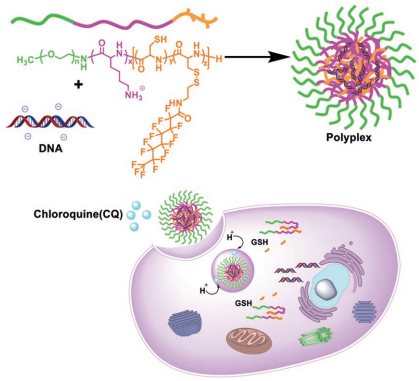
# Fluorinated PEG-Polypeptide Polyplex Micelles Have Good Serum-Resistance and Low Cytotoxicity for Gene Delivery

Ting Wu, Longhai Wang, Shenggang Ding,* Yezi You*

A novel PEGylation polypeptide, poly(ethylene glycol)-*b*-poly(L-lysine)-*b*-poly(L-cysteine) (PEG-PLL-PCys) triblock copolymer is synthesized via the sequential ring-opening polymerization of amino acid *N*-carboxyanhydrides initiated by methoxypolyethylene glycol amine (mPEG-$NH_2$, $M_w$ is 2 kDa). Subsequently, the obtained polypeptide is partially conjugated with fluorocarbon chains via disulfide exchange reaction. PLL segment can condense plasmid DNA through an electrostatic force to form a complex core, PEG segment surrounding the complex like a corona can prevent the complex from precipitation and reduce the adsorption of serum, while PCys segment with fluorocarbon can enhance the cellular uptake and the stability of the formed polyplex micelles in physiological conditions. Experiment results exhibit that the fluorinated polypeptides have low cytotoxicity and good gene transfection efficiency even in the presence of 50% fetal bovine serum.




## 1. Introduction

Gene therapy has attracted extensive attention during the past three decades as a promising approach for the treatment of a broad variety of human diseases, ranging from genetic disorders[1] to severe combined immunodeficiency[2] and cancer.[3] In this regard, original research used viral carriers to deliver the therapy gene to the targeting cells due to their high transfection efficiency,[4] however, their further applications are limited by immunogenicity,[5] toxicity, and difficulty of large-scale production.[6] Recently, the principal focus of research is turned to design effective nonviral vectors that condense and protect deoxyribonucleic acid (DNA) for gene therapy. Cationic lipids and polymers are promising gene carriers due to their limited immunogenicity for clinical application as well as the flexibility and diversity of their chemical design.[7]

However, nonviral gene delivery also faces a set of barriers. One of the challenges is the possible degradation of the genes by endonucleases in the physiological fluids and extracellular environment.[8] It has been found that the half-life of plasmid deoxyribonucleic acid (pDNA) is only several minutes by an intravenous injection of gene into mice.[9] Hence, encapsulation pDNA in a robust nanoparticulate vector is desirable to protect them from degradation by serum endonucleases and increase circulation time.[8] For example, mixing cationic lipids and DNA can spontaneously assemble into liposomes or lipoplexes.[10] Similarly, cationic polymers can condense

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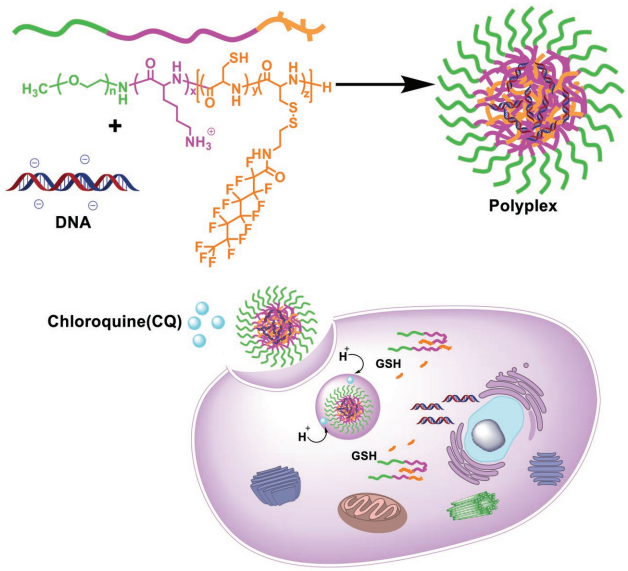
negatively charged DNA through electrostatic interaction and form condensed complexes (polyplexes).[11] However, electrostatic interaction among positively charged polyplexes is highly decreased in the presence of salts, thereby resulting in colloidal instability. Lipoplexes or polyplexes tend to break apart or aggregate in biological fluids which contain serum components and salts.[12] In particular, aggregation of polyplexes in the blood can lead to rapid clearance by circulating macrophages during systemic delivery and inhibit localization to the desired tissues. Moreover, it may cause embolism in lung capillaries.[13]

Poly(ethylene glycol) (PEG)-shielding has become a leading functional strategy to improve stability of polyplexes in physiological fluids. PEG polymers have been approved by the Food and Drug Administration for clinical use due to their low toxicity and no mimmunogenicity.[14] PEG can be conjugated with cationic polymers to form the outer corona, the resulting steric stabilization can prevent protein absorption and lengthen circulation time.[15] PEGylation also reduces opsonization and complement activation.[16] The hydrophilicity and phagocytosis of PEGylated polyplexes are affected by many parameters, such as density of PEG on the particle surface, the chain length and shape.[14] According to Gref et al. findings, the molecular weight between 2 and 5 kDa is optimal for reducing protein adsorption.[17] PEGylation, however, can cause the steric hindrance which leads to weaker binding and influences cellular uptake, decreasing the transfection efficiency.[18]

In recent years, fluoroalkyl compounds have gained extensive attention due to their unique properties, including bioinertness, thermal, and chemical stability, high rigidity, hydrophobicity, and lipophobicity.[19] Fluorination can enhance the affinity of polymers to cell membrane[19a] and the endosome/lysosome membrane.[20] Therefore, fluorination is beneficial to cellular uptake and endosomal escape of the polymers.[21] Moreover, fluorous compounds are reported to have low toxicity and good biocompatibility.[22]

Here, we report a new polypeptide (PEG-PLL-PCys) conjugated with fluorocarbon chains. As shown in Scheme 1, PLL segment can condense plasmid DNA through an electrostatic force to form a complex core, PEG segments surrounding the complex like a corona prevent the complex from precipitation and reduce the adsorption of serum, PCys segment with fluorocarbon chains not only improve the cellular uptake, but also enhance the stability of the consequent polyplex micelles in physiological conditions. The transfection results show that the fluorinated polypeptides have remarkably serum resistance and good gene transfection efficiency even in the presence of 50% fetal bovine serum (FBS).




*Scheme 1.* Schematic illustration of the formation of polyplex micelles and intracellular plasmid DNA delivery.

## 2. Experimental Section

### 2.1. Materials

Methoxypolyethylene glycol amine (mPEG-$NH_2$) ($M_w$ is 2 kDa, Aladdin), *N,S*-dicarbobenzoxy-L-cysteine (Z-Cys-Z) (Macklin), $N^6$-(carbobenzyloxy)- L-lysine (Z-Lys-OH) (Adamas), triphosgene (>98%, Energy Chemical), phosphorus pentachloride (>98%, Aladdin), pyridine disulfide (>98%, TCI), 2-mercaptoethylamine hydrochloride (98%, Sigma), pentadecafluorooctanoyl chloride (>97%, Sigma), perfluorooctanoic acid (>97%, Sigma), were used as received. *N, N′*-Dimethylformamide (DMF), tetrahydrofuran (THF), and toluene were distilled before use.

### 2.2. Synthesis of $N^6$-carbobenzyloxy-L-lysine *N*-carboxyanhydride (Lys(Z)-NCA)

Lys(Z)-NCA was synthesized using the Fuchs–Farthing method.[23] In brief, Lys(Z) (4.0 g, 14.2 mmol) was dispersed in dried THF (60 mL) at 50 °C. To this solution, triphosgene (2.0 g, 6.8 mmol) was then added. After 3 h, the solution was concentrated and precipitated into n-hexane, Lys(Z)-NCA was recrystallized from THF/n-hexane (7/9, v/v).

### 2.3. Synthesis of *S*-benzyloxycarbonyl-L-cysteine *N*-carboxyanhydride (Cys(Z)-NCA)

Cys(Z)-NCA was synthesized according to the reported literature.[24] In brief, to an ice-cooled solution of *N,S*-dibenzyloxycarbonyl-L-cysteine (Z-Cys-Z) (2.3 mg, 6.0 mmol) in toluene (25 mL), phosphorus pentachloride (1.6 g, 7.8 mmol) was added, and the reaction mixture was shaken for 40 min. Then the clear solution was heated to 50 °C for 1 h. The anhydride formed was precipitated with excess n-hexane.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### 2.4. Synthesis of Poly(ethylene glycol)-*b*-poly($N^6$-carbobenzyloxy-L-lysine)-*b*-poly (*S*-carbobenzoxy-L-cysteine) (PEG-PLL(Z)-PCys(Z))

PEG-PLL(Z)-PCys(Z) triblock copolymers were synthesized via sequential ring opening polymerization of Lys(Z)-NCA and Cys(Z)-NCA initiated by mPEG-$NH_2$ in anhydrous DMF. mPEG-$NH_2$ (0.2 g, 0.1 mmol) and Lys(Z)-NCA (1.5 g, 5.0 mmol) were dissolved in 8 mL anhydrous DMF and the reaction was kept for 3 d at 40 °C under stirring in the presence of $N_2$. Then the solution of Cys(Z)-NCA (0.8 mg, 3.0 mmol) in 5 mL anhydrous DMF was added, and the solution was kept for another 3 d at the same condition. The resulting mixture was precipitated twice in excessive diethyl ether and the product was dried under vacuum. The final product has ethylene glycol (EG) units of 44, Lys (Z) of 45, and Cys (Z) units of 13 calculated from its $^1$H NMR spectrum.

### 2.5. Deprotection of PEG-PLL(Z)-PCys(Z) Triblock Copolymers

Triblock copolymer PEG-PLL(Z)-PCys(Z) (1.0 g) was dissolved in $CF_3COOH$ (8 mL), subsequently, a 33% solution of HBr in HAc (0.5 mL) was added. After stirring the reaction for 3 h at 0 °C, the solution was added to excessive diethyl ether and the product was dried by vacuum.

### 2.6. Synthesis of *N*-(2-(2-pyridyldithio)ethyl) perfluorooctanamide

2-(2-Pyridyldithio)ethylamine hydrochloride (801.9 mg, 3.6 mmol) was dispersed in $CH_2Cl_2$ of 40 mL and cooled to −10 °C. Triethylamine (727.5 mg, 3.6 mmol) was added and stirred for 30 min. Pentadecafluorooctanoyl chloride (1.3 g, 3.0 mmol) dissolved in $CH_2Cl_2$ (10 mL) was added dropwise within 40 min at room temperature. After 3 h, the solution was washed with water twice. The organic layer was dried with anhydrous $Na_2SO_4$ and the volatiles were removed under vacuum. The crude product was purified by column chromatography (silica gel, hexane/dichloromethane = 3/1, v/v) to give the product as a colorless powder (1.7 g, yield: 80%).

### 2.7. Synthesis of Fluorinated Polypeptides

The fluorinated polypeptides were synthesized as follows. Triblock copolymer PEG-PLL-PCys ($M_n$ is 10 kDa) was dissolved in anhydrous DMF, and *N*-(2-(2-pyridyldithio)ethyl)perfluorooctanamide was added at different molar ratios. The reaction mixtures were kept stirring for 4 h at $N_2$ atmosphere at room temperature and then precipitated in diethyl ether, forming PEG-PLL-PCys-SS-$3C_7F_{15}$ and PEG-PLL-PCys-SS-$5C_7F_{15}$, respectively.

### 2.8. Characterization of Polymers and Polyplexes

The synthetic polymers were characterized by $^1$H NMR and $^{19}$F NMR (Supporting Information). All NMR were performed on a Bruker AV 400 NMR spectrometer using chloroform-d, dimethyl sulfoxide-$d_6$ (DMSO-$d_6$), and deuterium oxide as the solvent. Zeta potential and size of the fluorinated polypeptides/DNA polyplexes at different N/P ratios were characterized using dynamic light scattering (Malvern Zetasizer Nano ZS90). The morphology structures of the formed polyplexes were characterized using a JEM-2100F transmission electron microscopy (TEM, 100 KV).

### 2.9. Agarose Gel Electrophoresis Assay

Various cationic polymers (PEG-PLL-PCys, PEG-PLL-PCys-SS-$3C_7F_{15}$, and PEG-PLL-PCys-SS-$5C_7F_{15}$) were mixed with DNA at different nitrogen/phosphorus (N/P) ratios for 30 min. The polyplexes were electrophoresed onto a 0.9% agarose gels and subjected to electrophoresis for 60 min at 100 V in TAE (Tris base, acetic acid and EDTA) buffer. Then the gel was soaked in 0.5 μg mL$^{-1}$ ethidium bromide for 30 min and DNA bands were visualized by a UVP ED3 Imaging System.

### 2.10. Confocal Imaging

HeLa cells (10 000 cells per well) in 4-chamber glass dish were incubated overnight before experiment. The cells were treated with fluorinated polypeptides/DNA, PEG-PLL-PCys/DNA, PEI/DNA, and Lipofectamine 2000/DNA polyplexes (DNA labeled with Cy5), respectively. After incubating for 4 h, the nuclei were stained with DAPI for 20 min. Then the images were obtained by confocal laser scanning microscopy (Leica).

### 2.11. In Vitro Gene Transfection

Luciferase pDNA transfection efficiency was measured as follows: 293T cells were seeded into 96-well plates with the density of $8 \times 10^3$ cells per well in 100 μL of Dulbecco's modified eagle medium (DMEM) containing 10% FBS and incubated for 24 h before transfection experiment. The polyplexes (0.5 μg luciferase plasmid DNA per well) were prepared as follows: PEG-PLL-PCys-SS-$3C_7F_{15}$ and PEG-PLL-PCys-SS-$5C_7F_{15}$ were complexed with pDNA at N/P ratio of 30 for 30 min, PEI (N/P 10) and Lipofectamine 2000 (according to the product's protocols (Invitrogen)) were used as control. Then the polyplexes solution were added to each well containing $100 \times 10^{-6}$ M chloroquine and 100 μL of DMEM with 0%, 10%, 30%, and 50% FBS, respectively. After 4 h of incubation, the media were replaced with 200 μL fresh DMEM containing 10% FBS and the cells were cultured for another 48 h. Then the culture media were removed and cells were washed with phosphate buffer saline (PBS) buffer (pH 7.4) twice and then the cells were lysed with 100 μL of cell lysis buffer (Beyotime Biotechnology) overnight at −80 °C. Subsequently 30 μL thawing cell lysate was mixed with 50 μL of luciferase substrate for the determination of relative light units (RLU) using a luminometer (Thermo fisher, USA). The protein concentration in the cell lysate was measured by a bicinchoninic acid (BCA) assay kit (Pierce). The luciferase transfection results are expressed as mean RLU per milligram of cell protein of protein ± SD of triplicate experiments.

Green fluorescent protein (GFP) pDNA transfection efficiency was measured as follows: 293T cells were seeded into 96-well plates with the density of $8 \times 10^3$ cells per well in 100 μL of DMEM containing 10% FBS and incubated for 24 h before transfection experiment. The polyplexes (0.5 μg luciferase plasmid

DNA per well) were prepared as follows: PEG-PLL-PCys-SS-3$C_7F_{15}$ and PEG-PLL-PCys-SS-5$C_7F_{15}$ were complexed with pDNA at N/P ratio of 30 for 30 min, PEI (N/P 10) and Lipofectamine 2000 (according to the product's protocols (Invitrogen)) were used as control. Then the polyplexes solution were added to each well containing $100 \times 10^{-6}$ M chloroquine and 100 μL of DMEM with 0%, 10%, 30%, and 50% FBS, respectively. After 4 h of incubation, the media were replaced with 200 μL fresh DMEM containing 10% FBS and the cells were cultured for another 48 h. The expression of GFP plasmid in the cells was directly observed by a fluorescent microscopy (Olympus), and the transfection efficiency was quantitatively measured using flow cytometry.

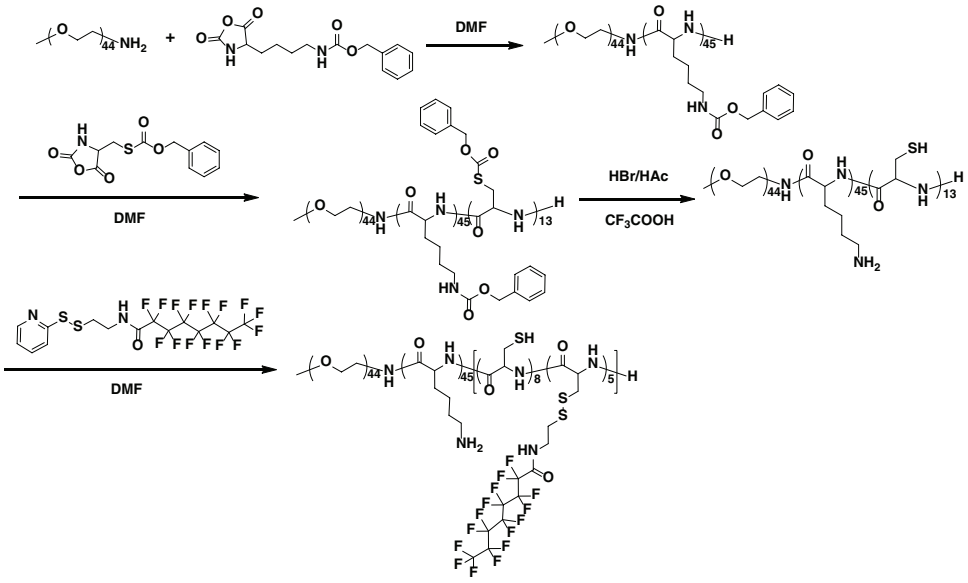
### 2.12. Cytotoxicity of Fluorinated Polypeptides

Cytotoxicity of fluorinated polypeptides was evaluated by MTT assay. Generally, 293T cells or MCF-7 cells were seeded into 96-well plates with the density of $1 \times 10^4$ cells per well in 100 μL of DMEM containing 10% FBS overnight. The cells were incubated with fluorinated polypeptides, PEI and Lipofectamine 2000 at different concentrations for 48 h. Subsequently, the culture media were removed and 20 μL of 5 mg $mL^{-1}$ MTT stock solution with a mixture of 100 mL DMEM was added to each well. The yielded formazan crystals were dissolved in 150 mL DMSO. Absorbance of the solution in each well was measured at wavelengths of 490 and 570 nm by a microplate reader.

## 3. Results and Discussion

### 3.1. Synthesis and Characterization of Fluorinated Polypeptides

The ring-opening polymerization of amino acid *N*-carboxyanhydrides initiated by primary amine group is the most active synthetic method for preparation of polypeptides,[25] and triblock copolypeptide PEG-PLL(Z)-PCys(Z) was synthesized by this method via the successive ring-opening polymerization of Lys(Z)-NCA and Cys(Z)-NCA initiated by mPEG-$NH_2$. Subsequently, a 33% solution of HBr in acetic acid was added to deprotect benzyloxycarbonyl (Z) groups of the PLL(Z) and PCys(Z) block, giving PEG-PLL-PCys. The resulting triblock copolypeptide has a block of 45 Lys units and a block of 13 Cys units which were calculated from its $^1$H NMR spectra (Figures S3 and S4, Supporting Information). Subsequently, the resulting polypeptide was partially conjugated with fluorocarbon chains via disulfide exchange reaction with *N*-(2-(2-pyridyldithio) ethyl)perfluorooctanamide as shown in Scheme 2, resulting in PEG-PLL-PCys-SS-3$C_7F_{15}$ and PEG-PLL-PCys-SS-5$C_7F_{15}$ (3 and 5 fluorocarbon chains were grafted onto PCys block, respectively). We measured the $^{19}$F NMR spectra of PEG-PLL-PCys-SS-3$C_7F_{15}$ and PEG-PLL-PCys-SS-5$C_7F_{15}$ with $CF_3COOH$ as an external standard as shown in Figures S9 and S10 (Supporting Information). It can be found that 3 and 5 fluorocarbon chains were attached to the polypeptides, respectively. We also measured the UV absorption spectroscopy of PEG-PLL-PCys, *N*-(2-(2-pyridyldithio) ethyl) perfluorooctanamid (pyridyldithio-$C_7F_{15}$) and the corresponding solution after the formation of PEG-PLL-PCys-SS-3$C_7F_{15}$ and PEG-PLL-PCys-SS-5$C_7F_{15}$ (Figure S11, Supporting Information). Pyridothione produced in the reaction of PEG-PLL-PCys with pyridyldithio-$C_7F_{15}$ has a strong absorption at wavelength of 380 nm. All the results showed that fluorinated polypeptides had been successfully synthesized.




*Scheme 2.* Synthesis route of the fluorinated polypeptide.



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### 3.2. Characterization of Fluorinated Polypeptides Polyplexes

The fluorinated triblock copolypeptides can condense DNA via electrostatic interaction, the pKa of PLL is about 9.4, therefore all primary amino groups of PLL are protonated in the neutral condition.[26] PLL block can condense DNA and form a complex core. PEG is hydrophilic and formed an outer corona, PCys segment with fluorocarbon can strengthen the binding capacity. The micelle morphology was characterized by TEM. From Figure 1A, it can be found that the polyplex micelles at N/P ratio of 10 is ≈80 nm, which is proper for the effective endocytosis. The zeta potential values of the formed polyplexes were demonstrated in Figure 1B at varying N/P ratios. The zeta potential transformed to positive value at the N/P ratio of 2, which demonstrated that the fluorinated polypeptides can bind with DNA at a low N/P ratio. The positive value increased with the increase of N/P ratios, and it was 40 mV at the N/P ratio of 30.

### 3.3. DNA Binding Capacity

In 1975, Laemmli first demonstrated the unusual capability of polylysine to condense DNA.[27] The DNA-binding ability of the fluorinated polypeptides was examined by agarose gel electrophoresis assay. As shown in Figure 2C, it was very clear that the fluorinated polypeptides were more efficient in DNA retardation compared with common polypeptides. The polypeptides with 5 fluorocarbon chains have a lower N/P ratio than that with 3 fluorocarbon chains to completely inhibit DNA migration demonstrating the introduction of fluorocarbon chains can enhance DNA binding ability.

### 3.4. Stability in the Presence of Heparin and Salts

Generally, the polyplexes are unstable in biological fluids which contain salts and serum components, in which the polyplexes tend to break apart or precipitate.[20] In this work, the stability of fluorinated polypeptides was investigated by agarose gel electrophoresis assay in the presence of heparin and salts, respectively. As shown in Figure 2A, at a heparin concentration of 100 μg mL$^{-1}$, bands corresponding to free DNA were clearly observed for the PEI/DNA polyplexes, while almost no free DNA was detected for PLL-PCys-SS-3$C_7F_{15}$/DNA and PEG-PLL-PCys-SS-5$C_7F_{15}$/DNA polyplexes. This result demonstrates the substantial stability of fluorinated polypeptides polyplexes in the presence of heparin. Moreover, the formed fluorinated polypeptides/DNA polyplexes were stable in the presence of NaCl. As shown in Figure 2C, the size of PEG-PLL-PCys-SS-5$C_7F_{15}$/DNA polyplexes micelles remained stable when treated with 200 × 10$^{-3}$ M NaCl. The excellent stability of fluorinated polypeptides/DNA polyplex micelles ascribes to that the high rigidity of fluorocarbon chains leads to a low surface energy in physiological fluids.[28] In addition, the steric stabilization of PEG and potential crosslinking of PCys segments also played roles in strengthening the stability of the polyplexes (as shown in Figure S12, Supporting Information).[29]

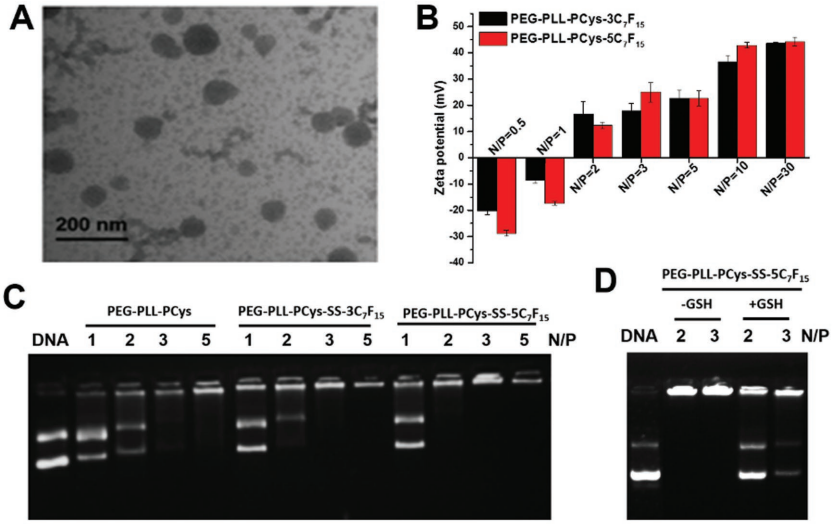


*Figure 1.* A) TEM image of the polyplexes formed from PEG-PLL-PCys-SS-5$C_7F_{15}$/DNA at the N/P ratio of 10. B) Zeta potential of the polyplexes formed from PEG-PLL-PCys-SS-3$C_7F_{15}$/DNA and PEG-PLL-PCys-SS-5$C_7F_{15}$/DNA at different N/P ratios. C) Agarose gel electrophoresis assay of PEG-PLL-PCys/DNA, PEG-PLL-PCys-SS-3$C_7F_{15}$/DNA, and PEG-PLL-PCys-SS-5$C_7F_{15}$/DNA polyplexes. D) Agarose gel electrophoresis assay of PLL-PCys-SS-5$C_7F_{15}$/DNA polyplexes treated with 0 or 20 × 10$^{-3}$ M glutathione (GSH).

### 3.5. In Vitro Gene Transfection and the Serum Resistance of Fluorinated Polypeptides Polyplexes

Cationic lipids and polymers used in vivo gene delivery must have a high stability to tolerate harsh conditions in biological fluids. Various blood components can decrease gene transfection efficiency by changing the properties of the polyplexes and dissociating the polyplexes.[30] In this work, the fluorinated polypeptides can condense pDNA well and form robust polyplex micelles which are stable in physiological fluids. The transfection efficiency and serum resistance of the fluorinated polypeptides were assessed in 293T cells with a medium containing 0%, 10%, 30%, and 50% FBS. Chloroquine (100 × 10$^{-3}$ M) was employed as an ensomolytic agent. As shown in

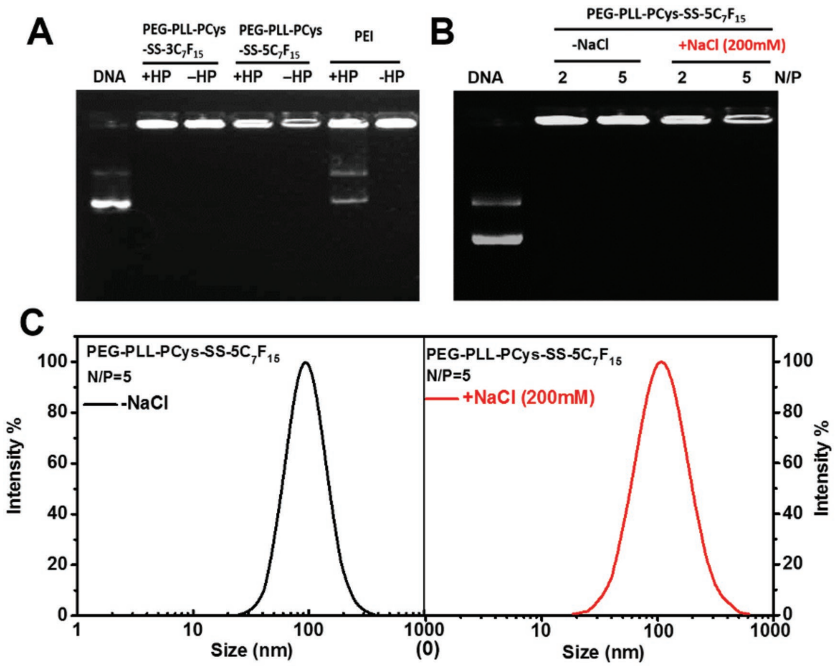


*Figure 2.* A) Agarose gel electrophoresis assay of PLL-PCys-SS-$3C_7F_{15}$/DNA, PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA, and PEI/DNA polyplexes at the N/P ratio of 10 with or without heparin (HP) at a HP concentration of 100 μg $mL^{-1}$. B) Agarose gel electrophoresis assay of PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes with 0 M and $200 \times 10^{-3}$ M NaCl. C) Size stability of PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes in NaCl solution at an N/P ratio of 5.

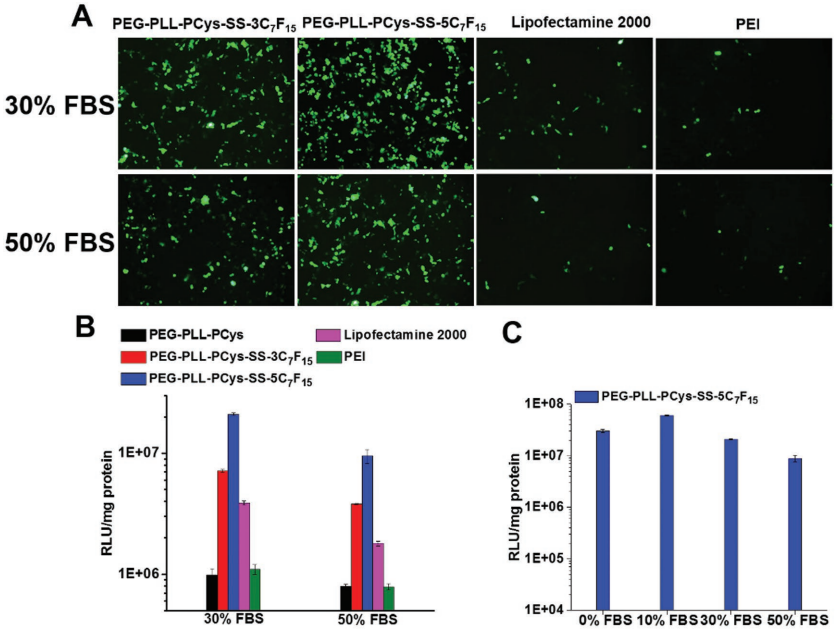


*Figure 3.* A,B) GFP expressions and luciferase transfection efficiency of PEG-PLL-PCys-SS-$3C_7F_{15}$/DNA and PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes at N/P ratio of 30 in 293T cells in mediums containing 30% and 50% FBS. PEI (N/P = 10) and Lipofectamine 2000 (according to the product's protocols (Invitrogen)) are used as control. C) Luciferase transfection efficiency of PEG-PLL-PCys-SS-$5C_7F_{15}$ at N/P ratio of 30 in 293T cells in mediums containing 0%, 10%, 30%, and 50% FBS, respectively.

Figure 3, the transfection efficiency of PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes changed very little with the increase of serum content and exhibited good GFP transfection efficiency (35%) even in the presence of 50% FBS (Figure S16, Supporting Information). In contrast, gene transfection efficiency of Lipofectamine 2000 and PEI were significantly decreased in the presence of FBS. The luciferase transfection efficiency of PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes was about ten times as that of Lipofectamine 2000 in the presence of 50% FBS. Also, the polypeptides with 5 fluorocarbon chains had much higher transfection efficiency than that with 3 fluorocarbon chains. The polypeptides with fluorocarbon chains showed better transfection efficiency than that without fluorocarbon chains in the presence of serums. These results strongly indicate the importance of fluorocarbon chains in the stabilization of the resulting micelles in physiological fluids and enhancement of the gene transfection efficiency in medium containing FBS.

### 3.6. Transfection and Serum-Resistance Mechanisms of Fluorinated Polypeptides

Cellular uptake and DNA release play important roles in efficient gene transfection. To explore the mechanisms of the high transfection efficiency of fluorinated polypeptides in high levels of serum, we compared the cellular uptake of PEG-PLL-PCys/DNA polyplexes with PEG-PLL-PCys-SS-$3C_7F_{15}$/DNA and PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes in HeLa cells by confocal laser scanning microscopy. From Figure S14 (Supporting Information), it can be found that fluorination remarkably increased the cellular uptake efficiency of Cy5-labeled DNA. And we also compared the cellular uptake of PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA with PEG-PLL-PCys/DNA, PEI/DNA and Lipofectamine 2000/DNA polyplexes in the presence of 50%



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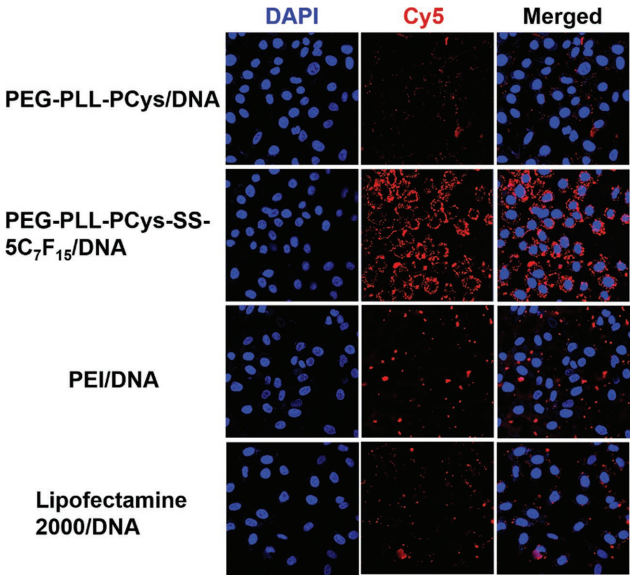


*Figure 4*. Confocal laser scanning microscopy images of HeLa cells treated with PEG-PLL-PCys/DNA polyplexes, PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes, PEI/DNA polyplexes, and Lipofectamine 2000/DNA polyplexes in the presence of 50% FBS (DNA was labeled with Cy5 and the nucleus were stained with DAPI solution for 20 min).

FBS. As shown in Figure 4, PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes exhibited much higher cellular uptake efficiency than those of Lipofectamine 2000/DNA and PEI/DNA polyplexes which is attributed to the high stability of fluorinated polypeptides/DNA polyplexes in physiological fluids. In this work, the fluorocarbon chains were grafted to the polypeptides by disulfide bonds. It is well-known that the disulfide bonds can be reduced after cell internalization since the intracellular environment is much more reductive. As shown in Figure 1D, when treated with $20 \times 10^{-3}$ M GSH, bands corresponding to free DNA were clearly observed for the PEG-PLL-PCys-SS-$5C_7F_{15}$/DNA polyplexes which can lead to DNA release.

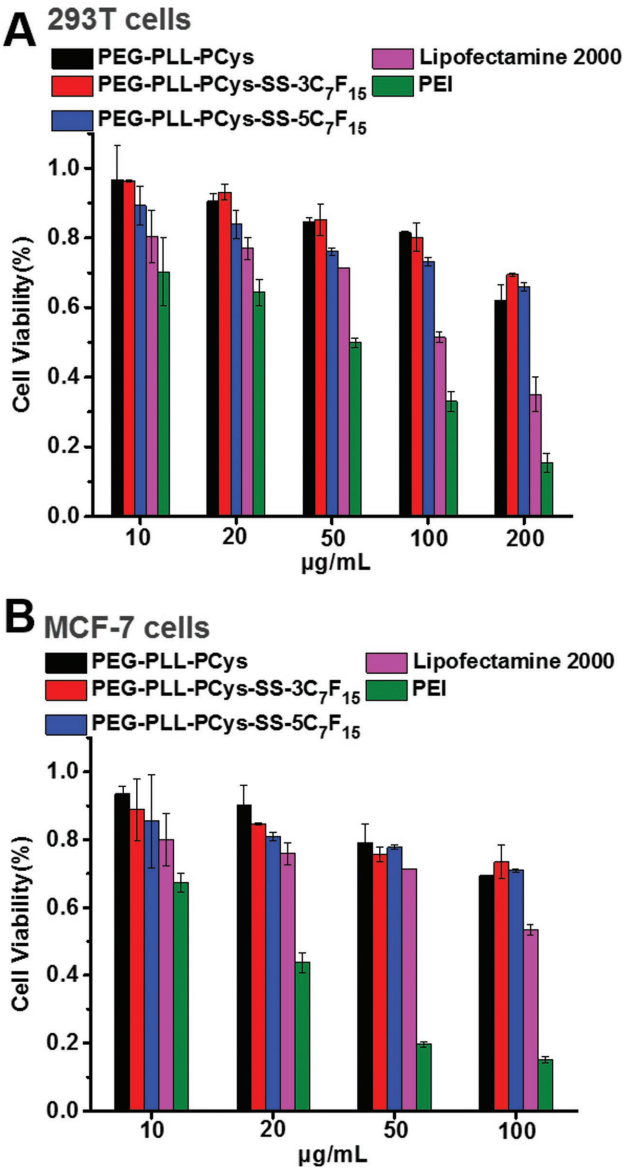


*Figure 5*. A,B) Cell viability of PEG-PLL-PCys, PEG-PLL-PCys-SS-$3C_7F_{15}$, PEG-PLL-PCys-SS-$5C_7F_{15}$, Lipofectamine 2000, and PEI solution in 293T cells and MCF-7 cells.

### 3.7. In Vitro Cytotoxicity of Fluorinated Polypeptides

The cytotoxicity of gene carriers is a very important factor for its further application. Synthetic polypeptides are biocompatible and biodegradable polymers linked by peptide bond, their structures are similar to natural proteins.[31] Fluorous compounds are also reported to have a relative low toxicity and good biocompatibility. Therefore, we predicted the fluorinated polypeptides may have a low cytotoxicity. In vitro cytotoxicity was examined by MTT method in 293T cells and MCF-7 cells for these fluorinated polypeptides along with Lipofectamine 2000 and commercial PEI-25kDa as control (Figure 5). PEI showed a quite strong dose-dependent cytotoxicity, and the cell viability dropped to about 30% above the concentration of 200 μg $mL^{-1}$. In contrast, the cytotoxicity of fluorinated polypeptides is low in the entire concentration range from 5 to 200 μg $mL^{-1}$. We also measured the cytotoxicity of PEG-PLL-PCys-SS-$5C_7F_{15}$ and PEG-PLL-PCys/perfluorooctanoic acid mixture to simulate the cytotoxicity caused by detachment of fluorocarbon chains in the cells. As shown in Figure S17 (Supporting Information), both PEG-PLL-PCys-SS-$5C_7F_{15}$ and PEG-PLL-PCys/perfluorooctanoic acid mixture are nearly nontoxic on the cells. All these results indicate a very low cytotoxicity of the fluorinated polypeptides in gene delivery.

## 4. Conclusions

In conclusion, a novel PEGylation polypeptide (PEG-PLL-PCys) conjugated with fluorocarbon chains was synthesized. PLL segment can condense plasmid DNA through an electrostatic force to form a complex core, PEG segment surrounding the complex like a corona can prevent the complex from precipitation and reduce the adsorption of serum, while PCys segment with fluorocarbon can enhance the binding capacity and lead to a significant increase in the stability of the resulting micelles in physiological fluids. The fluorinated polypeptides have a low cytotoxicity and show remarkably serum-resistance, they exhibit good gene transfection efficiency even in the presence of 50% FBS.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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