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PEGylated thermo-sensitive poly(amidoamine) dendritic drug delivery systems

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ABSTRACT

Thermo-sensitive dendrimers hold promise in various biomedical and pharmaceutical applications due to their stimuli-responsive properties. However, for such systems there are still certain unaddressed issues e.g. the undesired toxicity, immunogenicity and short blood circulation time. PEGylation is a potential approach to solve these above problems. The aims of this study were to engineer PEGylated thermo-sensitive dendritic derivatives and to investigate their temperature sensitivity and drug release behaviour therein. Linear poly(N-isopropylacrylamide) (PNIPAAm) and methoxy poly(ethylene glycol) (MPEG) were attached to the surface of polyamidoamine (PAMAM) dendrimers to generate PAMAM–*g*–PNIPAAm and PAMAM–*g*–PNIPAAm–*co*–PEG. PAMAM–*g*–PNIPAAm exhibited the lowest critical solution temperature (LCST) of ca. 32 °C, whereas PAMAM–*g*–PNIPAAm–*co*–PEG showed a LCST of ca. 35 °C. Indomethacin was used as a model molecule to examine the drug release profiles from both types of dendritic polymers. Results showed that such thermo-sensitive PAMAM derivatives could manipulate drug release simply by controlling the temperature above or below the LCST. At 37 °C a prolonged drug release was obtained for both systems with less than 30% of drug was released over 12 h, whilst the release rate is much faster at 30 °C and ca. 90% of drug was released over 12 h. The results obtained suggest that these thermo-sensitive PAMAM derivatives could be potential drug delivery systems to achieve controlled drug release.

1. Introduction

Dendrimers are well-defined, hyper-branched, synthetic macromolecules which have a history of over 30 years (Buhleier et al., 1978). Unlike conventional linear and branched polymers, dendrimers possess a tree-like architecture emanating from a central core from which the repeating branches are built up via a stepwise fashion. The number of repeated branching cycles determines the generation of dendrimers (e.g. G1.0, G2.0, G3.0, etc.) and hence the size of the dendrimers. Despite the diversity of core molecule, repeating branches and end groups, dendrimers are often divided into three regions: the initiator core, the interior (building branches) and the exterior (surface terminal groups). This unique structure not only presents cavities (i.e. interior) for encapsulation guest molecule, but also confers dendrimers molecular uniformity and surface functionality (Jansen et al., 1994). The molecular uniformity is highly desirable for improving synthetic reproducibility and minimising experimental and therapeutic variability (Astruc et al., 2010). The abundance of differing functional surfaces impart dendrimers numerous unique properties, e.g. aqueous solubility and stimuli (temperature and pH, etc.) responsivity. These properties make dendrimers favourable in many biomedical and pharmaceutical areas such as bio-imaging, tissue engineering, drug and gene delivery (Lee et al., 2005). By far poly(amidoamine) (PAMAM) dendrimer is the most intensively investigated.

In terms of drug delivery, the interior of PAMAM dendrimers exhibits high affinity for molecules with negative charges and is highly desirable as carriers of acidic drugs. It has been reported that PAMAM dendrimers could be efficient delivery systems with the benefits of enhanced drug solubility, prevention of drug degradation, increased circulation time, sustained/controlled drug release and potential drug targeting (Svenson, 2009). In addition, the advances in dendrimer surface engineering, i.e. the conjugation of functional groups to the chain ends of dendrimer surface, could provide stimuli-responsive properties to PAMAM dendritic delivery systems, which could add value to drug delivery efficiency and therapeutic efficacy (Kojima, 2010).

Among all the stimuli-responsive dendrimers, temperature-responsive systems are extremely promising with regard to drug delivery. Rendering dendrimers temperature-responsive usually involves grafting the terminal chain ends of dendrimers with temperature-sensitive groups, among which poly(N-isopropylacrylamide) is one of the best-known (Wei et al., 2007). PNIPAAm as a linear polymer is highly soluble in water at temperatures below ca. 32 °C, above which it becomes dehydrated and hence insoluble in water; this transition temperature is termed as lower critical solution temperature (LCST) (Zhang et al., 2004).

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Previous studies have successfully attached PNIPAAm to the dendrimer surfaces. For example, PNIPAAm was conjugated to the surfaces of poly(propylene imine) dendrimers forming a temperature-sensitive shell (Kimura et al., 2000; Zheng and Pan, 2006). In the same way, PNIPAAm was also introduced to other types of dendrimers (You et al., 2004).

Despite the advantages of thermo-sensitive dendritic drug delivery systems, there are still concerns regarding their toxicity, immunogenicity, and circulation time upon dose administration (Jevprasesphant et al., 2003). However, PEGylation (covalent linking polyethylene glycol (PEG) polymer chains with other molecules) is a popular approach to address these problems (Astruc et al., 2010; Jiang et al., 2010; Wong et al., 2010). For instance, Jevprasesphant et al. (2003) reported that the cytotoxicity of PAMAM dendrimers could be significantly deceased via surface PEGylation due to the shielding of the positive charge on the dendrimer surface by the PEG chains. Wang et al. (2009) showed that conjugation with PEG could effectively reduce the PAMAM-induced cell apoptosis by attenuating the production of reactive oxygen species. Kojima et al. (2010) demonstrated that PEGylayed PAMAM dendrimers exhibited longer blood retention and lower accumulation in other normal organs such as the kidneys than the non-PEGylated ones. In addition, PEGylation strategy has also been used previously to render the metal nanoparticle delivery systems non-toxic (Boyer et al., 2010). However, to our best knowledge, surface PEGylation has not been utilised in thermo-sensitive dendrimer delivery systems such as PAMAM dendrimers with a PNIPAAm shell.

As such, the aims of this study were to attach both thermo-sensitive PNIPAAm and biocompatible PEG to the periphery of PAMAM engineering long-circulating functional dendritic delivery systems and to investigate the effect of surface modification on the *in vitro* release profile of a model drug, indomethacin.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAM) and 4-nitrophenyl chloroformate were purchased from ACROS Organics (NJ, USA). HPLC grade tetrahydrofuran, indomethacin and methoxy poly(ethylene glycol) (MPEG) with a molecular weight (MW) of 2000 was provided by Sigma–Aldrich Chemie Gmbh (Munich, Germany). Analytical grade methanol, hexane, dichloromethane, diethyl ether, dimethyl sulfoxide, ethylenediamine (EDA), triethylamine (TEA), methyl acrylate (MA), 2-mercaptoethanol, and 2,2′-azobisisobutyronitrile were sourced from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Double distilled water from laboratory supply was used in the whole study unless otherwise stated.

2.2. Methods

2.2.1. Synthesis of PAMAM dendrimers

PAMAM dendrimers with EDA cores were synthesized using Tomalia's divergent growth approach (Esfand and Tomalia, 2001). Two consecutive chain-forming reactions (the exhaustive Michael addition and the exhaustive amidation reaction) were repeated alternately during the dendrimer synthesis. In the present study, G1.5 and G2.0 PAMAM dendrimers were obtained.

2.2.2. Synthesis of PAMAM–g–PNIPAAm

NIPAM monomers were purified by recrystallization with hexane prior to use. PNIPAAm with a terminal hydroxyl group (i.e. PNIPAAm–OH) was prepared by radical telomerisation (a polymerization reaction) of NIPAM using 2,2′-azobisisobutyronitrile (recrystallization with methanol prior to use) as the radical initiator and 2-mercaptoethanol as the chain transfer agent (Choi et al., 2006). PNIPAAm–OH was then diluted with water and dialysed using a dialysis tube (MW cut-off: 3400) against water for 5 days. The solution in the dialysis tube was collected and freeze-dried to get PNIPAAm–OH.

PNIPAAm-4-nitrophenyl carbonate was synthesised by reacting PNIPAAm–OH with 4-nitrophenyl chloroformate (Fig. 1). 4-Nitrophenyl chloroformate (7.2 mmol) and triethylamine (7.2 mmol) were added to 100 ml dichloromethane solution containing 3.6 mmol PNIPAAm; the mixture was stirred for 48 h at ambient temperature (25 ± 2 °C). The crude product was purified by recrystallization from diethyl ether to obtain PNIPAAm-4-nitrophenyl carbonate.

PNIPAAm-4-nitrophenyl carbonate (0.22 mmol) was put in a 10 ml solution of G2.0 PAMAM dendrimer (7 μmol) in dimethyl sulfoxide. The mixture was stirred for 7 days at ambient temperature, diluted with water and dialysed using a dialysis bag (MW cut-off: 14,000) against water for 36 h. The above crude compound was lyophilized and then eluted with water in a Sephadex G-75 column from Yuncheng, Inc. (Shanghai, China) to get the purified product of PAMAM–*g*–PNIPAAm (PAMAM dendrimers grafted with PNIPAAm).

2.2.3. Synthesis of PAMAM–g–PNIPAAm–co–PEG

The PAMAM dendrimers grafted with PNIPAAm and PEG (PAMAM–*g*–PNIPAAm–*co*–PEG) were synthesized via the formation of a urethane bond through the reaction of PAMAM–NH_2 with PNIPAAm-4-nitrophenyl carbonate and MPEG-4-nitrophenyl carbonate. MPEG-4-nitrophenyl carbonate was prepared by reacting MPEG with 4-nitrophenyl chloroformate and purified by recrystallization from diethyl ether (Yang et al., 2004) (Fig. 1). Briefly, PNIPAAm-4-nitrophenyl carbonate (0.22 mmol) and MPEG-4-nitrophenyl carbonate (0.11 mmol) were added to a solution of G2.0 PAMAM dendrimer (7 μmol) in dimethyl sulfoxide (10 ml); the mixture was stirred for 7 days at ambient temperature. Then the above solution was diluted with water and dialysed using a dialysis bag (MW cut-off: 14,000) against water for 36 h. The crude compound was lyophilized and then eluted with water in a Sephadex G-75 column to get the purified product of PAMAM–*g*–PNIPAAm–*co*–PEG.

2.2.4. FTIR and ^{1}H NMR

The dendrimer structures were analysed by Fourier transform infrared spectroscopy (FTIR) using a Tensor^{TM} 27 spectrometer (Bruker Optik GmbH, Ettlingen, Germany). Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) was also employed to characterise and confirm the structure of dendrimers and relevant intermediate products. The measurements were recorded using a 400 MHz Varian spectrometer (Varian, Inc., Walnut Creek, CA, USA).

2.2.5. Gel permeation chromatography

The average molecular weights of PNIPAAm–OH and PAMAM derivatives were determined by a gel permeation chromatographic (GPC) system equipped with a Waters 2414 detector (Waters Corporation, Milford, MA, USA). Tetrahydrofuran was used as an eluent at a flow rate of 0.3 ml/min through a Waters Ultrastyragel® column. Peak analysis was performed using Waters Millenium software program to calculate the MW of dendrimers according to a universal calibration curve generated by a polystyrene standard of narrow molecular weight distribution.

2.2.6. LCST determination

The LCST of the dendrimers was determined by measuring the absorbance of aqueous dendrimer solution (1.0 mg/ml) at 500 nm using a Cary100 UV–vis spectrometer (Varian, Inc., Walnut Creek,