

## Methods of the Synthesis of Silicon-Containing Nanoparticles Intended for Nucleic Acid Delivery

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### Article info

*Received:*

13 January 2018

*Received in revised form:*

24 March 2018

*Accepted:*

19 June 2018

**Keywords:**

synthesis  
silicon-containing nanoparticles  
nucleic acids  
delivery in cells

### Abstract

A promising new approach to the treatment of viral infections and genetic diseases associated with damaged or foreign nucleic acids in the body is gene therapy, i.e., the use of antisense oligonucleotides, ribozymes, deoxyribozymes, siRNA, plasmid DNA, etc. (therapeutic nucleic acids). Selective recognition of target nucleic acids by these compounds based on highly specific complementary interaction can minimize negative side effects, which occur with currently used low molecular weight drugs. To apply a new generation of therapeutic agents in medical practice, it is necessary to solve the problem of their delivery into cells. Silicon-containing nanoparticles are considered as promising carriers for this purpose due to their biocompatibility, low toxicity, ability to biodegradation and excretion from the body, as well as the simplicity of the synthesis and modification. Silicon-containing nanoparticles are divided into two broad categories: solid (nonporous) and mesoporous silicon nanoparticles (MSN). This review gives a brief overview of the creation of mesoporous, multilayer, and other silicon-based nanoparticles. The publications concerning solid silicon-organic nanoparticles capable of binding and delivering nucleic acids into cells are discussed in more detail with emphasis on methods for their synthesis. The review covers publications over the past 15 years, which describe the classical Stöber method, the microemulsion method, modification of commercial silica nanoparticles, and other strategies.

## 1. Introduction

A promising new approach to the treatment of viral infections and genetic diseases, such as hemophilia, Huntington's disease, asthma, cancer, etc. is gene therapy, i.e., the use of therapeutic nucleic acids. Gene therapy is an alternative method compared with currently used low molecular weight drugs, which often have low efficacy, are toxic, and cause unwanted side effects. The nucleic acid fragments (antisense oligonucleotides, ribozymes, deoxyribozymes, siRNA, plasmid DNA, etc.) have proved to be promising agents to affect nucleic acids (NA) within cells, in particular, damaged or foreign NA (for example, the genetic apparatus of viruses, pathogens, oncogenes, etc.). This new generation of drugs can be used for the treatment of diseases associated with nucleic

acids [1–4]. Selective recognition of target nucleic acids by NA-fragments based on highly specific complementary interaction can minimize negative side effects.

However, the negative charge of internucleotide phosphate groups in NA-fragments prevents their penetration through the cell membrane. A low internalization efficiency of NA-fragments and their low stability in biological media hampers the wide introduction of the approach in question in medical practice.

In recent years, many strategies have been proposed to solve the problem of the NA delivery in cells including the use of virus vectors, liposomes, cationic polymers of different nature, transporting peptides, etc. [5–9]. Each of the proposed strategies has its advantages and disadvantages (e.g., the immunogenicity of viral vectors, cytotoxicity of

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lipid nanoparticles, weak penetration of transport peptides, etc.). Therefore, the search for new ways to solve the problem of the NA delivery in cells remains an urgent task.

Ideal carriers for the NA delivery should meet criteria, such as ease of preparation and modification (for the introduction of functional groups), the efficiency of immobilization of NA or their fragments, high biocompatibility, low toxicity, and high efficiency of penetration through the cell membrane. Recent advances based on the use of inorganic nanoparticles offer impressive prospects in this area [10–12].

Nanoparticles of silicon dioxide are considered as promising carriers for the delivery of nucleic acids in cells [13, 14]. Silicon-containing nanoparticles are attractive as a biocompatible, nontoxic inert material, which is amenable to the introduction of various functional groups due to the presence of the silanol groups on their surface [15, 16]. Prospects of amino-modified SiO<sub>2</sub> nanoparticles as non-viral nanovectors for the delivery of plasmid DNA and the lesser toxicity of these nanoparticles in comparison with the widely used transfection agent lipofectamine are shown in work [17]. SiO<sub>2</sub> particles are bioinert and less toxic compared to cationic polymers used for the NA delivery; they are much more stable in physiological conditions than liposomes and other similar transporting nanoconstructions.

SiO<sub>2</sub> nanoparticles are also attractive from a biological point of view. Silicon is one of the microelements present in the human body, which indicates its biocompatibility [18]. Silica nanoparticles are mainly accumulated in the liver, kidneys, spleen, and lungs (depending on the size, shape, and mode of administration); they decompose to inert silicic acid under physiological conditions and are effectively excreted from the body through the kidneys [19–21]. It was shown that positively charged amino-modified silicon particles undergo faster excretion than neutral particles [22]. The researchers suggest that silica nanoparticles can degrade inside the lysosomes, and the dissolved products are subsequently released from the cells into the bloodstream and eventually excreted from the body through the kidneys.

The modification of silicon-containing nanoparticles by cationic molecules makes it possible to immobilize negatively charged nucleic acids through the electrostatic interaction. SiO<sub>2</sub> nanoparticles act not simply as a sedimentation agent but exhibit transfection effect [23].

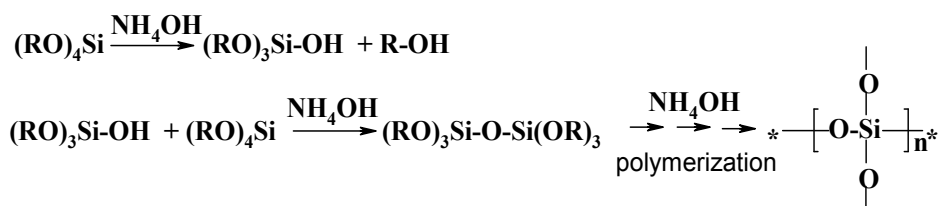
Silicon-containing nanoparticles (Si-nanoparticles) are divided into two broad categories: solid (nonporous) and mesoporous silicon nanoparticles (MSN), each of which is an amorphous structure. In this review, we do not consider hybrid nanoparticles, which consist of silicon along with other components (gold nanoparticles, iron oxide, etc.); this class of nanoparticles is considered in the review [24]. Numerous reviews [25–29] are devoted to mesoporous Si-nanoparticles as nucleic acid vehicles. Therefore, this review gives only a brief idea of the synthesis of such particles.

There are a sufficient number of reviews devoted to solid silicon-containing nanoparticles. These reviews consider many aspects related to Si-nanoparticles, i.e., production, biodistribution, toxicity, binding to various ligands, delivery of low molecular weight drugs, proteins, and genes, imaging and diagnostic use, bioimaging methods, use in medicine, release of drugs from nanoconstruction in the body, and use as fluorescent probes [13, 14, 16, 30]. The review of 2018 [31] covers history, classification, artificial and natural sources, toxic effects of nanostructured materials (including Si-nanoparticles). The growing interest of researchers, pharmacologists and physicians in the effective delivery of nucleic acids in cells *in vitro* and *in vivo* using nanoparticles requires careful consideration of existing approaches to the synthesis of nanomaterials suitable for use in medicine.

In contrast to the reviews published earlier, the main emphasis in this paper is on a detailed consideration of various methods for synthesizing silicon-containing nanoparticles, which can be used to deliver nucleic acids in cells. The review covers publications over the past 15 years concerning the synthesis of solid silicon-containing nanoparticles, a brief description of the creation of mesoporous, multilayer, and other Si-nanoparticles capable of binding and transporting nucleic acids to cells. In this review, we will consider different methods for the synthesis of these nanoparticles, i.e., the classical Stöber method, the microemulsion method, modification of commercial silica nanoparticles, and other strategies.

## 2. Classical method of synthesis of silicon-containing nanoparticles

The classical method for the synthesis of silicon-containing nanoparticles was proposed about 40 years ago [32]. The method consists in the hydrolysis of alkoxysilanes in alcoholic solutions in the presence of ammonia as a catalyst (Scheme 1).



Scheme 1

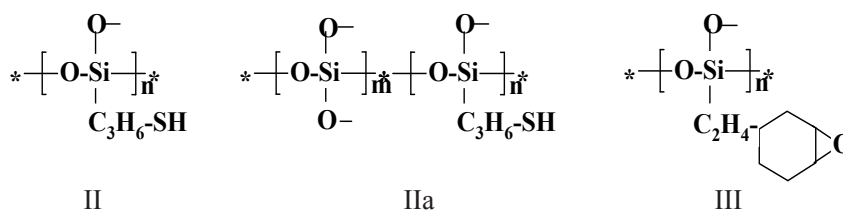
The growth and branching of the chain occur during hydrolysis. This scheme is a simplified depiction of a more complex process of hydrolysis of tetraalkylsilane, which is accompanied by condensation with the formation of Si-particles (I). The particle size (from 0.2 to 2  $\mu\text{m}$ ) depends on the alcohol used in the reaction (minimum size in methanol; maximum size in butanol) and on the nature of alkylsilane. The fastest reaction (1 min) occurs with the use of tetramethoxysilane with the formation of particles of a minimum size (0.2  $\mu\text{m}$ ), while tetrapentoxysilane requires 24 h to be completely hydrolyzed to form particles of about 2  $\mu\text{m}$  in size.

The Stöber method is still widely used in various modifications to produce particles with a diameter from tens of nanometers to several micrometers. Variation of the synthesis conditions (catalyst type, process temperature, and the ratio of the initial reactants) allowed one to synthesize silica sols with the particle size of 12–150 nm and high resistance to agglomeration [33]. The smallest non-agglomerated nanoparticles obtained by the Stöber

method have a diameter of 15–20 nm; these particles are more polydisperse (standard deviation of 10–20%) compared to larger particles (>200 nm, standard deviation <3%) [34].

The use of the classical method makes it possible to obtain silicon-containing nanoparticles with various functional groups, since there are a large number of different commercially available silanes. Aminomodified Si-nanoparticles are most often used for the immobilization of nucleic acids and their fragments. Modifying groups can be introduced in two ways, i.e., by copolymerization of silicon precursors, one of which contains functional groups, or post-synthetically by the treatment of the already formed particles with appropriate trialkoxysilane derivatives, which can react with the silanol groups on the particles.

Using the traditional Stöber method, the authors [35, 36] synthesized silicon-organic nanoparticles bearing the thiol groups during the separate or combined hydrolysis of 3-mercaptopropyltrimethoxy (or ethoxy) silane and tetraethoxysilane (tetraethylorthosilicate, TEOS).

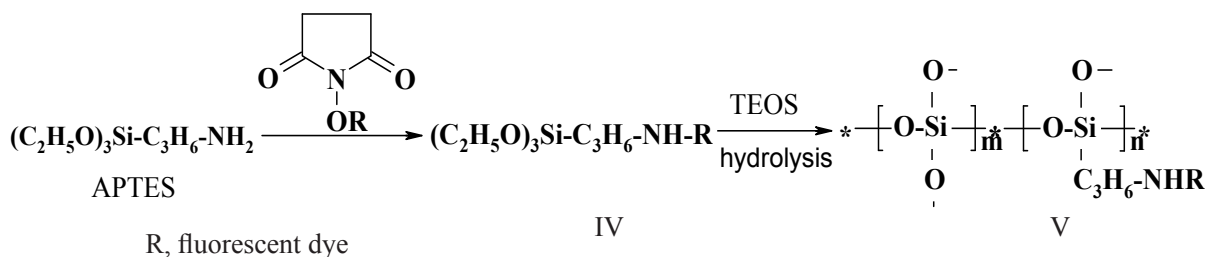


The particles (II) and (IIa) had a diameter of 40–700 nm and larger negative zeta potential (due to the presence of the SH groups) compared to the particles obtained by hydrolysis of tetraethoxysilane alone.

The same group of authors [37] synthesized Si-nanoparticles (III) containing the epoxy groups. They used 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane (EpoMS) as the starting compound. Depending on the reaction conditions (the presence/absence of ethanol, different concentrations of EpoMS), the authors obtained particles with the size of 100–1600 nm and zeta potential of about +46 mV. The epoxy groups have a high

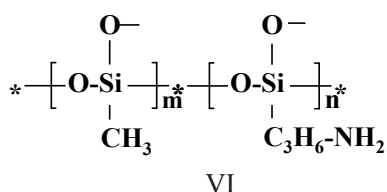
reactivity to amine-containing compounds, thus making it possible to introduce the amino groups by the treatment of the nanoparticles with ammonia. Since both epoxy and amine-containing nanoparticles have a positive charge, they can be used for the electrostatic binding to nucleic acids.

The fluorescently labeled Si-nanoparticles were synthesized by the Stöber reaction in the mixture of TEOS with conjugate (IV), which was pre-synthesized from 3-aminopropyltriethoxysilane (APTES) by the treatment with an N-hydroxysuccinimide ester of any fluorescent dye [35–38] (Scheme 2). The size of the resulting nanoparticles varied from 0.2 to 0.5  $\mu\text{m}$ .



Scheme 2

Nanoparticles (VI) were synthesized by the simultaneous hydrolysis of APTES and methyltrimethoxysilane in a volume ratio of 1 : 20 in the presence of aqueous ammonia (pH 11) and detergent TX-100 for 5 h [17].

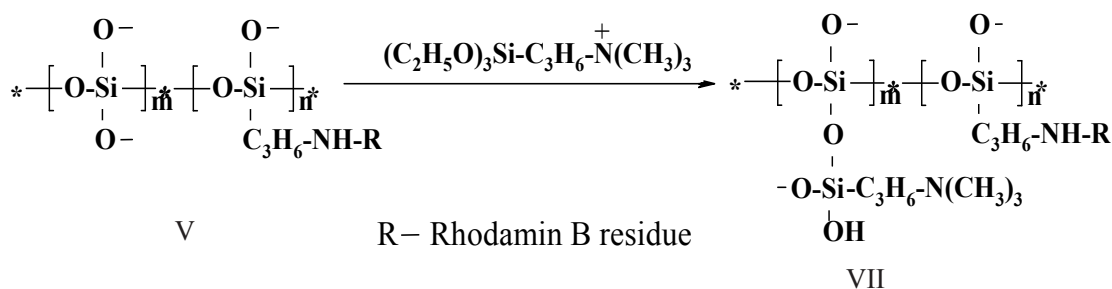


Particles (VI) had an average size of 170 nm and positive zeta potential (+41.8 mV), which made it possible to obtain complexes with plasmid DNA due to the electrostatic interaction between the positively charged amino groups on particles and negatively charged internucleotide phosphate groups in DNA. The authors showed that the resulting complexes of nanoparticles (VI) with

DNA are suitable for gene therapy because they have low toxicity and high transfection efficiency of cells.

The post-synthetic modification of the particles was used in [39, 40]. Silicon-containing water-soluble nanoparticles (V) were obtained similarly to Scheme 2 and treated with N-trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride to introduce the quaternary amino groups (Scheme 3).

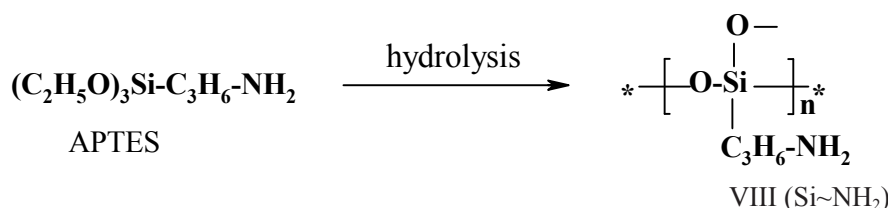
Nanoparticles (VII) (40–45 nm) were used for immobilization of plasmid DNA bearing the gene encoding the granulocyte-macrophage-colony-stimulating factor of dogs (GM-CSF). The authors showed that the formed GM-CSF/nanoparticles penetrate through the cell membrane, and after entering the cytoplasm, the plasmid is released from the nanoparticles and penetrates into the nucleus. The authors believe that the proposed nanocomposites can be used to correct acute leukopenia.



Scheme 3

The authors of this review synthesized the Si-nanoparticles by hydrolysis of APTES alone in an alkaline medium without the addition of

other alkoxy silanes [41] (Scheme 4). The resulting nanoparticles were shown to be low-molecular oligomers consisting of two-three monomer units.



Scheme 4

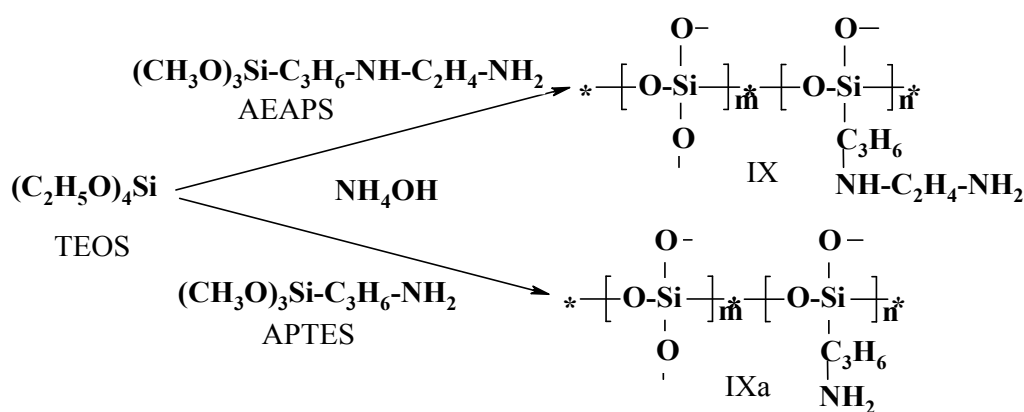
The nanocomplexes of oligodeoxyribonucleotides (ODN) with these nanoparticles were obtained due to the electrostatic binding. The size of nanoparticles (VIII) ( $\sim 1$  nm) and their complexes with oligonucleotides (2.5–3.5 nm depending on the oligonucleotide length) were evaluated by the method of dynamic light scattering. After the addition of ODN, the  $\xi$  value changes from a positive ( $\sim +8$  mV) to a negative value ( $\sim -14$  mV). We assume that the interaction of positively charged particles with negatively charged oligonucleotides produces a certain type of salt, where the particles act as counterions to the phosphate groups. The nanoparticles and their complexes with oligonucleotides are soluble in water. It is shown that the oligonucleotides in the resulting nanocomplexes retain the ability to form duplexes with complementary NA fragments, penetrate into the cells, and exhibit antiviral activity against influenza A virus in cell culture.

### 3. Synthesis of silicon-containing nanoparticles in microemulsion medium

Microemulsions are homogeneous mixtures of water, an organic solvent (oil), and a surfactant.

At the microscopic level, the mixtures consist of water droplets dispersed in an organic solvent and separated by monolayers of surfactants. Formed microemulsions consisting of nano-sized drops are often used as confined reaction media for the synthesis of nanoparticles. Microemulsions, unlike emulsions, are thermodynamically stable, while the content of droplets and molecules of surfactants is rapidly exchanged between different drops, thereby facilitating chemical reactions and providing the synthesis of particles ranging in size from tens to hundreds of nanometers [42].

In work [43], the nanoparticles were synthesized in the microemulsion, which was formed from a water/cyclohexane/Triton X-100/hexanol mixture. TEOS and AEAPS (3-(2-aminoethylamino)propyltrimethoxysilan) in the molar ratio of 3 : 1 were hydrolyzed in this mixture in the presence of ammonium hydroxide (24 h) (Scheme 5). Resulting nanoparticles (IX) were precipitated with acetone and thoroughly washed with alcohol and water. The preparation of the fluorescently-labeled nanoparticles was carried out by the same procedure using an aqueous solution of rhodamine isothiocyanate as the aqueous phase during the formation of the microemulsion.



Scheme 5

The particle size can be varied by changing the water/surfactant/TEOS ratio and reaction time. The formed spherical particles were uniform and had a size of  $\sim 25$  nm (a small number of particles with a large diameter (up to 100 nm) can be the result of agglomeration). The zeta potential value of  $+36.5$  mV in the neutral medium is due to protonation of the amino groups, which allows immobilizing negatively charged NA fragments on the particles. The authors immobilized antisense oli-

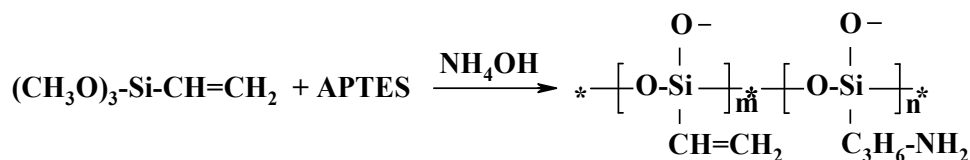
gonucleotides (ODN) on particles (IX) and showed the penetration of the complexes into cells. The particles protect oligonucleotides from hydrolysis by DNase 1. The cell culture experiments have shown that the Si~ODN nanocomplexes significantly increase the inhibitory activity of antisense oligonucleotides. Compared with liposomes, the resulting particles show better biocompatibility and the almost complete absence of toxicity at the concentrations necessary for the effective cell transfection.

The same group of authors [44] synthesized Si-nanoparticles (IXa) (~45 nm,  $\xi \approx +30$  mV in the neutral medium) during the simultaneous hydrolysis of TEOS and APTES with the 1 : 1 molar ratio in the water-organic emulsion medium. Resulting nanoparticles (IXa) were used for the immobilization of plasmid DNA carrying the green fluorescent protein (GFP) gene. It was shown that plasmid delivered into cells in the nanocomplex retained its functions even after the treatment with DNase 1, thus providing the GFP synthesis.

The microemulsion method was used in [45] for the synthesis of the fluorescently labeled Si-nanoparticles. Initially, the fluorescein isothiocyanate (FITC) was mixed with APTES to attach the fluorescein residue to the amino groups (similar to Scheme 2). Then, the resulting conjugate (IV) (R is the fluorescein residue) was introduced into a microemulsion system consisting of Triton X-100, n-hexanol, cyclohexane, deionized water, and TEOS. After 30 min of stirring,  $\text{NH}_4\text{OH}$  was added to the reaction mixture. After 24 h, nanoparticles of type (V) (R is the fluorescein residue) were precipitated from the emulsion with acetone and washed with alcohol and water. The particles were then treated with polyethyleneimine, and their size

increased from 50 to ~115 nm, and the zeta potential changed from the negative to the positive value (in water). Antisense oligonucleotides were electrostatically immobilized on the synthesized nanoparticles. The authors [45] did not observe the cytotoxic effect of the resulting nanocomplexes, and the immobilized oligonucleotides showed an antisense effect in the cellular system, i.e., inhibited the accumulation of the corresponding protein by 20%.

A large series of studies was performed for the synthesis and use of amine-containing Si-nanoparticles, the so-called ORMOSIL (ORganically MODified SILica) [13, 46–52]. Nanoparticles (X) of 20–30 nm in size were obtained by the hydrolysis of triethoxynylsilane (TEVS) with APTES in a microemulsion medium, which was formed by a mixture of aerosol (dioctyl sodium sulfosuccinate), butanol, dimethyl sulphoxide, and water (Scheme 6) [46]. After the hydrolysis, the micelle-forming components were removed by dialysis against water. The resulting nanoparticles were monodispersed (10–20 nm) and highly resistant to agglomeration. The presence of the organic groups increases the stability of particles in water systems and protects against precipitation.



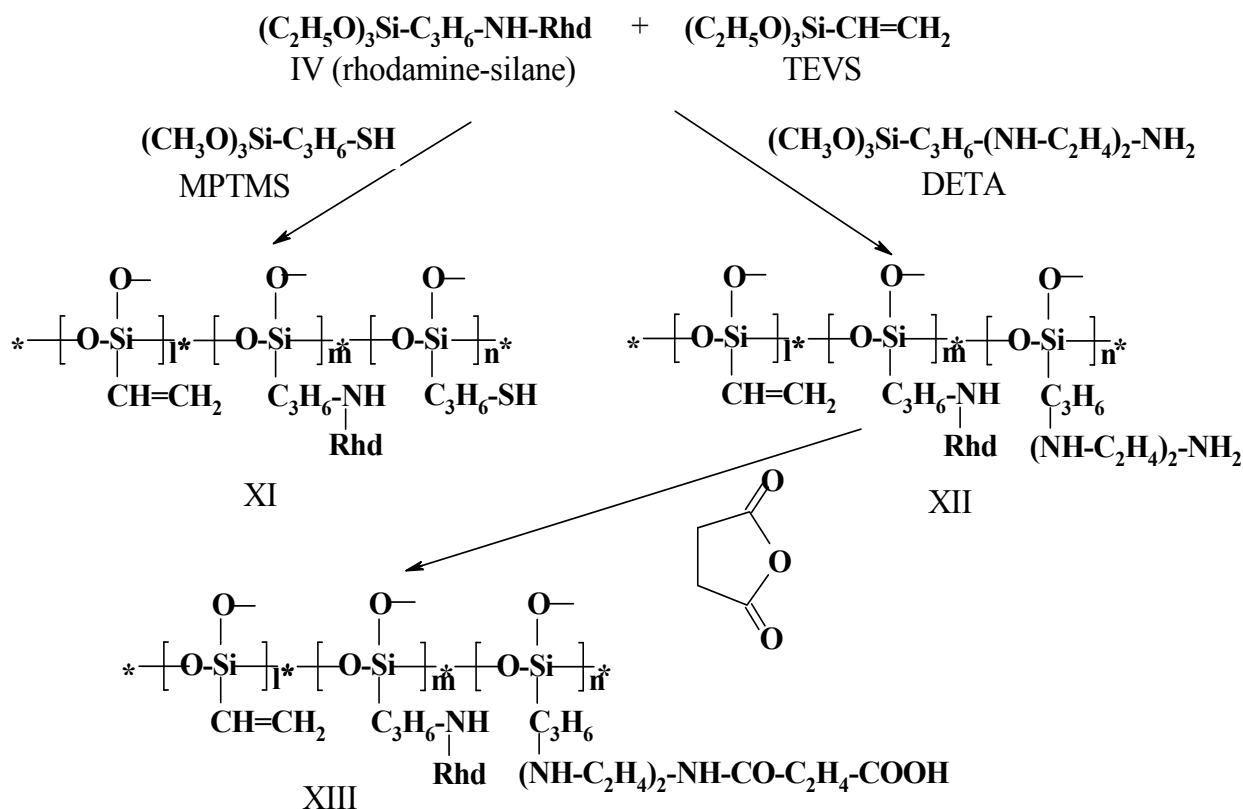
ORMOSIL, X  
Scheme 6

ORMOSIL particles (X) were shown to exhibit low cytotoxicity [47]. These nanoparticles were used to obtain complexes with plasmid or genomic DNA due to electrostatic binding. It was shown that nanoparticles protected DNA from hydrolysis. The complexes of the particles with DNA penetrate into the cells, and DNA after penetration was released from the complex and diffused into the cell nucleus [46]. It has been shown that the ORMOSIL nanoparticles with the immobilized plasmid DNA can be delivered to the brain cells *in vivo* and thus can be potential tools for gene therapy of various neuropathologies [47, 48].

The same group of the authors synthesized the ORMOSIL nanoparticles that contained covalently-bound fluorophore rhodamine B [49]. For this purpose, rhodamine-silane (IV) (R is the rhodamine residue) was used as a precursor. The

different functional group, such as SH,  $\text{NH}_2$ , and COOH, terminated ORMOSIL nanoparticles, covalently incorporating the fluorophore rhodamine, were synthesized in the nonpolar microemulsion system. Rhodamine-silane (IV), triethoxyvinylsilane (TEVS), and mercaptopropyltrimethoxysilane (MPTMS) or *N*-[3-(trimethoxysilyl)-propyl]diethylene triamine (DETA) were co-polymerized in the microemulsion medium (Scheme 7).

The carboxyl groups were introduced by the treatment of the amino-containing nanoparticles (XII) with succinic anhydride. Nanoparticles (XI–XIII) were purified by dialysis against water from the surfactant molecules and other unreacted compounds. The nanoparticles were separated from the Tween-80 micelles by spin-filtration (centrifugation in a microfuge membrane-filter). In this case, the Tween-80 micelles and nonconjugated



Scheme 7

rhodamine flow through the filter and are collected in the filtrate, and the nanoparticles remain on the membrane and are easily redispersed in water by sonication. Interestingly, rhodamine-silane (IV) itself is not polymerized (or only partially polymerized), i.e., does not form nanoparticles, although this derivative is co-polymerized with the other components in the mixture. Amino-containing nanoparticles (XII) were used for the electrostatic binding of the NA fragments. The carboxyl groups in nanoparticles (XIII) were utilized for the introduction of different bioactive molecules, e.g., transferrin and monoclonal antibodies, such as anti-claudin 4 and anti-mesothelin, for the targeted delivery to pancreatic cancer cells. The synthesized nanoparticles (20–25 nm) are monodisperse, stable in aqueous suspensions and retain the optical properties of the incorporated fluorophore. In the *in vitro* experiments, the synthesized nanocomplexes with DNA showed low toxicity and a high degree of the cellular uptake.

The authors believe that the proposed fluorescent ORMOSIL nanoparticles are potential candidates as efficient probes for optical bioimaging, both *in vitro* and *in vivo*.

Yokoi with co-workers [53] prepared spherical silicon-containing particles (12–23 nm) using aqueous solutions of lysine instead of ammonia as a catalyst and TEOS in an organic solvent. The resulting nanoparticles were calcined at 873 K to remove organic components (lysine and octane). Since the pKa values for R-COOH, R-NH<sup>3+</sup>, and R(CH<sub>2</sub>)<sub>4</sub>NH<sup>3+</sup> in *L*-lysine are 2.18, 8.90, and 10.28, respectively, and the isoelectric point is 9.74 (<http://www.chem.ucalgary.ca/courses/351/Carey5th/Ch27/ch27-1-4-2.html>), approximately 92% of R-(CH<sub>2</sub>)<sub>4</sub>NH<sub>2</sub> and 33% of R-NH<sub>2</sub> are protonated under the reaction conditions (pH 9.2). The *L*-lysine molecules can be attached to the surface of spherical nanoparticles during their growth due to the formation of hydrogen or ionic bonds between anionic silicates (SiO<sup>-</sup>) and protonated amino groups of lysine, which leads to the formation of particles with controlled size. The nanoparticles with a size of 12 to 23 nm can be obtained when using a mixture of D- and *L*-lysine isomers in different proportions. The size increased with increasing the proportion of the D-isomer, and monodisperse particles with the largest size (23 nm) were formed at the 1 : 1 ratio of these isomers.

*L*-lysine, *L*-histidine, and *L*-arginine belong to the basic amino acids. The use of *L*-arginine similar to *L*-lysine led to the formation of well-organized silicon-containing nanospheres. At the same time, *L*-histidine did not give the desired results, possibly because of low basicity. Attempts to use neutral amino acids (*L*-leucine and *L*-alanine) in combination with ammonia were also unsuccessful. Thus, the authors found an interesting ability of the main amino acids to form monodisperse spherical Si-nanoparticles.

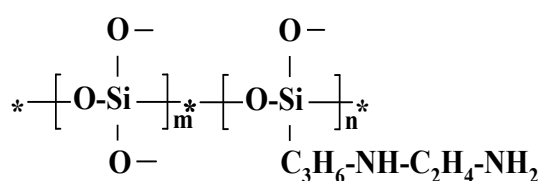
The Si-nanoparticles can be modified with polycations to enhance the immobilization of negatively charged nucleic acids and improve the penetration into cells [54]. A group of authors [55–56] developed a method for obtaining Si-nanoparticles modified with polylysine in a microemulsion medium. The microemulsion was prepared by mixing polyoxyethylene nonylphenyl ether, cyclohexane, and water. The hydrolysis and condensation of TEOS in this medium were initiated by the addition of  $\text{NH}_4\text{OH}$ . After completion of the reaction, the nanoparticles were precipitated with acetone and washed to remove surfactants. The dried nanoparticles were treated with a solution of  $\text{Na}_2\text{CO}_3$  (activation buffer) to ionize the hydroxyl groups, followed by mixing the activated nanoparticles with poly-*L*-lysine. The obtained Si~PL nanoparticles with immobilized polylysine were suspended in water and analyzed by transmission electron microscopy (TEM). The nanoparticles before and after the addition of polylysine were uniform in size with a diameter of ~20 nm. It should be noted that there is no clear proof of the addition of polylysine to Si nanoparticles in the cited papers [55–56].

The resulting Si~PL nanoparticles exhibit low cytotoxicity to HNE1 and HeLa cells. The death of ~40% of cells occurs only at a particle concentration of >500  $\mu\text{g/mL}$ . It was shown by polyacrylamide gel electrophoresis that oligodeoxynucleotides were immobilized on the nanoparticles. It turned out that the nanoparticles protected oligonucleotides from the action of nucleases. It was shown that the Si~PL-ODN(R) complexes bearing fluorescently-labeled oligonucleotides penetrate the cytoplasm of cells. It was also shown that oligonucleotides complementary to the *c-myc* proto-oncogene region delivered into cells in the complexes with nanoparticles inhibit the expression of the target gene, i.e., have an antisense effect. However, this effect is significant only in the serum-free medium, whereas the inhibition of expression becomes statistically insignificant in the presence of serum. This fact, according to the authors, can be explained by the interaction of polylysine in the PMS-NP-ODN complex with serum proteins, and this can lead to the formation of aggregates and impede the penetration of the complex into cells.

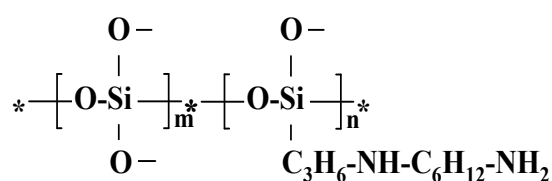
#### 4. Modification of commercial silica nanoparticles

In some works, commercial silicon-containing nanoparticles, usually silica particles, after functionalization are also used as carriers for delivery of nucleic acids in cells.

Commercial  $\text{SiO}_2$ -nanoparticles were modified with 3-(2-aminoethyl- or hexyl)-3-aminopropyltrimethoxysilane (AEAPS or AHAPS) [57]. The resulting amino-modified nanoparticles (XIV and XV, respectively) had a size of 10–100 nm and zeta potential from +7 to +31 mV.



XIV



XV

The nanoparticles formed the complex with plasmid DNA due to the electrostatic interaction. A high salt concentration and alkaline medium inhibited the immobilization of DNA on the nanoparticles. DNA in the complex with the nanoparticles appears to be protected from hydrolysis by DNases and can be delivered into cells.

The authors of the work [58] modified commercial Si-nanoparticles with AEAPS in a water-acetic acid mixture (20 h, 80 °C). The resulting nanoparticles XV were mixed with ethylene glycol, evaporated, dialyzed against water, and filtered through a 0.2  $\mu\text{m}$  filter. After the treatment with aminoalkoxysilane, nanoparticles XV contain the silanol

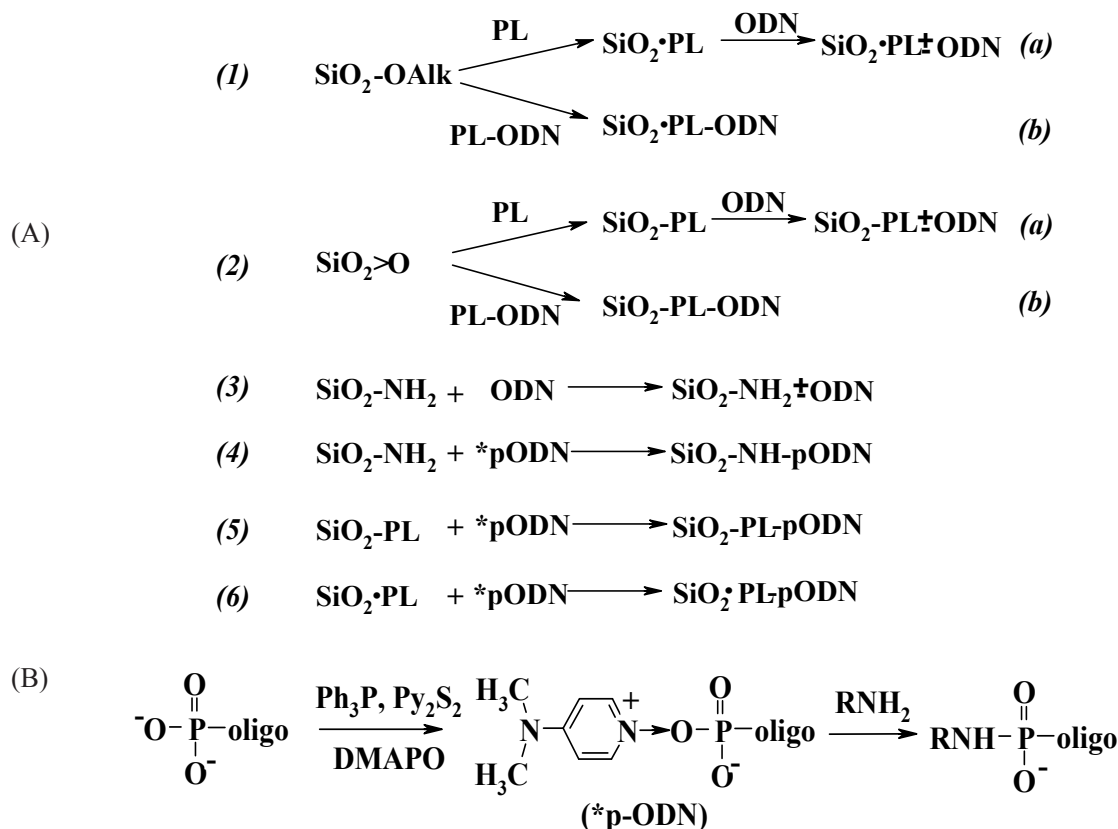


(Si-O<sup>-</sup>) and cationic amino (NH<sup>3+</sup>) groups. The particles tend to aggregate because of condensation of the silanol groups with the formation of Si-O-Si bridges [59]. The particles were lyophilized in the presence of lyoprotective agents (such as glucose, trehalose, mannitol, sorbitol, or glycerol) at -40 °C to prevent agglomeration. The hydroxyl groups of the sugar can react with the silanol groups to form ether or hydrogen bonds [60], which leads to the protection of the Si-OH groups from condensation with the active groups of adjacent particles, thus preventing agglomeration. The addition of excess water can cause partial destruction of the ether and hydrogen bonds between particles and sugar molecules. The lyophilized and resuspended in water particles had a size of 28 nm and a zeta potential of +46 mV, 35 mV, 0 mV, and -37 mV at pH 3.0, 4.0, 7.1, and 10, respectively. When glycerol was used, the nanoparticles were soluble in water. The authors showed that the pCMV plasmid immobilized on the resulting nanoparticles was delivered to the cells.

Commercial SiO<sub>2</sub> nanoparticles were modified with different alkoxy silanes and amines [61]. Zeta potential of the resulting nanoparticles varied at pH 7.4 from -38.8 mV for unmodified particles

to +20 mV and +49.8 mV for the particles treated with APTES and a mixture of glycidoxypropyltrimethoxysilane with ethylenediamine, respectively. DNA was shown to be quantitatively immobilized on the amino-modified nanoparticles, and the yield of immobilization was correlated with the amount of the functional groups on the particle surface. The nanoparticles that contained the shortest amino spacer provided the maximum extent of immobilization of DNA.

The authors of this review developed the methods of immobilization of oligonucleotides on commercial silica nanoparticles (10–20 nm) [62]. Polylysine (PL) or polylysine conjugates with an oligonucleotide (PL-ODN) were immobilized on silanized nanoparticles due to the affinity of polylysine to various surfaces [63] (Scheme 8A, reaction 1) PL and PL-ODN were attached to the nanoparticles bearing epoxy groups by the reaction with the amine groups of polylysine (Scheme 8A, reaction 2) Oligonucleotides were immobilized on the amino-containing nanoparticles due to the electrostatic interaction between the amino groups in the nanoparticles and internucleotide phosphate groups (Scheme 8A, reaction 3).



PL, polylysine; ODN, oligonucleotide; p\*, activated terminal phosphate of an oligonucleotide; Ph<sub>3</sub>P, triphenylphosphine; Py<sub>2</sub>S<sub>2</sub>, dipyridyl disulfide; DMAPO, 4-dimethylaminopyridine N-oxide.  
Scheme 8

It should be noted that in the cases represented by reactions *1a*, *1b*, *2a*, and *3*, oligonucleotides or their conjugates with polylysine are attached to nanoparticles noncovalently. The covalent binding is provided only in case of *2b* by the attachment of polylysine to the epoxy groups in the nanoparticles.

Another method involves the use of oligonucleotides containing the terminal phosphate activated with a dipyridyl disulfide/triphenylphosphine redox pair in the presence of a nucleophilic catalyst (4-dimethylaminopyridine *N*-oxide) [64] (Scheme 8B). The activated oligonucleotide can react with the amino groups on the nanoparticles (Scheme 8A, reaction *4*) or with the amino groups of the polylysine previously attached to the particles (Scheme 8A, reactions *5* and *6*).

In all cases, it was possible to obtain a sufficiently strong binding of oligonucleotides to the nanoparticles. The oligonucleotide in the composition of the nanocomposite prepared in Scheme 8A, *3* and targeted to the influenza A virus genome, suppressed the reproduction of the virus by ~40% in the cell system.

## 5. Mesoporous silicon nanoparticles

Recent studies have opened up the possibility of using mesoporous silicon nanoparticles (MSN) as carriers of nucleic acid-based drugs. MSN is a solid material with a porous structure like honeycombs, in which a relatively large number of bioactive molecules can be adsorbed. MSN is considered as a promising vehicle for the delivery of molecules due to the unique properties of the mesoporous structure, such as high surface area (>900 m<sup>2</sup>/g, high pore volume (>0.9 cm<sup>3</sup>/g), and adjustable pore diameter with narrow size distribution (2–10 nm) [65, 66]. MSN nanoparticle are chemically and thermally stable, exhibit low immunogenicity, and minimize undesirable inflammatory reactions [15]. Pores in mesoporous nanoparticles serve as storage tanks for biomolecules that can be released under certain conditions (stimuli-responsive release system) under the influence of heating, light, pH, ultrasound, chemical effects, etc. [26, 27].

MSN have ideal properties as transporters of nucleic acids (plasmids, siRNA, etc.) for gene therapy [27]. It was shown [67, 68] that the MSN particles with a functionalized surface are easily internalized by animal and plant cells without signs of toxicity. The biocompatibility of MSN was demonstrated in the reviews [25, 69]. The syn-

thesis of various types of MSN is described in numerous reviews [25, 65, 70–73]. The synthesis of MSN is simple, easy to scale, cost-effective, and manageable. Mesoporous silicon nanoparticles are promising for the development of universal drug delivery systems suitable for use in the clinic.

For the first time, the synthesis of these nanoparticles with a uniform pore size and extended ordered pore structure was carried out in the early 1990s with the use of surfactants as structure-forming agents [74]. The synthesis of MSN is as follows. The surfactant is mixed with water and alkali (NaOH or NH<sub>4</sub>OH), followed by the addition of tetraethylorthosilicate (TEOS) or sodium metasilicate (Na<sub>2</sub>SiO<sub>3</sub>), and the mixture is stirred at room temperature for at least 30 min. Then, if necessary, the mixture is heated at 100 °C for a certain time, and the solid product is separated by filtration. The dried product is suspended in water and reheated at 100 °C to remove the surfactant. At present, tangible progress has been made in the MSN synthesis with the use of various types of surfactants and sol-gel processing conditions. It has become possible to obtain nanoparticles with different size and pore structure and morphology. The most famous representatives of MSN are MCM-41 (Mobil Composite Matter number 41, with the hexagonal arrangement of mesopores), MCM-48 (with the cubic arrangement of mesopores), and MCM-50 (with plate structure) [75].

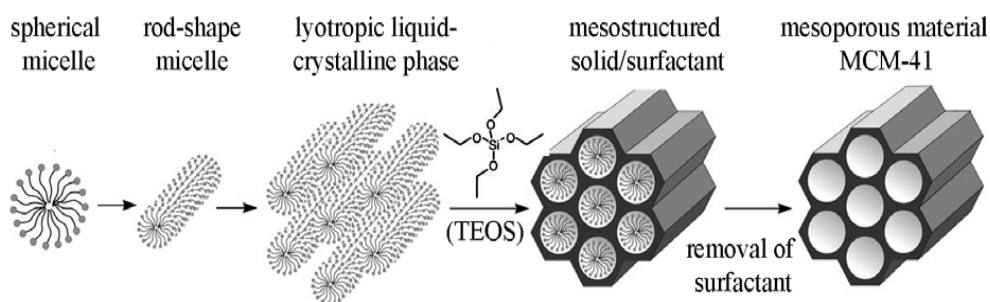
The topology and chemical structure of the inorganic skeleton are determined by the synthesis conditions (the nature of the surfactant, pH, temperature, salt concentration, etc.), with the type of surfactant and pH being the key factors [72]. The surfactants can be cationic, anionic, neutral, and nonionic. Silicon nanoparticles at pH 9 are negatively charged; they have a weak negative charge in a neutral or slightly acidic medium due to the predominance of the Si-OH group. Therefore, cationic surfactants (alkyltrimethylammonium bromides) are used under alkaline conditions during the synthesis of MCM-41 or MCM-48.

MCM-41 is the most intensely studied type of MSN for use in biomedicine [25]. Cetyltrimethylammonium bromide (CTAB) as a surfactant for the formation of liquid crystals and tetraethylorthosilicate (TEOS) or sodium metasilicate (Na<sub>2</sub>SiO<sub>3</sub>) as a silicon precursor, and alkali as a catalyst are used in the synthesis of MCM-41 [75]. At a critical concentration, CTAB is self-assembled into micelles, and TEOS (or Na<sub>2</sub>SiO<sub>3</sub>) condenses on the polar heads of the micelles to form silicon

walls around the micelle surface. After removal of the surfactant, the structure of MCM-41 (Scheme 9 [70]) is formed with a specific surface area  $>700 \text{ m}^2/\text{g}$  and with pores in the range of 1.6–10 nm.

Most of the work is devoted to the use of modified MSN containing functional groups. The introduction of functional groups in MSN is the cornerstone in the development of mesoporous materials as drug delivery systems. The main advantage of synthetic MSN is the possibility of their chemical modification to impart various functional properties [65]. The high density of the silanol groups

(Si-OH) in MSN makes it possible to modify the particles by grafting organic siloxanes. There are two ways of modification, i.e., direct functionalization [76] based on the addition of trialkoxysilane with the chosen functional groups to the reaction mixture during the MSN synthesis and post-synthetic functionalization [77] consisting in grafting functional groups after the removal of surfactants. It has been shown that the functionalization of MCM-41 by grafting the aminopropyl groups [78] is an excellent method for the subsequent immobilization of nucleic acids.



Scheme 9

As mentioned above, the recent synthesis, functionalization, and use of MSN (in particular for the delivery of nucleic acid fragments to cells) have been described in numerous reviews [25–29]. Therefore, we omit in this review a detailed discussion of the synthesis and functionalization of mesoporous silicon particles.

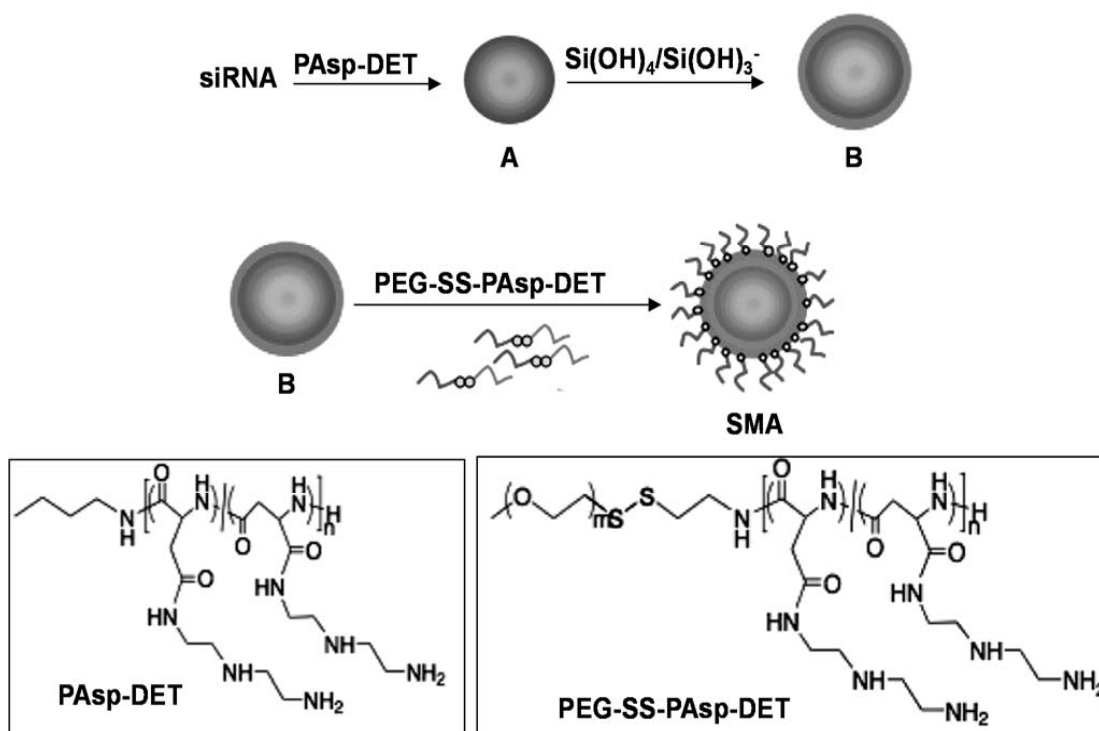
## 6. Other strategies

Cationic silicon-containing nanoparticles were prepared with the use of the  $\text{Ca}^{2+}$  cations [79]. Monodisperse nanoparticles are synthesized by the classical Stöber method with some modifications. Methyltrimethoxysilane was mixed with water, surfactant (Tween-80), and NaOH. The pH value was changed from 10.5 to 12.5 to obtain particles of different sizes. Particles with a diameter of 10 nm were obtained at pH 12.5. After 1 h of stirring, the calcium salt ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) was added, and the reaction mixture was kept for 24 h. The sol-gel synthesis technology in the presence of a surfactant makes it possible to obtain particles with a homogeneous dispersion even after the surface modification. Such a dispersion stability of particles is important for the *in vivo* use, since aggregation can lead to the premature removal of particles from the body. The NA molecules can be immobilized to the Ca-modified nanoparticles because calcium ions

have a high affinity for the internucleotide phosphate groups. Plasmid DNA gWiz GFP that carried the cytomegalovirus gene was added to the obtained nanoparticles. The resulting complexes with immobilized plasmid DNA were used for the efficient selective and non-toxic transfection of bone cells for the purpose of bone tissue regeneration.

The preparation of hollow spherical Si-nanoparticles bearing the protein or DNA molecules incorporated in the cavity is described [80]. The inclusion of unstable molecules into the hollow spheres of the nanoparticles protects them from the environment. DNA was incorporated into spherical particles during their formation. An aqueous solution of sodium silicate and double-stranded DNA was added to the surfactant solution (Tween 80 and Span-80) in hexane. The formed emulsion was precipitated with a solution of  $\text{NH}_4\text{Cl}$  or  $\text{NH}_4\text{HCO}_3$ ; the particles were filtered, washed and dried at  $60^\circ\text{C}$ . The particle size was 5–10  $\mu\text{m}$ . The authors [80] discussed a possible mechanism for encapsulating biomolecules and suggest that the designed system could be used to deliver biomolecules to cells. However, a sufficiently large particle size makes it doubtful. Moreover, as the authors themselves admit, they were able to obtain particles with only a small capacity for DNA.

A multi-layered delivery system (SMA, from the Smart Multilayer Assembly) was prepared by the layer-by-layer method [81] (Scheme 10).



Scheme 10

Small interfering RNA molecules (siRNA) were used as a core. The first polycationic layer served to destroy endosomes after delivery to the cells; the next protective layer consisted of  $\text{SiO}_2$ ; the last shell of polyethylene glycol additionally stabilized the entire structure.

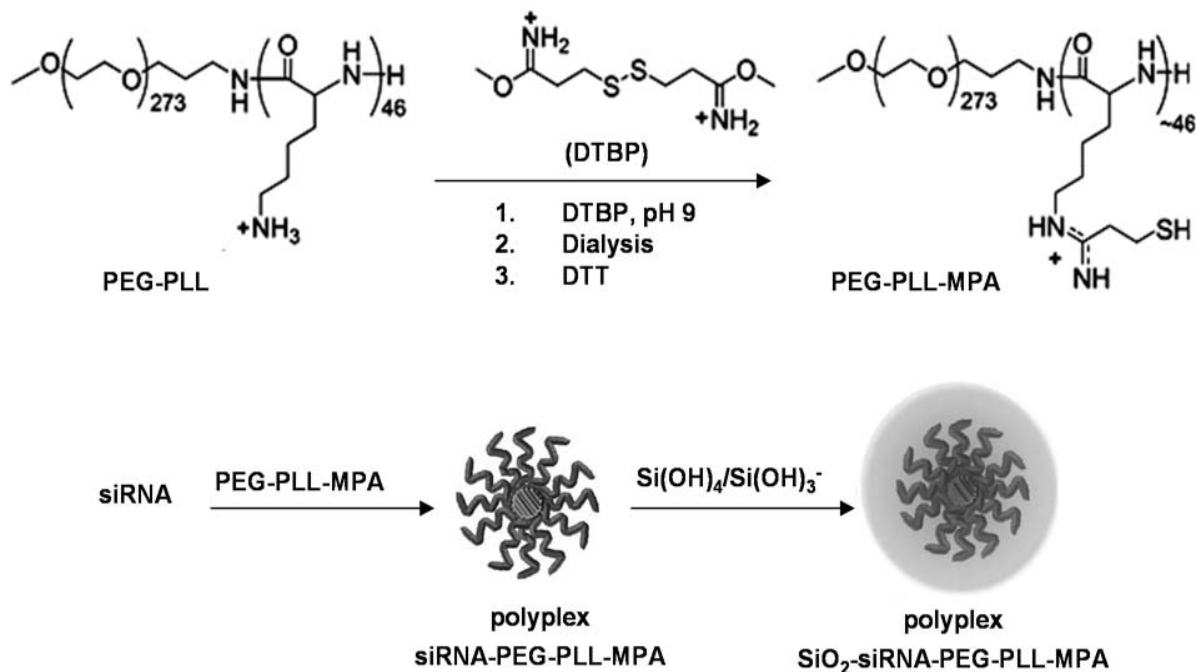
First, a mixture of siRNA with polycation (polyaspartamide containing the diethylenetriamine residue-PAsp-DET) is prepared, so that complex A has a positive charge to bind the silicon layer. The resulting complex (115 nm and  $\xi \approx +20$  mV) can penetrate the cell membrane. However, siRNA needs protection, which is provided by the silicon layer formed by incubation of the nanocomplex A with sodium silicate at pH 7.3. The size of particles B after this procedure is slightly increased ( $\sim 125$  nm), and the  $\xi$  value becomes negative (about  $-19$  mV), which confirms the formation of a negatively charged  $\text{SiO}_2$  layer. In the next step, the resulting structure is incubated with a copolymer of polyethylene glycol and polyaspartamide (PEG-SS-PAsp-DET). At a low concentration of the copolymer, large particles (several microns in diameter) are formed. As the concentration of PEG-SS-PAsp-DET increases, the particle size decreases, evidently due to the screening of the particles by the polymer. As a result, practically monodisperse spherical particles (SMA) with a diameter of 160 nm and a value of  $\xi \approx +4$  mV were obtained.

After delivery to the cells, the PEG layer can disintegrate under the action of disulfides, the silicon layer gradually dissolves, and siRNA is released from the nanocomplex.

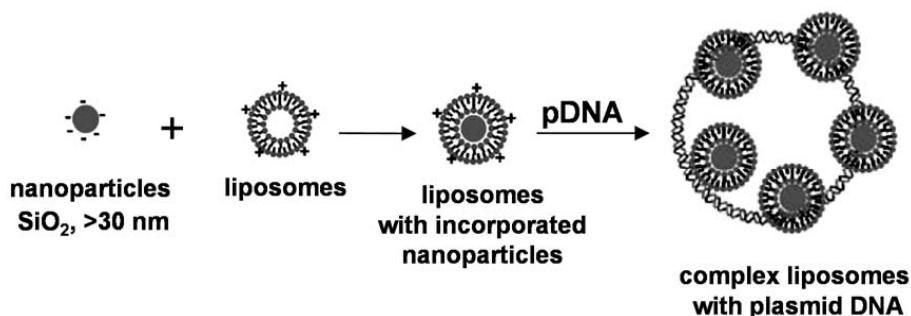
The same group of authors in the following work [82] also used PEG and  $\text{SiO}_2$  as protective layers for siRNA. In this work, the complex PEG-PLL-MPA (conjugate of polyethylene glycol with modified polylysine bearing SH groups) is pre-synthesized (Scheme 11), and then a polyplex is formed from the complex with siRNA.

The resulting polyplex ( $\sim 30$  nm) is incubated with sodium silicate, which leads to the formation of a  $\text{SiO}_2$  layer, and the particle size is increased to  $\sim 50$  nm. The ability of the created structures to penetrate into cells and the ability of delivered siRNA to influence gene expression *in vitro* and *in vivo* was shown in [81, 82]. Intravenous administration of the created siRNA-bearing system in mice with a subcutaneous tumor resulted in significant suppression of the genes in the tumor tissue without hematologic toxicity.

Another example of multilayered nanoparticles with the participation of silicon is presented in [83]. The authors studied the interaction of Si-nanoparticles (8–130 nm) with cationic liposomes and DNA (Scheme 12). The positively charged lipid layer includes Si-nanoparticles, and plasmid DNA is attached to the resulting complexes.



PEG-PLL, polyethylene glycol/polylysine copolymer; DTBP, dimethyl-3,3'-dithiobispropionimidate; DTT, dithiothreitol; PEG-PLL-MPA, polyethylene glycol/polylysine copolymer bearing the 1-(3-thiopropyl)amidinium group. Scheme 11

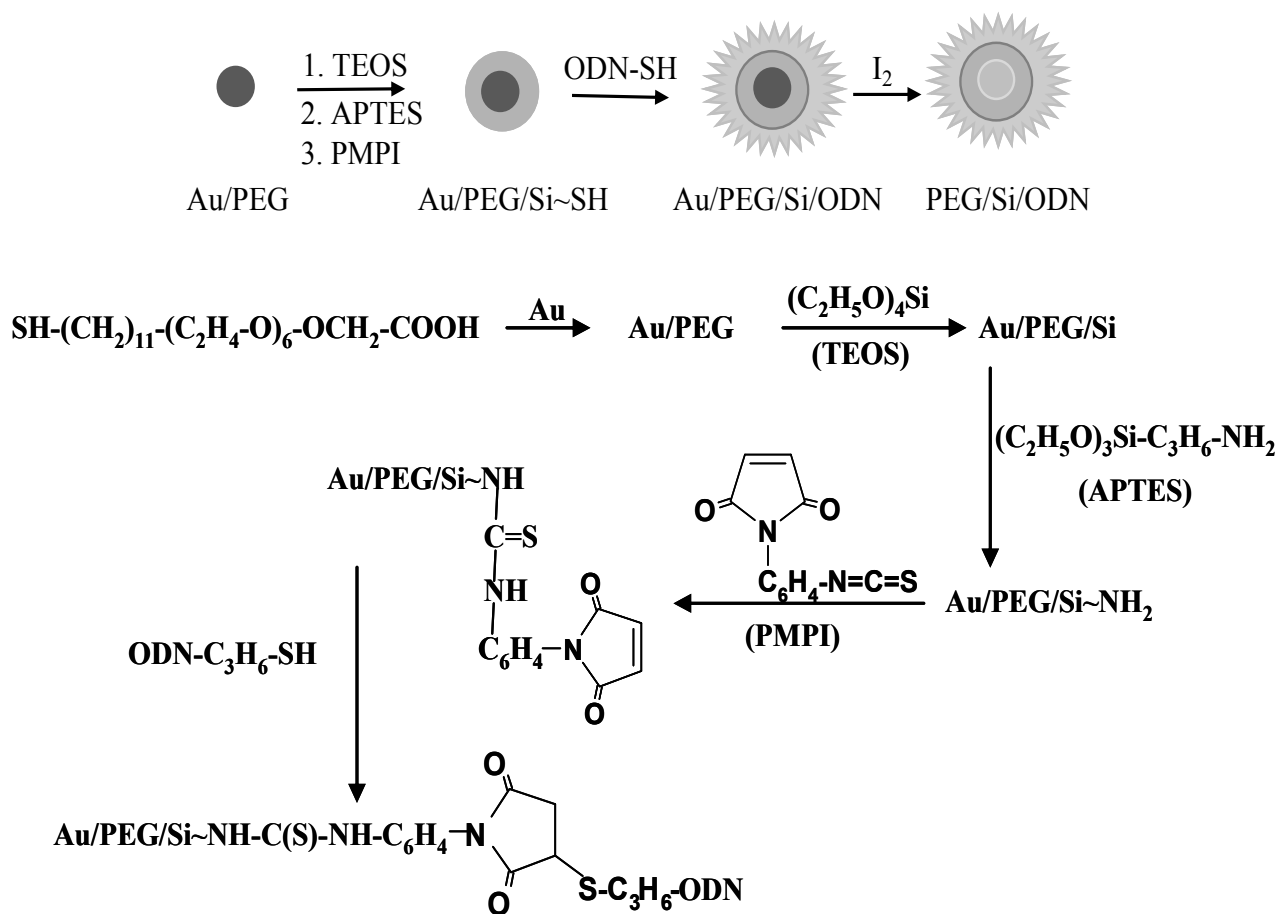


Scheme 12

It was shown that the transfection of cells depended on the size of the Si-nanoparticles and the composition of the lipid layer. The efficiency of transfection significantly decreases with increasing the particle size. The use of particles with a diameter of 30 nm resulted in the maximum penetration of DNA into the cells.

An original method for obtaining complexes of oligonucleotides with Si-nanoparticles (so-called spherical nucleic acids) is described in [84] (Scheme 13). Gold nanoparticles were stabilized with citrate and treated with polyethylene glycol containing the thiol groups at one end and the carboxyl groups at the other end:  $\text{SH-(CH}_2\text{)}_{11}\text{-(CH}_2\text{-CH}_2\text{-O)}_6\text{-OCH}_2\text{-COOH}$ . These particles were then coated with a thin layer (~15 nm) of the  $\text{SiO}_4$

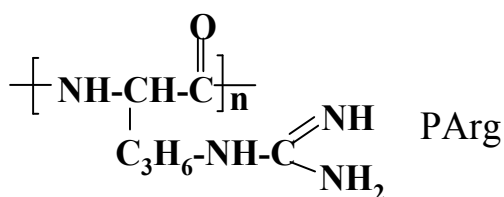
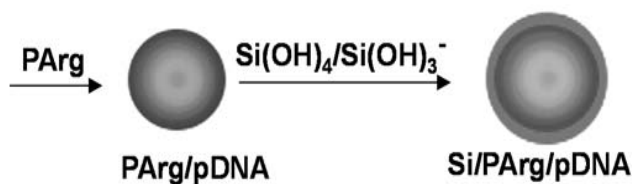
network, which was obtained by hydrolyzing triethoxysilane (TEOS) in the presence of  $\text{NH}_4\text{OH}$ . The thickness of the silicon layer can be easily controlled by changing the ratio of gold nanoparticles, water, and alkoxy silane. The resulting particles were treated with APTES to introduce the amino groups and then a heterobifunctional cross-linker with *p*-maleimidophenyl isothiocyanate (PMPI). As a result, the SH groups appeared on the surface of the particles, and oligonucleotides containing the thiopropyl groups were attached to the SH groups on the particle surface. The desired nanoparticles of spherical nucleic acids were obtained after the subsequent oxidation of the gold core with iodine.



PEG, polyethylene glycol; PMPI, *p*-maleimidophenyl isothiocyanate; ODN-C<sub>3</sub>H<sub>7</sub>-SH, oligonucleotide containing thiopropyl group at the 3' end.

Scheme 13

Particles called “silica-coated polyplexes” (Scheme 14) were described in [85]. Initially, polyarginine (PArg) was mixed with plasmid DNA encoding luciferase.



Scheme 14

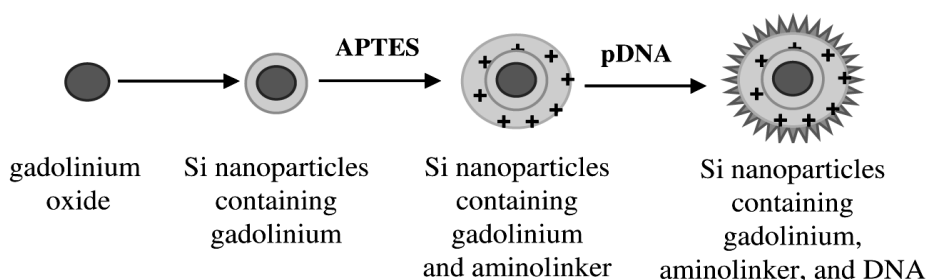
Polyarginine is chosen as a polyplex-forming polycation because of its high affinity for the sugar-phosphate backbone of DNA, as well as its ability to condense with sodium silicates to form the ionic and hydrogen bonds [86]. The resulting particles had a size of 100 nm and a zeta potential of +30 mV at pH 7.3. The Si/PArg/pDNA complex was obtained by mixing PArg/pDNA with the dilute sodium silicate solution that forms a silicon network on positively charged chains. The zeta potential of the particles decreases to -20 mV, and the size increases by 8–20 nm in comparison with the original polyplex.

It was shown that coating of the PArg/pDNA polyplex with silicon dioxide prevented aggregation of the nanoparticles. The Si/PArg/pDNA nanoparticles are stable under physiological conditions and gradually dissociate in the intracellular medium. The formation of luciferase in cells showed a sufficiently high transfection ability of the synthesized particles. The Si/PArg/pDNA

particles demonstrated the reduced cytotoxicity compared to the original PArg/pDNA polyplex. The authors believe that the resulting conjugates should degrade over time releasing oligonucleotides, which indicates the reversible nature of the silica-layer. These results demonstrated the utility of the silica-coating technique for polyplex-mediated gene delivery.

The authors of the work [87] synthesized silicon-containing nanoparticles decorated with gadolinium oxide (Scheme 15). Hexane, bis-2-(ethylhexyl) sodium sulfosuccinate (surfactant) and an aqueous solution of  $\text{Gd}(\text{NO}_3)_3$  were mixed to create microemulsion A. Microemulsion B consisted of a mixture of surfactant-hexane- $\text{NH}_4\text{OH}$ . The two microemulsions were mixed and stirred for 2 h at 4 °C, followed by the addition of tetramethyl orthosilicate (TMOS), and the stirring was continued for 72 h at room temperature. The resulting granules were separated by centrifugation, washed with hexane, and suspended in water with sonication. APTES and an ammonia solution were

added to the suspension to introduce the amino groups. After 16–20 h of stirring at 20–25 °C, the amino-modified nanoparticles were separated by centrifugation, washed, and suspended in PBS buffer (pH 6.0) under ultrasonic cooling. Excess APTES was removed by dialysis. The obtained spherical nanoparticles were practically monodisperse, and had a size of 40–50 nm at a 1 : 39 ratio of Gd to Si atoms. Plasmid DNA was electrostatically immobilized on the synthesized nanoparticles. DNA in the resulting complex was shown to be completely protected from hydrolysis by DNase 1. At a high concentration (50 and 100  $\mu\text{g}/\text{mL}$ ), the particles proved toxic to the cells. However, at the expected working concentration (10  $\mu\text{g}/\text{mL}$ ) the toxicity was not very high. The authors showed that the proposed nanoparticles are capable of transporting DNA into cells. The prepared nanocomplexes, which have paramagnetic properties, can deliver DNA to cells with an efficiency of ~75% compared to the commercial transfecting agent Polyfect®.



Scheme 15

## 7. Conclusions

As can be seen from the review, a huge number of works are devoted to the synthesis of silicon-containing nanoparticles, intended for the delivery of nucleic acids in cells.

The size of Si-nanoparticles is an important parameter that influences the transfection of cells, *in vivo* biodistribution, and the targeted action. The size of the Si-nanoparticles can be controlled by the synthesis conditions, i.e., the reagent concentration, the nature of the alkoxy silanes and the surfactant (in the microemulsion method), pH, and the reaction time. The presence of a large number of the silanol groups in nanoparticles makes it possible to modify  $\text{SiO}_2$  nanoparticles, both commercial and synthesized in the laboratory. At the same time, a wide variety of commercially available alkoxy silanes makes it possible to obtain Si-nanoparticles with various functional groups,

including amino-modified particles suitable for the immobilization of nucleic acids, which are usually bind to the amino groups in the nanoparticles due to electrostatic interaction.

Almost all studies have shown that silicon-containing nanoparticles are low-toxic, capable of transporting fragments of nucleic acids into cells and protecting them from the action of nucleases. An important property of Si-nanoparticles is their ability to decompose and release from the body by excretion. Most synthesis methods differ in the simplicity and availability of components. It can be hoped that they can find practical application in the future for obtaining effective systems for the nucleic acid delivery in cells *in vitro* and *in vivo*. As for the works on the creation of nanocomposites described in the section “Other strategies”, the complexity of the design and the multistage synthesis makes one doubt the practical applicability of the proposed methods.

Quite a lot of work is devoted to the introduction of silicon-containing nanoparticles for the delivery of fragments of nucleic acids (most often plasmid DNA and siRNA) *in vivo*. The authors who developed the synthesis of ORMOSIL particles have shown that a complex of particles with DNA can be used to regulate processes in nerve cells *in vivo*, i.e., for the treatment of brain diseases [13, 47, 48]. It has been shown that complexes of particles with plasmid DNA can correct dog leukopenia [39, 40]. Intravenous administration of particles carrying siRNA to mice with a subcutaneous tumor resulted in significant gene suppression in tumor tissue [81]. Mesoporous Si-nanoparticles proved to be effective carriers for various therapeutic agents. Only in the last four years, there have been many works concerning the use of MSN *in vivo* [88–92].

The properties of silicon-containing nanoparticles, i.e., the simplicity of the synthesis and modification, low toxicity, biocompatibility, and the ability to decompose and excrete, makes them promising nucleic acid vehicles for cell delivery. Despite the impressive results already achieved, researchers continue to improve the proposed methods and develop new strategies for the synthesis of silicon-containing nanoparticles.

## Acknowledgment

The review was supported by the grant no. 16-15-10073 from the Russian Scientific Foundation.

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