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Mesoporous Silica and Composite Nanostructures for Theranostics

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We discus methods for fabrication of silica and composite nanoparticles, which can be used in various biomedical applications. The most promising types of such nanostructures are hollow silica nanoshers, silica coated plasmon-resonant nanoparticles (gold nanorods and gold-silver nanocages) and nanorattles. Mesoporous silica shell can be doped by desirable targeting molecules. Here we present the results of formation of nanocomposites composed of gold nanorods and double-layer silica shell. The secondary mesoporous silica shell is doped with a photosensitizer (hematoporphyrine in our case). We demonstate some of promising theranostics applications of these nanocomposites for bioimaging and in vivo therapy of tumors.

Keywords: Mesoporous silica, Nanoparticles, Nanocomposites, Theranostics, Photodynamic therapy

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1. INTRODUCTION

Multifunctional nanocomposites that combine therapeutic, diagnostic, and sensing modalities in a single nanostructure are widely used in a new field of nanobiotechnology called theranostics [1-4]. Although the term “theranostics” has been employed for the first time quite recently [5], it is now rapidly growing and promising field at the crossroads of plasmonics and nanomedicine [6,7].

Silica is widely used for synthesis of nanoparticles and multifunctional nanocomposites owing to its biocompatibility and facility of preparation [8]. Amorphous silica is used, for example, as a core for nanoshells [9], whereas its mesoporous counterpart is also widely used owing to high volume of pores, which can serve as effective carriers in drug delivery applications. Nowadays mesoporous silica nanoparticles are replaced by more complex structures like hollow silica nanoparticles and nanocomposites, which can support several modalities in one nanoparticle. For example, such modalities can include plasmonic heat generation, visible and IR luminescence, and photodynamic generation of singlet oxygen [10]. Here we describe several types of silica-based nanostructures composed of plasmonic particles and double-layer silica shell. Such multifunctional nanocomposites can be used in promising theranostics applications [11] including bioimaging [12] and combined photodynamic (PD) and photothermal (PT) therapy of tumors [13].

2. FABRICATION OF HOLLOW SILICA SPHERES AND NANOCOMPOSITES

2.1 Hollow Silica Nanoparticles

The first step in fabrication of hollow silica nanoparticles is a formation of silica nanospheres using Stober method [14] (Fig. 1, a). According to the protocol by Fang et al. [15], silica nanoparticles were coated by cetyltrimethylammonium bromide (CTAB) and etched in alkaline conditions (Fig. 1, b, c). Two strategies of etching [15] lead to formation of various types of structures with worm-like (Fig. 1, b) and oriented pores in a silica shell (Fig. 1, c). Typically, the outer diameter of particles can be tuned within the range from 50 nm to 1200 nm. The shell thickness also can be varied from 15 nm to 70 nm.

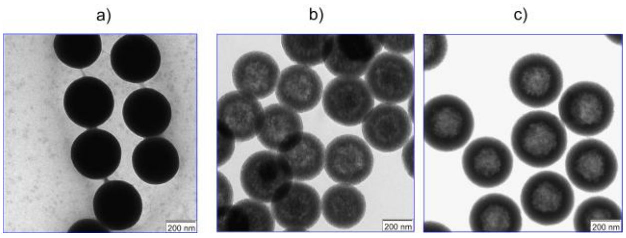

Fig. 1 – (a) Silica nanospheres formed by Stober method.(b) Hollow silica spheres obtained by alkaline etching using CTAB surface protection (c) Hollow silica spheres obtained by two-stages protocol including secondary mesoporous shell synthesis and alkaline etching. All scale bars are 200 nm

2.2 Nanocomposites Containing Plasmonic Nanoparticles Within Silica Shell

As a core of nanocomposites we used gold nanorods (NRs) and gold-silver nanocages (NCGs), which were coated by amorphous silica shell using modified Stober method [16]. The next step was coating of these nanoparticles by a mesoporous silica shell, containing fluorescent dye and photosensitizer hematoporphyrine [17] (Fig. 2 a). Incorporation of hematoporphyrine (HP) into silica shell was achieved by preliminary formation of HP-containing silane precursor, which was then added to the reaction medium. Amorphous transitional silica

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layer was necessary for prevention of electron transfer between dye molecules and metal surface, which could result in the fluorescence quenching.

In short, gold nanorods were synthesized by a seed-mediated growth method [18] with an original modifications described in our paper [19]. Gold-silver nanocages were synthesized by a two step protocol [20] modified in our paper [21]. The first step is a synthesis of siver nanocubes and the second step is a synthesis of gold-silver nanocages by the galvanic replacement reaction [22].

In a typical synthesis, AuNRs had an average length of 44 ± 7 nm and an average width of 11 ± 1 nm. The plasmon resonance wavelength was located near 810 nm. The fabricated Au-Ag NCGs had an average edge length of about 50 ± 5 nm and a plasmon resonance peak near 750-800 nm, depending on the Ag/Au conversion ratio.

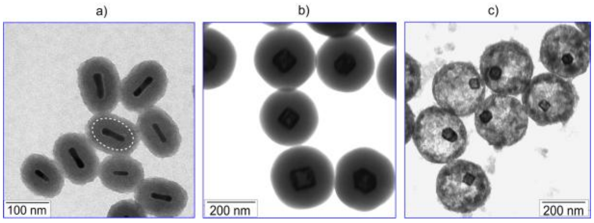

Fig. 2 – (a) Nanocomposites containing gold nanorods. White dotted line showed the boundary between primary amorphous silica shell and secondary mesoporous shell containing hematoporphyrine. (b) Gold-silver nanocages coated by amorphous silica shells. (c) Nanorattles, obtained by etching of thick silica shells fabricated on small gold-silver nanocages

2.3 Nanorattles

One of recently developed types of silica-based composite nanoparticles is so-called "nanorattles" [23], comprising a metal core, which can freely move inside the hollow silica shells. The strategy of their formation is similar to the method of fabrication of hollow silica nanoparticles.

In this work we fabricated nanorattles comprising gold-silver nanocages (Fig. 2, c). Silica etching was performed in alkaline conditions with poly(vinyl pyrrolidone) (PVP) for surface protection [23].

3. SILICA AND COMPOSITE NANOPARTICLES IN THERANOSTIC APPLICATIONS

Nanocomposites containing gold nanorods and hematoporphyrine in the secondary silica shell (Fig. 2a) can be used in bioimaging due to intense fluorescence of hematoporphyrine molecules. (Fig. 3b).

Straigtforward evidence for the successful functionalization of composite particles with HP is provided by the photo in the figure 3. Shown here are the cuvettes containing composite particles with attached HP (NCs), gold nanorods without HP (NRs) and free HP solution (HP). Under white light illumination, the first two cuvettes demonstrate a red color because of selective absorption of visible light near 515 nm and a third cuvette with HP solution is transparent. When irradiated with a UV lamp, the second and third cuvettes exhibit intense pink fluorescent emission, whereas the first cuvette retains nonfluorescent.

Such nanocomposites combine the PT and PD modalities can be used not only for antimicrobial treatment [17], but also for a combined therapy of cancer. We successively used these nanocomposites for *in vivo* therapeutics of cancer tumors transplanted into rats.

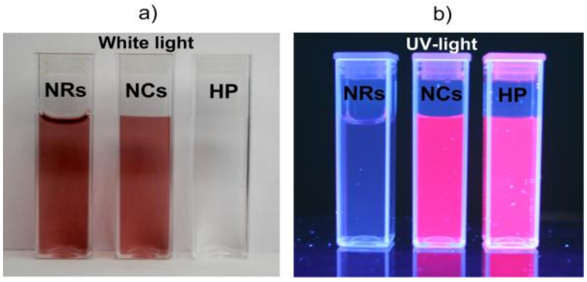

Fig. 3 – Suspensions of gold nanorods (NRs), nanocomosites containing gold nanorods, amorphous silica shell, and mesoporous silica shell with hematoporphyrine (NCs), and free hematoporphyrine solution (HP), irradiated by white light (a) and UV-light (b)

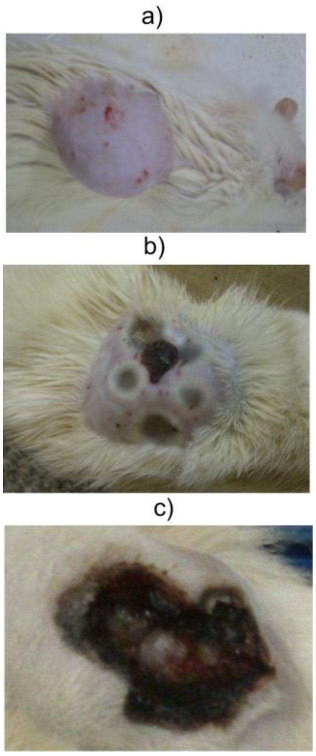

Fig. 4 – Images of (a) a tumor with volume of about 10 cm^3 before irradiation, (b) after 30 min of irradiation, and (c) after 3 h of irradiation. Combined PDT and PTT under 630 nm (0,86 mW/cm^2) and 810 nm (2,3 W/cm^2) irradiation

The rat liver cancer cells PC-1 (kindly provided by Blokhin Cancer Research Center, Moscow) were intramuscularly injected in rats, and tumors were allowed to grow up to volume of 10 cm^3. After intratumoral injection of suspension of nanocomposites into tumors, they we treated for skin clearance [24] followed by irradiation simultaneously by two lasers with wavelengths of 808 nm (2.3 W/cm^2, for photothermal therapy) and 630 nm (0.86 mW/cm^2, for photodynamic therapy).

Our results showed that combined usage of photodynamic and photothermal therapy is much more effective than only one of them. Figure 4 shows images of a tumor with volume of about 10 cm3 grafted on the back of the rat before irradiation (Fig.4, a) and 30 min (Fig. 4b) and 3 h (Fig. 4c) after.

4. CONCLUSIONS

In this work we have demonstrated the capability of synthesis of silica-based nanoparticles with various structures, such as amorphous, mesoporous and hollow. We have also synthesized nanocomposites with core-shell, core-double layered shell, and rattle-type structures.

Nanocomposites with a gold nanorod core and a photosensitizer HP-doped silica shell have been successfully fabricated. The visible fluorescence of bound HP molecules was not quenched due to metal-PS interaction because of separation from the nanorod core by amorphous silica shell. Under UV excitation, the nanocomposites reveal quite intense fluorescent emission of light that is visible by naked eye. These nanocomposites have been successively used in combined PT and PD therapy of grafted cancer tumors in rats in vivo. Thus, fabricated composites may be effectively used for theranostics of cancer deceases.

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