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Poly(amidoamine) dendrimer-grafted porous hollow silica nanoparticles for enhanced intracellular photodynamic therapy

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ABSTRACT

We report a novel photodynamic therapy (PDT) drug-carrier system, whereby third-generation (G3) polyamidoamine (PAMAM) was successfully grafted to the surface of porous hollow silica nanoparticles (PHSNPs), followed by the attachment of gluconic acid (GA) for surface charge tuning. The composite G3-PAMAM-grafted PHSNPs (denoted as G3-PHSNPs) with a diameter range of 100–200 nm and about 30 nm sized shell thickness retain bimodal pore structures (e.g. inner voids and porous structure of the shells) and PAMAM-functionalized outer layer with a large number of amino groups, allowing high loading efficacy of aluminum phthalocyanine tetrasulfonate ($AlPcS_4$) and its effective release to target tissue. The GA-induced G3-PHSNPs were evidenced to be able to favorably cross tumor cell walls and enter into the cell interior. The generation of singlet oxygen ($^1O_2$) from $AlPcS_4$-GA-G3-PHSNPs under visible light excitation was detected by the in situ electron spin resonance measurements and the oxidative reaction between the generated $^1O_2$ and a chemical probe. In vitro cellular experiments showed that the photosensitive GA-G3-PHSNPs exhibited a good biocompatibility in the dark and a higher killing efficacy against MCF-7 tumor cells upon irradiation as compared with free $AlPcS_4$, which implies that the preformed photosensitive drug-carrier system might be potentially applicable in PDT.

1. Introduction

Photodynamic therapy (PDT) has emerged as an important treatment modality for a variety of cancers in recent years [1]. PDT is based on the concept that certain therapeutic molecules called photosensitizers (PSs) are activated by light of appropriate wavelength, and then pass on their excess energy to nearby molecular oxygen to form reactive oxygen species such as singlet oxygen ($^1O_2$) and free radicals, which are toxic to cells and tissues. However, the clinical use of many PSs has been hampered by significant side effects, including prolonged photosensitivity and nonspecific damage to normal tissues due to low accumulation selectivity to specific cells or tissues, environmental degradation and hydrophobicity [2]. In this respect, biocompatible colloidal carriers for delivering PSs, such as liposomes [3], polymeric micelles [4] and nanoparticles [5,6], have been developed. These vehicles offer benefits of appropriate size for passive targeting to malignant tissues by enhanced permeability and retention effect, stable aqueous dispersion and stability by using surface modification, and protection of the PSs from environmental degradation [7]. Among these carriers, inorganic nanoparticles, especially porous silica nanoparticles with large surface area, tunable pore volume and uniform pore size, have been given more attention than others in PDT due to their lower biodegradability, which protects the PSs from degradation in systemic circulation and enables the repeated activation of PSs [8–10]. Furthermore, some studies have also shown less significant short-term cytotoxicity of porous silica nanoparticles [11]. More recent research achievements have demonstrated the potential of using silica-based nanoparticles as carriers for PDT [12]. Several successful examples of silica-based nanoparticles for one- [13] and two-photon [14] PDT applications have been described, in which the PS was physically entrapped inside the silica network or covalently attached to the carrier surface or interior. The physical encapsulation usually leads to a premature release of the PS from the carrier, and thus to reduced efficiency of treatment and to side effects, though covalent coupling can overcome these adverse issues. However, not all PS molecules can be incorporated into the silica matrix in the form of covalent bonds via a simple preparation procedure. Furthermore, in some cases, the stability of the particulate system via PS coupling will also be affected, resulting in a drop in the main cytotoxic agent $^1O_2$ productivity. Thus, the search is still on to develop an ideal silica-based drug delivery vehicle that can reduce the premature release of PS molecules from the carrier vehicle, and at the same time maintain the stability of this material system and the positive features of porous silica, with regard to its use in PDT.

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Poly(amidoamine) (PAMAM) dendrimers are nanoscale and perfect monodisperse macromolecules with a regular and hyperbranched three-dimensional architecture. PAMAM dendrimer conjugates have been used for delivering drugs [15], DNA [16], radionucleides [17], MRI contrast agents [18] and boron for neutron capture therapy [19]. They have also been used for PDT applications [20]. Dendrimers contain a large number of terminal functional groups with positive charge, which can not only provide spatial distribution of biospecific moieties, but also maintain superior dispersion stability even under physiological conditions. These features of dendrimers may indeed be considered as favorable factors to expand the functionality of porous silica particles for application in PDT. However, to date, dendrimer-grafted porous silica particles for PDT have not been yet reported.

In our previous work, we have fabricated novel porous hollow silica nanoparticles (PHSNPs) with adjustable particle size and shell thickness, high surface area and spacious inner voids, and demonstrated their potential as carriers for immobilization of enzyme and other drugs [21–24]. In the present study, motivated by the positive features of porous silica and dendrimer, we develop a new PDT drug-carrier system by grafting third-generation (G3) PAMAM to the surface of PHSNPs. The resulting G3-PAMAM-grafted PHSNPs (G3-PHSNPs) were characterized by various techniques including SEM, TEM, ^{13}C NMR, FTIR and zeta-potential measurements. One little trick is to anchor gluconic acid (GA) to G3-PAMAM-grafted PHSNPs to tune the surface charge close to neutral, which helps to prolong the circulation time [25]. The GA-modified G3-PHSNPs were then verified to be avidly uptaken by tumor cells in vitro. Aluminum phthalocyanine tetrasulfonate ($AlPcS_4$) with a cyclic tetrapyrrole structure and four sulfonic acid groups is water soluble and may be considered to be a promising second-generation photosensitizer as it shows excellent photodynamic activity [26]. Its principle advantage over Photofrin® or acid-mediated protoporphyrin, the sensitizers most used to date, lies in its stronger absorption at longer wavelengths of near 700 nm and thus allows deeper light penetration of tissue. Herein, using $AlPcS_4$ as a photosensitizer, the generation of $^1O_2$ by the $AlPcS_4$-GA-G3-PHSNPs on exposure to light of appropriate wavelength was determined by electron spin resonance (ESR) spectroscopy and a chemical approach. In addition, the phototoxic effects of photosensitive particles and free $AlPcS_4$ on the human breast adenocarcinoma (MCF-7) cells by irradiation were studied, and cell viability was estimated in 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium-bromide (MTT) assays. As a result, such hybrid photosensitive material is demonstrated to have potential as a carrier for intracellular PDT (see Fig. 1). The preparation procedure of the photosensitive silica material and its mechanism of killing tumor cells in PDT are shown in Fig. 1.

2. Experimental

2.1. Materials

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), MTT, 9,10-anthracenediyl-bis(methylene)-dimalonic acid (ABDA), 3-aminopropyltriethoxy silane (APTS) and fluorescein isothiocyanate (FITC) were purchased from Sigma Aldrich. Methyl acrylate (MA), ethylenediamine, gluconic acid (GA) and dimethyl sulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent, PR China. High-glucose Dulbecco's Modified Eagle's Medium (DMEM), 0.25% (w/v) trypsin–0.03% (w/v) ethylene diamine tetraacetic acid (EDTA) solution, and phosphate-buffered saline (PBS, 0.04 M, pH 7.4) were purchased from Invitrogen Corporation. $AlPcS_4$ was prepared according to the method previously reported in the literature [27]. Human breast adenocarcinoma (MCF-7) cells were from the American Type Culture Collection (ATCC). All chemicals used in the experiment were obtained from commercial sources as analytical reagents without further purification. Millipore water with a resistivity of 18.2 MΩ cm was used throughout the experiment.

2.2. Synthesis of PHSNPs

PHSNPs were fabricated via our previously reported method [28]. Briefly, a certain amount of 8.0 wt.% nanosized $CaCO_3$ (60–80 nm) suspension with surfactant was heated to 353 K under stirring,

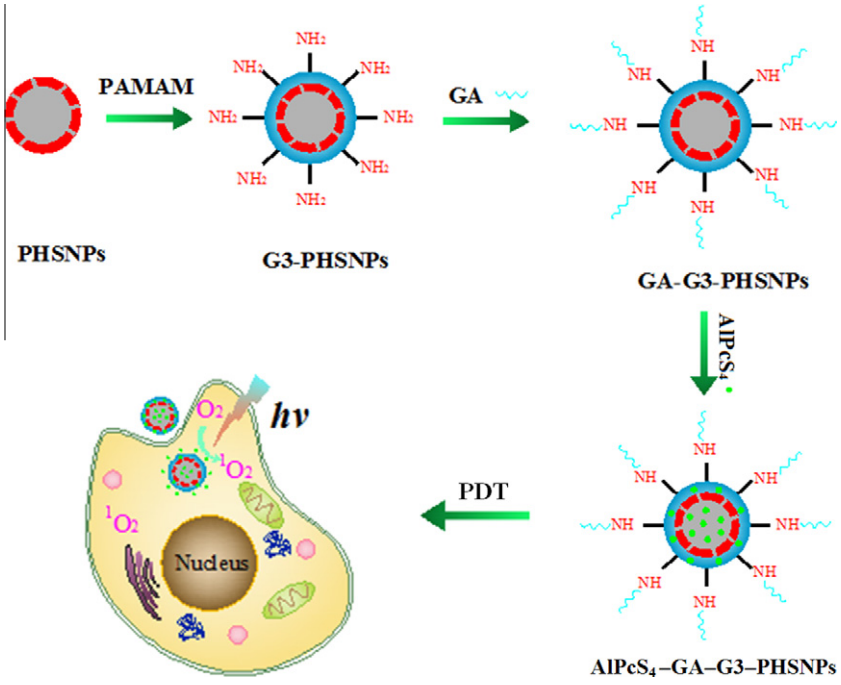

Fig. 1. Schematic representation of the preparation and the cytotoxicity of $^1O_2$ on cancer cells of GA-G3-PHSNPs embedded with an $AlPcS_4$ photosensitizer.

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