Study of Protoporphyrin IX-loaded silica hollow spheres for photodynamic therapy

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ABSTRACT

The PpIX-loaded silica hollow spheres were fabricated using DMDES (dimethyldiethoxysilane) which could be transformed into PDMS (polydimethylsiloxane) templates [1], and TEOS (tetraethoxysilane) as crosslinker. The morphology of the resulting samples was observed using transmission electron microscopy (TEM), and a hollow structure was clearly observed. The PpIX-containing silica structure was confirmed using fourier-transform infrared spectroscopy (FTIR). The FTIR peak around 1030 cm^{-1} was assigned to the characteristic peak of silica, indicated the shell was formed by silica. The singlet oxygen generation ability of the sample was confirmed using UV-Visible (UV-VIS) spectroscopy and singlet oxygen probe (DPA, diphenyl anthracene). After combined with singlet oxygen, the photo absorption of DPA at 376 nm would decrease. The resulting UV-VIS spectrum suggested that PpIX loaded in silica hollow spheres still generated singlet oxygen after UV irradiation [2].

Keywords: Protoporphyrin IX, photodynamic therapy, silica hollow sphere

1 INTRODUCTION

Photodynamic therapy (PDT) is an effective way for curing cancer, which combined two factors: a light excitation source and a photosensitizer (PS). It produces little or no cytotoxicity without light excitation, so the tumor cell-specific killing could be achieved by control the location and intensity of light irradiation. The tumor cell-killing ability of PDT was resulted from reactive oxygen species (ROS) produced by PSs upon the irradiation of light at particular wavelength. Among all kinds of ROS, singlet oxygen was believed to be the main specie that caused photo-cytotoxicity. Thus, the ability of generating singlet oxygen was a very important consideration for the agents used for PDT [3, 4].

Protoporphyrin IX (PpIX), one of the components of hemoglobin, was a naturally-occurring porphyrin derivative. PpIX could produce several kinds of ROS, including singlet oxygen upon the excitation of ultraviolet (UV) light. However, as many PSs, porphyrin derivatives were hydrophobic and tend to form clusters, which might cause blockage of blood capillaries. PSs might also induce mild cytotoxicity after direct contact with cell organelles [4-6].

In order to avoid these disadvantages, the concept of delivering PSs via a biocompatible drug carrier has been proposed in recent years [7, 8]. In this way, dark (i.e., without photo-irradiation) cytotoxicity in physiological environment could be avoided. For this purpose, silica was chosen as the material of drug carrier for PS delivery.

Silica demonstrated several characteristics which are advantageous to be a carrier material for PSs. First, it is relative transparent, thus light can penetrate and activate the PSs loaded in the shell or inside the carrier [9, 10]. Second, it's non-degradable, so the PpIX loaded in silica would not be released into the surrounding environment and cause dark cytotoxicity. Third, the biocompatibility of silica was good, and it would not have harmful effects to normal tissues. In this study, PpIX was loaded in the shell of silica hollow spheres with the expectation that PpIX-loaded silica hollow spheres (PpIX-SHS) might be employed as a PDT agent with ultrasound image contrast [9].

2 EXPERIMENTAL DETAILS

2.1 Materials

Tetraethoxysilane (TEOS), aqua ammonia (NH_4OH) Protoporphyrin IX (PpIX), Dimethyl sulfoxide (DMSO), 3-aminopropyl triethoxysilane (APTES), Ethanol (>99%), dimethyldiethoxysilane (DMDES), Diphenyl Anthracene (DPA).

2.2 Fabrication of silica hollow spheres

Silica hollow spheres were prepared following a previous study by Zoldesi et al using the monomer DMDES to form a initial template [1]. Briefly, DMDES was added to a diluted ammonium solution and magnetically stirred for a few minutes. Next, the solution was placed under room temperature overnight without stirring. DMDES in the ammonium solution would gradually transformed into PDMS during this process. TEOS was then added into the solution in order to crosslink the surface of PDMS templates. The outer surface of the PDMS template would grow as the degree of crosslinking increased. After the addition of TEOS, the solution was washed by ethanol for several times in order to remove the unnecessary PDMS in the shell and solution. Finally, the product was collected using the method of centrifugation.

2.3 Fabrication of PpIX-loaded silica hollow spheres

The PpIX-loaded silica hollow spheres were fabricated used a modified process of fabricating silica hollow spheres which mentioned in section 2.2. First, the PDMS templates were fabricated and TEOS was added to fabricate silica hollow spheres. Second, PpIX and APTES was then added into the above solution right after the addition of TEOS [1, 11]. The PpIX-loaded silica hollow spheres could be obtained after several hours of magneric stirring.

2.4 Singlet oxygen generation test

The singlet oxygen generation ability of the sample was confirmed using UV-Visible spectroscopy and singlet oxygen probe (DPA, diphenyl anthracene). DPA was a chemical reagent which could react with singlet oxygen and exhibited different UV absorbance before and after reaction with singlet oxygen. Since the photosensitizer Protoporphyrin IX used in this study had a light absorption peak at ultraviolet (UV) range, the light excitation source of singlet oxygen generation test would be a UV light source. PpIX-loaded silica hollow spheres were mixed with DPA before UV-irradiation, and the UV-Visible spectrum was obtained before and right after the irradiation of UV light. Absorption difference at 376 nm would be checked to confirm the singlet oxygen generation ability of PpIX-SHS. [2, 12, 13]

3 RESULTS AND DISCUSSION

The primary goal of this study was to prepare Protoporphyrin IX-loaded silica hollow spheres for photodynamic therapy. The morphology of silica hollow spheres and Protoporphyrin IX-loaded silica hollow spheres were observed by transmission electron microscope (TEM), as shown in Figure 1a and b, respectively. Spherical silica spheres with a hollow structure were clearly observed. Figure 1b shown black small particles attached to the surface of silica hollow spheres compared to Figure 1a. It was speculated that the black small particles were Protoporphyrin IX encapsulated by APTES and then attached to silica surface because of high affinity between silica and APTES. This speculation was confirmed by UV-excited fluorescence test. In Figure 2a, Protoporphyrin IX dissolved in DMSO shown visible red fluorescent light under UV irradiation. While Protoporphyrin IX could not be precipitated by the method of centrifugation in the solvent DMSO, silica hollow spheres and Protoporphyrin IX-loaded silica hollow spheres could be easily precipitated after centrifugation. In Figure 2b,centrifugation-precipitated Protoporphyrin IX-loaded silica hollow spheres shown visible red fluorescent light under UV irradiation, while supernatant shown no visible fluorescence. This result proved that the Protoporphyrin IX was binded to silica hollow spheres. The composition of Protoporphyrin IX-loaded silica hollow spheres were confirmed by fourier transform infrared spectroscopy (FTIR). As shown in Figure 3, a peak around 1000 cm^{-1} was assigned to silica, indicated that the shell was composed of biocompatible material silica. Finally, the singlet oxygen generation ability was confirmed by the method explained in section 2.4. In Figure 4, the UV-visible spectrum of DPA/PpIX-SHS mixture after UV irradiation shown significant decrease at 376 nm compared to the UV-visible spectrum of pure DPA after UV irradiation. This result suggested that Protoporphyrin IX could still be triggered and generated singlet oxygen in the silica shell by external UV excitation.

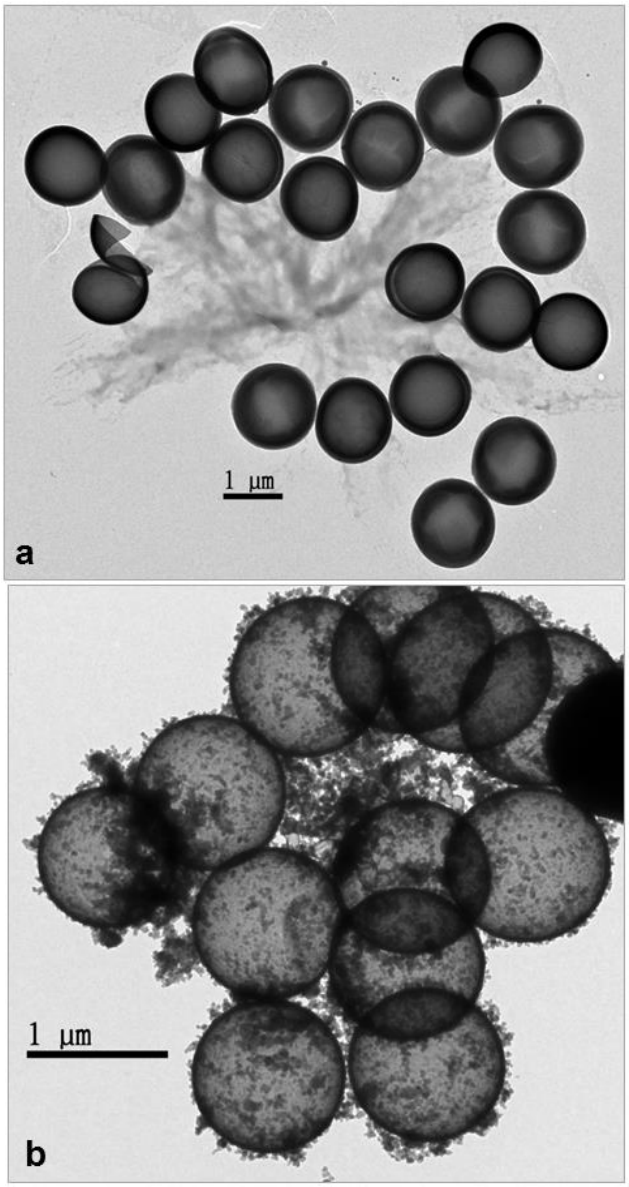

Figure 1: TEM images of (a) silica hollow spheres and (b) PpIX-loaded silica hollow spheres.

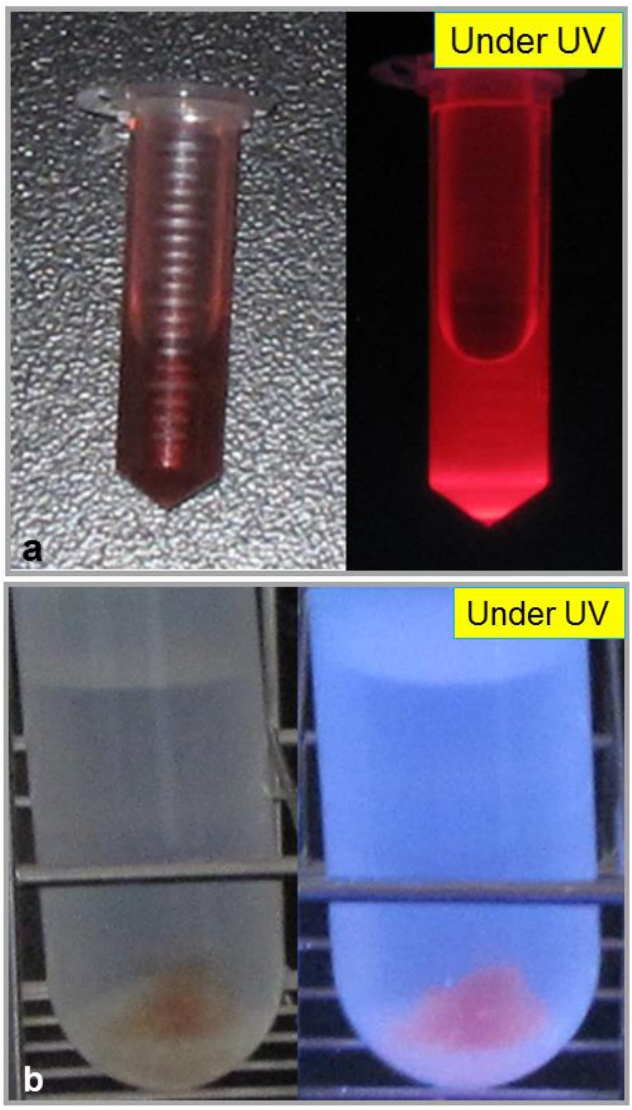

Figure 2: UV-excited fluorescence of (a) PpIX in DMSO, and (b) PpIX-loaded silica hollow spheres in DMSO.

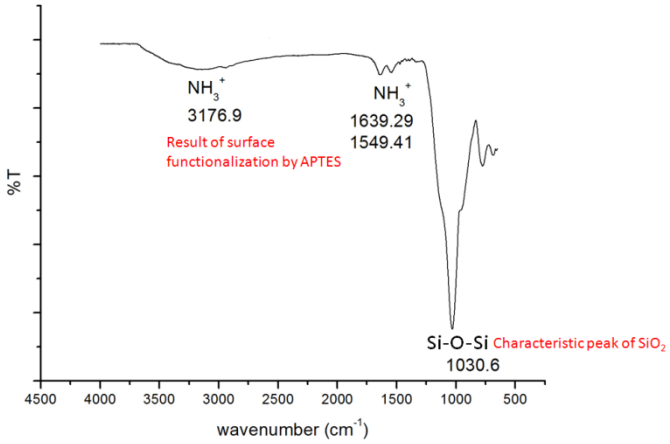

Figure 3: FTIR spectrum of PpIX-loaded silica hollow spheres.

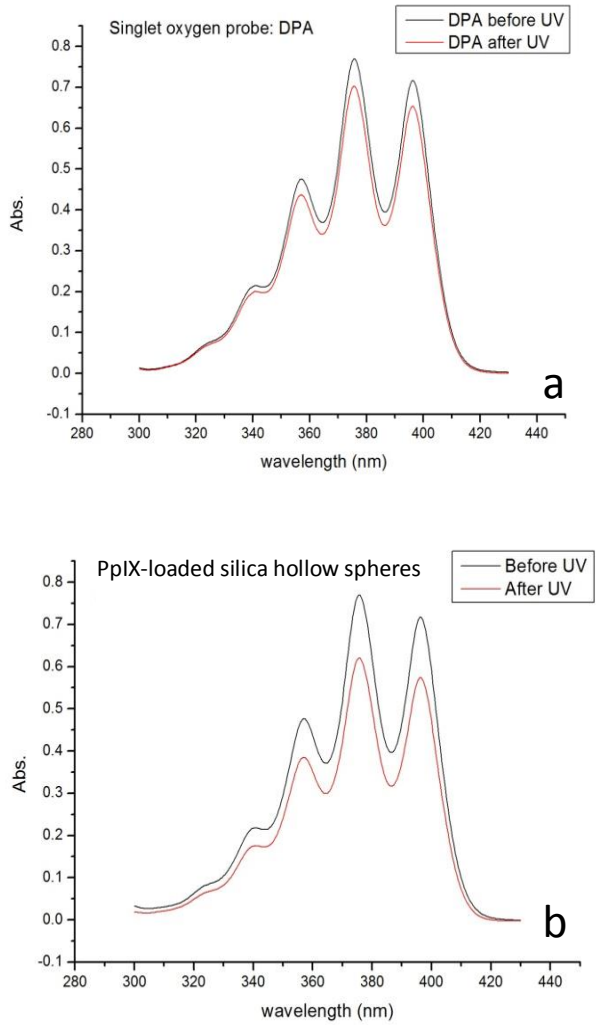

Figure 4: The UV-Visible spectrum of (a) singlet oxygen probe DPA and (b) DPA mixed with PpIX-loaded silica hollow spheres. (Black line: before UV irradiation, red line: after 10 minutes of UV irradiation.)

4 CONCLUSION

Silica is a biocompatible material which could be dispersed in aqueous enviroment. Combined silica and photosensitizers together for photodynamic therapy was expected to reduce the dark cytotoxicity and aggregation of photosensitizers in aqueous enviroment. Additionally, silica carriers would not block light penetration, so the Protoporphyrin IX loaded in the shell could be activated and generated singlet oxygen by the incident light. In conclusion, Protoporphyrin IX-loaded silica hollow spheres fabricated in this study has the potential to be applied as a safer and more efficient photodynamic therapy agent.

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