Biomaterials 125 (2017) 23–37

Contents lists available at ScienceDirect

Biomaterials

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

Molecularly organic/inorganic hybrid hollow mesoporous organosilica nanocapsules with tumor-specific biodegradability and enhanced chemotherapeutic functionality

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ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form
10 February 2017
Accepted 13 February 2017
Available online 15 February 2017

Keywords:
Mesoporous organosilica
Hollow
Cancer therapy
Biodegradability
Drug delivery

ABSTRACT

Based on the intrinsic features of high stability and unique multifunctionality, inorganic nanoparticles have shown remarkable potentials in combating cancer, but their biodegradability and biocompatibility are still under debate. As a paradigm, this work successfully demonstrates that framework organic-inorganic hybridization can endow the inorganic mesoporous silica nanocarriers with unique tumor-sensitive biodegradability and high biocompatibility. Based on a "chemical homology" mechanism, molecularly organic-inorganic hybridized hollow mesoporous organosilica nanocapsules (HMONs) with high dispersity and sub-50 nm particle dimension were constructed in mass production. A physiologically active disulfide bond (−S−S−) was directly incorporated into the silica framework, which could break up upon contacting the reducing microenvironment of tumor tissue and biodegrade accordingly. Such a tumor-specific biodegradability is also responsible for the tumor-responsive drug releasing by the fast biodegradation and disintegration of the framework. The ultrasmall particle size of HMONs guarantees their high accumulation into tumor tissue, thus causing the high chemotherapeutic outcome. This research provides a paradigm that framework organic-inorganic hybridization can endow the inorganic nanocarrier with unique biological effects suitable for biomedical application, benefiting the development of novel nanosystems with the unique bio-functionality and performance.

1. Introduction

The fast development of nano-biotechnology has catalyzed the generation of various material nanosystems, mainly including organic and inorganic materials. Organic carriers are featured with high biocompatibility and biodegradability, but their stability is low and functionality is usually single [1–3]. Comparatively, inorganic nanomaterials have high chemical/physiological stability and multifunctionality, but their biocompatibility and biodegradability are believed to be relatively low and still under debate [4,5]. Construction of organic/inorganic hybrid material nanosystems is expected to combine the advantage of traditional organic and inorganic nanocarriers and overcome their individual disadvantages accordingly, thus showing great promise for clinical translation [6–9].

Simple organic-inorganic hybridization by surface conjugation of nanoparticles (NPs) cannot change the intrinsic physiochemical/physiological features of initial nanocarriers. Only homogeneous hybridization within the framework is effective for endowing the nanosystems with new biological effects, which is still a big challenge unresolved. This work tries to establish a new paradigm of molecularly organic-inorganic hybridization within the framework of inorganic nanocarriers by directly incorporating physiologically-active organic groups into the matrix of inorganic NPs. We chose mesoporous silica NPs (designated as MSNs) [10,11] as the basic platform to conduct the organic-inorganic hybridization because silica-based nanosystems have been generally regarded to be biosafe for clinical translation, which has also been approved by U. S.

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http://dx.doi.org/10.1016/j.biomaterials.2017.02.018
0142-9612/

Food and Drug Administration (FDA) for clinical trials, such as "Cornell dots" [12]. The large mesopores of MSNs provide sufficient reservoirs for guest drug molecules [13], guaranteeing their applications in drug/gene delivery [14–24], molecular imaging [15–32] and even tissue engineering [33–35]. However, the biocompatibility and biodegradability of MSNs are still under debate due to their low biodegradability rate, causing the potential accumulation risk when they are used as intravenously injected drug carriers frequently. We expect that the organic-inorganic hybridization within the silica framework would result in much improved biocompatibility and biodegradation behavior to traditional MSNs, and bring with new functionalities to a certain extent where directly incorporating organic groups into silica framework may be effective.

The concept of direct incorporation of organic groups into mesoporous silica is not new, which has been well known as periodic mesoporous organosilicas (PMOs) [6,36–48]. However, the construction of PMOs with well-regulated key structural parameters for satisfying the strict demands of clinical application is greatly challenging. For instance, it has not been fully realized to synthesize PMOs smaller than 50 nm in particle diameter, which is critical to guarantee the high passive-tumor accumulation by enhanced permeability and retention (EPR) effect. Various organic groups, such as $-CH_2-CH_2-$ [14],  [49], and −CH═CH− [50,51], have been successfully incorporated into the framework of MSNs. However, these organic groups are physiologically inert and not beneficial for biological application. For instance, the −CH═CH− hybridization within MSNs' framework unfortunately slowed down the biodegradation rates compared to pure MSNs [52]. To fabricate PMOs with small-enough particle size, uniform morphology, monodispersity and enhanced biocompatibility/biodegradability, we herein report on a new and versatile synthetic strategy to fabricate small particle-sized (30–40 nm) hollow mesoporous organosilica nanocapsules (designated as HMONs) with specific molecularly disulfide (−S−S−) bonded hybridized framework. The hollow nanostructure was constructed to provide a large room for guest drug molecule encapsulations for enhanced drug-loading capacity of HMONs [53–56]. Therefore, this kind of carrier will enable largely reduced carrier amount used for sufficient drug doses. In addition, the disulfide bond has been extensively demonstrated to be highly active in physiological conditions, especially in reducing microenvironment of tumor tissues [57]. It has been demonstrated that the introduction of disulfide bonds into the silica framework could improve the biodegradation of organosilica nanosystems [14,57–60], which could also realize the controlled release of anticancer drugs or proteins. Disulfide bond-bridged MONs with either rod or spherical morphologies have been demonstrated to be biodegradable [57,58], but their relatively large particle sizes unfavor their further *in vivo* applications. Protein-incorporated organosilica nanoparticles were recently synthesized for intracellular protein delivery [59], but these nanoparticles had no nanoporous structure. In addition, all these reported MONs and solid orgnaosilica nanoparticles possessed no hollow nanostructure [57–59]. We recently also demonstrated the successful fabrication of large pore-sized MONs for intranuclear gene delivery [14]. The intringing performance of molecular organic-inorganic dislufide-bond hybridization encourages us to further optimize the nanostructure (hollow nanostructure) and key parameters (sub-50 nm particle size) for *in vivo* evaluations and applications.

In this work, the biodegradation behavior and the corresponding biodegradation mechanism of as-synthesized HMONs with disulfide bond-hybridized silsesquioxane framework will be investigated in details, for the first time as far as we know. Furthermore, the high drug-loading capacity, reducing microenvironment-trigged drug delivery and high *in vitro* and *in vivo* drug-delivery outcomes based on HMONs will be demonstrated systematically. It is highly expected that these HMONs with physiologically active-group hybridization are potentially promising for clinical translations in combating cancer.

2. Experimental

2.1. Materials

Tetrathylorthosilicate (TEOS), triethanolamine (TEA) and ammonia solution (25–28%) were bought from Sinopharm Chemical Reagent Co. Bis(3-triethoxysilylproyl) disulfide (BTDS) was obtained from Lark Chemical Technology Co., LTD. Phosphate buffer solution (PBS) was purchased from Shanghai Ruicheng Bio-Tech Co., LTD. Doxorubicin (DOX) was purchased from Beijing HuaFeng United Technology Co., LTD. Glutathione (GSH) and cetanecyltrimethyl cmmonium chloride (CTAC) were obtained from Sigma-Aldrich. Methoxy PEG silane (M-SLN-2000) was purchased from Jenkem Technology Co., LTD. Lyso-Tracker Red was bought from Beyotime Biotechnology. Deionized water was used in all experiments. All chemicals were used without other treatments.

2.2. Cell culture

Breast cancer line 4T1 cells were cultured in DMEM culture medium containing 10% fetal bovine serum (FBS, Ruicheng Bio-Tech Co., LTD., Shanghai), 100 units/mL penicillin and 100 μg/mL streptomycin. The cells were incubated at 37 °C in a 5% CO_2 in air atmosphere. For subculturing and harvesting cells, 0.25% trypsin (Ruicheng Bio-Tech Co., LTD., Shanghai) was used in all experiments.

2.3. Animals

Healthy female Kunming mice (~20 g) and 4-week old female BALB/c nude mice (~15 g) were obtained from Shanghai Experimental Animal Center (Shanghai). All animal experiments were conducted under the guideline approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.4. Synthesis of small particle-sized HMONs

The mesoporous silica NPs were initially synthesized using the conventional sol-gel process. Then, the organic-inorganic hybrid layer was coated onto the surface of MSNs. Finally, ammonia solution was used as an etching agent to etch away the inner core. Typically, CTAC aqueous solution (20 g, 10 wt%) and TEA solution (0.8 g, 10 wt%) were pre-mixed at 95 °C, then TEOS (1 mL) was added dropwise. MSNs were obtained after reaction for 1 h. The outer MONs' layer was coated by further adding a mixture of TEOS and BTDS for a further 4 h reaction. The white product was then collected by centrifugation and washed with ethanol for three times, followed by removing the CTAC soft templates *via* extraction in ethanol and concentrated HCl (37%) mixture ($V_{ethanol}$: V_{HCl} = 10: 1) at 78 °C for 12 h. The extraction was repeated for three times, and the product was then washed with ethanol for three times to obtain MONs@SiO_2. For etching process, MONs@SiO_2 solution (1 mL), which was initially dispersed in water (20 mL) to keep the colloidal solution, was taken out, followed by adding ammonia solution (0.4 mL). The final HMONs were formed after etching at 95 °C for 3 h.