Modified silica particles for gene delivery

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Abstract

In somatic gene therapy, new concepts for the transfer of DNA into specific cell nuclei are of interest. Inorganic nanoparticles have an interesting potential as DNA carrier system due to the possibility to tailor their surface reactivity and the electrical surface potential (zeta potential) that can be obtained by the surface modification. SiO_2 nanoparticles have been chosen for reasons of low toxicity. In order to obtain positively charged nanoparticles, basic surface groupings have been selected. Different types of alkoxysilanes and amines have been tested for surface modification of SiO_2 nanoparticles. The zeta potential at pH = 7.4 could be varied from − 38.8 mV (unmodified 10 nm particles) to + 20 mV (gamma-aminopropyl-triethoxysilane) and to + 49.8 mV (glycidoxypropyltrimethoxysilane and ethylendiamine). DNA could be completely immobilized at the nanoparticle surface and nanoparticle/DNA ratios between 2 and 15 (w/w) were obtained. The interaction of DNA with the particle surface correlates with an increasing number of modifier molecules on the particle surface.

Keywords: Silica; Nanoparticles; Surface modification; Aminosilanes; Gene transfer

1. Introduction

Gene therapy opens a wide variety of new medical applications if appropriate gene vectors are made available. Viral vectors are widely used, but non-viral vectors provide advantages like easy preparation and reduced risk of immune response [1]. Many systems have been studied in the past, including peptides, liposomes, organic polymers, and dendrimers [2,3]. However, organic polymers also show toxic effects at concentrations needed for an effective transfection [4,5].

Inorganic nanoparticles provide new and promising aspects as gene and drug transfer vehicles. They have the potential to overcome systemic barriers to build drugs with low solubility in water through appropriate surface modification and drug targeting becomes possible. Amorphous silica nanoparticles are favoured since SiO_2 is a nontoxic compound present in a lot of systems, which can be tailored with a variety of surface modifiers, allowing to adjust properties like zeta potential and surface reactivity. SiO_2 nanoparticles are available in different diameters. It was shown elsewhere that they are suitable as gene transfer vectors after surface modification with aminosilanes [6,7]. Gold nanoparticles also were used for the transfection of mammalian cells [8].

The mechanism of the interaction between the gene vector properties and DNA was studied for liposomes and organic nanoparticles [5,9]. The chemical structure of polymers determine the formation of polymer–DNA complexes with organic nanoparticles [10], but no systematic study was performed on the interaction between DNA and inorganic gene transfer vectors so far. Thus, the influence of the type of surface modifier like chain length or surface concentration on nanoparticle–DNA interaction is not known. These open questions have to be answered to understand and optimise gene transfer efficiency.

The aim of the study presented in this paper is to show the effect of surface modifications with alkoxysilanes and different amines on nanoparticle properties and on nanoparticle–DNA interaction. The scientific approach was to synthesize different modifiers, react the modifiers with colloidal silica and analyse the particle properties (zeta potential, hydrodynamic diameter, elementar composition) in order to correlate them with the nanoparticle–DNA interaction.

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2. Materials and methods

2.1. Surface modification

Commercially available silica nanoparticles (Levasil 300/30, Bayer) were modified with 3-glycidoxypropyltrimethoxysilane (GPTS) and amines as follows: 8 g Levasil 300/30 (2.4 g nanoparticles in water) were mixed with 150 ml distilled water. Consecutively, 16.6 mmol of the amine and 16.6 mmol GPTS were added to this solution and mixed for 3 days at room temperature (22 °C). Due to low solubility of 1,6-diaminohexane in water, only 1 mmol modifier was added to the same amount of particles for SIN25.

For the modification with 3-triethoxysilylpropylisocyanate (IPS) and amines (batches SIN28 and SIN29), 16.6 mmol amine and 16.6 mmol IPS were separately dissolved in 20 ml THF. The IPS containing solution was slowly added to the amine containing solution and the mixture was heated under reflux at 70 °C for 21 h. The solvent was evaporated and the remaining solid was dissolved in pure ethanol. For surface modification of SiO_2 nanoparticles, the ethanolic solution was added to a mixture of 8 g Levasil 300/30 and 150 ml water and stirred for 3 days at room temperature.

After surface modification, 60 ml ethylenglycol was added to the solution given above, rotated for 2 h under vacuum at 45 °C and dialysed in deionised water until the conductivity remained constant. The product was filtered (0.2 μm sterile filter) and stored at room temperature (22 °C).

For one modifier, both Levasil 300/30 (9 nm, SAP20) and Levasil 50/50 (50 nm, SIN32) were used to examine the effect of the particle size. In the case of Levasil 50/50, 4.8 g of the particle solution were used to keep the nanoparticles concentration constant (2.4 g), all the other components were used as given above.

The components used for modification of the different batches are given in Table 1.

2.2. Particle characterization

CHN analysis was performed with a CHN analyser RC-900 (Leco, St. Joseph, MI, USA). Zeta potential titration was performed with the Zetasizer 2000 HS (Malvern, UK). The instrument was checked routinely with a -50 mV standard to give a zeta potential of -50 ± 5 mV. The particles were diluted 1:6 in distilled water, the pH was adjusted to <3 with 1 M HCl and titration was performed automatically with 0.1 M NaOH from pH 3 to pH = 10, performing 14 zeta potential measurements at different pH values.

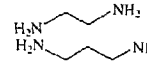
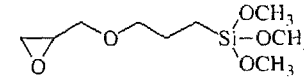
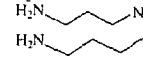
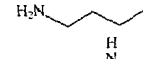
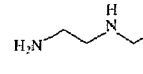
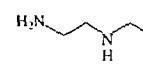
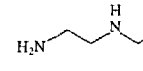
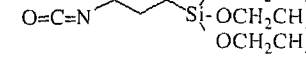
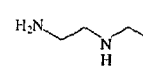
The electrophoretic mobility shift assay (EMSA) was used to study the interaction between nanoparticles and DNA. Nanoparticle–DNA complexes were prepared by mixing nanoparticles with DNA in water in different ratios. The β-galactosidase expression plasmid DNA pCMVbeta was used for these experiments, DNA concentration was fixed at 10 μg ml^{-1}. Nanoparticle–DNA complexes were prepared at ratios between 1 and 100 (w/w). Agarosegel electrophoresis was performed in a 1% (w/v) gel, ethidium bromide included for visualisation, for 90 min at 100 V. Images were taken using a UV transilluminator and a Geldoc2000 gel documentation system (Bio-Rad, Germany), band integration and background correction was performed using Molecular Analyst version 1.1 software (Bio-Rad, Germany). As a result, the electrophoretic mobility shift assay gives the nanoparticle/DNA ratio (w/w) at which no mobile DNA is detected—thus, a low value means high interaction.

3. Results and discussion

3.1. Nanoparticle modification

Since DNA is a highly anionic polyelectrolyte, it does not interact with negatively charged SiO_2-nanoparticles unless

Table 1
Alkylsilanes and amines applied for surface modification; silica nanoparticles of about 9 nm diameter were used for all batches (Levasil 300/30) except SIN32, where 50 nm particles were used (Levasil 50/50)

Particle batch	Amino component	Silane component
SAP19	ethylendiamine 	glycidoxypropyltrimethoxysilane 
SAP20, SIN32	1,3-diaminopropane 	
SIN24	1,4-diaminobutane	
SIN25	1,6-diaminohexane[a] 	
SIN27	triethylentetramine 	
SIN30	pentaethylenhexamine 	
SIN28	triethylentetramine 	3-triethoxysilylpropylisocyanate 
SIN29	pentaethylenhexamine 	

[a] Only 1/16 of the modifier added for synthesis due to low solubility in water.

their surface is modified to achieve positive zeta potentials. Therefore, the surface of silica nanoparticles was modified with different alkoxysilanes and amines (Table 1) and the effects of the modification on particle properties and nanoparticle–DNA interaction were characterised.

One would expect that the nanoparticle–DNA interaction is stronger with increasing positive zeta potential, i.e. with increasing number of amines (ammonium) groups on the particle surface. Two amino components (triethylentetramine and pentaethylenehexamine) were coupled with two different silane components (GTPS and IPS) for modification of the particles to give batches SIN27 to SIN30. These modifiers contain between four and seven nitrogen atoms per molecule. To see if there is a steric effect, the spacer length was varied for diamines: ethylenediamine (SAP19), 1,3-diaminopropane (SAP20) and 1,4-diaminobutane (SIN24) were used in combination with GPTS as surface modifiers. SIN25 particles contain GPTS and 1,6-diaminohexane, but less modifier was added in this reaction and a low degree of surface occupancy is expected. Furthermore, two silica particles of different sizes were modified with the same modifier, to examine the effect of particle size on particle properties and DNA binding capacity: SAP20 with 9 nm and SIN32 with 50 nm diameter.

The characterization of modified SiO_2 particles by solid-state ^{29}Si NMR reveals a high condensation degree (>90%) of T-units of the silane modifier. Additional solid-state ^{29}Si NMR cross-polarization experiments with variable contact time support the assumption that the silane compound is bound to the silica nanoparticles. The absence of epoxide signals in solid-state ^{13}C NMR spectra indicates the ring opening reaction, very likely caused by the amines, leading to a structure like proposed in Fig. 1. Further evidence for the reaction between the epoxide and the amino compounds is given by the chemical analysis and the zeta potential titrations: the particles contain up to 5.8% nitrogen and the zeta potentials show a change of about +80 mV at pH = 7.4 (Table 2).

The important physical and chemical properties of the surface modified particle batches are shown in Table 2. The zeta potential of the unmodified SiO_2-nanoparticles at pH = 7.4 changes from −39.8 and −44.7 mV to values ranging from +15.2 to +49.8 mV. The zeta potential curves (Fig. 2) show that the isoelectric point of the modified particles except SIN25 is above pH = 10. The lower i.e.p. of SIN 25 can be explained by the lower content of surface modifier. Due to the zeta potential at pH = 7.4, all particles form suspensions in aqueous solutions at room temperature.

The elemental analysis shows C (>20%) and H (>4%) contents for all Levasil 300/30 particles modified with GPTS and an amino component in expected range, but lower values for the particles where IPS was used as silane component (SIN 28, SIN29), whereas the N content of SIN29 is high due to the amino groups of the modifiers molecule.

3.2. Nanoparticle–DNA interaction

The results of the electrophoretic mobility assays are given in Table 2. As expected, DNA does not interact with unmodified silica particles but with all modified particles with positive zeta potentials at neutral pH.

As already described, the number of nitrogen atoms/modifier molecule was increased systematically in order to detect the influence on DNA binding. Contrary to the expectations, the particle–DNA interaction decreases with increasing number of amino group in the modifier molecule of the particles and with increasing number of nitrogen atoms/modifier molecule. Also, no correlation can be seen between the DNA binding and the zeta potential at neutral pH. SiO_2 nanoparticles modified with the previously synthesized modifier molecule (IPS + amine compound) are less effective in DNA binding in comparison with the synthesis by adding amine and GPTS to the silica particles.

The DNA binding of the batches SAP19, SAP20 and SIN24 is complete at nanoparticle/DNA ratios 2, 2 and 3 (w/w), respectively, whereas the larger nanoparticle

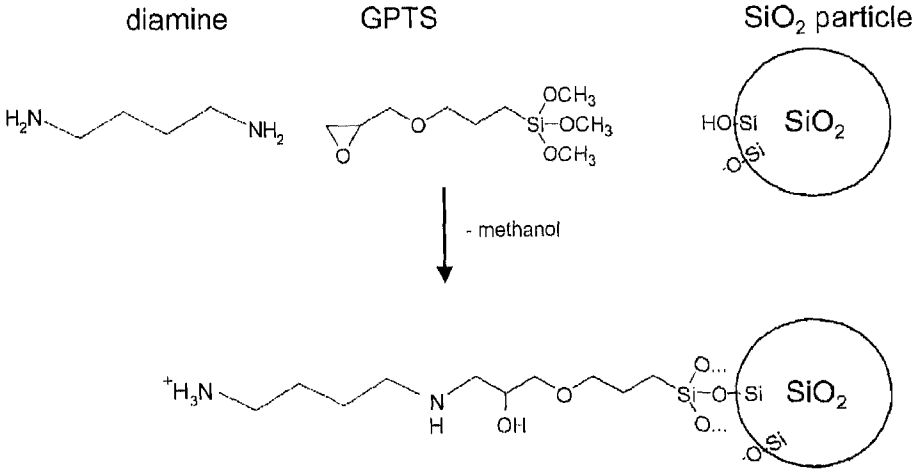


Fig. 1. Proposed surface modification reaction for silica particles (scheme).

Table 2
Physico-chemical properties of surface-modified SiO_2-nanoparticles and of the not modified particles (Levasil 300/30 and Levasil 50/50) in water and their interaction with DNA in electrophoretic mobility shift assay (EMSA)

	N atoms/modifier and spacer length (diamines)	Zeta potential at pH = 7.4 (mV)	Hydrodynamic diameter (PCS) (nm)	C content (%)	H content (%)	N content (%)	Nanoparticle/DNA ratio (EMSA) (w/w)
Levasil 300/30	0	−39.8	6.5	0.590	0.775	0.010	neg.
Levasil 50/50	0	−44.7	75.3				neg.
SAP19	2 with $(CH_2)_2$	+49.8	39.0	24.92	4.900	3.180	2
SAP20	2 with $(CH_2)_3$	+33.9	60.4	22.50	4.460	2.930	2
SIN24	2 with $(CH_2)_4$	+44.1	106.5	22.00	4.119	2.499	3
SIN25[a]	2 with $(CH_2)6$	+15.2	29.7	3.63	1.089	0.392	15
SIN27	4	+30.7	85.4	22.94	4.679	4.045	3
SIN28	5	+39.8	116.9	9.98	2.289	3.354	6
SIN29	7	+39.0	181.9	15.59	3.294	5.794	8
SIN30	6	+38.7	123.1	22.96	4.616	4.909	3
SIN32	2 with $(CH_2)_3$	+49.4	195.8	13.71	2.740	1.400	15

Levasil 300/30 particles of about 9 nm diameter were modified with amine and alkylsilane compounds given in Table 1, except for SIN 32 which is based on the Levasil 50/50 (about 50 nm) modified with the same modifiers as SAP20.
The ratio nanoparticle/modifier was kept constant except for SIN25 (1/16 of the modifier added). No nanoparticle–DNA interaction is detected with unmodified SiO_2 particles.
[a] Only 1/16 of the modifier added for synthesis due to low solubility in water.

analogue of SAP20, namely SIN32 on Levasil 50/50 basis, binds at a nanoparticle/DNA ratio of 15 only. This is also found for SIN25, a particle with low degree of surface occupancy due to low amount of added modifier. In conclusion, the spacer length does not exhibit a strong effect on the DNA binding of the modified particles.

All modifications except SIN25 were performed with the same ratio of SiO_2 particles and modifier molecules. However, the resulting degree of occupancy is different (number of modifier molecules/m^2 particle surface, calculated on the basis of the C content of the particles) and decreases with increasing modifiers size. A correlation seems to exist between the degree of occupancy and the particle–DNA interaction (Fig. 3). Particles coated more densely by amino-

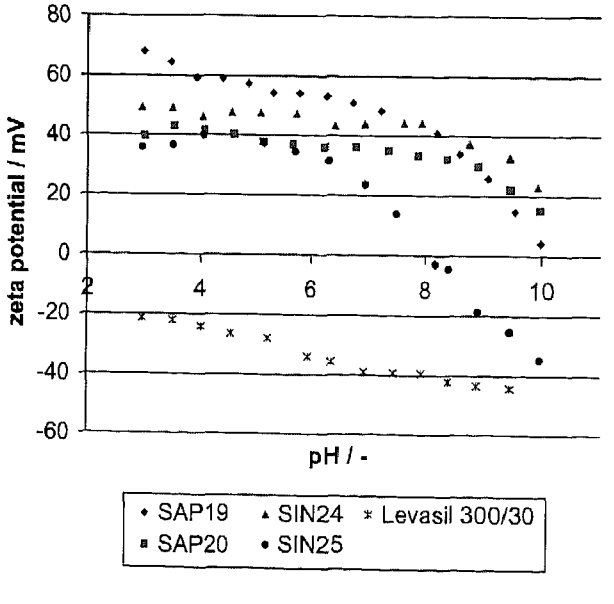


Fig. 2. Zeta potential titration curve of the unmodified particles (Levasil 300/0, Bayer) and of surface modified particles with different modifiers: SAP 19 (GPTS + ehtylendiamine), SAP20 (GPTS + 1,3-diaminopropane), SIN24 (GPTS + 1,4-diaminobutane), and SIN25 (GPTS + 1,6-diaminobutane); the ratio of nanoparticles to modifier was kept constant except for SIN 25 (1/16 of modifier).

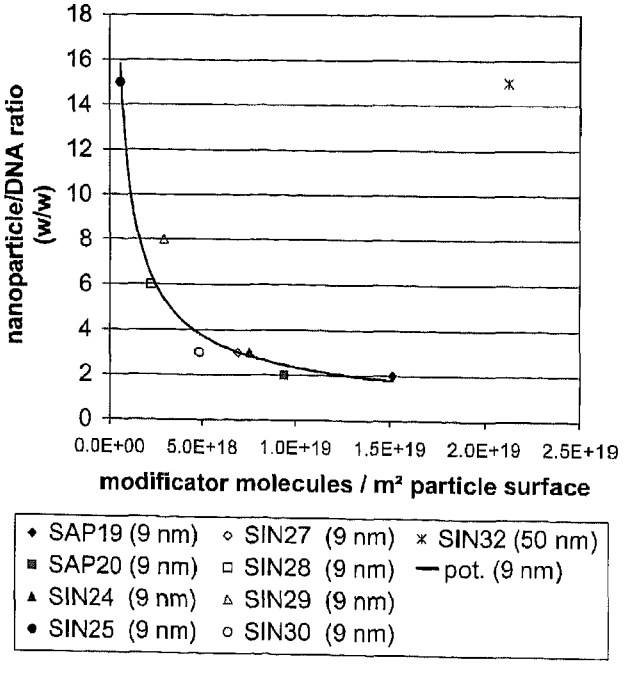


Fig. 3. Nanoparticle/DNA ratio (w/w) as a result of the electrophoretic mobility shift assay (EMSA) in relationship to the degree of occupancy calculated on the basis of carbon content; the EMSA gives the nanoparticle/DNA ratio at which 100% immobilisation is found—low values imply good interaction. The ratio nanoparticle/modifier was kept constant except for SIN 25 (marked, 1/16 of modifier added); the modifiers used are given in Table 1.

silanes of low molecular weight interact stronger with DNA than particles with a less dense coating of larger molecules, even if they contain more amino group.

As shown in the literature, for a synthetic cationic organic polymer, the nanoparticle/DNA ratio of 3 (w/w) was found to be optimal for transfection efficiency [5]. The minimal nanoparticle/DNA ratios at which the DNA is totally immobilised in this study are in good agreement with this value. For the interaction of DNA with organic polymers and liposomes, the structure of the polymer was shown to determine the affinity of DNA binding and the electrostatic interaction was shown to force the plasmid to change its structure [9,10]. The correlation between small hydrodynamic diameter and nanoparticle–DNA interaction was noted previously for organic nanoparticles [10]. In the case of SiO_2 nanoparticles, the interaction between the plasmid and surface-modified nanoparticle seems to be enhanced by a dense coating with small molecules rather than by a layer with larger molecules and less occupancy degree.

4. Conclusion

It could be shown that the concept of surface modification of SiO_2 nanoparticles can be extended to surface modifiers with different kinds of amino groups. However, as results of immobilizing of DNA show, the short chain modification resulted in the highest amount of fixed DNA. Since the detailed structure of the larger surface modifiers is not known, the DNA–modifier interaction is not quite clear so far.

The study was performed with model plasmid DNA described in the experimental part, which has 7140 base pairs. The effect of DNAs of different sizes could not be investigated in this paper but has to be considered in future work.

Nevertheless, the formation of DNA–nanoparticle complexes described in this paper may be an important step for gene transfer. The future work should be focused on the gene transfer to evaluate the relation between DNA binding affinity to particles and gene transfer efficiency.

Acknowledgements

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