Biotechnol. Prog. **2005,** *21,* 532–537

Cellular Fate of a Modular DNA Delivery System Mediated by Silica Nanoparticles

Richard A. Gemeinhart,* Dan Luo,‡ and W. Mark Saltzman†

School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, New York 14850-5201

Development of efficient molecular medicines, including gene therapeutics, RNA therapeutics, and DNA vaccines, depends on efficient means of transfer of DNA or RNA into the cell. Potential problems, including toxicity and immunogenicity, surrounding viral methods of DNA delivery have necessitated the use of nonviral, synthetic carriers. To better design synthetic carriers, or transfection reagents, the modular design of viruses has inspired a modular approach to DNA and RNA delivery. Each modular component can be designed to circumvent each of the many barriers. The modular approach will allow modification of individual components for a specific application. By utilizing a dense silica nanoparticle to form a ternary complex, transfection efficiency of a DNA−transfection reagent complex was increased by a factor of approximately 10 by concentrating the DNA at the surface of cells. Surface modification of the silica nanoparticles allowed determination of the cellular uptake mechanism with only minor alteration of transfection efficiency. Nanoparticles are internalized by an endosome-lysosomal route followed by perinuclear accumulation. The modification mechanism confirms that surface modification of the modular system can allow specific moieties to be incorporated into the modular system without significant alteration of the transfection efficiency. By showing that the modular system based upon concentration of DNA at the level of the cell can be used to increase transfection efficiency, we have shown that further modification of the system may better target DNA delivery and overcome other barriers of DNA expression.

Introduction

Gene therapeutics, siRNA therapeutics, anti-sense DNA therapeutics, and DNA vaccines are dependent upon delivery of poly(nucleic acid) sequences. The most effective methods currently used are viral in origin (infection). Synthetic procedures (transfection) of poly(nucleic acid) delivery have many advantages over infection mechanisms. These advantages include a lack of immunogenicity and low cytotoxicity. Unfortunately, the efficiency of synthetic delivery methods do not compare to viral systems (*1*). To overcome this deficiency, many groups are examining methods to increase the efficiency of transfection (*2*).

Chemical transfection mechanisms, including lipid-based, polymer-based, and chemical-based systems, are typically based upon two-component systems where the carrier, or transfection reagent, is complexed with DNA. Regardless of the transfection reagent, DNA is condensed by a cationic moiety, which protects the DNA in the extracellular milieu and masks the charge of the DNA to allow cellular uptake. The mechanism of uptake of DNA is different for each transfection reagent, but many transfection reagents are internalized by cells by endosomal uptake. The end fate of endosomal contents is the secondary lysosome having a pH of approximately 5.0 and elevated protease and nuclease activity. Several transfection reagents co-opt the endosomal-lysosomal transition to allow DNA to enter the cytoplasm by a mechanism described as a proton sponge (*3*). Both poly(amido amine) dendrimers and fractured poly(amido amine) dendrimer enhance lysosomal escape of DNA (*4*, *5*). Dendrimer and fractured dendrimer also enhance nuclear penetration of DNA in many cells, potentially due to the cationic nature that is similar to natural nuclear localization sequences, although there is some disagreement about the specificity of the localization (*6*). This transfection reagent, as well as others, exhibit several methods of enhancement of DNA expression but typically have one major and several minor methods of enhancement (*7*).

Even with the multiple levels of enhancement that typical transfection reagents exhibit, no chemical method of DNA delivery rivals the virus. A virus contains many levels of cellular and intracellular targeting that synthetic systems lack. A virus, however, does not use a single component to accomplish all of the extracellular and intracellular targeting necessary to accomplish the high level of expression. A virus typically includes cell recognition epitopes, endosomal disruptive peptides, intracellular translocation domains, and nuclear localization signals. Each aspect of the virus is modular and slight variations in each module contribute to the many subtypes of viruses including the variations of the common cold. By slightly modifying the modules for replica-

* To whom correspondence should be addressed. Present Address: 833 South Wood Street (MC 865), Departments of Biopharmaceutical Sciences and Bioengineering, University of Illinois, Chicago, IL 60612-7231. Tel: (312) 996-2253. Fax: (312) 996-2784. Email: rag@uic.edu.

† Present address: Department of Biomedical Engineering, Yale University, P.O. Box 208284, New Haven, CT 06520.

‡ Present Address: Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853-5701.

10.1021/bp049648w CCC: $30.25
Published on Web 01/26/2005

tion, the virus is able to overcome our cellular barriers. Borrowing the modular aspect of a virus, a ternary, modular DNA delivery system has been developed utilizing DNA, dendrimer, and silica nanoparticles (*8*, *9*). The specifics of the system have been guided by a mathematical model that describes optimal nanoparticle number and size. Silica nanoparticles are utilized due to their dense nature which concentrated DNA at the surface of cells growing in static culture. Concentration of DNA allows efficient DNA uptake by an endosomal-lysosomal route, as confirmed earlier by the temperature dependence of transfection efficiency (*8*). It has been established that DNA does enter cells (*10*) but which components of the complex and the end fate of this complex have not been examined. This study examined a method for modifying the surface of the silica nanoparticles to determine the fate of the silica following concentration of DNA at the surface of the cell. The primary objective of the work was to determine the fate of the nanoparticles, thus a fluorescent imaging agent was incorporated on the nanoparticles. It was, however, recognized that the modification could alter the transfection efficiency which predicated examination a series of modified nanoparticles. Based upon these studies, this modification scheme could be utilized for adding a cellular targeting or internalization signal which would further build upon the modular nature of this system.

Materials and Methods

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Nanosphere Labeling. Silica nanoparticles (Polysciences, Inc., Warrington, PA) were concentrated by centrifugation at 3500 rpm for 5 min in a glass conical centrifuge tube, followed by repeated washing with 100% ethanol. Silane solutions (United Chemical Technologies, Bristol, PA) of aminopropyl triethoxy silane (APTES) and methyl triethoxy silane (MTES) were added to the nanoparticles and allowed to incubate at room temperature for at least 2 h (See Supporting Information for scheme). All modifications used constant molar concentration of silane with each modification having differing amounts of APTES and MTES; A100M0 was modified in a solution of 100% APTES, and A50M50 was modified by 50% APTES and 50% MTES on a molar basis. The nanoparticles were recovered and washed repeatedly in ethanol followed by water. This resulted in nanoparticles with varying surface amino and methyl group content. Fluorescent labeling of the nanoparticles with Alexa Fluor 488 (AF488) was conducted using a slight modification of the manufacturer's protocol. AF488-succinimide (Molecular Probes, Eugene, OR) was dissolved in phosphate buffered saline and nanoparticles were added. After 2 h, the dispersion was added to a conical glass centrifuge tube and recovered. Multiple washings with deionized water were used to remove unbound AF488 and succinimide. Nanoparticles were sterilized in 70% ethanol and then maintained under sterile conditions for transfection.

Characterization of Nanoparticles. Nanoparticles were examined following drying of an aqueous sample on appropriate sample stubs. Samples were coated with 10 nm palladium/gold (Desk II, Denton Vacuum Sputter Coater) prior to examination (Stereoscan 440, Leica Cambridge, Cambridge, UK). A minimum of 1000 particles in at least 5 random areas have been measured from three samples of each modification to determine the size of the nanoparticles. Dilute suspensions of nanoparticles in phosphate buffered saline were sampled (Lazer Zee Meter, Pen Kem, Inc., Bedford Hills, NY) to determine ζ-potential and compared to standard potential samples (Laszlo Kovaks, Mohegan Lake, NY.). The relative amine content of each sample was also determined using the ninhydrin colorimetric assay with glycine as a standard (*11*).

Cell Culture. Chinese hamster ovarian cells (CHO; ATCC no. CCL-61, Manassas, VA) were cultured in DMEM medium with 10% fetal calf serum (Sigma, St. Louis, MO) at 37 °C in 5% CO_2. For subculture and flow cytometry, the cells were trypsinized, centrifuged, and suspended at appropriate concentrations in media or phosphate buffered saline.

Transfection. Transfections with partially fractured dendrimer (SuperFect; Qiagen, Valencia, CA) were carried out according to manufacturer's protocols with one additional step added for addition of silica nanoparticles. Briefly, cells were seeded at a density of 1×10^4 cells per well in 96-well plates. Twenty-four hours later, plasmid DNA (p-Vax-LacZ1; Invitrogen, Carlsbad, CA) was mixed with dendrimer for 5 min at room temperature. Then, the dendrimer−DNA complexes were incubated with silica nanoparticles for at least 5 min and added to cells in log-growth phase for 2 h under normal culture conditions. Following aspiration of the DNA−dendrimer−silica complex, cells were cultured at 37 °C and 5% CO_2 for 48 h in fresh growth medium.

Cells were then lysed using MPER (Pierce, Rockford, IL) and split onto two plates. On the first plate, the enzyme activity was assayed using β-gal assay kit (Promega, Madison, WI) and compared to β-gal protein standards. The second plate was used to determine total protein content of cells as a method of standardizing protein production. Total cellular protein was determined using the bicinchoninic acid (BCA) technique (Pierce) as described by the manufacturer (*12*). The transfection efficiency was determined according to eq 1, where the control is SuperFect-DNA transfected cells. This efficiency is proportional to the theoretical efficiency as previously described (*8*, *9*).

$$E = \frac{[\beta\text{-gal}]_{\text{sample}}}{[\text{protein}]_{\text{sample}}} \frac{[\text{protein}]_{\text{control}}}{[\beta\text{-gal}]_{\text{control}}} \qquad (1)$$

Flow Cytometry. Silica nanoparticles were diluted to 5×10^4 particles/mL in phosphate buffered saline and examined by flow cytometry to confirm fluorescent content (FACS Calibur Benchtop Flow Cytometer, Becton Dickinson). At least 10 000 nanoparticles were counted for each condition and 3 independent runs of each sample were reviewed. Equivalent conditions and system parameters were used for each test in order to obtain quantitative data of fluorescent activity of each run.

Lysosomal Tracking and Microscopy. A pH-sensitive dye, LysoTracker Red (Molecular Probes), was used to determine the location of lysosomes within cells. The manufacturer's protocol was observed without modification. Briefly, at specific time points, dye was diluted in serum-free media. Media was removed from cells and the dye-containing media was incubated on the cells for 1 h at 37 °C. The dye-containing media was aspirated and fresh serum containing media was place on the cells.

Living cells were examined in fluorescent and light microscopy (BX-50; Olympus) and gray scale images were acquired with CCD camera (Pentax) using standard optical filters for the specified fluorescent probe. Images were analyzed and pseudocolor images produced using MetaMorph software.

Table 1. Change in ζ-Potential and Amine Content Following Modification of Silica Nanoparticles with APTES/MTES (without AF488) and with APTES/MTES and AF488 (with AF488)[a]

	change in ζ-potential[b] (mV)		amine content[c] (μmol/g)	
sample	without AF488	with AF488	without AF488	with AF488
A100M0	+15.9 ± 1.3	+8.1 ± 1.0	321 ± 13	294 ± 10
A75M25	+11.3 ± 1.0	+7.3 ± 1.0	203 ± 5	191 ± 5
A50M50	+10.3 ± 1.3	+9.9 ± 1.1	111 ± 4	105 ± 1
A25M75	+11.5 ± 1.1	+7.2 ± 1.6	18 ± 2	22 ± 2
A0M100	+5.8 ± 1.0	+0.0 ± 1.0	0 ± 1	1 ± 3

[a] All measurements are the average ± SD of three independent samples and were taken at pH 7.4 in standard phosphate buffered saline. [b] Change in ζ-potential is presented as increase in ζ-potential compared to that of unmodified nanoparticles [c] Amine content is presented as equivalent moles of glycine per mass of silica nanoparticles.

Results

Modification of Nanoparticles. Chemical modification of silica nanoparticles using silane reagents resulted in an increase in ζ-potential and amine content for all samples compared to the unmodified nanoparticles (Table 1). Following fluorescent conjugation, silica nanoparticles also had an increase in ζ-potential compared to unmodified control. There was, however, a decrease in ζ-potential compared to the nonfluorescent silica nanoparticles at a specified modification. Silica nanoparticles modified with 100% MTES had a slight increase in ζ-potential, and with fluorescent modification no change in ζ-potential. The intermediate APTES modifications (A75M25, A50M50, and A25M75) did not have significantly different ζ-potential despite significantly greater amine content as evidenced by amine density on the nanoparticles. A slight decrease in amine content was found when AF488 was added to the nanoparticles for all modified nanoparticles.

The size of the nanoparticles was not altered following modification using silane or fluorescent molecules. Unmodified nanoparticles and A50M50-AF488 nanoparticles were not significantly different in size (Figure 1). All nanoparticles has a mean radius of 230 ± 10 nm. Images of all samples were examined, but only A50M50-AF488 modified and unmodified silica nanoparticles are presented for brevity.

Fluorescent dye was confirmed to be present on silica nanoparticles using light microscopy in initial studies. Little background fluorescence was present and individual nanoparticles were visualized indicating that the nanoparticles were completely dispersed. After several periods, the AF488-modified nanoparticles were sedimented using centrifugation and the supernatant was collected and examined qualitatively using a UV-lamp. Little fluorescent content was present in the supernatants at one month, and the silica nanoparticles were clearly fluorescent at all times.

To further confirm fluorescence on all nanoparticles, flow cytometry was utilized. Unlabeled nanoparticles were examined by flow cytometry, and no fluorescent signal was detected greater than 2 arbitrary fluorescent units. Silica nanoparticles with APTES or MTES modification exhibited similar lack of fluorescent presence to the nanoparticles as received from the vendor. Silica nanoparticles labeled with only MTES did not exhibit any fluorescence even after undergoing the conjugation reaction with AF488 (Figure 2A). When APTES was used in any combination with MTES followed by conjugation of AF488, all nanoparticles exhibit a fluorescent character

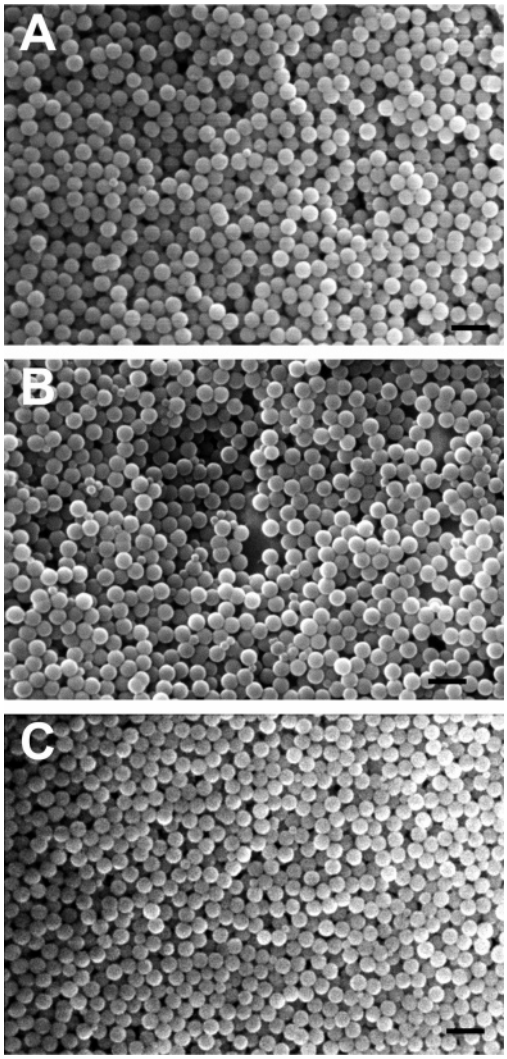

Figure 1. Representative scanning electron micrographs of unmodified silica nanoparticles (A) and 50% aminopropyl triethoxy silane, 50% methyl triethoxy silane modified silica nanoparticles before (B) and after Alexa Fluor 488 conjugation (C). Scale bar is 1 μm.

with no nanoparticle having a fluorescent intensity less than 9 arbitrary fluorescent units (Figure 2B−E). The modification of the four APTES-containing nanoparticles was not significantly different as each nanoparticle modification exhibited an average fluorescence of approximately 50 arbitrary fluorescent units and a range from 8 to 110 arbitrary fluorescent units.

Transfection Efficiency. Silica nanoparticle−DNA−dendrimer complex has been shown to increase transfection efficiency by an order of magnitude (*8*, *9*). The modified silica nanoparticles were equally able to increase the efficiency of transfection, the degree of increase varied little between the modifications. Following prior protocols, the efficiency of modified silica nanoparticles was similar to the unmodified nanoparticles. The efficiency enhancement obtained with unmodified nanoparticles at pH ∼7.4 was between 4 and 5 which was below that obtained for unmodified nanoparticles. It was expected that ionic interaction of the nanoparticle, DNA, and dendrimer contributed to the decrease in efficiency. To determine if the ionic nature of the complex contributed to the transfection efficiency, complexes were formed in media at various pH values prior to transfection which will alter the ionic interactions of the complex. Modified nanoparticles (A50M50-AF488) exhibited an efficiency

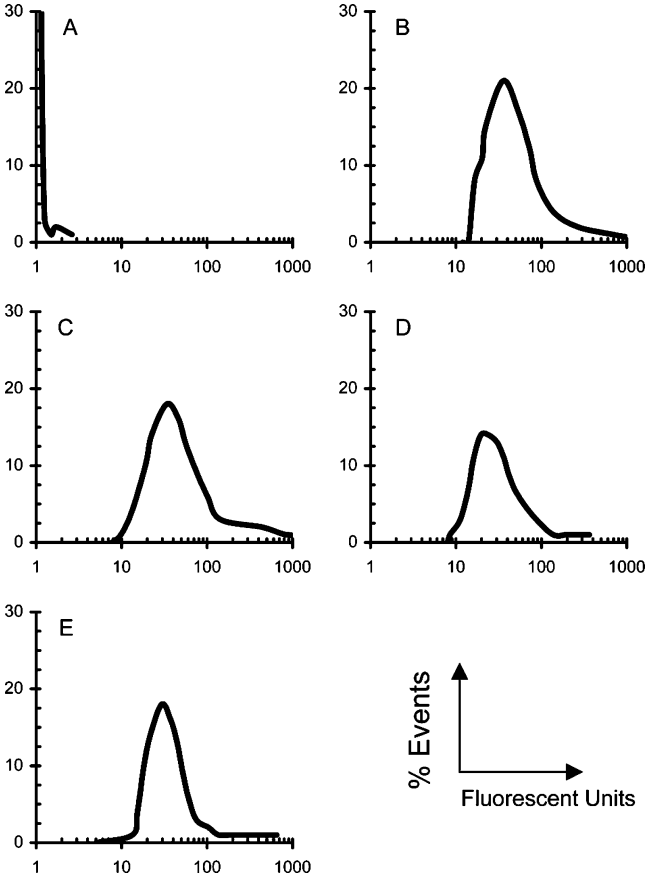

Figure 2. Flow cytometry analysis of modified silica nanoparticles. Each silica nanoparticle formulation was modified with aminopropyl triethoxy silane (APTES) and/or methyl triethoxy silane (MTES): mole fraction of APTES of 0.0 (A), 0.25 (B), 0.5 (C), 0.75 (D), and 1.0 (E). Following silane treatment all samples shown were modified with Alexa Fluor 488 succinimide. Unmodified silica particles exhibited phenomena similar to curve A. A single experiment is presented, but all samples were examined in triplicate.

enhancement of approximately 4.75, but the efficiency enhancement was greatly increased by conducting the complexation at a slightly lower pH (Figure 3). At a pH of 6.5, the efficiency was actually greater than unmodified silica nanoparticles (*8*, *9*).

Cellular Internalization. Light microscopy confirmed that nanoparticles were associated with CHO cells (Figure 4). The fluorescent nanoparticles localized in regions near, but not in the nucleus of the cell. Exact number of nanoparticles cannot be determined using this method, but from intensity and distribution of nanoparticles, many cells do take up multiple nanoparticles. There is a wide distribution of nanoparticle number in the cells with some cells exhibiting no fluorescence and other cells exhibiting a very high fluorescence. Since all nanoparticles are AF488 labeled, cells that do not exhibit fluorescence do not contain any nanoparticles.

The method of internalization of nanoparticles, and complexed DNA, was confirmed to be via an endosomal-lysosomal route (Figure 5). Nanoparticles (Figure 5A, green) are present in many cells and tend to concentrate in specific areas of the cell. A decreased pH is identified by a red-white fluorescence of the lysosomal dye, LysoTracker Red. Lysosomes are identified as concentrated fluorescence in a localized area of the cell (Figure 5B, red). By merging the pseudocolor images of nanoparticles (green) and lysosomal dye (red), an image was obtained showing the overlap of red and green (yellow).

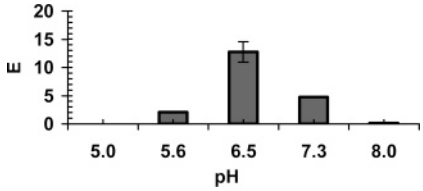

Figure 3. Efficiency of transfection of CHO cells with DNA–silica nanoparticle–dendrimer ternary complex. Silica nanoparticles were modified with 50% aminopropyl triethoxy silane, 50% methyl triethoxy silane modified silica nanoparticles followed by Alexa Fluor 488 conjugation. The pH of the complexation media is indicated, and the cells were transfected in a mixture of the complexation media and media containing serum. Efficiency (*E*) is the ratio of protein production per total protein production of the treatment and the protein production per total protein production of dendrimer–DNA complex treated cells; therefore, dendrimer–DNA complex has an efficiency of 1.0.

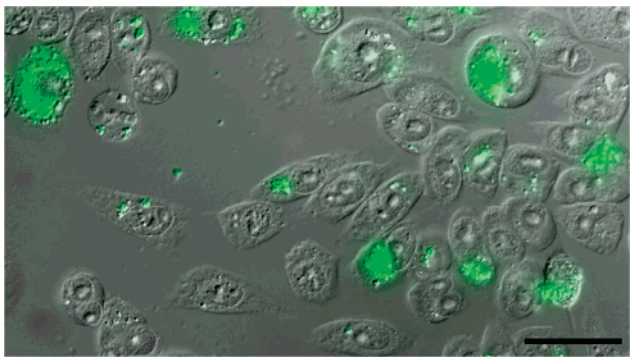

Figure 4. Pseudocolor DIC and fluorescent microscopy image of CHO cells transfected with A50M50-AF488 silica nanoparticle–DNA–dendrimer ternary complex. The green fluorescence is due to AF488 conjugated to the silica nanoparticles.

Nanoparticles are almost exclusively located in regions that were stained to be lysosomes. Many lysosomes do not contain nanoparticles, but a localization of nanoparticles near the nucleus in regions of acidity is marked. Again, no nanoparticles are present in the nuclear regions of the cells indicating that the nanoparticles do not enter the nucleus but plasmid DNA was able to enter the nucleus.

Discussion and Conclusions

In this study, a ternary transfection system was investigated which localized DNA near the surface of cells and enhanced protein production with little toxicity. This self-assembled, ternary system did not adversely increase transfection time or toxicity. Nanoparticles sediment DNA under culture conditions, greatly enhancing the transfection efficiency of the dendrimer used. Earlier studies have confirmed that this enhancement is nearly universal in nature (*8*, *9*). Luo et al. proposed a theory and a model for the efficiency enhancement, but neither the theory or model necessitated cellular uptake of the sedimentation agent (nanoparticles). We hypothesized that nanoparticles internalization and transport was responsible for the transfection efficiency increase. To test this hypothesis, nanoparticles were fluorescent labeled. Since the fluorescent label methodology may influence transfection, a series of modified nanoparticles were examined under different transfection conditions.

The earlier studies also suggested an endosomal-lysosomal mechanism of uptake of DNA–dendrimer complex which is confirmed by these studies. The nanoparticles have been shown by flow cytometry and confocal microscopy to enter cells (*10*), but the mechanism or

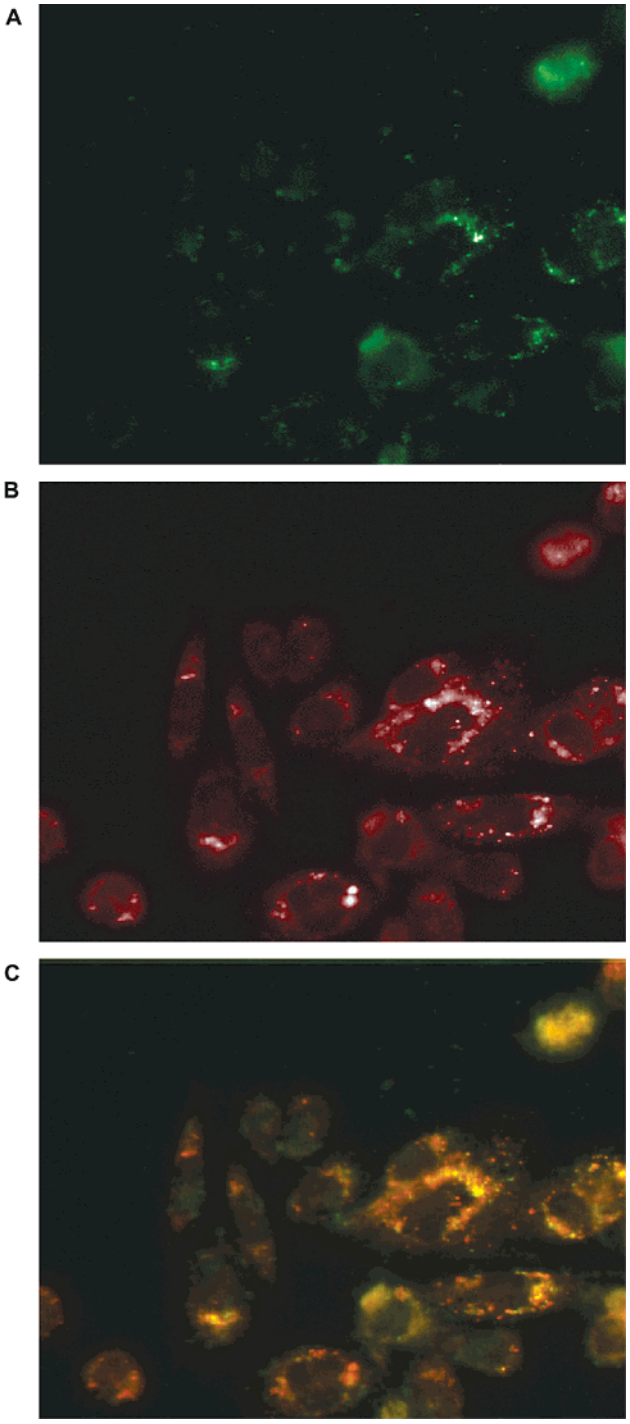

Figure 5. Pseudocolor fluorescent micrographs of CHO cells transfected with A50M50-AF488 silica nanoparticle−DNA−dendrimer complex and labeled with LysoTracker Red. The silica nanoparticles (green) are associated with punctuate regions in the cells (A) and the lysosomes of the cells (red) are also punctuate in these cells. The combined image indicated greatest combined intensity in the lysosomal compartments (C).

extent was not known. In fact, until this study, no confirmation of location or method of uptake of nanoparticles had been published. Cellular uptake of DNA and nanoparticles has been confirmed by light microscopy and fluorescent markers for organelles, specifically lysosomes and endosomes. The mathematical model presented in earlier studies only describes the sedimentation affect on transfection efficiency or the chemistry of the nanoparticle. A later modification did take into account possible interactions of the chemistry of the nanoparticle with the DNA−transfection reagent complex, but this still does not account for cellular internalization or cellular localization effects. Since the chemistry of the nanoparticle may affect cellular internalization as well as complex formation and complex dissociation the model will need to be adapted to accurately account for these affects. Unfortunately, our current study does not identify at which point the silica nanoparticle−DNA−dendrimer complex dissociates. By examining the localization of the nanoparticles, DNA, and dendrimer, the dissociation of the complex could be examined; however, this has not been done at this point. Since protein is produced, the complex does dissociate to some degree in the cells as evidenced by differing locations of DNA (nuclear) and the nanoparticles. Also, the end fate of the silica nanoparticles is perinuclear, the nanoparticles may play some role in cellular localization of complexed DNA. The mathematical model does not support or refute this possibility; only experimental determination of the temporal distribution of the components of the complex will definitively determine if the DNA is complexed with the nanoparticles at various points after entry into the cell.

Interestingly, other researchers have investigated silica nanoparticles as a stand-alone transfection reagent (*13*, *14*). In their systems, the nanoparticles were synthesized ranging in diameter from 10 to 145 nm. These nanoparticles were substantially smaller than the mathematical model would predict to be effective at transfection (*8*, *9*). The silica used by Kneuer et al. was modified in a manner similar to our experiments; however, a secondary amine and a primary amine were present, which alters the interaction of the DNA with the nanoparticles compared to a surface with only a primary amine. Therefore, the transfection efficiency of the silica nanoparticle-DNA-dendrimer or silica nanoparticle-DNA presented in this study cannot be compared directly with the results presented by Kneuer et al. Their result did, however, confirm that transfection could be accomplished with silica nanoparticle and that the toxicity of nanoparticles was minimal even at high concentration.

As confirmed by Kneuer et al., the surface chemistry did play a role in transfection; however, very little difference in transfection efficiency was observed between the different modifications. Since all nanoparticles maintained a negative ζ-potential, the interaction between the nanoparticles and the DNA−dendrimer complex is expected to be predominantly between the dendrimer and the nanoparticle. Some bridging of the complex is possible between the APTES groups and DNA, but it is expected that these are minimal due to the electronegativity of the nanoparticles. Regardless, the difference in ζ-potential between the nanoparticles is minimal when compared to the absolute value, −45 mV (*10*), are not significant, which explains the similarity in transfection efficiency between the unmodified and modified nanoparticles.

In conclusion, the use of modular transfection systems may be a better method of utilizing the ideas learned from the virus and should be applied to future DNA delivery systems. The virus uses several functionally independent tools to evade the human host, identify appropriate cells, enter the cells, escape the cellular defenses, enter the nucleus, and express the appropriate proteins necessary for reproduction. We have identified a method for improving the efficiency of an existing transfection reagent by simple complexation with a dense particle. The initial modification of the silica nanoparticles decreased transfection efficiency when the complexation was conducted in unmodified media; however, when the complexation media was appropriately buff-

ered, the transfection was greatly enhanced even when transfection was conducted at neutral pH. The chemical modification chosen is easy, reproducible, and can be utilized for further modification. Similar chemistry can be used to attach peptides, proteins, antibodies, or small molecules recognized by a specified cell type. The better choice of silane could even improve transfection efficiency by interacting with DNA in a manner similar to the transfection reagent. By utilizing a modular approach to DNA delivery, each of the barriers to expression of the desired protein can be breached individually by a single transfection complex. Tailoring the complex can be done at several levels each controlled in the individual laboratory or experiment for the cell type chosen. Therefore, the results obtained continue to confirm that a modular approach to DNA delivery may be useful in many aspects of DNA delivery including nonviral gene therapeutics, siRNA therapeutics, anti-sense DNA therapeutics, and DNA vaccines.

Acknowledgment

We thank Nadya Belcheva for helpful discussions, and Chris Anker and Ashwini Pai for technical assistance. This work was supported by the Cornell University Technology Development Fund (1999) and the National Science Foundation Grant 9986446.

Supporting Information Available: The scheme for silane modification of silica nanoparticles and the structures of APTES, MTES, and AF488. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Phillips, A. J. The challenge of gene therapy and DNA delivery. *J. Pharm. Pharmacol.* **2001**, *53*, 1169−1174.
(2) Luo, D.; Saltzman, W. M. Synthetic DNA delivery systems. *Nat. Biotechnol.* **2000**, *18*, 33−37.
(3) Boussif, O.; Lezoualch, F.; Zanta, M. A.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J. P. A Versatile vector for gene and oligonucleotide transfer into cells in culture and in-vivo−polyethylenimine. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7297−7301.
(4) Haensler, J.; Szoka, F. C. Polyamidoamine cascade polymers mediate efficient transfection of cells in culture. *Bioconjugate Chem.* **1993**, *4*, 372−379.
(5) Tang, M. X.; Redemann, C. T.; Szoka, F. C. In vitro gene delivery by degraded polyamidoamine dendrimers. *Bioconjugate Chem.* **1996**, *7*, 703−714.
(6) Chan, C. K.; Jans, D. A. Enhancement of polylysine-mediated transferrinfection by nuclear localization sequences: Polylysine does not function as a nuclear localization sequence. *Hum. Gene Ther.* **1999**, *10*, 1695−1702.
(7) Pouton, C. W.; Seymour, L. W. Key issues in nonviral gene delivery. *Adv. Drug Deliv. Rev.* **2001**, *46*, 187−203.
(8) Luo, D.; Saltzman, W. M. Enhancement of transfection by physical concentration of DNA at the cell surface. *Nat. Biotechnol.* **2000**, *18*, 893−895.
(9) Luo, D.; Han, E.; Belcheva, N.; Saltzman, W. M. A self-assembled, modular DNA delivery system mediated by silica nanoparticles. *J. Controlled Release* **2004**, *95*, 333−341.
(10) Guo, C.; Gemeinhart, R. A. Assessment of a modular transfection system based upon cellular localization of DNA. *Mol. Pharm.* **2004**, *1*, 309−316.
(11) Moore, S.; Stein, W. H. Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.* **1948**, *176*, 367−388.
(12) Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. Measurement of protein using bicinchoninic acid. *Anal. Biochem* **1985**, *150*, 76−85.
(13) Kneuer, C.; Sameti, M.; Haltner, E. G.; Schiestel, T.; Schirra, H.; Schmidt, H.; Lehr, C. M. Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA. *Int. J. Pharm.* **2000**, *196*, 257−261.
(14) Kneuer, C.; Sameti, M.; Bakowsky, U.; Schiestel, T.; Schirra, H.; Schmidt, H.; Lehr, C. M. A nonviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells in vitro. *Bioconjugate Chem.* **2000**, *11*, 926−932.

Accepted for publication December 8, 2004.

BP049648W