Colloids and Surfaces B: Biointerfaces 153 (2017) 2–9

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

# Design and synthesis of temperature-responsive polymer/silica hybrid nanoparticles and application to thermally controlled cellular uptake

Yuki Hiruta*,[1], Ryo Nemoto[1], Hideko Kanazawa*

*Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato, Tokyo 105-8512, Japan*

ARTICLE INFO

*Article history:*
Received 14 October 2016
Received in revised form 30 January 2017
Accepted 31 January 2017
Available online 4 February 2017

*Keywords:*
Temperature-responsive polymer
Hybrid material
Nanoparticle
Fluorescence imaging
Cellular uptake

ABSTRACT

This study reports the development of temperature-responsive polymer/silica hybrid nanoparticles and their application to temperature-dependent intracellular uptake of hydrophobic encapsulated fluorescence molecules. Amphiphilic diblock copolymer comprising a temperature-responsive segment, poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) [P(NIPAAm-*co*-DMAAm)] and a trimethyoxysilyl-containing hydrophobic segment was synthesized (**PBM-*b*-ND**); this amphiphilic diblock copolymer self-assembled in an aqueous solution, and temperature-responsive polymer/silica hybrid fluorescence nanoparticles were fabricated via a base-catalyzed sol–gel process. The fluorescence probe rhodamine DHPE or boron dipyrromethene derivative was encapsulated into the polymer core with a silica network in a stable manner. Other types of polymer/silica hybrid fluorescence nanoparticles were also developed using either homo-PNIPAAm (**PBM-*b*-N**) or homo-PDMAAm (**PBM-*b*-D**) segments, instead of P(NIPAAm-*co*-DMAAm). While **PBM-*b*-D** did not exhibit a temperature-dependent phase transition (hydrophilic characteristic), **PBM-*b*-N** and **PBM-*b*-ND** exhibited temperature-dependent phase transition (hydrophilic/hydrophobic) at 32 °C and 38 °C, respectively. The cellular uptake of **PBM-*b*-N** was clearly observed at both 37 °C and 42 °C, while the cellular uptake of **PBM-*b*-D** was minimal at these temperatures. On the other hand, significant enhancement in the intracellular uptake of **PBM-*b*-ND** was observed at 42 °C, compared to its uptake at a lower temperature of 37 °C. These results indicated that temperature-responsive polymer/silica hybrid nanoparticle, **PBM-*b*-ND** demonstrate potential for applications in theranostics with cancer therapy via the combination of local drug delivery and local hyperthermia, as well as for monitoring treatment effectiveness with fluorescence imaging.



## 1. Introduction

Organic–inorganic hybrid materials exhibit interesting properties, with the organic moieties offering enormous functional variations to the robust inorganic substrates [1]. Thus, these materials have been widely used for catalysis [2], sensors [3,4], separation [5] and biomedical applications [6]. Organic–inorganic nanoparticles (NPs) can have multiple functionalities, such as magnetic, optical, and surface plasmon resonance properties, on the same entity [7,8]. Hence, particularly for biomedical applications, these NPs have attracted immense attention for use in nanotherapeutics and nanodiagnostics. Au nanocages exhibiting the surface plasmon resonance phenomenon can be applied to generate hyperthermia [9]. Iron oxide NPs can be applied in magnetic resonance imaging (MRI) [10]. The target species can be incorporated onto the NP surface [11].

Silica NPs exhibit desirable physical and chemical properties such as optical transparency in the visible region; in addition, these NPs serve as robust hosts for embedding organic functional compounds, which in turn enables these NPs to become promising fluorescent materials [12]. Fluorescent dye molecules embedded into silica NPs are protected from the external environment, as well as immobilized, resulting in enhanced fluorescence quantum yields [13]. Furthermore, multiple dyes can be doped into these NPs [14,15]. The solubility limit of some water-insoluble fluorescent dye molecules is settled. Furthermore, the silica NP surface can be modified with organoalkoxysilane, and easily functionalized [16,17]. Poly(*N*-isopropylacrylamide) (PNIPAAm) exhibits a temperature-dependent phase transition at its lower critical solution temperature (LCST). Homo-PNIPAAm exhibits the LCST at around 32 °C [18], and the LCST of PNIPAAm-based polymers can be precisely controlled to near-body temperature for biomedical

* Corresponding authors.
*E-mail addresses:* hiruta-yk@pha.keio.ac.jp (Y. Hiruta), kanazawa-hd@pha.keio.ac.jp (H. Kanazawa).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.colsurfb.2017.01.052
0927-7765/

applications by copolymerization with hydrophilic co-monomers such as *N*,*N*-dimethylacrylamide (DMAAm) [19]. Furthermore, the temperature-responsive characteristics of PNIPAAm-based polymers can be changed in response to external stimuli, such as pH [20], ions [21] and light [22], by copolymerization with a stimuli-responsive monomer.

Previously, our group has reported the successful synthesis of PNIPAAm-based temperature, and pH responsive fluorescent polymer probes and applied them to temperature, and pH controlled intracellular uptakes [23,24]. In addition, Chilkoti and co-workers described methods for solid tumor targeting using temperature-responsive polymers and local hyperthermia [25]. This work achieved enhanced delivery of these polymers to solid tumors, in an in vivo model, facilitated by local heating of these tumors. NPs exhibiting long circulation times and the enhanced permeation and retention (EPR) effect [26], as well as multiple functionalities, are advantageous for use in imaging, as well as for use as drug carriers in biomedical applications. If the temperature-responsive property can be imparted to NPs, tumor-selective therapeutics and diagnostics will be expected. In this paper, temperature-responsive polymer/silica hybrid fluorescent NPs were developed, with different phase transition temperatures depending on the PNIPAAm/DMAAm copolymer composition ratio, and exploited for temperature-controlled cellular uptake.

## 2. Experimental

### 2.1. Materials and chemicals

*N*-Isopropylacrylamide (NIPAAm) provided by KJ Chemicals Co. (Tokyo, Japan) was purified by recrystallization from *n*-hexane and dried at 25 °C in vacuo. Butyl methacrylate (BMA), *N*,*N*-dimethylacrylamide (DMAAm), 2,2′-azobisisobutyronitrile (AIBN), 2-aminoethanol, dehydrated benzene, 1,4-dioxane, and dimethylformamide (DMF) were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). BMA and DMAAm were distilled under reduced pressure. AIBN was recrystallized from methanol. 3-(Trimethoxysilyl)propyl methacrylate (MSPM), 2-cyano-2-propyl benzodithioate (CPBD), and maleimide were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Lissamine rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (rhodamine DHPE) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). 1,3,5,7-Tetramethyl-8-phenyl-boron dipyrromethene (BODIPY) was prepared according to a previously reported procedure [27].

### 2.2. Synthesis of poly[butyl methacrylate-co-3-(trimethoxysilyl)propyl methacrylate] (**PBM**)

BMA (9.60 g, 67.5 mmol) and MSPM (1.86 g, 7.49 mmol) were dissolved in benzene (50 mL). AIBN (35 mg, 0.21 mmol) and CPBD (195 mg, 0.88 mmol), serving as the radical initiator and chain-transfer reagent, respectively, were added to the solution. The reaction mixture was degassed by bubbling with nitrogen for 30 min and heated at 65 °C. After the reaction was allowed to continue for 15 h, the reaction solution was then poured into methanol to precipitate the polymer. Finally, the crude product was further purified by repeated precipitation from a chloroform solution into methanol, followed by drying to afford a pink solid.

### 2.3. Diblock co-polymerization of P(NIPAAm-co-DMAAm) with PBM (**PBM-*b*-ND**)

**PBM-*b*-ND** was synthesized as follows. NIPAAm (3.32 g, 29.3 mmol) and DMAAm (735 mg, 7.41 mmol) were dissolved in 1,4-dioxane (10 mL). AIBN (3.7 mg, 0.023 mmol) and **PBM** (1.00 g, 0.081 mmol), serving as the macro-chain transfer agent, were added to the solution. The reaction mixture was degassed by bubbling with nitrogen for 30 min and heated at 70 °C. After the reaction mixture was allowed to continue for 19 h, the reaction solution was poured into hexane–diethyl ether to precipitate the polymer. Finally, the crude product was further purified by repeated precipitation from an acetone solution into hexane–diethyl ether, followed by drying to afford a pale pink solid.

**PBM-*b*-N** and **PBM-*b*-D** were synthesized by a similar procedure but by changing the molar ratios. Their reaction compositions were as follows: **PBM-*b*-N** = NIPAAm (4.03 g, 35.6 mmol), 1,4-dioxane (10 mL), AIBN (3.1 mg, 0.019 mmol), and **PBM** (0.97 g, 0.079 mmol); and **PBM-*b*-D** = DMAAm (4.03 g, 40.7 mmol), 1,4-dioxane (10 mL), AIBN (2.7 mg, 0.016 mmol), and **PBM** (1.00 g, 0.081 mmol).

### 2.4. Synthesis of polymer/silica hybrid nanoparticles

10 mg of an amphiphilic diblock copolymer (**PBM-*b*-ND**, **PBM-*b*-N**, or **PBM-*b*-D**), and 0.1 mg rhodamine DHPE or BODIPY was dissolved in 1 mL of DMF. This DMF solution was poured into 9 mL of water with stirring. The mixture was allowed to stir for an additional 4 h. Then, 20 mg of 2-aminoethanol and 5 mg maleimide were added. Finally, DMF and the free fluorescent dye were removed by dialysis against deionized water using a 12–14,000 Da MWCO dialysis membrane (Spectra/Por, Spectrum Laboratories, CA, USA). Dialysis was continued until no fluorescent dye leakage was observed (usually 3 days).

### 2.5. Characterization of polymers

$^1$H NMR spectra were recorded at 500 MHz on a Varian INOVA-500 spectrometer. Molecular weights of the polymers were determined by gel permeation chromatography (GPC; GPC-8020 system: column, TSK-GEL; mobile phase, DMF containing 10 mM of LiCl; TOSOH, Tokyo, Japan), and poly(ethylene oxide) standards were used for calibration.

### 2.6. Characterization of temperature-responsive polymer/silica hybrid nanoparticles

The phase transition temperature of polymer/silica hybrid NPs was determined by measuring their optical transmittance in a PBS buffer solution (100 μg/mL). Optical transmittance was measured at 500 nm at various temperatures using a UV–vis spectrophotometer (V-630, JASCO, Tokyo, Japan). Temperature was controlled using an ETC-717 controller (JASCO) and a PT-31 Peltier system (Krüss, Hamburg, Germany); the heating rate was 0.1 °C/min. Hydrodynamic diameters of polymer/silica hybrid NPs were determined by dynamic light scattering (Zetasizer Nano Series, Malvern Instruments Ltd., Malvern, UK) at 30–42 °C.

### 2.7. Cell culture

Human cervical cancer HeLa cells (RIKEN BRC CELL BANK) were cultured as subconfluent monolayers in a 75 cm$^2$ culture flask with a vent cap in minimum essential medium Eagle (MEM) with non-essential amino acids (NEAA), supplemented with 10% fetal bovine serum (FBS), 50 units/mL of penicillin, and 50 μg/mL of streptomycin, in a humidified incubator at 37 °C with 5% $CO_2$.

### 2.8. Temperature-dependent intracellular uptake of fluorescent polymer/silica hybrid nanoparticles

HeLa cells were seeded into 35 mm glass bottom dishes ($5 \times 10^4$ cells, 2 mL/dish) and cultured for 1 day at 37 °C in MEM with NEAA supplemented with 10% FBS in a humidified atmosphere