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Synthesis and Properties of Sulfonium Polyelectrolytes for Biological Applications

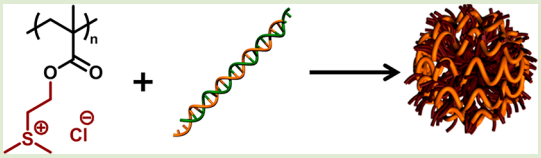
Sean T. Hemp,[†] Michael H. Allen, Jr.,[†] Adam E. Smith,[‡] and Timothy E. Long*[,†]

[†]Department of Chemistry and Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, Virginia 24061, United States

[‡]Department of Chemical Engineering, The University of Mississippi, University, Mississippi 38677, United States

Supporting Information

ABSTRACT: Sulfonium macromolecules displayed for the first time nucleic acid binding and transfection in vitro. Conventional and controlled radical polymerization techniques coupled with subsequent alkylation generated a sulfonium homopolymer, poly(DMSEMA), and a sulfonium diblock copolymer, poly(OEG-*b*-DMSEMA). DNA gel shift assays probed the ability of sulfonium macromolecules to complex nucleic acids, and luciferase assays examined the transfection efficiency and cytotoxicity of both sulfonium macromolecules. Poly(DMSEMA) and poly(OEG-*b*-DMSEMA) bound pDNA at a charge ratio of 1, and both induced significant luciferase expression in HeLa cells under serum-free conditions. Colloidal stability studies using dynamic light scattering highlighted the excellent colloidal stability of poly(OEG-*b*-DMSEMA) under salt and serum conditions due to the sterically stabilizing OEG block. Sulfonium macromolecules offer an alternate route to design cationic macromolecules for nonviral nucleic acid delivery, and future work will aim to add functionality to create more efficient delivery vehicles.



Prior to 2012, researchers relied solely on nitrogen-based cationic macromolecules for nonviral nucleic acid delivery. Common nitrogen-based cations that were utilized for electrostatic complexation and compaction of negatively charged nucleic acids included ammoniums,[1] pyridiniums,[2] imidazoliums,[3] and guanidiniums.[4] Cationic macromolecules typically examined in the literature include polyethyleneimine (PEI),[5] poly[2-(dimethylamino)ethyl methacrylate],[6] poly(vinyl imidazolium)s,[7] and chitosan.[8] These macromolecules encapsulate nucleic acids to generate nanoparticles called polyplexes, and they mediate cellular uptake and endosomal escape to enter the cell.[9] In 2012, Long et al. first described the utilization of phosphonium macromolecules for nonviral nucleic acid delivery.[10] They demonstrated improved nucleic acid binding and transfection using phosphonium macromolecules compared to ammonium analogues. Phosphonium-based diblock copolymers containing a stabilizing block consisting of oligo(ethylene glycol) methyl ether methacrylate (OEG) or 2-methacryloyloxyethyl phosphorylcholine demonstrated enhanced colloidal stability due to steric repulsion of the stabilizing block.[11] Fréchet et al. demonstrated the elegant synthesis of phosphonium acrylates, which displayed excellent siRNA-mediated gene knockdown compared to ammonium acrylates.[12] Kumar et al. also recently reported a triphenylphosphonium-modified PEI that exhibited enhanced pDNA and siRNA delivery compared to linear PEI.[13]

The synthesis of sulfonium cations normally relies on the quaternization of thioethers using activated halides or other techniques to drive quaternization to high conversions.[14] Sulfonium cations display inherent instability due to the poor nucleophilicity of the initial thioether,[15] and there are few reports detailing sulfonium polyelectrolytes. Hatch et al. reported the synthesis of poly(vinylbenzyl sulfonium)s wherein the sulfonium instability resulted in cross-linking during polymerization.[15] Novak et al. alkylated poly(*p*-phenylene sulfide) to generate poly(*p*-phenylene sulfonium)s suitable as photoresists.[16] Bailey and Combe examined sulfonium polyacrylates as potential flocculants.[17] *S*-Methylmethionine and *S*-adenosyl methionine, sulfonium-containing amino acids, occur naturally in biology and have multiple biological functions.[18−20] Kramer and Deming detailed multiple synthetic pathways to quaternize poly(L-methionine), and they reported a library of alkylated poly(L-methionine)s with varying functional groups.[14] Their postpolymerization functionalization led to high quaternization levels and stable, water-soluble sulfonium poly(L-methionine)s.

Herein, we report the unprecedented synthesis and examination of sulfonium macromolecules for nonviral nucleic acid delivery. Conventional and controlled radical polymerization created a thioether-containing homopolymer and diblock copolymer. Postpolymerization alkylation generated a sulfonium homopolymer and sulfonium diblock copolymer suitable for nucleic acid complexation and delivery. DNA gel shift assays and dynamic light scattering studies examined plasmid DNA (pDNA) binding and polyplex colloidal stability. Luciferase transfection assays directly examined transfection efficiency and cytotoxicity of sulfonium macromolecules. Further expansion of cation choice to include sulfonium cations will enable researchers to select from a broader library of delivery vehicles for nonviral nucleic acid delivery.

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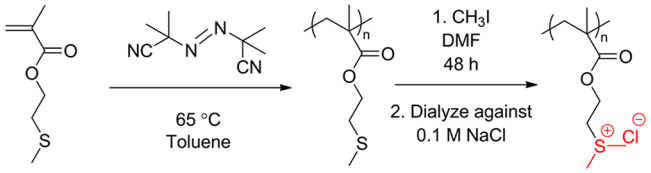
Conventional free radical polymerization of 2-(methylthio)-ethyl methacrylate (MTEMA) shown in Scheme 1 readily

Scheme 1. Conventional Free Radical Polymerization and Subsequent Quaternization to Achieve a Sulfonium-Containing Homopolymer, Poly(DMSEMA)



synthesized a poly(MTEMA) homopolymer (THF SEC, M_n = 17 300 g/mol, PDI = 2.24). Reversible-addition fragmentation chain transfer (RAFT) polymerization enabled the synthesis of a thioether-containing diblock copolymer (Scheme 2). RAFT polymerization of OEG using a trithiocarbonate chain transfer agent (CTA) created a well-defined poly(OEG) macroCTA (Aqueous SEC, M_n = 13 600 g/mol, PDI = 1.04). Subsequent chain extension of the poly(OEG) macroCTA with MTEMA generated the desired diblock copolymer, poly(OEG-*b*-MTEMA). ^{1}H NMR determined the M_n of the MTEMA B block (12 800 g/mol) based on integration to the OEG A block, and aqueous SEC after alkylation of the diblock copolymer confirmed a well-defined diblock copolymer (PDI = 1.05). Thioethers typically require activated halides or other synthetic techniques to generate sulfonium cations due to the inherently poor nucleophilicity of thioethers. Quaternization of poly(MTEMA) and poly(OEG-*b*-MTEMA) with 10 equiv of methyl iodide successfully generated a sulfonium-containing homopolymer, poly(2-methacryloxyethyldimethylsulfonium chloride) (poly(DMSEMA)), and a sulfonium-containing diblock copolymer, poly(OEG-*b*-DMSEMA). ^{1}H NMR determined quaternization levels of 90% and 87% for poly(DMSEMA) and poly(OEG-*b*-DMSEMA), respectively.

DNA gel shift assays probed the ability of sulfonium-containing macromolecules to effectively bind pDNA. Classically, researchers utilize an N/P ratio to quantify nucleic acid binding of nitrogen-based systems wherein N corresponds to the moles of protonatable, protonated, or quaternized nitrogen atoms in the macromolecule while P corresponds to the moles of negatively charged phosphate units in the DNA backbone.[21] A charge ratio (± ratio) for other cation-based systems appropriately correlates the moles of cationic charge in the polymer to the moles of negative charge in the DNA. Figure 1 shows the DNA gel shift assays for poly(DMSEMA) and poly(OEG-*b*-DMSEMA). Both sulfonium macromolecules efficiently bound pDNA at a ± ratio of 1.

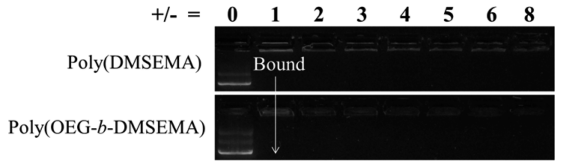
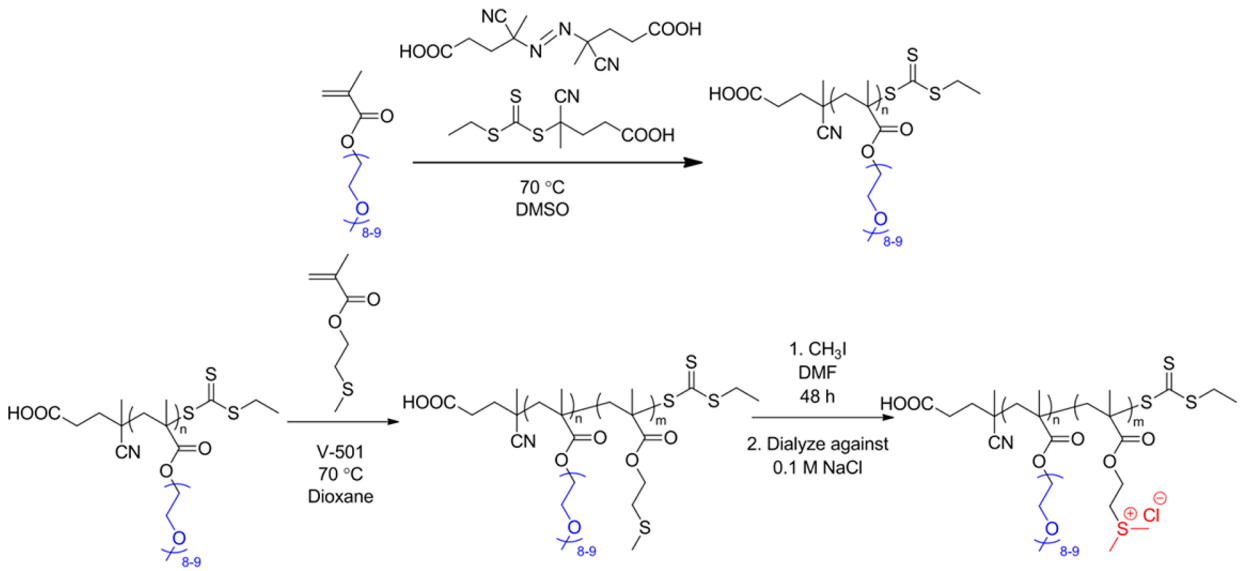


Figure 1. DNA gel shift assay demonstrating effective nucleic acid complexation at a charge ratio of 1 for both poly(DMSEMA) and poly(OEG-*b*-DMSEMA).

Luciferase transfections performed on HeLa cells in serum-free OMEM and serum-containing DMEM elucidated the delivery efficiency of sulfonium macromolecules compared to a common positive control, linear polyethyleneimine (Jet-PEI), and negative controls of cells or DNA only. Figure 2 shows the normalized luciferase expression (RLU/mg protein) and cell viabilities of HeLa cells for serum-free OMEM transfections. Both poly(DMSEMA) and poly(OEG-*b*-DMSEMA) successfully displayed significantly higher luciferase expression compared to negative controls ($p < 0.05$). Poly(DMSEMA) required a lower ± ratio of 6 to efficiently transfect HeLa cells compared to poly(OEG-*b*-DMSEMA), which required a higher ± ratio of 10 to achieve similar transfection levels as poly(DMSEMA). Poly(DMSEMA) demonstrated significantly more cytotoxicity compared to poly(OEG-*b*-DMSEMA), and as expected, cytotoxicity increased as the charge ratio increased. Transfections using Jet-PEI resulted in significantly higher luciferase expression compared to sulfonium macromolecules.

Scheme 2. RAFT Polymerization and Post-Polymerization Alkylation to Generate a Sulfonium-Containing Diblock Copolymer, Poly(OEG-*b*-DMSEMA)



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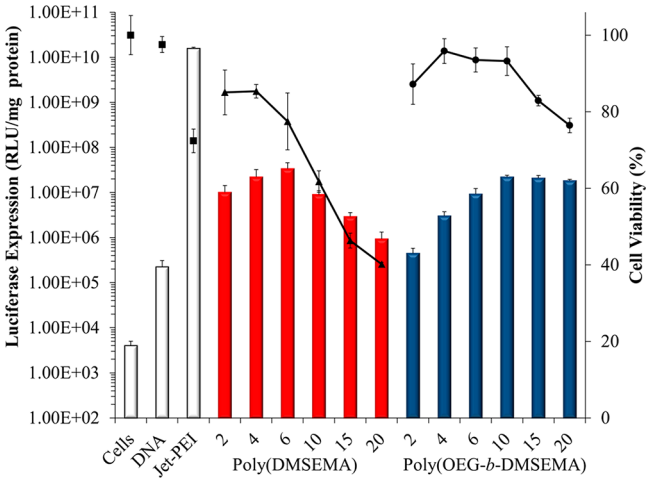

Figure 2. Luciferase expression and cell viability of HeLa cells for poly(DMSEMA) and poly(OEG-*b*-DMSEMA) under serum-free OMEM transfection conditions. The histogram bars correspond to the luciferase expression, while the data points correlate to cell viability.

Jet-PEI and other delivery vehicles with protonatable sites rely on the proton sponge effect to achieve endosomal escape for efficient transfection.[22] Both sulfonium macromolecules presumably struggled to escape the endosome due to their lack of protonatable sites, therefore lowering their transfection ability compared to Jet-PEI. Future incorporation of protonatable sites and other functionality will aim to improve the transfection of sulfonium macromolecules. Other potential hindrances for transfection using sulfonium macromolecules include lower cellular uptake or low nucleic acid release in the cytosol. Figure 3 illustrates the luciferase expression and cell viabilities of HeLa cells during serum transfections. The presence of serum during the transfection negatively affected the capability of both sulfonium macromolecules to deliver pDNA effectively. Serum typically hinders successful transfection due to association of negatively charged proteins to positively charged polyplexes and cationic free polymer in solution.[23] Serum transfections led

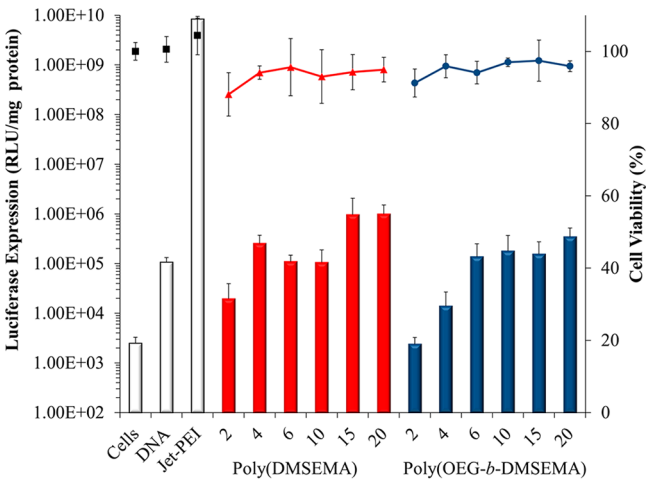

Figure 3. Luciferase expression and cell viability of HeLa cells for poly(DMSEMA) and poly(OEG-*b*-DMSEMA) under serum-containing DMEM transfection conditions. The histogram bars correspond to the luciferase expression, while the data points correlate to cell viability.

to higher cell viabilities with all delivery vehicles displaying minimal cytotoxicity.

Nanoparticle colloidal stability is imperative for in vivo applications where nanoparticle size and surface chemistry directly influence circulation times and biodistribution.[24] Colloidal stability studies directly compared the colloidal stability of poly(DMSEMA) (± ratio = 6), poly(OEG-*b*-DMSEMA) (± ratio = 10), and Jet-PEI (N/P = 5) polyplexes at their optimal transfection formulation. Conditions examined for polyplex colloidal stability included water, serum-free OMEM, and serum-containing DMEM. All three delivery vehicles exhibited colloidal stability in water, likely due to charge repulsion of the positively charged polyplexes (Figure 4). Zeta potentials for poly(DMSEMA) and poly(OEG-*b*-DMSEMA) were 22 and 18 mV, respectively.

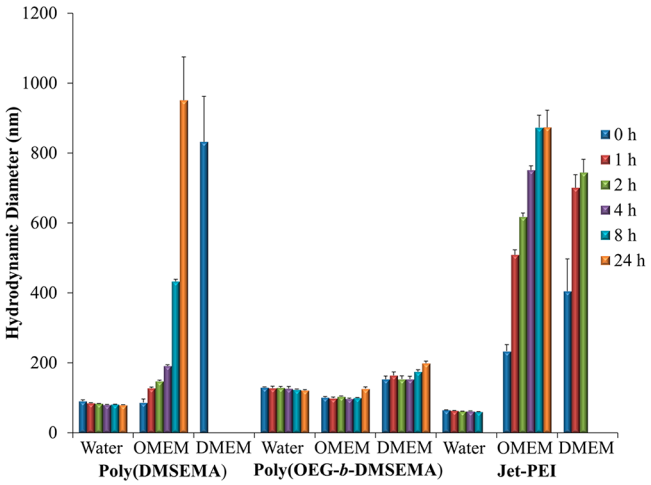

Figure 4. Colloidal stability of poly(DMSEMA) (± ratio = 6), poly(OEG-*b*-DMSEMA) (± ratio = 10), and Jet-PEI (N/P = 5) under various conditions (water, serum-free OMEM, and serum-containing DMEM).

Poly(DMSEMA) and Jet-PEI both demonstrated poor colloidal stability in serum-free OMEM, steadily increasing in hydrodynamic diameter over 24 h. The presence of salt neutralized the surface charge of the polyplexes, inducing aggregation.[25] Poly(OEG-*b*-DMSEMA) polyplexes resisted aggregation under salt conditions due to the steric stabilizing OEG block and displayed excellent colloidal stability over 24 h. Serum conditions also induced polyplex aggregation for poly(DMSEMA) and Jet-PEI polyplexes due to the association of negatively charged proteins to the polyplex surface.[26] Poly(OEG-*b*-DMSEMA) polyplexes exhibited enhanced colloidal stability in serum conditions compared to poly(DMSEMA). The sulfonium diblock copolymer ultimately displayed outstanding colloidal stability under both salt and serum conditions.

Conventional and controlled radical polymerization with postpolymerization quaternization successfully synthesized a sulfonium-containing homopolymer and diblock copolymer. Sulfonium macromolecules were shown for the first time to efficiently complex nucleic acids and deliver them in vitro to HeLa cells. Poly(DMSEMA) and poly(OEG-*b*-DMSEMA) exhibited significantly higher luciferase expression under serum-free OMEM conditions compared to negative controls, demonstrating maximal transfection at charge ratios of 6 and 10, respectively. Higher charge ratios were necessary for

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poly(OEG-*b*-DMSEMA) to achieve similar luciferase expression compared to poly(DMSEMA). Poly(OEG-*b*-DMSEMA) displayed lower cytotoxicities compared to poly(DMSEMA). Serum lowered the transfection efficiency for both sulfonium macromolecules while improving cell viabilities. Both poly(DMSEMA) and Jet-PEI exhibited poor colloidal stability in salt and serum conditions, while poly(OEG-*b*-DMSEMA) resisted aggregation and remained colloidally stable over 24 h. Future work will aim to broaden the sulfonium macromolecule library to provide buffering capacity and other functionality to enhance transfection.

EXPERIMENTAL SECTION

Materials. Methyl iodide (99%), sodium chloride (≥99%), and 2-(methylthio)ethyl methacrylate (MTEMA) (96%) were obtained from Sigma-Aldrich and used as received. OEG (485 g/mol) was purchased from Sigma-Aldrich and passed through neutral alumina to remove the inhibitor. α,α'-Azoisobutyronitrile (AIBN) and 4,4′-azobis(4-cyanopentanoic acid) (V-501) were obtained from Sigma-Aldrich and recrystallized from methanol. 4-Cyano-4-(ethylsulfanylthiocarbonyl)-sulfanylpentanoic acid (CEP) was synthesized according to previous literature.[27]

Analytical Methods. ^{1}H NMR spectroscopy was performed on a Varian Unity 400 operating at 400 MHz in CD_3OD or $CDCl_3$. The aqueous size-exclusion chromatography (SEC) instrumentation consisted of a Waters 1515 isocratic HPLC pump, a Waters 717plus autosampler, two Waters Ultrahydrogel linear columns, one Waters Ultrahydrogel 250 column, a Wyatt MiniDAWN light scattering (LS) detector, and a Waters 2414 refractive index (RI) detector operating at a flow rate of 0.8 mL/min. The aqueous eluent was a ternary mixture of 54/23/23 (v/v/v) water/methanol/acetic acid with 0.1 M sodium acetate. Absolute molecular weight of the OEG macroCTA was determined with a d*n*/d*c* of 0.1156 mL/g determined offline using a Wyatt Opti-lab T-rEX differential refractometer. THF SEC was operated at 40 °C and a 1 mL/min flow rate using a Waters autosampler, a Waters 2410 RI detector, a Wyatt MiniDAWN LS detector, and three 5 μm PLgel Mixed-C columns. Relative molecular weights were determined using polystyrene standards.

Polymer Synthesis. MTEMA (3.75 g, 23.4 mmol), AIBN (39.8 mg, 0.242 mmol, 1 mol %), and toluene (38 mL) were added to a 100 mL, round-bottomed flask with a magnetic stir bar. The solution was purged with argon for 30 min and subsequently heated at 65 °C for 24 h. The polymer was precipitated from methanol and dried in vacuo at 60 °C for 24 h to obtain a white solid.

OEG (40.10 g, 82.7 mmol), CEP (868 mg, 3.30 mmol), V-501 (184 mg, 0.656 mmol), and DMSO (330 mL) were added to a 500 mL, round-bottomed flask with a magnetic stir bar. The solution was sparged with argon for 1 h and then heated at 70 °C for 250 min. The polymer was dialyzed against water (MWCO = 3500 g/mol) for 2 days and subsequently lyophilized to obtain a yellow oil. Subsequent chain extension with MTEMA generated the desired diblock copolymer. Poly(OEG) macroCTA (268 mg, 0.02 mmol, 13 600 g/mol), V-501 (2.19 mg, 0.008 mmol), MTEMA (632 mg, 3.95 mmol), and dioxane (7.89 mL) were added to a 25 mL, round-bottomed flask with a magnetic stir bar. The solution was sparged with argon for 30 min and then heated at 70 °C for 4 h. The polymer solution was dialyzed against methanol (MWCO = 3500 g/mol) for 2 days and then concentrated in vacuo. The polymer was dried in vacuo at 60 °C for 24 h.

Both polymers were quaternized following a similar protocol, and the quaternization of poly(MTEMA) follows as an example. Poly(MTEMA) (504 mg, 3.15 mmol) was treated with 10 equiv of methyl iodide (2.0 mL, 32.1 mmol) in 17 mL of DMF for 48 h. The resulting solution was dialyzed against 0.1 M NaCl (MWCO = 3500 g/mol) for 2 days to exchange the counterion to Cl^- and then dialyzed against water for 2 days. The polymer solution was lyophilized to obtain a white solid.

DNA Binding Assay. Plasmid DNA (0.2 μg, Aldevron, gWiz-Luc) was diluted into an appropriate amount of water to achieve a total volume of 28 μL after polymer addition. The polymer solution (1 mg/mL) was subsequently added to achieve various charge ratios (ratio of positively charged sulfonium in polymer to negatively charged phosphates in DNA). The polyplexes were incubated for 30 min and subsequently electrophoresed at 70 V for 30 min in a 1 wt % agarose gel stained with SYBR green I (6 μL). The gel was imaged using a MultiDoc-it Digital Imaging System (UVP).

Dynamic Light Scattering. Plasmid DNA (2.0 μg in 100 μL of water) was complexed with the required amount of polymer solution for a specific charge ratio in 200 μL of total water. The polyplexes were incubated for 30 min and subsequently diluted into 800 μL of water or serum-free Opti-MEM (OMEM). Dynamic light scattering (DLS) using a Malvern Zetasizer Nano monitored polyplex hydrodynamic diameters over a 24 h period. All measurements reported were an average of three measurements. Serum stability required significantly higher pDNA concentrations to differentiate polyplexes from serum proteins in solution. pDNA (20.0 μg in 100 μL of water) was complexed with the necessary amount of polymer to achieve a desired charge ratio in 200 μL of total water. The resulting solution was incubated for 30 min and then diluted with 800 μL of serum-containing Dulbecco's Modified Eagle Media (DMEM). DLS monitored polyplex hydrodynamic diameters over 24 h, and each value reported was an average of three measurements.

Cell Culture. Human cervical cancer cells (HeLa cells) were obtained from ATCC and were cultured in serum-containing DMEM (10% FBS) with 100 U/mL of penicillin and 100 μg/mL of streptomycin in 95% humidity and 5% CO_2 at 37 °C. All reagents for subcultivation were obtained from MediaTech.

Luciferase and Cytotoxicity Assay. HeLa cells (500 μL, 200 000 cells/mL) were seeded in 24-well plates and incubated for 24 h. Polyplexes were prepared using pDNA (5 μg in 250 μL of water) and the required amount of a polymer solution to obtain a total water volume of 500 μL at specific charge ratios. Jet-PEI polyplexes were prepared at an N/P ratio of 5 and applied to cells according to the manufacturer's protocols. Each well was aspirated and washed with 300 μL of HBSS. Serum-free OMEM (400 μL) or serum-containing DMEM (400 μL) was added to each well, and then 100 μL of each polyplex solution was added to the wells (1 μg of pDNA/well). The cells were transfected for 4 h. Then the transfection media was aspirated, and 500 μL of serum-containing DMEM was added to each well. The cells were incubated for 44 h, rinsed with 300 μL of PBS, and then lysed using 120 μL of a 1x Promega lysis buffer. Each plate was incubated for 30 min at 37 °C and subjected to two freeze−thaw cycles to fully lyse the cells. Luciferase activity was quantified using a Promega luciferase assay kit and a Promega GloMax 96 Microplate Luminometer. Total protein in the lysates was determined using a Pierce BCA protein assay kit following manufacturer protocols. Luciferase expression was normalized using the protein concentration, and cell viabilities were determined based on the protein concentration relative to the cells only control. Experiments were performed in quadruplicate, and the Student's *t* test was utilized for statistical analysis.

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectroscopy and size-exclusion chromatography of polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: telong@vt.edu. Tel.: (540)231-2480. Fax: (540)231-8517.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Synatschke, C. V.; Schallon, A.; Jérôme, V.; Freitag, R.; Müller, A. H. E. *Biomacromolecules* **2011**, *12* (12), 4247−4255.
(2) San Juan, A.; Letourneur, D.; Izumrudov, V. A. *Bioconjugate Chem.* **2007**, *18* (3), 922−928.
(3) Pack, D. W.; Putnam, D.; Langer, R. *Biotechnol. Bioeng.* **2000**, *67* (2), 217−223.
(4) Funhoff, A. M.; van Nostrum, C. F.; Lok, M. C.; Fretz, M. M.; Crommelin, D. J. A.; Hennink, W. E. *Bioconjugate Chem.* **2004**, *15* (6), 1212−1220.
(5) Ahn, H. H.; Lee, J. H.; Kim, K. S.; Lee, J. Y.; Kim, M. S.; Khang, G.; Lee, I. W.; Lee, H. B. *Biomaterials* **2008**, *29* (15), 2415−2422.
(6) Layman, J. M.; Ramirez, S. M.; Green, M. D.; Long, T. E. *Biomacromolecules* **2009**, *10*, 1244−1252.
(7) Allen, M. H.; Green, M. D.; Getaneh, H. K.; Miller, K. M.; Long, T. E. *Biomacromolecules* **2011**, *12* (6), 2243−2250.
(8) Koping-Hoggard, M.; Tubulekas, I.; Guan, H.; Edwards, K.; Nilsson, M.; Varum, K. M.; Artursson, P. *Gene Ther.* **2001**, *8*, 1108−1121.
(9) Mintzer, M. A.; Simanek, E. E. *Chem. Rev.* **2009**, *109* (2), 259−302.
(10) Hemp, S. T.; Allen, M. H.; Green, M. D.; Long, T. E. *Biomacromolecules* **2012**, *13* (1), 231−238.
(11) Hemp, S. T.; Smith, A. E.; Bryson, J. M.; Allen, M. H.; Long, T. E. *Biomacromolecules* **2012**, *13* (8), 2439−2445.
(12) Ornelas-Megiatto, C.; Wich, P. R.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2012**, *134* (4), 1902−1905.
(13) Bansal, R.; Tripathi, S. K.; Gupta, K. C.; Kumar, P. *J. Mater. Chem.* **2012**, *22* (48), 25427−25436.
(14) Kramer, J. R.; Deming, T. J. *Biomacromolecules* **2012**, *13* (6), 1719−1723.
(15) Hatch, M. J.; Meyer, F. J.; Lloyd, W. D. *J. Appl. Polym. Sci.* **1969**, *13* (4), 721−744.
(16) Novak, B. M.; Hagen, E.; Hoff, S.; Viswanathan, A. *Macromolecules* **1994**, *27* (7), 1985−1986.
(17) Bailey, F. E.; La Combe, E. M. *J. Macromol. Sci., Part A: Chem.* **1970**, *4* (6), 1293−1300.
(18) Toennies, G. *J. Biol. Chem.* **1940**, *132* (1), 455−456.
(19) Lu, S. C. *Int. J. Biochem. Cell Biol.* **2000**, *32* (4), 391−395.
(20) Bourgis, F.; Roje, S.; Nuccio, M. L.; Fisher, D. B.; Tarczynski, M. C.; Li, C.; Herschbach, C.; Rennenberg, H.; Pimenta, M. J.; Shen, T.-L.; Gage, D. A.; Hanson, A. D. *Plant Cell Online* **1999**, *11* (8), 1485−1497.
(21) Chan, P.; Kurisawa, M.; Chung, J. E.; Yang, Y.-Y. *Biomaterials* **2007**, *28* (3), 540−549.
(22) Behr, J.-P. *Chimia Int. J. Chem.* **1997**, *51*, 34−36.
(23) Dash, P. R.; Read, M. L.; Barrett, L. B.; Wolfert, M. A.; Seymour, L. W. *Gene Ther.* **1999**, *6*, 643−650.
(24) Farokhzad, O. C.; Langer, R. *ACS Nano* **2009**, *3* (1), 16−20.
(25) Prevette, L. E.; Lynch, M. L.; Kizjakina, K.; Reineke, T. M. *Langmuir* **2008**, *24* (15), 8090−8101.
(26) de Wolf, H. K.; Luten, J.; Snel, C. J.; Oussoren, C.; Hennink, W. E.; Storm, G. *J. Controlled Release* **2005**, *109* (1−3), 275−287.
(27) Convertine, A. J.; Benoit, D. S. W.; Duvall, C. L.; Hoffman, A. S.; Stayton, P. S. *J. Controlled Release* **2009**, *133* (3), 221−229.