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## First approach on transfection ability of novel amphiphilic water soluble degradable poly( $\epsilon$ -caprolactone)-g-poly(L-lysine) copolymers

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**Abstract:** The potential of novel amphiphilic water soluble and degradable poly( $\epsilon$ -caprolactone)-g-poly(L-lysine) as carriers for DNA transfection has been investigated. Two graft copolymers having the same proportion of lysine units but different structures have been synthesized following two grafting techniques. The chemical composition of these copolymers, their expected architectures and their behaviour in aqueous solutions have been studied. The benefits resulting from the use of these degradable polycationic structures as well as their ability to form polyplexes are discussed. Finally, preliminary transfection assays of MCF-7 cells by pRL-TK plasmid using poly( $\epsilon$ -caprolactone)-g-poly(L-lysine) copolymers as carriers are reported.

**Keywords:** biodegradable polymer, poly( $\epsilon$ -caprolactone), poly(L-lysine), transfection, graft copolymer

### Introduction

Transfection is a well-known technique that relies on the delivery of therapeutic DNA into the nucleus of a target cell where the exogenous gene of interest is expressed, which results in the expected therapeutic effect. However, DNA is a large hydrophilic molecule with an overall negative charge that does not easily pass biological barriers and can be degraded by deoxyribonuclease. To overcome these drawbacks and to obtain acceptable gene expression levels, the use of carriers is required. Historically, viral vectors such as retroviruses, adenoviruses and adeno-associated viruses, were firstly developed [1-4]. They showed efficient gene transfer *in vitro* and *in vivo* as compared to non-viral vectors, but their use is associated with several severe drawbacks: limitations of the size of DNA that can be associated, targeting of dividing cells only, random DNA insertion, risks of replication or recombination with wild-type viruses and possible host immune reaction [5-8].

As a consequence, an increased attention has been devoted to non-viral systems such as cationic liposome/DNA complexes [9-14] and cationic polymer/DNA complexes [15-23] also called polyplexes. Among the synthetic polymers, poly(L-lysine) (PLL) has been widely studied. It has been demonstrated that PLL binds DNA efficiently. An increase of gene expression is obtained by targeting the DNA/PLL polyplex to the site of action by addition of ligands that can mediate cell specific

recognition and internalization, namely antibodies [24-28], saccharidic moieties [29-32] or folate moieties [33, 34]. However, despite good transfection efficiencies, the positively charged surface of PLL/DNA complex restrains its use, as it is responsible for the cytotoxicity and the rapid clearance from blood stream of the polyplex following the non-specific adsorption of serum proteins. To overcome this drawback PLL was associated with neutral hydrophilic poly(ethylene glycol) (PEG), which is widely used for the preparation of stealth long time circulating objects. Different structures have been synthesized, especially block PLL-*b*-PEG [33, 35-38], multiblock (PLL-*b*-PEG)<sub>n</sub> [39] and comb-like PLL-*g*-PEG [40-44] copolymers. Another approach was proposed by Brown *et al.* [35, 45] who showed that amphiphilic structures based on derivatized PLL present two advantages compared to PLL. The first one is a higher biocompatibility, thanks to a lower cytotoxicity due to a lower amount of available amine groups allowed to interact with cell anions at the surface of the micelle-like objects formed by the polyplex. The second one is a better release from acidic endosomal environment without adjunction of chloroquine [31, 46] or endosomolytic agents [46-48] which are normally necessary to obtain high transfection efficiencies with PLL.

Recent works showed that biocompatible and preferably biodegradable polymers yielded harmless degradation products [48-50]. In the family of degradable polymers, aliphatic polyesters are the most widely used for biomedical applications. However, very few examples of aliphatic polyesters-based structures are described in literature for gene delivery applications. Lim *et al.* [51,52] described a PLL-analogue polyester, namely poly( $\alpha$ -[4-aminobutyl]-L-glycolic acid) (PAGA), which exhibited higher transfection efficiency than PLL and showed no cytotoxicity. Another example was given by Lynn *et al.* who synthesized a non cytotoxic poly( $\beta$ -aminoester) [53]. Poly(D,L-lactic acid-co-glycolic acid) (PLAGA) and poly(D,L-lactic acid) (PLA) are used in the preparation of micro- and nanoparticles embedding and protecting DNA [54-56], or in the preparation of comb-like structures like PLL-*g*-PLAGA graft copolymer [57,58]. Only one structure based on poly( $\epsilon$ -caprolactone) (PCL) aimed at gene delivery is described by Shuai *et al.* [59] who prepared a histidine-PEI-*g*-(PCL-*b*-PEG) terpolymer.

Considering the limited number of examples listed above, and keeping in mind the advantages of degradable amphiphilic polymers for gene delivery, we focused on new amphiphilic water-soluble and degradable PCL-*g*-PLL copolymers. The aim of this work is to give a initial idea of the *in vitro* transfection possibilities offered by these new potential carriers through preliminary tests and to validate our approach before undertaking more systematic studies.

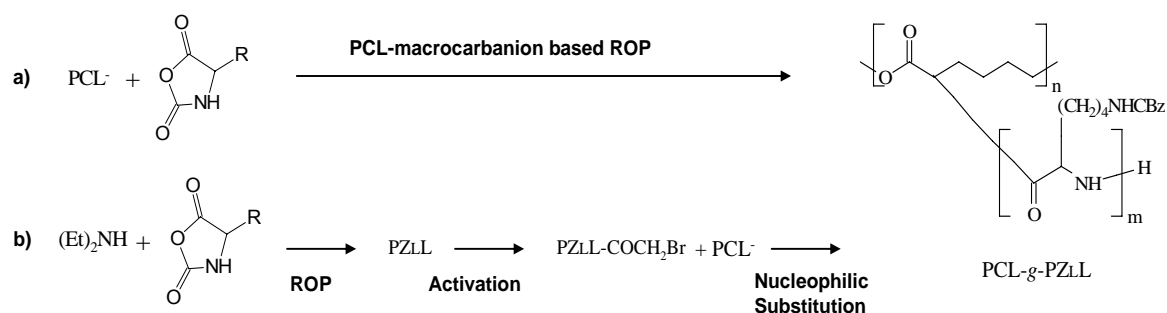
## Results and Discussion

As discussed in the introduction, it was expected that poly( $\epsilon$ -caprolactone)-*g*-poly(L-lysine) should bring advantages for gene delivery applications. The amphiphilic character of PCL-*g*-PLL copolymers should help transfection [45], while the degradability of these structures meets the last requirement of new synthetic vectors that should degrade and lead to harmless degradation products. Another advantage is that cytotoxicity of polycations is depending on the chain length of the macromolecule [60]. As a result, a strategy to decrease toxicity is to link polycationic moieties with degradable bonds. A high charge density is kept and thus good DNA-condensing properties of the long cationic macromolecules, while a lower toxicity is

insured due to the degradability, which leads only to short and less toxic cationic oligomers after transfection [61].

### Synthesis and analysis of the copolymers

The synthesis of poly( $\epsilon$ -caprolactone)-*g*-poly(L-lysine) (PCL-*g*-PLL) copolymers has been recently described elsewhere [62]. Two strategies have been used, based on a grafting “onto” or a grafting “from” methods. In the grafting “onto” method a Z-protected PLL (Z-PLL) was activated in a brominated form by reaction with bromoacetyl chloride, and then reacted on a macropolymer derived from PCL by anionic activation in the presence of lithium diisopropylamide (LDA). In the grafting “from” method an anionic polymerization of the N-carboxy anhydride of lysine was initiated by the same macropolymer. Scheme 1 illustrates these two synthetic pathways leading to copolymers with a hydrophobic degradable PCL backbone and hydrophilic degradable PLL side chains. The water-soluble copolymers obtained are referred as PCL-*g*-PLL “onto” and PCL-*g*-PLL “from” depending on the synthesis strategy. Thanks to  $^1\text{H}$  NMR analysis in D-TFA the compositions of these copolymers were found to be 40% of  $\epsilon$ -CL and 60% of L-Lysine units for both structures. SEC analyses in THF were carried out, giving  $\overline{M}_n = 23500$  for PCL-*g*-PLL “onto” and  $\overline{M}_n = 7200$  for PCL-*g*-PLL “from”.



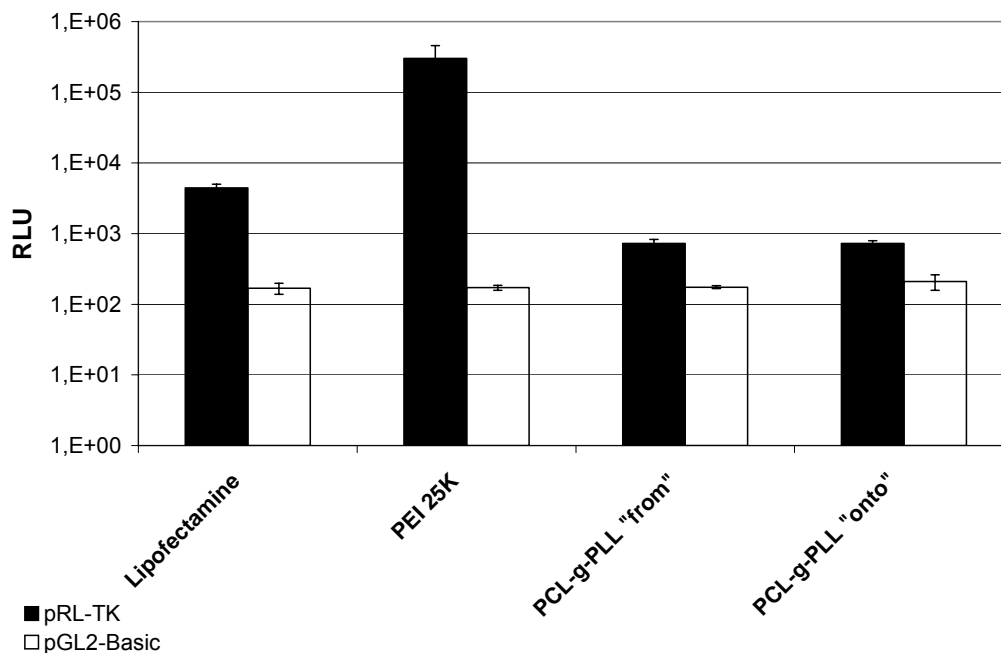
**Scheme 1.** Synthesis of poly( $\epsilon$ -caprolactone)-*g*-poly(N-Z-L-Lysine) copolymers (PCL-*g*-PZLL) following a) the grafting “from” strategy or b) the grafting “onto” strategy. (PCL<sup>-</sup> = macropolymer derived from PCL, R =  $-(\text{CH}_2)_4\text{-NH-CO-O-CH}_2\text{-C}_6\text{H}_4$ , ROP = Ring Opening Polymerization)

Analyses of PCL-*g*-PLL copolymers by  $^1\text{H}$  NMR and size exclusion chromatography (SEC) showed that architectures were dependent on the synthetic pathway [60]. While the overall composition was the same for the two copolymers, 40% of  $\epsilon$ -CL units and 60% of L-Lysine units, it was demonstrated that structures were different. According to the grafting “onto” method, the PCL backbone was grafted by an average number of 2 long PLL chains ( $\overline{DP}_n \approx 230$ ), whereas it was expected that in the grafting “from” method PCL backbone is grafted by many short chains of amino-protected PLL. Studies of PCL-*g*-PLL copolymers in distilled water confirmed this assumption. In PCL-*g*-PLL “from” copolymer, big sized aggregates ( $\varnothing \approx 500$  nm) were obtained in distilled water because of the long PLL chains expansion. In PCL-*g*-PLL “onto”, smaller particles in water ( $\varnothing \approx 100$  nm) were obtained [62].

From these different architectures and related behaviour in aqueous medium, it was expected that both copolymers should lead to different transfection efficiencies and cytotoxicities. It is noteworthy that PCL-g-PLL “from” contained short polycationic PLL chains which should decrease the overall toxicity of the copolymer during degradation.

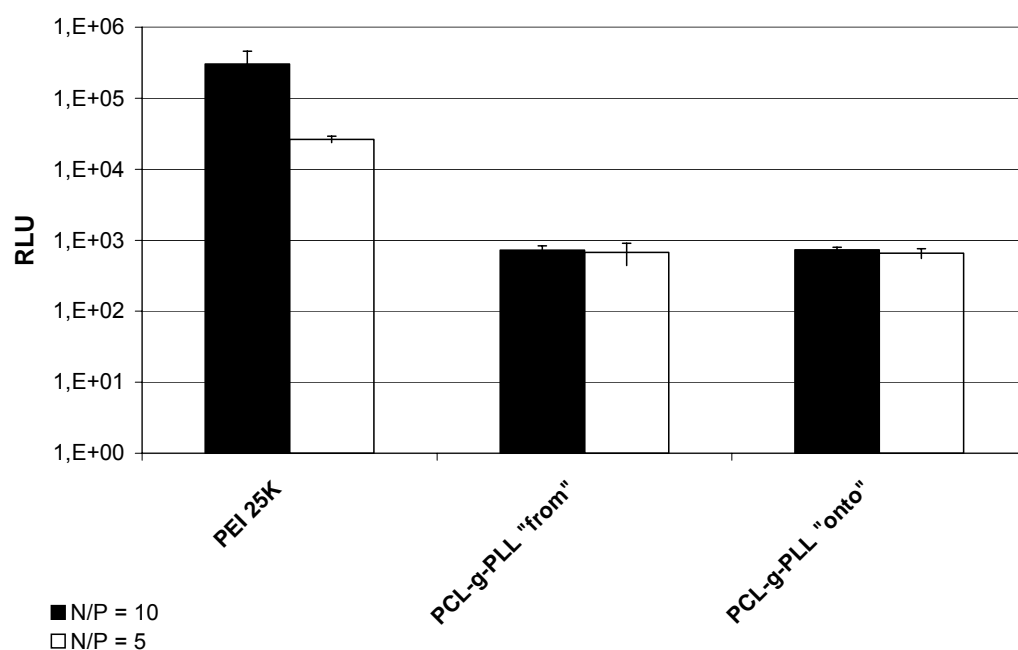
### Complexes formation, cytotoxicity and transfection efficiency

This study being devoted to a preliminary validation of new PCL-g-PLL as gene carrier, cytotoxicity was evaluated only by visual observation through a binocular microscope and comparison with non transfected wells. No counting of cell populations has been done. After a 2.5-hour transfection time, no dead cells were detected. Dividing cells were even observed in wells transfected with PCL-g-PLL showing good cell viability. In comparison, no dividing cells were detected in the PEI containing wells used as reference. Complexes with diameters 10 folds smaller than the cell diameter were also observed. Complexes with PCL-g-PLL were a little bigger than those obtained with free PEI. Blessing *et al.* [63], who used the same PEI according to the same protocol reported diameters of around 500 nm. At the end of the transfection period, typically 5 hours, no variation of diameter was observed. After removal of the complexes, incubation was prolonged for 24 hours. Cells transfected with PCL-g-PLL were not affected and an increase of the covered surface was observed, which is a good indication of the absence of acute toxicity, which should be confirmed in the future by MTT assays.



**Fig. 1.** MCF-7-MF transfection with pRL-TK (black bars) and pGL2-Basic (white bars) plasmids complexed by PEI 25K, poly( $\epsilon$ -caprolactone)-g-poly(L-Lysine) “from” and poly( $\epsilon$ -caprolactone)-g-poly(L-Lysine) “onto”. Lipofectamine™ Transfection Reagent used as a negative control according to Invitrogen™ recommendations. Polymer (nitrogen)/DNA(phosphate) ratio of 10 (N/P = 10) in HBS. Luciferase activity is shown as mean  $\pm$  SD of triplicate.

Transfection efficiency of PCL-*g*-PLL copolymers in MCF-7 cells was compared to two well known vectors, namely PEI 25K, a branched synthetic cationic polymer known to be highly efficient, and Lipofectamine™ Transfection Reagent, a cationic liposomal vector used as a routine vector for *in vitro* transfections. Two plasmids were transfected, pRL-TK that contain a cDNA encoding *Renilla Luciferase*, and pGL2-Basic which has no eukaryotic promoter and that was used as a “blank” plasmid to evaluate the residual luminescence of the lysate in the absence of *Renilla Luciferase*. Copolymer structure and N/P ratio were investigated, N standing for the number of amine functions in the cationic carrier and P the number of phosphate functions in the plasmid. As these transfection tests were the first to be achieved with PCL-*g*-PLL copolymers, a large excess of amine functions was retained (N/P = 5 or 10) to insure a good complex formation. It is known that high N/P ratios favoured transfection in the case of PEI-based polyplexes, but highly cationic charged surfaces can cause cytotoxicity. As previously described no toxicity was observed using N/P = 5 or 10 in PCL-*g*-PLL copolymers.



**Fig. 2.** MCF-7-MF transfection with pRL-TK plasmid complexed by PEI 25K, PCL-*g*-PLL “from” and PCL-*g*-PLL “onto” at a polymer(nitrogen)/DNA(phosphate) ratio N/P of 5 (white bars) or 10 (black bars) in HBS. Luciferase activity is shown as mean ± SD of triplicate.

Figure 1 shows the transfection efficiency of the different carriers. It is noteworthy that blank experiments realized with pGL2-basic gives the same amount of residual luminescence with a good reproducibility, thus indicating that values could be considered as a convenient background (RLU ≈ 1×10<sup>2</sup>). Considering pRL-TK transfection, PEI 25K is by far the most efficient vector with 3 decades higher RLU values compared to blank experiments, 2 decades higher RLU values compared to Lipofectamine™ Transfection Reagent, and 2.5 decades higher RLU values



compared to both PCL-*g*-PLL copolymers. It can be observed that despite the cationic character of PCL-*g*-PLL copolymers which makes them close to PEI, lower transfection efficiencies ( $RLU \approx 1 \times 10^3$ ) are obtained as compared to the routine-used liposomal system ( $RLU \approx 5 \times 10^3$ ). However, transfection occurred with degradable PCL-*g*-PLL copolymers with one-decade higher RLU values than pGL2-Basic blank experiments.

The influence of the ratio N/P on the transfection efficiency is shown in Figure 2 for N/P= 5 and 10. As expected, transfection efficiency of PEI 25K is higher when N/P =10:  $RLU \approx 3 \times 10^5$  for N/P = 10 and  $RLU \approx 3 \times 10^4$  for N/P = 5. Results are different with PCL-*g*-PLL copolymers, where RLU is not depending on N/P ( $RLU=1 \times 10^3$ ). It should be noticed that PCL-*g*-PLL copolymers were tested without chloroquine, which is always used with PLL derivatives in gene delivery applications to enhance exocytosis, thus comparison with results reported in the literature should be interpreted with precaution. Chloroquine was not added because we would like to obtain the true efficiency of the copolymer for transfection, but future studies should include chloroquine. At last, considering the copolymers structures, no difference was found in transfection results for either PCL-*g*-PLL “from” or PCL-*g*-PLL “onto” copolymers. If the two architectures lead to different physico-chemical properties, they seem to have no influence on transfection efficiency.

## Conclusions

New degradable poly( $\epsilon$ -caprolactone)-*g*-poly(L-lysine) copolymers, synthesized by either the grafting “from” or the grafting “onto” method, have been tested for transfection. They were used as vectors for gene delivery on MCF-7 cells transfected with pRL-TK, a plasmid coding for *Renilla Luciferase* expression. Data showed that these compounds are able to induce transfection and, although more accurate experiments should be carried out to confirm these results, they show no toxicity. However, even if transfection was assessed, efficiency of both types of PCL-*g*-PLL was a little lower than the one of liposomal Lipofectamine™ Transfection Reagent and far below PEI efficiencies. Despite the mild transfection efficiency reported herein, it is of interest that the new proposed PCL-*g*-PLL structures can be used as degradable vectors. Their transfection efficiency could potentially be increased by adding chloroquine, as it is classically done. However, this strategy was not retained since chloroquine cannot be envisioned for *in vivo* applications. The mild transfection efficiency obtained confirmed the validity of our approach and more specific studies will be carried out in the future to fully characterize these degradable systems (size, stability, toxicity etc). Furthermore, the structure of PCL-*g*-PLL could be further modified by anionic modification of PCL backbone, for example to graft endosomolytic moieties (like histidine) or PEG chains onto the copolymer skeleton in order to enhance the overall transfection efficiency.

## Experimental Part

### Materials

Poly( $\epsilon$ -caprolactone) ( $\overline{M}_n = 42500$  g/mol;  $\overline{M}_w = 80000$  g/mol ), lithium diisopropyl amide (LDA, 2M in THF/*n*-heptane/Ethylbenzene), diethylamine ( $Et_2NH$ ,  $\geq 98\%$ ), branched polyethylenimine with an average molecular weight of 25 kDa (PEI 25K), NaCl  $\geq 99\%$ , HCl 37% solution and HEPES were obtained from Aldrich (St. Quentin

Fallavier, France). N- $\epsilon$ -benzyloxycarbonyl-L-Lysine (N- $\epsilon$ -Z-Lys, >98%), bromoacetyl chloride (>95%) and ammonium chloride (NH<sub>4</sub>Cl) were obtained from Fluka (St. Quentin Fallavier, France). Triphosgene, hydrobromic acid in glacial acetic acid (33% wt), trifluoroacetic acid (TFA, >99%) and anhydrous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> were obtained from Acros Organics (Noisy-le-Grand, France). Ethyl acetate was obtained from Carlo-Erba (Val de Reuil, France), MgSO<sub>4</sub> was obtained from Prolabo (Paris, France), dichloromethane, methanol, dioxane and heptane were obtained from Riedel de Haën (St. Quentin Fallavier, France). All these products were used as received. THF was obtained from Acros Organics (Noisy-le-Grand, France) and distilled on benzophenone/sodium until the formation of a deep blue color. Cell culture medium, Dulbecco's modified Eagle's medium (DMEM), PBS, penicillin, streptomycin, L-glutamine and Lipofectamine™ Transfection Reagent were obtained from Invitrogen (Cergy Pontoise, France). Foetal calf serum was obtained from PAA Laboratories (Les Mureaux, France). pRL-TK plasmid and pGL2-Basic plasmid were kindly provided by Dr P. Roux (Research Center of Macromolecular Biochemistry, Montpellier, France).

### *Synthesis of poly( $\epsilon$ -caprolactone)-g-poly(L-lysine) copolymers*

The syntheses of poly( $\epsilon$ -caprolactone)-g-poly(L-lysine) copolymers (PCL-g-PLL) following either the grafting “onto” method or the grafting “from” method has been described elsewhere [62].

### *Preparation of plasmid solutions and complexes formation*

PEI 25K was dissolved in HBS (HEPES buffered saline: 145 mM NaCl /20 mM HEPES pH 7.2), neutralized with HCl solution to pH = 7.3, and used as a 0.43 mg/mL working solution (10 mM of amine functions, assuming a molecular weight of 43 Da for the repeating unit). PCL-g-PLL copolymers, under bromhydrate form, were dissolved in distilled water and lyophilized. PCL-g-PLL “from” and “onto” were dissolved in HBS buffer (10 ml) and used as 0.17 or 0.34 mg/mL working solutions (0.6 mM and 1.2 mM of amine functions, assuming a molecular weight of 171 Da of the repeating unit in the bromhydrate form).

Solutions of plasmid used with polycationic polymers were prepared from diluted solutions of pRL-TK (0.45  $\mu$ g/ $\mu$ L, 1.35 mM) and pGL2-Basic (1.22  $\mu$ g/ $\mu$ L, 3.66 mM) by dilution in HBS to obtain a 0.1 mM final phosphate concentration. Feedstock solutions of plasmid used with Lipofectamine™ transfection reagent were pRL-TK (1 $\mu$ g/ $\mu$ L, 3mM of phosphate functions) and pGL2-Basic (1.22  $\mu$ g/ $\mu$ L, 3.66mM of phosphate functions).

One hour before transfection, complexes were prepared by mixing plasmid solutions with polymer solutions or lipofectamine as summarized in Table 1. For Lipofectamine™ transfection reagent, complexes formation was achieved according to suppliers protocol in DMEM free from serum and antibiotic. Preparation of polyplexes was adapted from literature [63] in order to study the influence of two amine / phosphate (N/P) ratios, namely 5 and 10. All solutions were gently shaken prior to use and incubated 15-20 minutes at room temperature. In all cases, final pH was in the range 7.2-7.3.



### Cells and cell culture

MCF-7 cells, a human breast adenocarcinoma cell line, were grown according to the ATCC's recommendations. Briefly, cells were maintained at 37 °C in a 5% CO<sub>2</sub> humidified atmosphere, in DMEM containing 10% FCS supplemented with L-glutamine, streptomycin (0.1 mg/ml) and penicillin (20 units/ml).

### Transfection assay

One day before transfection, MCF-7 cells were trypsinated and plated at 360 000 cells/well on a 6-well tissue-culture plate. One hour before transfection assay, culture medium was removed, wells were washed twice with PBS, and 1 ml of free form serum and antibiotic DMEM was added. Transfection complexes were then added to the cells by pouring 100 µL of the solutions as described in Table 1. Three wells were transfected by each complex type. After 5 hours incubation, transfection medium was removed, cells were washed twice with PBS, and fresh DMEM containing 10% FCS supplemented with L-glutamine, streptomycin and penicillin was added. Cells were grown for additional 24 hours, washed twice with PBS and lysed 15 minutes with 1X passive lysis buffer. Luciferase activity was then assessed using the Dual-luciferase<sup>®</sup> reporter assay (Promega, Charbonnières, France) according to the manufacturer's recommendations. 20 µL of supernatant was added to 100 µL of luciferase substrate. After 2 s delay, luminescence was measured for a period of 10 s using a luminometer. Values are given as relative luciferase units (RLU, Firefly/Renilla).

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### References

- [1] Flotte, T. R.; Carter B. *J. Gene Ther.* **1995**, 2, 357.
- [2] Mah, C.; Byrne B. J.; Flotte T. R. *Clin. Pharmacokinet.* **2002**, 41, 901.
- [3] Rots, M. G.; Curiel D. T.; Gerritsen W. R.; Haisma H. J. *J. Control. Release* **2003**, 87, 159.
- [4] Salmons, B.; Guenzburg W. H. *Hum. Gene Ther.* **1993**, 4, 129.
- [5] Jolly, D. *Cancer Gene Ther.* **1994**, 1, 51.
- [6] Muzyczka, N. *Curr. Top. in Microbiol.* **1992**, 158, 97.
- [7] Yei, S.; Mittereder N.; Tang K.; O'Sullivan C.; Trapnell B. C. *Gene Ther.* **1994**, 1, 192.
- [8] Yei, S.; Mittereder N.; Wert S.; Whitsett J. A.; Wilmott R. W.; Trapnell B. C. *Hum. Gene Ther.* **1994**, 5, 731.
- [9] Audouy, S. A. L.; de Leij L. F. M. H.; Hoekstra D.; Molema G. *Pharm. Res.* **2002**, 19, 1599.
- [10] Dass, C. R.; Choong P. F. M. *J. Control. Release* **2006**, 113, 155.
- [11] Fillion, M. C.; Phillips N. C. *Int. J. Pharm.* **1998**, 162, 159.
- [12] Friend, D. S.; Papahadjopoulos D.; Debs R. J. *Biochim. Biophys. Acta-Biomembranes* **1996**, 1278, 41.
- [13] Kawakami, S.; Fumoto S.; Nishikawa M.; Yamashita F.; Hashida M. *Pharm. Res.* **2000**, 17, 306.
- [14] Woodle, M. C.; Newman M. S.; Cohen J. A. *J. Drug Target.* **1994**, 2, 397.
- [15] Brown, M. D.; Schatzlein A. G.; Uchegbu I. F. *Int. J. Pharm.* **2001**, 229, 1.

- [16] De Smedt, S. C.; Demeester J.; Hennink W. E. *Pharm. Res.* **2000**, 17, 113.
- [17] Godbey, W. T.; Wu K. K.; Mikos A. G. *J. Control. Release* **1999**, 60, 149.
- [18] Godbey, W. T.; Mikos A. G. *J. Control. Release* **2001**, 72, 115.
- [19] Kabanov, A. V.; Kabanov V. A. *Bioconjugate Chem.* **1995**, 6, 7.
- [20] Kichler, A.; Behr J. P.; Erbacher P. "Polyethylenimines: a family of potent polymers for nucleic acid delivery", in : *Nonviral Vectors for Gene Therapy*, Huang, L.; Hung, M-C.; Wagner, E.; Eds., Academic Press, London, **1999**, p191.
- [21] Lemieux, P.; Vinogradov S. V.; Gebhart C. L.; Guerin N.; Paradis G.; Nguyen H. K.; Ochietti B.; Suzdaltseva Y. G.; Bartakova E. V.; Bronich T. K.; St Pierre Y.; Alakhov V. Y.; Kabanov A. V. *J. Drug Target.* **2000**, 8, 91.
- [22] Merdan, T.; Kopecek J.; Kissel T. *Adv. Drug Deliver. Rev.* **2002**, 54, 715.
- [23] Park, T. G.; Jeong J. H.; Kim S. W. *Adv. Drug Deliver. Rev.* **2006**, 58, 467.
- [24] Chen, J.; Gamou S.; Takayanagi A. *Shimizu N.FEBS Letters* **1994**, 338, 167.
- [25] Cristiano, R. J.; Roth J. A. *Cancer Gene Ther.* **1996**, 3,4.
- [26] Schachtschabel, U.; Pavlinkova G.; Lou D.; Kohler H. *Cancer Gene Ther.* **1996**, 3, 365.
- [27] Suh, W.; Chung J. K.; Park S. H.; Kim S. W. *J. Control. Release* **2001**, 72, 171.
- [28] Xu, B.; Wiehle S.; Roth J. A.; Cristiano R. J. *Gene Ther.* **1998**, 5, 1235.
- [29] Dizhe, E. B.; Akifiev B. N.; Missul B. V.; Orlov S. V.; Kidgotko O. V.; Sukonina V. E.; Denisenko A. D.; Perevozchikov A. P. *Biochemistry- Moscow* **2001**, 66, 55.
- [30] Han, J.; Il Yeom Y. *Int. J. Pharm.* **2000**, 202, 151.
- [31] Midoux, P.; Mendes C.; Legrand A.; Raimond J.; Mayer R.; Monsigny M.; Roche A. C. *Nucleic Acids Res.* **1993**, 21, 871.
- [32] Thurnher, M.; Wagner E.; Clausen H.; Mechtler K.; Rusconi S.; Dinter A.; Birnstiel M. L.; Berger E. G.; Cotten M. *Glycobiology* **1994**, 4, 429.
- [33] Leamon, C. P.; Weigl D.; Hendren R. W. *Bioconjugate Chem.* **1999**, 10, 947.
- [34] Mislick, K. A.; Baldeschwieler J. D.; Kayyem J. F.; Meade T. J. *Bioconjugate Chem.* **1995**, 6, 512.
- [35] Brown, M. D.; Schaetzlein A.; Brownlie A.; Jack V.; Wang W.; Tetley L.; Gray A. I.; Uchegbu I. F. *Bioconjugate Chem.* **2000**, 11, 880.
- [36] Harada Shiba, M.; Yamauchi K.; Harada A.; Takamisawa I.; Shimokado K.; Kataoka K. *Gene Ther.* **2002**, 9, 407.
- [37] Katayose, S.; Kataoka K. *Bioconjugate Chem.* **1997**, 8, 702.
- [38] Wolfert, M. A.; Schacht E. H.; Toncheva V.; Ulbrich K.; Nazarova O.; Seymour L. W. *Hum. Gene Ther.* **1996**, 7, 2123.
- [39] Ahn, C. H.; Chae S. Y.; Bae Y. H.; Kim S. W. *J. Control. Release* **2004**, 97, 567.
- [40] Choi, Y. H.; Liu F.; Park J. S.; Kim S. W. *Bioconjugate Chem.* **1998**, 9, 708.
- [41] Choi, Y. H.; Liu F.; Choi J. S.; Kim S. W.; Park J. S. *Hum. Gene Ther.* **1999**, 10, 2657.
- [42] Lee, H.; Jeong J. H.; Park T. G. *J. Control. Release* **2002**, 79, 283.
- [43] Toncheva, V.; Wolfert M. A.; Dash P. R.; Oupicky D.; Ulbrich K.; Seymour L. W.; Schacht E. H.; *Biochim. Biophys. Acta-Gen. Subjects* **1998**, 1380, 354.
- [44] Wang, W.; Tetley L.; Uchegbu I. F. *J. Colloid Interf. Sci.* **2001**, 237, 200.
- [45] Brown, M. D.; Gray A. I.; Tetley L.; Santovena A.; Rene J.; Schatzlein A. G.; Uchegbu I. F. *J. Control. Release* **2003**, 93,193.
- [46] Akinc, A.; Langer R. *Biotechnol. Bioeng.* **2002**, 78, 503.
- [47] Fisher, K. J.; Wilson J. M. *Biochemical J.* **1997**, 321, 49.
- [48] Pack, D. W.; Putnam D.; Langer R. *Biotechnol. Bioeng.* **2000**, 67, 217.
- [49] Dang, J. M.; Leong K. W. *Adv. Drug Deliver. Rev.* **2006**, 58, 487.
- [50] Han, S. O.; Mahato R. I.; Sung Y. K.; Kim S. W. *Mol. Ther.* **2000**, 2, 302.

- [51] Lim, Y. B.; Han S. O.; Kong H. U.; Lee Y.; Park J. S.; Jeong B.; Kim S. W. *Pharm. Res.* **2000**, 17, 811.
- [52] Lim, Y. B.; Kim C. h.; Kim K.; Kim S. W.; Park J. S. *J. Am. Chem. Soc.* **2000**, 122, 6524.
- [53] Lynn, D. M. Langer R. *J. Am. Chem. Soc.* **2000**, 122, 10761.
- [54] Hedley, M. L.; "Gene delivery using poly(lactide-co-glycolide) microspheres", in: *Polymeric Gene Delivery*, CRC Press, Boca Raton **2005**, p451.
- [55] Maruyama, A.; Ishihara T.; Kim J. S.; Kim S. W.; Akaike T. *Bioconjugate Chem.* **1997**, 8, 735.
- [56] Wang, D.; Robinson D. R.; Kwon G. S.; Samuel J. *J. Control. Release* **1999**, 57, 9.
- [57] Jeong, J. H.; Park T. G. *J. Control. Release* **2002**, 82, 159.
- [58] Jeong, J. H.; Byun Y.; Park T. G. *J. Biomater. Sci. Polym. Ed.* **2003**, 14, 1.
- [59] Shuai, X.; Merdan T.; Unger F.; Wittmar M.; Kissel T. *Macromolecules* **2003**, 36, 5751.
- [60] Jeong, J. H.; Song S. H.; Lim D. W.; Lee H.; Park T. G. *J. Control. Release* **2001**, 73, 391.
- [61] Ahn, C. H.; Chae S. Y.; Bae Y. H.; Kim S. W. *J. Control. Release* **2002**, 80, 273.
- [62] Nottelet, B.; El Ghzaoui A.; Coudane J.; Vert M. *Biomacromolecules* **2007**, 8, 2594.
- [63] Blessing, T.; Kursu M.; Holzhauser R.; Kircheis R.; Wagner E. *Bioconjugate Chem.* **2001**, 12, 529.