Carbohydrate Polymers 184 (2018) 9–19

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Novel organic/inorganic hybrid flower-like structure of selenium nanoparticles stabilized by pullulan derivatives

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ARTICLE INFO

Keywords:
Hybrid flower
Selenium nanoparticles
Pullulan
Cytotoxicity
Formation mechanism

ABSTRACT

We proudly present the first organic/inorganic hybrid pullulan/SeNPs hybrid microflower material obtained using a simple and bio-inspired strategy. The chemical structures of pullulan, folic acid decorated cationic pullulan (FA-CP) were designed for stabilizing selenium nanoparticles (SeNPs). SeNPs stabilized by FA-CP hybrid microflowers were observed after the addition of a cysteine hydrochloride solution into the solution mixture of Na_2SeO_3 and FA-CP. We suggested that the concentrations of cysteine and FA-CP were the key factors for the formation of flower-like structure. In addition, the formation mechanism of the microflowers was tentatively identified as anisotropic hierarchical growth. The microflowers exhibited effective drug adsorption with the loading capacity of 142.2 mg g^{-1} for doxorubicin which was three times higher than that for the doxorubicin-loaded spherical SeNPs and showed more potent activity against cancer cells while showing less toxicity against normal cells. These data demonstrated that the microflower-like FA-CP/SeNPs structure could be a candidate anticancer drug template in drug delivery systems.

1. Introduction

Pullulan is a non-ionic polysaccharide which shows superior properties, namely, water solubility, non-toxicity, biocompatibility and low cost (Bae & Na, 2010; Rekha & Sharma, 2007; Sallustio et al., 2004). Pullulan can be easily derivatized in order to extend its applications by grafting different chemical structures onto its backbone (Lee et al., 2015; Lee et al., 2015; Scomparin et al., 2011; Souguir et al., 2007). Pullulan hydroxyls were used in numerous chemical reactions leading to a large number of derivatives. Regarding drug delivery applications, the use of pullulan derivatives with nonmetal nanoparticles (NPs) as an organic/inorganic hybrid material for cancer treatment has not been reported. Therefore, in this work we seek to design the pullulan molecule to provide the desired properties for the control of the shape and morphology of these hybrid materials.

Many different studies have reported the use of solely organic materials such as chitosan, dextran and PEG-PCL block co-polymer for cancer treatment application (Dass & Choong, 2008; Dragojevic et al., 2015). Although the encapsulation or conjugation of anticancer drugs with the polymers can stabilize and solubilize the drugs, the problems of small release and low loading of anticancer drugs were observed (Duncan, 2003; Hoare & Kohane, 2008). In addition, inorganic materials and especially metal nanoparticles such as gold, silver and metal oxide (iron oxide and cerium oxide) have received much attention recently due to their use in cancer therapy as drugs delivery vehicles (Frezza et al., 2010; Pasut & Veronese, 2007) and as anticancer agents (Muhammad & Guo, 2014; Vinardell & Mitjans, 2015). However, the easy aggregation of nanoparticles (Hosokawa et al., 2012; Keller et al., 2010) was the main problem for their use alone. Thus, the combination of organic and inorganic components to form organic/inorganic hybrid materials can address the problems associated with the use of each component by itself. Recently, controllable structure, porosity, surface to volume ratio, and functionalities of organic/inorganic hybrid material at the nano-scale has received considerable attention for a wide range of biological application in catalysis, drug delivery, and biosensing (Ge et al., 2012; Islam, Choi, Choi, & Lee, 2015; Lyu et al., 2014; Lu & Yang, 2016; Wang, Wang et al., 2013; Wang, Zhang et al., 2013). In addition, the design and synthesis of these materials for tailoring the structure of flower-like hybrid nanomaterial or hybrid nanoflower is challenging for their applications especially for biomaterial organic/inorganic hybrid. The cooperation between the inorganic components and biomaterial molecules play an especially key role for the functional

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https://doi.org/10.1016/j.carbpol.2017.12.029
Received 15 August 2017; Received in revised form 23 November 2017; Accepted 12 December 2017
Available online 20 December 2017
0144-8617/

enhancement of these hybrid materials (Koley et al., 2016). The study performed by Ge et al. (2012) showed that biomolecules-immobilized protein-inorganic hierarchical nanostructure exhibited an enhanced stability in solution. Moreover, greater activity and durability compared to free molecules were also observed in many studies (Ge et al., 2012; Lin, Xiao, Wang et al., 2014; Lin, Xiao, Yin et al., 2014; Manesh et al., 2010; Sun et al., 2014). In drug delivery systems (DDS), the structure of the carriers can greatly affect the DDS performance. Generally, the drug delivery vehicles should provide a high surface area, high drug loading efficiency and good biocompatibility for enabling a high potential in biomedical area (Islam et al., 2015; Yang, Hao, Zhao, Du, & Wang et al., 2013). In 2015, Isam et al. introduced the synthesis of polyelectrolyte-mediated hierarchical mesoporous calcium silicates with a large surface area resulting in an extremely high loading capacity for anionic drugs and proteins. This can be applied as a template for drug carriers.

In this work, we propose to design an organic/inorganic hybrid flower as a soft template for DDS with high drug loading capacity. Many studies have been reported for various metals such as copper (Ge et al., 2012; Lin et al., 2014; Lin, Xiao, Yin et al., 2014; Koley et al., 2016; Sun et al., 2014; Zhu et al., 2013), calcium (Lyu et al. 2014; Wang et al., 2014) and manganese (Zhang et al., 2015) for the organization of hierarchically structured nanomaterials. However, nanoflowers employing non-metal selenium nanoparticles have not been investigated.

Selenium nanoparticles (SeNPs) have excellent bioavailability, high biological activity, and low toxicity (Tan et al., 2009; Wu et al., 2012; Yang et al., 2012). They have attracted a great deal of attention as drug carriers (Huang et al., 2013; Liu et al., 2012; Yu et al., 2016). Numerous reports have been devoted to the controllable preparation of SeNPs with various morphologies such as nanowires, nanoribbons, nanotubes, nanoplate, nanospheres, and cubic-liked structures (Cao et al., 2004; Chen et al., 2009, 2010; Kumar et al., 2014; Luesakul et al., 2016; Yin et al., 2005). Moreover, the presence of a biopolymer or a functionalized biopolymer in the nanoparticles synthesis process can help enhance the stability (Xia et al., 2015; Zhang, Wang, & Zhang, 2010) and control the size (Peng et al., 2007; Song et al., 2016) and shape (Dobias et al., 2011; Luesakul et al., 2016; Zhang et al., 2010) to provide the unique properties of these nanomaterials. In this work, we focused on the synthesis of hybrid material using the SeNPs as the inorganic material and pullulan as the organic material instead of proteins that are widely used in hybrid nanoflower because pullulan can be functionalized to have the desired properties. The selectivity and stability of SeNPs should be improved by focusing on the modification of pullulan to stabilize the synthesized SeNPs and specifically target the cancer cells. Previous studies suggested that the nanoparticles stability can be enhanced by fabrication with positive charge (Yu et al., 2012), while the selectivity can improve by surface functionalization with targeting molecule such as folic acid (Huang et al., 2013; Lee et al., 2015; Lee, Cheon et al., 2015; Scomparin et al., 2011;). The FA-CP was introduced by quaternization to obtain the positive charge and functionalized folic acid (FA) on the pullulan chain. The modified pullulan was used as the organic substance for the organic/inorganic hybrid material.

Here, we reported the fabrication of SeNPs stabilized by pullulan derivative and unexpected selenium microflowers were observed. To the best of our knowledge, this represents the first report of pullulan/SeNPs organic/inorganic hybrid material and is one of only a few examples of novel 3D flower-like structures. We investigate the parameters for the synthesis of flower-shaped SeNPs stabilized by pullulan derivative with a drug loading capability. The formation mechanism of the microflowers was tentatively elucidated. Moreover, the applicability of hybrid microflowers as a template for drug transport was explored.

2. Materials and methods

2.1. Materials

Pullulan with average molecular weight, Mw, of 50–70 kDa and (3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (Quat-188) were provided by TCI, Japan. Folic acid, *N,N′*-Dicyclohexylcarbodiimide (DCC), 4-Dimethylaminopyridine. (DMAP), sodium selenite, L-cysteine hydrochloride and MTT (3-(4,5-imethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye were purchased from Sigma-Aldrich Co., USA. Cellulose dialysis tubing (Membrane Filtration Products, Inc., USA) with the molecular weight cutoff of 12–14 kDa was used to purify all modified pullulan samples. The doxorubicin hydrochloride anticancer drug was obtained from Beijing Packbuy M&C Co., Ltd.

2.2. Synthesis of folic acid decorated cationic pullulan (FA-CP)

2.2.1. Synthesis of cationic pullulan (CP)

1 g of pullulan was dissolved in 10 mL deionized (DI) water. NaOH solution (0.25 g in 3.5 mL) was added to the pullulan solution. Then, 0.5 mL of Quat-118 used as the quaternizing agent was added dropwise into the reaction mixture and stirred at 60 °C for 24 h. The mixture was neutralized before dialysis with distilled water for 2 days and the dialyzed solution was then concentrated under vacuum using a rotary evaporator. The CP product was collected and dried at 60 °C in an oven.

2.2.2. Synthesis of folic acid decorated cationic pullulan (FA-CP)

One gram of CP was dissolved in 30 mL of DMSO under stirring at room temperature until the clear solution was produced. 200 mg of folic acid (FA) was activated in 5 mL DMSO with 60 mg of DCC and 20 mg DMAP for 60 min at room temperature. The folic acid solution was added into the pullulan solution and the reaction proceeded at room temperature for 24 h. The mixture was dialyzed against a phosphate buffer with pH 7.4 for 3 days and then dialyzed continually against DI water for 4 days. The dialyzed solution was then concentrated and purified by precipitation from acetone. The modified sample was collected and dried for several days at 60 °C in an oven. The molecular structure of FA-CP is shown in Fig. 1.

2.3. Characterization of pullulan derivatives

2.3.1. ^{1}Nuclear magnetic resonance spectroscopy

^{1}H NMR spectra of pullulan, CP, and FA-CP were recorded in D_2O on a 400 MHz NMR spectrometer (Varian Mercury 400MHz Spectrometer). The degree of quaternization (%DQ) of pullulan and the degree of substitution (%DS) of FA were determined from ^{1}H NMR spectra by comparing the ratio of the areas under the proton peaks using Eq. (1) for the %DQ (Yuan et al., 2014) and Eq. (2) for the %DS of FA (Varshosaz et al., 2014), respectively.

$$\%DQ = \left[\frac{[H10]}{[H1]} x \frac{1}{9}\right] x100 \quad (1)$$

$$\%DS_{FA} = \left[\frac{[H11-H12]}{[H1]} x \frac{1}{2}\right] x100 \quad (2)$$

2.3.2. Fourier transformed infrared spectroscopy (FT-IR)

To determine the functional chemical groups through their characteristic frequencies, the synthesized product was examined by FT-IR spectroscopy (Nicolet6700) in the wavelength region from 400 to 4000 cm^{-1}.