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Polymer-coated mesoporous silica nanoparticles for the controlled release of macromolecules

Sanjib Bhattacharyya *, Henson Wang, Paul Ducheyne

Center for Bioactive Materials and Tissue Engineering, Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA

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ABSTRACT

With the goal of achieving constant release of large biological molecules over an extended period of time we focused on hybrid inorganic/organic nanoparticles. We synthesized poly(ethylene glycol) (PEG)-coated mesoporous silica nanoparticles (MSNs) with incorporated trypsin inhibitor (TI), a model protein molecule for growth factors. Due to the goal of incorporating large protein molecules the pore size of the as-synthesized MSNs was expanded by a hydrothermal treatment prior to TI incorporation. In vitro release from the MSNs without the thin polymer film shows an initial burst followed by continuous release. In the case of polymer-coated MSNs the initial burst release was completely suppressed and approximate zero order release was achieved for 4 weeks.

1. Introduction

Controlled release of therapeutics is often preferred over traditional therapeutic administration in which the drug is delivered as a bolus, as this may result in drug concentrations exceeding the maximum tolerated dose upon initial administration, and may have a limited time frame of efficacy as the concentration in the body is depleted. A delivery method that provides a nearly constant drug concentration within the therapeutic window would allow more effective disease treatment and would minimize potential toxicity risks. Additionally, controlled release may reduce the dosage frequency and offer site-specific delivery of the therapeutics.

The development of biocompatible, controlled release systems for macromolecules has evolved to the point that biologically active factors can be delivered to a targeted site [1]. It remains a critical issue with most controlled release systems, though, to slowly release drugs of large molecular weight such as proteins, including growth factors [2]. In fact, many controlled release formulations release an initial large bolus of drug immediately upon placement in an in vitro medium or an in vivo site, before the release achieves a stable profile. This phenomenon is typically referred to as "burst release", which leads to high initial drug delivery and also reduces the effective lifetime of the therapeutic [1–6].

Among controlled release materials polymers, bioactive ceramics and glasses have been widely investigated as materials for use as both artificial bone graft substitutes and controlled release materials [7–15]. However, when bioactive calcium phosphates were used as carriers for biologically active molecules they met with mixed success [7,16–19]. Most of these systems also suffered from the phenomenon of burst release [20].

A class of oxide-based controlled release systems has shown much greater promise. They are low temperature processed silica sol–gel materials. These materials, originally developed for engineering applications, have also been studied for both the entrapment and sustained release of biologically active compounds [21–29]. Some of the important benefits associated with the sol–gel processing of these silica-based glasses are the excellent biocompatibility, as demonstrated in vivo [25], and the ability to control the release kinetics [26].

Yet another group of oxide materials are the mesoporous silica materials characterized by a large specific surface area (>800 $m^2 g^{-1}$), a large pore volume and a pore size in the range 2–40 nm [30–32]. In addition, they also display a narrow pore size distribution. By virtue of these properties applications in the fields of catalysis, lasers, sensors and solar cells have been pursued successfully [30–32].

Recent breakthroughs in the synthesis of mesoporous silica materials with high specific surface areas (>800 $m^2 g^{-1}$) and tunable pore sizes (2–10 nm) have led to the development of a series of new delivery systems, with various guest molecules, such as pharmaceutical drugs, fluorescent imaging agents, and others. Many molecules have been incorporated into the mesopores and were then released in a controlled fashion [33–35].

Recently reports have appeared describing functional mesoporous silica materials in which the pore surface was decorated with

* Corresponding author. Tel.: +1 215 898 8382.
E-mail address: sanjib@seas.upenn.edu (S. Bhattacharyya).

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organic or inorganic moieties that served as gatekeepers to regulate the release of guest molecules triggered by a variety of external stimuli, such as chemical stimuli [36–38], temperature variations [39], redox reactions [40] or photo-irradiation [41]. These studies highlighted the potential of utilizing mesoporous materials with decorated pore surfaces for many controlled release applications. Another interesting recent finding related to the fact that mesoporous silica nanoparticles can be efficiently internalized into cells, are non-toxic, not affecting cell viability, growth or differentiation, and can escape from endolysosomal vesicles and resist lysosomal degradation [42,43].

The current status of the field of controlled release from silica-based materials points both to excellent advances, but also to significant limitations. Opportunity is associated with biocompatibility, room temperature processing, controlled nanopore size, and molecular control of the release trigger. However, long-term near zero order release of large biological therapeutic molecules (in the range 10 kDa and higher) from nanoparticles for targeted delivery has not yet been achieved. Herein we describe a unique method that achieves this elusive goal of releasing a molecule in excess of 20 kDa molecular weight for 4 weeks in vitro. We describe the various critical processing steps, their role and their interdependency, and we demonstrate near zero order release kinetics for 4 weeks, without any initial burst release.

2. Methods and materials

2.1. Overview

We synthesized composite hybrid drug delivery systems which combine a robust inorganic mesoporous silica nanosphere (MSNs) core with a grafted biocompatible poly(ethylene glycol) (PEG)polymer surface. Fig. 1 is a schematic drawing summarizing the various consecutive steps of synthesis. We first created nanoparticles. Due to the goal of incorporating large protein molecules the pore size of the as-synthesized MSNs was then expanded by a hydrothermal treatment. This was followed by macromolecule incorporation. As a model protein we used trypsin inhibitor (TI) (molecular weight 23 kDa). In fact, it has been successfully used as a model molecule before [22] and has a size similar to growth factors such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF) and bone morphogenetic proteins (BMPs), which are biological molecules with documented effects in bone and other tissues. TI was incorporated within the pores of the MSNs. Herein we report the in vitro release kinetics from this organic/inorganic hybrid system over 4 weeks, and compare them with release from nanoparticles that reflect several control conditions, namely MSNs without the polymer film and non-pore-expanded MSNs.

2.2. Materials

Tetra-ethylorthosilicate (TEOS), octadecyltrimethylammonium bromide (C_{18}TAB), TI, sodium hydroxide pellets, poly(ethyleneglycol)-bis-amine (PEG-amine) (molecular weight 3 kDa), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), 3-(triethoxylsilyl) propyl-succinic anhydride (TESPA), and dimethylhexadecylamine (DMH) were obtained from Sigma Aldrich (St. Louis, MO) and used without further purification.

2.3. Synthesis of carboxylated MSNs

The MSNs were prepared as follows 1.00 g of C_{18}TAB was dissolved in 480 ml of distilled water at 75 °C. The solution was made basic (pH∼12) by the addition of 3.5 ml of 2.00 M sodium hydroxide. After reaching a stable pH of 12, 5 ml of TEOS and 1 ml of TESPA was added drop-wise. The reaction temperature was maintained at 75 °C for 2 h, giving rise to a white precipitate. The mixture was filtered and washed several times with deionized water until the supernatant reached neutral pH (∼7). The samples were then dried under vacuum at 80 °C for 2 days. Non-carboxylated MSNs were synthesized in exactly the same way as described above without the addition of TESPA.

2.4. Pore expansion of MSNs

0.8 g of dried carboxylated MSNs was added to an aqueous emulsion of 0.5 g of DMH in 30 ml of water and stirred for 15 min. The emulsion was transferred toa Teflon-coated pressure vessel and kept in an oven at 110 °C for 3 days. Then it was filtered and washed several times with deionized water to remove unreacted DMH, and the samples dried at 80 °C for 1 day.

2.5. Removal of surfactant (CTAB and DMH)

The surfactants (C_{18}TAB and DMH) were removed as follows. 0.8 g of pore-expanded MSNs were refluxed in 100 ml of methanol containing 1 ml of concentrated hydrochloric acid for 48 h. The samples were washed several times with ethanol and water, and dried in an oven at 80 °C for 2 days. These were the final MSNs which were used for loading with TI and for polymer coating.

2.6. Trypsin inhibitor loading

40 mg of TI was dissolved in 0.1 M acetic acid in two separate vials. 200 mg of pore-expanded MSNs was added to one vial, while 200 mg of MSNs without pore expansion was added to the other vial (these latter specimens served as controls) with stirring for 24 h. The samples were centrifuged and washed with water in order to remove acetic acid. Finally, both samples were dried at 37 °C for 3 days. The actual amount of TI incorporated within the MSNs was calculated from the difference in concentration of TI in the supernatant after centrifugation and the initial concentration in the solution used for loading. A 16 wt.% loading of TI was achieved for pore-expanded MSNs and 9.5 wt.% for non-expanded MSNs.

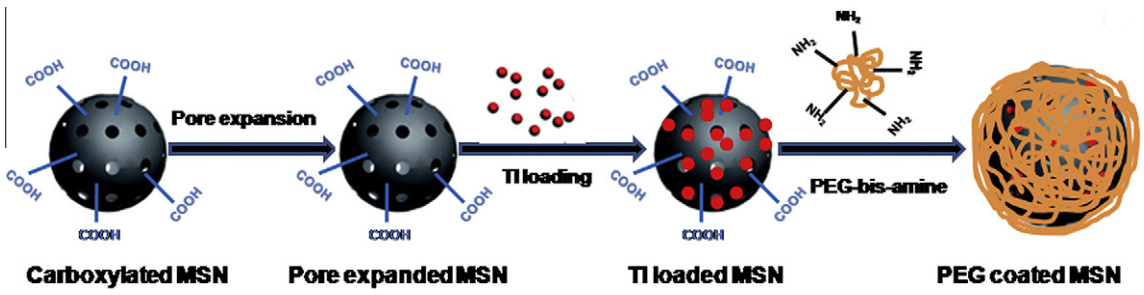

Fig. 1. Schematic diagram of the various steps used in synthesizing the hybrid mesoporous silica nanoparticles (MSNs). The steps involve: synthesizing carboxylated mesoporous silica nanoparticles; expanding the pores; loading the macromolecules; coating the MSNs with a poly(ethylene glycol) film.