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Silica nanoparticles as a delivery system for nucleic acid-based reagents

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Abstract

The transport of nucleic acid-based reagents is predicated upon developing structurally stable delivery systems that can preferentially bind and protect DNA and RNA, and release their cargo upon reaching their designated sites. Recent advancements in tailoring the size, shape, and external surface functionalization of silica materials have led to increased biocompatibility and efficiency of delivery. In this Feature Article, we highlight recent research progress in the use of silica nanoparticles as a delivery vehicle for nucleic acid-based reagents.

Introduction

Silica has proven to be a valuable material for biomedical research. One application has been to use silica nanoparticles as drug delivery vehicles. In particular, mesoporous silica nanoparticles (MSNs) formed by polymerizing silica in the presence of surfactants have many advantages for intracellular delivery, such as large surface area, tunable pore sizes and volumes, and encapsulation of drugs, proteins and biogenic molecules. Moreover, they can be tailored with a variety of surface functional groups to increase biocompatibility and generate efficient and versatile agents for molecular delivery.¹ For controlled delivery systems, it has been shown that silica is able to store and gradually release therapeutics such as antibiotics^{2–4} and other clinically-relevant compounds.^{5–7} In our own lab, we have incorporated a hydrophobic anticancer drug, camptothecin (CPT), into the pores of fluorescent mesoporous silica nanoparticles (FMSNs) and delivered the drug into a variety of human cancer cells to induce cell death.⁵ We have suggested that MSNs might be used as a vehicle to overcome the insolubility problem of many anticancer drugs.⁵ Hybrids of silica and other organic/inorganic materials have also been successfully used in various applications, including enzyme encapsulation,⁸ magnetic bioseparations,^{9,10} magnetic labeling and imaging,^{11,12} and cell labeling and tracking.^{7,13,14}

Silica materials themselves have therapeutic potential based on their intrinsic properties. For example, in Saos-2 cells that are similar to osteoblasts, bio-silica matrices stimulate increased hydroxyapatite deposition. This indicates that synthesized bio-silica is a promising route for tooth reconstruction *in vivo*.¹⁵ Similarly, MSNs affect gene expression in human mesenchymal stem cells by inducing transient osteogenic signals.¹⁶ In addition, silica nanoparticles have been used to track cells. Nanoparticles conjugated to quantum dots and functionalized with amino and phosphonate groups have been used as probes to label T-lymphocytes. By conjugating them to luminophores, MSNs have been tracked and suggested to be effective delivery vehicles.¹⁷

Recent studies opened up the possibility of using MSNs as a delivery vehicle for nucleic acid-based reagents. This is important because it provides a new way to treat genetic diseases, illnesses caused by abnormalities in genes. Although they are too numerous to list, some well known genetic disorders are hemophilia, Huntington's disease, asthma, and cancer. Some diseases, such as cancer, are caused by both a genetic predisposition and environmental factors. A potential approach to the treatment of genetic disorders is gene therapy whereby a working gene replaces its dysfunctional counterpart, thereby curing inherited and acquired diseases. This enables the body to produce the appropriate gene products to eliminate the underlying cause of the disease. The treatment potential of nanoparticle-mediated gene therapy has been of particular interest in the field of nanomedicine and cancer.¹⁸ Current treatment methods, such as chemotherapy, rely on the use of cytotoxic drugs; however, such therapy has limited efficacy due to the use of suboptimal dosages of those therapeutic agents in attempt to prevent both acute and chronic, unwanted side-effects. Gene therapy is a welcome alternative treatment because it only targets the defects that gives rise to major symptoms, and therefore avoids complications associated with chemotherapy.

In order to bind negatively charged nucleic acids and improve uptake, silica materials are typically modified with positively charged organic adjuncts, such as poly-L-lysine (PLL)¹⁹ or polyethylenimine (PEI).^{20–22} PEI confers increased gene delivery to nanoparticles due to its 'proton sponge effect' allowing endosomal escape.^{21,23,24} To be an effective delivery system, silica nanocarriers must be biocompatible, possess high affinity for their particular payload, sequester their payload from the outer environment, and avoid premature release of their contents. DNA and RNA would decompose in the highly acidic environment of the stomach if the carrier could not offer the necessary protection. As a practical therapy, the nucleic acid carrier must not degrade or leak until it reaches its intended target, ensuring the release of high local concentrations of the cargo.²⁵

In this Feature Article, we discuss various attempts to deliver nucleic acid-based reagents using silica nanoparticles. They hold the promise to encapsulate and protect a payload of therapeutic compounds, transport them to specific locations in the body, and release them in response to either external or cellular stimuli. Thus, biocompatible silica nanoparticles represent a new solution to gene delivery.

Silica nanoparticles with external surface modifications are capable of transporting DNA to affect gene expression

In the past decade, viral-mediated delivery (infection) has been the primary method of introducing DNA into mammalian cells (Fig. 1(a)). However, due to growing concerns over the toxicity and immunogenicity of viral DNA delivery systems, DNA transport *via* non-viral routes has become more desirable and beneficial. For example, nucleic acid-based reagents were delivered through the use of polycation–DNA complexes by Wagner and colleagues.²⁶ Such cationic adjuncts were applied to silica nanoparticles to bind, protect, and deliver DNA (Fig. 1(c)). Synthesized silica nanoparticles with covalently linked cationic external surface modifications were produced through a variety of methods. Kneuer *et al.* produced them by modification of commercially available silica particles (IPAST) or in-house synthesized silica particles with either *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane or *N*-(6-aminoethyl)-3-aminopropyltrimethoxysilane.²⁷ These nanoparticles were sized between 10 and 100 nm and displayed surface charge potentials from +7 to +31 mV at pH 7.4.²⁷ He *et al.* synthesized amino-modified silica nanoparticles (45 ± 4 nm) by using the synchronous hydrolysis of tetraethoxysilane and *N*-(β-aminoethyl)-γ-aminopropyltriethoxysilane in water-in-oil micro-emulsion.²⁸ Tan *et al.* utilized a similar method to develop uniform core/shell nanoparticles (5–400 nm), consisting of a silica layer coating and magnetite core.²⁹ These particles are surface-modified externally so that disulfide coupling chemistry can be used for

immobilization of oligonucleotides onto silica nanoparticles.²⁹ All three of these nanoparticles possessed the ability to electrostatically bind, condense, and protect plasmid DNA from cleavage.^{27–29}

Another study has shown that colloidal silica particles with covalently attached cationic external surface modifications with aminoalkylsilanes could transfect plasmid DNA *in vitro* successfully.³⁰ β -Galactosidase was chosen as the genetic payload, and its activity following delivery in Cos-1 cells was used to measure transfection efficiency. The use of silica–silane–DNA nanoplexes resulted in elevated expression of their DNA cargo.³⁰ When chloroquine was used in conjunction with DNA nanoplexes, transfection rates increased.³⁰ This may be due to induction of endosomolysis or delaying endosomal decomposition.^{30,31} Mechanistically, silica nanoparticles concentrate DNA at the surface of cells. An elevated local concentration of DNA allows efficient DNA uptake by an endosomal–lysosomal route, as confirmed previously by temperature-dependent transfection efficiency.³² This suggests that nanocomplexes are internalized by endocytosis and routed to the endosomal/lysosomal compartment (Fig. 2).

A study demonstrated the importance of temperature- and energy-dependence in the uptake of FMSNs into human cancer cells.³³ Lower temperatures significantly impeded cellular uptake of FMSNs in PANC-1 cells, thus raising the possibility that FMSNs enter cells in an energy-dependent manner.³³ Metabolic inhibitors, including sodium azide (which depletes intracellular ATP), sucrose (which suppresses coated pit function) and bafilomycin A (which inhibits v-ATPase function), suppressed the uptake of FMSNs into PANC-1 cells.³³ This suggests that FMSN uptake is regulated by energy-dependent, clathrin-mediated endocytosis that depends upon a V-ATPase-dependent transport mechanism.³³ The endocytosis inhibitor, nocadazole, also significantly disrupted FMSN uptake, suggesting that a dynamic microtubule network is also necessary.³³

Polyamidoamine (PAMAM) dendrimer-capped mesoporous silica nanospheres have also successfully served as nonviral gene transfection agents.³⁴ PAMAMs were covalently attached to the external surface of the nanospheres, and then complexed with plasmid DNA (pEGFP-C1).³⁴ Agarose gel electrophoresis confirmed complexation between nanospheres and pEGFP-C1 and showed that the plasmid DNA is protected against enzymatic cleavage.³⁴ Significant GFP expression was observed in fluorescence confocal micrographs of human epithelial carcinoma cells (HeLa) treated with pEGFP-C1-carrying nanoplexes.³⁴ Thus, although there is a strong electrostatic interaction binding negatively charged DNA to positively charged PAMAM nanospheres, it is somehow overcome in mammalian cells to allow delivery and transgene expression. However, the mechanism that governs this release remains unclear.

DNA delivery has been accomplished in plants using silica nanoparticles

Research has been done to deliver DNA to plants through the use of nanoparticles. MSNs used for animal systems are not applicable to a plant system because of the plant cell wall. However, Torney *et al.* circumvented this problem by utilizing the gene gun system in order to trigger gene expression in plant cells and intact leaves.³⁵ Externally surface-modified MSNs with approximate diameter of 100–200 nm were used to bind plasmid DNA. The pores of these fluorescein-doped MSNs were capped by surface-functionalized gold nanoparticles (10–15 nm in size) which not only acted as a biocompatible capping agent,³⁶ but more importantly added weight to each individual MSN to increase the density of the resulting complex material. This increase in MSN density improved transformation efficiency and the appearance of MSNs inside plant cells.³⁵ The advantage of using MSNs with the gene gun is that both the DNA and small effector molecules can be delivered at the same time. Here, the chemical inducer that activates transgene expression (β -oestradiol) was contained inside the gold-capped structure. After bombardment into the plant cell, the effector molecules were released from the gold-

capped structure by incubating the plant tissues on media containing dithiothreitol, a chemical that reduces the disulfide bonds that attach the gold caps to the MSNs. The encapsulated β -oestradiol was subsequently released in a controlled manner to trigger the expression of co-delivered GFP transgene in the cell.^{35,37} Further customization of pore size and external surface functionalization may also lead to improved targeting and delivery of multiple compounds as well.

Adsorption of DNA into very large pores of silica nanoparticles

It has been reported that MCM-41-type MSNs can be internalized *in vitro* by animal and plant cells and show minimal signs of cytotoxicity.^{25,38} However, the largest pore diameter available up until now has been 6 nm,³⁹ which limits MSN's use as a transporter for large molecules such as proteins and nucleic acids. The advantage of widening the size of the pores of functionalized MSNs is a route to enhance the performance of the adsorption process.⁴⁰ A study was done using acid-prepared mesoporous silica (APMS) nanoparticles, which have a distinct spherical shape. Mg^{2+} , Ca^{2+} , and Na^{+} promoted DNA adsorption onto external silica surfaces by mediating the electrostatic repulsion between the negatively charged external silica surface and DNA molecules. The synthesis of APMS is complete in less than two hours, and the particle size and pore diameter of APMS are easily controlled simply by altering a set of standard reaction conditions. The diameter of the pore was found to affect the amount of DNA that could be loaded into APMS. Materials with pores greater than 5.4 nm were found to be more favorable toward DNA adsorption, as the molecules could likely enter the pores without significant intermolecular interactions. Likewise, other authors have reported on the benefit of widening the pore size of mesoporous materials for bioimmobilization processes.^{39–43}

Gao *et al.* synthesized MSNs with controlled diameter (~70–300 nm) and with very large, uniform regular pores of 20 nm (Fig. 1(d)) by a lower temperature (10 °C) synthetic method in the presence of a dual surfactant system.⁴⁴ After external surface modification with aminopropyl groups, very high adsorption of DNA molecules took place. Using spectrophotometry, the authors learned that their MSN was able to adsorb more DNA per unit surface area than any other previously reported silica-based materials. Also, because of the large pore size, intermolecular interactions among DNA molecules and diffusion limitations were expected to be minimized. Finally, these large pore MSNs also conferred protection from enzymatic degradation as confirmed by agarose gel electrophoresis following exposure to restriction endonucleases.⁴⁴

Organic/inorganic silica hybrid nanoparticles in DNA transport

Organic/inorganic hybrid particles (Fig. 1(e)) are attractive as delivery vectors because they can be loaded with either hydrophilic or hydrophobic biomolecules, their preparation avoids the use of hydrophobic solvents such as cyclohexane, their external organic groups prevent particle precipitation in aqueous systems, and their external surfaces can be modified with targeting molecules.¹⁴

ORMOSIL (organically modified silane) such as *n*-octyl-triethoxysilane has been found to aggregate in the form of normal micelles as well as reverse micelles in which the triethoxysilane moieties are hydrolyzed to form a hydrated silica network while the *n*-octyl groups are held together through hydrophobic interaction. These nanoparticles are spherical in shape with an average diameter of below 100 nm. Hybrids like ORMOSIL nanoparticles have the potential to overcome many limitations of their solely inorganic counterparts. The resulting micellar cores can be loaded with therapeutic compounds like drugs, proteins, and nucleic acids.⁴⁵

Hydrated ORMOSIL nanoparticles based on the triethoxyvinylsilane (VTES) precursor have been synthesized in the nonpolar core of dioctyl sodium sulfosuccinate (Aerosol-OT)/DMSO/

water microemulsions. Hybrid amino-functionalized ORMOSIL nanoparticles have also been synthesized by a synchronous hydrolysis of VTES and 3-aminopropyltriethoxysilane (APTES). By varying the concentrations of Aerosol-OT and VTES, nanoparticles of various sizes (10–100 nm) were produced.¹⁴

External surface amino functionalization allows these silica nanoparticles to electrostatically bind to negatively charged DNA and protect it from enzymatic degradation as shown by agarose gel electrophoresis.¹⁴ Binding between DNA and the amino adjuncts on the external surface of these ORMOSIL nanoparticles was also confirmed by FRET analysis.¹⁴ It was postulated that as soon as the genetic material is released into the cytoplasm of the cell, it migrates to the nucleus.⁴⁶ This was later confirmed by confocal microscopy with DNA labeled with EMA, a fluorescent dye, and loaded onto unlabeled hybrid nanoparticles. The fluorescent DNA was optically tracked as it was delivered into Cos-1 cells and subsequently to the nucleus.¹⁴ Finally, to confirm that the transfected DNA was still functional following transport, pEGFP was transfected into cells using hybrid nanoparticles and the resulting GFP fluorescence was confirmed by localized spectroscopy.¹⁴

Organic/silica hybrids as a non-viral vector for gene delivery have already been applied successfully to *in vivo* models. The same ORMOSIL nanoparticles, functionalized with amino groups and complexed with pEGFP, were used to counter neuropathy, stimulate compensatory mechanisms, and aid neurogenesis. The adult mammalian Central Nervous System (CNS) possesses limited potential to generate new neurons, making it vulnerable to long-term injury and a perfect candidate for gene therapy.⁴⁷ However, the subventricular zone (SVZ) of the lateral ventricle (LV) retains the capacity for neurogenesis.⁴⁸ The SVZ contains a population of neural progenitor cells that can potentially give rise to neurons, astrocytes, and oligodendrocytes.^{49–51} Neural stem/progenitor cells (NSPCs) of the SVZ have been shown to proliferate once treated *in vitro* with exogenous FGF2. This suggests that stimulation of FGF receptors by exogenous FGF could influence the proliferation of the NSPC cells *in vivo*.⁵² *In vitro*, it has been found that nuclear accumulation of FGFR1 accompanies differentiation of the neural progenitor cells and is sufficient to induce cell differentiation.^{47,53} The following study aimed to determine if the same was true in an *in vivo* model.

The ORMOSIL nanoparticles complexed with pEGFP were injected into mice ventral midbrain and into the lateral ventricle. This allowed the authors to visualize and track the successfully transfected neuronal cells in the substantia nigra and areas surrounding the lateral ventricle using confocal fluorescent microscopy.⁵⁴ Mice then received intraventricular injections of ORMOSIL/FGFR1 complexes followed by BrdUrd injection. BrdUrd injection was used in this study because it is likely to primarily tag the faster-proliferating progenitor cells, and thus serve as a marker for successful FGFR1 delivery by ORMOSIL nanoparticles. FGFR1 and BrdUrd immunostaining was found to be increased in the SVZ of mice transfected with FGFR1, indicating a change in the replication cycle of progenitor cells in the SVZ.⁵⁴ Thus, organic/silica hybrid nanoparticles can be used to deliver FGFR1 into mammalian cells.

MSNs coupled to mannosylated PEI (MPS) have also proven to be effective non-viral transfection agents. Mannosylated PEI (MP) was synthesized by a thiourea linkage reaction between the isothiocyanate group of α -D-mannopyranosylphenyl-isothiocyanate and the primary amine group of PEI. The particle sizes of MPS/DNA complexes were analyzed by dynamic light scattering to investigate the degree of compaction with DNA. The sizes ranged from 60 to 130 nm, and the morphology was spherical with little aggregation.⁵⁵

The purpose of MP functionalization was to target macrophage cells with mannose receptors and enhance transfection efficiency. These MPS were able to form complexes with DNA, protect against DNase I, and release DNA. Furthermore, the low cytotoxicity of MPS in Raw

264.7 macrophage cells and HeLa (a human cervical cancer cell line) suggests that it is a safe gene vector. MPS also led to greater transfection efficiency in macrophage cells over HeLa cells, exhibiting mannose receptor-mediated gene delivery.⁵⁵ Thus, MPS is another silica hybrid nanocontainer that can safely be used in gene therapy.

Silica nanoparticles can transport antisense oligonucleotides (ASOs)

Antisense oligonucleotides (ASOs) are short nucleotide sequences of DNA that are reverse complements of the nucleotide sequence of their target mRNA. They inhibit gene expression at both mRNA and protein levels by Watson–Crick base-pairing, where the oligo single-stranded DNA binds to its complementary mRNA.^{56–58} Like siRNA, ASOs show great potential as a molecular tool and therapeutic agent against diseases with underlying genetic components, such as viral infections and cancer. However, like siRNA, they show poor intracellular uptake and stability.^{59,60} They also interfere with normal cellular function in a non-sequence-specific manner.⁶¹ These deficiencies limit their use as potential therapeutics.

To overcome these obstacles, PLL-modified silica nanoparticles (PMS-NP) have been used to bind and protect ASOs. PMS-NP (20 ± 2 nm) were prepared in a microemulsion system, using polyoxyethylene nonylphenyl ether/cyclohexane/ammonium hydroxide.⁶² The delivery of PMS-NP-ASO complexes was evaluated in human nasopharyngeal carcinoma cells (HNEI) and HeLa cells and the results were compared with free ASOs by fluorescence microscopy and flow cytometry.⁶² The specific blocking effects of antisense constructs designed against the proto-oncogene, *c-myc*, were examined by determining mRNA levels with reverse transcription-PCR (RT-PCR). Furthermore, HNEI and HeLa cells were treated with various concentrations of PMS-NP in the presence of serum-free media, after which serum-containing media was added. The results indicated that PMS-NP displayed significantly low cytotoxicity.⁶² Only at concentrations >500 $\mu\text{g/mL}$ does PMS-NP show cytotoxic effects. Overall, PMS-NP complexes were able to bind, protect, and deliver ASOs to cells where they exerted ASO-specific gene inhibition.⁶²

In a follow-up study, positively charged amino silica nanoparticles (NH_2SiNPs) were also effective as ASO carriers.⁶³ The NH_2SiNPs were synthesized by a microemulsion method utilizing synchronous hydrolysis of tetraethyl orthosilicate (TEOS) and *N*-(β -aminoethyl)- γ -aminopropyltriethoxysilane (AEAPS). The NH_2SiNPs with an average diameter of 25 nm could combine with ASOs to form a bioconjugate favorable for cellular uptake. This was visualized by using fluorescein isothiocyanate (FITC)-labeled ASOs and NH_2SiNPs doped with rhodamine 6G isothiocyanate (RITC) as fluorescent signal detectors. Compared to liposomes, NH_2SiNPs were reported to be more biocompatible and had almost no cytotoxicity at the concentrations required for efficient transfection. Further, they were able to protect ASOs from degradation by DNase I.⁶³ MTT assays and western blot analysis showed that the NH_2SiNPs greatly improve the inhibition efficiency of ASOs in HeLa and A549 cells.⁶³

Silica nanoparticles may be useful for transporting small interfering RNA (siRNA)

Gene inhibition through targeted delivery of sequence-specific siRNA is a promising method of gene therapy. siRNA, or short interfering RNA, is a class of 20–25 nucleotide-long double-stranded RNA molecules that are involved in the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene. RNAi is a form of post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous to the silenced gene. siRNAs are generated from the cleavage of longer dsRNAs by the action of ribonuclease III family enzymes. The siRNA duplexes specifically suppress expression of genes in mammalian cells.⁶⁴

siRNA is expected to be a powerful tool to inhibit gene function because it is easily applicable to virtually any therapeutic target including intracellular and transcription factors. However, poor intracellular uptake, instability, and non-specific immune stimulation are obstacles associated with current methods of siRNA oligonucleotide delivery.⁶⁵ Because nanoparticles have been shown to be effective DNA vectors, it is only logical that they be applied to siRNA and antisense therapeutics. At the time that this Feature Article was written, silica nanoparticles have not been used to transport siRNA or antisense oligonucleotides. However, because of silica's biocompatibility and ease of functionalization, we expect numerous attempts to be made in the future.

So far, synthetic polymers have achieved success in *in vitro* and *in vivo* silencing. Cationic polymeric nanoparticles were produced by chemical synthesis of tripartite polymer conjugates. For example, the nanoparticles consisted of PEI that is PEGylated with an Arg-Gly-Asp (RGD) peptide ligand.⁶⁶ The resulting nanoplex was about 100 nm in size. The purpose of this conjugate was to target tumors expressing integrins and deliver siRNA inhibiting vascular endothelial growth factor receptor-2 (VEGF R2) and thereby inhibit tumor angiogenesis. Cell delivery and activity of this nanoparticle was found to be siRNA sequence-specific, depended on the presence of peptide ligand and could be competed by free peptide. Intravenous administration into tumor-bearing mice gave selective tumor uptake, siRNA sequence-specific inhibition of protein expression within the tumor and inhibition of both tumor angiogenesis and growth rate.⁶⁶ Similarly, cationic liposomal nanoparticles have been introduced into athymic mice with human xenograft tumors in which Raf-1, a protein serine/threonine kinase, is constitutively active. These liposomes delivered Raf-1 siRNA and showed anti-tumor efficacy in this *in vivo* model.⁶⁷

Use of silica nanoparticles to enhance transfection efficiency

An interesting example of the use of silica nanoparticles for gene delivery is to enhance chemical transfection protocols (Fig. 1(b)). The non-viral DNA delivery system can consist of DNA, transfection reagents, and nanoparticles as modular components. Each module can be designed to overcome roadblocks such as attenuated transfection efficiency, cellular uptake, cytotoxicity, and protein expression.⁶⁸ In this modular approach, silica nanomaterials are used as a transfection enhancer rather than the primary mode of delivery.⁶⁹ Dense inorganic silica nanoparticles, which by themselves do not deliver DNA, are able to enhance DNA transfection mediated by other commonly used transfection reagents.³² This three-component transfection system consists of silica nanoparticles, DNA, and transfection reagents. Each component functions so that barriers to DNA delivery, such as low uptake of DNA by cells or lack of nuclear targeting, can be tackled individually. The particular role of silica nanoparticles is to enhance uptake by physical concentration at the cell surface.³² Customizing the complex can be done at several levels, thereby conferring a wide-range of versatility to the use of silica materials in nucleic acid therapy.

SEM provided visual confirmation for the assembly of the three-component complex.⁶⁸ Only in the presence of DNA did the complex form, and although some aggregation of the particles was observed, complexation appeared to prevent this event from occurring. Agarose gel electrophoresis further showed that the DNA was retained by the nanoplex.⁶⁸

In Cos-7 cells, enhancement of transfection was monitored by the increase in β -galactosidase activity using plasmid DNA, pVax-LacZ1, as the genetic payload.⁶⁸ Transfection enhancement with silica nanoparticles and Superfect as the transfection reagent was dependent on the concentration and size of the particles. In addition, the degree of enhancement was dependent on the transfection reagent used.⁶⁸ Likewise, the three-component system developed by Gemeinhart *et al.* consisted of silica nanoparticles functionalized with silanes, DNA, and

dendrimers as transfection reagents to enhance lysosomal escape of DNA.^{70,71} Dendrimers also enhance nuclear penetration of DNA in many cells because of its cationic nature similar to nuclear localization signal sequences.⁷² β -galactosidase activity was again used to measure transfection efficiency, and silica nanoparticles were again found to elevate transfection efficiency.⁷³

Gemeinhart *et al.* elaborated upon the nanoparticle-uptake mechanism by hypothesizing that nanoparticle internalization and transport was responsible for increases in transfection efficiency.⁷³ By using fluorescently-labeled nanoparticles (230 ± 10 nm) in flow cytometry and confocal microscopy, they were able to track the nanoparticles during transfection. It had already been suggested by earlier studies that an endosomal/lysosomal uptake route was how nanoparticles gained entry into a cell.⁷⁴ This hypothesis was confirmed in this follow-up study by light microscopy and identified that fluorescently-labeled nanoparticles tend to localize near the nucleus of CHO cells.⁷³ Gemeinhart *et al.* confirmed through fluorescent micrographs that nanoparticles deliver nucleic acids by entering cells *via* an endosomal/lysosomal route. Specifically, they localize to lysosomes and endosomes as indicated by the overlap of LysoTracker red-labeled lysosomes and green nanoparticles.⁷³

Biocompatibility of silica nanoparticles

Biocompatibility is of utmost importance when designing a delivery system. Since intravenous (IV) administration appears to be the most promising route for nanoparticle delivery, any cytotoxic effects that nanoparticles may have should be eliminated. The hemolysis behavior of amorphous and MSNs (100–300 nm) was investigated in rabbit red blood cells.⁷⁵ It was found that amorphous nanoparticles, but not MSNs, showed hemolytic toxicity. However, replacement of external surface silanol groups on the amorphous nanoparticles with a positively charged 3-aminopropyl functionality reduced toxicity.⁷⁵ A prior study had also concluded that commercial colloidal and laboratory-synthesized silica nanoparticles (20–400 nm) cause no significant genotoxicity.⁷⁶

The biodistribution and urinary excretion of external surface-modified silica nanoparticles (SiNPs) has also been studied in mice *in situ* using *in vivo* optical imaging, *ex vivo* organ optical imaging, TEM imaging, and energy-dispersed X-ray spectrum analysis of urine samples.⁷⁷ IV administration of these SiNPs followed by fluorescence tracing *in vivo* indicated that silica nanoparticles (SiNPs) are all cleared from systemic blood circulation, but that both the clearance time and subsequent biological organ deposition are dependent on the external surface functionalizations of the SiNPs.⁷⁷ PEG-SiNPs exhibited comparatively longer blood circulation times and lower uptake by the reticuloendothelial system organs than OH-SiNPs and COOH-SiNPs. Bladder and excretion analysis revealed that all three types of IV-injected SiNPs with a size of ~45 nm were partly excreted.⁷⁷

Nanoparticle aggregation is a hurdle that needs to be overcome before nanoparticles can be applied *in vivo*. Clusters of nanoparticles may not be filtered and excreted in living organisms, leading to chronic toxicity and other negative side effects in biodistribution. In order to reduce aggregation of nanoparticles, one solution has been to include a final incubation period in 3-hydroxysilylpropyl methylphosphonate during the synthesis process of fluorescent mesoporous silica nanoparticles (FMSNs).³³ The external FMSN surface was modified with inert and hydrophilic phosphonate group to prevent aggregation caused by the interparticle hydrogen bonding interaction between the anionic silanol groups and the unreacted cationic amine groups.³³

Cationic modification, such as PEI or PLL, allow for the electrostatic binding of DNA to silica nanoparticles; however, these groups are themselves cytotoxic and peaks in transfection efficiency often correlate with pronounced reductions in cell viability.^{21,22,70,78} Thus far, the

processes that mediate *in vitro* toxicity of polycations are not understood. It has been proposed that the cytotoxicity and transfection efficiency of PEI is affected by its molecular weight and shape.^{22,79} Other studies have demonstrated that high molecular weight PEI exhibits high transfection efficiency and cytotoxicity, while low molecular weight PEI shows attenuated transfection efficiency and cytotoxicity.^{80–82} Recent approaches to reduce the toxicity of polymer-based transfection systems include the optimization of synthesis^{22,83} or the modification of the polymer side chains by glycolylation.⁸⁴ This is an ongoing process and research continues on how polycations and silica structures can be modified to improve their biocompatibility.

Conclusions and future directions

In this Feature Article, we describe in detail the recent progress of utilizing silica nanomaterials as a delivery vehicle for nucleic acid-based reagents. MSNs have the potential to transport multiple therapeutic reagents simultaneously, treating diseases with a multifaceted and integrative approach. In this way, tumors can be targeted through the transport of anti-cancer drugs, such as CPT, and the delivery of siRNA to inhibit the underlying genetic component of oncogenesis. The steps by which silica materials can be transformed into intracellular nanocarriers has already been established, although future improvements in targeting and controlled release mechanisms are necessary. We have described here the passive diffusion of DNA, siRNA, and antisense oligonucleotides out of silica nanoparticles to affect gene inhibition, but an active mechanism to drive their effusion from nanopores would improve their therapeutic efficiency. For example, MSNs modified by azo-benzene derivatives, capable of storing small molecules and release them following light irradiation, have been fabricated and characterized.⁸⁵ Another biocompatible controlled-release motif is the snap-top-covered silica nanocontainer (SCSN). In general, the snap-top contains guest molecules stored within nanopores, but release the guests following cleavage of the stopper cap.⁸⁶ Targeting to specific tissues is also at the forefront of current research, especially in cancer therapeutics since their use can potentially avoid adverse reactions resulting from systemic drug release and absorption. Finding a particular cancer surface marker that is not expressed in normal healthy tissue has retarded progress in this area. However, progress continues to be made. For instance, the targeting and imaging of MDA-MB-231 human breast cancer cells using arginine-glycine-aspartic acid (RGD) peptide-labeled fluorescent silica nanoparticles has been achieved.⁸⁷ We look forward to seeing many research breakthroughs that will lead to the further optimization of silica nanoparticles as a medium for gene therapeutics.

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Biographies

Christopher Hom received his BSc in molecular biology from Cornell University in 2006. He subsequently joined UCLA's Department of Biology and Chemistry in 2006 as a research assistant in tumor suppressor biology. He is currently a Ph.D. candidate in Fuyuhiko Tamanoi's research group in the Department of Microbiology, Immunology, and Molecular Genetics at

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Jie Lu received his M.D. degree from Tongji Medical University in China in 1996 and his Ph.D. in medical science from the University of Tokyo in Japan in 2005. He worked as a research resident and invited scientist at Japan National Cancer Center from 2002 to 2006. He joined Professor Tamanoi's lab in the Department of Microbiology, Immunology, and Molecular Genetics at UCLA in 2006. His current research centers on the development of controllable drug delivery nanomachines using mesoporous silica nanomaterials for cancer therapy in cancer cells as well as in animal models.

Fuyuhiko Tamanoi received his Ph.D. from Nagoya University in 1977, and carried out his postdoctoral studies at Harvard Medical School from 1977 to 1980. He was a senior staff investigator and section head at Cold Spring Harbor Laboratory from 1980 to 1985. He was appointed as an Assistant Professor and then Associate Professor at the University of Chicago. In 1994, he moved to UCLA and was promoted to Full Professor in 1997. He is currently Vice-Chair of the Dept. of Microbiology, Immunology & Molecular Genetics, and Research Director at California NanoSystems Institute. In that capacity, he oversees research programs and promotes new initiatives. His own work on nanotechnology involves development of mechanized nanoparticles for targeted delivery and controlled release of anti-cancer drugs.

Fig. 1. Schematic representations of the techniques used for delivery of nucleic acid-based reagents into plant and mammalian cells. Nucleic acid molecules theoretically bind to positively-charged chemical modifications on the external surface of MSNs and hybrid nanoparticles.

Fig. 2. Schematic of the temperature- and energy-dependent process of endocytosis mediated by clathrin-coated pits. The exact mechanism and timing surrounding the release of nucleic acids from MSNs (indicated by the number 8) is currently unknown; however, transgene expression has been detected indicating that a dissociation event has occurred.