Supporting Information

Materials

L-Arginine, L-Arginine hydrochloride, *p*-toluenesulfonic acid monohydrate, succinyl chloride, adipoyl chloride, sebacoyl chloride, di-ethylene glycol (DEG), tri-ethylene glycol (TEG), tetra-ethylene glycol (TTEG), poly (ethylene glycol) (M_n= 300), poly (ethylene glycol) (M_n= 600), triethylamine and *p*-nitrophenol were all purchased from Sigma Chemical (St. Louis, MO) and used without further purification. Organic solvents like methanol, toluene, ethyl acetate, acetone, 2-propanol and dimethyl sulfoxide (DMSO) were purchased from VWR Scientific (West Chester, PA) and were purified by standard methods before use. Other chemicals and reagents if not otherwise specified were purchased from Sigma (St. Louis, MO).

Linear polyethylenimine (PEI) with a weight average molecular weight (M_w) of 25,000, ethidium bromide, MTT, and all buffers were purchased from Sigma (St. Louis, MO). Dulbecco's modified eagle medium (DMEM), penicillin–streptomycin (PS, 100 U/mL), trypsin–EDTA (TE, 0.5 % trypsin, 5.3 mM EDTA tetra-sodium), fetal bovine serum (FBS) were obtained from Gibco BRL (Rockville, MD). Cell lines (rat aortic SMC A10, BAEC endothelial cells), rat primary smooth muscle cells (RSMC), human umbilical vein endothelial cells (HUVEC)), rat mesenchymal stem cells (MSC) and rat bone marrow cells (BM)) were obtained from American Type Culture Collection (ATCC, Manassas, VA) or Professor Bo Liu's lab at Surgery Department of University of Wisconsin at Madison. DNA size marker N3014 was purchased from New England Lab (Woburn, MA). A Qiagen endotoxin-free plasmid Maxi kit was purchased from Qiagen (Valencia, CA). Lipofectamine2000® was purchased from Invitrogen (Carlsbad, CA). Promega Luciferase Assay Kit containing luciferase cell culture lyses reagent and luciferase substrates were obtained from Promega (Madison, WI).

Synthesis and Characterization of Monomers

An example of the synthesis of monomer **IIa** is given here: L-arginine (0.04 mol) and di-ethylene glycol (0.02 mol) were directly mixed in a three neck round bottom flask with toluene (b.p. 110 ºC) (400 mL) and *p*-toluenesulfonic acid monohydrate (0.082 mol). The solid-liquid reaction mixture was heated to 130 ºC and reflux for 24 hr after 2.16 mL (0.12 mol) of water was generated. The reaction mixture (viscous solid) was then cooled to room temperature. Toluene was decanted. The dried reacted mixture was finally purified by repeated precipitation in 2-propanol for three times. 2-propanol was decanted, and then the white sticky mass was dried in vacuum.

The following are some ^{1}H-NMR and FTIR details for the Monomers **IIa** (S salt type). The ^{1}H-NMR data for the Monomers **IIb** (Cl salt type) are the same as the corresponding S salt type Monomer **IIa**, except the difference of peak intensity of some groups:

Arg-2E-S: Yield of purified product: 81%. Appearance: white powder. IR (cm^{-1}): 1735 [-C(O)-], 1177 [-O-], 1127 [-CH2-O-CH2-]; ^{1}HNMR (DMSO-d6, ppm, δ): 1.61 [4H, -CH_2-C**H**$_2$-CH_2-NH-], 1.77 [4H, -OC(O)-CH(NH_3^+)C**H**$_2$-$(CH_2)_2$-], 2.29 [6H, **H**$_3$C-Ph-SO_3-], 3.10 [4H, -$(CH_2)_2$-C**H**$_2$-NH-], 3.60 [4H, -(O)C-O-CH_2-C**H**$_2$-O-], 4.07 [2H, $^+$$H_3$N-C**H**(R)-C(O)-O-], 4.32 [4H, -(O)C-O-C**H**$_2$-], 7.13, 7.48 [16H, Ph], 7.59 [10H, -CH_2-N**H**(N**H**$_2^+$)-N**H**$_2$], 8.42 [6H, $^+$**H**$_3$N-CH(R)-C(O)-O-];

Arg-3E-S: Yield of purified product: 85%. Appearance: white powder. IR (cm^{-1}): 1736 [-C(O)-], 1178 [-O-], 1125 [-CH2-O-CH2-]; ^{1}HNMR (DMSO-d6, ppm, δ): 1.63 [4H, -CH_2-C**H**$_2$-CH_2-NH-], 1.78 [4H, -OC(O)-CH(NH_3^+)C**H**$_2$-$(CH_2)_2$-], 2.28 [6H, **H**$_3$C-Ph-SO_3-], 3.12 [4H, -$(CH_2)_2$-C**H**$_2$-NH-], 3.55-65 [8H, -(O)C-O-CH_2-C**H**$_2$-O-C**H**$_2$-], 4.09 [2H, $^+$$H_3$N-C**H**(R)-C(O)-O-], 4.31 [4H, -(O)C-O-C**H**$_2$-], 7.15, 7.49 [16H, Ph], 7.62 [10H, -CH_2-N**H**(N**H**$_2^+$)-N**H**$_2$], 8.47 [6H, $^+$**H**$_3$N-CH(R)-C(O)-O-];

Arg-4E-S: Yield of purified product: 87%. Appearance: white powder. IR (cm^{-1}): 1734 [-C(O)-], 1179 [-O-], 1124 [-CH2-O-CH2-]; ^{1}HNMR (DMSO-d6, ppm, δ): 1.62 [4H, -CH_2-C**H**$_2$-CH_2-NH-], 1.79 [4H, -OC(O)-CH(NH_3^+)C**H**$_2$-$(CH_2)_2$-], 2.27 [6H, **H**$_3$C-Ph-SO_3-], 3.11 [4H, -$(CH_2)_2$-C**H**$_2$-NH-], 3.60-70 [12H, -(O)C-O-CH_2-C**H**$_2$-O-C**H**$_2$-C**H**$_2$-], 4.08 [2H, $^+$$H_3$N-C**H**(R)-C(O)-O-], 4.30 [4H, -(O)C-O-C**H**$_2$-], 7.17, 7.50 [16H, Ph], 7.63 [10H, -CH_2-N**H**(N**H**$_2^+$)-N**H**$_2$], 8.49 [6H, $^+$**H**$_3$N-CH(R)-C(O)-O-];

Arg-6E-S: Yield of purified product: 89 %. Appearance: white viscous solid. IR (cm^{-1}): 1737 [-C(O)-], 1177 [-O-], 1127 [-CH2-O-CH2-]; ^{1}HNMR (DMSO-d6, ppm, δ): 1.63 [4H,-CH_2-C**H**$_2$-CH_2-NH-], 1.80 [4H,-OC (O)-CH (NH_3^+) C**H**$_2$-$(CH_2)_2$-], 2.29 [6H, **H**$_3$C-Ph-SO_3-], 3.14 [4H,-$(CH_2)_2$-C**H**$_2$-NH-], 3.60-70 [20H, -(O)C-O-CH_2-C**H**$_2$-O-(C**H**$_2$-C**H**$_2$-)$_2$], 4.10 [2H,$^+$$H_3$N-C**H**(R)-C(O)-O-], 4.32 [4H, -(O)C-O-C**H**$_2$-], 7.16, 7.50 [16H, Ph], 7.64 [10H, -CH_2-N**H**(N**H**$_2^+$)-N**H**$_2$], 8.50 [6H, $^+$**H**$_3$N-CH(R)-C(O)-O-];

Arg-12E-S: Yield of purified product: 84 %. Appearance: white viscous solid. IR (cm^{-1}): 1737 [-C(O)-], 1177 [-O-], 1124 [-CH2-O-CH2-]; ^{1}HNMR (DMSO-d6, ppm, δ): 1.61 [4H,-CH_2-C**H**$_2$-CH_2-NH-], 1.78 [4H,-OC(O)-CH(NH_3^+)C**H**$_2$-$(CH_2)_2$-], 2.29 [6H, **H**$_3$C-Ph-SO_3-], 3.10 [4H, -$(CH_2)_2$-C**H**$_2$-NH-], 3.60-70 [44H, -(O)C-O-CH_2-C**H**$_2$-O-(C**H**$_2$-C**H**$_2$-)$_5$], 4.09 [2H, $^+$$H_3$N-C**H**(R)-C(O)-O-], 4.31 [4H, -(O)C-O-C**H**$_2$-], 7.15, 7.49 [16H, Ph], 7.61 [10H, -CH_2-N**H**(N**H**$_2^+$)-N**H**$_2$], 8.47 [6H, $^+$**H**$_3$N-CH(R)-C(O)-O-];

Synthesis of Polymers

An example of the synthesis of 2-Arg-2E-S via solution polycondensation is given here. Monomers **NSu** (1.0 mmol) and **Arg-2E-S** (1.0 mmol) in 1.5 mL of dry DMSO were mixed well by vortexing. The mixture solution was heated up with stirring to obtain a uniformed solution mixture. Triethylamine (0.31 mL, 2.2 mmol) was added drop by drop to the mixture while heating up to 75 °C with vigorous stirring until a complete dissolution of the monomers. The solution color turned into yellow after several minutes. The reaction vial was then kept for 48 hrs at 75 °C in a thermostat oven without stirring. The 2-Arg-2E-S polymer in the reaction solution was precipitated out by adding cold ethyl acetate, decanted, dried, re-dissolved in methanol and re-precipitated in cold ethyl acetate for further purifications. Repeat the purification for 2 times before drying *in vacuum* at room temperature. The prepared Arg-PEEAs were white solid powder (for EG with number 2, 3 and 4) or transparent/yellow viscous solid (for EG with number 6 and 12). All of them were obtained in high yields (80~90 %).

Characterizations

The prepared monomers and polymers were characterized by various standard methods. For Fourier transform infrared (FTIR) characterization, the samples were ground into powders and mixed with KBr at a sample/KBr ratio of 1:10 (w/w). FTIR spectra were then obtained with a Perkin-Elmer (Madison, WI) Nicolet Magana 560 FTIR spectrometer with Omnic software for data acquisition and analysis. ^{1}H-NMR spectra were recorded with a Varian Unity Inova 400-MHz spectrometer (Palo Alto, CA). Deuterated water (D_2O-*d2*; Cambridge Isotope Laboratories, Andover, MA) with tetramethylsilane as an internal standard or deuterated dimethyl sulfoxide (DMSO-*d6*; Cambridge Isotope Laboratories) was used as the solvent. MestReNova software was used for the data analysis. The solubility of Arg-PEEAs in common organic solvents at room temperature was assessed by using 2.0 mg/mL as a solubility criterion to determine whether Arg-PEEA polymer is soluble or not in a solvent. The quantitative solubility of Arg-PEEAs in distilled water at room temperature was measured by adding distilled water drop by drop until a clear solution was obtained. For electrophoresis assay, the N3014 DNA maker solution without Arg-PEEA was used as a blank control. The N3014 DNA marker was visualized by an UV illumination (FOTO/UV 300 Transilluminator). The migration of DNA from the Arg-PEEA/DNA complex was recorded by a digital camera (Panasonic WV-BP330). For the molecular weight (MW) measurement, polymers were prepared at a concentration of 1.0 mg.mL^{-1} in a 0.1 % (w/v) LiCl in DMAc solution. The sample MWs were determined from a standard curve generated from polystyrene standards with MWs ranging from 841.7 kDa to 2.93 kDa that were chromatographed under the same conditions as the samples. The standard curve was generated from a 3rd order polynomial fit of the polystyrene standard MWs.

Cell Culture

In this report, the following cells were used for tests: cell lines [Bovine aortic endothelial cells (BAEC)], primary cells [Rat smooth muscle cells (RSMC)], Human umbilical vein endothelial cells (HUVEC)] and stem cells (Rat Mesenchymal stem cells (MSC)]. All the cells were grown exactly as the recommended ATCC protocols. The cell lines were used from passages 6 to 12, and primary cells and stem cells were used from passages 2-5. Media were changed every 2 days. Cells were grown to 70 % confluence before splitting, harvesting or

transfection.

DNA preparation

The luciferase encoding reporter plasmids, COL (-772) /LUC and green fluorescence protein encoding reporter plasmid DNA (GFP) were all provided by Dr. Bo Liu's lab at Surgery Department of University of Wisconsin at Madison. All plasmids were prepared using Qiagen endotoxin-free plasmid Maxi kits according to the supplier's protocol.

Transfection Protocol

First, the transfection protocol for Arg-PEEAs was studied and optimized in terms of cell type, cell density, buffer types, transfection time, transfection media, and temperature. After optimization, all transfection experiments were carried out according to the optimized protocol. The details for the optimized transfection protocol for Arg-PEEAs are given below. For cell lines, such as rat aortic SMC A10 cells, the cells were seeded in 0.5 mL complete DMEM (10 % FBS, 1 % HEPES, 1 % penicillin-streptomycin) at 30 x 10^3 per well in a 24-well plate 24 hours before transfection (70 % confluent at transfection). Before transfection, the cell culture media was removed and the cells were washed with PBS buffer twice. Then 1.0 mL warmed serum free DMEM media (without antibiotics) was added into each well. For Lipofectamine2000®, the media was used according to the manufacturer's recommendation. The formulated Arg-PEEA/DNA nanoparticle solution was then added into each well. The plasmid DNA amount was fixed at 1 μg per well for 24-well cell culture plate. The transfection mixtures were immediately and slightly piped up and down for a few seconds, the cells were transected for 4 h at 37 °C (5 % CO_2) in an incubator, and then the media solution was removed. After that, 0.5 mL of complete DMEM (10 % FBS, 1 % HEPES, 1 % penicillin-streptomycin) were added into each well and kept incubated at 37 °C (5 % CO_2) in an incubator. After 48 hours, cells were harvested for luciferase reading. Triplicate results were obtained in each data point. The main differences between transfection of cell lines and primary cells/stem cells were the transfection time and cell culture media. For transfection time: 4 h was needed for cell lines and 12-16 h was needed for primary and stem cells; for cell culture media, the transfection media were the media recommended by ATCC without serum, the media before and after transfection were the media recommended by ATCC.

Luciferase assay protocol

Briefly, cells from each well of a 24-well plate were lysed in 100 μL lysis buffer, transferred to a micro-tube, and then centrifuged at 10,000 g for 2 min. Supernatants were collected and analyzed for luciferase activity. In a typical experiment, 20 μL of supernatant was added to luminometric tubes containing 100 μL of luciferase substrate (Promega). Light emission was measured with a Dual-luciferase detection system for periods of 5 sec, and the relative light units (RLUs) were determined. Triplicate results were used in each experiment. RLUs were normalized to the protein contents of each sample measured by spectrophotometric analysis.

Evaluation of Cytotoxicity of the Arg-PEEA/DNA Nanoparticles

The cytotoxicity evaluation of the Arg-PEEA/DNA nanoparticles was performed by MTT assay. The cultured cells were seeded at an appropriate cell density concentration (3,000 cells/well) in 96-well plates and incubated overnight in a 5 % CO_2 incubator at 37 °C. The cells were, then, treated with various Arg-PEEA/DNA nanoparticle solutions for 4 h or 12 h. The media was removed after that and complete DMEM was then added into each well for 44 h or 36h incubation (total time: 48 h). The cells treated with normal cell culture media only were used as the negative control (NC). PEI and Lipofectamine2000® treated cells (same time as the Arg-PEEA/DNA nanoparticles) were used as the positive controls. After 48 h incubation (total treat time) of the treated cells at 37 °C and 5 % CO_2, 15 μL of MTT solution (5 mg/mL) was added to each well, followed by 4 h incubation at 37 °C, 5 % CO_2. Then the cell culture medium was carefully removed and 150 μL of acidic isopropyl alcohol (with 0.1 M HCl) was added to dissolve the formed formazan crystal. OD was measured at 570 nm (subtract background reading at 690 nm) using a VersaMax Tunable Microplate reader. The cell viability (%) was calculated according to the following equation: Viability (%) = $(OD_{570\ (sample)}-OD_{620\ (sample)})/(OD_{570\ (control)}-OD_{620\ (control)}) \times 100$ %; where the $OD_{570\ (control)}$ represented the measurement from the wells treated with medium only, and the $OD_{570\ (sample)}$ from the wells treated with various Arg-PEEA and Arg-PEEA/plasmid DNA complexes. Thus, the cell viability was expressed as the percentage of the blank negative control. Triplicates were used in each experiment.

Statistics

Where appropriate, the data are presented as mean ± standard deviation calculated over at least three data points. JMP software (version 8.0, from SAS Company) was used for data analysis. Significant differences compared to control groups were evaluated by unpaired Student's t-test or Dunnet test at p 0.05, and between more than two groups by Tukey's test with or without one-way ANOVA analysis of variance.

Table S1 List of prepared *p*-toluenesulfonic acid salt of L-arginine diester monomer (**IIa** and **IIb**) from oligoethylene glycols

Monomer (**IIa** & **IIb**)	y	Naming
Arg-2E-S	2	tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) diesters of diethylene glycol
Arg-3E-S	3	tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) diesters of triethylene glycol
Arg-4E-S	4	tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) diesters of tetraethylene glycol
Arg-6E-S	6	tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) diesters of PEG300
Arg-12E-S	12	tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) diesters of PEG600
Arg-2E-Cl	2	di-*p*- toluenesulfonic acid di-hydrochloride acid salt of bis (L-arginine) diesters of diethylene glycol
Arg-3E-Cl	3	di-*p*- toluenesulfonic acid di-hydrochloride acid salt of bis (L-arginine) diesters of triethylene glycol
Arg-4E-Cl	4	di-*p*- toluenesulfonic acid di-hydrochloride acid salt of bis (L-arginine) diesters of tetraethylene glycol
Arg-6E-Cl	6	di-*p*- toluenesulfonic acid di-hydrochloride acid salt of bis (L-arginine) diesters of PEG300
Arg-12E-Cl	12	di-*p*- toluenesulfonic acid di-hydrochloride acid salt of bis (L-arginine) diesters of PEG600

Table S2 Arg-PEEAs (x-Arg-yEG-z) polymers prepared by different combinations of monomers (**I**) and (**IIa** or **IIb**)

I	**IIa**				
	Arg-2EG-S	**Arg-3EG-S**	**Arg-4EG-S**	**Arg-6EG-S**	**Arg-12EG-S**
NSu	2-Arg-2EG-S	2-Arg-3EG-S	2-Arg-4EG-S	2-Arg-6EG-S	2-Arg-12EG-S
NA	4-Arg-2EG-S	4-Arg-3EG-S	4-Arg-4EG-S	4-Arg-6EG-S	4-Arg-12EG-S
NS	8-Arg-2EG-S	8-Arg-3EG-S	8-Arg-4EG-S	8-Arg-6EG-S	8-Arg-12EG-S
I	**IIb**				
	Arg-2EG-Cl	**Arg-3EG-Cl**	**Arg-4EG-Cl**	**Arg-6EG-Cl**	**Arg-12EG-Cl**
NSu	2-Arg-2EG-Cl	2-Arg-3EG-Cl	2-Arg-4EG-Cl	2-Arg-6EG-Cl	2-Arg-12EG-Cl
NA	4-Arg-2EG-Cl	4-Arg-3EG-Cl	4-Arg-4EG-Cl	4-Arg-6EG-Cl	4-Arg-12EG-Cl
NS	8-Arg-2EG-Cl	8-Arg-3EG-Cl	8-Arg-4EG-Cl	8-Arg-6EG-Cl	8-Arg-12EG-Cl

Table S3. Water solubility at room temperature of Arg-PEEAs (x-Arg-yEG-S and x-Arg-yEG-Cl)

Polymer Name	**2-Arg-2EG-S**	**2-Arg-3EG-S**	**2-Arg-4EG-S**	**2-Arg-6EG-S**	**2-Arg-12EG-S**
Solubility(mg/mL)	200±10	100±10	40±5	40±5	100±10
Polymer Name	**4-Arg-2EG-S**	**4-Arg-3EG-S**	**4-Arg-4EG-S**	**4-Arg-6EG-S**	**4-Arg-12EG-S**
Solubility(mg/mL)	100±10	60±5	15±2	15±2	20±2
Polymer Name	**8-Arg-2EG-S**	**8-Arg-3EG-S**	**8-Arg-4EG-S**	**8-Arg-6EG-S**	**8-Arg-12EG-S**
Solubility(mg/mL)	15±2	10±2	6±1	6±1	10±1
Polymer Name	**2-Arg-2EG-Cl**	**2-Arg-3EG-Cl**	**2-Arg-4EG-Cl**	**2-Arg-6EG-Cl**	**2-Arg-12EG-Cl**
Solubility(mg/mL)	200±10	200±10	80±5	60±5	100±10
Polymer Name	**4-Arg-2EG-Cl**	**4-Arg-3EG-Cl**	**4-Arg-4EG-Cl**	**4-Arg-6EG-Cl**	**4-Arg-12EG-Cl**
Solubility(mg/mL)	150±10	100±10	50±5	30±2	50±5
Polymer Name	**8-Arg-2EG-Cl**	**8-Arg-3EG-Cl**	**8-Arg-4EG-Cl**	**8-Arg-6EG-Cl**	**8-Arg-12EG-Cl**
Solubility(mg/mL)	40±5	30±5	15±2	10±2	15±2

Table S4. Relative transfection efficiency of Arg-PEEAs with Lipofectamine2000® transfection value set at 100.

2-Arg-2EG-S 105	2-Arg-3EG-S 112	2-Arg-4EG-S 85	2-Arg-6EG-S 97	2-Arg-12EG-S 78
4-Arg-2EG-S 21	4-Arg-3EG-S 35	4-Arg-4EG-S 44	4-Arg-6EG-S 41	4-Arg-12EG-S 17
8-Arg-2EG-S 73	8-Arg-3EG-S 115	8-Arg-4EG-S 107	8-Arg-6EG-S 81	8-Arg-12EG-S 69
2-Arg-2EG-Cl 87	2-Arg-3EG-Cl 130	2-Arg-4EG-Cl 91	2-Arg-6EG-Cl 195	2-Arg-12EG-Cl 75
4-Arg-2EG-Cl 31	4-Arg-3EG-Cl 27	4-Arg-4EG-Cl 14	4-Arg-6EG-Cl 39	4-Arg-12EG-Cl 22
8-Arg-2EG-Cl 103	8-Arg-3EG-Cl 98	8-Arg-4EG-Cl 94	8-Arg-6EG-Cl 140	8-Arg-12EG-Cl 75