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SUPPLEMENTARY INFORMATION

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Biodegradable poly(amine-co-ester) terpolymers for targeted gene delivery

Materials.

ε-Caprolactone (CL, 99%), 12-dodecanolide (DDL, 98%), ω-pentadecalactone (PDL, 98%), 16-hexadecanolide (HDL, 97%), diethyl sebacate (DES, 98%), *N*-methyldiethanolamine (MDEA, 99+%), *N*-phenyldiethanolamine (PDEA, 97%), and diphenyl ether (99%) were purchased from Aldrich Chemical Co. and were used as received. Immobilized *Candida antarctica* lipase B (CALB) supported on acrylic resin or Novozym 435, chloroform (HPLC grade), dichloromethane (99+%), hexane (97+%), methanol (98%), and chloroform-*d* were also obtained from Aldrich Chemical Co. The lipase catalyst was dried at 50 °C under 2.0 mmHg for 20 h prior to use.

Instrumental Methods.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 500 spectrometer. The chemical shifts reported were referenced to internal tetramethylsilane (0.00 ppm) or to the solvent resonance at the appropriate frequency. The number and weight average molecular weights (M_n and M_w, respectively) of polymers were measured by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a model 1515 isocratic pump, a 717 plus autosampler, and a 2414 refractive index (RI) detector with Waters Styragel columns HT6E and HT2 in series. Empower II GPC software was used for running the GPC instrument and for calculations. Both the Styragel columns and the RI detector were heated and maintained at 40 °C temperature during sample analysis. Chloroform was used as the eluent at a

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flow rate of 1.0 mL/min. Sample concentrations of 2 mg/mL and injection volumes of 100 µL were used. Polymer molecular weights were determined based on a conventional calibration curve generated by narrow polydispersity polystyrene standards from Aldrich Chemical Co.

Synthesis and Purification of Lactone-DES-MDEA Terpolymers.

General procedures for CALB-catalyzed terpolymerization of lactone with DES and MDEA and the procedures for isolation and purification of the formed terpolymer products are described in the text of the paper. The ^{1}H and ^{13}C NMR resonance absorptions of the polymers are shown below. Table S1 summarizes the yield, composition, molecular weight (M_w), polydispersity (M_w/M_n), and nitrogen content of all purified Lactone-DES-MDEA terpolymers.

CL-DES-MDEA terpolymer (I): ^{1}H NMR ($CDCl_3$; ppm) 1.29 (br.), 1.34-1.39 (m), 1.60 (br.), 2.25-2.30 (m), 2.32 (s), 2.67 (t), 4.04 (t), 4.15 (t); ^{13}C NMR ($CDCl_3$; ppm) 24.48, 24.53, 24.84, 24.89, 25.46, 25.48, 28.32, 29.02-29.06 (m), 33.97, 34.02, 34.15, 34.20, 42.83, 55.91, 61.91, 61.97, 63.92, 64.01, 173.21, 173.31, 173.50, 173.60, plus a small absorption at 14.22 ppm.

DDL-DES-MDEA terpolymer (II): ^{1}H NMR ($CDCl_3$; ppm) 1.27-1.29 (br.), 1.61 (m, br.), 2.26-2.31 (m), 2.34 (s), 2.69 (t), 4.05 (t), 4.16 (t); ^{13}C NMR ($CDCl_3$; ppm) 24.87, 24.91, 24.95, 25.00, 25.93, 28.66, 29.06, 29.08, 29.14, 29.25, 29.27, 29.43, 29.50, 34.21, 34.24, 34.31, 34.35, 42.88, 55.95, 61.95, 64.35, 173.63, 173.68, 173.81, 173.86, plus a small absorption at 14.25 ppm.

PDL-DES-MDEA terpolymer (III): ^{1}H NMR ($CDCl_3$; ppm) 1.26-1.29 (br.), 1.61 (m, br.), 2.26-2.32 (m), 2.34 (s), 2.69 (t), 4.05 (t), 4.16 (t), plus a small absorption (triplet) at 3.57 ppm due to $–CH_2CH_2OH$ end groups; ^{13}C NMR ($CDCl_3$; ppm) 24.84, 24.90, 24.92, 24.99, 25.92, 28.65, 29.03, 29.07, 29.12, 29.25, 29.28, 29.47, 29.53, 29.58-29.63 (m), 34.13, 34.18, 34.23,

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Table S1. Characterization of Purified Lactone-DES-MDEA Terpolymers

Name[a]	Lactone/DES/MDEA (Feed Molar Ratio)	Lactone/Sebacate/MDEA (Unit Molar Ratio)[b]	Isolated Yield (%)	M_w[c]	M_w/M_n[c]	Nitrogen Content (wt%)
PMSC	0:50:50	0:50:50	-	31800	2.3	4.9
I-10%CL	10:90:90	10:90:90	85	18400	1.9	4.7
I-20%CL	20:80:80	20:80:80	80	19100	1.9	4.5
I-40%CL	40:60:60	40:60:60	83	18400	1.8	3.9
I-60%CL	60:40:40	60:40:40	81	17800	1.8	3.1
I-80%CL	80:20:20	80:20:20	86	20300	2.0	1.9
II-10%DDL	10:90:90	10:90:90	82	24900	1.9	4.6
II-20%DDL	20:80:80	20:80:80	80	29300	2.0	4.2
II-40%DDL	40:60:60	40:60:60	81	25800	1.8	3.4
II-60%DDL	60:40:40	60:40:40	84	47400	2.1	2.4
II-80%DDL	80:20:20	80:20:20	87	40600	2.1	1.3
III-10%PDL	10:90:90	10:90:90	81	30700	2.1	4.5
III-20%PDL	20:80:80	20:80:80	83	38700	2.3	4.1
III-40%PDL	40:60:60	40:60:60	85	33300	2.1	3.1
III-61%PDL	60:40:40	61:39:39	83	34500	2.3	2.1
III-82%PDL	80:20:20	82:18:18	88	41700	2.7	1.0
IV-10%HDL	10:90:90	10:90:90	80	25700	1.8	4.5
IV-20%HDL	20:80:80	20:80:80	81	26600	1.9	4.0
IV-40%HDL	40:60:60	40:60:60	83	31200	2.2	3.1
IV-61%HDL	60:40:40	61:39:39	86	37400	2.2	2.0
IV-80%HDL	80:20:20	80:20:20	89	59000	2.1	1.1

a. The polymer names are abbreviated or simplified. PMSC: poly(*N*-methyldiethyleneamine sebacate). Polymers I, II, III, and IV represent CL-DES-MDEA, DDL-DES-MDEA, PDL-DES-MDEA, and HDL-DES-MDEA terpolymers, respectively. Each polymer is denoted with x% lactone indicating the lactone unit content [mol% vs. (lactone + sebacate) units] in the polymer.

b. Measured by ^{1}H NMR spectroscopy.

c. Measured by GPC using narrow polydispersity polystyrene standards.

DOI: 10.1038/NMAT3187

34.28, 42.77, 55.87, 61.86, 64.27, 173.44, 173.50, 173.62, 173.68, plus two small absorptions at 14.22 and 61.58 ppm due to –CO–O*C*H$_2$*C*H$_3$ terminal groups.

HDL-DES-MDEA terpolymer (IV): ^{1}H NMR ($CDCl_3$; ppm) 1.26-1.29 (br.), 1.60 (m, br.), 2.25-2.31 (m), 2.32 (s), 2.68 (t), 4.05 (t), 4.15 (t); ^{13}C NMR ($CDCl_3$; ppm) 24.86, 24.91, 24.94, 25.00, 25.92, 28.65, 29.05, 29.07, 29.14, 29.25, 29.28, 29.47, 29.53, 29.59-29.65 (m), 34.18, 34.23, 34.28, 34.34, 42.86, 55.94, 61.94, 64.33, 173.57, 173.64, 173.75, 173.82, plus a small absorption at 14.24 ppm.

Synthesis of Purified PDL-DES-MDEA-PDEA Copolymer

A reaction mixture containing PDL/DES/MDEA/PDEA monomers in a molar ratio of 1:9:7.2:1.8, Novozym 435 catalyst (10 wt% vs. total monomer), and diphenyl ether (200 wt% vs. total monomer) was stirred at 90 ºC under 1 atmosphere of nitrogen for 19 h. Subsequently, the reaction pressure was reduced to 1.2 mmHg and the reaction was continued at 90 ºC for additional 72 h. The resultant, liquid PDL-DES-MDEA-PDEA copolymer was purified according to a procedure similar to that used for purification of PDL-DES-MDEA terpolymer III-10% PDL as described above. This copolymer consists of four repeat units: PDL, sebacate, *N*-methyldiethyleneamine, *N*-phenyldiethyleneamine; and its composition was determined by ^{1}H NMR spectroscopy.

PDL-DES-MDEA-PDEA copolymer: yield = 87%; 10 mol% PDL vs. (PDL+sebacate); 20 mol% PDEA vs. (MDEA+PDEA); M_w = 243000; M_w/M_n = 1.8; ^{1}H NMR ($CDCl_3$; ppm): 1.26-1.29 (br.), 1.61 (m, br.), 2.25-2.31 (m), 2.33 (s), 2.68 (t), 3.58 (t, −CH_2−CH_2−N(Ph)

−C*H*$_2$−CH$_2$−), 4.05 (t), 4.15 (t), 4.22 (t, −C*H*$_2$−CH$_2$−N(Ph)−CH$_2$−C*H*$_2$−), 6.69 (t), 6.75 (d), 7.20 (t).

Study on Polymer Chain Growth during Lactone-DES-MDEA Terpolymerization

Terpolymerization of PDL with DES and MDEA was selected as a typical example for polymer chain growth study. The reaction mixture contained 2:3:3 (molar ratio) PDL/DES/MDEA comonomers, Novozym 435 catalyst (10 wt% vs. total monomer), and diphenyl ether solvent (200 wt% vs. total monomer). The copolymerization reactions were carried out at 60, 70, 80, and 90 ºC temperatures in two stages: first stage oligomerization under 1 atmosphere pressure of nitrogen for 19 h, followed by second stage polymerization under 1.4 mm Hg vacuum for 72 h. To monitor the polymer chain growth, aliquots were withdrawn at various time intervals during the second stage polymerization. The formed polymers were then dissolved in HPLC-grade chloroform and filtered to remove the enzyme catalyst. Polymer products were not fractionated by precipitation prior to analysis of molecular weight. The filtrates containing whole products were analyzed by GPC using narrow polydispersity polystyrene standards to measure polymer molecular weights. Figure S1(A) shows the changes in polymer molecular weight (M_w) vs. polymerization time for the copolymerization at different temperatures. For all reactions, continuous chain growth was observed during the 72 hour polymerization period. For example, at 4, 21, 31, 47, 55, 72 h, the products formed at 90 ºC had M_w values of 12700, 19300, 21100, 26100, 30200, and 39700, respectively. Among these reactions, the copolymer molecular weight at a given reaction time was found to increase with increasing reaction temperature from 60 to 90 ºC. Thus, at 72 h, the resultant copolymers of the reactions at 60, 70, 80, and 90 ºC possessed M_w values of 13300, 19200, 32300, and 39700,

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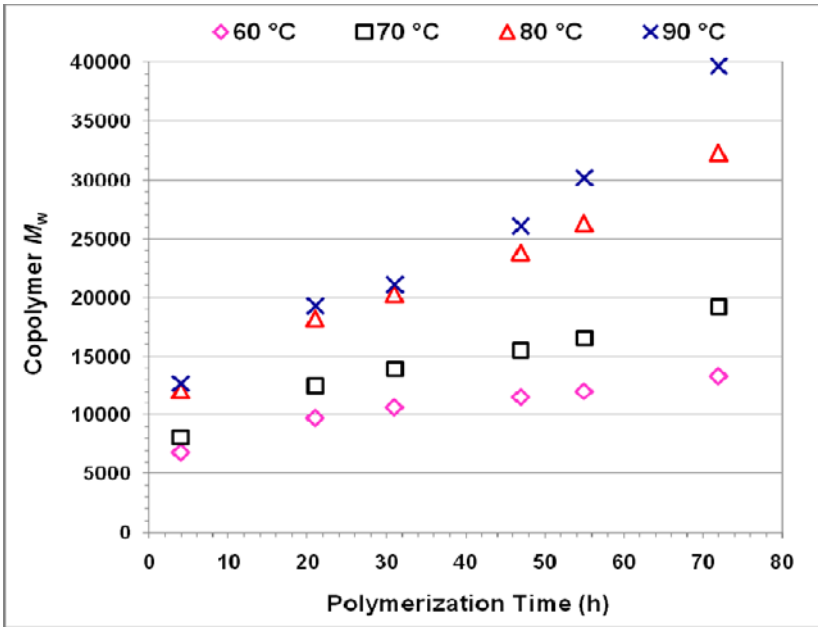
correspondingly. These results indicate that the molecular weight of the PDL-DES-MDEA terpolymers could be readily controlled by adjusting the reaction time and/or reaction temperature. The product polydispersity (M_w/M_n) vs. molecular weight (M_w) for the copolymerization reactions is delineated in Figure S1(B). The polydispersity values of all products follow a similar trend, which changed from 1.5 to 1.7 with increasing polymer molecular weight (M_w) from 6800 to 19000, and remained fairly constant at 1.8 in the molecular weight range between 19000 and 40000. Furthermore, NMR analysis showed that during the copolymerization reactions, byproduct ethanol was formed and condensed in the dry ice trap between the reactors and vacuum pump.

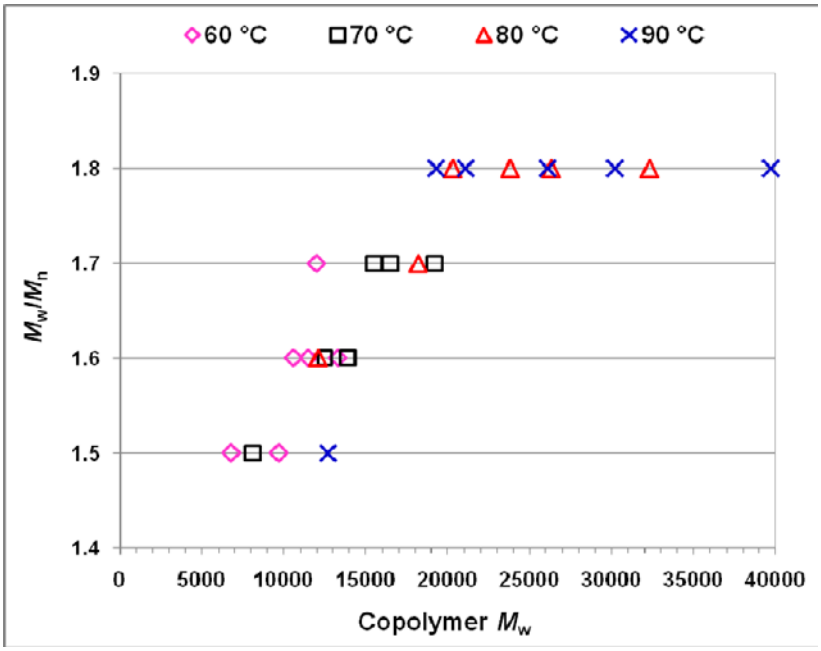
To determine whether the polymerization reactions were indeed catalyzed by CALB, control experiments were performed without the lipase. The control reaction was carried out at 90 ºC in diphenyl ether under identical conditions (stage 1: 2:3:3 PDL/DES/MDEA monomer ratio, under 1 atmosphere pressure of nitrogen for 19 h; stage 2: 1.4 mmHg for 72 h). GPC analysis showed that the product had a M_w of less than 800. This demonstrates that CALB catalyzes lactone-DES-MDEA terpolymerization.

Structural Characterization of Lactone-DES-MDEA Terpolymers

The structure and composition of the lactone-DES-MDEA terpolymers were determined by ^{1}H and ^{13}C NMR spectroscopy. NMR resonance absorptions were assigned by comparing signals of the terpolymers to those of reference polymers, poly(lactone) homopolymers[S1-S3] and poly(*N*-methyldiethyleneamine sebacate) (PMSC)[S4], and by observing changes in signal



(A)



(B)

Figure S1. Variations of product molecular weight and polydispersity during the copolymerization of PDL with DES and MDEA (polymerization conditions: 2:3:3 PDL/DES/MDEA, 1.4 mmHg pressure)

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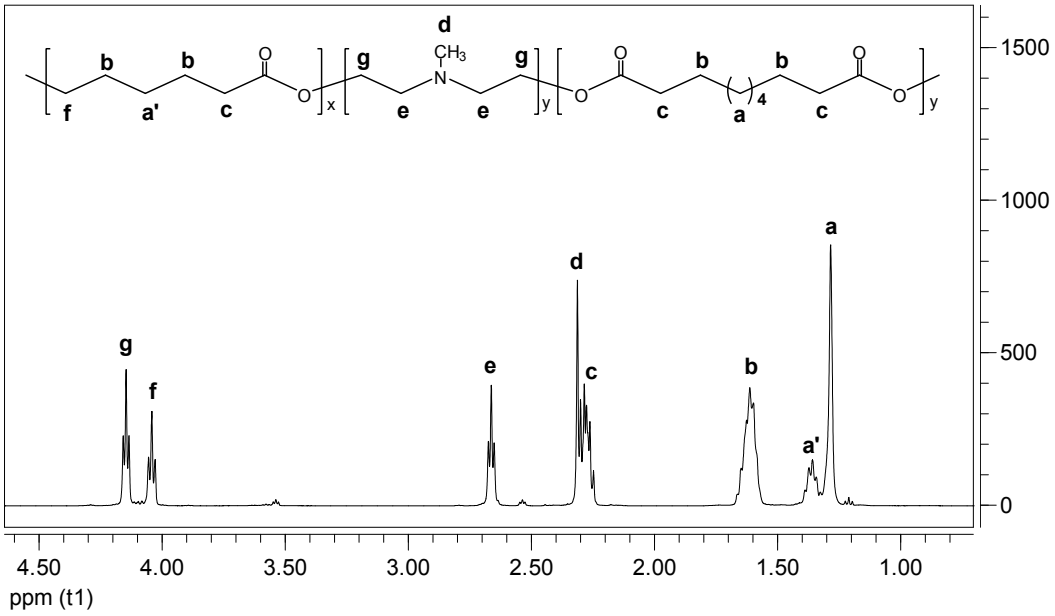
intensities among the terpolymers synthesized from various lactone/DES/MDEA monomer feed ratios.

The proton NMR spectra of the lactone-DES-MDEA terpolymers showed that the copolymers contained three different types of repeating units: lactone, *N*-methyldiethyleneamine, and sebacate. The structural assignments of the ^{1}H resonance absorptions for the terpolymers I, II, III, and IV are depicted in Figure S2. The molar ratios of lactone to *N*-methyldiethyleneamine to sebacate units in the terpolymers were calculated from proton resonance absorptions: number of lactone units from methylene absorption at 4.05 (± 0.01) ppm, number of *N*-methyldiethyleneamine units from absorptions at 4.15 (± 0.01) or 2.68 (± 0.01) ppm, and number of sebacate units from absorption at 1.60 (± 0.01) ppm after subtracting contribution from lactone units.

The above structural assignments for the terpolymers I-IV were further supported by the ^{13}C NMR spectra of the polymers. All terpolymers exhibited four ester carbonyl resonance absorptions at 173.2-173.9 ppm due to two diads of lactone unit and two diads of sebacate unit. For terpolymers II, III, and IV that contain large ($\geq C_{12}$) lactone units, the four resonance peaks with decreasing chemical shift are attributable to lactone*-lactone, sebacate*-lactone, lactone*-MDEA, and sebacate*-MDEA diads, respectively. For CL-DES-MDEA terpolymer (or terpolymer I), the carbon-13 absorbances at 173.60, 173.50, 173.31, and 173.21 ppm are ascribable to CL*-MDEA, sebacate*-MDEA, CL*-CL, and sebacate*-CL diads, correspondingly. For typical examples, Figure S3 shows the carbonyl resonances of terpolymers III-40% PDL, III-82% PDL, I-40% CL, and I-80% CL. Furthermore, for terpolymers II, III, and



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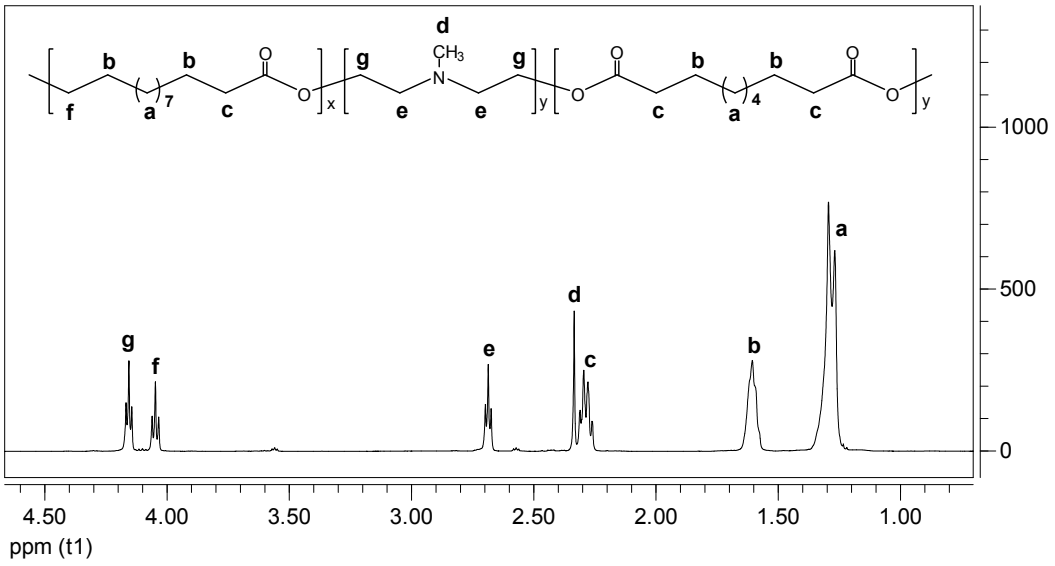

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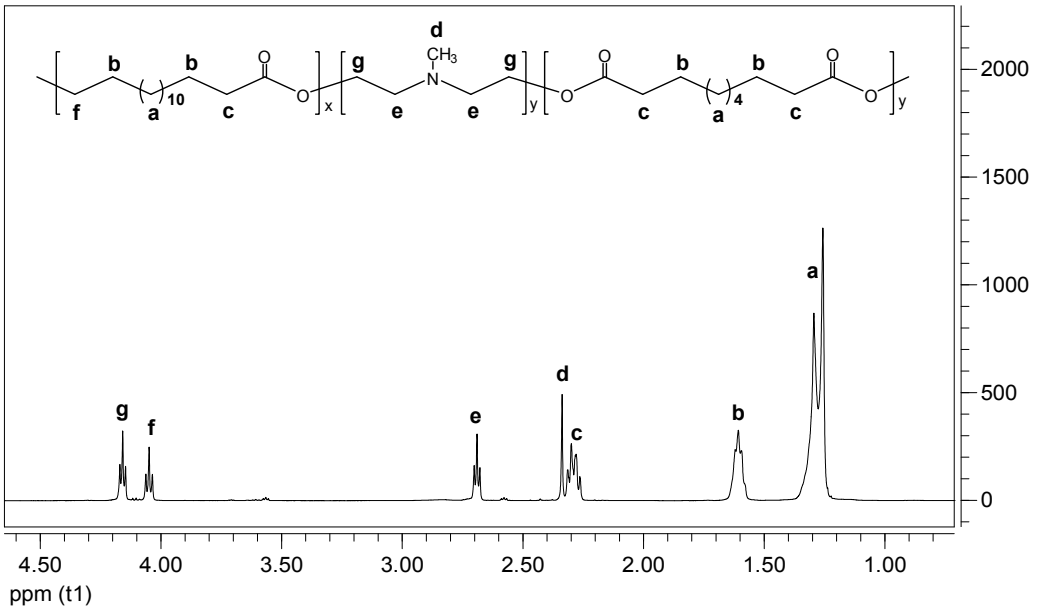

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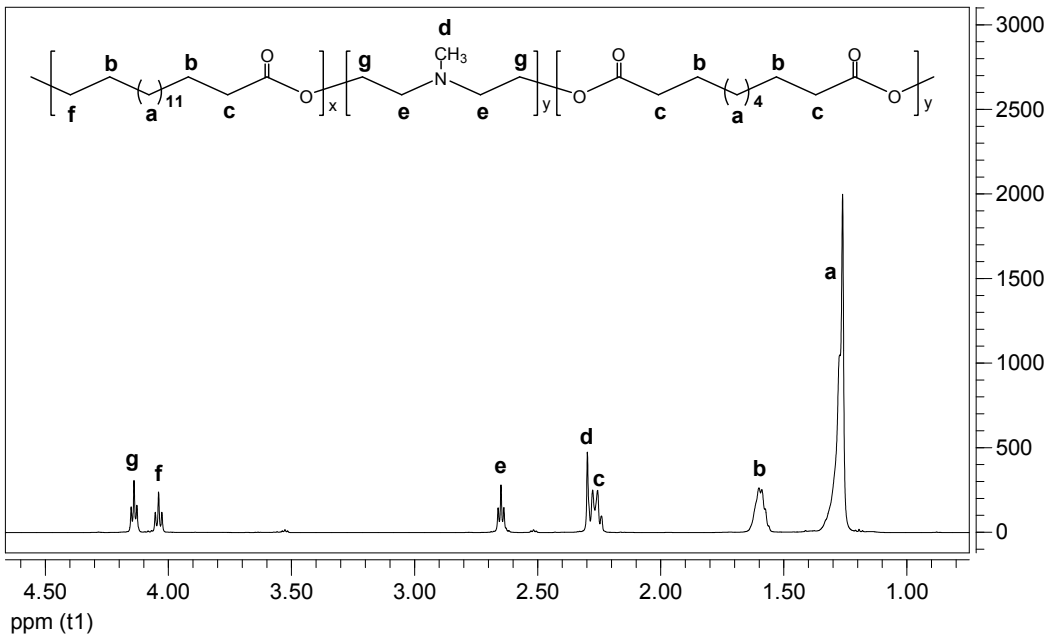

(D)

Figure S2. ^{1}H NMR spectrum of (A) I-60% CL, (B) II-60% DDL, (C) III-61% PDL, and (D) IV-61% HDL (solvent: $CDCl_3$).

IV, the –CH_2O– group of the lactone units and the –CH_2O– group of the MDEA units resonated at 64.3 (± 0.05) and 61.9 (± 0.05) ppm, respectively. It was found that increasing lactone content in the polymers resulted in higher resonance intensity at 64.3 ppm, but lower absorbance intensity at 61.9 ppm. However, terpolymer I showed four resonance absorptions of the –CH_2O– groups at 64.01, 63.92, 61.97, and 61.91 ppm, which are attributable to CL*-CL, CL*-sebacate, MDEA*-CL, and MDEA*-sebacate diads, correspondingly. In support of this structural assignment, it was observed that increasing CL content in terpolymer I increases the absorption intensity at 64.01 ppm, but decreases the absorption intensity at 61.91 ppm. On the other hand, the intensities of the two resonances at 63.92 and 61.97 ppm were comparable regardless of the polymer composition. For all four terpolymers, the resonance absorptions of the methyl and methylene groups adjacent to the nitrogen in MDEA units appeared at 42.8 (± 0.08) and 55.9 (± 0.05) ppm, respectively.

To determine the repeat unit sequence distributions in the terpolymers, the abundance of lactone*-lactone (L*-L), lactone-MDEA (L*-M), sebacate*-lactone (S*-L), and sebacate*-MDEA (S*-M) diads in the polymers was measured by ^{13}C NMR spectroscopy (see Figure S3 for typical carbon-13 resonance absorptions of the diads), and the obtained experimental values were then compared to theoretical diad distribution values calculated for random copolymers with same compositions. For a completely random lactone-DES-MDEA terpolymer, distributions of L*-L, L*-M, S*-L, and S*-M diads can be calculated by the following equations:

DOI: 10.1038/NMAT3187

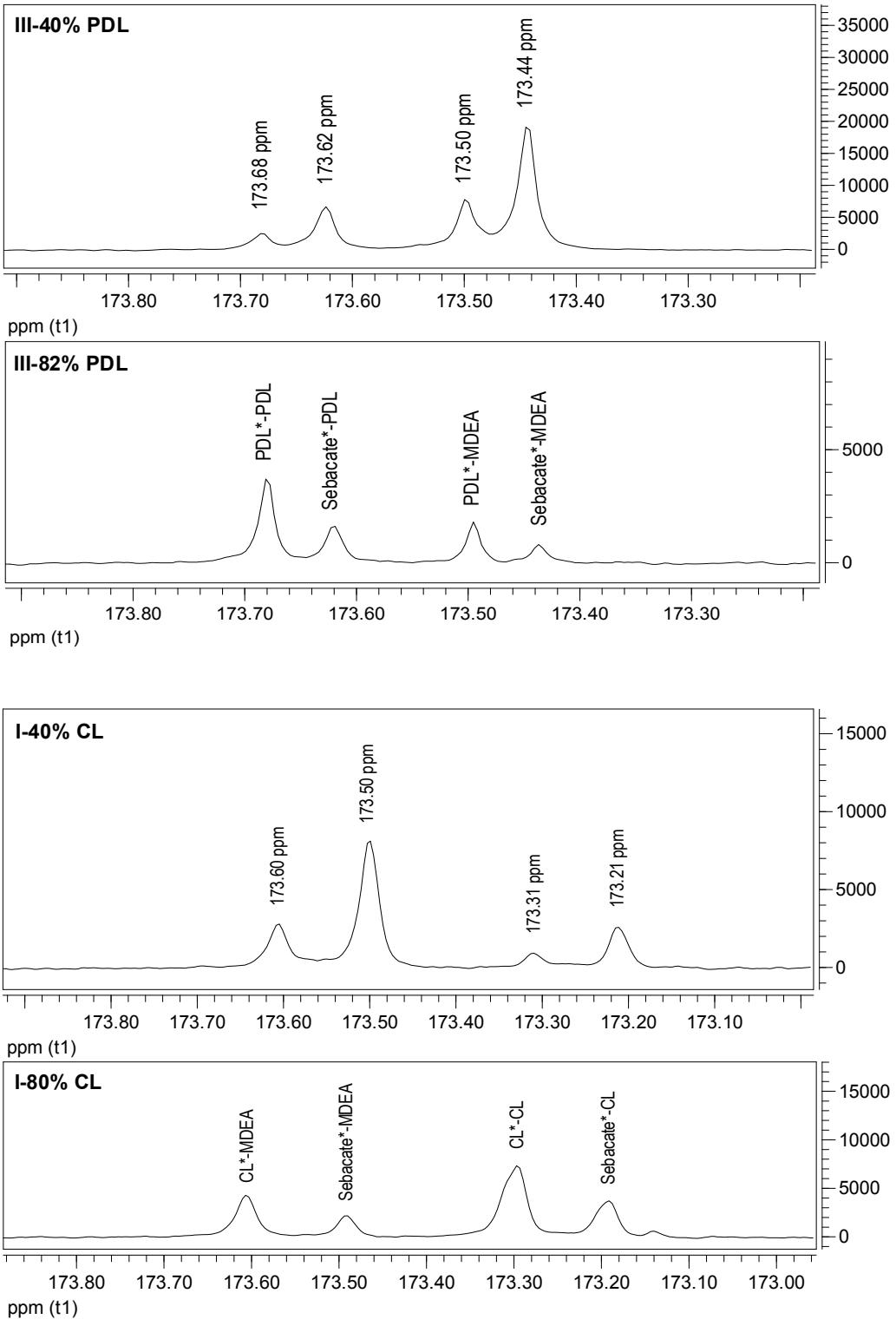

Figure S3. The carbonyl carbon-13 resonance absorptions of III-40% PDL, III-82% PDL, I-40% CL, and I-80% CL. (solvent: $CDCl_3$)

$$\text{L*-L distribution} = f_L \times f_L$$

$$\text{L*-M distribution} = f_L \times (2f_M)$$

$$\text{S*-L distribution} = (2f_S) \times f_L$$

$$\text{S*-M distribution} = (2f_S) \times (2f_M)$$

Where f_L, f_S, f_M are correspondingly molar fractions of L, S, and M repeating units in the terpolymers. It needs to be noted that in the above formulae, molar fractions of S and M units are doubled because both ends of the units can form an ester linkage of a diad, while only one end of L units can serve the purpose. Table S2 summarizes the measured diad distributions of terpolymers I, II, III, and IV with different compositions, as well as the values calculated for random copolymers. For all copolymers studied, the experimental values match remarkably well with the calculated values. Thus, the lactone-DES-MDEA terpolymers synthesized using CALB catalyst contain lactone, sebacate, and *N*-methyldiethyleneamine repeat units randomly distributed in the polymer chains, and can be described as poly(lactone-*co*-*N*-methyldiethylenamine-*co*-sebacate). These results are consistent with previous reports showing that enzymatic PDL-diethyl succinate-1,4-butanediol terpolymers also possessed random chain structures.[S5,S6]

DOI: 10.1038/NMAT3187

Table S2. Diad Distributions of Lactone-DES-MDEA Terpolymers: Comparison between Experimental Values and Theoretical Values Calculated for Random Copolymers

Polymer [a]	L/S/M [b] (unit ratio)	L*-L		L*-M		S*-L		S*-M	
		meas [c]	calc [d]	meas [c]	calc [d]	meas [c]	calc [d]	meas [c]	calc [d]
I-20% CL	20:80:80	0.01	0.01	0.10	0.10	0.10	0.10	0.80	0.79
I-40% CL	40:60:60	0.06	0.06	0.19	0.19	0.19	0.19	0.56	0.56
I-60% CL	60:40:40	0.18	0.18	0.25	0.25	0.25	0.25	0.33	0.33
I-80% CL	80:20:20	0.45	0.45	0.22	0.22	0.22	0.22	0.11	0.11
II-20% DDL	20:80:80	0.02	0.01	0.11	0.10	0.11	0.10	0.76	0.79
II-40% DDL	40:60:60	0.06	0.06	0.20	0.19	0.20	0.19	0.54	0.56
II-60% DDL	60:40:40	0.18	0.18	0.25	0.25	0.25	0.25	0.32	0.33
II-80% DDL	80:20:20	0.46	0.45	0.22	0.22	0.22	0.22	0.10	0.11
III-40% PDL	40:60:60	0.06	0.06	0.18	0.19	0.20	0.19	0.56	0.56
III-82% PDL	82:18:18	0.48	0.48	0.22	0.21	0.20	0.21	0.10	0.09
IV-20% HDL	20:80:80	0.01	0.01	0.10	0.10	0.11	0.10	0.78	0.79
IV-40% HDL	40:60:60	0.06	0.06	0.19	0.19	0.20	0.19	0.55	0.56
IV-61% HDL	61:39:39	0.18	0.19	0.25	0.25	0.25	0.25	0.32	0.32
IV-80% HDL	80:20:20	0.45	0.44	0.23	0.22	0.23	0.22	0.09	0.11

a. See Table S1 for meanings of the polymer abbreviations.
b. Unit abbreviations: L for lactone; S for sebacate; M for *N*-methyldiethyleneamine.
c. Measured from ^{13}C NMR spectra.
d. Calculated for a copolymer with statistically random unit distribution in the polymer chains.

In vitro transfection and characterization

Human embryonic kidney 293 (HEK293) cell line and lung cancer cell line A549 were obtained from ATCC (American Type Culture Collection). Cells were grown in DMEM medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) in a 37 ºC incubator containing 5% CO_2. For *in vitro* transfection, DNA polyplexes with weight ratio of 100:1 were used unless otherwise noted. Polymers were dissolved in DMSO at 25 mg/ml. For preparing DNA polyplexes for transfection in 24 well plates, 4 μl of polymer solution (25 mg/ml in DMSO) was first diluted in 50 μl sodium acetate buffer (25 mM, pH = 5.2). After brief vortexing, the polymer solution was mixed with the same volume of a DNA solution containing 1 μg DNA and vortexed for additional 10 seconds. The polymer/DNA mixture was incubated at room temperature for 10 min and then added to cells, which were seeded in 24-well plates at density of 75,000 cells/well in 500 μl of medium one night before transfection. Transfection using Lipofectamine 2000 (Invitrogen Corp.) was performed using the procedures provided by the manufacturer. PEI transfection was performed using the standard protocol by keeping the weight ratio of PEI to DNA at 3. The same amount of DNA was used in experiments comparing lactone-DES-MDEA terpolymer with Lipofectamine 2000 and PEI. For luciferase gene transfection, plasmid DNA expression luciferase, pGL4.13 (Promega) was used. Two days after transfection, the culture medium was removed and the cells were washed with cold PBS. Two hundred micro-liter Report Lysis Buffer (Promega) was added to each well. With a freeze-thaw cycle, cell lysate was collected. After a quick spin, 20 μl was subjected to luciferase assay using Luciferase Assay Reagent according to the standard protocol described in manufacturer manual (Promega). Additional 25 μl was used to quantify protein

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content using Pierce BCA protein assay kit (Pierce, Thermo Scientific). Luciferase signal was divided by the amount of total protein for comparison. Internal controls were used for normalization for group by group comparison. For experiments to detect cytotoxicity due to TRAIL, plasmid pEGFP-TRAIL[40] (Addgene) was used. Control plasmid, pEGFP, was obtained by removing TRAIL gene from pEGFP-TRAIL. Cell proliferation was determined by a standard MTT assay five days after transfection. The particle size and zeta potential of freshly prepared polyplexes were measured by ZetaPals dynamic light scattering (Brookhaven Instruments Corp). The morphology of polyplexes, which was stained with uranyl acetate, was visualized using FEI Tencai Biotwin TEM at 80Kv. Images were taken using Morada CCD and iTEM (Olympus) software.

Preparation of coated polyplexes for *in vivo* evaluations

To prepare polyplexes for *in vivo* gene delivery, 40 µl polymer solution in DMSO (50 mg/ml) was diluted to 40 µl NaAc buffer. After brief vortexing, the polymer solution was mixed with 80 µl NaAc buffer containing 0.25 mg/ml plasmid DNA, followed by a vigorous vortex for 10 second. Coating polyplexes with peptide polyE-mRGD was conducted 10 min after incubation at room temperature, by adding 40 µl buffer containing peptide at 2.5 mg/ml and allowing further incubation for 5 min. Peptide polyE-mRGD (EEEEEEEEEEEEEEE–GGGGGG–RGDK) was synthesized at the W.M. Keck Facility at Yale University. Immediately before injection, another 40 µl buffer containing 30% glucose was added. Two hundred microliter of the resulted mixture was then injected through tail vein of each mouse.

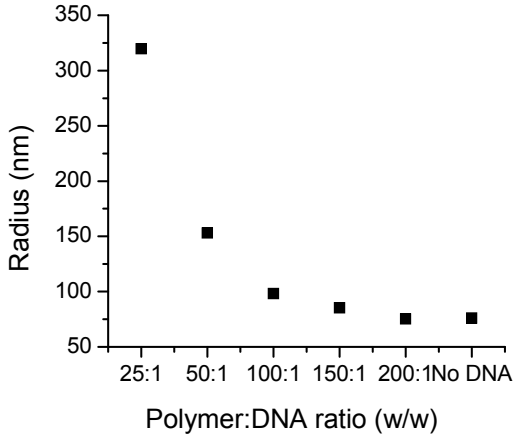

Figure S4. Size of III-20% PDL/DNA complexes with different polymer-to-DNA ratios. Polyplexes were formed by methods described in main text. Size was determined by Zetasizer (Malvern).

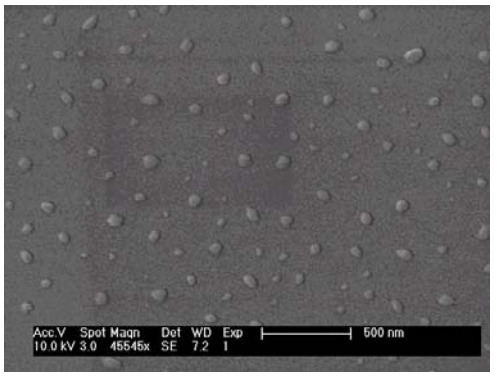

Figure S5. Visualization of III-20% PDL/DNA polyplexes at 100:1 weight ratio using a XL30 ESEM scanning electron microscope (FEI Company). Scale bar represents 500 nm. The polyplex nanoparticles were placed on a round cover glass mounted on an aluminum stub using carbon adhesive tape. After drying at room temperature, the stub was sputter-coated with a mixture of gold and palladium (60:40) under low pressure of argon using a Dynavac Mini Coater and subjected to SEM analysis.

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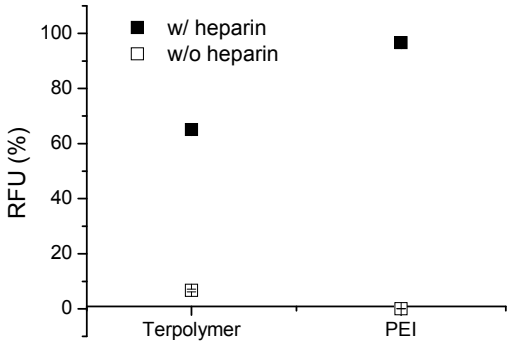

Figure S6. Release of DNA from III-20% PDL/DNA complexes and PEI/DNA complexes by heparin. Polyplexes were formed by methods described in main text. Heparin was added to polyplexes solution with a final concentration of 2% w/v. The values are expressed as a percentage of the fluorescence obtained with naked DNA. Each value is the mean of triplicates.

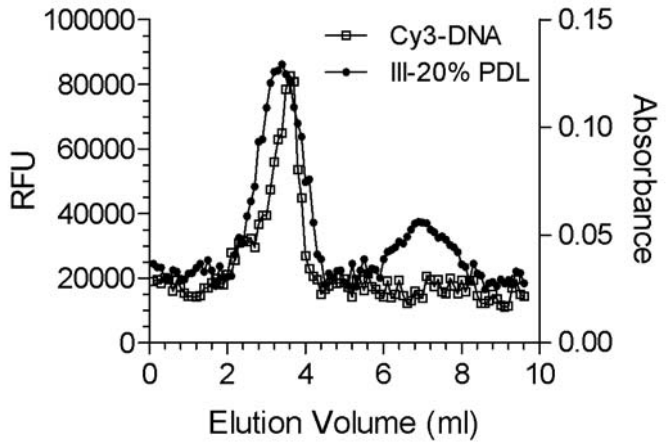

Figure S7. Size exclusion chromatography to determine the amount of III-20% PDL polymer associated with or without DNA when mixed with DNA at a 100:1 weight ratio. Size exclusion chromatography was performed using a Sepharose CL-2B column (14.5 x 50 mm, 8.3 ml column volume) at a flow rate of 0.4 ml/min. Twenty percentage of N-methyldiethyleneamine in III-20% PDL polymer was replaced with N-phenyldiethyleneamine, which allows sensitive detection of polymer based on UV absorption. Plasmid DNA was labeled with Cy3 Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Mirus Bio LLC) following manufacturer's protocol. Columns were pre-conditioned with 100 µg free III-20% PDL to prevent non-specific interactions between the polymer and column. After column equilibriation, fractions (0.1 ml) were collected using a phosphate buffered saline (pH 7.4) elution buffer. Elution samples were analyzed by absorbance at 300 nm and spectrofluorescence (ex/em: 550/570). Elution fractions were monitored for both III-20% PDL polymer (●) and Cy3-DNA (□) content after loading III-20% PDL/Cy3-DNA polyplexes on a Sepharose CL-2B column. Shown are representative data from three separate experiments. Amount of DNA-associated polymer was determined by area under curve analysis.

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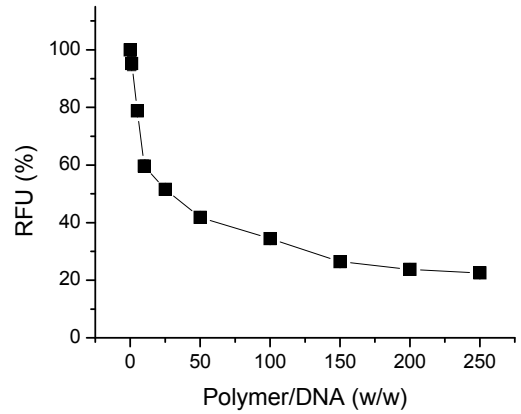

Figure S8. Ethidium bromide exclusion assay using III-20% PDL. The values are expressed as a percentage of the fluorescence relative to the initial fluorescence without polymer.

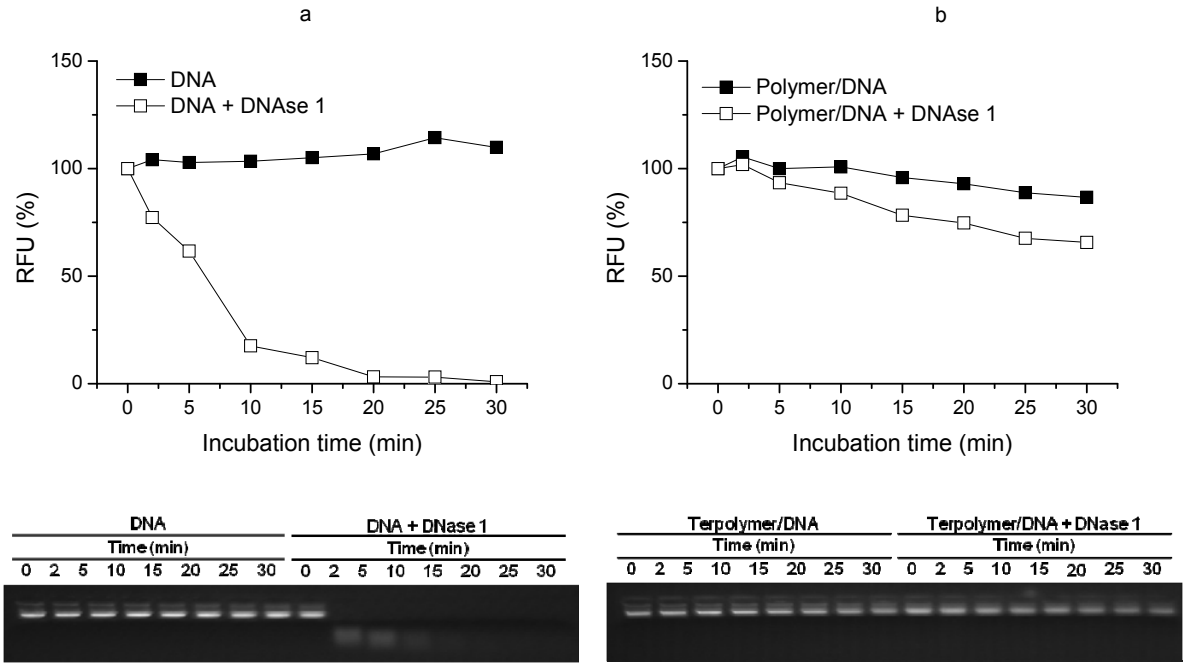

Figure S9. Protection of DNA from enzymatic degradation by III-20% PDL. Naked DNA (a) or III-20% PDL/DNA complexes (b) were subjected to DNase degradation at 37°C for indicated time. Residual DNA was quantified used PicoGreen (Invitrogen). The values are expressed as a percentage of the fluorescence obtained at time 0 min. In the bottom panels, below each graph, the corresponding agarose gel electrophoresis is shown.

a. b.

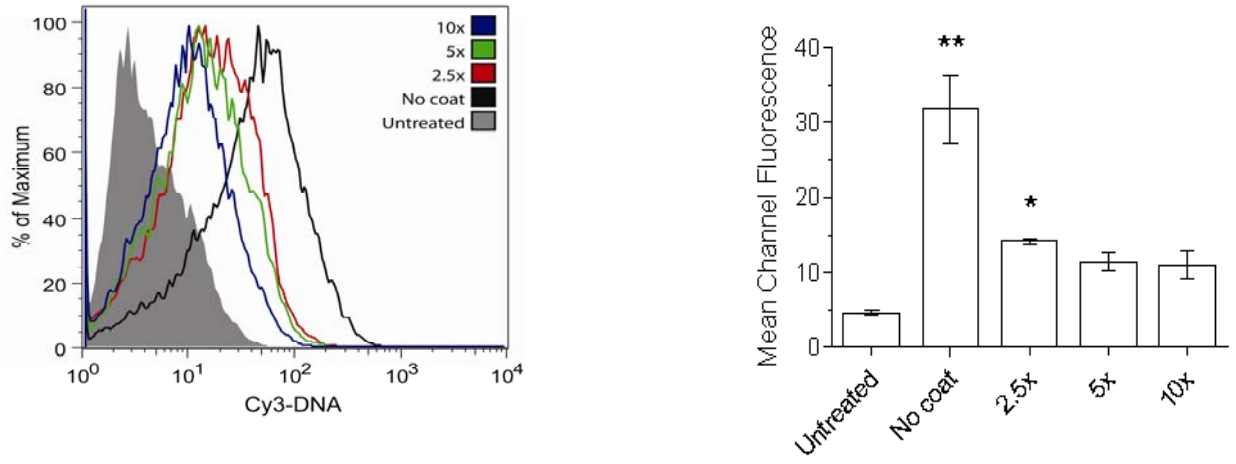

Figure S10. Titration of III-20% PDL/Cy3-DNA polyplexes with peptide polyE-mRGD decreased cellular uptake. a. Cell internalization of III-20% PDL/Cy3-DNA polyplexes was monitored using flow cytometry. HEK293 cells were incubated with III-20% PDL/Cy3-DNA polyplexes using the same conditions as the transfection procedure. After incubating for 4 h at either 37°C, cells were washed with PBS and harvested using enzyme-free cell dissociation buffer (Gibco). Cells were washed with 1% BSA in PBS and then analyzed on a BD Biosciences FACScan. b. Quantification of cell association with coated and uncoated polyplexes. HEK293 cells were incubated for 4 hours with III-20% PDL/Cy3-DNA polyplexes coated with various polyE-mRGD densities. n = 3, **p < 0.005, *p<0.05 using student's t-test with respect to polyplex with 10x coat.

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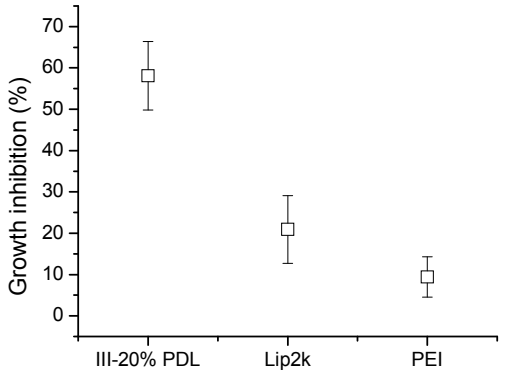

Figure S11. Toxicity of III-20% PDL/TRAIL, Lip2k/TRAIL and PEI/TRAIL complexes on A549 tumor cells. Toxicity was determined five days after treatment by standard MTT assay.

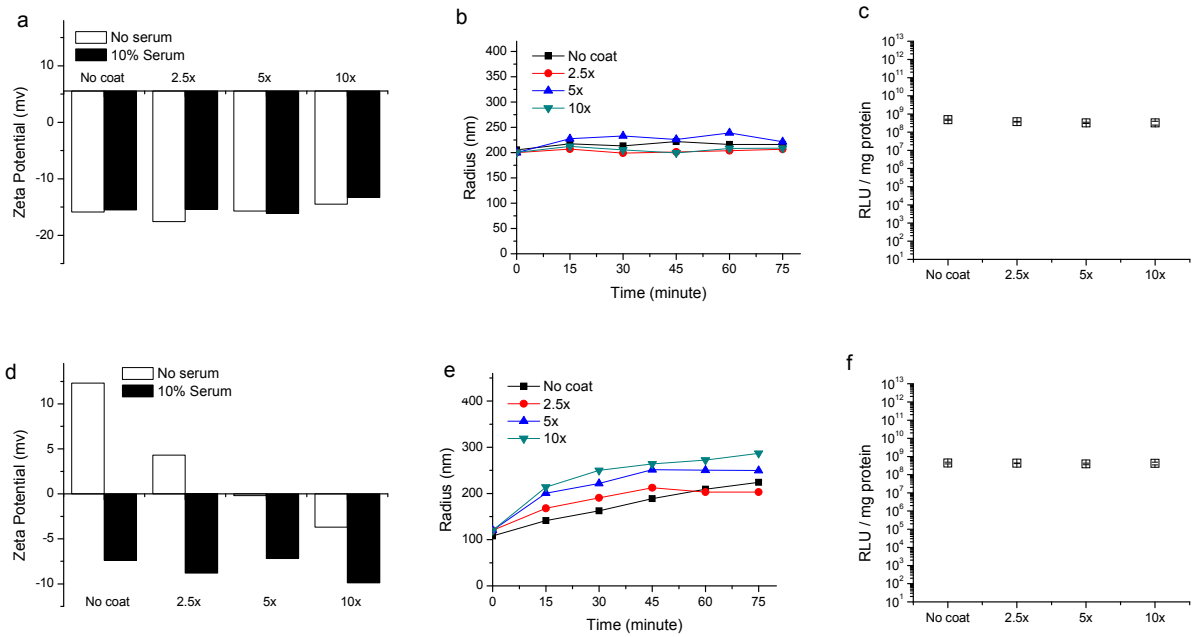

Figure S12. Effects of coating on Lip2k/DNA complexes (a-c) and PEI/DNA complexes (d-f). Coating was performed in Opti-MEM medium. Zeta potential (a, d), size (b, e) and gene delivery efficiency (c, f) was measured using the methods as described in main text.

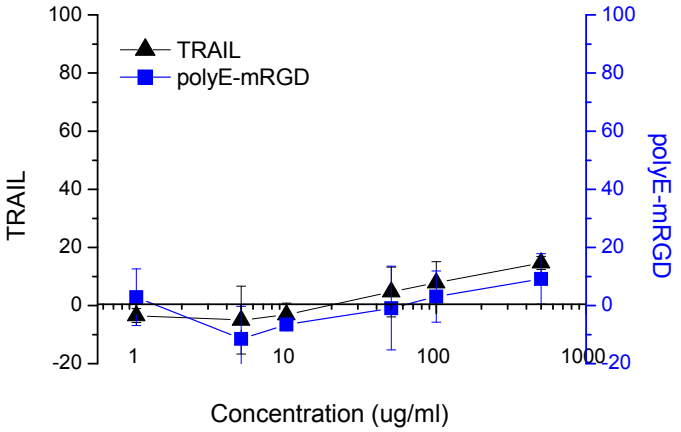

Figure S13. Toxicity of naked DNA and polyE-mRGD. A549 cells were treated with naked DNA or polyE-mRGD at indicated concentrations. Toxicity was determined five days after treatment by standard MTT assay.

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