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Dendronized mesoporous silica nanoparticles provide an internal endosomal escape mechanism for successful cytosolic drug release

Veronika Weiss [a, 1], Christian Argyo [a, 1], Adriano A. Torrano [a], Claudia Strobel [b], Stephan A. Mackowiak [a], Alexandra Schmidt [a], Stefan Datz [a], Tim Gatzenmeier [a], Ingrid Hilger [b], Christoph Bräuchle [a, *], Thomas Bein [a, *]

[a] Department of Physical Chemistry and Center of NanoScience (CeNS), University of Munich (LMU), Gerhard-Ertl-Building, Butenandtstraße 5-13, 81377 München, Germany
[b] Department of Experimental Radiology, Institute of Diagnostic and Interventional Radiology I, Jena University Hospital, Friedrich-Schiller-University Jena, Erlanger Allee 101, 07747 Jena, Germany

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ABSTRACT

Mesoporous silica nanoparticles (MSNs) attract increasing interest in the field of gene and drug delivery due to their versatile features as a multifunctional drug delivery platform. Here, we describe poly(-amidoamine) (PAMAM) dendron-functionalized MSNs that fulfill key prerequisites for a controllable intracellular drug release. In addition to high loading capacity, they offer 1) low cytotoxicity, showing no impact on the metabolism of endothelial cells, 2) specific cancer cell targeting due to receptor-mediated cell uptake, 3) a redox-driven cleavage of disulfide bridges allowing for stimuli-responsive cargo release, and most importantly, 4) a specific internal trigger based on the high buffering capacity of PAMAM dendrons to provide endosomal escape.

1. Introduction

In recent years, mesoporous silica nanoparticles (MSNs) have been intensively studied as drug delivery vehicles, due to their excellent material features such as good biocompatibility, large cargo capacity, and versatile organic surface functionalization [1]. Inorganic nanocarriers such as MSNs provide a multiplicity of advantages for drug delivery applications including selective size and shape control, tunable pore sizes, and large pore volume and surface area for high loading capacities [1–3]. Previous work in our group has focused on the development of core–shell silica nanoparticles [4,5]. Selective post-synthesis functionalization of the silica surface allows for the attachment of stimuli-responsive agents such as pH-responsive polymers [6], magnetic particles [7], or light-responsive molecules [8,9]. Furthermore, MSNs as host system for therapeutic agents provide protection of guest molecules from degradation and from detection by the immune system [1].

In general, an ideal drug delivery platform has to meet several requirements to achieve specific drug delivery [10–12], including biocompatibility [13–15], specific targeting [16,17], and stimuli-responsive drug release behavior [18–20]. Nano-sized drug delivery systems such as multifunctional MSNs encounter many challenges on their way towards reaching their desired target and efficiently releasing their cargo. In particular, the endosomal entrapment is a major obstacle for drug delivery, faced by MSNs that are internalized by cells via endocytosis [21]. Especially for membrane impermeable or immobilized cargo molecules, the nanocarriers must enter the cytosol to achieve efficient delivery to the targeted cell compartments. Several strategies have already been described to address the demanding task of endosomal escape, including pore formation, membrane fusion, photo-activated membrane rupture, and the proton sponge effect [9,22–24].

The latter is supposed to be a promising automatic strategy for endosomal release of the nanocarriers. The mechanism of the proton sponge effect follows an intrinsic osmotic swelling during

* Corresponding authors.
E-mail addresses: christoph.braeuchle@cup.uni-muenchen.de (C. Bräuchle), bein@lmu.de (T. Bein).
[1] V. Weiss and C. Argyo contributed equally to this work.

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endosomal acidification caused by the buffering capacity of modified nanocarriers such as cationic polymers [25–27]. Ultimately, this results in rupture of the endosomal membrane. Furthermore, a destabilization of the membrane caused by such positively charged vehicles has been proposed [28].

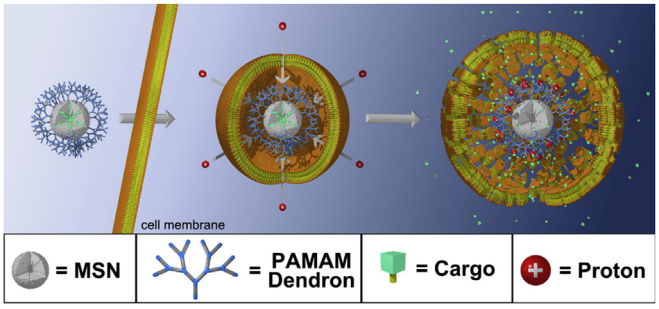
Poly(amidoamine) (PAMAM) dendrons or dendrimers provide high buffering capacity and have been found to be suitable for gene delivery exhibiting extraordinary stability in forming complexes with DNA [29–32]. Resulting transfection efficiency was explicitly attributed to an activated proton sponge mechanism. Dendron-coated mesoporous particles have also been used for intracellular delivery of plasmid-DNA [33,34].

Here we establish newly designed multifunctional core–shell MSNs coated with PAMAM dendron structures on the outer surface. Several important functional properties of PAMAM dendron-coated MSNs are specifically due to their internal mesoporous structure. For example, the specific synthesis route established offers precise control over the location of functional groups within the particle volume and allows for the facile synthesis of a bifunctional system [4]. These systems provide a successful mechanism for endosomal escape and subsequent cytosolic drug release from the silica nanocarriers. PAMAM dendron-coated MSNs feature a high buffering capacity acting as a potential trigger for a pH-responsive endosomal escape mechanism conceivably via the proton sponge effect (Scheme 1). To the best of our knowledge, we created for the first time a multifunctional drug delivery platform based on the combination of polycationic PAMAM dendrons as endosomal escape agents with a redox-responsive drug release mechanism (disulfide bridges inside mesopores) and the possibility to attach cancer cell targeting ligands to the MSN nanocarriers.

2. Experimental

2.1. Materials

Propargylamine (Aldrich, 98%), *N*,*N*-diisopropylethylamine (DIPEA, Sigma–Aldrich, ≥ 99%), copper(I) iodide (Aldrich, 99.999%), (3-aminopropyl) triethoxysilane (APTES, Aldrich, 99%), tetraethyl orthosilicate (TEOS, Fluka, > 98%), triethanolamine (TEA, Aldrich, 98%), cetyltrimethylammonium chloride (CTAC, Fluka, 25% in H_2O), (3-mercaptopropyl) trimethoxysilane (MPTMS, Gelest, 95%), methanethiosulfonate 5(6)-carboxy-X-rhodamine (MTS-ROX, Biotium), colchicine methanethiosulfonate (MTS-Col, Santa Cruz Biotechnology), 4,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma Aldrich), folic acid (FA, Sigma), Atto 633 maleimide (ATTO-TEC), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Fluka, 97%), *N*-hydroxysulfosuccinimide (sulfo-NHS, Sigma Aldrich, >98.5%), poly (ethylene glycol) bisamine (PEG_{2000}-bisNH_2, M_W 2000, Sigma Aldrich), *N*-succinimidyl oxycarbonylethyl methanethiosulfonate (NHS-3-MTS, Santa Cruz Biotechnology), and oxalic acid were used as received. Methyl acrylate (Aldrich, 99%) and 1,2-ethylendiamine (Aldrich, 99.5%) were freshly distilled prior to use. Ethanol (EtOH, absolute), *N*,*N*-dimethyl formamide (DMF, Sigma Aldrich, anhydrous) and methanol (MeOH, anhydrous, Sigma) were used as solvents without further purification. Bidistilled water was obtained from a Millipore system (Milli-Q Academic A10). (3-azidopropyl)trimethoxy silane (AzTMS) was freshly prepared as previously reported [35].



Scheme 1. Schematic illustration of the proposed intrinsic endosomal escape mechanism. PAMAM dendron-coated mesoporous silica nanoparticles are internalized into a cancer cell via endocytosis. Endosomal acidification leads to intrinsic osmotic swelling caused by the high buffering capacity of the dendron-coated MSNs. Subsequently, endosomal membrane rupture occurs which provides access to the cytosol. In this reductive environment the immobilized (disulfide bridges) cargo molecules can be released.

2.2. Dendronized MSNs (MSN-D3)

Colloidal mesoporous silica nanoparticles (MSN-D3) were prepared according to a synthesis procedure published by Cauda et al. [4]. The amount of functionalized silane was calculated to be 1% of total silica. A mixture of TEA (14.3 g, 95.6 mmol), TEOS (1.56 g, 7.48 mmol) and MPTMS (92.3 mg, 87.3 mL, 0.47 mmol) was heated for 20 min under static conditions at 90 °C in a polypropylene reactor. Afterwards, a solution of cetyltrimethylammonium chloride (CTAC, 2.41 mL, 1.83 mmol, 25 wt% in H_2O) in H_2O (21.7 g, 1.21 mmol) was preheated to 60 °C and added quickly to the TEOS solution. The reaction mixture was stirred vigorously (700 rpm) for 20 min while cooling down to room temperature. Subsequently, TEOS (138.2 mg, 0.922 mmol) was added in four equal increments every three minutes. The reaction was stirred for further 30 min. After this time, a mixture of TEOS (19.2 mg, 92.2 μmol) and a dendron-functionalized trialkoxysilane (S3, cf. SI) (92.2 μmol) was added. Furthermore, the mixture of TEOS and PAMAM silane was dissolved in a solution of 2 mL methanol and 1 mL water briefly before the addition. The reaction was stirred at room temperature overnight. The suspension was diluted 1:1 with absolute ethanol, the colloidal MSNs were separated by centrifugation (19,000 rpm, 43,146 rcf, 20 min) and redispersed in absolute ethanol. The template extraction was performed by heating the samples under reflux at 90 °C (oil bath) for 45 min in a solution of ammonium nitrate (2 wt% in ethanol) followed by 45 min under reflux at 90 °C in a solution of 10 mL conc. HCl (37%) in 90 mL ethanol. The extracted MSNs were collected by centrifugation after each extraction step and finally washed with 100 mL absolute ethanol. The resulting MSNs were stored in an ethanol/water solution (2:1).

2.3. Cargo loading

MSNs (1 mg) were incubated for 2 h at room temperature in the dark with MTS-ROX (5 μL, 5 mg/mL in DMF), MTS-Col (50 μL, 5 mg/mL in DMF), or MTS-DAPI for a one-step covalent attachment of the cargo molecules to the internal surface of the mesopores via disulfide bridges. MTS-DAPI was prepared in-situ by mixing DAPI (10 μL, 5 mg/mL in DMF) and NHS-3-MTS (100 μg) in an aqueous solution for 1 h at room temperature in the dark. This reaction mixture was subsequently added to MSN-D3. The particles were washed five times (centrifugation, 4 min, 14,000 rpm, 16,837 rcf) with water and were finally redispersed in 1 mL H_2O.

2.4. Cargo loading and release quantification

The loading and release capacities of the particles were determined with UV–Vis spectroscopy on a Thermo Scientific PeQLab Nanodrop 2000c. First, in order to quantify the loading capacity, 0.5 mg of MSN-D3 were loaded with 500 μL MTS-ROX (100 μg/mL in ethanol) for 24 h. The absorption of the supernatant after