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Co-loading of photothermal agents and anticancer drugs into porous silicon nanoparticles with enhanced chemo-photothermal therapeutic efficacy to kill multidrug-resistant cancer cells

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ABSTRACT

The development of nanoparticles-based drug delivery systems with a high therapeutic efficacy is necessary to treat multidrug-resistant (MDR) cancer cells. Herein, photothermal agents (IR820 dyes) and anticacner drugs (doxorubicin, DOX) were successively incorporated into amino-terminated porous silicon nanoparticles (NH_2-PSiNPs) *via* electrostatic attractions, to prepare DOX/IR820/NH_2-PSiNPs nanocomposites with a high loading amount of DOX (13.3%, w/w) and IR820 (18.6%, w/w), respectively. Meanwhile, DOX molecules were also directly loaded into NH_2-PSiNPs to form DOX/NH_2-PSiNPs nanocomposites (DOX, 18.7%, w/w). Compared with low release percentage (20.3%) of DOX molecules from DOX/NH_2-PSiNPs in acidic environments under NIR laser irradiation, DOX/IR820/NH_2-PSiNPs had dual pH/NIR light-triggered release and their release percentage could reach 88.1% under the same conditions. Furthermore, cellular interactions tests demonstrated that DOX/IR820/NH_2-PSiNPs could delivery more DOX molecules into the nuclei of MDR cancer cells, with efficient intracellular release triggered by NIR light, in contrast to DOX/NH_2-PSiNPs. Finally, DOX/IR820/NH_2-PSiNPs exhibited an enhanced chemo-photothermal therapeutic efficacy (cell viability, 38.4%) of killing MDR cancer cells *in vitro*, compared with 85.4% of free DOX and 75.9% of DOX/NH_2-PSiNPs. Therefore, based on PSiNPs-based nanocarriers conjugated with photothermal agents and anticancer drugs, NIR light-triggered drug delivery system with higher efficacy of combined chemo-photothermal therapy would have important potential on MDR cancer treatments in future.

1. Introduction

Until now, multidrug resistance (MDR) that frequently occurs in most cancers still remains a major impediment to successful chemotherapy on clinic [1]. As one of the most important mechanisms of MDR in cancer cell, the overexpression of ATP-binding cassette transporters (ABC) like P-glycoprotein (P-gp) in cell membrane can excrete a broad range of anticancer drugs out of cells, and reduce the accumulation and retention of intracellular drugs, resulting in the failure of chemotherapy in more than 90% of patients [2–4]. To avoid serious side effects or even toxicity caused by simply increasing dosage of anticancer drugs in body, a large amount of studies have been carried out to develop inhibitors of ABC transporters, and restore enough concentration of intracellular drugs for killing MDR cancer cells [5,6]. Considering low efficacy and toxic side effects of P-gp inhibitors on clinic [7,8], drug delivery systems based on nanocarriers have been designed as an innovative strategy to reverse MDR in cancer cells through bypassing P-gp efflux pump. These nanocarriers (*e.g.*, micelles, vesicles, or inorganic nanoparticles, *etc.*) could efficiently delivery anticancer drugs into cells *via* endocytosis independent from P-gp pathway [4,9–12]. Among various nanoparticles-based drug delivery systems, photothermal nanocarriers containing near-infrared (NIR) cyanine dyes, polydopamine, gold nanoparticles, or upconversion nanoparticles exhibited competitive advantages in overcoming MDR cancer, due to their synergistic anticancer activity including chemotherapy and photothermal therapy [13–19]. These photothermal nanoparticles can transduce NIR light into hyperthermia, which can further induce P-gp denaturation in cell membrane, inhibit DNA repair of intracellular nuclei, and even directly lead to the necrosis of MDR cancer cells [20]. Besides, hyperthermia can also improve cellular internalization of nanoparticles with increas-

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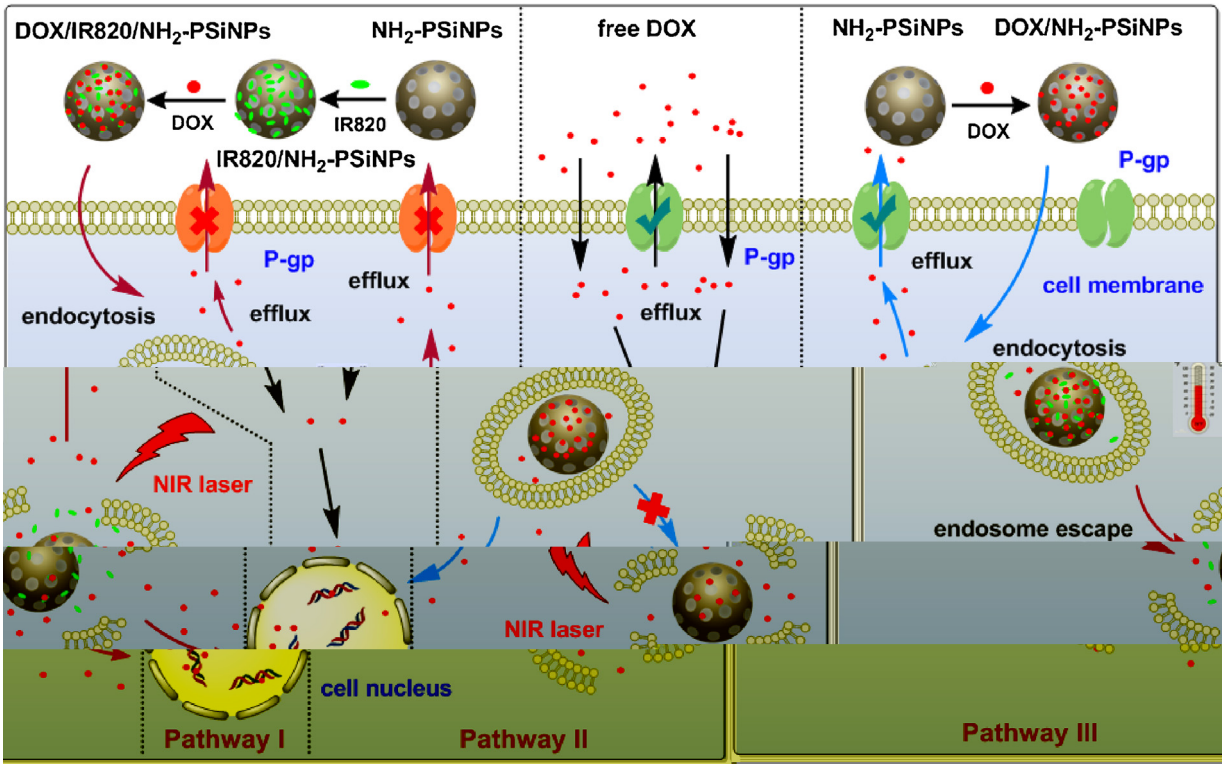

Fig. 1. Schematic fabrication process of PSiNPs-based nanocomposites, and the pathway of intracellular DOX molecules entering nuclei of MDR cancer cells including free DOX (I), DOX/NH_2-PSiNPs (II), and DOX/IR820/NH_2-PSiNPs (III).

ing the permeability and fluidity of cell membrane, facilitate the escape of anticancer drugs from endosome into cytoplasm, and eventually enhance the accumulation of anticancer drugs localized in intracellular nuclei of MDR cancer cells [21,22]. Therefore, the construction of nanoparticles-based drug delivery systems integrated with distinguish photothermal therapeutic features is highly desired for treating MDR cancer in future.

As a multifunctional nanoplatform with tunable photoluminescence (PL), tailored surface functionalization, versatile loading capability, excellent biodegradability and biocompatibility, porous silicon nanoparticles (PSiNPs) have exhibited potential applications on cancer theranostics, such as tumor imaging, chemotherapy, photodynamic therapy, gene therapy and immunotherapy [23–32]. Especially, the progress about chemo-photothermal therapy based on PSiNPs or PSiNPs-based nanocomposites had been reported in these references [33–35], which showed that their therapeutic efficacy was similar with that based on traditional photothermal gold (or carbon)-based nanocarriers [36–38]. Moreover, one of our latest studies also showed NIR light-triggered release of anticancer drugs (doxorubicin, DOX) from PSiNPs conjugated with IR820 dyes in drug-sensitive cancer cells [39]. However, it still remained unknown whether this PSiNPs-based delivery system was effective for killing drug-resistant cancer cells. Herein, it was further employed to investigate its anticancer activity for MDR cancer cells *in vitro*. First, PSiNPs were aminosilanized under microwave irradiation to fabricate amino-terminated PSiNPs (NH_2-PSiNPs) with positive surface charges. DOX/IR820/NH_2-PSiNPs or DOX/NH_2-PSiNPs nanocomposites were prepared by electrostatic assembly of NH_2-PSiNPs, IR820 dyes and DOX molecules, which was shown in Fig. 1. And then DOX release from DOX/IR820/NH_2-PSiNPs or DOX/NH_2-PSiNPs nanocomposites was assessed under different conditions such as the changing of pH value and NIR laser, respectively. Furthermore, after cellular uptake, intracellular release and nuclei distribution of DOX molecules from PSiNPs-based nanocomposites in drug-sensitive (MCF-7) or drug-resistant cancer cells (MCF-7/ADR) was monitored by confocal imaging and flow cytometry, respectively. Finally, the viability of MCF-7 or MCF-7/ADR cells incubated with free DOX, DOX/IR820/NH_2-PSiNPs or DOX/NH_2-PSiNPs was quantitatively analyzed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) staining under different conditions.

2. Experimental

2.1. Preparation and characterization of PSiNPs-based nanocomposites

Single-crystal p^+-type silicon wafers (8–10 Ω cm resistivity, Hefei Kejing Materials Technology Co. Ltd., China) were boiled in 3:1 (v/v) concentrated H_2SO_4/30% H_2O_2 for 30 min and repeatedly rinsed with deionized (DI) water (≥18 MΩ cm resistivity, Millipore). To prepare porous silicon, these clean silicon wafers were incubated in 40% HF/ethanol electrolyte (1:1, v/v), and then were electrochemically etched at the current intensity of 100 mA/cm^2 for 15 min. Subsequently, freshly-prepared porous silicon samples were fractured into PSiNPs after 3 h ultrasonication in ethanol. After 5 min microwave heating at 120 °C in ethanol, 1 mg PSiNPs were immersed in 1 mL toluene containing 20 μL 3-aminopropyl triethoxysilane (APTES, Sigma-Aldrich Chemicals, USA), microwave heated at 80 °C for 1 h, and then washed three times with ethanol to fabricate NH_2-PSiNPs. 1 mg NH_2-PSiNPs was immersed in 1 mL IR820 dyes aqueous solution (250 μg/mL, Sigma-Aldrich Chemicals, USA) at room temperature for 30 min, and then repeatedly washed with DI water to prepare IR820/NH_2-PSiNPs. Subsequently, 1 mg IR820/NH_2-PSiNPs was incubated in 1 mL DOX aqueous solution (200 μg/mL, Sigma-Aldrich Chemicals, USA) at room temperature for 20 h, and then repeatedly washed with water to prepare DOX/IR820/NH_2-PSiNPs. In addition, 1 mg NH_2-PSiNPs was directly immersed in 1 mL DOX aqueous solution (200 μg/mL) at room tem-