA as an intermediate reagent. AADA has one protected side and an amine group in the other side. The later functional group can react with ester forming amide group, while the protected side can be converted to aldehyde group by acidic hydrolysis.

The synthesis of new aldehyde terminal containing dendrimers in the proposed new method has two major advantages over current methods: the aldehyde terminal group does not undergo cross-linking reactions, and unlike amine group which has a positive charge at physiological pH, aldehyde group is neutralized at this condition. The toxicity of dendrimers is attributed to the interaction of surface cationic charge of dendrimers with negatively charged biological membranes *in vivo*. Neutralization of the surface charge can mask the cationic charge of dendrimer surface and reduce the cytotoxicity of the dendrimer making them more compatible for clinical applications. Other surface engineering methods such as PEGylation, acetylation and folate conjugation have been used to mask the cationic charge of dendrimers and minimize their toxicity (Duncan and Izzo 2005).

The strategy of masking of peripheral cationic charge of dendrimers by surface engineering through FGI method used in this study to minimize cytotoxicity was successful. The novel G1 and G2 aldehyde-terminated dendrimers synthesized in the present study were much less toxic than the corresponding amine-terminated polymers. The cytotoxicity increased with the increase the dendrimer generation with both amine and aldehyde-terminated dendrimers; however, this was much more significant with the former dendrimer which has a positive charge at physiological pH.

The amine-terminated dendrimers displayed concentration-dependent toxicity on MCF-7 cell lines. Their cytotoxic effects were much higher than those of the aldehyde-terminated dendrimers. No significant loss of viability was observed with G1 aldehyde-terminated dendrimers even at higher concentrations. Although G2 aldehyde-terminated dendrimer also reduced the number of viable cells in a concentration-dependent manner, the cells were much more resistant to higher concentrations of the G2 aldehyde-terminated polymer. Only 17% loss of cell viability was observed with G2 aldehyde-terminated dendrimer at 600 μM, whereas the corresponding value with G2 amine-terminated dendrimer was 76.13%.

Conclusion

PAMAM dendrimers are a unique family of dendritic polymers, which have recently been successfully used in the field of biomedical, such as drug delivery, gene delivery, cancer diagnosis, etc. The future of dendrimer as drug delivery module relies on fine modification of three component of dendrimer architecture i.e. core, branches and surface groups which made these carrier unique among existing nanoscale carrier system. It is expected that new methods and strategies for synthesizing biocompatible dendrimers will be developed. Dendritic boxes, which have been studied for their carrier properties, may also emerge useful in reducing toxicity of dendrimers. Unfortunately there are only few information on the in vivo toxicity as well as in vivo performance of these unique carriers and hence it is expected that more focused experiments will be performed in this direction(Jain *et al* 2010).

These results showed that synthesis of dendrimers with surface modifications cause increase of the cell increase at the cell viability.

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Ethical issues

None to be declared.

Conflict of interests

The authors declare no conflict of interests.

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References

Boas U, Christensen JB and Heegaard PMH. **2006**. *Dendrimers in Medicine and Biotechnology New Molecular Tools.* The Royal Society of Chemistry.

Cory A, Owen T, Barltrop J and Cory J. **1991**. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. *Cancer communications,* 3(7), 207-212.

Kaparissides C, Alexandridou S, Kotti K and Chaitidou S. **2005**. Recent Advances in Novel Drug Delivery Systems. *Journal of nanotechnology online*, doi: 10.2240/azojono0111

D'Emanuele A, Jevprasesphant R, Penny J and Attwood D. **2004**. The use of a dendrimer-propranolol prodrug to bypass efflux transporters and enhance oral bioavailability. *Journal of Controlled Release,* 95(3), 447-453.

Duncan R and Izzo L. **2005**. Dendrimer biocompatibility and toxicity. *Advanced Drug Delivery Reviews,* 57(15), 2215-2237.

Fre´chet JMJ and Tomalia DA. **2001**. *Dendrimers and Other Dendritic Polymers.* John Wiley & Sons Ltd.

Freshney RI. **1982**. Culture of animal cells: a manual of basic technique. *Liss AR, editor,* 4.

Hamidi A, Rashidi MR, Asgari D, Aghanejad A and Davaran S. **2012**. Covalent Immobilization of Trypsin on a Novel Aldehyde-Terminated PAMAM Dendrimer. *Bulletin of the Korean Chemical Society* (In press).

Jain K, Kesharwani P, Gupta U and Jain NK. **2010**. Dendrimer toxicity: Let's meet the challenge. *International Journal of Pharmaceutics,* 394(1-2), 122-142.

Jevprasesphant R, Penny J, Jalal R, Attwood D, Mckeownb NB and D’Emanuele A. **2003** The influence of surface modification on the cytotoxicity of PAMAM dendrimers. *International Journal of Pharmaceutics,* 252, 263-266.

Kafil V and Omidi Y. **2011**. Cytotoxic Impacts of Linear and Branched Polyethylenimine Nanostructures in A431 Cells. *BioImpacts,* 1(1), 23-30.

Kitchens KM, El-Sayed MEH and Ghandehari H. **2005**. Transepithelial and endothelial transport of poly (amidoamine) dendrimers. *Advanced Drug Delivery Reviews*, 57, 2163-2176.

Majoros IJ and Jr. JRB. **2008**. *Dendrimer-Based Nanomedicine.* Pan Stanford Publishing Pte. Ltd.

Malik N, Wiwattanapatapee R, Klopsch RK. Lorenz HF, Weener JW, Meijer EW *et al.* **2000**. Dendrimers: Relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125 labelled polyamidoamine dendrimers in vivo. *J. Contr. Release,* 65, 133-148.

Uhrich KE, Cannizzaro SM and Langer RS. **1999**. Polymeric Systems for Controlled Drug Release. *Chem. Rev.,* 99, 3181-3198.

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