Highly Efficient siRNA Delivery from Core-Shell Mesoporous Silica Nanoparticles with Multifunctional Polymer Caps

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Supporting Information

Materials

Fluorocarbon surfactant (FC-4, yick-vic chemicals), block copolymer surfactant (Pluronic F127, Sigma-Aldrich), 1,2,4-trimethylbenzene (TMB, Sigma-Aldrich, 98%) tetraethyl orthosilicate (TEOS, Fluka, > 98%), triethanolamine (TEA, Aldrich, 98%), cetyltrimethylammonium chloride (CTAC, Fluka, 25% in H_2O), mercaptopropyl triethoxysilane (MPTES, Sigma-Aldrich, > 95%), aminopropyl triethoxysilane (APTES, Sigma-Aldrich, 99%), phenyltriethoxysilane (PhTES, Sigma-Aldrich, 98%), toluene (Sigma-Aldrich, anhydrous), ammonium fluoride (NH_4F, Sigma, >98%), triethanolamine (TEA, Aldrich, 98%), 1,3,5-triisopropylbenzene (TiPB, Fluka, 96%), sulfo-N-hydroxysuccinimide (sulfo-NHS, Aldrich, 98%), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC, Sigma, 97%), N,N-dimethylformamide (DMF, Sigma-Aldrich, anhydrous), succinic anhydride (Fluka), cystamine dihydrochloride (Aldrich), NHS-ATTO-633 (ATTO-Tec), Mal-ATTO-633 (ATTO-Tec)
siRNA, Axolabs: GFPsiRNA: sense 5`-AuAucAuGGccGAcAAGcAdTsdT-3`, antisense 5`-UGCUUGUCGGCcAUGAuAUdTsdT-3´; ctrl siRNA: sense 5´-AuGuAuuGGccuGuAuuAGdTsdT-3´, antisense 5´-CuAAuAcAGGCcAAuAcAUdTsdT-3´
RPMI-1640, folate free (Life technologies), collagen (Biochrom), fetal calf serum (FCS, Life technologies), antibiotics (Biochrom), luciferin (Promega), lysis buffer (Promega),
oligomer 454 was synthesized as described before[1], oligomer 356 was synthesized as described before[2] GelRed™ (Biotum, Hayward, U.S.A.),
1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) ((DOTAP),18:1 TAP, Avanti Polar Lipids).
Millipore water was used in all experiments.

Methods

LP-MSN-Synthesis and post-synthetic grafting procedures

LP-MSN synthesis

The synthesis of the siliceous LP-MSN nanoparticles was performed similarly to a previous report.[3] 1.4 g of the fluorocarbon surfactant was dissolved together with 0.5 g of the block copolymer surfactant F127 in 60 g of a 0.02 M HCl solution at 60 °C under stirring at 800 rpm for 2 hours. The solution was cooled down to 15 °C in a thermostat and 0.5 g of TMB was added as pore extension agent. This mixture was stirred for 2 hours before 3 g of TEOS were subsequently added under stirring, which was continued for 24 hours. Finally, the mixture was filled into a 100 mL Teflon-lined autoclave and heated under autogenous pressure at 150 °C for another 24 hours. The product was centrifuged after cooling at 20 000 rpm for 15 minutes and the supernatant was exchanged for 30 g of a 2 M HCl solution. A second heat treatment was performed for 2 days at 140°C.

Template extraction

The sample was retrieved by centrifugation and the supernatant was exchanged for 35 mL of a 2 M HCl/ethanolic extraction solution and was refluxed at 90 °C for 3 hours. This process was repeated once before the sample was washed 2 times in 50 mL ethanol/water solution and was finally stored in ethanol for further use.

LP-MSN grafting

Grafting with APTES and PhTES was performed with varying molar ratios. Usually, 200 mg (3.3 mmol) SiO_2 of the parent LP-MSN sample was suspended in 20 mL of toluene under dry nitrogen in a Schlenk-flask and the silane coupling agents were added under nitrogen flow. A maximum of 20 mol% (0.66 mmol) of combined silane coupling agents was added to this solution and refluxed at 90 °C for 4 hours and washed 3 times in ethanol. The following molar ratios were used for the LP-MSN samples:

LP-1: no grafting, parent sample for LP-2 to LP-4
LP-2: APTES (0.66 mmol or 155 µL)
LP-3: APTES 0.44 mmol (103 µL) together with 0.22 mmol PhTES (53 µL)
LP-4: APTES 0.22 mmol (50 µL) together with 0.44 mmol PhTES (107 µL)
LP-5: APTES 0.22 mmol (50 µL) together with 0.44 mmol PhTES (107 µL)
LP-6: APTES 0.22 mmol (50 µL) together with 0.44 mmol PhTES (107 µL)
(LP-6 was prepared via cocondensation)

The resulting degree of functionalization was estimated from thermogravimetric measurements and was determined by elemental analysis as listed in Table 1.

MP-MSN-Synthesis with co-condensation

Co-condensation of the core-shell LP-MSN samples was achieved as follows: Solution 1: 100 mg NH_4F (2.7 mmol), 21.7 mL H_2O (1.12 mol), 2.97 ml TiPB (12 mmol) and 2.41 mL of a 25% CTAC solution (1.83 mmol) were mixed in a polypropylene reactor and heated to 60 °C under stirring. A second solution was prepared containing 12.77 mL TEA (97 mmol) to which was added 1.96 mL TEOS (8.8 mmol) combined with 0.2 mL APTES (0.86 mmol). This solution was heated to 90 °C under static conditions for 1 h and was subsequently added under strong stirring to solution 1. The combined

solutions were allowed to cool to room temperature under stirring. After 20 minutes we added 0.1 mL TEOS (0.45 mmol) dropwise and stirred the solution for another 30 minutes. After this time the ingredients for the shell layer were added, consisting of a premixed solution of 22 µL MTES (0.11 mmol) and 22 µL TEOS (0.10 mmol). The condensation reaction was allowed to continue over night.

Subsequently, this solution was mixed with an additional 50 ml ethanol for 15 minutes, and the sample was collected by centrifugation. Template extraction followed immediately, as described for the LP-MSN samples.

The silane concentrations used in samples MP-1 to MP3 relate to the following mol% of silane coupling agents with respect to the total amount of silanes used:
MP1: core 0.2 mL APTES (0.86 mmol, or 9 mol%), shell 22 µL MTES (0.11 mmol or 1 mol%)
MP2: core 0.2 mL APTES (0.86 mmol, or 9 mol%), shell 22 µL MTES (0.11 mmol or 1 mol%)
MP3: core 0.1 mL APTES (0.43 mmol, or 4.3 mol%), shell 44 µL MTES (0.22 mmol or 2 mol%)

In order to achieve larger pores in sample MP-1, we centrifuged the reaction mixture after stirring over night at room temperature, exchanged the supernatant against 50 mL of a 1:1 H_2O/ethanol solution and continued stirring for 2 more days at room temperature. Template extraction was subsequentially performed as described above. Sample composition was estimated from TGA measurements, and the sulfur content was confirmed by EDX.

Synthesis of MP-MSN-S-S:

The amino groups present in the parent MSN samples were transformed first into carboxy groups by dissolving 100 mg succinic anhydride in 8 mL dry DMF and adding 20 mg of the MSN sample to the solution, which was stirred for 3 hour at room temperature. Samples were washed 3 times in ethanol and stored in ethanol (MSN-COOH).
To 5 mg of MSN-COOH suspended in 5 ml ethanol were added 5 µL EDC and 5 mg NHS-sulfo, and the mixture was stirred for 1 hour. This was followed by the dropwise addition of 0.5 mL of an ethanolic solution containing 24 mg cystamine dihydrochloride. This solution was stirred over night, and the MSN sample was washed 3 times by centrifugation and resuspension in ethanol, resulting in samples MP-MSN-S-S.

siRNA loading and desorption
siRNA concentrations were determined by UV measurements performed with the Nanodrop 2000c spectrometer (Thermo Scientific), with the nucleic acid module (sample volume 1.5 µL). siRNA adsorption was performed with aliquots of MSN samples, usually amounts of 100 µg that were exposed to 100 µL siRNA solutions (either in water or MES buffer solution at pH = 5) of predetermined concentration. Samples were vortexed and shaken at 37 °C for defined adsorption times between 15 minutes to several hours. Subsequently, samples were centrifuged (14000 rpm, 7 minutes) and the supernatant was measured again with the Nanodrop to determine the adsorbed amount by difference calculations.
To study the desorption process, the supernatant from the loading process was taken off by micropipette and was replaced with 100 µL PBS buffer desorption solution at pH = 7.4. The cumulative desorption was measured in the supernatant solution after centrifugation at preset time intervals. Samples were vortexed and again shaken after each measurement without change of the buffer solution.

Polymer attachment

a) block copolymer (oligomer)

The oligomer 454 was attached to MP-MSN samples after loading with siRNA. The oligomer was added directly to the loading solution after the complete siRNA uptake had been confirmed by Nanodrop analysis and after redispersion of the sample. Usually, 50 µg of oligomer 454 were added to 100 µg MSN and shaken for 1 h at 37 °C. This was followed by a 7 minute centrifugation at 14000 rpm. The supernatant was taken off (and measured as a reference in the cell transfection experiments) and was replaced with PBS buffer at pH = 7.4. Cell transfection was performed shortly thereafter.

b) DOTAP

A DOTAP layer was attached to MP-MSN samples after loading with siRNA. Here, the supernatant loading solution was removed, and to 100 µg of MSN sample 25 µL of a 30 wt% DOTAP solution (2.5 mg/mL in 60:40 H_2O:EtOH) was added by micropipette and was carefully redispersed with the pipette tip, followed by short sonification for 2 seconds. 225 µL cold water (4°C) was subsequently added and again mixed with the pipette for 30 seconds. A 2-fold washing in 100 µl sterile PBS at pH = 7.4 (centrifugation for 3.5 minutes at 14000 rpm) (was performed to remove excess lipid. The final sample was kept in PBS buffer for cell transfection.

Characterization

Nitrogen sorption measurements

Nitrogen sorption measurements were performed on a Quantachrome Instruments NOVA 4000e. All samples (15-20 mg) were heated to 393 K overnight while being evacuated (10 mTorr) to remove any adsorbates before nitrogen sorption was measured at 77 K. The BET (Brunauer-Emmett-Teller) surface areas were calculated from the corresponding nitrogen sorption isotherms in the range p/p_0 = 0.05 – 0.2. Pore size distribution curves were obtained using the non-local density functional theory (NLDFT) by applying either the equilibrium model or the adsorption model for cylindrical pores with nitrogen on silica as the reference module. Pore volumes were determined in LP-MSN samples at a relative pressure p/p_0 = 0.98 and in MP-MSN at a relative pressure p/p_0 = 0.8 to avoid inclusion of the interparticle textural porosity that is usually observed with nanoparticle powders (visible as a second hysteresis loop; see MP-MSN in Fig. 2).

Infrared spectroscopy

FTIR measurements were performed on a ThermoScientific Nicolet iN10 (MX) IR microscope equipped with a MTC-A liquid nitrogen cooled detector. Samples (about 1 mg) were prepared from an ethanolic solution that was dropped onto a non-transparent reflective sample holder and dried before measurements. Spectra were taken in transflection mode using a gold disc as reference. Spectra are all baseline subtracted, cleared from the atmospheric CO_2 absorption and normalized to the silica-based mode at 1075 cm^{-1}.

Raman spectroscopy

Raman spectra were obtained with a combined FTIR/Raman instrument (Bruker Equinox 55 with FRA-106 Raman attachment), providing excitation with an Nd:YAG (YAG: yttrium–aluminum–garnet) laser at 1064 nm. Spectra were collected in back reflection with a laser power of 100 mW.

Thermogravimetry
TGA measurements were performed with the silane-grafted LP-MSN and the co-condensed MP-MSN samples (about 10 mg of dried powder) on a Netzsch STA 440 thermobalance (heating rate of 10 K/min in a stream of synthetic air of about 25 mL/min). The loading of functional groups was determined in comparison to the purely siliceous parent samples.

Transmission electron microscopy (TEM)
TEM measurements were performed on a Jeol JEM-2011 microscope operating at 200 kV with a CCD detection unit. Samples were dispersed in ethanol and one drop of the resulting solution was then dried on a carbon-coated copper grid.

Zeta potential measurements
Zeta potential measurements were performed on a Malvern Zetasizer-Nano instrument equipped with a 4 mW He-Ne-Laser (λ=633 nm) and an avalanche photodiode detector coupled to a zetasizer titration system (MPT-2). pH titration was performed with about 0.5 mg of samples diluted in 10 mL bi-distilled water, with diluted NaOH and HCl solutions serving as titrants.

Cell Microscopy

For imaging, cells were seeded into 8-well ibiTreat slides (ibidi) at densities of 5000-10 000 cells per well the day prior to particle incubation. 3.5 µg of particles were added per well (300 µL volume) and removed after an incubation time of 45 minutes at 37°C by exchanging the medium. Imaging was performed 22 h and 48 h after incubation at 37 °C under a 5% CO_2 humidified atmosphere on live cells using spinning disc microscopy (Zeiss Cell Observer SD utilizing a Yokogawa spinning disk unit CSU-X1). The objective was a 1.40 NA 63x Plan apochromat oil immersion objective (Zeiss). Cy5 was imaged with 639 nm and Atto 488 with 488 nm laser excitation, respectively. For two color detection a dichroic mirror (560 nm, Semrock) and band-pass filters 525/50 and 690/60 (both Semrock) were used in the detection path. Separate images for each fluorescence channel were acquired using two separate electron multiplier charge coupled device (EMCCD) cameras (PhotometricsEvolve™). Cell membranes were stained with wheat germ agglutinin Alexa Fluor 488 conjugate at a final concentration of 5 µg/ml. Lysosomes were stained with CellLight® Lysosomes-GFP, BacMam 2.0. The day before imaging 6 µl of the reagent were added per well of cells.

siRNA binding assay

A 2.5% agarose gel was prepared by dissolving agarose in TBE buffer (Trizmabase 10.8 g, boric acid 5.5 g, disodium EDTA 0.75 g, and 1 L of water) under heating. After cooling down to about 50 °C and addition of GelRed™ (1:10 000) the agarose gel was cast into the electrophoresis unit. Samples containing 500 ng siRNA were prepared as described above and placed into the sample pockets after 4 µL of loading buffer (prepared from 6 mL of glycerol, 1.2 mL of 0.5 M EDTA, 8 mL of H_2O, 0.02 g of bromophenol blue) had been added. Electrophoresis was performed at 120 V for 40 min if not stated otherwise.

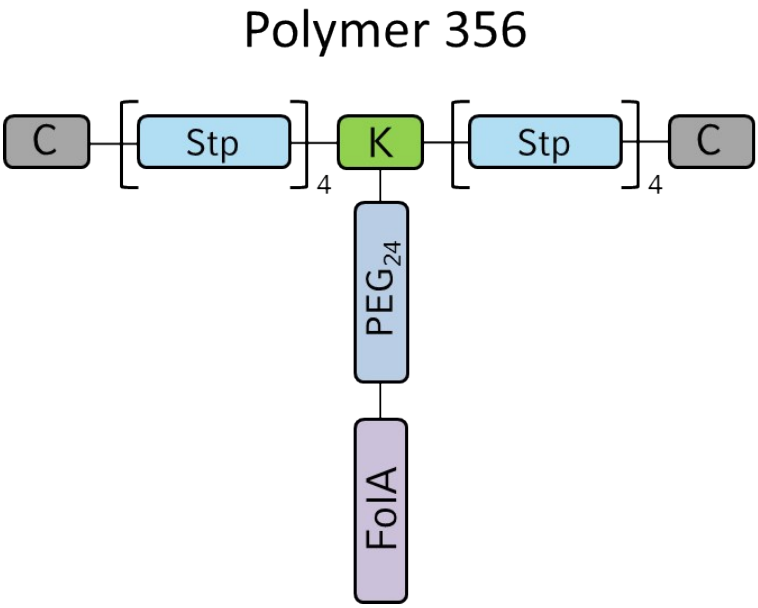
Cell culture

Human KB/eGFPLuc cells stably expressing luciferase (eGFP-luciferase fusion gene under the control of the CMV promoter) were cultivated in folate-free RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin.

Gene silencing with siRNA

Gene silencing experiments were performed in KB/eGFPLuc cells. The siRNAs employed here were either siRNA against eGFP for silencing the eGFPLuc fusion protein or its negative control sequence siCtrl. As internal standard (100% control) we used cells that were treated only with a buffer solution (HBG: 20 mM HEPES buffered, 5% glucose, pH 7.4). Silencing experiments were performed in triplicates in 96-well plates. 24 h prior to transfection plates were coated with collagen and 4000 cells/well were seeded. Before transfection, the medium was replaced with 80 µL fresh growth medium. 20 µl of MSN suspension (containing usually 10 µg MSN, but also as low as 0.06 µg MSN in PBS buffer at pH = 7.4, prepared as described above) were added to each well and incubated at 37 °C. The medium was replaced after the indicated incubation time. 48 h after initial transfection, cells were treated with 100 µL cell lysis buffer per well. Luciferase activity in 35 µl cell lysate was measured using a Centro LB 960 plate reader luminometer (Berthold Technologies, Bad Wildbad, Germany) and a luciferin-LAR (1 M glycylglycine, 100 mM $MgCl_2$, 500 mM EDTA, DTT, ATP, coenzyme A) buffer solution. The relative light units (RLU) were presented as percentage of the luciferase gene expression obtained with buffer treated control cells.

Structure of positive control block copolymer 356[2]



Scheme S1: Sequence of the polymer 356 used for polyplex formation and applied as positive control in cell experiments: C-Stp_4-K(PEG24-FolA)-Stp_4-C. Details are published elsewhere.[2]

Positive control polymer 356 is composed of eight succinoyl tetraethylene pentamine (stp) units, of which each carries three basic amino groups, while the terminal amino and carboxy groups are modified with cysteine (C) units for siRNA polyplex stabilization. The polymer center contains a branching lysine (K) that is further coupled to folic acid via a polyethylene glycol polymer. The final positive control polyplex formulation is prepared with this polymer and si- or Ctrl-RNA that are modified with the lytic peptide Inf7.

Cell viability assay

24 h prior to transfection plates were coated with collagen and 4000 KB/eGFP Luc cells/well were seeded. Before transfection, medium was replaced with 80 µL fresh growth medium. 20 µl of MSN solution (prepared as described above with either eGFP-siRNA or control siRNA) were added to each well and incubated at 37 °C. Medium was replaced after the indicated incubation time. After 48 h cellTiter-Glo (Promega, Mannheim, Germany) assay was performed according to the manufacturer's protocol. Cell viability was normalized to buffer-treated cells. All experiments were performed in triplicates.

S1: Adsorption-desorption studies of siRNA in LP-MSN

LP-MSN were prepared with APTES and PhTMS silane coupling reagents to result in the following concentrations of functional groups throughout the particle body:
LP-2 with ca. 9 mol% NH_2, LP-3 with ca. 4 mol% Ph and 6 mol% NH_2, LP-3 with ca. 6 mol% Ph and 3 mol% NH_2. 1 mg of these samples was exposed to three successive aliquots of a loading solution containing each 16 µg siRNA. The loaded samples were centrifuged and first redispersed in water, resulting in nearly no RNA desorption within 3 hours. A medium exchange for PBS showed a cumulative release up to about 5 % of the adsorbed siRNA, while larger elution amounts were only observed after additional medium exchanges after 3 and 24 h increasing the total RNA release to about 25% after 4 days.

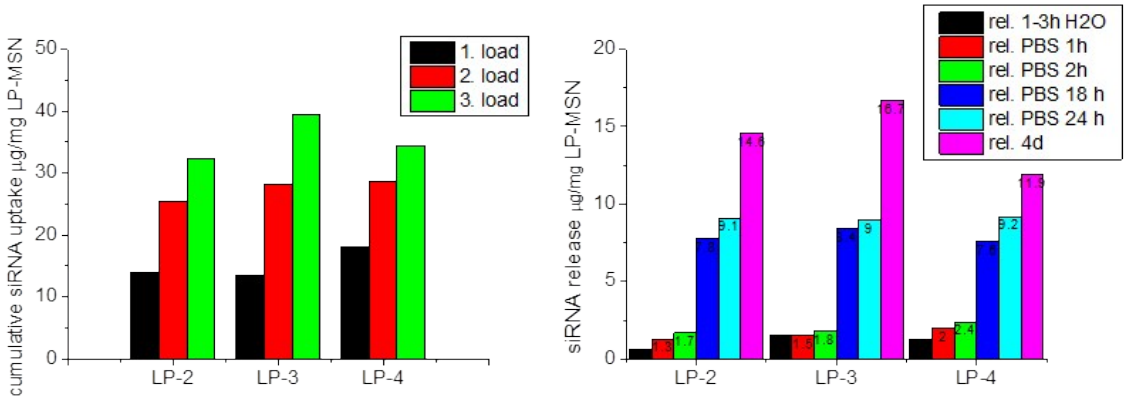


Figure S1: Cumulative siRNA adsorption and associated release diagrams of sample LP-2, LP-3 and LP-4.

S2: Transforming amino groups into disulfide-coupled amino groups

100 mg succinic anhydride, dissolved in 8 mL dry DMF was administered under nitrogen atmosphere to an ethanolic solution of 20 mg of sample S1 and was reacted at room temperature under stirring for 3 hours. The transformation of the amino groups into carboxy groups results in a shift of the IEP from about 6 to 4.6 as shown in Figure S2a). Additionally, carboxy groups are seen in the FTIR-spectrum by a C=O stretching frequency at 1714 cm^{-1}. This sample was subsequently conjugated with cystamine dihydrochloride in the following way: 5 µL EDC and 5 mg NHS-sulfo were added to a solution of 5 mg of the sample in 5 ml ethanol and stirred for 1 hour. An ethanolic solution of 24 mg cystamine was added dropwise under stirring and reacted overnight. The sample was retrieved by centrifugation and washed with ethanol 3 times. The conversion into now amino-terminated cleavable residues is seen by a return of the IEP to a higher value of 5.9 in the zeta potential curve. Furthermore, the C=O stretching bond has vanished and typical amide stretching frequencies of 1655 and 1542 cm^{-1} are seen for the amide I and amide II bonds in the FTIR.

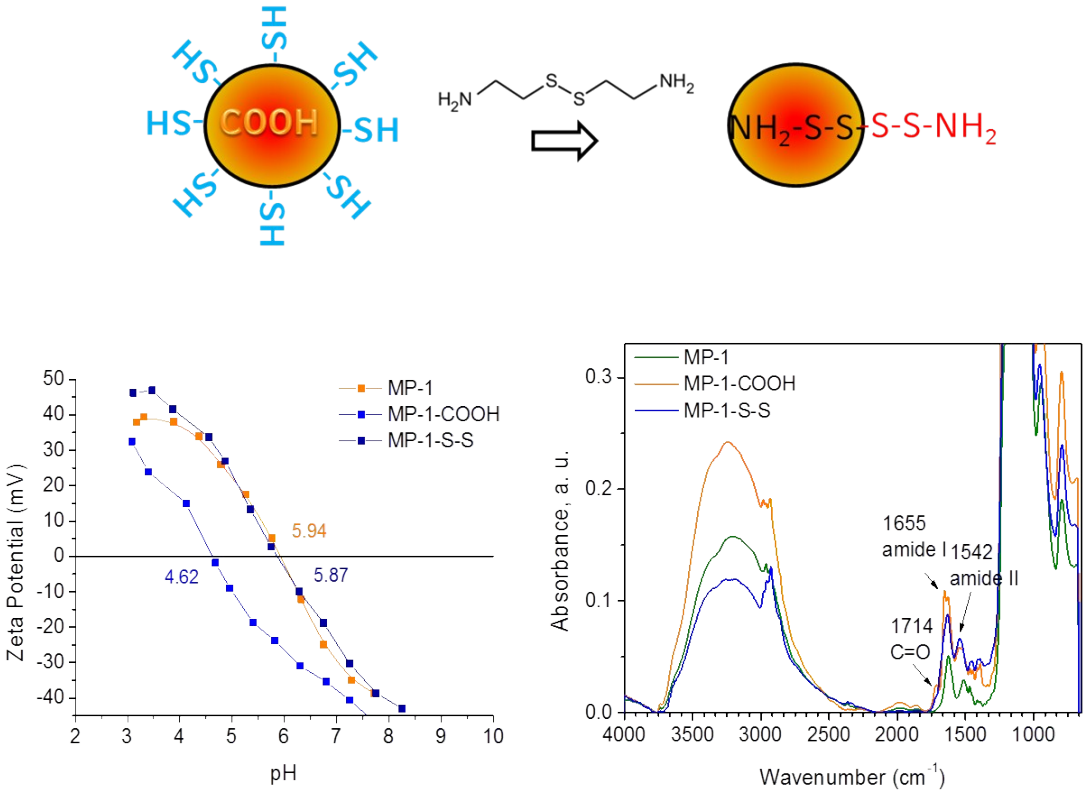


Figure S2: Transforming covalently coupled amino groups into disulfide-coupled amino groups: left) zeta-potential measurement of the parent sample MP-1, after carboxylation and disulfide coupling of amino groups via cystamine addition; right) the corresponding FTIR measurements.

S3: siRNA in sample MP-3 with lower amino group concentration (core 4 mol% NH_2, shell 2 mol% SH)

Adsorption was performed in MES buffer at pH=5 and the release was performed with PBS buffer at pH=7.4. The siRNA loading process is substantially slowed down compared to samples MP-1 and MP-2 with 9% amino groups, however, 93% of the offered RNA was absorbed after 5 h amounting to 72 µg/mg with the highest tested siRNA loading solution of 80 µg/mL. The diminished charge in this sample led to an easier elution of RNA in PBS buffer. Now, 60-70% were immediately released (measured after 15 minutes) and 68 to 87% of free RNA were measured after 1 day.

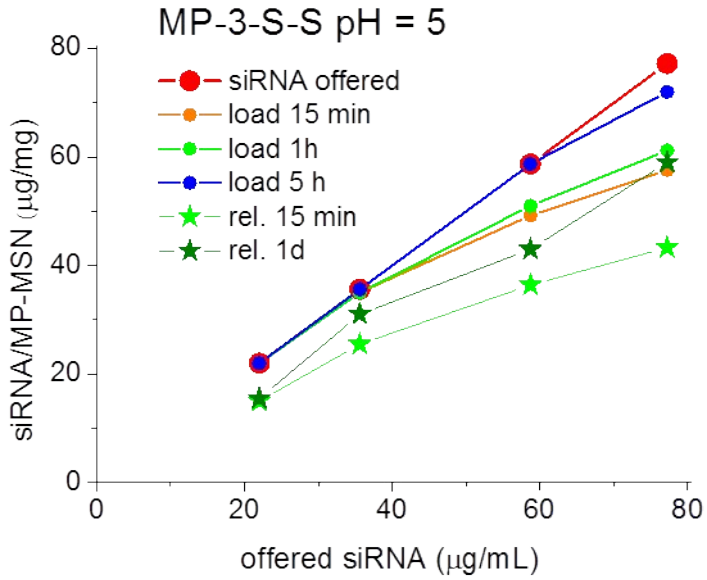


Figure S3a: Cumulative siRNA sorption and release curves of sample MP-3-S-S containing 4 mol% NH_2 in the core and 2 mol% SH in the shell, without polymer capping.

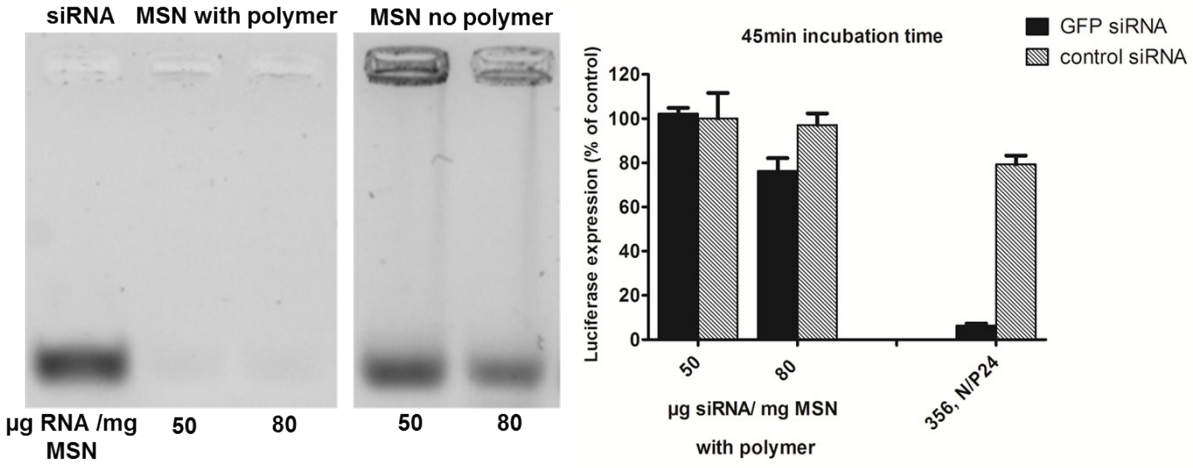


Figure S3b: Gel electrophoresis and transfection of RNA-loaded sample MP-3-S-S.

Sample MP-3-S-S with 4 mol% NH_2 and 2 mol% SH is loaded with 50 and 80 µg siRNA/mg MSN. Gel electrophoresis is shown with and without being capped with the block-copolymer. SiRNA is still present in the carrier system in the uncapped state, while the electrostatic interaction with the cationic polymer extracted the siRNA from the carrier and was detected in the supernatant solution.

Luciferase knockdown is thus only experienced to a very small extent in the higher loaded sample in the experiments.

S4: Spectroscopic evidence for mercapto groups and their transformation into disulfide-coupled amino groups

The mercapto S-H stretching frequency is seen at 2576 cm^{-1} in the Raman spectrum of the parent samples MP-1 and MP-3, however, it is better visible in sample MP-3 due to a higher concentration of 2 mol% mercapto groups in the shell. After reaction with cystamine this indicative band has completely vanished. Consequently, the IEP of the parent sample MP-3 shifts from 4.8 to 6.2 after cystamine binding.

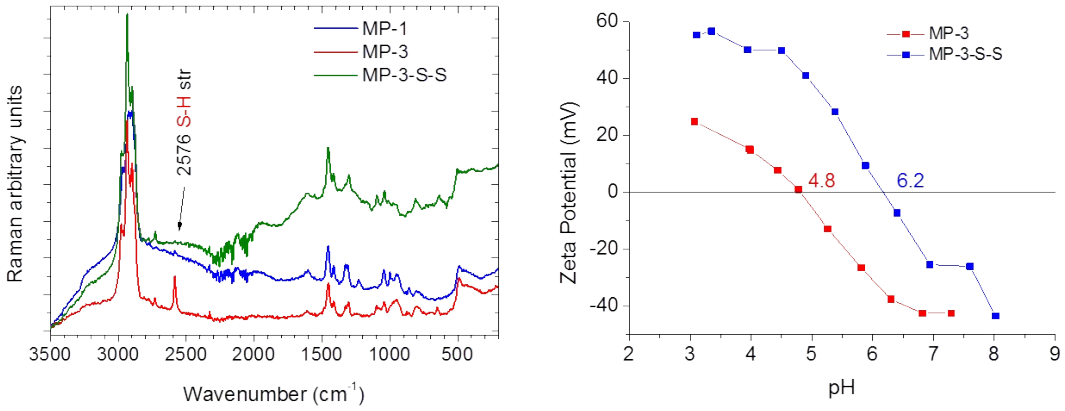


Figure S4: Raman spectroscopy of sample MP-1 and MP-3 and MP-3 after reaction with cystamine and zeta potential measurements of MP-3 before and after reaction with cystamine.

S5: Comparative adsorption isotherms

siRNA sorption is compared in samples in different medium-pore MSN samples. It shows that our samples are very efficient in siRNA sorption, which is almost quantitative even after only 1 h equilibration time. When disulfide-bridged amino groups were post-synthetically introduced into the mesopores, siRNA sorption is markedly retarded. This effect is stronger with a higher amino group surface coverage (sample MP-1-S-S). However, as indicated by our kinetic results shown in Figure 4, equilibrium conditions depend strongly upon the pore morphology and may not be reached after 1 h or even 1 day. Thus, even in MP-1-S-S we can reach a complete uptake of offered RNA amounting to 80 µg/mg (80 µg/mL equil. conc.; maximum concentration evaluated) after 5 h or to 380 µg/mg after 1 d in sample MP-1 as can be seen in Figure 4.

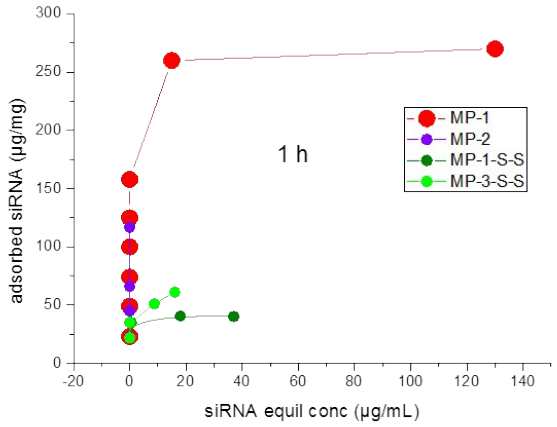


Figure S5: siRNA loading curves of different medium-pore samples after 1 h loading time.

S6: Evidence for polymer attachment to siRNA loaded MSNs

The IEP of the parent compound MP-1 (purple) shifts from 5.9 after loading with siRNA (blue) to 5.1 and returns to 6.4 after attachment of the cationic polymer (green); the zeta potential of the pure polymer is shown in addition (orange; IEP 7.6)

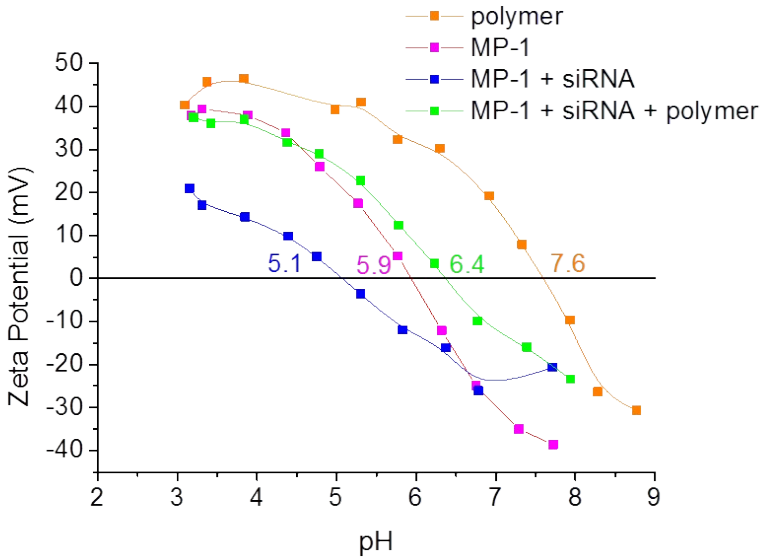


Figure S6: Zeta potential measurements of sample MP-1 before and after RNA loading and polymer capping.

S7: Knock-down efficiencies of block-copolymer 454 capped medium-pore MSNs with different pore-sizes

SiRNA loadings were varied from 5 wt%, over 10 wt% to 13 wt% siRNA, while keeping the total amount of siRNA at 0.5 µg/well constant (less particles /well). A sample without block copolymer attachment is also shown. An incubation time of 45 minutes was used, followed by a read out after 48 h. These results are very similar to those obtained with sample MP-1 under the same conditions as shown in Fig. 7 in the main text, which are added here again for easier comparison.

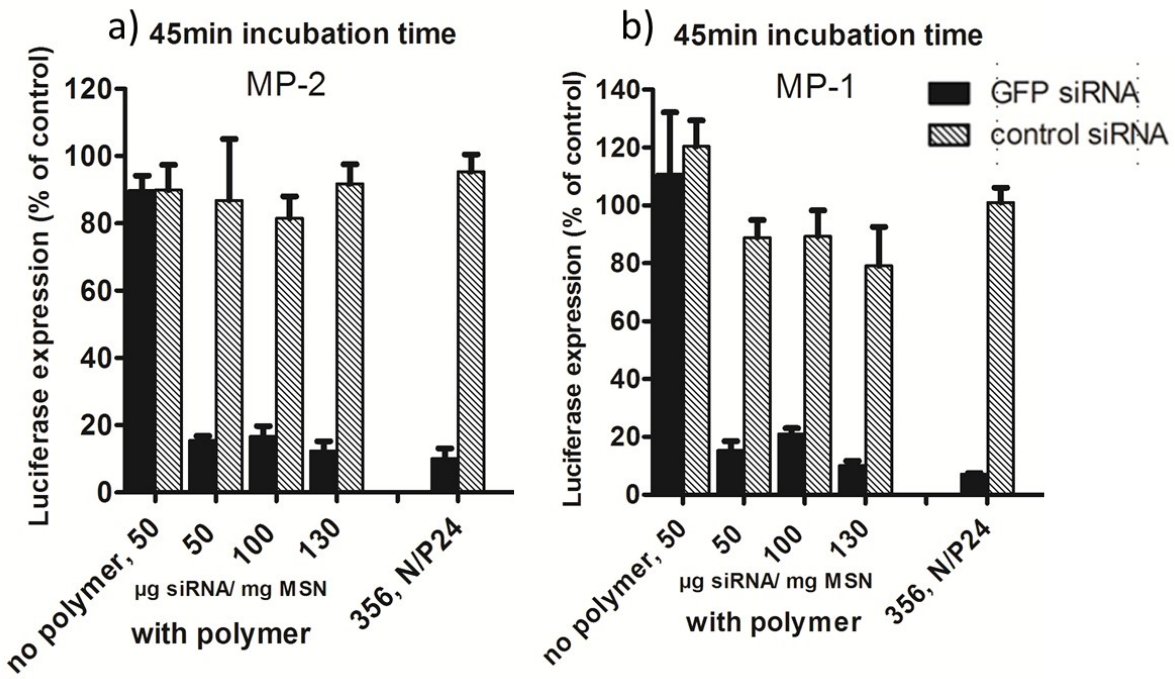


Figure S7: Cell transfections with a) sample MP-2 with 3.9 nm pores in comparison to b) sample MP-1 with 4.7 nm pores.

S8: Knock-down efficiencies of block-copolymer 454 capped large-pore MSN

Compared to the MP-samples the transfection efficiency is markedly reduced when the large-pore sample LP-2 was used for siRNA delivery. The highest loading capacity of this sample was only 88 µg RNA/mg MSN in LP-2, the LP sample with the highest amino group density. However, even using this higher RNA loading for cell transfection could not improve the efficacy, indicating that the desorption process in LP-samples is restricted.

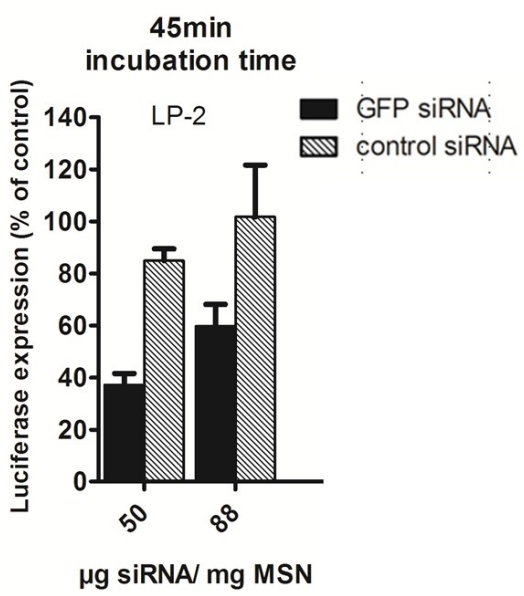
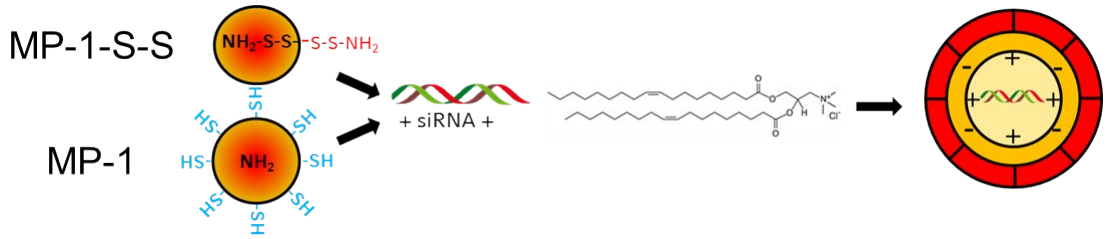


Figure S8: KB-cell transfection performed with polymer capped large-pore sample LP-2. Incubation time 45 minutes, read out after 48 h. Cells were exposed to 0.5 µg siRNA/well while the siRNA concentration was varied between 50 and 88 µg RNA/mg MSN (particle concentration was decreased from 10 µg to 6 µg per well).

S9: Knock-down efficiencies of DOTAP-capped MSNs



Scheme 9: scheme for sample assembly of MP-1 and MP-1-S-S covered with a **DOTAP** cationic lipid layer

DOTAP was administered to MP-1-S-S subsequently to siRNA loading. Samples were centrifuged and the supernatant containing surplus DOTAP was removed and exchanged for a PBS buffer solutions. These samples were used for cell transfection experiments shown below. Cells were incubated for 4 h before the medium was exchanged. Read-out was performed after 48 or 72 h (S8).
Samples were additionally treated with chloroquine or the endosomolytic peptide INF-7 for comparison. The Luciferase activity was reduced to over 80% and neither chloroquine nor INF-7 was necessary to increase the efficacy. It is noted that the toxicity was increased upon INF-7 addition.

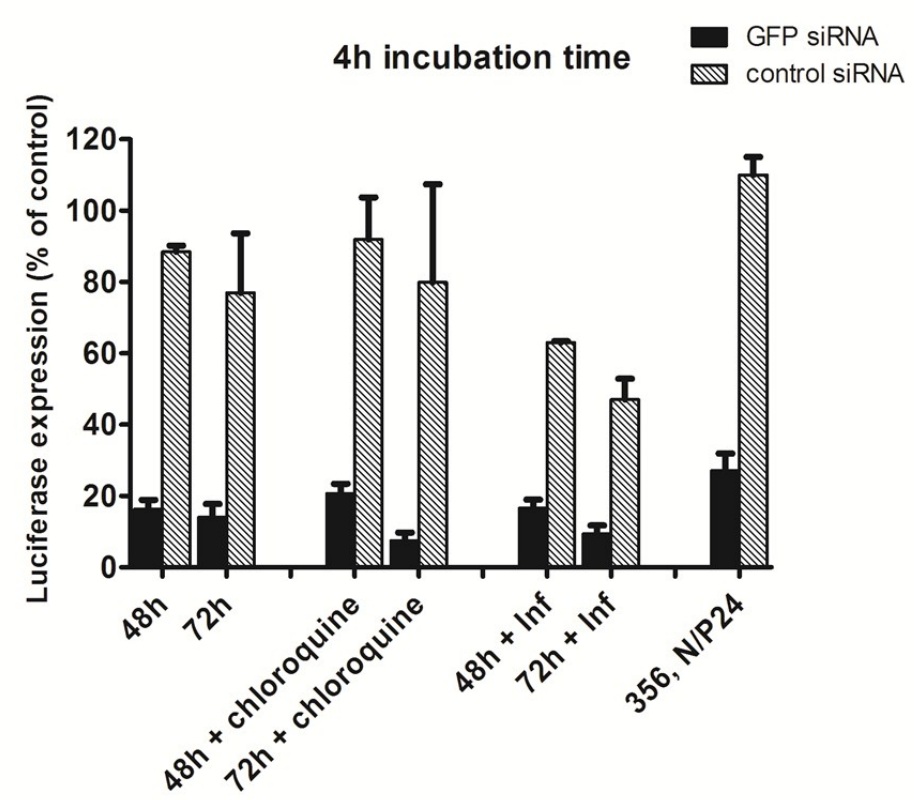


Figure S9: Transfection with DOTAP covered sample MP-1-S-S.

SiRNA concentration effects were measured with sample MP-1 and MP-1-S-S in combination with DOTAP. The loading with siRNA was increased from 5 wt% to 10 wt% and even to 200 wt% while the total siRNA amount administered per well was kept at 0.5 µg/well.

S10: cell studies with MCF-7 cell line

The transfection efficiency of our MP-1 system was investigated further in MCF-7 cells. Here we compare different incubation times of 45 minutes and 4 h, DOTAP-capped particles and polymer capped particles as well as different RNA loading schemes. It is shown that the DOTAP capped system is less efficient than the novel polymer-capped MSN system, which results in up to 60 % transfection efficiencies. Slight improvements were seen when the first particle exposure time was increased from 45 minutes (a) to 4 h (b) and when the total siRNA amount/well was increased from 0.5 µg (a) up to 1.5 µg (b).

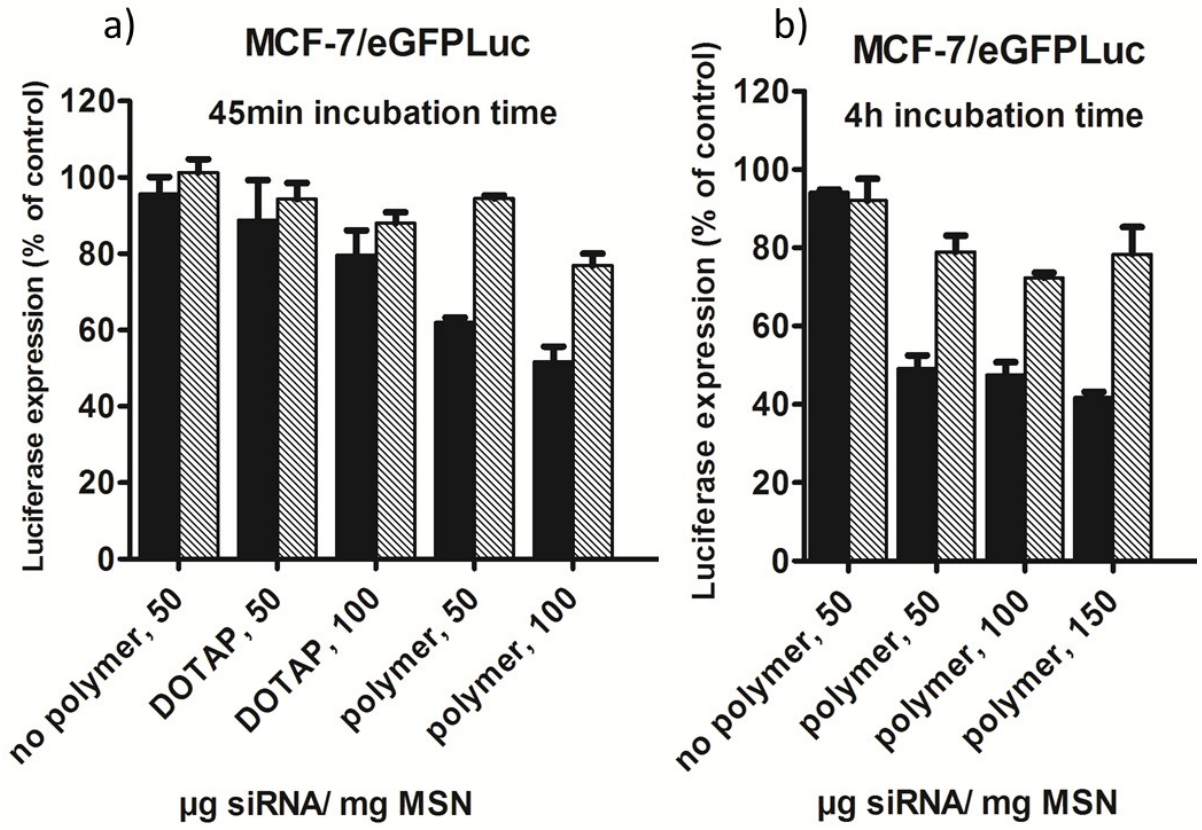
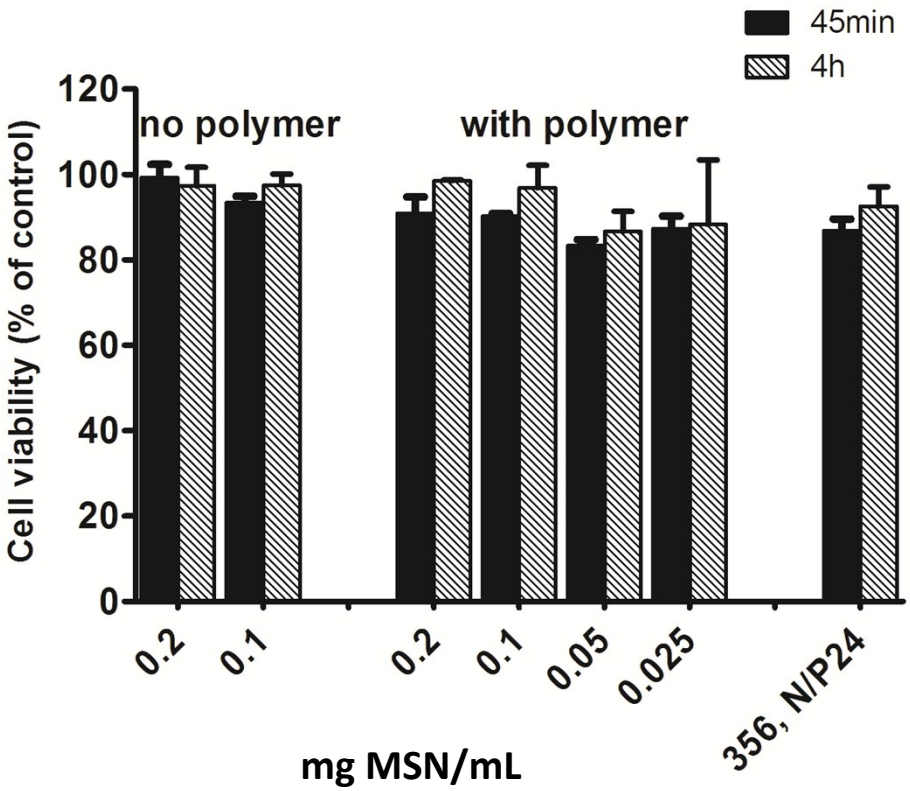


Figure S10: Transfection efficacies of DOTAP and polymer capped MP-1 in MCF-7 cells.
(a) Incubation time of 45 minutes, read out after 48 h. Cells were exposed to 0.5 µg siRNA/well while the siRNA concentration was varied between 50 and 100 µg RNA/mg MSN (particle concentration was decreased from 10 µg to 5 µg per well). (b) MCF-7 transfection with polymer-capped MP-1. Incubation time of 4h, read out after 48 h. Cells were exposed to 10 µg MSN particles per well. siRNA loading ranged from 50 µg siRNA/mg MSN to 100 and 150 µg siRNA/mg MSN, thus the siRNA concentration varied from 0.5 µg over 1 µg to 1.5 µg siRNA/well.

S11: Toxicity assay

Figure S11: Cell titer glo toxicity assay of siRNA-loaded sample MP-1. Samples were measured with and without block-copolymer attachment and incubated either 45 minutes or 4 h with the particles before the medium was exchanged. Final read-out was performed after 48 h.



S12: Cell microscopy

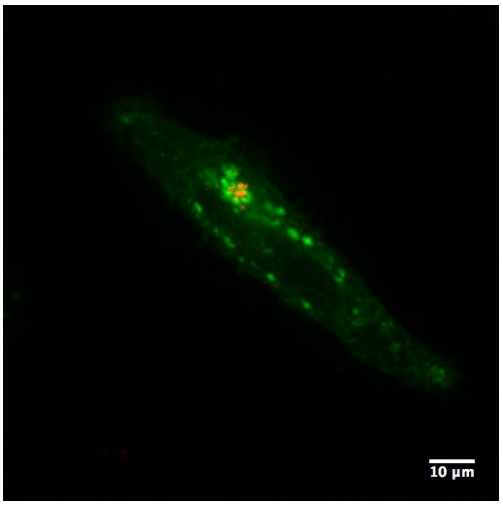


Figure S12: siRNA (red) in MP-1 with polymer attached taken up by a KB cell. After 48 h we observe co-localization with lysosomes (green), the late stage of endosomes. The green channel is slightly shifted to the lower left as compared to the red channel for better visualization.

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