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Communication

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Preparation of Cationic Nanogels for Nucleic Acid Delivery

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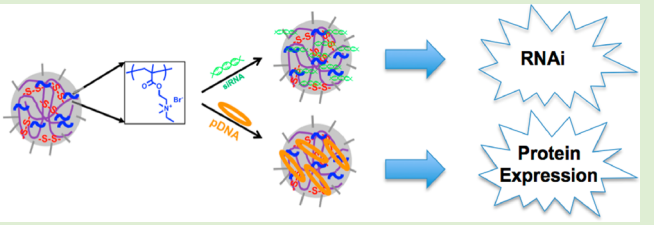
Supporting Information

ABSTRACT: Cationic nanogels with site-selected functionality were designed for the delivery of nucleic acid payloads targeting numerous therapeutic applications. Functional cationic nanogels containing quaternized 2-(dimethylamino)-ethyl methacrylate and a cross-linker with reducible disulfide moieties (qNG) were prepared by by electron transfer (AGET) atom transfer radical polymerization (ATRP) in an inverse miniemulsion. Polyplex formation between the qNG and nucleic acid exemplified by plasmid DNA (pDNA) and short interfering RNA (siRNA duplexes) were evaluated. The delivery of polyplexes was optimized for the delivery of pDNA and siRNA to the *Drosophila* Schneider 2 (S2) cell-line. The qNG/nucleic acid (i.e., siRNA and pDNA) polyplexes were found to be highly effective in their capabilities to deliver their respective payloads.



Short interfering RNA(siRNA) and plasmid DNA (pDNA) have emerged as important agents in both basic research and therapeutic strategies.[1−4] They affect the biosynthesis of their targeted proteins either through the introduction of specific units, resulting in synthesis of new proteins, in the case of pDNA, or targeting of messenger RNAs (mRNAs) for RNA interference (RNAi), as in the case of siRNA.[5,6] The selective delivery of siRNA and pDNA has been a challenge due to their degradation in the presence of nuclease and their anionic charge that hinders their cell permeability.[7,8] Solutions include cationic carrier systems, such as cationic lipids or polymers, which generate complexes via electrostatic interactions to form of lipoplexes or polyplexes, respectively, and thus enhance the transfection of nucleic acids into cells.[9−24] Nevertheless, the design of efficient polyplex-based siRNA and pDNA delivery systems for transfection is itself a challenge that limits realizing the full potential of siRNA and pDNA for therapeutic applications.[4,8,25−28]

Previously, the preparation of biodegradable cross-linked nanogels (NGs),[11] comprising an oligo(ethylene oxide) methacrylate (OEOMA) backbone was demonstrated using by electron transfer atom transfer radical polymerization (AGET ATRP) in inverse miniemulsion.[29−31] This method was successfully used to prepare protein−polymer hybrid NGs[32,33] and NGs for delivery of small molecules[34] and carbohydrates.[35] However, in order to use these materials for effective nucleic acid delivery, it is necessary to have site-specific incorporation of cationic monomers, into the predominant OEOMA NGs for polyplex formation, but currently this has not been accomplished. ATRP[36−39] is a versatile polymerization method that can be applied for the synthesis of diverse and complex polymeric architectures,[40−44] including cationic and quaternized nanogels (qNGs). In this communication, we report the synthesis of a biodegradable qNG via the copolymerization of OEOMA and a cationic monomer, quaternized dimethylaminoethyl methacrylate,[4545] by AGET ATRP in inverse miniemulsion. A disulfide-based cross-linker[9,30,46−48] was utilized to take advantage of the different concentrations of reducing agent contained in the extracellular (~1 μM) versus intracellular (~10 mM) matrix[49,50] and facilitate the biodegradation of the NG after transfection of the nucleic acid cargo. The qNGs were studied for the complexation and delivery of a pDNA that codes for a firefly luciferase protein and siRNA that targets a renilla luciferase mRNA for a dual luciferase reporter assay. The *Drosophila* Schneider 2 (S2) cell line was used as the model because of its importance and prevalence in basic biological research and challenges in successfully delivering both siRNA and pDNA to these cells.[51]

The biodegradable qNG were prepared in a water-in-oil inverse miniemulsion utilizing AGET ATRP (Scheme 1). $Cu^{(II)}Br_2$/tris(2-pyridylmethyl)amine (TPMA) was used as the ATRP catalytic species, poly(ethylene glycol) methyl ether 2-bromoisobutyrate ($PEGMI_{2000}$, M_n = 2000) as a macroinitiator, oligo(ethylene oxide)methacrylate ($OEOMA_{300}$, M_n = 300),

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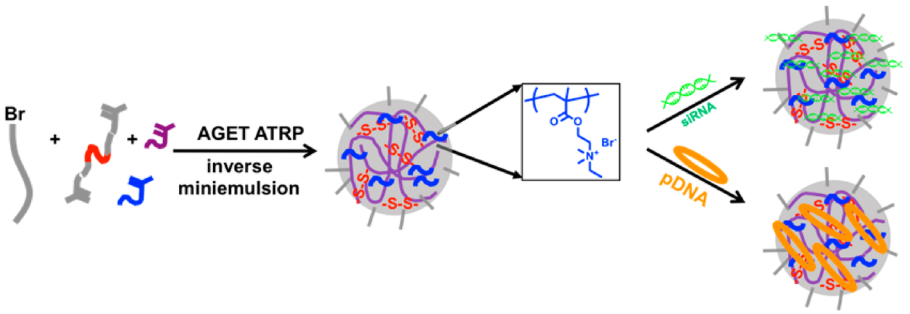
Scheme 1. Synthesis of Cationic NGs for Nucleotide Delivery of siRNA and pDNA Using AGET ATRP



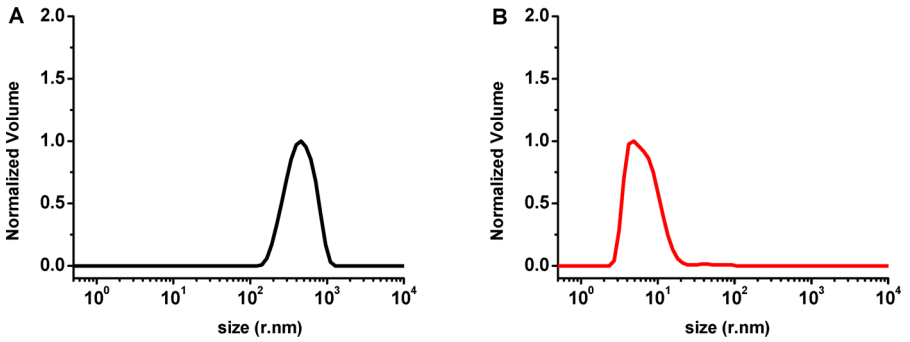


Figure 1. Normalized volume distribution of cationic NGs prepared by AGET ATRP in inverse miniemulsion measured using DLS. (A) Volume distribution of purified qNG prepared from $PEOMI_{2000}$:$OEOMA_{300}$:Q-DMAEMA:DMA:Cu(II)Br_2:Ascorbic Acid: 1/290/20/4/0.5/0.6/0.3, 55 mg $PEGOH_{2000}$, in 5% Span80 in cyclohexane for 24 h at 30 °C. Size = 275 nm, PDI = 0.164, ζ potential = 43.7 mV ±4.1. (B) Volume distribution of qNG after incubation with 10 mM glutathione for 4 days.

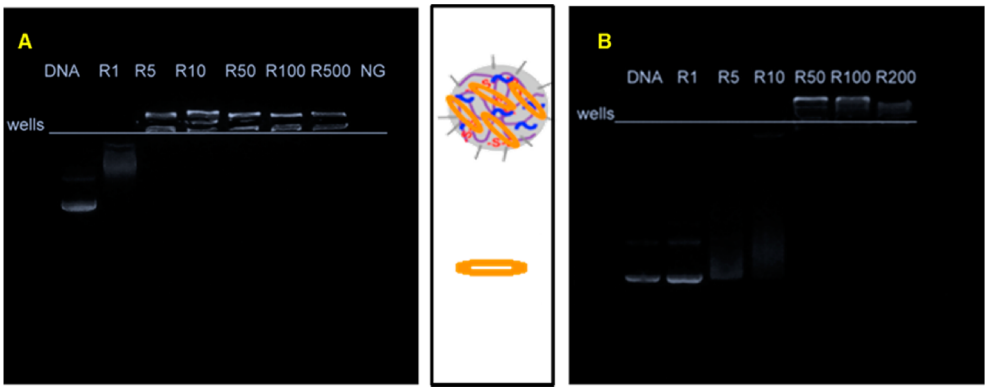


Figure 2. Agarose gel electrophoresis analysis of polyplex formation and disassociation of qNG and pDNA (LacZ-plasmid). Electrophoresis was conducted for 30 min, at 100 V, and the gels were stained with EtBr and imaged with UV-transillumination. (A) Polyplex formation, 500 ng of plasmid was incubated with varying amounts of qNG (R1-R500) for 1 h at 25 °C and then loaded onto a 1% agarose gel electrophoresis. (B) Preformed polyplexes of qNG:pDNA (R1-R200) were incubated with 0.015 $\mu g/\mu L$ Heparin sulfate for 30 min and then examined by gel electrophoresis using a 0.5% agarose gel.

and quaternized dimethylaminoethyl methacrylate (Q-DMAEMA, DMAEMA was quaternized with ethyl bromide) as comonomers, dithiopropionyl poly(ethylene glycol) dimethacrylate (DMA) (M_n =1260) as a cross-linking agent, and $PEG_{2000}OH$ as a stabilizer. They were dissolved in 1.40 mL of ultrapure water and added to a 25 mL solution of 0.05% (w/w) of Span-80 in cyclohexane. The reaction mixture was emulsified using ultrasonication to form stable water-in-oil droplets. The mixture was degassed with nitrogen and a degassed solution of ascorbic acid was injected into the emulsion to convert $Cu^{(II)}Br_2$ to $Cu^{(I)}Br$ and initiate the AGET ATRP. The polymerization was carried out for 24 h at 30 °C. The qNGs were purified by precipitation into THF, then washed several times with ultrapure water followed by dialysis (25 k MWCO membrane) against water to remove all unreacted reagents.

The qNGs were characterized by dynamic light scattering (DLS) and zeta potential, revealing a particle size of ca. 275 nm in diameter with a CV of 0.164 and 43.7 mV ±4.1, respectively (Figure 1A). To determine the biodegradability of these qNGs under reducing conditions, a 1 mg/mL solution of qNG in 10 mM glutathione[9] was prepared, and its particle size was monitored using DLS. The resulting degraded particles had a volume distribution of ca. 4 nm in diameter, indicating a successful REDOX mediated degradation of the qNG (Figure 1B).

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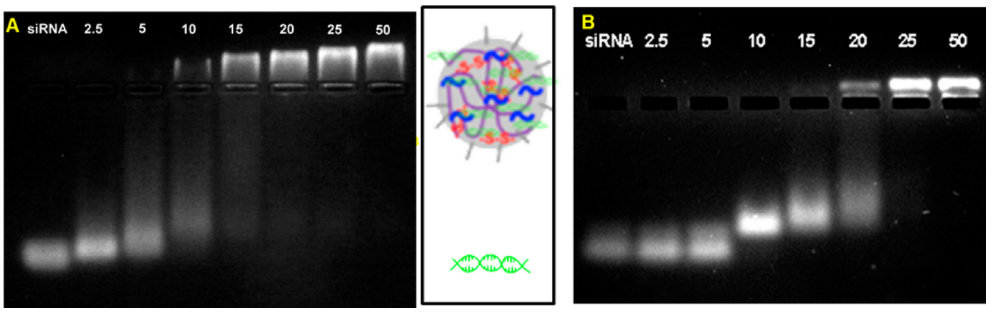

Figure 3. Agarose gel electrophoresis complexation assay measured at varying qNG:siRNA weight ratios. (A) Polyplexes were prepared by incubating 500 ng of siRNA with varying amounts of qNG for 1 h at 4 °C, then loaded onto a 2% agarose gel in Tris/Borate/EDTA buffer. After electrophoresis (30 min, 100 V) the gel was stained with EtBr and imaged with UV-transillumination. (B) Preformed polyplexes of qNG:siRNA (R1-R50) were incubated with 0.05 $\mu g/\mu L$ Heparin sulfate for 30 min and then examined by gel electrophoresis using a 3% agarose gel.

The ability of the qNG to complex both pDNA and siRNA was investigated using agarose gel shift assay. This assay was used to determine the weight ratio, R (i.e., NG:siRNA or NG:pDNA), at which qNG totally complexed pDNA and siRNA, respectively. Five hundred nanograms of pDNA was hybridized with qNG for 1 h at 25 °C (in nuclease-free ultrapure water pH = 7) and then loaded onto a 1% agarose gel. Following electrophoresis, the gels were stained with ethidium bromide (EtBr) and imaged. For pDNA, a ratio of R5 (i.e., $weight_{qNG}$:$weight_{pDNA}$ = 5:1) showed a lack of band migration of the qNG:pDNA polyplex. This indicated a near total complexation of the DNA with the qNG (Figure 2A). A polyplex disassociation study was conducted, by adding heparin sulfate to the precomplexed qNG:pDNA polyplexes, to determine the reversibility of the complexation between qNG and pDNA.[52] At ratios less than R50, the polyplexes could be disassociated using heparin sulfate (0.015 $\mu g/\mu L$) (Figure 2B). This result suggests that, although pDNA could be complexed, it could be released, when a small excess of qNG was used to complex the pDNA. Preliminary characterization of qNG:pDNA polyplexes using DLS and ζ potential analysis was conducted (Figure SI-1, Supporting Information). No aggregates were observed in the volume % distribution of qNG:pDNA polyplexes at R values (w/w) of R2−R0.5. The ζ potential of qNG:pDNA polyplexes for these R values are negative, indicating successful pDNA complexation. qNG:siRNA polyplexes were studied by incubating 500 ng of siRNA with varying ratios of qNG for 1 h at 4 °C (in nuclease-free ultrapure water pH = 7). Band migration of these polyplexes was studied by electrophoresis on a 2% agarose gel followed by EtBr staining and imaging. At a weight ratio of 15:1 qNG:siRNA (i.e., R15), no band migration was observed (Figure 3A), indicating total complexation of siRNA. Heparin sulfate-mediated qNG:siRNA polyplex disassociation was studied by incubating of preformed polyplexs at ratios of R2.5−R50 with heparin sulfate and analyzing with a 3% agarose gel (Figure 3B). The decomplexation studies of qNG:siRNA polyplexes indicate that, at a ratios less than R25, the siRNA can be released from the qNG via polyelectrolyte displacement. Once the qNG's ability to complex nucleotides was determined, the delivery of the polyplexes of qNG and siRNA and pDNA to S2 cells was studied.

Next, we tested the ability of the qNG to act as gene delivery (pDNA) agents using S2 cells. A firefly luciferase reporter plasmid (FLuc) polyplex was prepared at different weight ratios of qNG:pDNA and transfected into S2 cells. Different polyplex formation ratios were used to identify the conditions leading to maximal firefly luciferase signal after 24 h. Of the polyplexes tested, the R30 polyplex resulted in a maximum emission of the FLuc reporter gene signal (Figure 4). A paired Students t-Test

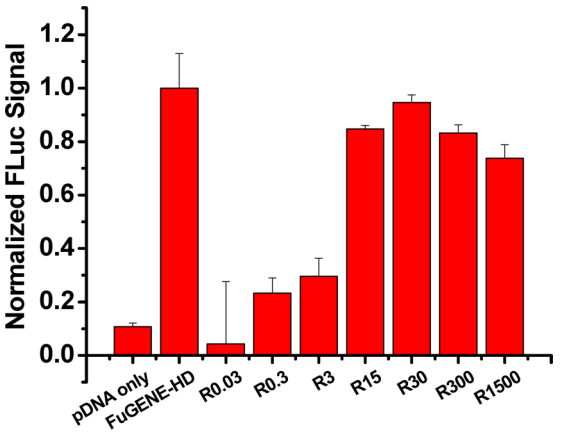

Figure 4. pDNA delivery using a firefly luciferase reporter assay: Graph of FLuc activity in S2 cells after 24 h of treatment with (N = 3): pDNA with no transfection reagent, i.e., pDNA with no FuGENE or qNG (negative control, red bar), 20 ng FLuc plasmid with FuGene-HD (positive control) or a weight ratio of qNG:pDNA(20 ng) polyplexes at (R1500, R300, R30, R15, R3, R0.3 and R0.03) (experimental group).

was used to compare the efficacies of the qNg:pDNA polyplexes and FuGENE-HD. The polyplexes prepared at R values of 300, 30, and 15 did not differ significantly from pDNA transfected with Fugene-HD (p > .05).

The qNGs ability to deliver siRNA was tested using a dual luciferase reporter assay (Dual-Glo luciferase reporter assay, Promega)[53] with S2 cells transfected with both firefly luciferase and Renilla luciferase (RLuc). Three hours after reporter plasmids were transfected, polyplexes of qNG and siRNA against RLuc were formed at R100, R20, R2, R1, R0.2 and R0.02, which were used to determine the optimal siRNA transfection R values. After 24 h, the post siRNA transfection RLuc and FLuc signals were measured, and the RLuc knockdown was quantified and normalized to the FLuc signal and a control well (N = 3). When no transfection reagent (i.e., no Fugene-HD or qNG) was used, the siRNA was inactive (Red bar), suggesting the initial transfection agent used to deliver the plasmid had been washed away. Maximum RLuc reporter signal knockdown was observed at a ratio of R0.2 (Figure 5). A paired Students t-Test was used to compare

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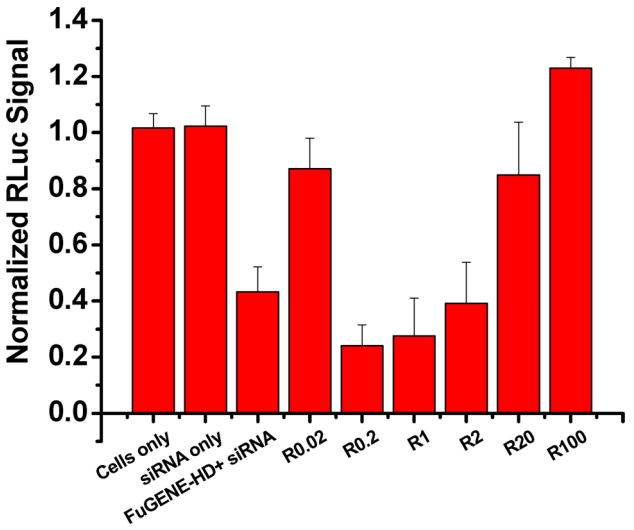

Figure 5. siRNA delivery using a dual-Luciferase reporter assay. Normalized to FLuc, RLuc activity in S2 cells after 24 h treatment with siRNA with no transfection reagent, i.e., siRNA with no FuGENE or qNG (negative control), 9 pmol of duplex siRNA with FuGene-HD (positive control), or a weight ratio of qNG:siRNA at R100, R20, R2, R0.2 and R0.02 (experimental group).

qNg:siRNA polyplexes efficiency compared to FuGENE-HD. The efficacy of the polyplexes prepared at R values of 2, 1, and 0.2 were found not to differ significantly from the efficacy of siRNA transfected with Fugene-HD ($p > .05$). This result underscores the utility of the qNGs for siRNA delivery.

CONCLUSIONS

We have demonstrated that well-defined qNGs (275 nm, PDI 0.164) can be prepared using AGET ATRP in inverse miniemulsion. The disulfide cross-linker conferred biodegradability to the qNG, causing it to undergo a REDOX-mediated degradation with glutathione, a model biological reducing agent. Moreover, the qNGs complexed pDNA and siRNA at relatively low weight ratios of qNG to DNA (5:1) and qNG to siRNA (15:1), as determined by agarose gel electrophoresis. Further, the NGs provided a robust delivery system for pDNA (~5 kb) as well as siRNA. On the basis of the electrophoresis disassociation experiments, we hypothesize that a balance between tight binding of nucleic acids to the qNG and ability to dissociate must be achieved for effective release and delivery of complexed material. In order to characterize the ability of different ratios of qNG to transfect siRNA, a dual-luciferase reporter assay was utilized to rapidly and accurately screen knockdown efficiency. A maximum reporter knockdown was obtained at R0.2, with efficacy suggesting more effective transfection than siRNA-Fugene-HD. For pDNA transfection, the maximum firefly luciferase reporter signal was observed at R30. These results confirm that qNGs are a promising platform for pDNA and siRNA delivery and future studies will include clinically relevant mammalian cells treated with the polyplexes.

ASSOCIATED CONTENT

Supporting Information

See Supporting Information (SI) for detailed NG synthesis, siRNA and pDNA transfection experiments, materials, methods, and additional studies of qNG:pDNA polyplex size and zeta potential characterizations. The qNG's effect on MC3T8 cell viability can also be found in the SI. This information is available free of charge via the Internet at http://pubs.acs.org/.

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Dykxhoorn, D. M.; Lieberman, J. The silent revolution: RNA interference as basic biology, research tool, and therapeutic. *Annu. Rev. Med.* **2005**, *56*, 401−23.

(2) Hamilton, A. J.; Baulcombe, D. C. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **1999**, *286*, 950−2.

(3) Fire, A.; Xu, S.; Montgomery, M. K.; Kostas, S. A.; Driver, S. E.; Mello, C. C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **1998**, *391*, 806−11.

(4) Verma, I. M.; Somia, N. Gene therapy - Promises, problems and prospects. *Nature* **1997**, *389*, 239−42.

(5) Lu, Q. L.; Bou-Gharios, G.; Partridge, T. A. Non-viral gene delivery in skeletal muscle: A protein factory. *Gene Ther.* **2003**, *10*, 131−42.

(6) Li, Z.; Rana, T. M. Molecular mechanisms of RNA-triggered gene silencing machineries. *Acc. Chem. Res.* **2012**, *45*, 1122−31.

(7) Aagaard, L.; Rossi, J. J. RNAi therapeutics: Principles, prospects and challenges. *Adv. Drug Delivery Rev.* **2007**, *59*, 75−86.

(8) Li, S.; Huang, L. Nonviral gene therapy: Promises and challenges. *Gene Ther.* **2000**, *7*, 31−34.

(9) Cho, H. Y.; Srinivasan, A.; Hong, J.; Hsu, E.; Liu, S.; Shrivats, A.; Kwak, D.; Bohaty, A. K.; Paik, H.-j.; Hollinger, J. O.; Matyjaszewski, K. Synthesis of biocompatible PEG-based star polymers with cationic and degradable core for siRNA delivery. *Biomacromolecules* **2011**, *12*, 3478−86.

(10) Cho, H. Y.; Gao, H.; Srinivasan, A.; Hong, J.; Bencherif, S. A.; Siegwart, D. J.; Paik, H.-j.; Hollinger, J. O.; Matyjaszewski, K. Rapid cellular internalization of multifunctional star polymers prepared by atom transfer radical polymerization. *Biomacromolecules* **2010**, *11*, 2199−203.

(11) Duncan, R.; Ringsdorf, H.; Satchi-Fainaro, R. Polymer therapeutics: Polymers as drugs, drug and protein conjugates and gene delivery systems: Past, present and future opportunities. *Adv. Polym. Sci.* **2006**, *192*, 1−8.

(12) Heath, W. H.; Senyurt, A. F.; Layman, J.; Long, T. E. Charged polymers via controlled radical polymerization and their implications for gene delivery. *Macromol. Chem. Phys.* **2007**, *208*, 1243−49.

(13) Siegwart, D. J.; Whitehead, K. A.; Nuhn, L.; Sahay, G.; Cheng, H.; Jiang, S.; Ma, M. L.; Lytton-Jean, A.; Vegas, A.; Fenton, P.; Levins, C. G.; Love, K. T.; Lee, H.; Cortez, C.; Collins, S. P.; Li, Y. F.; Jang, J.; Querbes, W.; Zurenko, C.; Novobrantseva, T.; Langer, R.; Anderson, D. G. Combinatorial synthesis of chemically diverse core-shell nanoparticles for intracellular delivery. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 12996−3001.

(14) Li, S.; Rizzo, M. A.; Bhattacharya, S.; Huang, L. Characterization of cationic lipid-protamine-DNA (LPD) complexes for intravenous gene delivery. *Gene Ther.* **1998**, *5*, 930−37.
(15) Sokolova, V.; Epple, M. Inorganic nanoparticles as carriers of nucleic acids into cells. *Angew. Chem., Int. Ed.* **2008**, *47*, 1382−95.
(16) Liu, Y.; Huang, L. Designer Lipids Advance Systemic siRNA Delivery. *Mol. Ther.* **2010**, *18*, 669−70.
(17) Shim, M. S.; Kwon, Y. J. Controlled delivery of plasmid DNA and siRNA to intracellular targets using ketalized polyethylenimine. *Biomacromolecules* **2008**, *9*, 444−55.
(18) Lee, J. S.; Green, J. J.; Love, K. T.; Sunshine, J.; Langer, R.; Anderson, D. G. Gold, poly(beta-amino ester) nanoparticles for small interfering RNA delivery. *Nano Lett.* **2009**, *9*, 2402−06.
(19) Duzgunes, N.; De Ilarduya, C. T.; Simoes, S.; Zhdanov, R. I.; Konopka, K.; Pedroso de Lima, M. C. Cationic liposomes for gene delivery: Novel cationic lipids and enhancement by proteins and peptides. *Curr. Med. Chem.* **2003**, *10*, 1213−20.
(20) Xiong, F.; Mi, Z.; Gu, N. Cationic liposomes as gene delivery system: Transfection efficiency and new application. *Pharmazie* **2011**, *66*, 158−64.
(21) Tian, H.; Tang, Z.; Zhuang, X.; Chen, X.; Jing, X. Biodegradable synthetic polymers: Preparation, functionalization and biomedical application. *Prog. Polym. Sci.* **2012**, *37*, 237−80.
(22) Zhang, J.; Li, X.; Li, X. Stimuli-triggered structural engineering of synthetic and biological polymeric assemblies. *Prog. Polym. Sci.* **2012**, *37*, 1130−176.
(23) Boyer, C.; Priyanto, P.; Davis, T. P.; Pissuwan, D.; Bulmus, V.; Kavallaris, M.; Teoh, W. Y.; Amal, R.; Carroll, M.; Woodward, R.; St Pierre, T. Anti-fouling magnetic nanoparticles for siRNA delivery. *J. Mater. Chem.* **2010**, *20*, 255−65.
(24) McAllister, K.; Sazani, P.; Adam, M.; Cho, M. J.; Rubinstein, M.; Samulski, R. J.; DeSimone, J. M. Polymeric nanogels produced via inverse microemulsion polymerization as potential gene and antisense delivery agents. *J. Am. Chem. Soc.* **2002**, *124*, 15198−15207.
(25) Whitehead, K. A.; Langer, R.; Anderson, D. G. Knocking down barriers: Advances in siRNA delivery. *Nat. Rev. Drug Discovery* **2009**, *8*, 129−38.
(26) Vaishnaw, A. K.; Gollob, J.; Gamba-Vitalo, C.; Hutabarat, R.; Sah, D.; Meyers, R.; de Fougerolles, T.; Maraganore, J. A status report on RNAi therapeutics. *Silence* **2010**, *1*, 14.
(27) Xu, L.; Anchordoquy, T. Drug delivery trends in clinical trials and translational medicine: Challenges and opportunities in the delivery of nucleic acid-based therapeutics. *J. Pharm. Sci.* **2011**, *100*, 38−52.
(28) O'Rorke, S.; Keeney, M.; Pandit, A. Non-viral polyplexes: Scaffold mediated delivery for gene therapy. *Prog. Polym. Sci.* **2010**, *35*, 441−58.
(29) Oh, J. K.; Tang, C.; Gao, H.; Tsarevsky, N. V.; Matyjaszewski, K. Inverse miniemulsion ATRP: A new method for synthesis and functionalization of well-defined water-soluble/cross-linked polymeric particles. *J. Am. Chem. Soc.* **2006**, *128*, 5578−84.
(30) Oh, J. K.; Siegwart, D. J.; Lee, H.-i.; Sherwood, G.; Peteanu, L.; Hollinger, J. O.; Kataoka, K.; Matyjaszewski, K. Biodegradable nanogels prepared by atom transfer radical polymerization as potential drug delivery carriers: Synthesis, biodegradation, in vitro release, and bioconjugation. *J. Am. Chem. Soc.* **2007**, *129*, 5939−45.
(31) Oh, J. K.; Bencherif, S. A.; Matyjaszewski, K. Atom transfer radical polymerization in inverse miniemulsion: A versatile route toward preparation and functionalization of microgels/nanogels for targeted drug delivery applications. *Polymer* **2009**, *50*, 4407−23.
(32) Peeler, J. C.; Woodman, B. F.; Averick, S.; Miyake-Stoner, S. J.; Stokes, A. L.; Hess, K. R.; Matyjaszewski, K.; Mehl, R. A. Genetically encoded initiator for polymer growth from proteins. *J. Am. Chem. Soc.* **2010**, *132*, 13575−77.
(33) Averick, S. E.; Magenau, A. J. D.; Simakova, A.; Woodman, B. F.; Seong, A.; Mehl, R. A.; Matyjaszewski, K. Covalently incorporated protein-nanogels using AGET ATRP in an inverse miniemulsion. *Polym. Chem.* **2011**, *2*, 1476−78.
(34) Oh, J. K.; Drumright, R.; Siegwart, D. J.; Matyjaszewski, K. The development of microgels/nanogels for drug delivery applications. *Prog. Polym. Sci.* **2008**, *33*, 448−77.
(35) Oh, J. K.; Siegwart, D. J.; Matyjaszewski, K. Synthesis and biodegradation of nanogels as delivery carriers for carbohydrate drugs. *Biomacromolecules* **2007**, *8*, 3326−31.
(36) Averick, S.; Simakova, A.; Park, S.; Konkolewicz, D.; Magenau, A. J. D.; Mehl, R. A.; Matyjaszewski, K. ATRP under biologically relevant conditions: Grafting from a protein. *ACS Macro Lett.* **2012**, *1*, 6−10.
(37) Matyjaszewski, K.; Xia, J. H. Atom transfer radical polymerization. *Chem. Rev.* **2001**, *101*, 2921−90.
(38) Matyjaszewski, K. Atom transfer radical polymerization (ATRP): Current status and future perspectives. *Macromolecules* **2012**, *45*, 4015−39.
(39) Xu, F. J.; Yang, W. T. Polymer vectors via controlled/living radical polymerization for gene delivery. *Prog. Polym. Sci.* **2011**, *36*, 1099−131.
(40) Averick, S.; Paredes, E.; Li, W. W.; Matyjaszewski, K.; Das, S. R. Direct DNA conjugation to star polymers for controlled reversible assemblies. *Bioconjugate Chem.* **2011**, *22*, 2030−37.
(41) Averick, S. E.; Paredes, E.; Grahacharya, D.; Woodman, B. F.; Miyake-Stoner, S. J.; Mehl, R. A.; Matyjaszewski, K.; Das, S. R. Protein-polymer, A hybrid mediated by DNA. *Langmuir* **2012**, *28*, 1954−58.
(42) Nese, A.; Kwak, Y.; Nicolay, R.; Barrett, M.; Sheiko, S. S.; Matyjaszewski, K. Synthesis of poly(vinyl acetate) molecular brushes by a combination of atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization. *Macromolecules* **2010**, *43*, 4016−19.
(43) Nese, A.; Lebedeva, N. V.; Sherwood, G.; Averick, S.; Li, Y. C.; Gao, H. F.; Peteanu, L.; Sheiko, S. S.; Matyjaszewski, K. pH-Responsive fluorescent molecular bottlebrushes prepared by atom transfer radical polymerization. *Macromolecules* **2011**, *44*, 5905−10.
(44) Siegwart, D. J.; Oh, J. K.; Matyjaszewski, K. ATRP in the design of functional materials for biomedical applications. *Prog. Polym. Sci.* **2012**, *37*, 18−37.
(45) Jaeger, W.; Bohrisch, J.; Laschewsky, A. Synthetic polymers with quaternary nitrogen atoms: Synthesis and structure of the most used type of cationic polyelectrolytes. *Prog. Polym. Sci.* **2010**, *35*, 511−77.
(46) Tsarevsky, N. V.; Matyjaszewski, K. Reversible redox cleavage/coupling of polystyrene with disulfide or thiol groups prepared by atom transfer radical polymerization. *Macromolecules* **2002**, *35*, 9009−14.
(47) Roy, D.; Cambre, J. N.; Sumerlin, B. S. Future perspectives and recent advances in stimuli-responsive materials. *Prog. Polym. Sci.* **2010**, *35*, 278−301.
(48) Oliveira, M. A. M.; Boyer, C.; Nele, M.; Pinto, J. C.; Zetterlund, P. B.; Davis, T. P. Synthesis of biodegradable hydrogel nanoparticles for bioapplications using inverse miniemulsion RAFT polymerization. *Macromolecules* **2011**, *44*, 7167−7175.
(49) Anderson, M. E. Glutathione: An overview of biosynthesis and modulation. *Chem. Biol. Interact.* **1998**, *111*, 1−14.
(50) Manickam, D. S.; Li, J.; Putt, D. A.; Zhou, Q. H.; Wu, C.; Lash, L. H.; Oupicky, D. Effect of innate glutathione levels on activity of redox-responsive gene delivery vectors. *J. Controlled Release* **2010**, *141*, 77−84.
(51) Rogers, S. L.; Rogers, G. C. Culture of Drosophila S2 cells and their use for RNAi-mediated loss-of-function studies and immunofluorescence microscopy. *Nat. Protoc.* **2008**, *3*, 606−11.
(52) Arigita, C.; Zuidam, N. J.; Crommelin, D. J. A.; Hennink, W. E. Association and dissociation characteristics of polymer/DNA complexes used for gene delivery. *Pharm. Res.* **1999**, *16*, 1534−41.
(53) Ruby, J. G.; Jan, C. H.; Bartel, D. P. Intronic microRNA precursors that bypass Drosha processing. *Nature* **2007**, *448*, 83−6.