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Timed-Release Polymers as Novel Transfection Reagents

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Development of novel agents that mediate nucleic acid delivery into cells has widespread application from basic cell biology to gene therapy. Enabling subsequent gene expression relies on the efficient delivery of DNA into the nucleus. In this work, we have developed a series of polymers designed to release DNA, via a self-catalysed hydrolysis mechanism, in a time-dependent manner to test if release of DNA near the time of cell division (which typically occurs every 24 h in mammalian cells) would result in an increase in levels of gene expression. We utilize a transient gene expression system to test our delivery potential. Our results show that the polymers are able to bind to DNA for up to 24 h and in some cases 48 h before release, thus providing sufficient time for endosomal escape and transport to the nucleus. Polymer A-C3, which bound DNA for up to 48 h, was able to achieve the highest levels of transfection efficiency. Using a GFP reporter gene, up to 95% of cells were positive for gene expression, which was much greater than the commercially available Freestyle Max. This work demonstrates a link between protection of DNA against degradation and high levels of transfection, indicating that protection of DNA is also a limiting factor in successful transfection. We postulate that due to the strong binding of the polymers to the DNA and the large size of the polyplexes, which are significantly larger than the nuclear pores, entry into the nucleus occurs through passive transport during cell division and nuclear envelope breakdown.

Introduction

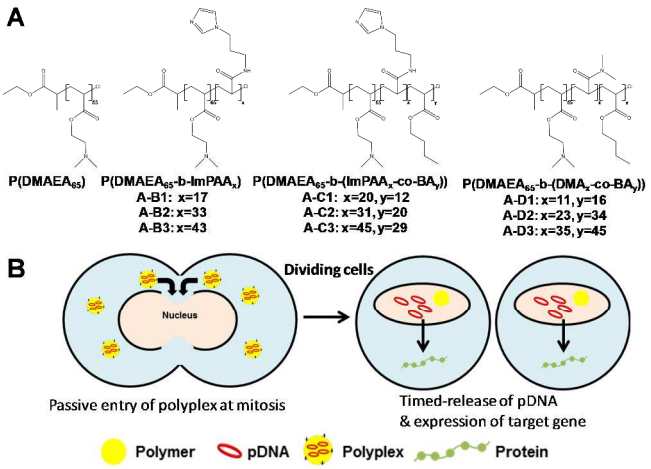
Over the past couple of decades there has been considerable work on the delivery of genetic material into mammalian cells for a wide range of applications, ranging from basic cell biology to recombinant protein production to gene therapy. The production of recombinant proteins usually occurs via stable cell line expression which can take several months (usually 6-12).[1] An alternative method is transient gene expression, which can take only weeks to produce a target protein,[2] the system used in this study. The delivery of genetic material can occur through physical, chemical or biological methods. Physical methods include techniques such as electroporation or microinjection, chemical methods use synthetic transfection reagents such as calcium phosphate, cationic liposomes and cationic polymers, and biological methods use viral vectors, to transport the genetic material across the cell membrane.[3-6] Due to safety concerns, cationic polymers and liposomes are preferred over viral vectors for use as transfection reagents. A variety of different cationic polymers and liposomes have been studied including chitosan-based vectors[7, 8], polyethylenimine (PEI) [9, 10], poly(amino) acids (PAA) [11], poly(dimethylamino ethyl methacrylate)[12, 13], Poly(L-lysine)[14-17] and N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA).[18]

For a transfection reagent to be effective in the delivery of genetic materials into mammalian cells, it must have certain characteristics which include the ability to bind and protect the DNA from nuclease degradation, be rapidly taken-up by cells, escape the endosome and then deliver the DNA by itself or as a complex across the nuclear membrane.[19-22] Since 1995 when PEI was first discovered by Boussif et al., it has been intensively studied for the above characteristics and its ability as a transfection reagent.[9, 23] PEI is able to efficiently bind and condense plasmid DNA (pDNA) to form PEI/pDNA polyplexes that favourably interact with the negatively charged cell-surface heparin sulphate proteoglycans to facilitate cellular uptake via endocytosis.[24] The polyplexes are then trafficked into acidic endosomes where escape into the cytosol occurs via the buffering capacity of the various amine side groups on PEI (denoted as the proton sponge effect).[25, 26] The polyplex must then deliver the DNA to the nucleus where transcription of the DNA can take place. The mechanism for nucleus translocation using PEI as the transfection reagent is not fully understood. It has been proposed that cellular division is required,[27, 28] which

contradicts the observation of PEI within the nucleus of non-dividing cells.[29-31] Despite the fact that PEI has been reported to achieve high transfection efficiencies using a wide variety of cells and works in a wide range of conditions and is relatively cheap compared to proprietary compounds such as Lipofectamine and Freestyle Max (both lipid-based transfection reagents), which are also very widely used, it also has several disadvantages including structural variation, polydispersity and even high levels of toxicity.[32, 33] Therefore, the ideal transfection reagent should include the above mention characteristics as well as be homogeneous with no batch to batch variations (i.e. consistent defined structure) and be non-toxic to cells, possibly by being able to degrade into benign non-toxic by-products that can be expelled from the cell.

After endosome escape, the protection and transportation of the DNA into the nucleus are considered to be the next major bottlenecks for successful transfection. It has been reported that the half-life of pDNA within HeLa cells is estimated to be 1-2 h.[34] The two main mechanisms proposed for nuclear entry are either passive entry when the nuclear membrane is temporarily disintegrated during cell division, or by active transport through the highly regulated nuclear pores (approximately 25 nm).[35] Therefore it would be highly advantageous for the transfection reagent to be able to either cross the nucleus membrane still bound to the DNA, or to have a timed-release characteristic that bound the DNA, and therefore providing maximum protection, until time of cell division, which in mammalian cells is generally every 24 h (see Scheme 1B).

In this study, we used a novel polymer, poly(2-dimethylaminoethyl acrylate) (PDMAEA, pKa ~ 7.1), in combination with other polymers as shown in Scheme 1A made by single electron transfer-living radical polymerization (SET-LRP)[36-40] to deliver pDNA to HEK293 cells, and study the transfection efficiency through GFP production. This polymer self-degrades through a self-catalysed hydrolysis mechanism to a negatively charge and nontoxic poly(acrylic acid) in a time-dependent manner (i.e. from a cationic to anionic polymer), in which degradation is independent of both the molecular weight of the polymer and pH of the environment,[41] allowing a predictable release time of negatively charged biomolecules regardless of cellular environment. PDMAEA has recently been incorporated as part of block copolymers for the self-assembly of timed-release nanoparticles.[42, 43] This polymer has also been shown to bind strongly to and release siRNA at a defined time (~17 h), independent of the environmental conditions.[44] The advantage of this polymer is its ability to release where external or environmental triggers are not accessible or can be variable. The resultant polymer after degradation is non-toxic, thus avoiding problems of toxic accumulation. In the case of siRNA delivery, the inclusion of PDMAEA in the block copolymer resulted in down regulation of the polo-like kinase 1 pathway, an in vitro model for the bone cancer osteosarcoma, and complete cell death.[45, 46] Here, our aim was to use the time-release characteristic of PDMAEA incorporate into a variety of block copolymers to study the binding and release of pDNA. We further wanted to determine, due to the release time of 17-24 h of PDMAEA, whether protecting and then releasing pDNA at mitosis (see Scheme 1B) will result in increased levels of transfection efficiency and recombinant protein yield.



Scheme 1. (A) Chemical structures and number of repeating units of P(DMAEA) (A), P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3), and P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3). (B) Proposed mechanism for nucleus entry of polymer/pDNA complexes during mitosis.

Experimental

Synthesis of novel nanoparticle polymers

Information regarding the synthesis of P(DMAEA), A-B1-3, A-C1-3 and A-D1-3 polymers can be found in the reference.[45]

Size and zeta potential measurements

Dynamic light scattering (DLS) experiments were performed using a Zetasizer 3000HS (Malvern). The Z-average diameter was measured five times for each sample and zeta potential three times. pDNA (16 µg) was mixed with polymers at different N/P ratios (5-50) in a total volume of 2 ml, vortex and left to complex at room temperature for 30 minutes before measurement.

Agarose gel DNA retardation assays

The binding capabilities of the polymers to pDNA were assessed using electrophoresis. pDNA (1 µg) was mixed with polymers at different N/P ratios (0.5-20) vortex, and left to complex at room temperature for 30 minutes. The resulting polyplexes were mixed with 2 µl DNA loading dye before loading onto a 1% agarose gel stained with SYBR Safe (Life Technologies). Gels were run at 90 V for 30 minutes before visualised using ChemiDoc MP (BioRad).

DNase I protection assays

The ability of the nanoparticle polymers to protect the pDNA against nuclease was examined using DNase I. Polyplexes were prepared the same as DNA retardation assays but were treated with 1 µl of DNase I (1000 units/ml in a buffer containing 100 mM Tris, 25m M MgCl2 and 5 mM CaCl2) and incubated at different time intervals, 30 or 120 minutes. After incubation 5 µl of 100 mM EDTA was added and incubated for a further 10 minutes at 75 °C to inactivate DNase I. The mixture was further incubated for 2 h with 10 µl of heparin (5 mg/ml) to dissociate the polyplexes. Samples were run on a 1% agarose gel stained with SYBR Safe (Life Technologies) at 90 V for 30 minutes. The released pDNA was visualised using ChemiDoc MP (BioRad).

Transfection studies

Cell density day of transfection for HEK293 cells was 2x106 cells/ml. Polyplexes were prepared in sterile water (25% of the initial culture volume) at different N/P ratios (5-30) using 2 µg DNA (pEGFP-C1) per millilitre of culture. Polyplexes were vortex for 30 seconds and left at room temperature for 30 minutes before addition to the cells. Freestyle Max (Life Technologies) was prepared according to the manufactures protocols except cell culture volume was 2 ml. Transfected cultures were incubated at 37 °C in a humidified 7.5% CO2 atmosphere on an orbital shaker at 140 rpm until 4 h post-transfection where cultures were fed with an equal volume of Freestyle 293 supplemented with 0.4% ACA (v/v). The cultures were then incubated at 37 °C. For endosomal escape assays cells were pre-incubated with a final concentration of 100 µM chloroquine 1 h before transfection. Chloroquine remained present for the entire experiment. Transfection efficiency was measured 48 h post-transfection via the FACSAria II (BD, San Jose, CA). Ten thousand events were measured per sample. Cell density and viability were measured using an automated trypan blue exclusion assaying using the Cedex HiRes Cell Counter (Innovatis AG, Bielefield, Germany).

Trafficking experiments

HEK293 cells were prepared as above. Polyplexes were prepared in the usual way except the pDNA was labelled with Cy5 using the Label-IT kit (Mirus Bio, Madison WI). At 4 h post-transfection wells were washed with PBS and then with 0.5 M Glycine pH2.2 in PBS. Cells were then fixed with 4% (w/v) paraformaldehyde in PBS for 20 minutes at room temperature followed by 3 x 1 minute washes with PBS. Cells were mounted on glass sides with VECTASHIELD HardSet Mounting Medium with DAPI (Vector Laboratories) and coverslip place over top. Slides were left overnight at 4 °C and visualised under confocal microscope LSM 710 (ZEISS, Goettingen, Germany).

Results and discussion

Polymer/DNA Binding

The homopolymer PDMAEA and three sets of diblock copolymers, with PDMAEA being the first block, were prepared by SET-LRP. The second block for each series was either ImPAA, BA, or a combination of both.[45] In each series, the number of monomer units was kept constant, and the number of monomer units of the second block were increased (see Scheme 1A).

For the polymers to be successful transfection reagents, they must have the ability to bind and condense pDNA, as well as protect and release it on-demand. The binding ability of the polymers was determined by an agarose gel DNA retardation assay. Polymer and pDNA were mixed together to form polymer/pDNA complexes or polyplexes, and the binding ability measured by running the polyplexes on an agarose gel. Migration of pDNA is hindered when completely bound to the polymer, and in some cases pDNA can be seen within the wells of the agarose gel. By varying the N/P ratios (i.e. the molar ratio of nitrogen atoms on the polymer to phosphate atoms on the DNA backbone), we determined the minimal N/P ratio required for complete binding and polyplex formation. All polymers bound strongly to pDNA at N/P ratios as low as 2.5, determined from the little or no migration of pDNA through the gel, or the observed pDNA in the wells of the gel (Figure 1 and Supplementary Figure S1).

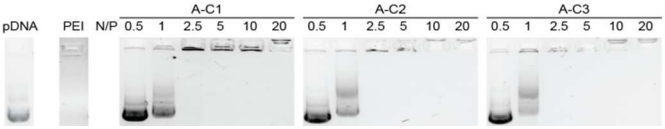


Figure 1. Agarose gel DNA retardation assay of P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3)/pDNA complexes and PEI/pDNA complex. Complexes formed using 1 µg of pDNA at different N/P ratios (0.5-20). PEI/pDNA complexed at 4:1 (w:w). Complexes incubated at room temperature for 30 minutes (PEI 4 h) before running on a 1% agarose gel at 90 V for 30 minutes.

Polymer/DNA Sizing

The size and overall charge of the polyplex (i.e. polymer complexed to pDNA) play an important role in cellular entry. The hydrodynamic diameter and zeta potential of the polyplexes were measured using dynamic light scattering (DLS). The N/P ratio that allowed good formation of polyplexes ranged from 5 to 50, as determined from the binding studies in Figure 1. The PEI polyplex (4:1 w:w to pDNA) gave a hydrodynamic diameter of 121.8 nm with a polydispersity index (PDIDLS) of 0.301, representing a broad size distribution. It should be noted that a PDIDLS value less than 0.1 represent narrow size distribution. All the polymers gave sizes ranging from 100 to 200 nm with broad size distributions (Table 1). The only narrow size distributions were observed for the A-B series polyplexes (i.e. PDIDLS < 0.1) at the higher N/P ratios. The zeta potential for all the polyplexes ranged from +21 to +36, supporting the formation of positively charged polyplexes (see Supplementary Table S1). These values are comparable to the zeta potential of the PEI complex (+35.2 mV). This, together with the similar size, suggests that our

polymer polyplexes have very similar physical characteristics to the PEI polyplex.

Table 1. Size of P(DMAEA) (A), P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3), and P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3) complexes with pDNA (16 μg) in water at different N/P ratios (5, 25 and 50). PEI/pDNA complex 4:1 (w:w) 121.8 nm (0.301). Dh data reported as an average of five Z-average measurements.

N/P Ratio	Z- average hydrodynamic diameter, D_h (nm) (PDI in parentheses)									
	A	A-B1	A-B2	A-B3	A-C1	A-C2	A-C3	A-D1	A-D2	A-D3
5	156.2 (0.119)	108.9 (0.215)	93.9 (0.257)	99.2 (0.271)	113.7 (0.108)	130.0 (0.104)	157.3 (0.133)	112.3 (0.393)	185.2 (0.295)	170.9 (0.405)
25	351.2 (0.240)	148.1 (0.091)	129.0 (0.097)	132.6 (0.122)	140.5 (0.193)	136.4 (0.184)	129.0 (0.187)	115.3 (0.255)	142.9 (0.212)	125.2 (0.197)
50	1305.6 (0.578)	203.5 (0.052)	199.9 (0.090)	134.2 (0.064)	129.4 (0.162)	135.5 (0.258)	128.6 (0.234)	129.0 (0.178)	135.4 (0.188)	144.5 (0.215)

Polymer/DNA Release

One of the objectives of this work was to determine if releasing pDNA in a time-dependent manner could provide insight into the mechanism of transfection. Our previous work, using a polyplex of siRNA and A-C3, demonstrated that the siRNA could be fully released after 17 h, which is close to the time of 24 h for a mitotic event. By varying the release time using the range of designed polymers in this work, we wanted to investigate whether delivery into the nucleus occurred via passive transport (i.e. entry during mitosis) or via entry through the nuclear pores.

The polyplexes were complexed in water and incubated at 37 °C for 4, 24, 48, 72 and 168 h, where the unbound pDNA was monitored by an agarose gel (Figure 2). Surprisingly, PEI showed no release of pDNA even after 168 h. Polymer A showed release of pDNA after 24 h and complete release at 168 h determined by the dark band corresponding to the free pDNA (see first lane in Figure 2). The dark smear on the gel prior to this time (between 24 to 72 h) represents partial release of the pDNA. The A-B series showed partial release after 48 h, and complete release at 72 h. The A-C series, with the exception of A-C1, partially released pDNA after 48 h with complete release well after 168 h. The same trend was observed for the A-D series. The subtleties in the release times suggest that all polymers strongly bind at 24 h, and only A-C2 and A-C3 show little release after 48 h. These results demonstrate that the different polymers have different release rates due to the different copolymer compositions of the polymers and the size of the polyplex. For example, A-C3 formed small 20 nm particles in water that grew to 200 nm when complexed with siRNA, whereas the A-B and A-D series polymers were fully water soluble (~9 nm) and formed polyplexes of ~200 nm.[45]

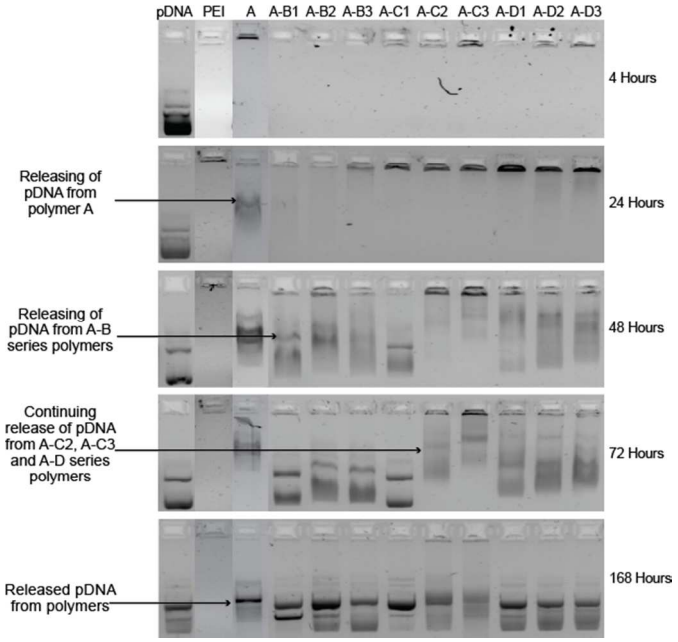
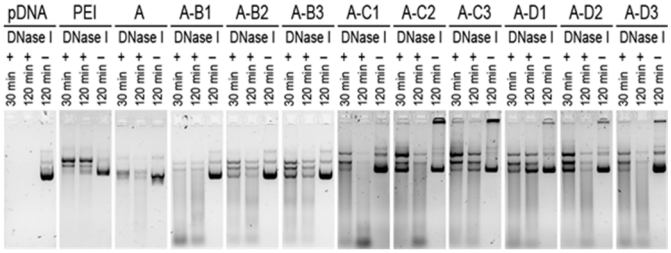


Figure 2. Time-release of pDNA from P(DMAEA) (A), P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3) and P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3) at N/P10, and PEI at 4:1 (w:w). Samples run at time points indicated on a 1% agarose gel at 90 V for 30 minutes.

Polymers/DNA Protection

Protection of the pDNA before nucleus entry is of vital importance due to the many cellular enzymes that degrade and significantly lower the efficiency of the pDNA. The ability of the polymers to protect the pDNA from DNase I was determined using the following procedure: (i) the polyplex was exposed to DNase I for 2 h, (ii) after this time the DNase I was inactivated, and (iii) the protected pDNA was released from the polymer using heparin, and the amount of pDNA measured on

an agarose gel (see Figure 3). The levels of pDNA was measured after 30 and 120 min (denoted as + in Figure 3) and compared to that in the absence of DNase I (denoted as - in Figure 3). It can be seen that PEI provides excellent protection of pDNA; the two higher bands most probably represent conformational changes to pDNA.[47] Most of the polymers provided some level of protection. The A-B series and A-D2 and A-D3 provided a low level of protection, whereas the A-C1 and A-C2 gave no protection. Polymer A-C3 and A-D1 showed similar levels of protection compared to PEI. The results



suggest that stronger binding of the pDNA to the cationic polymer provides a higher level of protection against nucleases found within the cell.

Figure 3. DNase I protection assays. P(DMAEA) (A), P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3), and P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3) polymers complexed with pDNA at N/P 20 and PEI complex with pDNA at 4:1(w:w). Complexes exposed to DNase I for either 30 or 120minutes before inactivation and release of pDNA by heparin. Released pDNA assessed on a 1% agarose gel at 90 V for 30 minutes.

Polymer/DNA Endosome Escape and Gene Expression

Our polymers have shown the ability to be able to bind, condense, protect and release pDNA. The next step is to determine if the polyplexes are able to cross the negatively charged cell membrane, escape the endosome, enter the nucleus, and produce protein (i.e. transfection). The addition of Cy5-labelled pDNA to the polymers allowed us to visualize the uptake of the polyplexes into the cell. The confocal images showed the cell nucleus in blue, and pDNA in red (Figure 4). Our results showed that polymers A, A-B3, A-C3, and A-D3/pDNA polyplexes were able to cross the cell membrane, and become internalised, as pDNA can be seen within the cell as indicated by the red areas.

Internalisation via endocytosis (believed to be the main method for internalisation of polyplexes) is only half the story in terms of successful transfection. Once in the cell, the polyplexes must escape the endosome, transverse the cytosol, enter the nucleus, and release the pDNA. Based on our previous work, the A-C series showed excellent endosome escape when complexed to siRNA, while A, A-B and A-D series were less efficient.[45] The combination of the ImPAA and BA monomers in the second block worked together to bind to the endosome membrane and allow escape. We decided to test all the polymers for their ability to escape the endosome and enter the nucleus in HEK293 cells. By adding chloroquine, a lysosomotropic agent that causes the endosomes to swell and burst, releasing all its contents into the cytosol, we could determine whether gene expression was directly related to the level of endosome escape.

Transfection was performed in cells pre-treated with chloroquine for an hour and compared with untreated cells. The efficiency of endosomal escape and nuclear entry was measured using green fluorescence protein (GFP) expression by flow cytometry, with transfection efficiency measured as a percentage of cells expressing GFP. The addition of chloroquine did not induce any toxic effects to the cells as cell viability remained similar between the non-treated and treated cells (Figure 5B, D and F). All polymers from the A-B, A-C, and A-D series were able to achieve transfection without the addition of chloroquine, while polymer A required the addition of chloroquine to achieve transfection (Figure 5A, C and E and Supplementary Figure S2A). For the A-B series polymers, an increase in transfection efficiency can be seen in the cells treated with chloroquine compared with those not treated. Polymers A-C2 and A-C3 are highly efficient at endosomal escape, as the difference between efficiency between treated and non-treated cells is minimal. These polymers were able to achieve GFP expression in more than 95% of cells. At an N/P ratio of 5, the A-D series polymers in the presence of chloroquine displayed an increased in efficiency compare with cells in the absence of chloroquine, and no difference was observed at higher N/P ratios.

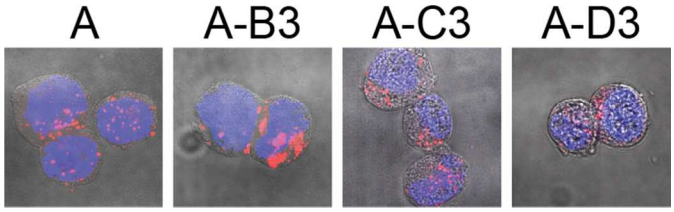


Figure 4. Fixed-cell confocal microscopy 4 h post-transfection showing internalisation of Cy5-pDNA (red) complexes of P(DMAEA) (A), P(DMAEA-b-ImPAA) (A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C3), and P(DMAEA-b-(DMA-co-BA)) (A-D3) in HEK293 cells. Cells were stained with DAPI to visualise the nucleus (blue).

Our results demonstrated that the addition of chloroquine increased transfection efficiency in the A-B series polymers at all N/P ratios tested and A-D series polymers as the lowest N/P ratio tested, suggesting that escape from the endosome was a limiting factor for these two polymer series. The second blocks of ImPAA or BA were unable to promote efficient endosome escape. For the A-C series polymers, escape from the endosome was efficient as no significant increase in transfection was observed in cells treated with chloroquine, suggesting that the combination of ImPAA and BA promotes efficient escape, similar to the results obtained by the siRNA knockdown

study.[45] By adding chloroquine, we reduced the possibility of endosome escape being the limiting factor in transfection; therefore, differences in transfection efficiencies between the polymer series must be due to either entry into the nucleus, or pDNA protection. We found that all polymers were able to bind the pDNA until 24 h, after which time release begins, and was of sufficient time to escape the endosome and travel to the nucleus for passive entry during mitosis. Our results show that the longer it took for the polymers to release the pDNA an increased in gene expression levels was observed, with polymer A-C3 (N/P=5) found to be the best transfection agent. These strongly support that by strongly binding with little or no release and protecting the pDNA, the polymer provided the best opportunity for entry into the nucleus during mitosis. The sizes of the polyplexes were significantly greater than the nuclear pores, which on a physical basis discounts delivery directly through these pores. However, the mechanism is complex, and delivery through the pores cannot be discounted.

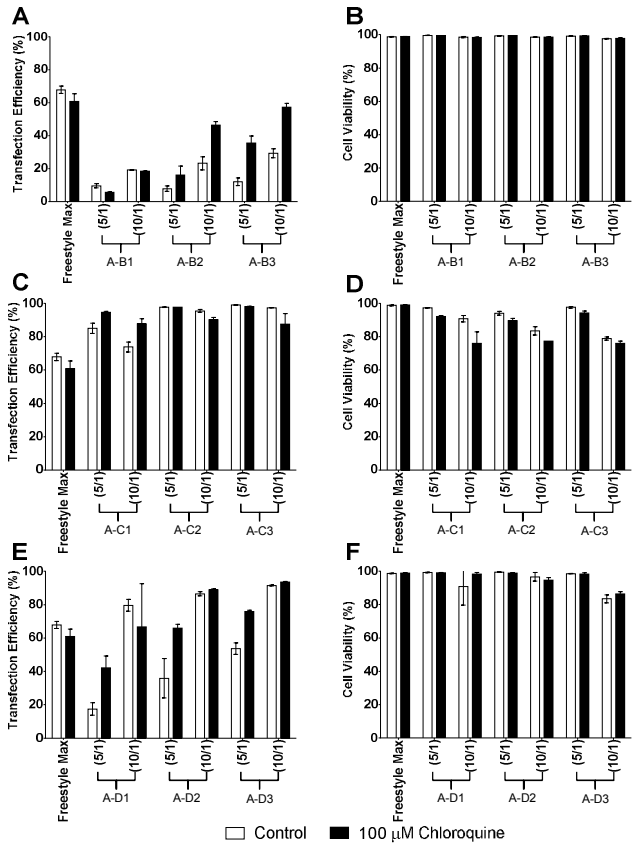


Figure 5. Endosomal escape assays for P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3), and P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3) at various N/P ratios in HEK293 cells pre-treated with and without 100 μM chloroquine. Results measured 48 h post-transfection. (A), (C) and (E) transfection efficiency (B), (D) and (F) cell viability. Data reported as the mean ± standard error of the mean of two replicates. N/P ratios are in parenthesis.

Efficient gene expression requires optimal ratios of plasmid to polymer

Optimum N/P ratios for each of the polymers were determined for efficient transfection with minimal cellular toxicity. A range of N/P ratios (5-30) were tested for each polymer and transfection efficiency measured 48 h later by flow cytometry (Figure 6). Cellular toxicity of the polymers was considered to be an issue when the cell viability dropped below 90%. Each of the polymers within the A-B series at an N/P ratio of 20 and 30 displayed similar results with A-B3 able to achieve around 50% transfection efficiency (Figure 6 and Supplementary Figure S3). This indicated that the optimum N/P ratio lies within this range with little cell death observed at these N/P ratios. For A-C series, little difference in transfection efficiency was observed at N/P ratios 5 and 10 but at the higher ratio, cell death became a significant issue with cell viability dropping below 80% for A-C3 (Figure 6 and Supplementary Figure S4). For the A-D series polymers higher levels of transfection efficiency could be achieved at N/P ratio of 10 and above, but once again at this ratio toxicity became a major issue, resulting in an optimum N/P ratio of 5 (Figure 6 and Supplementary Figure S5).

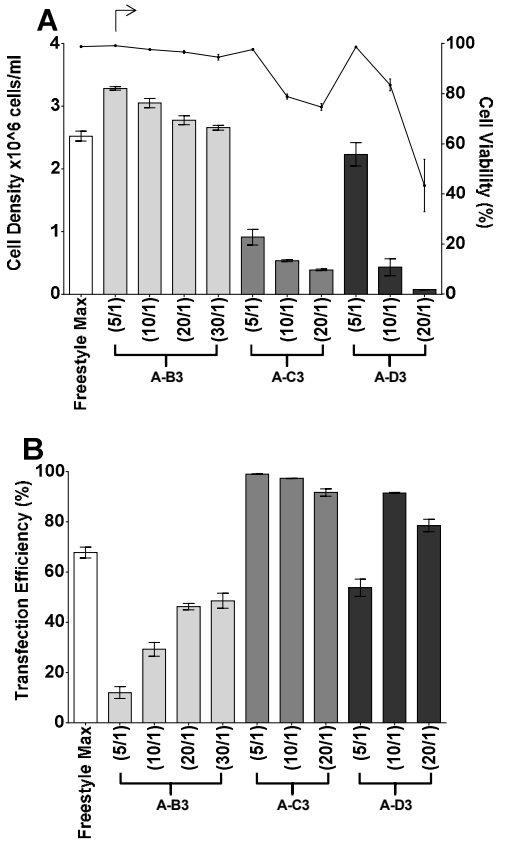


Figure 6. Transfection efficiency and cellular viability of P(DMAEA-b-ImPAA) A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C3), and P(DMAEA-b-(DMA-co-BA)) (A-D3) at various N/P ratios in HEK293 cells 48 h post-transfection. (A) Cell density and viability and (B) transfection efficiency. Data reported as the mean ± standard error of the mean of two replicates. N/P ratios are in parenthesis.

Conclusions

This work described the abilities of novel polymers as transfection reagents for transient gene expression. The polymers all contain the same first block, PDMAEA, which was able to bind and then release pDNA in a time-dependent manner. Our results showed that release of pDNA occurred after 24 h, which was sufficient time for the polymer/pDNA complexes to escape the endosome and travel to the nucleus and enter when the nuclear membrane is disintegrated during cell division. This is believed to be the main method of entry as the size of the polyplexes (100-200 nm) makes them virtually impossible to passively enter the nucleus through the nuclear pores (~25 nm), although other modes of active nucleus entry cannot ruled out. Our best polymer A-C3, which has the combination of ImPAA and BA blocks, was efficient at endosome escape determined from the high transfection efficiency even with the addition of chloroquine. A-C3 also provided the best protection against DNase I compared to the other polymers, as well as it bound the pDNA the longest period of time. We were able to achieve transfection efficiency of 95% with A-C3, which was much greater than the commercially available Freestyle Max. The polymer A-C3 provides sufficient protection of the pDNA until nucleus internalisation occurs. Current transection regents rely on a stochastic and unpredictable program of DNA release after cellular entry, the polymers developed in this work offer a tuneable and controlled process of DNA release. These properties make these reagents an attractive options for the timed release of DNA. For example, these timed-release polymers could be used to deliver DNA in slow growing cells or cells that are difficult to transfect using traditional methods (e.g. neurons). Moreover these polymers have a defined structure that can be readily be modified depending on circumstances (i.e. addition of ligands for use in targeting specific cell types), and are inexpensive to produce compared to the commercially available lipid-based transfection reagents, Lipofectamine and Freestyle Max.

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Notes and references

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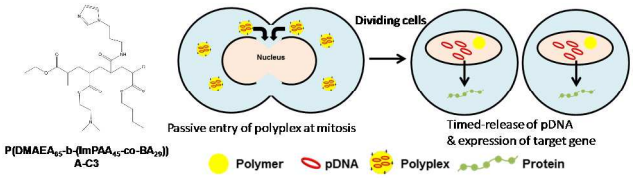

Timed-release polymer with 95% gene expression, which was greater than a commercial transfection reagent.

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