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pH-Responsive Dendritic Core–Multishell Nanocarriers

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ABSTRACT

In this paper we describe novel pH-responsive core–multishell (CMS) nanocarrier (pH-CMS), obtained by introducing an aromatic imine linker between the shell and the core. At a pH of 5 and lower the used imine linker was rapidly cleaved as demonstrated by NMR studies. The CMS nanocarriers were loaded with the dye Nile red (NR) and the anticancer drug doxorubicin (DOX), respectively. The transport capacities were determined using UV/Vis spectroscopy, and the sizes of the loaded and unloaded CMS nanocarriers were investigated using dynamic light scattering (DLS). We could show that CMS nanocarriers efficiently transported NR in supramolecular aggregates, while DOX was transported in a unimolecular fashion. After cellular uptake the DOX-loaded pH-responsive nanocarriers showed higher toxicities than the stable CMS nanocarriers. This is due to a more efficient DOX release caused by the cleavage of the pH-labile imine bond at lower pH within the intracellular compartments.

1. Introduction

Most clinically used drugs encounter the problem of short half-life times in blood and a high overall clearance rate. Due to their small size they rapidly diffuse into tissue and are therefore distributed throughout the whole body, which is the main reason for undesired side effects. New drug-delivery concepts rely on the use of polymeric drug delivery systems (DDSs) [1–3].

Among these one can find polymer conjugates [4,5], macromolecular prodrugs [6], and drug-delivery systems based on polymeric core–shell architectures [7]. The most famous examples of such core–shell architectures are polymeric micelles [8,9] and liposomes [10,11]. Nowadays, unimolecular core–shell particles have also become increasingly interesting because they do not fall apart upon dilution. For example, our group recently developed an efficient unimolecular core–shell architecture which consists of a dendritic hydrophobic poly(ethylene) core and a grafted, dendritic, hydrophilic polyglycerol (dPG) shell [12]. Furthermore, we developed a new type of unimolecular liposome-like multishell system which is based on a hydrophilic dPG core, a hydrophobic inner alkyl shell, and a hydrophilic outer poly(ethylene glycol) methyl ether (mPEG) shell. This system is characterized by its high solubility in a wide range of solvents and its ability to encapsulate hydrophobic as well as hydrophilic guest molecules [13]. The transport of guest molecules did not occur via unimolecular core–multishell (CMS) nanocarriers but via the formation of CMS aggregates [14]. Compared to polymer-drug conjugates, like for example PAMAM-based (poly(amidoamine)) star HPMA (*N*-(2-hydroxypropyl)methacrylamide) [15,16] or dPG-PEG polymer-drug conjugates [17], the loading of these architectures with active agents does not require a synthetic step. On the other hand, the loading capacities of DDS using simple entrapment as loading technique are usually lower than the ones for drug conjugates. The CMS nanocarriers have already been used in biomedical applications, e.g., for the modulation of the copper level in eukaryotic cells and the *in vivo* targeting of a F9 teratocarcinoma tumor [18,19]. Additionally, we were able to show that the nanocarriers can passively target tumors based on the enhanced permeability and retention (EPR) effect [20,21] which is one of the major benefits of macromolecular DDSs. The CMS nanocarriers specifically accumulated in tumor tissue and therefore delivered their guest molecules more selectively to the desired site of action [18]. Furthermore, the skin penetration of the hydrophilic dye rhodamin B and the hydrophobic dye Nile red could be greatly improved in comparison to other pharmaceutical formulations [22,23]. The CMS nanocarriers were also able to enhance the delivery of an electron paramagnetic resonance spin label into the upper layers of the stratum corneum [24].

One of the drawbacks of some DDS is their inability to release the encapsulated guest after reaching the desired site of action. For this reason, many DDSs have been developed which are able to release their cargo upon action of an external stimulus like light, ultrasound, magnetic

Abbreviations: DDS, drug delivery system; dPG, dendritic polyglycerol; mPEG, poly(ethylene glycol) methyl ether; PAMAM, poly(amido amine); HPMA, *N*-(2-hydroxypropyl)methacrylamide; CMS, core–multishell; EPR, enhanced permeation and retention; PEI, poly(ethylene imine); pH-CMS, pH-responsive CMS nanoparticles, C18diCOOH, 1,18-octadecanedioic acid; $mPEG_{1000}$, mPEG with an average number averaged molecular weight of 1000 g/mol; GPC, gel permeation chromatography; DLS, dynamic light scattering; DOX · HCl, doxorubicin hydrochloride, DOX, doxorubicin, free base; DMF, dimethylformamide; NR, Nile red; THF, tetrahydrofurane; SEC, size exclusion chromatography; DMEM, Dulbecco's Modified Eagle Medium; PBS, phosphate buffered saline; rt, room temperature; THBA, trihydroxybenzaldehyde; MWCO, molecular weight cut-off; RTCA, real time cell analysis.

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field, or a change in temperature, redox potential, or pH of the environment [25]. The use of pH-sensitive DDSs is of special interest since pH gradients are found in many biological systems. In some tumor tissues the pH easily drops from the physiological pH of 7.4 to values of 6 and lower [26]. During cellular uptake the pH-value can even drop to values of around 4–5 in the late lysosomes [27]. By taking this into consideration, different pH-cleavable core–shell type architectures have been developed within the last years, e.g., pH-responsive polymeric micelles [28,29] and polymersomes [30,31]. Our group reported a number of unimolecular pH-responsive core–shell DDSs based on dendritic poly(ethylene imine) (PEI) and dPG using different functional groups to attach pH-cleavable shells. These dendritic architectures released various guest molecules at pH values between 5 and 7 depending on the functional group used for the attachment of the shell [32–36]. Only few examples of pH-sensitive DDSs based on unimolecular core–multishell architecture have been reported so far. For instance, Shen et al. described a pH-sensitive core–double shell system based on PAMAM dendrimers [37] and Tian et al. developed a triple-shell DDS which is responsive to pH and temperature [38].

In order to combine the benefits of our unimolecular CMS nanocarriers with the ability to release the cargo due to a drop in pH, we introduced an aromatic imine bond for attachment of the double shell (pH-CMS, Fig. 1). The aromatic imine bond can be hydrolyzed at the required pH-value to achieve the cleavage of the carrier after cellular uptake. Additionally, it allows for further fine-tuning of the release due to the possibility of introducing substituents at the aromatic ring to adjust the pH to the required value for the cleavage of the shell [39].

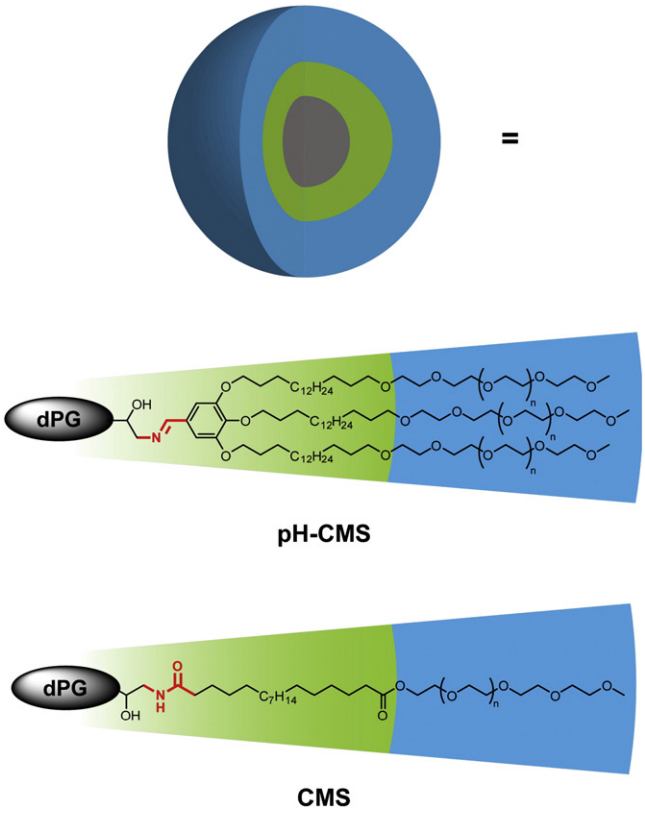

Fig. 1. Schematic structure of a core–multishell nanoparticle based on a dendritic polyglycerol core (dPG, gray), an inner hydrophobic alkyl shell (green), and an outer hydrophilic shell made of poly(ethylene glycol) methyl ether with an average number averaged molecular weight of 1000 g/mol ($mPEG_{1000}$, blue). The pH-sensitive CMS nanoparticle (pH-CMS) is obtained by introduction of an aromatic imine functionality which is derived from a trihydroxybenzaldehyde derivative.

2. Experimental section

2.1. General

Reactions requiring dry conditions were carried out in dried Schlenk glassware under argon. Analytical grade solvents and chemicals were purchased from Acros or Sigma Aldrich and used as received. The 1,18-octadecanedioic acid (C18diCOOH) was a kind gift of Cognis. Sephadex LH-20 was purchased from GE Healthcare. Dry solvents were obtained from a MBraun SPS-800 solvent purification system. dPG amine (M_n 10,000 g/mol) was prepared with a degree of amination of 70% analogous to the published method [40]. Dendritic CMS nanocarriers with $mPEG_{1000}$ (mPEG with an average number averaged molecular weight of 1000 g/mol) as the outer shell were synthesized as described in the literature [13,23].

NMR spectra were recorded on a Jeol ECX 400 or a Jeol Eclipse 500 MHz spectrometer. Proton and carbon NMR were recorded in ppm and were referenced to the indicated solvents [41]. NMR data were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), integration, and coupling constants (s) in Hertz (Hz). Multiplets (m) were reported over the range (ppm) at which they appear at the indicated field strength. Mass spectrometry was performed on an Agilent 6210 ESI-TOF spectrometer. Gel permeation chromatography (GPC) data was obtained by measurements using an Agilent 1100 solvent delivery system with pump, manual injector, and an Agilent differential refractometer. Three 30 cm Suprema columns (PPS: Polymer Standards Service GmbH, Germany; Suprema 100 Å, 1000 Å, 3000 Å with 5 and 10 μm particle size) were used to separate aqueous polymer samples using water with 0.05% NaN_3 as the mobile phase at a flow rate of 1 $mL \cdot min^{-1}$. The columns were operated at ambient temperature with the RI detector at 50 °C. The calibration was performed by using linear pullulan calibration standard (PPS GmbH, Germany). Measurements were carried out under highly diluted conditions (10 mg/mL, injected volume 20 μL). WinGPC Unity software from PSS was used for data acquirement and interpretation. UV/Vis spectra were recorded on a Scinco S-3100 UV/Vis spectrometer. The dynamic light scattering (DLS) measurements for the size determination were performed on a Malvern Zetasizer Nano equipped with a He–Ne laser (633 nm) using backscattering mode (detector angle 173°). The samples were filtered through 0.2 μm regenerated cellulose syringe filters prior to the DLS measurement and left for 24 h to equilibrate. 100 μL of the solution to be analyzed was added to a disposable microcuvette (Plastibrand) with a round aperture. The autocorrelation functions of the backscattered light fluctuation were analyzed using Zetasizer DTS software from Malvern to determine the size distribution by intensity and volume. The measurements were performed at 25 °C, equilibrating the system on this temperature for 120 s.

2.2. Preparation of DOX

Doxorubicin hydrochloride (DOX · HCl) was transferred into the free base doxorubicin (DOX) similar to the published method [42]. DOX · HCl was dissolved in dimethylformamide (DMF) and stirred with 2 eq. of triethylamine overnight.

2.3. Loading of CMS nanocarriers

Stock solutions of the stable CMS nanocarriers with concentrations of 1 and 5 mg/mL in dist. water were prepared. The solution of the pH-CMS nanocarriers with the same concentrations was always freshly prepared before usage. A 5 mg/mL guest (Nile red (NR) or DOX) stock solution in tetrahydrofuran (THF) or DMF in case of DOX was prepared. 200 μL of the guest stock solution was transferred into small sample vials and the solvent was evaporated. Afterwards, 3 mL of the different CMS nanocarrier stock solutions or dist. water as control was added. The