Colloids and Surfaces B: Biointerfaces 149 (2017) 297–300

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Improved controlled release of protein from expanded-pore mesoporous silica nanoparticles modified with co-functionalized poly(*n*-isopropylacrylamide) and poly(ethylene glycol) (PNIPAM-PEG)

Erick Yu[a,b], Allison Lo[b], Linda Jiang[b], Bradley Petkus[b], Nazar Ileri Ercan[b,1], Pieter Stroeve[b,*]

[a] Department of Materials Science and Engineering, University of California Davis, Davis, CA, 95616, United States
[b] Department of Chemical Engineering, University of California Davis, Davis, CA, 95616, United States

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form 14 October 2016
Accepted 16 October 2016
Available online 17 October 2016

Keywords:
Poly(*n*-isopropylacrylamide)
Poly(ethylene glycol)
Protein delivery
Mesoporous silica nanoparticles

ABSTRACT

Novel pore-expanded mesoporous silica nanoparticles (MSNs) with pore sizes of approximately 11 nm were synthesized and modified with thermoresponsive, poly(*n*-isopropylacrylamide) (PNIPAM) gating groups on the nanoparticle exterior surface and in addition with poly(ethylene-glycol) (PEG) within the porous interior to minimize protein adsorption. PEG traditionally has been grafted to the nanoparticle exterior to minimize non-specific binding and interactions with the biological environment, but due to the templating mechanism of MSN synthesis, both the pore interior and nanoparticle surface can be separately modified. Here, an improved control release behavior of bovine hemoglobin (BHb) was observed after PEGylating the interior porous framework, compared to the release BHb from unmodified MSNs. This can be attributed to the reduced protein denaturation on PEGylated silica that was observed using circular dichroism spectroscopy.

1. Introduction

MSNs as a drug delivery agent has been explored in the past two decades but it has only been recently further modified as a potential agent for the delivery of proteins [1–4] and genetic material [5–7]. The pore size of typical MCM41, or SBA type MSNs. is the limiting factor in the effective entrapment and delivery of larger peptide and protein species. To work around this, hollow MSNs have been used to deliver larger compounds [8], as with other pore-expanded MSNs but with sizes upward of 200–300 nm [6]. In an earlier work [9] pore-expanded, core-shell MSNs were synthesized that combined both a superparamagnetic core with a mesoporous silica shell. It was observed however that the presence of the iron oxide core reduced the effectiveness of the pore-expansion process with TMB. In other instances however [6], depending on when and how the MSN sol-gel is modified with trimethylbenzene (TMB), pore sizes upwards of 20 nm can be achieved, although with compromises in the porous structure of the MSN. Nonetheless, the potential in producing large pore MSNs capable of delivering large proteins on the order of 5–10 nm is desirable for many therapeutic drug delivery applications.

One consideration involving peptide and protein delivery is the structural stability of the species, which determines the activity and effectiveness of the peptide or protein after its release into the local environment. Protein adsorption onto surfaces has been explored in planar [10] and spherical studies [11], but its adsorption into porous silica environments has not been as thoroughly discussed. In particular, it was observed that proteins do in fact deform and change their secondary structures when adsorbed onto glass surfaces [12]. Several methods to reduce the negative effects of this include modifying the surface with polymers such as PEG, which has long been noted to reduce non-specific binding of proteins onto surfaces [13,14]. However, designing a robust MSN platform for protein delivery that also exclusively modifies the internal porous structure with PEG is ideal for reasons stated above.

In the scope of designing nanoparticles for protein delivery, a gating mechanism is necessary to eliminate any premature release of the species. PNIPAM has been previously demonstrated [15] to be an effective gating mechanism and chosen here. The lower critical solution temperature (LCST) of PNIPAM is typically 31–32 °C, which is situated between room and physiological temperature, making it ideal for a thermo-responsive gating mechanism for drug delivery

* Corresponding author.
E-mail address: pstroeve@ucdavis.edu (P. Stroeve).
[1] Present address: Chemical Engineering Department, Bogazi University, Bebek, 34342, Istanbul, Turkey.

http://dx.doi.org/10.1016/j.colsurfb.2016.10.033
0927-7765/

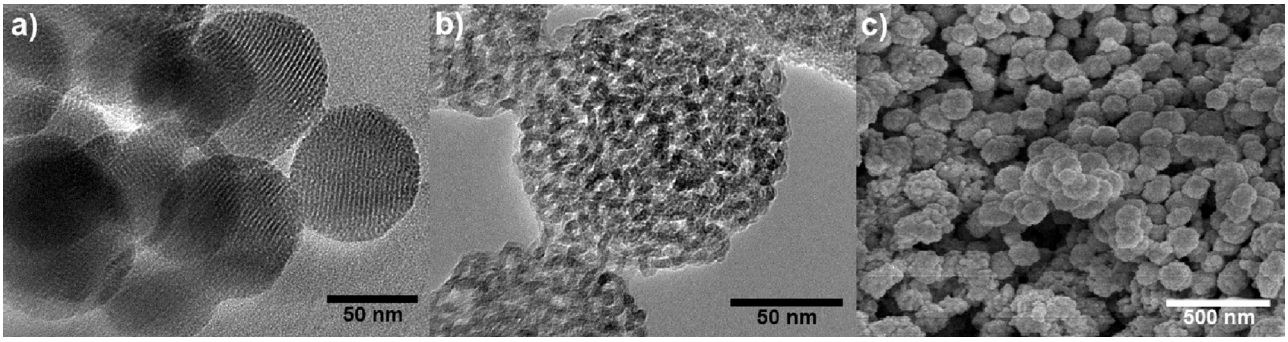

Fig. 1. (a) TEM of the MSNs, (b) expanded-pore, PNIPAM-PEG-MSNs, and (c) SEM images of the PNIPAM-PEG-MSNs.

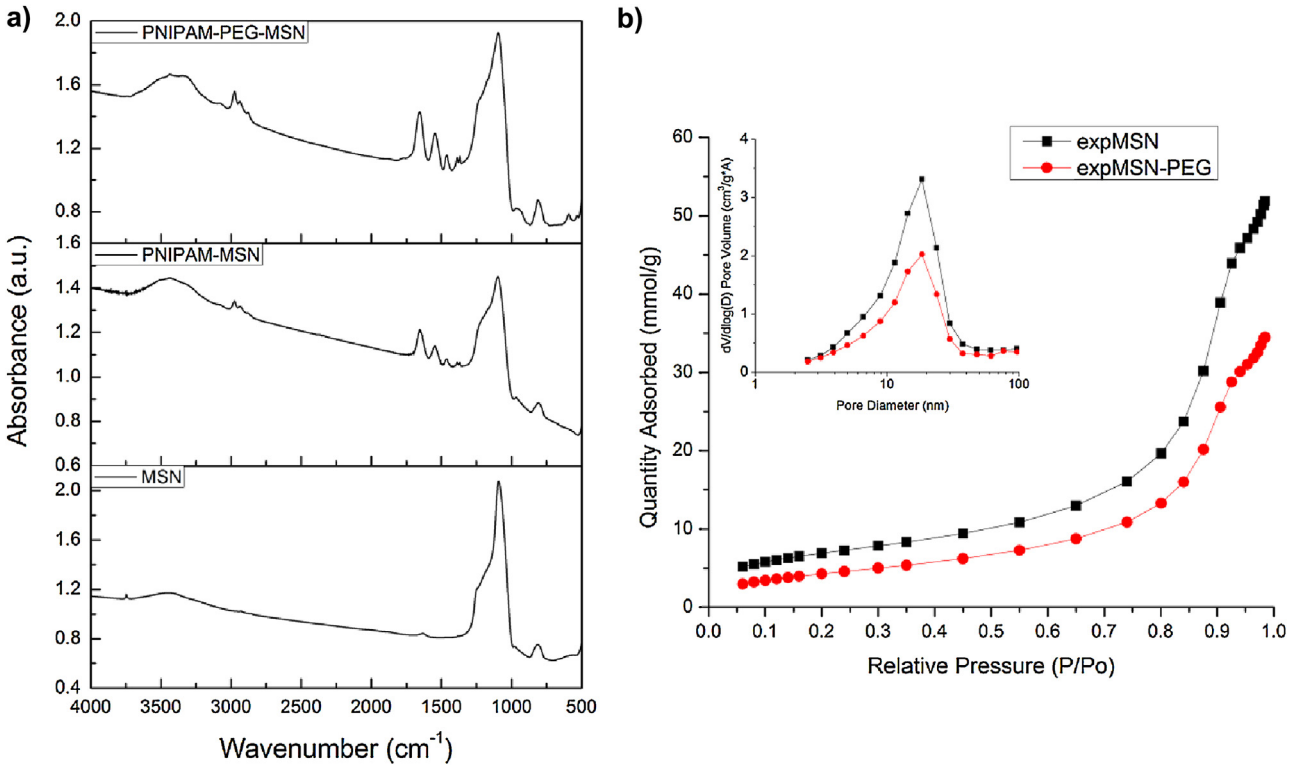

Fig. 2. (a) IR spectra of the MSN, PNIPAM-MSN, and PNIPAM-PEG-MSNs. (b) Nitrogen gas adsorption curves and pore size distribution of the expanded pore MSNs (expMSN) and PEGylated, expanded pore MSNs (expMSN-PEG). The adsorption isotherm and pore size distribution data are from single measurements.

media. Due to a straightforward and well-controlled functionalized methodology, PNIPAM is robust and can be combined with many other functionalization methods [16]. As such, a co-functionalized PEG/PNIPAM MSN is illustrated here for the delivery of protein, with bovine hemoglobin (BHb) as the model protein.

2. Materials and methods

Expanded-pore MSNs were produced by the sol-gel process, followed by a hydrothermal expansion process using TMB, similar to a previous work [17]. Briefly, first 1.75 mL of 2 M NaOH was added to 240 mL of DI water and increased to 80 °C. Afterwards, 500 mg of CTAB was slowly dissolved in the solution. 2.5 mL of TEOS was then added dropwise and the reaction ran for 2 h. The reaction was then cooled and aged overnight. The centrifuged MSNs were dispersed into 10 mL each of ethanol, water, and TMB, and placed into a Teflon autoclave. The hydrothermal pore expansion was carried out at 140 °C for 3 days and afterwards, the MSNs were collected rinsed in ethanol. To functionalize the exterior surface of the MSNs, a 3-aminopropyltriethoxysilane/α-bromoisobutyryl bromide (BIBAPTES) precursor was synthesized as done by Vasani et al. [18]. Atom transfer radical polymerization (ATRP) of PNIPAM was done using a previous methodology [9] with half the *n*-isopropylacrylamide (NIPAM) content. Prior to PEGylating the pore interior, the remaining surfactant template was removed through refluxing in 1 M ethanolic-HCl for 24 h. Afterwards, approximately 25 mg of PNIPAM-MSNs were added to 10 mL of EtOH and 146 uL of PEG-silane (Gelest SIM6492.7). The reaction was run for 7 h at 37 °C. For protein release studies, BHb loading was done with a 1:1:0.1 ratio of BHb (mg) to PNIPAM-PEG-MSNs (mg) to PBS 0.25× (mL) for 24 h.

3. Results and discussion

The pore-expansion process of MCM41-type MSNs has yielded a variety of porous structures in literature, depending on the pore-expansion agent and synthesis conditions. In the work done here, Fig. 1a and b show the microstructure of MCM41 MSNs before