<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Hyperbranched PEGmethacrylate linear pDMAEMA block copolymer as an efficient non-viral gene delivery vector</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>Mathew, Asha; Cao, Hongliang; Collin, Estelle; Wang, Wenxin; Pandit, Abhay</td>
</tr>
<tr>
<td><strong>Publication Date</strong></td>
<td>2012-06-02</td>
</tr>
<tr>
<td><strong>Publisher</strong></td>
<td>Elsevier</td>
</tr>
<tr>
<td><strong>Item record</strong></td>
<td><a href="http://hdl.handle.net/10379/3004">http://hdl.handle.net/10379/3004</a></td>
</tr>
<tr>
<td><strong>DOI</strong></td>
<td><a href="http://dx.doi.org/DOI">http://dx.doi.org/DOI</a> 10.1016/j.ijpharm.2012.05.010</td>
</tr>
</tbody>
</table>
Pharmaceutical Nanotechnology

Hyperbranched PEGmethacrylate linear pDMAEMA block copolymer as an efficient non-viral gene delivery vector

Asha Mathew 1, Hongliang Cao 1, Estelle Collin 1, Wenxin Wang*, Abhay Pandit**

Network of Excellence for Functional Biomaterials, NFB Building, IDA Business Park, Newcastle Road, Dangan, National University of Ireland, Galway, Ireland

1. Introduction

Gene therapy introduced in the early 1970's (Aposhian, 1970; Rogers, 1971; Osterman et al., 1971), is a promising approach for the treatment of genetic disorders, mutation and intractable diseases, and its success relies on the transfection capabilities of its vectors. Three main types of gene delivery systems have been described so far: viral and non-viral vectors and the direct injection of genetic materials into the tissues using “gene guns” (Goverdhana et al., 2005; Verma and Weitzman, 2005; Young et al., 2006; Lee et al., 2008; Itaka and Kataoka, 2009; Maurer, 2010). Viral vectors show high transfection efficiency. However, their application is limited by their toxicity, difficulty in pharmaceutical processing and scale-up as well as the possibility of the reversion of an engineered virus to a wild type (Young et al., 2006). Even though the idea of using non-viral cationic vectors as transfection agents was introduced in 1973 (Henner et al., 1973), this area was not fully explored until the mid to late 1990's (Abdallah et al., 1995, Frederiksen et al., 1999). Non-viral vectors have increasingly drawn attention due to their large scale manufacture, transport, storage, reduced immunogenic response and the possibility of modifications and their capacity to carry large inserts (Romano et al., 2000). In this regard, cationic polymers such as poly(ethyleneimine) (PEI), poly-l-lysine (PLL), poly(β-amino esters), poly-2-dimethylaminoethyl methacrylate (pPDMAEMA) and ethylene dimethacrylate (EGDMA) block were designed and synthesized via deactivation enhanced atom transfer radical polymerisation (DE-ATRP) for efficient gene delivery. Using this unique structure, with a linear pDMAEMA block, which efficiently binds to plasmid DNA (pDNA) and hyperbranched polyethylene glycol (PEG) based block as a protective shell, we were able to maintain high transfection levels without sacrificing cellular viability even at high doses. The transfection capability and cytotoxicity of the polymers over a range of pDNA concentration were analysed and the results were compared to commercially available transfection vectors such as polyethylene imine (branched PEI, 25 kDa), partially degraded poly(amido amine) dendrimer (dPAMAM; commercial name: SuperFect®) in fibroblasts and adipose tissue derived stem cells (ADSCs).

© 2012 Elsevier B.V. All rights reserved.
progress in polymerisation methods, it has become possible to design and prepare well-defined polymers by controlled/living polymerisations (Goto and Fukuda, 2004). Atom transfer radical polymerisation (ATRP) has been widely employed for the preparation of well-defined biomaterials (Zhang et al., 2007). Wang et al. (2007) introduced the deactivation enhanced atom transfer radical polymerisation (DE-ATRP) approach for the synthesis of hyperbranched copolymers. In this approach a halogen-CuI/halogen-CuII mixture was used to enhance the deactivation of the polymerisation process which leads to a slow polymer growth. This method is advantageous for synthesis of soluble hyperbranched polymer instead of gelation.

Herein a new hyperbranched polymeric system with a linear pDMAEMA block and a hyperbranched polyethylene glycol methyl ether methacrylate (PEGEMA) and ethylene dimethacrylate (EDGMA) block was synthesised via DE-ATRP technique and analysed for transfection capability and cytotoxicity over a range of plasmid DNA (pDNA) dosages. The rationale behind the synthesis and use of this new polymer is that the polymer structure with a linear pDMAEMA unit can efficiently bind pDNA whereas the PEG based hyperbranched unit will form the micelle-like structure in the water solution because of its amphiphilicity to protect the polyplex and enhance its viability thereby reducing the cytotoxicity while maintaining its transfection efficiency at high dosages. To prove this concept, two hyperbranched polymers one with a high PEG content (termed IpD-b-b-P/E 1) and another with low PEG content (termed IpD-b-P/E 2) were synthesized and compared with commercially available transfection vectors such as branched PEI (25 kDa) (gold standard for non viral gene delivery vector), partially degraded poly(amido amine) dendrimer (dPAMAM; commercial name: SuperFect®), and also with linear pDMAEMA. These polymers were tested on fibroblast cells which are generally easy to transfect and also on a primary cell line: adipose tissue derived stem cells (ADSCs) which are generally considered as “difficult to transfect cells” (Wang et al., 2008; Uchida et al., 2009).

2. Materials and methods

2.1. Chemicals

All reagents including HPLC and analytical solvents, synthesis and cell culture reagents were obtained from Sigma–Aldrich Chemical Co. unless otherwise stated. These reagents were used with a purity of more than 98%, and were used without further purification. pDNA, Gausia Luciferase kit was purchased from New England BioLabs and PicoGreen™ kit from Invitrogen. Dialysis membrane (MWCO 8000) was used as received from Spectrapor. dPAMAM was purchased from QIAGEN.

2.2. Synthesis of poly(2-dimethyl-aminoethylmethacrylate) (pDMAEMA)

pDMAEMA was synthesized via ATRP as follows: DMAEMA (15 g, 9.54 × 10⁻² mol), ethyl-α-bromoisoobutyrte (EBiB) (0.146 g, 7.51 × 10⁻⁴ mol), N,N,N,N′,N′-pentamethyldiethylenetriamine (PMDETA) (0.0649 g, 3.75 × 10⁻⁴ mol) and tetrahydrofuran (THF) (15 ml) was added to a two-necked round bottom flask. After sealing the reaction flask, the mixture was purged with nitrogen for 20 min. Copper chloride(I) (0.0371 g, 3.75 × 10⁻⁴ mol) was immediately added. The reaction flask was then sealed and immersed in an oil bath at 50 °C. The reaction was stopped after 2.5 h at a monomer conversion of 78%. The polymer was precipitated in hexane and was then dried under vacuum.

2.3. Synthesis of poly(2-dimethyl-aminoethylmethacrylate) (pDMAEMA)-block-polyethylene glycol methyl ether methacrylate (PEGEMA)/ethylene dimethacrylate (EDGMA)
	pDMAEMA-b-PEGEMA/EDGMA was synthesized via deactivation enhanced atom transfer radical polymerisation, with feed ratio 1:90:10, pDMAEMA (7.51 × 10⁻⁴ mol) was dissolved in THF (40 ml) in a two-necked round bottom flask. PEGEMA (28.538 g, 6.008 × 10⁻² mol), EDGMA (2.9779 g, 1.502 × 10⁻² mol), PMDETA (0.0324 g, 1.87 × 10⁻⁴ mol) were added thereafter. After sealing the reaction flask, the mixture was purged with nitrogen for 20 min. Ascorbic acid (0.0066 g, 3.75 × 10⁻⁵ mol) was immediately added. The reaction flask was then sealed and immersed in an oil bath at 50 °C. The reaction was stopped at 60% conversion for IpD-b-P/E 1 and 28% conversion for IpD-b-P/E 2. The polymer was precipitated in hexane and was dried under vacuum. Copolymer was purified using membrane dialysis for three days against distilled water and was then freeze dried and stored at −20 °C until use.

2.4. Gel permeation chromatography (GPC) characterisation

Number average molecular weight (Mn), weight average molecular weight (Mw) and polydispersity (Mw/Mn) were obtained by gel permeation chromatography (Varian 920-LC) with a RI detector. The columns (30 cm PolargelM, two in series) were eluted by dimethyl formamide (DMF) and calibrated with polyethylene methacrylate standards. All calibrations and analyses were performed at 40 °C at a flow rate of 1 ml/min. All samples were dissolved in DMF, and passed through 0.2 μm filter before injection to demonstrate the absence of gelation.

2.5. Nuclear magnetic resonance (NMR) analysis

¹H NMR was carried out on a 300 MHz Bruker DPX300 and was analysed using MestReC™ processing software. The chemical shifts were referenced to the lock deuterated chloroform (CDCl₃). The final polymer composition was calculated by the integral values provided by the ¹H NMR.

2.6. Agarose gel electrophoresis

pDNA binding ability of the hyperbranched polymers was examined by agarose gel electrophoresis. The polymer/pDNA complexes containing luciferase plasmid were prepared at varying polymer to pDNA weight ratios (1:1–15:1). 10 μg of pDNA (50 μl) was added to the polymer solutions (in phosphate buffered saline (PBS)) of different concentration (final volume 100 μl). The mixtures were then allowed to incubate at room temperature for 30 min. Thereafter, the complexes were loaded into individual wells of 0.7% agarose × TAE gel containing 10 μl Sybersafe™ and were electrophoresed at 100 V for 90 min. Naked pDNA was used as the control. The gel was then visualized on a UV transilluminator (Syngene).

2.7. Particle size and zeta potential analysis

Polymer/pDNA complexes were prepared as described previously, with weight ratios (1:1–15:1). After 30 min of incubation, the complex solutions were diluted 11 times in PBS (1×). Prior to analysis, the diluted complex solutions were allowed to stabilize for 30 min. The particle size and charge of the polymer/pDNA complexes were measured using Zetasizer Nano-ZS90 (Malvern Instrument Ltd., UK). The particle size and zeta potential measurements were repeated for five runs for each sample, and the data were reported as the average of five readings.
2.8. Cell culture experiments

The transfection ability and effect of polymers on cellular metabolic activity of the synthesized polymer was evaluated on two different cell types. Human Fetal Foreskin Fibroblast cells were purchased from Centre of Applied Microbiology and Research (CAMR). Adipose derived stem cells (ADSCs) were extracted from rabbit adipose tissue as follows. Adipose tissue was digested by collagenase type I at 0.025% for 1 h under agitation at 37 °C. The enzymatic reaction was stopped by addition of complete medium (Dulbeco's Modified Eagle Medium (DMEM), 10% Fetal Bovine Serum (FBS), 1% Penicillin Streptomycin (P/S)). The stromal fraction was collected by centrifugation 5 min at 1200 rpm, resuspended and filtered on a cell strainer 70 mm (Fisher). After 24 h incubation at 37 °C in a humidified atmosphere of 5% CO2, cells were washed to eliminate the contaminant cells (blood cells and adipocytes). Medium was changed every two to three days and cells were maintained sub-confluent. Adipogenic, chondrogenic and osteogenic differentiation assays were conducted to confirm the nature of the extracted cells.

2.9. Cell Metabolic activity: alamarBlue™ assay

Cellular metabolic activity was assessed by the alamarBlue™ assay, wherein dye reduction was considered proportional to the metabolic activity of the cells. Cells were seeded with a cell density of 6500 cells/well for fibroblasts, 6000 cells/well for ADSC cells in a 96-well tissue culture plate and grown overnight. Cells were washed and polymers with different concentrations (5, 25, 50, 100 µg/ml) were loaded on the cell and incubated for 48 h in medium containing serum. alamarBlue™ assay was conducted according to the manufacturer’s instructions. After 3-h incubation, absorbance at 550 and 595 nm was determined on a microplate reader (VarioskanFlash-4.0.0.53) and the percentage reduction of the dye was calculated. alamarBlue™ assay was also performed on fibroblasts (6000 cells/well) and ADSCs (6500 cells/well) after transfection.

2.10. In vitro gene transfection

The in vitro gene transfection ability of the polymer/pDNA complexes was assessed via G-luciferase activity. Under usual cell culture sterile conditions, cells were seeded in a 96-well plate with a cell density of 6500 cells/well for fibroblasts and 6000 cells/well for ADSCs for 24 h before the transfection experiment. pDNA (1 µg per well) was mixed with 50 µl polymer solutions with different weight ratios from 1:1 to 20:1. The mixture was then incubated for 30 min at room temperature to allow the formation of polymer/pDNA complexes, and 150 µl of serum medium was added to the polyplex solution. The cells were washed with HBSS. Complexes were then added into the well and incubated with the cells for 48 h at 37 °C. After 48 h at 37 °C, the cells were analysed for G luciferase activity.

2.11. Statistics

Results are expressed as mean ± standard deviation. Statistical significance was assessed using the analysis of variance (ANOVA). p values of <0.05 were considered statistically significant. In all studies, the minimum sample size was three.

Linear pDMAEMA was synthesized via normal ATRP (Scheme 1a). The molecular weights of the polymers were characterized by GPC. The molecular weight of linear pDMAEMA block was restricted to approximately 8000 Da. Then, this linear pDMAEMA was used as a macro initiator for DE-ATRP of PEGMEMA and EGDMA to produce a PEG based hybrid polymer with a hyperbranched structure (Scheme 1b). Two hyperbranched polymers, one with a high PEG content (lpD-b-P/E 1), and another with low PEG content (lpD-b-P/E 2), were synthesized. A small amount of reducing agent was added, thus being different from DE-ATRP technique introduced by Wang et al. (2007). This was to facilitate a controlled polymer growth without gelation over a period of time (Cao et al., 2011). Ascorbic acid was used as a reducing agent for the production of active CuI species, while CuII remaining in the system controls growth of the polymer chain. GPC clearly demonstrates the controlled polymer growth of lpD-b-P/E 1 over a period of time (Fig. 1). The molecular weight lpD-b-P/E 1 was around 38,000 Da and that of lpD-b-P/E 2 was around 14,000 Da. Low polydispersity index (PDI) in both cases demonstrates the controlled growth of the polymer chain (Table 1). The chemical structure of final hyperbranched polymer was determined using 1H NMR (Fig. 2). The characteristic peaks at chemical shifts of 6.1 and 5.6 ppm are attributed to the vinyl functional groups in the hyperbranched polymer. The degree of branching within the polymer structure was analysed via the following equations (Table 1):

\[
\text{DMAEMA = integrals of } j/6
\]

\[
\text{PEGMEMA = integrals of } l/30
\]

\[
\text{Linear EGDMA = integrals of } d \text{ or } e
\]

The molar ratio of pDMAEMA to PEGMEMA to EGDMA for lpD-b-P/E 1 was found to be 1:12.215:1.104 with 1.05% of branched EGDMA (Fig. 2a) and 1:0.879:1.12 with 33.3% of branched EGDMA for lpD-b-P/E 2 (Fig. 2b). At the early stage of the reaction, EGDMA adds up to the polymer block leading to high branching ratio.

Both lpD-b-P/E 1 and lpD-b-P/E 2 are able to form complexes with pDNA via electrostatic interactions (Scheme 1c). G luciferase (Gluc) pDNA binding ability of the hyperbranched polymers was examined by agarose gel electrophoresis and was compared with controls at their optimum weight ratios, e.g. PEI and pPAMAM to pDNA at 2:1 and 8:1 (w/w ratio) respectively. It was seen
that lpD-b-P/E 1 complexed with G-lucpDNA at 10:1 (w/w), whereas lpD-b-P/E 2 complexed at 2:1 (w/w ratio) (Fig. 3) and linear pDMAEMA as early as 1:1 (w/w ratio) (Fig. s1c, see supporting materials). lpD-b-P/E 1 has a larger PEG based unit than lpD-b-P/E 2. Therefore at a particular w/w ratio, the number of free cationic groups present in lpD-b-P/E 1 is less than lpD-b-P/E 2, which might be the reason why both the polymers complex at different w/w ratios. Since linear pDMAEMA has no PEG unit attached to it, the number of free cations present at a particular weight ratio is higher than lpD-b-P/E 1 and lpD-b-P/E 2. This is the reason why linear pDMAEMA complexes as early as 1:1 (w/w ratio). It should be noted that polymer/plasmid weight ratios were used in this study for increased accuracy over the commonly used N/P quotation because of differences arising in M_w values across varying GPC instruments/calibration. Moreover, w/w ratio is more relevant to this particular study as the maximum safe polyplex loading was investigated so that the final administration weight of the polyplex can be assessed.

The DNase I protection assay showed the capability of lpD-b-P/E 1 and lpD-b-P/E 2 and linear pDMAEMA to protect pDNA from degradation by endonucleases (Fig. s1). For lpD-b-P/E 1 polyplexes, the stability against DNase degradation increased with increasing w/w ratio (Fig. s1a). This might be because at lower w/w ratios complexes formed are weak and degrade easily under such an environment. However, lpD-b-P/E 2 was able to form tight complexes even at lower weight ratios (Fig. s1b) showing no degradation under DNase 1. In contrast, the naked pDNA which was used as a control was fully degraded by DNase 1 in 30 min. This suggests that both lpD-b-P/E 1 (at high w/w ratios) and lpD-b-P/E 2 (at all ratios) can form stable complexes and can circulate in blood for a longer time without degradation. The polyplex stability over periods of 2, 7 and 14 days was also determined (Fig. s2) and both the polymers lpD-b-P/E 1 and lpD-b-P/E 2 were seen to maintain their stability for two weeks. The stability of these polyplexes is critical to ensure safe delivery of the therapeutic plasmid to its target in vivo. Although linear pDMAEMA formed tight complexes even at lower weight ratios (Fig. s1c) showing no degradation under DNase 1, the polyplexes were not stable in PBS for two weeks (Fig. s2). This suggests that PEG based hyperbranched unit acts like a protective shield to the polyplexes against salt thereby increasing their stability.

Polyplexes were further characterized for size and charge analysis using Zetasizer and TEM respectively. The surface charge of lpD-b-P/E 1 (Fig. 4a) remained constant at different weight ratios (5:1–20:1, w/w ratio). High degree of PEGylation of lpD-b-P/E 1 shielded the polyplex surface charge leading to reduced zeta potential. However, the surface charge of lpD-b-P/E 2 (Fig. 4b) and linear

Table 1
Weight average molecular weight (M_w), number average molecular weight (M_n) and PDI of pDMAEMA and hyperbranched polymer (lpD-b-P/E 1 and lpD-b-P/E 2) determined by GPC.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>M_w (Da)</th>
<th>M_n (Da)</th>
<th>PDI</th>
<th>DMAEMA:PEGDMAEMA:EGDMA (molar ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1 (pDMAEMA)</td>
<td>8000</td>
<td>7500</td>
<td>1.07</td>
<td>1</td>
</tr>
<tr>
<td>Block 2 (lpD-b-P/E 1)</td>
<td>37,900</td>
<td>44,300</td>
<td>1.17</td>
<td>1:12.215:1.104</td>
</tr>
<tr>
<td>Block 2 (lpD-b-P/E 2)</td>
<td>13,700</td>
<td>16,300</td>
<td>1.19</td>
<td>1:0.879:1.12</td>
</tr>
</tbody>
</table>

Fig. 2. $^1$H NMR spectra of (a) lpD-b-P/E 1 and (b) lpD-b-P/E 2.

pDMAEMA (Fig. 4c) increased with increasing weight ratio demonstrating increased electrostatic interaction between the polymer and the plasmid leading to a stronger bond formation.

The size of the polyplexes decreased as the polymer to pDNA weight ratio increased because the higher the w/w ratio the stronger the bond (Fig. 4). The size of linear pDMAEMA polyplexes was much larger than lpD-b-P/E 1 and lpD-b-P/E 2. In contrast to linear pDMAEMA, hyperbranched polymers (lpD-b-P/E 1 and lpD-b-P/E 2), forms micelle like structure when complexed with a plasmid (Scheme 1c). This is the reason why hyperbranched polymers showed reduced size when compared to linear pDMAEMA. Fig. 3 demonstrates the decrease in size of the polyplexes as the ratio increases. At 10:1 (w/w ratio), the size of lpD-b-P/E 1 was around 200–250 nm, while as the size of lpD-b-P/E 2 was found to be around 50–80 nm (Fig. 3b), which also supports the results from Zetasizer.

The effect of polymers on cellular metabolic activity of fibroblasts and ADSCs was analysed via alamarBlue™ reduction method and compared to PEI and dPAMAM (Fig. 5). It was seen that at all concentrations both the hyperbranched polymers showed a significantly higher cellular metabolic activity when compared to PEI and at higher concentration (100 μg/ml), and significantly higher than dPAMAM. However, cellular metabolic activity of lpD-b-P/E 2 was significantly reduced at a higher concentration (100 μg/ml) (Fig. 5) when compared to lpD-b-P/E 1, which indicates that high PEGylation for the polymer can significantly decrease the cytotoxicity of the polymer, and was further quantified via cell proliferation.
assay (PicoGreen™)(Fig. 5). The results show that both lpD-b-P/E 1 and lpD-b-P/E 2 had no impact on cell proliferation in either of the cell lines. Both PEI and dPAMAM significantly reduced the DNA concentration above 5 µg/ml concentration when compared to control cells with no treatment. lpD-b-P/E 1 and lpD-b-P/E by contrast had less impact on DNA concentration levels compared to PEI and dPAMAM suggesting that they had less impact on cell proliferation.

Transfection capability of the hyperbranched polymers was assessed using fibroblast cells and ADSCs. To analyse transfection efficiency for the polymer, G-loc transfection assay kit was used as per protocol. A wide range of weight ratios of polyplexes was analysed for each polymer and the optimum weight ratio for each polymer was determined (Fig. 5). This step is crucial as the optimum weight ratio of the polymer is different on different cells. PEI showed its optimal transfection at 2:1 (w/w ratio) for fibroblasts and 1:1 (w/w ratio) for ADSCs, while dPAMAM showed its optimal transfection at 8:1 (both in fibroblast and ADSCs). lpD-b-P/E 1 showed its optimal transfection at 8:1 (w/w ratio) in fibroblasts and 10:1 (w/w ratio) in ADSCs. lpD-b-P/E 2 had its optimal transfection at 10:1 (w/w ratio) in both cell lines. Linear pDMAEMA showed its optimal transfection at 20:1 (w/w ratio) (in both cell lines) (data not shown). Fluorescent microscopic images of ADSCs also confirmed transfection with lpD-b-P/E 1 and lpD-b-P/E 2 (Fig. 6).

In order to determine the maximum amount of pDNA that the polymer could deliver for a specific number of cells without affecting both transfection and viability, the optimal weight ratio was selected for each polymer, and the transfection capability and cellular metabolic activity was determined as a function of increasing pDNA concentration (Fig. 6). lpD-b-P/E 1 showed no toxic effects at higher pDNA concentration in either cell lines without compromising transfection efficiency. lpD-b-P/E 1 showed at least a fifteen fold higher cellular metabolic activity when compared to PEI, two fold higher when compared to dPAMAM, and six fold higher than linear pDMAEMA. On the other hand, lpD-b-P/E 2 showed a significantly higher level of toxicity (p < 0.05) when compared to vehicle treated cells, but nine fold lower than PEI, 1.5 fold lower than dPAMAM and four to eight fold lower than linear pDMAEMA. PEI is the gold standard among the polymeric genes reported. dPAMAM, is widely used as a transfecting agent. Both these polymers showed toxicity issues at high doses. PEI showed less than 20% cellular metabolic activity above 10 µg pDNA concentration, which resulted in no transfection at these doses. dPAMAM showed less than 50% cellular metabolic activity at 50 µg pDNA concentration. Linear pDMAEMA with no modification showed minimal transfection at all pDNA concentrations. Large polyplex size of linear pDMAEMA (Fig. 4c) (around 400 nm at its optimal transfection w/w ratio i.e. 20:1), makes it difficult to internalize efficiently leading to low transfection. Also, linear pDMAEMA showed less than 30% cellular metabolic activity above 25 µg pDNA concentration, which also resulted in reduced transfection level. Both hyperbranched polymers showed above 70% cellular metabolic activity even at 50 µg pDNA concentration. This might be due to this unique structure i.e. with a linear pDMAEMA unit and a PEG based hyperbranched unit. The linear pDMAEMA with the tertiary amine group could easily bind to the phosphate groups of pDNA and form a strong complex which will lead to high transfection efficiency. PEG based hyperbranched unit, by contrast, enhanced the cellular metabolic activity thereby reducing the cytotoxicity while maintaining its transfection efficiency at high doses.

The transfection efficiency of PEI and dPAMAM complexes does not increase proportionally with increasing pDNA dose. These polymers increase toxicity with increasing dose, which indirectly affects their transfection ability. Both lpD-b-P/E 1 and lpD-b-P/E 2 complexes do not affect the toxicity at high doses as compared to PEI.
and dPAMAM, and therefore lpD-b-P/E 1 and lpD-b-P/E2 maintain their transfection efficiency even at a high dose. This polymeric system can prove favourable for sustained gene therapy applications where long-term and a high DNA dose is required. High doses of polyplex can be loaded into nanospheres carriers for controlled release.

4. Conclusion

A unique structure comprising of a linear pDMAEMA block and hyperbranched PEGMEMA and EGDMA block was synthesized using DE-ATRP approach. This is the first time that a polymer of this structure has been synthesized where the pDMAEMA unit was able to bind pDNA very efficiently while the hyperbranched unit acted as a shield. This enabled the polymer to maintain high transfection levels without sacrificing cellular viability. Furthermore, unlike the situation with commercially available transfection agents, the cellular was not affected at high doses. This polymeric system can prove favourable for sustained gene therapy applications, where long-term and a high DNA dose is required. Moreover, the free vinyl groups present could be utilized to bind specific antibodies for targeted delivery.

Acknowledgements

The authors would like to thank IRCSET (Irish Research Council for Science, Engineering and Technology), funded by the National Development Plan, for providing research funding. The authors also thank Dr. Oliver Carroll for his help on laboratory experiments and Antony Sloan for his careful help in finalizing the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2012.05.010.

References
