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Amphiphilic Poly[(propylene glycol)-*block*-(2-methyl-2-oxazoline)] Copolymers for Gene Transfer in Skeletal Muscle

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*Amphiphilic triblock copolymers such as poly(ethylene glycol-*b*-propylene glycol-*b*-ethylene glycol) PE6400 (PEG_{13}-PPG_{30}-PEG_{13}) have been recently shown to promote gene transfer in muscle. Herein we investigated the effect of a chemical change of the PEG moiety on the transfection activity of these compounds. We synthesized new amphiphilic copolymers in which the PEG end blocks are replaced by more hydrophilic poly(2-methyl-2-oxazoline) (PMeOxz) chains of various lengths. The resulting triblock PMeOxz-PPG-PMeOxz compounds were characterized by NMR, SEC, TGA, and DSC techniques and assayed for in vivo muscle gene transfer. The results confirm both the block structure and the monomer unit composition (DP_{PG}/DP_{MeOxz}) of the new PPG_{34}-$PMeOxz_{41}$ and PPG_{34}-$PMeOxz_{21}$ triblock copolymers. Furthermore, in vivo experiments show that these copolymers are able to significantly increase DNA transfection efficiency, despite the fact that their chemical nature and hydrophilic character are different from the poloxamers. Overall, these results show that the capacity to enhance DNA transfection in skeletal muscle is not restricted to PEG-PPG-PEG arrangements.*

Introduction

Direct gene transfer into skeletal muscle is particularly attractive for several reasons:[1] This tissue is easily accessible and constitutes about 30% of the normal adult body mass; it has an abundant vascular blood supply, which provides an efficient transport system for secreted proteins into the circulatory system; and because muscle fibers are terminally differentiated cells, gene transfer into muscle tissue should allow long-term expression.

Although intramuscular injection of naked DNA generates detectable transgene expression in mice[2] and humans,[3] the levels are probably too low for obtaining a beneficial therapeutic effect, with the exception of genetic immunization. Therefore, there is a crucial need to develop methods that allow efficient gene transfer into muscle. Over the past years, several nonviral approaches were shown to promote higher transgene expression in muscle relative to naked DNA. Notably, most of these are "physical" methods, such as electroporation,[4] ultrasound,[5] or hydrodynamic intravascular injection.[6] In the field of gene transfer, the amphiphilic triblock copolymers poly(ethylene glycol-*b*-propylene glycol-*b*-ethylene glycol) (also called poloxamers or Pluronics; PEG-PPG-PEG),[1] such as the PE6400 copolymer (PEG_{13}-PPG_{30}-PEG_{13}), belong to the rare group of compounds that have been described to increase gene transfer in muscle.[7,8] These synthetic copolymers are an important class of surfactants and have numerous industrial applications as detergents, dispersion stabilizers, foaming agents, emulsifiers, and lubricants; pharmaceutical applications include drug solubilization and controlled release. Interestingly, several poloxamers are already approved for human use by the US and European Pharmacopoeia Commissions.[9,10]

In aqueous solution, poloxamers can adopt micelle structures with a core formed by the hydrophobic PPG and a corona formed by the hydrated PEG blocks. The use of these copolymers for drug delivery[11] has several advantages: by modifying their structure it is possible to vary the critical micellar concentration (CMC) over a very broad range, and thus the stability of micelles and their circulation in the body can be regulated; drug partitioning can also be optimized.[12,13]

However, it remains unclear how these polymers function and whether there is a direct relationship between the potential of these vectors to effect DNA transfection into skeletal and cardiac muscle and their chemical or amphiphilic character. Therefore, there is considerable interest in designing new amphiphilic compounds by changing the chemical nature and hydrophilicity of the end blocks of the poloxamers. In this context, we synthesized copolymers based on PPG as the central block, and more hydrophilic poly(methyloxazoline) (PMeOxz)

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chains as end blocks. The synthetic route initially involved the tosylation of commercially available PPG (DP = 34).[14] The tosylated PPG was then used as macroinitiator of the polymerization of the 2-methyl-2-oxazoline to lead to triblock copolymers with hydrophilic end blocks of various degrees of polymerization. The structure of the resulting PMeOxz-PPG-PMeOxz polymers was confirmed by NMR spectroscopy, size-exclusion chromatography (SEC), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC). Interestingly, results of the in vivo transfection assays indicate that these copolymers are able to enhance gene transfer into muscle by almost 20-fold.

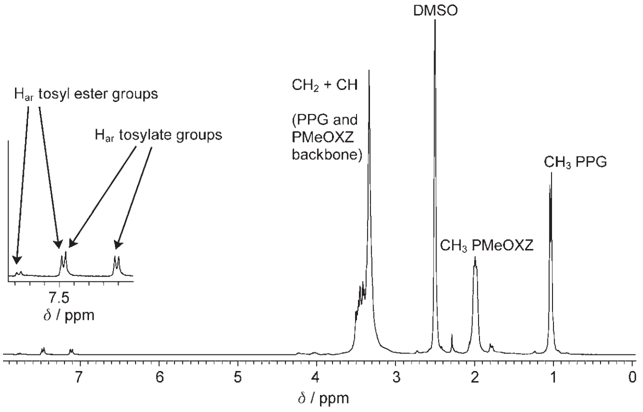
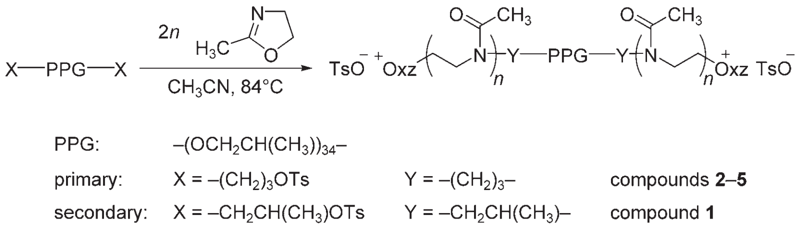


Figure 1. ^{1}H NMR spectrum (300 MHz) of PPG$_{34}$-PMeOxz$_{41}$ block copolymer **4** in [D$_6$]DMSO.

Results and Discussion

Synthesis of poly[(propylene glycol)-*b*-(2-methyl-2-oxazoline)] block copolymers (PPG$_{34}$-PMeOxz$_n$)

The synthesis of triblock copolymers containing both PPG and PMeOxz sequences was carried out by polymerization of the 2-methyl-2-oxazoline (MeOxz) monomer (M) initiated by primary and secondary tosylated polypropylene glycol (PPG-diTs) macroinitiators (I), which we previously described.[14] We used different molar feed ratios $[M]_0/[I]_0$ to obtain copolymers with PMeOxz sequences of various lengths (Scheme 1). After polymerization and evaporation of solvent and unreacted monomer, ^{1}H NMR analysis of the crude products was performed in [D$_6$]DMSO. The spectra show the peaks of methyl protons assigned to the PPG and PMeOxz blocks at $\delta = 1.0$ and 2.0 ppm, and a broad signal that corresponds to the methylene protons of the backbone of PPG and PMeOxz at around $\delta = 3.3$ ppm (Figure 1). Furthermore, the presence of the aromatic signal at $\delta = 7.7$ ppm characteristic of remaining initiator sites indicated a partial initiation in all runs, regardless of the PPG-diTs and the feed ratio used. The initiation contents obtained were thus evaluated for each experiment from the ratio of this peak area to that of the signal PPG shown at $\delta = 1.0$ ppm (Table 1 and S1, Supporting Information).



Scheme 1. Synthesis of triblock copolymers PMeOxz-PPG-PMeOxz by polymerization of MeOxz initiated by primary and secondary PPG-diTs.

A comparison of runs **1** and **3** indicates that the initiation and propagation rate is lower if a secondary macroinitiator is used in place of the primary PPG-diTs, implying that the latter is more efficient in initiating the polymerization of MeOxz (Table 1). However, in a previous study using PEG$_{2kDa}$-Ts as macroinitiator in the same polymerization conditions as in run **2**, we found the initiation step was complete after 10 h,[15] in contrast to that observed with the primary PPG-di(CH$_2$)$_3$-Ts despite a longer reaction time. This result suggests that the hydrophobic character of the PPG backbone decreases the reactivity of the macroinitiator in the initiation step.

Interestingly, by using the primary PPG-diTs, monomer incorporation was complete in all cases, and the initiation content was always greater than 70% (Table 1). Consequently, the copolymerization reaction produced a mixture of diblock PPG-PMeOxz and triblock PMeOxz-PPG-PMeOxz copolymers of various chain lengths. The content of these two species (x_{di} and x_{tri}) in the crude products was evaluated with NMR by using the peak areas present at $\delta = 7.7$ and 1.0 ppm (Table 1, Figure 1, and S1). To decrease the amount of diblock copolymers, the crude products were purified by selective precipitation in diethyl ether, as the PPG but not the PMeOxz block is soluble in this solvent.

For run **2** with copolymers having long polyoxazoline blocks, two successive precipitations in diethyl

Table 1. Polymerization of MeOxz (M) initiated by the primary and secondary tosylated PPGs (I).

Run	I	$[M]_0/[I]_0$[a]	*t* [h]	Yield [%]	Initiation Content [%][b]	Triblock x_{tri} [%][c]	Diblock x_{di} [%][c]
1	secondary	15	41	66	48	0	96[d]
2	primary	50	25	100	87	76[e]	24[e]
3	Primary	14	25	100	70	45	55

[a] Initial concentration of tosyl ester initiator sites: $[I]_0 = 7.0\times10^{-2}$ M for run **1** and $[I]_0 = 6.4\times10^{-2}$ M for runs **2** and **3**. [b] Determined by ^{1}H NMR with an accuracy of ±5% (for detailed calculations, see S1, Supporting Information). [c] Mole fraction of diblock PPG-PMeOxz and triblock PMeOxz-PPG-PMeOxz chains as determined by NMR with an accuracy of ±5% (see S1). [d] This compound contains 4% unreacted macroinitiator. [e] Compound **2** contains a residual amount of PMeOxz homopolymer not detected (≈5% (mol/mol), that is, ≤3% (*w/w*) of the product; estimate made from both NMR and yield results of the two precipitated fractions isolated during the purification process).

ether allowed us to isolate compound **4** with 85% triblock content in the second ether layer (Table 2 and S2, Supporting Information). A small amount of free PMeOxz was also recovered in the precipitates collected during the purification of the crude polymer **2**. The presence of this by-product could be the result of some transfer reaction to monomer during the polymerization process.[16] The same purification procedure was not successful when applied with the crude copolymer **3**. It required the successive use of diethyl ether and THF/hexane as precipitation solvents to obtain the purified rich triblock compound **5** (Table 2 and S2).

Table 2. Structure and composition of the purified PPG_{34}-$PMeOxz_n$ block copolymers **4** and **5**.

Run	Starting Crude Copolymer	n[a]	$PMeOxz_n$-PPG_{34}-$PMeOxz_n$ x_{tri}[b]	PPG_{34}-$PMeOxz_n$ x_{di}[b]
4	**2**	41	85	15
5	**3**	21	72	28

[a] DP of each PMeOxz block of the copolymers **4** and **5** determined by ^1H NMR with an accuracy of ±5% (for detailed calculations, see S1, Supporting Information). [b] Mole fraction of diblock and triblock chains in the purified compounds, determined by ^1H NMR with an accuracy of ±5% (see S1).

NMR spectroscopic analysis of the mole fractions of diblock and triblock in the purified compounds showed that it was possible to isolate the rich triblock copolymers PPG_{34}-$PMeOxz_{41}$ **4** and PPG_{34}-$PMeOxz_{21}$ **5**, with a triblock content at least equal to or greater than 70% and free of PMeOxz (Table 2). Moreover, the ratio of peak integrations corresponding to the methyl group of the repeating MeOxz monomer units at $\delta = 2.0$ ppm and to the tosylate counter-ions of the oxazolinium chain ends at $\delta = 7.1$ ppm allowed determination of the average degree of polymerization (DP_{MeOxz}) of the polyoxazoline chains (Figure 1 and S1). The results show that the average DP of each PMeOxz sequence of compounds grows with the ratio $[M]_0/[I]_0$ (Table 2).

Molecular weight determination and thermal characterization of the copolymers

A comparative analysis by SEC of the starting polymer PPG and the purified compounds was performed in DMF. The traces obtained confirmed the formation of block copolymers, as they were shifted toward higher molecular weight values relative to that of the macroinitiator PPG (Figure 2). However, the M_n values were found to be about 2-fold smaller than those expected according to NMR. This result can be explained by the presence of strong hydrophobic interactions within the PPG block in this highly polar solvent, which probably causes the shrinkage of copolymer chains and a decrease in their hydrodynamic size. This assumption seems to be confirmed by the 3-fold decrease of the molar mass of PPG_{34} when measured in DMF as compared with that obtained in THF, which fits well with the DP of the chain determined by NMR.

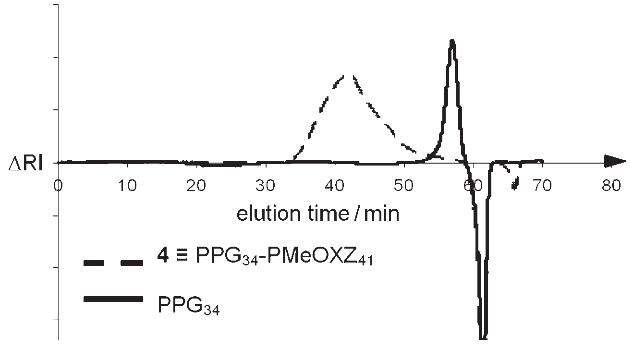


Figure 2. SEC traces of PPG_{34}-$PMeOxz_{41}$ **4** and PPG_{34} in DMF. M_{nSEC} (Ip): 4900 g mol^{-1} (1.41) (-----), 750 g mol^{-1} (1.51) (——).

TGA was carried out to confirm the block structure of the compounds. Figure 3 shows the weight loss curves for PPG_{34}-$PMeOxz_{41}$ (compound **4**), PPG, a PMeOxz homopolymer with a DP close to that of the polyoxazoline sequence of the copolymer, and for the mixture of the single chains of similar weight composition as that found in the copolymer. The plot shows one-step thermal degradation for the copolymer. This transition is different from those measured for the mixture of its respective free counterparts or those observed for each pure homopolymer. This result thus clearly demonstrates the covalent binding of both sequences. Notably, the thermal degradation of PMeOxz is not complete because it remains as a non-degraded carbon residue quantitatively evaluated to about 10% (*w*/*w*) of the sample.

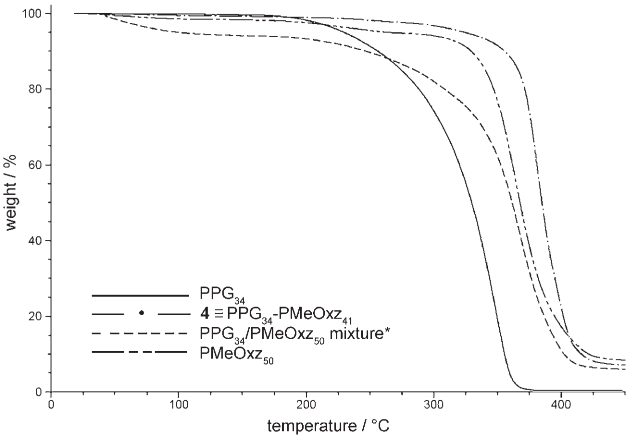


Figure 3. TGA curves obtained at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere for PPG_{34}-$PMeOxz_{41}$ **4**, $PMeOxz_{50}$, PPG_{34}, and a mixture of $PPG_{34}/PMeOxz_{50}$ (*: similar weight composition to that of the copolymer).

DSC was carried out to obtain glass transition temperatures (T_g) for the PPG and PMeOxz segments in the copolymers and their free counterparts. Figure 4 shows the DSC thermograms for the PPG_{34}-$PMeOxz_{41}$ **4** and PPG_{34}-$PMeOxz_{21}$ **5** copolymers and the mixture of $PPG/PMeOxz_{50}$ homopolymers for comparison. First, it was found that the copolymers present two glass

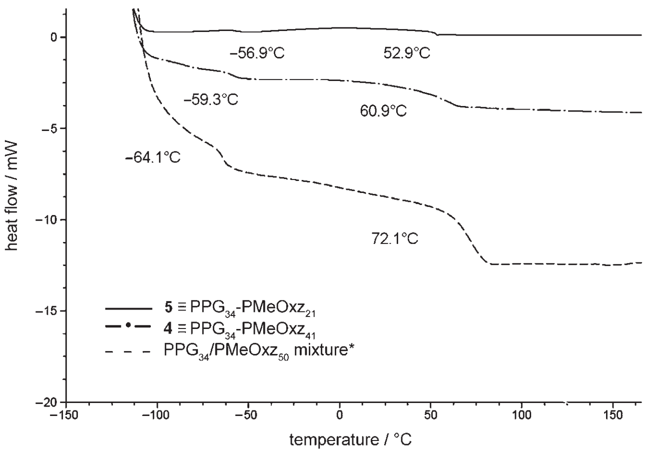


Figure 4. DSC second heating curves (20 °C min^{-1}) of PPG_{34}-$PMeOxz_{41}$ **4**, PPG_{34}-$PMeOxz_{21}$ **5**, and a mixture of PPG_{34}/$PMeOxz_{50}$ (*: similar weight composition to that of the copolymer **4**).

transitions, as in the case of the mixture, which indicates that phase separation occurs between the PPG and PMeOxz blocks within the samples. On the other hand, the T_g of the PMeOxz sections is gradually decreased in comparing the free homopolymer (72.1 °C) with compound **4** (60.9 °C) or **5** (52.9 °C). This difference may be caused by the presence of the amorphous phases formed by PPG which could induce a decrease in crystallinity. Clearly, this effect is more significant as the DP of the polyoxazoline blocks decreases. Moreover, we observed that copolymers **4** and **5** have clear glass transitions for the PPG blocks in the temperature range −56.9 °C to −59.3 °C. These T_g values were slightly higher than that of PPG (−64.1 °C), because the copolymers have PMeOxz end blocks that form crystalline phases which restrict the molecular motion of the PPG middle portion. This same behavior has already been observed for the PPG sequence during DSC analysis of PPG-PEG-PPG compounds with crystalline PEG chains.[17] It should be noted that the higher T_g value obtained for the PPG block of compound **5** (−56.9 °C) relative to that of **4** (−59.3 °C) could be explained, to some extent, by partial mixing of crystalline and amorphous phases in the case of the former polymer because of its lower DP_{MeOxz}.

Gene transfer study

Following the recent findings that poloxamers injected simultaneously with DNA into muscles are able to increase the transfection efficiency, we asked whether our copolymers also possess this property. Owing to its higher purity (Table 2), we were particularly interested in compound **4**. Despite the absence of positive charges, we first wanted to check whether the copolymer is able to interact with plasmid DNA. Therefore, we mixed increasing amounts of polymer **4** (from 0.01 to 0.5 % (*w/v*)) with a constant amount of DNA and tested whether **4** is able to alter the migration of DNA toward the cathode during agarose gel electrophoresis. Compound **4** did not change the migration profile of DNA, even at the highest concentration tested (data not shown). Next, we evaluated the transfection efficiency of **4** in vitro. Concentrations of copolymers between 0.01 and 0.5 % (*w/v*) were tested on HEK293 cells with 2 μg $well^{-1}$ of a plasmid containing the luciferase gene. The expression levels of luciferase 40 h after transfection indicate that **4** did not promote gene transfer (not shown). Taken together, these results are in good agreement with results obtained using commercially available PEG-PPG-PEG poloxamers.[8,18] Indeed, the study conducted with PE6400, for example, could not demonstrate an interaction between the copolymer and DNA by using gel electrophoresis, nor did the authors find that the polymer mediated gene transfer in vitro.[8]

To evaluate whether copolymer **4** is able to increase gene expression in muscle, plasmid DNA (5 μg) encoding luciferase was combined with increasing concentrations of **4** and injected into the tibialis anterior of 8-week-old female Balb/c mice. The muscles were harvested 7 days after injection, and the luciferase activity was measured. The results obtained (Figure 5 A) show that under optimal conditions (0.035 % *w/v*) the expression efficacy was found to be up to 18-fold higher than that of naked DNA. The same experiments made with copolymer **5** gave levels of transfection similar to those obtained with compound **4**, despite a weaker hydrophilicity. Next, we compared the efficiency of copolymer **4** with that of the commercially available poloxamer PE6400. Remarkably, the results show that the efficiency of both polymers is very similar (Figure 5 B).

Finally, we evaluated the toxicity of copolymer **4** in the absence and presence of DNA using the murine muscle cell line C2C12. The MTT assay indicates that our copolymer is poorly cytotoxic, whereas PE6400 used above 0.1 % significantly decreases cell viability (Figure 5 C).

Conclusions

Amphiphilic triblock copolymers consisting of poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG) blocks arranged in a PEG_x-PPG_y-PEG_x structure, including PE6400, were recently described to facilitate gene delivery in skeletal muscle tissue. For the study presented herein, we synthesized and characterized the new poly((propylene glycol)-*b*-(2-methyl-2-oxazoline)) PMeOxz-PPG-PMeOxz block copolymers, analogues of poloxamers in which the PEG portions were replaced by the more hydrophilic polymethyloxazoline (PMeOxz) chains. Interestingly, we show that these compounds are also able to promote gene transfer in muscle. These results show that the capacity to enhance DNA transfection is not restricted to PEG-PPG-PEG arrangements, but that there is great flexibility in the design of amphiphilic triblock-based DNA vectors. There remain, however, many particular questions concerning the way these amphiphilic copolymers act to increase DNA delivery.

Experimental Section

Materials: CH_3CN (sds) and 2-methyl-2-oxazoline (Aldrich) were dried over calcium hydride and then distilled. The PPG-diTs (DP_{PG}: 34; M_{nSEC} (lp) (THF): primary 2300 g mol^{-1} (1.17), secondary 2000 g mol^{-1} (1.21)) were synthesized as previously described.[14]

Diethyl ether (sds), THF (sds), and hexanes (sds) were used as received.

Copolymer characterization: Polymer analysis was performed by NMR spectroscopy (Brucker 300 MHz, [D$_6$]DMSO, 25 °C); SEC (Waters, columns: set of two WATO25861 in series, DMF, refractive index detection (ΔRI), flow rate: 2 mL min^{-1}, calibration of columns with PEG standards); TGA (TA Instruments Q50, flow rate: 100 mL min^{-1}, samples heated at 10 °C min^{-1} from room temperature to 480 °C in a dynamic nitrogen atmosphere); and DSC (TA Instruments Q100 equipped with a cooling accessory and calibrated with indium, flow rate: 50 mL min^{-1}. The following protocol was used for each sample: heating from room temperature to 180 °C at 20 °C min^{-1}, then quenching from 180 °C to −100 °C (40 °C min^{-1}) using liquid nitrogen, and finally reheating from −100 °C to 180 °C. Data were collected during the second heating run. Transition temperatures were taken as the midpoint of the heat capacity change).

Synthesis of poly((propylene glycol)-*b*-(2-methyl-2-oxazoline)) block copolymers (PPG$_{34}$-PMeOxz$_n$): As an example, compound **5** (PPG$_{34}$-PMeOxz$_{21}$) was obtained as follows: the primary ditosylated PPG macroinitiator (1.09 g, 0.47 mmol) was dried under vacuum and dissolved in dried acetonitrile (13.5 mL). MeOxz (1.15 mL, 13.5 mmol) was added and stirred at 84 °C for 25 h. After evaporation, the crude product was dissolved in methylene chloride and precipitated twice in diethyl ether. After centrifugation, the precipitated product was purified by two selective precipitations in a mixture of THF/hexanes (3:10 and 5:5 *v/v*). Finally, the polymer was dried in vacuo. Yield: 12 % (0.25 g); ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.0$ (d, 3 H, (CH_3)CH-CH$_2$), 2.0 (br s, 3 H, N-CO-CH_3), 2.3 (s, 3 H, CH_3-Φ), 3.3 (br s, (102 + 2*n* H), N(COMe)-CH_2-CH_2 and (CH$_3$)C*H*-CH_2), 7.1 and 7.5 (d, 4 H, Φ). $M_{n\mathrm{SEC}}$ (Ip) (DMF) 2200 g mol^{-1} (1.68).

DNA retardation assay: DNA binding was studied by agarose gel retardation assay. Plasmid DNA (1 μg) and increasing amounts of copolymer **4** were each diluted in an aqueous solution of NaCl (25 μL, 150 mM) and mixed. After incubation for 15 min, samples (20 μL) were electrophoresed through a 1 % agarose gel using Tris-borate-EDTA (TBE) buffer (EDTA = ethylenediaminetetraacetic acid). The DNA was visualized after ethidium bromide staining.

Cell culture: Dulbecco's modified Eagle medium (DMEM; Gibco-BRL) was supplemented with L-glutamine (2 mM), penicillin (100 units mL^{-1}), streptomycin (100 μg mL^{-1}), and fetal calf serum (FCS, 10 %). Transformed human embryonic kidney cells (HEK293; American Type Culture Collection, ATCC) were used for the transfection experiments. C2C12, a subclone of the C2 mouse myoblast cell line, was obtained from ATCC. The cells were routinely propagated in DMEM supplemented with FCS (20 %, *v/v*), penicillin (100 units mL^{-1}), and streptomycin (100 μg mL^{-1}). For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) assays, 5×10^4 cells well^{-1} were put into 24-well plates, and differentiation into myotubes was induced by using medium containing 5 % horse serum for one week.

Transfection experiments: Cells plated in 24-well plates were transfected when confluency reached 50–80 %. DNA complexes were generated as follows: plasmid DNA (4 μg) and increasing amounts of polymer **4** were each diluted in an aqueous solution of NaCl (100 μL, 150 mM) and gently mixed. After 15 min incubation

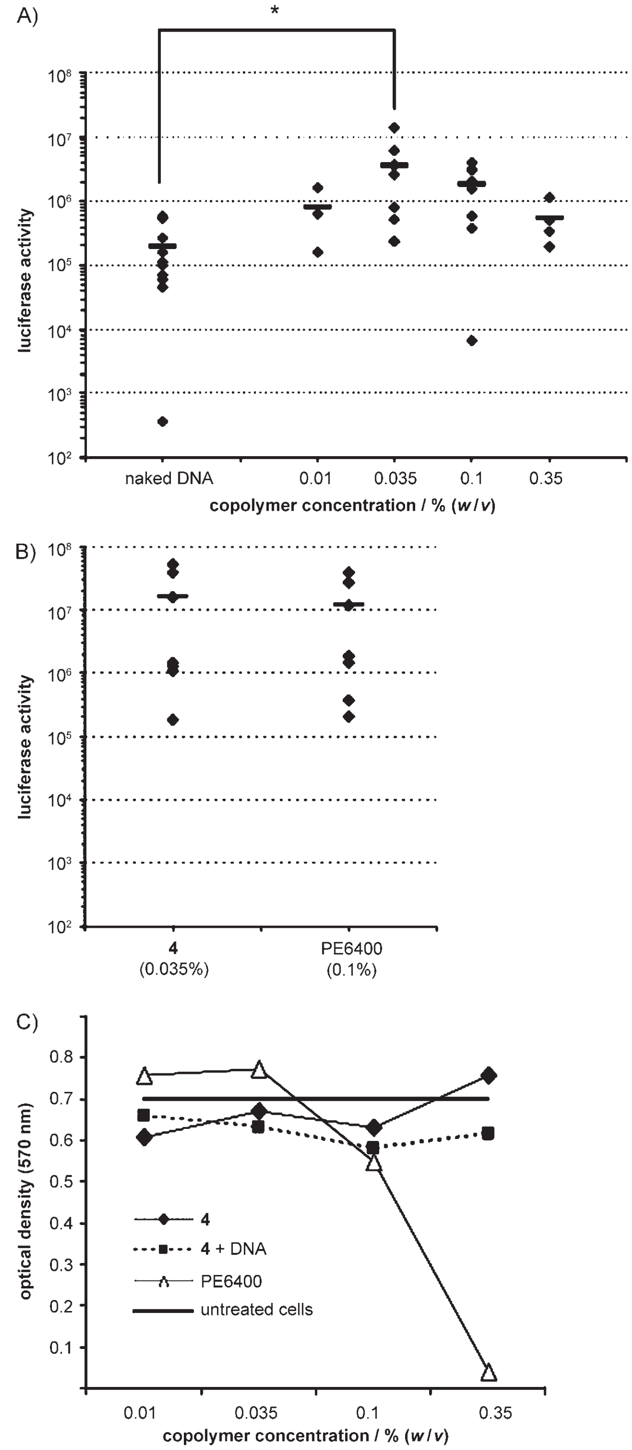


Figure 5. A) Evaluation of in vivo transfection efficiency. Complexes were prepared in a solution of NaCl (150 mM) containing CMV-Luc plasmid mixed with increasing concentrations of copolymer: 0.01, 0.035, 0.1, and 0.35 % (*w/v*), as indicated. Tibial anterior muscles were then slowly injected with 35 μL solution containing 5 μg DNA, and 7 days later the luciferase levels [activity expressed as total light units (10 sec)$^{-1}$ (mg protein)$^{-1}$] were measured from the processed muscles. Depending on the conditions, between 3 and 10 tibialis anterior muscles were injected (each ◆ represents one muscle). The horizontal bar indicates the average luciferase activity per condition. Statistical comparisons were made for 0.035 % of **4**–DNA versus naked DNA: *$p < 0.05$. B) Comparison between copolymer **4** and PE6400. By using the concentration giving the highest transfection efficiency in vivo, we compared copolymer **4** (used at 0.035 % *w/v*) to the commercially available poloxamer PE6400 (used at the *w/v* ratio of 0.1 %). Each ◆ represents one muscle, and the horizontal bar indicates the average luciferase activity per condition. C) The MTT cell viability assay was performed as described in the experimental section using murine C2C12 myotubes.

the mixture was diluted with serum-free medium to a final volume of 1 mL, and then 0.5 mL was transferred into each well of the duplicate. After incubation for 3 h at 37 °C, the medium was removed and replaced with fresh medium containing 10% FCS. Luciferase activity was measured 40 h after transfection, as previously described.[19]

Cell viability assay: Cytotoxicity was evaluated with the MTT assay. Briefly, C2C12 myotubes in 24-well plates were incubated in serum-free medium with increasing amounts of copolymers with or without plasmid (2 μg). After 4 h the cell culture medium was removed and replaced by serum-free DMEM containing 0.5 mg mL^{-1} MTT. After incubation at 37 °C for 2 h, the medium was removed and DMSO (200 μL) was added to each well to dissolve the formazan crystals produced from the reduction of MTT by viable cells. Absorbance was then measured at 570 nm. Untreated cells were used as control.

In vivo experiments: Animal experiments were performed according to institutional guidelines for animal care and use. DNA complexes were formed in 150 mM NaCl. DNA and the polymer were each diluted in 20 μL and then gently mixed. After incubation at room temperature for 15 min, 35 μL of polymer/5 μg CMV-Luc solution were slowly injected into the tibialis anterior of female Balb/C mice (8 weeks old, Iffa-Credo). Mice were sacrificed 7 days post-administration. For the measurement of luciferase levels in muscle, the following protocol was used: lysis buffer (500 μL) containing a cocktail of protease inhibitors (Sigma) was added to the collected organs. Each organ was then homogenized for about 30 s with an Ultra-turrax (Ika), and the homogenate was centrifuged for 10 min at 8000 *g* at 4 °C. An aliquot (5 μL) of the supernatant was used for the luciferase assay. Luciferase background was subtracted from each value, and the transfection efficiency is expressed as total light units (10 sec)$^{-1}$ (mg protein)$^{-1}$ and the values are the means of duplicates. Protein content was measured by using the BioRad (Bradford) protein assay.

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Keywords: amphiphiles · DNA · gene expression · macromolecular chemistry · polymerization

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