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REVIEW

Mesoporous Silica Nanoparticles as a Carrier Platform for Intracellular Delivery of Nucleic Acids

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Abstract—Virus-mediated gene delivery has been, to date, the most successful and efficient method for gene therapy. However, this method has been challenged because of serious safety concerns. Over the past decade, mesoporous silica nanoparticles (MSNs) have attracted much attention for intracellular delivery of nucleic acids. Delivery of cellular plasmid DNA (pDNA) is designed to replace the function of a defective gene and restore its normal function in the cell. Delivery of small interfering RNAs (siRNAs) can selectively knockdown genes by targeting specific mRNAs. The biocompatibility and unique structures of MSNs make these nanoparticles ideal candidates to act as biomolecule carriers. This concise review highlights current progress in the field of nucleic acid delivery using MSNs, specifically for delivery of pDNA, siRNA, and combinatorial delivery of nucleic acids and drugs. The review describes important design parameters presently being applied to MSNs to administer drugs and therapeutic nucleic acids.

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Inorganic nanoparticles such as iron oxide and silica have been extensively studied for a variety of biological applications. These include use as clinical agents in imaging based detection, diagnosis, and treatment of diseases. Silica nanoparticles have gained much attention in biomedical applications because it is relatively easy to tune their physicochemical properties for specific purposes. For example, size, shape, and porosity can be easily controlled, while surface modifications can be introduced to alter the chemical properties of silica nanoparticles [1]. Silica nanoparticles have high biological stability and low toxicity, and the exposed silanol groups on their surface enable versatile functionalization and modifications [2]. Silica nanoparticles are divided into two major categories, solid and mesoporous silica nanoparticles (MSNs). The different types of silica nanoparticles, for example solid, mesoporous, shaped, etched, and hollow, are synthesized via different methods [3, 4]. MSNs show potential as biomolecule delivery vehicles because of their unique mesoporous structure and physical properties such as high surface area, large pore volume, tunable pore diameter, and narrow size distribution [5]. Importantly, MSNs have low immunogenicity, minimizing unfavorable inflammatory reactions [6]. Several reports have described the synthesis of MSNs, including by, for example, liquid crystal templating, evaporation-induced self-assembly, and sol–gel [7-9]. The major advantage of using synthetic MSNs is that their surface can be chemically modified to enhance their biomolecule cargo-carrying and other functional capacities [10].

MSNs have been used in a wide range of applications, most notably for bioimaging and drug delivery [11]. Furthermore, the cellular mechanisms enabling drug delivery by MSNs have been well studied. They involve stimuli-responsive triggers such as pH [12], redox-activation or redox-modulated motifs [13-15], and supramolecular switches [16] to enable selective and sustained drug delivery into the cell. Most studies to date have utilized doxorubicin [17-20] rather than other drugs such as desipramine, camptothecin, itraconazole, and ibuprofen [21-24] as a model anti-cancer molecule for studying MSN-mediated drug delivery into cells. In addition, combinatorial delivery of multiple drugs using MSNs has been demonstrated [25, 26].

There is growing evidence that MSNs can be used as nucleic acid carriers for gene therapy. MSNs, unlike other nanoparticles, possess the ideal qualities to serve as nucle-

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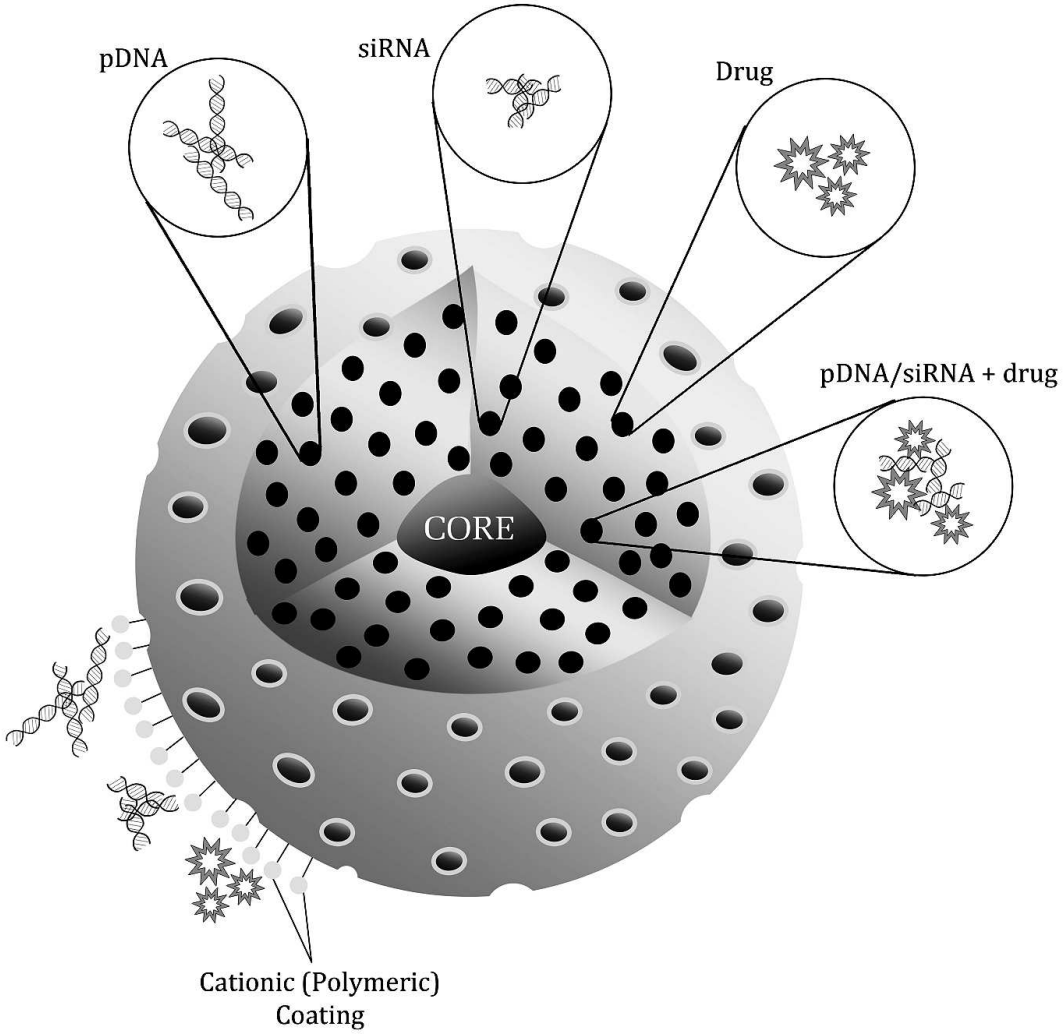
ic acid carriers. Several reviews have discussed the use of MSNs as a drug delivery platform in extensive detail [5, 11, 27-29]. With the growing body of evidence addressing use of MSNs for nucleic acid delivery, a review of this topic deserves special attention. Therefore, our concise review discusses current advances in the use of MSNs for gene delivery.

MSNs: A POTENTIAL INTRACELLULAR NUCLEIC ACID DELIVERY PLATFORM

Gene delivery introduces foreign nucleic acids into target cells solely for therapy. Plasmid DNAs (pDNAs) bearing essential genes to correct the function of a target gene (e.g. an oncogenic gene) have been used for gene therapy to treat certain diseases. Small interfering RNAs (siRNAs) are attractive as anti-cancer therapies because they can alter expression of activated oncogenes, cell cycle regulators, or proapoptotic genes, all potentially critical for tumorigenesis or tumor survival. Several studies have described attempts to load nucleic acids into MSN preparations for delivery into cells (figure).

pDNA delivery. Intracellular delivery of pDNA enhances expression of therapeutic genes by using a strong constitutive promoter to replace the defective gene and restore normal function. Several studies have investigated the feasibility of using monodispersed MSNs loaded with pDNA for delivery into cells (Table 1). Early work by Radu et al. [30] showed that a polyamidoamine (PAMAM) dendrimer coating provided a positively charged surface for complexing to DNA, while leaving the pores free to encapsulate drugs and dyes. This study also showed that MSNs were not toxic and that they pro-



Schematic representation of the general features of MSNs for nucleic acid and drug delivery. The surface is typically covered with cationic polymers or other molecules containing positively charged terminal functional groups to complex the nucleic acid. The pores of MSNs can hold nucleic acids, drugs, or a combination of both

tected the DNA against enzymatic cleavage. Other polymers including polyethyleneimine (PEI) [31] and poly(allylamine hydrochloride) (PAH) [32] are also excellent coatings to promote complexation of DNA on the MSN surface. Both cationic polymeric coatings enhanced DNA loading onto MSNs and transfection efficiency. These MSNs have pore sizes of less than 10 nm, and the smaller pore sizes cannot usually accommodate larger DNA molecules. Therefore, cationic surfaces, which help bind the negatively charged DNA, are introduced through positively charged terminal groups of polymers coating the nanoparticle surface. Other ways to introduce a positive charge on the MSN surface are to conjugate a small molecule or lipid containing terminal amine groups. Kim et al. [33] used aminopropyl-triethoxysilane (APTES) to impart a positive charge through its amine group, while Dengler et al. [34] used positively charged lipid bilayers. The surface charges for each system were +23 and +24 mV, respectively, and both systems had similar particle and pore sizes (200-230 nm particles, 2-5 nm pores). Similarly, both studies showed promising cellular transfection in applications related to stem cell and spinal cord therapy. Alternatively, histidine was used to functionalize MSNs [35]. Even though the resulting particles showed lower uptake *in vivo*, there was good internalization *in vitro* compared with that of amino-functionalized MSNs. Some studies have shown that a larger pore size (~up to 20 nm) could also improve DNA loading capacity, independent of cationic polymer coating [36, 37]. When compared to MSNs with smaller pores, the larger pore MSNs were shown to completely incorporate plasmids, while smaller pores had pDNA piled onto the MSN surfaces [36]. Another study showed that loading of pDNA into pores was enhanced for a larger pore MSN compared to those with smaller pores, even at a lower concentration of the large pore MSN [37]. While no particular surface charge has been shown to be the ideal, there is a consensus that a positive charge (at least +5 to +30 mV zeta potential before DNA complexation) is necessary for ensuring efficient encapsulation and delivery of DNA, even when the pore size is small.

siRNA delivery. siRNAs can selectively knock down target genes by targeting specific mRNAs. Many studies of siRNA delivery with MSNs used cationic polymeric coatings, such as PEI, similar to those used in DNA delivery studies (Table 1). Hom et al. [38] showed that siRNA was attached to the positively charged PEI external surface of MSNs via electrostatic interactions. This study also showed that the PEI coating promoted endosomal escape of the siRNA into the cytosol, necessary for much more effective target gene knockdown efficiency, in pancreatic cancer cells. Another group also used PEI-coated MSNs for siRNA delivery into breast cancer cells [50]. They observed reduced tumorigenic activity as a result of intracellular siRNA mediated knockdown of the target, TWIST 1, a transcription factor regulating angiogenesis. PEI can also be functionalized with other molecules prior to attachment to MSNs, including acetaldehyde-cysteine (AC) [47] or cyclodextrin [46]. AC-PEI coating enables controlled release of the siRNA inside cells via a GSH-triggered disulfide bond cleavage of the AC-PEI once inside the cytoplasm where GSH is present in much higher concentrations than outside the cell. This AC moiety provides a structural response to the intracellular microenvironment. In addition, it is autofluorescent, enabling MSNs to be tracked intracellularly [47]. The cyclodextrin moiety in cyclodextrin-PEI-coated MSNs reduced the charge-induced cytotoxicity of PEI for breast cancer cells [46]. Furthermore, cyclodextrin-PEI-coated MSNs showed good endosomal escape with good particle retention within the tumor *in vivo* [46]. In these studies, there appears to be no correlation between pore size and surface charge against efficacy of gene encapsulation and delivery, although in general these factors as well as pore morphology do play some role in adsorption and desorption of siRNA into the MSNs. In a recent study by Möller et al., even medium-sized pores (4 nm) with highly positively charged internal pore surfaces gave exceptionally high siRNA loadings [52].

Addition of a targeting peptide such as KALA, a cationic amphipathic cell-penetrating peptide, to the MSN surface produced enhanced endosomolytic function. Li et al. [43] and Chen et al. [48] synthesized MSNs coated with PEI and then subsequently conjugated to KALA peptides. Even with the small MSN pores (<5 nm, comparable to siRNA size), the highly positive surface charges (+25 mV [43] and +23.6 mV [48]) allowed for siRNA complexation and resulted in excellent delivery into target cells, with effective gene silencing and tumor inhibition.

Other surface coatings have also been explored as alternatives to PEI. Non-polymeric coatings such as APTES provide positively charged amine groups, and MSNs coated with APTES or polyethylene glycol (PEG) promoted efficient siRNA delivery into HeLa cells [40]. In these particles, most of the siRNA was encapsulated within the large MSN pores (23 nm), with PEG also added to minimize agglomeration. These MSNs, however, could be administered only intratumorally because of their tendencies to aggregate *in vivo*. siRNA is susceptible to degradation before reaching its intended target, and so should be protected. One way to do this has been by the addition of a tannic acid coating which covers the pores, but is pH responsive and only releases the siRNA when in the acidic conditions of the cytoplasm [51]. Successful delivery into the cytoplasm of KHOS cells *in vitro* was observed. Poly-2-dimethylaminoethyl methacrylate (PDMAEMA) is another polymer alternative that was demonstrated to enhance transfection efficiency. Though PDMAEMA is cytotoxic on its own, its toxicity is reduced when it is stably associated with MSNs [45]. However, the PDMAEMA-coated MSNs have poorer

Table 1. Particle and pore sizes and surface coatings in nucleic acid delivery systems with MSNs

Nucleic acid	Surface coating	Size, nm	Zeta potential, mV	Cell type tested	References
pDNA	PAMAM	particle: 250; pore: 2.7	–	HeLa CHO	[30]
	PAH	particle: 150; pore: 9.8	+20	PC1	[32]
	mannosylated PEI	particle: 60-130; pore: not measured	+5 to +50	RAW 264.7 HeLa	[31]
	aminopropyl groups	particle: 70-300; pore: 20	–	–	[36]
	amino-functionalized MSN	particle: 300-400; large pore: 23; small pore: 2	+10 to +20	HeLa	[37]
	amine groups	particle: 205; pore: 2.6	+23	MSC PC12 HeLa CHO	[33]
	lipid bilayer	particle: 230; pore: 2-5	+24	RAW 264.7 HEK293	[34]
	APTES and L-histidine	particle: 100-180; pore: 2.5	+5	HEK293T7	[35]
siRNA	PEI	particle: 100; pore: 2.5	–	PANC-1	[38]
	PEGylated liposomes	particle: 165; pore: 23-30	+12	Hep3B	[39]
	APTES and PEG	particle: 200; pore: 23	+8	HeLa	[40]
	PEI	particle 63; pore: not measured	+48	HeLa	[41]
	poly-L-lysine (and APTES)	particle: 100-200; pore: 28	PLL +2 APTES +3	HeLa KHOS	[42]
	Fe_3O_4 core with mesoporous silica shell, and coated with PEI	particle: 50; pore: 3.6	+25	A549	[43]
	PEI	particle: 50; pore: 3.7	–	A549	[44]
	PDMAEMA	particle: 100-150; pore: 10	+27	HeLa-Luc	[45]
	cyclodextrin and PEI-functionalized MSN	particle: 105; pore: not measured	+47	MDA-MB231	[46]
	PEI	particle: 178 (without siRNA); pore: 19	+34	KHOS	[47]
	PEI, PEG and KALA peptide	particle: 50; pore: 2.6	+23	A549 L02 PC-3 HCCLM-3	[48]
	PEI	particle: 200; pore: 11	+51	KHOS	[49]
	PEI	particle: 127; pore: 2-3	+44	MDA-MB435S	[50]
	APTES + tannic acid	particle: 150; pore: 12	+21	KHOS	[51]
	cationic block copolymer	particle: 150; pore: 4	+30 to +40	KB	[52]

intracellular endosomal escape in HeLa cells compared with the commercial transfection agent Lipofectamine 2000. Another study compared DNA loading capacities of two functionalized sets of MSNs, one with low molecular weight poly-L-lysine (PLL) and the other with APTES [42]. Both had nearly equal DNA loading capacities even though the PLL had fewer amine groups, therefore a lower positive charge, than APTES (+1.90 mV for PLL compared to +3.23 mV for APTES). The DNA loading capacity of the PLL–MSNs was attributed to the existence of two types of interactions between PLL and DNA – electrostatic and specific binding of PLL to the adenine-thymine (A-T) sequence – compared with only the electrostatic interaction in APTES. Interactions between PLL and the cell surface were also favorable in HeLa and KHOS cells, leading to better cellular uptake and internalization and, hence, enhanced siRNA delivery. There is perhaps a relationship between larger pore size and the ability to utilize polymers that result in lower surface charge.

PEGylated liposomes have also been used to coat MSNs after encapsulating siRNA in the pores (23-30 nm), producing good transfection efficiency in Hep3B cells [39]. MSNs were synthesized with magnetic cores, forming core-shell or yolk-shell structures. The magnetic cores enabled controlled delivery of siRNAs to target sites guided by an external magnetic field. Several studies showed that, by synthesizing magnetic iron oxide cores with a mesoporous silica shell coated with PEI, successful intracellular siRNA delivery and efficient endosomal escape was achieved in HeLa [41], A549 [44], and KHOS [49] cells. All these examples demonstrate that positive charge is necessary for gene complexation, but more studies are required on optimal pore size.

Co-delivery of nucleic acids and drugs. In recent years, there has been remarkable progress using MSNs as combinatorial delivery systems. With MSNs, nucleic acids have been delivered together with drugs to improve effectiveness of the drug being delivered, as well as to maximize delivery of the drug into the cell (Table 2). The traditional method of drug/gene delivery involved injection of a gene-carrying virus combined with administration of a therapeutic drug directly into the tumor. However, this usually resulted in low drug efficacy because of differences in the pharmacokinetics of the small molecule drug and the nucleic acid. MSNs are highly advantageous for such co-delivery systems because of their large surface area, well-established surface chemistry facilitating surface modifications, and porous structures enabling encapsulation of both drugs and nucleic acids [53]. An early example of co-delivery of a drug/nucleic acid combination was described by Chen et

Table 2. Particle and pore sizes and surface coatings in systems for co-delivery of drugs with nucleic acids using MSNs

Surface coating	Size, nm	Zeta potential, mV	Nucleic acid	Co-delivered drug	Cell type tested	Reference
PAMAM	particle: 200 pore: 2.88	–	siRNA	doxorubicin	A2780/AD	[54]
PEI	particle: 100-120 pore: 2-2.5	+30 to +35	siRNA	doxorubicin	KB-V1	[58]
PEG-LHRH peptide (luteinizing hormone-releasing hormone)	particle: 160-180 pore: 2.83-2.99	–	siRNA	doxorubicin cisplatin	A549	[26]
Poly-L-arginine-grafted MSNs	particle: 35-60 pore: 2.6	+20 to +32	pDNA	doxorubicin	HeLa A549	[60]
PEI-PEG copolymer	particle: 50 pore: not measured	+25	siRNA	doxorubicin	MCF-7 (multi-drug resistant)	[59]
PEI conjugated to folic acid	particle: 185 pore: 2.6	+28	siRNA	doxorubicin	HeLa (high folic acid expression) MCF-7	[56]
Ethylenediamine-modified β-cyclodextrin	particle: 150 pore: 2.7	+20	siRNA	doxorubicin	HeLa	[55]
PEI-PLL copolymer	particle: 249 pore: 2.6	+26	siRNA	doxorubicin	MDA-MB-231	[57]

al. [54]. They evaluated delivery of doxorubicin, an apoptosis-inducing agent, along with a siRNA targeted at *Bcl-2*, a gene regulating apoptotic cell death, into A2780/AD ovarian cancer cells. Bcl-2 siRNA was coupled to PAMAM-coated MSNs via electrostatic interactions between the negatively charged RNA and the positively charged dendrimer, while doxorubicin was loaded into the pores of the MSN. When the co-delivery system was tested in cells, doxorubicin co-delivered with siRNA was more cytotoxic than that delivered without siRNA. Similar findings were observed in other cell types including HeLa [55, 56], A549 [26], and MDA-MB-231 [57] cells, also using Bcl-2-targeted siRNA and doxorubicin in combination, albeit with slight differences to polymer/coatings. Furthermore, in *in vivo* biodistribution studies, Taratula et al. [26] showed that the MSNs, when inhaled, were localized and retained in the lungs of mice. Other siRNA types were tested in combination with doxorubicin [58, 59]. P-glycoprotein (Pgp) siRNA with doxorubicin was utilized to overcome multidrug resistance in two types of cancer cells, KB-V1 [58] and MCF-7 [59]. In both studies, PEI was the cationic polymer coating selected for siRNA complexation, whereas the pores were coated with phosphonate to electrostatically bind doxorubicin within them. These MSNs produced efficient intracellular co-delivery of doxorubicin and siRNA. Like previous examples of systems delivering nucleic acids only, alternative coatings were investigated for combined delivery systems. Kar et al. [60] grafted poly-L-arginine to the surface of MSNs to complex pDNA, with doxorubicin in the pores. Not only does poly-L-arginine impart a positive charge to complex DNA, but also MSNs prepared in this manner showed excellent cell penetration in both HeLa and A549 cells. In general, these studies showed that the drugs are encapsulated in the pores (which were small, <5 nm), and the genetic material complexed to the surface of the particle through positively charged groups/polymers.

Current research advances make nucleic acid delivery into cells using MSNs a foreseeable possibility. Furthermore, combination therapies delivering both nucleic acids and drugs into target cells showed enhanced therapeutic effects, making this a strategy of increasing interest for cancer therapy. Most of the studies to date have used doxorubicin as the model drug agent and, overall, the efficiency of MSN-mediated intracellular delivery of drugs was very high. The clinical value of MSNs for delivering therapeutic nucleic acids in combination with different chemotherapeutic drugs warrants continued investigation. Results so far indicate that, indeed, combination delivery of nucleic acids and drugs using MSNs has advantages compared with delivering either agent alone for inhibiting tumor growth. For example, the co-delivery of chemotherapeutic drugs and therapeutic nucleic acids is a potential approach to overcome drug resistance in cancer therapy. With increasingly successful research employing anti-cancer agents, future studies should explore the possibility of using MSNs to treat other disorders besides cancer, such as autoimmune and metabolic diseases. Study design should focus on the rational selection of nucleic acid and drug ratios, including the interactions of the two agents within MSNs. These factors have often been overlooked. Clarifying this selection process would allow optimization of combinatorial formulations to achieve maximum therapeutic efficiencies. In conclusion, MSNs have the potential to be an ideal platform for the efficient, safe delivery of nucleic acids and drugs into patients.

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