Acta Biomaterialia 6 (2010) 2212–2218

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Polymeric micelle-templated synthesis of hydroxyapatite hollow nanoparticles for a drug delivery system

Feng Ye *, Haifeng Guo, Haijiao Zhang, Xiulan He

School of Materials Science and Engineering, P.O. Box 433, Harbin Institute of Technology, Harbin 150001, People's Republic of China

ARTICLE INFO

Article history:
Received 23 June 2009
Received in revised form 30 November 2009
Accepted 4 December 2009
Available online 22 December 2009

Keywords:
Hydroxyapatite
Hollow nanoparticles
Polymeric micelle
pH-controlled release
Drug delivery system

ABSTRACT

Hydroxyapatite (HA) hollow nanoparticles (HNPs) have great potential in nanoscaled delivery devices due to their small size, excellent biocompatibility and expected high capacity. However, the preparation of HA HNPs for their application in a drug delivery system has rarely been reported because HA has a complicated crystal structure and it is difficult to obtain stable HA HNPs with hollows that are only nanoscaled in size. In the present study, HA HNPs were successfully produced through a novel polymeric micelle-templating method. The micelles were structured with completely insoluble Pluronic P123 molecules at cloud point as the core and Tween-60 molecules as the shell by the hydrophobic interaction of the alkyl chains with the insoluble P123 core. The morphology of the HA HNPs could be transformed from nanospheres to nanotubes by adding citric acid as a cosurfactant. The prepared HA HNPs had a much higher drug payload than traditional nanoparticles, using vancomycin as the model drug. Most importantly, the HA nanotubes were coupled with a layer of citrate molecules on the HA surfaces, which could further improve the drug load efficiency and could form an excellent pH-controlled open/closed gate for drug release with the addition of cationic polyelectrolytes.

1. Introduction

Recently, the synthesis of inorganic hollow nanoparticles (HNPs) has attracted much attention in the chemistry and materials communities because of their low density, large specific area and pore volume, small size, mechanical and thermal stabilities, and surface permeability [1]. Such HNPs have a wide variety of potential applications in cosmetics, catalysis, coatings, composite materials, dyes, ink, artificial cells and fillers. Their hollow structure can also be used as a microencapsulate for drugs in the pharmaceutical field [2]. Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), the main mineral component of bones and teeth, is native to the human body. HA crystals have been widely used in delivery systems for genes [3], proteins [4] and various drugs [5,6] due to their nontoxicity and excellent biocompatibility, as have been experimentally proven by recent reports [7–9]. However, HA crystals have a limited drug load capacity. HA mesoporous nanoparticles or HNPs have greater potential in a nanoscaled delivery system due to their promising high capacity. Recently, Yang et al. [10] reported that luminescent HA mesoporous nanoparticles possessed a high loading for ibuprofen (over 40 wt.%), though the carrier had uncontrolled release kinetics which made it have a burst release of ibuprofen of over 50% within 1 h. However, to our knowledge, the preparation and application of HA HNPs in drug delivery systems have been reported only rarely. The reason for this might be that HA has a complicated crystal structure (in comparison to common oxides, such as SiO_2) and it is very difficult to obtain stable HA HNPs whose hollow part is only tens of nanometers through traditional methods.

It is well known that poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) block copolymer exhibits peculiar behaviors in aqueous solution [11–13]. Below the characteristic temperature – the critical micellar temperature (CMT), both the PEO and PPO blocks are soluble in water, and the copolymer molecules remain in the form of unimers in solution. At the CMT the PPO blocks become insoluble and form spherical micelles, with PPO blocks as the hydrophobic core and the hydrated PEO blocks as the shell. Meanwhile, the PEO blocks start to lose hydrated water with increasing temperature, and at the temperature called the cloud point (CP), the PEO blocks become too insoluble and the copolymers get phase-separated from the water. The behaviors of block copolymer in water between the CMT and the CP have been extensively used to template various mesoporous materials [14–18], while its characteristics below CMT and at CP have often been used in cloud point extractions [19,20]. Actually the behaviors below CMT and at CP of triblock copolymer–Pluronic P123 ($EO_{20}PO_{70}EO_{20}$), i.e. completely insoluble at CP and totally soluble below CMT, can be adjusted for the preparation of HA HNPs by the use of Tween-60, whose CP is high-

* Corresponding author. Tel.: +86 451 86413921; fax: +86 451 86413922.
E-mail address: yf306@hit.edu.cn (F. Ye).

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doi:10.1016/j.actbio.2009.12.014

er than that of P123. The details are shown in Scheme 1: at low temperature (but above the CMT of P123) P123 and Tween-60 molecules form micelles with a core–shell structure in the presence of ethanol and a phosphorous source; at the CP of P123, the P123 molecules in these micelles become insoluble, but the Tween-60 molecules remain soluble; therefore, the insoluble P123 molecules become the condensed cores of new micelles and Tween-60 molecules form the shells by the hydrophobic interaction of their alkyl chains with the insoluble P123 core. When Ca^{2+} is added, HA forms within the shells of the micelles, and the cores act as template for the hollow parts of HA HNPs; the templates can then be easily removed by water and ethanol solution at low temperature (<CMT) because P123 is totally soluble below its CMT. In addition, polar citric acid was used as a cosurfactant to modify the shape of the micelles and ultimately to regulate the morphology of HA HNPs as the polar additive can associate with nonionic surfactant molecules, resulting in a change in the critical packing parameters of the surfactants.

In this report, HA HNPs were successfully produced through the above-designed polymeric micelle-templating method. With adding citric acid as the cosurfactant, the morphology of HA HNPs transformed from nanospheres to nanotubes. The obtained HA HNPs had a much higher drug payload than traditional nanoparticles, using vancomycin as the model drug. Most importantly, the HA nanotubes were coupled with a layer of citrate molecules upon the HA surfaces, which could further improve the drug load efficiency and could form an excellent pH-controlled open/closed gate for drug release with the addition of cationic polyelectrolytes.

2. Materials and methods

2.1. Investigation on the cloud points of surfactants

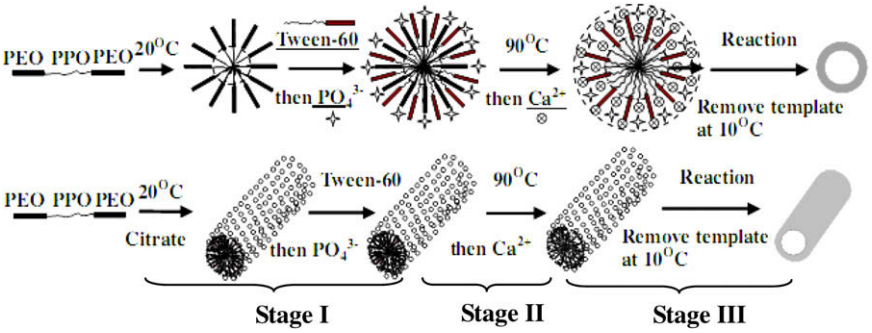
The cloud point of P123 aqueous solutions with different additives (ethanol, Tween-60 and citric acid) were investigated by raising the temperature at a rate of 2 °C min^{-1}. The cloudy phenomena of solutions were recorded by digital camera. The digital images are shown in Supplementary Fig. S1.

2.2. Preparation of HA HNPs

According to Scheme 1, calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) and phosphorous acid (H_3PO_4, 85%) were used as the calcium and phosphorous sources for HA, respectively. A mixture of the nonionic surfactants Pluronic $EO_{20}PO_{70}EO_{20}$ (P123; EO = ethylene oxide, PO = propylene oxide) and polyoxyethylene (20) sorbitan monostearate (Tween-60) was utilized as the template agent, with citric acid as the cosurfactant. All chemicals were analytical grade and used as purchased without further purification. Based on the analysis results of the CP for P123 in different additives (Supplementary Fig. S1), the temperatures at stages I and II in Scheme 1 were valued as 20 and 90 °C, respectively. Two groups of samples were prepared (designated S1 and S2). For S1, 2.9 g of P123 was dissolved in 50 ml of deionized water plus 10 ml of ethanol and the solution was stirred to form micelles at 20 °C. The solution was supplemented with 1.31 g Tween-60 (in 20 ml of water) and 0.1 mol PO_4^{3-}, then continuously stirred for 1 h. Next, the refluxing flask containing the mixed solution was put directly into a water bath at 90 °C. Subsequently 0.167 mol Ca^{2+} (39.438 g of $Ca(NO_3)_2{\cdot}4H_2O$ dissolved in 30 ml of water, pH 9) was slowly added to the solution, which was left to react for 48 h. The solution was continuously stirred and refluxed throughout the whole process. After the reaction was completed, the precipitate was eluted with ethanol and water six times at 10 °C because the CMT of P123 was around 15 °C. The obtained products were dried at 100 °C for 12 h and calcined at 300 °C for 3 h. For S2, 10 g of citrate acid was added to the P123 solution to change the micelle shape and ultimately regulate the morphology of the HA HNPs, using ammonia to make the solution's pH 9 in the first stage. The rest of the process was the same as for S1.

2.3. Drug load and in vitro release

The resultant HA HNPs were used as carriers for vancomycin. Typically, 0.4 g of HA HNPs was added to 20 ml of phosphate-buffered solution (PBS, 10 mM) contained 0.4 g vancomycin, which was then stirred at 37 °C for 12 h. For HA nanotubes coupled with a layer of citrate, 0.04 g of 40 wt.% cationic polyelectrolyte (poly(dimethyldiallyl ammonium) chloride, PDDA) was added to the PBS solution before the final completion of drug loading, to associate with the citrate carboxyl of the HA nanotubes and form an open/close gate. After being centrifuged (5000 rpm) and washed three times to remove the drug molecules adsorbed on the outer surface of the HA HNPs, the obtained products were freeze-dried and then vancomycin-loaded HA HNPs carriers were prepared. In order to determine the drug payload of these HA carriers, 0.02 g (M_C) of vancomycin-loaded HA HNPs was dissolved in 10 mM PBS solution (pH 1) and the concentration of vancomycin in the resultant solution was measured by ultraviolet–visible (UV–vis) spectroscopy at a wavelength of 280 nm. The PBS solutions with different concentrations of vancomycin (0.1, 0.2, 0.25, 0.4, 0.6, 0.8 and 1 g l^{-1}) were also measured by UV–vis spectroscopy to evaluate the Lambert–Beer mass absorption coefficient (ζ) of the vancomycin in the PBS solution, as shown in Supplementary Fig. S2. The product of the concentration and volume of the resultant solution is equal to the mass (M_V) of vancomycin-loaded in the HA HNPs. The drug payload ratio (L) for the HA HNP carrier was calculated as: $L = M_V/M_C \times 100\%$, and the loaded efficiency of the drug was $E = L/(1 - L) \times 100\%$ due to the mass of vancomycin and HA in the loading process was the same. The actual value of L was the



Scheme 1. Fabrication of hollow HA nanoparticles from P123 and Tween-60 core–shell structured micelles templates.

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